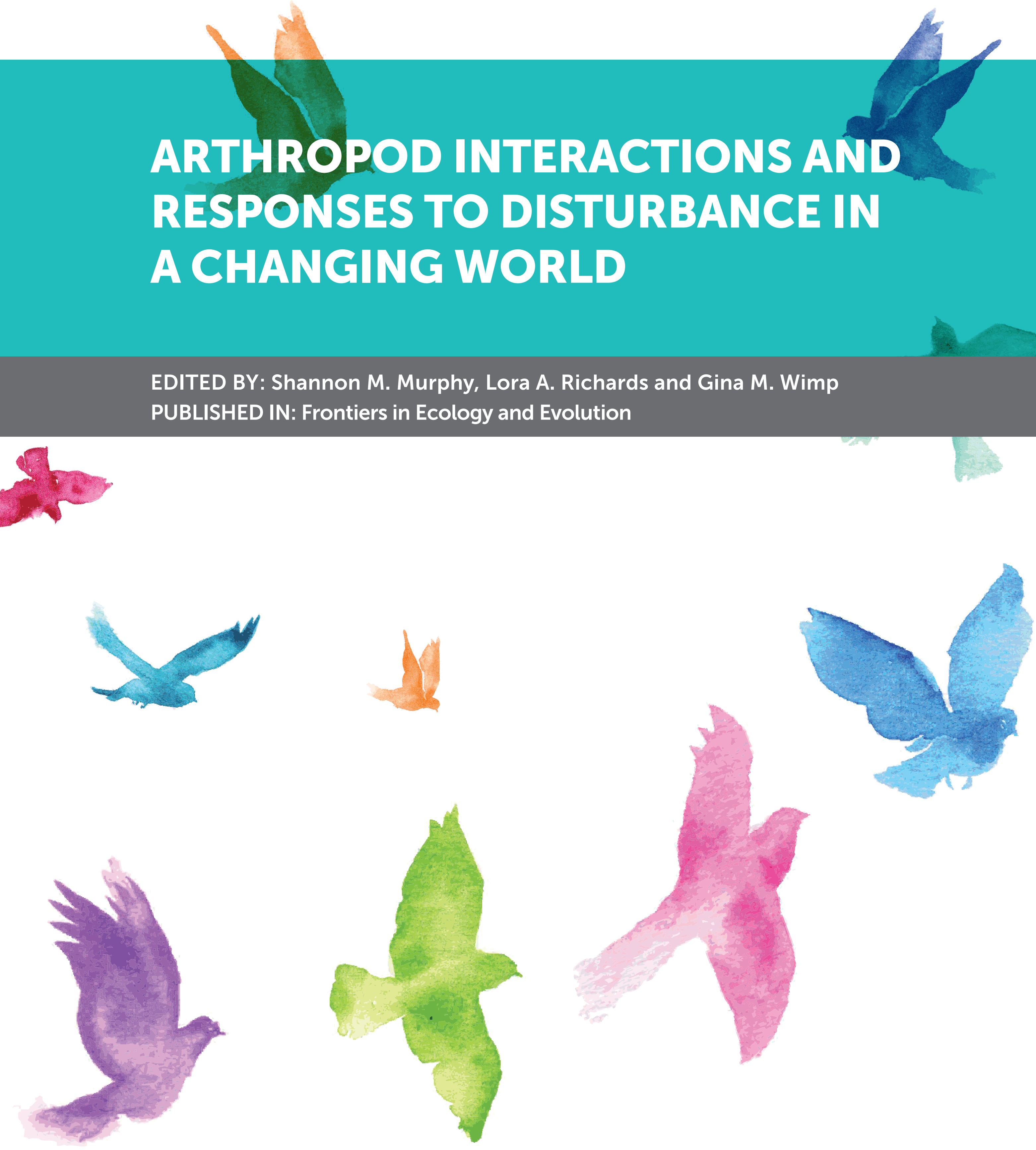
ARTHROPOD INTERACTIONS AND RESPONSES TO DISTURBANCE IN A CHANGING WORLD

EDITED BY: Shannon M. Murphy, Lora A. Richards and Gina M. Wimp
PUBLISHED IN: Frontiers in Ecology and Evolution



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88963-759-1
DOI 10.3389/978-2-88963-759-1

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

ARTHROPOD INTERACTIONS AND RESPONSES TO DISTURBANCE IN A CHANGING WORLD

Topic Editors:
Shannon M. Murphy, University of Denver, United States
Lora A. Richards, University of Nevada, Reno, United States
Gina M. Wimp, Georgetown University, United States

Citation: Murphy, S. M., Richards, L. A., Wimp, G. M., eds. (2020). Arthropod Interactions and Responses to Disturbance in a Changing World. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88963-759-1

Table of Contents

frontiers
in Ecology and Evolution

EDITORIAL
published: 14 April 2020
doi: 10.3389/fevo.2020.00093

Editorial: Arthropod Interactions and Responses to Disturbance in a Changing World

Shannon M. Murphy[1]*, *Lora A. Richards*[2] and *Gina M. Wimp*[3]

[1] *Department of Biological Sciences, University of Denver, Denver, CO, United States,* [2] *Department of Biology, University of Nevada, Reno, NV, United States,* [3] *Department of Biology, Georgetown University, Washington, DC, United States*

Keywords: disturbance, global change, species interactions, land-use change, wildfire, climate, range expansions, invasion

Editorial on the Research Topic

Arthropod Interactions and Responses to Disturbance in a Changing World

INTRODUCTION

Global environmental change is affecting insect communities worldwide. Recently there has been a debate in the literature about whether insect populations are declining precipitously as an "insect apocalypse" (Hallmann et al., 2017; Lister and Garcia, 2018; Sanchez-Bayo and Wyckhuys, 2019; Salcido et al., 2020). However, some have argued that the insect apocalypse is an overstatement, since supporting research has been restricted to specific sites and/or does not account for other disturbances (e.g., Thomas et al., 2019; Willig et al., 2019). In order to understand whether and how insect populations are changing over time, we need to understand the mechanistic drivers of declining insect diversity. Each of the articles in this issue examines how species interactions, diversity, and community composition are changing as a result of anthropogenic disturbance. Understanding the impacts of global change on population dynamics, ecological communities, biodiversity, and ecosystem processes thus requires a multi-trophic perspective.

LAND-USE CHANGE

Natural vegetation on every continent except Antarctica has been removed by human activities, leaving fragmented patches of suitable habitat across the landscape (Saunders et al., 1991). While human activities are negatively impacting biodiversity via multiple mechanisms, habitat fragmentation is widely considered to be the primary factor leading to species extinction worldwide (Wilson, 2002). Notably, habitat change is thought to be the primary driver of what some authors describe as the "insect apocalypse" (e.g., Sanchez-Bayo and Wyckhuys, 2019). Species that are dependent on natural habitats may be impacted not only by the size of habitat fragments, but also by increasing isolation among patches and altered species interactions along habitat edges (Debinski and Holt, 2000; Fahrig, 2003; Ewers and Didham, 2006; Murphy et al., 2016). While the focus of land use change has primarily been on species in natural habitats, managed habitats constitute roughly 34% of total land mass (Ramankutty et al., 2008). More importantly, ∼45% of insect species losses are thought to be the result of intensified agriculture, deforestation, and urbanization, while practices associated with agriculture, such as pesticide and fertilizer use, are thought to account for another ∼23% of insect species losses (Sanchez-Bayo and Wyckhuys, 2019). Thus, managed habitats and the practices associated with such management could account for two-thirds of insect declines. It is therefore essential that we understand how different land use practices may impact biodiversity and ecosystem services.

Edited and reviewed by:
Dennis Murray,
Trent University, Canada

****Correspondence:***
Shannon M. Murphy
shannon.m.murphy@du.edu

Specialty section:
This article was submitted to Population and Evolutionary Dynamics, a section of the journal Frontiers in Ecology and Evolution

Received: *18 February 2020*
Accepted: *20 March 2020*
Published: *14 April 2020*

Citation:
Murphy SM, Richards LA and Wimp GM (2020) Editorial: Arthropod Interactions and Responses to Disturbance in a Changing World. Front. Ecol. Evol. 8:93. doi: 10.3389/fevo.2020.00093

Organisms at higher trophic levels often display higher sensitivity to land use change. While habitat modifications are known to be a primary driver of species losses, fragmentation differentially impacts species with different life histories, trophic strategies, and levels of resource specialization. Organisms at higher trophic levels are often more susceptible to resource fragmentation because they are dependent on dynamics at lower trophic levels (Holt et al., 1999), however the level of susceptibility can be dependent on diet breadth. Anderson et al. found that while caterpillars were not affected by the size of a forest patch, resource specialization in the caterpillar prey affected higher trophic levels. In fragmented habitats, parasitism on specialist caterpillars decreased, but the authors found no change in parasitism on generalist caterpillars. Thus, resource specialization leads to stronger responses to habitat fragmentation in prey, with subsequent effects on their predators and parasites. Not only does habitat alteration affect herbivore natural enemies, but it can also affect native pollinator communities, with consequences for pollination services. While agricultural intensification can reduce the resources necessary for native pollinators, Kremen et al. found that the creation of native-plant hedgerows may mitigate these impacts on native pollinators. Kremen et al. found that native, perennial hedgerows provide floral and nesting resources necessary to native pollinators, particularly as the hedgerows age. While agricultural intensification can lead to the loss of beneficial species such as natural enemies and pollinators, such intensification can also lead to herbivore outbreaks. Such outbreaks have long been associated with extensive monocultures used in farming, but Le Gall et al. present compelling evidence that locust swarming is exacerbated by grazing practices, not cropland practices.

Even when habitats are not directly modified, nutrient runoff and pollution from agriculture and urbanization can alter natural communities. For example, Wimp et al. found nutrient subsidies at a landscape-level alter the trophic structure of the arthropod community by changing the relative abundances of various feeding groups. Herbivores had a negative response to increasing plant nitrogen density; specialist predators tracked their herbivore prey and thus also responded negatively to nitrogen density. However, generalists were not negatively affected by nitrogen density and indeed some generalist predators responded positively to nitrogen density. Such inputs of nutrients from agricultural and urban habitats into nearby natural systems are increasing, and differential responses by organisms with different feeding strategies and diet breadth can alter community composition.

While urbanization can affect organisms in nearby natural systems, arthropods living within cities are directly affected by human activities. The review by Miles et al. highlights the numerous abiotic factors that are altered in urban habitats (e.g., urban heat island effects, chemical and light pollution, and water availability), which in turn affect plant resources and herbivores. For example, salt is used for road deicing in the winter at northern latitudes, but soil salinization can decrease the ability of plants to take-up water from the soil. Bouraoui et al. found that salt pollution negatively impacted the growth and physiology of lime trees growing in urban greenspaces, which in turn negatively impacted arthropod biodiversity. Herbivores are thus directly affected by altered abiotic conditions in urban habitats, and indirectly via changes in their host plant. According to Miles et al., such alterations to critical resources not only affect herbivore population dynamics, but also interactions with predators. Because abiotic conditions and biotic interactions may be altered in urban habitats, Jamieson et al. argue the large number of bee samples collected near universities may lead to a distorted view of worldwide pollinator resources and biodiversity patterns. Such biased sampling may also affect our overall view of human impacts on arthropod biodiversity.

WILDFIRE

Climate change is known to be altering global fire regimes, primarily because of changes in precipitation regimes. However, how these increasingly frequent and severe fires are affecting species interactions and eco-evolutionary processes is not well-understood and there is a clear need for experimental work (Koltz et al., 2018). Further, there are entire taxa for which we know very little about how they respond to altered fire regimes, such as native bees, particularly because fire could impact resources they use for nesting or plants they pollinate. Burkle et al. addressed these critical knowledge gaps by studying the abundance and diversity of native bees, their floral and nesting resources, solitary bee nesting success, and traits of bees and plants in burned and unburned areas of three separate wildfires in Montana, USA. Their study is unique as they simultaneously measured not only the abundance and diversity of two interacting trophic levels important for pollination services, but also bee nesting success and trait variation, which are critical for understanding plant-pollinator interactions after wildfire. Burkle et al. found higher density and diversity of native bees and floral resources in burned areas, including areas that burned with high-severity wildfires, for two of the three wildfires that they studied. Notably, they found that nesting resources, such as bare ground and coarse woody debris, were higher in burned areas, and nesting success of solitary bees was also higher in burned areas. The results from Burkle et al. demonstrate that even large, high-severity wildfires can create conditions that support native bees and the resources they need to thrive, but unburned areas help maintain critical trait variation. Thus, having a landscape that is a mosaic of burned and unburned areas helps to conserve biodiversity of native bees.

Dell et al. used empirical metrics to quantify redundancy and resiliency in the fire-dependent longleaf pine ecosystem through measures of interaction diversity pre and post-fire to support the idea of response diversity (sensu, Elmqvist et al., 2003). While the concept of interaction diversity has been discussed for decades, especially in the context of conservation and global change, Dell et al. are the first to use empirical data to quantify interaction diversity at ecologically relevant scales to examine its role in resiliency. This was accomplished by recording interaction diversity parameters across a time since fire gradient as well as at hierarchical spatial scales. They found that local scale patterns of interaction diversity are associated with short-term resilience and broader scale patterns confer longer-term resilience. These findings are important, because they demonstrate that fire not only maintains species diversity but it also maintains the important interactions that contribute to ecosystem function and services, such as biological control. As global change continues to alter disturbance cycles as well as species richness, these relationships between disturbance and diversity are important to understand. Murphy et al. focused specifically on how fire severity affects insect-plant interactions, which are key drivers of forest ecosystem dynamics. Using wild forest fires of variable severity in Colorado, USA, they investigated how fire severity affects herbivore damage and plant quality. Murphy et al.. found that increasing fire severity decreased herbivore damage and altered plant quality by lowering water content, increasing C:N ratio, and increasing toughness. Interestingly, they found that the direct effect of fire was stronger than the plant-mediated effect on herbivore damage. The results from Murphy et al.. demonstrate that fire severity can have profound impacts on interactions between herbivores and their host plants with severity significantly affecting host plant quality. Understanding the outcomes of these complex interactions will be critical in order to predict the effects of increasing fire severity and frequency on communities in response to global climate change.

TEMPERATURE AND PRECIPITATION

Long term deviations in temperature and precipitation from historical patterns is the most direct effect of climate change on arthropods. Recent attention to insect declines has highlighted the negative consequences of increasing temperature and precipitation anomalies on insect species richness as well as the associated multitrophic interactions (Salcido et al., 2020). Arthropod life cycles and community composition are closely timed with seasonal climate patterns, leading to high susceptibility of arthropod populations changes in these patterns. Through long term continuous data collections, we are beginning to understand the underpinnings of climate variability on arthropod populations. Marquis et al. combined 20 years of caterpillar data on oak trees in southeastern Missouri to find that spring frosts and summer droughts caused a 62–99% decrease in caterpillar abundance. The magnitude of the effect varied according to caterpillar body size, feeding guild, and the type of weather event. However, the strength of these findings lie in their ability to monitor insect recovery after the extreme weather event, with most populations recovering in 1–5 years. This insight will increase our ability to make predictions on population resilience under future climate change models. Similar to oak tree caterpillar communities, Abarca et al. found that the timing of extreme weather events, in this case heatwaves, can have devastating effect on the populations of the Baltimore Checkerspot (*Euphydryas phaeton phaeton*). While summer heatwaves, which often reach an organism's critical thermal maximum, would intuitively have detrimental effects on the populations, surprisingly winter heatwaves during the overwintering life stage were the most damaging, with 75–100% mortality. Interestingly, larvae feeding on a native host plant had greater body mass and were better able to survive summer heatwaves compared to larvae feeding on an introduced host. As the frequency of extreme weather events are predicted to increase, long term data, and mechanistic life stage specific experiments have become increasingly important in our understanding of the consequences of climate change on insect diversity and abundance. Beyond weather anomalies, changes in the timing and duration of seasons can alter the phenological timing of arthropod emergence and potentially create an asynchrony with food availability (Visser and Gienapp, 2019). For pollinators, asynchrony and arthropod declines can cascade through the ecosystem as they provide fundamental services to their community. With this in mind, Slominski and Burkle combined field and lab based approaches to determine how temperature and season length influence mortality, phenology, and body condition of two solitary bee species. Their results suggest that the adult-wintering species may suffer higher mortality and reduced fitness under climate change due to earlier spring onset. Additionally, differences in sex-specific responses may alter sex ratios further contributing to population declines.

Changes in temperature and precipitation can have far reaching effects on the interactions between individuals, within species and across trophic levels. Macchiano et al. investigated sex-specific thermal responses in courtship activity of two sympatric species of treehoppers. They found both species-specific and sex-specific differences. For one species, male and female courtship activity changed similarly with temperature with females more likely to signal. In the other species, male and female activity responded differently, with females more likely to display signaling at very lowest and highest temperatures and males signaling more at mid temperatures. These results suggest that mating may be constrained in response to changing temperatures. Climate effects on the interactions between species, such as between hosts and parasitoid, have expanded our understanding of the consequences of altering population control mechanisms (e.g., Stireman et al., 2005). However, very little is known about these interactions in the Arctic, which is rapidly warming. Koltz et al. leveraged 18 years of data found in museum samples to investigate wolf spider egg sac parasitism in the Arctic. They found that parasitism was common in the southern site and not found in the northern site. In the south, the highest parasitism frequency was during the peak of the growing season, suggesting that the timing and window of vulnerability may shift as the Arctic warms. Trophic interactions also make understanding species responses to climate change more complex. In their review, Szczepaniec and Finke summarize the role of drought on a tripartite interaction between plants, pathogens, and insect vectors. Increasing incidence of droughts can increase a plant's susceptibility to pathogens due to cross talk between the signaling pathway of salicylic acid and the hormone abscisic acid, with some exceptions. Although there was no overall pattern in sap feeding insect performance on plants with a water deficit, changes in feeding behavior had indirect effects on pathogen transmission. More studies are needed that combine molecular, behavioral, and ecological techniques to further understand the mechanisms and consequences of drought on plant-pathogen-insect vector interactions and the implications for agroecosystems and pest outbreaks.

RANGE EXPANSIONS AND INVASIONS

Since Margaret Davis' pioneering work on North American plant range shifts after the last glaciation (e.g., Davis, 1981; Davis and Shaw, 2001), we have known that species can move in response to changing climatic conditions. However, the rate at which species are shifting their ranges currently and the rate at which climate envelopes are predicted to change in the near future is unprecedented (IPCC, 2014). Many species are shifting their ranges poleward (e.g., Parmesan et al., 1999) or upwards in elevation (e.g., Parmesan and Yohe, 2003), but novel species interactions may constrain range shifts (e.g., Jankowski et al., 2010). Further, not all species are moving poleward, but instead are shifting their ranges to maintain their climatic niche (e.g., VanDerWal et al., 2013). Further, range expansions propagated by human introductions of exotic species can significantly affect native ecological communities (Mooney and Hobbs, 2000).

Global change drivers that affect species range boundaries offer a new opportunity to study the evolution of species boundaries over rapid evolutionary timescales. This is particularly true for insects, whose geographic ranges, behaviors and life history traits are temperature dependent. Larson et al. review the potential for climate change to influence gene flow and species boundaries between closely related insect species, focusing on studies that have tracked changes in climate and insect distributions and/or have evaluated temperature dependent reproductive barriers between species. Manfredini et al. ask the question of why some social insects have become invasive species (e.g., fire ants and yellowjackets) while other social insects are in decline (e.g., bee pollinators). Why are some species thriving while others are on the brink of extinction? Social insects form highly cooperative colonies, characterized by different castes of individuals (such as queens and workers). These insects are renowned for their amazing flexibility in terms of their ability to produce different castes as well as adjust their behavior to meet colony needs. In their review, Manfredini et al. explore whether differences in their ability to flexibly adapt to their environments (termed phenotypic plasticity) can help to explain both the invasive nature and decline of different groups of social insects. Social insects are not the only introduced species with significant effects on ecosystems. Exotic plant species can have large impacts on ecological communities by altering plant-herbivore interactions. The study by Carper et al.. suggests that the impacts of novel host chemistry across larval development are likely complex and dependent on variation in host plant chemistry, and on stage-specific relationships between sequestration and defense. This research represents some of the first steps to understanding the mechanisms driving tradeoffs in defense strategies across caterpillar development, and how introduced hosts impact tritrophic interactions.

CONCLUSIONS

Collectively the papers in our Research Topic explore some of the many ways in which species interactions, diversity, and community composition are changing as a result of anthropogenic disturbance. One of our goals with our Research Topic was to highlight research from a diverse group of authors, and we feel that we have succeeded. Authors in our topic include not only professors across ranks from Assistant to Full, but also undergraduate students (7%), graduate students (18%), post-doctoral fellows (15%), and scientists not found in traditional academic positions (14%). Women in particular are often underrepresented in invited research forums (e.g., Nittrouer et al., 2018; Emma et al., 2019) and we are proud that the contributions to our Research Topic represent a balance between male and female contributing authors (55% of our authors identify as female and comprise 68% of our first authors and 53% of our last authors). While currently unusual, this kind of diversity and inclusion in STEM is imperative as we try to understand the new facets of how anthropogenic global change is affecting natural systems. Groups formed by diverse participants are effective at problem solving (Hong and Page, 2004) and thus the inclusion of a diverse pool of scientists will help us further our understanding of the possible impacts of global change. The publication of this volume will inform and hopefully stimulate more studies on how anthropogenic disturbances are changing arthropod interactions.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

Davis, M. B. (1981). "Quaternary history and the stability of forest communities," in *Forest Succession*, eds D. C. West, H. H. Shugart, and D. B. Botkin (New York, NY: Springer), 132–153. doi: 10.1007/978-1-4612-5950-3_10

Davis, M. B., and Shaw, R. G. (2001). Range shifts and adaptive responses to Quaternary climate change. *Science* 292, 673–679. doi: 10.1126/science.292.5517.673

Debinski, D. M., and Holt, R. D. (2000). A survey and overview of habitat fragmentation experiments. *Conserv. Biol.* 14, 342–355. doi: 10.1046/j.1523-1739.2000.98081.x

Elmqvist, T., Folke, C., Nystrom, M., Peterson, G., Bengtsson, J., Walker, B., et al. (2003). Response diversity, ecosystem change, and resilience. *Front. Ecol. Environ.* 1, 488–494. doi: 10.1890/1540-9295(2003)001[0488:RDECAR]2.0.CO;2

Emma, G. T., Jayabalasingham, B., Collins, T., Geertzen, J., Bui, C., and Dominici, F. (2019). Gender disparities in invited commentary authorship in 2459 medical journals. *JAMA Network Open* 2:e1913682. doi: 10.1001/jamanetworkopen.2019.13682

Ewers, R. M., and Didham, R. K. (2006). Confounding factors in the detection of species responses to habitat fragmentation. *Biol. Rev.* 81, 117–142. doi: 10.1017/S1464793105006949

Fahrig, L. (2003). Effects of habitat fragmentation on biodiversity. *Ann. Rev. Ecol. Syst.* 34, 487–515. doi: 10.1146/annurev.ecolsys.34.011802.132419

Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., et al. (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS ONE* 12:e0185809. doi: 10.1371/journal.pone.0185809

Holt, R. D., Lawton, J. H., Polis, G. A., and Martinez, N. D. (1999). Trophic rank and the species–area relationship. *Ecology* 80, 1495–1504. doi: 10.1890/0012-9658(1999)080[1495:TRATSA]2.0.CO;2

Hong, L., and Page, S. E. (2004). Groups of diverse problem solvers can outperform groups of high-ability problem solvers. *Proc. Natl. Acad. Sci. U.S.A.* 101, 16385–16389. doi: 10.1073/pnas.0403723101

IPCC (2014). Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, eds Core Writing Team, R. K. Pachauri, and L. A. *Meyer (Geneva: IPCC)*, 151.

Jankowski, J. E., Robinson, S. K., and Levey, D. J. (2010). Squeezed at the top: interspecific aggression may constrain elevational ranges in tropical birds. *Ecology* 91, 1877–1884. doi: 10.1890/09-2063.1

Koltz, A. M., Burkle, L. A., Pressler, Y., Dell, J. E., Vidal, M. C., Richards, L. A., et al. (2018). Global change and the importance of fire for the ecology and evolution of insects. *Curr. Opin. Insect Sci.* 29, 110–116. doi: 10.1016/j.cois.2018.07.015

Lister, B. C., and Garcia, A. (2018). Climate-driven declines in arthropod abundance restructure a rainforest food web. *Proc. Natl. Acad. Sci. U.S.A.* 115, E10397–E10406. doi: 10.1073/pnas.1722477115

Mooney, H. A., and Hobbs, R. J. (2000). *Invasive Species in a Changing World*. Washington, DC: Island Press.

Murphy, S. M., Battocletti, A. H., Tinghitella, R. M., Wimp, G. M., and Ries, L. (2016). Complex community and evolutionary responses to habitat fragmentation and habitat edges: what can we learn from insect science? *Curr. Opin. Insect Sci.* 14, 61–65. doi: 10.1016/j.cois.2016.01.007

Nittrouer, C. L., Hebl, M. R., Ashburn-Nardo, L., Trump-Steele, R. C. E., Lane, D. M., and Valian, V. (2018). Gender disparities in colloquium speakers at top universities. *Proc. Natl. Acad. Sci. U.S.A.* 115, 104–108. doi: 10.1073/pnas.1708414115

Parmesan, C., Ryrholm, N., Stefanescu, C., Hill, J. K., Thomas, C. D., Descimon, H., et al. (1999). Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature* 399, 579–583. doi: 10.1038/21181

Parmesan, C., and Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421, 37–42. doi: 10.1038/nature01286

Ramankutty, N., Amato, T. E., Monfreda, C., and Foley, J. A. (2008). Farming the planet: 1. Geographic distribution of global agricultural lands in the year 2000. *Glob. Biogeochem. Cycles* 22:GB1003. doi: 10.1029/2007GB002952

Salcido, D. M., Forrister, M. L., Garcia Lopez, H., and Dyer, L. A. (2020). Loss of dominant caterpillar genera in a protected tropical forest. *Sci. Rep*. 10, 422–432. doi: 10.1038/s41598-019-57226-9

Sanchez-Bayo, F., and Wyckhuys, K. A. G. (2019). Worldwide decline of the entomofauna: a review of its drivers. *Biol. Conserv.* 232, 8–27. doi: 10.1016/j.biocon.2019.01.020

Saunders, D. A., Hobbs, R. J., and Margules, C. R. (1991). Biological consequences of ecosystem fragmentation: a review. *Conserv. Biol.* 5, 18–32. doi: 10.1111/j.1523-1739.1991.tb00384.x

Stireman, J. O., Dyer, L. A., Janzen, D. H., Singer, M. S., Lill, J. T., Marquis, R. J., et al. (2005). Climatic unpredictability and parasitism of caterpillars: Implications of global warming. *Proc. Natl. Acad. Sci. U.S.A.* 102, 17384–17387. doi: 10.1073/pnas.0508839102

Thomas, C. D., Jones, T. H., and Hartley, S. E. (2019). "Insectageddon": a call for more robust data and rigorous analyses. *Glob. Change Biol.* 25, 1891–1892. doi: 10.1111/gcb.14608

VanDerWal, J., Murphy, H. T., Kutt, A. S., Perkins, G. C., Bateman, B. L., Perry, J. J., et al. (2013). Focus on poleward shifts in species' distribution underestimates the fingerprint of climate change. *Nat. Clim. Change* 3, 239–243. doi: 10.1038/nclimate1688

Visser, M. E., and Gienapp, P. (2019). Evolutionary and demographic consequences of phenological mismatches. *Nat. Ecol. Evol.* 3, 879–885. doi: 10.1038/s41559-019-0880-8

Willig, M. R., Woolbright, L., Presley, S. J., Schowalter, T. D., Waide, R. B., Scalley, T. H., et al. (2019). Populations are not declining and food webs are not collapsing at the Luquillo Experimental Forest. *Proc. Natl. Acad. Sci. U.S.A.* 116, 12143–12144. doi: 10.1073/pnas.1820456116

Wilson, E. O. (2002). *The Future of Life*. New York, NY: Vintage Books.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 12 September 2018
doi: 10.3389/fevo.2018.00135

Forest Fire Severity Affects Host Plant Quality and Insect Herbivore Damage

Shannon M. Murphy, Mayra C. Vidal†, Timothy P. Smith, Claudia J. Hallagan, E. Dale Broder†, Dexter Rowland and Laurel C. Cepero*

Department of Biological Sciences, University of Denver, Denver, CO, United States

Edited by:
Pavel Kindlmann,
Charles University, Czechia

Reviewed by:
Karolína Bílá,
Global Change Research Institute,
Czech Academy of Sciences, Czechia
Zdenka Krenova,
Global Change Research Institute,
Czech Academy of Sciences, Czechia

***Correspondence:**
Shannon M. Murphy
shannon.m.murphy@du.edu

†Present Address:
Mayra C. Vidal,
Department of Biology, Syracuse University, Syracuse, NY, United States
E. Dale Broder,
Department of Biology, St. Ambrose University, Davenport, IA, United States

Specialty section:
This article was submitted to Population and Evolutionary Dynamics,
a section of the journal Frontiers in Ecology and Evolution

Received: *27 June 2018*
Accepted: *22 August 2018*
Published: *12 September 2018*

Citation:
Murphy SM, Vidal MC, Smith TP, Hallagan CJ, Broder ED, Rowland D and Cepero LC (2018) Forest Fire Severity Affects Host Plant Quality and Insect Herbivore Damage. Front. Ecol. Evol. 6:135. doi: 10.3389/fevo.2018.00135

Climate change models predict increased forest fire occurrence and severity in the near future. Forest fire disturbance affects multiple ecological interactions, but there is little evidence for how naturally-occurring fires affect plant quality and herbivore damage, which is important because plants and herbivorous insects comprise most of the diversity in natural ecosystems and are responsible for a variety of ecosystem services. We surveyed three fires in the Rocky Mountains to investigate the effects of fire severity on wax currant (*Ribes cereum*), an important source of food and cover for wildlife in Colorado. We measured plant quality and herbivore damage; we found that fire severity had a significant negative effect on both measures. Notably, high severity fires decreased herbivore damage by about 50%. Furthermore, we found that the effect of fire on insect herbivore damage is mostly direct, but that indirect effects mediated through changes in plant quality are also significant. Our results have important implications for the effects of climate-driven increases in fire severity on plant-insect interactions, illustrating strong direct and weaker indirect negative effects of fire severity in a forest ecosystem.

Keywords: fire severity, herbivore damage, Lepidoptera, plant quality, *Polygonia gracilis zephyrus*, Rocky Mountains, wax currant, *Ribes cereum*

INTRODUCTION

Forest fires affect ecological interactions, population and community structure, as well as nutrient cycling, and thereby act as an important disturbance that shapes ecosystems (Veblen et al., 1994; Van Langevelde et al., 2003; Kay et al., 2008). Fires can significantly change an organism's habitat including the availability of food, water, and shelter, and these habitat alterations can drastically affect the animal communities in that area (Koltz et al., 2018). It is important to understand how community interactions are affected by fire because fire activity is predicted to increase in the near future due to global climate change (Flannigan et al., 2000). Climate change models project a 25–50% increase in the area burned in the United States through the year 2100, and an increase in fire severity of 10–50% across much of the US by 2060. As annual temperature increases due to climate change, more fires will occur, which will lead to changes in forest assembly (Flannigan et al., 2000; Dale et al., 2001; Clark et al., 2017). For example, lower montane forests are predicted to experience a lower frequency of fires that are more severe (Rocca et al., 2014). Changes in severity and frequency of forest fires may lead to more appreciable changes in an ecosystem. However, few studies so far investigated how fire severity might affect insect-plant interactions, which are a key driver of forest ecosystem dynamics.

Fire affects herbivores including mammalian grazers (Green et al., 2015) and arthropods (Knight and Holt, 2005), but the impacts of fire on arthropod communities are especially important to understand since arthropod herbivores are a key driver of forest ecosystems. For example, insects play important roles in shaping succession and biodiversity (McCullough et al., 1998), nutrient cycling and ecosystem processes (Kay et al., 2008), and even restructuring pollinator networks (Peralta et al., 2017). Fire may reduce arthropod abundance (in spittlebugs, Martin et al., 1999; in caterpillars, Diniz et al., 2011) or have no effect (Christie and York, 2009). But more often, fire can have positive effects on arthropods, increasing abundance and affecting community structure (in spruce beetle, Bebi et al., 2003; in ground beetles, Gandhi et al., 2008; in butterflies, Scandurra et al., 2014; in grasshoppers, Joubert et al., 2016). These effects depend on the arthropods and plants studied, the type of fire (e.g., wildfire, prescribed, surface, crown, etc. McCullough et al., 1998) as well as the frequency and severity of the fire disturbance (Schowalter, 2012). The complexity of these outcomes is further confounded by species interactions; for example, fire alone may not affect insect communities, but fire in conjunction with grazing mammals can reduce arthropod richness and abundance (Bailey and Whitham, 2002; Jonas and Joern, 2007) and can interact with time since fire to reduce density of a pollinator species (Moranz et al., 2014).

Fire may also indirectly affect herbivores by altering the plants they consume (Wan et al., 2014); thus it is critical that we understand how fire affects plants. First, fire directly changes biomass and size structure of the plant community (Higgins et al., 2007). Fire also shapes plant communities; low-severity fires boost plant species abundance and richness while high-severity fires may have the opposite effect (Pourreza et al., 2014). Fire can also affect plant growth, for example causing trees to produce more leaves (Lopes and Vasconcelos, 2011). Important for herbivores, fire also changes host plant quality (e.g., increasing crude protein content in leaves, Greene et al., 2012). While fire does not always change plant chemistry (Christie and York, 2009), fire can affect soil properties like soil carbon and water holding capacity (Kitzberger et al., 2005), which can affect plant nutrient content (Huang and Boerner, 2007). In some cases, fire causes plants to increase chemical defenses against herbivores (Wan et al., 2014).

While fire directly shapes plant communities, insect herbivores are in turn affected by their host plants. Plant community composition affects the herbivore community (Haddad et al., 2001) and herbivore density after fire is positively correlated with plant cover (e.g., Hahn and Orrock, 2015). Host plant quality is perhaps most important for our understanding of how fire, plants, and herbivores interact. Host plant quality directly affects insect herbivore fitness (Awmack and Leather, 2002); a recent meta-analysis showed that insect herbivores have greater fitness on better quality plants (Vidal and Murphy, 2018). Plant quality may also affect immune defense (Klemola et al., 2007), insect life history traits like whether to enter diapause (Hunter and McNeil, 1997), and even the distribution of insect herbivores (Egan and Ott, 2007). Plant quality can influence herbivores through three common measures of quality: toughness, water content and ratio of carbon to nitrogen (C:N) in leaves. For instance, a generalist herbivore was found to have lower pupal mass when reared on tougher leaves (Barnes and Murphy, 2018), and tougher leaves usually have fewer digestible components, which can inhibit larval feeding (Feeny, 1970). Herbivory seems to be positively related to water content (Faeth, 1985), possibly because low water content can make leaves less succulent and attractive to insect herbivores (Feeny, 1970). Finally, insect herbivores are usually limited by the nitrogen content of their host plants, and thus a lower C:N ratio corresponds with higher insect fitness (Mattson, 1980).

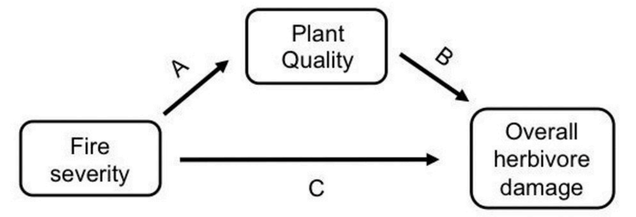


FIGURE 1 | A conceptual model of how herbivore damage may be affected by fire severity either indirectly via changes in host plant quality (i.e., fire severity directly affects host plant quality **[path A]**, which then affects herbivore damage **[path B]**), or directly via changes to the habitat **[path C]**.

As herbivores are directly affected by plant quality traits, the effect of fire on insect herbivores could be direct or indirectly mediated by changes in plant quality (**Figure 1**). Fire is known to affect host plant quality (**Figure 1** path A, e.g., McCullough et al., 1998; Kay et al., 2007, 2008; Greene et al., 2012; Wan et al., 2014; Hood et al., 2015), and host plant quality affects herbivore fitness, abundance, and diversity (**Figure 1** path B, e.g., Vidal and Murphy, 2018, and references therein). However, these indirect effects of fire on herbivores via plant quality are not well understood (but see Christie and York, 2009; Kim and Holt, 2012; Hahn and Orrock, 2015). Additionally, fire may directly affect insects, either positively or negatively, through fire or heat mortality or through changes in habitat structure (**Figure 1** path C, e.g., Kim and Holt, 2012). All of the studies that have tried to tease apart the direct and indirect effects of fire on insects have studied prescribed fires, not wildfires. For example, Vogel et al. (2010) found that the indirect effect of fire through changes in vegetation composition had a stronger impact on the abundance of prairie butterflies than the direct impact of fire. Kim and Holt (2012) found that fire affected insect communities indirectly through change in habitat structure instead of directly via changes in host plant quality, and Kay et al. (2007) found a strong indirect effect of fire through canopy openness. These studies used time since fire or fire frequency to explore the variable effects of fire, but prescribed fires are generally less severe than wildfires (e.g., Dooley and Treseder, 2012; Stephan et al., 2015). Given that fire severity is projected to increase with climate change (Flannigan et al., 2000; Dale et al., 2001; IPCC, 2014; Rocca et al., 2014; Clark et al., 2017), it is important to understand if severity influences herbivores directly, or indirectly via plant quality or habitat structure.

It remains untested if fire severity affects host plant quality and herbivore damage using wildfires, which vary significantly in severity from prescribed fires. We studied three wildfires in the Colorado Rocky Mountains and measured herbivore damage on a dominant understory plant in these forests, wax currant (*Ribes cereum*), which occurs in unburned, low severity, and high severity burn areas of each fire that we studied. We measured herbivore damage caused by all chewing herbivores as well as by a specific herbivore, the Zephyr Anglewing butterfly (*Polygonia gracilis zephyrus*; hereafter *Polygonia*). As *Polygonia* larvae feed on their host plant, they leave behind a unique stripping pattern that is easily identifiable (Stout, 2008), which allowed us to differentiate their damage from damage by other herbivores. Here we test if fire severity affects herbivore damage directly (**Figure 1** path C) or indirectly through change in host plant quality (**Figure 1** paths A,B) in order to answer the important question of how fire severity affects insect herbivores.

METHODS

Study Location and Site Selection

We surveyed three forest fires within Pike National Forest in the Front Range of Colorado that burned between 1996 and 2002: Buffalo Creek (1996), High Meadows (2000), and Hayman (2002). We surveyed plants for herbivore damage and collected samples to measure plant quality in July 2016, so time since burn varied from 16 to 22 years. Our study sites were located in a subalpine forest ecosystem dominated mainly by Douglas fir and Ponderosa pine trees with mixed vegetation in the low herbaceous layer that included our focal host plant, wax currant (*R. cereum*). Wax currant plants are usually killed by fires, but seeds in the soil benefit from fire and new plants germinate well, especially after short duration, low severity fires (Marshall, 1995). Wax currants sprout quickly after fires and as they are an alternate host for white pine blister rust (*Cronartium ribicola*), their recovery from fire is thought to be important to the pathology of this rust and its implications on white pine (Zambino, 2010). Wax currant is a useful indicator species of historical fire regime along the Front Range (Keith et al., 2010).

To assess the impact of fire severity on plant quality and herbivore damage, we identified three regions within each fire that varied in fire severity: unburned, low severity, and high severity. We determined fire severity using data overlays provided by the United States Forest Service (MTBS Data Access: Fire Level Geospatial Data, 2016, April-last revised). Each of the fires was heterogeneous with regard to fire severity (**Figure 2**) and thus we were able to select sites that were interspersed so that severity types were not clustered together geographically. We visited each site to ensure that the site matched the severity determined by satellite imagery. We characterized high severity sites as completely burned with mostly fallen trees, few standing trees, and little to no canopy cover. We characterized low severity sites as having some standing, scorch-marked trees, and some fully burned fallen trees. We characterized unburned sites as sites with heavy canopy cover and no evidence of burn damage. We selected new sites if, after ground-truthing, the sites did not reflect these characteristics. However, for both the Hayman and High Meadows fires we could find only two unburned sites that had wax currant plants or that did not have active cattle grazing; thus, we had a total of 25 sampled sites (**Table 1**).

Host Plant Density

We surveyed the density of wax currant plants at each site by haphazardly choosing a focal wax currant plant that was located at the approximate center of the site. We then used a transect tape to measure 25 m in each cardinal direction from the central plant, and those points were marked with flags, creating four triangular quadrants (**Supplementary Figure 1**). To measure density, we counted the number of wax currant plants in each quadrant, calculated a mean number of plants/m^2 and then averaged those values across the four quadrants to calculate a mean number of plants/m^2 per site.

Host Plant Quality and Herbivore Damage

We sampled wax currant plants to measure plant quality and herbivore damage at each site. We aimed to sample 5 plants per site and used the same 4 transects that we established to measure plant density. The first plant that we sampled at each site was the focal plant at the center of the quadrant. For the other 4 plants, we chose the plant that was the closest to the 10 m mark along each of the 4 transects. Not all sites had 5 wax currant plants and if a site had fewer plants, we sampled all of them at the site ($n = 118$ plants sampled; **Table 1**).

To measure herbivore damage, we haphazardly chose two stems from each plant and counted the number of branches coming off of each stem. We then noted the presence or absence of herbivore damage on each branch along the stem. We calculated the percent damage for each plant as the number of branches with herbivore damage divided by the total number of branches per stem. We also noted whether the herbivore damage was due to *Polygonia* larvae, which leave a unique striping pattern on leaves that is easily identifiable. We also measured the maximum height and width of each plant as well as the number of stems as a measure of plant size.

To assess plant quality, we haphazardly selected two branches from each plant (these branches were not the same as those used to measure herbivore damage), removed the branches from the plant with clippers, and immediately placed them in a cooler filled with ice. We transported samples to the laboratory at the University of Denver and froze them (-20°C) until testing. In the lab, we thawed the plant samples, rinsed them with water to remove dirt, and then let them dry for 15 min. We then randomly selected 10 leaves from each plant by choosing every tenth leaf along both branches. We measured the wet mass of all 10 leaves as a group (to the nearest 0.01 mg) and then haphazardly selected 5 leaves to measure leaf toughness. For each of these 5 leaves, we measured the length of each leaf from the tip of the leaf along the central vein to the base of the stem and measured width across the widest section of the leaf (to the nearest 0.5 mm). We measured toughness using a modified version of the sand-pouring method described by Feeny (1970). We attached a safety pin through the leaf 0.1 cm up from the base of the stem, along the central vein, and then attached the safety pin to a cup by a string. We poured sand into the cup until the safety pin broke all the way through

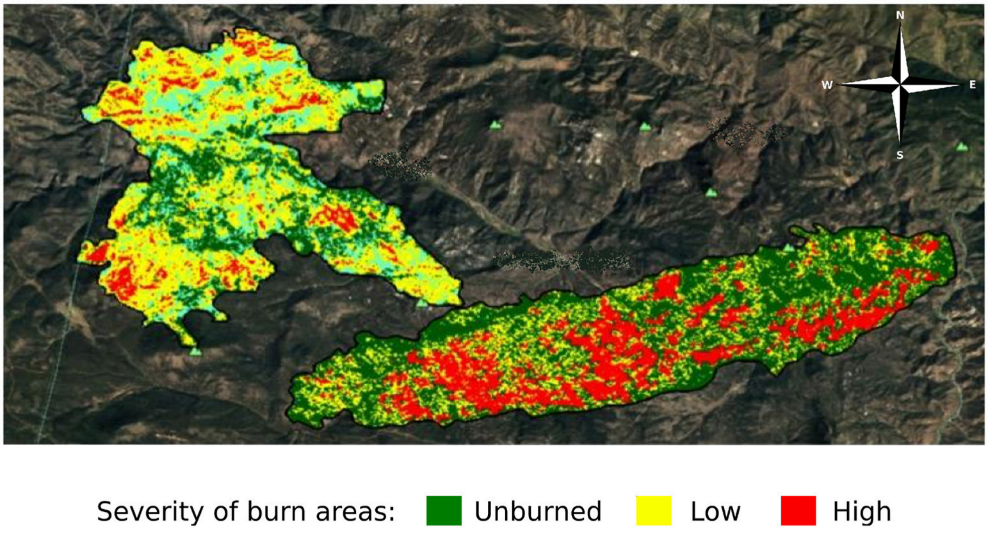


FIGURE 2 | Satellite imagery of the area burned and severity of the High Meadows fire (left) and the Buffalo Creek fire (right) in Colorado; Hayman fire not shown (MTBS Data Access: Fire Level Geospatial Data, 2016, April-last revised). Dark green indicates unburned areas, yellow indicates low severity burn areas, and red indicates high severity burn areas.

the leaf and weighed the sand. We calculated a mean value per plant using all of the leaf toughness values. After measuring toughness on five leaves, we dried all 10 leaves from each plant for 3 days at 60°C and then weighed them as a group once dry. We calculated percent water by subtracting dry mass from fresh mass and dividing by fresh mass. We performed all water content and leaf toughness mass measurements using a Scout Pro Ohaus Balance (Ohaus Corporation, Pine Brook, NJ USA). For each plant, we ground the dry leaves using a Retsch MM 400 Model mixer mill (Retsch GmbH, Haan, Germany), weighed them using a Mettler-Toledo XP6 microbalance (Mettler-Toledo, Columbus, OH), and rolled them into tin capsules (Elementar Americas). We sent the samples to Cornell University Stable Isotope Laboratory to be analyzed for %N and %C using an elemental analyzer-stable isotope ratio mass spectrometer system (Thermo Delta V Advantage IRMS and Carlo Erba NC2500 EA systems).

Data Analysis

We analyzed our data in two ways, using model selection and mediation analysis. We first used the model selection approach to determine which factors influence plant quality and herbivore damage. In those models, we included all variables that could explain the response variables of interest and using backward selection we could infer which of the variables best explained the changes in plant quality and herbivore damage. We performed 3 independent model selections to analyze: (1) the effect of fire severity and plant characteristics on plant quality (**Figure 1**, path A), (2) the effect of plant quality on insect herbivore damage (**Figure 1**, path B), and (3) the effect of plant quality, plant characteristics, and fire severity on herbivore damage (**Figure 1**, paths B+C). For all our models, site was included as a random factor. To test the effect of fire severity and plant characteristics on plant quality (model selection 1), we had three response variables: toughness, water content, and C:N ratio. For each response variable, we performed a backward model selection starting with all the predictor variables included, which were fire identity, fire severity, the interaction between fire identity and fire severity, plant size, leaf size, and density of *Ribes* plants at the site. The variables that measure plant size are colinear with each other, as well as the measures of leaf size, which may result in wrong interpretations of statistical significance (Graham, 2003). Therefore, we performed principal component analyses for the variables that measured plant size and leaf size to deal with their collinearity and used the eigenvalues that explained most of the variance in our models (as suggested by Graham, 2003). For leaf size we used the variables leaf width and leaf length to perform the PCA. In our models, we used the first eigenvalue axis that explained 93.4% of the variance in the data. For plant size, we used height, width, and length of each plant measured. In this case, the first axis explained 70.1% and the second explained 18.8% of the variance, and thus we used both in our models. All PCAs were performed using JMP 11®. Plant density was not normally distributed, but the log of density was, which we used in our analysis.

To test if plant quality had an effect on herbivore damage (model selection 2), we had 2 response variables that we tested separately: overall herbivore damage and herbivore damage by *Polygonia*. *Polygonia* herbivore damage did not follow a normal distribution, so we used the log transformed data of *Polygonia* damage + 1. The fixed effects were toughness, C:N ratio, and percent water content. To test the effect of plant quality, plant characteristics, and fire severity on herbivore damage (model selection 3), we had two response variables: overall herbivore damage and herbivore damage by *Polygonia*. The fixed effects for the starting model were toughness, percent water, C:N ratio,

TABLE 1 | GPS coordinates for each of the sampling locations and number of wax currant plants sampled at each site.

Fire	Site	Unburned	Low severity	High severity
Buffalo Creek	1	39° 20.390′ N	39° 20.955′ N	39° 21.090′ N
		105° 20.238′ W	105° 19.074′ W	105° 18.914′ W
		$n = 5$	$n = 5$	$n = 5$
	2	39° 20.755′ N	39° 21.156′ N	39° 21.244′ N
		105° 15.260′ W	105° 19.058′ W	105° 19.315′ W
		$n = 5$	$n = 5$	$n = 5$
	3	39° 20.617′ N	39° 20.708′ N	39° 21.514′ N
		105° 20.191′ W	105° 18.970′ W	105° 18.913′ W
		$n = 3$	$n = 5$	$n = 5$
Hayman	1	39° 5.451′ N	39° 8.001′ N	39° 6.158′ N
		105° 7.101′ W	105° 9.887′ W	105° 7.848′ W
		$n = 5$	$n = 5$	$n = 5$
	2	39° 17.918′ N	39° 8.490′ N	39° 6.815′ N
		105° 16.474′ W	105° 10.452′ W	105° 9.031′ W
		$n = 5$	$n = 2$	$n = 5$
	3	NA	39° 10.525′ N	39° 7.703′ N
			105° 10.018′ W	105° 9.943′ W
			$n = 5$	$n = 5$
High Meadows	1	39° 21.081′ N	39° 22.152′ N	39° 22.118′ N
		105° 23.646′ W	105° 22.094′ W	105° 22.422′ W
		$n = 3$	$n = 5$	$n = 5$
	2	39° 21.105′ N	39° 22.177′ N	39° 22.708′ N
		105° 22.806′ W	105° 21.554′ W	105° 22.182′ W
		$n = 5$	$n = 5$	$n = 5$
	3	NA	39° 22.991′ N	39° 22.323′ N
			105° 21.997′ W	105° 22.709′ W
			$n = 5$	$n = 5$

For each of the three fires (Hayman, High Meadow, and Buffalo Creek), sites were classified by severity (unburned, low severity, and high severity).

density of plants (log transformed), size of plants ($PCA1_{plantsize}$ and $PCA2_{plantsize}$), size of leaves ($PCA1_{leafsize}$), fire severity, fire identity, and the interaction between fire severity and identity. For the backward model selection, we removed step by step the factor that least explained the data, and we compared the reduced model with the previous model using F-statistics. If the reduced model was not significantly different from the extended model, we removed the factor from the analysis and kept the reduced model. The same procedure of backward model selection was performed for groups 1, 2, and 3 of response variables. We further tested if fire severity had an effect on plant density, as a measure of possible change on habitat structure or resource availability. We used log density as the response variable, fire severity as the fixed effect, and fire identity as the random effect.

For the second analysis method, we used mediation analysis with only the variables that were shown to affect the response variables with our model selection. Using this approach, we can directly investigate which factor influenced herbivore damage more: if fire severity had a direct effect on herbivore damage or if the effect of fire severity on herbivore damage was mediated by the effect of fire severity on plant quality (**Figure 1**). To test that, we used a mediation analysis to parse out the relative contribution of plant quality and fire severity on herbivore damage. For our mediation analysis, we used only the variables that were significant in the model selections for groups 1, 2, and 3 explained above, thus beside giving evidence of the factors that explain herbivore damage and plant quality, the model selection approach provides support for choosing the variables to include in the mediation analysis. For instance, in the mediation analysis for both overall herbivore damage and herbivore damage by *Polygonia*, we included the effect of fire severity, fire identity, leaf size, and plant size to measure the effect of fire severity on C:N ratio (see results below). However, we were only interested in the effect of fire severity, so we report only the coefficient (or estimate) for the effect of severity on C:N ratio. Then we performed other models to test the effect of C:N ratio alone, fire severity alone, and C:N together with fire severity on herbivore damage. Since our results show that unburned and low severity fires did not differ from each other, and were both different from high severity fire, we combined unburned and low severity into one category. Therefore, our coefficients for the mediation analysis show the difference from

TABLE 2 | Model summary for the effect of each explanatory variable on three measures of host plant quality: leaf toughness, water content, and C:N ratio.

	Effect on toughness			Effect on % water			Effect on C:N		
Included variable	**Estimated coefficient**	**St. error**	***t*-value**	**Estimated coefficient**	**St. error**	***t*-value**	**Estimated coefficient**	**St. error**	***t*-value**
Intercept	37.63	1.72	21.85*	50.7	1.41	35.04*	36.8	0.79	46.64*
FIRE SEVERITY									
Low	−6.07	2.01	−3.00*	4.66	2.02	2.3*	−3.87	0.98	−3.94*
Unburn	−6.99	2.21	−3.16*	4.72	2.18	2.16*	−4.3	1.05	−4.08*
FIRE IDENTITY									
Hayman	−5.08	1.94	−2.64*	–	–	–	−3.87	0.89	−3.82*
High Meadows	−3.23	1.99	−1.63	–	–	–	−4.3	0.94	−1.75
Leaf size	1.5	0.6	2.5*	–	–	–	−0.72	0.27	−2.15*
Plant size	–	–	–	–	–	–	−0.7	0.32	−2.71*

Asterisks represent $P < 0.05$. The final model for toughness included fire severity, fire identity, and leaf size. For percent water, the only fixed effect included was fire severity. For C:N ratio, the variables included were fire severity, fire identity, leaf size, and plant size. The variable levels are compared to the intercept; in the case of fire severity, the intercept is the high severity fire, whereas for fire identity the intercept was the Buffalo Creek fire.

low and unburned to high severity fires. All analyses were performed in R environment 3.4.3 (R Development Core Team, 2011) using the package *lme4* with *lmer* function (Bates et al., 2015). To perform pairwise comparisons between the different fire severities in each final model from our model selection done with groups A and C, we used the package *multcomp* with the *glht* functions (Hothorn et al., 2008). For the model comparisons, we used package *pbkrtest* with *KRmodcomp* function (Halekoh and Hojsgaard, 2014), which gives the *p*-value for the model comparisons with a Kenward-Roger adjustment. We deposited our data in the Dryad Repository: doi: 10.5061/dryad.t36p0cd (Murphy et al., 2018).

RESULTS

Effect of Fire on Plant Quality

We found that increased fire severity decreased plant quality (**Table 2**). Leaves were tougher [**Figure 3A**, $F_{(2,\ 23.81)} = 6.15$, $P = 0.002$], had greater C:N ratio [**Figure 3B**, $F_{(2,\ 27)} = 10.25$, $P < 0.001$], and lower water content [**Figure 3C**, $F_{(2,\ 21.78)} = 3.44$, $P = 0.05$] in places with high severity fire compared to areas with low severity fire or unburned areas. Toughness and C:N ratio of *Ribes* leaves were also influenced by fire identity [toughness: $F_{(2,\ 20.69)} = 3.53$, $P = 0.023$; C:N: $F_{(2,\ 21.17)} = 7.26$, $P = 0.004$] and leaf size [toughness: $F_{(1,\ 106.07)} = 6.12$, $P = 0.015$; C:N: $F_{(1,\ 107.59)} = 4.5$, $P = 0.036$]. Furthermore, C:N ratio was influenced by plant size [$\text{PC1}_{\text{plantsize}}$ only, $F_{(1,\ 109.25)} = 7.09$, $P = 0.009$]. We found no effect of fire severity on *Ribes* plant density [$F_{(2,\ 20.5)} = 1.78$, $P = 0.2$].

Effect of Plant Quality on Herbivore Damage

Overall herbivore damage and herbivore damage by *Polygonia* were explained only by the plant quality measure of C:N ratio [overall herbivore damage: $F_{(1,\ 101.5)} = 4.16$, $P = 0.044$; *Polygonia*: $F_{(1,\ 108.8)} = 6.83$, $P = 0.01$]. Overall herbivore damage and herbivore damage by *Polygonia* were greater on plants with low C:N ratio [overall herbivore damage: estimate $= -0.64 \pm 0.31$, $t = -2.05$, $P = 0.043$; *Polygonia*: estimate $= -0.05 \pm 0.02$, $t = -2.67$, $P = 0.009$].

Effect of Plant Quality and Fire on Herbivore Damage

Overall herbivore damage and damage by *Polygonia* were both 50% lower in sites with high severity fire than in unburned sites, and were similar between unburned and low severity sites (**Figure 4**). When we performed our model selection with both plant quality and fire effects on herbivore damage, we found that overall herbivore damage and *Polygonia* damage were affected only by fire severity [Overall: $F_{(2,\ 22)} = 18.61$, $P < 0.0001$, *Polygonia*: $F_{(2,\ 21.82)} = 7.21$, $P = 0.004$; **Table 3**].

From our mediation analysis, both total herbivore damage and damage by *Polygonia* were mainly affected directly by fire severity (**Figure 5**). The effect of fire mediated through host plant quality (C:N ratio) was not nearly as strong as the direct effect of fire severity on herbivore damage. When we included C:N in the model with fire severity, the coefficient of fire severity only changed slightly compared with fire severity alone (for overall herbivore damage the coefficient went from 29.08 without C:N to 31.04 with C:N, while for *Polygonia* herbivore damage the coefficient went from 8.03 to 9.09).

DISCUSSION

Fire severity directly impacted host plant quality as well as herbivore damage, and the direct effect of fire severity on herbivore damage was stronger than the indirect effect via plant quality. Although plant quality differed across fires (e.g., Buffalo Creek, Hayman, or High Meadows), the effect of fire severity on plant quality was stronger than fire identity, and fire identity did not influence herbivore damage. We found an inverse relationship between fire severity and host plant quality; increasing fire severity decreased plant quality through an increase in C:N ratio and toughness, and a decrease in water content. However, of these quality measures, the only one

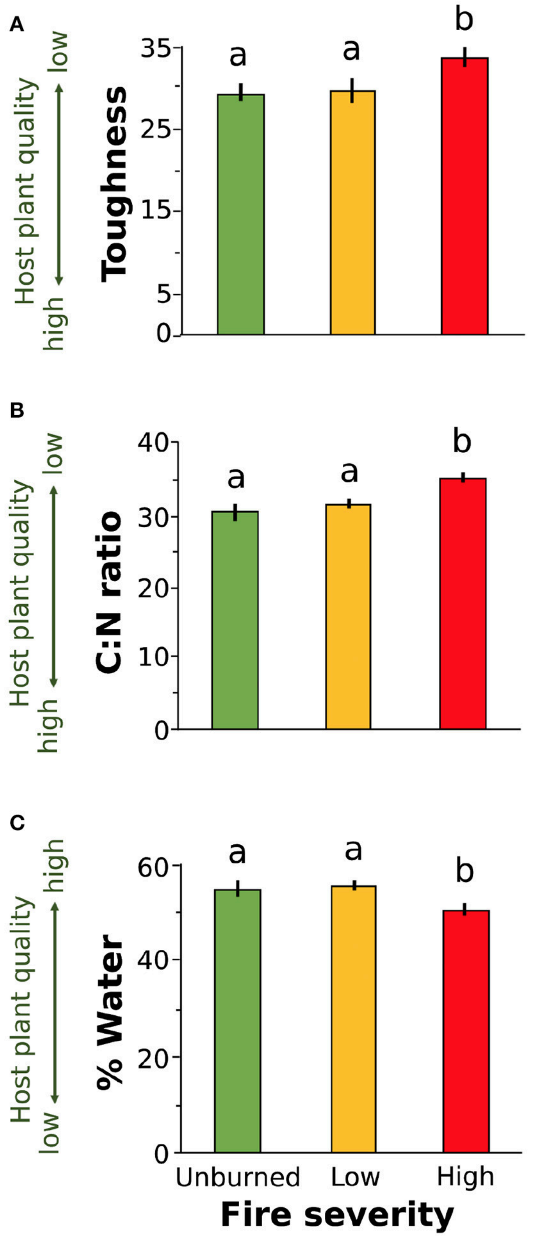


FIGURE 3 | Plant quality measures for leaves collected from wax current plants growing in unburned, low severity, and high severity fire sites: **(A)** toughness, **(B)** carbon to nitrogen ratio, and **(C)** percent water. Increased leaf toughness and C:N ratio reduce host plant quality for herbivores **(A,B)** while increased percent water increases host plant quality **(C)**. Means are given ±SE and letters represent pairwise comparisons within host plant quality measure ($P < 0.05$).

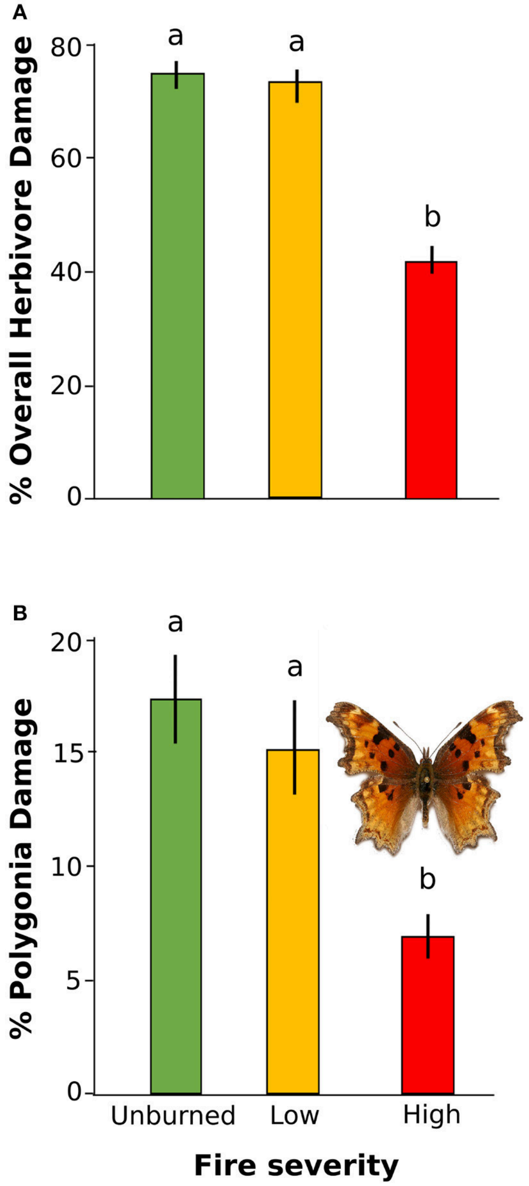


FIGURE 4 | Percent herbivore damage on wax currant plants in unburned, low severity, and high severity fire sites for **(A)** overall herbivore damage and **(B)** herbivore damage by *Polygonia gracilis zephyrus*. Means are given ±SE and letters represent pairwise comparisons within type of herbivore damage ($p < 0.05$).

that predicted herbivore damage by herbivores in general and specifically *Polygonia* was the C:N ratio. Kim and Holt (2012) studied time since fire rather than fire severity in prescribed burns in the Florida scrub, but similar to our results found that changes in habitat structure from fire had a stronger effect on herbivores than changes in plant quality. Although we did not find an effect of fire severity on plant density, we found that insect herbivores were strongly affected by fire severity through mechanisms other than change in plant quality. Thus, our results demonstrate that severity of forest wildfire influences herbivores through strong direct effects but also indirectly through changes in plant quality.

We focused on severity for this study because models predict that fire severity will be one of the biggest responses to climate change (IPCC, 2014), but previous work tends to focus on time since fire, which is a measure of recovery time of the community (e.g., Kay et al., 2007; Vogel et al., 2010; Kim and Holt, 2012).

TABLE 3 | Model summary for the effect of fire severity on overall herbivore damage and damage by the butterfly *Polygonia gracilis zephyrus* to wax currant plants.

	Effect on overall herbivore damage			Effect on *Polygonia* damage		
Included variable	**Estimated coefficient**	**St. error**	***t*-value**	**Estimated coefficient**	**St. error**	***t*-value**
Intercept	38.64	5.95	6.49*	1.69	0.17	10.11*
SEVERITY						
Low	43.43	8.47	5.13*	0.66	0.24	2.75*
Unburn	36.42	8.54	4.26*	0.94	0.26	3.59*

Asterisks represent $P < 0.05$. The fire severity levels are compared to the intercept that is the high severity fire.

Notably, the effect of fire severity was greater than that of fire identity suggesting that while our fires happened at three different time points, time since fire does not account for the effects of fire severity; our three fires occurred between 16 and 22 years ago, which may not be a long enough difference to detect a time-since-fire effect. During post-fire succession, wax current densities can depend on land management (Bock et al., 1978), but plants usually thrive and can remain at high densities for decades post-fire (Coop and Schoettle, 2009). Fire severity has different effects on herbivores than time since fire, because increasing severity significantly affects habitat structure (e.g., increase in canopy openness), soil moisture, and nutrients, as well as which species can survive (New, 2014; Koltz et al., 2018; Pressler et al. in review). These impacts could be responsible for the strong direct effect of severity on herbivore damage that we found in our study, which was stronger than the indirect negative effect mediated through plant quality. Fire severity can influence the survival of insects both above and belowground; for example, belowground insects suffer high mortality in high fire severity that greatly increase the temperature of the soil (New, 2014; Pressler et al. in review). The negative effect of a high severity fire can also affect higher trophic levels that then influence the insect herbivore community (Koltz et al., 2018). Some natural enemies are less mobile (e.g., spiders), or pupate or nest in the soil (e.g., some wasps and ants), and thus may suffer high mortality in severe fire events. The potential negative effect of fire on natural enemies would lead to positive effects on herbivores due to enemy release (Pausas and Parr, 2018). These complex but critical interactions would be missed if we only considered time since fire and not fire severity. Moving forward, it is necessary to consider the different measures of fire disturbance (severity, frequency, time since fire, and time of year of fire) and how they interact with each other when exploring the effect of fire on herbivores.

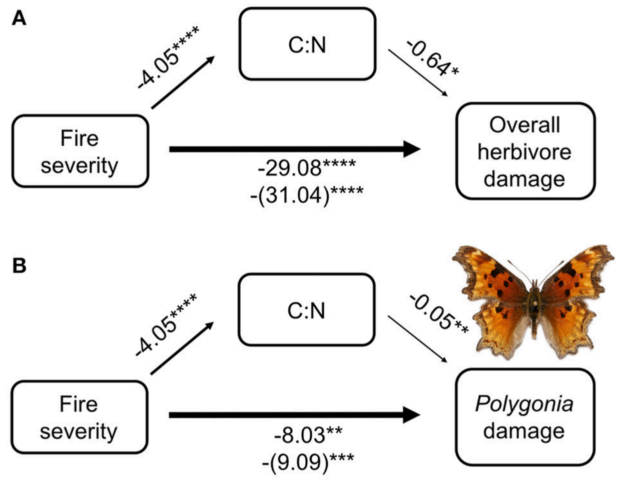


FIGURE 5 | Mediation analysis of fire severity, C:N ratio, and herbivore damage on wax currant plants for **(A)** overall herbivore damage and **(B)** herbivore damage by *Polygonia gracilis zephyrus*. Numbers are model coefficients (coefficients in parentheses are the model that considers both C:N and fire severity) and asterisks indicate significant effects at $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

While less important than the direct effect of fire severity, plant quality also influenced herbivore damage, especially through changes in C:N ratio. The effects of fire on plants (e.g., Lopes and Vasconcelos, 2011; Wan et al., 2014) and the effect of plant quality on herbivore damage (e.g., Greene et al., 2012; Hahn and Orrock, 2015) have been previously documented, but the complex interaction among these processes is critical in order to understand how communities will respond to increase in fire severity. These relationships are even more complex since fire can also influence how herbivores affect plant growth (Conway and Johnstone, 2017), plant chemistry, and decomposition (Kay et al., 2008). While we connected fire severity, plant quality, and herbivore damage, other work has suggested that the soil community should also be considered. For example, fire reduces microbial abundance (Dooley and Treseder, 2012) as well as soil fungal abundance (Holden et al., 2013). Additionally, fire severity affects the reduction in microbial biomass and respiration so that more severe fires have larger negative impacts on the soil community (Holden et al., 2016). This reduction in both fungal and microbial biomass reduces decomposition rates in post-fire soils (Holden et al., 2015), directly affecting the resources available to plants, and thus plant quality, as well as altering the carbon cycle of the ecosystem.

While our work sheds light on the complex interactions among fire, plants, and herbivores, it also inspires questions for future exploration. First, we measured damage to plants holistically as a community level estimate of total damage, but individual specialist herbivore species likely have unique responses to their host plants and to fire. We did, however, examine damage from a common herbivore in our system, *Polygonia* larvae, and found identical patterns comparing this species' herbivore damage to our community level measure. It is currently unknown if different insect herbivore species will respond similarly to changes in fire severity, though we might expect life history traits like ability to disperse from or avoid fire (e.g., enter diapause), as well as evolutionary history with fire to shape species-specific patterns (Koltz et al., 2018). This is an important question for future work. Additionally, because only one dominant understory shrub was present in all of our sites, we focused on wax currant. Future work should compare our results to herbivores that feed on other plant species, such as grasses or trees. Finally, we might expect some processes, like recolonization rates, to be driven by an interaction between fire severity and burn size, which we did not measure, and future work should explore this interaction.

Global climate change is altering fire regimes worldwide with projected large changes to fire severity (Flannigan et al., 2000; Dale et al., 2001; IPCC, 2014; Rocca et al., 2014; Clark et al., 2017), but how these changes will affect insect communities and species interactions is largely unknown (Koltz et al., 2018). Ours is the first study to investigate how fire severity affects plant-herbivore interactions, as measured by plant quality and herbivore damage, in naturally occurring forest wildfires. Our results demonstrate that severity can have profound impacts on interactions between herbivores and their host plants with severity significantly affecting host plant quality, but with an even larger direct effect on insect herbivores, likely through changes in habitat structure. Thus, our results suggest that increasing severity of wildfires will significantly influence how herbivores interact with their host plants and may impact long-term population sizes and community structure.

DATA ACCESSIBILITY STATEMENT

We deposited our data in the Dryad Repository: doi: 10.5061/dryad.t36p0cd (Murphy et al., 2018).

AUTHOR CONTRIBUTIONS

LC and SM conceived and designed the experiments; LC, TS, DR, and SM conducted the fieldwork; TS, DR, CH, and SM conducted the labwork; EB, MV, CH, and SM conceptualized the framework for the research; CH and TS managed the data; MV analyzed the data; SM, EB, MV, CH, and TS wrote the paper.

ACKNOWLEDGMENTS

We thank the University of Denver Undergraduate Research Center for funding this research as well as a NSF GRFP that funded LC. We are grateful to Eva Horna Lowell for help with the fieldwork, Bailey Rojewski and April Vollmer for help with the labwork, and Cathy Durso for help with the statistical analysis. We thank the University of Denver Ecology and Evolution (DUEEBs) group for comments that greatly improved an earlier draft of this manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2018.00135/full#supplementary-material

REFERENCES

Awmack, C. S., and Leather, S. R. (2002). Host plant quality and fecundity in herbivorous insects. *Annu. Rev. Entomol.* 47, 817–844. doi: 10.1146/annurev.ento.47.091201.145300

Bailey, J. K., and Whitham, T. G. (2002). Interactions among fire, aspen, and elk affect insect diversity: reversal of a community response. *Ecology* 83, 1701–1712. doi: 10.1890/0012-9658(2002)083[1701:IAFAAE]2.0.CO;2

Barnes, E. E., and Murphy, S. M. (2018). Time-lagged intraspecific competition in temporally separated cohorts of a generalist insect. *Oecologia* 186, 711–718. doi: 10.1007/s00442-018-4067-9

Bates, D., Machler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48. doi: 10.18637/jss.v067.i01

Bebi, P., Kulakowski, D., and Veblen, T. T. (2003). Interactions between fire and spruce beetles in a subalpine Rocky Mountain forest landscape. *Ecology* 84, 362–371. doi: 10.1890/0012-9658(2003)084[0362:IBFASB]2.0.CO;2

Bock, J. H., Raphael, M., and Bock, C. E. (1978). A comparison of planting and natural succession after a forest fire in the northern Sierra Nevada. *J. Appl. Ecol.* 15, 597–602. doi: 10.2307/2402613

Christie, F. J., and York, A. (2009). No detectable impacts of frequent burning on foliar C and N or insect herbivory in an Australian eucalypt forest. *Appl. Veg. Sci.* 12, 376–384. doi: 10.1111/j.1654-109X.2009.01033.x

Clark, J. A., Loehman, R. A., and Keane, R. E. (2017). Climate changes and wildfire alter vegetation of Yellowstone National Park, but forest cover persists. *Ecosphere* 8:e01636. doi: 10.1002/ecs2.1636

Conway, A. J., and Johnstone, J. F. (2017). Moose alter the rate but not the trajectory of forest canopy succession after low and high severity fire in Alaska. *For. Ecol. Manage.* 391, 154–163. doi: 10.1016/j.foreco.2017.02.018

Coop, J. D., and Schoettle, A. W. (2009). Regeneration of Rocky Mountain bristlecone pine (*Pinus aristata*) and limber pine (*Pinus flexilis*) three decades after stand-replacing fires. *For. Ecol. Manage.* 257, 893–903. doi: 10.1016/j.foreco.2008.10.034

Dale, V. H., Joyce, L. A., McNulty, S., Neilson, R. P., Ayres, M. P., Flannigan, M. D., et al. (2001). Climate change and forest disturbances. *Bioscience* 51, 723–734. doi: 10.1641/0006-3568(2001)051[0723:CCAFD]2.0.CO;2

Diniz, I., Higgins, B., and Morais, H. (2011). How do frequent fires in the Cerrado alter the lepidopteran community? *Biodivers. Conserv.* 20, 1415–1426. doi: 10.1007/s10531-011-0034-z

Dooley, S. R., and Treseder, K. K. (2012). The effect of fire on microbial biomass: a meta-analysis of field studies. *Biogeochemistry* 109, 49–61. doi: 10.1007/s10533-011-9633-8

Egan, S. P., and Ott, J. R. (2007). Host plant quality and local adaptation determine the distribution of a gall-forming herbivore. *Ecology* 88, 2868–2879. doi: 10.1890/06-1303.1

Faeth, S. H. (1985). Quantitative defense theory and patterns of feeding by oak insects. *Oecologia* 68, 34–40. doi: 10.1007/BF00379470

Feeny, P. (1970). Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology* 51, 565–581. doi: 10.2307/1934037

Flannigan, M. D., Stocks, B. J., and Wotton, B. M. (2000). Climate change and forest fires. *Sci. Total Environ.* 262, 221–229. doi: 10.1016/S0048-9697(00)00524-6

Gandhi, K. J., Gilmore, D. W., Katovich, S. A., Mattson, W. J., Zasada, J. C., and Seybold, S. J. (2008). Catastrophic windstorm and fuel-reduction treatments alter ground beetle (Coleoptera: Carabidae) assemblages in a North American sub-boreal forest. *For. Ecol. Manage.* 256, 1104–1123. doi: 10.1016/j.foreco.2008.06.011

Graham, M. H. (2003). Confronting multicollinearity in ecological multiple regression. *Ecology* 84, 2809–2815. doi: 10.1890/02-3114

Green, D. S., Roloff, G. J., Heath, B. R., and Holekamp, K. E. (2015). Temporal dynamics of the reponses by African mammals to prescribed fire. *J. Wildl. Manage.* 79, 235–242. doi: 10.1002/jwmg.827

Greene, L., Hebblewhite, M., and Stephenson, T. (2012). Short-term vegetation response to wildfire in the eastern Sierra Nevada: implications for recovering an endangered ungulate. *J. Arid Environ.* 87, 118–128. doi: 10.1016/j.jaridenv.2012.06.001

Haddad, N. M., Tilman, D., Haarstad, J., Ritchie, M., and Knops, J. M. (2001). Contrasting effects of plant richness and composition on insect communities: a field experiment. *Am. Nat.* 158, 17–35. doi: 10.1086/320866

Hahn, P. G., and Orrock, J. L. (2015). Land-use legacies and present fire regimes interact to mediate herbivory by altering the neighboring plant community. *Oikos* 124, 497–506. doi: 10.1111/oik.01445

Halekoh, U., and Hojsgaard, S. (2014). A Kenward-Roger approximation and parametric bootstrap methods for tests in linear mixed models the R package pbkrtest. *J. Stat. Softw.* 59, 1–32. doi: 10.18637/jss.v059.i09

Higgins, S. I., Bond, W. J., February, E. C., Bronn, A., Euston-Brown, D. I., Enslin, B., et al. (2007). Effects of four decades of fire manipulation on woody vegetation structure in savanna. *Ecology* 88, 1119–1125. doi: 10.1890/06-1664

Holden, S. R., Berhe, A. A., and Treseder, K. K. (2015). Decreases in soil moisture and organic matter quality suppress microbial decomposition following a boreal forest fire. *Soil Biol. Biochem.* 87, 1–9. doi: 10.1016/j.soilbio.2015.04.005

Holden, S. R., Gutierrez, A., and Treseder, K. K. (2013). Changes in soil fungal communities, extracellular enzyme activities, and litter decomposition across a fire chronosequence in Alaskan boreal forests. *Ecosystems* 16, 34–46. doi: 10.1007/s10021-012-9594-3

Holden, S. R., Rogers, B. M., Treseder, K. K., and Randerson, J. T. (2016). Fire severity influences the resopnse of soil microbes to a boreal forest fire. *Environ. Res. Lett.* 11:035004. doi: 10.1088/1748-9326/11/3/035004

Hood, S., Sala, A., Heyerdahl, E. K., and Boutin, M. (2015). Low-severity fire increases tree defense against bark beetle attacks. *Ecology* 96, 1846–1855. doi: 10.1890/14-0487.1

Hothorn, T., Bretz, F., and Westfall, P. (2008). Simultaneous inference in general parametric models. *Biom. J.* 50, 346–363. doi: 10.1002/bimj.200810425

Huang, J., and Boerner, R. E. (2007). Effects of fire alone or combined with thinning on tissue nutrient concentrations and nutrient resorption in *Desmodium nudiflorum*. *Oecologia* 153, 233–243. doi: 10.1007/s00442-007-0733-z

Hunter, M. D., and McNeil, J. N. (1997). Host-plant quality influences diapause and voltinism in a polyphagous insect herbivore. *Ecology* 78, 977–986. doi: 10.1890/0012-9658(1997)078[0977:HPQIDA]2.0.CO;2

IPCC (2014). "Climate change 2014: synthesis report," in *Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change,* eds R. K. Pachauri, L. A. Meyer, and Core Writing Team (Geneva: IPCC).

Jonas, J. L., and Joern, A. (2007). Grasshopper (Orthoptera: Acrididae) communities respond to fire, bison grazing and weather in North American tallgrass prairie: a long-term study. *Oecologia* 153, 699–711. doi: 10.1007/s00442-007-0761-8

Joubert, L., Pryke, J. S., and Samways, M. J. (2016). Positive effects of burning and cattle grazing on grasshopper diversity. *Insect Conserv. DiverS.* 9, 290–301. doi: 10.1111/icad.12166

Kay, A. D., Mankowski, J., and Hobbie, S. E. (2008). Long-term burning interacts with herbivory to slow decomposition. *Ecology* 89, 1188–1194. doi: 10.1890/07-1622.1

Kay, A. D., Schade, J. D., Ogdahl, M., Wesserle, E. O., and Hobbie, S. E. (2007). Fire effects on insect herbivores in an oak savanna: the role of light and nutrients. *Ecol. Entomol.* 32, 754–761. doi: 10.1111/j.1365-2311.2007.00925.x

Keith, R. P., Veblen, T. T., Schoennagel, T. L., and Sherriff, R. L. (2010). Understory vegetation indicates historic fire regimes in ponderosa pine-dominatedecosystems in the Colorado Front Range. *J. Veg. Sci.* 21, 488–499. doi: 10.1111/j.1654-1103.2009.01156.x

Kim, T. N., and Holt, R. D. (2012). The direct and indirect effects of fire on the assembly of insect herbivore communities: examples from the Florida scrub habitat. *Oecologia* 168, 997–1012. doi: 10.1007/s00442-011-2130-x

Kitzberger, T., Raffaele, E., Heinemann, K., and Mazzarino, M. J. (2005). Effects of fire severity in a north Patagonian subalpine forest. *J. Veg. Sci.* 16, 5–12. doi: 10.1111/j.1654-1103.2005.tb02333.x

Klemola, N., Klemola, T., Rantala, M. J., and Ruuhola, T. (2007). Natural host plant quality afects immune defence of an insect herbivore. *Entomol. Exp. Appl.* 123, 167–176. doi: 10.1111/j.1570-7458.2007.00533.x

Knight, T. M., and Holt, R. D. (2005). Fire generates spatial gradients in herbivory: an example from a Florida sandhill ecosystem. *Ecology* 86, 587–593. doi: 10.1890/04-1069

Koltz, A. M., Burkle, L. A., Dell, J. E., Pressler, Y., Richards, L. A., Vidal, M. C., et al. (2018). Global change and the importance of fire on the ecology and evolution of insects. *Curr. Opin. Insect Sci.* 29, 110–116. doi: 10.1016/j.cois.2018.07.015

Lopes, C. T., and Vasconcelos, H. L. (2011). Fire increases insect herbivory in a neotropical savanna. *Biotropica* 43, 612–618. doi: 10.1111/j.1744-7429.2011.00757.x

Marshall, K. A. (1995). "Ribes cereum," in *Fire Effects Information System,* [Online]. U.S. Department of Agriculture; Forest Service; Rocky Mountain Research Station. Available online at: https://www.fs.fed.us/database/feis/plants/shrub/ribvel/all.html

Martin,-R., M., Cox, J. R., Ibarra,-F., F., Alston, D. G., Banner, R. E., and Malecheck, J. C. (1999). Spittlebug and buffelgrass responses to summer fires in Mexico. *J. Range Manage.* 52, 621–625. doi: 10.2307/4003632

Mattson, W. J. (1980). Herbivory in relation to plant nitrogen content. *Annu. Rev. Ecol. Syst.* 11, 119–161. doi: 10.1146/annurev.es.11.110180.001003

McCullough, D. G., Werner, R. A., and Neumann, D. (1998). Fire and insects in northern and boreal forest ecosystems of North America. *Annu. Rev. Entomol.* 43, 107–127. doi: 10.1146/annurev.ento.43.1.107

Moranz, R. A., Fuhlendorf, S. D., and Engle, D. M. (2014). Making sense of a prairie butterfly paradox: the effects of grazing, time since fire, and sampling period on regal fritillary abundance. *Biol. Conserv.* 173, 32–41. doi: 10.1016/j.biocon,.2014.03.003

MTBS Data Access: Fire Level Geospatial Data (2016). *MTBS Project [Online]*. USDA Forest Service/U.S. Geological Survey. Available online at: http://mtbs.gov/data/individualfiredata.html

Murphy, S. M., Vidal, M. C., Smith, T. P., Hallagan, C. J., Broder, E. D., Rowland, D., et al. (2018). Data from: forest fire severity affects host plant quality and insect herbivore damage. *Dryad Digit. Repository.* doi: 10.5061/dryad.t36p0cd. [Epub ahead of print].

New, T. R. (2014). *Insects, Fire and Conservation.* Switzerland: Springer International Publishing.

Pausas, J. G., and Parr, C. L. (2018). Towards an understanding of the evolutionary role of fire in animals. *Evol. Ecol.* 32, 113–125. doi: 10.1007/s10682-018-9927-6

Peralta, G., Stevani, E. L., Chacoff, N. P., Dorado, J., and Vázquez, D. P. (2017). Fire influences the structure of plant-bee networks. *J. Anim. Ecol.* 86, 1372–1379. doi: 10.1111/1365-2656.12731

Pourreza, M., Hosseini, S. M., Sinegani, A. A. S., Matinizadeh, M., and Alavai, S. J. (2014). Herbaceous species diversity in relation to fire severity in Zagros oak forests, Iran. *J. Forestry Res.* 25, 113–120. doi: 10.1007/s11676-014-0436-3

R Development Core Team (2011). *R: A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing. Available online at: http://www.r-project.org.

Rocca, M. E., Brown, P. M., MacDonald, L. H., and Carrico, C. M. (2014). Climate change impacts on fire regimes and key ecosystem services in Rocky Mountain forests. *For. Ecol. Manage.* 327, 290–305. doi: 10.1016/j.foreco.2014.04.005

Scandurra, A., Magliozzi, L., Aria, M., and D'Aniello, B. (2014). Short-term effects of fire on Papilionoidea (Lepidoptera) communities: a pilot study in Mediterranean maquis shrubland. *Ital. J. Zool.* 81, 599–609. doi: 10.1080/11250003.2014.953218

Schowalter, T. (2012). Insect responses to major landscape-level disturbance. *Annu. Rev. Entomol.* 57, 1–20. doi: 10.1146/annurev-ento-120710-100610

Stephan, K., Kavanagh, K. L., and Koyama, A. (2015). Comparing the influence of wildfire and prescribed burns on watershet nitrogen biogeochemistry using 15N natural abundance in terrestrial and aquatic ecosystem components. *PLoS ONE* 10:e0119560. doi: 10.1371/journal.pone.0119560

Stout, T. (2008). Utah Lepidopterist. *Bull. Utah Lepidopterists' Soc.* 15, 1–3.

Van Langevelde, F., Van De Vijver, C. A., Kumar, L., Van De Koppel, J., De Ridder, N., Van Andel, J., et al. (2003). Effects of fire and herbivory on the stability of savanna ecosystems. *Ecology* 84, 337–350. doi: 10.1890/0012-9658(2003)084[0337:EOFAHO]2.0.CO;2

Veblen, T. T., Hadley, K. S., Nel, E. M., Kitzberger, T., Reid, M., and Villalba, R. (1994). Disturbance regime and disturbance interactions in a Rocky Mountain subalpine forest. *J. Ecol.* 82, 125–135. doi: 10.2307/2261392

Vidal, M. C., and Murphy, S. M. (2018). Bottom-up vs. top-down effects on terrestrial insect herbivores: a meta-analysis. *Ecol. Lett.* 21, 138–150. doi: 10.1111/ele.12874

Vogel, J. A., Koford, R. R., and Debinski, D. M. (2010). Direct and indirect responses of tallgrass prairie butterflies to prescribed burning. *J. Insect Conserv.* 14, 663–677. doi: 10.1007/s10841-010-9295-1

Wan, H. Y., Rhodes, A. C., and St Clair, S. B. (2014). Fire severity alters plant regeneration patterns and defense against herbivores in mixed aspen forests. *Oikos* 123, 1479–1488. doi: 10.1111/oik.01521

Zambino, P. J. (2010). Biology and pathology of *Ribes* and their implications for management of white pine blister rust. *For. Pathol.* 40, 264–291. doi: 10.1111/j.1439-0329.2010.00658.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 25 October 2018
doi: 10.3389/fevo.2018.00170

Pollinator Community Assembly Tracks Changes in Floral Resources as Restored Hedgerows Mature in Agricultural Landscapes

Claire Kremen[1*†], *Leithen K. M'Gonigle*[1,2†] *and Lauren C. Ponisio*[1,3]

[1] Department of Environmental Science, Policy, and Management, University of California, Berkeley, Berkeley, CA, United States, [2] Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada, [3] Department of Entomology, University of California, Riverside, Riverside, CA, United States

Intensive agriculture reduces wild pollinator abundance, diversity and pollination services, while depending critically on wild pollinators for crop pollination. Floral enhancements such as hedgerows (native, perennial flowering trees and shrubs) can enhance pollinator colonization, persistence, occupancy, and species richness within intensive agricultural landscapes. However, little is known about the specific features of hedgerows that promote pollinator communities in such landscapes. Understanding how pollinator communities respond to local changes in site conditions as hedgerows mature, such as the availability of floral or nesting resources, can help guide the design of more effective hedgerows that promote pollinators and/or pollination services. In an intensively-managed agricultural region of California, we found that pollinator community attributes responded principally to the enhancement of floral diversity as hedgerows mature, as well as to surrounding natural habitat. Once hedgerows matured, this relationship leveled off, suggesting either saturation of community assembly processes, or greater importance of floral density/display relative to diversity. Although we did not find any relationships between measures of pollinator community diversity and nesting resources, such resources are notably difficult to measure. Surrounding natural habitat also affected species and functional richness at hedgerows, particularly for solitary bees that nest above ground. Such species are known to be particularly sensitive to the negative effects of agriculture. Thus, hedgerows in combination with natural habitat may reverse some of the community disassembly provoked by intensive agriculture.

Keywords: habitat restoration, floral enhancement, pollinator, functional diversity, agro-ecosystem, bee, California, intensive agriculture

Edited by:
Shannon Murphy,
University of Denver, United States

Reviewed by:
Jane Memmott,
University of Bristol, United Kingdom
Mary A. Jamieson,
Oakland University, United States

***Correspondence:**
Claire Kremen
ckremen@berkeley.edu

† These authors have contributed equally to this work

Specialty section:
This article was submitted to Population and Evolutionary Dynamics, a section of the journal Frontiers in Ecology and Evolution

Received: *02 July 2018*
Accepted: *05 October 2018*
Published: *25 October 2018*

Citation:
Kremen C, M'Gonigle LK and Ponisio LC (2018) Pollinator Community Assembly Tracks Changes in Floral Resources as Restored Hedgerows Mature in Agricultural Landscapes. Front. Ecol. Evol. 6:170. doi: 10.3389/fevo.2018.00170

1. INTRODUCTION

Agricultural intensification is a global land use change (Ramankutty et al., 2018) that has large impacts on arthropod biodiversity and the ecosystem functions and services provided by arthropods within those ecosystems (Gurr et al., 2016). Native pollinators, which provide valuable pollination services to crops similar in magnitude to those of managed bees (Kleijn et al., 2015) even when managed honey bees are present (Garibaldi et al., 2013; Rader et al., 2016), have reduced abundance and diversity in larger fields that are more simplified (Kovács-Hostyánszki et al., 2017). However, these are the landscapes where pollination services are in highest demand (Koh et al., 2016). Re-diversifying such landscapes with habitat enhancements such as hedgerows or wildflower strips can support pollinator communities (Garibaldi et al., 2014), potentially increasing the supply of pollinators to adjacent crops (Blaauw and Isaacs, 2014; Morandin et al., 2016, but see Sardiñas and Kremen, 2015). Although such plantings may chiefly support generalist pollinators (Kleijn et al., 2015), some evidence suggests that well-targeted plantings can promote diverse communities of pollinators including more specialized species (Kremen and M'Gonigle, 2015; M'Gonigle et al., 2015, 2017; Ponisio et al., 2016), as well as supporting other arthropod-mediated services such as pest control (Gurr et al., 2016; Morandin et al., 2016). Habitat plantings in farmland may thus enhance both arthropod conservation and ecosystem services. However, much remains to be known about how to target farmland plantings to reverse the disturbance of agricultural intensification and promote arthropod conservation and/or ecosystem services (Williams et al., 2015; Kovács-Hostyánszki et al., 2017; Williams and Lonsdorf, 2018).

In intensively-managed production systems in California, hedgerow plantings, implemented to enhance pest control, game birds, and/or other ecosystem services, are made up of diverse, perennial native shrubs, grasses, and forbs that provide a sequence of floral resources (Bugg et al., 1998; Garbach and Long, 2017; Long et al., 2017). Our previous studies examined how these hedgerows affect pollinator populations and communities, focusing on time since restoration in two types of studies: before-after-control-impact (Kremen and M'Gonigle, 2015; M'Gonigle et al., 2015) or space-for-time substitution (Ponisio et al., 2016, 2017). These studies have demonstrated that hedgerows have a strong positive impact on pollinator occupancy rates, local and regional species richness, functional diversity, occurrence of resource specialists, and plant-pollinator network characteristics. Additional studies demonstrated that hedgerows promote spillover of individuals into adjacent fields (Morandin and Kremen, 2013), leading to enhanced pest control and pollination services (Morandin et al., 2016), although pollination improvements were not observed for all crops (Sardiñas and Kremen, 2015).

Thus, in California, hedgerows appear to promote the conservation of native pollinators in intensively-managed landscapes and, in some crops, pollination services. Existing studies have not yet examined, however, which aspects of hedgerow maturation are responsible for the observed changes in pollinator community composition. Understanding how pollinator communities respond to local changes in site conditions as hedgerows mature, such as the availability of floral or nesting resources, can help guide the design of more effective hedgerows that promote pollinators and/or pollination services.

Here, we used data collected over 9 field seasons within a 10 year period in a before-after-control-impact design, to ask how metrics of pollinator community composition change in response to local site characteristics (floral and nesting resources) and to surrounding landscape characteristics (area of natural habitat). Comparing against unmanaged field edges (controls), we asked: (1) How do floral and nesting resources change with years post-restoration and surrounding landscape characteristics? (2) How do bee community responses (abundance, species richness, diversity, evenness and functional diversity) change with local (i.e., hedgerow) and surrounding landscape characteristics, and years post-restoration? (3) How long after hedgerow planting does it take to observe consistent changes in bee community composition relative to initial conditions? We were also interested in determining whether crop-visiting bees show similar or distinct responses to the entire bee community.

We expected to find that floral and nesting resources were enhanced as hedgerows matured relative to controls and that bee community responses would track these changes, leading to greater abundance, richness, diversity and functional diversity, but lower evenness as rarer species colonized the hedgerows. Because so little natural habitat occurs in the landscape we studied (0–4.5% natural cover in regions extending 1,500 m out from our sites), we did not expect to see an effect of natural habitat on bee community metrics. Finally, we expected that inclusion of local site characteristics (e.g., floral and/or nesting resources) would substitute for hedgerow maturation (years post-restoration, or *ypr*), leading to its non-inclusion in final models. Further, we expected changes in bee community composition relative to initial conditions would only be consistent at least 4 years post-planting, at which point shrubs are sufficiently mature that they produce large floral displays.

2. MATERIALS AND METHODS

2.1. Study Sites and Collection Methods

Located in the Central Valley of California (Yolo County), our study area is an intensively-managed landscape comprising conventional (i.e., using an array of synthetic pesticides and fertilizers, irrigation and mechanization) field crops, grape vineyards, and orchards (**Figure S1**). Field crops typically rotate annually; common crops in the region are safflower, tomato, wheat, grass, sunflower, melon. Common orchard crops include almond, walnut and plum. We selected five farm edges for restoration and 10 non-restored control edges. Hedgerows were planted in 2007 and 2008 with native perennial shrubs and trees (e.g., *Cercis occidentalis*, *Ceanothus* spp., *Rosa californica*, *Heteromeles arbutifolia*, *Sambucus mexicana*, *Eriogonoum* spp., *Baccharis* spp., *Salvia* spp., and others; see **Table S1** for a complete list of shrub plantings by site). Hedgerow restorations were approximately 350 m long and 3–6 m wide, bordering large crop fields (ca. 30 hectare). In 2008, nine regularly-spaced small plots (1 × 8 m) were seeded with forb mixtures made up of nine locally-native annual or perennial forb species within the genera *Escholtzia, Grindelia, Lupinus, Lotus, Phacelia, Trifolium* spp., as described in detail in Wilkerson et al. (2014). Hedgerows were irrigated and weeded for 3 years, after which no further management was needed (except for some replanting in 2009 of species or individuals that did not take; see **Figure S2** for an example of a restoration prior to and 6 years after restoration). Pollinator sampling at each restoration site began 1 year prior to restoration, except at one site where sampling began earlier in the same year that restoration occurred (see **Table S2** for an overview of sampling history per site). Restoration and control sites were sampled in each year of the study to achieve a before-after-control-impact design (Underwood, 1994; Kremen and M'Gonigle, 2015).

Control sites were selected to match conditions surrounding restoration sites. For each restoration site, we selected two control sites adjacent to the same crop type (rotational field crop, orchard, pasture, or vineyard), within the same landscape context (i.e., within 1–3 km of the restoration site), but > 1 km from all other study sites. Control sites were generally weedy field edges and reflect a variety of unmanaged crop field edges found in the region as the pre-restored condition (**Figure S1**). Such edges typically contain a variety of non-native forbs, grasses, shrubs, and trees and may at times be tilled, treated with pesticides, or left alone. The most common flowering plants at these sites are *Convolvulus arvensis*, *Brassica* spp., *Lepidium latifolium*, *Picris echioides*, and *Centaurea solstitialis*. Many of these species have also established at restoration sites.

We sampled pollinator communities at each restoration and control site three or more times per year from 2006 until 2014 (with a few exceptions; see **Table S2**). All sampling was conducted between April and August. Dates of sampling were spread evenly across this time period. Sites were selected in random order for sampling and, once all sites had been sampled, the process began again for the next sample round. Due to logistical constraints, no sampling was conducted in 2010.

Insect pollinators were netted in timed samples along a 350 m transect, pausing the timer while handling specimens and identifying the plant species from which each specimen was collected, until 1 h of netting time had elapsed. Surveys were only conducted under sunny conditions when the temperature was above 21 °C and wind speed was below 2.5 m/s. While all insect visitors that contacted the reproductive parts of the flower were collected, here we focus our analyses only on wild bees (non-honey bees). Specimens were identified to species (or morpho-species for some bee specimens in the genera *Lasioglossum*, *Nomada*, and *Sphecodes*) by an expert taxonomist (Robbin Thorp, Professor Emeritus, UC Davis).

2.2. Functional Traits

Traits selected for assessing functional diversity were resource capture and use traits, as described previously (Kremen and M'Gonigle, 2015; Ponisio et al., 2016). Qualitative traits for bees included sociality, nesting location, nesting habit, lecty (degree of pollen specialization), and whether the species is known to pollinate crops. Following Burkle et al. (2013), we classified bees as social (including primitively social to eusocial) or solitary based on Michener (2000). Following Williams et al. (2011), we classified nesting location as above- or below-ground and nesting habit as constructing a nest (excavator) or using a pre-existing cavity (renter). Nesting location was based on Krombein et al. (1979), Michener (2000), Cane et al. (2007), and Sheffield et al. (2011), and nesting habit was based on Michener (2000). Lecty was classified as oligo (specialist) or poly (generalist) lectic, based on Krombein et al. (1979). Bee species were classified as crop visitors if they had been detected as visitors during studies conducted in the sample area on the main pollinator-dependent crops grown in the region (watermelon, tomato, sunflower, and almond, Kremen et al., 2004; Greenleaf and Kremen, 2006a,b; Klein et al., 2012; Brittain et al., 2013a,b; Sardiñas et al., 2016). We omitted the five cleptoparasitic species (13 specimens) from the functional trait analysis because such species depend on their host species for pollen and nest construction and thus cannot be scored for several of the qualitative characteristics.

Quantitative traits for bees included mean body size and floral resource specialization. For body size, we used log-transformed inter-tegular distance as a proxy for mobility (Greenleaf et al., 2007), measuring from one to five specimens under a dissecting microscope. We calculated floral resource specialization and regional abundance based on a more inclusive data set (this data set plus additional data collected in the same study area on an additional 48 hedgerow and control sites using identical sampling methods within the same set of sample years; Morandin and Kremen, 2013; Ponisio et al., 2017, C. Kremen, unpublished data). For floral resource specialization, for each bee species in our data, we calculated the metric d' , which measures the deviation of the observed interaction frequency from a null model in which all partners interact in proportion to their abundances (Blüthgen et al., 2006); thus, it is not confounded with abundance as is the total number of interaction partners (Winfree et al., 2014). It ranges from 0 for generalist species to 1 for specialist species. In contrast to lecty, measurements of d' includes floral visits both for pollen to provision larvae and for nectar and pollen for adult food, reflecting both larval and adult diet breadth.

3. MEASURING SITE AND LANDSCAPE CHARACTERISTICS

3.1. Floral Diversity

In order to investigate how restoration affects pollinator communities via changes in floral composition, we calculated a Shannon diversity index for the plants blooming at each site. On, or within several days of, each bee survey date, we surveyed 50 1m^2 quadrats for the presence of blooming plant species, and used the incidence of each blooming species among those 50 quadrats as a proxy for its abundance when calculating the Shannon diversity index (see **Figure S3** for further details about quadrat placement). For each site, this metric was calculated at each sample date within a year (for pollinator community composition analyses) or across all dates within a year (for pollinator functional diversity analyses, see below).

3.2. Nesting Resources

During one of the floral diversity surveys each year, we also noted several characteristics previously associated with nesting resources for above or below-ground nesting bees (Potts et al., 2005; Sardiñas and Kremen, 2014). In each of the 50 1 m^2 quadrats, we estimated the percentages of the quadrat comprising exposed bare ground or covered by dead wood as indicators of below or above-ground nesting habitat, respectively. We then used the average percentage across all 50 quadrats as a metric of "percent bare ground" or "percent dead wood" at each site in each year.

3.3. Natural Cover

We used a habitat classification method developed in Karp et al. (2016) to quantify the extent of semi-natural habitat surrounding a site. This method accounts for nearby natural habitat likely having a greater impact on a focal site than natural habitat that is farther away (see also Miguet et al., 2017). It does this by weighting each cover type fragment according to its distance from the focal site, and summing the resultant distance-weighted areas. Weights are calculated using a Gaussian function with a decay rate α, such that each fragment is assigned a weight of $W = e^{-d^2/(2*\alpha^2)}$, where d is the distance from the focal site. Reducing α lowers the weight assigned to distant fragments relative to the weight assigned to near fragments. Here, we chose a decay rate equal to $\alpha = 350$ which translates to effects of natural habitat beyond 1 km being negligible, which fits within typical bee foraging movements (Greenleaf et al., 2007; Kennedy et al., 2013). To determine the area of surrounding natural habitat within a 1 km radius of each hedgerow, we classified aerial imagery data from the National Agricultural Imagery Program (NAIP, 1 m^2 resolution, https://www.fsa.usda.gov/programs-and-services/aerial-photography/imagery-programs/naip-imagery/) for Yolo County using heads-up digitizing in ArcGIS (version 10.1, Environmental Systems Research Institute, Redlands, CA, USA) to identify patches of semi-natural habitat, which in this intensively-farmed landscape were restricted to riparian and riparian-scrub habitats. For samples collected prior to 2010, we developed cover scores from 2006 NAIP data, and for those collected during or after 2010, we developed cover scores from 2012 NAIP data.

3.4. Statistical Analyses

All continuous predictors were centered prior to analysis to allow direct comparison of the magnitude of effect sizes. Sample day and day^2 were included as explanatory variables in all models (except where noted otherwise) to account for seasonality (M'Gonigle et al., 2015), as were random effects of site and year to account for non-independence among samples collected from the same site or within the same year.

3.4.1. Floral and Nesting Resources

To investigate whether floral or nesting resources changed as a result of restoration, we modeled floral diversity (see above), dead wood, or bare ground as a function of the number of years post-restoration (*ypr*) for each site in each year. Values of *ypr* are initially 0 for all sites and then, for hedgerow sites, increase by +1 each year following planting. For example, a site restored in 2007 would have a value of $ypr = 0$ in 2006 and 2007, and a value of 1 in 2008, 2 in 2009, and so on. Use of a quantitative predictor such as *ypr* permits greater flexibility in analyses then a classic before-after coding scheme. A before-after coding is better suited to analyzing a pulse disturbance, whereas we studied a press disturbance (the maturation of hedgerows and their effects on site characteristics and flower visitor communities).

3.4.2. Community and Functional Composition

To investigate whether pollinator community composition tracked changes in floral resources over time, we used generalized linear mixed models (GLMM) to model aggregate abundance (Negative Binomial error), species richness (Gaussian error, Chao1 estimator, transformed as (natural log+1); Chao, 2006), species diversity (Gaussian error, Shannon index) and species evenness (Gaussian error, Evar; 82 of 412 total samples had ≤ 1 species and, thus, had to be dropped, Smith and Wilson, 1996) as a function of floral diversity, natural cover, *ypr*, and the interactions of *ypr* with floral diversity and natural cover. Transformations were used when necessary to achieve normality. After constructing the full model with these terms, we used Likelihood Ratio Tests to compare successively simpler models, dropping the least significant interaction or main effect at each step (Zuur et al., 2009). We wished to determine if changes in floral diversity or natural cover could substitute for *ypr* or, if not, whether they interacted with *ypr*. We were also interested in whether changes in above or below ground nesting resources led to changes in the corresponding groups of pollinators; however, since neither resource (dead wood or bare ground) showed a significant response to restoration (see section 4), we do not present models investigating responses of above or below ground bee communities to these variables.

We also used GLMM to analyze functional diversity (Gaussian error) except that samples at each site were combined within each year. We used functional dispersion (Fdis, square-root transformed, one sample with 0 species was dropped) as our metric of functional diversity. Fdis measures how communities fill an *n*-dimensional trait space independently of species richness (Laliberté and Legendre, 2010). Fdis is the sum of the abundance-weighted distances of each species from the community centroid in the n-dimensional trait space, normalized by abundance. Pooling data from multiple site visits within each year ensured that samples contained enough species to calculate Fdis. The first three samples per site within each year were pooled for analysis (see **Table S2**), in order to maintain consistency among sites in sample effort and seasonal timing. Model structure was the same as above, except that within-season effects (e.g., sample day and day^2) were excluded because they were no longer meaningful.

Finally, in order to determine how long after restoration significant changes in community and functional metrics are detectable, we replaced *ypr* with a categorical years-post-restoration predictor (*yprF*) in those original models in which *ypr* was retained as a significant main or interactive effect. Here, we omitted the floral diversity and natural cover co-variates, but retained seasonal and random effects, in order to focus on the temporal component.

4. RESULTS

Across the 9 sample years and 15 sites, we collected 7,179 specimens comprising 101 bee species (including 4 morphospecies containing a total of 11 specimens). Forty-two species in the sample (41.5%) were known to visit crops common to the region, and comprised the majority of individuals in

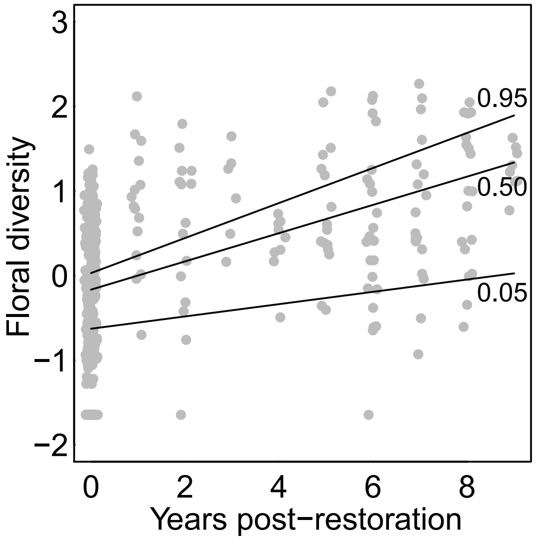


FIGURE 1 | Effect of restoration on floral diversity. Points show Shannon diversity values for each sample at each site in each year. Lines show model-estimated curves for different possible values of adjacent natural cover (from bottom to top, curves correspond to the 5th, 50th, and 95th quantiles for levels of natural cover observed in our data-set).

the sample (6,125 specimens, 85.3%). The subset of data used for functional diversity analysis included 5,718 specimens comprising 84 species, after removing those species for which trait data was incomplete (16 species including cleptoparasites, 44 specimens in total) and some samples to standardize sampling across sites (1,417 specimens).

Floral diversity increased linearly and significantly in response to *ypr* (effect size = 0.147; $p < 0.001$) and the amount of natural cover in the surrounding landscape (effect size = 0.21; $p < 0.037$). A positive interaction between *ypr* and the amount of natural habitat cover (effect size = 0.042; $p = 0.021$) meant that floral diversity increased faster with *ypr* for sites with greater surrounding natural habitat cover (**Figure 1**). Neither the amount of dead wood nor the amount of bare ground increased with *ypr* or surrounding natural cover.

Species abundance increased significantly with floral diversity, while species evenness declined (**Table 1**, **Figures 2a,c**). Species richness, species diversity and functional dispersion increased with floral diversity and *ypr*, but these factors had a negative interaction, such that species richness in new and maturing hedgerows increased with increasing floral diversity, while mature hedgerows had a stable or negative response to increased floral diversity (**Table 1**, **Figures 2b,d**, **3**). Surrounding natural cover also had a weak, but significant, positive effect on species richness ($p = 0.046$) and functional dispersion ($p = 0.034$) (**Table 1**).

A consistent positive change in species richness and diversity occurred at 5 years post restoration and beyond (**Figures 4A,B**). Notably, there was also a significant positive change at 2 years post restoration. Since plants were young at this stage and provided few to no blooms, this increase may indicate that the sites selected for hedgerow plantings were already somewhat higher in bee richness then control sites. We found significant changes in functional dispersion only at 4 and 8 years compared to the initial community (**Figure 4C**).

5. DISCUSSION

Bee species abundance and evenness tracked changes in floral diversity (**Figure 2**), and no other variables were explanatory. As predicted, abundance responded positively to greater floral diversity, while evenness declined, probably due to the addition of a greater number of rare species. Bee species diversity, richness, and functional diversity (as measured by functional dispersion) also responded positively to increases in floral diversity, but only in young and maturing hedgerows. In contrast, these quantities declined in response to floral diversity at the most mature hedgerows. This effect was more pronounced for functional diversity than for species diversity or richness. At sites with the highest floral diversity levels (i.e., more mature hedgerows, **Figure 1**), higher floral diversity may be associated with reduced densities of preferred or total floral resources, leading to reduced visitation. For example, Hegland and Boeke (2006), studying a semi-natural grassland in Norway, found that floral visitor occurrence, richness, and activity all responded more strongly to total floral density than floral diversity. Williams et al. (2015), studying the response of pollinator communities to wildflower plantings in farmland, similarly found that, when using plant mixes that provide continuous bloom, pollinator abundance and richness responded to floral display size rather than to floral diversity. Alternatively, the changes in visitor response to floral diversity with hedgerow maturation may reflect a saturating community assembly process with increased floral resources. In experimentally assembled plant communities of varying floral richness, Ebeling et al. (2008) found that pollinator community richness saturated as floral density increased over 15 percent or floral richness increased over nine species.

Floral diversity of hedgerows increased as hedgerows matured, and this pattern was stronger when hedgerows were nearer to natural habitats (**Figure 1**). An increase in floral diversity associated with natural habitat could occur if native or non-native plant species colonize from nearby semi-natural habitat patches. The majority of species that colonized hedgerow plantings (i.e., non-planted species that were not present in floral samples in the year prior to planting) were non-native; these species could have been present in the seed bank, or have colonized either from surrounding agricultural or semi-natural habitats. Semi-natural habitats may provide refugia for weed species; in Europe, greater complexity of the surrounding landscape had a positive effect on weed species diversity of both vegetation and seed bank within agricultural fields (Roschewitz et al., 2005). Alternatively, floral diversity at hedgerows may be responding to other factors that are correlated negatively or positively with semi-natural habitat. Since our floral diversity metric measured the diversity of what was blooming at each sampling event (as opposed to plant species diversity), changes in the floral diversity metric

TABLE 1 | Effect sizes and standard errors (parentheses) for GLMM results of pollinator community response to years post restoration (*ypr*), floral diversity and natural cover, showing the covariates retained in the final, most parsimonious model.

	Predictor				
Response	**ypr**	**Floral diversity**	**Natural cover**	**Floral diversity * ypr**	**Natural cover * ypr**
Species abundance	–	0.31(0.061)***	–	–	–
Species evenness (evar)	–	−0.043(0.013)**	–	–	–
Species diversity (Shannon)	0.039(0.017)*	0.13(0.037)***	–	−0.035(0.012)**	–
Species richness (chao1)	0.076(0.023)**	0.19(0.048)***	0.13(0.066)*	−0.039(0.016)*	–
Functional dispersion	0.010(0.0039)*	0.032(0.0098)**	0.019(0.0091)*	−0.0069(0.0033)*	–
Functional dispersion – reduced†	–	0.027(0.0071)***	–	–	–

Significance is indicated by (p < 0.05), (**p < 0.01), and (***p < 0.001).* †*In the reduced model, Functional dispersion was calculated omitting traits of nest location and sociality (see text).*

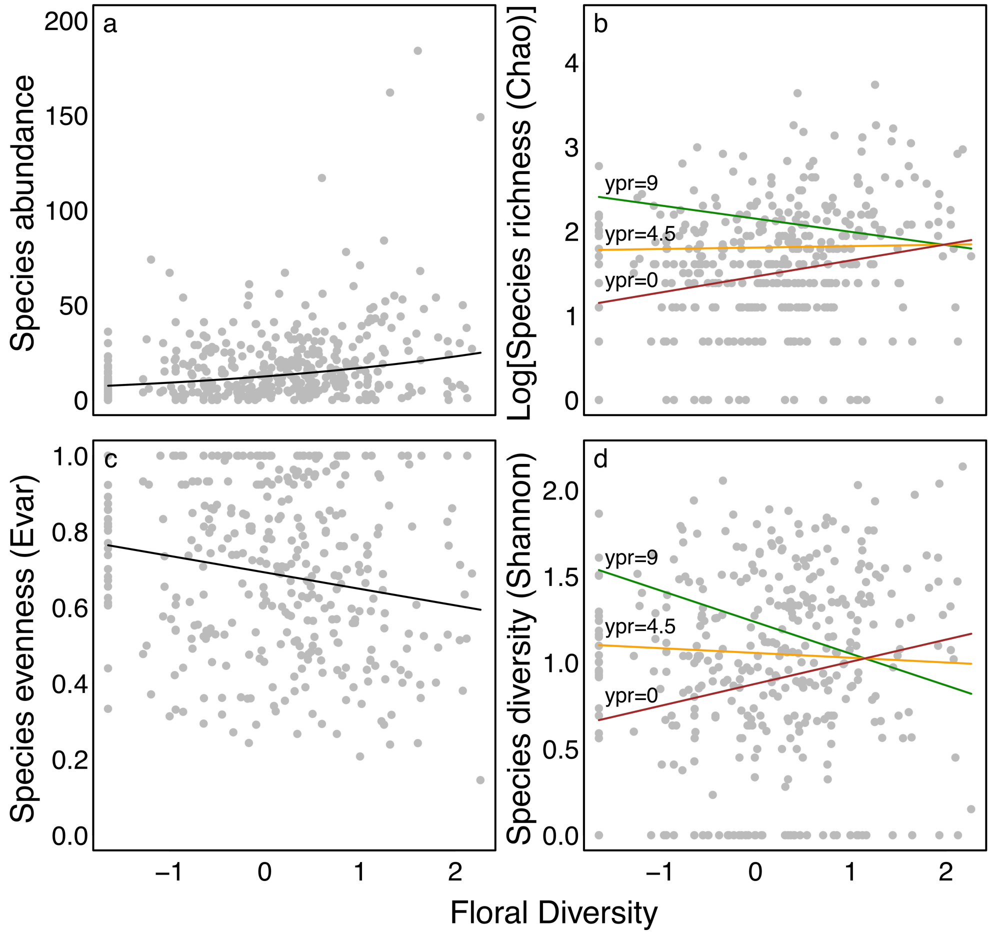


FIGURE 2 | Effect of floral diversity on **(a)** abundance, **(b)** estimated species richness, **(c)** species evenness, and **(d)** species diversity in bees. Points show calculated values of each metric for each sample at each site within each year and lines show curves for best fit models, as presented in **Table 1**. Curves in **(b,d)** show the predicted effect of floral diversity for for restorations of different ages (*ypr*) with colors corresponding to 0 (the minimum; red), 4.5 (the mean; orange), and 9 (the maximum; green). All other parameters were set to their mean values.

may reflect slight site-to-site alterations in the timing or duration of flowering, rather than addition of species. Such changes in flowering phenology might be due to micro-climate, soil type, or urban heat-island effects (Dunne et al., 2003; Neil and Wu, 2006; Dahlgren et al., 2007) that could be correlated to the proportion and distribution of nearby natural habitat.

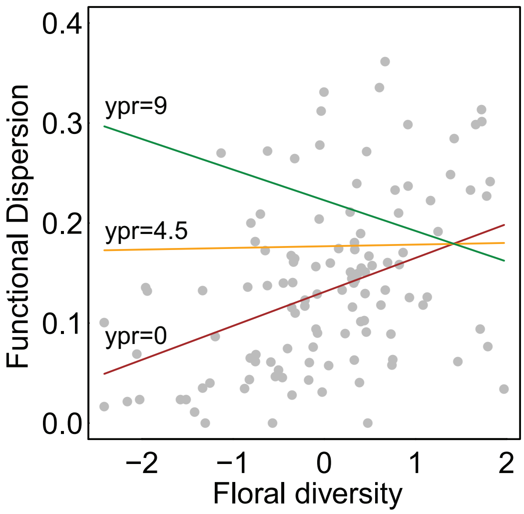


FIGURE 3 | Effect of natural cover on trait functional dispersion. Points show calculated values for each site in each year and lines show model-estimated curves. Curves show different restoration ages (*ypr*) with colors corresponding to 0 (the minimum; red), 4.5 (the mean; orange), and 9 (the maximum; green). All other parameters were set to their mean values. Unlike **Figure 2**, data from multiple visits were pooled for each site within each year.

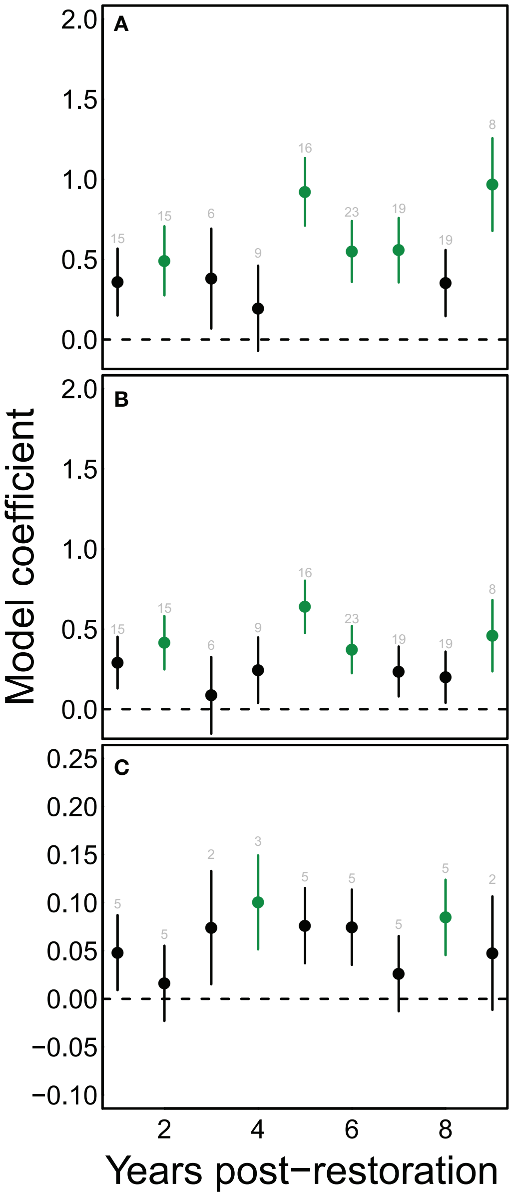


FIGURE 4 | Restoration has significant effects on bee communities chiefly after the first 5 years of restoration. Points show mean model estimates for the effect of each value of *yprF* on **(A)** estimated species richness, **(B)** Shannon diversity, and **(C)** functional dispersion. Vertical bars denote the standard error of this estimate. Green coloring indicates values that are significantly different from the starting condition (*yprF*=0). Text above each point indicates the number of samples for that value on the horizontal axis.

In contrast to floral diversity, we did not observe a significant relationship between the amount of dead wood or bare ground as hedgerows matured. Because above-ground nesting bees respond positively to hedgerow additions (Kremen and M'Gonigle, 2015), we expected that hedgerows would enhance woody material and nesting sites. In contrast, the lack of change in bare ground with hedgerow maturation corroborates our earlier results comparing 8 mature hedgerow sites and 8 controls, in which we found no changes in 4 indicators of ground-nesting resources (bare exposed ground, slope variability, surface soil compaction and soil particle size) or in the nesting rates of ground-nesting bees (Sardiñas et al., 2016). It may be that the quantification of dead wood used here is not an accurate proxy for the amount of above-ground wood or the availability of above-ground nesting sites. The proxies used in our study and previously by Sardiñas et al. (2016) were recommended by Potts et al. (2005) based on a study in Israel, but these resources may be less applicable in other regions. Until better methods for estimating nesting resources are developed and validated, data on proxies for nesting site resources should be carefully interpreted.

Finally, contrary to our expectations, local and landscape site characteristics did not entirely replace years post restoration as an explanatory variable, since bee species diversity, richness, and functional diversity still responded positively to this variable. The length of time since planting may be a proxy for other unmeasured variables that are enhanced with time, such as nesting resources, which we may have failed to measure adequately. Examining the response to years post restoration in more detail (**Figure 4**) shows that bee community responses to shrub-based habitat enhancements take time—as we expected, consistent increases in species richness and other metrics relative to non-restored conditions generally occurred after five or more years had passed since restoration. The more variable behavior of functional diversity may reflect a weaker ability to detect changes in this metric, because of lower resolution in the metric (fewer functional types than species), and lower power (annual vs. per sample calculation of functional dispersion metric). The bee community responses that we observed over the 9 years following planting may not represent saturated community responses. In other studies in which we compared these "maturing" sites with both "mature" ($\gg$10 years) hedgerows and unrestored controls, we found that mature hedgerows displayed greater beta and functional diversity (Ponisio et al., 2016) than maturing hedgerows. However both maturing and mature hedgerows displayed substantial turnover in pollinators and plant-pollinator interactions, indicating the dynamic nature of these communities (Ponisio et al., 2017).

Crop-visiting species made up the majority of records in our sample, primarily due to the dominance of two species in bee communities, *Lasioglossum (Dialictus) incompletum* and *Halictus tripartitus*. These two species dominate not only hedgerow and control sites within agriculture but also nearby natural habitats (Forrest et al., 2015). Not surprisingly, therefore, all significant responses that we observed for the entire bee community were also observed for the subset of crop-visiting species, except for minor changes in the model for functional dispersion. Hedgerows, therefore, enhance communities of crop-visiting species and have the potential to support pollination services, although the amount of support is likely to be crop, scale, and context specific (Sardiñas and Kremen, 2015).

Bee species richness and functional diversity were both positively associated with the amount of surrounding natural cover. Natural habitat patches may supply colonists to maturing and mature hedgerows, and may specifically enrich certain functional types. To query this hypothesis, we examined the correlations between average trait states at our hedgerow and control sites (i.e., trait values weighted by relative abundances) and surrounding natural habitat. Sociality and nest location were significantly correlated to natural cover, with solitary, above-ground nesting bees more likely to be associated with natural cover. After removing sociality and nest location traits and re-calculating the functional dispersion metric, we no longer found any significant effect of surrounding natural habitat but only floral diversity (**Table 1**). These findings suggest that nearby natural habitat may contribute solitary and above-ground nesting colonists to hedgerow sites, and aligns with a study conducted in the same region showing that farmed land, whether organic or conventional, imposes strong filters on bee functional types, weeding out bees with above-ground nesting and solitary traits found in adjacent natural habitats (Forrest et al., 2015). Through habitat creation and subsequent community assembly, hedgerow plantings, in combination with nearby natural habitat remnants, may reverse some of the functional and community disassembly that occurs as natural habitats are converted to farmlands.

DATA AVAILABILITY STATEMENT

The datasets analyzed for this study can be found in the https://github.com/leithen/hedgerow_traits.

AUTHOR CONTRIBUTIONS

CK designed the study; CK, LM, and LP collected data; CK and LM analyzed data with LP's input. CK wrote the manuscript and all authors contributed to revisions.

FUNDING

This work was supported by funding from the Army Research Office (W911NF-11-1-0361 to CK), the Natural Resources Conservation Service (CIG-69-3A75-12-253, CIG-69-3A75-9-142, CIG-68-9104-6-101, and WLF-69-7482-6-277 to The Xerces Society), the National Science Foundation (DEB-0919128 to CK), the U.S. Department of Agriculture (USDA-NIFA 2012-51181-20105 to Michigan State University), USDA-NIFA and NSF predoctoral fellowships to LP and The Natural Sciences and Engineering Research Council of Canada (Discovery Grant to LM). Publication made possible in part by support from the Berkeley Research Impact Initiative (BRII) sponsored by the UC Berkeley Library.

ACKNOWLEDGMENTS

We thank the growers and land owners that allowed us to work on their property and Dr. Robbin Thorp (Professor Emeritus, University of California, Davis) for identifying our bee specimens. We would also like to thank T. Shih, M. VanDyke, K. Ullman, C. Locke, H. Wallis, S. Kaiser, K. Cutler, H. Sardiñas, and the many field and lab assistants that have helped over 10 years of data collection and specimen processing.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2018.00170/full#supplementary-material

REFERENCES

Blaauw, B. R., and Isaacs, R. (2014). Flower plantings increase wild bee abundance and the pollination services provided to a pollination-dependent crop. *J. Appl. Ecol.* 51, 890–898. doi: 10.1111/1365-2664.12257

Blüthgen, N., Menzel, F., and Blüthgen, N. (2006). Measuring specialization in species interaction networks. *BMC Ecol.* 6:9. doi: 10.1186/1472-6785-6-9

Brittain, C., Kremen, C., and Klein, A. M. (2013a). Biodiversity buffers pollination from changes in environmental conditions. *Global Change Biol.* 19, 540–547. doi: 10.1111/gcb.12043

Brittain, C., Williams, N., Kremen, C., and Klein, A. M. (2013b). Synergistic effects of non-Apis bees and honey bees for pollination services. *Proc. R. Soc. B* 280:20122767. doi: 10.1098/rspb.2012.2767

Bugg, R. L., Anderson, J. H., Thomsen, C. D., and Chandler, J. (1998). "Farmscaping in California: hedgerows, roadside plantings and wild plants for biointensive pest management," in *Enhancing Biological Control: Habitat Management to Promote Natural Enemies of Agricultural Pests*, eds C. H. Pickett and R. L. Bugg (Berkeley, CA: University of California Press), 339–374.

Burkle, L. A., Marlin, J. C., and Knight, T. M. (2013). Plant-pollinator interactions over 120 years: loss of species, co-occurrence, and function. *Science* 339, 1611–1615. doi: 10.1126/science.1232728

Cane, J. H., Griswold, T., and Parker, F. D. (2007). Substrates and materials used for nesting by North American *Osmia* bees (hymenoptera: Apiformes: Megachilidae. *Ann. Entomol. Soc. Am.* 100, 350–358. doi: 10.1603/0013-8746(2007)100[350:SAMUFN]2.0.CO;2

Chao, A. (2006). "Species estimation and applications," in *Encyclopedia of Statistical Sciences* (John Wiley & Sons). doi: 10.1002/0471667196.ess5051

Dahlgren, J. P., von Zeipel, H., and Ehrlén, J. (2007). Variation in vegetative and flowering phenology in a forest herb caused by environmental heterogeneity. *Am. J. Bot.* 94, 1570–1576. doi: 10.3732/ajb.94.9.1570

Dunne, J. A., Harte, J., and Taylor, K. J. (2003). Subalpine meadow flowering phenology responses to climate change: integrating experimental and gradient methods. *Ecol. Monograph.* 73, 69–86. doi: 10.1890/0012-9615(2003)073[0069:SMFPRT]2.0.CO;2

Ebeling, A., Klein, A. M., Schumacher, J., Weisser, W. W., and Tscharntke, T. (2008). How does plant richness affect pollinator richness and temporal stability of flower visits? *Oikos* 117, 1808–1815. doi: 10.1111/j.1600-0706.2008.16819.x

Forrest, J. R. K., Thorp, R. W., Kremen, C., and Willams, N. M. (2015). Contrasting patterns in species and functional-trait diversity of bees in an agricultural landscape. *J. Appl. Ecol.* 52, 706–715. doi: 10.1111/1365-2664.12433

Garbach, K., and Long, R. F. (2017). Determinants of field edge habitat restoration on farms in California's sacramento valley. *J. Environ. Manage.* 189, 134–141. doi: 10.1016/j.jenvman.2016.12.036

Garibaldi, L. A., Carvalheiro, L. G., Leonhardt, S. D., Aizen, M. A., Blaauw, B. R., Isaacs, R., et al. (2014). From research to action: enhancing crop yield through wild pollinators. *Front. Ecol. Environ.* 12, 439–447. doi: 10.1890/130330

Garibaldi, L. A., Steffan-Dewenter, I., Winfree, R., Aizen, M. A., Bommarco, R., Cunningham, S. A., et al. (2013). Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science* 339, 1608–1611. doi: 10.1126/science.1230200

Greenleaf, S. S., and Kremen, C. (2006a). Wild bee species increase tomato production and respond differently to surrounding land use in Northern California. *Biol. Cons.* 133, 81–87. doi: 10.1016/j.biocon.2006.05.025

Greenleaf, S. S., and Kremen, C. (2006b). Wild bees enhance honey bees' pollination of hybrid sunflower. *Proc. Natl. Acad. Sci. U.S.A.* 103, 13890–13895. doi: 10.1073/pnas.0600929103

Greenleaf, S. S., Williams, N. M., Winfree, R., and Kremen, C. (2007). Bee foraging ranges and their relationship to body size. *Oecologia* 153, 589–596. doi: 10.1007/s00442-007-0752-9

Gurr, G. M., Lu, Z., Zheng, X., Xu, H., Zhu, P., and Chen, G. et al. (2016). Multi-country evidence that crop diversification promotes ecological intensification of agriculture. *Nat. Plants.* 2:16014. doi: 10.1038/nplants.2016.14

Hegland, S. J., and Boeke, L. (2006). Relationships between the density and diversity of floral resources and flower visitor activity in a temperate grassland community. *Ecol. Entomol.* 31, 532–538. doi: 10.1111/j.1365-2311.2006.00812.x

Karp, D. S., Moses, R., Gennet, S., Jones, M. S., Joseph, S., M'Gonigle, L. K., et al. (2016). Agricultural practices for food safety threaten pest control services for fresh produce. *J. Appl. Ecol.* 53, 1402–1412. doi: 10.1111/1365-2664.12707

Kennedy, C. M., Lonsdorf, E., Neel, M. C., Williams, N. M., Ricketts, T. H., Winfree, R., et al. (2013). A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. *Ecol. Lett.* 16, 584–599. doi: 10.1111/ele.12082

Kleijn, D., Winfree, R., Bartomeus, I., Carvalheiro, L. G., Henry, M., Isaacs, R., et al. (2015). Delivery of crop pollination services is an insufficient argument for wild pollinator conservation. *Nat. Commun.* 6:7414. doi: 10.1038/ncomms8414

Klein, A. M., Brittain, C., Hendrix, S. D., Thorp, R., Williams, N., and Kremen, C. (2012). Wild pollination services to California almond rely on semi-natural habitat. *J. Appl. Ecol.* 49, 723–732. doi: 10.1111/j.1365-2664.2012.02144.x

Koh, I., Lonsdorf, E. V., Williams, N. M., Brittain, C., Isaacs, R., Gibbs, J., et al. (2016). Modeling the status, trends, and impacts of wild bee abundance in the United States. *Proc. Natl. Acad. Sci. U.S.A.* 113, 140–145. doi: 10.1073/pnas.1517685113

Kovács-Hostyánszki, A., Espíndola, A., Vanbergen, A. J., Settele, J., Kremen, C., and Dicks, L. V. (2017). Ecological intensification to mitigate impacts of conventional intensive land use on pollinators and pollination. *Ecol. Lett.* 20, 673–689. doi: 10.1111/ele.12762

Kremen, C., and M'Gonigle, L. K. (2015). Small-scale restoration in intensive agricultural landscapes supports more specialized and less mobile pollinator species. *J. Appl. Ecol.* 52, 602–610. doi: 10.1111/1365-2664.12418

Kremen, C., Williams, N. M., Bugg, R. L., Fay, J. P., and Thorp, R. W. (2004). The area requirements of an ecosystem service: crop pollination by native bee communities in California. *Ecol. Lett.* 7, 1109–1119. doi: 10.1111/j.1461-0248.2004.00662.x

Krombein, K. V., Hurd, J. P. D., Smith, D. R., and Burks, B. D. (1979). *Catalog of Hymenoptera in America North of Mexico*. Washington, DC: Smithsonian Institution Press.

Laliberté, E., and Legendre, P. (2010). A distance-based framework for measuring functional diversity from multiple traits. *Ecology* 91, 299–305. doi: 10.1890/08-2244.1

Long, R. F., Garbach, K., and Morandin, L. A. (2017). Hedgerow benefits align with food production and and sustainability goals. *Calif. Agricult.* 71, 117–119. doi: 10.3733/ca.2017a0020

M'Gonigle, L. K., Ponisio, L. C., Cutler, K., and Kremen, C. (2015). Habitat restoration promotes pollinator persistence and colonization in intensively-managed agriculture. *Ecol. Appl.* 25, 1557–1565. doi: 10.1890/14-1863.1

M'Gonigle, L. K., Williams, N. M., Lonsdorf, E., and Kremen, C. (2017). A tool for selecting plants when restoring habitat for pollinators. *Conserv. Lett.* 10, 105–111. doi: 10.1111/conl.12261

Michener, C. D. (2000). *The Bees of the World*. Baltimore, MD: Johns Hopkins University Press.

Miguet, P., Fahrig, L., and Lavigne, C. (2017). How to quantify a distance-dependent landscape effect on a biological response. *Methods Ecol. Evol.* 8, 1717–1724. doi: 10.1111/2041-210X.12830

Morandin, L. A., and Kremen, C. (2013). Hedgerow restoration promotes pollinator populations and exports native bees to adjacent fields. *Ecol. Appl.* 23, 829–839. doi: 10.1890/12-1051.1

Morandin, L. A., Long, R. F., and Kremen, C. (2016). Pest control and pollination cost-benefit analysis of hedgerow restoration in a simplified agricultural landscape. *J. Econ. Entomol.* 109, 1020–1027. doi: 10.1093/jee/tow086

Neil, K., and Wu, J. (2006). Effects of urbanization on plant flowering phenology: a review. *Urban Ecosyst.* 9, 243–257. doi: 10.1007/s11252-006-9354-2

Ponisio, L. C., Gaiarsa, M. P., and Kremen, C. (2017). Opportunistic attachment assembles plant-pollinator networks. *Ecol. Lett.* 20, 1261–1272. doi: 10.1111/ele.12821

Ponisio, L. C., M'Gonigle, L. K., and Kremen, C. (2016). On-farm habitat restoration counters biotic homogenization in intensively managed agriculture. *Global Change Biol.* 22, 704–715. doi: 10.1111/gcb.13117

Potts, S. G., Vulliamy, B., Roberts, S., O'Toole, C., Dafni, A., Ne'eman, G., et al. (2005). Role of nesting resources in organising diverse bee communities in a Mediterranean landscape. *Ecol. Entomol.* 30, 78–85. doi: 10.1111/j.0307-6946.2005.00662.x

Rader, R., Bartomeus, I., Garibaldi, L. A., Garratt, M. P. D., Howlett, B. G., Winfree, R., et al. (2016). Non-bee insects are important contributors to global crop pollination. *Proc. Natl. Acad. Sci. U.S.A.* 113, 146–151. doi: 10.1073/pnas.1517092112

Ramankutty, N., Mehrabi, Z., Waha, K., Jarvis, L., Kremen, C., Herrero, M., et al. (2018). Trends in global agricultural land use: implications for environmental health and food security. *Annu. Rev. Plant. Biol.* 69, 789–815. doi: 10.1146/annurev-arplant-042817-040256

Roschewitz, I., Gabriel, D., Tscharntke, T., and Thies, C. (2005). The effects of landscape complexity on arable weed species diversity in organic and conventional farming. *J. Appl. Ecol.* 42, 873–882. doi: 10.1111/j.1365-2664.2005.01072.x

Sardiñas, H. S., and Kremen, C. (2014). Evaluating nesting microhabitat for ground-nesting bees using emergence traps. *Basic Appl. Ecol.* 15, 161–168. doi: 10.1016/j.baae.2014.02.004

Sardiñas, H. S., and Kremen, C. (2015). Pollination services from field-scale agricultural diversification may be context-dependent. *Agric. Ecosyst. Environ.* 207, 17–25. doi: 10.1016/j.agee.2015.03.020

Sardiñas, H. S., Ponisio, L. C., and Kremen, C. (2016). Hedgerow presence does not enhance indicators of nest-site habitat quality or nesting rates of ground-nesting bees. *Restoration Ecol.* 24, 499–505. doi: 10.1111/rec.12338

Sheffield, C. S., Ratti, C., Packer, L., and Griswold, T. (2011). Leafcutter and mason bees of the genus *Megachile* Latreille (hymenoptera: Megachilidae) in Canada and Alaska. *Can. J. Arthropod. Identif.* 18, 1–107. doi: 10.3752/cjai.2011.18

Smith, B., and Wilson, J. T. (1996). A consumer's guide to evenness indices. *Oikos* 76, 70–82.

Underwood, A. J. (1994). On beyond BACI: sampling designs that might reliably detect environmental disturbances. *Ecol. Appl.* 4, 3–15. doi: 10.2307/1942110

Wilkerson, M. L., Ward, K. L., Williams, N. M., Ullmann, K. S., and Young, T. P. (2014). Diminishing returns from higher density restoration seedings suggest trade-offs in pollinator seed mixes. *Restor. Ecol.* 22, 782–789. doi: 10.1111/rec.12141

Williams, N. M., Cariveau, D., Winfree, R., and Kremen, C. (2011). Bees in disturbed habitats use, but do not prefer, alien plants. *Basic Appl. Ecol.* 12, 332–341. doi: 10.1016/j.baae.2010.11.008

Williams, N. M., and Lonsdorf, E. V. (2018). Selecting cost-effective plant mixes to support pollinators. *Biol. Cons.* 217, 195–202. doi: 10.1016/j.biocon.2017.10.032

Williams, N. M., Ward, K. L., Pope, N., Isaacs, R., Wilson, J., May, E. A., et al. (2015). Native wildflower plantings support wild bee abundance and diversity in agricultural landscapes across the United States. *Ecol. Appl.* 25, 2119–2131. doi: 10.1890/14-1748.1

Winfree, R., Williams, N. M., Dushoff, J., and Kremen, C. (2014). Species abundance, not diet breadth, drives the persistence of the most linked pollinators as plant-pollinator networks disassemble. *Am. Nat.* 183, 600–611. doi: 10.1086/675716

Zuur, A., Ieno, E. N., Walker, N., Saveliev, A. A., and Smith, G. M. (2009). *Mixed Effects Models and Extensions in Ecology with R*. New York, NY; London: Springer.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 01 May 2019
doi: 10.3389/fevo.2019.00145

Interaction Diversity Maintains Resiliency in a Frequently Disturbed Ecosystem

Jane E. Dell[1,2]*, Danielle M. Salcido[1], Will Lumpkin[1], Lora A. Richards[1], Scott M. Pokswinski[3], E. Louise Loudermilk[2], Joseph J. O'Brien[2] and Lee A. Dyer[1]

[1] Ecology, Evolution, and Conservation Biology, Department of Biology, University of Nevada, Reno, NV, United States, [2] US Forest Service, Center for Forest Disturbance Science, Southern Research Center, Athens, GA, United States, [3] Wildland Fire Science Program, Tall Timbers Research Station, Tallahassee, FL, United States

Frequently disturbed ecosystems are characterized by resilience to ecological disturbances. Longleaf pine ecosystems are not only resilient to frequent fire disturbance, but this feature sustains biodiversity. We examined how fire frequency maintains beta diversity of multi-trophic interactions in longleaf pine ecosystems, as this community property provides a measure of functional redundancy of an ecosystem. We found that beta interaction diversity at small local scales is highest in the most frequently burned stands, conferring immediate resiliency to disturbance by fire. Interactions become more specialized and less resilient as fire frequency decreases. Local scale patterns of interaction diversity contribute to broader scale patterns and confer long-term ecosystem resiliency. Such natural disturbances are likely to be important for maintaining regional diversity of interactions for a broad range of ecosystems.

Keywords: interaction diversity, tri-trophic interaction, resilience, response diversity, scale-dependency, *Pinus palustris*, prescribed fire

Edited by:
Gina Marie Wimp,
Georgetown University, United States

Reviewed by:
Francesco Pomati,
Swiss Federal Institute of Aquatic Science and Technology, Switzerland
Serena Rasconi,
Wasser Cluster Lunz, Austria

Correspondence:
Jane E. Dell
jane.dell@nevada.unr.edu

Specialty section:
This article was submitted to Population and Evolutionary Dynamics,
a section of the journal Frontiers in Ecology and Evolution

Received: *06 November 2018*
Accepted: *15 April 2019*
Published: *01 May 2019*

Citation:
Dell JE, Salcido DM, Lumpkin W, Richards LA, Pokswinski SM, Loudermilk EL, O'Brien JJ and Dyer LA (2019) Interaction Diversity Maintains Resiliency in a Frequently Disturbed Ecosystem.
Front. Ecol. Evol. 7:145.
doi: 10.3389/fevo.2019.00145

INTRODUCTION

Disturbances are significant features of ecosystems, with frequency and intensity being important for shaping not only community composition, structure, and function but also serving as selective forces in the evolution of life history strategies, especially in disturbance-prone ecosystems (Sousa, 1984; Seidl et al., 2016). Resiliency and high beta diversity are critical features of many of these frequently disturbed ecosystems (Elmqvist et al., 2003; Larson et al., 2013). Regular disturbance events maintain a diverse and functional ecosystem state in disturbance-dependent systems and within this context, resiliency is defined as ecosystem recovery to pre-disturbance levels of factors such as diversity, population measures, and nutrient cycling immediately post-disturbance; i.e., persistence of an ecologically stable state (Peterson et al., 1998; Gunderson, 2000). Conversely, a disruption of the disturbance regime, such as reduced frequency, represents a transformational and longer-term perturbation where ecosystem structure and function shift and push such systems to unpredictable or unstable, alternative states (Beisner et al., 2003; Bowman et al., 2016). In this context, removal of disturbance erodes the basin of ecological stability or long-term resilience of a disturbance-adapted ecosystem.

Resiliency requires a minimum level of underlying species and functional diversities to allow for multiple pathways toward post-disturbance responses (Peterson et al., 1998; Seidl et al., 2016). The return to pre-disturbance function due to functional redundancies provided by biological diversity is also known as response diversity (**Figure 1**). Elmqvist et al. (2003) define response diversity as the diversity of responses to disturbance among different assemblages of species that contribute to equivalent ecosystem functions. However, response diversity is not simply equivalent to species richness for different broad taxa or at different trophic levels, because ecological communities are comprised of species that interact in different functional ways. For instance, a broad diet breadth or shared basal resources provide functional redundancy and are indicative of response diversity and trophic network stability (Pilosof et al., 2017; **Figure 1**). As such, quantification of response diversity requires measured metrics such as interaction diversity, defined as the richness and relative abundance of species interactions in a community, in order to understand the effects of disturbance on the interactions between species (Dyer et al., 2010). Critical ecosystem functions, such as pollination, population control of herbivores by natural enemies, and seed dispersal are dependent upon a broad range of biotic interactions at small scales, the loss of which can precipitate species extinctions and loss of ecological function (Kremen et al., 2007; Valiente-Banuet et al., 2015; **Figure 1**). Therefore, it is also important to consider interaction diversity, as a primary contributor to ecosystem resilience and a critical component of response diversity. While species richness and potential interactions are necessarily positively correlated, diversity of species and diversity of interactions can have different effects on ecosystem function and stability (Pardikes et al., 2018). Like other diversity metrics, interaction diversity across the landscape has alpha, beta, and gamma components that can differ substantially from species diversity.

As with any metric exploring patterns of biodiversity, concepts of scale are necessary to consider when examining the causes and consequences of the richness and turnover of interacting species (Bowman et al., 2016; Giron et al., 2018). Patterns observed at larger scales represent the combined processes occurring at smaller scales, but it is not always clear how patterns at nested scales relate to one another. Species richness differs among local and regional scales (Rahbek and Graves, 2001; Witman et al., 2004) partly because regional and local diversity are shaped by different processes. For plant-insect networks, regional processes are affected more by large-scale evolutionary and historical factors, such as speciation, dispersal, extinction, and biogeographical history, while local processes include ecological effects such as, biotic interactions, resource availability, and disturbance. Furthermore, interaction networks are not static, and patterns in interaction diversity are unlikely to be constant across the landscape or at different spatial extents (Dáttilo et al., 2019). At smaller local scales, trait-distributions, environmental conditions and species abundance will affect the potential of two co-occurring species to interact (Poisot et al., 2015). Regionally, interaction diversity values can change substantially depending on the scale at which they are examined (Pardikes et al., 2018).

In this study we focused on trophic interactions between host plants, arthropod herbivores, and their parasitoid enemies in a frequently disturbed ecosystem across a large fire-adapted forest ecosystem. Disturbance by fire has been a part of terrestrial ecosystems since the Silurian Period and is an essential process for maintaining both ecosystem function and biological diversity in fire dependent ecosystems (Pausas and Keeley, 2009), such as the frequently burned longleaf pine (*Pinus palustris* Mill., Kirkman et al., 2004; O'Brien et al., 2008; Mitchell et al., 2009). In the absence of fire, competitive advantage is given to faster growing, non-fire dependent broadleaved vegetation, resulting in a closed canopy, extensive habitat degradation, and reductions in plant diversity (Mitchell et al., 2009; Noss et al., 2014). The removal of fire from the landscape initiates a shifting ecosystem trajectory where fire-adapted species are replaced by other species assemblages, yielding an alternative stable state (Beisner et al., 2003; Barrios et al., 2011).

Our primary objective was to quantify interaction diversity across a time since fire gradient, in order to assess the effect of longer fire return intervals on biotic community interactions and potential for resiliency in longleaf pine forests. We posit that resiliency will be greatest in ecosystems where there is functional redundancy, (i.e., high response diversity), and that this functional redundancy is greatest when levels of beta interaction diversity (for multiple scales) are maintained (**Figure 1**). Higher levels of turnover in interactions are indicative of increased ecological function (Lepesqueur et al., 2018) such that a reduction in beta diversity represents a homogenization of interactions which may reduce ecosystem function by affecting productivity, resilience to disturbance, and vulnerability to biological invasion (Balata et al., 2007; Dell et al., 2019). As frequent fire maintains high-levels of plant diversity and ecosystem function, we predict that large-scale interaction diversity will be higher in frequently burned stands than in stands with longer times since fire. Second, to understand the way interaction diversity varies with scale, we investigated how these patterns vary at both the small, plot-level vs. broader, regional-level scales. Many understory plant species have a patchy distribution in longleaf pine because of fine-scale variation in fuel and fire heterogeneity (Menges and Hawkes, 1998; Dell et al., 2017), and diversity of these plants is best quantified at small spatial scales, therefore we expect that interaction diversity will also vary and patterns will change with increasing spatial scale. Due to the connectivity between these spatial scales any such local scale patterns of interaction diversity will contribute to broader scale patterns and confer long-term ecosystem resiliency for the region.

MATERIALS AND METHODS

Study Area

Research was conducted in longleaf pine forests across the Gulf Coastal Plain during 2013 to 2016. Sites included Eglin Air Force Base and Blackwater River State Forest located in the Florida panhandle and Solon Dixon Forestry Education Center and Conecuh National Forest in southern Alabama. The fire regime in longleaf pine ecosystems is characterized by high-frequency, low-intensity surface fires with return intervals of 1–5 years (Mitchell et al., 2009). Numerous longleaf pine stands within the region are actively managed by prescribed fires with a target of an 18-month to 2-year fire return interval (Hiers et al., 2007). However, there exist stands within all management

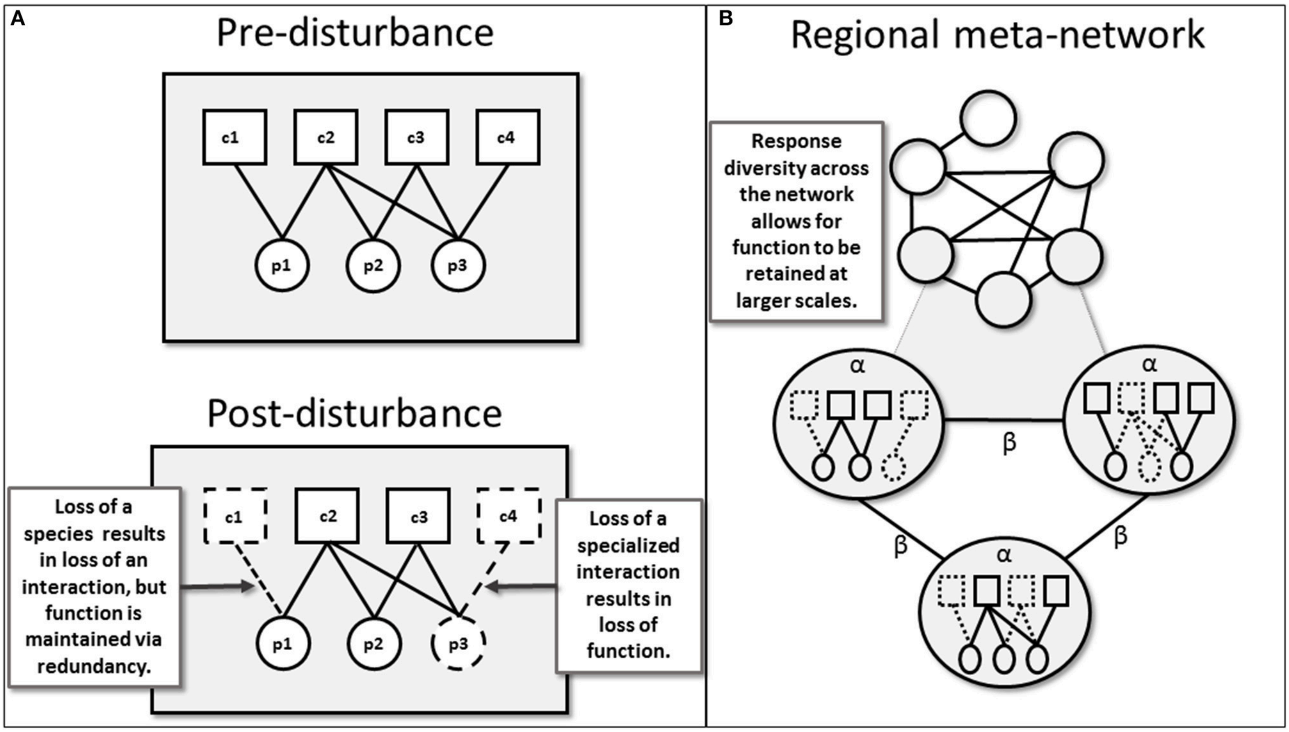


FIGURE 1 | (A) Pre-disturbance and post-disturbance metawebs, displaying the full regional pool of species and potential interactions. Here nodes represent individual species of primary producers (circles) and herbivores (squares), while edges (links) represent interactions between species. Post-disturbance, the loss of species and interactions are indicated by dashed edges. In this case the loss of a single species (c1) also results in the loss of an interaction, however ecological function (i.e., nutrient cycling via consumption of this plant species) is maintained as a redundant interaction occurs with another herbivore species (c2). Conversely, the loss of a specialized interaction may result in the loss of ecological function. In this example, the interaction between a plant (p3) and herbivore (c4) no longer occurs, reducing functional diversity and eventual loss of partner species (p3-c4). **(B)** The regional meta-network, for which nodes represent plots and edges represent shared interactions between plots. Three plots are enlarged so that we may examine the corresponding local networks of interactions. While α-diversity of species and interactions are calculated within each plot, β-diversity is calculated between plots. Focusing in on shared interactions between three individual plots illustrates the turnover of interactions between local plots (high β-diversity), this β-diversity summarizes variation in post-disturbance responses, which provides ecological resiliency.

areas that have not experienced burning for longer periods of time including up to several decades. Sampling includes both frequently burned and infrequently burned areas as well as an intermediate transitional state.

Field Collection

We established sixty-seven, 30-m diameter plots in forested stands that varied in the time since last disturbance by fire (**Figure 2**). Based on available fire history records and vegetative indicator species associated with known fire return intervals, plots were placed into a burn category; frequently burned (fire return interval (FRI): 1–5 years, $n = 49$), intermediately burned (FRI: 5–25 years, $n = 9$), and infrequently burned (FRI: >25 years, $n = 9$).

Throughout the entire growing season, Lepidopteran larvae were sampled within each plot using beat sheets and visual searches in a standardized format moving clockwise around the plot. Within each fire return interval type, we also generally collected caterpillars in stands similar in burn frequency and adjacent to standardized plots to further expand the trophic network within each fire return interval category. Caterpillars were reared out to adulthood or eclosion of a parasitoid. Host plant associations were based on the vegetation from which the caterpillars were collected and confirmed through feeding in the laboratory. Host plants and arthropods were identified to species or were assigned a morphospecies based on morphological characteristics, behavior, and host plant record following Wagner (2005). Sampled arthropods were deposited into the research collection at the University of Nevada, Reno Museum of Natural History.

Quantification of Diversity

Diversity was estimated for species and interactions at two scales; the plot-level or local scale and the broader, regional-level scale. Interaction diversity was based on the richness and abundance of interactions between species, where richness is the number of unique interactions and abundance the total number of each interaction (Dyer et al., 2010; **Figure 3**). The frequencies of unique bipartite interactions were quantified between plants and caterpillars as well as between caterpillars and parasitoids. Additionally, the frequency of tri-trophic interactions between plants, caterpillars, and parasitoids were

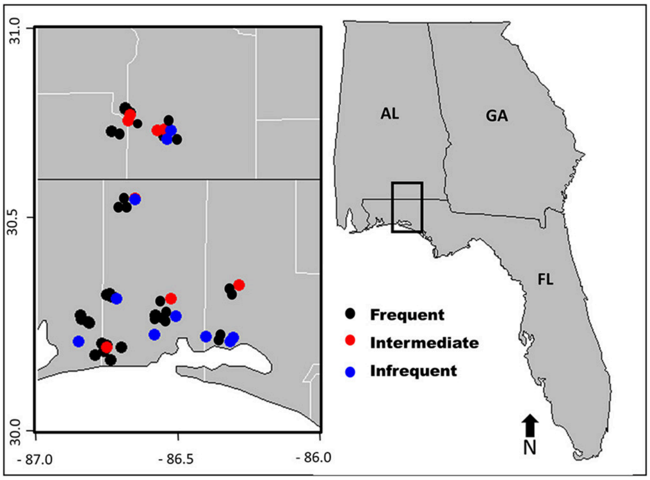


FIGURE 2 | Reference map of Southeastern United States with study area highlighted in black rectangle. Within the study area, individual plot locations are indicated by colored dots corresponding to each of the three fire return intervals.

included to capture emergent properties on network structure (Dyer et al., 2010; Pilosof et al., 2017; **Figure 3**). The richness and associated frequencies of these interaction types were used to estimate interaction diversity. Alpha diversity of species and their interactions was calculated for each plot and summarized using mean alpha diversity to compare across each burn category. Local beta diversity was calculated as the turnover of species or interactions among plots within a fire return interval category. To obtain estimates of variance for local beta diversity within fire return interval categories, we randomly sampled plots within each category and calculated turnover among subsampled plots. We reiterated this process 100 times, each time taking a subsample of plots relative to the total number of plots within the fire return interval following Chao et al. (2008). Additionally, we estimated alpha and beta diversity at the regional scale. The regional level represents each fire return interval category, and regional networks utilized all data, including interactions recorded within a plot and data from generally collected species and interactions within each burn category over the entire range of the study. In other words, all of the interaction data collected from a region was synthesized into a single network matrix for that region (rather than creating a meta-network of smaller matrices). This regional approach, using all the interaction data from a large area is the traditional method for estimating networks (Dunne, 2006; Poisot et al., 2015), and it is in contrast to our fine-scale approach of constructing networks from individual plots. It should be noted that longleaf pine forests in this study may have frequently burned stands adjacent to infrequently burned stands, therefore our use of the term regional does not infer a singular spatial aggregation, but rather a broader character-state organization. All diversity estimates are reported as effective number of species or interactions using the inverse Simpson diversity index (1/D) and represent independent measures of alpha and beta following Jost (2007). Documented interactions were used to create and visualize trophic networks for all data and for each fire return interval category (**Figure S1**).

Statistical Analysis

To determine if interaction and species diversity across the burn gradient showed similar patterns within plots, we utilized analysis of variance, with fire return interval category as an independent variable, and with alpha and beta diversity parameters as response variables. We performed separate univariate ANOVAs for each diversity component at the local scale. *Post-hoc* analyses utilizing Tukey's test were performed to identify differences between fire return interval types for each diversity parameter.

To address unequal sampling efforts in terms of number of plots within each fire return interval category, we performed sample-based rarefaction for species and interaction richness. We also calculated non-parametric asymptotic estimators at equal sample coverage levels following Chao et al. (2014) to allow for community comparison across the fire return interval gradient. Discriminant function analyses were conducted to detect differences between species and interactions within fire return interval categories. All analyses were performed in R (v.3.2.3, R Core Team, 2013).

RESULTS

The collective sampling effort resulted in a trophic network between 64 host plant species, 183 caterpillar species, and 47 parasitoid species. Combined, there were 1,415 individual interactions between species comprised of 468 unique interactions. Sixty six percent of all interactions were detected only once, and only 2% of interactions occurred over 20 times. Within all fire return interval categories, the majority of herbivorous interactions tended to be between one caterpillar and one host plant species. However, some individual plant species were consumed by numerous herbivores and the percentage of caterpillars with more than two host plants had an inverse relationship with time since fire (**Table 1**). Parasitoid species tended to have a more specialized diet breadth, generally interacting with only a single host species. Each fire return interval category had certain plant species that were involved in a disproportionate number of interactions (**Table 1**). These highly connected species, such as the host plant *Quercus laevis* (turkey oak) connected to 24% of the entire network, are also referred to as network hubs (**Figure S1**). However, the overall connectivity of turkey oak within each fire return interval category decreased with time since fire, reducing from 24% connectivity in frequently burned networks to 12 and 7% in intermediate and infrequently burned networks, respectively.

Large Scale Patterns

Dividing the entire network into regions of similar fire return intervals: frequently, intermediately, and infrequently burned yielded variable patterns in both species and interaction diversity (**Table 2**, **Figure 4**). At this larger scale, species alpha diversity increased with longer fire return intervals. However, frequently burned areas had the greatest parasitoid and herbivore species diversity. Parasitoids made up 15% of species richness in

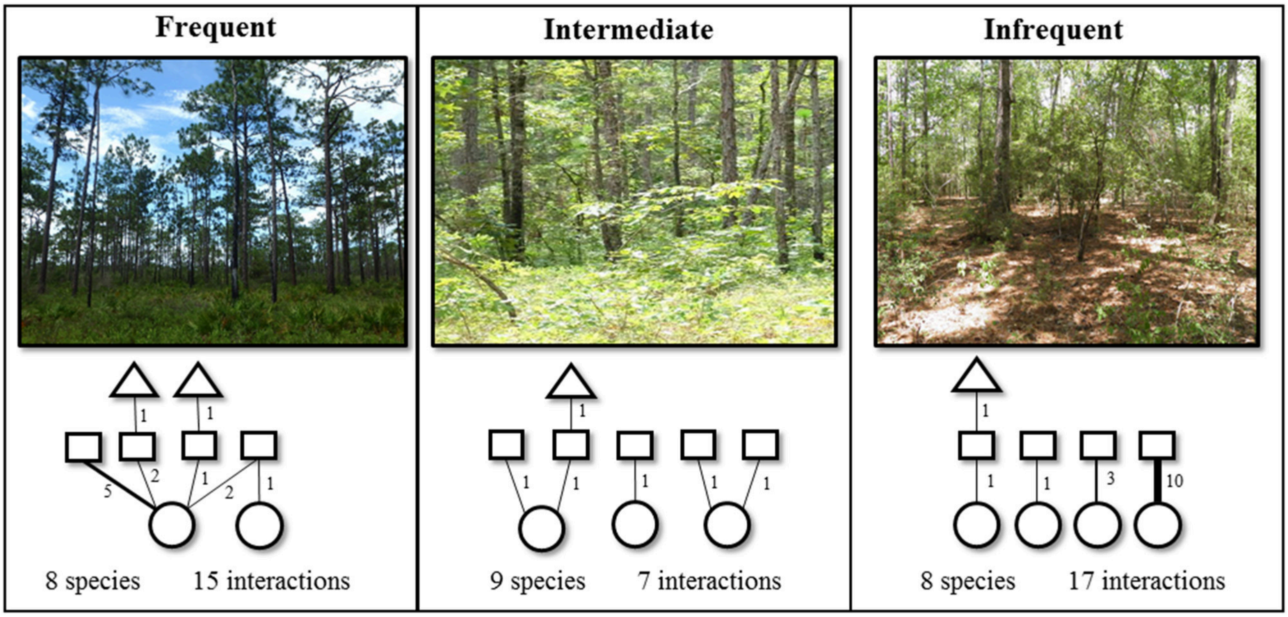


FIGURE 3 | Photo representation of each fire return interval (FRI) category and a sample interaction diversity network found within a single plot in each FRI type. Interactions and their abundances are indicated by solid lines and corresponding numbers, representing interactions between host plants (circles) and herbivores (squares), or between herbivores and parasitoids (triangles), respectively. Each tri-trophic interaction linking a plant, herbivore, and their parasitoid is considered an additional single interaction. Interaction richness and abundance are used to quantify interaction diversity components. Local-scale diversity metrics were calculated with individual plots while regional-scale diversity metrics represent an aggregation of interaction diversity plots and general collections within each FRI category.

frequently burned areas while only 8% in the infrequently burned stands (**Table 2**). The diversity of interactions did not have a clear pattern across the burn gradient with frequent and infrequently burned regions having higher interaction diversity than intermediately burned regions. Beta diversity between the fire return interval regions was higher for interactions than for turnover in species (**Table 2**).

Small Scale Patterns

At the local level, alpha species diversity was significantly higher in intermediately burned plots than in frequently burned plots with infrequently burned plots not significantly different than either [$F_{(2,63)} = 6.48$, $P = 0.003$; **Figure 4A**]. Beta species diversity was significantly higher in infrequently burned plots compared to intermediately burned plots but was not different compared to frequently burned plots [$F_{(2,297)} = 202.3$, $P < 0.001$]. Interaction richness was greatest within infrequently burned plots, while alpha interaction diversity was significantly greater in intermediate burned plots than in frequently burned plots but did not differ between infrequent plots [$\mathrm{F}_{(2,63)} = 5.03$, $P = 0.01$; **Figure 4B**]. In contrast, beta interaction diversity, or the turnover of interactions, was significantly higher in frequently burned plots, almost double the beta diversity of plots in intermediately and infrequently burned stands [$F_{(2,297)} = 820.6$, $P < 0.001$].

Rarefaction analyses illustrated that richness of both species and interactions was highest within infrequently burned plots as compared to intermediately and frequently burned plots (**Figure S2**). Comparing Chao's asymptotic estimates of species richness at an equal level of coverage of 20 samples, the most species were found within infrequently burned plots ($\mathrm{Chao1}_{\mathrm{infrequent}} = 149$) followed by intermediate ($\mathrm{Chao1}_{\mathrm{intermediate}} = 89$), and frequently burned plots ($\mathrm{Chao1}_{\mathrm{frequent}} = 78$). Interaction richness was also highest in infrequently burned plots compared to intermediately and frequently burned plots in both rarefaction compared at equal sampling effort and comparison of Chao's asymptotic estimates of interaction richness ($\mathrm{Chao1}_{\mathrm{frequent}} = 75$, $\mathrm{Chao1}_{\mathrm{intermediate}} = 110$, and $\mathrm{Chao1}_{\mathrm{infrequent}} = 172$). Compositional differences between burn interval categories was confirmed through the discriminant function analysis where the first discriminant function explained 99% of the variance and differentiated interactions and species in frequently burned forests from intermediate and infrequently burned forests, with an opposite relationship at the local (species: $b = 0.75$; interactions: $b = 0.83$) and regional (species: $b = -0.72$; interactions: $b = -0.64$) scale.

DISCUSSION

We found that the relationship between fire return interval and biodiversity was scale dependent for both species and interactions, as measured by richness, and both alpha and beta diversity components (**Table 2**). Frequently burned stands were more diverse at a regional-level scale in species and interaction richness as well as interaction alpha diversity. However, these

TABLE 1 | Relative connectivity of the most linked plant and herbivore species in each tri-trophic network within each fire return interval category.

Frequent		**Intermediate**		**Infrequent**	
Plant	**Connectivity (%)**	**Plant**	**Connectivity (%)**	**Plant**	**Connectivity (%)**
Quercus laevis	24	*Quercus laevis*	12	*Vaccinium arboreum*	7
Diospyros virginiana	12	*Quercus marilandica*	10	*Ilex vomitoria*	6
Quercus incana	8	*Quercus margaretta*	9	*Quercus laevis*	6
Smilax auriculata	4	*Ilex vomitoria*	6	*Vitis rotundifolia*	6
Vaccinium arboreum	4	*Quercus incana*	5	*Vaccinium stamineum*	5
Herbivore	**Connectivity (%)**	**Herbivore**	**Connectivity (%)**	**Herbivore**	**Connectivity (%)**
Gelechiidae 8	3	*Gelechiidae* 3	5	*Hypeagyris esther*	3
Hyperstrovia flaviguttata	3	*Gelechiidae* 10	4	*Noctuidae* 5	3
*Gelechiidae*3	3	*Anisota stigma*	3	*Geometridae* 22	3
*Erebidae*1	2	*Megalopyge crispate*	2	*Thysanopyga intractata*	2
*Noctuidae*5	1	*Hyperstrovia flaviguttata*	2	Gelechiidae 4	2
Host plants per species		**Host plants per species**		**Host plants per species**	
1	70%	1	80%	1	72%
2	10%	2	11%	2	22%
>2	20%	>2	9%	>2	6%

Individual species (i.e., node) connectivity is measured as the percentage of total network links connected to the node in the network. As a summary of diet breadth for each fire return interval category, the percentage of herbivore species that consume 1, 2, or >2 host plants are reported.

TABLE 2 | Diversity measures for both species and interactions calculated at the regional and local scales for each fire return interval category.

Scale	**Fire return interval**	**Richness [P/H/E]**	**α**	**β**	**Richness**	**α**	**β**
Regional	Frequent	170 [37/108/25]	14.11		245	29.83	
	Intermediate	115 [30/67/18]	12.28	2.11	143	8.93	2.80
	Infrequent	145 [38/95/12]	28.18		158	20.44	
Local	Frequent	6.84^{a} (0.59) [2.31/4.04/0.5]	4.39^{a} (0.29)	5.42^{b} (0.63)	5.44^{a} (0.54)	3.97^{a} (0.35)	15.29^{b} (1.39)
	Intermediate	9.33^{ab} (1.45) [3.11/5.22/0.8]	7.26^{b}(1.20)	4.27^{a} (0.38)	7.55^{ab} (1.39)	7.13^{b} (1.32)	8.04^{a} (0.44)
	Infrequent	13.57^{b} (2.31) [3.89/6.11/1.0]	6.63^{ab} (1.37)	5.57^{b} (0.88)	11.29^{b} (2.61)	5.57^{ab} (1.45)	9.00^{a} (0.21)

Alpha and beta diversity components were estimated using inverse Simpson's index (1/D). Variance around local beta values estimated by bootstrapping. The regional scale represents each fire return interval category, and regional networks utilized all data, including interactions recorded within a plot and data from generally collected species and interactions within each burn category over the entire range of the study. Bracketed values in species richness represent plants (P), herbivores (H), and parasitoids (E), respectively, with local levels indicating mean plot values. All other local level means are reported (±SE). Letters denote significant differences between local fire return interval categories based on Tukey's test at $P < 0.05$.

patterns were not consistent when scaling down to the local, plot-level scale. The higher levels of richness among species and interactions, and the higher alpha interaction diversity at local scales in infrequently and intermediately burned stands appeared to be driven by rare species and specialized, single interactions. Shrubby growth forms of hardwood species in longleaf pine forests are maintained by frequent fire, so as fire is removed from the landscape, these species grow and eventually close out the canopy (Hiers et al., 2007). This leads to a depauperate understory of shade tolerant and fire-sensitive plants (Kirkman et al., 2004; Mitchell et al., 2006). As these key plant species are removed due to lack of fire, the increase of fire-sensitive species promotes new interactions.

Frequently burned stands in our study area have more open canopies (Dell et al., 2017), and the characteristic vegetation and associated specialist consumers within more closed canopy stands are not found outside of areas that have not burned in decades as indicated by the segregation of species along the fire return interval gradient. While the assemblages of plants, herbivores, and parasitoids occurring in infrequently burned stands and are characterized by higher richness in comparison to plots that burn more often, interactions between these species are constrained at the local scale. Thus, the lower levels of interaction beta diversity within infrequently burned plots are indicative of the same specialized interactions occurring in individual plots which results in reduced variation in response to disturbance.

One of the most interesting patterns of diversity in the longleaf pine system was the high beta diversity of interactions in frequently burned plots compared to other plots. Lower species richness in frequently burned stands might usually predict similar assemblages in any given plot at the local scale, but this was not the case. While species and interaction richness

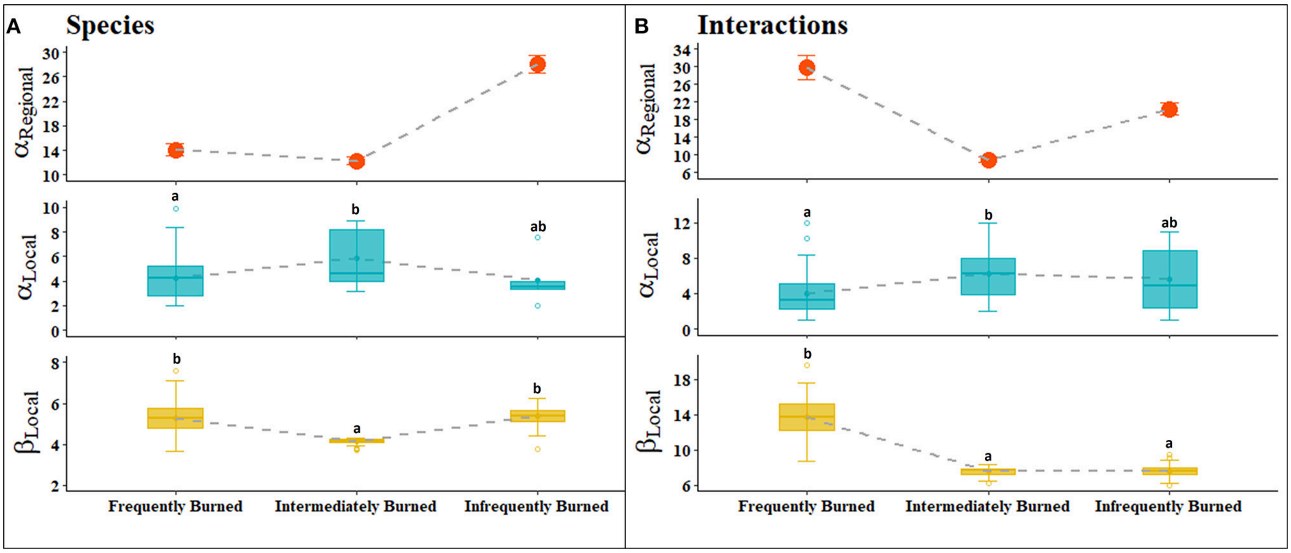


FIGURE 4 | (A) Species diversity components and **(B)** interaction diversity components calculated from frequently, intermediately, and infrequently burned sites at hierarchical spatial extents. The broader, regional-level alpha diversity (top panel) represents diversity calculated at each fire return interval category. Our use of the term regional does not infer a singular spatial aggregation, but rather a broader level organization. Local level alpha diversity (middle panel) and beta diversity (bottom panel) represent diversity components calculated at the plot level. Solid lines indicate median value and points connected to dashed lines indicate mean value within local level panels. Letters denote significant differences between local fire return interval categories based on Tukey's test at $P < 0.05$. Diversity values are reported in (1/D).

were lower than in plots without fire, the increased turnover of interactions between plots reveals that stands that burn more often harbor slightly more generalized consumers, an attribute that confers greater potential resiliency to disturbance with increased response diversity (Peterson et al., 1998; Elmqvist et al., 2003). Fire maintains high response diversity by keeping the ecosystem in a state dominated by longleaf pine and a species-rich, fire-adapted ground cover. In frequently burned forests 20% of the herbivores had a more generalized diet breadth (i.e., > 2 host plant species, **Table 1**), which provides functional redundancy. In this case, the decreased local alpha diversity can facilitate increased local beta diversity (Chase and Myers, 2011), contributing to greater gamma or regional interaction diversity in frequently burned forests—supporting our predictions of frequent fire positively affecting interaction diversity and varying across scale. Focusing in on shared interactions between individual plots illustrates the turnover of interactions between local plots (high β-diversity), this β-diversity summarizes variation in post-disturbance responses regionally, which provides the potential for ecological resiliency.

Furthermore, redundant interactions that may be interchangeable can contribute to sustained ecosystem function (Valiente-Banuet et al., 2015). Higher interaction beta diversity and lower species richness suggest redundant interactions via a rewiring of interacting species in frequently burned forests and may confer resiliency by way of maintenance of ecological function (Lepesqueur et al., 2018). This high degree of interaction turnover may provide an advantage to species adapted to frequently disturbed longleaf pine ecosystem. For example, more generalized diet breadth can be beneficial for individuals post-fire when there is high variability in local plant species composition (García et al., 2016). Response diversity depends on examining multiple spatiotemporal scales to assess full resiliency potential, which may not be evident if only one scale is examined. Regional or ecosystem-level networks represent an aggregation of numerous snapshots in space and time. Thus, there are dynamic processes occurring over time in networks of realized interactions that are not captured in our static presentation of trophic networks in this system. However, application of a multilayer network perspective allows for associative connections between individual plots (single layer) and the larger scale (multiple layers) by way of shared species and interactions (Pilosof et al., 2017). Therefore, the information we gain by analyzing diversity of interactions are still informative for assessing the impact of fire return interval on the biotic communities. Specifically, contributions to both immediate and long-term resiliencies are found at local and regional-level scales, respectfully.

The relative ecological importance of connectivity in these longleaf networks becomes more apparent when focusing on dynamics of individual species or management of particular species. The relative connectivity of highly connected species, or hubs, has an inverse relationship with fire return interval (**Table 1**). For example, turkey oak (*Quercus laevis*), a host plant to many herbivores, was represented in 24% of all network links in frequently burned forests compared to only 12 and 6% in intermediate and infrequently burned forests, respectively. While highly connected networks are more resilient to perturbation, the loss of highly connected species would have significant impact on the remaining network and in simulations, eventually leads to network collapse (Bascompte and Jordano, 2014). Therefore, maintenance of hub species is an important management consideration. Removal of turkey oak has often been the inappropriate target of intense management in longleaf pine ecosystem (Hiers et al., 2014; Loudermilk et al., 2016). However, our results highlight that the important contributions of turkey oak to functioning networks in longleaf pine forests.

CONCLUSION

Disturbances, including natural perturbations such as fire, insect outbreaks, and hurricanes, increase habitat heterogeneity which in turn increases the realization of interactions locally and regionally. Variation in interactions is a consequence of varying species abundances, trait distributions and local environmental conditions across the landscape due to variation in disturbance frequency, intensity, duration, and extent (Bowman et al., 2016). Understanding patterns of interaction diversity within disturbance-dependent networks requires carefully collected data at the appropriate scale at which interactions occur, as well as relevant positions along the disturbance gradient. No biological network is static, and large published webs that are assembled from species inventories (e.g., Bascompte and Jordano, 2014) or that examine interactions over large gradients (e.g., Dyer et al., 2007; Forister et al., 2015) are misleading in many ways because identities of interactions often vary across the landscape (Fox and Morrow, 1981; Dáttilo et al., 2019). These metawebs (sensu Dunne, 2006) represent potential interactions within the regional species pool. At finer scales, such as those examined in our longleaf pine plots, networks are comprised of realized interactions. At these local scales, environmental conditions, community composition, and phenologies differ (Chase and Myers, 2011; Garzon-Lopez et al., 2014; Poisot et al., 2015), and the large static network of potential interactions does not exist. Local scale patterns are particularly important in the longleaf pine ecosystem because fine scale heterogeneity in soils, fuels, fire, and dispersal affect plant diversity and community assembly processes (Dell et al., 2017). The processes maintaining assemblages of species and interactions within longleaf pine networks may be deterministic and niche-based at larger scales (entire ecosystems across the landscape), neutral or stochastic at small scales (1-10 m^2 patches; Dell, 2018), or governed by both niche and neutral processes as described by the continuum hypothesis (Gravel et al., 2006), presenting an exciting opportunity for future research.

AUTHOR CONTRIBUTIONS

LD and LR designed the study. JD, DS, LR, SP, EL, JO, and LD collected data. JD, DS, and WL analyzed data with LD's input. JD, DS, and LD wrote the manuscript and all authors contributed to revisions.

FUNDING

This work was made possible through funding by the Strategic Environmental Research and Development Program of the Department of Defense (RC-2243) and the Earthwatch Institute. LD and LR were also supported by DEB-1442103. The funding agencies have no involvement in this study nor publication process.

ACKNOWLEDGMENTS

We would like to thank the dedicated volunteers from the Earthwatch Institute for assistance with caterpillar collection in the field and rearing in the laboratory.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00145/full#supplementary-material

REFERENCES

Balata, D., Piazzi, L., and Benedetti-Cecchi, L. (2007). Sediment disturbance and loss of beta diversity on subtidal rocky reefs. *Ecology* 88, 2455–2461. doi: 10.1890/07-0053.1

Barrios, B., Arellano, G., and Koptur, S. (2011). The effects of fire and fragmentation on occurrence and flowering of a rare perennial plant. *Plant. Ecol*. 212, 1057–1067. doi: 10.1007/s11258-010-9886-7

Bascompte, J., and Jordano, P. (2014). *Mutualistic Networks*. Princeton: Princeton University Press.

Beisner, B. E., Haydon, D. T., and Cuddington, K. (2003). Alternative stable states in ecology. *Front. Ecol. Environ*. 1, 376–382. doi: 10.1890/1540-9295(2003)001[0376:ASSIE]2.0.CO;2

Bowman, D. M. J. S., Perry, G. L. W., Higgins, S. I., Johnson, C. N., Fuhlendorf, S. D., and Murphy, B. P. (2016). Pyrodiversity is the coupling of biodiversity and fire regimes in food webs. *Phil. Trans. R. Soc. B* 371:20150169. doi: 10.1098/rstb.2015.0169

Chao, A., Gotelli, N. J., Hsieh, T. C., Sander, E. L., Ma, K. H., Colwell, R. K., et al. (2014). Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. *Ecol. Monogr*. 84, 45–67. doi: 10.1890/13-0133.1

Chao, A., Jost, L., Chiang, S. C., Jiang, Y.-H., and Chazdon, R. L. (2008). A two-stage probabilistic approach to multiple-community similarity indices. *Biometrics* 64, 1178–1186. doi: 10.1111/j.1541-0420.2008.01010.x

Chase, J. M., and Myers, J. A. (2011). Disentangling the importance of ecological niches from stochastic processes across scales. *Phil. Trans. R. Soc. B* 366, 2351–2363. doi: 10.1098/rstb.2011.0063

Dáttilo, W., Vizentin-Bugoni, J., Debastiani, V. J., Jordano, P., and Izzo, T. J. (2019). The influence of spatial sampling scales on ant-plant interaction network architecture. *J. Anim. Ecol*. 1–12. doi: 10.1111/1365-2656.12978

Dell, J. E. (2018). *Untangling Patterns of Plant and Arthropod Diversity in A Fire-Adapted Ecosystem: Dynamic Relationships Between Fire, Scale, and Trophic Interactions in Longleaf Pine Forests.* Doctoral Dissertation. Reno, NV: University of Nevada, Reno.

Dell, J. E., Pokswinski, S. P., Richards, L. A., Hiers, J. K., Williams, B. W., O'Brien, J. J., et al. (2019). Maximizing the monitoring of diversity for management activities: Additive partitioning of plant species diversity across a frequently burned ecosystem. *Forest Ecol. Manag*. 432, 409–414. doi: 10.1016/j.foreco.2018.09.022

Dell, J. E., Richards, L. A., O'Brien, J. J., Loudermilk, E. L., Hudak, A. T., Pokswinski, S. M., et al. (2017). Overstory-derived surface fuels mediate plant species diversity in frequently burned longleaf pine forests. *Ecosphere* 8:e01964. doi: 10.1002/ecs2.1964

Dunne, J. A. (2006). "The network structure of food webs" in *Ecological Networks: Linking Structure to Dynamics in Food Webs*, eds. M. Pascual and J.A Dunne. (Oxford: Oxford University Press), 27–86.

Dyer, L. A., Singer, M. S., Lill, J. T., Stireman III., J. O., Gentry, G. L., et al. (2007). Host specificity of Lepidoptera in tropical and temperate forests. *Nature* 448, 696–699. doi: 10.1038/nature05884

Dyer, L. A., Walla, T. R., Greeney, H. F., Stireman, I. I. I., J. O., and Hazen, R. F. (2010). Diversity of interactions: a metric for studies of biodiversity. *Biotropica* 42, 281–289. doi: 10.1111/j.1744-7429.2009.00624.x

Elmqvist, T., Folke, C., Nystrom, M., Peterson, G., Bengtsson, J., Walker, B., et al. (2003). Response diversity, ecosystem change, and resilience. *Front. Ecol. Environ*. 1, 488–494. doi: 10.1890/1540-9295(2003)001[0488:RDECAR]2.0.CO;2

Forister, M. L., Novotny, V., Panorska, A. K., Baje, L., Basset, Y., Butterill, P. T., et al. (2015). Global distribution of diet breadth in insect herbivores. *Proc. Natl. Acad. Sci. USA*. 112, 442–447. doi: 10.1073/pnas.1423042112

Fox, L. A., and Morrow, P. A. (1981). Specialization: species property or local phenomenon? *Science* 211, 887–893.

García, Y., Castellanos, M. C., and Pausas, J. G. (2016). Fires can benefit plants by disrupting antagonistic interactions. *Oecologia* 182, 1165–1173. doi: 10.1007/s00442-016-3733-z

Garzon-Lopez, C. X., Jansen, P. A., Bohlman, S. A., Ordonez, A., and Olff, H. (2014). Effects of sampling scale on patterns of habitat association in tropical trees. *J. Veg. Sci*. 25, 349–362. doi: 10.1111/jvs.12090

Giron, D., Dubreuil, G., Bennett, A., Dedeine, F., Dicke, M., Dyer, L. A., et al. (2018). Promises and challenges in insect–plant interactions. *Entomol. Exp. et Appl*. 166, 319–343. doi: 10.1111/eea.12679

Gravel, D., Canham, C. D., Beaudet, M., and Messier, C. (2006). Reconciling niche and neutrality: the continuum hypothesis. *Ecol. Lett*. 9, 399–409. doi: 10.1111/j.1461-0248.2006.00884.x

Gunderson, L. (2000). Ecological resilience–in theory and application. *Annu. Rev. Ecol. Syst*., 31, 425–439. doi: 10.1146/annurev.ecolsys.31.1.425

Hiers, J. K., O'Brien, J. J., Will, R. E., and Mitchell, R. J. (2007). Forest floor depth mediates understory vigor in xeric Pinus paulustris ecosystems. *Ecol. Appl*. 17, 806–814. doi: 10.1890/06-1015

Hiers, J. K., Walters, J. R., Mitchell, R. J., Varner, J. M., Conner, L. M., Blanc, L. A., et al. (2014). Ecological value of retaining pyrophytic oaks in longleaf pine ecosystems. *J. Wildl. Manage*. 78, 383–393. doi: 10.1002/jwmg.676

Jost, L. (2007). Partitioning diversity into independent alpha and beta components. *Ecology* 88, 2427–2439. doi: 10.1890/06-1736.1

Kirkman, L. K., Goebel, C. P., and Palik, B. J. (2004). Predicting plant species diversity in a longleaf pine landscape. *Ecoscience* 11, 80–93. doi: 10.1080/11956860.2004.11682812

Kremen, C., Williams, N. M., Aizen, M. A., Gemmill-Herren, B., LeBuhn, G., Minckley, R., et al. (2007). Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change. *Ecol. Lett*. 10, 299–314. doi: 10.1111/j.1461-0248.2007.01018.x

Larson, A. J., Belote, R. T., Cansler, A., Parks, S. A., and Dietz, M. S. (2013). Latent resilience in ponderosa pine forest: effects of resumed frequent fire. *Ecol. Appl*. 23, 1243–1249. doi: 10.1890/13-0066.1

Lepesqueur, C., Scherrer, S., Vieira, M. C., Almeida-Neto, M., Salcido, D. M., Dyer, L. A., et al. (2018). Changing interactions among persistent species as the major driver of seasonal turnover in plant-caterpillar interactions. *PLoS ONE* 13:e0203164. doi: 10.1371/journal.pone.0203164

Loudermilk, E. L., Hiers, J. K., Pokswinski, S., O'Brien, J. J., Barnett, A., and Mitchell, R. J. (2016). The path back: Oaks *(Quercus spp*.) facilitate longleaf pine (*Pinus palustris*) seedling establishment in xeric sites. *Ecosphere* 7:e01361. doi: 10.1002/ecs2.1361

Menges, E. S., and Hawkes, C. V. (1998). Interactive effects of fire and microhabitat on plants of Florida scrub. *Ecol. Appl*. 8, 935–946. doi: 10.1890/1051-0761(1998)008[0935:IEOFAM]2.0.CO;2

Mitchell, R. J., Hiers, J. K., O'Brien, J., and Starr, G. (2009). Ecological forestry in the southeast: understanding the ecology of fuels. *J. Forest*. 107, 391–397.

Mitchell, R. J., Hiers, J. K., O'Brien, J. J., Jack, S. B., and Engstrom, R. T. (2006). Silviculture that sustains: the nexus between silviculture, frequent prescribed fire, and conservation of biodiversity in longleaf pine forests of the southeastern United States. *Can. J. Forest Res*. 36, 2724–2736. doi: 10.1139/x06-100

Noss, R. F., Platt, W. J., Sorrie, B. A., Weakley, A. S., Means, D. B., Costanza, J., et al. (2014). How global biodiversity hotspots may go unrecognized: Lessons from the North American Coastal Plain. *Divers. Distrib*. 21: 236–244. doi: 10.1111/ddi.12278

O'Brien, J. J., Hiers, J. K., Callaham, M. A., Mitchell, R. J., and Jack, S. B. (2008). Interactions among overstory structure, seedling life history traits and fire in frequently burned neotropical pine forests. *Ambio* 37, 542–547. doi: 10.1579/0044-7447-37.7.542

Pardikes, N., Lumpkin, W., Hurtado, P. J., and Dyer, L. A. (2018). Simulated tri-trophic networks reveal complex relationships between species diversity and interaction diversity. *PLoS One*, 13:e0193822. doi: 10.1371/journal.pone.0193822

Pausas, J. G., and Keeley, J. E. (2009). A burning story: the role of fire in the history of life. *BioScience* 59, 593–601. doi: 10.1525/bio.2009.59.7.10

Peterson, G., Allen, C., and Holling, C. (1998). Ecological resilience, biodiversity, and scale. *Ecosystems* 1, 6–18. doi: 10.1007/s100219900002

Pilosof, S., Porter, M. A., Pascual, M., and Kefi, S. (2017). The multilayer nature of ecological networks. *Nat. Ecol. Evol*. 1, 1–9. doi: 10.1038/s41559-017-0101

Poisot, T., Stouffer, D. B., and Gravel, D. (2015). Beyond species: why ecological interaction networks vary through space and time. *Oikos* 124. 243–251. doi: 10.1111/oik.01719

R Core Team (2013). *R: A Language and Environment for Statistical Computing*. Available online at: http://www.R-project.org/ (accessed April 27, 2018).

Rahbek, C., and Graves, G. R. (2001). Multiscale assessment of patterns of avian species richness. *Proc. Natl. Acad. Sci. U.S.A*. 98, 4534–4539. doi: 10.1073/pnas.071034898

Seidl, R., Spies, T. A., Peterson, D. L., Stephens, S. L., Hicke, J. A., and Angeler, D. (2016). REVIEW: Searching for resilience: addressing the impacts of changing disturbance regimes on forest ecosystem services. *J. Appl. Ecol*. 53, 120–129. doi: 10.1111/1365-2664.12511

Sousa, W. P. (1984). The role of disturbance in natural communities. *Annu. Rev. Ecol. Syst.* 15, 353–391. doi: 10.1146/annurev.es.15.110184.002033

Valiente-Banuet, A., Aizen, M. A., Alcántara, J. M., Arroyo, J., Cocucci, A., Galetti, M., et al. (2015). Beyond species loss: the extinction of ecological interactions in a changing world. *Funct. Ecol.* 29, 299–307. doi: 10.1111/1365-2435.12356

Wagner, D. L. (2005). *Caterpillars of Eastern North America*. Princeton: Princeton University Press.

Witman, J. D., Etter, R. J., and Smith, F. (2004). The relationship between regional and local species diversity in marine benthic communities: a global perspective. *Proc. Natl. Acad. Sci. U.S.A.* 101, 15664–15669. doi: 10.1073/pnas.0404300101

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor is currently co-organizing a Research Topic with one of the authors, LR, and confirms the absence of any other collaboration.

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 04 June 2019
doi: 10.3389/fevo.2019.00193

Heatwaves and Novel Host Consumption Increase Overwinter Mortality of an Imperiled Wetland Butterfly

Mariana Abarca, Elise A. Larsen and Leslie Ries*

Department of Biology, Georgetown University, Washington, DC, United States

Disruptive effects of climate change include range shifts, phenological mismatches among consumers and producers, and population declines. While these biological alterations have been widely documented, studies identifying specific mechanisms linking climate change to population declines are scarce. Extreme events, such as heatwaves can have devastating effects on living organisms and are increasing in frequency as Earth warms. Hence, understanding the effects of heatwaves on insects is necessary to inform conservation efforts and to develop predictions of population dynamics under future climate scenarios. Here, we experimentally evaluated the effects of heatwaves on the survival and phenology of the Baltimore Checkerspot (*Euphydryas phaeton phaeton*), a wetland butterfly with imperiled populations that has incorporated a novel host. We performed laboratory manipulations (implementing realistic temperature regimes) to assess the effect of heatwaves during summer and winter on the survival and phenology of *E. p. phaeton.* In addition, we analyzed historical temperature records to quantify the incidence of heatwaves within *E. p. phaeton*'s range to assess their potential role in the decline of southeastern populations. We found that winter heatwaves with maximum temperatures of 20°C can have more devastating effects on survival than summer heatwaves (up to 41°C). Eggs endured acute heat stress during summer with no significant effects on phenology and survival; similarly, pre-overwintering larvae were robust to heatwave exposure, as only the most intense heatwave treatment reduced their survival (37% reduction compared to control conditions). By contrast, dormant larvae were the most vulnerable stage, as they lost from 2 to 6% of their body mass after a three-day summer heatwave. Furthermore, their exposure to winter heatwaves resulted in 75 to 100% mortality. Feeding on the native host provided higher resilience under thermal stress than feeding on the invasive, recently acquired host. Finally, both heatwave incidence and severity have increased in the southern range of *E. p. phaeton* in the period from 1894 to 2011. We show that warm winter days induced severe mortality, providing a mechanistic explanation of how climate change can trigger population declines in *E. p. phaeton* and other insects.

Keywords: novel host, dormancy, climate change, baltimore checkerspot, *Euphydryas phaeton*, invasive, *Plantago lanceolata*

Edited by:
Lora A. Richards,
University of Nevada, Reno,
United States

Reviewed by:
Fernando Colchero,
University of Southern
Denmark, Denmark
Shu-Jun Wei,
Beijing Academy of Agricultural and
Forestry Sciences, China

***Correspondence:**
Mariana Abarca
ma782@georgetown.edu

Specialty section:
This article was submitted to
Population and Evolutionary
Dynamics,
a section of the journal
Frontiers in Ecology and Evolution

Received: *02 March 2019*
Accepted: *13 May 2019*
Published: *04 June 2019*

Citation:
Abarca M, Larsen EA and Ries L
(2019) Heatwaves and Novel Host
Consumption Increase Overwinter
Mortality of an Imperiled Wetland
Butterfly. Front. Ecol. Evol. 7:193.
doi: 10.3389/fevo.2019.00193

INTRODUCTION

Disruptive effects of climate change such as range shifts, phenological advances (Parmesan and Yohe, 2003; Parmesan, 2007), local extinctions (Parmesan, 2006), and phenological mismatches among consumers and producers (Gordo and Sanz, 2005; Parmesan, 2007; Yang and Rudolf, 2010; Ovaskainen et al., 2013) have been widely documented. Despite the growing number of studies linking altered phenological patterns (Parmesan, 2007) and decrements in arthropod abundance to altered temperature regimes (Lister and Garcia, 2018), studies identifying the specific mechanisms that result in insect decline (e.g., death, emigration) are scarcer (Cahill et al., 2012). Extreme events, such as heatwaves are becoming more common as a result of climate change (Hansen et al., 2012), and can have detrimental effects on both human populations (Xu et al., 2016) and ecosystem dynamics (Ummenhofer and Meehl, 2017). Temperature extremes can trigger changes in plant mortality, community composition and productivity; which in turn affect carbon cycling (Frank et al., 2015; Ummenhofer and Meehl, 2017). Arthropods are not exempt from susceptibility to extreme events; studies in the laboratory have shown that summer heat waves (5 days at constant 42°C) diminished male reproduction and sperm function in the flour beetle *Triboleum castaneum* not only in the generation experiencing them, but also in their offspring (Sales et al., 2018). Studies with lepidopterans showed that exposure to heatwaves of tropical butterflies resulted in prolonged development time, and a reduction of both pupal mass and immune function (Fischer et al., 2014). Furthermore, exposure of immature stages of the moth *Plutella xylostella* to high temperatures resulted in decreased adult lifespan, fecundity, and altered oviposition patterns (Zhang et al., 2015b). Despite experimental evidence of detrimental effects of temperature extremes on individuals, attribution of insect population declines to specific extreme events remains challenging, as it would require careful monitoring of both extreme events and insect populations. The frequency, intensity and duration of heatwaves are predicted to increase as Earth's mean temperature keeps rising (Meehl and Tebaldi, 2004; Perkins et al., 2012; Christidis et al., 2015). Thus, it is important to understand their effects on insects, which are experiencing generalized population declines (Hallmann et al., 2017) in which climate change has been identified as a key contributing factor (Lister and Garcia, 2018; Sánchez-Bayo and Wyckhuys, 2019).

The high temperatures that characterize summer heatwaves (∼40°C) challenge insect physiological limits and can result in death by impaired metabolic activity (Addo-bediako et al., 2000; Chown, 2001). However, unseasonably warm temperatures throughout the year can also have deleterious effects, even if they fall well below the upper physiological limits of insects. For example, warm spells during winter can result in reduced overwinter survival and they can also trigger altered phenological patterns such as early onset of spring activities (Williams et al., 2015). Early spring activity increases the risk of exposure to harsh spring weather (Augspurger, 2013) and can trigger phenological mismatches among consumers and producers (Kudo and Ida, 2013). Food scarcity resulting from altered phenological patterns has been identified as the most important factor by which climate change results in local extinctions of animal populations (Cahill et al., 2012). Hence, unseasonal weather at any time of the year has the potential to be detrimental.

Deleterious effects of climate change on animal populations are often exacerbated by other environmental problems that affect the abundance and quality of food sources (Dirzo et al., 2014). Altered plant community composition, resulting from habitat destruction and colonization by invasive species, exposes herbivorous insects to novel hosts, often triggering host switches or expansions (Moran and Alexander, 2014). The incorporation of novel hosts into insect diets can have deleterious effects on insect populations because foliage quality often differs among novel and native hosts (Rosenwald et al., 2017). In addition, novel hosts have different phenological patterns; which in turn can affect the seasonality of their consumers (Batalden and Oberhauser, 2015); For oligophagous insects, host plant choice can drastically affect fitness, as it affects development time, fecundity (Awmack and Leather, 2002) and their relationships with predators (Bowers, 1980). Furthermore, feeding on high quality hosts can mitigate thermal stress (Diamond and Kingsolver, 2010). Thus, host quality is an important factor to consider when assessing the effects of climate change on herbivorous insects.

Here, we evaluate the effects of summer and winter heatwaves on the performance of a wetland, oligophagous butterfly, the Baltimore Checkerspot (*Euphydryas phaeton phaeton*, Drury Nymphalidae: Melitaeini). While northern populations of *E. p. phaeton* are stable (Bowers and Richardson, 2013), severe declines have been documented in the southern edge, where it is considered endangered (Durkin, 2009; Frye et al., 2013). Because *E. p. phaeton* have only one generation per year, temperature regimes experienced by each ontogenetic stage differ drastically (**Figure 1**). In our experiments, we matched abnormally warm periods with the corresponding ontogenetic stage that is most likely to experience them. Acknowledging differences in thermal sensitivity among ontogenetic stages is necessary to avoid biases when assessing species' vulnerability to climate change (Levy et al., 2015). In addition, we considered the effect of nutrition at mitigating thermal stress. North eastern populations of *E. p. phaeton* have two primary hosts: the native *Chelone glabra* L. (white turtlehead) and the invasive *Plantago lanceolata* L. (English plantain, Plantaginaceae), which was recently acquired as a host (Stamp, 1979). We performed a series of laboratory manipulations, implementing realistic temperature regimes, to determine the susceptibility to heatwaves of different ontogenetic stages of *E. p. phaeton* (eggs, active larvae, dormant larvae), and to assess the role of host plant at mitigating thermal stress. Finally, we analyzed historical temperature records from weather stations in two sites within *E. p. phaeton's* range to assess whether heatwave incidence, duration and intensity have recently increased.

METHODS

Study System

Euphydryas phaeton are univoltine butterflies native to Eastern North America. Two subspecies have been described: *E. p. phaeton* (Drury), in the east, and *E. p. ozarkae* (Masters), in

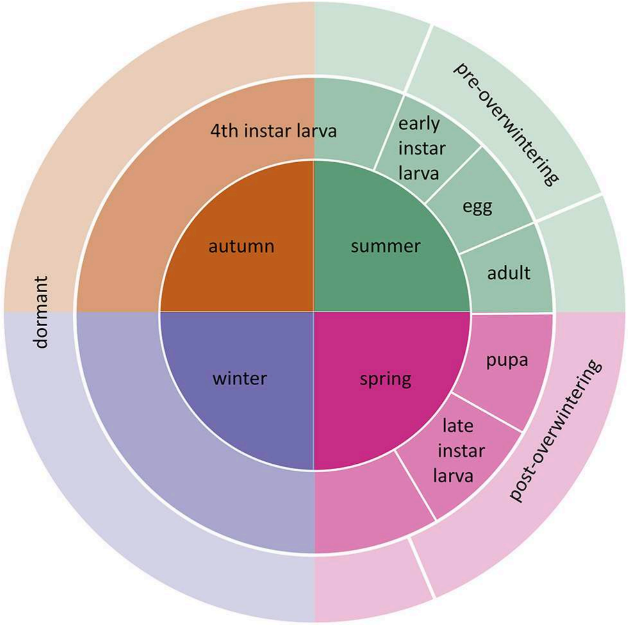


FIGURE 1 | Life cycle of *E. p. phaeton* and the corresponding season when each stage occurs. Counterclockwise, eggs are laid in summer, they hatch into pre-overwintering larvae, which turn into dormant larvae upon molting into 4th instar. Larvae remain dormant during part of the summer, autumn, and winter. Post-overwintering larvae resume feeding and pupate the following spring and adults start the next life cycle.

the montane southwestern part of their range (Robertson, 2015). Adults mate and lay eggs in large clusters during summer, eggs hatch within 3 weeks, larvae feed until the third instar, molt, and fourth instar larvae remain in a state of low metabolic activity from late-summer until the following spring, when they pupate and emerge as adults. Thus, eggs and young larvae (1st to 3rd instar) develop while exposed to hot summer temperatures. By contrast, 4th instar larvae experience a broad temperature range throughout their dormancy, which lasts about 8 months, from the end of summer to the following spring, when they complete development (**Figure 1**). Northern populations of *E. p. phaeton* have two oviposition hosts; the native *C. glabra*, and the invasive *P. lanceolata*, which was introduced from Eurasia and was first reported in North America about 190 years ago (Cavers et al., 1980). *Euphydryas p. ozarkae* has an additional oviposition host, the hemiparasitic *Aureolaria flava*, L. Farw. (Smooth Yellow Foxglove, Orobanchaceae, (Robertson, 2015). The incorporation of *P. lanceolata* to *E. p. phaeton*'s diet has allowed for colony persistence in areas where *C. glabra* is absent or disappearing (Bowers and Richardson, 2013); however, feeding on *P. lanceolata* has some potential costs including higher predation risk due to enhanced palatability to birds (Bowers, 1980), lower growth rate and food efficiency conversion (Bowers et al., 1992) and the risk of death by haying due to grass management practices (Bowers and Richardson, 2013). Herbivore host preferences do not necessarily track foliage quality, as generalists often use hosts according to their relative abundance and not their nutritional value (Mason et al., 2011). Changes in abundance of *P. lanceolata* due to land management practices has already lead to local extinctions of a closely related species, *E. editha* (Singer and Parmesan, 2018). While northern populations of *E. p. pheaton* use *P. lanceolata* as both oviposition and secondary (post-diapause) host (Bowers and Richardson, 2013), populations in Maryland, USA have been reported to use *P. lanceolata* solely as a secondary host (Pers. Comm. Jen Selfridge). For our experiments, we maintained a captive colony of *E. p. phaeton* with founders from Cape Cod, MA, a population that feeds primarily on *P. lanceolata*. This colony is supplemented with eggs collected from wild females of the same population every year; we indicate in each assay whether wild-caught or lab-reared individuals were included.

Experiment Design

To evaluate the effects of heatwaves on *E. p. phaeton* throughout their lifecycle, we performed two sets of experimental manipulations, simulating summer and winter conditions. We used growth chambers (models 136 VL and 130 VL, Percival Scientific, Perry, IA, USA) to expose individuals to either typical or heatwave conditions during the season they naturally occur. Because *E. p. phaeton* lay eggs in clusters, we were able to control for genetic variation in many of our experiments by splitting clutches of siblings onto the different treatments of a given assay. We exposed eggs, pre-overwintering (1st to 3rd instar) and dormant larvae (4th instar) to summer heatwaves and dormant larvae to winter heatwaves. We used different individuals in each assay to avoid potential cumulative effects of repetitive heatwave exposure. In all treatments, temperature oscillated daily approximating a sinusoidal function. We used generalized linear and mixed effects models (GLM & GLMM) to compare performance estimates (e.g., survival, development time, larval mass) among experimental conditions. GLMMs allowed us to incorporate both the fixed effects of interest (e.g., temperature regime) as well as random effects (e.g., of clutch). We modeled survival over a specific interval as a Bernoulli trial and thus used a binomial distribution and logit link function; we modeled development time as the count of days to complete a given life stage and thus used a Poisson distribution with a log link function(Abarca et al., 2018). Finally, to analyze larval mass we implemented a gamma distribution with an inverse function, as mass data exhibited this distribution. We performed all analyses in R version 3.4.3 (R Core, 2017), using package lme4 (Bates et al., 2015). For model validation of GLM and GLMM, we visually inspected residuals and tested the significance of predictors using type II deviance test, implemented in the Anova function of R package car (Fox and Weisberg, 2011).

Summer Heatwave Experiments

To assess the effects of summer heatwaves we performed three assays, each for a different ontogenetic stage: eggs, active larvae (2nd instar) and dormant larvae (4th instar).We implemented a typical summer temperature regime in the southeastern range of 26 ± 5°C (mean ± amplitude), which was maintained for control individuals and interrupted by a 3-day heatwave with either a large 31 ± 10°C or a small 36 ± 5°C amplitude, resulting in three treatments: control (26 ± 5°C), low (31 ± 10°C) and high (36 ± 5°C). Note that the maximum temperature of both heatwave treatments was 41°C (**Figure 2**).

To assess the effect of heatwave exposure on eggs, we evenly split six clutches, from six different wild-caught females (egg mass size varied from 180 to 300 eggs) and assigned siblings to each of the three treatments. Eggs were laid in an oviposition cage in the field (Cape Cod MA, early July, 2018) and transferred to laboratory control conditions 1 day after oviposition. Exposure to heatwave treatments (control, low, high) occurred 4 days later (5-day old eggs). After heatwave exposure, all clutches were maintained on *C. glabra* under control (26 ± 5) conditions until the end of August. We recorded hatching success (proportion of eggs that hatched), egg and larval development time (to hatch and to 4th instar, respectively) and survival to the onset of dormancy (4th instar). We assessed the effects of temperature regime on egg and larval development time and survival to dormancy onset using GLMMs. We compared development time among temperature regimes using two models, one from oviposition to eclosion and one from hatching to the onset of dormancy (4th instar), both using a Poisson error distribution (for the count of number of days needed) and log link function (**Table 1**). We used a binomial error distribution and logit link function in modeling survival to the onset of dormancy. In all models we included temperature regime as a fixed factor and clutch as a random factor.

To assess the effects of summer heatwaves on active, pre-overwintering larvae, we followed a similar approach. We split five clutches from five different wild females into three groups, one for each summer temperature regime; we maintained neonates on *C. glabra* and exposed 7-day old larvae (2nd instar) to the corresponding regime (control, low, high). We recorded development time and survival to the onset of dormancy (4th instar). We used GLMM analogous to those described above to compare survival and development time among temperature regimes (see **Table 1**).

Finally, to test whether exposure of dormant larvae to summer heatwaves results in mass loss, we weighed 4th instar larvae from each of four clutches before and after exposure to each of the three summer treatments (control, low and high). Larvae included in this assay had fed on either *C. glabra* (2 clutches) or *P. lanceolata* (2 clutches). We split groups of siblings among the three treatments for an initial sample size of 240; however, a group from one of the clutches was lost to causes independent from experimental treatments, leaving a final sample size of 220. We kept caterpillars from each sibling group together because larvae are gregarious in nature; our observations in the lab showed that when separated and maintained individually, they engage in abnormal behaviors which could result in mass loss, such as excessive movement and silking. While keeping larvae together was necessary to reduce stress, it prevented the identification of individual caterpillars. Thus, to calculate mass loss, we ranked larvae by weight and assumed the rankings were consistent before and after treatment (that is, the heaviest caterpillar before the heatwave would also be the heaviest individual after the heatwave). We compared percent mass loss among treatments with a GLMM including clutch as a random effect and temperature regime, host, and their interaction as fixed effects, using a gamma error distribution (inverse link

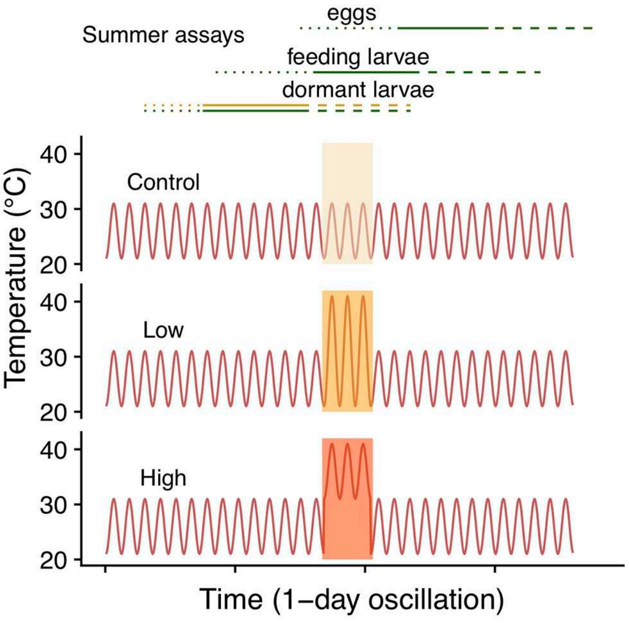


FIGURE 2 | Summer heatwave temperature regimes. Lines in the top panel correspond to the different sets of clutches included in each of the three summer assays. Lines designate eggs (dotted), feeding (solid) and dormant (dashed) larvae. Colors indicate host, green for *C. glabra* and yellow for (*P. lanceolata*), only the dormant larvae assay included both hosts.

TABLE 1 | GLMM summaries for effects of heatwave exposure during summer on survival, development time and mass of *E. phaeton* at different ontogenetic stages: eggs, pre-overwintering (pre), and dormant larvae. All models included clutch as a random intercept.

Life stage Figure	**Response**	**Fixed factors**	**DF**	**χ^2**	***P***	**Error distribution, link function**
Eggs						
	Survival	regime	2	4.83	0.08	binomial, logit
	Development time (eggs)	regime	2	0.41	0.81	Poisson, log
	Development time (1st- 4th)	regime	2	0.63	0.73	Poisson, log
Larvae (pre) **Figure 5A**	Survival	regime	2	64.807	<0.0001	binomial, logit
Larvae (pre) **Figure 5B**	Development time	regime	2	0.9	0.95	Poisson, log
Dormant larvae **Figure 6** (inset)	Mass	host	1	6.44	0.01	gamma, inverse
Dormant larvae **Figure 6**	Mass loss (% of body mass)	regime	2	111.24	<0.0001	gamma, inverse
		host	1	0.46	0.5	
		regime × host	2	8.69	0.013	

function; see **Table 1**). We built a similar model to compare initial mass among caterpillars from different hosts (*C. glabra* vs. *P. lanceolata*), including host as explanatory variable and initial mass as the response variable (**Table 1**).

Winter Heatwave Experiment

To evaluate the effect of winter heatwaves on *E. p. phaeton* survival, we subjected dormant larvae (4th instar) to each of three winter regimes: cold (0 ± 5°C), mild (4 ± 5°C), or heatwaves (4 ± 5°C interrupted every 10 days by a two-day period at 15 ± 5°C; **Figure 3**). For this assay we used groups of siblings ($N = 12$ groups), and groups of larvae from multiple clutches ($N = 5$ groups). These 17 groups of larvae did not participate in any other assays, and were raised under uniform laboratory conditions feeding on either *P. lanceolata* (4 groups) or *C. glabra* (13 groups) during their whole pre- overwintering stage (instars 1 to 4). We kept all larvae at realistic temperatures for a gradual transition from fall to winter conditions. Total sample size by regime was: cold: $N = 714$; mild: $N = 719$; heatwaves: $N = 719$ larvae. Exposure to these winter temperature regimes started on December 15th 2017 and ended on March 16th 2018, when all larvae were supplied with *P. lanceolata* foliage and exposed to uniform spring conditions (**Figure 3**). We counted the number of individuals who survived to spring (were alive and moving on March 19th) and who initiated feeding and growing (had fed and were still alive on March 28th). We compared survival among treatments as a Bernoulli trial (binomial error distribution, logit link function) within a GLM for each date in which survival was assessed (see **Table 2**).

Host Plant and Thermal Stress

To test whether host plant choice can affect *E. p. phaeton* performance under thermal stress, we exposed pre-overwintering larvae to stressfully low temperatures while feeding on either *P. lanceolata* or *C. glabra* following a fully factorial design. We split each of five laboratory- reared egg masses of at least 200 viable eggs into groups of 50 hatchlings and assigned them to the four treatments resulting from the combination of two hosts (*C. glabra, P. lanceolata*) and two spring temperature regimes (cold vs. warm spells): All individuals were kept at a base regime of 20 ± 5°C and were exposed to either warm (4 days at 22.5 ± 2.5°C) or cold spells (4 days at 12.5 ± 2.5°C) twice during larval development (**Figure 4**). Upon the onset of dormancy (molt to 4th instar), we transferred all larvae to 26 ± 5°C and weighed them using a balance (Mettler Toledo MX5). We recorded development time and survival to the end of August (2 months after reaching 4th instar). We compared development time and survival among treatments using GLMMs as described above (see **Table 3** for error distributions and link functions). While pre-overwintering larvae are not likely to experience temperatures as low as our regimes, this assay allowed us to both assess the role of host plant at mitigating thermal stress, and to obtain groups of caterpillars of variable size (body mass). Having a gradient of larval body sizes allowed us to test whether dormant caterpillar mass affects the likelihood of survival to the end of summer. To do this,

TABLE 2 | GLM summaries for effects of heatwave exposure during winter on survival of pre-overwintering larvae.

Life stage Figure	Response	Fixed factors	DF	χ^2	*P*	Error distribution, link function
Dormant larvae **Figure 7**	survival (dormancy termination)	host	1	24.65	< 0.0001	GLM, binomial, logit
		regime	2	839.23	< 0.0001	
		regime × host	2	16.19	< 0.001	
Dormant larvae **Figure 7**	Survival (feed and grow)	host	1	47.082	< 0.0001	GLM, binomial, logit
		regime	2	286.447	< 0.0001	
		regime × host	2	9.936	< 0.01	

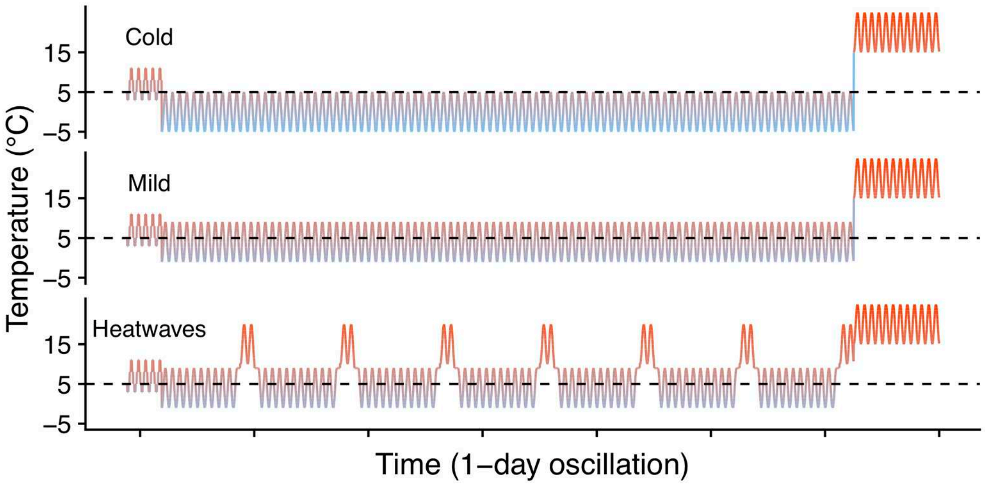


FIGURE 3 | Winter heatwave temperature regimes. Dashed lines indicate 5°C to facilitate visual comparison among regimes.

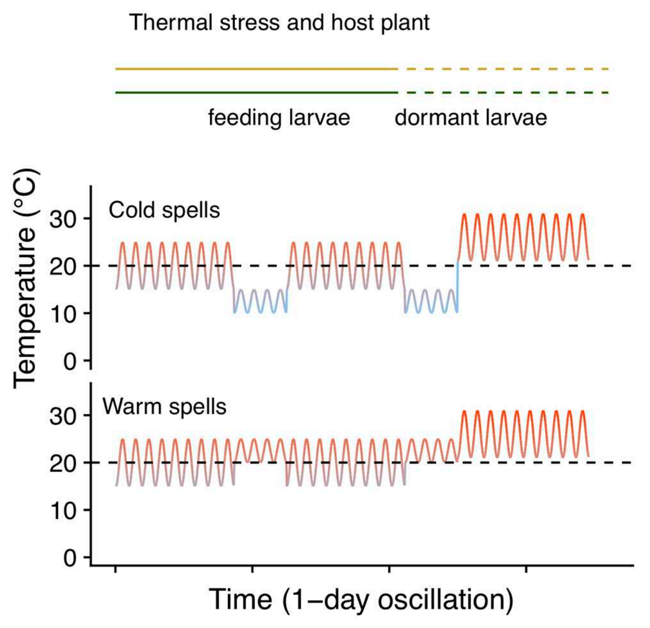


FIGURE 4 | Temperature regimes of the thermal stress and host plant assay. Lines in the top indicate the timing at which feeding (solid) and dormant (dashed) larvae experienced each regime. Dashed black lines indicate 20°C to facilitate comparison.

TABLE 3 | GLMM and logistic regression summaries for the combined effects of cold stress and host plant on survival and development time of pre-overwintering larvae.

Life stage Figure	Response	Fixed factors	DF	χ^2	*P*	Error distribution, link function
Pre-overwintering larvae	survival	regime	1	13.19	0.001	GLMM, binomial, logit
		host	1	15.14	0.001	
		regime × host	1	7.98	0.005	
Pre-overwintering larvae	development time to 4th	regime	1	1.49	0.22	GLMM, Poisson, log
		host	1	0.03	0.87	
		regime × host	1	0.04	0.95	
Pre-overwintering	4th instar mass	regime	1	2.056	0.15	GLMM, gamma, inverse
		host	1	2.070	0.15	
		regime × host	1	26.05	<0.0001	
Pre-overwintering larvae **Figure 8**	survival	4th instar mass	1	152.97	<0.001	logistic regression

we performed a logistic regression including the mean mass per group ($N = 16$ groups) as independent variable and survival to the end of August (survivors/total) as the dependent variable (**Table 3**).

Historical Temperature Analysis

To determine whether heatwave incidence and intensity have recently increased within *E. p. phaeton*'s range, we gathered daily temperature data from two weather stations, one in the northeastern part of the range (located at Brockton, MA: 42.04756 N,−71.00819W) and one in the southeastern part of the range (located on the eastern shore at Chestertown, MD: 39.2166N,−76.0519W). We selected these weather stations because they had the longest periods recorded (1894 to 2011) within the regions of interest. We defined heatwave after Russo et al. (2014), as three or more consecutive days with a maximum temperature above the 90th percentile of a reference period. To set the reference periods we divided each season (spring, summer, autumn, winter) into 6 sub-seasons of 15 or 16 days and calculated the 90th percentile value for location and sub-season using historical data (1894 to 1913). We subsequently used these reference values to identify heatwaves and quantify their yearly incidence. To test whether heatwave incidence (number of heatwaves), intensity (maximum temperature during a heatwave) and duration (mean heatwave length by season) have changed over time, we performed two sets of GLMs: one per region, for each response variable (incidence, intensity, and duration) including year and season as predictors (**Table 4**). We used a Poisson distribution (log link function) to analyze counts of heatwave incidence and a Gamma distribution (inverse link function) for heatwave intensity. To analyze heatwave duration, we used a quasipoisson distribution (log link function) because mean heatwave length is the average of multiple day counts, so it was not discrete.

We found that winter warm spells, characterized by maximum temperatures of 20°C resulted in severe mortality of *E. p. phaeton* larvae in the laboratory. Thus, we separately assessed the incidence of heatwaves during the first part of winter (excluding most of March), hereafter "early winter." This period reflects when host plant foliage is less likely to be available. For this analysis, we counted the heatwaves occurring from day 353 (December 21st) of each year to day 63 (March 4th) of the following year. To assess whether "early winter" heatwave frequency has increased over time we performed two GLMs, one per region, including year as predictor (**Table 4**).

RESULTS

Summer Heatwave Experiments

Exposure of eggs to summer heatwaves of a maximum temperature of 41°C did not have a significant effect on survival to the onset of dormancy (**Table 1**), or development time to both hatching and the onset of dormancy (**Table 1**). More than 90% of the eggs hatched, and about 50% of individuals survived to the onset of dormancy (4th instar) regardless of experimental treatment. Eggs from all temperature regimes hatched about 14 days after being laid (14 [median], 1 [interquartile interval]) and larvae entered dormancy about a month after oviposition (30, 3). By contrast, exposure of 2nd instar larvae (7-day old) to the high heatwave regime (36 ± 5°C) resulted in a 37% decrease in survivorship when compared to those under control and low heatwave conditions (**Table 1**, **Figure 5A**); however, there were no significant differences in development time (**Table 1**, **Figure 5B**) as larvae from all summer regimes entered dormancy about 17 days after hatching.

Larvae fed on the native *C. glabra* were 12% larger (7.26 mg [median], 1.52 [interquartile interval]) at the onset of dormancy than those fed on *P. lanceolata* (**Figure 6**). Furthermore, host plant mediated the effects of heatwave exposure on dormant 4th instar larvae. Individuals maintained under control conditions lost about 3% of their body mass regardless of host plant. However; under heatwave conditions, larvae feeding on *P. lanceolata* lost up to 6% of their mass, and only 3% when feeding on *C. glabra* (**Table 1**, **Figure 6**).

Winter Heatwave Experiment

Exposure to winter heatwaves resulted in severe mortality, as <15% of larvae subjected to this treatment survived to resume activity the following spring (**Figure 7**, **Table 2**). By contrast individuals exposed to the cold treatment had much higher survivorship to both resuming activity the following spring (March 19th, 87%) and to feed and grow (March 28th, 50%). Individuals in the mild winter treatment exhibited intermediate survivorship (∼ 40% and 25%). Notably, the likelihood of winter survival was influenced by host plant use during the pre-overwintering period; the probability of survival was consistently larger for larvae that had fed on *C. glabra* (**Table 2**, **Figure 7**). No larvae that had fed on *P. lanceolata* during the pre-overwintering period and were exposed to winter heatwaves survived.

TABLE 4 | GLM summaries for the analysis of heatwave incidence, intensity and duration in MD and MA during the period of 1894 to 2011.

Region	Response	Independent variables	DF	χ^2	*P*	Error distribution, link function
MD	incidence	year	1	61.42	<0.0001	GLM, poison, log
		season	3	81.5	<0.0001	
	intensity	year	1	20.60	<0.0001	GLM, gamma, inverse
		season	3	902.13	<0.0001	
	duration	year	1	6.56	0.01	GLM, quasipoisson, log
		season	3	14.02	<0.01	
	early winter incidence	year	1	10.01	<0.01	GLM, Poisson, log
MA	incidence	year	1	11.21	<0.001	GLM, poison, log
		season	3	8.12	0.044	
	intensity	year	1	0.12	0.73	GLM, gamma, inverse
		season	3	659.93	<0.001	
	duration	year	1	1.4	0.24	GLM, quasipoisson, log
		season	3	1.8	0.61	
	early winter incidence	year	1	11.87	<0.001	GLM, Poisson, log

Host Plant and Thermal Stress

We found no effect of temperature regime, host, or their interaction on development time of pre-overwintering larvae (1st to 4th instar), as it took them 18 days (median; interquartile range = 2) to reach the dormancy stage regardless of host (**Table 3**). By contrast, all three factors significantly affected caterpillar survival to the end of summer (**Table 3**), as caterpillars feeding on *C. glabra* and experiencing warm spells had larger survival odds (75%) than those in all other host and temperature combinations (50%; **Table 3**).

Analyses of pre-overwintering dormant larval mass showed that for larvae experiencing cold spells, those feeding on *C. glabra* were 20% larger than those feeding on *P. lanceolata* at the end of summer (a significant effect of the host by temperature regime interaction, but not of each factor separately; **Table 3**). Finally, there was a significant relationship between larval mass and summer survival, as colonies that had larger mean larval mass also had a higher proportion of survivors, **Table 3**, **Figure 8**).

Historical Temperature Analysis

We found that the incidence of heatwaves has increased since 1894 in both MA and MD (**Figure 9**, **Table 4**). Heatwave intensity (**Figure 10**, **Table 4)** and duration (**Table 4**) exhibited a significant increase in MD only. In Maryland, heatwave intensity (maximum temperature during a heatwave) during summer varied between 30 to 40°C and during winter between 13.9 and

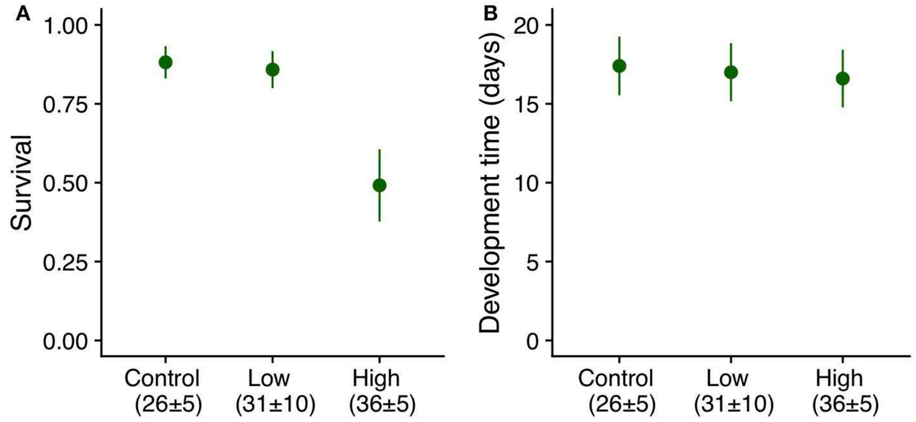


FIGURE 5 | (A) Survival (LS means ± SE) and **(B)** development time (LS means ± SE) from hatching to 4th instar of *E. phaeton* individuals exposed to summer heatwave regimes as 2nd instar (7-day old) larvae. There were significant differences among treatments in survival, but not in development time (**Table 1**).

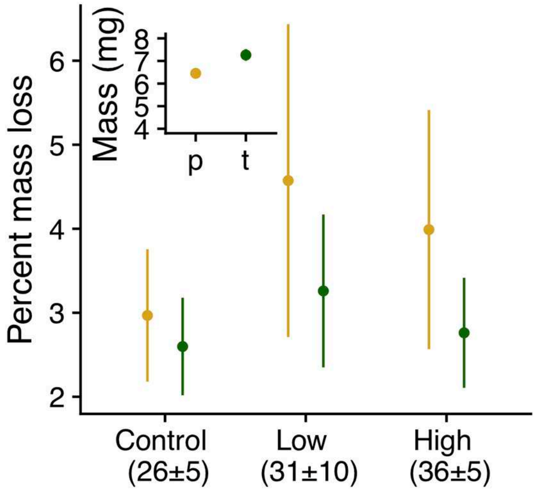


FIGURE 6 | Mass (inset, LS means ± SE , $\chi^2 = 6.44$, $P = 0.01$) and percent mass lost (interaction: $\chi^2 = 8.69$, $P = 0.013$, host: $\chi^2 = 0.46$, $P = 0.5$, regime: $\chi^2 = 111.23$, $P < 0.0001$) by dormant *E. phaeton* larvae during exposure to summer heatwaves. Larvae had previously fed on *C. glabra* (c, green) or *P. lanceolata* (p, yellow).

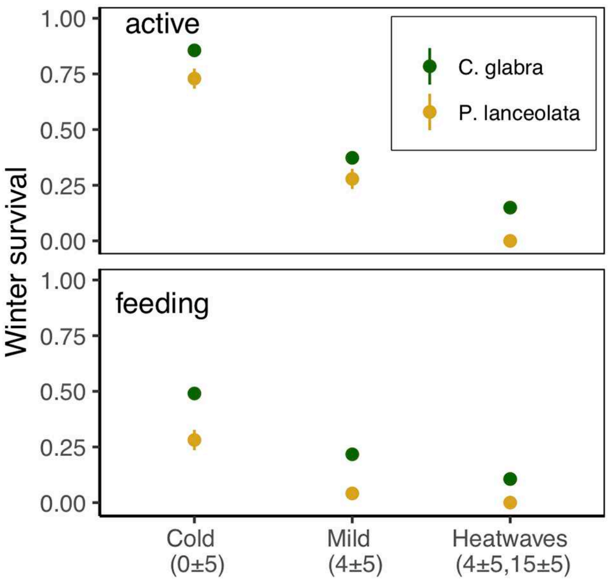


FIGURE 7 | Survival of dormant *E. phaeton* larvae exposed to cold, mild, or heatwave winter regimes at two different times, March 19th, 2018 (active) and March 28th, 2018 (feeding).

30.6°C. In MA ranges were 30 to 40°C in summer and 8.3 to 28.9°C in winter. Most heatwaves had a duration of 3 to 5 days in both regions. When early winter (excluding most of March) was considered separately, annual incidence of heatwaves during the first part of winter was consistently under 4 per winter in MA, but in MD increased from 2 in the 1890's to 5 in recent years (**Table 4**).

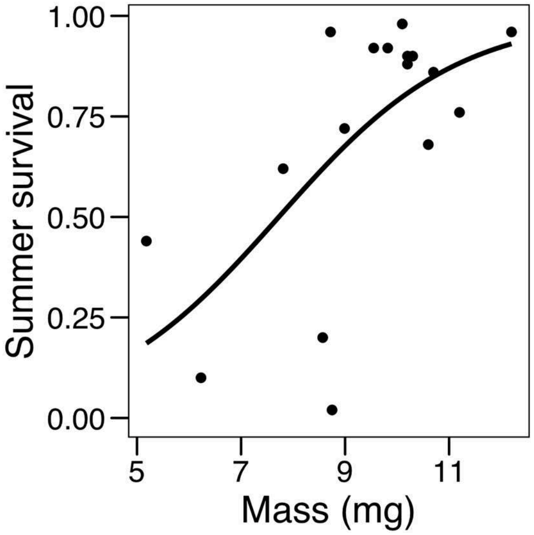


FIGURE 8 | Logistic regression showing the effect of mean larval mass on summer survival (proportion of dormant larvae surviving to the end of August).

DISCUSSION

This study identifies winter heatwaves as a potential factor contributing to the decline of southeastern populations of *E. p. phaeton*. Winter heatwaves resulted in severe larval mortality in the laboratory and historical temperature records show an increment in the incidence, intensity and duration of these events within the southeastern range of *E. p. phaeton*, where population declines have been observed (Durkin, 2009; Frye et al., 2013). Thus, we conclude that climate change, and more specifically, winter heatwaves, should be considered a serious threat to *E. p. phaeton* populations. Our results are consistent with predictions that increased temperature variation in mid-latitudes would negatively impact insects inhabiting these regions (Kingsolver et al., 2013). Careful monitoring of overwintering *E. p. phaeton* populations would be necessary to attribute demographic changes to specific heatwave events in the wild.

We found dormant larvae (4th instar) to be particularly vulnerable to heatwaves. These larvae rely on biomass accumulated during early instars to persist for a period of over 7 months in which they experience a broad range of thermal conditions. Exposure of dormant larvae to summer heatwaves resulted in mass loss **(Figure 6)**, which can increase pre-winter mortality, as the odds of larval survival to the end of summer decreased with size (**Figure 8**). Hence, summer heatwaves can result in fewer and smaller larvae surviving until the onset of winter. Because biomass is an important predictor of larval overwinter success (Smith, 2002), summer heatwaves can also result in increased larval susceptibility to unfavorable winter conditions. Furthermore, winter heatwaves drastically reduced survival of dormant caterpillars **(Figure 7)**. Dormant larvae exposed to warm temperatures during winter activate and move around; thus, mortality was probably due to energy

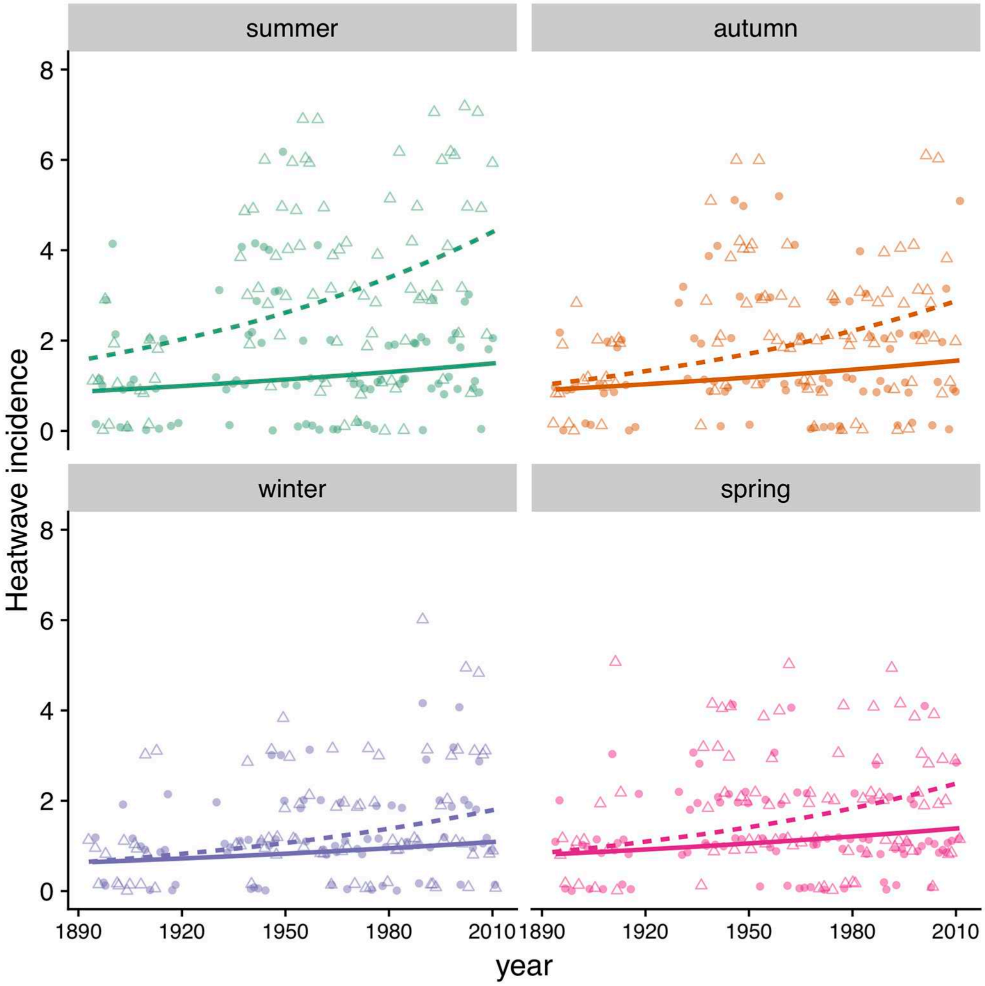


FIGURE 9 | Heatwave incidence (count) by season in MD (triangles, dashed line) and MA (circles, solid line). Symbols correspond to data points and lines to model predictions (**Table 4**).

reserve depletion resulting from increased metabolic activity. This phenomenon has been observed in multiple taxa including vertebrates (Williams et al., 2015) and gallflies (Irwin and Lee, 2003). By contrast, eggs (which are only present during summer) showed no negative effects on survival or phenology after exposure to summer heatwaves. The egg stage of *E. phaeton* has a long duration compared to other summer butterflies whose eggs often hatch after 2 to 10 days (James and Nunnallee, 2011); for example, eggs of *Epargyreus clarus* hatch after ∼ 5 days in summer temperature regimes (Abarca et al., 2018) and *Papilio polyxenes* eggs hatch after ∼ 6 days (Blau, 1981) and it is also more resilient to heatwaves than other lepidopterans; exposure to heatwaves resulted in increased egg mortality in both *Ostrinia furnacalis* (Zhou et al., 2018) and *Manduca sexta* (Potter et al., 2011). Pre-overwintering larvae were somewhat resilient to heatwaves, as neither of the regimes (low, high) affected their phenology (**Figure 5**). However, the high regime (36 ± 5 °C) did result in reduced survival. This regime was characterized by a relatively high minimum temperature of 31°C, which is not characteristic of the MD area, where minimum summer heatwave temperatures range within 21°C and 27°C. Minimum temperatures during heatwaves have been found to have larger effects on mortality than maximum temperatures (Hajat et al., 2002). Note that while summer heatwaves had little to no effect on the survival and phenology of *E. p. phaeton* eggs and early-instar larvae; we cannot discard the occurrence of sublethal effects, such as reduced fertility (Addo-bediako et al., 2000; Zhang et al., 2015a) and immune function (Chown, 2001) which have been reported to occur in response to heatwaves.

The winter temperature regimes we implemented were conservative compared to actual winter temperatures recorded in the MD area. In the laboratory, we exposed larvae to a heatwave regime with a maximum temperature of 20°C. Maximum winter heatwave temperatures commonly exceed this threshold in MD, but not in MA (**Figure 9**). Thus, the winter conditions MD caterpillars currently experience in the wild are likely to be harsher than our heatwave regime. Current *E. p. phaeton* populations in MD are limited to the northern

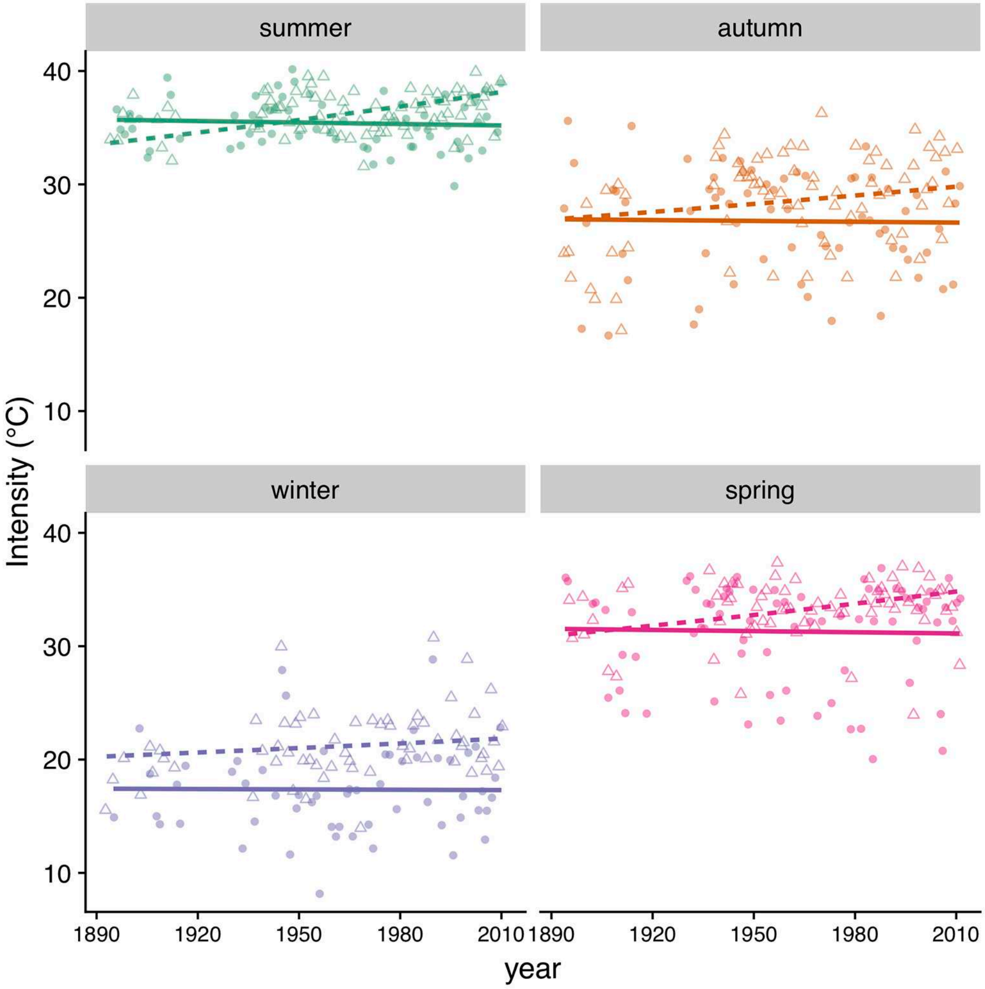


FIGURE 10 | Heatwave intensity (maximum temperature) by season in MD (triangles, dashed line) and MA (circles, solid line). Symbols correspond to data points and lines to model predictions (**Table 4**).

Piedmont, west to the Appalachian Plateau (Pers. Comm. Jen Selfridge) where temperatures are presumably lower than the Chestertown weather station used in this study, which is more representative of Coastal Plain, valleys and urban areas. This distribution pattern is consistent with what would be expected under a scenario in which winter heatwaves are a main mortality source in the wild. Further, niche models identify temperature as a main driver of this species distribution (Czachura et al., in preparation) and climate change as a potential threat (Frye et al., 2013). Given the imperiled status of *E. p. phaeton* in MD, we used individuals from a MA population in our experiments. This constitutes a potential limitation of our study, as MD populations could be more resilient to heatwaves than those in MA due to local adaptation. However, we argue that it is unlikely for MD populations to be robust to winter heatwaves because maximum thermal physiological limits are well conserved within taxa (Chown, 2001). In addition, the heatwave effects we found on survival were so dramatic (survival of <15 %), that even if twice as many MD individuals survived irregular winters, populations would still experience severe losses. Future experiments would elucidate whether northern and southern populations of *E. p. phaeton* significantly differ in their thermal thresholds and susceptibility to winter heatwaves.

We found that host plant had an important role at mediating the effects of thermal stress. Larvae feeding on *C. glabra* attained larger sizes, were less vulnerable to mass loss during summer heatwaves and, more importantly, all survivors under the winter heatwave regime had fed on *C. glabra*. This is consistent with previous work showing that host plant identity (Diamond and Kingsolver, 2010; Abarca et al., 2018) and nutrient content (Andersen et al., 2009), can mediate insect responses to thermal stress; and that resource acquisition before overwintering significantly affects survival (Pullin, 1987). For example, Vallières et al. (2015) showed that both population of origin and host plant species mediated the effect of fall heatwaves on the survival of the hemlock looper (*Lambdina fiscellaria*).Thus, the costs of feeding on suboptimal hosts are increased under thermal stress, which may be the reason why *P.*

lanceolata has not been adopted as a primary host (oviposition) by *E. p. phaeton* populations in MD, where pre-overwintering larvae have been reported to feed exclusively on *C. glabra*. Future studies would investigate this possibility. Additionally, heatwave incidence and intensity are likely to increase throughout *E. p. phaeton*'s range, and the reliance of northern populations on the more widespread novel host, *P. lanceolata,* may become maladaptive under future thermal regimes. Generalist herbivores often use hosts according to their relative abundance and not their nutritional value (Mason et al., 2011); the intersection of limited *C. glabra* availability and climate change imposes a significant threat to *E. p. phaeton*'s long-term persistence. Finally, it is important to note that warmer temperatures can reduce host quality (Bauerfeind and Fischer, 2013), which is a potential effect of summer heatwaves that we did not investigate but that can further imperil not only *E. p. phaeton* but other arthropod populations.

We showed that winter heatwaves, characterized by temperatures well under physiological limits can have more devastating effects than summer heatwaves characterized by maximum temperatures that are physiologically stressful. Our results are consistent with others showing that irregular winter patterns are detrimental (Bale and Hayward, 2010), particularly for species that overwinter as larvae (Williams et al., 2012). Populations of *E. p. phaeton* in MD face multiple threats such as habitat destruction, urbanization and host plant (*C. glabra*) scarcity (Frye et al., 2013) that can be mitigated through conservation efforts. We recommend those efforts to consider local thermal regimes and be concentrated in relatively cool areas, were winter heatwaves are less likely to result in severe overwinter mortality.

DATA AVAILABILITY

All datasets generated for this study are included in the **Supplementary Files** as **Data Sheets 1–7**.

AUTHOR CONTRIBUTIONS

MA, LR, and EL conceived and designed the experiments. LR and MA collected colony founders. MA and EL carried out the experiments and analyzed data. MA wrote the manuscript with input and edits from EL and LR.

FUNDING

Funding for this project was generously provided by Georgetown University.

ACKNOWLEDGMENTS

We thank Greg Breed for his help developing *E. phaeton* rearing techniques and Douglas Taron for advice on caterpillar husbandry. We thank Madeline Lee & Declan Mirabella for their assistance in the laboratory and with data management. We also thank Jennifer Selfridge (former J Frye) for her kind support and information on the current status of *E. p. phaeton* populations in MD.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00193/full#supplementary-material

Data Sheet 1 | Historical temperature records.

Data Sheet 2 | Summer heatwave experiments: eggs.

Data Sheet 3 | Summer heatwave experiments: feeding larvae.

Data Sheet 4 | Summer heatwave experiments: dormant larvae.

Data Sheet 5 | Winter heatwave: dormant larvae.

Data Sheet 6 | Host plant and thermal stress: phenology and survival.

Data Sheet 7 | Host plant and thermal stress: caterpillar mass.

REFERENCES

Abarca, M., Larsen, E. A., Lill, J. T., Weiss, M., Lind, E., and Ries, L. (2018). Inclusion of host quality data improves predictions of herbivore phenology. *Entomol. Exp. Appl.* 8, 1–13. doi: 10.1111/eea.12715

Addo-bediako, A., Chown, S. L., and Gaston, K. J. (2000). Thermal tolerance, climatic variability and latitude. *Proc. R. Soc. B Biol. Sci.* 267, 739–745. doi: 10.1098/rspb.2000.1065

Andersen, L. H., Kristensen, T. N., Loeschcke, V., Toft, S., and Mayntz, D. (2009). Protein and carbohydrate composition of larval food affects tolerance to thermal stress and desiccation in adult Drosophila melanogaster. *J. Insect Physiol.* 56, 336–340. doi: 10.1016/j.jinsphys.2009.11.006

Augspurger, C. K. (2013). Reconstructing patterns of temperature, phenology, and frost damage over 124 years: Spring damage risk is increasing. *Ecology* 94, 41–50. doi: 10.1890/12-0200.1

Awmack, C. S., and Leather, S. R. (2002). Host Plant Quality and Fecundity in Herbivorous Insects. *Annu. Rev. Entomol.* 47, 817–844. doi: 10.1146/annurev.ento.47.091201.145300

Bale, J. S., and Hayward, S. A. L. (2010). Insect overwintering in a changing climate. *J. Exp. Biol.* 213, 980–994. doi: 10.1242/jeb.037911

Batalden, R. V., and Oberhauser, K. S. (2015). "Potential changes in eastern North American monarch migration in response to an introduced milkweed, *Asclepias curassavica*," in *Monarchs in a Changing World: Biology and Conservation of an Iconic Insect*, eds. K. S. Oberhauser, K. R. Nail, and S. Altizer (Ithaca, New York: Cornell University press), 215–222.

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48. doi: 10.18637/jss.v067.i01

Bauerfeind, S. S., and Fischer, K. (2013). Increased temperature reduces herbivore host-plant quality. *Glob. Change Biol.* 19, 3272–3282. doi: 10.1111/gcb.12297

Blau, W. S. (1981). Life history variation in the black swallowtail butterfly. *Oecologia* 48, 116–122. doi: 10.1007/BF00346997

Bowers, M. D. (1980). Unpalatability as a defense strategy of Euphydryas phaeton (Lepidoptera: Nymphalidae). *Evolution* 34, 586–600. doi: 10.1111/j.1558-5646.1980.tb04846.x

Bowers, M. D., and Richardson, L. L. (2013). Use of two oviposition plants in populations of Euphydryas phaeton Drury (Nymphalidae). *J. Lepid. Soc.* 67, 299–300. doi: 10.18473/lepi.v67i4.a7

Bowers, M. D., Stamp, N. E., and Collinge, S. K. (1992). Early stage of host range expansion by a specialist herbivore, euphydryas phaeton (Nymphalidae). *Ecology* 73, 526–536. doi: 10.2307/1940758

Cahill, A., E., Aiello-Lammens, M. E., Fisher-Reid, M. C., Hua, X., et al. (2012). How does climate change cause extinction? *Proc. R. Soc. B Biol. Sci.* 280, 20121890–20121890. doi: 10.1098/rspb.2012.1890

Cavers, P. B., Bassett, I. J., and Crompton C. W. (1980). The biology of canadian weeds. 47. plantago lanceolata L. *Can. J. Plant Sci.* 60, 1269–1282. doi: 10.4141/cjps80-180

Chown, S. L. (2001). Physiological variation in insects: hierarchical levels and implications. *J. Insect Physiol.* 47, 649–660. doi: 10.1016/S0022-1910(00)00163-3

Christidis, N., Jones, G. S., and Stott, P. A. (2015). Dramatically increasing chance of extremely hot summers since the 2003 European heatwave. *Nat. Clim. Chang.* 5, 46–50. doi: 10.1038/nclimate2468

Diamond, S. E., and Kingsolver, J. G. (2010). Environmental dependence of thermal reaction norms : host plant quality can reverse the temperature-size rule. *Am. Nat.* 175, 1–10. doi: 10.1086/648602

Dirzo, R., Young, H. S., Galetti, M., Ceballos, G., Isaac, N. J. B., and Collen, B. (2014). Defaunation in the Anthropocene. *Science* 345, 401–406. doi: 10.1126/science.1251817

Durkin, P. M. (2009). Efforts to restore the baltimore checkerspot (Euphydryas phaeton) in Maryland. *News Lepid. Soc.* 51, 3–5.

Fischer, K., Klockmann, M., and Reim, E. (2014). Strong negative effects of simulated heat waves in a tropical butterfly. *J. Exp. Biol.* 217, 2892–2898. doi: 10.1242/jeb.106245

Fox, J., and Weisberg, S. (2011). *An R companion to Applied Regression*. Thousand Oaks, CA: Sage Available online at: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

Frank, D., Reichstein, M., Bahn, M., Thonicke, K., Frank, D., Mahecha, M. D., et al. (2015). Effects of climate extremes on the terrestrial carbon cycle: Concepts, processes and potential future impacts. *Glob. Chang. Biol.* 21, 2861–2880. doi: 10.1111/gcb.12916

Frye, J., Durkin, P., Gibbs, D., Gibbs, R., and Lustig, M. (2013). *Conservation and Management of the Baltimore Checkerspot (Euphydryas phaeton Drury) in Maryland: Strategies for Statewide Monitoring and for Wetland Restoration, Captive Breeding and Release in the Piedmont Region*. Annapolis.

Gordo, O., and Sanz, J. J. (2005). Phenology and climate change: a long-term study in a Mediterranean locality. *Oecologia* 146, 484–495. doi: 10.1007/s00442-005-0240-z

Hajat, S., Kovats, R. S., Atkinson, R. W., and Haines, A. (2002). Impact of hot temperatures on death in London: A time series approach. *J. Epidemiol. Commun. Health* 56, 367–372. doi: 10.1136/jech.56.5.367

Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., et al. (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS ONE* 12, 1–21. doi: 10.1371/journal.pone.0185809

Hansen, J., Sato, M., and Reto, R. (2012). Perception of climate change. *Proc. Natl. Acad. Sci.* 109, E2415–E2423. doi: 10.1073/pnas.1205276109

Irwin, J. T., and Lee, R. E. (2003). Cold winter microenvironments conserve energy and improve overwintering survival and potential fecundity of the goldenrod gall fly, Eurosta solidaginis. *Oikos* 100, 71–78. doi: 10.1034/j.1600-0706.2003.11738.x

James, D. G., and Nunnallee, D. (2011). *Life Histories of Cascadia Butterflies*. 1st ed. Corvallis, OR: Oregon State University Press.

Kingsolver, J. G., Diamond, S. E., and Buckley, L. B. (2013). Heat stress and the fitness consequences of climate change for terrestrial ectotherms. *Funct. Ecol.* 27, 1415–1423. doi: 10.1111/1365-2435.12145

Kudo, G., and Ida, T. Y. (2013). Early onset of spring increases the phenological mismatch between plants and pollinators. *Ecology* 94, 2311–2320. doi: 10.1890/12-2003.1

Levy, O., Buckley, L. B., Keitt, T. H., Smith, C. D., Boateng, K. O., Kumar, D. S., et al. (2015). Resolving the life cycle alters expected impacts of climate change. *Proc. R. Soc. B* 282, 1813. doi: 10.1098/rspb.2015.0837

Lister, B. C., and Garcia, A. (2018). Climate-driven declines in arthropod abundance restructure a rainforest food web. *Proc. Natl. Acad. Sci. U.S.A.* 115, E10397–E10406. doi: 10.1073/pnas.1722477115

Mason, P. A., Wilkes, S. R., Lill, J. T., and Singer, M. S. (2011). Abundance trumps quality: Bi-trophic performance and parasitism risk fail to explain host use in the fall webworm. *Oikos* 120, 1509–1518. doi: 10.1111/j.1600-0706.2011.19053.x

Meehl, G. A., and Tebaldi, C. (2004). More intense, more frequent, and longer lasting heat waves in the 21st century. *Science* 305, 994–997. doi: 10.1126/science.1098704

Moran, E. V., and Alexander, J. M. (2014). Evolutionary responses to global change: lessons from invasive species. *Ecol. Lett.* 17, 637–649. doi: 10.1111/ele.12262

Ovaskainen, O., Skorokhodova, S., Yakovleva, M., Sukhov, A., Kutenkov, A., Kutenkova, N., et al. (2013). Community-level phenological response to climate change. *Proc. Natl. Acad. Sci. U. S. A.* 110, 13434–13439. doi: 10.1073/pnas.1305533110

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annu. Rev. Ecol. Evol. Syst.* 37, 637–669. doi: 10.1146/annurev.ecolsys.37.091305.110100

Parmesan, C. (2007). Influences of species, latitudes and methodologies on estimates of phenological response to global warming. *Glob. Chang. Biol.* 13, 1860–1872. doi: 10.1111/j.1365-2486.2007.01404.x

Parmesan, C., and Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421, 37–42. doi: 10.1038/nature01286

Perkins, S. E., Alexander, L. V., and Nairn, J. R. (2012). Increasing frequency, intensity and duration of observed global heatwaves and warm spells. *Geophys. Res. Lett.* 39, 1–5. doi: 10.1029/2012GL053361

Potter, K. A., Davidowitz, G., and Arthur Woods, H. (2011). Cross-stage consequences of egg temperature in the insect Manduca sexta. *Funct. Ecol.* 25, 548–556. doi: 10.1111/j.1365-2435.2010.01807.x

Pullin, A. S. (1987). Adult feeding time, lipid accumulation, and overwintering in Aglais urticae and Inachis io (Lepidoptera: Nymphalidae). *J. Zool.* 211, 631–641. doi: 10.1111/j.1469-7998.1987.tb04476.x

R Core, T. (2017). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Robertson, S. M. (2015). Life history, oviposition characteristics, and management of the Ozark Baltimore Checkerspot (Euphydryas phaeton ozarke).

Rosenwald, L. C., Lill, J. T., Lind, E. M., and Weiss, M. R. (2017). Dynamics of host plant selection and host-switching by silver-spotted skipper caterpillars. *Arthropod. Plant. Interact.* 11, 833–842. doi: 10.1007/s11829-017-9538-0

Russo, S., Dosio, A., Graversen, R. G., Sillmann, J., Carrao, H., Dunbar, M. B., et al. (2014). Magnitude of extreme heat waves in present climate and their projection in a warming world. *J. Geophys. Res.* 119, 12500–12512. doi: 10.1002/2014JD022098

Sales, K., Vasudeva, R., Dickinson, M. E., Godwin, J. L., Lumley, A. J., Michalczyk, Ł., et al. (2018). Experimental heatwaves compromise sperm function and cause transgenerational damage in a model insect. *Nat. Commun.* 9, 1–11. doi: 10.1038/s41467-018-07273-z

Sánchez-Bayo, F., and Wyckhuys, K. A. G. (2019). Worldwide decline of the entomofauna: a review of its drivers. *Biol. Conserv.* 232, 8–27. doi: 10.1016/j.biocon.2019.01.020

Singer, M. C., and Parmesan, C. (2018). Lethal trap created by adaptive evolutionary response to an exotic resource. *Nature* 557, 238–241. doi: 10.1038/s41586-018-0074-6

Smith, R. J. (2002). Effect of larval body size on overwinter survival and emerging adult size in the burying beetle, *Nicrophorus investigator*. *Can. J. Zool.* 80, 1588–1593. doi: 10.1139/z02-151

Stamp, N. E. (1979). New oviposition plant for Euphydryas phaeton (Nymphalidae). *J. Lepid. Soc.* 33, 203–204.

Ummenhofer, C. C., and Meehl, G. A. (2017). Extreme weather and climate events with ecological relevance: a review. *Philos. Trans. R. Soc. B Biol. Sci.* 372. doi: 10.1098/rstb.2016.0135

Vallières, R., Rochefort, S., Berthiaume, R., Hébert, C., and Bauce, É. (2015). Effect of simulated fall heat waves on cold hardiness and winter survival of hemlock looper, Lambdina fiscellaria (Lepidoptera: Geometridae). *J. Insect Physiol.* 73, 60–69. doi: 10.1016/j.jinsphys.2014.12.001

Williams, C. M., Hellmann, J., and Sinclair, B. J. (2012). Lepidopteran species differ in susceptibility to winter warming. *Clim. Res.* 53, 119–130. doi: 10.3354/cr01100

Williams, C. M., Henry, H. A. L., and Sinclair, B. J. (2015). Cold truths: How winter drives responses of terrestrial organisms to climate change. *Biol. Rev.* 90, 214–235. doi: 10.1111/brv.12105

Xu, Z., FitzGerald, G., Guo, Y., Jalaludin, B., and Tong, S. (2016). Impact of heatwave on mortality under different heatwave definitions: a systematic review and meta-analysis. *Environ. Int.* 89–90, 193–203. doi: 10.1016/j.envint.2016.02.007

Yang, L. H., and Rudolf, V. H. W. (2010). Phenology, ontogeny and the effects of climate change on the timing of species interactions. *Ecol. Lett.* 13, 1–10. doi: 10.1111/j.1461-0248.2009.01402.x

Zhang, W., Chang, X. Q., Hoffmann, A. A., Zhang, S., and Ma, C. S. (2015a). Impact of hot events at different developmental stages of a moth: the closer to adult stage, the less reproductive output. *Sci. Rep.* 5, 1–9. doi: 10.1038/srep10436

Zhang, W., Rudolf, V. H. W., and Ma, C. S (2015b). Stage-specific heat effects: timing and duration of heat waves alter demographic rates of a global insect pest. *Oecologia* 179, 947–957. doi: 10.1007/s00442-015-3409-0

Zhou, J., cheng, Liu, Q., quan, Han, Y., xu, and Dong, H. (2018). High temperature tolerance and thermal-adaptability plasticity of Asian corn borer (Ostrinia furnacalis Guenée) after a single extreme heat wave at the egg stage. *J. Asia. Pac. Entomol.* 21, 1040–1047. doi: 10.1016/j.aspen.2018.07.024

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

PERSPECTIVE
published: 06 June 2019
doi: 10.3389/fevo.2019.00194

Geographic Biases in Bee Research Limits Understanding of Species Distribution and Response to Anthropogenic Disturbance

Mary A. Jamieson[1]*, **Adrian L. Carper**[2], **Caleb J. Wilson**[1,3], **Virginia L. Scott**[4] and **Jason Gibbs**[5]

[1] Department of Biological Sciences, Oakland University, Rochester, MI, United States, [2] Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO, United States, [3] Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC, United States, [4] Entomology Collection, University of Colorado Museum of Natural History, Boulder, CO, United States, [5] Department of Entomology, University of Manitoba, Winnipeg, MB, Canada

OPEN ACCESS

Edited by:
Shannon Murphy,
University of Denver, United States

Reviewed by:
Ruben Martin-Blazquez,
University of Illinois at Urbana-Champaign, United States
Sarah Jane Cusser,
Michigan State University, United States

***Correspondence:**
Mary A. Jamieson
mjamieson@oakland.edu

Specialty section:
This article was submitted to Population and Evolutionary Dynamics,
a section of the journal Frontiers in Ecology and Evolution

Received: *07 March 2019*
Accepted: *13 May 2019*
Published: *06 June 2019*

Citation:
Jamieson MA, Carper AL, Wilson CJ, Scott VL and Gibbs J (2019) Geographic Biases in Bee Research Limits Understanding of Species Distribution and Response to Anthropogenic Disturbance. Front. Ecol. Evol. 7:194. doi: 10.3389/fevo.2019.00194

Habitat loss and degradation due to agricultural intensification and urbanization are key threats facing wild pollinators, especially bees. However, data on the distribution and abundance of most of the world's 20,000+ bee species is lacking, making it difficult to assess the effects of anthropogenic disturbance through time. Moreover, there are geographic biases in the study of bees creating gaps in our understanding of species distributions and regional patterns of diversity. Research efforts are often focused around cities or field stations associated with universities and other research institutions. In this perspectives paper, we provide examples of geographic bias in knowledge regarding bee species distributions using recently collected data from Michigan and Colorado, USA—two states with published species checklists. We illustrate how a limited sampling effort can advance knowledge about bee species distributions, yielding species occurrence records at local and regional scales. Given the implications of geographic biases, we recommend future research efforts focus on poorly sampled geographic regions, especially those affected by anthropogenic disturbance, in order to expand our understanding of human impacts on wild bee species. Sampling across a broader geographic area will provide critical information for taxonomy and predictive models of bee species distributions and diversity. We encourage researchers to plan future studies with consideration of strategies to avoid oversampling local bee populations, the taxonomic expertise required to identify specimens, and resources necessary to voucher specimens.

Keywords: bee decline, land-use change, pollinator conservation, global change, bee communities

INTRODUCTION

Pollinators play a key ecological role in terrestrial habitats, contributing to reproduction in more than 85% of flowering plants (Ollerton et al., 2011) and thereby supporting food webs worldwide. Furthermore, pollinators, in particular bees, benefit ~75% of the world's leading crops and contribute significantly to global food production (Klein et al., 2007; Potts et al., 2010). Unfortunately, in recent years, researchers have documented significant declines in the abundances of managed and wild bee species (Goulson et al., 2015). While a number of risk factors threaten bees, habitat loss and degradation due to agricultural intensification and urbanization are leading causes of bee decline (Winfree et al., 2009; Cariveau and Winfree, 2015; Goulson et al., 2015).

Species traits, such as dispersal ability, nesting habits, and diet breadth, influence how bees respond to anthropogenic disturbances (Cariveau and Winfree, 2015; Harrison and Winfree, 2015; Normandin et al., 2017, but see Bartomeus et al., 2018). For example, while cavity-nesting species are often positively influenced by urbanization (e.g., Cane et al., 2006; Bates et al., 2011; Fitch et al., 2019), ground-nesting species are typically more negatively affected (e.g., Kearns and Oliveras, 2009; Geslin et al., 2016). Moreover, some functional and taxonomic groups appear to be especially sensitive to environmental perturbations. Notably, recent studies have shown negative effects of urbanization and agricultural intensification on bumble bee abundance, population size, and geographic range size (e.g., Cameron et al., 2011; Kerr et al., 2015; Glaum et al., 2017; Hamblin et al., 2017; Jacobson et al., 2018). Bumble bee decline is especially concerning due to the ecological and economic importance of these pollinators. The availability of data on bumble bee species' relative abundances and historical distributions have been critical to the detection of their decline.

In recent years, researchers have increasingly recognized the value of pollination services provided by wild bee species in natural and agricultural systems (Losey and Vaughn, 2006; Slagle and Hendrix, 2009; Garibaldi et al., 2013; Klein et al., 2018; Winfree et al., 2018). A recent model of pollinator abundance on the landscape predicted worrisome mismatches between pollinator dependent crops and pollinator abundance (Koh et al., 2016). However, this study, based largely on expert opinion, also modeled large areas of uncertainty in bee abundance in the United States of America (USA). Thus, there is a need to understand how land-use change influences wild bees given continued increases in agricultural intensification and urbanization. The population status of most bee species is uncertain due to a lack of long-term or even baseline data (Bartomeus et al., 2013; Goulson et al., 2015). Relative to other parts of the world, the USA is a well-studied area with respect to pollinators (Archer et al., 2014). In this paper, however, we argue that even in the USA, there are geographic biases in bee research that limit our understanding of population dynamics, taxonomy, species distributions, and regional patterns of species diversity. We propose that limited sampling efforts, especially in understudied geographic regions, can contribute substantially to our knowledge of bees and their responses to anthropogenic disturbances.

THE IMPORTANCE OF BEE RESEARCH IN UNDERSTUDIED REGIONS

Bee research is often centered in areas surrounding universities and research stations, which results in a lack of information in areas beyond these research centers (see e.g., Scott et al., 2011; Gibbs et al., 2017a). In the USA, numerous state checklists have demonstrated a paucity of species occurrence records across many counties and larger regions within these states where certain bee species may be expected to occur (e.g., Donovall and VanEnglesdorp, 2010; Jean, 2010; Scott et al., 2011; Dibble et al., 2017; Gibbs et al., 2017a). Furthermore, published species inventories are lacking for most states in the USA, and available checklists are usually from the eastern half of country (e.g., Michigan, Indiana, New York, Pennsylvania, and Maine). The species richness in these states, however, is substantially less than many states in the western USA (Scott et al., 2011; Carril et al., 2018). Large tracts of the USA, such as the High Plains and Great Plains are poorly sampled, even though they may have some of the greatest habitat loss across the country (Samson et al., 2004). With limited sampling effort, diverse bee communities can be found in both pristine (e.g., Grundel et al., 2011) and in anthropogenically disturbed habitats (e.g., Camilo et al., 2017). For example, a single day of collecting in Bellaire, Michigan added 50 new bee species records to Antrim County, bringing the total known species from 34 to 84 in 2016 (Gibbs unpublished data). Given the variability in bee species' response to anthropogenic change (Cariveau and Winfree, 2015), increased sampling in understudied regions affected by human disturbances is important for understanding which species are most likely at risk of decline (e.g., Bates et al., 2011; Banaszak-Cibicka and Zmihorski, 2012).

Species distribution and diversity models are important for documenting, evaluating, and predicting how bees respond to environmental changes, including climate change (e.g., Kerr et al., 2015) and land-use change (e.g., Bennett et al., 2014; Koh et al., 2016). Many regional models could be improved, however, by better sampling across a broader geographic range. Moreover, given that exotic bees may negatively affect native species (Russo, 2016), research evaluating the dispersal and population trends of exotic bees relative to native bees is needed. For example, the introduced bee, *Anthidium manicatum*, has rapidly spread across North American (Gibbs and Sheffield, 2009) and may competitively exclude native bees in some habitats (Miller et al., 2002, but see Soper and Beggs, 2013). Predictive models have been developed to estimate areas of high abundance for *A. manicatum*, with variable success (Strange et al., 2011; Graham and MacLean, 2018). Greater sampling across understudied regions could improve predictive habitat models for research examining bee community response to environmental change as well as exotic species spread.

To demonstrate the value of research in historically understudied areas, we summarize new species occurrence records from two recent studies examining the influence of anthropogenic disturbance on bee communities. Both studies occurred in states with published species checklists. We explore how new species records at the local (i.e., county) and regional (i.e., multi-county and statewide) level add to our knowledge of bee species distributions. Furthermore, we propose that research in other understudied areas could find similar or even greater numbers of new records. Finally, we conclude with recommendations for guiding future bee surveys and community studies.

Bee Community Study in Northeastern Colorado

Bee diversity is greater in the western half of the USA as compared with the eastern half, and vast areas of the West have been historically poorly sampled (e.g., Scott et al., 2011; see Carril et al., 2018). Colorado is currently the only state in the West with a recently published statewide checklist of bee species (Scott et al., 2011). Boulder County, home to the University of Colorado, is the best-documented county in the state, with 562 bee species recorded to date (Goldstein and Scott, 2015). Similarly, counties surrounding other universities and research institutions, including Larimer County, home to Colorado State University, have been extensively sampled—with 439 species recorded (Scott et al., 2011). In comparison, the bee community in eastern Colorado has been poorly inventoried relative to the central and western regions of the state where research has been historically focused (Scott et al., 2011; **Figure 1A**). In the Colorado Eastern Plains, 13 counties had fewer than 100 species recorded as of the 2011 statewide checklist, and most counties had substantially fewer species (mean = 39 ± 27 SD).

The first study we present to demonstrate the value of a limited sampling effort in understudied regions involved bee surveys at 32 sites located across five counties in northeastern Colorado (study region $\approx$ 25,000 km^2). Our sites covered an area known as the Eastern or High Plains region, and included grassland habitats that were either enrolled in the U.S. Department of Agriculture Conservation Reserve Program or used for low-intensity grazing. The goal of this study was to provide baseline data on bee communities that could be used in the future to evaluate effects of projected land-use change due to increased bioenergy production in the region. In this study, bees were surveyed once per month for a 24-h period using passive and active sampling methods (bowl and vane traps alongside hand-netting) from June-September of 2013 and May-September of 2014.

From 9 days of collecting, this study added 425 new county-level species occurrence records, 97 occurrence records in the five county survey region, and 15 new state records for bees not previously known to occur in Colorado (**Table 1A**; **Table S1**; **Figure 1A**). We increased the number of bees known to occur in Washington county, a relatively large county (6,537 km^2) in CO, from 5 to 127 species. One species newly recorded in Colorado, *Cemolobus ipomoea*, was found ~1,000 km west of its previously known western range limit in Missouri, according to publicly available georeferenced specimens [e.g., DiscoverLife.org, GBIF.org (GBIF, 2018; Carper et al., 2019)]. While not a new species for Colorado, a specimen of *Centris ceasalpiniae* that we collected from Washington county is only the 3rd record in the state and is now the most northern record for this species in North America. Similarly, specimens collected in this study expanded the known geographic range for at least a few *Lasioglossum* species. Based on this 2-year study, we conclude that the regional bee species diversity in eastern Colorado, and the broader High Plains Region more generally, is likely much greater than previously documented.

This new survey of bees in northeastern Colorado added significantly to our knowledge of bee species that occur in that part of the state. The study added a number of new species

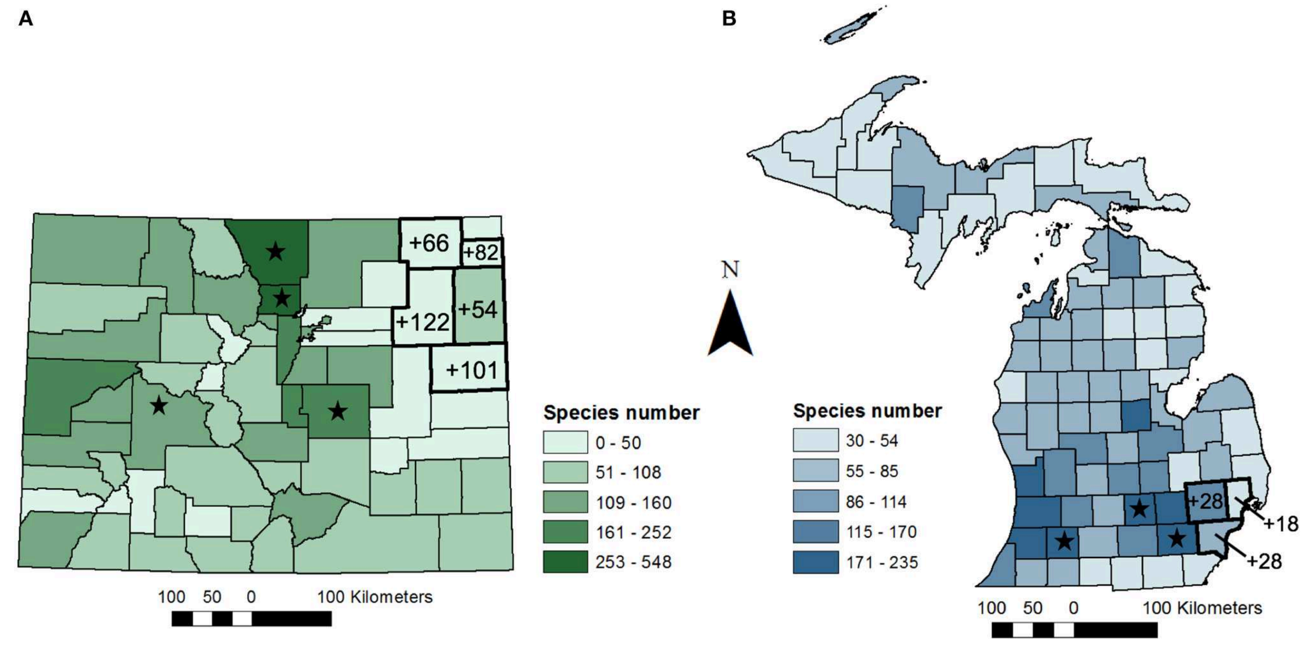


FIGURE 1 | Bee species numbers by county for **(A)** Colorado (modified from Scott et al., 2011) and **(B)** Michigan (modified from Gibbs et al., 2017a). Values on maps show the number of new occurrence records for bee species by county from **(A)** a two-year study in Colorado (N = 32 sites across five counties) and **(B)** a one-year study (N = 15 sites across three counties). Shades of green and blue reflect species numbers from Scott et al. (2011) and Gibbs et al. (2017a), respectively. Stars highlight counties with universities and research stations where researchers have contributed a significant number of specimens to museum collections.

occurrence records for the state of Colorado, including multiple species of *Perdita*, *Melissodes*, and *Lasioglossum* (**Table S1**). Furthermore, this project contributed to expanding the known geographic range limit for several species. It should be noted, however, that there are taxonomic impediments to surveys such as this, particularly in the western USA, as well as many countries throughout the world. Although this survey involved only 9 days of collecting, it took 3 years to get specimens processed and identified by multiple taxonomic specialists, and some specimens remain identified only at a morphospecies level. Other western USA bee surveys have faced similar issues (e.g., Carril et al., 2018). For certain diverse groups (e.g., *Nomada, Sphecodes,* and some *Lasioglossum*), taxonomic keys are lacking, making identifications impossible without taxonomic expertise. Even with taxonomic expertise, some groups are not fully resolved and new species are still being described. If we had identifications for all of our collected specimens, we anticipate that this study would contribute many more new species occurrence records for Colorado and some undescribed species. The need for additional series of specimens to support species descriptions underscores the importance of surveying in such undersampled regions.

Bee Community Study in Southeastern Michigan

While Michigan's bee fauna is relatively well-documented in comparison to some states in the eastern USA, many counties in the northern and eastern parts of the state remain poorly sampled (Gibbs et al., 2017a). Similar to Colorado, two of the most well-studied counties in Michigan (Ingham and Washtenaw Counties) are those that are home to the two major state universities (**Figure 1B**). Extrapolating from these two counties, most counties in southern Michigan only have one to two-thirds of their bee fauna documented (Gibbs et al., 2017a). Here, we present new county and state records from a recent study conducted across three counties (Wayne, Oakland, and Macomb) in southeastern Michigan to further illustrate how a limited sampling effort can contribute to enhancing knowledge of bee distributions (study region $\approx$ 5,600 km^2). This 3-month study (June-August 2017) involved a once per month 48-h passive and active sampling (bowl traps and hand-netting) of bee communities at 15 farms and community gardens located across a tri-county region (Wilson and Jamieson unpublished data; **Figure 1B**). The goal of this study was to evaluate bee response to urbanization and floral resource availability in order to better inform urban agriculture practices. Historic records indicate that the three counties surveyed in our study had been undersampled.

Despite the proximity of the surveyed region to counties that are home to two major state universities, this study yielded 74 new county-level species records, with 31 new occurrence records to the overall study region (**Table 1B**; **Figure 1B**). Furthermore, this study added two new state-level species records—*Chelostoma rapunculi* and *C. campanularum*. Both of these species are cavity-nesting specialists on *Campanula* (Campanulaceae) that were accidentally introduced into North America (Käpyl, 1978; Eickwort, 1980). These species have previously been reported in central New York State, USA and southern Ontario, Canada (Eickwort, 1980; Buck et al., 2005). To our knowledge, these records are the farthest west these species have been recorded. Results from the study presented here suggest that surveys in these areas have the potential to expand information on bee species distributions and regional species richness.

Efforts to comprehensively document state and county checklists invariably fall short of their ultimate goal. Species distributions fluctuate through time and sampling protocols are always limited in extent and duration. Nonetheless, checklists can invigorate interest in a region and emphasize deficits in our understanding of species distributions. A decade ago, a checklist of Pennsylvanian bees identified 398 species for the state (Donovall and VanEnglesdorp, 2010). The fauna for the state is now known to be at least 450 species (Kilpatrick et al., 2019). Similarly, new records continue to emerge in well-documented states, such as Colorado and Michigan, because bees in some geographic regions of these states have been poorly sampled, as shown above. More recent collection efforts in Michigan continue to find new state and county records (T. Wood in *litt.*). By surveying bees in undersampled regions as identified in statewide or regional checklists, sampling efforts can be better directed to fill gaps in our knowledge of bee distribution and improve baseline data for future ecological and taxonomic studies.

CONSIDERATIONS FOR FUTURE BEE COMMUNITY SURVEYS

Continued long-term monitoring efforts in well-studied areas is essential for evaluating changes in bee populations and communities over time. We propose that future research expanding into historically undersampled areas, in particular those undergoing environmental change, is also critical. For example, grasslands in the Great Plains of the USA have experienced significant land-use change due to agricultural intensification (Wright and Wimberly, 2013; Johnston, 2014). Pollination services provided by bees in this area are critical for ecosystems and agriculture in this region. Yet, we know little about bee communities throughout large areas of the Great Plains. When possible, research investigating land-use change effects on bees should include a range of disturbance levels or undisturbed sites for comparison. Likewise, surveys before and after disturbance are ideal for assessing environmental change effects.

In general, standardized protocols for bee sampling and specimen vouchering would help facilitate comparisons across studies. Nationwide surveys could enhance species inventorying and monitoring efforts to detect bee declines. Lebuhn et al. (2013) proposed that a standardized and nationwide survey network could be implemented across the USA to provide a better understanding of bee species trends over time. There are a number of challenges, however, with respect to such large-scale monitoring efforts, including logistical challenges for taxonomists and museums as well as issues with collection biases of pan traps that may not detect rare species or some species sensitive to disturbance (see further discussion in

TABLE 1 | New occurrence records for bee species by county and study region for surveys conducted in (A) northeastern Colorado and (B) southeastern Michigan.

(A) Two-year study in northeastern Colorado

County	# species previously recorded[a]	# species collected	# new occurrence records	# species recorded to date
Kit Carson	30	113	101	131
Logan	39	84	66	105
Phillips	11	88	82	93
Washington	5	124	122	127
Yuma	81	84	54	135
Study region	**119**	**153**	**97**	**216**

(B) One-year study in southeastern Michigan

County	# species previously recorded[b]	# species collected	# new occurrence records	# species recorded to date
Macomb	47	24	18	65
Oakland	133	90	28	161
Wayne	109	74	28	137
Study region	**179**	**107**	**31**	**210**

*See **Tables S1**, **S2** for species lists. New occurrence records are for species with positive identifications; no morphospecies or undetermined specimens are included here. Study region refers to species occurrences from all counties combined.*

[a] *Numbers from Scott et al. (2011).*

[b] *Numbers from Gibbs et al. (2017a).*

Lebuhn et al., 2015 and Tepedino et al., 2015). Tepedino et al. (2015) recommend that surveys should aim to evaluate how specific anthropogenic disturbances affect bee populations in select areas—in particular, areas undergoing rapid environmental change, such as the oil shale land in the western USA. Specimens collected for such studies could expand our knowledge of species distributions while answering ecological questions and addressing conservation solutions.

To inform conservation efforts, we need to understand how bees interact with their natural and modified habitats—in particular with respect to resource-use and ecological interactions.

In parallel with targeted ecological studies, there is an urgent need to sample more broadly to fill gaps in knowledge regarding species distributions as well as patterns of abundance and diversity over space and time. This type of survey work is more typically associated with taxonomic studies that focus on collecting the maximum number of species rather than repeated collections in a few sites with the goal of collecting species in their relative abundance. We hope examples above help demonstrate that much work remains in order to complete a basic inventory of bee species, let alone the arduous task of monitoring those species. Here we highlight a need for bee research in understudied areas and outline key concerns and considerations for future projects.

Firstly, in addition to considering the statistical power needed for addressing research questions, researchers should consider the effect of sampling large numbers of bees on local bee populations. Active netting and pan traps are unlikely to have strong effects on bee populations when used for targeted surveys (Gezon et al., 2015). However, the increased use of blue vane traps in regular surveys may be more problematic (Gibbs et al., 2017b). Blue vane traps are larger, more apparent, and may collect certain taxa, in particular larger apid bees, at levels that are potentially harmful on a local scale (Gibbs et al., 2017b). It would be foolhardy to limit collection efforts given the critical need to inventory and monitor bee populations; however, we suggest that blue vane traps may be best deployed for short periods (i.e., 24 h or less), especially in ecologically sensitive regions or seasons.

Secondly, bee species of some genera are notoriously difficult to accurately identify, and large monitoring projects could create a tremendous amount of work for a small number of bee taxonomists (Tepedino et al., 2015). As funding for bee research continues to increase worldwide, we hope to see greater funding and training opportunities for emerging bee taxonomists in addition to continued advances in DNA barcoding and molecular tools for species identifications (Packer et al., 2009; see Gonzalez et al., 2013; and Sheffield et al., 2017 for further discussion). DNA barcoding entire collections or representative samples as standard practice in ecological surveys could have two potential benefits: (1) reducing the pressure on taxonomic experts for routine identifications and (2) contributing data for taxonomists to further revise species concepts. It should be noted that DNA barcodes are not foolproof. Not all specimens will barcode successfully and DNA barcodes do not always discriminate closely related species (Meyer and Paulay, 2005; Meier et al., 2006; Gibbs, 2018), but neither does morphology (Packer et al., 2009). A helpful step that researchers can take prior to planning a community survey is to contact the appropriate taxonomists in advance of a project and write support for taxonomic expertise or DNA barcoding into grant applications. Since DNA barcoding databases may not be comprehensive or may require interpretation, taxonomic expertise is always recommended.

Finally, prior to planning a new study, researchers must consider where and how bee specimens will be vouchered. Large samples of bee specimens can require significant time, space, and resources to voucher in museums. Thus, researchers should discuss plans for vouchering specimens with collections managers. Museum specimens are the backbone of bee research—vital to the compilation of state, regional, and worldwide species lists as well as the development of taxonomic revisions, identification keys, and as reference material for specimen identification. Given that some bee taxa require revisions or present problems for accurate identification, vouchering of specimens in museum collections is imperative. Furthermore, museum specimens are good resources for documenting species declines over time (Colla et al., 2012b; Bartomeus et al., 2013, 2018; Burkle et al., 2013; Jacobson et al., 2018), or lack there of (Colla et al., 2012a), and are thus invaluable for directing conservation efforts. Colla and Packer (2008) used museum specimens to document the decline in *Bombus affinis* Cresson in southern Ontario over an ∼30-year period. Burkle et al. (2013) also used historic data to evaluate changes in plant-pollinator networks in Illinois, USA. Resampling an area after 120 years, Burkle et al. (2013) found that 50% of bee species from the regional study were locally extirpated. They attributed this effect to land-use and climate change that resulted in spatial and temporal mismatches between forbs and bees, in particular rare and specialized bee species. Such longitudinal studies are critical for assessing changes in bee communities, and these studies benefit from surveys over broad geographic areas.

CONCLUSIONS

We conclude that current gaps in knowledge and research biases make it difficult to monitor changes in bee communities over time and space, which presents challenges in evaluating and modeling the effects of anthropogenic disturbances on bees. We contend, however, that targeted sampling efforts in understudied areas have the potential to substantially improve our knowledge of bee species distributions, range limits, and geographic patterns of species diversity. A better understanding of such patterns can aid in identifying species of concern, such as declining or introduced species, and help direct future conservation efforts.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the **Supplementary Files**.

AUTHOR CONTRIBUTIONS

All authors contributed to writing and development of perspectives presented in this paper. MJ, AC, and CW designed experiments, implemented field research, and conducted data analyses. VS and JG contributed occurrence data and identifications for CO and MI bee species.

FUNDING

Research was supported by funding from the Foundation for Food and Agriculture (Award No. 430876) and the USDA National Institute for Food and Agriculture (Award No. 2013-67009-20375) provided to MJ in addition to an Oakland University Graduate Student Provost Award to CW.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00194/full#supplementary-material

REFERENCES

Archer, C. R., Pirk, C. W. W., Carvalheiro, L. G., and Nicolson, S. W. (2014). Economic and ecological implications of geographic bias in pollinator ecology in the light of pollinator declines. *Oikos* 123, 401–407. doi: 10.1111/j.1600-0706.2013.00949.x

Banaszak-Cibicka, W., and Zmihorski, M. (2012). Wild bees along an urban gradient: winners and losers. *J. Insect Conserv.* 16, 331–343. doi: 10.1007/s10841-011-9419-2

Bartomeus, I., Ascher, J. S., Gibbs, J., Danforth, B. N., Wagner, D. L., Hedtke, S. M., et al. (2013). Historical changes in northeastern US bee pollinators related to shared ecological traits. *Proc. Natl. Acad. Sci. U.S.A.* 110, 4656–4660. doi: 10.1073/pnas.1218503110

Bartomeus, I., Cariveau, D. P., Harrison, T., and Winfree, R. (2018). On the inconsistency of pollinator species traits for predicting either response to land-use change or functional contribution. *Oikos* 127, 306–315. doi: 10.1111/oik.04507

Bates, A. J., Sadler, J. P., Fairbrass, A. J., Falk, S. J., Hale, J. D., and Matthews, T. J. (2011). Changing bee and hoverfly pollinator assemblages along an urban-rural gradient. *PLoS ONE* 6:23459. doi: 10.1371/journal.pone.0023459

Bennett, A. B., Meehan, T. D., Gratton, C., and Isaacs, R. (2014). Modeling pollinator community response to contrasting bioenergy scenarios. *PLoS One* 9:e110676. doi: 10.1371/journal.pone.0110676

Buck, M., Paiero, S. M., and Marshall, S. A. (2005). New records of native and introduced aculeate hymenoptera from ontario, with keys to eastern canadian species of *Cerceris* (Crabronidae) and Eastern Nearctic Species of *Chelostoma* (Megachilidae). *J. Entomol. Soc. Ontario.* 136, 37–52.

Burkle, L. A., Marlin, J. C., and Knight, T. M. (2013). Plant-pollinator interactions over 120 years: loss of species, co-occurrence, and function. *Science* 339, 1611–1615. doi: 10.1126/science.1232728

Cameron, S. A., Lozier, J. D., Strange, J. P., Koch, J. B., Cordes, N., Solter, L. F., et al. (2011). Patterns of widespread decline in North American bumble bees. *Proc. Natl. Acad. Sci. U.S A.* 108, 662–667. doi: 10.1073/pnas.1014743108

Camilo, G. R., Muñiz, P. A., Arduser, M. S., Spevak, M., and Louis, S. (2017). A checklist of the bees (Hymenoptera: Apoidea) of St. Louis. *J. Kansas Entomol. Soc.* 90, 175–188. doi: 10.2317/0022-8567-90.3.175

Cane, J. H., Minckley, R. L., Kervin, L. J., Roulston, T. H., and Neal, M. (2006). Complex responses within a desert bee guild (Hymenoptera: Apiformes) to urban habitat fragmentation. *Ecol. Appl.* 16, 632–644. doi: 10.1890/1051-0761(2006)016[0632:CRWADB]2.0.CO;2

Cariveau, D. P., and Winfree, R. (2015). Causes of variation in wild bee responses to anthropogenic drivers. *Curr. Opin. Insect Sci.* 10, 104–109. doi: 10.1016/j.cois.2015.05.004

Carper, A. L., Schwantes, C. J., and Jamieson, M. A. (2019). A new state record of the rare bee, *Cemolobus ipomoeae* (Hymenoptera, Apidae), from Colorado, USA. *J. Kansas Entomol. Soc.* 91, 171–175. doi: 10.2317/0022-8567-91.2.171

Carril, O. M., Griswold, T., Haefner, J., and Wilson, J. S. (2018). Wild bees of Grand Staircase-Escalante National Monument: richness, abundance, and spatio-temporal beta-diversity. *PeerJ* 6, e5867. doi: 10.7717/peerj.5867

Colla, S. R., Ascher, J. S., Arduser, M., Cane, J., Deyrup, M., Droege, S., et al. (2012a). Documenting persistence of most Eastern North American bee species (Hymenoptera: Apoidea: Anthophila) to 1990 – 2009. *J. Kansas Entomol. Soc.* 85, 14–22. doi: 10.2317/JKES110726.1

Colla, S. R., Gadallah, F., Richardson, L., Wagner, D., and Gall, L. (2012b). Assessing declines of North American bumble bees (*Bombus* spp.) using museum specimens. *Biodivers. Conserv.* 21, 3585–3595. doi: 10.1007/s10531-012-0383-2

Colla, S. R., and Packer, L. (2008). Evidence for decline in eastern North American bumblebees (Hymenoptera: Apidae), with special focus on *Bombus affinis* Cresson. *Biodivers. Conserv.* 17, 1379–1391. doi: 10.1007/s10531-008-9340-5

Dibble, A. C., Drummond, F. A., Stubbs, C., Veit, M., and Ascher, J. S. (2017). Bees of maine, with a state species checklist. *Northeast. Nat.* 24, 1–48. doi: 10.1656/045.024.m1503

Donovall, L. R. I., and VanEnglesdorp, D. (2010). A checklist of the bees (Hymenoptera: Apoidea) of Pennsylvania. *J. Kansas Entomol. Soc.* 83, 7–24. doi: 10.2317/JKES808.29.1

Eickwort, G. C. (1980). Two european species of *Chelostoma* established in New York State (Hymenoptera: Megachilidae). *Psyche* 87, 315–323.

Fitch, G., Glaum, P., Simao, M., C., Vaidya, C., and Matthijs, J. (2019). Changes in adult sex ratio in wild bee communities are linked to urbanization. *Sci. Rep.* 9:3767. doi: 10.1038/s41598-019-39601-8

Garibaldi, L. A., Steffan-Dewenter, I., Winfree, R., Aizen, M. A., Bommarco, R., Cunningham, S. A., et al. (2013). Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science* 339, 1608–1611. doi: 10.1126/science.1230200

GBIF (2018). *GBIF Occurrence Download, Cemolobus ipomoea*. Available online at: https://doi.org/10.15468/dl.f9fcnx (accessed March 1, 2019).

Geslin, B., Le Feon, V., Folschweiller, M., Flacher, F., Carmignac, D., Motard, E., et al. (2016). The proportion of impervious surfaces at the landscape scale structures wild bee assemblages in a densely populated region. *Ecol. Evol.* 6, 6599–6615. doi: 10.1002/ece3.2374

Gezon, Z. J., Wyman, E. S., Ascher, J. S., Inouye, D. W., and Irwin, R. E. (2015). The effect of repeated, lethal sampling on wild bee abundance and diversity. *Methods Ecol. Evol.* 6, 1044–1054. doi: 10.1111/2041-210X.12375

Gibbs, J. (2018). DNA barcoding a nightmare taxon: assessing barcode index numbers and barcode gaps for sweat bees. *Genome* 61, 21–31. doi: 10.1139/gen-2017-0096

Gibbs, J., Ascher, J. S., Rightmyer, M. G., and Isaacs, R. (2017a). The bees of Michigan (Hymenoptera: Apoidea: Anthophila), with notes on distribution, taxonomy, pollination, and natural history. *Zootaxa* 4352, 1—160. doi: 10.11646/zootaxa.4352.1.1

Gibbs, J., Joshi, N. K., Wilson, J. K., Rothwell, N. L., Powers, K., Haas, M., et al. (2017b). Does passive sampling accurately reflect the bee (Apoidea: Anthophila) communities pollinating apple and sour cherry orchards? *Environ. Entomol.* 46, 579–588. doi: 10.1093/ee/nvx069

Gibbs, J., and Sheffield, C. S. (2009). Rapid range expansion of the wool-carder bee, *Anthidium manicatum* (Linnaeus) (Hymenoptera : Megachilidae), in North America. *J. Kansas Entomol. Soc.* 82, 21–29. doi: 10.2317/JKES805.27.1

Glaum, P., Simao, M. C., Vaidya, C., Fitch, G., and Iulinao, B. (2017). Big city Bombus : using natural history and land-use history to find significant environmental drivers in bumble-bee declines in urban development. *R. Soc. Open Sci.* 4:170156. doi: 10.1098/rsos.170156

Goldstein, P. Z., and Scott, V. L. (2015). Taxonomic and behavioral components of faunal comparisons over time: the bees (Hymenoptera: Anthophila) of boulder county, colorado, past and present. *Proc. Entomol. Soc. Washingt.* 117, 290–346. doi: 10.4289/0013-8797.117.3.290

Gonzalez, V. H., Griswold, T., and Engel, M. S. (2013). Obtaining a better taxonomic understanding of native bees: where do we start? *Syst. Entomol.* 38, 645–653. doi: 10.1111/syen.12029

Goulson, D., Nicholls, E., Botías, C., and Rotheray, E. L. (2015). Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* 347, 1255957. doi: 10.1126/science.1255957

Graham, K. K., and MacLean, M. G. (2018). Presence-only modeling is ill-suited for a recent generalist invader, *Anthidium manicatum*. *Ecol. Indic.* 89, 56–62. doi: 10.1016/j.ecolind.2018.02.002

Grundel, R., Jean, R. P., Frohnapple, K. J., Gibbs, J., Glowacki, G. A., and Pavlovic, N. B. (2011). A survey of bees (Hymenoptera: Apoidea) of the indiana dunes and Northwest Indiana, USA. *J. Kansas Entomol. Soc.* 84, 105–138. doi: 10.2317/JKES101027.1

Hamblin, A. L., Youngsteadt, E., López-Uribe, M. M., and Frank, S. D. (2017). Physiological thermal limits predict differential responses of bees to urban heat-island effects. *Biol. Lett.* 13:20170125. doi: 10.1098/rsbl.2017.0125

Harrison, T., and Winfree, R. (2015). Urban drivers of plant-pollinator interactions. *Funct. Ecol.* 29, 879–888. doi: 10.1111/1365-2435.12486

Jacobson, M. M., Tucker, E. M., Mathiasson, M. E., and Rehan, S. M. (2018). Decline of bumble bees in northeastern North America, with special focus on *Bombus terricola*. *Biol. Conserv.* 217, 437–445. doi: 10.1016/j.biocon.2017.11.026

Jean, R. P. (2010). *Studies of bee diversity in indiana the influence of collection methods on species capture and a state checklist based on museum collections*. (Dissertation). Indiana State University, Terre Haute, IN, United States.

Johnston, C. A. (2014). Agricultural expansion: Land use shell game in the U.S. *Northern Plains. Landsc. Ecol.* 29, 81–95. doi: 10.1007/s10980-013-9947-0

Käpylä, M. (1978). Bionomics of five wood-nesting solitary species of bees (Hym., Megachilidae), with emphasis on flower relationships. *Biol. Rev. Rep. Univ.* 5, 3–89.

Kearns, C. A., and Oliveras, D. M. (2009). Environmental factors affecting bee diversity in urban and remote grassland plots in Boulder, Colorado. *J. Insect. Conserv.* 13, 655–665. doi: 10.1007/s10841-009-9215-4

Kerr, J. T., Pindar, A., Galpern, P., Packer, L., Potts, S. G., Roberts, S. M., et al. (2015). Climate change impacts on bumblebees converge across continents. *Science* 349, 177–180. doi: 10.1126/science.aaa7031

Kilpatrick, S. K., Gibbs J., Mikulas, M. M., Spichiger, S., Ostiguy, N., Biddinger, D., et al. (2019). *Checklist of the Bees of Pennsylvania*. Available online at: https://lopezuribelab.com/checklist-bees-pennsylvania/ (accessed March 1, 2019).

Klein, A.-M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., et al. (2007). Importance of pollinators in changing landscapes for world crops. *Proc. Biol. Sci.* 274, 303–313. doi: 10.1098/rspb.2006.3721

Klein, A. M., Boreux, V., Fornoff, F., Mupepele, A. C., and Pufal, G. (2018). Relevance of wild and managed bees for human well-being. *Curr. Opin. Insect Sci.* 26, 82–88. doi: 10.1016/j.cois.2018.02.011

Koh, I., Lonsdorf, E. V., Williams, N. M., Brittain, C., Isaacs, R., Gibbs, J., et al. (2016). Modeling the status, trends, and impacts of wild bee abundance in the United States. *Proc. Natl. Acad. Sci. U. S. A.* 113, 140–145. doi: 10.1073/pnas.1517685113

Lebuhn, G., Droege, S., Connor, E. F., Gemmill-Herren, B., Potts, S. G., Minckley, R. L., et al. (2013). Detecting insect pollinator declines on regional and global scales. *Conserv. Biol.* 27, 113–120. doi: 10.1111/j.1523-1739.2012.01962.x

Lebuhn, G., Droege, S., Connor, E. F., Gemmill-Herren, B., Potts, S. G., Minckley, R. L., et al. (2015). Evidence-based conservation: reply to Tepedino et al. *Conserv. Biol.* 29, 283–285. doi: 10.1111/cobi.12438

Losey, J. E., and Vaughn, M. (2006). The economic value of ecological services provided by insects. *Bioscience* 56, 311–323. doi: 10.1641/0006-3568(2006)56[311:TEVOES]2.0.CO;2

Meier, R., Shiyang, K., Vaidya, G., and Ng, P. K. L. (2006). DNA barcoding and taxonomy in diptera: a tale of high intraspecific variability and low identification success. *Syst. Biol.* 55, 715–728. doi: 10.1080/10635150600969864

Meyer, C. P., and Paulay, G. (2005). DNA barcoding: error rates based on comprehensive sampling. *PLoS Biol.* 3, e422. doi: 10.1371/journal.pbio.0030422

Miller, S. R., Gaebel, R., Mitchell, R. J., and Arduser, M. (2002). Occurrence of two species of old world bees, *Anthidium manicatum* and *A. oblongatum* (Apoidea: Megachilidae), in northern Ohio and southern Michigan. *Gt. Lakes Entomol.* 35, 65–69.

Normandin, É., Vereecken, N. J., Buddle, C. M., and Fournier, V. (2017). Taxonomic and functional trait diversity of wild bees in different urban settings. *PeerJ* 5:e3051. doi: 10.7717/peerj.3051

Ollerton, J., Winfree, R., and Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos* 120, 321–326. doi: 10.1111/j.1600-0706.2010.18644.x

Packer, L., Gibbs, J., Sheffield, C., and Hanner, R. (2009). DNA barcoding and the mediocrity of morphology. *Mol. Ecol. Resour.* 9, 42–50. doi: 10.1111/j.1755-0998.2009.02631.x

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., and Kunin, W. E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends Ecol. Evol.* 25, 345–353. doi: 10.1016/j.tree.2010.01.007

Russo, L. (2016). Positive and negative impacts of non-native bee species around the world. *Insects* 7:E69. doi: 10.3390/insects7040069

Samson, F. B., Knopf, F. L., and Ostlie, W. R. (2004). Great Plains ecosystems: past, present, and future. *Wildl. Soc. Bull.* 32, 6–15. doi: 10.2193/0091-7648(2004)32[6:GPEPPA]2.0.CO;2

Scott, V. L., Ascher, J. S., Griswold, T. L., and Nufio, C. R. (2011). The bees of colorado. *Nat. Hist. Invent. Color.* 23, vi-100. doi: 10.1016/S1472-6483(10)60037-5

Sheffield, C. S., Heron, J., Gibbs, J., Onuferko, T. M., Oram, R., Best, L., et al. (2017). Contribution of DNA barcoding to the study of the bees (Hymenoptera: Apoidea) of Canada: progress to date. *Can. Entomol.* 149, 736–754. doi: 10.4039/tce.2017.49

Slagle, M. W., and Hendrix, S. D. (2009). Reproduction of *Amorpha canescens* (Fabaceae) and diversity of its bee community in a fragmented landscape. *Oecologia* 161, 813–823. doi: 10.1007/s00442-009-1429-3

Soper, J., and Beggs, J. R. (2013). Assessing the impact of an introduced bee, *Anthidium manicatum,* on pollinator communities in New Zealand. *New Zeal. J. Bot.* 51, 213–228. doi: 10.1080/0028825X.2013.793202

Strange, J. P., Koch, J. B., Gonzalez, V. H., Nemelka, L., and Griswold, T. (2011). Global invasion by *Anthidium manicatum* (Linnaeus) (Hymenoptera: Megachilidae): Assessing potential distribution in North America and beyond. *Biol. Invasions* 13, 2115–2133. doi: 10.1007/s10530-011-0030-y

Tepedino, V. J., Durham, S., Cameron, S. A., and Goodell, K. (2015). Documenting bee decline or squandering scarce resources. *Conserv. Biol.* 29, 280–282. doi: 10.1111/cobi.12439

Winfree, R., Aguilar, R., Vásquez, D. P., LeBuhn, G., and Aizen, M. A. (2009). A meta-analysis of bees' responses to anthropogenic disturbance. *Ecology* 93, 2068–2076.

Winfree, R., Reilly, J. R., Bartomeus, I., Cariveau, D. P., Williams, N. M., and Gibbs, J. (2018). Species turnover promotes the importance of bee diversity for crop pollination at regional scales. *Science* 359, 791–793. doi: 10.1126/science.aao2117

Wright, C. K., and Wimberly, M. C. (2013). Recent land use change in the Western Corn Belt threatens grasslands and wetlands. *Proc. Natl. Acad. Sci. U.S.A.* 110, 4134–4139. doi: 10.1073/pnas.1215404110

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 07 June 2019
doi: 10.3389/fevo.2019.00208

Host Plant Effects on Immune Response Across Development of a Specialist Caterpillar

Adrian L. Carper, Micaela Enger and M. Deane Bowers*

Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO, United States

Some herbivorous insects sequester chemicals from their host plants, which can serve as a defense against predators. However, sequestration can also be costly, impacting immunological responses important for other types of natural enemies, such as parasites and pathogens. These costs could also vary across herbivorous insect development, as both immune function and sequestration abilities change, although few studies have assessed variation in the cost of sequestration across life stages. The buckeye, *Junonia coenia* (Nymphalidae), is a specialist butterfly that sequesters iridoid glycosides from its host plants, including the introduced weed, *Plantago lanceolata* (Plantaginaceae). To determine the immunological costs of sequestration and how the immune response varies with host plant and across development, we reared caterpillars on a native host plant, *Mimulus guttatus* (Phrymaceae), which does not contain iridoid glycosides, and on *P. lanceolata*, which does. We assayed immune function across 3rd, 4th, and 5th instar caterpillars, estimating both hemocyte density and the ability of hemocytes to encapsulate foreign bodies by challenging the immune system with nylon filaments. For caterpillars reared on *P. lanceolata*, we then explored the relationships between iridoid glycoside sequestration and immune function across instars. We found that while hemocyte density tended to increase with instar regardless of host plant, caterpillars reared on *P. lanceolata* had lower encapsulation ability, and encapsulation decreased with increasing sequestration of iridoid glycosides, though patterns varied between instars and experimental periods. Interestingly, immune challenged caterpillars sequestered more iridoid glycosides than unchallenged caterpillars, suggesting that caterpillars responded to immune challenge by sequestering or retaining more iridoids, even though that may decrease their ability to encapsulate. These results suggest that sequestration can have important consequences for immune function across caterpillar development, and that the incorporation of novel hosts may affect defense against natural enemies.

Keywords: immune, ecoimmunology, sequestration, iridoid, *Plantago*, *Junonia*

OPEN ACCESS

Edited by:
Gina Marie Wimp,
Georgetown University, United States

Reviewed by:
Kelli Hoover,
Pennsylvania State University,
United States
Liza Holeski,
Northern Arizona University,
United States

****Correspondence:***
Adrian L. Carper
adrian.carper@colorado.edu

Specialty section:
This article was submitted to
Population and Evolutionary
Dynamics,
a section of the journal
Frontiers in Ecology and Evolution

Received: *07 March 2019*
Accepted: *20 May 2019*
Published: *07 June 2019*

Citation:
Carper AL, Enger M and Bowers MD
(2019) Host Plant Effects on Immune
Response Across Development of a
Specialist Caterpillar.
Front. Ecol. Evol. 7:208.
doi: 10.3389/fevo.2019.00208

INTRODUCTION

Herbivorous insects are exposed to many different natural enemies throughout their development, including predators, parasites, parasitoids, and pathogens (Rosenheim, 1998; Vidal and Murphy, 2018), and employ a variety of strategies to defend themselves against these enemies. For example, some insects use chemical defenses that are sequestered from the plants on which they feed (Brower et al., 1968; Bowers, 1990). However, while sequestration can be an effective defense against predators, the process of sequestration can also be costly (Bowers, 1992; Hartmann, 2004; Smilanich et al., 2009a; Zvereva and Kozlov, 2016). Sequestration or detoxification of plant secondary metabolites (PSMs) may involve several processes to store or metabolize the compounds; for example, compounds may be rendered harmless by the activity of gut enzymes (Dobler et al., 2011), or the compounds may be transferred from the gut to certain organs for storage (Poreddy et al., 2015). The mechanisms required for those processes are energetically costly and may impact other physiological processes. For instance, the immune system functions to target parasites, parasitoids, and pathogens that have entered the insect's body (Barbosa and Caldas, 2007) using specific immune cells to target and kill foreign bodies (Beckage, 2008). However, while a substantial amount of research has been conducted on the chemical defenses of herbivorous insects, until recently there has been less focus on how sequestration might impact immune function (see Smilanich et al., 2009a).

Ecoimmunology is an interdisciplinary field combining ecology and immunology that has been advancing because of its applications in fields such as disease ecology and biological control (Stanley et al., 2012). Smilanich et al. (2009a) showed that larvae feeding on plants with high amounts of PSMs have weaker immune responses compared to those fed on plants with lower amounts, suggesting that host plant chemistry is important not only for insect chemical defenses, but also for the effectiveness of the immune responses. This raises the possibility of trade-offs in defense strategies against multiple natural enemies, if the costs of sequestering PSMs outweigh the benefits of immune defenses or vice versa. Such trade-offs may become even more important in the presence of ecological perturbations, such as human-induced environmental change, which can lead to changes in host plant quality (Jamieson et al., 2015; Decker et al., 2018), range shifts of host plants or natural enemies (Jeffs and Lewis, 2013), the introduction of novel hosts (Jahner et al., 2011), and subsequently insect defense against natural enemies (Gherlenda et al., 2016).

Moreover, as an insect develops, the suite of natural enemies encountered often changes, necessitating different strategies in response to attacking enemies (Stamp, 1986). For example, younger and smaller stages may be more susceptible to predators (Memmott et al., 2000) and their immune system may not be fully developed until later stages (Brodeur and Vet, 1995; Gillespie et al., 1997), when parasitoids and pathogens may be more frequently encountered. Insects have an innate immune system with both humoral and cellular defenses, the latter consisting of three components, phagocytosis, nodule formation, and encapsulation (Strand, 2008). The cellular responses are primarily used to defend against enemies that have invaded the insect, such as parasitoid eggs, parasites, and pathogens (Beckage, 2008). Early larval instars have been shown to have weaker immune responses, suggesting that one component of the immune response, hemocyte production and differentiation may be important later in development (Brodeur and Vet, 1995; Gillespie et al., 1997). The activity of the immune response itself may also vary across development. For example, in *Bombyx mori* (Bombycidae), hemocyte adhesion, and subsequently phagocytosis have been shown to increase against invaders as larvae age (Wago and Ichikawa, 1979). Given that selective pressures from natural enemies change over larval development, larvae could exhibit stage-specific investment in chemical and immune defensive strategies. Previous studies have investigated how sequestering PSMs can affect insect herbivore preference and performance, parasitoid success, and immune response (Dyer, 1995; Smilanich et al., 2009b). However, changes in immune responses over insect development and how it relates to PSM sequestration has been little studied.

To determine how the sequestration of PSMs impacts the immune function of caterpillars across development, we reared larvae of the sequestering specialist, *Junonia coenia* (Nymphalidae) (common buckeye) on two hosts: one, an introduced weed (Cavers et al., 1980), containing sequesterable PSMs (*Plangato lanceolata*, Plantaginaceae) and the other, a native host plant species, without (*Mimulus guttatus*, Phrymaceae). We then compared immune functions of different instar caterpillars between host plants, and explored the relationship between sequestration and immune function across caterpillar development. Specifically we asked: (1) does the immune response of this specialist caterpillar change with larval instar, (2) if so, does the host plant on which caterpillars feed affect this response, and (3) does the relationship between sequestration and immune function vary across caterpillar development.

METHODS

Study System

Junonia coenia (Nymphalidae), the common buckeye, is a specialist butterfly whose larvae feed on a variety of host plant species, most of which contain a specific group of PSMs, iridoid glycosides (Bowers, 1984). Iridoid glycosides (hereafter IGs) serve as feeding stimulants for the larvae and as oviposition stimulants for adult females (Bowers, 1984; Bowers and Puttick, 1986; Pereyra and Bowers, 1988; Bowers et al., 1992a). Larvae have been recorded feeding on plants in five different families containing IGs: Scrophulariaceae, Plantaginaceae, Verbenaceae, Acanthaceae, and Cornaceae (Bowers, 1984). Previous research has shown that *J. coenia* larvae sequester relatively high levels of IGs, ranging from 10 to 20% in newly molted individuals (Bowers and Collinge, 1992) and making them unpalatable to potential predators (Bowers, 1992).

Plantago lanceolata (Plantaginaceae, hereafter *Plantago*) is a common weed incorporated into the diet of many native North American insect herbivores, including buckeyes (Bowers and Collinge, 1992). It is native to Eurasia but has been distributed all over the world (Cavers et al., 1980). *Plantago* contains two IGs, aucubin and catalpol (Duff et al., 1965), that can be deterrent to generalist herbivores and non-adapted specialist herbivores (Bowers and Puttick, 1988). Buckeye larvae also feed on the native yellow monkey flower, *Mimulus guttatus* (Phrymaceae, hereafter *Mimulus*), which is found in wetlands across western North America and is their only host plant that does not contain IGs (Kooiman, 1970; Holeski et al., 2010). Although, *Mimulus* does contain phenylpropanoid glycosides; one of these, verbascoside, serves as a feeding stimulant for buckeye larvae, and can also be deterrent to generalist herbivores (Holeski et al., 2013).

Experimental Design

To explore how a novel host plant impacts sequestration and immune function, we used greenhouse grown *Mimulus* and *Plantago* as alternate hosts for buckeye caterpillars. Plants were grown from seed in a growth chamber at 25° Celsius until large enough to be transferred to a greenhouse where the temperature ranged from 10° to 21°C. Plants were watered daily and fertilized with a 24-8-16 water soluble fertilizer (Miracle-Gro, The Scotts Company, LLC) twice per week. Given that chemical analyses indicated *Plantago* plants were very low in IGs when the experiment was first conducted in January (designated Experiment 1) (see Results), we repeated the entire experiment in September (designated Experiment 2) when plants had higher IG levels, allowing for qualitative comparison between times when host plants are low or high in IG concentrations.

Caterpillars for the experiment were reared from a source colony originating from adult butterflies collected around Sacramento, California. The adults were kept in 60 × 60 × 60 cm butterfly tents (BugDorm), where they were allowed to mate and oviposit on either *Plantago* or *Mimulus* plants. First instar larvae were collected from the host plants and placed into clear, round plastic petri dishes, 14 cm in diameter, and placed in a growth chamber kept on a 14:10 LD cycles, at 25°C light and 20°C dark. When larvae molted to the second instar, individuals were put into 60 ml plastic cups with plastic lids in order to monitor when they molted into third instar to begin immune assays.

Immune Assays

We compared two metrics of the immune response between caterpillars reared on each host plant: hemocyte density and encapsulation. Hemocytes are the primary cellular response of the insect immune system (Lavine and Strand, 2002), and all hemocyte types are involved in the immune response (Strand et al., 2006). Encapsulation is the process by which hemocytes in the insect's body cavity attach to foreign objects (Pech and Strand, 1996) too large to be killed by phagocytosis (Gillespie et al., 1997). After hemocytes adhere to the foreign object, they die and melanize (Smilanich et al., 2009b), killing cells (such as parasitoid eggs or pathogens) by asphyxiation and with cytotoxic molecules (Nappi and Christensen, 2005). Encapsulation can thus be measured by quantifying the melanization on the foreign object using photo imaging software (Smilanich et al., 2009b).

Once larvae reared on each of the two host plants reached the middle of the appropriate instar (∼2 days after molting to third, fourth, or fifth; $N = 15$ on each host plant/instar combination), they were weighed to the nearest 0.01 mg, then placed into a freezer at −29°C for 1–2 min in order to slow the caterpillar's movement while being assayed (Smilanich et al., 2009b). Each caterpillar was then placed on a watch glass so that it was lying on its right side, and Parafilm used to hold the caterpillar in place. A clean insect pin was used to make a small hole in a designated spot between the 4th and 5th proleg in each caterpillar. After removing the pin, we sampled 10 uL of hemolymph using a Gilson Pipet set to 10 microliters. For some smaller 3rd instars where 10 uL was unable to be collected (due to small size), we sampled 5 uL. Hemolymph samples were immediately added to an equal amount of anticoagulant, and stored on ice until hemocytes were counted after filament insertion (see below). Anticoagulant was prepared using 0.684 grams of EDTA, 0.346 grams of citric acid, and 180 mL of phosphate-buffered saline (as in Smilanich et al., 2018). We counted all hemocytes using a hemocytometer (Sigma-Aldrich Bright-Line) under a compound light microscope following Triggs and Knell (2012), and calculated cell density per mL of hemolymph accordingly.

To measure encapsulation we inserted nylon filaments (**Figure 1**) as a proxy for parasitoid eggs or larvae (Smilanich et al., 2009b). Filaments were made using 0.20 mm diameter

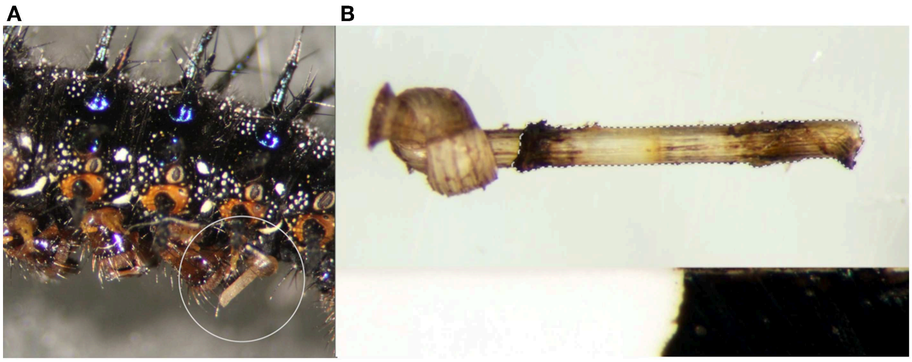


FIGURE 1 | Nylon filaments were inserted just beneath the cuticle **(A)** behind the 4th abdominal proleg and imaged after 24 h **(B)** to measure the degree of melanization. Hashed lines represent the area of filament within the body of the caterpillar that was quantified.

monofilament fishing line (Berkley Trilene XL Smooth Casting). The filaments were prepared by first lightly sanding a length of fishing line, then knots were tied along it and 2 mm lengths trimmed from the edge of each knot. An alternate method was also used in January, where sections of sanded monofilament were heated next to a butane lighter to melt and expand one end, then trimmed to 2 mm. Immediately after hemolymph was drawn from each caterpillar, a single filament was inserted into the wound by sliding it in laterally just under the cuticle of the caterpillar. The expanded end of the monofilament or knot held these inserts in place while the caterpillars were returned to the growth chamber, allowed to feed on their respective host plant, and given 24 h for encapsulation to occur (Smilanich et al., 2009b). Afterward, the filament was removed, and either placed in 70% ethanol in a 1.5 mL eppendorf tube in Experiment 1 (January) or immediately frozen (no ethanol) in Experiment 2 (September).

We photographed filaments using a Canon Rebel XTi DSLR camera (Canon, U.S.A, Inc) mounted on a dissecting microscope. All photographs were taken at 16x magnification, and analyzed using Adobe Photoshop CC 2017 (Abobe, Inc.). In January, photographs were analyzed by setting the image mode to grayscale with 0 as white and 255 as black. The 1.5 mm tail end of the nylon monofilament was selected using the "magic wand" tool so that only the part that was inserted into the caterpillar was measured, and then we calculated the mean white value using the histogram tool. In September, we included a black and white standard with each filament during imaging to help standardize imaging measurements. The black and white standard was created using a small section of acid free archival, alcohol proof label paper, half of which was covered in black India ink. For each image, we set the black and white balance based on this standard, and measured the entire length of filament within the body of the caterpillar. We then calculated mean white values as above. Given that the mean measures the average amount of white per pixel across the filament, the inverse of the mean represents the average darkness of the filament; the darker the color the higher the degree of encapsulation (Smilanich et al., 2009b). We used this mean as a quantitative estimate of immune response.

IG Quantification and Sequestration

After 24 h of feeding and immediately after removing filaments, all caterpillars fed *Plantago* were placed in 1.5 mL of 95% methanol in 2 mL centrifuge tubes and frozen. To prepare samples for IG quantification, larvae were removed from the freezer and transferred into 15 mL glass test tubes. Whole caterpillars were ground with sand in five mL of methanol, left for 24 h for IGs to extract, then all particulates were filtered, and the remaining methanol evaporated (Knerl and Bowers, 2013). We then added 1 mL of 0.500 mg/mL phenyl-β-D-glucopyranoside (PBG) as an internal standard and 3 mL of water. To remove lipophilic substances, samples were treated with 3 ml of ether; vortexed, and the water and ether layers separated by centrifuging the samples for 4 min. The ether layer (containing lipophilic substances) was discarded (Bowers and Stamp, 1997) and the process repeated three times. The water layer, containing IGs and sugars, was evaporated, and then 1.00 ml of methanol was added and the sample left overnight. A 0.100 ml aliquot was then removed, the methanol evaporated, and the sample derivatized using Tri-Sil Z (Sigma Aldrich Chemical Company) (Bowers and Stamp, 1997).

To measure the iridoid glycoside content of the plant material fed to the larvae, we sampled leaves from five different *Plantago* plants in January and 10 different plants in September. Each set of leaves was dried at 50°C for 48 h, ground to a fine powder, and 25 mg weighed and extracted in methanol for 24 h. Plant sample preparation was then identical to caterpillar sample preparation. IG concentrations were reported as percent dry weight because of the variation in water content in plants and caterpillars (Knerl and Bowers, 2013). We calculated a dry weight conversion factor for 3rd, 4th, and 5th instar caterpillars, by weighing sets of 10 larvae fresh, then again after drying at 50°C for 48 h. We then multiplied the fresh weights of all caterpillars by the respective conversion factor to estimate dry weight.

We used gas chromatography (GC) to quantify IG concentrations of source plants of *Plantago* used for feeding and for caterpillars reared on *Plantago* during the experiment, following standard protocols (Bowers and Stamp, 1997). For IG quantification, we used an Agilent 7890A gas chromatograph equipped with a DB-1 column (30 m, 0.320 mm, 0.25 μm particle size), using flame-ionization detection. The temperature program used an initial temperature of 200°C held for 1 min, followed by a 3 min increase to 260°C, which was held for 8 min, followed by a 3 min increase to a final temperature of 320°C, held for 10 min. Amounts of aucubin and catalpol were quantified using ChemStation B-03-01 software after calibration using a standard containing known amounts of PBG, aucubin, and catalpol.

Sequestration and Immune Challenge

Since caterpillars store IGs in hemolymph (Bowers, 2003), removing hemolymph for counting hemocytes could directly reduce IGs in immune-challenged caterpillars. Therefore, we conducted two additional experiments to determine the impacts of our immune treatments on IG concentration. For the first, during both immune experiments (Experiment 1 and Experiment 2), we reared 15 additional caterpillars per instar on *Plantago* to serve as a comparative unmanipulated control for IG quantification. These were reared in the same way as other experimental groups but were not assayed for an immune response, thus no hemolymph was removed. In Experiment 1(January), these unchallenged controls were set up immediately after the initial experiment, and thus plants fed to those larvae could differ somewhat in their IG content compared to plants fed to caterpillars used for the immune assays (see Quintero et al., 2014) leading to differences in caterpillar IG content, although differences were likely to be small. In Experiment 2 (September), therefore, we set up the experiment so that unchallenged trials were conducted simultaneously with challenged trials.

In a third experiment (Experiment 3), we wanted to examine the consequences of hemolymph sampling and the immune assays for caterpillar sequestration in more detail. Therefore, the following March, we used 5th instar caterpillars fed on *Plantago* and had a control with three different immune assay treatments ($N = 15$ for each treatment). The first treatment group had 10 uL hemolymph drawn and was then immediately frozen for IG analysis to determine the direct effect of sampling hemolymph; the second had 10 ul hemolymph drawn and was then allowed 24 h to feed and recover before being frozen for IG analyses, allowing us to account for compensation for IG loss over 24 h; the third was treated as in the immune experiment, with 10 uL hemolymph drawn, a nylon filament inserted, and the caterpillar allowed 24 h to feed before removing the filament and freezing for IG analyses, to test for response to immune challenge. The control group was reared alongside other treatments but frozen without any immune assay. We expected immediate hemolymph draws should reduce IG concentration relative to unassayed controls. If caterpillars compensated for IG loss, they should sequester the same or more IGs after 24 h. Adding in the nylon filament should allow us to detect if immune challenge alters sequestration, either reducing or increasing IG concentrations relative to 24 h post-hemolymph draw treatments.

Statistical Analyses

All analyses were conducted in R version 3.51 (R. Core Team, 2017) and separate analyses were conducted for January and September. We compared hemocyte density between host plants and instars using two-way analysis of variance (ANOVA), with host plant, instar, and their interaction as fixed effects. We log-transformed hemocyte density to meet the assumption of normality. Given that hemocyte density should be predictive of encapsulation, we included it as a factor in analyses of melanization. To determine if host plant affected the relationship between hemocyte density and melanization, we analyzed melanization using a full-factorial ANCOVA, including host plant, and instar as main effects, hemocyte density as a covariate, and all two-way and three-way interactions. For caterpillars reared on *Plantago*, we compared the percent total IG sequestration and the proportion of IGs sequestered that was catalpol across larval instars using separate one-way ANOVAs, with larval instar as a fixed factor. We logit transformed both metrics of sequestration to meet the assumption of normality. To determine the effects of IG sequestration on immune response across development, we analyzed melanization of caterpillars fed *Plantago* using ANCOVA, with total IG concentration, cell density, and instar as main effects, and all two- and three-way interactions. We used only total percent IGs sequestered in ANCOVAs, given that Experiment 1 indicated that the proportion of catalpol sequestered was negatively correlated with total IG sequestration ($T_{41} = -3.07$, $p = 0.004$, $r = -0.43$). To determine if changes in sequestered IGs were indeed the results of immune challenge, we compared the different levels of immune treatment with ANOVA and Tukey *Post Hoc* tests. We included challenge type as a fixed effect, and logit transformed all IG measures. Seven caterpillars that died as a result of immune challenge during the experiment were excluded from analyses of melanization, given that they were unable to mount an immune response.

RESULTS

Immune Assays

Experiment 1 (January)

Hemocyte density varied across larval development in January, increasing from third to fifth instar [**Figure 2A**, $F_{(1,84)} = 17.75$, $p < 0.001$] and was 36% higher in individuals fed *Plantago* vs. *Mimulus* [**Figure 2A**, $F_{(1,84)} = 8.34$, $p = 0.005$]. However, there was no significant interaction between instar and host plant [$F_{(1,84)} = 0.601$, $p = 0.550$], suggesting that the effect of host plant on hemocyte density was consistent across caterpillar development. Despite consistently higher hemocyte density when fed *Plantago*, there was no main effect of host plant on melanization and no overall relationship between hemocyte density and melanization (see **Table 1**). However, melanization varied significantly between instars and there was a significant interaction between instar and host plant (**Figure 2B**), wherein melanization increased across instars in caterpillars reared on *Mimulus*, but not when reared on *Plantago*. There was also a significant interaction between instar and hemocyte density, where melanization increased with increasing hemocyte density in third and fourth instars but not in fifth (**Figure 2C**). However, there was no interaction between host plant and hemocyte density and no three-way interaction between host plant, instar, and hemocyte density, suggesting little indirect effect of host plant on immune function through altered hemocyte function.

Experiment 2 (September)

Hemocyte density was 23% higher on average in September than January (373 vs. 303 cells per mL on average, respectively) and results differed. We found no differences in hemocyte density between caterpillar instars ($F_{(2,84)} = 2.00$, $p = 0.142$), no effect of host plant [$F_{(1,84)} = 0.85$, $p = 0.359$], and no interaction between host plant and caterpillar instar [**Figure 3A**, $F_{(2,84)} = 0.29$, $p = 0.749$, **Figure 3A**]. While there was no main effects of either host plant or instar on melanization, there was a significant interaction between host plant and instar (**Table 2**), with 5th instars having 16% less melanization on average when raised on *Plantago* vs. *Mimulus*, a pattern similar to January (**Figure 3B**). However, we found no other two-way or three-way interactions (**Table 2**).

IG Sequestration in *Plantago*-Reared Caterpillars

Experiment 1 (January)

Caterpillars sequestered both aucubin and catalpol and in concentrations more than twice that of *Plantago* plants (**Figure 4A**). In January, the percent dry weight total IGs sequestered varied across larval instars [$F_{(2,42)} = 28.03$, $P < 0.001$], with 4th instars sequestering the highest levels of IGs, over three times the concentrations of 3rd instars, and more than 80% higher than 5th instars (**Figure 4A**). ANCOVA revealed no direct effects of IG sequestration on hemocyte density or melanization (**Table 3**). However, there was a significant interaction between total IG sequestration and hemocyte density on melanization, but the relationships did not vary by caterpillar instar as there were no two- or three-way interactions. Immune challenged caterpillars sequestered a mean of 76% more total IGs per dry

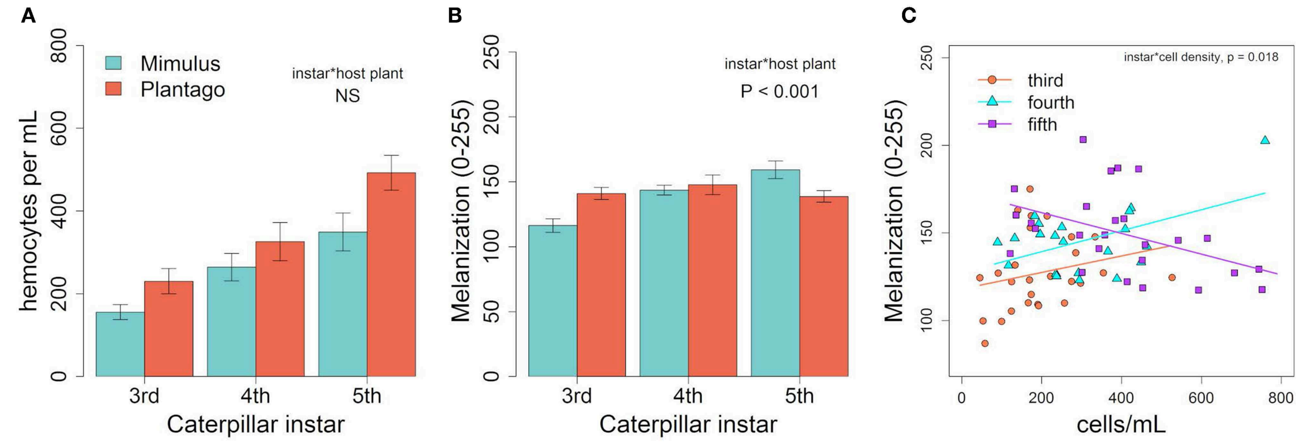


FIGURE 2 | In January, **(A)** hemocyte density varied by instar and was higher on *Plantago* though the interaction was not significant. **(B)** There was significant interaction between host plant and instar, suggesting melanization increased with instar when fed *Mimulus* but did not change when fed *Plantago*. **(C)** A significant interaction between instar and hemocyte density suggested that melanization increased with cell density in third and fourth instars, but decreased with increasing cell density in fifth instar caterpillars. Lines represent predicted linear relationships for each instar. Bars represent ± 1 SE.

TABLE 1 | Results from ANCOVA in the January experiment included a main effect of caterpillar developmental instar on melanization, as well as interactions between host plant and instar and instar and hemocyte density.

Factor	Df	Sum Sq	Mean Sq	*F*-value	Pr(>F)
Host plant	1	198	198	0.55	0.460
Instar	2	7,440	3,720	10.39	**<0.001**
Hemocyte density	1	91	91	0.26	0.616
Host plant*Instar	2	6,630	3,315	9.26	**<0.001**
Host plant*Hemocyte density	1	80	80	0.22	0.639
Instar*Hemocyte density	2	3,085	1,542	4.31	**0.018**
Host plant*Instar*Hemocyte	2	842	421	1.18	0.316
Residuals	62	22,202	358	–	–

Bold values indicate significant effects.

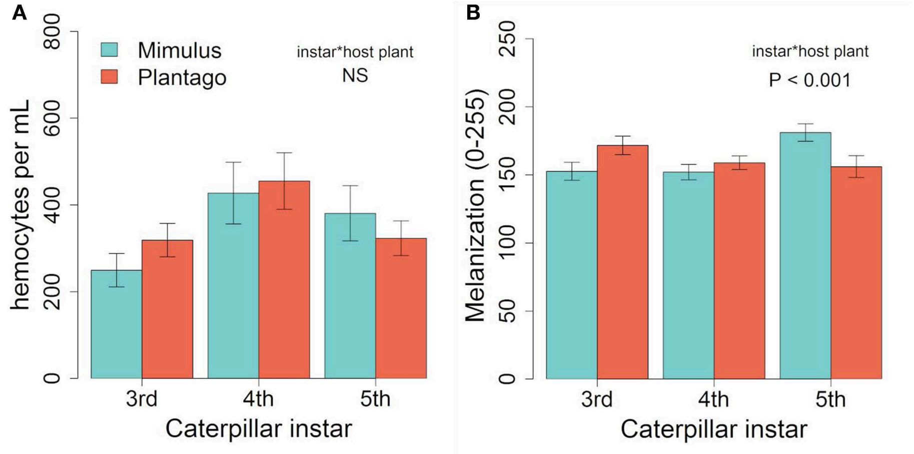


FIGURE 3 | In September, **(A)** we found no significant effects of host plant or instar on hemocyte density. However, similar to January **(B)** melanization varied across instars when reared on *Mimulus* but not when reared on *Plantago*. Bars represent ± 1 SE.

weight than unchallenged caterpillars [$F_{(1,84)} = 14.13$, $P < 0.001$, **Figure 4A**]. Although sequestration varied by instar [$F_{(2,84)} = 22.26$, $P < 0.001$], and there was a significant interaction between instar and immune challenge [$F_{(2,84)} = 9.06$, $P < 0.001$], with fourth instars sequestering over two and half times more IGs than controls, much more than either third or fifth instars

TABLE 2 | In September ANCOVA indicated only an interaction between host plant and caterpillar developmental instar on melanization.

Factor	Df	Sum Sq	Mean Sq	*F*-value	Pr(>*F*)
Host plant	1	21	21	0.04	0.853
Instar	2	2,366	1,183	2.00	0.143
Hemocyte density	1	1,520	1,520	2.57	0.113
Host plant*Instar	2	6,284	3,142	5.31	**0.007**
Host plant*Hemocyte density	1	80	80	0.14	0.714
Instar*Hemocyte density	2	1,015	507	0.86	0.428
Host plant*Instar*Hemocyte	2	893	447	0.76	0.474
Residuals	72	42,587	592	–	–

Bold values indicate significant effects.

(**Figure 4**). The proportion of total IGs sequestered that was catalpol was also 35% lower in immune challenged compared to unchallenged caterpillars [$F_{(1,82)} = 20.28$, $P < 0.001$]. Though the proportion of catalpol varied by instar [$F_{(2,82)} = 14.5$, $P <$ 0.001], and there was a significant interaction between instar and immune challenge [$F_{(2,82)} = 6.29$, $P = 0.003$], with third instars sequestering similar proportions between challenged and control caterpillars (0.37 and 0.38, respectively), and fourth and fifth instars sequestering roughly half the proportion of catalpol when immune challenged (0.15 vs. 0.29 and 0.17 vs. 0.37, respectively, **Figure 4A**).

Experiment 2 (September)

In September, caterpillars sequestered over 3.5 times more IGs than in January (8.89% dry weight on average compared to 2.53% dry weight in January). While IG sequestration was much higher, it was also more variable, and there was no significant difference in total IG sequestration between instars [$F_{(2,38)} =$ 1.12, $P = 0.336$, **Figure 4B**]. Similar to January, ANCOVA revealed little direct effects of either instar, hemocyte density, or IG sequestration on melanization (**Table 4**). However, there was a significant interaction between sequestration, hemocyte density, and instar, suggesting that, although melanization tended to increase with hemocyte density, it declined with sequestration, and the relationship varied across caterpillar development. Similar to results from January, immune challenged caterpillars sequestered 75% more IGs than unchallenged controls [$F_{(1,86)}$ $= 9.02$, $P = 0.004$] but the interaction between instar and immune challenge was not significant [$F_{(1,86)} = 2.63$, $P = 0.110$, **Figure 4B**]. The proportion of catalpol sequestered also varied across instars [$F_{(1,84)} = 25.95$, $P < 0.001$] and treatment [$F_{(1,84)}$ $= 19.41$, $P < 0.001$], although there was a significant interaction between instar and immune challenge [$F_{(1,84)} = 7.10$, $P =$ 0.009], driven mostly by 4th and 5th instars sequestering 43 and 17% higher proportions of catalpol, compared to 3rd instars which sequestered 19% lower proportion catalpol when immune challenged (**Figure 4B**).

Experiment 3 (March)

In this experiment comparing the different immune challenge treatments with a control, sequestered IGs were generally low, although caterpillars sequestered IGs at nearly five times higher concentrations than the *Plantago* on which they were reared (**Figure 5**). We found a significant effect of immune challenge treatment on total IG sequestration [$F_{(3,57)} = 6.43$, $P < 0.001$, **Figure 5**], but not on the proportion of total IGs sequestered that was catalpol [$F_{(3,57)} = 1.03$, $P = 0.387$). *Post-hoc* tests showed that as expected, drawing hemolymph led to a 25% reduction in IG concentrations on average, although this difference was not significant (**Figure 5**). After 24 h IG concentrations were similar to controls, and these three treatments were not significantly different (**Figure 5**). Interestingly, when challenged with hemolymph withdrawal and insertion of a filament, caterpillars sequestered 47% more IGs than controls, which was a significant difference.

DISCUSSION

Overall, we found that caterpillars reared on an introduced host had higher levels of cellular defenses than when reared on a native host, but that those defenses do not appear to translate into increased immune function. Moreover, the relationships between host plant, cellular defenses, and melanization vary through time, and are likely driven in part by sequestration of plan secondary metabolites. Still, these results suggest that introduced host plants can impact native herbivores through stage-specific immunological responses, and that variation in introduced host plant chemistry can mediate the relationships between sequestration and immune function. Interestingly, these results also indicated that immune challenge can change caterpillar sequestration abilities, suggesting that chemically-mediated defense against natural enemies could play some role in the potential switch to introduced hosts. That the patterns detected varied across the course of the study and corresponded to differences in host plant chemistry suggest that the ecological conditions that herbivores and their host plants experience likely have complex consequences for sequestration and defense.

In general, while stage-specific (e.g., Meylaers et al., 2007; Stoepler et al., 2013) and within stadium (e.g., Hoover et al., 2002; McNeil et al., 2010) immunity in insects has been observed, intra-annual variation and across different instars is less well-understood. The results from immune assays in Experiment 1 (January) suggested that immunity, as measured by hemocyte density, varies across instars, increasing with caterpillar development. In contrast, hemocyte density was 23% higher on average and much more variable in

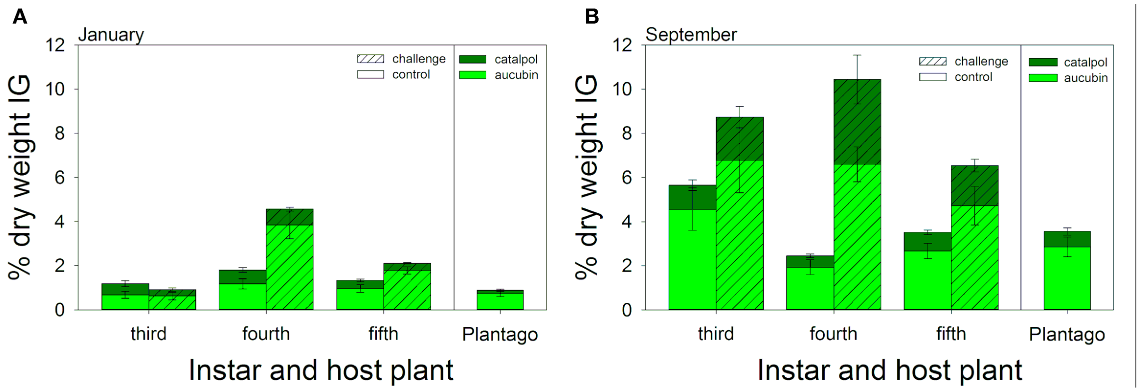


FIGURE 4 | Composite figures illustrate multiple significant interactions between caterpillar instar and immune challenge on both total iridoid glycoside sequestrations (bar height) and the proportion of sequestered IGs that was catalpol (dark green). Results from the January **(A)** and September **(B)** experiments are included: right panels illustrate chemistry of sampled *Plantago* used for feeding during each experimental period. Bars represent ± 1 SE. See Results for statistical tests.

TABLE 3 | For caterpillars reared on *Plantago* in January, ANCOVA showed an interaction between percent dry weight total IGs sequestered and hemocyte density on melanization.

Factor	Df	Sum Sq	Mean Sq	*F* value	Pr(>*F*)
% IGs	1	119	119	0.47	0.499
Instar	2	347	173	0.69	0.514
Hemocyte density	1	1	1	0.004	0.951
% IGs*Instar	2	826	412	1.64	0.218
% IGs*Hemocyte density	1	1,585	1,585	6.28	**0.020**
Instar*Hemocyte density	2	1,601	800	3.17	0.062
% IGs*Instar*Hemocyte density	2	922	460	1.83	0.185
Residuals	22	5,555	252		

Bold values indicate significant effects.

Experiment 2 (September), resulting in no significant differences between instars. This could be due in part to differences in caterpillar response to host plants between time periods: *J. coenia* larvae feeding on *Plantago* in January had higher hemocyte density than those feeding on *Mimulus*; although, in September, there were no differences. Host plant quality may vary across a growing season (Boege and Marquis, 2005; Hanley et al., 2007; Barton and Koricheva, 2010; Quintero et al., 2014) and differences between alternative hosts could be magnified by seasonal variation in host plant quality or chemistry. Alternatively, hemocyte density may not be the best metric of immunity. For example, Bauer et al. (1998) found no differences in hemocyte density across *Pieris brassicae* development, although total hemocytes increased with instar, in line with dramatic increases in hemolymph volume as caterpillars grew (Bauer et al., 1998). Still, the fact that encapsulation similarly changed in magnitude between time periods, with encapsulation being 13% higher in September, suggests that hemocyte density was an underlying mechanism driving immune function.

Moreover, results from our encapsulation assays suggest that immune function, as measured through melanization, varied across caterpillar instars depending on host plant. In both Experiment 1 and 2, melanization increased across larval instars when reared on *Mimulus*, but not when reared on *Plantago*. The results were that third and fourth instar caterpillars feeding on *Plantago* had higher immune function than those feeding on *Mimulus*, while fifth instars fed Mimulus had the highest immune function and more so than fifth instars reared on *Plantago*. This suggests that tradeoffs in immune function across caterpillar development are likely host plant specific, although we detected no three-way interaction between host plant, instar, and hemocyte density in January or September. It should be noted that melanization is the result of both cellular and humoral responses of the insect immune system (Strand, 2008), although we did not measure humoral responses. However, changes in immune function over caterpillar development can be driven by hemocyte differentiation within the immune system occurring later in development (Brodeur and Vet, 1995; Gillespie et al., 1997), potentially an evolutionary response to pressure from parasitoids targeting larger caterpillars (Memmott et al., 2000). We found a significant interaction between instar and hemocyte density in January, but melanization actually decreased with hemocyte density in fifth instars, while increasing with hemocyte density in third and fourth instars. Hemocyte titers have been shown to decline during rapid growth of final caterpillar instars (Beetz et al., 2008). That fifth instars in Experiment 2 (September) had lower hemocyte densities, could suggest that fifth instars

TABLE 4 | For caterpillars reared on *Plantago* in September, ANCOVA revealed a three-way interaction between percent dry weight total IGs sequestered, hemocyte density, and caterpillar instar on melanization.

Factor	Df	Sum Sq	Mean Sq	*F* value	Pr(>*F*)
% IGs	1	716	716	1.28	0.267
Instar	2	1,583	792	1.41	0.259
Hemocyte density	1	542	542	0.97	0.333
% IGs*Instar	2	631	316	0.56	0.576
% IGs*Hemocyte density	1	1,402	1,402	2.50	0.124
Instar*Hemocyte density	2	429	215	0.38	0.685
% IGs*Instar*hemocyte density	2	3,926	1,963	3.50	**0.043**
Residuals	30	16,819	561		

Bold values indicate significant effects.

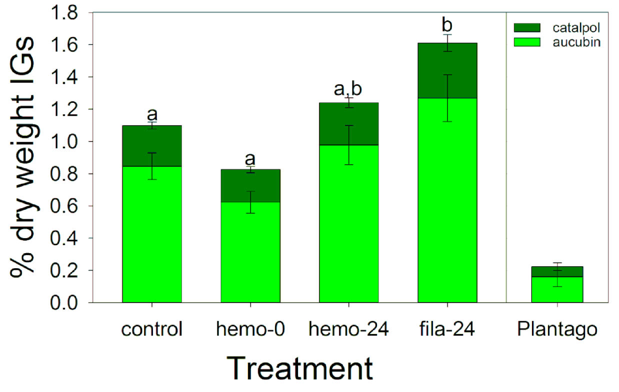


FIGURE 5 | Iridoid glycoside sequestration during the March experiment testing the effects of immune challenge showed no significant difference in the proportion catalpol sequestered. However, total IG sequestration was similar between controls and caterpillars where hemolymph was sampled, but increase when immune challenged with a nylon filament. Letters are significant groups from a Tukey *post-hoc* test of % dry weight IGs. Bars represent ± 1 SE.

exhibit compensatory growth to immune challenge instead of investing in immune defense. Given that the development of immune function in our study varied as a result of host plant, one impact of introduced hosts could therefore be through altering stage-specific susceptibility to natural enemies.

Plant chemistry is a fundamental component of host quality for sequestering herbivores and can impact immunity, posing potential tradeoffs in defense against natural enemies (Gentry and Dyer, 2002). In buckeyes for example, Smilanich et al. (2009a) demonstrated that larvae fed on a diet with higher levels of iridoid glycosides are more poorly defended against parasitoids while being better protected against predators. The results from our study showed that IG sequestration varies across instars and time periods, and this can have implications for immune function, although relationships may be complex and vary through time. In general, IG sequestration was low in January, just 2.5% dry weight on average, compared to 8.9% dry weight on average in September. This was driven by extremely low IG concentrations of *Plantago* in January compared to September (0.89 vs. 4.8% dry weight IG, respectively), and lower than previous studies (Bowers and Stamp, 1992). By comparison, Lampert and Bowers (2010) found that buckeye larvae in the fourth instar sequestered 5 to 15% dry weight IGs, and larvae can sequester up to up to 25% of their dry weight (Theodoratus and Bowers, 1999). Regardless, in both January and September, fourth instar immune challenged caterpillars had the highest concentrations of IGs relative to third and fifth instars, which could suggest that the costs of sequestering IGs vary across caterpillar development. Moreover, the relationships between sequestration and immune function varied across time periods. We found little evidence of IGs directly impacting immune function in either time periods. However, when IGs were higher in September, we found a significant three-way interaction between sequestration, caterpillar instar, and hemocyte density on melanization. This interaction suggested that the effect of hemocyte density on melanization could be impacted by high IG sequestration and that any costs of sequestration on immune function likely vary across instars. This could be illustrative of stage-specific tradeoffs in sequestration and immune function.

Interestingly, we also found that immune challenged caterpillars from both Experiments 1 and 2 actually sequestered higher IG concentrations relative to unchallenged controls, and in all instars except third instars in Experiment 2 (**Figure 4**). Furthermore, it appears that this is not due to the methods we used, given the results of Experiment 3, in which we compared several different immune challenge treatments (**Figure 5**). The results of that experiment showed a short-term reduction in IGs due to hemolymph loss (although the difference was not significant), followed by a return to control levels after 24 h (during which time caterpillars were feeding). Most importantly, our results showed that when hemolymph was removed and a filament inserted (immune challenged), after 24 h caterpillars had sequestered significantly higher concentrations of IGs. The mechanism driving this is unclear; it could be due to compensatory feeding or an increase in active transport of IGs from the gut to the hemolymph. Alternatively, it may not be an adaptive response, but due to underlying regulatory pathways. For example, in pollen beetles injected with bacteria and yeast, there were concurrent upregulation of stress and detoxification-related genes along with known immune-related genes (Vogel et al., 2014). Still, that fourth instars exhibited higher responses than third and fifth, and in both the January and September experiments, suggests that potential costs associated with sequestration vary across caterpillar development. Further experiments to tease apart mechanisms driving such stage-specific tradeoffs in sequestration and defense could help improve our understanding of herbivore response to introduced hosts.

Without doubt, exotic plant species can have large impacts on ecological communities across the globe (Daehler, 1998; Mack et al., 2000), including through altering herbivore-host plant interactions (Bowers et al., 1992b), impacting the physiology, growth, and fitness of herbivores (Dyer, 1995;

Knerl and Bowers, 2013), and with particularly large effects on larval stages with limited mobility (Graves and Shapiro, 2003). These in turn, can also affect tritrophic interactions, depending on how natural enemies respond to host plant mediated chemical defenses (Knerl and Bowers, 2013), but also potentially on how tradeoffs associated with chemical defenses vary across herbivore development. The results of this study suggest that the impacts of novel host chemistry across larval development are likely complex and dependent on variation in host plant chemistry, and on stage-specific relationships between sequestration and defense. Future studies manipulating host plant chemistry could help disentangle the mechanisms driving tradeoffs in defense strategies across caterpillar development and how introduced hosts impact tritrophic interactions.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

The initial research was conceptualized by ME and MB. ME and AC conducted experiments. AC conducted statistical tests and graphical representation of results. All authors contributed to manuscript preparation.

FUNDING

This research was supported by grants from the University of Colorado Undergraduate Research Opportunity Program, the Marion and Gordon Alexander Fellowship, and the National Science Foundation (award #IOS 1456338).

ACKNOWLEDGMENTS

We thank N. Pepen and F. Hasandokht for assisting with experiments, A. Smilanich and N. Muchoney for advice on immunological assays, and the Bowers lab for comments and suggestions on the manuscript.

REFERENCES

Barbosa, P., and Caldas, A. (2007). Do larvae of species in macrolepidopteran assemblages share traits that influence susceptibility to parasitism? *Environ. Entomol.* 36, 329–336. doi: 10.1093/ee/36.2.329

Barton, K. E., and Koricheva, J. (2010). The ontogeny of plant defense and herbivory: characterizing general patterns using meta-analysis. *Am. Natural.* 175, 481–493. doi: 10.1086/650722

Bauer, E., Trenczek, T., and Dorn, S. (1998). Instar-dependent hemocyte changes in *Pieris brassicae* after parasitization by *Cotesia glomerata*. *Entomol. Exp. Et Appl.* 88, 49–58. doi: 10.1046/j.1570-7458.1998.00345.x

Beckage, N. E. (2008). *Epilogue: Pathways into the Future of Insect Immunology in Insect Immunity.331-336.* San Diego, CA: Academic Press.

Beetz, S., Holthusen, T. K., Koolman, J., and Trenczek, T. (2008). Correlation of hemocyte counts with different developmental parameters during the last larval instar of the tobacco hornworm, *Manduca sexta*. *Arch. Insect Biochem. Physiol.* 67, 63–75. doi: 10.1002/arch.20221

Boege, K., and Marquis, R. J. (2005). Facing herbivory as you grow up: the ontogeny of resistance in plants. *Trends Ecol. Evol.* 20, 441–448. doi: 10.1016/j.tree.2005.05.001

Bowers, M. D. (1984). Iridoid glycosides and host-plant specificity in larvae of the buckeye butterfly, *Junonia coenia* (Nymphalidae). *J. Chem. Ecol.* 10, 1567–1577. doi: 10.1007/BF00988425

Bowers, M. D. (1990). "Recycling plant natural products for insect defense," in *Insect Defenses: Adaptive Mechanisms and Strategies of Prey and Predators,* eds D. L. Evans and J. O. Schmidt (Albany, NY: SUNY Press), 353–386.

Bowers, M. D. (1992). "The evolution of unpalatability and the cost of chemical defense in insects," in *Insect Chemical Ecology: an Evolutionary Approach,* eds B. D Roitberg and M. B. Isman (New York, NY: Chapman and Hall), 216–244.

Bowers, M. D. (2003). "Coevolution of insects and plants," in *Encyclopedia of Plant and Crop Science,* eds M. Kogan and S. Rao (New York, NY: Marcel Dekker Inc), 289–291. doi: 10.1081/E-EPCS-120010481

Bowers, M. D., and Collinge, S. K. (1992). Fate of iridoid glycosides in different life stages of the buckeye, *Junonia coenia* (Lepidoptera, Nymphalidae). *J. Chem. Ecol.* 18, 817–831. doi: 10.1007/BF00988322

Bowers, M. D., Collinge, S. K., Gamble, S. E., and Schmitt, J. (1992a). Effects of genotype, habitat, and seasonal-variation on iridoid glycoside content of *Plantago lanceolata* (Plantaginaceae) and the implications for insect herbivores. *Oecologia* 91, 201–207. doi: 10.1007/BF00317784

Bowers, M. D., and Puttick, G. M. (1986). Fate of ingested iridoid glycosides in lepidopteran herbivores. *J/ Chem. Ecol.* 12, 169–178. doi: 10.1007/BF01045600

Bowers, M. D., and Puttick, G. M. (1988). Response of generalist and specialist insects to qualitative allelochemical variation. *J. Chem. Ecol.* 14, 319–334. doi: 10.1007/BF01022549

Bowers, M. D., and Stamp, N. E. (1992). Chemical variation within and between individuals of *Plantago lanceolata* (Plantaginaceae). *J. Chem. Ecol.* 18, 985–995. doi: 10.1007/BF00980057

Bowers, M. D., and Stamp, N. E. (1997). Fate of host-plant iridoid glycosides in lepidopteran larvae of Nymphalidae and Arctiidae. *J. Chem. Ecol.* 23, 2955–2965. doi: 10.1023/A:1022535631980

Bowers, M. D., Stamp, N. E., and Collinge, S. K. (1992b). Early stage of host range expansion by a specialist herbivore, *Euphydryas phaeton* (Nymphalidae). *Ecology* 73, 526–536. doi: 10.2307/1940758

Brodeur, J., and Vet, L. E. M. (1995). Relationships between parasitoid host-range and host-defense: a comparative-study of egg encapsulation in 2 related parasitoid species. *Physiol. Entomol.* 20, 7–12. doi: 10.1111/j.1365-3032.1995.tb00794.x

Brower, L. P., Ryerson, W. N., Coppinger, L., and Glazier, S. C. (1968). Ecological chemistry and palatability spectrum. *Science* 161, 1349–1350. doi: 10.1126/science.161.3848.1349

Cavers, P. B., Bassett, I. J., and Crompton, C. W. (1980). The biology of canadian weeds.47. *Plantago lanceolata L. Can. J. Plant Sci.* 60, 1269–1282. doi: 10.4141/cjps80-180

Daehler, C. C. (1998). The taxonomic distribution of invasive angiosperm plants: ecological insights and comparison to agricultural weeds. *Biol. Conserv.* 84, 167–180. doi: 10.1016/S0006-3207(97)00096-7

Decker, L. E., de Roode, J. C., and Hunter, M. D. (2018). Elevated atmospheric concentrations of carbon dioxide reduce monarch tolerance and increase parasite virulence by altering the medicinal properties of milkweeds. *Ecol. Lett.* 21, 1353–1363. doi: 10.1111/ele.13101

Dobler, S., Petschenka, G., and Pankoke, H. (2011). Coping with toxic plant compounds: the insect's perspective on iridoid glycosides and cardenolides. *Phytochemistry* 72, 1593–1604. doi: 10.1016/j.phytochem.2011.04.015

Duff, R. B., Bacon, J. S. D., Mundie, C. M., Farmer, V. C., Russell, J. D., and Forreste, Ar. (1965). Catalpol and methylcatalpol: naturally occurring glycosides in *Plantago* and *Buddleia* species. *Biochem. J.* 96, 1–5. doi: 10.1042/bj0960001

Dyer, L. A. (1995). Tasty generalists and nasty specialists? antipredator mechanisms in tropical lepidopteran larvae. *Ecology* 76, 1483–1496. doi: 10.2307/1938150

Gentry, G. L., and Dyer, L. A. (2002). On the conditional, nature of neotropical caterpillar defenses against their natural enemies. *Ecology* 83, 3108–3119. doi: 10.1890/0012-9658(2002)083[3108:OTCNON]2.0.CO;2

Gherlenda, A. N., Haigh, A. M., Moore, B. D., Johnson, S. N., and Riegler, M. (2016). Climate change, nutrition and immunity: effects of elevated CO2 and temperature on the immune function of an insect herbivore. *J. Insect Physiol.* 85, 57–64. doi: 10.1016/j.jinsphys.2015.12.002

Gillespie, J. P., Kanost, M. R., and Trenczek, T. (1997). Biological mediators of insect immunity. *Annual Rev. Entomol.* 42, 611–643. doi: 10.1146/annurev.ento.42.1.611

Graves, S. D., and Shapiro, A. M. (2003). Exotics as host plants of the California butterfly fauna. *Biol. Conserv.* 110, 413–433. doi: 10.1016/S0006-3207(02)00233-1

Hanley, M. E., Lamont, B. B., Fairbanks, M. M., and Rafferty, C. M. (2007). Plant structural traits and their role in anti-herbivore defence. *Perspect. Plant Ecol. Evol. Syst.* 8, 157–178. doi: 10.1016/j.ppees.2007.01.001

Hartmann, T. (2004). Plant-derived secondary metabolites as defensive chemicals in herbivorous insects: a case study in chemical ecology. *Planta* 219, 1–4. doi: 10.1007/s00425-004-1249-y

Holeski, L. M., Chase-Alone, R., and Kelly, J. K. (2010). The genetics of phenotypic plasticity in plant defense: trichome production in *Mimulus guttatus*. *Am. Natural.* 175, 391–400. doi: 10.1086/651300

Holeski, L. M., Keefover-Ring, K., Bowers, M. D., Harnenz, Z. T., and Lindroth, R. L. (2013). Patterns of phytochemical variation in *Mimulus guttatus* (yellow monkeyflower). *J. Chem. Ecol.* 39, 525–536. doi: 10.1007/s10886-013-0270-7

Hoover, K., Grove, M. J., and Su, S. (2002). Systemic component to intrastadial developmental resistance in *Lymantria dispar* to its baculovirus. *Biol. Control* 25, 92–98. doi: 10.1016/S1049-9644(02)00041-5

Jahner, J. P., Bonilla, M. M., Badik, K. J., Shapiro, A. M., and Forister, M. L. (2011). Use of exotic hosts by lepidoptera: widespread species colonize more novel hosts. *Evolution* 65, 2719–2724. doi: 10.1111/j.1558-5646.2011.01310.x

Jamieson, M. A., Schwartzberg, E. G., Raffa, K. F., Reich, P. B., and Lindroth, R. L. (2015). Experimental climate warming alters aspen and birch phytochemistry and performance traits for an outbreak insect herbivore. *Glob Change Biol.* 21, 2698–2710. doi: 10.1111/gcb.12842

Jeffs, C. T., and Lewis, O. T. (2013). Effects of climate warming on hostparasitoid interactions. *Ecol. Entomol.* 38, 209–218. doi: 10.1111/een.12026

Knerl, A., and Bowers, M. D. (2013). Incorporation of an introduced weed into the diet of a native butterfly: consequences for preference, performance and chemical defense. *J. Chem. Ecol.* 39, 1313–1321. doi: 10.1007/s10886-013-0355-3

Kooiman, P. (1970). Occurrence of iridoid glycosides in Scrophulariaceae. *Acta Botan. Neerland.* 19, 329–330. doi: 10.1111/j.1438-8677.1970.tb00655.x

Lampert, E. C., and Bowers, M. D. (2010). Host plant species affects the quality of the generalist *Trichoplusia ni* as a host for the polyembryonic parasitoid *Copidosoma floridanum*. *Entomol. Exp. Appl.* 134, 287–295. doi: 10.1111/j.1570-7458.2009.00956.x

Lavine, M. D., and Strand, M. R. (2002). Insect hemocytes and their role in immunity. *Insect Biochem. Molecul. Biol.* 32, 1295–1309. doi: 10.1016/S0965-1748(02)00092-9

Mack, R. N., Simberloff, D., Lonsdale, W. M., Evans, H., Clout, M., and Bazzaz, F. A. (2000). Biotic invasions: causes, epidemiology, global consequences, and control. *Ecol. Appl.* 10, 689–710. doi: 10.1890/1051-0761(2000)010[0689:BICEGC]2.0.CO;2

McNeil, J., Cox-Foster, D., Slavicek, J., and Hoover, K. (2010). Contributions of immune responses to developmental resistance in *Lymantria dispar* challenged with baculovirus. *J. Insect Physiol.* 56, 1167–1177. doi: 10.1016/j.jinsphys.2010.03.020

Memmott, J., Martinez, N. D., and Cohen, J. E. (2000). Predators, parasitoids and pathogens: species richness, trophic generality and body sizes in a natural food web. *J. Anim. Ecol.* 69, 1–15. doi: 10.1046/j.1365-2656.2000.00367.x

Meylaers, K., Freitak, D., and Schoofs, L. (2007). Immunocompetence of *Galleria mellonel*la: sex- and stage-specific differences and the physiological during cost of mounting an immune response metamorphosis. *J. Insect Physiol.* 53, 146–156. doi: 10.1016/j.jinsphys.2006.11.003

Nappi, A. J., and Christensen, B. M. (2005). Melanogenesis and associated cytotoxic reactions: applications to insect innate immunity. *Insect Biochem. Molecul. Biol.* 35, 443–459. doi: 10.1016/j.ibmb.2005.01.014

Pech, L. L., and Strand, M. R. (1996). Granular cells are required for encapsulation of foreign targets by insect haemocytes. *J. Cell Sci.* 109, 2053–2060.

Pereyra, P. C., and Bowers, M. D. (1988). Iridoid glycosides as oviposition stimulants for the buckeye butterfly, *Junonia coenia* (Nymphalidae). *J. Chem. Ecol.* 14, 917–928. doi: 10.1007/BF01018783

Poreddy, S., Mitra, S., Schöttner, M., Chandran, J., Schneider, B., Baldwin, I. T., et al. (2015). Detoxification of hostplant's chemical defence rather than its anti-predator co-option drives β-glucosidase-mediated lepidopteran counteradaptation. *Nat. Commun.* 6:8525. doi: 10.1038/ncomms9525

Quintero, C., Lampert, E. C., and Bowers, M. D. (2014). Time is of the essence: direct and indirect effects of plant ontogenetic trajectories on higher trophic levels. *Ecology* 95, 2589–2602. doi: 10.1890/13-2249.1

R. Core Team (2017). *R: A Language and Environment For Statistical Computing*. R Foundation for Statistical Computing. Vienna, Austria

Rosenheim, J. A. (1998). Higher-order predators and the regulation of insect herbivore populations. *Ann. Rev. Entomol.* 43, 421–447. doi: 10.1146/annurev.ento.43.1.421

Smilanich, A. M., Dyer, L. A., Chambers, J. Q., and Bowers, M. D. (2009a). Immunological cost of chemical defence and the evolution of herbivore diet breadth. *Ecol. Lett.* 12, 612–621. doi: 10.1111/j.1461-0248.2009.01309.x

Smilanich, A. M., Dyer, L. A., and Gentry, G. L. (2009b). The insect immune response and other putative defenses as effective predictors of parasitism. *Ecology* 90, 1434–1440. doi: 10.1890/08-1906.1

Smilanich, A. M., Langus, T. C., Doan, L., Dyer, L. A., Harrison, J. G., Hsueh, J., et al. (2018). Host plant associated enhancement of immunity and survival in virus infected caterpillars. *J. Invert. Pathol.* 151, 102–112. doi: 10.1016/j.jip.2017.11.006

Stamp, N. E. (1986). Physical constraints of defense and response to invertebrate predators by pipevine caterpillars *Battus philenor* Papilionidae. *J. Lepidopterists' Soc.* 40, 191–205.

Stanley, D., Haas, E., and Miller, J. (2012). Eicosanoids: exploiting insect immunity to improve biological control programs. *Insects* 3, 492–510. doi: 10.3390/insects3020492

Stoepler, T. M., Castillo, J. C., Lill, J. T., and Eleftherianos, I. (2013). Hemocyte density increases with developmental stage in an immune-challenged forest caterpillar. *PLoS ONE* 8:e0157882. doi: 10.1371/journal.pone.0070978

Strand, M. R. (2008). The insect cellular immune response. *Insect Sci.* 15, 1–14. doi: 10.1111/j.1744-7917.2008.00183.x

Strand, M. R., Beck, M. H., Lavine, M. D., and Clark, K. D. (2006). *Microplitis demolitor* bracovirus inhibits phagocytosis by hemocytes from *Pseudoplusia includens*. *Arch. Insect Biochem. Physiol.* 61, 134–145. doi: 10.1002/arch.20107

Theodoratus, D. H., and Bowers, M. D. (1999). Effects of sequestered iridoid glycosides on prey choice of the prairie wolf spider, *Lycosa carolinensis*. *J. Chem. Ecol.* 25, 283–295. doi: 10.1023/A:1020894729188

Triggs, A., and Knell, R. J. (2012). Interactions between environmental variables determine immunity in the Indian meal moth *Plodia interpunctella*. *J. Anim. Ecol.* 81, 386–394. doi: 10.1111/j.1365-2656.2011.01920.x

Vidal, M. C., and Murphy, S. M. (2018). Bottom-up vs. top-down effects on terrestrial insect herbivores: a meta-analysis. *Ecol. Lett.* 21, 138–150. doi: 10.1111/ele.12874

Vogel, H., Badapanda, C., Knorr, E., and Vilcinskas, A. (2014). RNA-sequencing analysis reveals abundant developmental stage-specific and immunity-related genes in the pollen beetle *Meligethes aeneus*. *Insect Molecul Biol.* 23, 98–112. doi: 10.1111/imb.12067

Wago, H., and Ichikawa, Y. (1979). Changes in the phagocytic rate during the larval development and manner of hemocytic reactions to foreign cells in *Bombyx mori*. *Appl. Entomol. Zool.* 14, 397–403. doi: 10.1303/aez.14.397

Zvereva, E. L., and Kozlov, M. V. (2016). The costs and effectiveness of chemical defenses in herbivorous insects: a meta-analysis. *Ecol. Monogr.* 86, 107–124. doi: 10.1890/15-0911.1

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 02 July 2019
doi: 10.3389/fevo.2019.00252

Wildfires Influence Abundance, Diversity, and Intraspecific and Interspecific Trait Variation of Native Bees and Flowering Plants Across Burned and Unburned Landscapes

Laura A. Burkle[1]*, *Michael P. Simanonok*[1], *J. Simone Durney*[1], *Jonathan A. Myers*[2] and *R. Travis Belote*[3]

[1] Department of Ecology, Montana State University, Bozeman, MT, United States, [2] Department of Biology, Washington University in St. Louis, St. Louis, MO, United States, [3] The Wilderness Society, Bozeman, MT, United States

Edited by:
Gina Marie Wimp,
Georgetown University, United States

Reviewed by:
Bertrand Schatz,
Centre National de la Recherche Scientifique (CNRS), France
Diane Lynn Larson,
United States Geological Survey, United States

***Correspondence:**
Laura A. Burkle
laura.burkle@montana.edu

Specialty section:
This article was submitted to Population and Evolutionary Dynamics, a section of the journal Frontiers in Ecology and Evolution

Received: *19 February 2019*
Accepted: *17 June 2019*
Published: *02 July 2019*

Citation:
Burkle LA, Simanonok MP, Durney JS, Myers JA and Belote RT (2019) Wildfires Influence Abundance, Diversity, and Intraspecific and Interspecific Trait Variation of Native Bees and Flowering Plants Across Burned and Unburned Landscapes. Front. Ecol. Evol. 7:252. doi: 10.3389/fevo.2019.00252

Wildfire regimes are changing in the western United States, yet the ways in which wildfires influence native bees, the resources they depend on for food and nesting, or the traits that influence their interactions with plants are poorly understood. In burned and unburned areas in Montana, USA, we investigated the abundance and diversity of native bees, floral and nesting resources, nesting success, and traits of flowers and bees. In two of the three localities studied, burned areas, including areas that burned with high-severity wildfires, supported higher density and diversity of native bees and the flowers they depend on for food and larval provisioning. Burned areas also had more bare ground for ground-nesting bees and more available coarse woody debris for cavity-nesting bees than unburned areas. Moreover, cavity-nesting bees were completely unsuccessful at nesting in artificial nesting boxes in unburned areas, while nesting success was 40% in burned areas. Mean bee intertegular distance (a trait strongly correlated with tongue length, foraging distance, and body size) was similar between burned and unburned areas. However, wildfires influenced both interspecific and intraspecific trait variation of bees and plants. Intraspecific variation in bee intertegular distance was higher in unburned than burned areas. Both interspecific and intraspecific variation in floral traits important for interactions with pollinators were generally higher in burned than unburned areas. Thus, wildfires generally increased the density and species diversity of bees and flowers as well as trait variation at both trophic levels. We conclude that wildfires—even large, high-severity wildfires—create conditions that support native bees and the resources they need to flourish, but that unburned areas maintain trait variation in landscape mosaics with heterogeneous fire conditions.

Keywords: community assembly, fire management, functional trait diversity, Northern Rockies Ecoregion, pollination, pyrodiversity, wild bees, wildfire disturbance regime

INTRODUCTION

Among the many threats posed to biodiversity by global environmental change, changes to natural disturbance regimes are likely to have some of the most profound impacts on animals and plants and the ecosystem services they provide (Hessburg et al., 2015; Johnstone et al., 2016). Wildfires have played a critical role in maintaining the structure and function of many ecosystems worldwide (Bond and Keeley, 2005; Bowman et al., 2009). However, humans have altered natural wildfire regimes through fire suppression and climate change (Westerling et al., 2006). Despite the importance of wildfires in natural ecosystems, neither ecologists nor land managers fully understand how or why wildfire affects biodiversity and ecosystem services across complex landscapes that vary in environmental conditions (Burkle et al., 2015). This lack of understanding is particularly true for native bees, which provide pollination services essential to the recovery of plant communities in post-fire landscapes (e.g., Potts et al., 2006; Van Nuland et al., 2013; Heil and Burkle, 2019).

Several key gaps limit our understanding of how wildfires influence native bees, the resources they depend on for food and nesting, and the traits that influence their interactions with plants. First, previous studies of responses of native bees to wildfire focused primarily on floral resources, whereas the effects of wildfire on bee nesting habitat and offspring success remain poorly understood (reviewed in Koltz et al., 2018). Fire can directly cause bee mortality (Love and Cane, 2016), but it has been suggested that individuals of many ground-nesting bee species may survive fires (Cane and Neff, 2011). Wildfires can open forest canopies and increase space and resources for understory flowering plants, and native bees are attracted to burned areas with abundant floral resources (e.g., Van Nuland et al., 2013). Such increases in bee and floral abundance and diversity tend to peak soon (1–5 years) after fire (Potts et al., 2003a). As forest succession proceeds, floral resources often decline, with concurrent declines in pollinator abundance (especially diet-generalist bees; Peralta et al., 2017), resulting in successional changes for both trophic levels (e.g., Potts et al., 2003a,b, 2006; Pauw, 2007; Moretti et al., 2009; Lazarina et al., 2016; Heil and Burkle, 2018). Fire and post-fire succession can also affect community composition, both taxonomically and functionally (e.g., Moretti et al., 2009; Lazarina et al., 2016, 2017; Simanonok, 2018). For bees, the quality and proximity of nesting habitat, including bare ground, stems, standing dead wood and nesting cavities, can be enhanced or reduced by fire, likely influencing bee community composition (e.g., Potts et al., 2005; Moretti et al., 2009; Lazarina et al., 2016; Simanonok, 2018). The responses of different functional groups of pollinators to fire vary in magnitude and direction, and there is high spatial variation in the effects of fire on pollinator diversity (e.g., Moretti et al., 2004; Grundel et al., 2010; Lazarina et al., 2016, 2017). Fire can subsequently affect the interactions between bees and flowering plants (Peralta et al., 2017).

Second, studies of bee and plant responses to wildfire often focus on changes in species diversity. However, changes in other key components of biodiversity—such as variation in functional traits within and among bee and plant species—can provide important insights into mechanisms of community assembly, the maintenance of biodiversity, and effects of environmental change on ecosystem functioning and services (Weiher and Keddy, 1995; McGill et al., 2006; Funk et al., 2008). Interspecific and intraspecific variation in functional traits may be especially useful for understanding the assembly, diversity, and dynamics of local communities or regions that contain individuals or species with functionally-redundant traits (Fukami et al., 2005; Spasojevic et al., 2016, 2018). For example, when fire disturbance selects for (or filters out) individuals with traits that confer tolerance to fire or increase fitness in post-fire environments, fire may cause burned communities to converge in trait values (environmental filtering), even when local species diversity remains relatively unchanged. This can result in a shift in mean trait values, lower trait variation, or both in burned relative to unburned communities (Pausas and Verdú, 2008; Cavender-Bares and Reich, 2012). Alternatively, fire may cause burned communities to diverge in trait values when it increases heterogeneity in environmental conditions or species sorting among communities (Myers et al., 2015), resulting in higher trait variation in burned relative to unburned communities. Despite the importance of traits for species interactions, the degree to which wildfire decreases or increases intraspecific vs. interspecific trait variation in two interacting trophic levels (i.e., bees and flowering plants) is poorly understood.

Third, the wildfire history and current wildfire regimes of a region also likely influence floral and nesting resources. Most studies of bee responses to fire have been conducted in fire-prone Mediterranean ecosystems or ecosystems with a long history of human-induced fire disturbance. In other regions, such as western North America, forests with a build-up of fuel have been retained by fire suppression, but the occurrence of large-scale wildfires is now increasing (Hessburg and Agee, 2003; Westerling et al., 2006). These conditions result in forests with large patches of multi-storied contiguous canopy cover proximal to large areas burned by wildfires (Hessburg et al., 2015), in a region where the native bee fauna is poorly known (Reese et al., 2018). Given that bees are central place foragers with limited foraging distances from their nests (Westrich, 1996; Gathmann and Tscharntke, 2002), these large-scale wildfires may affect the quality and proximity of nesting and foraging resources in ways that differ from better-studied systems. Thus, there is a critical need to assess the effects of wildfire on native bees in this region and to begin to explore the mechanisms by which wildfire may act on bees (i.e., via effects on floral or nesting resources).

In this study, we examined how wildfire influenced the density and diversity of native bees, floral and nesting resources, nesting success, and traits of bees and flowering plants among unburned areas and areas burned by wildfires in three localities in western North America (Montana, USA). We expected to observe greater density, richness, and nesting success of bees in burned areas, reflecting—in part—greater density and richness of floral resources in post-wildfire areas (Burkle et al., 2015). Our predictions about nesting resources were more complex. We expected nesting resources (the amount of bare ground and coarse woody debris) to reflect the interplay between (a) the power of wildfire to clear understory vegetation and burn built-up fuel (i.e., more bare ground and less coarse woody debris) and (b) the rate and extent of regrowth of understory plants after the canopy is cleared as well as the availability of dead wood in post-fire environments (i.e., less bare ground and more coarse woody debris).

In all three of our study localities, burned areas included sites burned with a mixture of low, medium, and high wildfire severity. Given this heterogeneity in local fire conditions, we expected i) mean trait values of bees and flowering plants to differ between burned and unburned areas, and (ii) higher interspecific and intraspecific trait variation in burned than unburned areas. For bees, we predicted higher mean values of intertegular distance (the width between the wing bases on the dorsal side of the thorax)—a trait strongly correlated with tongue length, foraging distance, and body size (e.g., Cariveau et al., 2016)—in burned than unburned areas, potentially reflecting the ability of larger bees to disperse farther (Warzecha et al., 2016) after large-scale wildfires. For flowering plants, we predicted higher mean values of specific leaf area in unburned areas that have more canopy cover and lower understory light levels (sensu Evans and Poorter, 2001) and where shade-tolerant species may persist (i.e., carbon-gain hypothesis; Valladares and Niinemets, 2008). For other plant traits (plant height, flower size, and floral display), we expected differences between burned and unburned areas to likely reflect the interplay between increased sunlight and decreased soil moisture in burned areas and subsequent shifts in plant resource allocation to growth and reproduction. Finally, we explored the degree to which our predictions regarding wildfire were supported across all three study localities, which vary in other key characteristics including climate and rates of forest succession following wildfire (Burkle et al., 2015).

METHODS

Study System and Sampling Design

We selected three study localities with similar recent histories of wildfire that span a regional gradient of climate, productivity, and rate of succession: Helena (low productivity and slow succession), Paradise (moderate productivity and succession), and Whitefish (high productivity and fast succession) (Burkle et al., 2015) (**Figure S1**). These localities also represent different forest types: ponderosa-pine forests dominate in Helena, lodgepole-pine and Douglas-fir in Paradise, and western-larch, lodgepole-pine and mixed-conifer in Whitefish. Within each locality, we identified a large wildfire perimeter within which a fire occurred between 2001 and 2007 (**Table S1**). Within each wildfire perimeter, we used Monitoring Trends in Burn Severity (mtbs.gov) data to select two units (15-ha each) that experienced mixed-severity fire and two units that experienced high-severity fire. We included both mixed- and high-severity units in order to (a) span the full range of fire severities created by these wildfires and (b) be able to compare differences in bees and flowering plants between mixed- and high-severity units in other studies (Burkle et al., 2015; LaManna et al., in review). In this study, we focused on overall differences among burned and unburned units, where burned units included both mixed- and high-severity units. In each locality, we selected two unburned units (15-ha each) in close proximity to the wildfires (mean distance = 5.75 km) with similar environmental characteristics (e.g., slope, aspect, elevation, forest type) to the burned units. All unburned units had no wildfire in at least 60 years.

Within each unit, we established nine circular sampling plots (25 m in diameter; 491 m^2) randomly stratified using a generalized random tessellation stratified (GRTS) survey design (Kincaid and Olsen, 2011), for a total of 160 plots. Specifically, there were 54 plots each in Paradise and Whitefish and 52 plots in Helena (2 of the plots originally selected as unburned in Helena were burned and could not be replaced), with 16–18 unburned plots and 36 burned plots (18 mixed-severity plots and 18 high-severity plots, pooled as burned plots for analyses) in each locality. For each mixed-severity fire unit, GRTS was used to stratify the nine plots such that three were low-severity, three medium-severity, and three high-severity. The median and range of fire severities of mixed-severity plots were similar across localities; the same was true for high-severity plots (Burkle et al., 2015). Additional details on site selection and characteristics are provided in Burkle et al. (2015). These plots were used to evaluate the density, diversity, and traits of native bees, floral resources, and nesting resources across 3–4 years. Plots in Helena were surveyed twice in 2013, 12 times in 2014, 9 times in 2015, and 7 times in 2016. Plots in Paradise were observed twice in 2013, 9 times each in 2014 and 2015, and 5 times in 2016. Plots in Whitefish were observed twice in 2013, and 7 times each in 2014 and 2015. Whitefish plots were not observed in 2016. Plots from Helena and Paradise were used to investigate traits of flowers and bees. A subset of the Paradise plots (described below) were selected to investigate solitary bee nesting success.

Density and Species Richness of Native Bees

At each plot, we censused the frequency and identity of native bees by hand-netting bees observed to contact the reproductive parts of flowers for 20 min weekly throughout the growing season in each year, for a total of 1,258 h of observations across all plots and years. Bees were killed and identified to species or, if not yet described by keys for this region, to morphospecies. We calculated the mean bee density (i.e., average number of bees captured in a plot during a 20-min observation period) and mean bee species richness in an observation period for each plot across all observation years. To account for differences in sampling among years, we weighted the mean value in each year by multiplying the mean value times the proportion of all censuses conducted in that year (i.e., 2013 observation values received less weight in the overall plot mean because there were fewer observations in that year). Observations were performed when there were flowers present in the plot (see *Floral resources* below). If observations were performed but no bees were observed, zeros were recorded and included in subsequent analyses. Bee density and richness were ln+1 transformed for normality of model residuals.

Floral Resources

Across the center of each plot, we established a 25 × 2 m band transect, oriented along topographic contours to minimize changes in elevation within transects. Within each band transect, we quantified the number of open flowers of each plant species on each observation day. We also recorded the identity of all flowering plant species in bloom in each plot on each observation day. We calculated the mean floral density and the mean flowering plant species richness on an observation day for each plot across all observation years, weighting these averages by the number of censuses in each year. Floral density was log+1 transformed for normality of model residuals. With these metrics, we specifically targeted a subset of the plant community in order to quantify floral resources; the effects of wildfire on the whole plant community, including forbs (regardless of whether they were blooming), grasses and woody plants can be found in Burkle et al. (2015).

Nesting Resources

For each plot, we measured nesting resources for ground-nesting bees by quantifying percent cover of bare ground and for cavity-nesting bees by quantifying coarse woody debris (CWD) (e.g., Potts et al., 2005; Grundel et al., 2010). We recorded whether bare ground or CWD was present every 10 cm along the diameter of each plot (25 m line transect) using a point-intercept method. In the field, we classified CWD into five diameter classes of downed logs following a modified method of Davis et al. (2016), and we grouped them together for analysis given the possibility that CWD more generally may be used as a nesting resource (e.g., Vázquez et al., 2011; Westerfelt et al., 2015; Carper and Bowers, 2017). Counts of bare ground and CWD were ln-transformed for normality of residuals.

Nesting Success

We used a subset of the plots in Paradise to assess nesting success ($N = 18$ plots). This subset included 12 burned plots and 6 unburned plots. Due to logistical constraints, we were unable to measure nesting success across all three localities. Instead, we used one locality, Paradise, as a focal study site to compare nesting success among burned and unburned plots. A nesting box was affixed to the snag nearest to the center of each plot in early June 2016. When no standing snags were present, the largest piece of coarse woody debris (for 2 of 18 plots) nearest to the center of the plot was used. Nest boxes were placed with their cavity openings facing southeast and ca. one meter from the ground whenever possible. Nesting boxes were constructed out of pine or poplar, and each box had 16 drilled cavities into which cardboard bee nesting tubes were placed. Four sizes of tubes were used in each box (3, 4, 5, and 6 mm) to maximize the number of species which could potentially nest in the boxes. Nest boxes were checked at least every other week from June through August; occupied nesting tubes were removed and replaced with unused empty tubes. Occupied tubes were then individually stored in plastic bottles with 1.5 mm air holes and overwintered in ambient conditions (i.e., an uninsulated outdoor structure in Bozeman, MT) from September 2016 until emergence was first noted in April 2017. Once bees began to emerge, tubes were moved to room temperature lab conditions and checked twice per week from April to August 2017 for new emergence. After emergence, bees were frozen and identified to species. For each plot, we calculated the proportion of nesting tubes from which bees emerged and nesting bee species richness.

Functional Trait of Bees

All bee individuals (5,098) and species (260) captured visiting flowers of selected plant species from Helena and/or Paradise (see *Traits of flowering plants*, below) were used for functional-trait measurement. We had time and resources to rigorously measure traits at two of the localities, and thus, traits were not measured in Whitefish. Queen bumblebees were not collected, and, thus, were not included in trait measurement. Specifically, we measured the intertegular distance (ITD, the width between the wing bases on the dorsal side of the thorax; Cariveau et al., 2016) of each individual bee because it is highly correlated with tongue length, body mass and foraging distance (Cane, 1987; Greenleaf et al., 2007; Cariveau et al., 2016). Thus, ITD represents functional traits related to interactions with flowers, reproductive success, and dispersal, among others. The ITD (mm) of each individual was measured in ocular units with a Leica S6E microscope equipped with a calibrated reticle ruler. We calculated the mean ITD of all measured bee individuals captured from each plot (mean $N = 51$ individuals per plot; range = 3–150 individuals per plot).

Functional Traits of Flowering Plants

We selected a subset of flowering plant species present in Helena and/or Paradise for functional-trait measurement. As above, we were unable to measure traits in Whitefish. Because we were interested in flowering plants as floral resources for bees (i.e., assessing the bees perspective on flowering-plant traits), we selected those species that were observed to interact with at least one bee species and were (1) present in both Helena and Paradise or (2) present in both burned and unburned areas of Helena or Paradise. This totaled 4,099 individuals of 81 plant species.

We measured plant height, flower number, flower volume, and specific leaf area (SLA) of up to 5 randomly selected flowering individuals of each selected species, stratified across each plot, in 2017. Measurements were collected every week, alternating between Helena and Paradise, throughout the growing season to allow for measurements during peak flowering for each species. Plant height was measured from the ground to the top of the inflorescence of a flowering individual, and can be important for plant reproduction as taller plants are more visible and often attract more pollinators (e.g., Fornoff et al., 2017). The number of flowers was counted on a randomly selected inflorescence, and is a measure of plant attractiveness to pollinators (Conner and Rush, 1996; Coux et al., 2016). Flower volume was calculated from the length, width, and depth (mm) with digital calipers, and is important for plant reproduction as larger flowers typically attract more pollinators (e.g., Stang et al., 2006). For specific leaf area (SLA), we collected a leaf from the base of each individual measured, pressed it, and weighed (mg) to obtain dry mass. The leaf was also photographed and processed with ImageJ to measure surface area, and SLA was calculated as leaf surface area per dry mass. Leaf rachis and petiolules (for species with compound leaves) and petioles (for all species) were included in measurements of leaf area and dry mass. SLA is important for plant energy use and potential relative growth rate (Cornelissen et al., 2003; Vieira et al., 2013; Fornoff et al., 2017). Though SLA may not be important for pollinator attraction or rewards, we included this commonly-measured trait to gain information on the plant perspective of the potential effects of wildfire and how these effects may differ from those on plant traits important for bees. We calculated the mean trait values of plant height, flower number (square-root transformed for normality of residuals), flower volume (log-transformed), and SLA (log-transformed) across all individuals of all species measured for each plot mean $N = 39$ individuals per plot; range = 5–75 individuals per plot). We did not weight these mean trait values by their proportional abundances (i.e., community-weighted means) because traits were measured on a subset of species in each plot, though weighting was used for calculation of inter- and intra-specific variation (below).

Inter- and Intra-specific Variation in Bee and Plant Traits

To test how wildfire influenced inter- and intra-specific variation in bee and flowering plant traits, we first standardized each trait separately using Z-scores and then followed Leps et al. (2006). Specifically, to determine the interspecific variation (i.e., the among-species variation) of a trait for each plot, we calculated the weighted mean deviation of the average trait value of each species from the trait mean of all individuals of all species in the plot. For intra-specific variation, we calculated the weighted mean within-species trait variance across all species in each plot. For both inter- and intra-specific variation, weighting was incorporated as the proportion of individuals (i.e., bee individuals or flowers) of a given species in each plot. It was not possible to perform paired *t*-tests of variance for species common to burned and unburned areas of Helena or Paradise (i.e., control for potential differences in community composition) due to too few replicate species with two or more individuals in a plot. Interspecific variation in plant height, flower volume, and SLA was log-transformed, and interspecific variation in the number of flowers per inflorescence was cube-root transformed for normality of residuals. Intraspecific variation in bee ITD was cube-root transformed and intraspecific variation in all plant traits was log-transformed for normality of residuals.

Statistical Methods

We tested the effects of wildfire (burned vs. unburned), locality, and their interaction on the density and species richness of bees and flowers, nesting resources, trait values of bees and flowering plants, and inter- and intra-specific variation in traits of bees and flowering plants across plots using separate mixed-effects ANOVA models, including unit within locality as a nested random effect. Significant ANOVAs were followed by Tukey HSD tests. For plant traits, we first used a MANCOVA including all traits. Following a significant MANCOVA for mean trait values [Wilks $\lambda = 0.071$, $F_{(12,262)} = 37.45$, $P < 0.0001$], interspecific variation in plant traits [Wilks $\lambda = 0.29$, $F_{(12,262)} = 12.93$, $P < 0.0001$], and intraspecific variation in plant traits [Wilks $\lambda = 0.38$, $F_{(12,254)} = 9.26$, $P < 0.0001$], we proceeded with a separate two-way ANOVA for each trait.

No statistical analyses were performed on nesting success (i.e., proportion emergence or bee species richness in nesting boxes) because no bees successfully emerged from nesting boxes in unburned plots (see section Results).

In order to help distinguish patterns in traits between burned and unburned areas due to differences in species composition from those due to shifts in trait distributions of individual species, we also performed a paired *t*-test for each trait in each of the two localities, including only the bee or plant species present in both burned and unburned areas in Helena or in Paradise (i.e., common species). There were 84 bee species in common between burned and unburned areas of Helena, 17 bee species in common in Paradise, 26 plant species in common in Helena, and 12 plant species in common in Paradise. This analysis of the effects of wildfire on matched species pairs was restricted to within each locality because there were too few species common to burned and unburned plots in both Helena and Paradise.

RESULTS

Density and Species Richness of Native Bees

Overall, we observed 281 bee species from 32 genera and 5 families (Reese et al., 2018). Across localities, dominant bee genera included *Bombus* (i.e., bumble bees like *Bombus bifarius* and *B. appositus*), *Osmia* (mason bees), *Megachile* (leafcutter bees), and *Lasioglossum* (sweat bees).

Bee density and species richness were higher in burned than unburned plots (**Table 1**). Moreover, these patterns differed among localities (locality × burn interaction; **Table 1**). Bee density was 100 and 155% higher in burned plots of Helena and Paradise, respectively, compared to unburned plots (**Table 1**; **Figure 1A**). Likewise, bee species richness was 85 and 120% higher in burned plots of Helena and Paradise, respectively, compared to unburned plots (**Table 1**; **Figure 1B**). Bee density and richness were similar in burned and unburned plots of Whitefish (**Table 1**; **Figures 1A,B**). Bee density and richness were generally higher in Helena than in Paradise or in Whitefish (**Table 1**; **Figures 1A,B**).

Floral Resources

Overall, we observed 239 flowering plant species from 128 genera and 40 families. Widespread flowering plant species included *Achillea millefolium*, *Campanula rotundifolia*, *Rosa woodsii*, and *Symphoricarpos albus*.

Floral density and species richness were higher in burned than unburned plots (**Table 1**), but these patterns differed among localities (locality × burn interaction; **Table 1**). Floral density was 510% and 450% higher in burned than unburned plots of Helena and Paradise, respectively (**Table 1**; **Figure 1C**). Floral species richness was 80 and 68% higher in burned plots of Helena and Paradise, respectively, compared to unburned plots (**Table 1**; **Figure 1D**). Floral density and richness were similar in burned and unburned plots of Whitefish (**Table 1**; **Figures 1C,D**). Floral species richness was generally highest in Helena, intermediate in Paradise, and lowest in Whitefish (**Table 1**; **Figure 1D**).

TABLE 1 | Results of two-way ANOVAs testing the effects of wildfire (i.e., burn status as burned or unburned), locality (i.e., Helena, Paradise, or Whitefish) and their interaction on native bee density and species richness, floral density and species richness, and nesting resources (i.e., bare ground and coarse woody debris [CWD]) measured across plots.

Response	Source	DF	*F*	*P*
NATIVE BEES				
Bee density	Burn status	1,154	134.96	**<0.0001**
	Locality	2,154	83.21	**<0.0001**
	Locality × Burn	2,154	25.52	**<0.0001**
Bee richness	Burn status	1,154	159.36	**<0.0001**
	Locality	2,154	82.30	**<0.0001**
	Locality × Burn	2,154	32.02	**<0.0001**
FLORAL RESOURCES				
Floral density	Burn status	1,154	62.53	**<0.0001**
	Locality	2,154	2.33	0.10
	Locality × Burn	2,154	8.42	**0.0003**
Floral richness	Burn status	1,154	69.20	**<0.0001**
	Locality	2,154	89.93	**<0.0001**
	Locality × Burn	2,154	40.71	**<0.0001**
NESTING RESOURCES				
Bare ground	Burn status	1,154	38.89	**<0.0001**
	Locality	2,154	3.88	**0.023**
	Locality × Burn	2,154	9.11	**0.0002**
CWD	Burn status	1,154	48.81	**<0.0001**
	Locality	2,154	25.66	**<0.0001**
	Locality × Burn	2,154	6.02	**0.0030**

P-values < 0.05 are in boldface.

Nesting Resources

Nesting resources (bare ground & coarse-woody debris) were higher in burned than unburned plots (**Table 1**), but these patterns differed among localities (locality × burn interaction; **Table 1**). The availability of nesting resources was higher in burned plots of Helena and Paradise compared to unburned plots, but similar between burned and unburned plots of Whitefish (**Table 1**). Specifically, bare ground was 195 and 330% higher in burned plots of Helena and Paradise relative to unburned plots (**Figure 1E**), and CWD was 525 and 150% higher in burned areas of Helena and Paradise (**Figure 1F**). Bare ground was generally higher in Helena and Paradise than in Whitefish (**Table 1**; **Figure 1E**), while CWD was higher in Whitefish than in Helena or Paradise (**Table 1**; **Figure 1F**).

Nesting Success

Bees emerged from nesting tubes in burned plots, but not from unburned plots. Across all nesting blocks, 262 nesting tubes contained offspring cells, from which 230 adult bees of 10 species emerged. Bee individuals were primarily *Megachile lapponica* (107) and *Hoplitis albifrons argentifrons* (87). No emergence was observed from any of the 47 nesting tubes collected from unburned plots that contained offspring cells, while bee emergence was 48.4% (i.e., bee offspring emerged successfully from 104 of 215 nesting tubes) in burned areas. An average of 3.0 bee species nested in boxes in burned plots.

Functional Trait of Bees

Mean ITD of all species was 28% greater in burned plots compared to unburned plots in HE, but was similar between burned and unburned plots in PV (**Table 2**; **Figure 2A**). In both Helena and Paradise, there was no difference in mean bee ITD of species common to burned and unburned areas (Helena paired t-test: $t = 1.19$, $N = 84$ species, $P = 0.24$; Paradise paired t-test: $t = 0.37$, $N = 17$ species, $P = 0.72$).

Across Helena and Paradise, burned plots had 24% higher interspecific variation in bee ITD than unburned plots (**Table 3**; **Figure 2B**). Burned plots had 47% higher intraspecific variation in bee ITD than unburned plots in Helena, but unburned plots had 40% higher intraspecific variation than burned plots in Paradise (**Table 4**; **Figure 2C**). Overall, interspecific variation in bee ITD was much higher than intraspecific variation (**Figures 2B,C**).

Functional Traits of Flowering Plants

Mean values of flowering plant traits generally differed between burned and unburned plots. The main effect of wildfire on the height of flowering individuals was positive: mean flowering plant height across all measured individuals and species was 16% higher in burned plots compared to unburned plots (**Table 2**; **Figure 3A**). The mean number of flowers per inflorescence was 115 and 450% higher in burned plots compared to unburned plots in Helena and Paradise, respectively (**Table 2**; **Figure 3B**). Mean flower volume was similar between burned and unburned plots in Helena, but was 193% higher in unburned plots compared to burned plots in Paradise (**Table 2**; **Figure 3C**). Mean SLA was similar between burned and unburned plots in Helena, but was 95% higher in unburned plots compared to burned plots in Paradise (**Table 2**; **Figure 3D**).

Mean flowering plant height of species present in both burned and unburned areas (i.e., common species) was marginally higher (by 9%) in unburned areas compared to burned areas in Helena (paired t-test: $t = 1.85$, $N = 26$ species, $P = 0.075$), and was 9% higher in unburned areas compared to burned areas in Paradise ($t = 2.75$, $N = 12$ species, $P = 0.019$). Mean number of flowers per inflorescence of common species was marginally higher (by 15%) in burned areas compared to unburned areas in Helena (paired t-test: $t = 1.86$, DF $= 25$, $P = 0.074$), but was similar between burned and unburned areas of Paradise ($t = 0.93$, DF $= 1$, $P = 0.37$). There was no difference in flower volume of common species between burned and unburned areas in Helena (paired t-test: $t = 1.35$, DF $= 25$, $P = 0.19$) or in Paradise ($t = 1.46$, DF $= 11$, $P = 0.17$). Mean SLA of common species was marginally higher (by 7%) in unburned areas compared to burned areas in Helena (paired t-test: $t = 1.84$, DF $= 25$, $P = 0.078$), and was 99% higher in unburned areas in Paradise ($t = 6.22$, DF $= 11$, $P < 0.0001$).

There were main effects of wildfire on interspecific variation in plant height and in the number of flowers per inflorescence (**Table 3**): across Helena and Paradise, burned plots had more variation in these traits than unburned plots (**Figures 4A,B**). There was similar interspecific variation in flower volume between burned and unburned plots in both Helena and Paradise (**Table 3**; **Figure 4C**). In Helena, there was no difference in

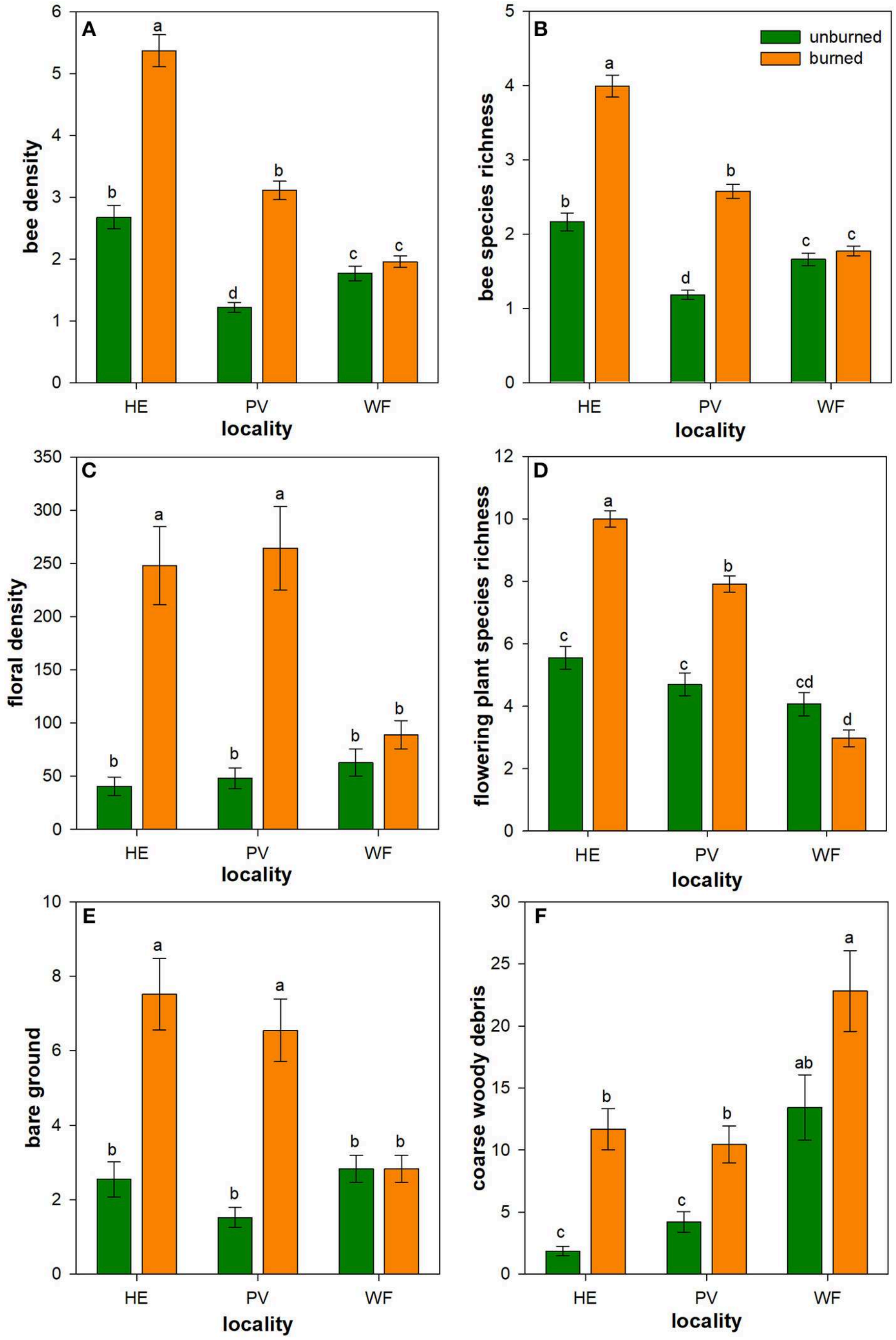


FIGURE 1 | Influence of wildfire on bees, floral resources, and nesting resources. Back-transformed least square means (± 1SE) of bee density **(A)**, bee species richness **(B)**, floral density **(C)**, flowering plant species richness **(D)**, bare ground **(E)**, and coarse woody debris (CWD) **(F)** were higher in burned plots (orange) than in unburned plots (green) in Helena (HE) and Paradise (PV), but not in Whitefish (WF). Letters represent significant differences in means at $\alpha = 0.05$. $N = 16$–18 unburned plots per locality; $N = 36$ burned plots (mixed-severity and high-severity fires) per locality.

interspecific variation in SLA between burned and unburned plots, but in Paradise, interspecific variation in SLA was higher in unburned plots than burned plots (**Figure 4D**).

In Helena, burned and unburned plots had similar intraspecific variation in plant height, while in Paradise, intraspecific variation in plant height in burned plots was higher than in unburned plots (**Table 4**; **Figure 4E**). In both Helena and Paradise, intraspecific variation in the number of flowers per inflorescence was higher in burned plots compared to unburned plots (**Table 4**; **Figure 4F**). There was similar intraspecific variation in flower volume between burned and unburned plots in both Helena and Paradise (**Table 4**; **Figure 4G**). In Helena and Paradise, intraspecific variation in SLA was higher in unburned plots compared to burned plots (**Table 4**; **Figure 4H**).

Overall, interspecific variation in plant traits was greater than their intraspecific variation across plots. One exception to this pattern was the relatively greater mean intraspecific variation in SLA across plots in Helena.

DISCUSSION

Areas burned by wildfires supported assemblages of native bees with greater density and species diversity than unburned areas in two of the three study localities. In these two localities, we also observed greater density of floral resources, species diversity of floral resources, and nesting resources in burned areas, indicating that wildfire acts to enhance multiple aspects of habitat important for bee success. Higher bee success in

TABLE 2 | Results of two-way ANOVAs testing the effects of wildfire (i.e., burn status as burned or unburned), locality (i.e., Helena or Paradise) and their interaction on the mean values of bee and plant traits of all individuals and species measured across plots.

Response	Source	DF	*F*	*P*
BEE TRAITS				
Bee intertegular distance	Burn status	1,95	13.71	**0.0004**
	Locality	1,95	41.71	**<0.0001**
	Locality × Burn	1,95	11.17	**0.0012**
PLANT TRAITS				
Plant height	Burn status	1,102	8.47	**0.044**
	Locality	1,102	4.51	**0.036**
	Locality × Burn	1,102	0.14	0.71
Flowers per inflorescence	Burn status	1,102	97.61	**<0.0001**
	Locality	1,102	5.95	**0.0164**
	Locality × Burn	1,102	8.56	**0.0042**
Flower volume	Burn status	1,102	5.34	**0.023**
	Locality	1,102	10.71	**0.0015**
	Locality × Burn	1,102	9.86	**0.0022**
Specific leaf area	Burn status	1,102	118.87	**<0.0001**
	Locality	1,102	245.08	**<0.0001**
	Locality × Burn	1,102	61.63	**<0.0001**

P-values < 0.05 are in boldface.

TABLE 3 | Results of two-way ANOVAs testing the effects of wildfire (i.e., burn status as burned or unburned), locality (i.e., Helena or Paradise) and their interaction on the mean interspecific variation in bee and plant traits of all individuals and species measured across plots.

Response	Source	DF	*F*	*P*
BEE TRAITS				
Bee intertegular distance	Burn status	1,95	5.17	**0.025**
	Locality	1,95	29.97	**<0.0001**
	Locality × Burn	1,95	0.058	0.81
PLANT TRAITS				
Plant height	Burn status	1,102	8.48	**0.0044**
	Locality	1,102	0.39	0.53
	Locality × Burn	1,102	3.12	0.081
Flowers per inflorescence	Burn status	1,102	57.87	**<0.0001**
	Locality	1,102	10.30	**0.0018**
	Locality × Burn	1,102	0.72	0.40
Flower volume	Burn status	1,102	0.32	0.57
	Locality	1,102	12.34	**0.0007**
	Locality × Burn	1,102	1.34	0.25
Specific leaf area	Burn status	1,102	12.81	**0.0005**
	Locality	1,102	41.29	**<0.0001**
	Locality × Burn	1,102	14.22	**0.0003**

P-values < 0.05 are in boldface.

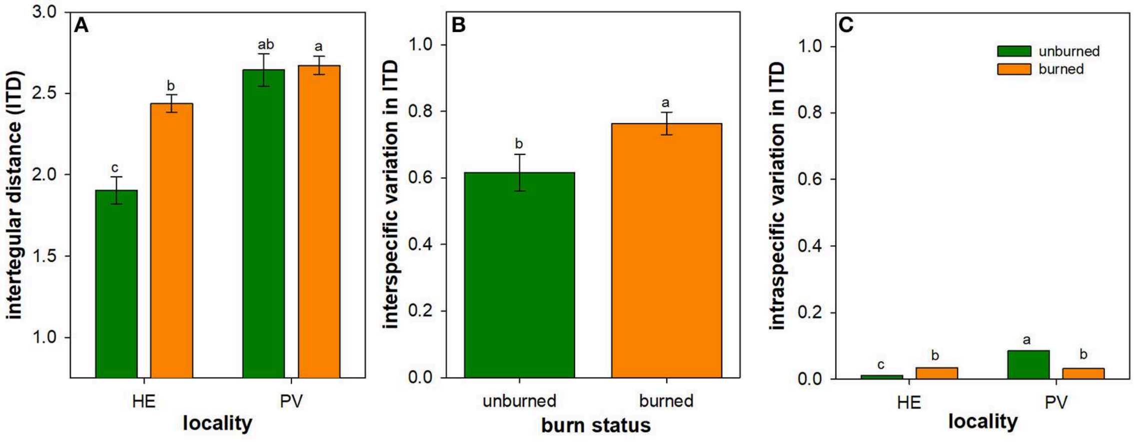


FIGURE 2 | Influence of wildfire on a bee trait and its intraspecific and interspecific variation. Least square mean (± 1SE) bee intertegular distance (ITD) (in mm) **(A)**, and back-transformed least square mean interspecific **(B)** and intraspecific **(C)** variation in bee ITD across all individuals of all species measured in burned (orange) and unburned (green) plots. Scaling is consistent between **(B,C)** for ease of comparison. Only the main effect of burn status is illustrated in panel B due to the lack of interactive effect between locality and burn status in interspecific variation in ITD (**Table 3**). Letters represent significant differences in means at α = 0.05. *N* = 16–18 unburned plots per locality; *N* = 36 burned plots (mixed-severity and high-severity fires) per locality.

TABLE 4 | Results of two-way ANOVAs testing the effects of wildfire (i.e., burn status as burned or unburned), locality (i.e., Helena or Paradise) and their interaction on the mean intraspecific variation in bee and plant traits of all individuals and species measured across plots.

Response	Source	DF	*F*	*P*
BEE TRAITS				
Bee intertegular distance	Burn status	1,88	0.52	0.47
	Locality	1,88	47.02	**<0.0001**
	Locality × Burn	1,88	53.81	**<0.0001**
PLANT TRAITS				
Plant height	Burn status	1,102	22.62	**<0.0001**
	Locality	1,102	1.94	0.17
	Locality × Burn	1,102	8.27	**0.0049**
Flowers per inflorescence	Burn status	1,102	62.67	**<0.0001**
	Locality	1,102	7.19	**0.0086**
	Locality × Burn	1,102	7.34	**0.0079**
Flower volume	Burn status	1,102	0.37	0.54
	Locality	1,102	4.79	**0.031**
	Locality × Burn	1,102	1.15	0.29
Specific leaf area	Burn status	1,102	14.29	**0.0003**
	Locality	1,102	3.95	**0.049**
	Locality × Burn	1,102	1.83	0.18

P-values < 0.05 are in boldface.

burned areas was also confirmed by the presence of bees nesting in these areas and the complete lack of successful bee nesting in unburned areas. However, although interspecific and intraspecific trait variation of bees and plants was often higher in burned areas, representing important functional diversity, some unburned areas harbored trait variation as well, indicating that a landscape mosaic containing both burned and unburned areas represents the greatest reservoir of trait variation for both trophic levels. Given that, to our knowledge, this is one of the first studies to measure intraspecific trait variation in bees and that we found some divergent patterns between bees and flowering plants in the patterns of trait variation after wildfire, additional consideration of this topic will likely yield important insights into bee-forb interaction structure and function. Overall, despite some variability in bee and plant responses to wildfire across this wide biogeographic region in the Northern Rockies, wildfires create environmental conditions that are generally conducive to a wide variety of flowering plants and nesting habitat, which support native bees with diverse functional traits. Together, these results suggest that areas burned by wildfires likely also support more complex networks of bee-flower interactions than unburned areas.

Density and Diversity of Native Bees, Floral Resources, Nesting Resources, and Nesting Success

Our study confirms that, like previously-studied fire-prone systems (e.g., Potts et al., 2003a, 2005; Lazarina et al., 2016, 2017), the bee fauna of conifer forests of the western U.S. can benefit from wildfires, including those that burned with high severity. It is interesting to note that despite low bee density and, thus, diversity observed in unburned forests, these areas are likely harboring much of the full species pool from which bees recruit to burned areas, either by flying in or emerging from nests not destroyed by fire (sensu Love and Cane, 2016). Enhanced bee density, diversity, and nesting success in burned areas was accompanied by floral and nesting habitat, suggesting that both of these resources contribute to bee success after wildfire. The fact that both bare ground (supporting ground-nesting bee species) and coarse woody debris (supporting cavity-nesting bee species) were higher in burned compared to unburned areas indicates that species in these two major functional groups of bees had the potential to establish reproductive populations in burned areas, potentially contributing to the enhanced interspecific variation in ITD observed in burned areas (see section discussion below).

Whitefish, the locality for which we observed no differences in native bee diversity or in floral or nesting resources between burned and unburned areas, is also where forest succession is rapid. Young trees already dominate many burned areas in Whitefish (Burkle et al., 2015), suggesting that the window of time conducive to native bees after wildfire, if any, is short in this region and had passed before our sampling began. Such areas with rapid tree regeneration deserve additional investigation, particularly in the years immediately following wildfire, to better understand the effects on native bees.

Traits of Bees

Bees in Helena were, on average, bigger (i.e., higher intertegular distance; proxy for body size, Cariveau et al., 2016) in burned plots compared to unburned plots, while they were similarly sized regardless of wildfire in Paradise, indicating that wildfires in some environmental contexts may select for larger bees. For example, bee species with larger body sizes and thus longer potential flight ranges to reach the center of a large burn may be advantageous in the Helena landscape. This result is especially interesting in light of the pattern that Helena harbored smaller bees than Paradise overall, perhaps related to Helena's lower productivity. Given that the mean ITD of individuals of common bee species were similar between burned and unburned areas of Helena, we can conclude that the overall effect of wildfire on bee body size was driven by differences in species composition between burned and unburned areas (LaManna et al. unpublished data), with burning selecting for species with larger body size, and not recruitment of especially large individuals. Thus, it is likely that the environmental conditions important for bees are strongly contrasting between burned and unburned areas of Helena, and differential species sorting by body size is occurring. In particular, bumble bees (*Bombus* sp.) are likely contributing to these patterns as they are large bodied, requiring relatively large quantities of nectar and pollen for individual and colony success, and thus may be less likely to inhabit unburned areas. By contrast, the similar ITD profiles between burned and unburned areas in Paradise may indicate that recruiting into burned areas was relatively easier for bee species with smaller body sizes. Alternatively, it is possible the species pool present in Paradise simply has few small-bodied species, and thus the body size profiles of burned plots more closely reflects those of unburned plots. These differences between Helena and Paradise may also be indicative of the slower speed of succession in Helena (which

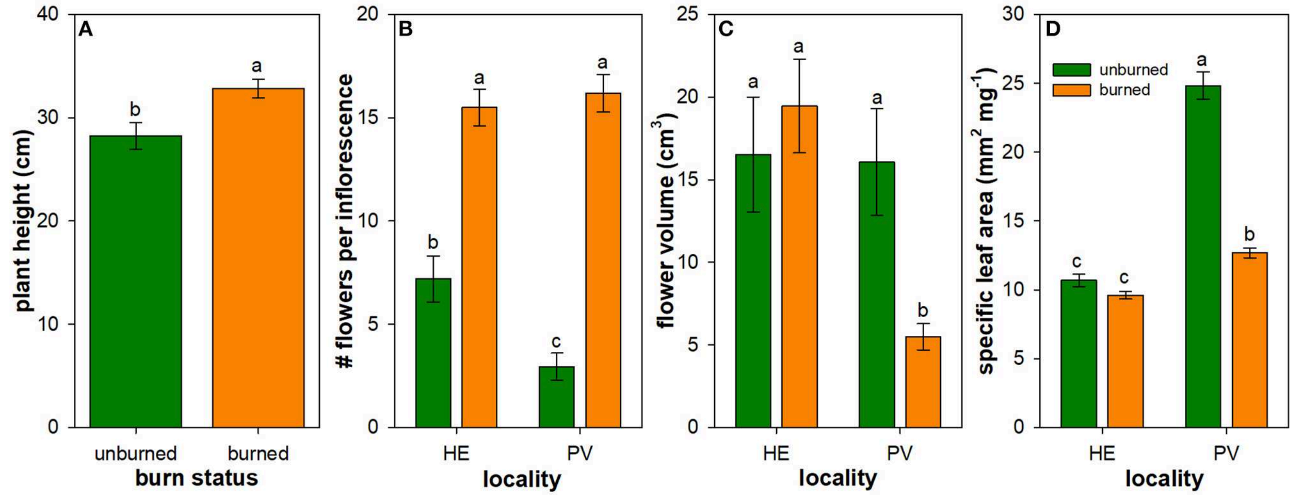


FIGURE 3 | Influence of wildfire on plant traits: plant height **(A)**, number of flowers per inflorescence **(B)**, flower volume **(C)**, and specific leaf area **(D)**. Least square mean (± 1SE) plant traits across all individuals of all species measured in burned (orange) and unburned (green) plots. Back-transformed least square means are shown for number of flowers per inflorescence, flower volume, and specific leaf area (SLA). Only the main effect of burn status is illustrated in panel A due to the lack of interactive effect between locality and burn status on plant height (**Table 3**). Letters represent significant differences in means at $\alpha = 0.05$. *N* = 16–18 unburned plots per locality; *N* = 36 burned plots (mixed-severity and high-severity fires) per locality.

is very dry and unproductive; trees have not regrown, Burkle et al., 2015) compared to Paradise (which is wetter and more productive), and it is possible that the difference in bee size observed in Helena may attenuate with time.

Across localities, there was higher interspecific variation in bee body size in burned areas compared to unburned areas, suggesting that these post-wildfire landscapes pose a less restrictive environmental filter to native bees than do unburned landscapes. This pattern is consistent with the patterns in bee species richness that we observed, albeit weaker, and confirms that burned landscapes provide more conducive habitat to a wider range of bee species than unburned landscapes. Interestingly, however, Helena and Paradise displayed contrasting patterns in the effect of wildfire on intraspecific variation in bee body size, with higher within-species body size variation in burned communities in Helena but higher variation in unburned communities in Paradise. While the overall magnitude of intraspecific variation was small relative to that of interspecific variation, these patterns in intraspecific variation in bee body size have important implications for interactions with flowers, including pollination effectiveness (Willmer and Finlayson, 2014). Furthermore, higher between-individual variation in the body size of a bee species can allow the population to be more generalized in foraging even if individuals remain specialized, resulting in more links per species (i.e., higher degree) in a community-level interaction network (sensu Bolnick et al., 2011). Higher average degree across species would result in a more-connected interaction network that would, in theory, be more robust to disturbances, species losses, and other environmental changes (e.g., Dunne et al., 2002; Memmott et al., 2004). Thus, in Paradise, where bee density and richness is particularly low in unburned areas, the relatively high intraspecific variation in body size may allow for the maintenance of bee-flower interaction networks and continued pollination services. These findings carry weight as this is one of the first studies to evaluate intraspecific trait variation in bees at the community level, and additional studies in this vein are warranted (Classen et al., 2017; Rumeu et al., 2018).

Traits of Plants

Wildfire influenced plant traits important for pollination and for plant growth (i.e., SLA). Across Helena and Paradise, plants in burned areas were taller and had more flowers per inflorescence than in unburned areas, while in Paradise only, plants in unburned areas had larger flowers and higher specific leaf area. These results suggest that post-wildfire conditions promote tall plants and flower production. After wildfire has cleared the canopy in previously forested areas, sunlight and open space are initially plentiful, allowing the establishment of a dense and diverse understory flowering plant community (e.g., Swanson et al., 2011). However, once established, there is likely competition for light and selection for taller plants (e.g., Selaya et al., 2008; Butterfield and Callaway, 2013). Because we found that plant individuals of common species were taller in *unburned* areas, species sorting for taller plant species may be particularly strong in burned areas. With adequate sunlight in burned areas, plants likely achieve a resource status that permits reproduction (e.g., Kilkenny and Galloway, 2008), and resources are allotted to flower production and reproduction. In Helena, the enhanced floral displays in burned areas seem to be due to a combination of both species composition (selection for species with larger floral displays) and plant-level resource allocation (individuals of common species had marginally more flowers per inflorescence), while in Paradise, the pattern appeared to be mainly driven by

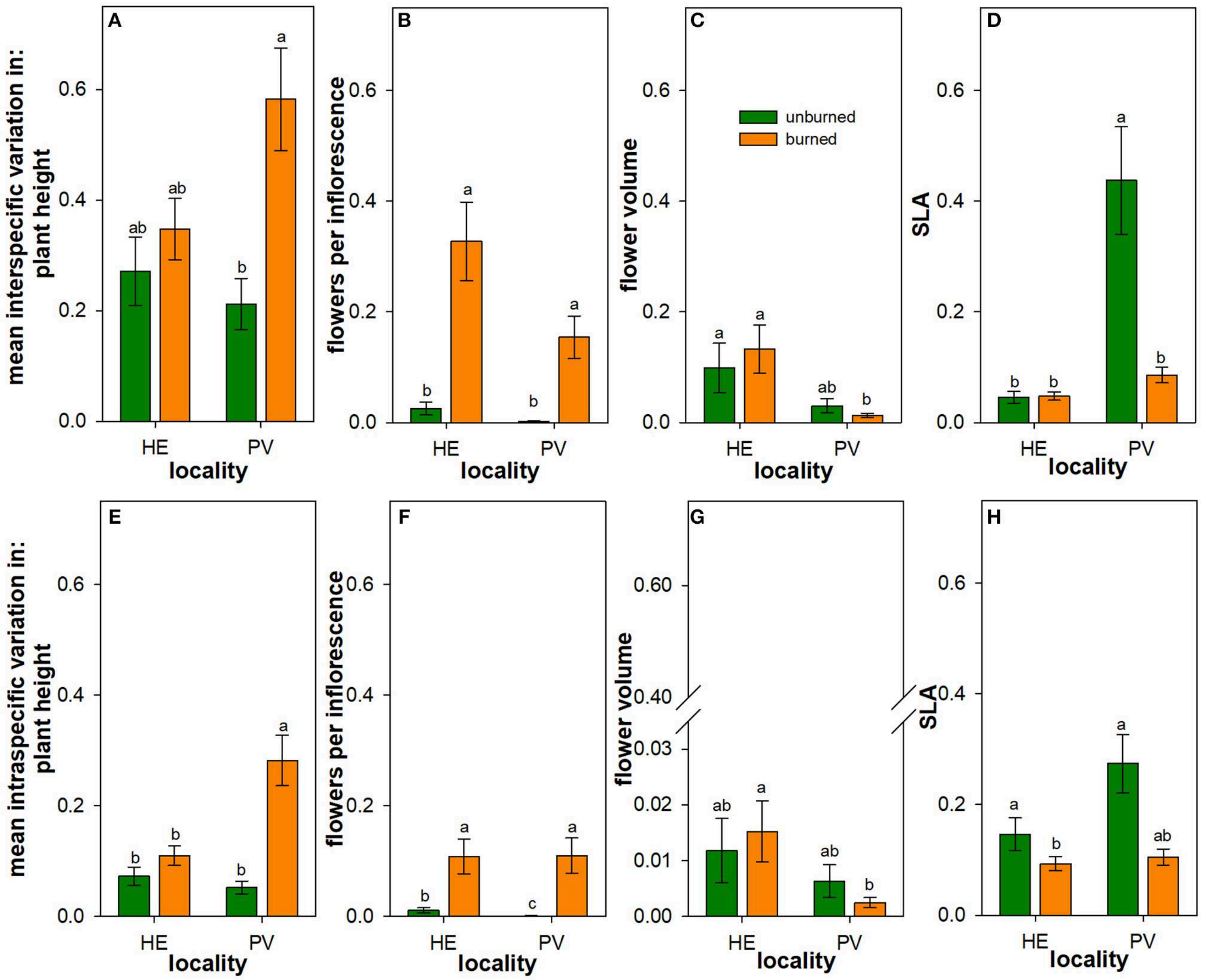


FIGURE 4 | Influence of wildfire on intraspecific and interspecific variation in plant traits. Back-transformed least square means (± 1SE) of the interspecific variation **(A–D)** and intraspecific variation **(E–H)** in plant height **(A,E)**, number of flowers per inflorescence **(B,F)**, flower volume **(C,G)**, and specific leaf area (SLA) **(D,H)** across all individuals of all species in burned (orange) and unburned (green) plots. Letters represent significant differences in means at $\alpha = 0.05$ based on transformed values. Scaling is consistent across panels for ease of visual comparison, though note the broken y-axis in panel to accommodate the extremely low intraspecific variation in flower volume. $N = 16$–18 unburned plots per locality; N = 36 burned plots (mixed-severity and high-severity fires) per locality.

species composition. Likewise, the pattern of smaller flowers in burned areas of Paradise appeared to be driven by species composition, not individuals of common species producing smaller flowers in burned areas. Because burned areas in Paradise may be particularly hotter and drier relative to unburned areas, it is possible that sorting of species with small flowers occurs because flowers can be very expensive in terms of the water budget of a plant (e.g., Galen et al., 1999; Galen, 2000; Carroll et al., 2001). The higher SLA in unburned areas of Paradise is consistent with plants growing in low light conditions (e.g., Björkman, 1981), and the physiological responses of individuals of common species contributed to this pattern, though we cannot rule out differences in species composition between burned and unburned areas as well [i.e., potential for shade-tolerant species with higher SLA in unburned areas; (Valladares and Niinemets, 2008)]. The lack of difference in flower size and SLA between burned and unburned areas of Helena may indicate that the environmental conditions influencing these traits are more similar between burned and unburned areas of Helena than they are in Paradise, that species composition swamps out any signal in these traits, or that the relatively unproductive conditions of Helena impose stronger selection on these traits at the regional scale compared to any selective effects of fire at more local scales.

Across localities, burned plots had more interspecific variation in plant height and flowers per inflorescence than unburned plots, indicating that these burned areas posed a less restrictive environmental filter to flowering plants—based on these traits—than unburned areas. Again, these patterns are consistent with patterns in flowering plant species richness. By contrast, the high inter- and intraspecific variation in SLA in unburned areas of

Paradise suggests that the conditions here permit species and individuals with a wide range of values for this trait. Similar to the patterns in interspecific variation, intraspecific variation in plant height (in Paradise only) and in flowers per inflorescence was higher in burned areas than in unburned areas, and thus may help promote complexity and robustness in plant-pollinator networks after wildfire. The lack of differences in interspecific variation in flower size between burned and unburned areas may point to a lack of species sorting based on this trait, or trade-offs with other traits for which there is stronger sorting. In addition, the lack of differences in intraspecific variation in flower size between burned and unburned areas may indicate similar strengths of evolutionary selection for consistency (or lack thereof) in flower size within species regardless of community and environmental context (e.g., Cresswell, 1998).

Summary of Bee and Plant Traits

Like bees, interspecific variation in plant traits was generally greater than their intraspecific variation across plots, though the relative effects of wildfire were of similar magnitude between bees and plants for both inter- and intraspecific variation. While bees have potentially greater dispersal abilities (flying), plants have seed banks that allow them to recruit immediately after fire (Keeley and Fotheringham, 2000), potentially contributing to the general consistency of wildfire's effects on trait variation across trophic levels. One exception to this pattern was the relatively greater mean intraspecific variation in SLA compared to interspecific variation in SLA across plots in Helena.

Implications for Land Management

Ecologists have long recognized the importance of fire in maintaining species diversity, composition, and structure of forests (Larson, 2016). In recent decades, fire policy has begun to shift from full suppression tactics to management and use. However, few fires are allowed to burn as managed wildfires (North et al., 2015), and public communications of fire is still mostly negative (e.g., Jacobson et al., 2001; Donovan and Brown, 2007). Our work provides insights into fire's role in maintaining plant and pollinator diversity across landscapes. Fire increases the diversity and abundance of flowering plants and pollinators, likely within a window of time post-wildfire related to the system-specific rate of succession. Nonetheless, unburned areas near recent fires support pollinator traits important for the maintenance of landscape-scale trait diversity, and this remains true despite variable land-use, climate, and community composition across our study wildfires. Therefore, landscape mosaics of adjacent unburned and burned lands likely lead to overall species diversity and ecological function (Belote, 2015). Forest and fire management goals should include objectives aimed at maintaining this landscape heterogeneity (Hessburg et al., 2015).

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

LB, RB, and JM conceived of the study. LB, MS, and JD collected the data. LB analyzed the data and wrote the first manuscript draft. All authors contributed to manuscript revisions.

FUNDING

The National Science Foundation (DEB 1256788 and 1256819 to LB, RB, and JM) and the Joint Fire Science Program Graduate Research Innovation Award (to MS) provided financial support.

ACKNOWLEDGMENTS

We thank F. Ambrose, E. Baker, B. Bode, L. Clark, J. Cutter, E. Ehrlich, T. Hall, L. Hamburg, L. Heil, C. Herron-Sweet, G. Hoffman, M. Lavin, S. Lewis, B. Malotky, P. Mitchell, J. Neville, C. Qubain, R. Quire, E. Reese, L. Roberts, M. Rockwell, E. Sawyer, A. Starcheski, C. Seymour, C. Welch, and J. Wray for help in the field and lab, M. Spasojevic for insightful discussions on functional traits, and to E. Reese, T. Griswold, C. Delphia, S. Burrows, J. Gibbs, H. Ikerd, M. Orr, and K. Wright for bee species identifications.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00252/full#supplementary-material

REFERENCES

Belote, R. T. (2015). "Contemporary patterns of burn severity heterogeneity from fires in the Northwestern U.S.," in *Proceedings of the Large Wildland Fires Conference*, eds R. E. Keane, M. Jolly, and R. Parsons, K. Riley (Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station), 252–256.

Björkman, O. (1981). "Responses to different quantum flux densities," in *Physiological Plant Ecology I: Responses to the Physical Environment Encyclopedia of Plant Physiology*, eds O. L. Lange, P. S. Nobel, C. B. Osmond, and H. Ziegler (Berlin, Heidelberg: Springer Berlin Heidelberg), 57–107.

Bolnick, D. I., Amarasekare, P., Araújo, M. S., Bürger, R., Levine, J. M., Novak, M., et al. (2011). Why intraspecific trait variation matters in community ecology. *Trends Ecol. Evol.* 26, 183–192. doi: 10.1016/j.tree.2011.01.009

Bond, W. J., and Keeley, J. E. (2005). Fire as a global 'herbivore': the ecology and evolution of flammable ecosystems. *Trends Ecol. Evol.* 20, 387–394. doi: 10.1016/j.tree.2005.04.025

Bowman, D. M. J. S., Balch, J. K., Artaxo, P., Bond, W. J., Carlson, J. M., Cochrane, M. A., et al. (2009). Fire in the earth system. *Science* 324, 481–484. doi: 10.1126/science.1163886

Burkle, L. A., Myers, J. A., and Belote, R. T. (2015). Wildfire disturbance and productivity as drivers of plant species diversity across spatial scales. *Ecosphere* 6, 1–14. doi: 10.1890/ES15-00438.1

Butterfield, B. J., and Callaway, R. M. (2013). A functional comparative approach to facilitation and its context dependence. *Funct. Ecol.* 27, 907–917. doi: 10.1111/1365-2435.12019

Cane, J. H. (1987). Estimation of bee size using intertegular span (apoidea). *J. Kansas Entomol. Soc.* 60, 145–147.

Cane, J. H., and Neff, J. L. (2011). Predicted fates of ground-nesting bees in soil heated by wildfire: thermal tolerances of life stages and a survey of nesting depths. *Biol. Conserv.* 144, 2631–2636. doi: 10.1016/j.biocon.2011.07.019

Cariveau, D. P., Nayak, G. K., Bartomeus, I., Zientek, J., Ascher, J. S., Gibbs, J., et al. (2016). The allometry of bee proboscis length and its uses in ecology. *PLoS ONE* 11:e0151482. doi: 10.1371/journal.pone.0151482

Carper, A. L., and Bowers, M. D. (2017). *The Conservation Value of Woody Debris for Cavity-nesting Bees on Boulder County Open Space*. Boulder, CO: Boulder County Open Space Final Report.

Carroll, A. B., Pallardy, S. G., and Galen, C. (2001). Drought stress, plant water status, and floral trait expression in fireweed (*Epilobium angustifolium*) (Onagraceae). *Am. J. Bot.* 88, 438–446. doi: 10.2307/2657108

Cavender-Bares, J., and Reich, P. B. (2012). Shocks to the system: community assembly of the oak savanna in a 40-year fire frequency experiment. *Ecology* 93, S52–S69. doi: 10.1890/11-0502.1

Classen, A., Steffan-Dewenter, I., Kindeketa, W. J., and Peters, M. K. (2017). Integrating intraspecific variation in community ecology unifies theories on body size shifts along climatic gradients. *Funct. Ecol.* 31, 768–777. doi: 10.1111/1365-2435.12786

Conner, J. K., and Rush, S. (1996). Effects of flower size and number on pollinator visitation to wild radish, *Raphanus raphanistrum*. *Oecologia* 105, 509–516. doi: 10.1007/BF00330014

Cornelissen, J. H. C., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D. E., et al. (2003). A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Aust. J. Bot.* 51, 335–380. doi: 10.1071/BT02124

Coux, C., Rader, R., Bartomeus, I., and Tylianakis, J. M. (2016). Linking species functional roles to their network roles. *Ecol. Lett.* 19, 762–770. doi: 10.1111/ele.12612

Cresswell, J. E. (1998). Stabilizing selection and the structural variability of flowers within species. *Ann. Bot.* 81, 463–473. doi: 10.1006/anbo.1998.0594

Davis, C. R., Belote, R. T., Williamson, M. A., Larson, A. J., and Esch, B. E. (2016). A rapid forest assessment method for multiparty monitoring across landscapes. *J. Foresty* 114, 125–133. doi: 10.5849/jof.14-118

Donovan, G. H., and Brown, T. C. (2007). Be careful what you wish for: the legacy of Smokey Bear. *Front. Ecol. Environ.* 5, 73–79. doi: 10.1890/1540-9295(2007)5[73:BCWYWF]2.0.CO;2

Dunne, J. A., Williams, R. J., and Martinez, N. D. (2002). Network structure and biodiversity loss in food webs: robustness increases with connectance. *Ecol. Lett.* 5, 558–567. doi: 10.1046/j.1461-0248.2002.00354.x

Evans, J. R., and Poorter, H. (2001). Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant Cell Environ.* 24, 755–767. doi: 10.1046/j.1365-3040.2001.00724.x

Fornoff, F., Klein, A.-M., Hartig, F., Benadi, G., Venjakob, C., Schaefer, H. M., et al. (2017). Functional flower traits and their diversity drive pollinator visitation. *Oikos* 126, 1020–1030. doi: 10.1111/oik.03869

Fukami, T., Bezemer, T. M., Mortimer, S. R., Putten, W. H., and van der (2005). Species divergence and trait convergence in experimental plant community assembly. *Ecol. Lett.* 8, 1283–1290. doi: 10.1111/j.1461-0248.2005.00829.x

Funk, J. L., Cleland, E. E., Suding, K. N., and Zavaleta, E. S. (2008). Restoration through reassembly: plant traits and invasion resistance. *Trends Ecol. Evol.* 23, 695–703. doi: 10.1016/j.tree.2008.07.013

Galen, C. (2000). High and dry: drought stress, sex-allocation trade-offs, and selection on flower size in the alpine wildflower *Polemonium viscosum* (Polemoniaceae). *Am. Natural.* 156, 72–83. doi: 10.1086/303373

Galen, C., Sherry, R. A., and Carroll, A. B. (1999). Are flowers physiological sinks or faucets? Costs and correlates of water use by flowers of *Polemonium viscosum*. *Oecologia* 118, 461–470. doi: 10.1007/s004420050749

Gathmann, A., and Tscharntke, T. (2002). Foraging ranges of solitary bees. *J. Anim. Ecol.* 71, 757–764. doi: 10.1046/j.1365-2656.2002.00641.x

Greenleaf, S. S., Williams, N. M., Winfree, R., and Kremen, C. (2007). Bee foraging ranges and their relationship to body size. *Oecologia* 153, 589–596. doi: 10.1007/s00442-007-0752-9

Grundel, R., Jean, R. P., Frohnapple, K. J., Glowacki, G. A., Scott, P. E., and Pavlovic, N. B. (2010). Floral and nesting resources, habitat structure, and fire influence bee distribution across an open-forest gradient. *Ecol. Appl.* 20, 1678–1692. doi: 10.1890/08-1792.1

Heil, L. J., and Burkle, L. A. (2018). Recent post-wildfire salvage logging benefits local and landscape floral and bee communities. *Forest Ecol. Manag.* 424, 267–275. doi: 10.1016/j.foreco.2018.05.009

Heil, L. J., and Burkle, L. A. (2019). The effects of post-wildfire salvage logging on plant reproductive success and pollination in *Symphoricarpos albus*, a fire-tolerant shrub. *Forest Ecol. Manag.* 432, 157–163. doi: 10.1016/j.foreco.2018.09.013

Hessburg, P. F., and Agee, J. K. (2003). An environmental narrative of Inland Northwest United States forests, 1800–2000. *Forest Ecol. Manag.* 178, 23–59. doi: 10.1016/S0378-1127(03)00052-5

Hessburg, P. F., Churchill, D. J., Larson, A. J., Haugo, R. D., Miller, C., Spies, T. A., et al. (2015). Restoring fire-prone Inland Pacific landscapes: seven core principles. *Landscape Ecol.* 30, 1805–1835. doi: 10.1007/s10980-015-0218-0

Jacobson, S. K., Monroe, M. C., and Marynowski, S. (2001). Fire at the wildland interface: the influence of experience and mass media on public knowledge, attitudes, and behavioral intentions. *Wildlife Soc. Bull.* 29, 929–937. Available online at: https://www.jstor.org/stable/3784420

Johnstone, J. F., Allen, C. D., Franklin, J. F., Frelich, L. E., Harvey, B. J., Higuera, P. E., et al. (2016). Changing disturbance regimes, ecological memory, and forest resilience. *Front. Ecol. Environ.* 14, 369–378. doi: 10.1002/fee.1311

Keeley, J. E., and Fotheringham, C. J. (2000). Role of fire in regeneration from seed. *Seeds* 2, 311–330. doi: 10.1079/9780851994321.0311

Kilkenny, F. F., and Galloway, L. F. (2008). Reproductive success in varying light environments: direct and indirect effects of light on plants and pollinators. *Oecologia* 155, 247–255. doi: 10.1007/s00442-007-0903-z

Kincaid, T. M., and Olsen, A. R. (2011). *spsurvey: Spatial Survey Design and Analysis*. Vienna: R Foundation for Statistical Computing. Available online at: http://www.r-project.org/

Koltz, A. M., Burkle, L. A., Pressler, Y., Dell, J. E., Vidal, M. C., Richards, L. A., et al. (2018). Global change and the importance of fire for the ecology and evolution of insects. *Curr. Opin. Insect Sci.* 29, 110–116. doi: 10.1016/j.cois.2018.07.015

Larson, A. J. (2016). Introduction to the article by Elers Koch: the passing of the Lolo Trail. *Fire Ecol.* 12, 1–6. doi: 10.4996/fireecology.1201001

Lazarina, M., Sgardelis, S. P., Tscheulin, T., Devalez, J., Mizerakis, V., Kallimanis, A. S., et al. (2017). The effect of fire history in shaping diversity patterns of flower-visiting insects in post-fire Mediterranean pine forests. *Biodivers. Conserv.* 26, 115–131. doi: 10.1007/s10531-016-1228-1

Lazarina, M., Sgardelis, S. P., Tscheulin, T., Kallimanis, A. S., Devalez, J., and Petanidou, T. (2016). Bee response to fire regimes in Mediterranean pine forests: the role of nesting preference, trophic specialization, and body size. *Basic Appl. Ecol.* 17, 308–320. doi: 10.1016/j.baae.2016.02.001

Leps, J., De Bello, F., Lavorel, S., and Berman, S. (2006). Quantifying and interpreting functional diversity of natural communities: practical considerations matter. *Preslia* 78, 481–501.

Love, B. G., and Cane, J. H. (2016). Limited direct effects of a massive wildfire on its sagebrush steppe bee community. *Ecol. Entomol.* 41, 317–326. doi: 10.1111/een.12304

McGill, B. J., Enquist, B. J., Weiher, E., and Westoby, M. (2006). Rebuilding community ecology from functional traits. *Trends Ecol. Evol.* 21, 178–185. doi: 10.1016/j.tree.2006.02.002

Memmott, J., Waser, N. M., and Price, M. V. (2004). Tolerance of pollination networks to species extinctions. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* 271, 2605–2611. doi: 10.1098/rspb.2004.2909

Moretti, M., De Bello, F., Roberts, S. P. M., and Potts, S. G. (2009). Taxonomical vs. functional responses of bee communities to fire in two contrasting climatic regions. *J. Anim. Ecol.* 78, 98–108. doi: 10.1111/j.1365-2656.2008.01462.x

Moretti, M., Obrist, M. K., and Duelli, P. (2004). Arthropod biodiversity after forest fires: winners and losers in the winter fire regime of the southern Alps. *Ecography* 27, 173–186. doi: 10.1111/j.0906-7590.2004.03660.x

Myers, J. A., Chase, J. M., Crandall, R. M., and Jiménez, I. (2015). Disturbance alters beta-diversity but not the relative importance of community assembly mechanisms. *J. Ecol.* 103, 1291–1299. doi: 10.1111/1365-2745.12436

North, M. P., Stephens, S. L., Collins, B. M., Agee, J. K., Aplet, G., Franklin, J. F., et al. (2015). Reform forest fire management. *Science* 349, 1280–1281. doi: 10.1126/science.aab2356

Pausas, J. G., and Verdú, M. (2008). Fire reduces morphospace occupation in plant communities. *Ecology* 89, 2181–2186. doi: 10.1890/07-1737.1

Pauw, A. (2007). Collapse of a pollination web in small conservation areas. *Ecology* 88, 1759–1769. doi: 10.1890/06-1383.1

Peralta, G., Stevani, E. L., Chacoff, N. P., Dorado, J., and Vázquez, D. P. (2017). Fire influences the structure of plant–bee networks. *J. Anim. Ecol.* 86, 1372–1379. doi: 10.1111/1365-2656.12731

Potts, S., Vulliamy, B., Dafni, A., Ne'eman, G., and Willmer, P. (2003b). Linking bees and flowers: how do floral communities structure pollinator communities? *Ecology* 84, 2628–2642. doi: 10.1890/02-0136

Potts, S. G., Petanidou, T., Roberts, S., O'Toole, C., Hulbert, A., and Willmer, P. (2006). Plant-pollinator biodiversity and pollination services in a complex Mediterranean landscape. *Biol. Conserv.* 129, 519–529. doi: 10.1016/j.biocon.2005.11.019

Potts, S. G., Vulliamy, B., Dafni, A., Ne'eman, G., O'Toole, C., Roberts, S., et al. (2003a). Response of plant-pollinator communities to fire: changes in diversity, abundance and floral reward structure. *Oikos* 101, 103–112. doi: 10.1034/j.1600-0706.2003.12186.x

Potts, S. G., Vulliamy, B., Roberts, S., O'Toole, C., Dafni, A., Ne'eman, G., et al. (2005). Role of nesting resources in organising diverse bee communities in a Mediterranean landscape. *Ecol. Entomol.* 30, 78–85. doi: 10.1111/j.0307-6946.2005.00662.x

Reese, E. G., Burkle, L. A., Delphia, C. M., and Griswold, T. (2018). A list of bees from three locations in the Northern Rockies Ecoregion (NRE) of western Montana. *Biodivers. Data J.* 6:e27161. doi: 10.3897/BDJ.6.e27161

Rumeu, B., Sheath, D. J., Hawes, J. E., and Ings, T. C. (2018). Zooming into plant-flower visitor networks: an individual trait-based approach. *PeerJ* 6:e5618. doi: 10.7717/peerj.5618

Selaya, N. G., Oomen, R. J., Netten, J. J. C., Werger, M. J. A., and Anten, N. P. R. (2008). Biomass allocation and leaf life span in relation to light interception by tropical forest plants during the first years of secondary succession. *J. Ecol.* 96, 1211–1221. doi: 10.1111/j.1365-2745.2008.01441.x

Simanonok, M. P. (2018). *Plant-pollinator Network Assembly after Wildfire*. Available online at: https://scholarworks.montana.edu/xmlui/handle/1/14863 (accessed February 8, 2019).

Spasojevic, M. J., Catano, C. P., LaManna, J. A., and Myers, J. A. (2018). Integrating species traits into species pools. *Ecology* 99, 1265–1276. doi: 10.1002/ecy.2220

Spasojevic, M. J., Turner, B. L., and Myers, J. A. (2016). When does intraspecific trait variation contribute to functional beta-diversity? *J. Ecol.* 104, 487–496. doi: 10.1111/1365-2745.12518

Stang, M., Klinkhamer, P. G., and van der Meijden, E. (2006). Size contraints and flower abundance determine the number of interaction in a plant-flower visitation web. *Oikos* 112, 111–121. doi: 10.1111/j.0030-1299.2006.14199.x

Swanson, M. E., Franklin, J. F., Beschta, R. L., Crisafulli, C. M., DellaSala, D. A., Hutto, R. L., et al. (2011). The forgotten stage of forest succession: early-successional ecosystems on forest sites. *Front. Ecol. Environ.* 9, 117–125. doi: 10.1890/090157

Valladares, F., and Niinemets, Ü. (2008). Shade tolerance, a key plant feature of complex nature and consequences. *Annu. Rev. Ecol. Evol. Syst.* 39, 237–257. doi: 10.1146/annurev.ecolsys.39.110707.173506

Van Nuland, M. E., Haag, E. N., Bryant, J. A. M., Read, Q. D., Klein, R. N., Douglas, M. J., et al. (2013). Fire promotes pollinator visitation: implications for ameliorating declines of pollination services. *PLoS ONE* 8:e79853. doi: 10.1371/journal.pone.0079853

Vázquez, D. P., Alvarez, J. A., Debandi, G., Aranibar, J. N., and Villagra, P. E. (2011). Ecological consequences of dead wood extraction in an arid ecosystem. *Basic Appl. Ecol.* 12, 722–732. doi: 10.1016/j.baae.2011.08.009

Vieira, M. C., Cianciaruso, M. V., and Almeida-Neto, M. (2013). Plant-Pollinator coextinctions and the loss of plant functional and phylogenetic diversity. *PLoS ONE* 8:e81242. doi: 10.1371/journal.pone.0081242

Warzecha, D., Diekötter, T., Wolters, V., and Jauker, F. (2016). Intraspecific body size increases with habitat fragmentation in wild bee pollinators. *Landscape Ecol.* 31, 1449–1455. doi: 10.1007/s10980-016-0349-y

Weiher, E., and Keddy, P. A. (1995). Assembly rules, null models, and trait dispersion: new questions from old patterns. *Oikos* 74, 159–164. doi: 10.2307/3545686

Westerfelt, P., Widenfalk, O., Lindelöw, Å., Gustafsson, L., and Weslien, J. (2015). Nesting of solitary wasps and bees in natural and artificial holes in dead wood in young boreal forest stands. *Insect Conserv. Divers.* 8, 493–504. doi: 10.1111/icad.12128

Westerling, A. L., Hidalgo, H. G., Cayan, D. R., and Swetnam, T. W. (2006). Warming and earlier spring increase western US forest wildfire activity. *Science* 313:940. doi: 10.1126/science.1128834

Westrich, P. (1996). "Habitat requirements of central European bees and problems of partial habitats," in *The Conservation of Bees*, eds A. Matheson, S. L. Buchmann, C. O'Toole, P. Westrich, and I. H. Williams (London: Academic Press), 1–16.

Willmer, P. G., and Finlayson, K. (2014). Big bees do a better job: intraspecific size variation influences pollination effectiveness. *J. Pollinat. Ecol.* 14, 244–254. doi: 10.26786/1920-7603(2014)22

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 03 July 2019
doi: 10.3389/fevo.2019.00250

Dominant Arctic Predator Is Free of Major Parasitoid at Northern Edge of Its Range

Amanda M. Koltz[1], Lauren E. Culler[2,3], Joseph J. Bowden[4,5], Eric Post[6] and Toke T. Høye[5,7]*

[1] *Department of Biology, Washington University in St. Louis, St. Louis, MO, United States,* [2] *Environmental Studies Program, Dartmouth College, Hanover, NH, United States,* [3] *Institute of Arctic Studies, Dartmouth College, Hanover, NH, United States,* [4] *Canadian Forest Service—Atlantic Forestry Centre, Corner Brook, NL, Canada,* [5] *Arctic Research Centre, Aarhus University, Aarhus, Denmark,* [6] *Department of Wildlife, Fish, and Conservation Biology, University of California, Davis, Davis, CA, United States,* [7] *Department of Bioscience Kalø, Aarhus University, Aarhus, Denmark*

Parasitoids can affect host population dynamics with community-level consequences. In the Arctic, a high diversity of parasitoids relative to potential hosts suggests that parasitoids may exert strong selection pressure on arthropods, but the extent to which these interspecific linkages drive arthropod population dynamics remains unclear. Wolf spiders are dominant and ecologically important arctic predators that experience high rates of egg sac parasitism by wasps. We investigated potential changes in egg sac parasitism rates at two rapidly warming sites in Greenland: a high-arctic site (18 years of data, 1,088 egg sacs) and a low-arctic site (5 years of data, 538 egg sacs). While up to 13% of egg sacs were parasitized annually in the low-arctic site, we found no evidence of it at the high-arctic site despite the presence of congeneric parasitoid species at both locations. The surprising lack of parasitism in the north suggests that populations of this widespread spider species have different eco-evolutionary histories and may respond differentially to climate change.

Keywords: Arctic, Hymenoptera, Greenland, parasitism, parasitoid, wasp, wolf spider, *Pardosa glacialis*

Edited by:
Gina Marie Wimp,
Georgetown University, United States

Reviewed by:
Clay Cressler,
University of Nebraska-Lincoln,
United States
Marcelo Oliveira Gonzaga,
Federal University of Uberlandia, Brazil

***Correspondence:**
Amanda M. Koltz
akoltz@wustl.edu

Specialty section:
This article was submitted to Population and Evolutionary Dynamics,
a section of the journal Frontiers in Ecology and Evolution

Received: *19 February 2019*
Accepted: *17 June 2019*
Published: *03 July 2019*

Citation:
Koltz AM, Culler LE, Bowden JJ, Post E and Høye TT (2019) Dominant Arctic Predator Is Free of Major Parasitoid at Northern Edge of Its Range. Front. Ecol. Evol. 7:250.
doi: 10.3389/fevo.2019.00250

INTRODUCTION

Host-parasite interactions may play a particularly important role in food web dynamics in the Arctic, where arthropod communities exhibit a high diversity of parasitoids relative to overall arthropod diversity (Stahlhut et al., 2013). Parasite impacts on arthropod hosts may even increase in the future, because parasitoids are disproportionately benefitting from rapidly warming temperatures relative to other arthropod groups (Koltz et al., 2018c). Unfortunately, while vertebrate host-parasite dynamics are relatively well-described in the Arctic (e.g., Moerschel, 1999; Hagemoen and Reimers, 2002; Kutz et al., 2002), little is currently known about parasitism among arctic arthropods (Kukal and Kevan, 1987; Bowden and Buddle, 2012a, but see Avery and Post, 2013; Ernst et al., 2016). Addressing this important gap in our knowledge of arctic natural history is critical because arthropods represent the most biodiverse group of multi-cellular organisms in this region, and as herbivores, detritivores, pollinators, and predators, arthropods play important roles in maintaining the health and functioning of arctic ecosystems (Høye and Culler, 2018). Here, we compare the strength of host-parasite interactions in one of the most abundant arctic arthropod groups for two sites in Greenland.

Wolf spiders inhabit nearly all terrestrial ecosystems and are dominant predators in the Arctic (Wyant et al., 2011; Bowden et al., 2018; Koltz et al., 2018a). They commonly prey upon decomposers and thereby indirectly affect decomposition rates and nutrient cycling in the tundra (Koltz et al., 2018b) and elsewhere (Lawrence and Wise, 2000; Lensing and Wise, 2006). Additionally, wolf spiders themselves are preyed upon by a variety of vertebrates (e.g., birds, Wirta et al., 2015) and parasitic wasps, including egg predators and egg parasitoids which target their egg sacs (both generally referred to here as parasitoids; Bowden and Buddle, 2012a). Female wolf spiders display maternal care by tethering their egg sacs to their abdomens for one to several weeks. During this incubation time, eggs develop into juvenile spiders and ultimately leave the egg sac. Egg sacs are conspicuous, being blue-gray or silver in color and large compared to a female's body (**Figure 1A**). Given the vulnerability of exposed egg sacs and the near global distribution and high local abundances of wolf spiders, it is not surprising that egg sac parasitism is commonly observed in temperate (Edgar, 1971; Cobb and Cobb, 2004) and arctic ecosystems (Bowden and Buddle, 2012a). In some areas of the Arctic, wasps from the genus *Gelis,* whose juveniles ultimately consume or destroy the entire contents of egg sacs, are found in as many as half of all sampled wolf spider egg sacs (Bowden and Buddle, 2012a). Such high rates of parasitism could impose major selective pressures on the phenology, physiology, and behavior (e.g., Godfray et al., 1994; Fellowes and Godfray, 2000; Low et al., 2014) of wolf spiders, with potential consequences for their population dynamics and ecosystem functioning.

Wolf spiders in the Arctic are becoming larger as a consequence of the longer growing seasons that have resulted from recent climate change (Høye et al., 2009). Because female spider body size is positively associated with the number of eggs per egg sac (Bowden and Buddle, 2012b), larger female spider bodies are expected to result in higher fecundity (Marshall and Gittleman, 1994) and potentially higher adult wolf spider abundances. Similarly, access to more eggs and/or reproductive females should presumably favor parasitoid wasps that feed upon wolf spider eggs, but the extent to which parasitoid behavior and abundances are being affected by changes in their typical prey are currently unknown.

We investigated the spatial and long-term temporal variability of egg sac parasitism in the dominant species of wolf spider across most of Greenland, *Pardosa glacialis* (Lycosidae) (Høye and Hammel, 2010; Bowden et al., 2018) at two sites that are warming rapidly under climate change. Specifically, we quantified parasitism using museum samples of wolf spider egg sacs that were collected over 18 years between 1996 and 2014 in Zackenberg, Greenland, a High-Arctic site (Meltofte and Rasch, 2008). Additionally, in 5 years between 2010 and 2017, we assessed parasitism rates on *P. glacialis* egg sacs in Kangerlussuaq, Greenland, a low-arctic site (Post, 2013). We affirmed the identities of parasitoid species by using DNA barcoding methods on a portion of parasitized egg sacs. We also measured the body sizes of a subset of egg sac – carrying female spiders to test whether parasitic wasps preferentially parasitize egg sacs produced by larger individuals (Bowden and Buddle, 2012a). Prior studies have shown that the abundance of parasitoid wasps in Zackenberg is higher in years with warmer summers (Koltz et al., 2018c). Given that body sizes, and presumably fecundity, of wolf spiders are increasing at Zackenberg as well (Høye et al., 2009), we predicted a general increase in parasitism rates over our 18-year study period. Additionally, we predicted that overall parasitism rates should be higher at Kangerlussuaq than at Zackenberg because growing seasons and hence, potential temporal overlap in host and parasitoid phenology are longer at Kangerlussuaq.

MATERIALS AND METHODS

Study Sites

We sampled female arctic wolf spiders (*Pardosa glacialis*) and their egg sacs from two locations in Greenland (**Figure 2**). Samples from the first site were collected near the Zackenberg Research Station (74.28°N 20.34°W), which is located in Northeast Greenland in the high-arctic and is at the most northern range of *P. glacialis* (Dondale and Redner, 1990). The second site is an inland, mountainous tundra system in West Greenland near Kangerlussuaq (67.11°N 50.37°W) and is considered low-arctic (Bliss, 1981). Both sites are characterized by continental climates with cold winters and generally dry conditions, although overall climatic conditions are harsher at Zackenberg. Over the study period, the average temperature in July at Zackenberg was 6.5°C, whereas the average July temperature in Kangerlussuaq was 13.2°C (**Figure S1**). There are general warming trends of similar magnitude at Zackenberg and Kangerlussuaq (Zackenberg: +9.0 summed degree days/year between 1996 and 2017; Kangerlussuaq: +4.6 summed degree days/year between 1996 and 2013; **Figure S1**). *P. glacialis* is the only wolf spider species present in the samples collected at Zackenberg and is the most commonly collected species in Kangerlussuaq (In 2017 at Kangerlussuaq, 89% of all wolf spiders collected were *P. glacialis*, 11% were *Arctosa insignita* and <1% were *Pardosa groenlandica*, L. Culler unpublished data).

Wolf spider egg sacs from Zackenberg were extracted from samples of ground-dwelling arthropods that were collected using pitfall traps from late May through at least the end of August from 1996 to 2014 (excluding 2010) as part of the BioBasis Monitoring Programme (Jensen et al., 2013). This sampling period includes almost the entire active period for both wolf spiders and potential parasitoid wasps; **Table S1** contains specific information on sampling dates and the number of trapping days per year. Sampling plots were located in three different habitat types: mesic heath, arid heath, and wet fen (Høye and Forchhammer, 2008). Pitfall traps were 10 cm in diameter, yellow, and 1/3 filled with water and a few drops of detergent. Traps were emptied once per week. All captured specimens were stored in 70% ethanol. Samples were subsequently sorted and counted by technicians from the Department of Bioscience at Aarhus University, Denmark and spiders were identified and measured by J.J.B.

We collected spiders in Kangerlussuaq in 2010, 2011, 2012, 2016, and 2017. In 2010-2012, sampling occurred from May 30

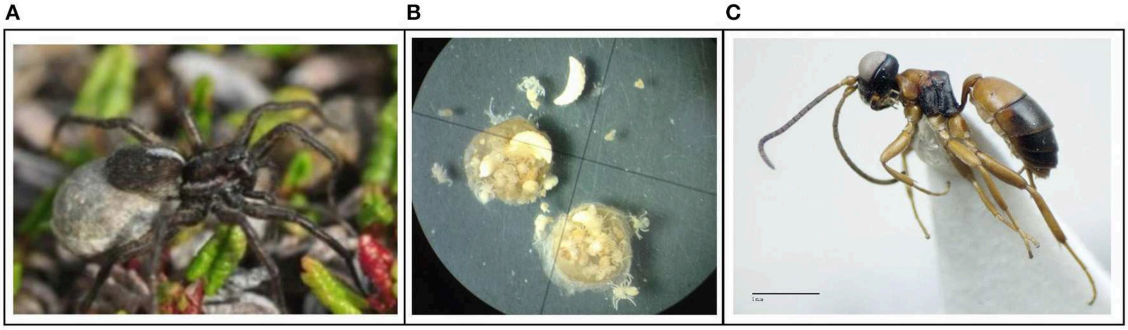


FIGURE 1 | Egg sac parasitism of wolf spiders by parasitoid wasps is common in arctic and temperate ecosystems, including in SW Greenland, where populations of *P. glacialis* are parasitized by the wasp *G. micrurus*. **(A)** Female *P. glacialis* with egg sac; **(B)** Egg sac contents with a mix of wolf spider eggs and parasitoid larvae; **(C)** adult parasitoid *G. micrurus*. Photo credits: **(A)** Oskar L.P. Hansen; **(B)** Lauren E. Culler; **(C)** Ika Osterblad, Station Linné/Swedish Museum of Natural History, License: CreativeCommons Attribution, Non-commercial ShareAlike (CC BY-NC-SA 3.0).

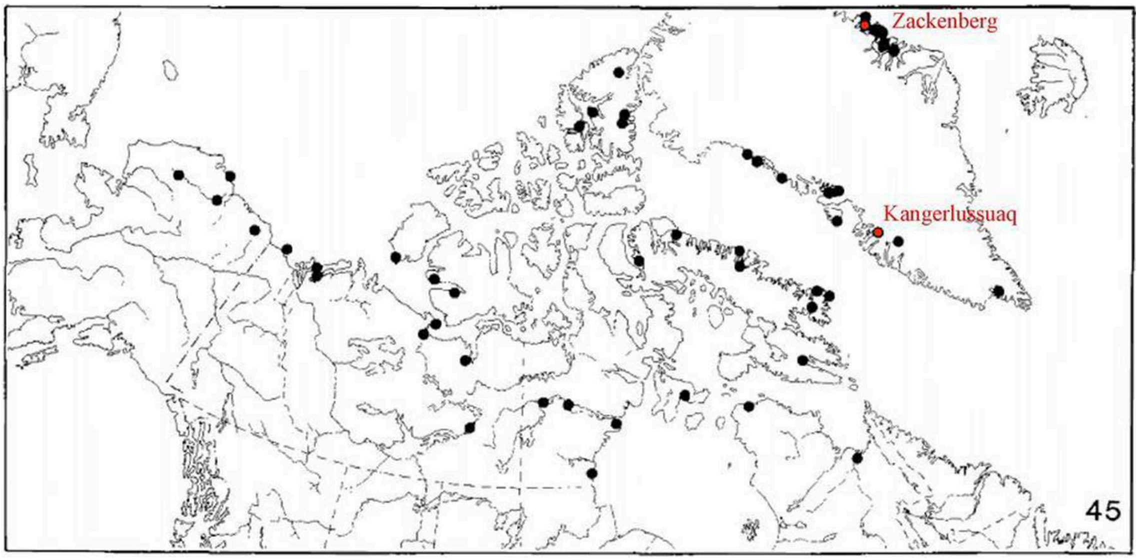


FIGURE 2 | Map of the distribution of the arctic wolf spider *Pardosa glacialis* (Figure modified and reproduced with permission from Dondale and Redner, 1990). Egg sacs of *P. glacialis* were sampled and inspected for evidence of parasitism from Zackenberg (74.47°N 20.57°W), a high-arctic site in NE Greenland, and Kangerlussuaq (67.11°N 50.37°W) in low-arctic W Greenland.

through July 21. During these 3 years, twenty pitfall traps were deployed in mixed dwarf shrub / graminoid tundra habitat at each of six trapping sites (**Table S2**) that were evenly distributed between north- and south-facing slopes. Of the 20 pitfall traps at each site, 10 were located within a large herbivore exclosure treatment and the other 10 were outside the treatment in open tundra. In 2011 and 2012, additional sticky traps were deployed within 10 meters from the established pitfall sampling plots. The contents of all traps were collected every 24–48 h, preserved in 70% ethanol, and sorted at Pennsylvania State University by Michael Avery before further processing. Sampling in Kangerlussuaq in 2016 and 2017 was part of two studies documenting the abundance and composition of ground-dwelling arthropods in habitats of varying vegetation cover and soil moisture (**Table S3**). Sampling was conducted over approximately 2.5 weeks during the peak of the summer season in 2016 (30 June to 16 July) and during a longer period of 9 weeks in 2017 (22 May to 25 July). In 2016, 72 pitfall traps were evenly distributed among dwarf shrub and unvegetated tundra at three distinct sites (total trap days = 1,152; **Table 1**, **Table S3**). In 2017, 144 pitfall traps were distributed among dwarf shrub, dry graminoid, wet graminoid, and unvegetated tundra at eight distinct sites (total trap days = 3,117; **Table 1**, **Table S3**). In each year, sample contents were collected every 4–5 days and preserved in 70% ethanol for later processing at Dartmouth College. Although these samples do not capture the entirety of the growing season, in combination with the sampling from 2010 to 2012, they provide the best information available on wolf spider phenology and potential overlap with parasitoids in the Kangerlussuaq area.

TABLE 1 | Number of sampled wolf spider (*Pardosa glacialis*) egg sacs and egg sac parasitism rates for each study year from the Greenland sites of Kangerlussuaq (southern site) and Zackenberg (northern site).

Year	Total trap days	*N* sampled egg sacs	*N* parasitized egg sacs	Parasitism rate	Probability of detecting 0% parasitism given sample size and 4.4% parasitism rate	Probability of detecting 0% parasitism given sample size and 11.47% parasitism rate
KANGERLUSSUAQ, GREENLAND						
2010	6,360	13	0	0%	0.558	0.205
2011	6,360	159	7	4.40%	0.001	0.000
2012	6,360	31	0	0%	0.247	0.022
2016	1,152	30	4	13.33%	0.258	0.026
2017	3,117	305	35	11.48%	0.000	0.000
ZACKENBERG, GREENLAND						
1996	1,257	45	0	0%	0.132	0.004
1997	2,411	136	0	0%	0.002	0.000
1998	2,317	113	0	0%	0.006	0.000
1999	1,354	159	0	0%	0.001	0.000
2000	1,584	100	0	0%	0.011	0.000
2001	1,474	83	0	0%	0.024	0.000
2002	1,526	15	0	0%	0.509	0.160
2003	1,540	86	0	0%	0.021	0.000
2004	1,661	66	0	0%	0.051	0.000
2005	1,840	48	0	0%	0.115	0.003
2006	1,512	57	0	0%	0.078	0.001
2007	2,312	18	0	0%	0.443	0.112
2008	1,523	15	0	0%	0.509	0.162
2009	2,321	29	0	0%	0.270	0.030
2011	2,505	34	0	0%	0.216	0.016
2012	2,209	23	0	0%	0.356	0.060
2013	2,510	26	0	0%	0.311	0.042
2014	2,154	35	0	0%	0.207	0.014

Probability values denote the likelihood of detecting zero parasitism for a given sample size of egg sacs assuming a parasitism rate of either 4.4% or 11.475%, which were the rates from Kangerlussuaq for years in which parasitism was detected from the lowest and highest sample sizes, respectively. Total trap days account for the number of trapping days and the number of deployed pitfall traps in a given year (also see ***Tables S1–S3****).*

Determining Egg Sac Parasitism Rates

We inspected a total of 1,088 wolf spider egg sacs from Zackenberg (high-arctic site) and 538 egg sacs from Kangerlussuaq (low-arctic site) for evidence of parasitism (**Table 1**). To investigate the extent to which sample sizes may have compromised our ability to detect egg sac parasitism events in certain sites and years, we evaluated the likelihood of observing a given number of events under different assumptions of parasitism rates. Specifically, we stochastically simulated 100,000 samples from a population with either 4.4 or 11.5% parasitism rates (i.e., the rates of parasitism observed in the southern site in years in which parasitism was detected and sampling effort was, respectively, smallest and largest) and compared the resulting distribution with the observed value.

Body Size as a Predictor of Egg Sac Parasitism

We used binary logistic regression models to test whether the egg sacs of larger females have higher probabilities of being parasitized. The body size of adult female *P. glacialis* that were clearly identified as egg sac carriers was quantified by measuring carapace width (Hagstrum, 1971) using digital calipers (Diesella, Kolding, Denmark). All egg-sac carrying adult females from Kangerlussuaq were measured in samples from 2010 to 2012 ($N = 203$). From Zackenberg, we were only able to measure female body sizes for 292 of the 1,088 samples, as egg sacs were frequently separated from the adult females. Sampling year and date at which the egg sacs were obtained were also included as predictors in our models. All statistical analyses were performed in RCoreTeam (2017).

Identification of Egg Sac Parasitoids

We submitted the parasitoid tissue (either eggs or larvae) from seven parasitized egg sacs that were collected from Kangerlussuaq in 2017 to LifeScanner (http://lifescanner.net/) for sequence analysis. LifeScanner is a DNA barcoding service that DNA barcodes user-provided samples using standardized sampling kits, a mobile app, and standardized laboratory workflow. The project is based at the Centre for Biodiversity Genomics (http://biodiversitygenomics.net/) at the University of Guelph;

Taxonomic identifications are generated through the Barcode of Life Data (BOLD) Systems ID Engine (Ratnasingham and Hebert, 2007).

RESULTS

At Kangerlussuaq, we found evidence of egg sac parasitism in 3 of the 5 sampling years. Rates of egg sac parasitism varied between 0 and 13.3% from these collection years (**Table 1**). In 2017, the year with the largest sample size ($N = 305$), parasitism was observed in 11.5% of all collected egg sacs (**Table 1**). Incidences of egg sac parasitism were most commonly detected in wet graminoid habitat at Kangerlussuaq (**Table S4**). While we did not find evidence of parasitism in egg sacs from Kangerlussuaq in 2010 or 2012, our stochastic simulations suggest that these absences are likely to be a product of low sample sizes in those years (**Table 1**). Results from the binomial generalized linear model indicated that incidences of egg sac parasitism at Kangerlussuaq were not significantly related to female body size, sampling date, or sampling year (all $p > 0.087$; **Table S5**).

The majority of parasitized egg sacs from Kangerlussuaq were found in mid-July. However, we also detected parasitism as early as June 21 in 1 year (**Table S4**). Each parasitized egg sac contained between 1 and 10 wasp eggs, larvae, or pupae (**Figure 1B**, **Table S4**). Of the seven samples from 2017 from Kangerlussuaq that were submitted for DNA barcoding, three specimens were successfully identified as *Gelis micrurus* (Forster, 1850; **Figure 1C**) with 100% confidence using the BOLD database (Ratnasingham and Hebert, 2013). The sequences and associated data for identified parasitoids (i.e., GPS coordinates of collection locality and images of parasitoid tissues) are available from BOLD (www.boldsystems.org) through the following Barcode Index Number: BOLD:ACF8182 and DOI: dx.doi.org/10.5883/DS-LSGNLD18. We note that *G. micrurus* is a new species record for the area, which is not included in the recent identification manual for Greenland (Böcher et al., 2015). Identification attempts for the other four samples were not successful, perhaps due to the limited amount of tissue submitted for wasp eggs and/or larvae.

In sharp contrast, we did not find any evidence of egg sac parasitism at Zackenberg, the high-arctic site, despite having sampled at that site over a longer time period (18 years) and generally having larger samples than at Kangerlussuaq (**Table 1**). Simulation tests indicate that failure to detect parasitized egg sacs would have been highly unlikely given our sample sizes if Zackenberg's parasitism rate was similar to either the highest or lowest parasitism rate measured at Kangerlussuaq (**Table 1**).

DISCUSSION

We found no evidence that the wolf spider *P. glacialis*, a dominant tundra predator in Greenland, has experienced any measurable predation pressure by egg sac parasitoids at the northern edge of its range over an 18-year period of the recent past. This finding is surprising because wolf spider egg sacs in general, are commonly parasitized across arctic (Bowden and Buddle, 2012a) and temperate (Edgar, 1971) ecosystems, and because our own measurements indicate that *P. glacialis* in particular, is exposed to parasitism in southwestern Greenland. Whether arctic wolf spiders have "escaped" parasitism as a result of local extinction of the parasitoid at the northern site or because this population of wolf spiders has never been exposed to parasitism is unclear. Nevertheless, our results demonstrate that geographic variability in the rate of parasitism is high for this widespread wolf spider species. In the context of a rapidly warming Arctic, such high variability in the strength of host-parasite species interactions also suggests that population-level responses to environmental change are unlikely to be uniform throughout a species' range.

Understanding what drives wolf spider population dynamics is important because these predators comprise a large proportion of worldwide spider abundance and diversity (World Spider Catalog, 2018). In the Arctic, this endeavor is even more relevant, because wolf spiders are one of the most widespread and locally abundant arthropods (Bowden and Buddle, 2010; Wyant et al., 2011; Hansen et al., 2016a; Koltz et al., 2018b). In terms of host-parasite dynamics, the absence of parasitism in Zackenberg is especially surprising when considering that this site hosts two wasp species (Wirta et al., 2016) of a genus known to parasitize wolf spiders in North America (Bowden and Buddle, 2012a) and Kangerlussuaq (this study). At least one of these species, however, is a Lepidopteran parasitoid (on the family Erebidae; Wirta et al., 2015) that is unlikely to prey on wolf spider egg sacs. Given that the population viability of a species is typically most fragile at the edge of its range (Lawton, 1993), it is possible that a local absence of parasitism has enabled *P. glacialis* to occur further north than would otherwise be expected. If that is the case, then the possibility of parasitoid range expansions under climate change (Chen et al., 2011) could threaten the continued persistence of this species in the northern High Arctic. Because wolf spiders generate trophic cascades at multiple levels within the food web that can ultimately affect important processes like decomposition and nutrient cycling (Wise et al., 1999; Scheu, 2001; Koltz et al., 2018b), such distributional changes, although seemingly insignificant, could have important ecological impacts.

There are a number of possible explanations for the local absence of a viable parasitoid of wolf spider egg sacs at Zackenberg, including the fact that Zackenberg has a more extreme climate than Kangerlussuaq (**Figure S1**). Likewise, slight differences in plant community composition and other abiotic conditions contribute to which arthropod species are present at a given site (Hansen et al., 2016a,b). Local geological and glacial histories also strongly affect the distribution of species, the interactions between them, and their co-evolutionary dynamics (Anderson and Ferree, 2010; Stewart et al., 2010; Yeakel et al., 2013; Ávila-Jiménez et al., 2019). Zackenberg is more recently deglaciated compared to other areas of the Arctic where wolf spider egg sac parasitism rates are high, such as NW North America (Tarasov et al., 2012; Lecavalier et al., 2014). Various barriers to dispersal, such as the Greenland ice sheet, may have prohibited potential egg sac parasitoids from accessing NE Greenland. Such dispersal barriers are likely to be particularly limiting to apterous, or wingless, arthropods, including females from many *Gelis* species, which may contribute to these parasitoids having more narrow spatial distributions.

The geographic variability in the strength of predation pressure by egg sac parasitoids on *P. glacialis* that we demonstrate here suggests that life history strategies of this wolf spider species and in turn, its population-level responses to climate change, may vary across its range. For example, although sampling date was not a significant predictor of parasitism (**Table S5**), our data suggest that incidences of egg sac parasitism occur during the peak of the growing season (**Table S4**). Early season production of egg sacs by female wolf spiders could thus be one strategy to avoid parasitism, but the timing and duration of this period is expected to shift as the Arctic continues to warm. The degree to which parasitism pressure plays a role in dictating various life history traits of this abundant predator and other arctic arthropods, including their phenology, behavior, and foraging decisions (e.g., Schmid Hempel, 2011), warrants further investigation, particularly where climate change may affect the timing and strength of these species interactions.

DATA AVAILABILITY

All datasets analyzed for this study are included in the manuscript and the **Supplementary Files**.

AUTHOR CONTRIBUTIONS

AK conceived of the idea for the research. LC collected field samples. AK, JB, LC completed the lab work. AK, TH, LC, JB, and EP wrote the paper.

FUNDING

AK was supported by a grant from Aarhus University Research Foundation and the National Geographic Society Committee for Research and Exploration. This research was also supported by U.S. National Science Foundation Award numbers 1106401 to AK, 1506155 to LC, and 1107381 and 0902125 to EP. Grace Stillwell, supported by Dartmouth's Women In Science Program, assisted with lab work. This work is a contribution to the Arctic Science Partnership (ASP), asp-net.org.

ACKNOWLEDGMENTS

We are grateful to Michael Avery and the Greenland Ecosystem Monitoring programme for providing us access to the samples for this study and to the Natural History Museum, Aarhus for curation of the Zackenberg specimens.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00250/full#supplementary-material

REFERENCES

Anderson, M. G., and Ferree, C. E. (2010). Conserving the stage: climate change and the geophysical underpinnings of species diversity. *PLoS ONE* 5:e11554. doi: 10.1371/journal.pone.0011554

Avery, M., and Post, E. (2013). Record of a Zoophthora sp.(Entomophthoromycota: Entomophthorales) pathogen of the irruptive noctuid moth Eurois occulta (Lepidoptera) in West Greenland. *J. Invertebr. Pathol.* 114, 292–294. doi: 10.1016/j.jip.2013.09.005

Ávila-Jiménez, M. L., Solhøy, T., Gwiazdowicz, D. J., Fjellberg, A., Dózsa-Farkas, K., Monson, F., et al., (2019). The terrestrial invertebrate fauna of Edgeøya, Svalbard: Arctic landscape community composition reflects biogeography patterns. *Polar Biol.* 42, 837–850. doi: 10.1007/s00300-019-02471-x

Bliss, L. (1981). 1.2 North American and Scandinavian tundras and polar deserts. *Tundra Ecosyst.* 25:8.

Böcher, J., Kristensen, N. P., Pape, T., and Vilhelmsen, L. (2015). *The Greenland Entomofauna: An Identification Manual of Insects, Spiders and Their Allies*. Leiden: Brill. doi: 10.1163/9789004261051

Bowden, J., and Buddle, C. (2012a). Egg sac parasitism of Arctic wolf spiders (Araneae: Lycosidae) from northwestern North America. *J. Arachnol.* 40, 348–350. doi: 10.1636/P11-50.1

Bowden, J., and Buddle, C. (2012b). Life history of tundra-dwelling wolf spiders (Araneae: Lycosidae) from the Yukon Territory, Canada. *Can. J. Zool.* 90, 714–721. doi: 10.1139/z2012-038

Bowden, J. J., and Buddle, C. M. (2010). Determinants of ground-dwelling spider assemblages at a regional scale in the Yukon Territory, Canada. *Ecoscience* 17, 287–297. doi: 10.2980/17-3-3308

Bowden, J. J., Hansen, O. L. P., Olsen, K., Schmidt, N. M., and Høye, T. T. (2018). Drivers of inter-annual variation and long-term change in High-Arctic spider species abundances. *Polar Biol.* 41, 1635–1649. doi: 10.1007/s00300-018-2351-0

Chen, I.-C., Hill, J. K., Ohlemüller, R., Roy, D. B., and Thomas, C. D. (2011). Rapid range shifts of species associated with high levels of climate warming. *Science* 333, 1024–1026. doi: 10.1126/science.1206432

Cobb, L. M., and Cobb, V. A. (2004). Occurrence of parasitoid wasps, *Baeus* sp. and *Gelis* sp., in the egg sacs of the wolf spiders Pardosa moesta and Pardosa sternalis (Araneae, Lycosidae) in southeastern Idaho. *Can. Field-Nat.* 118, 122–123. doi: 10.22621/cfn.v118i1.894

Dondale, C., and Redner, J. (1990). "Part-17: The insects and arachnids of Canada," in *The wolf spiders, Nurseryweb Spiders, and Lynx Spiders of Canada and Alaska. Araneae: Lycosidae, Pisauridae, and Oxyopidae.* Ottawa, ON: Agriculture Canada.

Edgar, W. D. (1971). Aspects of the ecology and energetics of the egg sac parasites of the wolf spider Pardosa lugubris (Walckenaer). *Oecologia* 7, 155–163. doi: 10.1007/BF00346357

Ernst, C. M., Hanelt, B., and Buddle, C. M. (2016). Parasitism of ground beetles (Coleoptera: Carabidae) by a new species of hairworm (Nematomorpha: Gordiida) in Arctic Canada. *J. Parasitol.* 102, 327–335. doi: 10.1645/15-863

Fellowes, M. D. E., and Godfray, H. C. J. (2000). The evolutionary ecology of resistance to parasitoids by Drosophila. *Heredity* 84:1. doi: 10.1046/j.1365-2540.2000.00685.x

Godfray, H., Hassell, M., and Holt, R. (1994). The population dynamic consequences of phenological asynchrony between parasitoids and their hosts. *J. Anim. Ecol.* 63, 1-10. doi: 10.2307/5577

Hagemoen, R. I. M., and Reimers, E. (2002). Reindeer summer activity pattern in relation to weather and insect harassment. *J. Anim. Ecol.* 71, 883–892. doi: 10.1046/j.1365-2656.2002.00654.x

Hagstrum, D. W. (1971). Carapace width as a tool for evaluating the rate of development of spiders in the laboratory and the field. *Ann. Entomol. Soc. Am.* 64, 757–760. doi: 10.1093/aesa/64.4.757

Hansen, R. R., Hansen, O. L. P., Bowden, J. J., Normand, S., Bay, C., Sørensen, J. G., et al., (2016a). High spatial variation in terrestrial arthropod species diversity and composition near the Greenland ice cap. *Polar Biol.* 39, 2263–2272. doi: 10.1007/s00300-016-1893-2

Hansen, R. R., Hansen, O. L. P., Bowden, J. J., Treier, U. A., Normand, S., and Høye, T. (2016b). Meter scale variation in shrub dominance and soil moisture structure Arctic arthropod communities. *PeerJ* 4:e2224. doi: 10.7717/peerj.2224

Høye, T. T., and Culler, L. E. (2018). Tundra arthropods provide key insights into ecological responses to environmental change. *Polar Biol.* 41, 1523–1529. doi: 10.1007/s00300-018-2370-x

Høye, T. T., and Forchhammer, M. C. (2008). Phenology of high-arctic arthropods: effects of climate on spatial, seasonal, and inter-annual variation. *Adv. Ecol. Res.* 40, 299–324. doi: 10.1016/S0065-2504(07)00013-X

Høye, T. T., and Hammel, J. U. (2010). Climate change and altitudinal variation in sexual size dimorphism of arctic wolf spiders. *Clim. Res.* 41, 259–265. doi: 10.3354/cr00855

Høye, T. T., Hammel, J. U., Fuchs, T., and Toft, S. (2009). Climate change and sexual size dimorphism in an Arctic spider. *Biol. Lett.* 5, 542–544. doi: 10.1098/rsbl.2009.0169

Jensen, L. M., Rasch, M., and Schmidt, N. M. (ed) (2013). *Zackenberg Ecological Research Operations, 18th Annual Report, 2012*. Aarhus University; DCE-Danish Centre for Environment and Energy, 122.

Koltz, A. M., Asmus, A., Gough, L., Pressler, Y., and Moore, J. C. (2018a). The detritus-based microbial-invertebrate food web contributes disproportionately to carbon and nitrogen cycling in the Arctic. *Polar Biol.* 41, 1531–1545. doi: 10.1007/s00300-017-2201-5

Koltz, A. M., Classen, A. T., and Wright, J. P. (2018b). Warming reverses top-down effects of predators on belowground ecosystem function in Arctic tundra. *Proc. Natl. Acad. Sci. U.S.A.* 115, E7541–E7549. doi: 10.1073/pnas.1808754115

Koltz, A. M., Schmidt, N. M., and Høye, T. T. (2018c). Differential arthropod responses to warming are altering the structure of Arctic communities. *R. Soc. Open Sci.* 5:171503. doi: 10.1098/rsos.171503

Kukal, O., and Kevan, P. G. (1987). The influence of parasitism on the life history of a high arctic insect, Gynaephora groenlandica (Wöcke) (Lepidoptera: Lymantriidae). *Can. J. Zool.* 65, 156–163. doi: 10.1139/z87-022

Kutz, S. J., Hoberg, E. P., Nishi, J., and Polley, L. (2002). Development of the muskox lungworm, Umingmakstrongylus pallikuukensis (Protostrongylidae), in gastropods in the Arctic. *Can. J. Zool.* 80, 1977–1985. doi: 10.1139/z02-197

Lawrence, K. L., and Wise, D. H. (2000). Spider predation on forest-floor Collembola and evidence for indirect effects on decomposition. *Pedobiologia* 44, 33–39. doi: 10.1078/S0031-4056(04)70026-8

Lawton, J. H. (1993). Range, population abundance and conservation. *Trends Ecol. Evol.* 8, 409–413. doi: 10.1016/0169-5347(93)90043-O

Lecavalier, B. S., Milne, G. A., Simpson, M. J., Wake, L., Huybrechts, P., Tarasov, L., et al., (2014). A model of Greenland ice sheet deglaciation constrained by observations of relative sea level and ice extent. *Quat. Sci. Rev.* 102, 54–84. doi: 10.1016/j.quascirev.2014.07.018

Lensing, J. R., and Wise, D. H. (2006). Predicted climate change alters the indirect effect of predators on an ecosystem process. *Proc. Natl. Acad. Sci. U.S.A.* 103, 15502–15505. doi: 10.1073/pnas.0607064103

Low, P. A., McArthur, C., and Hochuli, D. F. (2014). Dealing with your past: experience of failed predation suppresses caterpillar feeding behaviour. *Anim. Behav.* 90, 337–343. doi: 10.1016/j.anbehav.2014.02.020

Marshall, S. D., and Gittleman, J. L. (1994). Clutch size in spiders: is more better? *Funct. Ecol.* 8, 118–124. doi: 10.2307/2390120

Meltofte, H., and Rasch, M. (2008). The study area at Zackenberg. *Adv. Ecol. Res.* 40, 101–110. doi: 10.1016/S0065-2504(07)00005-0

Moerschel, F. M. (1999). Use of climatic data to model the presence of oestrid flies in carbiou herds. *J. Wildl. Manage.* 63, 588–593. doi: 10.2307/3802647

Post, E. (2013). *Ecology of Climate Change: The Importance of Biotic Interactions*. Princeton, NJ: Princeton University Press. doi: 10.23943/princeton/9780691148472.001.0001

Ratnasingham, S., and Hebert, P. D. (2007). BOLD: the barcode of life data system (http://www.barcodinglife.org). *Mol. Ecol. Notes* 7, 355–364. doi: 10.1111/j.1471-8286.2007.01678.x

Ratnasingham, S., and Hebert, P. D. (2013). A DNA-based registry for all animal species: the Barcode Index Number (BIN) system. *PLoS ONE* 8:e66213. doi: 10.1371/journal.pone.0066213

RCoreTeam (2017). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. Vienna.

Scheu, S. (2001). Plants and generalist predators as links between the below-ground and above-ground system. *Basic Appl. Ecol.* 2, 3–13. doi: 10.1078/1439-1791-00031

Schmid Hempel, P. (2011). *Evolutionary Parasitology: The Integrated Study of Infections, Immunology, Ecology, and Genetics* (Oxford; New York: Oxford University Press).

Stahlhut, J. K., Fernández-Triana, J., Adamowicz, S. J., Buck, M., Goulet, H., Hebert, P. D., et al., (2013). DNA barcoding reveals diversity of Hymenoptera and the dominance of parasitoids in a sub-arctic environment. *BMC Ecol.* 13:2. doi: 10.1186/1472-6785-13-2

Stewart, J. R., Lister, A. M., Barnes, I., and Dalén, L. (2010). Refugia revisited: individualistic responses of species in space and time. *Proc. R. Soc. Lond. B. Biol. Sci* 277, 661–671. doi: 10.1098/rspb.2009.1272

Tarasov, L., Dyke, A. S., Neal, R. M., and Peltier, W. R. (2012). A data-calibrated distribution of deglacial chronologies for the North American ice complex from glaciological modeling. *Earth Planet. Sci. Lett.* 315-316, 30–40. doi: 10.1016/j.epsl.2011.09.010

Wirta, H., Várkonyi, G., Rasmussen, C., Kaartinen, R., Schmidt, N. M., Hebert, P., et al., (2016). Establishing a community-wide DNA barcode library as a new tool for arctic research. *Mol. Ecol. Resour.* 16, 809–822. doi: 10.1111/1755-0998.12489

Wirta, H. K., Vesterinen, E. J., Hambäck, P. A., Weingartner, E., Rasmussen, C., Reneerkens, J., et al. (2015). Exposing the structure of an Arctic food web. *Ecol. Evol.* 5, 3842–3856. doi: 10.1002/ece3.1647

Wise, D., Snyder, W., Tuntibunpakul, P., and Halaj, J. (1999). Spiders in decomposition food webs of agroecosystems: theory and evidence. *J. Arachnol.* 27, 363–370.

World Spider Catalog (2018). *The World Spider Catalog, Version 18.5.in Museum.* Bern. Available online at: http://wsc.nmbe.ch/

Wyant, K. A., Draney, M. L., and Moore, J. C. (2011). Epigeal spider (Araneae) communities in moist acidic and dry heath tundra at Toolik Lake, Alaska. *Arctic Antarctic Alp. Res.* 43, 301–312. doi: 10.1657/1938-4246-43.2.301

Yeakel, J. D., Guimarães, P. R., Bocherens, H., and Koch, P. L. (2013). The impact of climate change on the structure of Pleistocene food webs across the mammoth steppe. *Proc. R. Soc. Lond. B Biol. Sci.* 280:20130239. doi: 10.1098/rspb.2013.0239

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

MINI REVIEW
published: 09 July 2019
doi: 10.3389/fevo.2019.00262

Plant-Vector-Pathogen Interactions in the Context of Drought Stress

Adrianna Szczepaniec[1]* *and Deborah Finke*[2]

[1] *Department of Entomology, Texas A&M AgriLife Research, Amarillo, TX, United States,* [2] *Division of Plant Sciences, University of Missouri, Columbia, MO, United States*

Plants regularly encounter stress, and their responses to abiotic and biotic stressors have been a focus of research for decades. Stress caused by drought is one of the most often studied abiotic stresses owing to the increase in the incidence of drought driven by climate change. Severe drought has been shown to elicit a whole-plant response guided by key phytohormones, which not only respond to water stress but also play a critical role in the response of plants to biotic stress imposed by herbivores and pathogens. This is especially relevant for insect-transmitted pathogen systems, where plants, herbivores, and pathogens are linked in a web of direct and indirect interactions. Few studies have thus far explored the complex nature of drought-mediated tripartite interactions, however, and our ability to generalize and predict how plants respond to herbivore-transmitted pathogens while simultaneously countering the consequences of drought remains limited. The goal of this mini-review is to assess the current state of the field regarding the molecular mechanisms underlying plant responses to the combined effects of drought and simultaneous herbivory and pathogen transmission and their ecological consequences. We discuss plant responses to drought, herbivory, and pathogens as distinct and concurrent stresses, and highlight the implications of the tripartite interactions on insect vector and pathogen suppression in agroecosystems. This review provides a framework for future research linking generalized molecular responses in drought-stressed plants to tripartite species interactions and the ecology of insect-transmitted pathogens in the context of modern agriculture and water deficit driven by climate change.

Keywords: herbivory, pathogen transmission, plant-insect interactions, plant-pathogen interactions, water deficiency, climate change

OPEN ACCESS

Edited by:
Lora A. Richards,
University of Nevada, Reno,
United States

Reviewed by:
Muthappa Senthil-Kumar,
National Institute of Plant Genome Research (NIPGR), India
Karsten Mody,
Darmstadt University of Technology, Germany

***Correspondence:**
Adrianna Szczepaniec
aszczepaniec@tamu.edu

Specialty section:
This article was submitted to Population and Evolutionary Dynamics,
a section of the journal
Frontiers in Ecology and Evolution

Received: *28 March 2019*
Accepted: *24 June 2019*
Published: *09 July 2019*

Citation:
Szczepaniec A and Finke D (2019) Plant-Vector-Pathogen Interactions in the Context of Drought Stress. Front. Ecol. Evol. 7:262. doi: 10.3389/fevo.2019.00262

INTRODUCTION

Climate change is driving an increase in incidence of drought at a global scale and extreme water deficiency is a significant impediment to plant fitness (Boyer, 1982, 2010; Bray et al., 2000). The impact of drought stress on plants extends beyond primary plant productivity, however, and results in profound changes in phytohormones and secondary metabolites involved in plant defenses (Asselbergh et al., 2008), the consequences of which for plant resistance to insects and pathogens are still poorly understood.

Plants commonly contend with multiple abiotic and biotic stresses simultaneously and must be capable of responding in a coordinated and dynamic fashion. This is particularly true for vector-borne plant pathogen systems where reciprocal direct and indirect feedback among plants, vectors, and pathogens results in changes in performance and behavior of the vectors and pathogens (**Figure 1**). Yet studies rarely measure the responses of plants to multiple stresses and even fewer studies have quantified the molecular mechanisms and ecological outcomes of tripartite plant-vector-pathogen interactions in the context of drought. However, the potential for non-additive effects of multiple stresses has contributed to a growing consensus that simplified investigations of pairwise interactions may not be sufficient (Mittler and Blumwald, 2010; Atkinson and Urwin, 2012; Atkinson et al., 2013; Prasch and Sonnewald, 2013; Bergès et al., 2018). Thus, the goal of this review is to outline the current state of the field and synthesize research on responses of drought-stressed plants to pathogen and herbivore attack. While reviews of the molecular mechanisms and consequences of single and multiple stresses have been assembled previously (e.g., Atkinson et al., 2015), this is the first synthesis of research focused specifically on the drought-mediated tripartite interactions among plants, insect vectors, and vector-borne pathogens. We highlight the general responses of plants to drought and biotic stress in order to contextualize the concepts of multi-stress responses. We examine the specific consequences of water deficit for plant interactions with pathogens and herbivores individually, and synthesize research on drought-mediated tripartite interactions. We also highlight the consequences of these responses for species interactions and for insect vector and pathogen suppression in agroecosystems. This mini-review aims to create a framework for linking key molecular mechanisms in plants under simultaneous drought and pathogen/vector attack to specific and predictable interactions among species.

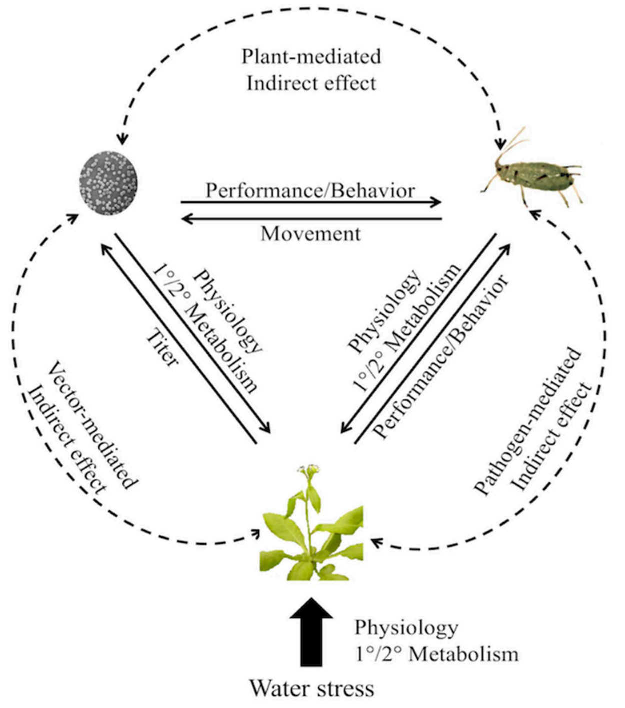


FIGURE 1 | Direct and indirect interactions among insect vectors, pathogens, and drought-stressed host plants, with examples of responding traits. Solid lines are direct effects, dashed lines are indirect effects. Arrows indicate the effect of one factor on another. Water deficit affects plant primary (differentiation) and secondary (defense) metabolism. These changes can alter reciprocal direct and indirect feedback among the plants, vectors, and pathogens and result in changes in performance and behavior of the vectors and pathogens.

OVERVIEW OF GENERAL MOLECULAR RESPONSES OF PLANTS TO BIOTIC STRESS AND DROUGHT

Plants utilize a diverse array of responses to attack by herbivores and pathogens. Metabolic responses are regulated by interconnected signaling pathways in which salicylic acid (SA) and jasmonic acid (JA) play a key role (Stout et al., 2006). The SA-mediated response pathway was originally implicated in plant resistance to pathogens (Kunkel and Brooks, 2002; Shah, 2003) and the JA-mediated pathway was thought to confer resistance to insects (Howe et al., 1996). We now know, however, that these pathways are intricate, interconnected (Walling, 2000; Koornneef and Pieterse, 2008; Thaler et al., 2010), and complicated by reciprocal down-regulation (i.e., "crosstalk") (Thaler et al., 2002) that requires plants to integrate and prioritize their defenses (Bostock, 2005). Consequently, plant-mediated indirect interactions between pathogens and herbivores can lead to either induced resistance or induced susceptibility as a result of a multiple-enemy attack (Bostock, 2005).

The JA- and SA-mediated pathways overlap with the pathways that regulate plant responses to drought regulated by the hormone abscisic acid (ABA) (Ramanjulu and Bartels, 2002; Bartels and Sunkar, 2005; Urano et al., 2009; Harb et al., 2010). ABA modulates plants responses to drought stress, and its accumulation results in increases of reactive oxygen species, changes in cell turgor, and stomatal closure (Lee and Luan, 2012). ABA also prioritizes plant responses to biotic stress (Asselbergh et al., 2008), and interactions among ABA, JA, and SA have been extensively studied (Lee and Luan, 2012; Kazan, 2015; Nguyen et al., 2016).

Abscisic acid can dramatically re-shape interactions between JA and SA (Yasuda et al., 2008; Fan et al., 2009; Kazan, 2015; Muñoz-Espinoza et al., 2015; Guo et al., 2016; Wei et al., 2018), which illustrates that plant responses to biotic stress are frequently altered under drought. In general, accumulations of ABA are accompanied by a decrease in SA (Yasuda et al., 2008; Fan et al., 2009; Muñoz-Espinoza et al., 2015; Guo et al., 2016; Liu et al., 2016; Nachappa et al., 2016). However, an increase in ABA can also result in concomitant rise in SA (Audenaert et al., 2002; Anderson et al., 2004; Mauch-Mani and Mauch, 2005). Interactions between ABA and JA are equally variable, and ABA can have synergistic and antagonistic interactions with JA (Asselbergh et al., 2008). For example, changes in proteins involved in JA synthesis and accumulation were noted in a diversity of plants exposed to water deficit (Fan et al., 2009; Harb et al., 2010; Bonhomme et al., 2012; Ahmad et al., 2016; Haider et al., 2017), while other studies have indicated lack of JA induction in drought-stressed plants (Muñoz-Espinoza et al., 2015; Nachappa et al., 2016). The exact nature of interactions among the defensive responses of plants to biotic and abiotic stresses remains understudied, however, despite their importance to plant-pathogen, plant-insect, and plant-pathogen-vector interactions (Asselbergh et al., 2008; Erb et al., 2011; Davis et al., 2015b; Nachappa et al., 2016).

PATHOGEN-PLANT INTERACTIONS IN WATER DEFICIT

In general, there is an inverse relationship between drought and resistance to pathogens driven by the negative cross talk between ABA and SA. For example, an ABA-mediated decrease in SA enhanced the susceptibility of Arabidopsis and tomato to *Pseudomonas syringae* (Mohr and Cahill, 2007) and tomato to *Botrytis cinereal* (Audenaert et al., 2002). Further, applications of exogenous ABA suppressed systemic acquired resistance (SAR) to pathogens, while induction of SAR decreased expression of ABA-biosynthesis genes, suggesting that the antagonistic interaction between these pathways is reciprocal (Yasuda et al., 2008). It is noteworthy that the crosstalk between ABA and SA can be accompanied by a synergism between ABA and JA in some (Fan et al., 2009), but not all cases (Anderson et al., 2004; Yasuda et al., 2008), emphasizing the complex and intricate links among these phytohormones.

There are instances, however, where ABA or drought stress in general promote enhanced plant resistance to pathogens. Stomatal closure induced by ABA to reduce transpiration can prevent pathogen entry (Melotto et al., 2006), and ABA signaling has been implicated in defense against necrotrophic pathogens, which induce JA-mediated defenses. This outcome suggests that instances of ABA-JA synergism can have positive outcomes for plant resistance and can vary across and within plant species (Asselbergh et al., 2008; Ramegowda and Senthil-Kumar, 2015).

INSECT VECTOR-PLANT INTERACTIONS IN WATER DEFICIT

Sap-feeding herbivores including insect vectors are predicted to respond positively to drought-stressed plants due to enhanced nutritional quality (Joern and Mole, 2005; Yan et al., 2015; Nachappa et al., 2016; Sconiers and Eubanks, 2017; Florencio-Ortiz et al., 2018). Specifically, physiological changes stemming from ABA-regulated osmotic adjustments that enable plants to maintain cell turgor by accumulating solutes (Ingram and Bartels, 1996; Harb et al., 2010) can benefit herbivores through increases in nitrogen and carbohydrate availability (Huberty and Denno, 2004). However, empirical support for this prediction is mixed.

Drought can have particularly complex and inconsistent effects on aphids, common vectors of phytopathogens. Aphids can respond positively (Tariq et al., 2012), negatively (Pons and Tatchell, 1995; Mcvean and Dixon, 2001; Hale et al., 2003; Simpson et al., 2012; Guo et al., 2016; Nachappa et al., 2016), or neutrally (Larsson and Björkman, 1993; Nachappa et al., 2016; Sconiers and Eubanks, 2017) to drought-stressed plants. The variable effects of drought on aphid fitness may be driven by the intensity of water deficit, whereby performance of a single aphid species increases under moderate stress but decreases under severe drought stress (Banfield-Zanin and Leather, 2015). Furthermore, aphids are highly susceptible to changes in osmotic pressure, which can constrain their ability to exploit elevated nutrients in drought-stressed plants, as was demonstrated in the lack of concomitant increase in performance of soybean aphids (*Aphis glycines*) exposed to soybean (*Glycine max*) under drought stress (Nachappa et al., 2016). Increased concentrations of JA-regulated glucosinolates have also been implicated in the lack of an enhanced aphid performance despite higher levels of nitrogen in stressed plants (Tariq et al., 2012).

Thrips and whiteflies are two other major insect vectors of phytopathogens, but few studies have explored the impact of drought on their fitness as vectors. Whiteflies, for example, were found to favor plants under low rates of irrigation (Paris et al., 1993; Skinner, 1996), while their oviposition was decreased by water deficit (Inbar et al., 2001). Similarly, thrips were more abundant on plants under water deficit and benefited from intermittent drought stress in cotton (Sconiers and Eubanks, 2017). Further, intense and severe drought was linked to outbreaks of *Thrips tabaci* in onion, which resulted in significant losses to crop productivity (Fournier et al., 1995).

The consequences of drought for vector fitness may also depend on its infection status. For instance, water deficit in tomato increased survival of *Bactericera cockerelli* (Hemiptera: Triozidae) infected with a bacterium *Candidatus* Liberibacter solanacearum, and resulted in a 60% increase in the number of adults (Huot and Tamborindeguy, 2017). Further, populations of infectious *Rhopalosiphum padi* aphids grew faster on wheat infected with Barley yellow dwarf virus compared to virus-free plants, and the effect was stronger when plants were under drought stress compared to unstressed plants (Davis et al., 2015a). This study did not explore how non-infected aphids responded to drought, which may further alter the outcome of plant-insect vector interactions (Hale et al., 2003). There is evidence that pathogens can lower the fitness of their insect vectors (Donaldson and Gratton, 2007; Nachappa et al., 2011; de Oliveira et al., 2014), and pathogen-mediated vector traits can interact with drought-mediated changes in plants. This was illustrated in the case of infectious soybean aphids, which unlike their virus-free counterparts, were unaffected by water status of the plants (Castle and Berger, 1993). Overall, water deficit exposure appears to have no effect or improve performance of non-infectious aphids, but further research is necessary to validate this effect.

PLANT-MEDIATED EFFECTS OF PHYTOPATHOGENS ON THEIR VECTORS

Increasing evidence documents that phytopathogens manipulate the performance and behavior of their insect vectors by altering the chemical or physical properties of their shared host plant (Casteel and Falk, 2016; Eigenbrode et al., 2018; Mauck et al., 2018). These phytopathogen-induced vector and host plant phenotypes are typically thought to be conducive to pathogen dissemination in the environment (Mauck et al., 2018). For example, pathogen-induced injury and altered volatile emissions can increase the attractiveness of infected plants to vectors (Eigenbrode et al., 2002; Jiménez-Martínez et al., 2004; Belliure et al., 2005; Bosque-Pérez and Eigenbrode, 2011; Hodge et al., 2011; Shapiro et al., 2012; Ogada et al., 2013). However, the exact nature of the interaction likely depends on the mechanism of pathogen transmission (Castle and Berger, 1993; Mauck et al., 2012; Eigenbrode et al., 2018). Pathogens that require long, uninterrupted feeding periods for efficient transmission may benefit by improving host plant quality and enhancing vector settling behavior (Belliure et al., 2005; Ogada et al., 2013). Alternatively, pathogens that require only a brief feeding probe for transmission may promote their own proliferation by inducing deceptive visual or chemical cues to attract vectors to unpalatable or low-quality plants from which vectors quickly emigrate after an initial feeding attempt (Purcell and Almeida, 2005; Mauck et al., 2010). While it is likely that drought will alter the plant-mediated effects of pathogens on their vectors, we currently lack the necessary understanding of the mechanistic basis of plant virus manipulation to predict these complicated multi-stress outcomes (Mauck et al., 2019).

INSECT TRANSMISSION OF PATHOGENS UNDER DROUGHT STRESS

The efficiency of pathogen transmission by insects is likely to be influenced by drought stress. Water deficit can directly affect plant resistance to pathogens and vectors via the overlap and crosstalk in the pathways regulated by the key phytohormones. However, water deficit can also affect plant infection risk indirectly by altering vector behaviors or preferences that are critical to transmission and are otherwise manipulated by vector-borne pathogens to their benefit in unstressed plants (reviewed in Mauck et al., 2016). For example, a loss in plant cell turgor pressure may alter feeding behaviors, such as the number of times a vector probes a plant (Krugner and Backus, 2014; Nachappa et al., 2016). Furthermore, low turgor pressure may enhance the plant-to-plant movement of vectors as they search for acceptable hosts, thereby increasing the number of plants contacted by individual vectors. These recent studies support the idea that drought may transiently and indirectly increase plant susceptibility to insect-transmitted pathogens, particularly if water deficit is severe and stimulates vector movement and probing frequency.

In fact, there is accumulating evidence to support the indirect effects of drought-stressed host plants on pathogen transmission. Drought stress increased aphid transmission of Cauliflower mosaic virus (CMV) and Turnip mosaic virus to *Brassica rapa* (van Munster et al., 2017). However, water deficit had the opposite effect on the aphid-transmitted Turnip yellow virus (TuYV) in Arabidopsis, whereby transmission of TuYV was significantly reduced due to lower viral accumulation in aphids feeding on drought-stressed plants (Yvon et al., 2017). Nachappa et al. (2016) found that incidence and transmission of the aphid-transmitted Soybean mosaic virus were also lower in soybean exposed to drought compared to well-watered plants, likely owing to a decrease in time required for the aphids to access phloem in the stressed plants. Further, drought affected aphid transmission of CMV across Arabidopsis accessions with varying fitness responses to water deficit, and the authors concluded that the consequences of drought for transmission and virulence were driven by the growth traits of the host plants (Bergès et al., 2018).

Thus, it appears that the effect of drought on vector feeding behavior and other fitness traits plays a key, albeit indirect, role in shaping the consequences of drought on transmission of vector-borne pathogens. It is also apparent that the interactions among the intensity and duration of drought, plant species, and vector and pathogen natural history traits are likely to be even more complex under field conditions.

DROUGHT-MEDIATED TRIPARTITE INTERACTIONS

Plants susceptible to insect-transmitted pathogens must contend simultaneously with the consequences of drought, herbivory, and pathogen infection and coordinate their defensive responses accordingly. Likewise, herbivores and pathogens employ strategies to overcome plant defenses and promote their own proliferation. The result is a complex web of direct and indirect effects among pathogens, vectors, and their shared host plants (**Figure 1**).

Predicting the outcomes of these complex interactions is a challenge as they are likely to be species-specific and driven by natural history traits and genetic diversity of each of the organisms. We speculate that changes in vector performance and behavior mediated by drought-induced modifications in plant primary and secondary metabolism are likely to increase plant disease risk. The overlap and crosstalk in the molecular responses of plants to biotic and abiotic stresses, however, will ultimately determine the outcome of tripartite interactions. Drought is likely to influence the overall number of vectors present in the environment and the likelihood that these vectors will encounter and feed upon host plants, which is the essential first-step in vector-borne pathogen transmission. However, the probability that an encounter between a vector and a host plant will result in infection is likely governed by the ability of the plant to mount a successful immune response while simultaneously countering the effects of herbivory and water limitation. Multiple comprehensive studies that integrate molecular, behavioral, and ecological studies within a single plant system are necessary in order to advance our understanding of the mechanisms and consequences of drought-mediated tripartite interactions.

IMPLICATIONS OF DROUGHT STRESS FOR SUPPRESSION OF INSECT VECTORS AND VECTOR-BORNE PATHOGENS

The tripartite interactions among plants, insect vectors, and pathogens take place within diverse communities, and the complex outcomes of these interactions can have broad implications for the management of agricultural systems. First, the ability to predict how deficit irrigation affects plant productivity, insect vectors, and the associated vector-borne pathogens will allow for effective exploitation of water as a means to suppress vectors and pathogens (Daane and Williams, 2003; Rousselin et al., 2017). Secondly, understanding the impact of drought stress on direct and indirect plant defenses will allow for predictions of the likelihood of pest outbreaks and proper remediation plans that incorporate irrigation as one of the pest management tactics. For example, drought may impair the competitive advantage of native herbivores during invasions by non-native herbivores, as was illustrated in wheat, where water deficit reduced the ability of the native aphid *R. padi* to inhibit the population growth of the invasive aphid, *Metopolophium festucae* Theobald subsp. *cerealum* (Foote et al., 2017). Incorporating irrigation to alter these interactions could lower the incidence and severity of outbreaks of non-native vectors and associated pathogens. Further, recruitment of natural enemies and their meaningful contribution to pest suppression can be affected by drought through altered attraction of predators to injured plants. The nearly universal drought-induced decrease in SA, which is important in indirect plant defenses, is likely to affect recruitment of predators through disrupted emission of volatiles (Martini and Stelinski, 2017).

CONCLUSIONS

Given the increases in incidence of drought across the globe, research exploring the mechanisms driving drought-mediated tripartite interactions on multiple levels of organization and across trophic levels is more critical than ever. Research should employ a species-specific approach to quantifying plant responses to multiple stresses and assessing their consequences from gene expression to the fitness and behavior of vectors, pathogens associated with them, and their natural enemies.

AUTHOR CONTRIBUTIONS

AS and DF contributed equally to the conceptualization and composition of the manuscript.

FUNDING

This work was supported by the USDA NIFA TEX09638 to AS and USDA NIFA MO-HAPS0006 to DF.

ACKNOWLEDGMENTS

The authors thank Punya Nachappa and Priya Voothuluru for discussion that inspired an earlier draft of this review.

REFERENCES

Ahmad, P., Rasool, S., Gul, A., Sheikh, S. A., Akram, N. A., Ashraf, M., et al. (2016). Jasmonates: Multifunctional roles in stress tolerance. *Front. Plant Sci.* 7:813. doi: 10.3389/fpls.2016.00813

Anderson, J. P., Badruzsaufari, E., Schenk, P. M., Manners, J. M., Desmond, O. J., Ehlert, C., et al. (2004). Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis. *Plant Cell* 16, 3460–3479. doi: 10.1105/tpc.104.025833

Asselbergh, B., De Vleesschauwer, D., and Höfte, M. (2008). Global switches and fine-tuning—ABA modulates plant pathogen defense. *Mol. Plant Microbe Interact.* 21, 709–719. doi: 10.1094/MPMI-21-6-0709

Atkinson, N. J., Jain, R., and Urwin, P. E. (2015). "The Response of "Plants to simultaneous biotic and abiotic stress," in *Combined Stresses in Plants: Physiological, Molecular, and Biochemical Aspects*, ed R. Mahalingam (Cham: Springer International Publishing), 181–201. doi: 10.1007/978-3-319-07899-1_9

Atkinson, N. J., Lilley, C. J., and Urwin, P. E. (2013). Identification of genes involved in the response of Arabidopsis to simultaneous biotic and abiotic stresses. *Plant Physiol.* 162, 2028–2041. doi: 10.1104/pp.113.222372

Atkinson, N. J., and Urwin, P. E. (2012). The interaction of plant biotic and abiotic stresses: from genes to the field. *J. Exp. Bot.* 63, 3523–3543. doi: 10.1093/jxb/ers100

Audenaert, K., Meyer, G. B. D., and Höfte, M. M. (2002). Abscisic acid determines basal susceptibility of tomato to *Botrytis cinerea* and suppresses salicylic acid-dependent signaling mechanisms. *Plant Physiol.* 128, 491–501. doi: 10.1104/pp.010605

Banfield-Zanin, J. A., and Leather, S. R. (2015). Reproduction of an arboreal aphid pest, *Elatobium abietinum*, is altered under drought stress. *J. Appl. Entomol.* 139, 302–313. doi: 10.1111/jen.12159

Bartels, D., and Sunkar, R. (2005). Drought and salt tolerance in plants. *Crit. Rev. Plant Sci.* 24, 23–58. doi: 10.1080/07352680590910410

Belliure, B., Janssen, A., Maris, P. C., Peters, D., and Sabelis, M. W. (2005). Herbivore arthropods benefit from vectoring plant viruses. *Ecol. Lett.* 8, 70–79. doi: 10.1111/j.1461-0248.2004.00699.x

Bergès, S. E., Vile, D., Vazquez-Rovere, C., Blanc, S., Yvon, M., Bédiée, A., et al. (2018). Interactions between drought and plant genotype change epidemiological traits of Cauliflower mosaic virus. *Front. Plant Sci.* 9:703. doi: 10.3389/fpls.2018.00703

Bonhomme, L., Valot, B., Tardieu, F., and Zivy, M. (2012). Phosphoproteome dynamics upon changes in plant water status reveal early events associated with rapid growth adjustment in maize leaves. *Mol. Cell. Proteomics* 11, 957–972. doi: 10.1074/mcp.M111.015867

Bosque-Pérez, N. A., and Eigenbrode, S. D. (2011). The influence of virus-induced changes in plants on aphid vectors: insights from luteovirus pathosystems. *Virus Res.* 159, 201–205. doi: 10.1016/j.virusres.2011.04.020

Bostock, R. M. (2005). Signal crosstalk and induced resistance: straddling the line between cost and benefit. *Annu. Rev. Phytopathol.* 43, 545–580. doi: 10.1146/annurev.phyto.41.052002.095505

Boyer, J. S. (1982). Plant productivity and environment. *Science* 218, 443–448. doi: 10.1126/science.218.4571.443

Boyer, J. S. (2010). Drought decision-making. *J. Exp. Bot.* 61, 3493–3497. doi: 10.1093/jxb/erq231

Bray, E. A., Bailey-Serred, J., and Weretilnyk, E. (2000). "Responses to abiotic stresses," in *Biochemistry and Molecular Biology of Plants* (Rockville, MD: American Society of Plant Physiologists), 1158–1203.

Casteel, C. L., and Falk, B. W. (2016). "Plant virus-vector interactions: more than just for virus transmission," in *Current Research Topics in Plant Virology*, eds

A. Wang and X. Zhou (Cham: Springer International Publishing), 217–240. doi: 10.1007/978-3-319-32919-2_9

Castle, S. J., and Berger, P. H. (1993). Rates of growth and increase of *Myzus persicae* on virus-infected potatoes according to type of virus-vector relationship. *Entomol. Exp. Applic.* 69, 51–60. doi: 10.1111/j.1570-7458.1993.tb01727.x

Daane, K. M., and Williams, L. E. (2003). Manipulating vineyard irrigation amounts to reduce insect pest damage. *Ecol. Applic.* 13, 1650–1666. doi: 10.1890/02-5328

Davis, T. S., Bosque-Pérez, N. A., Foote, N. E., Magney, T., and Eigenbrode, S. D. (2015a). Environmentally dependent host–pathogen and vector–pathogen interactions in the Barley yellow dwarf virus pathosystem. *J. Appl. Ecol.* 52, 1392–1401. doi: 10.1111/1365-2664.12484

Davis, T. S., Bosque-Pérez, N. A., Popova, I., and Eigenbrode, S. D. (2015b). Evidence for additive effects of virus infection and water availability on phytohormone induction in a staple crop. *Front. Ecol. Evol.* 3:114. doi: 10.3389/fevo.2015.00114

de Oliveira, C. F., Long, E. Y., and Finke, D. L. (2014). A negative effect of a pathogen on its vector? A plant pathogen increases the vulnerability of its vector to attack by natural enemies. *Oecologia* 174, 1169–1177. doi: 10.1007/s00442-013-2854-x

Donaldson, J. R., and Gratton, C. (2007). Antagonistic effects of soybean viruses on soybean aphid performance. *Environ. Entomol.* 36, 918–925. doi: 10.1093/ee/36.4.918

Eigenbrode, S. D., Bosque-Pérez, N. A., and Davis, T. S. (2018). Insect-borne plant pathogens and their vectors: ecology, evolution, and complex interactions. *Annu. Rev. Entomol.* 63, 169–191. doi: 10.1146/annurev-ento-020117-043119

Eigenbrode, S. D., Ding, H., Shiel, P., and Berger, P. H. (2002). Volatiles from potato plants infected with potato leafroll virus attract and arrest the virus vector, *Myzus persicae* (Homoptera: Aphididae). *Proc. R. Soc. Lond. Ser. B Biol. Sci.* 269, 455–460. doi: 10.1098/rspb.2001.1909

Erb, M., Balmer, D., Lange, E. S. D., Merey, G. V., Planchamp, C., Robert, C., et al. (2011). Synergies and trade-offs between insect and pathogen resistance in maize leaves and roots. *Plant Cell Environ.* 34, 1088–1103. doi: 10.1111/j.1365-3040.2011.02307.x

Fan, J., Hill, L., Crooks, C., Doerner, P., and Lamb, C. (2009). Abscisic acid has a key role in modulating diverse plant-pathogen interactions. *Plant Physiol.* 150, 1750–1761. doi: 10.1104/pp.109.137943

Florencio-Ortiz, V., Sellés-Marchart, S., Zubcoff-Vallejo, J., Jander, G., and Casas, J. L. (2018). Changes in the free amino acid composition of *Capsicum annuum* (pepper) leaves in response to *Myzus persicae* (green peach aphid) infestation. A comparison with water stress. *PLoS ONE* 13:e0198093. doi: 10.1371/journal.pone.0198093

Foote, N. E., Davis, T. S., Crowder, D. W., Bosque-Pérez, N. A., and Eigenbrode, S. D. (2017). Plant water stress affects interactions between an invasive and a naturalized aphid species on cereal crops. *Environ. Entomol.* 46, 609–616. doi: 10.1093/ee/nvx071

Fournier, F., Boivin, G., and Stewart, R. K. (1995). Effect of *Thrips tabaci* (Thysanoptera: Thripidae) on yellow onion yields and economic thresholds for its management. *J. Econ. Entomol.* 88, 1401–1407. doi: 10.1093/jee/88.5.1401

Guo, H., Sun, Y., Peng, X., Wang, Q., Harris, M., and Ge, F. (2016). Up-regulation of abscisic acid signaling pathway facilitates aphid xylem absorption and osmoregulation under drought stress. *J. Exp. Bot.* 67, 681–693. doi: 10.1093/jxb/erv481

Haider, M. S., Kurjogi, M. M., Khalil-Ur-Rehman, M., Fiaz, M., Pervaiz, T., Jiu, S., et al. (2017). Grapevine immune signaling network in response to drought stress as revealed by transcriptomic analysis. *Plant Physiol. Biochem.* 121, 187–195. doi: 10.1016/j.plaphy.2017.10.026

Hale, B. K., Bale, J. S., Pritchard, J., Masters, G. J., and Brown, V. K. (2003). Effects of host plant drought stress on the performance of the bird cherry-oat aphid, *Rhopalosiphum padi* (L.): a mechanistic analysis. *Ecol. Entomol.* 28, 666–677. doi: 10.1111/j.1365-2311.2003.00563.x

Harb, A., Krishnan, A., Ambavaram, M. M. R., and Pereira, A. (2010). Molecular and physiological analysis of drought stress in Arabidopsis reveals early responses leading to acclimation in plant growth. *Plant Physiol.* 154, 1254–1271. doi: 10.1104/pp.110.161752

Hodge, S., Hardie, J., and Powell, G. (2011). Parasitoids aid dispersal of a nonpersistently transmitted plant virus by disturbing the aphid vector. *Agric. For. Entomol.* 13, 83–88. doi: 10.1111/j.1461-9563.2010.00493.x

Howe, G. A., Lightner, J., and Ryan, C. A. (1996). An octadecanoid pathway mutant (JL5) of tomato is compromised in signaling for defense against insect attack. *Plant Cell* 8, 2067–2077. doi: 10.1105/tpc.8.11.2067

Huberty, A. F., and Denno, R. F. (2004). Plant water stress and its consequences for herbivorous insects: a new synthesis. *Ecology* 85, 1383–1398. doi: 10.1890/03-0352

Huot, O. B., and Tamborindeguy, C. (2017). Drought stress affects *Solanum lycopersicum* susceptibility to *Bactericera cockerelli* colonization. *Entomol. Exp. Appl.* 165, 70–82. doi: 10.1111/eea.12627

Inbar, M., Doostdar, H., and Mayer, R. T. (2001). Suitability of stressed and vigorous plants to various insect herbivores. *Oikos* 94, 228–235. doi: 10.1034/j.1600-0706.2001.940203.x

Ingram, J., and Bartels, D. (1996). The molecular basis of dehydration tolerance in plants. *Annu. Rev. Plant Biol.* 47, 377–403. doi: 10.1146/annurev.arplant.47.1.377

Jiménez-Martínez, E. S., Bosque-Pérez, N. A., Berger, P. H., Zemetra, R. S., Ding, H., and Eigenbrode, S. D. (2004). Volatile cues influence the response of *Rhopalosiphum padi* (Homoptera: Aphididae) to Barley yellow dwarf virus–infected transgenic and untransformed wheat. *Environ. Entomol.* 33, 1207–1216. doi: 10.1603/0046-225X-33.5.1207

Joern, A., and Mole, S. (2005). The plant stress hypothesis and variable responses by blue grama grass (*Bouteloua gracilis*) to water, mineral nitrogen, and insect herbivory. *J. Chem. Ecol.* 31, 2069–2090. doi: 10.1007/s10886-005-6078-3

Kazan, K. (2015). Diverse roles of jasmonates and ethylene in abiotic stress tolerance. *Trends Plant Sci.* 20, 219–229. doi: 10.1016/j.tplants.2015.02.001

Koornneef, A., and Pieterse, C. M. (2008). Cross talk in defense signaling. *Plant Physiol.* 146, 839–844. doi: 10.1104/pp.107.112029

Krugner, R., and Backus, E. A. (2014). Plant water stress effects on stylet probing behaviors of *Homalodisca vitripennis* (Hemiptera: Cicadellidae) associated with acquisition and inoculation of the bacterium *Xylella fastidiosa*. *J. Econ. Entomol.* 107, 66–74. doi: 10.1603/EC13219

Kunkel, B. N., and Brooks, D. M. (2002). Cross talk between signaling pathways in pathogen defense. *Curr. Opin. Plant Biol.* 5, 325–331. doi: 10.1016/S1369-5266(02)00275-3

Larsson, S., and Björkman, C. (1993). Performance of chewing and phloem-feeding insects on stressed trees. *Scand. J. Forest Res.* 8, 550–559. doi: 10.1080/02827589309382801

Lee, S. C., and Luan, S. (2012). ABA signal transduction at the crossroad of biotic and abiotic stress responses. *Plant Cell Environ.* 35, 53–60. doi: 10.1111/j.1365-3040.2011.02426.x

Liu, X., Song, Y., Xing, F., Wang, N., Wen, F., and Zhu, C. (2016). GhWRKY25, a group I WRKY gene from cotton, confers differential tolerance to abiotic and biotic stresses in transgenic *Nicotiana benthamiana*. *Protoplasma* 253, 1265–1281. doi: 10.1007/s00709-015-0885-3

Martini, X., and Stelinski, L. L. (2017). Drought stress affects response of phytopathogen vectors and their parasitoids to infection- and damage-induced plant volatile cues. *Ecol. Entomol.* 42, 721–730. doi: 10.1111/een.12439

Mauch-Mani, B., and Mauch, F. (2005). The role of abscisic acid in plant–pathogen interactions. *Curr. Opin. Plant Biol.* 8, 409–414. doi: 10.1016/j.pbi.2005.05.015

Mauck, K., Bosque-Pérez, N. A., Eigenbrode, S. D., De Moraes, C. M., and Mescher, M. C. (2012). Transmission mechanisms shape pathogen effects on host–vector interactions: evidence from plant viruses. *Funct. Ecol.* 26, 1162–1175. doi: 10.1111/j.1365-2435.2012.02026.x

Mauck, K. E., Chesnais, Q., and Shapiro, L. R. (2018). "Evolutionary determinants of host and vector manipulation by plant viruses," in *Advances in Virus Research Environmental Virology and Virus Ecology*, ed C. M. Malmstrom (Cambridge, MA: Academic Press), 189–250. doi: 10.1016/bs.aivir.2018.02.007

Mauck, K. E., De Moraes, C. M., and Mescher, M. C. (2016). Effects of pathogens on sensory-mediated interactions between plants and insect vectors. *Curr. Opin. Plant Biol.* 32, 53–61. doi: 10.1016/j.pbi.2016.06.012

Mauck, K. E., Kenney, J., and Chesnais, Q. (2019). Progress and challenges in identifying molecular mechanisms underlying host and vector manipulation by plant viruses. *Curr. Opin. Insect Sci.* 33, 7–18. doi: 10.1016/j.cois.2019.01.001

Mauck, K. E., Moraes, C. M. D., and Mescher, M. C. (2010). Deceptive chemical signals induced by a plant virus attract insect vectors to inferior hosts. *Proc. Natl. Acad. Sci. U.S.A.* 107, 3600–3605. doi: 10.1073/pnas.0907191107

Mcvean, R. I. K., and Dixon, A. F. G. (2001). The effect of plant drought-stress on populations of the pea aphid *Acyrthosiphon pisum*. *Ecol. Entomol.* 26, 440–443. doi: 10.1046/j.1365-2311.2001.00341.x

Melotto, M., Underwood, W., Koczan, J., Nomura, K., and He, S. Y. (2006). Plant stomata function in innate immunity against bacterial invasion. *Cell* 126, 969–980. doi: 10.1016/j.cell.2006.06.054

Mittler, R., and Blumwald, E. (2010). Genetic engineering for modern agriculture: challenges and perspectives. *Annu. Rev. Plant Biol.* 61, 443–462. doi: 10.1146/annurev-arplant-042809-112116

Mohr, P. G., and Cahill, D. M. (2007). Suppression by ABA of salicylic acid and lignin accumulation and the expression of multiple genes, in Arabidopsis infected with *Pseudomonas syringae* pv. tomato. *Funct. Integr. Genomics* 7, 181–191. doi: 10.1007/s10142-006-0041-4

Muñoz-Espinoza, V. A., López-Climent, M. F., Casaretto, J. A., and Gómez-Cadenas, A. (2015). Water stress responses of tomato mutants impaired in hormone biosynthesis reveal abscisic acid, jasmonic acid and salicylic acid interactions. *Front. Plant Sci.* 6:997. doi: 10.3389/fpls.2015.00997

Nachappa, P., Culkin, C. T., Saya, P. M., Han, J., and Nalam, V. J. (2016). Water stress modulates soybean aphid performance, feeding behavior, and virus transmission in soybean. *Front Plant Sci* 7:552. doi: 10.3389/fpls.2016.00552

Nachappa, P., Shapiro, A. A., and Tamborindeguy, C. (2011). Effect of '*Candidatus* Liberibacter solanacearum' on fitness of its insect vector, *Bactericera cockerelli* (Hemiptera: Triozidae), on tomato. *Phytopathology* 102, 41–46. doi: 10.1094/PHYTO-03-11-0084

Nguyen, D., Rieu, I., Mariani, C., and van Dam, N. M. (2016). How plants handle multiple stresses: hormonal interactions underlying responses to abiotic stress and insect herbivory. *Plant Mol. Biol.* 91, 727–740. doi: 10.1007/s11103-016-0481-8

Ogada, P. A., Maiss, E., and Poehling, H.-M. (2013). Influence of tomato spotted wilt virus on performance and behaviour of western flower thrips (*Frankliniella occidentalis*). *J. Appl. Entomol.* 137, 488–498. doi: 10.1111/jen.12023

Paris, H., Stoffella, P., and Powell, C. (1993). Sweet-potato whitefly, drought stress, and leaf silvering of squash. *Hortscience* 28, 157–158. doi: 10.21273/HORTSCI.28.2.157

Pons, X., and Tatchell, G. M. (1995). Drought stress and cereal aphid performance. *Ann. Appl. Biol.* 126, 19–31. doi: 10.1111/j.1744-7348.1995.tb05000.x

Prasch, C. M., and Sonnewald, U. (2013). Simultaneous application of heat, drought, and virus to Arabidopsis plants reveals significant shifts in signaling networks. *Plant Physiol.* 162, 1849–1866. doi: 10.1104/pp.113.221044

Purcell, A. H., and Almeida, R. P. P. (2005). "Insects as vectors of disease agents," in *Encyclopedia of Plant and Crop Science*, ed M. Dekker (London: Tayler & Francis). doi: 10.1081/E-EPCS-120010496

Ramanjulu, S., and Bartels, D. (2002). Drought- and desiccation-induced modulation of gene expression in plants. *Plant Cell Environ.* 25, 141–151. doi: 10.1046/j.0016-8025.2001.00764.x

Ramegowda, V., and Senthil-Kumar, M. (2015). The interactive effects of simultaneous biotic and abiotic stresses on plants: mechanistic understanding from drought and pathogen combination. *J. Plant Physiol.* 176, 47–54. doi: 10.1016/j.jplph.2014.11.008

Rousselin, A., Bevacqua, D., Sauge, M.-H., Lescourret, F., Mody, K., and Jordan, M.-O. (2017). Harnessing the aphid life cycle to reduce insecticide reliance in apple and peach orchards. A review. *Agron. Sustain. Dev.* 37:38. doi: 10.1007/s13593-017-0444-8

Sconiers, W. B., and Eubanks, M. D. (2017). Not all droughts are created equal? The effects of stress severity on insect herbivore abundance. *Arthropod Plant Interact.* 11, 45–60. doi: 10.1007/s11829-016-9464-6

Shah, J. (2003). The salicylic acid loop in plant defense. *Curr. Opin. Plant Biol.* 6, 365–371. doi: 10.1016/S1369-5266(03)00058-X

Shapiro, L., Moraes, C. M. D., Stephenson, A. G., and Mescher, M. C. (2012). Pathogen effects on vegetative and floral odours mediate vector attraction and host exposure in a complex pathosystem. *Ecol. Lett.* 15, 1430–1438. doi: 10.1111/ele.12001

Simpson, K. L. S., Jackson, G. E., and Grace, J. (2012). The response of aphids to plant water stress – the case of *Myzus persicae* and *Brassica oleracea* var. capitata. *Entomol. Exp. Applic.* 142, 191–202. doi: 10.1111/j.1570-7458.2011.01216.x

Skinner, R. H. (1996). Response of *Bemisia argentifolii* (Homoptera: Aleyrodidae) to water and nutrient stressed cotton. *Environ. Entomol.* 25, 401–406. doi: 10.1093/ee/25.2.401

Stout, M. J., Thaler, J. S., and Thomma, B. P. H. J. (2006). Plant-mediated interactions between pathogenic microorganisms and herbivorous arthropods. *Annu. Rev. Entomol.* 51, 663–689. doi: 10.1146/annurev.ento.51.110104.151117

Tariq, M., Wright, D. J., Rossiter, J. T., and Staley, J. T. (2012). Aphids in a changing world: testing the plant stress, plant vigour and pulsed stress hypotheses. *Agric. For. Entomol.* 14, 177–185. doi: 10.1111/j.1461-9563.2011.00557.x

Thaler, J. S., Agrawal, A. A., and Halitschke, R. (2010). Salicylate-mediated interactions between pathogens and herbivores. *Ecology* 91, 1075–1082. doi: 10.1890/08-2347.1

Thaler, J. S., Karban, R., Ullman, D. E., Boege, K., and Bostock, R. M. (2002). Cross-talk between jasmonate and salicylate plant defense pathways: effects on several plant parasites. *Oecologia* 131, 227–235. doi: 10.1007/s00442-002-0885-9

Urano, K., Maruyama, K., Ogata, Y., Morishita, Y., Takeda, M., Sakurai, N., et al. (2009). Characterization of the ABA-regulated global responses to dehydration in Arabidopsis by metabolomics. *Plant J.* 57, 1065–1078. doi: 10.1111/j.1365-313X.2008.03748.x

van Munster, M., Yvon, M., Vile, D., Dader, B., Fereres, A., and Blanc, S. (2017). Water deficit enhances the transmission of plant viruses by insect vectors. *PLoS ONE* 12:e0174398. doi: 10.1371/journal.pone.0174398

Walling, L. L. (2000). The myriad plant responses to herbivores. *J. Plant Growth Regul.* 19, 195–216. doi: 10.1007/s003440000026

Wei, T., Deng, K., Wang, H., Zhang, L., Wang, C., Song, W., et al. (2018). Comparative transcriptome analyses reveal potential mechanisms of enhanced drought tolerance in transgenic *Salvia miltiorrhiza* plants expressing AtDREB1A from Arabidopsis. *Int. J. Mol. Sci.* 19:E827. doi: 10.3390/ijms19030827

Yan, J., Lipka, A. E., Schmelz, E. A., Buckler, E. S., and Jander, G. (2015). Accumulation of 5-hydroxynorvaline in maize (*Zea mays*) leaves is induced by insect feeding and abiotic stress. *J. Exp. Bot.* 66, 593–602. doi: 10.1093/jxb/eru385

Yasuda, M., Ishikawa, A., Jikumaru, Y., Seki, M., Umezawa, T., Asami, T., et al. (2008). Antagonistic interaction between systemic acquired resistance and the abscisic acid–mediated abiotic stress response in Arabidopsis. *Plant Cell* 20, 1678–1692. doi: 10.1105/tpc.107.054296

Yvon, M., Vile, D., Brault, V., Blanc, S., and van Munster, M. (2017). Drought reduces transmission of Turnip yellows virus, an insect-vectored circulative virus. *Virus Res.* 241, 131–136. doi: 10.1016/j.virusres.2017.07.009

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

REVIEW
published: 23 July 2019
doi: 10.3389/fevo.2019.00263

A Global Review on Locusts (Orthoptera: Acrididae) and Their Interactions With Livestock Grazing Practices

Marion Le Gall[1]*, **Rick Overson**[1] and **Arianne Cease**[1,2]

[1] School of Sustainability, Arizona State University, Tempe, AZ, United States, [2] School of Life Sciences, Arizona State University, Tempe, AZ, United States

Edited by:
Lora A. Richards,
University of Nevada, Reno,
United States

Reviewed by:
Andrea Sciarretta,
University of Molise, Italy
Stephen Rogers,
University of Cambridge,
United Kingdom

***Correspondence:**
Marion Le Gall
marionlegall314@gmail.com

Specialty section:
This article was submitted to
Population and Evolutionary
Dynamics,
a section of the journal
Frontiers in Ecology and Evolution

Received: 02 March 2019
Accepted: 24 June 2019
Published: 23 July 2019

Citation:
Le Gall M, Overson R and Cease A
(2019) A Global Review on Locusts
(Orthoptera: Acrididae) and Their
Interactions With Livestock Grazing
Practices. Front. Ecol. Evol. 7:263.
doi: 10.3389/fevo.2019.00263

Connections between locusts and people date back millennia and locusts remain a major food security challenge today throughout the world. Locust biology is often linked to abiotic conditions like temperature and/or precipitation, fueling the perception that aside from active control, humans are not key players in the interaction locusts have with their environment. However, several studies have shown that land management practices like grazing heavily influences locust-human linkages. In this review we synthesize published research and reports on connections between locust outbreaks and ranching. For this, we conducted an extensive literature search using Google Scholar on the 19 species of grasshoppers that are currently considered to be locusts or non-model locusts. Species were sorted according to their feeding guilds: (1) forb- and/or tree-feeding locusts; (2) mix-feeding locusts (grasses, forbs, and/or trees); (3) grass-feeding locusts. We review their pest status, ecology, and relationship with grazing. We then discuss the overall data and draw general patterns on how locusts and locust control affect livestock grazing through various mechanisms (competition, nutritional preferences, pesticide use, nutrient cycling). We draw attention to "telecoupling" a process in which land management practices like grazing have ecological feedbacks on locust populations, which in turn affects food security in distant regions due to the migratory capacity of locusts. Finally, we present new perspectives for sustainable management practices that integrate understanding of land management.

Keywords: grasslands, plant-insect interactions, locust phase change, land use and land cover change, drylands, nutrients, grazing

INTRODUCTION

Connections between locusts and people date back millennia and remain a major food security challenge throughout the world today. For instance, in Senegal, West Africa, locust outbreaks are second only to drought in damaging agricultural productivity (D'Alessandro et al., 2015). The impressive capacity of locust swarms to rapidly move long distances and descend on communities unexpectedly has shrouded their source in mystery. Perhaps for this reason, locusts have often been viewed as a divine punishment or curse; both the Bible and the Qur'an describe locusts as one of the devastating plagues of Egypt (El-Mallakh and El-Mallakh, 1994).

The unpredictability and overwhelming nature of these outbreaks has also likely contributed to the perception that, outside of active treatment of outbreaks, people are passive recipients of swarms. However, increasing evidence suggests that human decisions about how we manage our land influence locust population dynamics (Cease et al., 2015). For example, in Inner Mongolia, China, heavy livestock grazing promotes Mongolian locust, *Oedaleus asiaticus,* outbreaks by lowering plant nitrogen content (Cease, 2012). Many other locust species also originate in areas used for livestock grazing. Yet, despite the known impacts of grazing on rangeland grasshoppers (Branson et al., 2006) and our longstanding connection with locusts, the potential to influence locust populations through land management practices has received relatively little attention. Here, we discuss how locust behavioral and migratory plasticity can lead to unique human-acridid linkages, outline the pest status, ecology, and interactions with grazing for all locusts, consider similarities and differences among species in various grazing systems, and highlight potential areas for further research.

At low densities, locusts, and grasshoppers are critical to grassland ecosystem functioning (Branson et al., 2006). They cycle nutrients, shape plant community structure, and are an important food source for many animals including spiders and birds (Wiens, 1973; Joern, 1986; Belovsky and Slade, 2000; Oedekoven and Joern, 2000; Sokol-Hessner and Schmitz, 2002; Schmitz, 2008). Humans also rear and consume locusts (Stoops et al., 2016; Osimani et al., 2017); they are the second most commonly eaten insect after crickets (Mignon, 2002; Osimani et al., 2017). At high densities, however, they compete with livestock for forage. In this scenario, locusts become highly problematic. Of the roughly 6,800 known acridid grasshopper species (Cigliano et al., 2017), 19 are currently considered locusts (Cullen et al., 2017). Locusts are grasshoppers that, when exposed to specific environmental cues, develop into either gregarious and swarming or solitarious phenotypes (Pener, 1991; Cullen et al., 2017). These phenotypes differ in behavior, morphology, and physiology; however, their component traits can be decoupled, and vary among species. Behavior can change within hours for some species but other traits, such as morphology, can take several generations to fully shift. This plasticity, termed locust phase polyphenism (Pener, 1991; Pener and Simpson, 2009), creates unique challenges. Gregarious locusts that originate in one region can migrate *en masse* hundreds of kilometers in a single night and plagues can span continents. For instance the desert locust, *Schistocerca gregaria*, can potentially affects 60 countries (Popov et al., 1991), and a single swarm can cover 1,200 km^2 (Mohamed Shaluf, 2007). Successful management of locust upsurges requires the infrastructure both for robust monitoring throughout a locust's range to aid in early detection and for rapid and targeted treatment of nymphal bands. Effective management strategies thus often require international cooperation and a strong consideration of scale (Lockwood et al., 2001; Toleubayev et al., 2007).

When in the gregarious phase, locusts group together and become more polyphagous. Numerous anecdotal reports suggest that not only will gregarious locusts eat more plant species, but also plants belonging to different families than the ones they typically feed on when in the solitarious phase (COPR, 1982). Solitarious desert locusts, *S. gregaria*, avoid eating a plant species containing the alkaloid hyoscyamine but gregarious locusts will actively consume it, likely to gain gut-content mediated toxicity and avoid predation (Despland and Simpson, 2005a). Additionally, in the laboratory, gregarious locusts are less discerning when faced with artificial diets with nutrient imbalances as compared to solitarious locusts (Simpson et al., 2002). This expanded palate may be due to several reasons. On one hand, migrating individuals may encounter increased food diversity and this breadth may allow gregarious individuals to redress nutrient imbalances (Clark et al., 2013). On the other hand, groups of gregarious locusts may cross vast areas with no food and a narrower host plant breadth may mean starvation. This diet expansion, in combination with aggregation, likely heightens agricultural impacts. Indeed, locusts can cause 80–100% crop losses across affected areas (Brader et al., 2006).

Locust impacts on people are well-documented, but humans also influence locust population dynamics and distributions directly through control and indirectly through land use/land cover change; however, the latter has not been reviewed and summarized. For instance, due to targeted treatment in breeding zones, the red locust, *Nomadacris septemfasciata* (Serville, 1838), has had fewer outbreaks since the 1929–1944 plague (COPR, 1982; Thindwa, 1999; FAO, News Article: Red Locust disaster in Eastern Africa prevented, 2009. The impacts of the Moroccan locust, *Dociostaurus maroccanus* (Thunberg, 1815), also seem to be diminishing, most likely because its habitat has been replaced by croplands (although there are notable local exceptions that we will discuss below) (Latchininsky, 1998). On the other hand, land use change may increase the pest status of other species. For example, early swarms of the Australian plague locust, *Chortoicetes terminifera*, in the late 1800's may have been promoted by the introduction of European livestock and agriculture to Australia (Deveson, 2012). Therefore, understanding the grassland-livestock-locust system will be an important contribution for solving pressing issues of food security and will provide an exploratory framework for revealing the pathways that connect human and ecological systems over large spatial distances (Cease et al., 2015).

LITERATURE REVIEW

To synthesize published research and reports on connections between locust outbreaks and ranching we carried out a literature search using Google Scholar. We searched for the binomial taxon names in quotations. Based on the raw count of hits from each binomial search we separated species into two groups: those with <1,000 hits (subset 1) and those with >1,000 hits (subset 2). For subset 1, all article and report titles were viewed, and irrelevant articles were discarded. For the remaining articles in subset 1, abstracts were individually reviewed. For species in subset 2, due to the volume of articles initially queried, we further filtered articles by forcing them to contain either the word "grazing" or "livestock" and subsequently followed the same procedure as for subset 1. Additionally, all pertinent references from COPR (1982) were reviewed. The seminal 1982 Locust and Grasshopper Agricultural Manual provides an excellent overview of the ecology and pest status of these (and many other) species. In this paper, there is some necessary overlap, but we focus on papers published after 1982 where possible and refer readers to the manual for reviews of older references. Results are presented by feeding guild (forb-and/or tree-feeding locusts; mix -feeding locusts; grass-feeding locusts), and taxonomic category. A third of the subfamilies in Acrididae have been found to be paraphyletic and to form four major clades (Clade A, B, C, and D) (Song et al., 2018). We thus included information on their subfamily and clades.

FORB-AND/OR TREE-FEEDING LOCUSTS

These forb or tree specialists are likely not strong competitors with livestock grazing on grasses, but they may be if livestock are limited to or prefer shrubs and trees (e.g., camels). Additionally, grazing areas may be preferable to these locusts, relative to cereal crop fields, presumably due to increased diversity of forbs and trees.

Subfamily Calliptaminae—Clade D

Calliptamus italicus (Linnaeus, 1758)

The Italian locust, or stout-bodied grasshopper

Pest status. This species has been reported as a pest since medieval times and "raging" outbreaks remain common to date (Stolyarov, 2000). In the Siberian steppes its density sometimes exceeded 2000 individuals m^{-2}. Economically important crops that are attacked include wheat, sunflowers, beans, and many more crop species (COPR, 1982).

Habitat and ecology. This locust can be found from the eastern Mediterranean region through the Siberian steppes to arid regions of northwestern China, and, in Europe, as far north as Germany (Louveaux et al., 1988; Sergeev and Van'kova, 2008). It inhabits primarily dry habitats including semi-desert steppes, rocky slopes, dry glades and pine forest edges, and shrubby terrain. It is also common in agrolandscapes (Sergeev and Van'kova, 2008) and degraded habitats (Louveaux et al., 1988). The Italian locust spends most of its time on the ground surface and is rarely found climbing on grass and forbs (Batáry et al., 2007). This species does not tolerate high humidity and eggs must be laid in soil containing <5% moisture (Louveaux et al., 1988). It has one generation per year. Eggs are laid during the summer and undergo diapause during the winter. Outbreaks are associated with hot and dry springs and summers (Stolyarov, 2000) and females avoid laying eggs near shrubs and trees because of their cooling effect on soil (Urech, 2003). In some countries, like China, its range is likely to expand with climate change (Qin et al., 2012). The Italian locust differs from some other locust species by having no color distinction between the different phases (Sergeev and Van'kova, 2008). To distinguish between phases, researchers rely on morphometric features: body size and the ratio of forewing length to hind femur length (gregarious individuals are bigger) (Sergeev and Van'kova, 2008).

Grazing interactions. Despite feeding primarily on forbs, Italian locust populations are sometimes associated with grazing fields and pastures and will attack forage including fodder grasses as well as alfalfa (COPR, 1982). In steppe habitats, this locust likely competes to some extent with cattle; however, the primary economic impact arises when it moves from grazing or natural areas to cropland. In Eastern Kazakhstan, even between the outbreaks, the locust density can be very high (up to 20–26 individuals m^{-2}), and this is especially true for fallow lands, overgrazed pastures, and belts of perennial grasses within crop-rotation fields. Interestingly this locust rarely inhabits fallow lands dominated by wheat grass (Sergeev and Van'kova, 2008), but has been described as a pest of wheat (Wilps et al., 2002). Intensive tillage of steppes near the Irtysh River in the late 1950s–early 1960s promoted larger Italian locust populations, which then spilled into fields with perennial grasses and grain crops, ecotones, and pastures (Sergeev and Van'kova, 2008), but the mechanism of this interactions is unknown.

Subfamily Cyrtacanthacridinae—Clade D

Anacridium melanorhodon (Walker, 1870)

The Sahelian tree locust

Pest status. This species is primarily a tree pest (e.g., gum arabic, apple trees, citrus trees, palm date, olive trees, mango trees), but young nymphs will feed on grass and adults will feed on crops including bulrush millet, sorghum, maize, rice, cassava, cotton during the winter (dry) season and when swarming (COPR, 1982). Of greatest economic importance is its impact on gum arabic production. Gum arabic is one of the main crops produced in the traditional rain-fed agricultural sector of Sudan and South Sudan and is especially important for the semi-nomadic people in the Savannah belt of the region (Haroon et al., 2011). It is a non-timber forest product harvested from gum arabic tree (mainly *Acacia senegal* var. *senegal*). Gum arabic provides on average 12% of the gross domestic product of the country and accounts for about 15–10 % of the income of the gum producers and other farmers in the gum belt across the Sudan. A study conducted in North Kordofan State in Sudan showed that gum arabic crop yield was more than halved by locusts (going from 273.9 to 93.8 kg/ha) and financial benefits plummeted from 292.6 Sudanese Pound (SDG) per hectare to losses of −21.2. SDG per hectare (Elamin et al., 2008). In addition, tree locust outbreaks can delay tree tapping from October to January/February because of defoliation.

Habitat and ecology. This locust is well-adapted to arid habitats and drought. It can resorb water from frass under hot and dry conditions and feeds almost exclusively at night (Abushama, 1970; COPR, 1982). It is physiologically adapted to feed on trees, containing phenols such as tannic and gallic acids and these compounds actually stimulate feeding. Typically, phenols are harmful to herbivores because they bind to leaf proteins and inhibit protein digestion post-consumption. To limit this effect, locusts use these ingested compounds as a source of phenolics in tanned cuticle, rather than obtaining them from the aromatic amino acids tyrosine and phenylalanine as most insects do (Evans and Bell, 1979; Bernays and Woodhead, 1982; Bernays et al., 1983). The aptly named Sahelian tree locust is distributed in the Sahelian zone from Cape Verde to Ethiopia, as well as parts of the Middle East (COPR, 1982).

Grazing interactions. Because it feeds on trees, this locust is probably not an important competitor for grass grazers, however it can compete with domestic cattle and camels when feeding on wild trees and shrubs (e.g., *Boscia senegalensis*) (COPR, 1982). In areas that are overstocked, the Sahelian tree locust can exacerbate the impact of livestock on shrubs and trees, with subsequent impacts on soil quality. In Sahelian sylvopastoral systems, woody vegetation can limit soil degradation by reducing top soil erosion (Breman and Kessler, 1997). On the other hand, the Sahelian tree locust may contribute significantly to nutrient cycling in silvopastoral systems. For example, a study quantified the nitrogen deposited during a locust outbreak in an *Acacia tortilis* woodland in Oman (Robinson, 2001). The average fecal deposition was 65.28 g m^{-2}. The mean fecal crude protein and N were respectively, 20.14 and 3.22 g per 100 g ash-free dry weight, or roughly 1.1 kg of fecal N per ha. For comparison, this single contribution by locusts equals about one-third of the total standing crop of detrital N under perennial vegetation in a hectare of the Mohave Desert of North America. These calculations, combined with the seasonal prevalence and diversity of grasshoppers in dry areas, suggests they are likely broadly important for nutrient cycling in arid systems.

MIX-FEEDING LOCUSTS (GRASSES, FORBS, AND/OR TREES)

Mix-feeding locusts tend to be highly polyphagous, eating broadly from many plant families. This adaptation allows them to persist in and migrate through many landscape types. It also makes plagues a significant threat to livestock forage and pastures, as well as a broad array of crops. Several of these species prefer overgrazed, or otherwise disturbed, habitat, potentially due to increased bare soil for laying eggs and thermoregulating.

Subfamily Cyrtacanthacridinae—Clade D

Schistocerca cancellata (Serville, 1838)

South American locust

Pest status. The first report of damage from *S. cancellata* was on cassava in 1538 in Buenos Aires (Gastón, 1969), and since that time, the list of damaged plants has grown to virtually every cultivated plant in Argentina including soybeans, sorghum, maize, peanut, and citrus, as well as pasture grass. From the 1800's to 1954, plagues increased in frequency and size causing millions of tons of crop and pasture losses in Argentina alone, and historically spanned from Argentina, Bolivia, Paraguay, Uruguay, Chile, and Brazil (COPR, 1982). Starting in the 1960's, consistent monitoring and pesticide application (including DDT) is thought to have led to successful control for the next six decades, with only three small outbreaks in 1961, 1989, and 2010 (Waloff and Pedgley, 1986; Medina et al., 2017). However, in 2015 a large upsurge began, likely promoted by elevated winter temperatures and rainfall (Medina et al., 2017). The outbreak has persisted to the present (March 2019), expanding from a small region in NE Argentina into much of the rest of country, as well as into neighboring Bolivia and Paraguay. Continued monitoring and pesticide application is underway in the tri-country response (Medina et al., 2017).

Habitat and ecology. This highly polyphagous locust prefers desert or semidesert areas and likely persists mainly in a central permanent zone (∼600 × 200 km) within Argentina (Köhler, 1962) that receives 250–400 mm of annual precipitation. Breeding populations are hypothesized to persist in this zone due to sufficient habitat heterogeneity, which acts as a refuge even during unfavorable times (COPR, 1982; Waloff and Pedgley, 1986). However, solitarious individuals at low density have been found consistently across Argentina as well as in a small region in Chile (M. Pocco & H. Song, pers. comm.). Work by Hunter and Cosenzo (1990) demonstrated a link between the occurrence and persistence of outbreaks in South America and the timing of rainfall particularly in the states of Catamarca and La Rioja. With a longer developmental time than *S. gregaria* (Pedgley, 1981), *S. cancellata* relies on rainfall between late spring and late summer (November and March, respectively) and generally spends the dry season (April–October) as nymphs, in a type of reproductive diapause (Barrera and Turk, 1983). However, Hunter and Cosenzo demonstrated that rainfall in the recession zone during winter months followed by a wet consecutive spring and summer, allows three generations per year and fuels dramatic population growth. This sequence is highly predictive of outbreaks from 1897 to 1954. Once upsurges were initiated with a three-generation year, they generally grew to maximum size over the following 2 years, and then diminished gradually over 8–15 years due to the effects of attenuating dry years that allowed for only a single generation in Catamarca and La Rioja.

Grazing interactions. The dominant vegetation of the permanent breeding zone is a wooded steppe of *Prosopis* and *Larrea* bushes and some areas are used for large-scale, low-density livestock grazing (pers. obs.); however, grazing impacts on the South American locust are unknown. Interestingly, recent experiments showed that gregarious marching bands of this species are carbohydrate (not protein) limited in the permanent zone, and even more so in its recent invasion area in southern Bolivia (Overson et al. unpublished data). These results suggest *S. cancellata* might prefer low nitrogen (low protein, high carbohydrate) plants, at least while migrating. Potentially, grazing practices that lower plant nitrogen content could promote South American locust outbreaks, similar to the Mongolian locust (Cease, 2012). However, despite their affinity for carbohydrates over protein (Overson et al. unpublished data), this species is remarkably polyphagous and has been recorded eating a variety of presumably high protein plants (e.g., *Prosopis*, soy, peanut). During upsurges, swarms expand throughout the larger species range resulting in widespread invasion into including both grazing and crop land. During the current (2015–2018) upsurge, damages were reported to natural pastures with only minor impact on crops in Argentina. In Bolivia, nymphal bands, and adults damaged soybeans, maize, sorghum, peanut, and citrus. In Paraguay, nymphs and adults were detected on natural pastures and shrublands (Medina et al., 2017).

Schistocerca gregaria flaviventris (Burmeister, 1838); *Schistocerca gregaria gregaria* (Forskål, 1775)

Desert locust

Taxonomic notes. The *S. gregaria flaviventris* subspecies is found in southern Africa, exists as solitarious phenotypes, and rarely undergoes phase change—outbreaks from 1934 to 1935 and 1948 being two limited exceptions (Chapuis et al., 2017). Therefore, our review focuses on *S. g. gregaria*, the phase-changing northern subspecies that forms massive swarms. This locust has been the focus of extensive life history and biology research (reviewed in COPR, 1982; Symmons and Cressman, 2001; Pener and Simpson, 2009; Cullen et al., 2017), including food selection and nutrient balancing (Maxwell-Darling, 1936; Chandra and Williams, 1983; Behmer et al., 2001).

Pest status. Desert locust swarms typically originate in desert regions mostly uninhabited by people across North Africa, the Middle East, and Southwest Asia. However, this species' expansive invasion zone is among the largest of any locust and includes many agricultural areas (Popov et al., 1991). *S. gregaria* has caused periodic devastation for millennia and was recorded as early as 2420 BC in Egyptian tombs (Nevo, 1996). This species remains a threat through modern times, although recent plagues persist for shorter time periods. For example, before 1963, plagues in the 1900s persisted 7–14+ years (Waloff and Green, 1976; Symmons and Cressman, 2001); the four plagues that occurred between 1965–2006 each lasted <3 years (Magor et al., 2008). This decline is correlated with implementation of preventative desert locust control (Magor et al., 2008; Symmons, 2009). Nevertheless, desert locust outbreaks and plagues remain costly. The last major plague was 2003–2005, costing over $500 million USD to control (Belayneh, 2005) and resulting in 80–100% crop losses in afflicted regions, predominantly Sub-Saharan Africa (Brader et al., 2006). These short-term devastating impacts on agriculture have long-term effects on livelihoods, including educational outcomes. A study based in Mali showed that children born during the 1987–1989 plague in villages hit by locusts were less likely to ever start school; the effect was greatest for young women (De Vreyer et al., 2014). Since 2005, and as of February 2019, no plagues have occurred, but there have been numerous localized outbreaks allowing formation of smaller swarms, including swarms originating from along the Red Sea in early 2019 (FAO Locust Watch: http://www.fao.org/ag/locusts). The desert locust eats a wide variety of crops and other plants, including a broad assortment vegetable and cereal crops, banana, citrus, groundnuts, fruit trees, coffee, and many others (reviewed in COPR, 1982). Due to its vast reach and significance to agriculture, this species is often considered the most dangerous migratory pest in the world (Steedman, 1990).

Habitat and ecology. The desert locust is well-adapted to live in a vast arid and unpredictable landscape. During recession years, *S. gregaria* live in a broad belt of arid and semi-arid habitat that spans from the western coast of Africa to northwest India and has an average annual of rainfall of roughly 0–400 mm. The rainfall is sporadic, unpredictable, and can vary as much as 70% above or below average. Outbreaks can arise unpredictably from many areas within the expansive recession zone if the areas receive rain in sufficient amounts and timing to develop habitat suitable for locust growth (Cressman, 2016). *S. gregaria* is multivoltine, with up to three generations per season under favorable conditions. Typically, it takes several years for outbreaks to develop into a plague, but plagues can subside within 6 months (Roffey and Magor, 2003). Locusts can travel 150 km in a day and typically migrate between seasonal breeding areas (Pedgley, 1981). Heavy rains can allow for population buildup within the recession zone. As vegetation rescinds, locusts aggregate, which can lead to gregarization.

Schistocerca gregaria research has been foundational to our understanding of locust phase polyphenism. Tactile stimulation on the hind femur (Rogers et al., 2003) or a combination of visual and olfaction stimuli from conspecifics (Roessingh et al., 1998) will induce behavioral phase change. This behavioral shift from the solitarious to gregarious phase is modulated by a pulse of serotonin (Anstey et al., 2009), can take place in 4 h, and involves an increase in activity and attraction to conspecifics (Rogers et al., 2014). There are several lines of evidence to suggest that this initial behavioral shift is an anti-predator strategy. *S. gregaria* (and several other, but not all, locust species) develop aposematic coloration when at high density and will accept foods containing the alkaloid hyoscyamine (Despland and Simpson, 2005a), which garners them "gut-content mediated toxicity" (Sword, 1999, 2001). At a small scale, clumped resources promote interactions and consequent gregarization (Bouaïchi, 1996; Collett et al., 1998; Despland and Simpson, 2000; Despland et al., 2000; Babah and Sword, 2004; Cisse et al., 2013, 2015), but this relationship may be the opposite at large, landscape-scales (Despland et al., 2004). In addition to resource distribution, food quality affects phase change. For example, *S. gregaria* given multiple options of imbalanced diets will switch more regularly among them to attain complementary nutrients, which increases the rate of contact with other locusts (Despland and Simpson 2000). The mechanisms and consequences of phase change are reviewed in depth in Cullen et al. (2017).

Grazing interactions. Gregarious swarms in the invasion zone have a much-expanded list of plants they will consume (COPR, 1982), leaving a broad range of crops and rangeland vulnerable. Abou-Ali and Belhaj (2008) explored the benefits and costs of locust control campaigns in Morocco, Sudan, and Eritrea from 1980 to 2000. The desert locust caused significant losses in crop and fodder, the latter decreasing livestock production. However, pesticides had negative effects on human, livestock, and environmental health. Considering these tradeoffs, as well as the cost of control, a contingent valuation method showed that, as an alternative to pesticides, farmers would be willing to pay an annual fee that would later be used to compensate affected farmers in the event of an invasion (Abou-Ali and Belhaj, 2008). For a comprehensive review on management strategies for *S. gregaria* to reduce damage to crops and livestock grazing areas, see Van Huis et al. (2007).

Field observations suggest that camel grazing accelerates gregarization, potentially by concentrating locusts onto ungrazed vegetation (Roffey and Popov, 1968). However, when in agricultural areas, *S. gregaria* tends to be more prevalent in cultivated areas, relative to grazing sites (Van Der Werf et al., 2005). Moreover, when in grazing areas, the desert locust may eat trees and shrubs and not be in direct competition with livestock (Wilps and Diop, 1997). This pattern might be explained by a preference for high nitrogen plants. *S. gregaria* grows faster and has higher survival rates when fed nitrogen-fertilized millet, in contrast to other locusts (Van Huis et al., 2008). The same study showed that *S. gregaria* is also more abundant in areas with lower grazing pressure and higher-nitrogen plants.

Schistocerca interrita (Scudder, 1899)

Peruvian locust

Taxonomic notes. The regional common name for this species "langosta migratoria" confuses taxonomic identification as the name is applied to *S. piceifrons peruviana* as well (Duranton et al., 2006).

Pest status. Until 1983–84, this species was known only as a non-swarming grasshopper in Peru until an upsurge connected to El Niño and the resulting extreme rainfall in the Lambayeque Desert in northern Peru (Duranton et al., 2001). El Niño years drive a pattern where the Lambayeque Desert becomes a productive breeding ground leading to upsurges, a phenomenon attributed to the two most recent outbreaks in the area as well. A large outbreak which spread throughout the Lambayeque and Cajamarca provinces in 1998 led to a campaign of government monitoring and spraying driving an end to the upsurge by 2002 with control efforts costing an estimated $2,057,000 USD (Morales R.S. pers. comm.). More recently, an anticipated outbreak due to El Niño occurred in 2017 with successful preventative treatment beginning in May via monitoring and spraying of focal areas totaling 7,769 hectares scattered throughout a much larger 320,000 hectare region (Morales R.S. pers. comm.).

Habitat and ecology. Schistocerca interrita is a mix-feeder that prefers dry wooded areas with sandy soils where plants in the genus *Caparus* and *Angularis* occur, as well as anthropogenically cleared areas (Duranton et al., 2006). Plants in the family Solanaceae such as *Exodeconus prostatus* as well as grasses are known food sources when solitarious individuals are at low densities (Morales R.S. pers. comm.). Nymphs are green at low density but develop black and yellow markings at high density. Basic ecology and population dynamics have not been well-studied (Song, 2011). Adults move seasonally to 2,800–3,300 m elevation tracking green vegetation during the dry season of winter. During the wetter summer, adults move to lower elevations (500–1,000 m) to mate and oviposit. The species is also possibly bivoltine, with a spring generation from eggs laid by seasonally descending adults at intermediate elevations around 1,800 m, and a second generation born at lower elevations (Duranton et al., 2006). After birth, nymphs move to higher elevations gradually as they develop through five (or sometimes six) instars. During winter, adults diapause and later become reproductively capable around September and onward into summer.

Grazing interactions. The Peruvian locust is known to have an affinity for areas which have been anthropogenically cleared for grazing and for overgrazed areas and these are of focus during monitoring and treatment (Morales R.S. pers. comm.).

Schistocerca piceifrons Peruviana (Lynch Arribalzaga, 1903); *Schistocerca piceifrons piceifrons* (Walker, 1870)

Central American locust

Taxonomic notes. Research and management of *S. piceifrons* has been confounded by a history of misidentifications and nomenclatural changes. Originally, the species was described as *Acridium patianum* and subsequently described as two different species by F.L. Arribálzaga: *S. peruviana* and then *S. urichi* (Lynch Arribalzaga, 1903, 1918). The species has also often been confused with either *S. gregaria* or *S. americana*. It was incorrectly referred to as *S. paranensis* in the literature (a name originally applied to the gregarious form of *S. cancellata*). One invasion in Guyana in 1917 led to printed identifications of four different names (Harvey, 1983). As currently described, *S. piceifrons* has two subspecies: one from Mexico and parts of Central America (*S. p. piceifrons*) and the other from Peru, Ecuador, Colombia, Venezuela, Panama, and Trinidad and Tobago (*S. p. peruviana*) (Harvey, 1983; Barrientos Lozano et al., 1992).

Pest status. Schistocerca piceifrons piceifrons has been responsible for high levels of agricultural damage historically throughout southern Mexico and northern Costa Rica making it one of the most important pests in that region (Harvey, 1983; Barrientos Lozano et al., 1992; Cullen et al., 2017). *S. piceifrons* has a long history as a pest, and has even been implicated in the downfall of the Mayan civilization (Flores Granados, 2011). Upsurges of *S. p. piceifrons* were recorded as early as 1611 with an average of 3–5 plagues per century thereafter, affecting every country in Central America (COPR, 1982). Plagues during 1922–23 almost certainly originated from resident populations of *S. piceifrons* in Campeche and Yucatán but this was not recognized due to confusion about the solitarious and gregarious phases (Harvey, 1983). Three permanent breeding areas were detected historically for *S. p. piceifrons* in Central America (Harvey, 1983), including one in the Yucatan which has been under strong management focus recently as a site of continual gregarization and invasion source for a large zone in the region including Veracruz, San Luis Potosí, Tamaulipas and Tabasco (Cullen et al., 2017). To this day, *S. p. piceifrons* continues to be one of the most damaging insect pests in all Mexico and Central America. Intensive monitoring and pesticide treatments are implemented to stave off outbreaks virtually every year for this species in Mexico throughout an area of 64 municipalities in the states of Campeche, Chiapas, Hidalgo, Oaxaca, Quintana Roo, San Luis Potosí, Tabasco, Tamaulipas, Veracruz and Yucatán with major damage reported on corn, soy, beans, peanut, cotton sugar cane, coco, citrus, and others (SENASICA-D GSV, 2016). The subspecies *S. piceifrons peruviana* is currently of management concern in Peru and is regularly monitored and sprayed but has not had any major eruptions of significant economic importance since a large upsurge from 1945 to 1948 and is less economically significant than the sympatric *S. interrita* in Peru (Morales R.S. pers. comm.). In addition to low volume spraying focusing on nymphs, manual collecting of individuals are used in control of this species, as well as poisonous baits, especially in areas where livestock poisoning is a concern (Morales R.S. pers. comm.).

Habitat and ecology. This species is a generalist herbivore distributed from southern Mexico throughout Central America and northern South America where annual rainfall is between 100 and 250 mm and there is both a distinct dry winter and no cold season. Dry tropical woodland is believed to be the native habitat (Harvey, 1983) and it is not found in appreciable numbers in mature woodlands. Habitats that can support higher densities contain bare earth, food plants (which are composed of variable plant communities from site to site) (Bredo, 1963), and tall shrubs for shelter (Harvey, 1983). In Mexico, the species is bivoltine with generations in spring and fall. The spring generation is shorter in duration (Hernández-Zul et al., 2013) and the fall generation goes into diapause as adults (Song, 2011). Nymphs are green at birth and at low density, but at high density exhibit black markings on a peach background (Hunter-Jones, 1967). Unlike findings with *S. gregaria* (Ellis and Ashall, 1957), nymphs are able to move through high grass without losing cohesion, even traveling by climbing shrubs and leaping from one to the next (Harvey, 1983). Gregarious adults become bright yellow when sexually mature (Harvey, 1983). Similar to other locusts, behavior of adults can change post-molt depending on density of conspecifics so that gregarious vs. solitarious morphology and physiology can be decoupled (Harvey, 1983). Life history characteristics have been studied both in the laboratory (Hunter-Jones, 1967) and two locations in the field (Bredo, 1963). Most individuals have six nymphal instars but some males have only five. This species is a strong generalist feeding on: maize, wheat, rice, palms, citrus, sunflower, soybeans lentil, wax myrtle, potato, tobacco, banana, sugarcane, sorghum, and more (COPR, 1982; Cullen et al., 2017). *S. p. piceifrons* appears to use the patchily distributed shrub *Pisonia aculeate* for refuge, as the plant remains green year-round. This plant potentially drives aggregation by concentrating locust densities (Poot-Pech et al., 2016; Cullen et al., 2017). Research on the environmental drivers of locust density in the Yucatán peninsula demonstrated a correlation between locust density and the presence of the grass *Panicum maximum*, but no correlation with soil type (Poot-Pech et al., 2018).

Grazing interactions. Over the past 30 years, the state of Yucatán has experienced a dramatic increase in the conversion of forest to grassland driven by ranching and agriculture. A 47% increase in grassland from 1981 to 2014 has greatly expanded suitable habitat in the region for *S. piceifrons* as well as its capacity for gregarization and swarming, making the Yucatán of prime importance for managing locust swarms (Poot-Pech, 2016). Additionally, the ranching practice of burning dried grass annually to promote regrowth in the spring encourages aggregation and thus gregarization by locusts by creating patchworks of bare soil that are desirable for oviposition. Earlier observations of *S. p. piceifrons* found them commonly in disturbed forest, as well as extensive plantations of sisal (Yucatán) that are harvested on 7-year cycles and harbor locusts during regrowth cycles. In Campeche, hopper bands were observed by Harvey in 1981 in pastureland with regrowth of trees and cleared roadsides. In these instances, nymphs were always marching downwind and tended to accumulate in shrubby weeds when moving across bare ground, likely maintaining or heightening gregarization. Well-maintained crops and pasture are apparently not suitable habitat (COPR, 1982).

Gomphocerinae - Clade B

Dociostaurus maroccanus (Thunberg, 1815)

Moroccan locust

Pest status. This locust was likely one of the grasshopper species described in the bible due to its spectacular plagues. It is highly polyphagous and attacks cereal, but also, vegetables, forage, oil producing crops, fruit trees, date palms, and even conifers (Latchininsky, 1998). Crop damage from *D. maroccanus* has been reported in more than 25 countries, often requiring military help for control (Latchininsky, 1998). Interestingly, it appears that outbreaks are less frequent in recent years, probably because croplands are replacing locust habitat. However, in some portion of its range, overgrazing can make locust problems worst (Latchininsky, 1998).

Habitat and ecology. The Moroccan locust lives in dry, degraded areas, with bare patches of soil and fragmented vegetation cover (Latchininsky and Launois Luong, 1992). It can be found from the Atlantic islands (Madeira and Canary islands), throughout the Mediterranean zone, to Afghanistan and South Kazakhstan (Latchininsky and Launois Luong, 1992). This species feeds on over 150 plant species belonging to 33 families, including 50 different crop species (Latchininsky and Launois Luong, 1992). This is unusual for a grasshopper belonging to the Gomphocerinae sub-family, whose members are typically grass feeders. Its feeding habits are reflected in its biology: it has a higher number of sensilla relative to other Gomphocerinae and is more similar in this regard to mix-feeding species belonging to the Oedipodinae sub-family (Ghadraoui et al., 2002).

Grazing interactions. Dociostaurus maroccanus has significant interactions with livestock as a competitor because it attacks forage crops (COPR, 1982) and can be extremely abundant in overgrazed pastures. Overgrazing likely promotes outbreaks of this species because it leads to fragmented vegetation cover and bare soil, which is the ideal habitat for the Moroccan locust (Latchininsky, 1998). Indeed, this locust tends to colonize areas where the natural vegetation has been disturbed and is patchy. In Algeria, such areas are concentrated around human settlements, earning Moroccan locusts the name djerad-el-adami ("man's locust") (Pasquier, 1934). Similarly, locust penetration into previously uninhabited arid areas of the Middle East usually follows the paths of nomadic herders of cattle and sheep (Skaf, 1972). Overgrazing throughout the range of *D. maroccanus* has become increasingly common (Uvarov, 1977) due cropland expansion and subsequent reduction in available grasslands for livestock grazing.

GRASS-FEEDING LOCUSTS

Most locust species are grass-feeders (12 out of 19 species). Grass-feeders theoretically only feed on plants belonging to the family Poaceae. However, this designation is nuanced on two levels. Firstly, grasses belongs to the fifth-largest plant family with over 10,000 species and are very ubiquitous in their distribution (Gibson, 2009). Secondly, following gregarization locust host-plant range expands considerably (Despland, 2005) and every single species described below has been reported feeding on plants outside of the Poaceae family during outbreaks. Grass-feeders are thus typically present in grassland and pastures where they compete with livestock. They readily feed on Poaceae of economic interest like cereals or forage, but they can also cause serious damage to non-grass crops during outbreaks.

Subfamily Cyrtacanthacridinae - Clade D

Austracris guttulosa (Walker, 1870) (Synonyms: *Nomadacris guttulosa*)

Spur-throated locust

Pest status. This locust is fairly common in southwestern Australia, but typically only forms swarms above 25°S latitude. It feeds on a wide range of crops with well-documented damage historically (COPR, 1982 and references therein), including sorghum, sunflowers, soybeans, millet, wheat, eucalyptus, banana, and citrus. Additionally, native trees (*Eucalytpus*, *Acacia*), shrubs (*Dodonaea*), and several grasses are consumed and allow the species to persist in marginal conditions (COPR, 1982). This species is of less economic importance than other Australian orthopteran pests and, as nymphs they are not considered economically viable to control unless in and around high-value crops. However, this species will cause economically relevant damage once a threshold of around 20 nymphs or 3 adults per m^2 is reached (DPI, 2018).

Habitat and ecology. This large brown locust is a tropical dryland locust with a lifecycle adapted to a prolonged, dry winter and wet summer. Observation and mandible morphology suggests a preference for grass over dicotyledons (Bullen, 1968; COPR, 1982). The species is univoltine and survives the dry season as sexually immature adults with low feeding rates that overwinter in large clusters in trees (Jenkins, 1968; COPR, 1982). Sexually mature individuals engage in long distance migrations under the cover of darkness in spring and summer from Oct–Jan. During autumn and winter young adults engage in shorter migratory flights (DPI, 2018). Adult females lay eggs on summer nights between Dec–Jan on cracking clay soils (COPR, 1982). Grass plains with clay-rich soil in the North Territory and Queensland support high levels of breeding (Bullen, 1968). In one irrigated area in Western Australia oviposition was concentrated along banks and cleared areas adjacent to irrigation canals (Bullen, 1968). Anthropogenically cleared areas such as roadsides are also used as oviposition sites (COPR, 1982).

Grazing interactions. The first swarms recorded in New South Wales (NSW) were in 1973, which was 8°-10° of latitude further south than its previously described breeding range (Casimir and Edge, 1979). That same summer, in March of 1974, swarms moved from sorghum fields in NSW into grazing country. Individual swarms throughout the grazing areas ranged in size from 200 to 800 ha where they roosted in and completely defoliated trees, in addition to eating pasture grass. One of three key factors implicated in the 1974 swarming event was the conversion of grazing land to cereal, oil-seed, and sorghum production in the preceding decade (Casimir and Edge, 1979). Nymphs and adults of this species are found abundantly in medium size forage grasses such as *Chloris gayana* which provide excellent shelter and food (COPR, 1982). Nymphs of this species often move into crops from nearby rangeland so effective management includes treating adjacent rangeland/pasture to avoid reinvasion (DPI, 2018).

Nomadacris septemfasciata (Audinet-Serville, 1883)

Red locust

Pest status. This species has been controlled by IRLCO (International Red Locust Control Organization), since the last great invasion of 1929–1944, which affected most African countries south of the equator (Bahana, 2000). Costs were measured in South Africa during two seasons, 1933–34 and 1934–35, and control alone was £933,000 (around 40 million USD today), even though most of the labor was unpaid (COPR, 1982). Infestations are now less frequent and are mainly limited to the reproduction areas, far from areas of cultivation (Thindwa, 1999). However, large outbreaks occurred between 1994 and 1996, and more recently in 2009 (FAO, News Article: Red Locust disaster in Eastern Africa prevented, 2009).

Habitat and ecology. The red locust is predominantly a grass feeder but will feed on shrubs and trees during outbreaks. It is mainly distributed throughout central and southern Africa; some isolated populations can also be found in the lake Chad basin, the central delta of the Niger River in Mali, and the Cape Verde Islands. Outbreak areas are mainly located in the Great Lakes region of East Africa, in Tanzania, Zambia, Malawi and Mozambique (COPR, 1982; Lecoq et al., 2011). They usually breed in seasonally-flooded plains where there is a mosaic of tall grasses and sedges (e.g., *Echinochloa*, *Hyperrhenia*, and *Cyperus* spp.) and shorter grasses (*Cynodon sp.*) (Burnett, 1951; Vesey-Fitzgekald, 1955; COPR, 1982). During outbreaks, swarms usually fly low and don't travel more than 20–30 km. Unlike many other locust species, red locusts appear to fly during the day and, in low density, into the wind until they reach areas characterized by large stands of the grass *Echinochloa.* High-density populations have been recorded to fly downwind, likely because the wind speeds were too high for upwind orientation (Chapman, 1959).

Grazing interactions. The red locust can compete with livestock for forage. In South Africa from 1933 to 1935, red locust damage to grazing areas for sheep and cattle, and to maize and sugar cane was valued at £20, 000 (over a million USD today); an additional £40,000 was lost due to decrease in animal product outputs through arsenic poisoning used for locust control. However, in recent years, likely due to control in the known breeding areas, red locust oubreaks are minimal and do not result in as much damage to crops and pastures, particularly since biopesticides and spray aircrafts are now used (Thindwa, 1999; FAO, News Article: Red Locust disaster in Eastern Africa prevented, 2009).

Patanga succincta (Johansson, 1763)

Bombay locust

Pest status. Thailand was largely unaffected by locust and grasshopper damage until the early 1960's when a Bombay locust outbreak had a significant impact on maize. This unprecedented outbreak may have been brought on by forest clearing and cereal production (Bullen, 1966). The species numbers and resulting damage grew considerably from 1961 to 1963 with enough individuals to damage 8,000 acres of maize worth £50,000 at the time. In Thailand today, it is both a major agricultural pest and one of the most popular insect food items commercially produced for human consumption (Phiriyangkul et al., 2015). Corn is grown to feed to locusts that are later sold as food commercially (Hanboonsong et al., 2013). Bombay locusts have been reported damaging a large variety of crops including: coconut, maize rice, bamboo, banana, betel nut, bulrush millet, cashew, cassava, castor, chinse cabbage, citrus, common millet, cowpea, cucumber, *Conax canniformis*, durian, fig, ginger millet, *Gardenia*, ginger, ground nut, guava, jujube, mango, mulberry, mung bean, mustard, oil palm, pigeon pea, rambutan, rubber, sorghum, soy, sugar cane, sweet potato, talipot, palm, tea, and tobacco (Roffey, 1979).

Habitat and ecology. The Bombay locust is distributed throughout the Middle East and Asia (COPR, 1982). It inhabits grassy plains of Asia up to about 1,500 m. In Rajasthan, India, it has been associated with *Cyperus tuberosus*, a sedge sometimes cultivated for its edible tubers. Historically, swarms were recorded in India from 1787 to 1796 and from 1901 to 1908, but since then only occasional swarms have been reported (COPR, 1982). These uncommon swarms inhabit forests of the Western Ghats during winter and, as temperatures rise, move north or east where they disperse and reproduce after rains. In these areas, Bombay locusts have been recorded to oviposit in grasslands, millet fields, and embankments between farm fields (COPR, 1982).

Grazing interactions. Conversion of forest to grass and cropland has likely increased Bombay locust populations in Thailand. Deforested fields with the grass "lalang" (*Imperata* sp.) support locust densities up to 20–50 per m^2. In Malaysia, high locust abundance occurs in land cleared for rice, because locusts feed on the grasses that initially grow there, but populations diminish with heavy weeding over time in rice fields and they are not a significant rice pest (COPR, 1982).

Subfamily Gomphocerinae—Clade B

Gomphocerus sibiricus (Linnaeus, 1767); Synonym *Aeropus sibiricus*

Siberian locust

Pest status. This locust is one of the most serious agricultural pests in the eastern regions of the European part of the former USSR: the southern forest, forest-steppe, and steppe zones of Kazakhstan and Siberia. It is also one of the most important pests of Xinjiang province in northwestern China (Yang and Wang, 2004). The larvae and adults severely damage summer sowings of wheat, rye, oat, barley, and to a lesser extent winter rye, corn, panicum, mogar, buckwheat, tobacco, potato, cabbage, mustard, hemp, flax, and other agricultural crops, and also pastures and haysag [AgroAtlas - Pests - *Aeropus sibiricus (L).* - Siberian locust (Siberian grasshopper)., 2003].

Habitat and ecology. Most locusts species are found in lower-elevation environments, however the Siberian locust range includes high mountains (between 2,000 and 2,800 m) ranging from West Siberia to the Atlantic coast (Gosálvez and López-Fernández, 1981). In the subalpine grasslands of Switzerland, it dominates habitats with short grasses (Spalinger et al., 2012). In the Irkutsk region of Russia, it is often associated with *Agropyrum cristatum* (crested wheatgrass) (Vinokurov and Rubtzov, 1930). It is capable of living in low altitude as well and can be found in desert steppes and meadow steppes. Siberian locusts can regulate the level of stress resistant substances, like linoleic acid, in response to fluctuating temperatures. This adaptation is important under the trend of climate warming as outbreaks tend to be more persistent during warmer weather (Li et al., 2014). The adults mate in August (Bouchard, 1998) and females lay egg pods containing 7–10 eggs, and oviposit an average of 11 times. The Siberian locust is univoltine and hatching usually takes place late the following spring (Il'enko, 1930).

Grazing interactions. In Switzerland, this species does not appear to be affected by wild ungulate grazing (Spalinger et al., 2012). However, sheep may impact population dynamics (Gueguen-Genest and Gueguen, 1987).

Subfamily Oedipodinae—Clade B

Aiolopus simulatrix (Walker, 1870); Synonym *Aiolopus savigny* (Krauss, 1890) (Moussi et al., 2011)

Sudan plague locust

Pest status. Several members of this genus are economically important and are widely distributed from agriculture rangelands to semi-desert regions (Bughio et al., 2014). In Sudan, *A. simulatrix* is the most devastating pest of cereal crops (Joyce, 1952; Song, 2011). In the Sahelian region, this locust migrates on long-range diurnal (Chapman, 1976) and nocturnal flights along with the Senegalese locust, *Oedaleus senegalensis*, following the winds associated with the Intertropical Convergence zone (Riley and Reynolds, 1983). It forms impressive migratory swarms, but the existence of hopper bands is not well-recorded (Song, 2011).

Habitat and ecology. The Sudan plague locust is a grass feeder, distributed in Anatolia, Southern Asia, and Central and Northern

Africa (Moussi et al., 2011; Kaya et al., 2015). A community analysis comparing acridids present in steppes or oasis in Algeria found that this locust was the only species present across these different vegetation assemblages. It was commonly found in halophilic vegetation (steppes) as well as irrigated cultures (oasis) (Moussi et al., 2011). It has one generation per year with adults peaking in June (Moussi et al., 2011) and it favors areas of bare clay soil with large cracks (Riley and Reynolds, 1983).

Grazing interactions. Given its natural and extensive presence in grasslands, the Sudan plague locust is very likely to compete with livestock for forage, although that effect, to our knowledge, has not been measured (Shen et al., 2009; Wang et al., 2010).

Ceracris kiangsu (Tsai, 1929)

Yellow-spined bamboo locust

Pest status. This locust is a pest of cereal crops and bamboo in South China, and is especially economically important in the Hunan province (COPR, 1982). Banana, maize, millet, rice, sorghum and other crops are also attacked (COPR, 1982 and references therein). In 2015, Laos experienced an unprecedented outbreak, which impacted 20,000 people (Locust control campaign in northern Laos, 2016).

Habitat and ecology. The yellow-spined bamboo locust can be found in India and Thailand but is mainly concentrated in southern China where it is a serious pest of grain crops (Uvarov, 1977) and bamboo leaves (COPR, 1982). Most eggs are laid on southeast facing mountain slopes at 300–400 m. Preferred host plants include many species of bamboo, but it will also feed on other grasses including wild grasses and cereal crops. It has also been recorded eating, palms, and a handful of herbaceous plants (COPR, 1982 and references therein). This locust engages in puddling behavior in the presence of human urine and is attracted to several componets of urine, especially NaCl (Shen et al., 2009). Ongoing research is exploring the potential of these results to develop baits for control in China (Yu et al., 2011a,b)

Grazing interactions. As C. *kiangsu* has principally been reported only as a pest of bamboo and cereal crops in Asia, no substantial interactions between grazing and this species are currently known.

Chortoicetes terminifera (Walker, 1870)

Australian plague locust

Pest status. The first outbreaks of the Australian Plague locust were recorded in the early 1870s, with earlier problems in the 1840s being attributed to the small plague grasshopper, *Austroicetes cruciata* (Saussure, 1888) (Deveson, 2012). Today the Australian plague locust is among the most significant and widespread agricultural pests in the country (Hunter, 2004). An extensive plague in the 1930s initiated a national research approach to address the "grasshopper problem" (Deveson, 2011). Another significant plague occurred in the 1950s, but it was the 1973–74 plague extending through New South Wales, northern Victoria, and southern South Australia that provided the impetus to develop the Australian Plague Locust Commission (APLC). Since then, there have been five plagues, roughly spaced every 10 years. One of the worst, and most recent, was the 2010 plague with about 2,000 landholders reporting high-density bands (Deveson, 2011). Since 2010, there have been some localized outbreaks, but the 2017–18 season had among the lowest locust numbers recorded by the APLC (Locusts - Department of Agriculture and Water Resources). Australian plague locusts impact a variety of crops including cereal crops, clover, cotton, potatoes, sugar cane, orchards, vegetables, and pasture grasses (COPR, 1982).

Habitat and ecology. *Ceracris terminifera* is distributed throughout the arid and semi-arid regions of Australia, which is most of the country, excluding the wetter coastal regions. A combination of molecular and survey data suggest populations are well-connected throughout its range (Deveson and Walker, 2005; Chapuis et al., 2011). Extensive research has been carried out on *C. terminifera*, starting in the 1930s and continuing through present time, including its migration and life history [e.g., (Key, 1945; Clark, 1950, 1965; Farrow, 1979b; Hunter, 1982; Drake and Farrow, 1983; Wright, 1983; Miller and Simpson, 2010; Graham et al., 2015; Woodman, 2017); reviewed in (Deveson, 2011)]. It is multivoltine with typically 2–3 generations per year; more generations per year leads to exponential growth and outbreaks.

Ceracris terminifera are adapted to arid and semi-arid environments with high temporal and spatial variability in precipitation (Hunter et al., 2001). This locust can persist over dry periods through embryonic diapause or as quiescent eggs or adults and can migrate several hundred kilometers in a single warm night or about 20 km during a day flight. While these migratory events can lead to unfavorable environments, e.g., flying into the ocean, or areas with no rainfall (Farrow, 1979), they are important for at least some percentage of migrants to find areas of localized rainfall and subsequent green vegetation (Hunter et al. 2001). Field studies revealed that sufficient rains, and corresponding green vegetation, were necessary to enable accumulation of lipids for long distant flight and that *C. terminifera* below a certain body lipid content did not migrate (Hunter et al., 1981). *C. terminifera* prefers grasses and is often found in perennial tussock grass communities (Hunter, 1989) but will feed on forbs and shrubs if water limited. Lack of water may induce mortality in more arid parts of its range (Bernays and Chapman, 1973). Access to grasses from which they can extract a carbohydrate-rich diet are also likely to be critical to maintain growth in field populations (Clissold et al., 2006), and to build up lipid stores for migration. Therefore, the composition of available vegetation, and not just the amount, is expected to play a significant role on the population dynamics and migratory potential of this species.

Ceracris terminifera exhibits behavioral phase polyphenism (Gray et al., 2009), with crowd-reared nymphs increasing activity and propensity to spend time near conspecifics (Cullen et al., 2012). Antennae tactile stimulation is the most important proximal cue for inducing gregarious behavior (Cullen et al., 2010). Crowd-reared nymphs tend to have increased melanization relative to solitary-reared nymphs; however, this species does not exhibit striking color differences between the phases (Rogers S., unpublished data, pers. comm.). There is some evidence that clumped resources promote gregarization, likely due to increased conspecific interactions. For example, an occupancy model showed there was a higher probability of dispersed populations leading to gregarious outbreaks in desert areas, where vegetation resources are more clumped, relative to grasslands (Veran et al., 2015).

Grazing interactions. The Australian plague locust is often found in livestock grazing areas due to its preference for grasses and low woody vegetation cover (Clark, 1950). Such rangelands are likely required to support population build up leading to outbreaks, which then spillover into proximate and distant grazing and crop lands (Watts et al., 1982; Symmons, 1984; Wright and Symmons, 1987). The importance of rangelands for *C. terminifera* populations may be due to a number of factors including bare ground areas for thermoregulation and oviposition, as well as plant species and quality optimal for locust growth. For example, *C. terminifera* tend to select sites for oviposition where there is about 50% bare ground and short vegetation (Clark, 1947). Indeed, historic deforestation and creation of new grasslands likely allowed the expansion of this species in the late 1800s through to present day (Deveson, 2011, 2012). In large numbers, *C. terminifera* can wipe out grazing paddocks, leaving limited forage for livestock and negatively impacting landholders. However, locust control can also negatively impact the livestock industry through the effects of pesticide residues on livestock (Hooper, 1998).

Gastrimargus musicus (Fabricius, 1775)

Yellow-winged locust—Australia

Pest status. This locust is an important pest in areas which receive summer rainfall in northern Australia where periodic plagues connected to increased rainfall historically devastated home gardens and farms (Jenkins, 1968). The species has also been recorded as an occasionally significant pest of sugar cane (Long and Hensley, 1972) and is known to attack cotton, maize, Monterey pine, pineapple, Rhodes grass, sorghum, Sudan grass, sugar cane, vine, orchards, and pasture (COPR, 1982). Sporadic outbreaks in recent decades have caused significant damage to pasture albeit at local scales (Kathy, 2006; Gartry and Edwards, 2016; Beavan, 2017; Stanley, 2018).

Habitat and ecology. The yellow-winged locust gets its name from conspicuous black and yellow hind wings. The species is found in coastal and subcoastal areas of Australia with more than 500 mm of annual rainfall and patchy habitat containing tall grass with bare ground. It reportedly prefers the pasture grasses *Cynodon dactylon* and *Paspalum dilatatum* (COPR, 1982; Bernays and Hamai, 1987) and is common in rangelands, pastures, parks, and lawns. At low densities individuals are usually predominantly green but when swarming they become brown. Number of generations per year depends on geography, with normally two generations in central Queensland but only one in the table lands of New South Wales (DPI, 2019).

Grazing interactions. Increases in population levels of this locust are most likely driven by deforestation and livestock grazing that result in a combination of tall and short grasses for shelter and food, and bare ground for oviposition (Uvarov, 1977). Egg pods are preferentially laid on bare, compact soil especially on overgrazed pasture (DPI, 2019). Continued sporadic outbreaks occasionally breaking into swarming over the last several decades have cause considerable damage to pasturelands with occasional swarming in Western Australia, but swarming frequency is less common, and swarms are more localized and slower moving relative to the Australian plague locust (DPI, 2019).

Locusta migratoria migratoria (Linnaeus, 1758); *Locusta migratoria migratorioides* (Reiche and Fairmaire, 1849)

Migratory locust

Taxonomic notes. The migratory locust is the most widely distributed grasshopper species in the world. Historically, many subspecies of *Locusta migratoria* were recognized based on their geographic range. A phylogeographic analysis (Ma et al., 2012) collapsed all subspecies into just two corresponding to the northern and southern lineages: the Asian Migratory Locust, *L. migratoria migratoria* (Linnaeus, 1758), and the African Migratory Locust, *L. migratoria migratorioides* (Reiche and Fairmaire, 1849).

Pest status. The migratory locust is present across the entire temperate and tropical Eastern hemisphere. Its northern limit corresponds roughly with the southern edge of the coniferous forest of Eurasia and it extends as far South as New Zealand. In the West it can be found as far as in the Azores and in the East as far as the Fiji (COPR, 1982).

Plagues from the Asian migratory locust were reported in China from 200 BC, typically in association with droughts and flood events (Stige et al., 2007; Tian et al., 2011). Its presence in Australia is more recent: before 1973 it was an uncommon species of the coastal and subcoastal region of Australia with a few scattered records from inland. In 1973–76, an important outbreak occurred in the Central Highlands district of Queensland (Farrow, 1979a). It is one of the most important agricultural pests in the countries of the former Soviet Union particularly Russia, Kazhakhstan, and Uzbekistan (Latchininsky, 2013). The Asian migratory locust can fly distances of over 1,000 km and infest very large areas, for example over 1 million ha in 1946 (Latchininsky, 2013). Outbreak densities can reach over 10,000 individuals m^{-2} for early stadium nymphs as reported in China in 2003 (Tanaka and Zhu, 2005).

Historically, the African migratory locust only gave rise to plagues in the Middle Niger flood plains and surrounding Sahelian steppes. There, conditions enable four to five generations to take place annually. Movements of swarms generally follow the movement of the inter-tropical convergence zone. Damages are typically reported for the whole invasion area, and usually concern cereals, but also other plants when grasses are not available (e.g., banana, pineapple leaves, palms, etc.). Recent rapid agricultural development, particularly broad-acre cultivation of cereal summer crops has caused population upsurges in new areas, which have occasionally led to outbreaks and plagues. Some outbreaks appear to have been a reaction to the initial disturbance and have never recurred while others recur when meteorological conditions are favorable (Farrow, 1987). Outbreaks have been reported in Mali, Chad, Sudan and Madagascar (Farrow, 1987).

In contrast to the desert locust, gregarization is a slower process for the migratory locust. Locusts are only partially gregarized even after 64 h of crowding (Guo et al., 2011; Ma et al., 2011, 2015). Similar to the desert locust, biogenic amines are involved in phase change polyphenism. However, their role is complex, and the functional significance of change in their concentration is not fully understood. For instance it appears that serotonin is linked to both behavioral solitarization and gregarization (Guo et al., 2011; Ma et al., 2011; Cullen et al., 2017). In the past two decades, advances in molecular research have demonstrated that full phase change is accompanied by extensive changes in gene expression (Kang et al., 2004; Guo et al., 2011).

Habitat and ecology. The Asian migratory locusts are typically associated with river deltas, and coastal plains that present intermittently flooded areas. They favor grasses such as *Panicum sp*. *Phragmites communis*, *Artemisia sp*. and *Polygonum*. In many areas, shifting cultivation practiced for a few years before abandoning the land has produced food and shelter, conditions that promotes transformation into the gregarious phase (Uvarov, 1936).

The African migratory locust favors area of 10–15% bare ground with mixed association of *Ctenium elegans*, *Cenchrus biflorus*, *Eragrostis tremula* for breeding in the late rainy season. The Niger flood plains provide special conditions because the retreating floods allow the locust to survive during the dry season but also to breed. Near the Lake Chad basin, *Cynodon* is the favored host plant. Shifting cultivation in the Sahel also led to the development of abandoned land with bare areas that retain millet, sorghum, and *Cenchrus biflorus* which are favored host plants.

Grazing interactions. In Mali, Chad, Sudan and Madagascar, people have likely increased migratory locust population through agricultural and pastoral activities. For instance, the Sahara Desert used to be unsuitable for this species, but human activities starting in the 1980's, particularly the introduction of irrigated crops under pivoting sprayers favored population increases (Benfekih et al., 2002; Benfekih and Petit, 2010). In the Touat region alone, cropped areas increased by more than 60% from 1984 to 1989 (Benfekih et al., 2002).

While the migratory locust is mostly considered a pest of cereal crops, a study in Tibet showed that early instars (1st and 2nd instar nymphs) fed mostly on pasture grass (Qing and Chunxian, 2008). After the 3rd instar they switched to barley and winter wheat. However, treating pastures and rangeland can cause serious health issues to livestock, as illustrated in China in the Xinjinan Uygur Autonomous region where infestation areas are huge and spraying by aircraft problematic because of livestock (Tanaka and Zhu, 2005).

Locustana pardalina (Walker, 1870)

Brown locust

Pest status. The brown locust is among the most serious pests in South Africa, attacking cereals and pastures (Price and Brown, 2000; Todd et al., 2002). Controlling the brown locust has cost the South African taxpayer millions of Rands annually. The massive outbreak in 1985–86 cost over R50 million to control (equivalent to $25 million USD at the time), while the outbreak in 1995–96 cost ∼R14 million ($3.5 million USD at the time). Due to locust control, the true swarming phase with extensive migrations has been limited in recent decades (COPR, 1982; Price and Brown, 2000; Todd et al., 2002). However, this species has been outbreaking more frequently than other locust species in South Africa, and these non-swarming outbreaks last for 7–11 years. De Villiers (1988) hypothesized that the lack of major swarms, and associated boom-bust population cycles, may increase the overall impacts of brown locusts on agriculture because outbreaks are found more consistently across years.

Habitat and ecology. This grass-feeding locust is found in the Karroo region of the Cape province of South Africa and neighboring parts of Free State and Namibia. It is primarily found in arid regions where rainfall is limited and erratic, the vegetation is sparse and short, and where it feeds mostly on dwarf grasses (COPR, 1982). Shifting rainfall patterns have been correlated with range expansion and contraction (Kieser et al., 2010). Increased summer rainfall (>150 mm) resulting in taller and more dense grasses was correlated with decreased locust populations, while drier conditions (100–150 mm) were associated with range expansion. During outbreaks the species used to spread throughout southern Africa, as far north as 16°S.

Grazing interactions. The brown locust has significant impacts on livestock pastures. In the 1970s, the most severe damage was in sheep grazing areas (Botha, 1974; COPR, 1982), when organochloride control options were tested but were shown to be impractical for sheep (Botha, 1974). Sheep grazing, in turn, may promote brown locust outbreaks. High sheep stocking rates since European colonization have been associated with land degradation in South Africa (Keay-Bright and Boardman, 2006) which creates shorter, sparser grasses that brown locusts prefer (COPR, 1982; Kieser et al., 2010). Currently, there is no active field monitoring program to locate hopper bands. Instead, control efforts rely on the legal requirement of farmers to report outbreaks on their property, after which broad-spectrum insecticides are applied on aggregated immature locusts or roosting mature swarms (Crooks and Cheke, 2014).

Oedaleus decorus asiaticus (Bey-Bienko, 1941)

Mongolian locust

Taxonomic notes. Bei-Bienko and Mishchenko (1951) distinguish *asiaticus* from *decorus*, but Ritchie (1981) maintains that *asiaticus* is a subspecies of *decorus* (*O. decorus asiaticus*) (Bei-Bienko and Mishchenko, 1951; Ritchie, 1981). We use *O. asiaticus* to maintain consistency with the literature that has focused on this species in China. Mitochondrial genome analyses have shown that *O. asiaticus* is closely related to the migratory locust, *L. migratoria* (Ma et al., 2009).

Pest status. Prior to the 1970's, *O. asiaticus* was rarely reported to cause agricultural damage. However, it is now considered a dominant pastoral pest across the northern Eurasian steppe (Jiang et al., 2003; Kang et al., 2007). For example, in 1999, an outbreak was recorded to span six million hectares in Inner Mongolia, China with densities of 100 individuals m^{-2}; in 2002, densities of over 1,000 *O. asiaticus* m^{-2} were reported (Wang, 2004). Feng et al. (1994) calculated that at a density of 11 individuals m^{-2}, Inner Mongolian grasshoppers consume about 15 % of the aboveground biomass or about 200 kg/ha of fresh forage annually, suggesting the outbreak numbers would decimate all vegetation. Northern and northeastern China is one of the most important grassland-based animal husbandry areas for the country (Li et al., 2008) and *O. asiaticus* and other grasshoppers compete with sheep and cattle for forage Accordingly, locusts are ranked as one of the most serious pests due to its capacity to devastate grassland productivity (China Ministry of Agriculture, 2012) and the country has implemented an ongoing management program including pesticides, biopesticides, and chickens as locust predators (Xu et al., 2014; Zhang and Hunter, 2017).

Habitat and ecology. Oedaleus asiaticus is found across the Eurasian steppe, including China, Mongolia, and Russia (Ritchie, 1981). It is primarily a grass feeder, especially *Stipa* spp. Several studies have demonstrated *O. asiaticus* is most abundant in *Stipa*-dominated landscapes and that it prefers and has the highest growth and survival when eating this grass (Han et al., 2008; Cease et al., 2012; Huang et al., 2015, 2016, 2017a). A gut transcriptome analysis revealed *Stipa krylovii* has a higher use efficiency by *O. asiaticus* relative to other common grasses, including *Leymus chinensis*, which is often co-dominant with *Stipa* in this species' range (Huang et al., 2017b). Accordingly, *O. asiaticus* has a greater olfactory response to plants in the Poaceae family relative to forb feeders (Chen and Kang, 2000). Several additional studies have investigated the nutritional ecology of this species. For example, field cage studies revealed that *O. asiaticus* maintains phosphorus homeostasis by changing absorption and excretion rates, but other mechanisms may be more important for nitrogen balance (Zhang et al., 2014). Based on a combination of field surveys in Inner Mongolia and climate data, *O. asiaticus* was most prominent on the temperate bunchgrass steppe at flat sites or sites with southeastern slopes at an elevation of 1300-1400 m, sites with chestnut soil containing about 60–70 % sand, and sites with moderate (30–50 %) vegetation coverage (Huang et al., 2015). It is univoltine with one generation per year, hatching in early June and undergoing five juvenile instars prior to molting into adults in mid-July (Ma et al., 2009; Cease et al., 2010). With climate warming, *O. asiaticus* is likely to hatch earlier, develop faster, and shift northward (Guo et al., 2009; Wu et al., 2012), though its capacity to withstand high temperatures as eggs may be limited (Hao and Kang, 2004).

Oedaleus asiaticus is a non-model locust (Song, 2011) with high-density outbreaks and swarms comprised predominantly of darker, brown morphs (Jiang et al., 2003). Brown morphs have increased relative investment in thorax and hind legs along with higher metabolic rates (Cease et al., 2010). Female brown morphs are larger, on average, than green morphs (males have not been compared), consistent with some locusts (*D. maroccanus*, *L. pardalina*, and *C. terminifera*), but in contrast of others (*S. gregaria*, *N. septemfasciata*, *L. migratoria*) (Uvarov, 1966, 1977; Cease et al., 2010). Higher densities induce *O. asiaticus* to increase some morphological traits predicted to enhance migration: larger wings and a shift in relative mass from abdomen to thorax (Cease et al., 2010).

Grazing interactions. Due to its competition with livestock (Liu et al., 2013a), many studies have looked at the relationship between livestock grazing and *O. asiaticus* density. In *Stipa grandis, Leymus chinensis,* and *Aneurolepidium chinense* steppes with 320–350 mm annual precipitation and 1,000–1,050 m in elevation in the Xilingol region of Inner Mongolia, stocking rate tends to decrease diversity and increase abundance of grasshoppers, particularly *O. asiaticus* (Kang and Chen, 1995; Xinghui and Hongchang, 1997; Cease et al., 2012) across a wider precipitation gradient (200–400 mm), livestock grazing had the greatest impact at the driest sites, where it similarly decreased grasshopper diversity and increased abundance of *O. asiaticus* (Hao et al., 2015). There are likely many factors driving this pattern, but several studies suggest host plant nutrition is a key driver. While livestock grazing can concentrate nitrogen due to livestock nitrogen excretion, in Inner Mongolia, continuous high stocking rates tend to decrease soil and plant nitrogen. The mechanism is two-fold: (1) increased wind erosion due to more bare soil, and (2) sheep are kept in sheepfolds at night where substantial manure is accumulated. The manure is burned for household fuel and thus the nutrients are not returned to the landscape (Giese et al. 2013). *O. asiaticus* prefer low nitrogen *Stipa grandis* collected from heavily grazed areas relative to ungrazed fields (Cease et al., 2012). This pattern matches with the species of host plants preferred by *O. asiaticus*. Counterintuitively, even though *Stipa* spp tend to have lower nitrogen and protein contents than *Leymus* and other common grasses, it is generally preferred by *O. asiaticus* (Liu et al., 2013a; Cease et al., 2017; Huang et al., 2017b). This is likely due to its low protein and high carbohydrate content; although secondary metabolites may also play a role (Li et al., 2019). These carbohydrate-biased diets increase growth rate, survival, and migratory capacity for *O. asiaticus* (Cease et al., 2012, 2017).

Oedaleus senegalensis (Krauss, 1877)

Senegalese locust, or Senegalese grasshopper

Pest status. This species was not widely recognized as a pest until 1974 when, together with other grasshopper species, it infested 3.5 million hectares in West Africa and was responsible for the loss of 368,000 tons of agricultural production (Bernardi, 1986). It is now considered a major pest in the Sahel area (Maiga et al., 2008) and very often dominates grasshopper communities. In central Sudan, adults can reach densities of over 3,400 green adults/ha and 5,500 brown adults/ha (Elamin et al., 2013). It attacks several subsistence crops, particularly millet.

The lifecycle and survival strategies of *O. senegalensis* have evolved to include migration following shifts in the intertropical convergence zone (ITCZ) and embryonic diapause in the dry season. The alternation between outbreaks and recession periods seems to be related to the high spatiotemporal rainfall variability in the Sahel and various models designed to monitor the population dynamics of this species have been published

(Launois, 1978, 1979; Holt and Colvin, 1997; Fisker et al., 2007; Axelsen, 2009; Maiga et al., 2009; Bal et al., 2015). However, the variability in outbreaks and phase change polyphenism are not well-understood (Maiga et al., 2008; Song, 2011).

Habitat and ecology. Oedaleus senegalensis is a grass feeding Sahelian grasshopper. Its distribution range covers the whole Indo-Saharan zone, from the Atlantic, including Cape Verde, to central India (Maiga et al., 2008). In the Sahel, this species is adapted to the long dry season and summer rains arising from the Intertropical Convergence Zone (ITCZ). It typically has three generations (G1 to G3) per year, which follow the rains north and then south again to stay in an optimal humidity range. Adults following the front of the ITCZ can migrate up to 350 km a night (Cheke, 1990). G3 adults return to the southern range and lay eggs that undergo a 7–8 month long embryonic diapause during the long dry season. Interestingly the hatching pattern of non-diapausing and diapausing eggs is quite different. Non-diapausing eggs hatch in high numbers over a brief period at the end of the rainy season, while diapausing eggs hatch in small numbers over a longer period during the rainy season. The latter most likely constitutes a "bet-hedging" adaptation to the unpredictability of the onset of summer rains—the first set of rain events are frequently followed by periods of drought (Maiga et al., 2010). No controlled studies on phase change have been done for *O. senegalensis.* However, nymphal marching bands and loose swarms have often been recorded (Batten, 1969), and high density outbreaks have been reported to have brown and black coloration as compared to low density populations with predominantly green coloration (Ritchie, 1978; Le Gall et al. under review). In the West African Sahelian region, *O. senegalensis* colonizes annual grass communities dominated by species such as *Cenchrus biflorus* (referred to as "cram-cram" in the francophone countries of the Sahel), *Aristida mutabilis*, *Aristida adscensionis* ("6 weeks threeawn"), *Eragrostis* spp ("lovegrass" or "canegrass"), *Dactyloctenium aegyptium* ("Egyptian crowfoot grass"), *Schoenefeldia gracilis*. They also consume a few woody and perennial species like *Aristida pallida* (Maiga et al., 2008).

Grazing interactions. While *O. senegalensis* is widely recognized as a major pest of cereal crops like millet, it is actually more abundant in grazed and fallowed fields (Amatobi et al., 1988; Toure et al., 2013; Word et al., 2019), where it is very likely to compete with livestock. In general cultivation and afforestation affect grass-feeding grasshoppers negatively, but *O. senegalensis* is less sensitive to it than some other species (Amatobi et al., 1988), perhaps because of its ability to feed on cereals.

DISCUSSION

In reviewing the livestock grazing interactions with the 19 grasshopper species currently considered locusts (Cullen et al., 2017), we found that land use has strong effects on many locust species (**Table 1**). This pattern is illustrated by correlations between outbreak frequency and changes in agricultural practices. However, the mechanisms underpinning these relationships are often unknown and the directionality is often species-specific. Most locusts originate in grasslands. These ecosystems are subject to expanding agriculture, urbanization, energy development, and desertification, making them among the most threatened biomes on Earth (Hoekstra et al., 2005). For some mix and grass-feeder species like the Moroccan locust or the red locust, this increase in anthropogenic change, alongside modern control practices, has led to a decrease in outbreak frequency (Thindwa, 1999; Benfekih et al., 2002). This also appears to have been the case for the Rocky Mountain grasshopper, *Melanoplus spretus*, which destroyed vast quantity of crops throughout the western and central portion of the United States and Canada during the mid-1800s. The species declined rapidly in the late 1800s and agricultural practices likes tillage, irrigation, introduction of cattle, plants, and birds into grasslands are thought to be responsible for its extinction (Lockwood and Debrey, 1990). However, in many other cases because cropland expansion includes cereals that are readily consumed by mix- and grass-feeders, it yielded an increase in outbreak frequencies, as seen with the Bombay locust (Bullen, 1966), the spur-throated locust (Casimir and Edge, 1979), and the Central American locust (Poot-Pech, 2016).

One mechanistic link between livestock grazing and locust populations is the conversion of wooded areas to open fields, including patchy grasslands, more desirable to locusts (Clark, 1950). For example, early swarms of the Australian plague locust, *Chortoicetes terminifera*, in the late 1800's may have been promoted by the introduction of European livestock and agriculture to Australia (Deveson, 2012). The Mongolian locust, *Oedaleus asiaticus*, was rarely reported to have economically important outbreaks prior to the 1970's; increased outbreaks may coincide with increased livestock populations in Inner Mongolia, China (Robinson et al., 2017). Thus, land use and cover change has potentially contributed to the emergence of new locust species that previously did not exhibit strong locust phase change characteristics, likely in conjunction with shifting climate. For instance, the Senegalese grasshopper was not reported as a pest until it first started outbreaking in the 1970's and is now considered the main pest of millet in the Sahel, exhibiting locust characteristics like density-dependent color change and migrations (Popov, 1980; Maiga et al., 2008). Similarly, the Peruvian locust was only known as a non-swarming grasshopper until an upsurge in Peru in 1983–84, subsequently gregarized individuals invaded several Peruvian states from 1997 to 2003 (Duranton et al., 2006). However, to our knowledge, land use/cover change has not been tested as a causal link to increased outbreak frequency in either species and more research is needed to uncover the potential mechanistic links.

Increasing grazing pressure and agricultural intensity may inadvertently be creating a nutritionally optimal environment for locusts through soil degradation. In addition to presenting bare areas favorable to egg laying and thermoregulation, degraded pastures may harbor plants with low protein:carbohydrate ratios that are favorable to locusts. Soil erosion typically decreases soil nitrogen resulting in low nitrogen plants (for a mechanistic description between grazing and plant nitrogen, see *O. asiaticus* section) (Jie et al., 2002; Giese et al., 2013). Since most

TABLE 1 | Worldwide Locust Species, adapted from Cullen et al. (2017).

Species, subfamilies, and clades by feeding guildes	Common name	Geographic range	Grazing interactions reported in the literature	Nutritional preferences
FORB- AND/OR TREE-FEEDING LOCUSTS				
Calliptamus italicus (Linnaeus, 1758) Calliptaminae Clade D	Italian locust	Europe and Central Asia	- Weak but present with livestock in pastures	NA
Anacridium melanorhodon (Walker, 1870) Cyrtacanthacridinae Clade D	Sahelian tree locust	North and Central Africa, Middle East	- Weak but present with livestock in pastures - Nutrient cycling	NA
MIX-FEEDING LOCUSTS (GRASSES, FORBS, AND/OR TREES)				
Schistocerca cancellata (Serville, 1838) Cyrtacanthacridinae Clade D	South American locust	South America	- Not reported	Carbohydrate-biased P1:C2 (Overson et al., in prep)
Schistocerca gregaria flaviventris (Burmeister, 1838) Cyrtacanthacridinae Clade D	Desert locust (southern race)	Southern Africa (N.B., typically non-swarming)	- Attack fodder - Non-target effects of chemical control - Camels accelerate gregarization	Carbohydrate-biased P1:C1.25 (Simpson et al., 2002)
Schistocerca gregaria gregaria (Forskål, 1775) Cyrtacanthacridinae Clade D	Desert locust (northern race)	Southern Europe, North Africa, Middle East, Indian subcontinent		
Schistocerca interrita (Scudder, 1899) Cyrtacanthacridinae Clade D	Peruvian locust	Peru	- Not reported	NA
Schistocerca piceifrons peruviana (Lynch Arribalzaga, 1903) Cyrtacanthacridinae Clade D	Central American locust	Peru, Ecuador, Colombia, Venezuela, Panama, Trinidad and Tobago	- Conversion of forest to grassland has increased capacity for gregarization	NA
Dociostaurus maroccanus (Thunberg, 1815) Gomphocerinae - Clade B	Moroccan locust	North Africa, Europe, Caucasus	- Attack fodder - Present with livestock in pastures (particularly if overgrazed)	NA
GRASS-FEEDING LOCUSTS				
Austracris guttulosa (Walker, 1870) Cyrtacanthacridinae Clade D	Spur-throated locust	Australia	- Attack fodder - Present with livestock in pastures when swarming	NA
Nomadacris septemfasciata (Audinet-Serville, 1883) Cyrtacanthacridinae Clade D	Red locust	Sub-Saharan and Southern Africa	- Attack fodder - Present with livestock in pastures - Non-target effects of chemical control	NA
Patanga succincta (Johansson, 1763) Cyrtacanthacridinae Clade D	Bombay locust	India, South-East Asia	- Conversion of forest to grassland has increased capacity for gregarization	NA
Gomphocerus sibiricus (Linnaeus, 1767) Gomphocerinae Clade B	Siberian locust	Europe, Caucasus, North Asia	- Present with livestock in pastures	NA
Aiolopus simulatrix (Walker, 1870) Oedipodinae	Sudan plague locust	North and Central Africa	- Present with livestock in pastures	NA
Ceracris kiangsu (Tsai, 1929) Oedipodinae Clade B	Yellow-spined bamboo locust	East Asia	- Not reported	NA
Chortoicetes terminifera (Walker, 1870) Oedipodinae Clade B	Australian plague locust	Australia	- Conversion of forest to grassland has increased capacity for gregarization - Present with livestock in pastures	Field populations are consistently carbohydrate-biased P1:C1.4 to P1:2.2C *(Cease et al, and Lawton et al. unpublished data)*
Gastrimargus musicus (Fabricius, 1775) Oedipodinae Clade B	Yellow-winged locust	Australia	- Conversion of forest to grassland has increased capacity for gregarization - Present with livestock in pastures	NA

(Continued)

TABLE 1 | Continued

Species, subfamilies, and clades by feeding guildes	Common name	Geographic range	Grazing interactions reported in the literature	Nutritional preferences
Locusta migratoria migratoria (Linnaeus, 1758) Oedipodinae Clade B	Asian migratory locust	West and Central Asia, Eastern Europe	- Early instars present with livestock in pastures	Carbohydrate-biased P1:C1.4 (Chambers et al., 1995)
Locusta migratoria migratorioides (Reiche and Fairmaire, 1849) Oedipodinae Clade B	African migratory locust	Mainland Africa and Atlantic Islands, Madagascar, South and East Asia, Australasia, Tibetan plateau		
Locustana pardalina (Walker, 1870) Oedipodinae Clade B	Brown locust	Southern Africa	- Present with livestock in pastures - Non-target effects of chemical control	NA
Oedaleus asiaticus (Bey-Bienko, 1941) Oedipodinae Clade B	Mongolian locust	Northern Asia	- Present with livestock in pastures (particularly if overgrazed)	Carbohydrate-biased P1:C2(Cease et al., 2012)
Oedaleus senegalensis (Krauss, 1877) Oedipodinae Clade B	Senegalese locust	North Africa, Middle East	- Present with livestock in pastures	Carbohydrate-biased P1:C1.6 (Le Gall et al, under review)

nitrogen in plants, particularly grasses, is found in the form of protein, and protein and carbohydrate tend to be negatively correlated, this translates into low protein, high carbohydrate plants (Loaiza et al., 2017). In contrast to the N-limitation hypothesis (White, 2012), emerging evidence suggests that locusts are more likely to be carbohydrate-limited in field populations. For example, marching bands of *S. cancellata* from the recent upsurge ate most readily from dishes containing carbohydrates and rarely stopped at dishes with protein (Medina et al. 2017; Overson et al. unpublished data). Over recent decades, many lab studies have shown multiple locust species select a carbohydrate-biased diet when given the choice and show higher performance around their self-selected low protein, high carbohydrate diet (Raubenheimer and Simpson, 1993; Chambers et al., 1995; Simpson and Raubenheimer, 2000; Simpson et al., 2002).

There are multiple factors that might explain why carbohydrate-biased diets are beneficial for locusts. First, carbohydrate-biased diets may be a requirement for long-distance flight (Hunter et al., 1981; Cease et al., 2017). A defining characteristic of locusts is their capacity to undergo spectacular migrations: swarms can fly hundreds of kilometers in a single night and plagues can span continents. These long-distance flights are fueled primarily from lipid reserves (Weis-Fogh, 1952; Jutsum and Goldsworthy, 1976), which are built with dietary carbohydrates (Walker et al., 1970; Jutsum and Goldsworthy, 1976; Hunter et al., 1981). Insect flying metabolic rates can be 20-100 times that of resting animals and are among the highest known (Rankin and Burchsted, 1992). This high energy demand means their food source pre-and during migration is critical to fueling flight. Second, carbohydrate-biased diets may be important for resistance against parasites and pathogens. For instance, Australian plague locusts are more likely to die of fungal infection when they are restricted to diets high in protein than when they are restricted to diets high in carbohydrates (Graham et al., 2014). Potentially because fungus are better at exploiting protein in the insect's hemolymph, or perhaps because immune function is correlated with lipid levels of the host (Graham et al., 2015). Finally, these diets may help locusts persist in an arid environment because consuming more carbohydrate enhances lipid stores, which can in turn be used as a water reserve, as shown in the migratory locust (Loveridge and Bursell, 1975).

Although the link between land use, plant nutrient content, and locust performance has only experimentally been demonstrated for *O. asiaticus* (Cease, 2012; Cease et al., 2015), correlation between plant nutrient content and locust density has also been shown for *O. senegalensis* (Word et al., 2019). The connection between plant nutritional landscape and locust outbreaks is thus a promising mechanism to explain the association between agricultural practices and locusts, and is an area for suggested research, particularly for non-model locusts (see **Table 1**). Furthermore, the presence of locusts in grazed areas leads to strong competition between locusts and livestock, particularly for mix- and grass-feeders. Even tree-feeders, like the Sahelian tree locust, were reported to compete with livestock by attacking shrubs that are a food source for domestic cattle and camels (COPR, 1982). This competition for resources between livestock and locusts has the potential to further degrade soils in over-grazed areas as shown for the brown locust in South Africa (Lea, 1958a,b; De Villiers, 1988; Price and Brown, 2000).

The interactions between decisions regarding land use and locust biology has rippling effects: increased grazing pressure creates optimal environments for locusts, leading locusts to reach higher densities, which promotes gregarization. Gregarization in turn increases locust diet breadth, leading to expansive and diverse crop damage (COPR, 1982; Despland, 2005). One potential explanation for the diet expansion is that gregarious locusts may be more likely to encounter a variety of host plants with varying nutrient contents and, by eating from among these diverse food sources, they can balance nutrient requirements (Simpson et al., 2002). Another explanation is eating toxic plants can serve as an anti-predatory strategy, which is linked to the development of density-dependent aposematic coloration seen in some locust species (e.g., *S. gregaria*, Sword et al., 2000; Sword, 2002; Cullen et al., 2017): Gregarious desert locusts are less deterred by plant secondary compounds and ingest more plant toxins than solitarious locusts, they are thus better defended against predators (Despland, 2005; Despland and Simpson, 2005b). Exploring how gregarious locusts expand their diet breadth is another understudied area.

In many cases livestock grazing has positive effects on locust populations, which can lead to food insecurity. However, grasshoppers, including locusts, are dominant herbivores and vital players in grassland ecosystems (Branson et al., 2006). Furthermore, chemical control of grasshoppers and locusts has many undesirable side-effects on livestock health (Botha, 1974; Tanaka and Zhu, 2005), human health (Houndekon and De Groote, 1998; Tingle et al., 2003), and predator communities (Balança and De Visscher, 1997). Therefore, management programs that rely on population monitoring and early intervention, such as chemical treatment as soon as populations start to increase, are more likely to be sustainable (Van Huis et al., 2007; Magor et al., 2008; Zhang et al., 2019). These programs have been aided by rapid progress made in remote sensing techniques that can help detect locust bands and/or potential locust habitat by distinguishing areas of green vegetation from bare soils. For instance, a combination of satellite data to estimate rainfall and vegetation cover, and field survey data to indicate locust outbreaks, can be used to develop predictive models (Cressman, 2013; Piou et al., 2013). These models assist managers in determining the probability of finding locusts in specific areas to more efficiently focus survey efforts.

With a greater understanding of the connections between agricultural practices and locust outbreaks, cultural control options may be viable components of future management programs. For example, habitat manipulation can slow nymphal development, reduce survival and reproduction, or decrease yearly variability in those life history traits (Onsager and Olfert, 2000; Branson et al., 2006). Manipulations to limit pest damage can include direct competition from mammalian herbivores such as livestock for grasshoppers (Onsager, 2000), altering food quality for terrestrial herbivores (Le Gall and Tooker, 2017), manipulating plant composition, and increasing the abundance of natural enemies of grasshoppers (Belovsky, 2000; Joern, 2000). These types of management practices are less developed for locusts but harbor great potential. For example, in West Africa, fallow fields often have more Senegalese grasshoppers than other field types (Toure et al., 2013; Word et al., 2019), likely due, in part, to vegetation nutritionally optimal for locusts growing in those areas (Le Gall et al., under review). Due to limited land availability, fields may be left fallow only when the soil has been depleted and yield is limited, resulting in a low-N environment and carbohydrate-biased plants. Because locusts require a particularly carbohydrate-biased diet (**Table 1**), bottom up control using knowledge of locust nutritional requirements may be a viable option (Le Gall and Tooker, 2017; Word et al., 2019). Practices that improve soil fertility, such as reducing grazing pressure in Inner Mongolia, China (Cease et al., 2012) or increasing soil organic matter and nitrogen on West African small-holder farms (Word et al., 2019) have the potential to alleviate locust problems. However, livestock and farm managers may not have the resources, knowledge, or incentives to implement practices to decrease locust populations. Therefore, cultural control options should be developed iteratively, in ways that integrate stakeholder perspectives, as well as the natural and social science (Cullen et al., 2017).

Such research linking land use to locust populations has revealed that this ecological feedback connects people across time and space producing both local and far-reaching environmental and social impacts (Cease et al., 2015), a process called *telecoupling* (Liu et al., 2013b; Kapsar et al., 2019). At a regional scale, locusts can spill from rangelands into croplands. For instance, the Australian plague locust is typically associated with grazing areas but during outbreaks will attack agricultural crops (Watts et al., 1982; Symmons, 1984; Wright and Symmons, 1987). Due to their powerful migratory capacity (>100 km traveled per day), locusts can link distant regions as well. Decisions about stocking rates in one region, for example, have the potential to initiate outbreaks that could lead to swarms invading distant regions. In addition to ecological links, distant regions are coupled through the economy (e.g., livestock markets) and policies. Indeed, because locusts connect distant regions and span continents, a major challenge is implementing consistent management efforts across broad regions (Cressman, 2016; Zhang et al., 2019). This requires many countries to work together from the level of the producer to national and international government organizations (Lockwood et al., 2001; Cease et al., 2015). This review highlights that humans are not passive players in their relationship with locusts. To effectively integrate land-use into management programs, important data on locust biology, such as nutritional preference and thermobiology, remain to be collected, particularly for non-model locust species, for which the expressions of density-dependent phase polyphenism in not well-understood (Song, 2011). In addition, this biology research needs to be linked to broader landscape patterns and their management, including producer decisions as well as market and government constraints.

AUTHOR CONTRIBUTIONS

ML wrote the discussion and sections for nine species and oversaw paper cohesion. RO performed the initial literature review and wrote the method section and sections for seven species. AC conceptualized the framework for the paper, wrote the introduction and sections for three species and edited all sections for consistency. All authors participated in revisions of the whole manuscript.

FUNDING

This work was supported by the National Science Foundation, United States [DEB-1313693 to AC].

REFERENCES

Abou-Ali, H., and Belhaj, M. (2008). "Cost benefit analysis of desert locusts' control: a multicountry perspective," in *Economic Research Forum Working Papers.* (Giza)

Abushama, F. T. (1970). Loss of water from the grasshopper *Poecilocerus hieroglyphicus* (Klug), compared with the tree locust *Anacridium melanorhodon melanorhodon* (Walker). *Zeitschrift Angew. Entomol.* 66, 160–167.

AgroAtlas - Pests - *Aeropus sibiricus (L).* - Siberian locust (Siberian grasshopper). (2003). Available online at: http://www.agroatlas.ru/en/content/pests/Aeropus_sibiricus/index.html (accessed April 15, 2019).

Amatobi, C. I., Apeji, S. A., and Oyidi, O. (1988). Effects of farming practices on populations of two grasshopper pests (*Kraussaria angulifra* Krauss and *Oedaleus senegalensis* Krauss (Orthoptera: Acrididae) in Northern Nigeria. *Int. J. Pest Manag.* 34, 173–179. doi: 10.1080/09670878809371237

Anstey, M. L., Rogers, S. M., Ott, S. R., Burrows, M., and Simpson, S. J. (2009). Serotonin mediates behavioral gregarization underlying swarm formation in desert locusts. *Science* 323, 627–630. doi: 10.1126/science.1165939

Axelsen, J. A. (2009). Simulation studies of Senegalese Grasshopper ecosystem interactions I: the ecosystem model. *Int. J. Pest Manag.* 55, 85–97. doi: 10.1080/09670870802601084

Babah, M. A. O., and Sword, G. A. (2004). Linking locust gregarization to local resource distribution patterns across a large spatial scale. *Environ. Entomol.* 33, 1577–1583. doi: 10.1603/0046-225X-33.6.1577

Bahana, J. W. (2000). "The role of the international Red Locust Control Organisation for Central and Southern Africa (IRLCOCSA) in the management of migratory pests," in *Workshop on Research Priorities for Migrant Pests of Agriculture in Southern Africa, Plant Protection Research Institute, Pretoria, South Africa, March 1999* (Chatham: Natural Resources Institute), 25–33.

Bal, A. B., Ouambama, Z., Moumouni, A., Dieng, I., Maiga, I. H., Gagare, S., et al. (2015). A simple tentative model of the losses caused by the Senegalese grasshopper, *Oedaleus senegalensis* (Krauss 1877) to millet in the Sahel. *Int. J. Pest Manag.* 61, 198–203. doi: 10.1080/09670874.2015.1031201

Balança, G., and De Visscher, M.-N. (1997). Impacts on nontarget insects of a new insecticide compound used against the desert locust *Schistocerca gregaria* (Forskal 1775). *Arch. Environ. Contamin. Toxicol.* 32, 58–62. doi: 10.1007/s002449900155

Barrera, M., and Turk, S. (1983). Estado actual de la langosta *Schistocera cancellata paranensis* (Burm.) en la Republica Argentina: neuvos aportes a su bioecologia. *Acta Zool. Lilloana* 27, 15–29.

Barrientos Lozano, L., Astacio Cabrera, O., Alvarez Bonilla, F., and Poot Martínez, O. (1992). *Manual técnico sobre la langosta voladora (Schistocerca piceifrons piceifrons Walker, 1870) y otros Acridoideos de Centro América y Sureste de México*. FAO, San Salvador (El Salvador) OIRSA, San Salvador (El Salvador).

Batáry, P., Orci, K. M., Báldi, A., Kleijn, D., Kisbenedek, T., and Erdos, S. (2007). Effects of local and landscape scale and cattle grazing intensity on Orthoptera assemblages of the Hungarian Great Plain. *Basic Appl. Ecol.* 8, 280–290. doi: 10.1016/j.baae.2006.03.012

Batten, A. (1969). The Senegalese grasshopper *Oedaleus senegalensis* Krauss. *J. Appl. Ecol.* 6, 27–45.

Beavan, K. (2017). *Grasshoppers descend on Central Australia after heavy rainfall.* ABC Rural. Available online at: https://www.abc.net.au/news/rural/2017-01-24/grasshoppers-swarm-outback-australia/8204992 (accessed February 28, 2019).

Behmer, S. T., Raubenheimer, D., and Simpson, S. J. (2001). Frequency-dependent food selection in locusts: a geometric analysis of the role of nutrient balancing. *Anim. Behav.* 61, 995–1005. doi: 10.1006/anbe.2000.1695

Bei-Bienko, G. Y., and Mishchenko, L. L. (1951). *Acridoidea of the USSR and Adjacent Countries*. Part II. Acridoidea of the USSR and adjacent countries.

Belayneh, Y. T. (2005). Acridid pest management in the developing world: a challenge to the rural population, a dilemma to the international community. *J. Orthopt. Res.* 14, 187–196. doi: 10.1665/1082-6467(2005)14[187:APMITD]2.0.CO;2

Belovsky, G. E. (2000). "Do grasshoppers diminish grassland productivity? A new perspective for control based on conservation," in *Grasshoppers and Grassland Health, NATO Science Series (Series 2.Environment Security)*, Vol 73, eds J. A. Lockwood, A. V. Latchininsky, and M. G. Sergeev (Dordrecht: Springer), 7–29. doi: 10.1007/978-94-011-4337-0_2

Belovsky, G. E., and Slade, J. B. (2000). Insect herbivory accelerates nutrient cycling and increases plant production. *Proc. Natl. Acad. Sci. U.S.A.* 97, 14412–14417. doi: 10.1073/pnas.250483797

Benfekih, L., Chara, B., and Doumandji-Mitiche, B. (2002). Influence of Anthropogenic Impact on the Habitats and Swarming Risks of *Dociostaurus maroccanus* and *Locusta migratoria* (Orthoptera, Acrididae) in the Algerian Sahara and the Semiarid Zone. *J. Orthopt. Res.* 11, 243–250. doi: 10.1665/1082-6467(2002)011[0243:IOAIOT]2.0.CO;2

Benfekih, L., and Petit, D. (2010). The annual cycle of Saharan populations of *Locusta migratoria cinerascens* (Orthoptera: Acrididae: Oedipodinae) in Algeria. *Ann. Soc. Entomol. France* 46, 351–358. doi: 10.1080/00379271.2010.10697674

Bernardi, M. (1986). *Le Problème des sauteriaux. Compte Rendu du Séminaire International du Projet CILSS de Lutte Intégrée, Niamey, Niger*. *6–13 décembre 1984*, 43–57.

Bernays, E. A., Chamberlain, D. J., and Woodhead, S. (1983). Phenols as nutrients for a phytophagous insect *Anacridium melanorhodon*. *J. Insect Physiol.* 29, 535–539. doi: 10.1016/0022-1910(83)90085-9

Bernays, E. A., and Chapman, R. F. (1973). The role of food plants in the survival and development of *Chortoicetes terminifera* (Walker) under drought conditions. *Austr. J. Zool.* 21, 575–592. doi: 10.1071/ZO9730575

Bernays, E. A., and Hamai, J. (1987). Head size and shape in relation to grass feeding in Acridoidea (Orthoptera). *Int. J. Insect Morphol. Embryol.* 16, 323–330. doi: 10.1016/0020-7322(87)90004-3

Bernays, E. A., and Woodhead, S. (1982). Incorporation of dietary phenols into the cuticle in the tree locust *Anacridium melanorhodon*. *J. Insect Physiol.* 28, 601–606. doi: 10.1016/0022-1910(82)90057-9

Botha, R.oss, and van Ark, P. (1974). Residues in sheep exposed to BHC treated Karoo-veld in the outbreak region of the brown locust *Locustana pardalina* (Walker). *Phytophylactica* 6, 235–248.

Bouaïchi, A. (1996). *The behavioural and environmental bases of gregarization in the desert locust Schistocerca gregaria (Forskal)*. (Ph D. Thesis), University of Oxford.

Bouchard, J. (1998). Évaluation de l'impact de produits toxiques sur le criquet de montagne *Gomphocerus sibiricus* (colchicine et 6-mercaptopurine). *Ann. Sci. Nat.* 19, 127–140. doi: 10.1016/S0003-4339(99)80002-1

Brader, L., Djibo, H., Faye, F. G., Ghaout, S., Lazar, M., Luzietoso, P. N., et al. (2006). *Towards a More Effective Response to Desert Locusts and Their Impacts on Food Security, Livelihoods and Poverty*. *Multilateral Evaluation of the 2003–05 Desert Locust Campaign.* Food and Agriculture Organisation, Rome.

Branson, D. H., Joern, A., and Sword, G. A. (2006). Sustainable management of insect herbivores in grassland ecosystems: new perspectives in grasshopper control. *Bio Sci.* 56, 743–755. doi: 10.1641/0006-3568(2006)56[743:SMOIHI]2.0.CO;2

Bredo, H. J. (1963). *Rapport de mission relatif a l'etude du Schistocerca paranensis Burm. en Amerique Centrale, Panama et Mexique*. 77. Rome, FAO.

Breman, H., and Kessler, J. J. (1997). The potential benefits of agroforestry in the Sahel and other semi-arid regions. *Eur. J. Agron.* 7, 25–33. doi: 10.1016/S1161-0301(97)00035-X

Bughio, B. A., Sultana, R., RIND, M., and Wagan, M. S. (2014). Some studies on the taxonomic status of the genus *Aiolopus Fieber* (Acrididae: Acridoidea: Orthoptera) from Pakistan. *J. Biodiv. Environ. Sci* 4, 410–417.

Bullen, F. T. (1966). Locusts and grasshoppers as pests of crops and pasture-a preliminary economic approach. *J. Appl. Ecol.* 147–168. doi: 10.2307/2401671

Bullen, F. T. (1968). *Report on an Outbreak of the Spur-Throated Locust (Austracris Guttulosa Walk.) in the East Kimberley Region of Western Australia*. CSIRO Division of Entomology.

Burnett, G. F. (1951). *Field Observations on the Behaviour of the Red Locust (Nomadacris Septemfasciata Serville) in the Solitary Phase.* Available online at: https://www.cabdirect.org/cabdirect/abstract/19510500702 (accessed March 12, 2018).

Casimir, M., and Edge, V. E. (1979). The Development and Impact of a Control Campaign against *Austracris guttulosa* in New South Wales. *Proc. Acad. Natl. Sci. U.S.A.* 25, 223–236. doi: 10.1080/09670877909412089

Cease, A. (2012). *Locust Outbreaks and Migration in the Asian Steppe: The Influence of Land Management Practices and Host Plant Nutrient Status*. Available online at: https://search.proquest.com/docview/1012769819/abstract/9493761F6ECC4FBFPQ/1 (accessed September 3, 2018).

Cease, A. J., Elser, J. J., Fenichel, E. P., Hadrich, J. C., Harrison, J. F., and Robinson, B. E. (2015). Living with locusts: connecting soil nitrogen, locust outbreaks, livelihoods, and livestock markets. *Bio Sci.* 65, 551–558. doi: 10.1093/biosci/biv048

Cease, A. J., Elser, J. J., Ford, C. F., Hao, S., Kang, L., and Harrison, J. F. (2012). Heavy livestock grazing promotes locust outbreaks by lowering plant nitrogen content. *Science* 335, 467–469. doi: 10.1126/science.1214433

Cease, A. J., Hao, S., Kang, L., Elser, J. J., and Harrison, J. F. (2010). Are color or high rearing density related to migratory polyphenism in the band-winged grasshopper, *Oedaleus asiaticus*? *J. Insect Physiol.* 56, 926–936. doi: 10.1016/j.jinsphys.2010.05.020

Cease, A. J., Harrison, J. F., Hao, S., Niren, D. C., Zhang, G., Kang, L., et al. (2017). Nutritional imbalance suppresses migratory phenotypes of the Mongolian locust (*Oedaleus asiaticus*). *R. Soc. Open Sci.* 4:161039. doi: 10.1098/rsos.161039

Chambers, P. G., Simpson, S. J., and Raubenheimer, D. (1995). Behavioural mechanisms of nutrient balancing in *Locusta migratoria* nymphs. *Anim. Behav.* 50, 1513–1523. doi: 10.1016/0003-3472(95)80007-7

Chandra, S., and Williams, G. (1983). Frequency-dependent selection in the grazing behaviour of the desert locust *Schistocerca gregaria*. *Ecol. Entomol.* 8, 13–21. doi: 10.1111/j.1365-2311.1983.tb00477.x

Chapman, R. F. (1959). Observations on the flight activity of the red locust, *Nomadacris Septemfasciata* (Serville). *Behaviour* 14, 300–333. doi: 10.1163/156853959X00126

Chapman, R. F. (1976). *A Biology of Locusts.* Available online at: https://www.cabdirect.org/cabdirect/abstract/19760541473 (accessed February 20, 2018).

Chapuis, M.-P., Popple, J.-A. M., Berthier, K., Simpson, S. J., Deveson, E., Spurgin, P., et al. (2011). Challenges to assessing connectivity between massive populations of the Australian plague locust. *Proc. R. Soc. B: Biol. Sci.* 278, 3152–3160. doi: 10.1098/rspb.2010.2605

Chapuis, M. P., Foucart, A., Plantamp, C., Blondin, L., Leménager, N., Benoit, L., et al. (2017). Genetic and morphological variation in non-polyphenic southern African populations of the desert locust. *Afr. Entomol.* 25, 13–24. doi: 10.4001/003.025.0013

Cheke, R. A. (1990). A migrant pest in the Sahel: the Senegalese grasshopper *Oedaleus senegalensis*. *Phil. Trans. R. Soc. Lond. B* 328, 539–553. doi: 10.1098/rstb.1990.0126

Chen, H. H., and Kang, L. (2000). Olfactory responses of two species of grasshoppers to plant odours. *Entomol. Exp. Applic.* 95, 129–134. doi: 10.1046/j.1570-7458.2000.00650.x

China Ministry of Agriculture (2012). *National Rangeland Monitoring Report 2011 (in Chinese)*. Beijing, China: China Ministry of Agriculture.

Cigliano, M. M., Braun, H., Eades, D. C., and Otte, D. (2017). *Orthoptera Species File*. Version 5.0/5.0.

Cisse, S., Ghaout, S., Mazih, A., Babah Ebbe, M. A. O., Benahi, A. S., and Piou, C. (2013). Effect of vegetation on density thresholds of adult desert locust gregarization from survey data in Mauritania. *Entomol. Exp. Applic.* 149, 159–165. doi: 10.1111/eea.12121

Cisse, S., Ghaout, S., Mazih, A., Ould Babah Ebbe, M. A., and Piou, C. (2015). Estimation of density threshold of gregarization of desert locust hoppers from field sampling in Mauritania. *Entomol. Exp. Appl.* 156, 136–148. doi: 10.1111/eea.12323

Clark, D. P. (1965). On the sexual maturation, breeding, and oviposition behaviour of the Australian plague locust, *Chortoicetes terminifera* (Walk.). *Austr. J. Zool.* 13, 17–46. doi: 10.1071/ZO9650017

Clark, L. R. (1947). *An Ecological Study of the Australian Plague Locust (Chortoicetes terminifera Walk.) in the Bogan-Macquarie Outbreak Area*. NSW.

Clark, L. R. (1950). On the abundance of the Australian Plague Locust *Chortoicetes terminifera* (Walker) in relation to the presence of trees. *Austr. J. Agric. Res.* 1, 64–75. doi: 10.1071/AR9500064

Clark, X., Clissold, F. J., Charleston, M. A., and Simpson, S. J. (2013). "Foraging in a nutritionally complex world: tests using agent-based models and locusts," (Oxford Univ Press Inc Journals Dept, 2001 EVANS RD, CARY, NC 27513 USA), E266–E266.

Clissold, F. J., Sanson, G. D., and Read, J. (2006). The paradoxical effects of nutrient ratios and supply rates on an outbreaking insect herbivore, the Australian plague locust. *J. Anim. Ecol.* 75, 1000–1013. doi: 10.1111/j.1365-2656.2006.01122.x

Collett, M., Despland, E., Simpson, S. J., and Krakauer, D. C. (1998). Spatial scales of desert locust gregarization. *Proc. Natl Acad. Sci. U.S.A.* 95, 13052–13055. doi: 10.1073/pnas.95.22.13052

COPR (1982). *The Locust and Grasshopper Agricultural Manual.* London: Overseas Pest Research. 690.

Cressman, K. (2013). Role of remote sensing in desert locust early warning. *J. Appl. Remote Sens.* 7:075098. doi: 10.1117/1.JRS.7.075098

Cressman, K. (2016). Desert locust. *Biol. Environ. Hazards Risks Disasters* 87–105. doi: 10.1016/B978-0-12-394847-2.00006-1

Crooks, W. T., and Cheke, R. A. (2014). Soil moisture assessments for brown locust *Locustana pardalina* breeding potential using synthetic aperture radar. *JARS* 8:084898. doi: 10.1117/1.JRS.8.084898

Cullen, D. A., Cease, A. J., Latchininsky, A. V., Ayali, A., Berry, K., Buhl, J., et al. (2017). "From molecules to management: mechanisms and consequences of locust phase polyphenism," in *Advances in Insect Physiology*, ed H. Verlinden (Oxford: Elsevier), 167–285. doi: 10.1016/bs.aiip.2017.06.002

Cullen, D. A., Sword, G. A., Dodgson, T., and Simpson, S. J. (2010). Behavioural phase change in the Australian plague locust, *Chortoicetes terminifera*, is triggered by tactile stimulation of the antennae. *J. Insect Physiol.* 56, 937–942. doi: 10.1016/j.jinsphys.2010.04.023

Cullen, D. A., Sword, G. A., and Simpson, S. J. (2012). Optimizing multivariate behavioural syndrome models in locusts using automated video tracking. *Anim. Behav.* 84, 771–784. doi: 10.1016/j.anbehav.2012.06.031

D'Alessandro, S., Fall, A. A., Grey, G., Simpkin, S., and Wane, A. (2015). *Senegal*. Washington, DC: Agricultural sector risk assessment.

De Villiers, W. M. (1988). On the plague dynamics of the brown locust Locustana pardalina (Walk.), in *Proceedings of the Locust Symposium* (Johannesburg: Bulletin of the South African Institute of Ecologists), 41–49.

De Vreyer, P., Guilbert, N., and Mesple-Somps, S. (2014). Impact of natural disasters on education outcomes: evidence from the 1987–89 locust plague in Mali. *J. Afr. Econ.* 24, 57–100. doi: 10.1093/jae/eju018

Despland, E. (2005). Diet breadth and anti-predator strategies in desert locusts and other Orthopterans. *Orth* 14, 227–233. doi: 10.1665/1082-6467(2005)14[227:DBAASI]2.0.CO;2

Despland, E., Collett, M., and Simpson, S. J. (2000). Small-scale processes in desert locust swarm formation: how vegetation patterns influence gregarization. *Oikos* 88, 652–662. doi: 10.1034/j.1600-0706.2000.880322.x

Despland, E., Rosenberg, J., and Simpson, S. J. (2004). Landscape structure and locust swarming: a satellite's eye view. *Ecography* 27, 381–391. doi: 10.1111/j.0906-7590.2004.03779.x

Despland, E., and Simpson, S. J. (2000). Small-scale vegetation patterns in the parental environment influence the phase state of hatchlings of the desert locust. *Physiol. Entomol.* 25, 74–81. doi: 10.1046/j.1365-3032.2000.00166.x

Despland, E., and Simpson, S. J. (2005a). Food choices of solitarious and gregarious locusts reflect cryptic and aposematic antipredator strategies. *Anim. Behav.* 69, 471–479. doi: 10.1016/j.anbehav.2004.04.018

Despland, E., and Simpson, S. J. (2005b). Surviving the change to warning colouration: density-dependent polyphenism suggests a route for the evolution of aposematism. *Chemoecology* 15, 69–75. doi: 10.1007/s00049-005-0296-6

Deveson, E. D. (2011). The search for a solution to Australian locust outbreaks: how developments in ecology and government responses influenced scientific research. *Hist. Records Austr. Sci.* 22, 1–31. doi: 10.1071/HR11003

Deveson, E. D. (2012). Naturae amator and the grasshopper infestations of South Australia's early years. *Transact. R. Soc. South Austr.* 136, 1–15. doi: 10.1080/03721426.2012.10887158

Deveson, E. D., and Walker, P. W. (2005). Not a one-way trip: historical distribution data for Australian plague locusts support frequent seasonal exchange migrations. *J. Orthopt. Res.* 14, 91–106. doi: 10.1665/1082-6467(2005)14[91:NAOTHD]2.0.CO;2

DPI (2018). *Spur-Throated Locust.* Department of Primary Industries and Regional Development. Available online at: https://www.agric.wa.gov.au/pest-insects/spur-throated-locust?nopaging=1 (accessed March 1, 2019).

DPI (2019). *Yellow-winged locusts. Department of Primary Industries and Regional Development*. Available online at: https://www.agric.wa.gov.au/pasture-management/yellow-winged-locusts (accessed February 28, 2019).

Drake, V. A., and Farrow, R. A. (1983). The nocturnal migration of the Australian plague locust, *Chortoicetes terminifera* (Walker) (Orthoptera: Acrididae):

quantitative radar observations of a series of northward flights. *Bull. Entomol. Res.* 73, 567–585. doi: 10.1017/S0007485300009172

Duranton, J.-F., Monard, A., and Morales, R. S. (2006). Contribution à l'étude de la bio-écologie de deux locustes péruviens, *Schistocerca cf. interrita* Scudder 1899 et *Schistocerca piceifrons peruviana* Lynch Arribalzaga 1903 (Orthoptera, Cyrtacanthacridinae). *J. Orthopt. Res.* 157–169. doi: 10.1665/1082-6467(2006)15[157:CLDLBD]2.0.CO;2

Duranton, J.-F., Monard, A., and Solano, R. (2001). *Outbreaks of Schistocerca interrita (Scudder, 1899) in Northern Peru*. Metaleptea.

Elamin, A. E., Abdalla, A. M., and El Naim, A. M. (2013). Studies on population dynamics of senegalese grasshopper (*Oedaleus senegalensis*) in Kordofan of Sudan. *World* 1, 85–89.

Elamin, H. M. A., Roth, M., and Taha, M. E. (2008). "The consequences of defoliation of gum arabic tree (*Acacia senegal*) by Sahelian tree locust (*Anacridium melanorhodon melanorhodon*) for the gum producers in North Kordofan State, Sudan," in *Conference on International Research On Food Security, Natural Resource Management and Rural Development* (Stuttgart: University of Hohenheim), 7–9.

Ellis, P. E., and Ashall, C. (1957). Field studies on diurnal behaviour, movement and aggregation in the desert locust (*Schistocerca gregaria* Forskål). *Anti-locust Bull.* 25, 4–94.

El-Mallakh, O. S., and El-Mallakh, R. S. (1994). Insects of the Qur'an. *Am. Entomol.* 40, 82–84. doi: 10.1093/ae/40.2.82

Evans, C. S., and Bell, E. A. (1979). Non-protein amino acids of *Acacia* species and their effect on the feeding of the acridids *Anacridium melanorhodon* and *Locusta migratoria*. *Phytochemistry* 18, 1807–1810. doi,: 10.1016/0031-9422(79)83057-5

FAO, News Article: Red Locust Disaster in Eastern Africa Prevented (2009). Available online at: http://www.fao.org/news/story/en/item/21084/icode/ (accessed March 12, 2018).

Farrow, R. A. (1979). *Causes of Recent Changes in the Distribution and Abundance of the Migratory Locust (Locusta migratoria L.) in Australia in Relation to Plagues. Report.* CSIRO Division of Entymology, 32–41.

Farrow, R. A. (1979a). *Causes of Recent Changes in the Distribution and Abundance of the Migratory Locust (Locusta migratoria L.) in Australia in Relation to Plagues*.

Farrow, R. A. (1979b). Population Dynamics of the Australian Plague Locust, *Chortoicetes terminifera* (Walker), in Central Western New South Wales. I. Reproduction and Migration in Relation to Weather. *Austr. J. Zool.* 27, 717–745. doi: 10.1071/ZO9790717

Farrow, R. A. (1987). Effects of changing land use on outbreaks of tropical migratory locust, *Locusta migratoria migratorioides* (R. *and F.). Int. J. Trop. Insect Sci.* 8, 969–975. doi: 10.1017/S1742758400023262

Feng, G. H., Fan, S. X., Liu, Q. F., Yang, Y. B., Ma, L. X., and Cao, G. S. (1994). The determination capacity for eaten of several species grasshoppers in grassland in outside cage condition. *Acta Agrestia Sinica* 3, 230–235.

Fisker, E. N., Bak, J., and Niassy, A. (2007). A simulation model to evaluate control strategies for the grasshopper *Oedaleus senegalensis* in West Africa. *Crop Protect.* 26, 592–601. doi: 10.1016/j.cropro.2006.04.026

Flores Granados, F. (2011). Las plagas de langosta en el área maya: ambiente e historia de una calamidad en la época prehispánica. *Península* 6, 27–46.

Gartry, L., and Edwards, T. (2016). *Summer rain brings locust swarms to Kimberley*. ABC News. Available online at: https://www.abc.net.au/news/2016-03-24/locusts-swarm-kimberley-town-halls-creek-pastoral-stations/7276048 (accessed February 28, 2019).

Gastón, J. (1969). *Sintesis Historica de la Langosta en la Argentina*. Secretaría de Estado de Agricultura y Ganadería.

Ghadraoui, L. E., Petit, D., Picaud, F., and Yamani, J. E. (2002). Relationship between labrum sensilla number in the Moroccan locust *Dociostaurus maroccanus* and the nature of its diet. *J. Orthopt. Res.* 11, 11–18. doi: 10.1665/1082-6467(2002)011[0011:RBLSNI]2.0.CO;2

Gibson, D. J. (2009). *Grasses and Grassland Ecology*. Oxford: Oxford University Press.

Giese, M., Brueck, H., Gao, Y. Z., Lin, S., Steffens, M., Kögel-Knabner, I., et al. (2013). N balance and cycling of Inner Mongolia typical steppe: a comprehensive case study of grazing effects. *Ecol. Monogr.* 83, 195–219. doi: 10.1890/12-0114.1

Gosálvez, J., and López-Fernández, C. (1981). Extra heterochromatin in natural populations of *Gomphocerus sibiricus* (Orthoptera: Acrididae). *Genetica* 56, 197–204. doi: 10.1007/BF00057560

Graham, R. I., Deacutis, J. M., Pulpitel, T., Ponton, F., Simpson, S. J., and Wilson, K. (2014). Locusts increase carbohydrate consumption to protect against a fungal biopesticide. *J. Insect Physiol.* 69, 27–34. doi: 10.1016/j.jinsphys.2014.05.015

Graham, R. I., Deacutis, J. M., Simpson, S. J., and Wilson, K. (2015). Body condition constrains immune function in field populations of female Australian plague locust *Chortoicetes terminifera*. *Parasite Immunol.* 37, 233–241. doi: 10.1111/pim.12179

Gray, L. J., Sword, G. A., Anstey, M. L., Clissold, F. J., and Simpson, S. J. (2009). Behavioural phase polyphenism in the Australian plague locust (*Chortoicetes terminifera*). *Biol. Lett.* 5, 306–309. doi: 10.1098/rsbl.2008.0764

Gueguen-Genest, M. C., and Gueguen, A. (1987). Effect of sheep grazing on population dynamic of the Siberian criquet *Gomphocerus sibericus* Finot Orthoptera Acrididae in an alpine grassland. *Comptes Rendus Acad. Sci. Ser. 3 Sci. Vie* 304, 443–447.

Guo, K. U. N., HAO, S.-G., Sun, O. J., and Kang, L. E. (2009). Differential responses to warming and increased precipitation among three contrasting grasshopper species. *Glob. Change Biol.* 15, 2539–2548. doi: 10.1111/j.1365-2486.2009.01861.x

Guo, W., Wang, X., Ma, Z., Xue, L., Han, J., Yu, D., et al. (2011). CSP and takeout genes modulate the switch between attraction and repulsion during behavioral phase change in the migratory locust. *PLoS Genet.* 7:e1001291. doi: 10.1371/journal.pgen.1001291

Han, J. G., Zhang, Y. J., Wang, C. J., Bai, W. M., Wang, Y. R., Han, G. D., et al. (2008). Rangeland degradation and restoration management in China. *Rangeland J.* 30, 233–239. doi: 10.1071/RJ08009

Hanboonsong, Y., Jamjanya, T., and Durst, T. B. (2013). *Six-Legged Livestock: Edible Insect Farming, Collection and Marketing in Thailand*. Bangkok: Food and Agriculture Organization of the United Nations, Regional Office for Asia and the Pacific.

Hao, S., Wang, S., Cease, A., and Kang, L. (2015). Landscape level patterns of grasshopper communities in Inner Mongolia: interactive effects of livestock grazing and a precipitation gradient. *Landsc. Ecol.* 30, 1657–1668. doi: 10.1007/s10980-015-0247-8

Hao, S.-G., and Kang, L. (2004). Postdiapause development and hatching rate of three grasshopper species (Orthoptera: Acrididae) in Inner Mongolia. *Environ. Entomol.* 33, 1528–1534. doi: 10.1603/0046-225X-33.6.1528

Haroon, W. M., Pages, C., Vassal, J.-M., Abdalla, A. M., Luong-Skovmand, M., and Lecoq, M. (2011). Laboratory and field investigation of a mixture of *Metarhizium acridum* and Neem seed oil against the Tree Locust *Anacridium melanorhodon melanorhodon* (Orthoptera: Acrididae). *Biocontrol Sci. Technol.* 21, 353–366. doi: 10.1080/09583157.2010.550678

Harvey, A. W. (1983). *Schistocerca piceifrons* (Walker)(Orthoptera: Acrididae), the swarming locust of tropical America: a review. *Bull. Entomol. Res.* 73, 171–184. doi: 10.1017/S0007485300008786

Hernández-Zul, M. I., Quijano-Carranza, J. A., Yáñez-López, R., Ocampo-Velázquez, R. V., Torres-Pacheco, I., Guevara-González, R. G., et al. (2013). Dynamic simulation model of central American locust *Schistocerca piceifrons* (Orthoptera: Acrididae). *Florida Entomol.* 96, 1274–1283. doi: 10.1653/024.096.0405

Hoekstra, J. M., Boucher, T. M., Ricketts, T. H., and Roberts, C. (2005). Confronting a biome crisis: global disparities of habitat loss and protection. *Ecol. Lett.* 8, 23–29. doi: 10.1111/j.1461-0248.2004.00686.x

Holt, J., and Colvin, J. (1997). A differential equation model of the interaction between the migration of the Senegalese grasshopper, *Oedaleus senegalensis*, its predators, and a seasonal habitat. *Ecol. Modell.* 101, 185–193. doi: 10.1016/S0304-3800(97)01981-9

Hooper, G. H. S. (1998). The changing environment of locust control in Australia. *J. Orthopt. Res.* 113–115. doi: 10.2307/3503505

Houndekon, V. A., and De Groote, H. (1998). "Health costs and externalities of pesticide use in locust and grasshopper control in the Sahel," in *Annual conference of the American Agricultural Economics Association* (Milwaukee, WI).

Huang, X., McNeill, M., and Zhang, Z. (2015). Quantitative analysis of plant consumption and preference by *Oedaleus asiaticus* (Acrididae: Oedipodinae) in changed plant communities consisting of three grass species. *Environ. Entomol.* 45, 163–170. doi: 10.1093/ee/nvv172

Huang, X., McNeill, M. R., Ma, J., Qin, X., Tu, X., Cao, G., et al. (2017b). Gut transcriptome analysis shows different food utilization efficiency by the grasshopper *Oedaleus asiaticus* (Orthoptera: Acrididae). *J. Econ. Entomol.* 110, 1831–1840. doi: 10.1093/jee/tox128

Huang, X., Wu, H., McNeill, M. R., Qin, X., Ma, J., Tu, X., et al. (2016). Quantitative analysis of diet structure by real-time PCR, reveals different feeding patterns by two dominant grasshopper species. *Sci. Rep.* 6:32166. doi: 10.1038/srep32166

Huang, X. B., McNeill, M. R., Ma, J. C., Qin, X. H., Tu, X. B., Cao, G. C., et al. (2017a). Biological and ecological evidences suggest *Stipa krylovii* (Pooideae), contributes to optimal growth performance and population distribution of the grasshopper *Oedaleus asiaticus*. *Bull. Entomol. Res.* 107, 401–409. doi: 10.1017/S000748531600105X

Hunter, D. M. (1982). Adult development in the Australian plague locust, *Chortoicetes terminifera* (Walker)(Orthoptera: Acrididae). *Bull. Entomol. Res.* 72, 589–598. doi: 10.1017/S0007485300008622

Hunter, D. M. (1989). The response of Mitchell grasses (*Astrebla* spp.) and Button grass (Dactyloctenium radulans (R. *Br.*)) to rainfall and their importance to the survival of the Australian plague locust, Chortoicetes terminifera (Walker), in the arid zone. *Austr. J. Ecol.* 14, 467–471. doi: 10.1111/j.1442-9993.1989.tb01456.x

Hunter, D. M. (2004). Advances in the control of locusts (Orthoptera: Acrididae) in eastern Australia: from crop protection to preventive control. *Austr. J. Entomol.* 43, 293–303. doi: 10.1111/j.1326-6756.2004.00433.x

Hunter, D. M., and Cosenzo, E. L. (1990). The origin of plagues and recent outbreaks of the South American locust, *Schistocerca cancellata* (Orthoptera: Acrididae) in Argentina. *Bull. Entomol. Res.* 80, 295–300. doi: 10.1017/S0007485300050495

Hunter, D. M., McCulloch, L., and Wright, D. E. (1981). Lipid accumulation and migratory flight in the Australian plague locust, *Chortoicetes terminifera* (Walker)(Orthoptera: Acrididae). *Bull. Entomol. Res.* 71, 543–546. doi: 10.1017/S0007485300010051

Hunter, D. M., Milner, R. J., and Spurgin, P. A. (2001). Aerial treatment of the Australian plague locust, *Chortoicetes terminifera* (Orthoptera: Acrididae) with *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes). *Bull. Entomol. Res.* 91, 93–99.

Hunter-Jones, P. (1967). Life history of the Central American Locust, *Schistocerca sp.*(Orthoptera: Acrididae), in the laboratory. *Ann. Entomol. Soc. Am.* 60, 468–477. doi: 10.1093/aesa/60.2.468

Il'enko, M. I. (1930). *The Development and Meta-Morphoses of the Siberian Grasshopper Gomphocerus sibiricus. The Development and Meta-morphoses of the Siberian Grasshopper Gomphocerus sibiricus.* Available online at: https://www.cabdirect.org/cabdirect/abstract/19310500191 (accessed April 27, 2018).

Jenkins, C. F. H. (1968). Grasshoppers and locusts in Western Australia. *J. Depart. Agric. West. Austr. Ser.* 4 9, 169–175.

Jiang, X., Maimaitiming, Zhang, L. (2003). Nocturnal Migration of Grasshopper (Acrididae: *Oedaleus asiaticus*). *Acta Agrest. Sin.* 1, 75–77.

Jie, C., Jing-Zhang, C., Man-Zhi, T., and Zi-Tong, G. (2002). Soil degradation: a global problem endangering sustainable development. *J. Geogr. Sci.* 12, 243–252. doi: 10.1007/BF02837480

Joern, A. (1986). Experimental study of avian predation on coexisting grasshopper populations (Orthoptera: Acrididae) in a Sandhills Grassland. *Oikos* 46, 243–249. doi: 10.2307/3565473

Joern, A. (2000). "What are the consequences of non-linear ecological interactions for grasshopper control strategies?," in *Grasshoppers and Grassland Health*, eds J. Lockwood, A. Latchininsky, and M. G. Sergeev (New York, NY: Springer), 131–144. doi: 10.1007/978-94-011-4337-0_9

Joyce, R. J. V. (1952). *The Ecology of Grasshoppers in east central Sudan. The Ecology of Grasshoppers in east central Sudan.* Available online at: https://www.cabdirect.org/cabdirect/abstract/19520500484 (accessed February 22, 2018).

Jutsum, A. R., and Goldsworthy, G. J. (1976). Fuels for flight in *Locusta*. *J. Insect Physiol.* 22, 243–249. doi: 10.1016/0022-1910(76)90032-9

Kang, L., Chen, X., Zhou, Y., Liu, B., Zheng, W., Li, R., et al. (2004). The analysis of large-scale gene expression correlated to the phase changes of the migratory locust. *Proc. Natl. Acad. Sci. U.S.A.* 101, 17611–17615. doi: 10.1073/pnas.0407753101

Kang, L., and Chen, Y. (1995). Dynamics of grasshopper communities under different grazing intensities in Inner Mongolian steppes. *Insect Sci.* 2, 265–281. doi: 10.1111/j.1744-7917.1995.tb00048.x

Kang, L., Han, X., Zhang, Z., and Sun, O. J. (2007). Grassland ecosystems in China: review of current knowledge and research advancement. *Philos. Trans. R. Soc. B Biol. Sci.* 362, 997–1008. doi: 10.1098/rstb.2007.2029

Kapsar, K. E., Hovis, C. L., Bicudo da Silva, R. F., Buchholtz, E. K., Carlson, A. K., Dou, Y., et al. (2019). Telecoupling Research: The First Five Years. *Sustainability* 11, 1033. doi: 10.3390/su11041033

Kathy, C. (2006). *Yellow winged locusts on the swarm in Qld*. ABC Rural. Available online at: http://www.abc.net.au/site-archive/rural/content/2006/s1590089.htm (accessed February 28, 2019).

Kaya, M., Erdogan, S., Mol, A., and Baran, T. (2015). Comparison of chitin structures isolated from seven Orthoptera species. *Int. J. Biol. Macromol.* 72, 797–805. doi: 10.1016/j.ijbiomac.2014.09.034

Keay-Bright, J., and Boardman, J. (2006). Changes in the distribution of degraded land over time in the central Karoo, South Africa. *Catena* 1, 1–14. doi: 10.1016/j.catena.2005.12.003

Key, K. H. L. (1945). Council for Scientific and Industrial Research, 127 p., 8, no. 186, p. 119–122.

Kieser, M., Thackrah, A., and Rosenberg, J. (2010). *Changes in the Outbreak Region of the Brown Locust in Southern Africa*. Available online at: http://gadi.agric.za/articles/Kieser_M/kieser_vol4_2002_locust.php (accessed May 1, 2019).

Köhler, P. (1962). Ecología de la zona central y de gregarización de la langosta en la República Argentina. *Idia* 7:108.

Latchininsky, A. V. (1998). Moroccan locust *Dociostaurus maroccanus* (Thunberg, 1815): a faunistic rarity or an important economic pest? *J. Insect Conserv.* 2, 167–178.

Latchininsky, A. V. (2013). Locusts and remote sensing: a review. *JARS* 7:075099. doi: 10.1117/1.JRS.7.075099

Latchininsky, A. V., and Launois Luong, M. H. (1992). *Le Criquet Marocain, Dociostaurus maroccanus (Thunberg, 1815), Dans la Partie Orientale de Son Aire de Distribution: Etude Monographique Relative a l'ex-URSS et Aux Pays Proches*. Montpellier: CIRAD-PRIFAS, 289.

Launois, M. (1978). *Modélisation Écologique et Simulation Opérationnelle en Acridologie: Application à Oedaleus senegalensis (Krauss, 1877)*. Paris: Ministère de la Coopéracion/GERDAT.

Launois, M. (1979). An ecological model for the study of the grasshopper *Oedaleus senegalensis* in West Africa. *Phil. Trans. R. Soc. Lond. B* 287, 345–355. doi: 10.1098/rstb.1979.0066

Le Gall, M., and Tooker, J. F. (2017). Developing ecologically based pest management programs for terrestrial molluscs in field and forage crops. *J. Pest Sci.* 90, 1–14. doi: 10.1007/s10340-017-0858-8

Lea, A. (1958a). Recent outbreaks of the brown locust, *Locustana pardalina* (Walk), with special reference to the influence of rainfall. *J. Entomol. Soc. South. Afr.* 21, 162–213.

Lea, A. (1958b). The continuing challenge of the brown locust. *J. Entomol. Soc. South. Afr.* 21, 03–15.

Lecoq, M., Chamouine, A., and Luong-Skovmand, M.-H. (2011). Phase-dependent color polyphenism in field populations of red locust nymphs (*Nomadacris septemfasciata* Serv.) in Madagascar. *Psyche*. 2011:105352. doi: 10.1155/2011/105352

Li, J., Li, S., Wang, D., and Ji, R. (2014). Changes in the contents of stress resistant substances in *Gomphocerus sibiricus* (Orthoptera: Acrididae) under high temperature stress. *Acta Entomol. Sin.* 57, 1155–1161.

Li, S., Huang, X., McNeill, M. R., Liu, W., Tu, X., Ma, J., et al. (2019). Dietary stress from plant secondary metabolites contributes to grasshopper (Oedaleus asiaticus) migration or plague by regulating insect insulin-like signaling pathway. *Front. Physiol.* 10:531. doi: 10.3389/fphys.2019.00531

Li, X. L., Yuan, Q. H., Wan, L. Q., and He, F. (2008). Perspectives on livestock production systems in China. *Rangeland J.* 30, 211–220. doi: 10.1071/RJ08011

Liu, G.-H., Hao, S.-G., Shao, X.-Q., Zhang, Y.-J., and Wang, S.-P. (2013a). Diet composition and trophic niche of *Oedaleus asiaticus* (Orthoptera: Acrididae) in natural grasslands under different grazlng pressure in Inner Mongolia, northern China. *Acta Entomol. Sin.* 56, 537–547.

Liu, J., Hull, V., Batistella, M., DeFries, R., Dietz, T., Fu, F., et al. (2013b). Framing sustainability in a telecoupled World. *Ecol. Soc.* 18:26. doi: 10.5751/ES-05873-180226

Loaiza, P. A., Balocchi, O., and Bertrand, A. (2017). Carbohydrate and crude protein fractions in perennial ryegrass as affected by defoliation frequency and nitrogen application rate. *Grass Forage Sci.* 72, 556–567. doi: 10.1111/gfs.12258

Lockwood, J. A., and Debrey, L. D. (1990). A solution for the sudden and unexplained extinction of the rocky mountain grasshopper (Orthoptera: Acrididae). *Environ. Entomol.* 19, 1194–1205. doi: 10.1093/ee/19.5.1194

Lockwood, J. A., Showler, A. T., and Latchininsky, A. V. (2001). Can we make locust and grasshopper management sustainable? *J. Orthoptera Res.* 10, 315–329. doi: 10.1665/1082-6467(2001)010[0315:CWMLAG]2.0.CO;2

Locust Control Campaign in Northern Laos, | FAO in Laos | Food and Agriculture Organization of the United Nations (2016). Available online at: http://www.fao.org/laos/news/detail-events/en/c/414367/ (accessed April 15, 2019).

Long, W. H., and Hensley, S. D. (1972). Insect pests of sugar cane. *Annu. Rev. Entomol.* 17, 149–176. doi: 10.1146/annurev.en.17.010172.001053

Louveaux, A., Peyrelongue, J. Y., and Gillon, Y. (1988). *Outbreaks of the Italian Locust (Calliptamus italicus (L.)) in Poitou-Charentes (France)*. Comptes Rendus de l'Academie d'Agriculture de France (France).

Loveridge, J. P., and Bursell, E. (1975). Studies on the water relations of adult locusts (Orthoptera, Acrididae). I. Respiration and the production of metabolic water. *Bull. Entomol. Res.* 65, 13–20. doi: 10.1017/S0007485300005708

Lynch Arribalzaga, E. (1903). La langosta voladora del Peru. *An. Mus. Nac. Hist. Nat. B Aires* 9, 1–5.

Lynch Arribalzaga, E. (1918). La langosta voladora de Colombia y Venezuela. *Physis* 4, 49–79.

Ma, C., Liu, C., Yang, P., and Kang, L. (2009). The complete mitochondrial genomes of two band-winged grasshoppers, *Gastrimargus marmoratus* and *Oedaleus asiaticus*. *BMC Genomics* 10:156. doi: 10.1186/1471-2164-10-156

Ma, C., Yang, P., Jiang, F., CHAPUIS, M.-P., Shali, Y., Sword, G. A., et al. (2012). Mitochondrial genomes reveal the global phylogeography and dispersal routes of the migratory locust. *Mol. Ecol.* 21, 4344–4358. doi: 10.1111/j.1365-294X.2012.05684.x

Ma, Z., Guo, W., Guo, X., Wang, X., and Kang, L. (2011). Modulation of behavioral phase changes of the migratory locust by the catecholamine metabolic pathway. *Proc. Natl. Acad. Sci. U.S.A.* 108, 3882–3887. doi: 10.1073/pnas.1015098108

Ma, Z., Guo, X., Lei, H., Li, T., Hao, S., and Kang, L. (2015). Octopamine and tyramine respectively regulate attractive and repulsive behavior in locust phase changes. *Sci. Rep.* 5:8036. doi: 10.1038/srep08036

Magor, J. I., Lecoq, M., and Hunter, D. M. (2008). Preventive control and Desert Locust plagues. *Crop Protect.* 27, 1527–1533. doi: 10.1016/j.cropro.2008.08.006

Maiga, I. H., Axelsen, J. A., Badji, K., and Kooyman, C. (2009). Simulation studies of Senegalese Grasshopper ecosystem interactions III: optimization of grasshopper control. *Int. J. Pest Manag.* 55, 113–120. doi: 10.1080/09670870802601092

Maiga, I. H., Lecoq, M., and Kooyman, C. (2008). Ecology and management of the Senegalese grasshopper *Oedaleus senegalensis* (Krauss 1877) (Orthoptera: Acrididae) in West Africa: review and prospects. *Ann. Soc. Entomol. France* 44, 271–288. doi: 10.1080/00379271.2008.10697563

Maiga, I. H., Lecoq, M., and Morand, S. (2010). Egg survival strategies of the Senegalese grasshopper during the dry season in the African Sahel. *Int. J. Pest Manag.* 56, 223–232. doi: 10.1080/09670870903464390

Maxwell-Darling, R. C. (1936). The outbreak centres of *Schistocerca gregaria*, Forsk., on the Red Sea coast of the Sudan. *Bull. Entomol. Res.* 27, 37–66. doi: 10.1017/S0007485300058107

Medina, H. E., Cease, A., and Trumper, E. (2017). The resurgence of the South American locust (*Schistocerca cancellata*). *Metaleptea* 37, 17–21.

Mignon, J. (2002). L'entomophagie : une question de culture? - Mignon Jacques. *Tropicultura* 20, 151–155.

Miller, G. A., and Simpson, S. J. (2010). 1582301. Isolation from a marching band increases haemocyte density in wild locusts (*Chortoicetes terminifera*). *Ecol. Entomol.* 35, 236–239. doi: 10.1111/j.1365-2311.2010.01180.x

Mohamed Shaluf, I. (2007). An overview on disasters. *Disaster Prev. Manag.* 16, 687–703. doi: 10.1108/09653560710837000

Moussi, A., Abba, A., Harrat, A., and Petit, D. (2011). Desert acridian fauna (Orthoptera, Acridomorpha): comparison between steppic and oasian habitats in Algeria. *Compt. Rendus Biol.* 334, 158–167. doi: 10.1016/j.crvi.2010.12.001

Nevo, D. (1996). The desert locust, *Schistocerca gregaria*, and its control in the land of Israel and the Near East in antiquity, with some reflections on its appearance in Israel in modern times. *Phytoparasitica* 24, 7–32. doi: 10.1007/BF02981450

Oedekoven, M. A., and Joern, A. (2000). Plant quality and spider predation affects grasshoppers (Acrididae): food-quality-dependent compensatory mortality. *Ecology* 81, 66–77. doi: 10.1890/0012-9658(2000)081[0066:PQASPA]2.0.CO;2

Onsager, J. A. (2000). Suppression of grasshoppers in the Great Plains through grazing management. *J. Range Manag.* 592–602. doi: 10.2307/4003152

Onsager, J. A., and Olfert, O. (2000). "What tools have potential for grasshopper pest management?," in *Grasshoppers and Grassland Health*, eds J. Lockwood, A. Latchininsky, and M. G. Sergeev (New York, NY: Springer), 145–156. doi: 10.1007/978-94-011-4337-0_10

Osimani, A., Garofalo, C., Aquilanti, L., Milanović, V., Cardinali, F., Taccari, M., et al. (2017). Transferable antibiotic resistances in marketed edible grasshoppers (*Locusta migratoria migratorioides*). *J. Food Sci.* 82, 1184–1192. doi: 10.1111/1750-3841.13700

Pasquier, R. (1934). Contributiona l'étude du Criquet marocain, Dociostaurus maroccanus Thunb., en Afrique mineure (lre note). *Bull. Soc. Hist. Nat. Afr. Nord.* 25, 167–200.

Pedgley, D. (1981). *Desert Locust Forecasting Manual.*. London: COPR.

Pener (1991). "Locust phase polymorphism and its endocrine relations," in *Advances in Insect Physiology*, ed P. D. Evans (London: Elsevier), 1–79. doi: 10.1016/S0065-2806(08)60091-0

Pener, M. P., and Simpson, S. J. (2009). "Locust phase polyphenism: an update," in *Advances in Insect Physiology*, eds S. J. Simpson and M. P. Pener (London: Academic Press), 1–272. doi: 10.1016/S0065-2806(08)36001-9

Phiriyangkul, P., Srinroch, C., Srisomsap, C., Chokchaichamnankit, D., and Punyarit, P. (2015). Effect of food thermal processing on allergenicity proteins in Bombay locust (*Patanga succincta*). *Int. J. Food Eng.* 1, 23–28. doi: 10.18178/ijfe.1.1.23-28

Piou, C., Lebourgeois, V., Benahi, A. S., Bonnal, V., Jaavar, M. H., Lecoq, M., et al. (2013). Coupling historical prospection data and a remotely-sensed vegetation index for the preventative control of Desert locusts. *Basic Appl. Ecol.* 14, 593–604. doi: 10.1016/j.baae.2013.08.007

Poot-Pech, M. A. (2016). "La langosta voladora Schistocerca piceifrons (Orthoptera: Acrididae): hacia un manejo sustentable. El Patrimonio, su Importancia y Conservación," in *Conociendo el Patrimonio, Cuerpo Académico Patrimonio y Desarrollo Sustentable, Edito-rial TECCIS* ed E. Fontes (San Francisco de Campeche), 58–66.

Poot-Pech, M. A., Ruiz-Sánchez, E., Ballina-Gómez, H. S., Gamboa-Angulo, M. M., and Reyes-Ramírez, A. (2016). Olfactory response and host plant feeding of the Central American Locust *Schistocerca piceifrons piceifrons* Walker to common plants in a gregarious zone. *Neotrop. Entomol.* 45, 382–388. doi: 10.1007/s13744-016-0385-y

Poot-Pech, M. A., Ruiz-Sánchez, E., Gamboa-Angulo, M., Ballina-Gómez, H. S., and Reyes-Ramírez, A. (2018). Population fluctuation of *Schistocerca piceifrons piceifrons* (Orthoptera: Acrididae) in the Yucatán Península and its relation with the environmental conditions. *Rev. Biol. Trop.* 66, 403–414. doi: 10.15517/rbt.v66i1.29502

Popov, G. B. (1980). *Studies on Oviposition, Egg Development and Mortality in Oedaleus senegalensis (Krauss), (Orthoptera, Acridoidea) in the Sahel*. London: Centre for Overseas Pest Research, 33–48.

Popov, G. B., Duranton, J.-F., and Gigault, J. (1991). *Etude Écologique des Biotopes du Criquet Pèlerin# Schistocerca gregaria#(Forskal, 1775) en Afrique Nord-Occidentale: Mise en Évidence et Description des Unités Territoriales Écologiquement Homogènes*. CIRAD-PRIFAS.

Price, R. E., and Brown, H. D. (2000). "A century of locust control in South Africa," in *Workshop on Research Priorities for Migrant Pests of Agriculture in Southern Africa* (Chatham) 37–49.

Qin, Y., Li, Z., Zhao, L., Fowler, G., and Fang, Y. (2012). "The current and future potential geographical distribution of the Italian locust, Calliptamus italicus (Linnaeus)(Orthoptera: Acrididae) in China," in *International Conference on Computer and Computing Technologies in Agriculture* (Beijing: Springer), 290–298. doi: 10.1007/978-3-642-36137-1_35

Qing, Y. Q. L. Z. L., and Chunxian, Y. G. F. C. J. (2008). Feeding habits and economic threshold of *Locusta migratoria tibetensis*. *Acta Phytophylac. Sin.* 5.

Rankin, M. A., and Burchsted, J. C. A. (1992). The cost of migration in insects. *Annu. Rev. Entomol.* 37, 533–559. doi: 10.1146/annurev.en.37.010192.002533

Raubenheimer, D., and Simpson, S. J. (1993). The geometry of compensatory feeding in the locust. *Anim. Behav.* 45, 953–964. doi: 10.1006/anbe.1993.1114

Riley, J. R., and Reynolds, D. R. (1983). A long-range migration of grasshoppers observed in the Sahelian zone of Mali by two radars. *J. Anim. Ecol.* 167–183. doi: 10.2307/4594

Ritchie, J. M. (1978). Melanism in *Oedaleus senegalensis* and other oedipodines (Orthoptera, Acrididae). *J. Nat. Hist.* 12, 153–162.

Ritchie, J. M. (1981). *A Taxonomic Revision of the Genus Oedaleus Fieber (Orthoptera: Acrididae)[Including Crop Pests]*. London: Bulletin of the British Museum. Natural History. Entomology.

Robinson, B. E., Li, P., and Hou, X. (2017). Institutional change in social-ecological systems: the evolution of grassland management in Inner Mongolia. *Glob. Environ. Change* 47, 64–75. doi: 10.1016/j.gloenvcha.2017.08.012

Robinson, M. D. (2001). Desert nitrogen cycles: fecal nitrogen from a population of the Sahelian Tree Locust in Oman. *Sultan Qaboos Univ. J. Sci.* 6, 33–38. doi: 10.24200/squjs.vol6iss1pp33-38

Roessingh, P., Bouaïchi, A., and Simpson, S. J. (1998). Effects of sensory stimuli on the behavioural phase state of the desert locust, *Schistocerca gregaria*. *J. Insect Physiol.* 44, 883–893. doi: 10.1016/S0022-1910(98)00070-5

Roffey, J. (1979). *Locusts and Grasshoppers of Economic Importance in Thailand*. London: Locusts and grasshoppers of economic importance in Thailand.

Roffey, J., and Magor, J. I. (2003). *Desert Locust Popualation Dynamics Parameters*. Technical Series-Desert Locust Field Reserch Stations (FAO).

Roffey, J., and Popov, G. (1968). Environmental and behavioural processes in a desert locust outbreak. *Nature* 219, 446. doi: 10.1038/219446a0

Rogers, S. M., Cullen, D. A., Anstey, M. L., Burrows, M., Despland, E., Dodgson, T., et al. (2014). Rapid behavioural gregarization in the desert locust, *Schistocerca gregaria* entails synchronous changes in both activity and attraction to conspecifics. *J. Insect Physiol.* 65, 9–26. doi: 10.1016/j.jinsphys.2014.04.004

Rogers, S. M., Matheson, T., Despland, E., Dodgson, T., Burrows, M., and Simpson, S. J. (2003). Mechanosensory-induced behavioural gregarization in the desert locust *Schistocerca gregaria*. *J. Exp. Biol.* 206, 3991–4002. doi: 10.1242/jeb.00648

Schmitz, O. J. (2008). Effects of predator hunting mode on grassland ecosystem function. *Science* 319, 952–954. doi: 10.1126/science,.1152355

SENASICA-D GSV. (2016). *Langosta Centroamericana [Schistocerca piceifrons piceifrons (Walker, 1870)*.

Sergeev, M. G., and Van'kova, I. A. (2008). Dynamics of the Italian locust *Calliptamus italicus* L. population in the southeast of the West Siberian Plain. *Contempor. Probl. Ecol.* 1, 204–209. doi: 10.1134/S1995425508020057

Serville, M. A. (1838). *Histoire Naturelle des Insectes Orthoptères (dans les Suites à Buffon)*. Paris: Roret.

Shen, K., Wang, H.-J., Shao, L., Xiao, K., Shu, J.-P., Xu, T.-S., et al. (2009). Mud-puddling in the yellow-spined bamboo locust, *Ceracris kiangsu* (Oedipodidae: Orthoptera): does it detect and prefer salts or nitrogenous compounds from human urine? *J. Insect Physiol.* 55, 78–84. doi: 10.1016/j.jinsphys.2008.10.011

Simpson, S. J., and Raubenheimer, D. (2000). The hungry locust. *Adv. Study Behav.* 29, 1–44. doi: 10.1016/S0065-3454(08)60102-3

Simpson, S. J., Raubenheimer, D., Behmer, S. T., Whitworth, A., and Wright, G. A. (2002). A comparison of nutritional regulation in solitarious- and gregarious-phase nymphs of the desert locust Schistocerca gregaria. *J. Exp. Biol.* 205, 121–129.

Skaf, R. M. (1972). "Problem of introducing modern methods of chemical control of locusts in the developing countries," in *International Study Conference on the Current and Future Problems of Acridology, London, 1970. Proceedings*.

Sokol-Hessner, L., and Schmitz, O. J. (2002). Aggregate effects of multiple predator species on a shared prey. *Ecology* 83, 2367–2372. doi: 10.1890/0012-9658(2002)083[2367:AEOMPS]2.0.CO;2

Song, H. (2011). Density-dependent phase polyphenism in nonmodel locusts: a minireview. *Psyche.* 2011:741769. doi: 10.1155/2011/741769

Song, H., Mariño-Pérez, R., Woller, D. A., and Cigliano, M. M. (2018). Evolution, diversification, and biogeography of grasshoppers (Orthoptera: Acrididae). *Insect Syst. Divers* 2, 1–25. doi: 10.1093/isd/ixy008

Spalinger, L. C., Haynes, A. G., Schuetz, M., and Risch, A. C. (2012). Impact of wild ungulate grazing on Orthoptera abundance and diversity in subalpine grasslands. *Insect Conserv. Diver.* 5, 444–452. doi: 10.1111/j.1752-4598.2011.00180.x

Stanley, M. (2018). *Locusts Swarm in the Pilbara and Kimberley, Costing Farmers Thousands*. ABC Rural. Available online at: https://www.abc.net.au/news/rural/2018-04-02/yellow-winged-locust-plague-swarms-northern-wa-stations/9603136 (accessed February 28, 2019).

Steedman, A. (1990). *Locust handbook*. Chatham: Natural Resources Institute.

Stige, L. C., Chan, K.-S., Zhang, Z., Frank, D., and Stenseth, N. C. (2007). Thousand-year-long Chinese time series reveals climatic forcing of decadal locust dynamics. *Proc. Natl. Acad. Sci. U.S.A.* 104, 16188–16193. doi: 10.1073/pnas.0706813104

Stolyarov, M. V. (2000). Strategy and tactics of gregarious locust control. *Zashchita Karantin Rastenii* 10, 17–19.

Stoops, J., Crauwels, S., Waud, M., Claes, J., Lievens, B., and Van Campenhout, L. (2016). Microbial community assessment of mealworm larvae (*Tenebrio molitor*) and grasshoppers (*Locusta migratoria migratorioides*) sold for human consumption. *Food Microbiol.* 53, 122–127. doi: 10.1016/j.fm.2015.09.010

Sword, G. A. (1999). Density-dependent warning coloration. *Nature* 397, 217. doi: 10.1038/16609

Sword, G. A. (2001). Tasty on the outside, but toxic in the middle: grasshopper regurgitation and host plant-mediated toxicity to a vertebrate predator. *Oecologia* 128, 416–421. doi: 10.1007/s004420100666

Sword, G. A. (2002). A role for phenotypic plasticity in the evolution of aposematism. *Proc. R. Soc. Lond. B Biol. Sci.* 269, 1639–1644. doi: 10.1098/rspb.2002.2060

Sword, G. A., Simpson, S. J., El Hadi, O. T. M., and Wilps, H. (2000). Density–dependent aposematism in the desert locust. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* 267, 63–68. doi: 10.1098/rspb.2000.0967

Symmons, P. (2009). A critique of "Preventive control and desert locust plagues." *Crop Protect.* 28, 905–907. doi: 10.1016/j.cropro.2009.04.012

Symmons, P. M. (1984). Control of the Australian plague locust, *Chortoicetes terminifera* (Walker). *Crop Protect.* 3, 479–490. doi: 10.1016/0261-2194(84)90029-2

Symmons, P. M., and Cressman, K. (2001). *Desert Locust Guidelines: Biology and Behavior*. Rome: Food and Agriculture Organization of the United Nations.

Tanaka, S., and Zhu, D.-H. (2005). Outbreaks of the migratory locust Locusta migratoria (Orthoptera: Acrididae) and control in China. *Appl. Entomol. Zool.* 40, 257–263. doi: 10.1303/aez.2005.257

Thindwa, H. P. (1999). Red Locust Population Monitoring and Control in Malawi, 1988–1998. *Int. J. Trop. Insect Sci.* 19, 351–354. doi: 10.1017/S174275840001897X

Tian, H., Stige, L. C., Cazelles, B., Kausrud, K. L., Svarverud, R., Stenseth, N. C., et al. (2011). Reconstruction of a 1,910-y-long locust series reveals consistent associations with climate fluctuations in China. *Proc. Natl. Acad. Sci. U.S.A.* 108, 14521–14526. doi: 10.1073/pnas.1100189108

Tingle, C. C. D., Rother, J. A., Dewhurst, C. F., Lauer, S., and King, W. J. (2003). "Fipronil: environmental fate, ecotoxicology, and human health concerns," in *Reviews of Environmental Contamination and Toxicology: Continuation of Residue Reviews*, ed. G. W. Ware (New York, NY: Springer New York), 1–66. doi: 10.1007/978-1-4899-7283-5_1

Todd, M. C., Washington, R., Cheke, R. A., and Kniveton, D. (2002). Brown locust outbreaks and climate variability in southern Africa. *J. Appl. Ecol.* 39, 31–42. doi: 10.1046/j.1365-2664.2002.00691.x

Toleubayev, K., Jansen, K., and van Huis, A. (2007). Locust control in transition: the loss and reinvention of collective action in post-soviet Kazakhstan. *Ecol. Soc.* 12, 38. doi: 10.5751/ES-02229-120238

Toure, M., Ndiaye, M., and Diongue, A. (2013). Effect of cultural techniques: Rotation and fallow on the distribution of *Oedaleus senegalensis* (Krauss, 1877)(Orthoptera: Acrididae) in Senegal. *Afr. J. Agric. Res.* 8, 5634–5638.

Urech, R. (2003). *Influence of spreading woody plants and surface cover on the distribution of calliptamus italicus and Oedipoda caerulescens (Saltatoria, Caelifera) in a steppe habitat* (Diplomarbeit). Universität Bern, Bern, Switzerland.

Uvarov, B. (1966). *Grasshoppers and Locusts: A Handbook of General Acridology. 1. Anatomy, Physiology, Development, Phase Polymorphism, Introduction to Taxonomy*. Cambridge: University Press.

Uvarov, B. (1977). *Grasshoppers and Locusts. A Handbook of General Acridology Vol. 2. Behaviour, Ecology, Biogeography, Population Dynamics*. Cambridge: Centre for Overseas Pest Research.

Uvarov, B. P. (1936). The oriental migratory locust (*Locusta migratoria manilensis*, Meyen 1835). *Bull. Entomol. Res.* 27, 91–104. doi: 10.1017/S0007485300058144

Van Der Werf, W., Woldewahid, G., Van Huis, A., Butrous, M., and Sykora, K. (2005). Plant communities can predict the distribution of solitarious desert locust *Schistocerca gregaria*. *J. Appl. Ecol.* 42, 989–997. doi: 10.1111/j.1365-2664.2005.01073.x

Van Huis, A., Cressman, K., and Magor, J. I. (2007). Preventing desert locust plagues: optimizing management interventions. *Entomol. Exp. Applic.* 122, 191–214. doi: 10.1111/j.1570-7458.2006.00517.x

Van Huis, A., Woldewahid, G., Toleubayev, K., and Van Der Werf, W. (2008). Relationships between food quality and fitness in the desert locust, *Schistocerca gregaria*, and its distribution over habitats on the Red Sea coastal plain of Sudan. *Entomol. Exp. Applic.* 127, 144–156. doi: 10.1111/j.1570-7458.2008.00682.x

Veran, S., Simpson, S. J., Sword, G. A., Deveson, E., Piry, S., Hines, J. E., et al. (2015). Modeling spatiotemporal dynamics of outbreaking species: influence of environment and migration in a locust. *Ecology* 96, 737–748. doi: 10.1890/14-0183.1

Vesey-Fitzgekald, D. F. (1955). "The vegetation of the outbreak areas of the red locust (*Nomadacris septemfasciata* Sers.) in Tanganyika and Northern Rhodesia. The Vegetation of the Outbreak Areas of the Red Locust (Nomadacris septemfasciata Sers)," in *Tanganyika and Northern Rhodesia.* Available online at: https://www.cabdirect.org/cabdirect/abstract/19560500242 (accessed March 12, 2018). doi: 10.1080/00306525.1955.9633036

Vinokurov, G. M., and Rubtzov, I. A. (1930). *Studies on the Ecology of Grasshoppers in the Irkutsk Region*. Irkutsk: Studies on the Ecology of Grasshoppers in the Irkutsk Region.

Walker, P. R., Hill, L., and Bailey, E. (1970). Feeding activity, respiration, and lipid and carbohydrate content of the male desert locust during adult development. *J. Insect Physiol.* 16, 1001–1015. doi: 10.1016/0022-1910(70)90229-5

Waloff, Z., and Green, S. M. (1976). Some temporal characteristics of desert locust plagues with a statistical analysis. *Antilocust Mem. Minist Overseas Dev. Antilocust. Res. Cent. Lond*. 1976:36.

Waloff, Z., and Pedgley, D. E. (1986). Comparative biogeography and biology of the South American locust, *Schistocerca cancellata* (Serville), and the South African desert locust, *S. gregaria flaviventris (Burmeister)(Orthoptera: Acrididae*): a review. *Bull. Entomol. Res.* 76, 1–20. doi: 10.1017/S0007485300015236

Wang, D. C. (2004). The study on the breakout and disserving of grasshopper in Sunitezuo Banner, Inner Mongolia. *Inner Mongolia Pratacult.* 16, 14–15.

Wang, H., Shao, L., Sju, J., Shen, K., Xia, K., Li, G., et al. (2010). Analysis of some human urine-borne phagostimulants to the yellow-spined bamboo locust, *Ceracris kiangsu*. *J. Nanjing Agric. Univ.* 2.

Watts, J. G., Huddleston, E. W., and Owens, J. C. (1982). Rangeland entomology. *Annu. Rev. Entomol.* 27, 283–311. doi: 10.1146/annurev.en.27.010182.001435

Weis-Fogh, T. (1952). Fat combustion and metabolic rate of flying locusts (*Schistocerca gregaria* Forskaal). *Phil. Trans. R. Soc. Lond. B* 237, 1–36. doi: 10.1098/rstb.1952.0011

White, T. C. (2012). *The Inadequate Environment: Nitrogen and the Abundance of Animals*. Berlin: Springer Science and Business Media.

Wiens, J. A. (1973). Pattern and process in grassland bird communities. *Ecol. Monogr.* 43, 237–270. doi: 10.2307/1942196

Wilps, H., and Diop, B. (1997). "Field investigations on *Schistocerca gregaria* (Forskaal) adults, hoppers and hopper bands," in *New Strategies in Locust Control*, eds S. Krall, R. Peveling, and D. Ba Diallo (Basel: Springer), 117–128. doi: 10.1007/978-3-0348-9202-5_16

Wilps, H., Levchenko, V., and Vernigor, A. (2002). Field trials with Imidacloprid (Confidor) on the Italian locust *Calliptamus italicus* in Kazakhstan. *J. Appl. Entomol.* 126, 436–443. doi: 10.1046/j.1439-0418.2002.00676.x

Woodman, J. D. (2017). Effects of substrate salinity on oviposition, embryonic development and survival in the Australian plague locust, *Chortoicetes terminifera* (Walker). *J. Insect Physiol.* 96, 9–13. doi: 10.1016/j.jinsphys.2016.10.001

Word, M. L., Hall, S. J., Robinson, B., Manneh, B., Beye, A., and Cease, A. J. (2019). Soil-targeted interventions could alleviate locust and grasshopper pest pressure in West Africa. *Sci. Total Environ*. 663, 632–643. doi: 10.1016/j.scitotenv.2019.01.313

Wright, D. E. (1983). *The Origin and Development of Major Plagues of Chortoicetes terminifera.* Oxford: Walker.

Wright, D. E., and Symmons, P. M. (1987). The development and control of the 1984 plague of the Australian plague locust, *Chortoicetes terminifera* (Walker). *Crop Protect.* 6, 13–19. doi: 10.1016/0261-2194(87)90022-6

Wu, T., Hao, S., Sun, O. J., and Kang, L. (2012). Specificity responses of grasshoppers in temperate grasslands to diel asymmetric warming. *PLoS ONE* 7:e41764. doi: 10.1371/journal.pone.0041764

Xinghui, Q., and Hongchang, L. (1997). Effect of livestock exclusion on abundance of grasshoppers on *Aneurolepidium chinense* and *Stipa grandis* steppes. *Chin. J. Appl. Ecol.* 4:013.

Xu, H., Su, H., Su, B., Han, X., Biswas, D. K., and Li, Y. (2014). Restoring the degraded grassland and improving sustainability of grassland ecosystem through chicken farming: a case study in northern China. *Agric. Ecosyst. Environ.* 186, 115–123. doi: 10.1016/j.agee.2014.02.001

Yang, L., and Wang, J. (2004). Meteorological factors on grasshopper bloom in Hami Area. *Chin. J. Ecol.* 6:009.

Yu, H.-P., Shen, K., Wang, Z.-T., Mu, L.-L., and Li, G.-Q. (2011a). Population control of the yellow-spined bamboo locust, *Ceracris kiangsu*, using urine-borne chemical baits in bamboo forest. *Entomol. Exp. Applic.* 138, 71–76. doi: 10.1111/j.1570-7458.2010.01076.x

Yu, H.-P., Wang, Z.-T., Xiao, K., Shao, L., and Li, G.-Q. (2011b). The presence of conspecific decoys enhances the attractiveness of an NaCl resource to the yellow-spined locust, *Ceracris kiangsu*. *Journal of insect science* 11, 45. doi: 10.1673/031.011.0145

Zhang, L., and Hunter, D. (2017). Management of locusts and grasshoppers in China. *Journal of Orthoptera Research* 26, 155. doi: 10.3897/jor.26.20119

Zhang, L., Lecoq, M., Latchininsky, A., and Hunter, D. (2019). Locust and Grasshopper Management. *Annual Review of Entomology* 64, 15–34. doi: 10.1146/annurev-ento-011118-112500

Zhang, Z., Elser, J. J., Cease, A. J., Zhang, X., and Yu, Q. (2014). Grasshoppers Regulate N: P Stoichiometric Homeostasis by Changing Phosphorus Contents *in*. doi: 10.1371/journal.pone.0103697

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 31 July 2019
doi: 10.3389/fevo.2019.00282

Deicing Salt Pollution Affects the Foliar Traits and Arthropods' Biodiversity of Lime Trees in Riga's Street Greeneries

*Dalinda Bouraoui [1,2,3], Gunta Cekstere [2,3], Anita Osvalde [2], Pierre Vollenweider [3] and Sergio Rasmann [1]**

[1] *Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland,* [2] *Institute of Biology, University of Latvia, Riga, Latvia,* [3] *Forest Dynamics, Swiss Federal Institute for Forest, Snow, and Landscape Research (WSL), Birmensdorf, Switzerland*

Street greeneries and other green spaces within the urban matrix can potentially reduce air pollution and increase urban biodiversity. Yet, these services can be negatively affected by anthropogenic stress factors. In the boreo-nemoral zone, large amounts of salts are spread each year for deicing the pavement. To address the effect of deicing salt on street lines of lime trees and how this cascades up to influence the surrounding arthropod biodiversity, we compared heavily salt-polluted, and less polluted sites in the city of Riga, Latvia. We analyzed the impairment of foliar functions and development of aphid colonies using a common garden experiment. We found marked variation in the soil physico-chemical properties in polluted vs. unpolluted sites, and the overall composition of arthropod communities, considering their abundance together with their diversity, significantly responded to site contamination. In a common garden experiment, we also showed that the exposure to increased salt levels in the soil caused functional as well as structural injuries within foliage and slowed down the development of aphid colonies. Finally, the damage inflicted by the lime tree aphids, especially in unpolluted sites, was positively correlated to the production of mucilage in the leaves, suggesting herbivore-induced mucilage production in leaves. The effects of de-icing salts thus appeared to cascade up through the trophic chain and to negatively affect not only the trees but also the associated arthropod biota. These findings point at the necessity of a wider screening of plant species and cultivars that not only better tolerate anthropogenic stress but also promote the biodiversity in cities.

Keywords: arthropod biodiversity, deicing salt, *Eucallipterus tiliae*, multidisciplinary functional approach, plant tolerance, urban ecology, urban soils

Edited by:
Shannon Murphy,
University of Denver, United States

Reviewed by:
Elsa Youngsteadt,
North Carolina State University,
United States
Steve Frank,
North Carolina State University,
United States

****Correspondence:***
Sergio Rasmann
sergio.rasmann@unine.ch

Specialty section:
This article was submitted to
Population and Evolutionary
Dynamics,
a section of the journal
Frontiers in Ecology and Evolution

Received: *28 February 2019*
Accepted: *11 July 2019*
Published: *31 July 2019*

Citation:
Bouraoui D, Cekstere G, Osvalde A, Vollenweider P and Rasmann S (2019) Deicing Salt Pollution Affects the Foliar Traits and Arthropods' Biodiversity of Lime Trees in Riga's Street Greeneries. Front. Ecol. Evol. 7:282. doi: 10.3389/fevo.2019.00282

INTRODUCTION

By 2050, 80% of the world population is expected to live in cities (Cohen, 2003). Supplying future communities with an adequate level of natural ecosystem exposure thus represents a challenging issue (Chen and Jim, 2008; Klemm et al., 2015). In the framework of urban environmental policies, the green infrastructure, such as parks and street greeneries established in cities all around the world, are key elements that mitigate, for example, urban noise and air pollution, and improve urban biodiversity (Costanza and Folke, 1997; Bolund and Hunhammar, 1999; Thaiutsa et al., 2008; Seamans, 2013). However, urban trees are affected by considerable biotic and abiotic airborne and soil borne stress factors (Vollenweider and Günthardt-Goerg, 2005). A widespread source of stress for urban trees at mid and higher latitudes relates to snow clearing using anti-icing and de-icing salts spread on roads and sidewalks in winter. For this purpose, various deicing chemicals and abrasives, such as chloride salts, acetates, formates, urea, glycols, and even agro-based deicers, are being routinely used (Fay and Shi, 2012). Among these latter compounds, NaCl is certainly one of those salts most frequently spread in streets, because of its inexpensiveness and deicing efficiency (Berkheimer and Hanson, 2006; Fay and Shi, 2012). Therefore, compared to non-urbanized zones, as a consequence of snowmelt, runoff, infiltration, and plowing, disproportionally high amounts of NaCl end up in the urban soils close to roads, and sidewalks (Blomqvist and Johansson, 1999; Bryson and Barker, 2002; Czerniawska-Kusza et al., 2004; Legros, 2007). Moreover, some 20–63% of deicing road salt can spread tens of meters away from pavement via air transport (Blomqvist and Johansson, 1999). Therefore, de-icing salts spread in streets can have far-reaching consequences for the urban ecosystems.

Salt spreading on streets has been shown to affect the soil physical and chemical properties. In the soil, excess Na^+ can bind to the negatively-charged aggregates of mineral and organic constituents of soils (the clay-humus complex), and in turn dislodge important plant nutrients such as Zn, K, Cu, Mn, or Ca ions, ultimately affecting soil aggregation (Legros, 2007). Compared to Na^+, Cl^- seems to interfere less with the soil chemistry (Dmuchowski et al., 2014). Hence, several studies demonstrate the link between Cl^- mobility and contamination of groundwater (Ramakrishna and Viraraghavan, 2005) and document the toxicity of this anion for the vegetation (Paludan-Müller et al., 2002; Cekstere et al., 2008; Marosz and Nowak, 2008; Kwasowski and Czyz, 2010; Cekstere and Osvalde, 2013). In other words, salt accumulation in soils generally results in plants to uptake higher doses of Cl^-, which has been directly correlated with increased leaf injury marks (Paludan-Müller et al., 2002) and a general decrease in tree vitality (Paludan-Müller et al., 2002; Dmuchowski et al., 2014).

Plant exposure to excessive salt contamination has short- as well as long-term effects. Physiological reactions to salt exposure include a reduction of shoot growth, as a possible consequence of root water shortage, and of leaf photosynthetic activity, in relation to decreased chlorophyll concentrations (Ashraf and Harris, 2004; Sudhir and Murthy, 2004). These effects can be mediated by a disturbed osmotic homeostasis, promoting "physiological drought," as a consequence of salt accumulation (Bernstein and Hayward, 1957; Dobson, 1991; Cekstere et al., 2015). In other words, salt-stressed plants modify their hormonal signaling, such as by reducing root-to-shoot transport of kinetin, and increasing abscisic acid (ABA) production, which results in plant displaying symptoms similar to drought (Davies et al., 1986; Bano et al., 1993; Pospíšilová et al., 2000); such as enhanced production of smaller, darker, thicker leaves, reduced stomatal density (Bernstein, 1975; Abrams, 1994; Shannon, 1997), or higher root:shoot ratio (Künnemann and Gad, 1997; Munns, 2002). Salt injury is first observed at the leaf tip in the form of discoloration and necrosis. It then extends to the whole leaf margin, with leaf yellowing reaching the main veins, prior to leaf abscission (Bernstein, 1975). These symptoms are also indicative of an acceleration of cell senescence (Inada et al., 1998; Sudhir and Murthy, 2004; Gunthardt-Goerg and Vollenweider, 2007; Negrão et al., 2017). By chronic salt exposure, more severe injury, extending to the tree crown and including twig dieback, complete defoliation and even tree death in extreme cases, has been reported (Shannon, 1997; Cekstere and Osvalde, 2013).

The effects of injuries, anatomical adjustments, and eco-physiological responses caused by increased salt stress in plants can cascade up to affect the plants' associated arthropod community. Theory suggests that the diversity and abundance of arthropods' communities should reflect, from the bottom-up, the diversity and abundance of herbivores (Hunter and Price, 1992). In this regard, the plant stress hypothesis (White, 1974, 1984) predicts that palatability of plants to herbivores will increase with abiotic stress, whereas the plant vigor hypothesis (Price, 1991) predicts instead that more vigorous plants—those that are least stressed—will be the most palatable. Both theories agree, however, that variation in abiotic conditions is likely to modify plant traits so as to produce variation in plant palatability. Reviews on the topics have outlined high levels of specificity of plant-arthropod guild interactions with regard to the foliage-mediated effects of salt stress (Koricheva et al., 1998; Huberty and Denno, 2004). Specifically, Larsson (1989), based on experimental evidence, predicted that phloem and cambium feeders may respond more positively to plant stress than herbivore or gall-forming insects. Along these lines, some leaf miners were observed to successfully complete their development on salt-stressed plants, despite a reduction of leaf feeding (Martel, 1998). However, while several studies partially agree with these findings, other found non-significant effects (Baczewska et al., 2011; Muñoz et al., 2014), or even documented decreased aphid population growth in response to higher Cl^- accumulation (e.g., Araya et al., 1991; Görür, 2006; Sienkiewicz-Paderewska et al., 2017). Hence, high salt content can injure leaves to a level that is actually toxic or inhibiting to the herbivores, and this should cascade up to affect the entire arthropod community living in the canopies.

The main objective in this study was to investigate the effects of soil salt pollution on foliage functional traits of lime trees, and relate them to the plant herbivore and arthropod diversity on lime tree canopies. Specifically, we had the following working hypotheses: (1) soil pollution with deicing salt causes physiological and structural injury to trees' foliage. (2) On salt-contaminated soils, salt accumulation in foliage of lime trees alters the abundance and diversity of arthropods established in the tree canopy, and (3) salt accumulation modifies the palatability to herbivores by changing physico-chemical properties of leaves.

MATERIALS AND METHODS

Site Selection and Conditions

The study was conducted in the city of Riga, Latvia (56.9489°N, 24.1064°E), located within the boreo-nemoral climate zone (Kottek et al., 2006). During the 1997–2016 reference period, mean precipitation amounted to 865 mm annually, the temperatures averaged +18.7○ C in summer (JJA) and −1.7○ C in winter (DJF) with freezing temperatures during 90 days (source: Latvian Environment, Geology, and Meteorology Center, state limited liability). As a consequence, large quantities of NaCl, amounting to 4.06 kg m^{-2} yearly, have been applied every winter for deicing the street pavement (Cekstere et al., 2008). With 39% of Riga's streets lined by lime trees (or linden; *Tilia* x *vulgaris* H.), this ornamental tree is likely the most commonly planted species in street greeneries. Past surveys have highlighted that a majority of trees show salt stress symptoms, which a large fraction needs then to be replaced due to high mortality rates (Cekstere et al., 2008).

Within a larger framework project on structural effects of deicing salt (Sciex-NMSch grant), 7 sites in Riga's downtown and one supplementary site in a National Botanical Garden (NBG) 20 km southeast of city center—and thus protected from salt contamination and urban air pollution—had been selected in September 2014 (**Table S1**). Altogether, these eight sites displayed a range of soil contamination representative of Riga's downtown (Cekstere et al., 2008; Cekstere and Osvalde, 2013). In the current study, these eight sites were also used to study foliar injury by aphids and relate it to NaCl contamination in foliage (see below). Out of the seven sites of Riga's downtown, we selected a subset of four, for characterizing changes in the physico-chemical properties of soil horizons and arthropod communities of tree canopy in response to salt contamination (**Table S1**, **Figure S1**). At each site, we selected a salt-polluted plot (P) and an uncontaminated plots (U), further away from salt pollution, but otherwise generally similar to the polluted plots close to the pavement. Thus, each of the U plots provided paired controls, being located in a tree alignment close (20–30 m) to the P plot but 5–50 m away from the street and thus preserved from contamination by salt sludge splashing and melting heaps of salt-contaminated snow.

Soil and Foliage Analyses From Polluted and Unpolluted Soils

The soil properties at each P and U plot were characterized by means of one 100 cm deep soil pit excavated in September 2015. The soil profile was described according to the "World reference base for soil resources" (IUSS Working Group WRB, 2015). For each horizon, the texture was assessed directly in the field and about 1 kg of soil was sampled for further physico-chemical analyses. In the laboratory, the soil samples were homogenized, dried at 40°C for 48 h, sieved at 2 mm, and ground using agate mortars for subsequent chemical analyses (see soil analyses, methods and results in **Figure S2**).

The levels of salt accumulation in the foliage of lime trees were characterized on the basis of 2014 data collection from the framework project, and completed in the case of P and U plots ($n = 3$ trees per plot) with supplementary assessments in 2015. At each plot, three trees with similar crown condition were randomly selected. One unshaded branch, about 50 cm long, in the lower part of crown canopy was pole-pruned at 3–5 m high within each tree on September 16, 2014 and July 20, 2015. The collected leaf samples were washed in distilled water, dried at 60°C and milled to powder using a laboratory mill (IKA, A11 basic, Germany). Leaf samples were then mineralized by dry-ashing, using concentrated HNO_3 vapors and the mineral fraction dissolved using either 3% HCl (Na^+) or distilled water (Cl^-) and analyzed by flame photometry (Na^+) and $AgNO_3$ titration (Cl^-) (Rinkis et al., 1987). Along with the leaf sampling of July 2015, three soil core samples (5 cm diameter, 20 cm deep) per site (24 samples in total) were also collected for soil Na^+/Cl^- concentration measurements. The concentration of Na^+/Cl^- in the soil samples was determined after 1M HCl/distilled water extraction, followed by flame photometry/$AgNO_3$ titration (Cekstere and Osvalde, 2013). See results for NaCl concentrations in leaves and soil in **Table S2**, and **Figure S1**).

De-icing Salt Impact on Arthropods' Biodiversity in the Street Trees' Canopies

At both paired plots within each of the four sites from the downtown subset, the biodiversity of arthropods in the canopy of each tree ($n = 3$ trees per plot) was assessed by means of passive trapping in July 2015. The custom-made passive traps consisted of a disposable yellow-painted aluminum chafing dish, (18 × 13 and 6 cm deep) filled with 500 ml of a salty solution (10 g/l NaCl and a few drops of liquid soap). Traps were left hanging for 48 h at 3–4 m high in the lower canopy. All trapped arthropods were stored in 70% ethanol prior to counting and classification at the "morpho-species" level. Because all P and U sites were located in the same micro-climatic conditions of open spaces with same sun exposure, and with no high building or other manmade barriers as interference, this approach specifically aimed at comparing P and U plots across sites. However, we did not aim at producing a comprehensive characterization of the lime tree-associated arthropod biodiversity in Riga, which would have required long term monitoring aver the entire season of growth (Basset et al., 1996).

We first estimated the effect of salt contamination on total abundance of arthropods trapped, the abundance of the most frequently trapped arthropod, the lime aphids (*Eucallipterus tiliae*, Homoptera, Aphididae), and the arthropod Shannon diversity [calculated with the *diversity* function of R *vegan* package (Oksanen et al., 2013)] using mixed-effect models [pollution as fixed factor and site as random factor, *lmer* function in the package lme4 (Bates et al., 2015)]. Second, we estimated the effect of salt contamination (P vs. U) on the amount of arthropods and species composition by means of permutational multivariate ANOVA (PERMANOVA). We used the *adonis* function in the *vegan* package for calculating dissimilarity indices between samples based on Euclidean distances, although results were robust to other distance metrics such as Bray-Curtis. Sites were included as strata in the model. Contribution of the different arthropod species to the groups' (P vs. U) separation was analyzed using Regularized Discriminant Analysis (RDA).

Structural Injury in *T. x vulgaris* Foliage by Arthropods

Structural injury was assessed using foliar samples excised from the 2nd or 3rd leaf from twig apex, within the lower canopy branch harvested at the eight Riga and NBG sites on September 16, 2014 (framework project). Two 10 mm in diameter disks from one leaf per tree (3 trees per site, 48 samples in total) were excised from the median and still asymptomatic part of leaf blade and immediately fixed by immersion in either LM- or EM-grade glutaraldehyde buffered at pH 7.0 using 0.067 M Soerensen phosphate buffer. The disks were then stored at 4°C in renewed fixing solution, waiting for further processing. For descriptive and quantitative histological assessments using light microscopy (LM), one leaf disk per tree was dehydrated with 2-methoxyethanol (3 changes), ethanol, n-propanol, n-butanol (Feder and O'brien, 1968), and embedded in Technovit 7,100 (Kulzer HistoTechnik) resin. At least 10, 1.5 μm thick, semi-thin sections were then cut using a Reichert UltraCut S ultramicrotome and stained in 1% acid fuchsine and 0.05% toluidine blue in acetate buffer pH 4.4 (Feder and O'brien, 1968), prior to mounting in DPX. Observations were performed using the 5×-100× objectives of a Leica microscope Leitz DMRB, and micrographs taken using the INFINITY 2-1R camera and Lumenera Infinity Analyze (release 6.4) software (Lumenera Corp., Ottawa, Canada). For analyzing cellular and subcellular changes on transmitted electron microscopy (TEM), the other 24 leaf disks samples were post-fixed in buffered 2% OsO_4, dehydrated by a series of graded ethanol, infiltrated by a series of graded propylene oxide/Epon 812 mixture (with DDSA, NMA, and DMP hardener) and embedded in Epon. Ultra-thin sections (70 nm) were cut using the aforementioned ultramicrotome, mounted on copper grids and stained using uranyl acetate 5% solution in water and lead citrate 2% solution in water (Reynolds procedure). Sections were observed using a Philips CM12 transmission electron microscope (TEM) and micrographs taken using the Gatan Microscopy Suite Software (Gatan Inc., Pleasanton, USA).

Biotic injury and the associated microscopic changes were diagnosed according to characteristic visible and microscopic symptoms (Fink, 1999; Vollenweider and Günthardt-Goerg, 2006; Gunthardt-Goerg and Vollenweider, 2007) and past experience (Cekstere et al., 2016; Vollenweider et al., 2016). Specifically, we quantified several structural markers related to aphid injury, including: percentage of the epidermis length filled with mucilage; frequency of the mucilage spills in the intercellular space; percentage length of necrosis in lower epidermis. After correcting biases to normality (using LOG for Muc_le and SQRT for the rest) in descriptors according to Legendre (1998), we plotted the variables using Principal Component Analysis (PCA, based on a correlation matrix). We next extracted the magnitude of the loadings for each variable and tested the correlation between the variables of the first three most important loadings (Cl^-, Muc_uE, and Muc_lE) and the first axis of the PCA using multivariate mixed effect linear models by including sites as random factor [function *lmer* function in the package lme4 (Bates et al., 2015)]. By including site as random factor, we could account for potential non-independence between the three trees at each site.

Effect of Experimental Salt Exposure on Tilia *x* Vulgaris Foliage and Resistance

To measure the effect of salt contamination on the development of lime tree aphids under controlled conditions, we performed a common garden experiment using 6 year-old seedlings of *Tilia x vulgaris*. After planting (spring of 2014) in 10 L plastic pot filled with commercial neutralized peat substrate (KKS-M2, Laflora Ltd., Latvia; pH 5.0 ± 0.3; nutrients: N 80, P 75, K 200, Ca 3,000, Mg 360, S 40, Fe 80, Mn 5.0, Zn 2.20, Cu 2.00, Mo 0.25, B 0.55 mg/L; electric conductivity EC of 1.60 mS/cm), the tree seedlings were left to acclimate in an open greenhouse of the Institute of Biology (UL) in Salaspils, Latvia (56°51'45.5"N 24°21'37.3"E). The plants were kept watered to field capacity (60–70% substrate moisture) and fertilized, according to assessments of substrate and leaf concentration of nutrients, with the addition of macro- and micronutrients being repeated thrice over the vegetation season. On July 1, 2015, a controlled salt exposure experiment with 5 treatments [$n = 8$ tree replicates per treatment; treatment levels: 0, 10, 20, 30, and 40 mM NaCl, selected based on our previous studies in the street greenery of Riga (Cekstere and Osvalde, 2013)] was started. Over 4 days, NaCl solutions (10 mM NaCl/L) were added to each pot (except the control) to gradually reach the target concentrations of the treatment according to scheme: trees in the 10 mM treatment received 10 mM NaCl solution on day 1 and plain water thereafter; trees in the 20 mM treatment—10 mM NaCl solution on days 1 and 2 and plain water thereafter; trees in the 30 mM treatment—10 mM NaCl solution on days 1, 2, and 3 and plain water thereafter; trees in the 40 mM treatment—10 mM NaCl solution on days 1, 2, 3, and 4. The gradual increment was used to avoid osmotic shock on plants. The treatments were randomized according to a pseudo-latin square design.

The effect of salt contamination on the chlorophyll fluorescence and concentration within seedling foliage was assessed in August 2015. For this, we used two tagged leaves from the first flush per plant. Measurements were performed ten times per leaf, and averaged to a single value per tree. The potential maximum quantum yield PSII (Fv/Fm) and performance index (Pindex) were determined using a HANSATECH Handy-PEA device (Hansatech Instruments Ltd., Pentney, United Kingdom). The chlorophyll content was measured using a chlorophyll meter SPAD-502 device (Konica Minolta, Chiyoda, Tokyo, Japan). The additional leaf functional traits (leaf dry weight, DW; leaf water content, WC; leaf area, LA; specific LA, SLA;) were measured using 8 leaves from first flush per seedling harvested on September 14–16, 2015, and averaged to a single value per tree. The LA estimates were obtained after scanning the leaves at 600 dpi (3,000 × 300 pixels images), using the transmitted light mode of a 2014 EPSON Perfection Scanner and the version 1.2 of Pixstat software (https://www.wsl.ch/en/services-and-products/software-websites-and-apps/pixstat.html). SLA was computed by dividing the LA by DW of each leaf. Finally, we measured the seedling trunk diameter at stem base on September 20, 2015, using a caliper. Because, we used similar size plants at the beginning of the experiment, differences in trunk diameter at the end of the experiment were meant to reflect NaCl treatment effect only. The effect of NaCl treatments (5 levels) on plants functional traits was analyzed using univariate ANOVAs, and differences among levels were assessed using Tukey's HSD *post-hoc* tests.

The effect of salt treatments from the common garden experiment on aphid development was tested by means of a no-choice cafeteria bioassay realized in July 2016. Prior to the bioassay, salt addition was repeated on May 6–9, using the same procedure as in 2015. One fully-expanded healthy leaf per plant with its petiole base wrapped in wet cotton cloth was placed in a Petri dish lined with humid filter paper and moved into a climate chamber (15–18°C, 55% Rh, and 16 h light). Two alate aphid females were immediately introduced in each Petri dish and the number of parthenogenetic offsprings on each leaf was counted after 5 days. Because we could not reach normality in the residual distribution, to test the effect of salt contamination on final aphid offsprings, we fitted an ANOVA using permutation tests [*aovp* function in the package lmPerm in R (Wheeler, 2010)]. Differences among salt levels were assessed using TukeyHSD *post-hoc* tests.

RESULTS

Soil and Foliage Contamination by Deicing Salts in Riga

Deriving from Baltic Ice Lake sandy deposits, the analyzed soils in Riga's downtown showed a primarily sandy texture (>84% sand), with the mean content of organic matter in the topsoil not exceeding 6.5%. As a consequence, the soil structure was generally weak or absent. The soil horizons contained a high proportion of manmade technogenic artifacts (10–40%), typical for anthroposoils (IUSS Working Group WRB, 2015). In general, the soil pH of all the P and U plots was neutral or slightly alkaline with pH_{H2O} ranging from 7.2 to 8.5 and pH_{KCl} from 6.8 to 7.1 (**Figure S2**). All polluted (P) plots had higher Na^+ and Cl^- concentrations than unpolluted (U) plots, from the topsoil and down till 60 cm deep. Average Na/Cl concentrations in the topsoil ranged between 97–455/15–74 mg/kg for P plots, and 11–92/10–46 mg/kg for U plots (**Table S2**). In sum, more than 5.5 times and 2.5 times higher Na and Cl concentrations, respectively were thus measured in the polluted soils compared to unpolluted soils (Cekstere and Osvalde, 2013). This resulted in significant increase in the soil electrical conductivity in polluted soils (max value of 1.09 mS/cm).

Within foliage (2014 assessments), the Na^+ and Cl^- concentrations at the seven urban sites ranged between 960–9,400 and 2,375–9,000, respectively, compared to 68–151 and 600–2,050 ppm, respectively, for the (unpolluted) NGB site. Na^+ and Cl^- concentrations were thus 37.7 and 5.4 times higher, respectively, in the polluted compared to unpolluted environments. Compared to soil concentration and considering the four plots only (2015 assessments), the foliage concentration of Na^+ and Cl^- (3,970 ± 1,954 and 5,281 ± 1,396 ppm, respectively) were 13.3 and 91.8 times larger than those measured in the topsoil (**Table S2**).

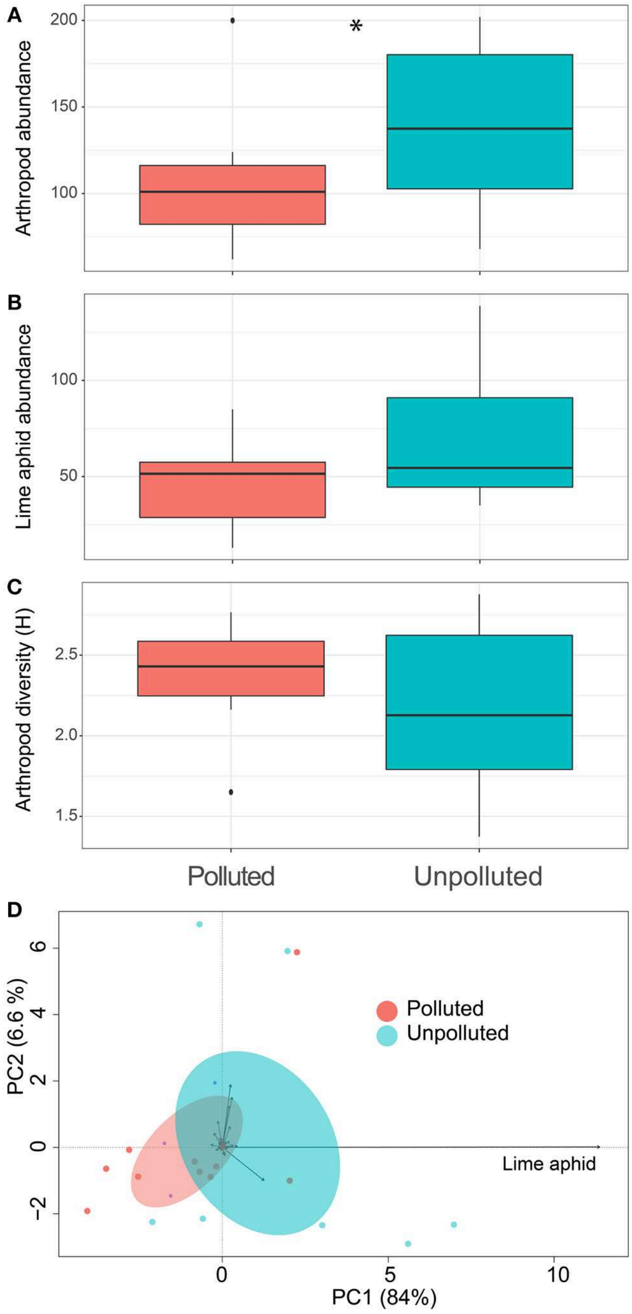


FIGURE 1 | Effect of salt pollution on lime trees associated arthropod diversity. Boxplots show the effect of soil pollution on **(A)** total arthropod abundance, **(B)** lime aphid abundance, and **(C)** arthropod Shannon diversity. Asterisks show significant differences among pollution sites (Chi-squared test, $p < 0.05$). Panel **(D)** shows the principal component arthropod's ordination of the different sites. Blue is unpolluted soil, while red indicates highly polluted soils. Ellipses show 95% confidence intervals around the pollution groups.

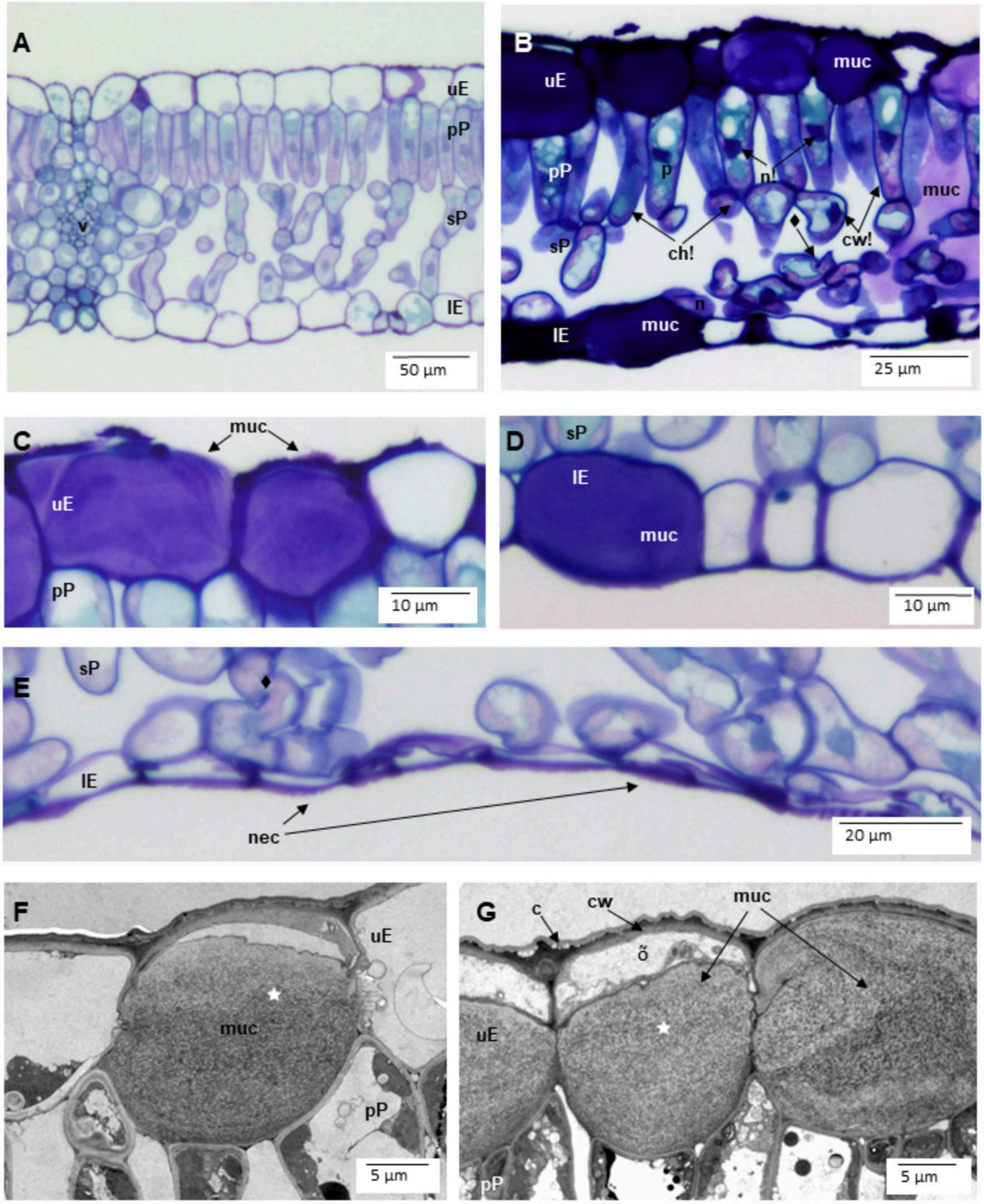


FIGURE 2 | Symptoms of biotic injury, primarily by aphids, in leaves of *Tilia x vulgaris* trees from the street greenery of Riga and National Botanic Garden (NBG) in Latvia. **(A)** nearly asymptomatic leaf tissues. **(B)** in the leaf blade, the severely affected leaf parts showed a higher frequency of mucilages (muc) within upper (uE), and lower (lE) epidermis as well as mucilage spills in the intercellular space of palisade (pP) and spongy (sP) parenchyma. Mesophyll cells showed cell wall thickening (cw!), nucleus (n!), and chloroplast (ch!) condensation as well as increased amounts of vacuolar phenolics (p). Some cells were partially cytorrhised (♦). **(C,D)** mucilage structure in upper **(C)** and lower **(D)** epidermis (lE). **(E)** cytorrhisis indicative of necrosis (nec) in lower epidermis. **(F,G)** mucilage within epidermal cells showed a mostly granular structure, with banding patterns (★). Cell death was evidenced by disrupted protoplasm remnants (ö). Other abbreviations: c cuticula. Na, Cl concentration: 2,660, 5,000 ppm **(A)**, 124, 5,000 ppm **(B)**, 72, 530 ppm **(C,D)**, 8,800, 6,896 ppm **(E)**, 4,180, 11,500 **(F)**, 5200, 10750 **(G)**. Technical specifications: **(A–E)**: 1.5 µm semi-thin cuttings stained with toluidine blue and acid fuchsine and observed in diascopic light microscopy; **(F,G)**: 70 nm ultra-thin sections stained with uranyl acetate and lead citrate and observed in transmission electron microscopy.

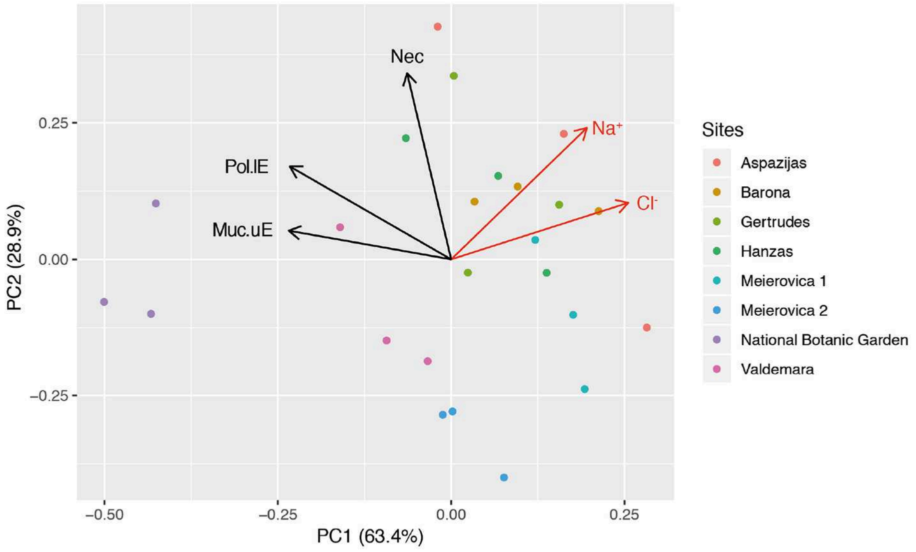


FIGURE 3 | Unconstrained ordination (PCA) of structural parameters indicative of biotic injury (Muc_lE, Muc_uE, Nec_lE) in the plane formed by the first and second principal components. Na/Cl contamination within lime tree foliage at the research sites: (i) uncontaminated, below 98/940 ppm, NBG; (ii) moderate, contamination range 118–3,100/3,000–7,570 ppm, Valdemara, Meierovica 2; (iii) severe, contamination range 2,660–6,600/5,000–11,500 ppm, Barona, Meierovica 1; (iv) very high, contamination range 5,400–13,600/5,250–16,750 ppm, Gertrudes, Hanzas, Aspazijas. Abbreviations for the descriptor variables: Muc_lE/uE percentage length of lower/upper epidermis filled with mucilages, Nec_lE percentage length of lower epidermis showing necrosis, *n* = 3 tress per site.

De-icing Salt Impact on Arthropods' Biodiversity in the Street Tree Canopy

Using passive traps we collected about 2,450 specimens that we could divide into 212 morphospecies, consisting of 8 mites (Acaria), 5 spiders (Aranea), 11 beetles (Coleoptera), 73 flies (Diptera), 81 wasps and bees (Hymenoptera), 8 butterflies (Lepidptera), 2 neuropteran, 4 barklice (Psocoptera), 4 thrips (Thysanoptera), and 2 caddisflies (Trichoptera) species were observed in lime trees in the summer of 2015. Overall, we found 36% more arthropod individuals at the U vs. P plots (**Figure 1A**, pollution effect; Chisq = 3.65, $p = 0.05$). With 47% of all individuals on average, the lime aphid *E. tiliae* (Homoptera, Aphididae) formed the arthropod species most frequently observed in the traps. In a statistically non-significant trend, *E. tiliae* also tended to be 1.5 times more abundant in U plots than P plots (**Figure 1B**, pollution effect; Chisq = 3.15, $p = 0.07$). The arthropod diversity, as measured by Shannon index, remained similar across pollution plots (**Figure 1C**, pollution effect; Chisq = 0.03, $p = 0.84$). However, changes in the arthropod communities, considering their abundance together with their diversity, significantly responded to site contamination [**Figure 1D**, PERMANOVA, pollution effect; $F_{(1,19)} = 1.55$, $p = 0.02$]. The strongest explanatory variable in the RDA was the lime aphid with 11.4% of the total contribution to the first axis of the ordination (**Figure 1D**).

Structural Injury in *T. x vulgaris* Foliage by Arthropods

The leaves sampled in Riga and at NBG were generally sticky and shiny because of aphid honeydew and showed a rather leathery texture (field observations). Disk cross-sections showed many injuries at tissue and cell level (**Figure 2**). Typical of biotic injury, their distribution and severity were very variable, even within a single leaf section (about 5 mm long). In the leaf blade, they included an increased frequency of mucilage filling in the upper (**Figures 2B,C,F,G** vs. **2A**) and lower (**Figures 2B,D** vs. **2A**) epidermis. This compound identification was confirmed by (1) cell wall-like hues after toluidine blue staining (**Figures 2B–D**), (2) accumulation in periplasm (**Figures 2F,G**), or (3) granular ultrastructure and banding patterns. On average, 39.9 ± 1.8% (range: 27.3–59.1%) and 7.2 ± 1.1% (range: 1.3–23.7%) of upper and lower epidermis segments in leaf cross-sections were filled with mucilage. The frequency of intercellular mucilage in mesophyll amounted to 1.7 ± 0.3 spills mm^{-1}. The PCA analysis showed that Cl (29.3), Muc_uE (27.4), and Muc_lE (23.3) were mostly contributing to the first axis of the ordination (**Figure 3**). Subsequent multivariate analysis identified a significant positive correlation between foliar salt contamination (Cl^-) and first axis of the PCA [$t_{(13)} = 51.27$, $p < 0.001$], and a negative correlation between Muc_lE and the first axis of the PCA [$t_{(13)} = 2.26$, $p = 0.04$]. In other words, the frequency of mucilage-filled cells is higher in leaves growing in unpolluted sites, where herbivory is likely higher (e.g., National Botanical Garden, **Figure 3**).

Responses in Foliage of *T. x vulgaris* to Experimental Salt Exposure

The salt treatments affected the leaf physiology as well as morphology of salt-exposed seedlings (**Figure 4**). As indicated by the monotonic reduction of chlorophyll concentration [**Figure 4A**; $F_{(4,32)} = 4.02$, $p = 0.01$] and fluorescence

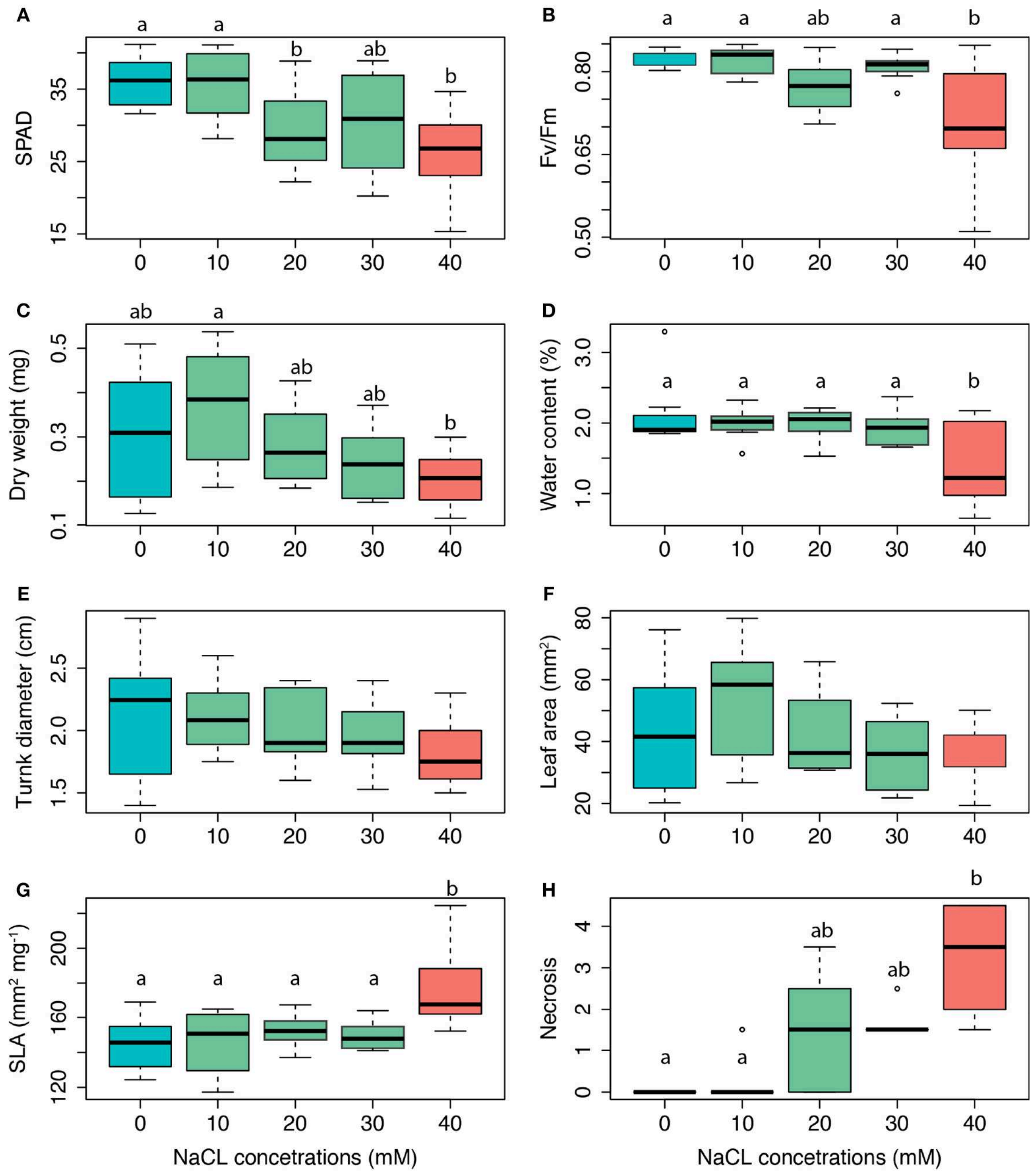


FIGURE 4 | Changes in functional traits of *Tilia x vulgaris* seedlings in response to four salt addition treatments (0–40 mM NaCl). **(A)** chlorophyll content (SPAD units), **(B)** Fv/Fm potential maximum quantum yield of PSII, **(C)** DW leaf dry weight (mg), **(D)** WC water content (%), **(E)** trunk diameter at the base (cm); **(F)** LA leaf area (mm^2), **(G)** SLA specific leaf area (mm^2 mg^{-1}), **(H)** leaf injury (necrosis %). Different letters indicate significant difference among the salt treatments (Tukey's HSD *post-hoc* test, $p < 0.05$).

[**Figure 4B**; $F_{(4,32)} = 4.90$, $p = 0.003$], both the chloroplast structure and functioning were impaired with increasing salt concentration. Significant correlation between the two latter variables (**Figure S3**) suggested a possible causal link. We found no effect of salt treatment on plant biomass [**Figure 4C**; $F_{(4,32)} = 2.36$, $p = 0.07$], but found that plants under high salt stress had almost half the amounts of water in their tissues than control plants [**Figure 4D**; $F_{(4,32)} = 3.12$, $p = 0.03$]. We also found no effect of salt stress on the diameter of the trunk, nor the leaf area (LA) (**Figures 4E,F**, $p > 0.05$, respectively). The most striking morphological change in response to salt exposure thus consisted in the monotonic increase of SLA [$F_{(4,32)} = 14.78$, $p < 0.001$; **Figure 4G**]. As a likely consequence of increasingly larger necrotic leaf rims [$F_{(4,32)} = 4.64$, $p = 0.004$; **Figure 4H**], the WC was decreased up to two third [$F_{(4,32)} = 3.12$, $p = 0.03$; **Figure 4D**], nevertheless not monotonically.

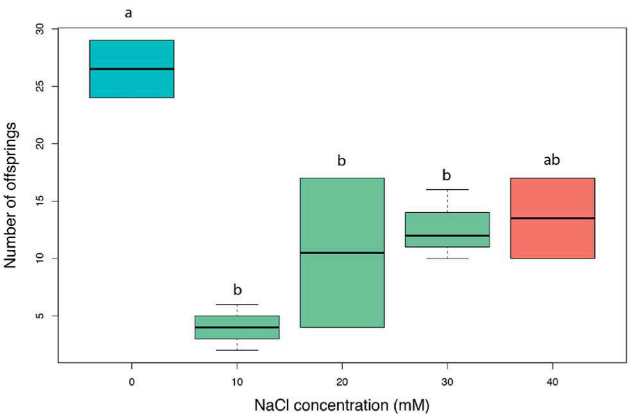


FIGURE 5 | Amount of lime aphid (*Eucallipterus tiliae*) offsprings on single detached leaves of *Tilia x vulgaris* leaves from seedlings exposed to 0–40 mM NaCl, after 5 days of colony development.

Development of Aphid Colonies as a Function of Salt Exposure

The exposure of lime tree seedlings to salt significantly reduced the development of aphid colonies [$F_{(4,7)} = 7.3$, nb of iterations $= 5{,}000$, $p = 0.01$; **Figure 5**]. After 5 days of development, the aphid offspring on salt-exposed vs. control leaves was 2.6 times lower. However, this offspring reduction was not proportional, in which all salt-addition treatments resulted in no variation in aphid development.

DISCUSSION

With this study we highlighted a cascade of events that starts with the application of de-icing salt to the streets of Riga in winter, followed by salt pollution in urban soils, and in the subsequent accumulation foliage of trees on the roadsides. Salt accumulation in trees modifies leaf-ecopyhsiological parameters to largely mimic drought and general stress injuries. Ultimately, this results in trees from salt-polluted habitats bearing lower arthropod numbers, including specialized herbivores (**Figure 6**). Below we discuss each step along the way.

Effect of Salt Pollution on Urban Soils

We found no effect of soil pollution on the soil physical structure. This result is likely driven by a general lack of soil structure in such sandy soils. The missing salt disruptive effect on soil structure therefore means that soil aggregation, porosity, and the soil moisture availability were also not affected by salt pollution; something that was shown in more structured urban soils of other cities (Tedeschi and Dell'Aquila, 2005; Legros, 2007; Rengasamy, 2010). Clearly, the most important consequence of salt pollution was the increase up to 10-folds in Na^+ and Cl^- soil concentrations, a recurrent observation for similar sampling along urban street greeneries (Czerniawska-Kusza et al., 2004; Cekstere et al., 2008; Fay and Shi, 2012; Dmuchowski et al., 2014). Given soil sampling in the fall (at the beginning of September) however, the concentration of salt contaminants during foliage development in spring was certainly underestimated, as a consequence of tree uptake and leaching (Cekstere et al., 2008). The highest concentration for Na^+ cations within the topsoil (A horizon) vs. deeper subsoil (C and Z horizon) for Cl^- anions was indicative of the fact that Na^+ ions are retained by the negatively charged argilo-humic soil complexes, whereas the free Cl^- ions remain in the soil solution (Cekstere et al., 2008; Fay and Shi, 2012). Since Na^+ and Cl^- concentrations were correlated to pH and EC, respectively, these parameters could show the same pattern of variation across the soil profile. In sum, salt contaminants spread on roadsides effectively infiltrate the surrounding urban soils, in turn modifying the soil physicochemical properties. The roadside plants collect these contaminants with their root systems and accumulate them in foliage during the subsequent growth season.

Effect of Salt Pollution on Plant Eco-Physiological Traits

On the experimentally salt-exposed seedlings, increasing leaf injury with increasing salt addition led to symptoms of leaves being morphologically similar to symptoms of leaves observed in the field, which confirmed the causal role and negative impact of salt accumulation on street-lining lime trees, as classically described by Bernstein (1975). In this instance, the *T. x vulgaris* species studied appears to be more salt-sensitive than other tree species commonly used in street greeneries (Paludan-Müller et al., 2002; Marosz and Nowak, 2008; Kwasowski and Czyz, 2010; Dmuchowski et al., 2013). For example, *Populus talassica* and *P. euphratica* showed salt symptoms after 8 days of exposure to 206 mM and 354 mM NaCl, respectively (Chen et al., 2001), vs. 7 days to 40 mM NaCl in the case of our *T. x vulgaris* common garden seedlings. Initial injury was observed for leaf dry matter content of Cl^- exceeding 0.4% (10 mM treatment), just above the normal level of Cl^- in Tilia leaves −0.3%—reported by Dmuchowski et al. (2014). Interestingly, such Cl^- leaf dry matter contents was sizably lower than that one previously observed causing leaf injury in foliage of *Tilia x vulgaris* from Riga's street greeneries (0.66% Cekstere et al., 2008). The main difference between these two studies is that our seedlings were exposed to NaCl after full foliage development. Lower sensitivity of street foliage may then relate to morphological and physiological adjustments during, for instance, leaf differentiation, thus conferring better stress tolerance (Bernstein, 1975; Abrams, 1994; Künnemann and Gad, 1997; Shannon, 1997; Munns, 2002). The negative impact of salt accumulation on leaf functional traits, primarily the photosynthetic apparatus, was in agreement with findings in other studies (Brugnoli and Lauteri, 1991; Maxwell and Johnson, 2000; Parida and Das, 2005; Volpe et al., 2011; Negrão et al., 2017). With chronic salt exposure and besides phenological adjustments, stress-related changes in morpho-anatomical properties, mostly missing in the case of late exposure of seedlings in the common garden experiment, may thus be expected in street greeneries.

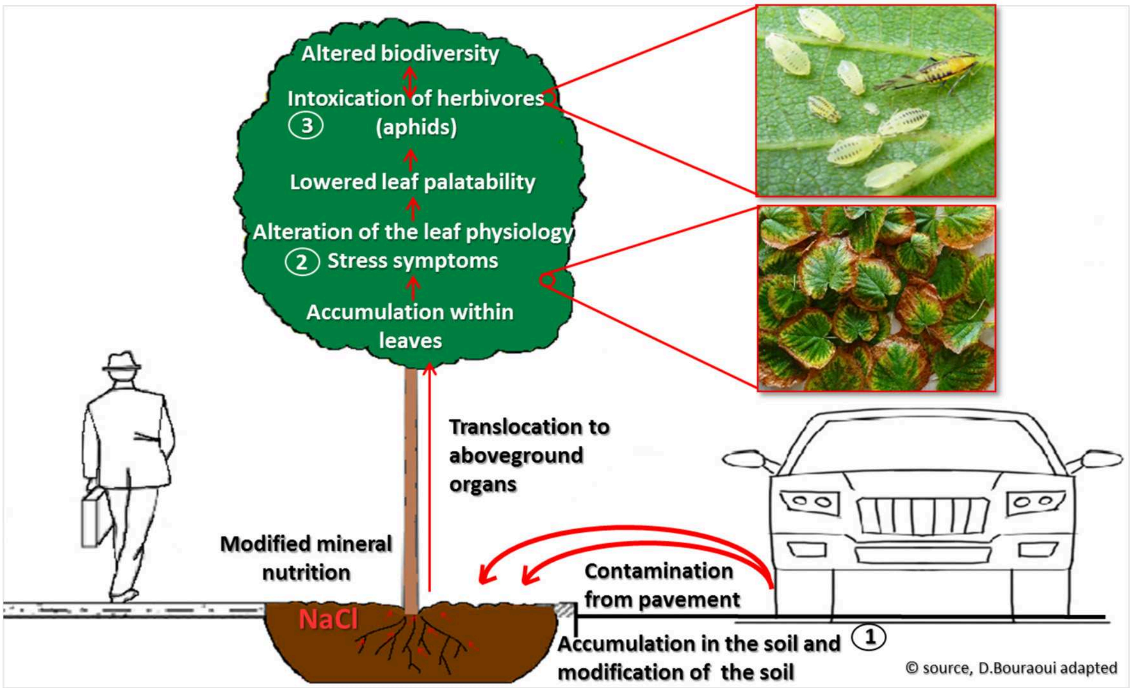


FIGURE 6 | Cascading effects de-icing salts on street greenery and biodiversity, from soil infiltration and contamination (1), to foliage injury (2), and to altered diversity of arthropods communities in the canopy (3).

Effect of Salt Pollution on Canopy Arthropod Community

We found that trees growing on salt-polluted plots sustained lower overall arthropod abundance, but not lower diversity (Shannon diversity index). In comparing the otherwise highly similar unpolluted and polluted plots, leaves from salt-stressed plant contained lower amounts of chlorophyll, and therefore, likely, lower amount of N (Wright et al., 2004). Moreover, the reduction in water content of leaves was also shown to interfere with the herbivores 'capacity to access drought-enhanced foliar nitrogen (Koricheva et al., 1998). Therefore, reduced arthropod abundance in salt-stressed plants could be partially explained by a reduction in N and water availability in the leaves, ultimately resulting in lower arthropod abundance overall. This assumption thus aligns with the plant vigor hypothesis (Price, 1991), and not with mere changes in microhabitat between U and P plots. The fact that the tree physiology, and not a potentially slightly warmer microclimate near the streets of the polluted trees drove our results, is supported by the fact that arthropods tend to gather in warmer microhabitats (Hodkinson, 2005). The warming effect would have resulted in more arthropods near the streets, and not the opposite as we observed. Finally, the incongruent relationship between insect abundance and diversity results were in turn somewhat surprising, since species diversity is generally positively linked to abundance (Bock et al., 2007), but not unusual for urban ecosystems (Marzluff, 2001; Shochat et al., 2006). In our case, diversity is the same across sites, or even tend to be higher in polluted sites. Since the specialist lime aphid abundance followed the expected trends, we could hypothesize that salt-polluted trees are less palatable for a more generalist arthropod community.

Structural Injury in *T. x vulgaris* Foliage by Arthropods

The microscopic analyses showed clear evidence of a reduction in aphid injury symptoms, paralleling with the decrease in aphid frequency, and with the increase of salt contamination in soils. Our findings are in good agreement with the research results conducted on *Tilia* "Euchlora" in street greenery (Sienkiewicz-Paderewska et al., 2017), where aphid's frequency was most strongly negatively affected by Cl^- content in the leaves. Interestingly, among the three quantified markers of biotic injury, the mucilage frequency in lower epidermis was the parameter best correlating with Cl^-, considered as more toxic than Na^+ (Paludan-Müller et al., 2002; Dmuchowski et al., 2014). This finding was also consistent with the development of aphid colonies on the lower leaf side, as generally observed in the field. While there is some evidence of epidermal mucilages production coincident with leaf water loss (Lyshede, 1977; Mariani et al., 1988; Günthardt-Goerg et al., 1993), we here provide a first, yet still indirect, evidence of mucilage spilling in relation to aphid injury (Fink, 1999; Gunthardt-Goerg and Vollenweider, 2007). In other words, we showed that leaf from salt-polluted plants that are less damaged by aphids produced lower amounts of mucilage. This finding thus indicates that aphid injury stimulates mucilage spilling, and should be seen as an inducible defense mechanisms against aphid feeding in plants (Fink, 1999). Mucilage accumulation can confer the injured leaves a more leathery texture and contribute to increasing the leaf mass per area, a typical herbivory deterring reaction (Westoby et al., 2002). The principal role of leaf epidermis mucilage may thus primarily relate to leaf defense and not water homeostasis; similarly to pathologically induced mucilages in other plant organs (Fink, 1999). This determinant role of pathogens vs. water homeostasis is particularly corroborated in the context of our experiment, with the frequency of epidermis mucilage being decreased whereas the "physiological drought" triggered by salt was increased (Dobson, 1991).

CONCLUSION

We showed that salt accumulation in the street greeneries of Riga caused leaf injury, and affected the arthropods associated with this urban street ecosystem. Street lime trees showed high NaCl concentrations in their foliage, with physiological and structural reactions similar to those observed in street greeneries of other affected cities. The arthropod communities living in close contact with the street greeneries also showed changes. Therefore, the advocated replacement of sensitive lime trees by more tolerant tree species needs testing regarding consequences for all ecosystem services, also including urban biodiversity. Especially in historical centers, more sensitive lime trees are likely to be maintained for cultural reasons. Comparisons of performances between lime tree species and cultivars are thus also needed. Promoting more tolerant lime trees may thus also help to maintain specific lime tree extended arthropod communities, as an integral part of the environmental value of the urban ecosystem at mid-latitudes.

DATA AVAILABILITY

This manuscript contains previously unpublished data. The name of the repository and accession number are not available.

AUTHOR CONTRIBUTIONS

DB performed the experiments, collected, and analyzed the data. GC designed the experiments and helped collecting and analyzing the data. AO supervised data collection and analysis. PV designed the experiments and supervised the data collection and analysis. SR designed the experiments, analyzed the data. DB and SR wrote the first draft of the manuscript. All authors contributed to the writing.

FUNDING

This work was supported by the Sciex-NMSch grant 14.038 to PV, Swiss National Science Foundation Grant 159869 to SR, COST action FP1204 (COST-STSM-ECOST-STSM-FP1204-010815-063102) to DB, and the Basic Research Funding (University of Latvia) for the project ZD2015/AZ81 to GC and AO.

ACKNOWLEDGMENTS

We thankfully acknowledge technical support by Terry Menard (light and electron microscopy), Patrick Schleppi (leaf morphology), Andis Karlsons (arthropod sampling, field works of soil), Guntis Tabors (field works of soil), and the Center for Microscopy and Image Analysis of the University of Zurich (transmission electron microscopy).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00282/full#supplementary-material

REFERENCES

Abrams, M. D. (1994). Genotypic and phenotypic variation as stress adaptations in temperate tree species: a review of several case studies. *Tree Physiol.* 14, 833–842. doi: 10.1093/treephys/14.7-8-9.833

Araya, F., Abarca, O., Zuñiga, G., and Corcuera, L. (1991). Effects of NaCl on glycine-betaine and on aphids in cereal seedlings. *Phytochemistry* 30, 1793–95. doi: 10.1016/0031-9422(91)85014-Q

Ashraf, M., and Harris, P. J. C. (2004). Potential biochemical indicators of salinity tolerance in plants. *Plant Sci.* 166, 3–16. doi: 10.1016/j.plantsci.2003.10.024

Baczewska, A. H., Dmuchowski, W., Gozdowski, D., Styczek, M., and Bragoszewska, P. (2011). "Influence of saline stress on the abundance of lime aphid (*Eucallipterus tiliae* L.) on the leaves of street trees-crimean linden," in *Proceedings of Ecopole,* 5, 3–19.

Bano, A., Dorffling, K., Bettin, D., and Hahn, H. (1993). Abscisic acid and cytokinins as possible root-to-shoot signals in xylem sap of rice plants in drying soil. *Funct. Plant Biol.* 20, 109–115. doi: 10.1071/PP9930109

Basset, Y., Springate, N. D., Aberlenc, H. P., and Delvare, G. (1996). "A review of methods for sampling arthropods in tree canopies," in *Canopy arthropods,* eds N.E. Stork, J. Adis and R.K. Didham (London, UK: Chapman and Hall), 35.

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H. B., and Singmann, H. (2015). lme4: linear mixed-effects models using Eigen and S4. *R Package Version* 2014, 1.1–9. doi: 10.18637/jss.v067.i01

Berkheimer, S. F., and Hanson, E. (2006). Deicing salts reduce cold hardiness and increase flower bud mortality of highbush blueberry. *J. Am. Soc. Horticult. Sci.* 131, 11–16. doi: 10.21273/JASHS.131.1.11

Bernstein, L. (1975). Effects of salinity and sodicity on plant growth. *Annu. Rev. Phytopathol.* 13, 295–312. doi: 10.1146/annurev.py.13.090175.001455

Bernstein, L., and Hayward, H. E. (1957). Physiology of salt tolerance. *Annu. Rev. Plant Physiol.* 9, 25–46. doi: 10.1146/annurev.pp.09.060158.000325

Blomqvist, G., and Johansson, E.-L. (1999). Airborne spreading and deposition of de-icing salt: a case study. *Sci. Total Environ.* 235, 161–168. doi: 10.1016/S0048-9697(99)00209-0

Bock, C. E., Jones, Z. F., and Bock, J. H. (2007). Relationships between species richness, evenness, and abundance in a southwestern savanna. *Ecology* 88, 1322–1327. doi: 10.1890/06-0654

Bolund, P., and Hunhammar, S. (1999). Ecosystem services in urban areas. *Ecol. Econom.* 29, 293–301. doi: 10.1016/S0921-8009(99)00013-0

Brugnoli, E., and Lauteri, M. (1991). Effects of salinity on stomatal conductance, photosynthetic capacity, and carbon isotope discrimination of salt-tolerant

(*Gossypium hirsutum* L.) and salt-sensitive (*Phaseolus vulgaris* L.) C(3) non-halophytes. *Plant Physiol.* 95, 628–635. doi: 10.1104/pp.95.2.628

Bryson, G. M., and Barker, A. V. (2002). Sodium accumulation in soils and plants along Massachusetts roadsides. *Commun. Soil Sci. Plant Anal.* 33, 67–78. doi: 10.1081/CSS-120002378

Cekstere, G., Karlsons, A., and Grauda, D. (2015). Salinity-induced responses and resistance in *Trifolium repens* L. *Urban For. Urban Green.* 14, 225–236. doi: 10.1016/j.ufug.2015.02.010

Cekstere, G., Nikodemus, O., and Osvalde, A. (2008). Toxic impact of the de-icing material to street greenery in Riga, Latvia. *Urban For. Urban Green.* 7, 207–217. doi: 10.1016/j.ufug.2008.02.004

Cekstere, G., and Osvalde, A. (2013). A study of chemical characteristics of soil in relation to street trees status in Riga (Latvia). *Urban For. Urban Green.* 12, 69–78. doi: 10.1016/j.ufug.2012.09.004

Cekstere, G., Osvalde, A., and Vollenweider, P. (2016). De-icing salt impact on leaves of street trees (*Tilia x vulgaris*) in Riga, Latvia. *Acta Biol. Universit. Daugavpil.* 16, 31–38.

Chen, S., Li, J., Wang, S., Hüttermann, A., and Altman, A. (2001). Salt, nutrient uptake and transport, and ABA of *Populus euphratica*; a hybrid in response to increasing soil NaCl. *Trees* 15, 186–194. doi: 10.1007/s004680100091

Chen, W. Y., and Jim, C. (2008). Assessment and valuation of the ecosystem services provided by urban forests. *Ecol. Plann. Manage. Urban For.* 53–83. doi: 10.1007/978-0-387-71425-7_5

Cohen, J. E. (2003). Human population: the next half century. *Science* 302, 1172–1175. doi: 10.1126/science.1088665

Costanza, R., and Folke, C. (1997). Valuing ecosystem services with efficiency, fairness and sustainability as goals. *Nat. Serv*. 49–70.

Czerniawska-Kusza, I., Kusza, G., and Duzynski, M. (2004). Effect of deicing salts on urban soils and health status of roadside trees in the Opole region. *Environ. Toxicol.* 19, 296–301. doi: 10.1002/tox.20037

Davies, W. J., Metcalfe, J., Lodge, T. A., and da Costa, A. R. (1986). Plant growth substances and the regulation of growth under drought. *Funct. Plant Biol.* 13, 105–125. doi: 10.1071/PP9860105

Dmuchowski, W., Baczewska, A. H., Gozdowski, D., and Bragoszewska, P. (2013). Effect of salt stress on the chemical composition of leaves of different trees species in urban environment. *Fresen. Environ. Bull.* 22, 987–994.

Dmuchowski, W., Baczewska, A. H., Gozdowski, D., Rutkowska, B., Szulc, W., Suwara, I., et al. (2014). Effect of salt stress caused by deicing on the content of microelements in leaves of linden. *J. Elementol.* 19, 65–79. doi: 10.5601/jelem.2014.19.1.588

Dobson, M. (1991). *De-icing Salt Damage to Trees and Shrubs*. London: HMSO; Forestry Commission.

Fay, L., and Shi, X. (2012). Environmental impacts of chemicals for snow and ice control: state of the knowledge. *Water Air Soil Poll.* 223, 2751–2770. doi: 10.1007/s11270-011-1064-6

Feder, N., and O'brien, T. P. (1968). Plant microtechnique: some principles and new methods. *Am. J. Bot.* 55, 123–142.

Fink, S. (1999). *Pathological and Regenerative Plant Anatomy*. Berlin, Stuttgart: Borntraeger.

Görür, G. (2006). Effects of heavy metal accumulation in host plants to cabbage aphid *(Brevicoryne brassicae)* morphology. *Ekologia* 25, 314–321.

Günthardt-Goerg, M. S., Matyssek, R., Scheidegger, C., and Keller, T. (1993). Differentiation and structural decline in the leaves and bark of birch (*Betula pendula*) under low ozone concentrations. *Trees* 7, 104–114. doi: 10.1007/BF00225477

Gunthardt-Goerg, M. S., and Vollenweider, P. (2007). Linking stress with macroscopic and microscopic leaf response in trees: new diagnostic perspectives. *Environ. Pollut.* 147, 467–488. doi: 10.1016/j.envpol.2006.08.033

Hodkinson, I. D. (2005). Terrestrial insects along elevation gradients: species and community responses to altitude. *Biol. Rev.* 80, 489–513. doi: 10.1017/S1464793105006767

Huberty, A. F., and Denno, R. F. (2004). Plant water stress and its consequences for herbivorous insects: a new synthesis. *Ecology* 85, 1383–1398. doi: 10.1890/03-0352

Hunter, M. D., and Price, P. W. (1992). Playing chutes and ladders—heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology* 73, 724–732.

Inada, N., Sakai, A., Kuroiwa, H., and Kuroiwa, T. (1998). Three-dimensional analysis of the senescence program in rice (*Oryza sativa* L.) coleoptiles. *Planta* 206, 585–597. doi: 10.1007/s004250050436

IUSS Working Group WRB (2015). *World Reference Base For Soil Resources 2014, Update 2015: International Soil Classification System for Naming Soils and Creating Legends for Soil Maps.* World Soil Resources Reports No. 106, 192. FAO, Rome.

Klemm, W., Heusinkveld, B. G., Lenzholzer, S., and van Hove, B. (2015). Street greenery and its physical and psychological impact on thermal comfort. *Landsc. Urban Plan.* 138, 87–98. doi: 10.1016/j.landurbplan.2015.02.009

Koricheva, J., Larsson, S., and Haukioja, E. (1998). Insect performance on experimentally stressed woody plants: a meta-analysis. *Annu. Rev. Entomol.* 43, 195–216. doi: 10.1146/annurev.ento.43.1.195

Kottek, M., Grieser, J., Beck, C., Rudolf, B., and Rubel, F. (2006). World map of the köppen-geiger climate classification updated. *Meteorol. Zeitschr.* 15, 259–263. doi: 10.1127/0941-2948/2006/0130

Künnemann, T.-D., and Gad, G. (1997). *Überleben Zwischen Land und Meer: Salzwiesen*. Oldenburg: Verlag Isensee.

Kwasowski, W., and Czyz, M. (2010). Reaction of lime trees (*Tilia* sp.) growing along the Zwirki i Wigury Street in Warsaw on soil salinity caused by chemical technology of snow removal. *Ecol. Quest.* 14:81. doi: 10.12775/v10090-011-0023-6

Larsson, S. (1989). Stressful times for the plant stress: insect performance hypothesis. *Oikos* 56, 277–283. doi: 10.2307/3565348

Legendre, P. (1998). *Numerical Ecology, Vol. 20*. Amsterdam: Elsevier Science & Technology.

Legros, J.-P. (2007). *Les Grands Sols Du Monde.* Lausanne: PPUR Presses Polytechniques.

Lyshede, O. B. (1977). Studies on the mucilaginous cells in the leaf of *Spartocytisus filipes* W.B. *Planta* 133, 255–260. doi: 10.1007/BF00380686

Mariani, P., Rascio, N., Baldan, B., Paiero, P., and Urso, T. (1988). Epidermal mucilage cells in leaves of *Salix* species. *Flora* 181, 137–145. doi: 10.1016/S0367-2530(17)30359-6

Marosz, A., and Nowak, J. S. (2008). Effect of salinity stress on growth and macroelements uptake of four tree species. *Dendrobiology* 59, 23–29.

Martel, J. (1998). Plant-mediated effects of soil salinity on a gall-inducing caterpillar Epiblema scudderiana (Lepidoptera: Tortrieidae) and the in?uence of feeding guild. *Eur. J. Entomol.* 95, 545–557.

Marzluff, J. M. (2001). "Worldwide urbanization and its effects on birds," in *Avian Ecology and Conservation in an Urbanizing World,* eds J.M. Marzluff, R. Bowman and R. Donnelly (Boston, MA: Springer US), 19–47. doi: 10.1007/978-1-4615-1531-9_2

Maxwell, K., and Johnson, G. N. (2000). Chlorophyll fluorescence—a practical guide. *J. Exp. Bot.* 51, 659–668. doi: 10.1093/jexbot/51.345.659

Munns, R. (2002). Comparative physiology of salt and water stress. *Plant Cell Environ.* 25, 239–250. doi: 10.1046/j.0016-8025.2001.00808.x

Muñoz, P. T., Torres, F. P., and Megías, A. G. (2014). Effects of roads on insects: a review. *Biodivers. Conserv*. 24, 659–682. doi: 10.1007/s10531-014-0831-2

Negrão, S., Schmöckel, S. M., and Tester, M. (2017). Evaluating physiological responses of plants to salinity stress. *Ann. Bot.* 119, 1–11. doi: 10.1093/aob/mcw191

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., et al. (2013). "*vegan: Community Ecology Package*". 2.0-10 ed. Available online at: http://vegan.r-forge.r-project.org/ (accessed January 04, 2013).

Paludan-Müller, G., Saxe, H., Pedersen, L. B., and Randrup, T. B. (2002). Differences in salt sensitivity of four deciduous tree species to soil or airborne salt. *Physiol. Plant.* 114, 223–230. doi: 10.1034/j.1399-3054.2002.1140208.x

Parida, A. K., and Das, A. B. (2005). Salt tolerance and salinity effects on plants: a review. *Ecotoxicol. Environ. Saf.* 60, 324–349. doi: 10.1016/j.ecoenv.2004.06.010

Pospíšilová, J., Synková, H., and Rulcová, J. (2000). Cytokinins and water stress. *Biol. Plant.* 43, 321–328. doi: 10.1023/A:1026754404857

Price, P. W. (1991). The plant vigor hypothesis and herbivore attack. *Oikos* 62, 244–251. doi: 10.2307/3545270

Ramakrishna, D. M., and Viraraghavan, T. (2005). Environmental impact of chemical deicers–a review. *Water Air Soil Pollut.* 166, 49–63. doi: 10.1007/s11270-005-8265-9

Rengasamy, P. (2010). Soil processes affecting crop production in salt-affected soils. *Funct. Plant Biol.* 37, 613–620. doi: 10.1071/FP09249

Rinkis, G., Ramane, H., and Kunicka, T. (1987). *Methods of Soil and Plant Analysis*. Riga: Zinatne.

Seamans, G. S. (2013). Mainstreaming the environmental benefits of street trees. *Urban For. Urban Green.* 12, 2–11. doi: 10.1016/j.ufug.2012.08.004

Shannon, M. C. (1997). Adaptation of plants to salinity. *Adv. Agron.* 60, 75–120. doi: 10.1016/S0065-2113(08)60601-X

Shochat, E., Warren, P. S., Faeth, S. H., McIntyre, N. E., and Hope, D. (2006). From patterns to emerging processes in mechanistic urban ecology. *Trends Ecol. Evol.* 21, 186–191. doi: 10.1016/j.tree.2005.11.019

Sienkiewicz-Paderewska, D., Dmuchowski, W., Baczewska, A. H., Bragoszewska, P., and Gozdowski, D. (2017). The effect of salt stress on lime aphid abundance on Crimean linden (*Tilia* 'Euchlora') leaves. *Urban For. Urban Green.* 21, 74–79. doi: 10.1016/j.ufug.2016.11.010

Sudhir, P., and Murthy, S. D. S. (2004). Effects of salt stress on basic processes of photosynthesis. *Photosynthetica* 42, 481–486. doi: 10.1007/S11099-005-0001-6

Tedeschi, A., and Dell'Aquila, R. (2005). Effects of irrigation with saline waters, at different concentrations, on soil physical and chemical characteristics. *Agricult. Water Manage.* 77, 308–322. doi: 10.1016/j.agwat.2004.09.036

Thaiutsa, B., Puangchit, L., Kjelgren, R., and Arunpraparut, W. (2008). Urban green space, street tree and heritage large tree assessment in Bangkok, Thailand. *Urban For. Urban Green.* 7, 219–229. doi: 10.1016/j.ufug.2008.03.002

Vollenweider, P., and Günthardt-Goerg, M. S. (2005). Diagnosis of abiotic and biotic stress factors using the visible symptoms in foliage. *Environ. Pollut.* 137, 455–465. doi: 10.1016/j.envpol.2005.01.032

Vollenweider, P., and Günthardt-Goerg, M. S. (2006). Erratum to "Diagnosis of abiotic and biotic stress factors using the visible symptoms in foliage" [Environ. Pollut. 137 (2005) 455–465]. *Environ. Pollut.* 140, 562–571. doi: 10.1016/j.envpol.2006.01.002

Vollenweider, P., Menard, T., Arend, M., Kuster, T. M., and Günthardt-Goerg, M. S. (2016). Structural changes associated with drought stress symptoms in foliage of Central European oaks. *Trees* 30, 883–900. doi: 10.1007/s00468-015-1329-6

Volpe, V., Manzoni, S., Marani, M., and Katul, G. (2011). Leaf conductance and carbon gain under salt-stressed conditions. *J. Geophys. Res.* 116:G04035. doi: 10.1029/2011JG001848

Westoby, M., Falster, D. S., Moles, A. T., Vesk, P. A., and Wright, I. J. (2002). Plant ecological strategies: some leading dimensions of variation between species. *Annu. Rev. Ecol. Syst.* 33, 125–159. doi: 10.1146/annurev.ecolsys.33.010802.150452

Wheeler, R. E. (2010). *"multResp() lmPerm"*. The R project for statistical computing. Available online at: http://www.r-project.org/ (accessed August 02, 2016).

White, T. C. (1974). A hypothesis to explain outbreaks of looper caterpillars, with special reference to populations of Selidosema suavis in a plantation of Pinus radiata in New Zealand. *Oecologia* 16, 279–301. doi: 10.1007/BF00344738

White, T. C. R. (1984). The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia* 63, 90–105. doi: 10.1007/BF00379790

Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., et al. (2004). The worldwide leaf economics spectrum. *Nature* 428, 821–827. doi: 10.1038/nature02403

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REVIEW
published: 16 August 2019
doi: 10.3389/fevo.2019.00310

Urbanization Shapes the Ecology and Evolution of Plant-Arthropod Herbivore Interactions

Lindsay S. Miles[1]*, *Sophie T. Breitbart*[1,2]*, *Helene H. Wagner*[1,2] *and Marc T. J. Johnson*[1,2,3]

[1] Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada, [2] Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON, Canada, [3] Centre for Urban Environments, University of Toronto Mississauga, Mississauga, ON, Canada

Urbanization is quickly changing natural and agricultural landscapes, with consequences for the herbivorous arthropods dwelling in or near cities. Here, we review the evidence for the effects of urbanization on the ecology and evolution of plant-herbivore interactions. We first summarize how abiotic factors associated with urbanization affect the ecology and evolution of herbivorous arthropods. Next, we explore how urbanization affects plant-herbivore interactions, by considering how urban environments may disrupt top-down and bottom-up ecological processes that affect herbivory. Abiotic changes in the urban environment, such as the urban heat island effect, have caused shifts in phenology for some herbivorous arthropods. Other abiotic changes in urban areas, including water availability, pollution, and habitat fragmentation, have resulted in changes to physiology, behavior, and population abundance. Native species richness tends to decline in urban areas; however, changes in abundance appear to be species specific. These shifts in ecology suggest that urbanization could affect both adaptive and non-adaptive evolution of herbivorous arthropods and their host plants in urban environments. However, plant-herbivore interactions may be dramatically altered if either arthropods or plants are unable to tolerate urban environments. Thus, while some species can physiologically acclimate or genetically adapt to the abiotic urban environment, many species are expected to decline in abundance. We conclude with suggestions for future research to advance our understanding of how urbanization alters the ecology and evolution of plant-herbivore interactions.

Keywords: urban, plant-arthropod herbivore interaction, ecological processes, eco-evolutionary feedbacks, herbivorous arthropod

Edited by:
Gina Marie Wimp,
Georgetown University, United States

Reviewed by:
Christofer Bang,
Arizona State University, United States
Holly Martinson,
McDaniel College, United States

Correspondence:
Lindsay S. Miles
lindsay.miles@utoronto.ca
Sophie T. Breitbart
sophie.breitbart@gmail.com

Specialty section:
This article was submitted to Population and Evolutionary Dynamics, a section of the journal Frontiers in Ecology and Evolution

Received: *30 April 2019*
Accepted: *31 July 2019*
Published: *16 August 2019*

Citation:
Miles LS, Breitbart ST, Wagner HH and Johnson MTJ (2019) Urbanization Shapes the Ecology and Evolution of Plant-Arthropod Herbivore Interactions. Front. Ecol. Evol. 7:310. doi: 10.3389/fevo.2019.00310

INTRODUCTION

The ecological and evolutionary interactions between plants and their herbivores are among the most important interactions in nature. Arthropods have consumed plants for over 415 million years (Labandeira, 2007), and their long history of coevolution has led to the diversification of a broad array of phenotypes and species among both arthropods and plants (Ehrlich and Raven, 1964; Thompson, 1994; Althoff et al., 2014). In contemporary communities, nearly 75% of the total biodiversity and biomass of extant macro-organisms is comprised of plants and their herbivores (Strong et al., 1984; Wilson, 2001). Herbivores are responsible for consuming 5–20% of the total biomass produced annually (Cyr and Pace, 1993; Agrawal, 2011; Turcotte et al., 2014), making

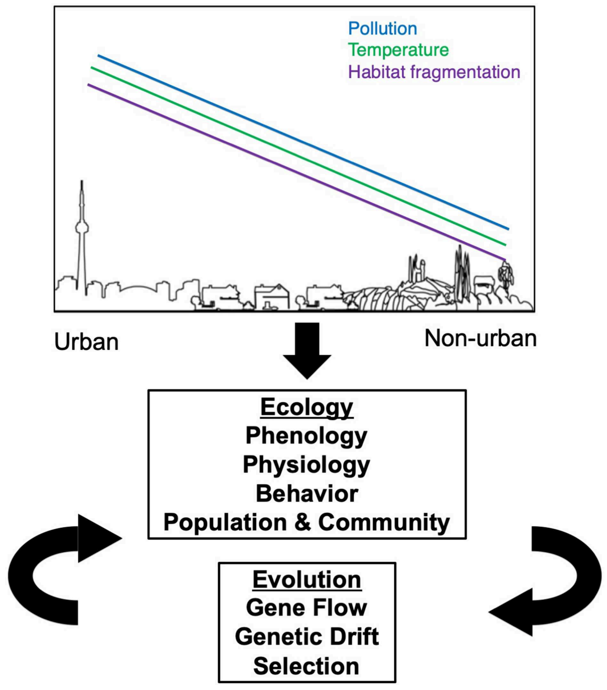


FIGURE 1 | Expected effects of urban environments on the ecology and evolution of herbivorous arthropods. Moving from non-urban to urban landscapes, there is an overall increase in the amount of pollution, temperature, and habitat fragmentation. Each of these three factors influence changes in the ecology of herbivorous arthropods, which in turn can influence their evolution in a feedback loop.

them among the most important drivers of ecological and ecosystem processes in natural and managed systems.

Urbanization alters natural and historically agricultural landscapes with the development of cities, suburbs and supporting infrastructure (McDonnell and Pickett, 1990; Faeth et al., 2005). The novel, human built environment shows commonalities in many features throughout the world, such as high human population densities, increased impervious surface (e.g., roads and buildings), elevated pollution (e.g., air quality, light, soil), elevated temperatures (Oke, 1973; Ziter et al., 2019), habitat degradation and fragmentation (Grimm et al., 2008; Seto et al., 2011, 2012). However, cities also vary in some characteristics, such as in city age, human population density, urban infrastructure, city size, local climate, socioeconomics, and government regulations (Grimm et al., 2008). These convergent and divergent changes to urban environments create completely novel ecosystems that are unlike any natural environment. These manifold changes to the biotic and abiotic environments in cities present new challenges to herbivorous arthropods, their predators, and host plants, which can influence both the ecology and evolution of plant-arthropod herbivore interactions in or near cities (**Figure 1**).

Here, we review evidence for the effects of urbanization on the ecology and evolution of arthropod herbivores and their interactions with plants. We start by summarizing advances in our understanding of how abiotic factors associated with urbanization affect the ecology and evolution of herbivorous arthropods. Next, we explore how urbanization affects plant-herbivore interactions by considering how urban environments may disrupt top-down and bottom-up ecological and evolutionary processes that affect herbivory.

URBAN FACTORS THAT CAN INFLUENCE ECOLOGY AND EVOLUTION

The urban heat island effect describes the phenomenon whereby cities are warmer than non-urban areas (Oke, 1973; Arnfield, 2003), because of the increased impervious surfaces (e.g., asphalt and concrete) and typically decreased tree cover in cities (Bowler et al., 2010; Ziter et al., 2019). The asphalt and concrete that traps heat can make cities warmer in the day and night throughout the year, which can also extend warm seasons. This urban warming can influence both thermal conditions and breeding seasons of herbivorous arthropods and their predators (Imhoff et al., 2010).

Light and chemical pollution impose stresses on urban-dwelling organisms (Isaksson, 2015). Light pollution alters natural light cycles, which can disrupt circadian rhythms (Gaston et al., 2013). Light pollution can also influence the behavior and, consequently, the survival of arthropods that are attracted to light. Chemical pollution encompasses the release of small particulate matter including harmful airborne gases (e.g., CO, O_3, nitrogen oxides) (Isaksson, 2015), which can alter an organism's physiology and behavior, and even increase mutation rates (Yauk and Quinn, 1996; Somers et al., 2002, 2004; Massad and Dyer, 2010). Chemical pollution can also provide nutrients to plants, which influences bottom-up effects on herbivorous arthropods (Martin and Stabler, 2002; Shochat, 2004; Raupp et al., 2010).

The expansion and development of urban areas degrades and fragments the non-urban landscape. This urban expansion decreases the total amount of available natural habitat and increases the isolation of remnant non-urban habitat patches within the urban matrix (Grimm et al., 2008). Organisms that are unable to cross the urban matrix can become isolated and are more likely to experience population declines (Grimm et al., 2008). Such population declines may dramatically alter bottom-up and top-down trophic dynamics within plant-herbivore communities (Hope et al., 2003; McKinney, 2008; Aronson et al., 2014; **Figure 2**).

HOW URBANIZATION AFFECTS HERBIVOROUS ARTHROPODS

Urbanization leads to changes in multiple abiotic factors that impact the ecology and evolution of herbivorous arthropods that dwell in or near cities (**Table 1**). Here, we review how urbanization changes the phenology, physiology, and behavior of populations of herbivorous arthropods, as well as their community assemblage. We then consider how urbanization influences both adaptive and non-adaptive evolution of herbivorous arthropods.

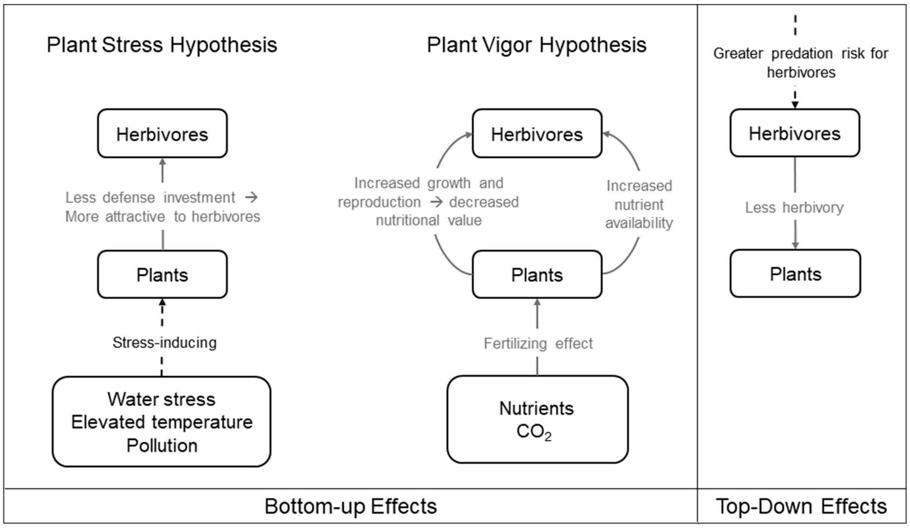


FIGURE 2 | Bottom-up and top-down effects in urban environments. Solid gray line indicates process that benefits recipient, dashed black line indicates process that harms recipient. The left panel illustrates the bottom-up effects on herbivorous arthropods under the two competing hypotheses: the Plant Stress Hypothesis, where plants subject to stress may decrease investment in defenses and thus experience greater herbivore damage, and the Plant Vigor Hypothesis, where plants with more growth are more attractive to herbivores and consequently suffer from greater herbivore attack. The right panel illustrates the top-down effects on herbivorous arthropods.

TABLE 1 | Examples of ecological and evolutionary responses to urbanization for herbivorous arthropods.

Response	Urban factor	Specific response	Species name	Climate type	References
Phenology	UH	Delayed emergence	Multiple	T	Diamond et al., 2014
	UH	Emerge early, long season	Cereal leaf beetle (*Oulema melanopus*)	T	Evans et al., 2013
	UH	Emerge early, short season	Tent caterpillar (*Malacosoma disstria*)	T	Schwartzberg et al., 2014
Physiology	UH	Higher thermal optimum	Multiple	T	Hamblin et al., 2017
	WA	Abundance increase and better breeding	Aphids (*Aphididae*)	A	Andrade et al., 2017
Behavior	P	Reduced flight to light	Ermine moth (*Yponomeuta cagnagella*)	T	Altermatt and Ebert, 2016
	WA	Increased water seeking	Multiple	T	McCluney et al., 2018
Population	HF	Increased abundance	Azalea lace bug (*Stephanitis pyrioides*)	T	Shrewsbury and Raupp, 2000
	HF	Increased abundance	Horse chestnut scale (*Pulvinaria regalis*)	T	Speight et al., 1998
	UH	Increased abundance	Scale insect (*Melanaspis tenebriscosa*)	Y	Dale and Frank, 2014, 2017
Community	HF	Decline in abundance and richness	Multiple	A	Bang and Faeth, 2011
	HF	Increased generalist and exotic	Multiple	T	Sattler et al., 2010
Evolution	HF	Gene flow (reduced), genetic drift (increased)	Woodland ground beetle (*Abax ater*)	T	Desender et al., 2005
	P	Selection	Peppered moth (*Biston betularia*)	T	van't Hof et al., 2011, 2016; Cook and Saccheri, 2013

Columns show the ecological or evolutionary response; the abiotic urban factor driving the response includes the urban heat island effect (UH), water availability (WA), pollution (P), and habitat fragmentation (HF); the specific response to the abiotic urban factor; the common and scientific name (when available); the climate type that the study was performed in as temperate (T) or arid (A); and the citation for the study.

Physiological Effects

Urbanization can alter water availability through changes in air temperature, humidity, and soil moisture (Hadley, 1994; Sabo et al., 2008; McCluney and Sabo, 2009; Chown et al., 2011; Chown and Duffy, 2015), which can have important physiological consequences for arthropods. Specifically, dehydration is lethal for many arthropods (Hadley, 1994). Water stress likely varies within and between cities, based on the heterogeneous nature of urbanization (Hope et al., 2003; Martin et al., 2004; Schwarz et al., 2015). For example, in the arid city of Phoenix, AZ, USA, aphid abundance within the city is positively correlated with the areas of the city that have increased water availability, a phenomenon caused by irrigation of lawns and parks within the city (Andrade et al., 2017). Because of supplemental water use, arid cities often have reduced water stress on average compared to temperate cities and compared to non-urban areas with similar climate (Buyantuyev and Wu, 2009). For example, during periods of drought, arthropods were less dehydrated in Phoenix, AZ, than arthropods in a temperate city like Raleigh, NC, because the arid city has increased water available compared to non-urban areas as described above. By contrast, temperate cities may also have elevated irrigation in places, but impervious surfaces quickly funnel water to storm drains and sewers instead of into the soil (McCluney et al., 2017). During periods of drought when the soil moisture is depleted, mobile arthropods moved to areas with more water sources. The herbivorous arthropods with reduced mobility significantly increased herbivory on host trees to meet their water demands (McCluney et al., 2018). Therefore, during periods of drought and higher temperatures in temperate cities, where there is less supplemental water available than in arid cities, there can be significant increases in herbivory relative to non-urban areas.

In addition to water availability, urban heat islands can have variable physiological effects on species depending on their thermal optima. Species with higher critical thermal optima tend to be better able to survive in urban areas compared to those with lower critical thermal optima (Youngsteadt et al., 2015; Hamblin et al., 2017). Consequently, species with lower critical thermal optima disproportionately experience population declines in urban areas (Hamblin et al., 2017). Although there seems to be significant variation in thermal tolerance between species, heat tolerance can also be sex-specific. Experimental heatwaves, similar to those found in urban areas, significantly reduced male fertility in the red flour beetle, *Tribolium castaneum* (Sales et al., 2018). Specifically, male reproductive output was 50% reduced following a heatwave which also carried over to the next generation, and males experienced an even greater reduction in reproduction following a second heatwave. By contrast, females experienced no change in reproductive output under the same heat stress (Sales et al., 2018). Herbivorous arthropods are not only subject to declines from experiencing increased maximum temperatures in urban areas, but also from extended warmer nighttime periods, which can exacerbate the detrimental effects of heat. For example, experimental warming at night reduced survival for the English grain aphid, *Sitobion avenae,* and reduced adult longevity and fecundity, which together reduced overall population abundance (Zhao et al., 2014). Thus, the diurnal and seasonal effects of elevated urban heat can have detrimental fitness consequences for herbivorous arthropods.

Phenological Shifts

Phenological shifts are expected to occur in herbivorous arthropods when there are phenological limitations associated with lower and upper thermal tolerance (**Figure 3**). When summer temperatures do not exceed the upper thermal tolerance of organisms, but nevertheless maintain longer warm periods, then we expect an overall longer season in which species are active. When summer temperatures exceed an organism's thermal tolerance, we expect shifts in phenology to earlier (spring) or later (fall) seasons, which may also be shorter if lower thermal tolerance limits do not shift equally. For these reasons, urban heat islands cause some arthropods to shift their phenology, emerging earlier in the spring or later in the fall. For example, in an analysis of 28 species of butterflies in the United Kingdom, those species found in urban areas emerged earlier than the species found in non-urban areas (Dennis et al., 2017). Although urban butterfly species emerged sooner, their total breeding time was generally shorter than that of rural species, indicating that urban butterfly species breed sooner but for a shorter period of time than rural ones (Dennis et al., 2017). Within species, a trend toward earlier emergence in urban areas was observed for 4 of 20 butterfly species studied in Ohio (USA), but another seven species showed significant shifts toward delayed emergence in urban compared to rural populations, and their breeding season was similarly shorter for these species (Diamond et al., 2014). While the breeding season may be shorter for many urban butterflies, other arthropods experience both earlier emergence and an extended breeding season. For example, in New Jersey (USA), urban and agricultural bee communities emerge sooner in the spring than non-urban bees and their breeding season is extended into the fall compared to non-urban bees that end their season in the summer (Harrison et al., 2018). Although the summer season was extended for urban bee communities in general, this extended season is likely species-specific as the bee community assemblages vary significantly between urban and non-urban habitats (Harrison et al., 2018). Therefore, it appears as though a species' thermal tolerance may be one indicator for whether and how urbanization will shift the phenology of an organism (**Figure 3**).

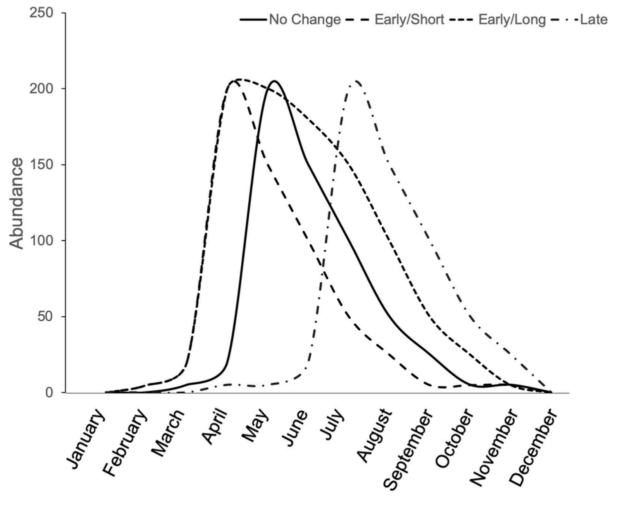


FIGURE 3 | Conceptual illustration of phenological shifts due to the urban heat island effect. Depending on the species' thermal optima and tolerance, the urban heat island effect can result in no change from the natural emergence time (solid line), earlier emergence, potentially with a shorter season (long dash), earlier emergence with an extended season (short dash), or a late emergence, potentially with a shorter season (dash-dot).

Behavioral Shifts

One way that herbivorous arthropods cope with novel urban environments is by changing behavior. Anthropogenic light pollution is inherent to all urban areas and it can disrupt an organism's circadian rhythm (Cinzano et al., 2001; Gaston et al., 2013). Attraction to light can have detrimental consequences for organisms through direct burning from the light source and increased exposure to predators (Warren, 1990; Jones and Francis, 2003). Several arthropod predators (e.g. toads) congregate around lights to take advantage of the flight-to-light behavior (Larsen and Pedersen, 1982). In response to the increased risks associated with flight-to-light in artificially lit urban areas, Altermatt and Ebert (2016) found that some herbivorous arthropods reduce their propensity to fly to light. Urban populations of the herbivorous small ermine moth, *Yponomeuta cagnagella*, avoid light more than non-urban populations (Altermatt and Ebert, 2016). However, reduced attraction to light may be species-specific and may also be influenced by variation in the lit environment. For example, when street lamps are at high densities, with spectrally diverse light, more moth species are attracted (Plummer et al., 2016). Large moth species are more attracted to light with lamps at high density, whereas small moth species are more attracted to UV-emitting lamps (Plummer et al., 2016). Therefore, the behavioral response to urban factors may be taxon specific. The attraction to light may be linked to body size, where larger arthropods are less timid and thus more attracted to light while smaller ones are more sensitive to predation and thus avoid light in urban areas. It is unclear whether these changes in behavior are the result of phenotypically plastic responses or evolved adaptations, which represents an important avenue for future research.

Population and Community Shifts

The multifarious changes in the environment due to urbanization frequently have large effects on the population ecology of herbivorous arthropods. On average, native species experience reduced abundance and extirpation in urban areas (McIntyre, 2000; Faeth et al., 2005, 2011; Sadler et al., 2006; Sánchez-Bayo and Wyckhuys, 2019). The urban heat island effect can also reduce the abundance of herbivorous arthropods. For example, wild bee abundance declined for every degree Celsius increase caused by urban warming in Raleigh, NC (Hamblin et al., 2018). However, the urban heat island effect can increase population abundance for several herbivorous arthropods, leading to pest outbreaks (Dale and Frank, 2014, 2018; Lahr et al., 2018; Meineke et al., 2018). The scale insect pest, *Melanaspis tenebricosa*, was most abundant in warmer patches in the city than in cooler patches, causing an infestation on urban street trees which resulted in significant tree damage (Dale and Frank, 2014). Given the differences in abundances among different species, these population responses may be tied to thermal tolerance limits such that the urban heat island causes temperatures beyond a species' tolerance.

Pollution, such as insecticides, will drastically reduce the total abundance of many arthropod species. Pest species, such as termites, are often the target of insecticide use in urban areas as they can cause $40 billion USD annually in damage to human built structures (Rust and Su, 2012). The insecticide use for termites has led to substantial declines in many species of pest termites and new technologies have been developed to reduce the impact of pesticides on non-target species (Su, 2002). Insecticide and herbicide use in agriculture and field margins have caused significant declines in moths and butterflies (Erhardt and Thomas, 1991; Hahn et al., 2015; Forister et al., 2016; Hallmann et al., 2017). In urban gardens, insecticide and herbicide use also causes population declines of local butterfly species (Muratet and Fontaine, 2015). Interestingly, when a targeted pesticide is used and successfully eliminates the target pest, non-target herbivorous pests can experience population outbreaks (Szczepaniec et al., 2011). Regardless of the type of pesticide used, there are typically overall population declines in both pest and non-pest species of herbivorous arthropods.

Environmental heterogeneity in the urban environment leads to reduced arthropod abundance (McIntyre et al., 2001; Rickman and Connor, 2003; Philpott et al., 2014; Vergnes et al., 2014; Smith et al., 2015; Egerer et al., 2017). For example, in Phoenix, AZ, the natural desert and agricultural locations had a significantly higher abundance of herbivorous arthropods than industrial locations (McIntyre et al., 2001). The reduction in abundance is correlated to both habitat type and percent impervious surface with desert having the lowest and industrial having the highest percent impervious surface within the city (McIntyre et al., 2001). The shifts in population abundance are likely species-specific, with pests experiencing "outbreaks" and natives in population decline. As we discuss in the next paragraph, such population shifts are expected to result in changes to the community assemblages within urban areas compared to non-urban habitats.

The total diversity of arthropods in cities can be comparable to the diversity in non-urban areas due to the presence of non-native taxa, which also alter the community composition (Bolger et al., 2000; McIntyre, 2000). Habitat fragmentation in particular has been identified as a driver of shifts in community assembly, whereby communities in habitats that are highly fragmented tend to have more generalist and invasive non-native species than communities with less habitat fragmentation (Bolger et al., 2000; Gibb and Hochuli, 2002; Sattler et al., 2010). Many plants in the urban environment are non-native (Pyšek, 1998; McKinney, 2004; Pickett et al., 2011). This can lead to an abundance of native generalist herbivores, whereas native specialist herbivorous arthropods are likely to experience population declines in urban areas due to a lack of their native host species (Burghardt et al., 2010).

Adaptive and Non-adaptive Evolution

The effects of urbanization on the ecology of arthropods also suggest that urban development could have large impacts on the evolution of arthropod populations. Urbanization can influence gene flow by either creating barriers, such as roads and habitat fragmentation that reduce gene flow (Holderegger and Di Giulio, 2010; Storfer et al., 2010), or habitat corridors and human-mediated dispersal that increase gene flow (Crispo et al., 2011; Arredondo et al., 2018; Miles et al., 2018a,b). Perhaps unsurprisingly, urbanization more frequently reduces gene flow for herbivorous arthropods (Desender et al., 2005; Davis et al., 2010; Schoville et al., 2013; López-Uribe et al., 2015; Vickruck and Richards, 2017) than it elevates it (Keller et al., 2004; Desender et al., 2005; Dronnet et al., 2005). In fact, when calculating the mean pairwise differentiation from the studies that measured the degree of population structure ($N = 6$; **Supplemental Table 1**), which is directly affected by the amount of gene flow, there is slightly higher and more variable genetic differentiation (measured by the fixation index F_{ST}) between urban populations than between non-urban populations; however, this trend is not significant [$t_{(5)} = 1.24$, $p = 0.26$; **Figure 4A**]. The paucity of studies that measure genetic differentiation between urban and non-urban populations of herbivorous arthropods may be due to the relatively new field of urban evolution and thus patterns of genetic differentiation may become clearer once there are more studies.

Similarly, urbanization typically increases the importance of genetic drift, due to habitat loss that reduces the total population size and fragmentation that reduces local population sizes and restricts gene flow, all of which can reduce the effective population size (N_e) (Johnson and Munshi-South 2017). This increased genetic drift results in reduced genetic diversity within urban populations of herbivorous arthropods (Keller et al., 2004; Desender et al., 2005; Vandergast et al., 2009; Schoville et al., 2013; López-Uribe et al., 2015; Vickruck and Richards, 2017). There is 18% less genetic diversity, measured as observed heterozygosity (H_O), in urban compared to non-urban populations across the studies that measured genetic diversity ($N = 8$); however, this trend is also non-significant [$t_{(7)} = 1.19$, $p = 0.27$; **Supplemental Table 1**; **Figure 4B**]. If a reduction in genetic diversity is supported more strongly with further studies, then the loss of genetic diversity within urban populations can have multiple negative consequences. Low genetic diversity within populations can increase inbreeding, which can lower fitness due to inbreeding depression and thus reduce local population viability through greater risk of local extinction (Young et al., 1993, 1996; Lowe et al., 2005). Reduced genetic diversity also reduces adaptive evolutionary potential for populations responding to changing environments (Young et al., 1996; Holderegger and Di Giulio, 2010). Therefore, non-adaptive evolution may have a strong influence on the persistence of urban populations for both short-term and long-term.

For populations of herbivorous arthropods that persist or even thrive in urban environments, it is likely that many have undergone rapid evolutionary change (i.e., adaptive evolution) in this new habitat. To date, the peppered moth, *Biston betularia*, is the only herbivorous arthropod that has been studied in the context of urban adaptation. The industrial pollution that discolored the habitat of the peppered moth, *B. betularia,* led to an increase in selective predation on the light-colored morph, thus increasing the frequency of the darker-colored morph until pollution mitigation efforts were put in place (Kettlewell, 1955, 1958; Brakefield and Liebert, 2000; Cook et al., 2005; van't Hof et al., 2011, 2016; Cook and Saccheri, 2013). While urbanization can cause direct selective pressures on organisms that live in urban habitats, urban landscapes may also indirectly select for species with specific characteristics. For example, flightless species of beetles are more commonly found in rural environments while species that are capable of flight are found more often in urban environments (Niemelä and Kotze, 2009; Vergnes et al., 2014). These differences in species composition indicate that the urban environment selects for dispersal traits that facilitate movement across highly fragmented urban areas (Vergnes et al., 2014; Aronson et al., 2016). However, it is unclear whether the urban environment acts as an agent of natural selection on the beetle populations. Given all of the factors associated with urbanization that potentially impose natural selection on herbivorous arthropods, there is a need for studies that investigate adaptive evolution. Much of the current literature on adaptive evolution in urban environments is in vertebrate populations, which are often of conservation concern (Rivkin et al., 2018).

HOW URBANIZATION AFFECTS PLANT-HERBIVORE INTERACTIONS

The relationship between urbanization and arthropod herbivory depends both on the degree of urbanization and an herbivore's feeding guild (i.e., leaf-chewing, piercing-sucking, gall-forming, etc.). Previous research has suggested a positive relationship between urbanization and herbivory (Cregg and Dix, 2001). For example, a study of herbarium specimens of four plant species, originating from a region (Northeastern US) that experienced substantial urban development over the past 100+ years, found increasing rates of herbivory through time (Meineke et al., 2018). Conversely, a previous meta-analysis identified no significant correlation between habitat fragmentation and herbivory when considering studies of urban and non-urban sites, even though the abundance and diversity of herbivores decreased (Rossetti et al., 2017). However, other studies suggest that leaf damage in response to fragmentation may be similar among urban and non-urban areas: Christie and Hochuli (2005) found greater herbivory among small remnant edges than among large remnant edges as well as interior sites of both small and large remnants in urban areas, while De Carvalho Guimarães et al. (2014) also found that non-urban edge sites experienced more herbivory than interior sites, especially from Lepidoptera and Orthoptera.

Given the scarcity of studies directly linking urbanization to levels of arthropod herbivory, there is no consensus for which patterns of urban herbivory will occur most often and in various types of cities. Small urban parks may be expected to experience greater herbivore damage than large parks under the predictions posited by Christie and Hochuli (2005) and De Carvalho Guimarães et al. (2014). However, urban parks may experience similar levels of herbivory damage regardless of size under the prediction posed by Rossetti et al. (2017). While relatively few cases have directly linked urbanization with herbivory, several studies shed light on the processes that shape plant-herbivore interactions in cities. These studies, discussed below, illuminate how bottom-up and top-down forces associated with urbanization influence herbivores by benefiting or harming them and their host plants.

Bottom-Up Effects

Urban environmental stressors on plants, including water stress, elevated temperature, and pollution, may affect arthropod herbivores directly, as described in Section "Urban Factors That Can Influence Ecology and Evolution," or indirectly via bottom-up effects. The Plant Stress Hypothesis (**Figure 2**) posits that plants subject to stress may decrease investment in defenses

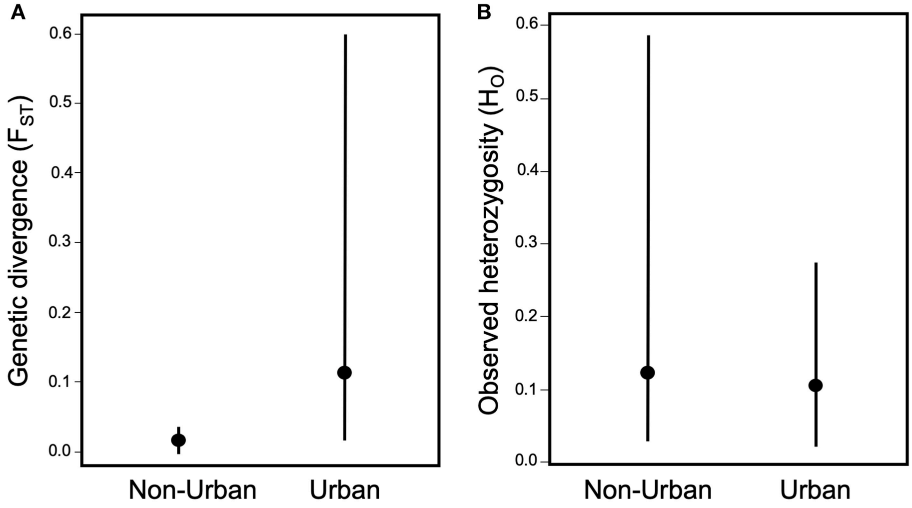


FIGURE 4 | Effect of urbanization on **(A)** genetic differentiation between populations measured as F_{ST}, and **(B)** genetic diversity within populations measured as observed heterozygosity (H_O). Measures of F_{ST} are the mean of pairwise values between urban populations and the mean of pairwise values between non-urban populations, extracted from the studies (**Supplemental Table 1**) of non-adaptive evolution in herbivorous arthropods. Measures of H_O are the mean values of urban populations and the mean values of non-urban populations extracted from the studies of non-adaptive evolution (**Supplemental Table 1**).

and thus endure greater herbivore damage (White, 1969, 1984; see Price, 1991 for review). Two aspects of urbanization, soil compaction and widespread impervious surface coverage, can prevent the absorption of nutrients and water into host plants and render them more susceptible to herbivore colonization (Speight et al., 1998; Huberty and Denno, 2004). Decreased water absorption may also cause plants to have concentrated phloem, with higher amino acid content than watered plants, which can be beneficial to some phloem-sucking insects such as aphids (White, 1984, 2009; Schmitz, 1996), though there is conflicting evidence regarding the effects of chronic water stress on leaf-chewing and phloem-sucking arthropods (Mattson and Haack, 1987; Huberty and Denno, 2004; Meineke and Frank, 2018). Elevated temperatures caused by the urban heat island effect further induces water stress in plants, which makes them grow less and become more susceptible to herbivory in some circumstances (Meineke and Frank, 2018). Water stress also induces stomatal closure, which conserves water but decreases photosynthesis, potentially reducing herbivory from phloem-suckers (Schaffer and Mason, 1990; Hawkes and Sullivan, 2001). As mentioned earlier, elevated urban temperatures that cause decreased arthropod hydration can also lead to increased herbivory (Sabo et al., 2008; McCluney and Sabo, 2009; McCluney et al., 2017). Sap-feeding herbivores may profit from intermittent host water stress (i.e., the Pulsed Stress Hypothesis) by way of increases in nitrogen produced by plants undergoing periodic episodes of stress and recovery (White, 1984; Huberty and Denno, 2004). Roadside herbivores may benefit from leaves with increased nutritional value on trees stressed by de-icing salts (Kropczynska et al., 1988). Fine particulate matter pollutants may further facilitate herbivore attacks, possibly as a result of compromised plant defense (Dohmen, 1985). Thus, the bottom-up effects of urbanization affecting plant defensive chemistry and physiology can benefit herbivores according to the predictions of the Plant Stress Hypothesis (White, 1984), which are associated with detrimental effects on plant growth and fitness.

Urbanization can also increase nutrient deposition and elevate atmospheric carbon dioxide in some circumstances, which can effectively fertilize urban plants and increase their fitness, with concomitant positive effects on herbivore populations. Although the two hypotheses are not mutually exclusive, the Plant Vigor Hypothesis (**Figure 2**) posits that plants exhibiting vigorous growth may be more susceptible to herbivores and consequently suffer from greater herbivore attack (Price, 1991). Given that plants that thrive in urban areas often prefer high levels of nutrients (Lososová et al., 2006), and they are often limited by nitrogen for growth (LeBauer and Treseder, 2008; Fay et al., 2015), the addition of nutrients from fossil fuel combustion and artificial fertilizers is likely to benefit some herbivores through increased host productivity and quality in specific contexts (Martin and Stabler, 2002; Shochat, 2004; Raupp et al., 2010). For instance, some woody plants exposed to increased nutrient availability benefit urban herbivores including mites, scales, and adelgids (Herms, 2002). Elevated carbon dioxide emission, which is characteristic of cities, can lead to elevated plant growth and reproduction (Bidart-Bouzat et al., 2005). However, these benefits may be counteracted if increased plant growth leads to greater herbivory (Bezemer and Jones, 1998; Stiling and Cornelissen, 2007; Bidart-Bouzat and Imeh-Nathaniel, 2008; Massad and Dyer, 2010). Thus, though multiple aspects of urbanization may enhance plant fitness, these benefits may be short-lived if increased plant vigor ultimately results in intensified herbivore attacks and consequently increases top-down pressure from herbivores.

Top-Down Tri-trophic Effects

Urbanization can also alter top-down tri-trophic cascades (**Figure 2**). Some insect-eating bird species such as rock pigeons, *Columba livia*, and the European starling, *Sturnus vulgaris*, experience high abundance in cities (Aronson et al., 2014), likely because of decreased predation risk (Faeth et al., 2005) and increased food availability (Marzluff et al., 2001), such as through supplementary feeding by humans (Orros and Fellowes, 2012). This increase in bird abundance has been linked to increased predation on urban arthropods (Marzluff et al., 2001; Shochat, 2004), which may also be responsible for decreased foliage loss to some trees (Kozlov et al., 2017). However, more work is needed to understand the role of urbanization in shaping insectivorous bird-prey interactions, as our knowledge is limited to very few studies that suggest that the role of increased bird predation in decreasing herbivore abundance may be weaker than previously thought (but see Faeth et al., 2005; Bang et al., 2012; Turrini et al., 2016). For example, urbanization reduced both insectivorous birds and the gall-forming fly, *Eurosta solidaginis*, disrupting the typical stabilizing selection of gall size that is found in non-urban areas and thus dampening tri-trophic effects (Start et al., 2018).

Predation from both urban birds and insects may help suppress herbivore outbreaks, though the reduction of herbivores' natural enemies could release herbivores from predator pressure and lead to their proliferation (Raupp et al., 2010). Other tri-trophic cascades reviewed by Aronson et al. (2016), including host-parasite and songbird-nest predator relationships, have been reported to collapse in urban areas (Rodewald et al., 2011; Fischer et al., 2012; Calegaro-Marques and Amato, 2014; Nelson and Forbes, 2014). However, some multi-trophic relationships may persist, as seen with the sustained stability of an ant-aphid mutualism despite existence in a harsh urban environment (Rocha and Fellowes, 2018). More work is needed to clarify the ways in which urbanization affects plant-herbivore-predator interactions, though the majority of studies on tri-trophic interactions suggest a disadvantage to herbivores and thus increase in urban plant fitness.

Evolution of Plant Defenses

Plant defense mechanisms play a large role in structuring the bottom-up and top-down community forces that mediate urban plant-herbivore interactions. Though our understanding of how urbanization influences the evolution of these defense mechanisms is limited, existing research suggests that urbanization can influence the evolution of plant chemical defenses. Presently this has only been studied in white clover, *Trifolium repens*, which exhibits a Mendelian polymorphism for the production of hydrogen cyanide (HCN), a toxic anti-herbivore chemical defense. At least two studies have shown that plant populations evolve decreased production of HCN within cities, but this evolution does not seem to be related to changes in herbivory. The decreased HCN is instead due to how the presence of the chemical defense affects cold tolerance (Thompson et al., 2016; Johnson et al., 2018). HCN-producing clover experience reduced frost tolerance, whereby lower minimum winter temperatures in cities select against HCN producing plants, in favor of genotypes that lack HCN (Thompson et al., 2016). In addition to chemical defenses, which include secondary metabolites such as cardenolides and tannins (Cipollini and Stiles, 1992), other plant defense mechanisms may be structural (e.g., wax layers, trichomes, thorns; Alba et al., 2011) or phenological, where a plant engages in quick regeneration of vulnerable tissues or avoids herbivory at certain life stages (Saltz and Ward, 2000). Conversely, indirect defenses may involve the recruitment of other organisms (e.g., predators, host-seeking herbivore parasites) to prey on attackers to liberate the plant from herbivory (Sabelis et al., 2001). Studies have also shown that, under relaxed predation threat from herbivores, plants may rapidly evolve a weakened resistance response while increasing their competitive ability (Agrawal et al., 2012; Züst et al., 2012). Future studies investigating the frequency or strength of these defensive strategies in an urban context, such as along an urban-rural gradient, would advance our understanding of the evolution of plant-herbivore interactions in response to urbanization.

CONCLUSIONS AND FUTURE DIRECTIONS

Identifying how the ecology and evolution of plant-herbivore interactions are impacted in urban environments will contribute to understanding the role humans play in shaping our environment. As the field is rapidly growing, emerging research cannot be easily simplified into overarching trends: many of the abiotic factors that influence urban herbivorous arthropods appear to be taxon-specific, and the literature suggests varying responses to top-down and bottom-up influences such that some communities stabilize quickly in response to urbanization while others do not. Below, we discuss four research priorities that, if addressed, would markedly improve our understanding of the relationship between urban plants and herbivorous arthropods.

Research in Tropical Cities

The majority of studies focusing on urban plant-herbivore interactions have been conducted in temperate or arid climates. In fact, Raleigh, NC (temperate) and Phoenix, AZ (arid) are overrepresented in much of the studies that identify how urbanization influences herbivorous arthropods and plant-herbivore interactions. One of the reasons that Phoenix, AZ, is overrepresented in this literature is because of the Long-Term Ecological Research (LTER) site, Central Arizona-Phoenix (CAP-LTER), which specifically researches how urbanization influences ecology (Kaiser, 1997). Recently, Magle et al. (2012) identified that the majority of urban wildlife and conservation studies were in North America and Europe, noting that there is a need for future research in tropical ecosystems. Currently, there remains a paucity of tropical urban studies that investigate urban ecology and evolution. This is particularly troublesome as the tropics in Asia, South America, and Africa, which contain some of the world's most prominent and important biodiversity hotspots (Myers et al., 2000; Mcdonald et al., 2008), are areas that are experiencing rapid human population growth and urbanization (Chace and Walsh, 2006).

Many of the conclusions have been taken from studies of temperate regions and one arid region. This limits the overall scope of the many influences that urbanization can have on the ecology and evolution of organisms. For example, water availability varies between types of cities, with temperate cities being less water scarce than arid cities. Perhaps in tropical cities there is less of a pressure for water availability because of natural rainfall. Not only is there a need for studies in the tropics, but also a quantification of the possible stressors that occur between urban and non-urban habitats in each of these ecotypes.

Evolutionary Dynamics

There is a paucity of studies that examine how urbanization affects the evolution of herbivorous arthropods. Perhaps most striking is that only the classic peppered moth has been studied in the context of human impacts on adaptive evolution of an herbivorous arthropod in cities (Kettlewell, 1955, 1958; Brakefield and Liebert, 2000; Cook et al., 2005; van't Hof et al., 2011, 2016; Cook and Saccheri, 2013). In recent reviews of evolution in urban environments, it has been noted that there are only a few studies that have identified evidence for adaptation to urbanization (Donihue and Lambert, 2015; Johnson and Munshi-South, 2017; Rivkin et al., 2018). Urban adaptation has been studied in birds (Yeh, 2004; Partecke and Gwinner, 2007; Mueller et al., 2014), mice (Harris et al., 2013), fish (Whitehead et al., 2010; Wirgin et al., 2011), and even one plant (Cheptou et al., 2008). Studies of urban adaptation continue with a bias toward vertebrates, particularly those of conservation concern (Rivkin et al., 2018). However, given the vastly different life histories, genome sizes, and even mutation rates between vertebrates and invertebrates (e.g., Allio et al., 2017), there remains a large gap in our knowledge of how arthropods may be adapting to the urban environment. Future studies should not only identify potentially adaptive phenotypes, but also investigate the underlying genomic basis of these adaptations. With rapid advances in genome sequencing (Ekblom and Galindo, 2011; Andrews et al., 2016), it is becoming easier and more cost-effective to incorporate more taxa into studies of genome evolution.

Reciprocal Feedbacks Between Ecology and Evolution

It is imperative to account for the interrelationship between ecological and evolutionary processes when studying urban plant-herbivore interactions. Though the fields of urban ecology and urban evolution are still young, it is essential to integrate these perspectives to gain a more complete picture of the eco-evolutionary feedbacks that shape plant-herbivore interactions.

The ecological impacts of urbanization on the evolution of organisms that live in cities is not well-understood, let alone the specific eco-evolutionary relationship between urban-dwelling plants and herbivorous arthropods. Most of our current knowledge of how urbanization affects the evolution of plant defense comes from a single system- white clover, *Trifolium repens*. It would be valuable to understand how urbanization influences the evolution of plants with varying defense mechanisms in a variety of species, especially those with coevolved specialist herbivores.

Eco-evolutionary feedbacks in urban settings have also been explored in the context of phenotypic selection. Start et al. (2018) describe a tri-trophic interaction among the plant goldenrod, *Solidago altissima*, a gall-forming parasitic fly, *Eurosta solidaginis*, and the fly's own predator community, composed of two bird and three parasitoid species. In the absence of urbanization, gall size has been shown to be under stabilizing selection; birds tend to attack larger galls while parasitoids colonize smaller galls (Weis and Abrahamson, 1985). However, the authors conclude that urbanization liberates gall size from this form of selection as gall abundance and avian predation, but not parasitoid presence, increased with distance from the urban center (Start et al., 2018). More research is needed to elucidate the consequences of urbanization on the evolution of multiple populations within communities, and how these ecological changes can then affect community dynamics and patterns.

Heterogeneity of the Urban Landscape

Land use change, habitat fragmentation, and disturbance, which are inherently high in urban areas, are expected to influence population dynamics, the diversity and composition of species, as well as adaptive and non-adaptive dynamics of populations (Rivkin et al., 2018). Although cities tend to exhibit similarities such as increased impervious surface, they can vary in the history of development, disturbance, vegetation cover, and climate (McDonnell et al., 2009; Niemelä, 2011). Additionally, cities are often a mosaic of habitats, with significant heterogeneity of land-use types and constant changes to the landscape within the city (Niemelä, 2011; Pickett et al., 2017). Currently, the consequences of heterogeneity within and between cities has been through the lens of population genetics in vertebrate taxa (reviewed in Rivkin et al., 2018). The effect of such habitat changes and disturbance has not been investigated in the context of plant-herbivore interactions and should be a priority for future research.

AUTHOR CONTRIBUTIONS

MJ conceived the article. LM and SB wrote the first draft. MJ and HW edited subsequent drafts. All authors wrote the final draft.

FUNDING

This work was funded by NSERC Discovery grants to MJ and HW, a Canadian Research Chair to MJ, and the NSERC CREATE program ADVENT/ENVIRO.

ACKNOWLEDGMENTS

We thank LR Rivkin for valuable comments on a draft version of this review. We would also like to thank the reviewers and the editor for their comments and suggestions to improve this review.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00310/full#supplementary-material

REFERENCES

Agrawal, A. A. (2011). Current trends in the evolutionary ecology of plant defence. *Funct. Ecol*. 25, 421–433. doi: 10.1111/j.1365-2435.2010.01796.x

Agrawal, A. A., Hastings, A. P., Johnson, M. T. J., Maron, J. L., and Salminen, J.-P. (2012). Insect herbivores drive real-time ecological and evolutionary change in plant populations. *Science* 338, 113–116. doi: 10.1126/science.1225977

Alba, J. M., Glas, J. J., Schimmel, B. C. J., and Kant, M. R. (2011). Avoidance and suppression of plant defenses by herbivores and pathogens. *J. Plant Interact*. 6, 221–227. doi: 10.1080/17429145.2010.551670

Allio, R., Donega, S., Galtier, N., and Nabholz, B. (2017). Large variation in the ratio of mitochondrial to nuclear mutation rate across animals: implications for genetic diversity and the use of mitochondrial DNA as a molecular marker. *Mol. Biol. Evol*. 34, 2762–2772. doi: 10.1093/molbev/msx197

Altermatt, F., and Ebert, D. (2016). Reduced flight-to-light behaviour of moth populations exposed to long-term urban light pollution. *Biol. Lett*. 12, 3–6. doi: 10.1098/rsbl.2016.0111

Althoff, D. M., Segraves, K. A., and Johnson, M. T. (2014). Testing for coevolutionary diversification: linking pattern with process. *Trends Ecol. Evol*. 29, 82–89. doi: 10.1016/j.tree.2013.11.003

Andrade, R., Bateman, H. L., and Kang, Y. (2017). Seasonality and land cover characteristics drive aphid dynamics in an arid city. *J. Arid Environ*. 144, 12–20. doi: 10.1016/j.jaridenv.2017.04.007

Andrews, K. R., Good, J. M., Miller, M. R., Luikart, G., and Hohenlohe, P. A. (2016). Harnessing the power of RADseq for ecological and evolutionary genomics. *Nat. Rev. Genet*. 17, 81–92. doi: 10.1038/nrg.2015.28

Arnfield, A. J. (2003). Two decades of urban climate research: a review of turbulence, exchanges of energy and water, and the urban heat island. *Int. J. of Climatol*. 23, 1–26. doi: 10.1002/joc.859

Aronson, M. F., La Sorte, F. A., Nilon, C. H., Katti, M., Goddard, M. A., Lepczyk, C. A., et al. (2014). A global analysis of the impacts of urbanization on bird and plant diversity reveals key anthropogenic drivers. *Proc. Royal Soc. B*. 281:20133330. doi: 10.1098/rspb.2013.3330

Aronson, M. F., Nilon, C. H., Lepczyk, C. A., Parker, T. S., Warren, P. S., Cilliers, S. S., et al. (2016). Hierarchical filters determine community assembly of urban species pools. *Ecology* 97, 2952–2963. doi: 10.1002/ecy.1535

Arredondo, T. M., Marchini, G. L., and Cruzan, M. B. (2018). Evidence for human-mediated range expansion and gene flow in an invasive grass. *Proc. R. Soc. B*. 285:20181125. doi: 10.1098/rspb.2018.1125

Bang, C., and Faeth, S. H. (2011). Variation in arthropod communities in response to urbanization: seven years of arthropod monitoring in a desert city. *Landsc. Urban Plan*. 103, 383–399. doi: 10.1016/j.landurbplan.2011.08.013

Bang, C., Faeth, S. H., and Sabo, J. L. (2012). Control of arthropod abundance, richness, and composition in a heterogeneous desert city. *Ecol. Monogr*. 82, 85–100. doi: 10.1890/11-0828.1

Bezemer, T. M., and Jones, T. H. (1998). Plant-insect herbivore interactions in elevated atmospheric CO_2: Quantitative analyses and guild effects. *Oikos* 82, 212–222. doi: 10.2307/3546961

Bidart-Bouzat, M. G., and Imeh-Nathaniel, A. (2008). Global change effects on plant chemical defenses against insect herbivores. *J. Integr. Plant Biol*. 50, 1339–1354. doi: 10.1111/j.1744-7909.2008.00751.x

Bidart-Bouzat, M. G., Mithen, R., and Berenbaum, M. R. (2005). Elevated CO_2 influences herbivory-induced defense responses of *Arabidopsis thaliana*. *Oecologia* 145, 415–424. doi: 10.1007/s00442-005-0158-5

Bolger, D. T., Suarez, A. V., Crooks, K. R., Morrison, S. A., Case, J., Suarez, V., et al. (2000). Arthropods in urban habitat fragments in Southern California: Area, age, and edge effects. *Ecol. Appl*. 10, 1230–1248. doi: 10.1890/1051-0761(2000)010[1230:AIUHFI]2.0.CO;2

Bowler, D. E., Buyung-Ali, L., Knight, T. M., and Pullin, A. S. (2010). Urban greening to cool towns and cities: a systematic review of the empirical evidence. *Landsc. Urban Plan*. 97, 147–155. doi: 10.1016/j.landurbplan.2010.05.006

Brakefield, P. M., and Liebert, T. G. (2000). Evolutionary dynamics of declining melanism in the peppered moth in The Netherlands. *Proc. R. Soc. B*. 267, 1953–1957. doi: 10.1098/rspb.2000.1235

Burghardt, K. T., Tallamy, D. W., Philips, C., and Shropshire, K. J. (2010). Non-native plants reduce abundance, richness, and host specialization in lepidopteran communities. *Ecosphere* 15, 1–22. doi: 10.1890/ES10-00032.1

Buyantuyev, A., and Wu, J. (2009). Urbanization alters spatiotemporal patterns of ecosystem primary production: a case study of the Phoenix metropolitan region, USA. *J. Arid Environ*. 73, 512–520. doi: 10.1016/j.jaridenv.2008.12.015

Calegaro-Marques, C., and Amato, S. B. (2014). Urbanization breaks up host-parasite interactions: a case study on parasite community ecology of rufous-bellied thrushes (*Turdus rufiventris*) along a rural-urban gradient. *PLoS ONE* 9:e103144. doi: 10.1371/journal.pone.0103144

Chace, J. F., and Walsh, J. J. (2006). Urban effects on native avifauna: a review. *Landsc. Urban Plan*. 74, 46–69. doi: 10.1016/j.landurbplan.2004.08.007

Cheptou, P.-O., Carrue, O., Rouifed, S., and Cantarel, A. (2008). Rapid evolution of seed dispersal in an urban environment in the weed *Crepis sancta*. *Proc. Natl. Acad. Sci. U.S.A*. 105, 3796–3799. doi: 10.1073/pnas.0708446105

Chown, S. L., and Duffy, G. A. (2015). Thermal physiology and urbanization: perspectives on exit, entry and transformation rules. *Funct. Ecol*. 29, 902–912. doi: 10.1111/1365-2435.12478

Chown, S. L., Sørensen, J. G., and Terblanche, J. S. (2011). Water loss in insects: an environmental change perspective. *J. Insect Physiol*. 57, 1070–1084. doi: 10.1016/j.jinsphys.2011.05.004

Christie, F. J., and Hochuli, D. F. (2005). Elevated levels of herbivory in urban landscapes: are declines in tree health more than an edge effect? *Ecol. Soc*. 10, 1–10. doi: 10.5751/ES-00704-100110

Cinzano, P., Falchi, F., and Elvidge, C. D. (2001). The first world atlas of the artificial night sky brightness. *Mon. Not. Royal Astron. Soc*. 328, 689–707. doi: 10.1046/j.1365-8711.2001.04882.x

Cipollini, M. L., and Stiles, E. W. (1992). Relative risks of microbial rot for fleshy fruits: significance with respect to dispersal and selection for secondary defense. *Adv. Ecol. Res*. 23, 35–91. doi: 10.1016/S0065-2504(08)60146-4

Cook, L. M., and Saccheri, I. J. (2013). The peppered moth and industrial melanism: evolution of a natural selection case study. *J. Hered*. 110, 207–212. doi: 10.1038/hdy.2012.92

Cook, L. M., Sutton, S. L., and Crawford, T. J. (2005). Melanic moth frequencies in Yorkshire, an old English industrial hot spot. *J. Hered*. 96, 522–528. doi: 10.1093/jhered/esi082

Cregg, B. M., and Dix, M. E. (2001). Tree moisture stress and insect damage in urban areas in relation to heat island effects. *J. Arboric*. 27, 8–17.

Crispo, E., Moore, J., Lee-Yaw, J. A., Gray, S. M., and Haller, B. C. (2011). Broken barriers: human-induced changes to gene flow and introgression in animals. *Bioessays* 33, 508–518. doi: 10.1002/bies.201000154

Cyr, H., and Pace, M. L. (1993). Magnitude and patterns of herbivory in aquatic and terrestrial ecosystems. *Nature* 361, 148–150. doi: 10.1038/361148a0

Dale, A. G., and Frank, S. D. (2014). Urban warming trumps natural enemy regulation of herbivorous pests. *Ecol. Appl*. 24, 1596–1607. doi: 10.1890/13-1961.1

Dale, A. G., and Frank, S. D. (2017). Warming and drought combine to increase pest insect fitness on urban trees. *PLoS ONE* 12:e0173844. doi: 10.1371/journal.pone.0173844

Dale, A. G., and Frank, S. D. (2018). Urban plants and climate drive unique arthropod interactions with unpredictable consequences. *Curr. Opin. Insect Sci*. 29, 27–33. doi: 10.1016/j.cois.2018.06.001

Davis, E. S., Murray, T. E., Fitzpatrick, N., Brown, M. J. F., and Paxton, R. J. (2010). Landscape effects on extremely fragmented populations of a rare solitary bee, *Colletes floralis*. *Mol. Ecol*. 19, 4922–4935. doi: 10.1111/j.1365-294X.2010.04868.x

De Carvalho Guimarães, C. D., Viana, J. P. R., and Cornelissen, T. (2014). A meta-analysis of the effects of fragmentation on herbivorous insects. *Environ. Entomol*. 43, 537–545. doi: 10.1603/EN13190

Dennis, E. B., Morgan, B. J. T., Roy, D. B., and Brereton, T. M. (2017). Urban indicators for UK butterflies. *Ecol. Indic*. 76, 184–193. doi: 10.1016/j.ecolind.2017.01.009

Desender, K., Small, E., Gaublomme, E., and Verdyck, P. (2005). Rural-urban gradients and the population genetic structure of woodland ground beetles. *Conserv. Genet*. 6, 51–62. doi: 10.1007/s10592-004-7748-3

Diamond, S. E., Cayton, H., Wepprich, T., Jenkins, C. N., Dunn, R. R., Haddad, N. M., et al. (2014). Unexpected phenological responses of butterflies to the interaction of urbanization and geographic temperature. *Ecology* 95, 2613–2621. doi: 10.1890/13-1848.1

Dohmen, G. P. P. (1985). Secondary effects of air pollution: enhanced aphid growth. *Environ. Pollution. Ser. A, Ecol. Biol.* 39, 227–234. doi: 10.1016/0143-1471(85)90099-6

Donihue, C. M., and Lambert, M. R. (2015). Adaptive evolution in urban ecosystems. *Ambio* 44, 194–203. doi: 10.1007/s13280-014-0547-2

Dronnet, S., Chapuisat, M., Vargo, E. L., Lohou, C., and Bagnères, A. G. (2005). Genetic analysis of the breeding system of an invasive subterranean termite, *Reticulitermes santonensis*, in urban and natural habitats. *Mol. Ecol.* 14, 1311–1320. doi: 10.1111/j.1365-294X.2005.02508.x

Egerer, M. H., Arel, C., Otoshi, M. D., Quistberg, R. D., Bichier, P., and Philpott, S. M. (2017). Urban arthropods respond variably to changes in landscape context and spatial scale. *J. Urban Ecol.* 3:jux001. doi: 10.1093/jue/jux001

Ehrlich, P. R., and Raven, P. H. (1964). Butterflies and plants: a study in coevolution. *Evolution* 18, 586–608. doi: 10.1111/j.1558-5646.1964.tb01674.x

Ekblom, R., and Galindo, J. (2011). Applications of next generation sequencing in molecular ecology of non-model organisms. *Heredity* 107, 1–15. doi: 10.1038/hdy.2010.152

Erhardt, A., and Thomas, J. A. (1991). "Lepidoptera as indicators of change in the semi-natural grasslands of lowland and upland Europe," in *The Conservation of Insects and their Habitats,* eds N. M. Collins and J. A. Thomas (London: Academic Press), 213–237.

Evans, E. W., Carlile, N. R., Innes, M. B., and Pitigala, N. (2013). Warm springs reduce parasitism of the cereal leaf beetle through phenological mismatch. *J. Appl. Entomol.* 137, 383–391. doi: 10.1111/jen.12028

Faeth, S. H., Bang, C., and Saari, S. (2011). Urban biodiversity: patterns and mechanisms. *Ann. N. Y. Acad. Sci.* 1223, 69–81. doi: 10.1111/j.1749-6632.2010.05925.x

Faeth, S. H., Marussich, W. A., Shochat, E., and Warren, P. S. (2005). Trophic dynamics in urban communities. *Bioscience* 55, 399–407. doi: 10.1641/0006-3568(2005)055[0399:TDIUC]2.0.CO;2

Fay, P. A., Prober, S. M., Harpole, W. S., Knops, J. M. H., Bakker, J. D., Borer, E. T., et al. (2015). Grassland productivity limited by multiple nutrients. *Nat. Plants* 1, 1–5. doi: 10.1038/nplants.2015.80

Fischer, J. D., Cleeton, S. H., Lyons, T. P., and Miller, J. R. (2012). Urbanization and the predation paradox: the role of trophic dynamics in structuring vertebrate communities. *BioScience* 62, 809–818. doi: 10.1525/bio.2012.62.9.6

Forister, M. L., Cousens, B., Harrison, J. G., Anderson, K., Thorne, J. H., Waetjen, D., et al. (2016). Increasing neonicotinoid use and the declining butterfly fauna of lowland California. *Biol. Lett.* 12:20160475. doi: 10.1098/rsbl.2016.0475

Gaston, K. J., Bennie, J., Davies, T. W., and Hopkins, J. (2013). The ecological impacts of nighttime light pollution: a mechanistic appraisal. *Biol. Rev.* 88, 912–927. doi: 10.1111/brv.12036

Gibb, H., and Hochuli, D. F. (2002). Habitat fragmentation in an urban environment: large and small fragments support different arthropod assemblages. *Biol. Conserv.* 106, 91–100. doi: 10.1016/S0006-3207(01)00232-4

Grimm, N. B., Faeth, S. H., Golubiewski, N. E., Redman, C. L., Wu, J., Bai, X., et al. (2008). Global change and the ecology of cities. *Science* 319, 756–760. doi: 10.1126/science.1150195

Hadley, N. F. (1994). *Water Relations of Terrestrial Arthropods*. San Diego, CA: Academic Press.

Hahn, M., Schotthöfer, A., Schmitz, J., Franke, L. A., and Brühl, C. A. (2015). The effects of agrochemicals on Lepidoptera, with a focus on moths, and their pollination service in field margin habitats. *Agric. Ecosyst. Environ.* 207, 153–162. doi: 10.1016/j.agee.2015.04.002

Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., et al. (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS ONE* 12:e0185809. doi: 10.1371/journal.pone.0185809

Hamblin, A. L., Youngsteadt, E., and Frank, S. D. (2018). Wild bee abundance declines with urban warming, regardless of floral density. *Urban Ecosyst.* 21, 419–428. doi: 10.1007/s11252-018-0731-4

Hamblin, A. L., Youngsteadt, E., López-Uribe, M. M., and Frank, S. D. (2017). Physiological thermal limits predict differential responses of bees to urban heat-island effects. *Biol. Lett.* 13:20170125. doi: 10.1098/rsbl.2017.0125

Harris, S. E., Munshi-South, J., Obergfell, C., and O'Neill, R. (2013). Signatures of rapid evolution in urban and rural transcriptomes of white-footed mice (*Peromyscus leucopus*) in the New York metropolitan area. *PLoS ONE* 8:e74938. doi: 10.1371/journal.pone.0074938

Harrison, T., Gibbs, J., and Winfree, R. (2018). Forest bees are replaced in agricultural and urban landscapes by native species with different phenologies and life-history traits. *Glob. Chang. Biol.* 24, 287–296. doi: 10.1111/gcb.13921

Hawkes, C. V., and Sullivan, J. J. (2001). The impact of herbivory on plants in different resource conditions: a meta-analysis. *Ecology* 82, 2045–2058. doi: 10.1890/0012-9658(2001)082[2045:TIOHOP]2.0.CO;2

Herms, D. (2002). Effects of fertilization on insect resistance of woody ornamental plants: reassessing an entrenched paradigm. *Environ. Entomol.* 31, 923–933. doi: 10.1603/0046-225X-31.6.923

Holderegger, R., and Di Giulio, M. (2010). The genetic effects of roads: a review of empirical evidence. *Basic Appl. Ecol.* 11, 522–531. doi: 10.1016/j.baae.2010.06.006

Hope, D., Gries, C., Zhu, W., Fagan, W. F., Redman, C. L., Grimm, N. B., et al. (2003). Socioeconomics drive urban plant diversity. *Proc. Natl. Acad. Sci. U.S.A.* 100, 8788–8792. doi: 10.1073/pnas.1537557100

Huberty, A. F., and Denno, R. F. (2004). Plant water stress and its consequences for herbivorous insects: a new synthesis. *Ecology* 85, 1383–1398. doi: 10.1890/03-0352

Imhoff, M. L., Zhang, P., Wolfe, R. E., and Bounoua, L. (2010). Remote sensing of the urban heat island effect across biomes in the continental USA. *Remote Sens. Environ.* 114, 504–513. doi: 10.1016/j.rse.2009.10.008

Isaksson, C. (2015). Urbanization, oxidative stress and inflammation: a question of evolving, acclimatizing or coping with urban environmental stress. *Funct. Ecol.* 29, 913–923. doi: 10.1111/1365-2435.12477

Johnson, M. T. J., and Munshi-South, J. (2017). Evolution of life in urban environments. *Science* 358:eaam8327. doi: 10.1126/science.aam8327

Johnson, M. T. J., Prashad, C. M., Lavoignat, M., and Saini, H. S. (2018). Contrasting the effects of natural selection, genetic drift and gene flow on urban evolution in white clover (*Trifolium repens*). *Proc. Royal Soc. B.* 285:20181019. doi: 10.1098/rspb.2018.1019

Jones, J., and Francis, C. M. (2003). The effects of light characteristics on avian mortality at lighthouses. *J. Avian Biol.* 34, 328–333. doi: 10.1111/j.0908-8857.2003.03183.x

Kaiser, J. (1997). Ecologists to study life in the fast lane. *Science* 278:375. doi: 10.1126/science.278.5337.375a

Keller, I., Nentwig, W., and Largiadèr, C. R. (2004). Recent habitat fragmentation due to roads can lead to significant genetic differentiation in an abundant flightless ground beetle. *Mol. Ecol.* 13, 2983–2994. doi: 10.1111/j.1365-294X.2004.02310.x

Kettlewell, H. B. (1955). Recognition of appropriate backgrounds by the pale and black phases of Lepidoptera. *Nature* 175, 943–944. doi: 10.1038/175943a0

Kettlewell, H. D. (1958). A survey of the frequencies of *Biston betularia* (L.)(Lep.) and its melanic forms in Great Britain. *J. Hered.* 12, 51–72. doi: 10.1038/hdy.1958.4

Kozlov, M. V., Lanta, V., Zverev, V., Rainio, K., Kunavin, M. A., and Zvereva, E. L. (2017). Decreased losses of woody plant foliage to insects in large urban areas are explained by bird predation. *Glob. Chang. Biol.* 23, 4354–4364. doi: 10.1111/gcb.13692

Kropczynska, D., Van De Vrie, M., and Tomczyk, A. (1988). Bionomics of *Eotetranychus tiliarium* and its phytoseiid predators. *Exp. Appl. Acarol.* 5, 65–81. doi: 10.1007/BF02053818

Labandeira, C. C. (2007). The origin of herbivory on land: initial patterns of plant tissue consumption by arthropods. *Insect Sci.* 14, 259–275. doi: 10.1111/j.1744-7917.2007.00152.x

Lahr, E. C., Dunn, R. R., and Frank, S. D. (2018). Getting ahead of the curve: cities as surrogates for global change. *Proc. Royal Soc. B.* 285:20180643. doi: 10.1098/rspb.2018.0643

Larsen, L. O., and Pedersen, J. N. (1982). The snapping response of the toad *Bufo bufo*, towards prey dummies at very low light intensities. *Amphib-Reptil.* 2, 321–327. doi: 10.1163/156853882X00248

LeBauer, D. S., and Treseder, K. K. (2008). Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* 89, 371–379. doi: 10.1890/06-2057.1

López-Uribe, M. M., Morreale, S. J., Santiago, C. K., and Danforth, B. N. (2015). Nest suitability, fine-scale population structure and male-mediated dispersal of a solitary ground nesting bee in an urban landscape. *PLoS ONE* 10:e0125719. doi: 10.1371/journal.pone.0125719

Lososová, Z., Chytrý, M., Kühn, I., Hájek, O., Horáková, V., Pyšek, P., et al. (2006). Patterns of plant traits in annual vegetation of man-made habitats in central Europe. *Perspect. Plant Ecol. Evol. Syst*. 8, 69–81. doi: 10.1016/j.ppees.2006.07.001

Lowe, A. J., Boshier, D., Ward, M., Bacles, C. F. E., and Navarro, C. (2005). Genetic resource impacts of habitat loss and degradation; Reconciling empirical evidence and predicted theory for neotropical trees. *J. Hered*. 95, 255–273. doi: 10.1038/sj.hdy.6800725

Magle, S. B., Hunt, V. M., Vernon, M., and Crooks, K. R. (2012). Urban wildlife research: past, present, and future. *Biol. Conserv*. 155, 23–32. doi: 10.1016/j.biocon.2012.06.018

Martin, C. A., and Stabler, L. B. (2002). Plant gas exchange and water status in urban desert landscapes. *J. Arid. Environ*. 51, 235–254. doi: 10.1006/jare.2001.0946

Martin, C. A., Warren, P. S., and Kinzig, A. P. (2004). Neighborhood socioeconomic status is a useful predictor of perennial landscape vegetation in residential neighborhoods and embedded small parks of Phoenix, AZ. *Landscape Urban Plan*. 69, 355–368. doi: 10.1016/j.landurbplan.2003.10.034

Marzluff, J. M., Bowman, R., and Donnelly, R. (2001). *Avian Ecology and Conservation in an Urbanizing World*. Boston, MA: Kluwer Academic. doi: 10.1007/978-1-4615-1531-9

Massad, T. J., and Dyer, L. A. (2010). A meta-analysis of the effects of global environmental change on plant-herbivore interactions. *Arthropod Plant Interact*. 4, 181–188. doi: 10.1007/s11829-010-9102-7

Mattson, W. J., and Haack, R. A. (1987). The role of drought in outbreaks of plant-eating insects. *Bioscience* 37, 110–118. doi: 10.2307/1310365

McCluney, K. E., Burdine, J. D., and Frank, S. D. (2017). Variation in arthropod hydration across US cities with distinct climate. *J. Urban Ecol*. 3:jux003. doi: 10.1093/jue/jux003

McCluney, K. E., George, T., and Frank, S. D. (2018). Water availability influences arthropod water demand, hydration and community composition on urban trees. *J. Urban Ecol*. 4:juy003. doi: 10.1093/jue/juy003

McCluney, K. E., and Sabo, J. L. (2009). Water availability directly determines per capita consumption at two trophic levels. *Ecology* 90, 1463–1469. doi: 10.1890/08-1626.1

Mcdonald, R. I., Kareiva, P., and Forman, R. T. T. (2008). The implications of current and future urbanization for global protected areas and biodiversity conservation. *Biol. Conserv*. 141, 1695–1703. doi: 10.1016/j.biocon.2008.04.025

McDonnell, M. J., Hahs, A. K., and Breuste, J. H. (2009). *Ecology of Cities and Towns: A Comparative Approach*. Cambridge, UK: Cambridge University Press. doi: 10.1017/CBO9780511609763

McDonnell, M. J., and Pickett, S. T. A. (1990). Ecosystem structure and function along urban-rural gradients: An unexploited opportunity for ecology. *Ecology* 71, 1232–1237. doi: 10.2307/1938259

McIntyre, N. E. (2000). Ecology of urban arthropods: a review and a call to action. *Ann. Entomol. Soc. Am*. 93, 825–835. doi: 10.1603/0013-8746(2000)093[0825:EOUAAR]2.0.CO;2

McIntyre, N. E., Rango, J., Fagan, W. F., and Faeth, S. H. (2001). Ground arthropod community structure in a heterogeneous urban environment. *Landsc. Urban Plan*. 52, 257–274. doi: 10.1016/S0169-2046(00)00122-5

McKinney, M. L. (2004). Measuring floristic homogenization by non-native plants in North America. *Glob. Ecol. Biogeogr*. 13, 47–53. doi: 10.1111/j.1466-882X.2004.00059.x

McKinney, M. L. (2008). Effects of urbanization on species richness: a review of plants and animals. *Urban Ecosyst*. 11, 161–176. doi: 10.1007/s11252-007-0045-4

Meineke, E. K., Classen, A. T., Sanders, N. J., and Davies, J. T. (2018). Herbarium specimens reveal increasing herbivory over the past century. *J. Ecol*. 107, 105–117. doi: 10.1111/1365-2745.13057

Meineke, E. K., and Frank, S. D. (2018). Water availability drives urban tree growth responses to herbivory and warming. *J. Appl. Ecol*. 55, 1701–1713. doi: 10.1111/1365-2664.13130

Miles, L. S., Dyer, R. J., and Verrelli, B. C. (2018a). Urban hubs of connectivity: contrasting patterns of gene flow within and among cities in the western black widow spider. *Proc. Royal Soc. B*. 285:20181224. doi: 10.1098/rspb.2018.1224

Miles, L. S., Johnson, J. C., Dyer, R. J., and Verrelli, B. C. (2018b). Urbanization as a facilitator of gene flow in a human health pest. *Mol. Ecol*. 27, 3219–3230. doi: 10.1111/mec.14783

Mueller, J. C., Edelaar, P., Carrete, M., Serrano, D., Potti, J., Blas, J., et al. (2014). Behaviour-related DRD 4 polymorphisms in invasive bird populations. *Mol. Ecol*. 23, 2876–2885. doi: 10.1111/mec.12763

Muratet, A., and Fontaine, B. (2015). Contrasting impacts of pesticides on butterflies and bumblebees in private gardens in France. *Biol. Conserv*. 182, 148–154. doi: 10.1016/j.biocon.2014.11.045

Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B., and Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature* 403, 853–858. doi: 10.1038/35002501

Nelson, A. E., and Forbes, A. A. (2014). Urban land use decouples plant-herbivore-parasitoid interactions at multiple spatial scales. *PLoS ONE* 9:e102127. doi: 10.1371/journal.pone.0102127

Niemelä, J. (2011). *Urban Ecology: Patterns, Processes, and Applications*. Oxford, UK: Oxford University Press. doi: 10.1093/acprof:oso/9780199563562.001.0001

Niemelä, J., and Kotze, D. J. (2009). Carabid beetle assemblages along urban to rural gradients: a review. *Landscape Urban Plan*. 92, 65–71. doi: 10.1016/j.landurbplan.2009.05.016

Oke, T. R. (1973). City size and the urban heat island. *Atmos. Environ. Pergamon Pres*. 7, 769–779. doi: 10.1016/0004-6981(73)90140-6

Orros, M., and Fellowes, M. (2012). Supplementary feeding of wild birds indirectly affects the local abundance of arthropod prey. *Basic Appl. Ecol*. 13, 286–293. doi: 10.1016/j.baae.2012.03.001

Partecke, J., and Gwinner, E. (2007). Increased sedentariness in European blackbirds following urbanization: a consequence of local adaptation? *Ecology* 88, 882–890. doi: 10.1890/06-1105

Philpott, S. M., Cotton, J., Bichier, P., Friedrich, R. L., Moorhead, L. C., Uno, S., et al. (2014). Local and landscape drivers of arthropod abundance, richness, and trophic composition in urban habitats. *Urban Ecosyst*. 17, 513–532. doi: 10.1007/s11252-013-0333-0

Pickett, S. T., Cadenasso, M. L., Grove, J. M., Boone, C. G., Groffman, P. M., Irwin, E., et al. (2011). Urban ecological systems: Scientific foundations and a decade of progress. *J. Environ. Manage*. 92, 331–362. doi: 10.1016/j.jenvman.2010.08.022

Pickett, S. T. A., Cadenasso, M. L., Rosi-Marshall, E. J., Belt, K. T., Groffman, P. M., Grove, J. M., et al. (2017). Dynamic heterogeneity: A framework to promote ecological integration and hypothesis generation in urban systems. *Urban Ecosyst*. 20, 1–14. doi: 10.1007/s11252-016-0574-9

Plummer, K. E., Hale, J. D., O'Callaghan, M. J., Sadler, J. P., and Siriwardena, G. M. (2016). Investigating the impact of street lighting changes on garden moth communities. *J. Urban Ecol*. 2:juw004. doi: 10.1093/jue/juw004

Price, P. W. (1991). The plant vigor hypothesis and herbivore Attack. *Oikos* 62, 244–251. doi: 10.2307/3545270

Pyšek, P. (1998). Alien and native species in Central European urban floras: a quantitative comparison. *J. Biogeogr*. 25, 155–163. doi: 10.1046/j.1365-2699.1998.251177.x

Raupp, M. J., Shrewsbury, P. M., and Herms, D. A. (2010). Ecology of Herbivorous Arthropods in Urban Landscapes. *Annu. Rev. Entomol*. 55, 19–38. doi: 10.1146/annurev-ento-112408-085351

Rickman, J. K., and Connor, E. F. (2003). The effect of urbanization on the quality of remnant habitats for leaf-mining Lepidoptera on *Quercus agrifolia*. *Ecography* 26, 777–787. doi: 10.1111/j.0906-7590.2003.03345.x

Rivkin, L. R., Santangelo, J. S., Alberti, M., Aronson, M. F. J., de Keyzer, C. W., Diamond, S. E., et al. (2018). A roadmap for urban evolutionary ecology. *Evol. Appl*. 18, 1–15. doi: 10.1111/eva.12734

Rocha, E. A., and Fellowes, M. D. E. (2018). Does urbanization explain differences in interactions between an insect herbivore and its natural enemies and mutualists? *Urban Ecosyst*. 21, 405–417. doi: 10.1007/s11252-017-0727-5

Rodewald, A. D., Kearns, L. J., and Shustack, D. P. (2011). Anthropogenic resource subsidies decouple predator-prey relationships. *Ecol. Appl*. 21, 936–943. doi: 10.1890/10-0863.1

Rossetti, M. R., Tscharntke, T., Aguilar, R., and Batáry, P. (2017). Responses of insect herbivores and herbivory to habitat fragmentation: a hierarchical meta-analysis. *Ecol. Lett*. 20, 264–272. doi: 10.1111/ele.12723

Rust, M. K., and Su, N. Y. (2012). Managing social insects of urban importance. *Annu. Rev. Ent*. 57, 355–375. doi: 10.1146/annurev-ento-120710-100634

Sabelis, M. W., Janssen, A., and Kant, M. R. (2001). The enemy of my enemy is my ally. *Science* 291, 2104–2105. doi: 10.1126/science.1059939

Sabo, J. L., McCluney, K. E., Marusenko, Y., Keller, A., and Soykan, C. U. (2008). Greenfall links groundwater to aboveground food webs in desert river floodplains. *Ecol. Monogr*. 78, 615–631. doi: 10.1890/07-1382.1

Sadler, J. P., Small, E. C., Fiszpan, H., Telfer, M. G., and Niemel,ä, J. (2006). Investigating environmental variation and landscape characteristics of an urban-rural gradient using woodland carabid assemblages. *J. Biogeogr*. 33, 1126–1138. doi: 10.1111/j.1365-2699.2006.01476.x

Sales, K., Vasudeva, R., Dickinson, M., Godwin, J., Lumley, A., Michalczyk, Ł., et al. (2018). Experimental heatwaves compromise sperm function and cause transgenerational damage in a model insect. *Nat. Commun.* 9:4771. doi: 10.1038/s41467-018-07273-z

Saltz, D., and Ward, D. (2000). Responding to a three-pronged attack: desert lilies subject to herbivory by dorcas gazelles. *Plant Ecol.* 148, 127–138. doi: 10.1023/A:1009895512145

Sánchez-Bayo, F., and Wyckhuys, K. A. (2019). Worldwide decline of the entomofauna: a review of its drivers. *Biol. Conserv*. 232, 8–27. doi: 10.1016/j.biocon.2019.01.020

Sattler, T., Duelli, P., Obrist, M. K., Arlettaz, R., and Moretti, M. (2010). Response of arthropod species richness and functional groups to urban habitat structure and management. *Landsc. Ecol*. 25, 941–954. doi: 10.1007/s10980-010-9473-2

Schaffer, B., and Mason, L. J. (1990). Effects of scale insect herbivory and shading on net gas exchange and growth of a subtropical tree species (*Guaiacum sanctum* L.). *Oecologia* 84, 468–473. doi: 10.1007/BF00328162

Schmitz, G. (1996). Urban ruderal sites as secondary habitats for phytophagous insects. *Verh. Ges. Okol.* 26, 581–585.

Schoville, S. D., Widmer, I., Deschamps-Cottin, M., and Manel, S. (2013). Morphological clines and weak drift along an urbanization gradient in the butterfly, *Pieris rapae*. *PLoS ONE* 8:e83095. doi: 10.1371/journal.pone.0083095

Schwartzberg, E. G., Jamieson, M. A., Raffa, K. F., Reich, P. B., Montgomery, R. A., and Lindroth, R. L. (2014). Simulated climate warming alters phenological synchrony between an outbreak insect herbivore and host trees. *Oecologia* 175, 1041–1049. doi: 10.1007/s00442-014-2960-4

Schwarz, K., Fragkias, M., Boone, C. G., Zhou, W., McHale, M., Grove, J. M., et al. (2015). Trees grow on money: Urban tree canopy cover and environmental justice. *PLoS ONE* 10:e0122051. doi: 10.1371/journal.pone.0122051

Seto, K. C., Fragkias, M., Gueneralp, B., and Reilly, M. K. (2011). A meta-analysis of global urban land expansion. *PLoS ONE* 6:e23777. doi: 10.1371/journal.pone.0023777

Seto, K. C., Güneralp, B., and Hutyra, L. R. (2012). Global forecasts of urban expansion to 2030 and direct impacts on biodiversity and carbon pools. *Proc. Natl. Acad. Sci U.S.A.* 109, 16083–16088. doi: 10.1073/pnas.1211658109

Shochat, E. (2004). Credit or debit? Resource input changes population dynamics of city-slicker birds. *Oikos* 106, 622–626. doi: 10.1111/j.0030-1299.2004.13159.x

Shrewsbury, P. M., and Raupp, M. J. (2000). Evaluation of components of vegetational texture for predicting azalea lace bug, *Stephanitis pyrioides* (Heteroptera: Tingidae), abundance in managed landscapes. *Environ. Entomol.* 29, 919–926. doi: 10.1603/0046-225X-29.5.919

Smith, L. S., Broyles, M. E. J., Larzleer, H. K., and Fellowes, M. D. E. (2015). Adding ecological value to the urban lawnscape. Insect abundance and diversity in grass-free lawns. *Biodivers. Conserv*. 24, 47–62. doi: 10.1007/s10531-014-0788-1

Somers, C. M., McCarry, B. E., Malek, F., and Quinn, J. S. (2004). Reduction of particulate air pollution lowers the risk of heritable mutations in mice. *Science* 304, 1008–1010. doi: 10.1126/science.1095815

Somers, C. M., Yauk, C. L., White, P. A., Parfett, C. L., and Quinn, J. S. (2002). Air pollution induces heritable DNA mutations. *Proc. Natl. Acad. Sci. U.S.A.* 99, 15904–15907. doi: 10.1073/pnas.252499499

Speight, M. R., Hails, R. S., Gilbert, M., and Foggo, A. (1998). Horse Chestnut Scale (*Pulvinaria regalis*) (Homoptera: Coccidae) and Urban host tree environment. *Ecology* 79, 1503–1513. doi: 10.1890/0012-9658(1998)079[1503:HCSPRH]2.0.CO;2

Start, D., Bonner, C., Weis, A. E., and Gilbert, B. (2018). Consumer-resource interactions along urbanization gradients drive natural selection. *Evolution* 72, 1863–1873. doi: 10.1111/evo.13544

Stiling, P., and Cornelissen, T. (2007). How does elevated carbon dioxide (CO_2) affect plant-herbivore interactions? A field experiment and meta-analysis of CO_2-mediated changes on plant chemistry and herbivore performance. *Glob. Change Biol.* 13, 1823–1842. doi: 10.1111/j.1365-2486.2007.01392.x

Storfer, A., Murphy, M. A., Spear, S. F., Holderegger, R., and Waits, L. P. (2010). Landscape genetics: where are we now? *Mol. Ecol.* 19, 3496–3514. doi: 10.1111/j.1365-294X.2010.04691.x

Strong, D. R., Lawton, J. H., and Southwood, R. (1984). *Insects on Plants*. Cambridge, MA: Harvard University Press.

Su, N. Y. (2002). Novel technologies for subterranean termite control. *Sociobiology* 40, 95–101.

Szczepaniec, A., Creary, S. F., Laskowski, K. L., Nyrop, J. P., and Raupp, M. J. (2011). Neonicotinoid insecticide imidacloprid causes outbreaks of spider mites on elm trees in urban landscapes. *PLoS ONE* 6:e20018. doi: 10.1371/journal.pone.0020018

Thompson, J. N. (1994). *The Coevolutionary Process*. Chicago, IL: University of Chicago Press. doi: 10.7208/chicago/9780226797670.001.0001

Thompson, K. A., Renaudin, M., and Johnson, M. T. J. (2016). Urbanization drives parallel adaptive clines in plant populations. *Proc. R. Soc. B*. 283:20162180. doi: 10.1098/rspb.2016.2180

Turcotte, M. M., Davies, T. J., Thomsen, C. J., and Johnson, M. T. (2014). Macroecological and macroevolutionary patterns of leaf herbivory across vascular plants. *Proc. Royal Soc. B*. 281:20140555. doi: 10.1098/rspb.2014.0555

Turrini, T., Sanders, D., and Knop, E. (2016). Effects of urbanization on direct and indirect interactions. *Ecol. Appl*. 26, 664–675. doi: 10.1890/14-1787

Vandergast, A. G., Lewallen, E. A., Deas, J., Bohonak, A. J., Weissman, D. B., and Fisher, R. N. (2009). Loss of genetic connectivity and diversity in urban microreserves in a southern California endemic Jerusalem cricket (Orthoptera: Stenopelmatidae: *Stenopelmatus* n. sp. "santa monica"). *J. Insect Conserv*. 13, 329–345. doi: 10.1007/s10841-008-9176-z

van't Hof, A. E., Campagne, P., Rigden, D. J., Yung, C. J., Lingley, J., Quail, M. A., et al. (2016). The industrial melanism mutation in British peppered moths is a transposable element. *Nature* 534, 102–105. doi: 10.1038/nature17951

van't Hof, A. E., Edmonds, N., Dalíkov,á, M., Marec, F., and Saccheri, I. J. (2011). Industrial melanism in British peppered moths has a singular and recent mutational origin. *Science* 332, 958–960. doi: 10.1126/science.1203043

Vergnes, A., Pellissier, V., Lemperiere, G., Rollard, C., and Clergeau, P. (2014). Urban densification causes the decline of ground-dwelling arthropods. *Biodivers. Conserv*. 23, 1859–1877. doi: 10.1007/s10531-014-0689-3

Vickruck, J. L., and Richards, M. H. (2017). Nesting habits influence population genetic structure of a bee living in anthropogenic disturbance. *Mol. Ecol*. 26, 2674–2686. doi: 10.1111/mec.14064

Warren, A. D. (1990). Predation of five species of Noctuidae at ultraviolet light by the Western Yellowjacket (Hymenoptera: Vespidae). *J. Lepidopterists' Soc*. 44:32.

Weis, A. E., and Abrahamson, W. G. (1985). Potential selective pressures by parasitoids on a plant- herbivore interaction. *Ecology* 66, 1261–1269. doi: 10.2307/1939179

White, T. C. R. (1969). An index to measure weather-induced stress of trees associated with outbreaks of psyllids in Australia. *Ecology* 50, 905–909. doi: 10.2307/1933707

White, T. C. R. (1984). The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia* 6, 90–105. doi: 10.1007/BF00379790

White, T. C. R. (2009). Plant vigour versus plant stress: a false dichotomy. *Oikos* 118, 807–808. doi: 10.1111/j.1600-0706.2009.17495.x

Whitehead, A., Triant, D. A., Champlin, D., and Nacci, D. (2010). Comparative transcriptomics implicates mechanisms of evolved pollution tolerance in a killifish population. *Mol. Ecol*. 19, 5186–5203. doi: 10.1111/j.1365-294X.2010.04829.x

Wilson, E. O. (2001). *The Diversity of Life*. Toronto, ON: Penguin.

Wirgin, I., Roy, N. K., Loftus, M., Chambers, R. C., Franks, D. G., and Hahn, M. E. (2011). Mechanistic basis of resistance to PCBs in Atlantic tomcod from the Hudson River. *Science* 331, 1322–1325. doi: 10.1126/science.1197296

Yauk, C. L., and Quinn, J. S. (1996). Multilocus DNA fingerprinting reveals high rate of heritable genetic mutation in herring gulls nesting in an industrialized urban site. *Proc. Natl. Acad. Sci*. *U.S.A*. 93, 12137–12141. doi: 10.1073/pnas.93.22.12137

Yeh, P. J. (2004). Rapid evolution of a sexually selected trait following population establishment in a novel habitat. *Evolution* 58, 166–174. doi: 10.1111/j.0014-3820.2004.tb01583.x

Young, A., Boyle, T., and Brown, T. (1996). The population genetic consequences of habitat fragmentation for plants. *Trends Ecol. Evol.* 11, 413–418. doi: 10.1016/0169-5347(96)10045-8

Young, A. G., Merriam, H. G., and Warwick, S. I. (1993). The effects of forest fragmentation on genetic variation in *Acer saccharum* Marsh. (sugar maple) populations. *J. Hered.* 71, 277–289. doi: 10.1038/hdy.1993.136

Youngsteadt, E., Dale, A. G., Terando, A. J., Dunn, R. R., and Frank, S. D. (2015). Do cities simulate climate change? A comparison of herbivore response to urban and global warming. *Glob. Change Biol.* 21, 97–105. doi: 10.1111/gcb.12692

Zhao, F., Zhang, W., Hoffmann, A. A., and Ma, C. S. (2014). Night warming on hot days produces novel impacts on development, survival and reproduction in a small arthropod. *J. An. Ecol.* 83, 769–778. doi: 10.1111/1365-2656.12196

Ziter, C. D., Pedersen, E. J., Kucharik, C. J., and Turner, M. G. (2019). Scale-dependent interactions between tree canopy cover and impervious surfaces reduce daytime urban heat during summer. *Proc. Natl. Acad. Sci. U.S.A.* 116:201817561. doi: 10.1073/pnas.1817561116

Züst, T., Heichinger, C., Grossniklaus, U., Harrington, R., Kliebenstein, D. J., and Turnbull, L. A. (2012). Natural enemies drive geographic variation in plant defenses. *Science* 338, 116–119. doi: 10.1126/science.1226397

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 28 August 2019
doi: 10.3389/fevo.2019.00314

Solitary Bee Life History Traits and Sex Mediate Responses to Manipulated Seasonal Temperatures and Season Length

Anthony H. Slominski and Laura A. Burkle*

Ecology Department, Montana State University, Bozeman, MT, United States

The effects of climate change on solitary bee species, the most diverse and abundant group of wild pollinators, remain poorly understood, limiting our ability to forecast consequences for bee-plant interactions and pollination services. Life history traits, such as overwintering life stage, sex, and body size may influence solitary bee responses to climate change by mediating the effects of temperature on physiological processes spanning fall, winter, and spring. Yet, most studies assessing the effects of temperature on solitary bees have focused on managed species and have isolated the effects of winter temperature. Here, we reared male and female individuals representing eight cavity-nesting solitary bee species that overwinter either as adults (i.e., *Osmia* spp.) or prepupae (i.e., *Megachile* spp.). Eight rearing treatments were used, in which we manipulated fall and spring temperature, fall duration, and the timing of spring onset. We measured pre-emergence mortality, pre-emergence weight loss, emergence timing, and post-emergence lifespan. We found that *Osmia* spp. responded primarily to the timing of spring onset, whereas *Megachile* spp. responded primarily to spring temperature. Early-spring onset increased both pre-emergence mortality and pre-emergence weight loss and reduced post-emergence lifespan in *Osmia* spp. In addition, treatments caused unequal shifts in the timing of emergence between male and female *Osmia* spp. By contrast, warmer spring temperature decreased weight loss, and increased lifespan in *Megachile* spp. These findings suggest that *Osmia* spp. may be more vulnerable to negative fitness consequences of climate change compared to *Megachile* spp., and that climate change may have implications for population-level sex-ratios and mating success in species of *Osmia*. This work helps build a mechanistic understanding of how life histories may mediate solitary bee responses to climate change, and how these responses may impact solitary bee fitness and plant-bee interactions.

Keywords: climate change, pollinators, overwinter, emergence, weight loss, lifespan

Edited by:
Gina Marie Wimp,
Georgetown University, United States

Reviewed by:
Theresa L. Pitts-Singer,
Pollinating Insect Biology, Management, Systematics Research (USDA-ARS), United States
Weronika Banaszak-Cibicka,
Poznan University of Life Sciences, Poland

****Correspondence:***
Anthony H. Slominski
anthony.slominski@montana.edu

Specialty section:
This article was submitted to Population and Evolutionary Dynamics,
a section of the journal Frontiers in Ecology and Evolution

Received: *01 March 2019*
Accepted: *06 August 2019*
Published: *28 August 2019*

Citation:
Slominski AH and Burkle LA (2019) Solitary Bee Life History Traits and Sex Mediate Responses to Manipulated Seasonal Temperatures and Season Length. Front. Ecol. Evol. 7:314. doi: 10.3389/fevo.2019.00314

INTRODUCTION

Temperature strongly influences most biological and ecological processes in plants and insects (Deutsch et al., 2008; Lambers et al., 2008), including processes, such as survival and phenology, that mediate plant-insect interactions. Thus, there is concern that climate change will affect plant-insect interactions (Tylianakis et al., 2008), and in turn, disrupt essential ecosystem services, like pollination (Hegland et al., 2009; Burkle et al., 2013; Vanbergen et al., 2013; Potts et al., 2016). Central to forecasting the consequences of climate-change for plant-pollinator interactions is a better understanding of the mechanisms, and underlying species traits, that mediate response patterns among plant and pollinator species (Cariveau and Winfree, 2015; Rafferty, 2017). While linking plant traits, such as flowering season and frost tolerance, with plant responses to climate change (Fitter and Fitter, 2002; Sherry et al., 2007; Cook et al., 2012; Gezon et al., 2016; Pardee et al., 2018) has improved our understanding of the consequences of climate change for plant species and plant-pollinator interactions, similar progress with pollinator species is lacking (Ogilvie and Forrest, 2017). In particular, few studies have examined the role of species traits in mediating solitary bee responses to climate change, the most diverse and abundant group of wild pollinators (but see Fründ et al., 2013).

Understanding how solitary bee species are responding to climate change is difficult in part because, unlike plants, there is little long-term data on phenology and species abundances to link with changes in climate (Ogilvie and Forrest, 2017). While studies incorporating temperature manipulations in the field impart realism (e.g., Forrest and Thomson, 2011; CaraDonna et al., 2018), controlled laboratory studies are better equipped to elucidate response mechanisms. However, solitary bee responses to temperature have only been investigated in a limited number of managed species (e.g., Bosch and Kemp, 2003, 2004; Sgolastra et al., 2010, 2011; O'Neill et al., 2014; Schenk et al., 2018a), and have primarily isolated responses to a single component of climate change (e.g., extended fall duration, winter temperature, or spring temperature, Bosch and Kemp, 2000, 2003; Sgolastra et al., 2011; Fründ et al., 2013; O'Neill et al., 2014; Schenk et al., 2018a; but see Bosch and Kemp, 2004). While such studies have provided an important foundation for understanding how temperature influences biological processes in solitary bees, bee life cycles can span several seasons or years (Forrest et al., 2019), and climate change is occurring year-round (Sparks and Menzel, 2002; Alexander et al., 2006; Burrows et al., 2011). In addition, the paucity of studies on non-managed species hinders our ability to generalize response patterns across species based on shared traits. Therefore, to gain a more accurate picture of how climate change is impacting solitary bees, and to avoid misleading conclusions based on temperature manipulations isolated within a single season, assessing responses to temperature across multiple seasons and life stages is required (Kingsolver et al., 2011).

For solitary bees, responses to climate change will likely be driven in part by the influence of temperature on biological processes occurring prior to emergence. For example, warmer temperatures can accelerate development (Radmacher and Strohm, 2010; O'Neill et al., 2014), reduce stored-energy reserves (Bosch and Kemp, 2003, 2004; O'Neill et al., 2011, 2014; Sgolastra et al., 2011; Schenk et al., 2018a), increase mortality prior to emergence (Bosch and Kemp, 2003; O'Neill et al., 2011), advance emergence (Forrest and Thomson, 2011), and reduce post-emergence lifespan (Bosch and Kemp, 2003, 2004; Bosch et al., 2010). In addition, elevated seasonal temperatures and shorter winters associated with climate change (Sparks and Menzel, 2002; Menzel et al., 2006) may shorten periods of cold exposure, which can disrupt diapause development (Hodek and Hodkova, 1988; Sgolastra et al., 2010) and cause increased pre-emergence energy consumption, increased winter mortality, and delayed emergence (Bosch and Kemp, 2003, 2004; Sgolastra et al., 2010). In turn, such effects on bee species could alter seasonal activity periods in bees (Memmott et al., 2007) and affect reproductive output (Bosch and Kemp, 2004; Bosch and Vicens, 2006), which could alter the composition and abundance of bee species in a community and influence bee-plant interactions.

Understanding solitary bee responses to climate change will ultimately depend on interplay among multiple biological mechanisms acting during different developmental stages. In insects and other organisms, the outcomes of this interplay can be complex (Post et al., 2008; Kingsolver et al., 2011), even counterintuitive (Cook et al., 2012), with responses depending on physiological processes associated with life-history traits (e.g., Diamond et al., 2011; Buckley and Kingsolver, 2012; Cook et al., 2012; Sgolastra et al., 2016; Schenk et al., 2018a). First, solitary bee species in temperate zones overwinter either as adults or as prepupae (Stephen et al., 1969). In both cases, bees overwinter on a fixed energetic budget and do not consume food until after they emerge. Overwintering life histories determine the seasonal timing of transitions between life stages, and may influence how physiological processes are impacted by temperature and season length. For example, adult-wintering species may have a higher metabolic demand during fall while in the nest (i.e., prior to overwintering) compared to prepupal-wintering species that overwinter in an immature life stage (Waters and Harrison, 2012). If so, fitness in adult-wintering species may be more sensitive to warmer, extended falls compared to prepupal-wintering species (Bosch et al., 2010). Further, cold exposure during winter can influence post-emergence fitness in adult-wintering species by affecting adult diapause development and termination (Sgolastra et al., 2010). By contrast, prepupal-wintering species develop into adults during spring and early summer and emerge in mid-summer. Therefore, prepupal-wintering species may be more sensitive to spring temperatures and less sensitive to fall and winter temperatures (Kemp and Bosch, 2000, 2001; Kemp et al., 2004; O'Neill et al., 2011). Second, body size and sex may influence the effects of temperature on pre-emergence energy consumption in solitary bees due to differences in the ratio of lipids to body mass entering winter (O'Neill et al., 2014). Size differences among species, and between male and female bees within species, may therefore mediate responses to temperature, particularly in adult-wintering solitary bee species during fall and winter.

Here, we used a combination of field and laboratory techniques that allowed us to investigate the influence of temperature and season length on mortality, stored-energy consumption, and the timing of emergence across multiple species of wild, cavity-nesting solitary bees that overwinter either as adults or prepupae. We investigated (1) how seasonal temperature interacts with the duration and timing of fall, winter, and spring to influence biological responses in cavity-nesting solitary bees, and (2) how overwintering life histories, body size, and sex mediate these responses. We reared eight cavity-nesting solitary bee species that overwinter either as adults or prepupae within one of eight treatment combinations (**Figure 1**). Treatments manipulated fall and spring temperature, the duration of fall, and the start of spring. We quantified the number of bees that died prior to emergence (i.e., pre-emergence mortality), weight loss during treatments (i.e., pre-emergence weight loss), the number of days it took bees to emerge after the start of the spring treatment (i.e., days to emergence), and lifespan after emergence without feeding (i.e., post-emergence lifespan).

Based on differences in overwintering life histories, as well as physiological responses to temperature observed in managed solitary bee species and other insects, we tested the following hypotheses. For adult-wintering species, we expected that elevated temperature, and extended fall duration would increase weight loss and pre-emergence mortality, and decrease both the number of days to emergence and post-emergence lifespan (Bosch and Kemp, 2004; **Figure 2**). We expected that these patterns for adult-wintering species would depend on sex and body size (**Figure 2**). For prepupal-wintering species, we hypothesized that elevated spring temperature would reduce the number of days to emergence, but we did not expect temperature during fall or the duration of fall to influence the timing of emergence (Kemp and Bosch, 2000; O'Neill et al., 2011; **Figure 2**). As a result of decreased days to emergence, and, in turn, less time spent in non-feeding life-stages, we predicted that elevated temperature would reduce pre-emergence weight loss and increase post-emergence lifespan in prepupal-wintering species (O'Neill et al., 2011; **Figure 2**). Because the prepupal life stage may be less sensitive to temperature (Pitts-Singer and James, 2009), we did not expect pre-emergence mortality in prepupal-wintering species to vary in this study, and we did not anticipate body size to mediate the effects of temperature. Finally, due to the potential influence of cold exposure during winter on adult diapause termination, we expected a shorter winter duration to increase both the number of days to emergence and pre-emergence weight loss in adult-wintering species, but not prepupal-wintering species (Bosch and Kemp, 2003; Sgolastra et al., 2010; but see Forrest et al., 2019; **Figure 2**).

MATERIALS AND METHODS

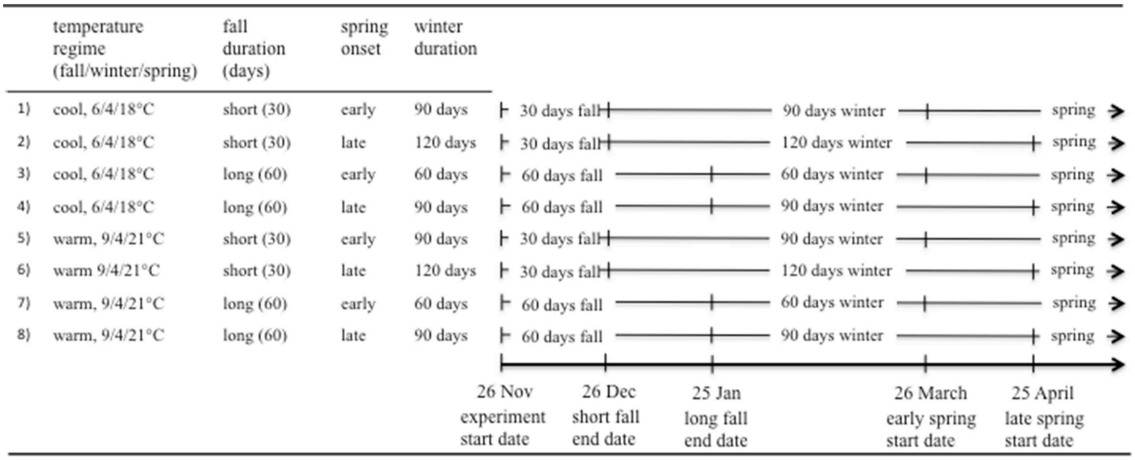
Focal Bee Species

We studied cavity-nesting solitary bees from the genus *Osmia* (mason bees) and the genus *Megachile* (leafcutter bees) (Hymenoptera: Megachilidae). In total, we studied eight species with at least 8 emerged male and 8 emerged female individuals per species. Six of the species overwinter as adults: *Osmia californica* Cresson, *Osmia coloradensis* Cresson, *Osmia lignaria* Say, *Osmia montana* Cresson, *Osmia tersula* Cockerell, and *Osmia vallicola* Cockerell, and two species overwinter as prepupae: *Megachile lapponica* Thomson and *Megachile relativa* Cresson (see **Table S1** for sample sizes). Species included in the study range in size from ∼6–20 mm long and are widespread and abundant pollinators of both wild plants and crops, making this a relevant suite of species to address the objectives of this study. In addition, as cavity-nesting species that nest above ground in existing cavities, these species are expected to be more susceptible to changing seasonal temperatures compared to species that nest below ground, due to the lack of insolating effects that nesting below the soil surface provides (Forrest and Thomson, 2011; Sgolastra et al., 2011).

Experimental Design

The solitary bees examined in this study were collected by placing trap-nests at six locations near Bozeman, Montana starting in March and April 2013 (see **Table S2** for descriptions of collection sites). Environmental conditions, not local genotypes, predicted adult emergence dates in several montane cavity-nesting bee species (Forrest and Thomson, 2011). Thus, we assumed that individuals of the same species collected at different locations in our study would not have pre-adapted sensitivities to local temperatures. After retrieving nests during September of 2013, we determined overwintering life-stage (i.e., adult or prepupae) by inspecting cocoons with a dissecting microscope. We assumed that each individual had developed to their overwintering life stage by this time, and that provisions had been consumed (Stephen and Torchio, 1961; Torchio, 1989). To avoid the disruption caused by opening cocoons, we used nesting materials, and cocoon sizes to approximate species; species identities were later confirmed after emergence.

To investigate how seasonal temperatures, as well as the duration and timing of seasons influenced responses in solitary bees, we reared individual bees inside gelatin capsules using 4.5 × 4.5 meter Conviron temperature-controlled chambers. Temperature treatments consisted of two regimes that manipulated fall and spring temperature, while exposing bees to the same winter temperature ("cool" regime: 6/4/18°C, "warm" regime: 9/4/21°C, fall/winter/spring; **Figure 1**). Temperature manipulations were based on seasonal average temperatures near Bozeman, MT (NOAA, https://www.ncdc.noaa.gov/) and were designed to simulate a projected 3°C increase in seasonal average temperatures caused by a doubling of atmospheric CO2 (IPCC, 2014). All treatments received the same winter temperature to isolate the effects of fall and spring temperature on physiological processes occurring during non-wintering periods of the solitary bee life cycle. Within each temperature regime, we used four factorial treatment combinations consisting of either a "short" or "long" fall duration and an "early" or "late" date of spring onset (**Figure 1**). We manipulated the duration of fall by moving bees between chambers set to fall and winter temperatures after 30 (short-fall duration) or 60 days (long-fall duration). Spring onset was manipulated by moving bees between chambers set to winter and spring temperatures on either 26 March (early-spring onset) or 25 April (late-spring onset). Fall duration and spring onset treatment combinations resulted in winter durations of either 60, 90, or 120 days. The start and end dates for each 90 day winter period depended on fall and spring treatment combinations, allowing an assessment of how fall duration and the date of spring onset interacted with winter duration. Historically (1971–2000), mean air temperatures near Bozeman, MT fall below 0°C from December through February (NOAA, https://www.ncdc.noaa.gov/). Using these data to roughly approximate the start and end dates of winter, we manipulated short-fall duration treatments to end during December and long-fall duration



	temperature regime (fall/winter/spring)	fall duration (days)	spring onset	winter duration
1)	cool, 6/4/18°C	short (30)	early	90 days
2)	cool, 6/4/18°C	short (30)	late	120 days
3)	cool, 6/4/18°C	long (60)	early	60 days
4)	cool, 6/4/18°C	long (60)	late	90 days
5)	warm, 9/4/21°C	short (30)	early	90 days
6)	warm 9/4/21°C	short (30)	late	120 days
7)	warm, 9/4/21°C	long (60)	early	60 days
8)	warm, 9/4/21°C	long (60)	late	90 days

FIGURE 1 | Description and timeline of experimental treatments. Treatments are 4 factorial combinations of fall duration ("short" or "long") and date of spring onset ("early" or "late"), nested within 2 temperature regimes that manipulated fall and spring temperature while holding winter temperature constant (i.e., "cool" regime: 6/4/18°C or "warm" regime: 9/4/21°C, fall/winter/spring). Fall and spring treatment combinations resulted in three winter durations (60, 90, 120 days), with the length of winter, as well as the start and end date of winter, determined by fall and spring treatment combinations.

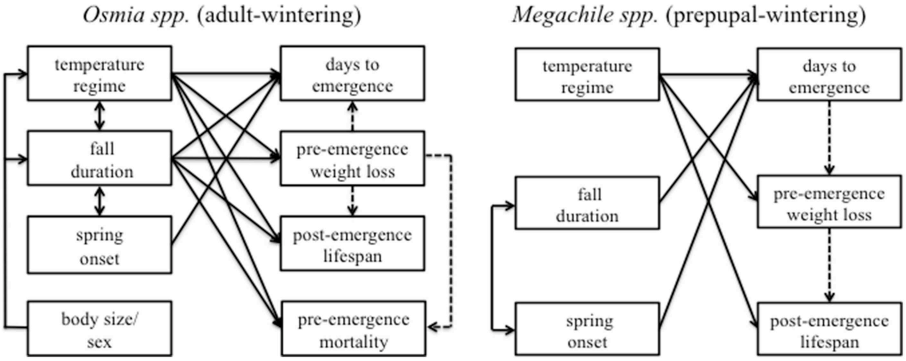


FIGURE 2 | Conceptual figure showing response variables (boxes on right side of each panel) and independent variables (boxes on left side of each panel) included in our hypotheses for *Osmia* spp. (i.e., adult-wintering species) and *Megachile* spp. (i.e., prepupal-wintering species). Solid directional arrows linking treatments and responses signify expectations of treatment effects. Arrows between explanatory variables show expected interactions, with arrow type (i.e., directional or double arrow) denoting the expected direction of the interaction (e.g., for adult-wintering bees, body size, and sex are expected to influence the effect of temperature and fall duration, but not vice versa, whereas fall duration, and temperature are expected to influence each other). Dashed lines represent expected correlations among response variables that we assessed with Piecewise SEM.

treatments to extend into January. For the spring treatment, we manipulated early-spring onset to begin during March (i.e., the first month of mean temperature above 0°C) and late-spring onset treatments to extend into April. We quantified bee weight prior to the start of the study by weighing each cocoon inside a labeled capsule (i.e., cocoon containing the bee plus all nesting materials and feces) to the nearest 0.1 mg using a Mettler Toledo NewClassic MF electronic balance. We distributed individuals from the same mother (i.e., cocoons from the same nesting tube), species, and nesting location equally across treatments. In total, we assigned 1,326 individual cocoons to treatments.

After moving bees into spring treatments, emergence was monitored twice each day (0800 and 1800) and the date on which bees emerged was recorded. We then calculated "days to emergence" as the number of days it took for a bee to emerge following the start of the spring treatment (early- vs. late-spring onset treatments differed by 30 days, but days to emergence were relative to the start of the spring treatment for all bees). We defined emergence as cocoons that had been torn open by a bee. Bees that did not emerge were inspected for the presence of parasitoids. Non-parasitized bees that appeared to be fully developed were identified to species, and used to analyze treatment effects on pre-emergence mortality. After emergence, we re-weighed the labeled capsules containing the emerged bee plus the cocoon, nesting materials, and feces. Post-emergence weights were recorded no more than 14 h after emergence and were used to calculate pre-emergence weight loss occurring during treatments. In some *Osmia* individuals, pre-emergence weight loss was negative, indicating that weight was *gained* between the start of treatments and emergence. Because *Osmia* individuals had reached a non-feeding life stage at the start of treatments, we speculate that moisture, either absorbed through the cuticle of the overwintering bees (May, 1972) or by the gelatin capsule encasing the bee cocoons, may have accounted for the observed weight gain. We considered any weight gain to be negligible and did not adjust or remove any data from our analysis of pre-emergence weight loss. Emerged bees were placed (still inside their capsule) in ambient indoor temperature (∼21°C) without feeding, where they were monitored once per day until death to determine post-emergence lifespan without feeding (Bosch and Kemp, 2000). We determined death by probing the intertegular space of the thorax (i.e., between the base of the wings) with forceps; if no movement was observed for three seconds, we assumed the bee was dead. Deceased bees were identified to species and sex was determined (McGavin, 1994; Michener, 2007; Sheffield et al., 2011; Rightmyer et al., 2013). Intertegular distance (i.e., body size) was quantified by measuring the distance (i.e., ocular units) between tegulae (i.e., base of the wings) using a calibrated reticle ruler and measurements were converting to millimeters (Cane, 1987; Cariveau et al., 2016). Weight loss was divided by intertegular distance and analyzed as the proportion of weight loss (mg) per unit body size (mm).

Statistical Analyses: Mixed-Effects and Generalized Linear Models

Statistical analyses were conducted using R statistical software version 3.4.3 (R Core Team, 2013). In total, we included eight cavity-nesting solitary bee species in the analyses. These species were represented by at least 8 emerged male and 8 emerged female individuals (i.e., 458 total bees; see **Table S2** for sample sizes in each treatment broken down by species and sex). Due to small sample sizes for some species, we could not analyze all response variables individually for each species. With the exception of pre-emergence mortality, and winter emergence (see below), we grouped species together by genus (i.e., *Osmia* spp. and *Megachile* spp.) and analyzed responses across species at the genus level. We investigated responses to fall and spring temperature, the duration of fall, the date of spring onset, and the duration of winter by analyzing four response variables individually for *Osmia* spp. and *Megachile* spp.: pre-emergence mortality, pre-emergence weight loss, days to emergence, and post-emergence lifespan. Days to emergence, post-emergence lifespan, and pre-emergence weight loss were analyzed using linear mixed-effects models ("nlme" package; Pinheiro et al., 2013). To improve normality, emergence was log-transformed in models fitted to *Osmia* spp. All linear mixed-effects models included a random intercept for "maternal identity" (i.e., bees from the same mother were contained in a single tube) nested within "collection location" (i.e., the field location of the trap-nest from which bees were collected). When necessary to achieve homogeneity of variances, we used the "varIdent" variance structure (Pinheiro et al., 2013). Only *O. coloradensis* and *O. tersula* had pre-emergence mortality numbers high enough to analyze (see Results). However, due to small sample sizes for *O. tersula* (**Table S2**), we combined pre-emergence mortality data for *O. tersula* with *O. coloradensis*, and analyzed them together. Only non-emerged bees that had developed into mature adults and had not been parasitized were included in the analysis of pre-emergence mortality. Pre-emergence mortality was analyzed using a generalized linear model with a "logit" link (R Core Team, 2013). In addition, a considerable number of *O. lignaria* emerged while still in the winter treatment. We assumed that emerging prior to the onset of spring would likely result in mortality, and were interested in whether treatments influenced this response. Thus, we analyzed the probability of *O. lignaria* emergence during winter using generalized linear models with a "logit" link (R Core Team, 2013).

All models included temperature (i.e., cool or warm), fall duration (i.e., short or long), spring onset (i.e., early or late), and sex as categorical independent variables. Given that body size could both influence the response to, and result from our treatments, we first fit the latter. We found treatment effects on body size for *Megachile* spp. but not *Osmia* spp. Therefore, we only included body size as a covariate in *Osmia* models. In each model, we included temperature × fall duration, temperature × spring onset, and fall duration × spring onset interactions to test for interactive effects of our treatment combinations. Interactions of sex and body size with each independent variable (i.e., temperature, fall, and spring) were included to assess, sex-, and size-specific responses to each treatment. A temperature × fall duration × spring onset three-way interaction was included to assess whether the mediating effects of winter duration on fall duration and spring onset depended on temperature. However, this interaction was not significant (i.e., $\alpha \leq 0.05$) in any models and we do not discuss it further. To determine the influence of each treatment and their interactions on each response variable, we used separate ANOVAs (R Core Team, 2013). We then evaluated our hypotheses with *post-hoc* tests using the "lsmeans" package (Lenth and Hervac, 2015), which tested for differences in means among pairwise comparisons between treatment levels (averaged over the other treatments) and generated an estimate of effect size. A "false discovery rate" p-value adjustment (Benjamini and Hochberg, 1995) was used when comparing means across multiple combinations of independent variables or covariates. Figures representing the model estimates (i.e., least square means) were produced with the ggplot2 package (Wickham, 2009).

Statistical Analyses: Piecewise SEM

To disentangle the direct and indirect effects of treatments on the response variables tested in this study (not including winter mortality), we fit Structural Equation Models (SEMs) separately for *Osmia* spp. and *Meagachile* spp. using the "PiecewiseSEM" package (Lefcheck, 2016). This approach allowed us to produce mechanistic models representing our *a priori* hypotheses for *Osmia* spp. and *Meagachile* spp. (**Figure 2**). The categorical independent variables temperature, fall duration, spring onset, and sex, were included in each PiecewiseSEM model fitted to days to emergence, pre-emergence weight loss, and post-emergence lifespan. As in each mixed-effects model, body size was only

TABLE 1 | ANOVA tables of the generalized linear models fitted separately to pre-emergence mortality and the probability of winter emergence (i.e., emerging prior to the initiation of spring treatments).

	Res. df	*Dev.*	*Res. Dev.*	*p*
Pre-Emergence Mortality				
Spring onset	1,242	8.52	147.19	<0.001
Body size	1,241	4.57	321.39	0.037
Winter Emergence				
Sex	1,243	27.32	262.08	<0.001
Spring onset	1,240	126.9	132.49	<0.001
Sex × temperature	1,238	7.01	124.66	0.008
Sex × fall duration	1,237	5.40	119.26	0.020
Temperature × fall duration	1,235	8.10	108.60	0.004

Pre-emergence mortality data was analyzed together for Osmia coloradensis and Osmia tersula. Winter emergence was analyzed for Osmia lignaria. Tables show only significant independent variables and interactions (α ≤ 0.005). See ***Table S3*** *for full ANOVA tables showing results for all independent variables and interactions fitted to pre-emergence mortality and winter emergence.*

included as an independent variable in the *Osmia* model. We included pre-emergence weight loss as an independent variable in the days to emergence and post-emergence lifespan models fitted to the *Osmia* data. In the *Megachile* models, we included days to emergence as an independent variable in the pre-emergence weight loss and post-emergence lifespan models. All models fitted within the PiecewiseSEM framework used the "lme" function in the "nlme" package (Pinheiro et al., 2013) and included a random intercept for maternal identity nested within collection location. Emergence and lifespan were log-transformed to improve normality. To facilitate comparisons among each link, coefficients were range standardized (Grace and Bollen, 2005). That is, each effect is reported as the percent of the range of values for a given response variable (when a variable is categorical), or as the percent change per one unit increase in a continuous explanatory variable. To highlight potentially distinct mechanistic pathways operating between *Osmia* spp. and *Megachile* spp., we only report significant links (i.e., $\alpha \leq 0.05$) and focus on indirect treatment effects to supplement the results of the mixed effects analyses.

RESULTS

Pre-emergence Mortality and Winter Emergence

Pre-emergence mortality was low (< 6%) in *O. lignaria*, *O. californica*, *O. montana*, *O. vallicola*, *M. lapponica*, and *M. relativa,* and higher in *O. coloradensis* (26%) and *O. tersula* (75%). *Osmia coloradensis* and *O. tersula* were more likely to survive and emerge when spring onset was late compared to early ($z = 3.01$, $p = 0.003$; (**Table 1** and **Figure 3**, see **Table S3** for ANOVA table showing full results for pre-emergence mortality). The probability of pre-emergence mortality decreased as body size increased (**Table 1**), but body size did not interact with the spring onset treatment to affect pre-emergence mortality (**Table S3**). Sex did not affect the probability of pre-emergence mortality (**Table S3**).

Osmia lignaria winter emergence (i.e., emergence prior to the start of the spring treatment) was influenced by the timing of spring onset, fall duration, and temperature, and differed between male and female bees (**Table 1**, see **Table S3** for ANOVA table showing full results for winter emergence). When spring onset was late and fall duration was long, males were more likely to emerge during winter compared to females ($z = 2.97$, $p < 0.001$; **Figure 3**). In addition, when spring onset was late and fall duration was long, male bees in the warm temperature treatment were more likely to emerge during winter compared to male bees in the cool treatment ($z = 2.23$, $p = 0.026$; **Figure 3**). Body size did not influence the likelihood of *O. lignaria* emergence during winter (**Table S3**).

Days to Emergence

In *Osmia* spp. (i.e., adult-wintering species), males emerged in 2.3 ± 0.4 fewer days relative to the start of the spring treatment compared to females ($t_{287} = 7.82$, $p < 0.001$; **Figure 4A** and **Table 2**, see **Table S4** for ANOVA tables showing full results for all response variables). The timing of spring onset influenced mean days to emergence in both male and female bees. When averaged across sex, bees in the early-spring onset treatment emerged in 1.4 ± 0.2 more days relative to the start of the spring treatment compared to the late-spring onset treatment ($t_{287} = 6.82$, $p < 0.001$; **Figure 4A** and **Table 2**). For females, the effect size of the spring onset treatment depended on fall duration (**Table 2**). On average, females emerged in the fewest days in the short-fall duration treatment when spring onset was late (i.e., 120 days winter), with the largest difference in days to emergence (3.6 ± 1.3 fewer days) occurring between the long-fall duration + early-spring onset treatment combination (i.e., 60 days of winter; $t_{287} = 3.67$, $p = 0.001$; **Figure 4A**).

Neither the temperature treatment nor body size influenced days to emergence in *Osmia* spp. (**Table S4**).

Male *Megachile* emerged in 3.2 ± 0.5 fewer days relative to the start of the spring treatment compared to females ($t_{38} = 6.50$, $p < 0.001$; **Figure 4D** and **Table 2**, see **Table S4** for ANOVA tables showing full results for all response variables). In contrast to *Osmia* spp., the temperature treatment had a strong effect on mean days to emergence in *Megachile* spp. (i.e., prepupal-wintering species; **Table 2**), causing bees in the warm treatment to emerge in 27.2 ± 0.4 fewer days on average compared to the cool treatment ($t_{38} = 60.42$, $p < 0.001$; **Figure 4D**). In addition, the spring onset treatment affected mean days to emergence in *Megachile* spp. (**Table 2**), but this effect was smaller compared to the temperature effect. On average, the late-spring onset treatment caused bees to emerge in 1.7 ± 0.5 fewer days relative to the start of the spring treatment compared to the early-spring onset treatment ($t_{38} = 3.73$, $p = 0.001$; **Figure 4D**).

The fall duration treatment did not affect days to emergence in *Megachile* spp. (**Table S4**).

Weight Loss

In *Osmia* spp., mean pre-emergence weight loss per mm of intertegular distance was greater in females compared to males (0.9 ± 0.3 mg/mm greater loss, $t_{252} = 3.06$, $p = 0.003$ **Figure 4B** and **Table 2**). When averaged across sex, the

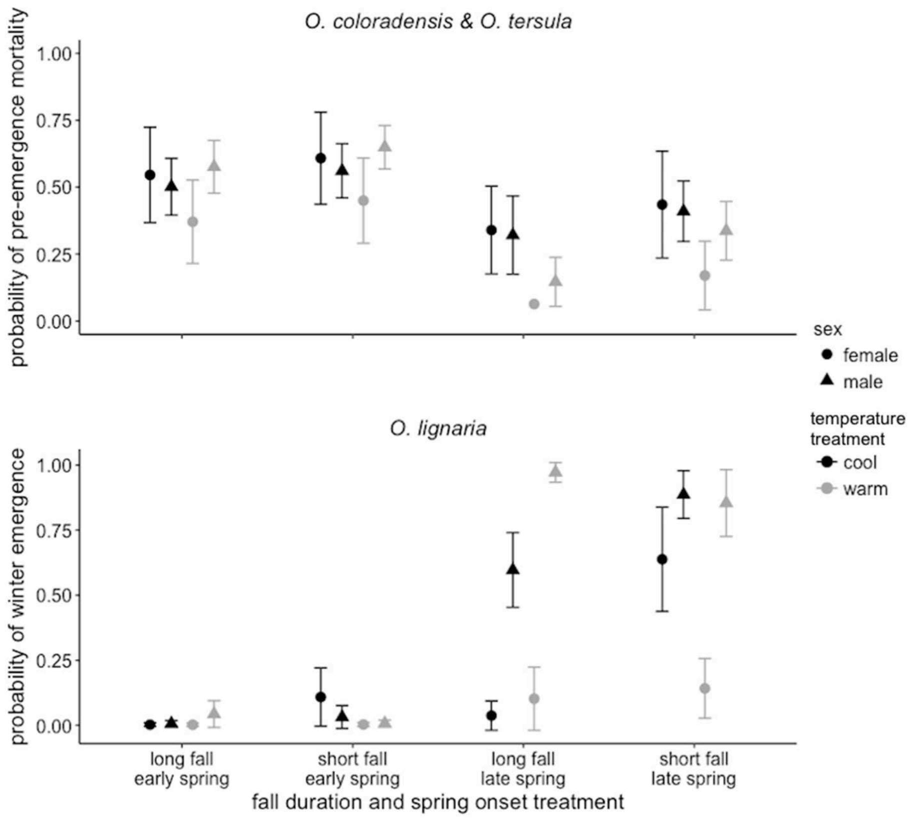


FIGURE 3 | The probabilities of pre-emergence mortality ± SE for *O. coloradensis* and *O. tersula* (top panel) and of winter emergence ± SE for *O. lignaria* (bottom panel). Probabilities ± SE shown for all treatment combinations separated by sex.

spring onset treatment affected mean pre-emergence weight loss (**Table 2**), with bees in the early-spring onset treatment losing 1.4 ± 0.2 mg/mm more weight on average compared to the late spring treatment ($t_{252} = 6.18$, $p < 0.001$; **Figure 4B**). The temperature treatment also affected mean pre-emergence weight loss, but only in females (**Table S4**). On average, female bees lost 1.3 ± 0.4 mg/mm more weight in the warm temperature treatment compared to the cool treatment ($t_{252} = 3.16$, $p = 0.004$; **Figure 4B**), whereas the effect of the temperature treatment on mean pre-emergence weight loss in males was not significant ($t_{252} = 0.90$, $p = 0.453$; **Figure 4B**). In addition, body size influenced mean pre-emergence weight loss (**Table 2**). Pre-emergence weight loss increased by 0.1 mg/mm per each 0.1 mm increase in intertegular distance ($t_{252} = 2.14$, $p = 0.033$; **Figure S1**).

There was no effect of the fall duration treatment on pre-emergence weight loss in *Osmia* spp. (**Table S4**).

In *Megachile* spp., the effect of temperature on pre-emergence weight loss was opposite to that observed in *Osmia* spp. and depended on the spring onset treatment (**Table 2**). In the late-spring onset treatment, temperature affected pre-emergence weight loss, with male *Megachile* losing 4.0 ± 1.2 mg/mm more weight in the cool temperature treatment compared to the warm treatment ($t_{38} = 3.44$, $p = 0.030$; **Figure 4E**), and females losing 6.1 ± 0.9 mg/mm more weight in the cool treatment compared to the warm treatment ($t_{38} = 6.83$, $p < 0.001$; **Figure 4E**). The difference in mean pre-emergence weight loss between the cool temperature treatment and the warm treatment was not significant for either male or female bees when spring onset was early (p-values > 0.095; **Figure 4E**). These patterns resulted in the greatest pre-emergence weight loss occurring in the cool treatment when spring onset was late for both male and female bees (average weight loss across sex: 13.9 ± 1.1 mg/mm; **Figure 4E**).

Neither fall duration nor sex influenced pre-emergence weight loss in *Megachile* spp. (**Table S4**).

Lifespan

In *Osmia* spp., mean post-emergence lifespan (without feeding) was 1.0 ± 0.2 days longer in male bees compared to female bees ($t_{279} = 4.48$, $p < 0.001$; **Figure 4C** and **Table 2**). Temperature affected mean post-emergence lifespan in *Osmia* spp., but the effect of temperature depended on spring onset, fall duration, and sex (**Table 2**). In the early-spring onset treatment, mean male post-emergence lifespan was 2.0 ± 0.3 days shorter in the warm temperature treatment compared to the cool treatment ($t_{279} = 6.28$, $p < 0.001$; **Figure 4C**). In the late-spring onset

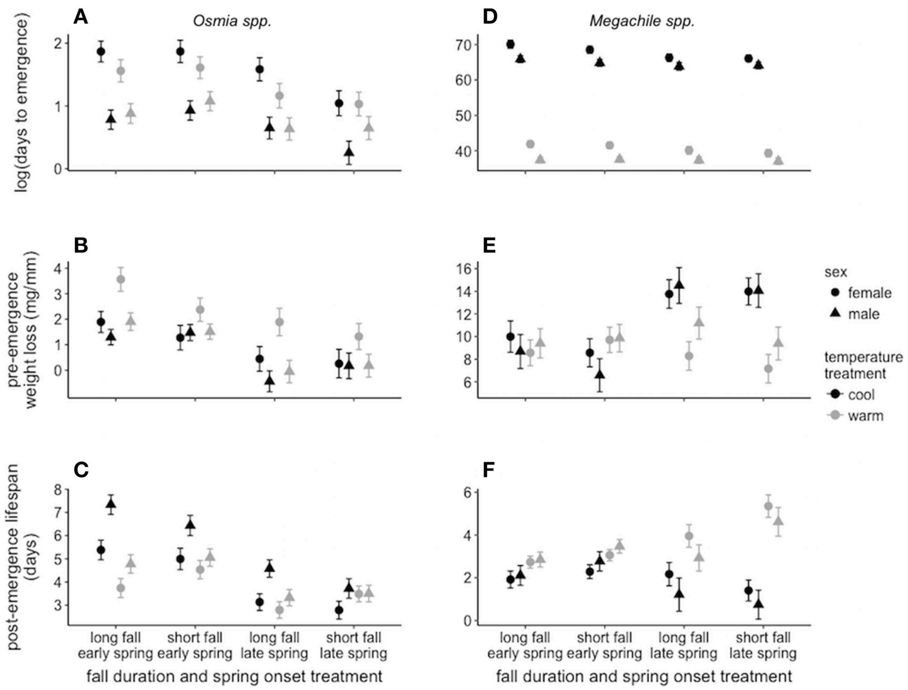


FIGURE 4 | Least square mean ± SE showing **(A)** *Osmia* spp. days to emergence, **(B)** *Osmia* spp. pre-emergence weight loss, **(C)** *Osmia* spp. post-emergence lifespan, **(D)** *Megachile* spp. days to emergence, **(E)** *Megachile* spp. pre-emergence weight loss, and **(F)** *Megachile* spp. post-emergence lifespan. Least square means ± SE shown for all treatment combinations separated by sex. Note negative weight loss values (i.e., weight gain) are likely due to moisture absorbed by the bee or the nesting materials during treatments.

TABLE 2 | ANOVA tables of the linear mixed-effects models fitted separately to *Osmia* spp. (i.e., adult-wintering species) and *Megachile* spp. (i.e., prepupal-wintering species).

***Osmia* spp.**	***df***	***F***	***p***	***Megachile* spp.**	***df***	***F***	***p***
Days To Emergence							
Sex	1,287	135.90	<0.001	Sex	1,38	137.53	<0.001
Spring onset	1,287	4206.00	<0.001	Temperature	1,38	4048.58	<0.001
Sex × spnng	1,287	4.88	0.028	Spring onset	1,38	9.48	0.004
Fall × spring	1,287	7.65	0.006				
Pre-Emergence Weight Loss							
Sex	1,252	33.68	<0.001	Temperature	1,38	16.90	<0.001
Temperature	1,252	7.38	0.007	Spring onset	1,38	13.15	0.001
Spring onset	1,252	58.86	<0.001	Temperature × spring	1,38	36.93	<0.001
Body size	1,252	13.94	<0.001				
Post-Emergence Lifespan							
Sex	1,279	25.44	<0.001	Temperature	1,34	1.34	<0.001
Temperature	1,279	20.05	<0.001	Temperature × spring	1,34	14.91	<0.001
Spring onset	1,279	116.26	<0.001				
Sex × temperature	1,279	11.41	0.001				
Sex × spring	1,279	8.28	0.004				
Temperature × fall	1,279	11.71	0.001				
Temperature × spring	1,279	15.42	<0.001				

Tables show only significant independent variables and interactions (α ≤ 0.05) affecting the response variables: days to emergence (relative to the start of the spring onset treatment), pre-emergence weight loss, and post-emergence lifespan (without feeding). See ***Table S4*** *for full ANOVA tables showing results for all independent variables and interactions fitted to these response variables.*

treatment, the effect of temperature on mean male post-emergence lifespan depended on fall duration (**Table 2**). In the late-spring onset + long-fall duration treatment combination, mean male *Osmia* post-emergence lifespan was 1.3 ± 0.3 days shorter in the warm temperature treatment compared to the cool treatment ($t_{279} = 3.93$, $p = 0.001$; **Figure 4C**). In contrast, the effect of the temperature treatment on mean male post-emergence lifespan was not significant in the late-spring onset treatment when fall duration was short ($t_{279} = 0.60$, $p = 0.558$; **Figure 4C**). For female *Osmia*, post-emergence lifespan was 1.6 ± 0.4 days shorter on average in the warm temperature treatment compared to the cool treatment, but only in the early-spring onset treatment when fall duration was long ($t_{279} = 4.02$, $p < 0.001$; **Figure 4C**).

In contrast to *Osmia* spp., mean post-emergence lifespan in *Megachile* spp. was shorter in the cool temperature treatment compared to the warm treatment (1.8 ± 0.3 days shorter, $t_{34} = 6.07$, $p < 0.001$; **Figure 4F**), but the effect size of the temperature treatment depended on the spring onset treatment (**Table 2**). In the cool temperature treatment, *Megachile* individuals lived 2.8 ± 0.5 days longer on average compared to the warm treatment when spring onset was late ($t_{34} = 5.60$, $p < 0.001$; **Figure 4F**), but only 0.8 ± 0.3 days longer when spring onset was early ($t_{34} = 2.74$, $p = 0.011$; **Figure 4F**). These patterns resulted in the longest mean post-emergence lifespan (4.3 ± 0.4 days) occurring in the warm treatment when spring onset was late, and the shortest mean post-emergence lifespan (1.2 ± 0.4 days) occurring in the cool treatment when spring onset was late.

Sex did not influence mean post-emergence lifespan in *Megachile* spp. (**Table S4**).

Piecewise Structural Equation Models

The *Osmia* model (i.e., adult-wintering species) did not differ significantly from the data (Fisher *C*-score = 0.94, $df = 2$, $p = 0.625$; **Figure 5**), indicating our model provided an appropriate fit (Lefcheck, 2016). Our model was most effective at describing days to emergence ($R^2c = 0.65$), followed by longevity ($R^2c = 0.49$), then weight loss ($R^2c = 0.16$).

There was a significant positive correlation between pre-emergence weight loss and the number of days to emergence relative to the start of the spring treatment, with a 29.5 ± 12.1% increase in days to emergence associated with each 1 mg/mm increase in weight loss ($p = 0.016$; **Figure 5**). The effect sizes of the significant ($\alpha \leq 0.005$) direct treatment effects on pre-emergence weight loss were small (> 0.03; **Figure 5**), which suggests that pre-emergence weight loss was more strongly influenced by the indirect effects of treatments via their influence on emergence timing, particularly early-spring onset. This also suggests that the timing of emergence responded more strongly to the direct effect of early-spring onset compared to the influence on pre-emergence weight loss. In addition, there was a significant negative correlation between pre-emergence weight loss and post-emergence lifespan, with post-emergence lifespan decreasing by 34.4 ± 7.4% ($p < 0.001$) per each 1 mg/mm increase in pre-emergence weight loss (**Figure 5**). Weak direct effects of treatments on pre-emergence weight loss suggest that post-emergence lifespan responded most strongly to the direct effect of early-spring onset (16 ± 1.8% decrease in lifespan compared to late-spring onset, $p < 0.001$) and to the indirect effect of early-spring onset via the effect of the early-spring treatment on the timing of emergence and pre-emergence weight loss (i.e., the early spring—emergence—weight loss—lifespan pathway; **Figure 5**).

The *Megachile* model (i.e., prepupal-wintering species) did not differ significantly from the data (fisher *C*-score = 1.01 $df = 4$, $p = 0.909$; **Figure 5**), suggesting an appropriate fit to the data (Lefcheck, 2016). Our model was most effective at describing emergence ($R^2c = 0.97$), followed by weight loss ($R^2c = 0.56$), and longevity ($R^2c = 0.46$).

Days to emergence relative the start of the spring treatment influenced pre-emergence weight loss, with a 13.5 ± 5.5% ($p = 0.017$) increase in pre-emergence weight loss per each one day increase in days to emergence (**Figure 5**). The warm treatment indirectly reduced pre-emergence weight loss by 9.4% compared to the cool treatment via the effect of the temperature treatment on days to emergence (**Figure 5**). In addition, there was a significant negative correlation between pre-emergence weight loss and post-emergence lifespan, with post-emergence lifespan decreasing by 33.3 ± 13.0% ($p = 0.014$) per each 1 mg/mm increase in weight loss (**Figure 5**). Thus, it appears that the effect of temperature on post-emergence lifespan in *Megachile* spp. was mediated by the indirect effect of temperature on days to emergence and weight loss (i.e., the temperature—emergence—weight loss—lifespan pathway; **Figure 5**).

DISCUSSION

Our ability to understand and forecast the consequences of climate change for plant-pollinator interactions has benefited from linking plant life history traits to response patterns observed among plants species (e.g., Fitter and Fitter, 2002; Sherry et al., 2007; Cook et al., 2012; Burkle et al., 2013; Kudo and Ida, 2013; Gezon et al., 2016; Pardee et al., 2018). However, such progress with pollinator species is lacking. Here, we investigated the influence of overwintering life history, sex, and body size on solitary bee responses to controlled temperature and season length manipulations that spanned fall, winter, and spring. There were negative direct and indirect effects of early spring onset, increased temperature, and a long fall season on winter emergence, pre-emergence mortality, pre-emergence weight loss, and post-emergence lifespan in *Osmia* spp. (i.e., adult-wintering species), but not *Megachile* spp. (i.e., prepupal-wintering species). This indicates that climate change may be more detrimental to the fitness of adult-wintering species compared to prepupal-wintering species. In addition, there were treatment effects on emergence that depended on overwintering life history and sex. Observed phenological responses suggest that there may be implications of climate change for phenological synchrony among plant and pollinator species, as well as intraspecific male-female interactions that can be better understood by considering pollinator overwintering life histories.

Reduced cold exposure during adult diapause experienced by *Osmia* spp. in the early-spring onset treatment likely delayed emergence relative to the start of the spring treatment and increased pre-emergence weight loss, as well as increased pre-emergence mortality in *O. coloradensis* and *O. tersula*. In

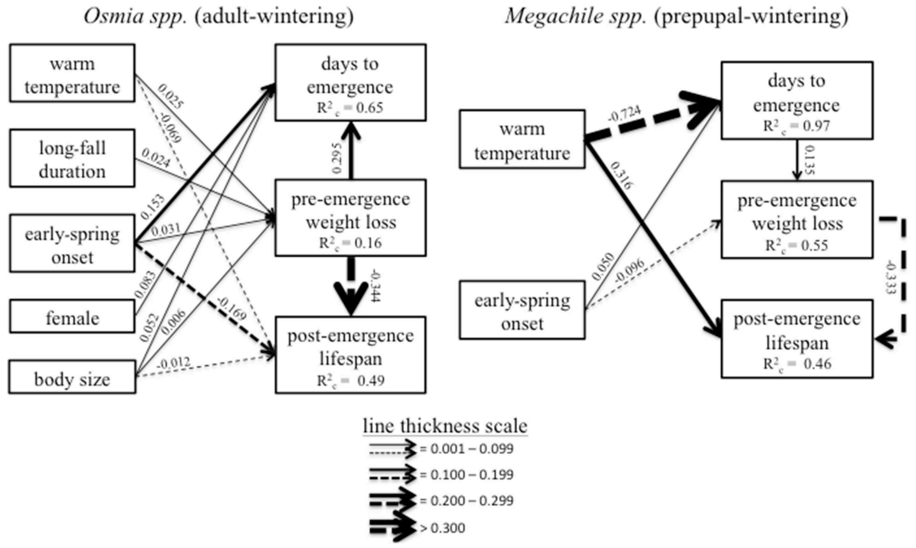


FIGURE 5 | Shows results of the Piecewise Structural Equation Analyses separately for *Osmia* spp. and *Megachile* spp. Directional arrows denote significant ($\alpha \leq 0.05$) effects. Percentages associated with each arrow are range standardized effect sizes (i.e., the percent of the range of values for a categorical response variable, or as the percent change per one unit increase in a continuous explanatory variable). Solid arrows represent a positive effect and dashed arrows represent a negative effect, with arrow thickness corresponding to the size of the effect. Effect sizes for categorical variables represent the effect of the stated level of the independent variable shown in each box compared to the other level of that variable. For example, in the *Megachile* spp. model, the warm temperature treatment caused a 72.4% decrease in the range of values for days to emergence in *Megachile* spp. compared to the cool temperature treatment.

laboratory studies on *Osmia lignaria*, shorter durations of cold exposure can delay adult diapause termination, resulting in delayed emergence relative to the start of spring incubation (i.e., exposure to warm temperatures that elicit emergence, Bosch and Kemp, 2003, 2004; Sgolastra et al., 2010). In both laboratory and field studies on *Osmia* spp., delayed emergence can cause rapid metabolic expenditure, resulting in depleted stored-energy reserves at emergence, and increased pre-emergence mortality (i.e., *O. lignaria*, *O. cornuta,* and *O. ribifloris,* Bosch and Kemp, 2003, 2004; Sgolastra et al., 2010; CaraDonna et al., 2018). Responses to early-spring onset observed across *Osmia* spp. in this study were consistent with previous studies, suggesting that responses to cold exposure during adult diapause may be similar among species of *Osmia*. This interpretation is further supported by structural equation analyses in this study, which revealed a positive correlation between the number of days to emergence and weight loss in *Osmia* spp. Taken together, our findings indicate that *Osmia* spp. may be vulnerable to increased pre-emergence energy depletion and higher rates of pre-emergence mortality under shorter winter seasons caused by climate change.

However, the duration of winter was equal (i.e., 90 days) in both the early-spring + short-fall treatment combination and the late-spring + long-fall treatment combination, indicating that the timing of the spring treatment, and not just the duration of the winter treatment, drove the observed responses by *Osmia* spp. in this study. In *O. lignaria*, the timing of adult diapause initiation may influence the timing of emergence by determining when cold exposure begins accumulating, and in turn, when diapause termination occurs. Given that adult diapause is initiated in *O. lignaria* at the end of summer relative to adult eclosion, and is not cued by cold temperatures (Sgolastra et al., 2010), *Osmia* spp. in this study likely initiated adult diapause prior to or shortly after the start of the fall treatment. In the fall treatment, temperatures in both the warm and cool treatments were within the range at which *Osmia* spp. can successfully overwinter in the laboratory (Bosch and Kemp, 2003, 2004; Sgolastra et al., 2010). Therefore, *Osmia* spp. likely accumulated cold exposure outside of the "winter" period, especially prior to the start of the spring treatment when spring onset was late compared to early. These explanations align with reduced cold exposure as the most plausible cause of the observed responses by *Osmia* spp. in the early-spring onset treatment.

While the warm temperature treatment did not affect the timing of *Osmia* emergence, it resulted in increased weight loss. This weight loss was likely due to increased energetic expenditure during fall (Bosch and Kemp, 2003, 2004; Sgolastra et al., 2011; Schenk et al., 2018a) and when emergence was delayed by the early-spring onset treatment. However, only female *Osmia* were susceptible to this temperature-induced weight loss, suggesting that female *Osmia* may be more vulnerable to increased pre-emergence energy consumption under climate change compared to males. We also found an effect of body size on weight loss, suggesting that larger bees may store and expend more energy compared to smaller bees (O'Neill et al., 2014). But, because body size did not interact with any treatments, larger bees may not be likely to experience disproportionate effects of climate change via increased energy consumption. Increased female weight loss in response to the warm treatment and the early spring treatment indicate that multiple response mechanisms operating within different life cycle stages of solitary bees may interact to affect pre-emergence energy consumption. Investigating these mechanisms will be important while continuing to build a better understanding of how pollinator species are responding to climate change. Here, both temperature and season length resulted in increased weight loss, whereas only early-spring onset affected the timing of emergence. This suggests that increased pre-emergence energy consumption may be a consistent consequence of climate change for *Osmia* spp., whereas shifts in the timing of emergence may depend more on variation in winter temperature and duration (Forrest and Thomson, 2011; CaraDonna et al., 2018), compared to fall temperature and duration.

When assessing mortality, it is possible that factors unrelated to our treatments, such as parasitism (Wcislo and Cane, 1996), pathogens (Evison and Jensen, 2018), or inadequate provision quantity (Torchio, 1985), may have caused pre-emergence mortality in this study. Alternatively, some species of *Osmia* demonstrate parsivoltinism (i.e., can overwinter for one *or* two years prior to emerging (Torchio and Tepedino, 1982; Forrest et al., 2019), which could account for non-emerged bees following one winter in this study. However, we favor energy depletion caused by delayed emergence as the most plausible explanation of increased pre-emergence mortality in *O. coloradensis* and *O. tersula* for several reasons. First, we excluded parasitized bees from the analysis of pre-emergence mortality. Second, we only analyzed non-emerged bees that had developed into adults, and development to the adult life stage is inconsistent with mortality caused by pathogens (Evison and Jensen, 2018), as well as the decision to forgo emergence following a single winter (Torchio and Tepedino, 1982; Forrest et al., 2019). Provision quantity could have influenced mortality, but care taken to distribute individuals from the same mother and collection location equally across treatments likely reduced any bias in provision quantities among treatments.

In contrast to *Osmia* spp., neither the timing of spring onset nor the duration of fall caused increased weight loss or increased pre-emergence mortality in *Megachile* spp. As expected, the timing of emergence and weight loss in *Megachile* spp. was affected primarily by temperature. Because prepupal-wintering bees develop into adults following prepupal diapause, warmer spring temperature likely accelerated developmental rates and advanced the timing of emergence relative to the start of the spring treatment, an interpretation that is consistent with previous laboratory studies on *Megachile rotundata* (Kemp and Bosch, 2000; O'Neill et al., 2011). Less pre-emergence weight loss in the warm temperature treatment compared to the cool treatment is also consistent with previous work on *M. rotundata*, in which body fat content increased with post-winter temperatures within the range used in this study (O'Neill et al., 2011). In this study, reduced pre-emergence weight loss in the warm temperature treatment was likely a result of accelerated development. *Megachile rotundata* that develop more quickly in the laboratory tend to emerge earlier, which results in less time spent in spring incubation temperatures and less stored-energy depletion prior to emergence (Kemp and Bosch, 2000; O'Neill et al., 2011). However, the effect of temperature on pre-emergence weight loss in this study only occurred in the late-spring onset treatment, despite similar emergence responses to temperature in both the early- and late-spring onset treatments. This surprising result implies that the timing of spring onset, not just the interplay between post-winter developmental rates and the timing of emergence, influenced weight loss in *Megachile* spp. To our knowledge, interplay between the magnitude and timing of post-winter temperature has not been previously shown to affect pre-emergence weight loss in *Megachile* spp. Further research is needed to understand how post-winter temperature may interact with the timing of spring onset to influence physiological processes in *Megachile spp.*

Pre-emergence mortality responded inconsistently across *Osmia spp.,* and was low in both species of *Megachile,* suggesting that increased pre-emergence mortality in response to shorter winters associated with climate change may be a species-specific response among species of *Osmia,* and not a widespread consequence for *Megachile* spp. On the other hand, increased weight loss in the early-spring onset treatment was observed across *Osmia* spp., and for female *Osmia* in the warm treatment. If pre-emergence weight loss is indicative of stored-energy consumption, the amount of energy available to *Osmia* spp. for fitness-related activities, such as gamete production, mating, nest construction, and offspring provisioning (Hahn and Denlinger, 2007; O'Neill et al., 2015) may be affected by climate change (Bosch et al., 2010). Although we cannot draw a causative link between increased pre-emergence weight loss and post-emergence fitness in this study, structural equation analyses revealed a significant negative correlation between pre-emergence weight loss and post-emergence lifespan without feeding in both *Osmia* spp. and *Megachile* spp. Post-emergence lifespan is an important predictor of fitness in solitary bees (Bosch and Vicens, 2006). Thus, if the observed relationship between pre-emergence weight loss and post-emergence lifespan holds in natural systems, our findings suggest that *Osmia* spp. may be vulnerable to post-emergence fitness consequences under climate change. This relationship was particularly strong in female *Osmia* in the early-spring onset treatment when fall was long, suggesting female *Osmia* may be disproportionately susceptible to post-emergence fitness consequences caused by shorter winter durations. Alternatively, solitary bees may be able to overcome any potential post-emergence fitness consequences of increased pre-emergence energy consumption by consuming nectar and pollen after they emerge (Sgolastra et al., 2016). More work is needed to understand how rearing conditions and post-emergence nectar and pollen availability may affect fitness in solitary bees.

In contrast to *Osmia* spp., a positive effect of early-spring onset and warmer temperatures on pre-emergence weight loss in *Megachile* spp. suggests that *Megachile spp.,* and possibly other species of solitary bees that overwinter as prepupae, may be less vulnerable to potential negative fitness consequences associated with increased stored-energy depletion under climate change, a conclusion reached by previous authors (Bosch et al., 2010; Fründ et al., 2013). Furthermore, the negative correlation between pre-emergence weight loss and post-emergence lifespan without feeding revealed by structural equation analyses in this study suggests that warmer springs may aid fitness in *Megachile* spp. Importantly, however, spring temperatures used to rear *Megachile* spp. in this study were at the lower end of the temperature range suitable for development of *M. rotundata* reared in the laboratory (O'Neill et al., 2011). In nature, post-winter development in *Megachile* spp. is likely influenced by spring onset as well as daily mean and maximum temperatures during summer (Kemp and Bosch, 2000), which are likely higher than the temperatures used in this study. This is important because higher post-wintering temperatures cause non-linear effects on development, emergence timing, and fat loss in *M. rotundata* in the laboratory (O'Neill et al., 2011; Yocum et al., 2012). If *M. lapponica* and *M. relativa* respond to temperature in a similar manner to *M. rotundata*, the effects of temperature on the timing of emergence and pre-emergence weight loss likely depend on the temperature and timing of summer. In future work, it will be important to incorporate spring *and* summer treatments into experimental examinations of how *Megachile* spp. are responding to climate change.

In addition to weight loss, shifts in the timing of emergence can have implications for fitness in solitary bees by impacting temporal synchrony among plant and pollinator species (Forrest, 2015). For *Osmia* spp., delayed emergence in response to the early-spring onset treatment is seemingly at odds with the widely documented pattern showing earlier emergence dates in spring-active pollinator species under climate change (e.g., Bartomeus et al., 2011; Burkle et al., 2013; Kudo and Ida, 2013). However, treatment effects on the timing of emergence in *Osmia* spp. in this study were small (ca. 2–3 days). Regardless of the timing of spring onset, *Osmia* spp. emerged in <7 days on average relative to the start of the spring treatment. This indicates that the timing of emergence in *Osmia* spp. is highly plastic, and responsive to spring warming, which is consistent with advancing emergence phenologies reported for spring-active pollinator species by other authors. The degree to which shifts in phenology among *Osmia* spp. will affect synchrony with flowering periods of spring-flowering plants will largely depend on whether the cues that drive flowering phenologies are the same as those that drive the timing of emergence in *Osmia* spp., and, if separate cues are used, whether climate-driven changes in these separate cues are correlated (Lambert et al., 2010).

For *Megachile* spp., our findings indicate that the timing of emergence will advance under warmer spring temperatures. However, because changes in summer temperature under climate change will also influence the timing of emergence in *Megachile* spp., our ability to speculate about the consequences of climate change for emergence phenologies among *Megachile* spp. is limited. Importantly, summer-flowering plant species can be more prone to maintain or delay their phenologies in response to climate change compared to spring-flowering plants (Fitter and Fitter, 2002; Sherry et al., 2007; Cook et al., 2012). Thus, it will be important to understand whether phenological responses to temperature in summer-active pollinators, such as *Megachile spp.*, will lead to divergent shifts in phenology with summer-flowering plants.

Interestingly, winter emergence in *O. lignaria* (i.e., emergence occurring prior to being transferred to the spring treatment) indicates that *O. lignaria* can emerge in the absence of perceiving spring warming (Bosch and Kemp, 2003). It could be that *O. lignaria* responded to depleted energy reserves by emerging, regardless of perceiving the onset of spring, a mechanism suggested to exist in some diapausing insects (Irwin and Lee, 2000; Hahn and Denlinger, 2007). A stronger effect of warm, extended fall conditions on winter emergence in males compared to females when spring onset was late suggests that emergence may be more constrained in male *O. lignaria*, potentially as a result of reduced energy availability at the start of the experiment (i.e., *O. lignaria* males entered the experiment with 8.5 mg/mm less mass compared to females; $t_{252} = 9.96$, $p < 0.001$). Emerging prior to the onset of spring would likely increase mortality in *O. lignaria* due to scarce or absent floral resource availability when they emerge (Schenk et al., 2018b), which could have important implications for *O. lignaria* populations under climate change. However, given that the likelihood of winter emergence was low when spring-onset was early suggests that climate change may not increase the occurrence of this response. On the other hand, sex-specific sensitivity to warmer, longer fall seasons could differentially alter the timing of male and female emergence, as well as disproportionately affect male mortality. Because *O. lignaria* populations have male-biased sex ratios (Torchio and Tepedino, 1980) and demonstrate protandry (i.e., males emerge prior to females), changes in population sex ratios driven by increased male mortality (Torchio and Tepedino, 1980), or changes in the mean population-level degree of protandry (Morbey and Ydenberg, 2001), could have ecological and evolutionary implications. This potential consequence of climate change deserves further investigation.

When speculating about the implications of our results for solitary bees under climate change, we acknowledge that the responses we observed under artificial temperature and season length manipulations may not accurately represent bee responses in natural settings. In addition to the potential for non-linear responses to temperature in *Megachile* spp. (O'Neill et al., 2011), bees typically experience fluctuating temperatures. In the laboratory, rearing solitary bees under fluctuating temperatures can cause different responses compared to constant temperatures (Kemp and Bosch, 2000; Rinehart et al., 2011). An important path forward will be to incorporate this variation into manipulative studies in the laboratory and in the field. In addition, we were largely unable to analyze responses at the species level, and our sample sizes differed by species. It is therefore possible that responses we observed when grouping species together by genus were driven by a subset of species. Assessing species-specific response patterns among species with shared traits will help build a better understanding of how pollinator life history traits mediate responses to climate change.

In summary, we demonstrated that overwintering life stage can mediate solitary bee responses to manipulated temperature and seasonal timing treatments that spanned fall, winter, and spring. Our findings suggest that *Osmia* spp., which overwinter as adults, may be more prone to increased stored-energy depletion prior to emergence compared to *Megachile* spp., which overwinter as prepupae. These results indicate that *Osmia* spp. may be more vulnerable to pre-emergence mortality and negative post-emergence fitness consequences of climate change compared to *Megachile* spp. In addition, sex-specific responses, particularly winter emergence by male *O. lignaria*, suggest that climate change may affect male and female emergence phenology and post-emergence fitness differently, with potential implications for population-level mating success and sex ratios. This study highlights the importance of combining experimental manipulations across multiple life cycle stages to help reveal the trait-mediated mechanisms by which climate change is impacting species. We urge future researchers to continue disentangling the complex roles of life history traits in mediating responses to climate change, so that we can better understand and preserve the species interactions that support essential ecosystem services such as pollination.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

AS and LB conceived the project and obtained funding and contributed to revisions. AS designed the project, collected and analyzed the data, and wrote the first draft of manuscript.

FUNDING

Montana Academy of Science, Montana Institute on Ecosystems, and Montana State University.

ACKNOWLEDGMENTS

We thank K. O'Neill, J. Hu, and J. Mangold for providing helpful comments on drafts of this manuscript, and D. Baumbauer for assistance with equipment integral to the project. We also thank the two reviewers for their time and contributions to this article.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00314/full#supplementary-material

REFERENCES

Alexander, L. V., Zhang, X., Peterson, T. C., Caesar, J., Gleason, B., Tank, A., et al. (2006). Global observed changes in daily climate extremes of temperature and precipitation. *J. Geophys. Res. Atmosph.* 111, 1–22. doi: 10.1029/2005JD006290

Bartomeus, I., Ascher, J. S., Wagner, D., Danforth, B. N., and Colla, S. (2011). Climate-associated phenological advances in bee pollinators and bee-pollinated plants. *Proc. Natl. Acad. Sci. U.S.A.* 108, 20645–20649. doi: 10.1073/pnas.1115559108

Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate - a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Seri. Methodol.* 57, 289–300. doi: 10.1111/j.2517-6161.1995.tb02031.x

Bosch, J., and Kemp, W. P. (2000). Development and emergence of the orchard pollinator *Osmia lignaria* (Hymenoptera: Megachilidae). *Environ. Entomol.* 29, 8–13. doi: 10.1603/0046-225X-29.1.8

Bosch, J., and Kemp, W. P. (2003). Effect of wintering duration and temperature on survival and emergence time in males of the orchard pollinator *Osmia lignaria* (Hymenoptera: Megachilidae). *Environ. Entomol.* 32, 711–716. doi: 10.1603/0046-225X-32.4.711

Bosch, J., and Kemp, W. P. (2004). Effect of pre-wintering and wintering temperature regimes on weight loss, survival, and emergence time in the mason bee *Osmia cornuta* (Hymenoptera: Megachilidae). *Apidologie* 35, 469–479. doi: 10.1051/apido:2004035

Bosch, J., Sgolastra, F., and Kemp, W. P. (2010). Timing of eclosion affects diapause development, fat body consumption and longevity in *Osmia lignaria*, a univoltine, adult-wintering solitary bee. *J. Insect Physiol.* 56, 1949–1957. doi: 10.1016/j.jinsphys.2010.08.017

Bosch, J., and Vicens, N. (2006). Relationship between body size, provisioning rate, longevity and reproductive success in females of the solitary bee *Osmia cornuta*. *Behav. Ecol. Sociobiol.* 60, 26–33. doi: 10.1007/s00265-005-0134-4

Buckley, L. B., and Kingsolver, J. G. (2012). Functional and phylogenetic approaches to forecasting species' responses to climate change. *Ann. Rev. Ecol. Evol. Syst.* 43, 205–226. doi: 10.1146/annurev-ecolsys-110411-160516

Burkle, L., Marlin, J. C., and Knight, T. M. (2013). Plant-pollinator interactions over 120 years: loss of species, co-occurrence, and function. *Science* 339, 1611–1615. doi: 10.1126/science.1232728

Burrows, M. T., Schoeman, D. S., Buckley, L. B., Moore, P., Poloczanska, E. S., Brander, K. M., et al. (2011). The pace of shifting climate in marine and terrestrial ecosystems. *Science* 334, 652–655. doi: 10.1126/science.1210288

Cane, J. H. (1987). Estimation of bee size using intertegular span (apoidea). *J. Kans. Entomol. Soc.* 60, 145–147.

CaraDonna, P. J., Cunningham, J. L., and Iler, A. M. (2018). Experimental warming in the field delays phenology and reduces body mass, fat content and survival: implications for the persistence of a pollinator under climate change. *Funct. Ecol.* 32, 2345–2356. doi: 10.1111/1365-2435.13151

Cariveau, D. P., Nayak, G. K., Bartomeus, I., Zientek, J., Ascher, J. S., Gibbs, J., et al. (2016). The allometry of bee proboscis length and its uses in ecology. *PLoS ONE* 11:e0151482. doi: 10.1371/journal.pone.0151482

Cariveau, D. P., and Winfree, R. (2015). Causes of variation in wild bee responses to anthropogenic drivers. *Curr. Opin. Insect Sci.* 10, 104–109. doi: 10.1016/j.cois.2015.05.004

Cook, B., Wolkovich, E. M., and Parmesan, C. (2012). Divergent responses to spring and winter warming drive community level flowering trends. *Proc. Natl. Acad. Sci. U.S.A.* 109, 9000–9005. doi: 10.1073/pnas.1118364109

Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., et al. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl. Acad. Sci. U.S.A.* 105, 6668–6672. doi: 10.1073/pnas.0709472105

Diamond, S., Frame, A. M., Martin, R. A., and Buckley, L. B. (2011). Species' traits predict phenological responses to climate change in butterflies. *Ecology* 92, 1005–1012. doi: 10.1890/10-1594.1

Evison, S. E. F., and Jensen, A. B. (2018). The biology and prevalence of fungal diseases in managed and wild bees. *Curr. Opin. Insect Sci.* 26,105–113. doi: 10.1016/j.cois.2018.02.010

Fitter, A., and Fitter, R. S. R. (2002). Rapid changes in flowering time in British plants. *Science* 296, 1689–1691. doi: 10.1126/science.1071617

Forrest, J. R. K. (2015). Plant-pollinator interactions and phenological change: what can we learn about climate impacts from experiments and observations? *Oikos* 124, 4–13. doi: 10.1111/oik.01386

Forrest, J. R. K., Cross, R., and CaraDonna, P. J. (2019). Two-year bee, or not two-year bee? How voltinism is affected by temperature and season length in a high-elevation solitary bee. *Am. Natural.* 193, 560–574. doi: 10.1086/701826

Forrest, J. R. K., and Thomson, J. D. (2011). An examination of synchrony between insect emergence and flowering in Rocky Mountain meadows. *Ecol. Monogr.* 81, 469–491. doi: 10.1890/10-1885.1

Fründ, J., Zieger, S. L., and Tscharntke, T. (2013). Response diversity of wild bees to overwintering temperatures. *Oecologia* 173, 1639–1648. doi: 10.1007/s00442-013-2729-1

Gezon, Z. J., Inouye, D. W., and Irwin, R. E. (2016). Phenological change in a spring ephemeral: implications for pollination and plant reproduction. *Glob. Chang. Biol.* 22, 1779–1793. doi: 10.1111/gcb.13209

Grace, J., and Bollen, K. (2005). Interpreting the results from multiple regression and structural equation models. *Bull. Ecol. Soc. Am.* 86, 283–295. doi: 10.1890/0012-9623(2005)86[283:ITRFMR]2.0.CO;2

Hahn, D., and Denlinger, D. L. (2007). Meeting the energetic demands of insect diapause: nutrient storage and utilization. *J. Insect Physiol.* 53, 760–773. doi: 10.1016/j.jinsphys.2007.03.018

Hegland, S., Nielsen, A., Lazaro, A., Bjerknes, A. L., and Totland, O. (2009). How does climate warming affect plant-pollinator interactions? *Ecol. Lett.* 12, 184–195. doi: 10.1111/j.1461-0248.2008.01269.x

Hodek, I., and Hodkova, M. (1988). Multiple role of temperature during insect diapause - a review. *Entomol. Exp. Appl.* 49, 153–165. doi: 10.1111/j.1570-7458.1988.tb02486.x

IPCC (2014). *Climate Change 2014: Synthesis Report Contribution of Working Groups I, II, and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change,* eds Core Writing Team, RK Pachauri and LA Meyer (Geneva: IPCC), 151.

Irwin, J. T., and Lee, R. E. (2000). Mild winter temperatures reduce survival and potential fecundity of the goldenrod gall fly, *Eurosta solidaginis* (Diptera: Tephritidae). *J. Insect Physiol.* 46, 655–661. doi: 10.1016/S0022-1910(99)00153-5

Kemp, W. P., and Bosch, J. (2000). Development and emergence of the alfalfa pollinator *Megachile rotundata* (Hymenoptera: Megachilidae). *Ann. Entomol. Soc. Am.* 93, 904–911. doi: 10.1603/0013-8746(2000)093[0904:DAEOTA]2.0.CO;2

Kemp, W. P., and Bosch, J. (2001). Postcocooning temperatures and diapause in the alfalfa pollinator *Megachile rotundata* (Hymenoptera: Megachilidae). *Ann. Entomol. Soc. Am.* 94, 244–250. doi: 10.1603/0013-8746(2001)094[0244:PTADIT]2.0.CO;2

Kemp, W. P., Bosch, J., and Dennis, B. (2004). Oxygen consumption during the life cycles of the prepupa-wintering bee *Megachile rotundata* and the adult-wintering bee *Osmia lignaria* (Hymenoptera: Megachilidae). *Ann. Entomol. Soc. Am.* 97, 161–170. doi: 10.1603/0013-8746(2004)097[0161:OCDTLC]2.0.CO;2

Kingsolver, J. G., Woods, H. A., Buckley, L. B., Potter, K. A., MacLean, H. J., and Higgins, J. K. (2011). Complex life cycles and the responses of insects to climate change. *Integr. Comp. Biol.* 51, 719–732. doi: 10.1093/icb/icr015

Kudo, G., and Ida, T. Y. (2013). Early onset of spring increases the phenological mismatch between plants and pollinators. *Ecology* 94, 2311–2320. doi: 10.1890/12-2003.1

Lambers, H., Chapin III, F. S., and Pons, T. L. (2008). *Plant Physiological Ecology*. New York, NY: Springer Science & Business Media.

Lambert, A. M., Miller-Rushing, A. J., and Inouye, D. W. (2010). Changes in snowmelt date and summer precipitation affect the flowering phenology of *erythronium grandiflorum* (glacier lily; Liliaceae). *Am. J. Bot.* 97, 1431–1437. doi: 10.3732/ajb.1000095

Lefcheck, J. S. (2016). PiecewiseSEM: piecewise structural equation modelling in R for ecology, evolution, and systematics. *Methods Ecol. Evol.* 7, 573–579. doi: 10.1111/2041-210X.12512

Lenth, R. V., and Hervac, M. Ó. (2015). *Lsmeans: Least-Squares Means R Package Version 216*. Available online at: https://cran.r-project.org/web/packages/lsmeans/index.html

May, D. G. K. (1972). Water uptake during larval development of a sweat bee, *Augochlora pura* (Hymenoptera: Apoidea). *J. Kansas Entomol. Soc.* 45, 439–449.

McGavin, G. C. (1994). The bee genera of North and Central-America - michener, cd, mcginley, rj, danforth, bn. *Nature* 370, 261–262. doi: 10.1038/370261b0

Memmott, J., Craze, P. G., Waser, N. M., and Price, M. V. (2007). Global warming and the disruption of plant-pollinator interactions. *Ecol. Lett.* 10, 710–717. doi: 10.1111/j.1461-0248.2007.01061.x

Menzel, A., Sparks, T. H., Estrella, N., Koch, E., Aasa, A., Ahas, R., et al. (2006). European phenological response to climate change matches the warming pattern. *Glob. Chang. Biol.* 12, 1969–1976. doi: 10.1111/j.1365-2486.2006.01193.x

Michener, C. D. (2007). *The Bees of the World, 2nd Edn*. Baltimore, MD: John Hopkins University Press.

Morbey, Y. E., and Ydenberg, R. C. (2001). Protandrous arrival timing to breeding areas: a review. *Ecol. Lett.* 4, 663–673. doi: 10.1046/j.1461-0248.2001.00265.x

Ogilvie, J. E., and Forrest, J. R. K. (2017). Interactions between bee foraging and floral resource phenology shape bee populations and communities. *Curr. Opin. Insect Sci.* 21, 75–82. doi: 10.1016/j.cois.2017.05.015

O'Neill, K. M., Delphia, C. M., and O'Neill, R. P. (2014). Oocyte size, egg index, and body lipid content in relation to body size in the solitary bee *Megachile rotundata*. *Peerj.* 2:e314. doi: 10.7717/peerj.314

O'Neill, K. M., Delphia, C. M., and Pitts-Singer, T. L. (2015). Seasonal trends in the condition of nesting females of a solitary bee: wing wear, lipid content, and oocyte size. *Peerj* 3:e930. doi: 10.7717/peerj.930

O'Neill, K. M., O'Neill, R. P., Kemp, W. P., and Delphia, C. M. (2011). Effect of temperature on post-wintering development and total lipid content of Alfalfa Leafcutting Bees. *Environ. Entomol.* 40, 917–930. doi: 10.1603/EN10320

Pardee, G. L., Inouye, D. W., and Irwin, R. E. (2018). Direct and indirect effects of episodic frost on plant growth and reproduction in subalpine wildflowers. *Glob. Chang. Biol.* 24, 848–857. doi: 10.1111/gcb.13865

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Development Core Team (2013). *nlme: Linear and Nonlinear Mixed Effects Models R Package Version,* 31–111.

Pitts-Singer, T. L., and James, R. R. (2009). Prewinter management affects *Megachile rotundata* (Hymenoptera: Megachilidae) prepupal physiology and adult emergence and survival. *J. Econ. Entomol.* 102, 1407–1416. doi: 10.1603/029.102.0402

Post, E. S., Pedersen, C., Wilmers, C. C., and Forchhammer, M. C. (2008). Phenological sequences reveal aggregate life history response to climatic warming. *Ecology* 89, 363–370. doi: 10.1890/06-2138.1

Potts, S. G., Imperatriz-Fonseca, V., Ngo, H. T., Aizen, M. A., Biesmeijer, J. C., Breeze, T. D., et al. (2016). Safeguarding pollinators and their values to human well-being. *Nature* 540, 220–229. doi: 10.1038/nature20588

R Core Team (2013). *R: A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing,Vienna, Austria. Available online at: https://www.r-project.org/

Radmacher, S., and Strohm, E. (2010). Factors affecting offspring body size in the solitary bee *Osmia bicornis* (Hymenoptera, Megachilidae). *Apidologie* 41, 169–177. doi: 10.1051/apido/2009064

Rafferty, N. E. (2017). Effects of global change on insect pollinators: multiple drivers lead to novel communities. *Curr. Opin. Insect Sci.* 23, 22–27. doi: 10.1016/j.cois.2017.06.009

Rightmyer, M. G., Griswold, T., and Brady, S. G. (2013). Phylogeny and systematics of the bee genus *Osmia* (Hymenoptera: Megachilidae) with emphasis on North American Melanosmia: subgenera, synonymies and nesting biology revisited. *Syst. Entomol.* 38, 561–576. doi: 10.1111/syen.12013

Rinehart, J. P., Yocum, G. D., West, M., and Kemp, W. P. (2011). A fluctuating thermal regime improves survival of cold-mediated delayed emergence in developing *Megachile rotundata* (Hymenoptera: Megachilidae). *J. Econ. Entomol.* 104, 1162–1166. doi: 10.1603/EC11062

Schenk, M., Krauss, J., and Holzschuh, A. (2018b). Desynchronizations in bee-plant interactions cause severe fitness losses in solitary bees. *J. Anim. Ecol.* 87, 139–149. doi: 10.1111/1365-2656.12694

Schenk, M., Mitesser, O., Hovestadt, T., and Holzschuh, A. (2018a). Overwintering temperature and body condition shift emergence dates of spring-emerging solitary bees. *Peerj* 6:e4721. doi: 10.7717/peerj.4721

Sgolastra, F., Arnan, X., Pitts-Singer, T. L., Maini, S., Kemp, W. P., and Bosch, J. (2016). Pre-wintering conditions and post-winter performance in a solitary bee: does diapause impose an energetic cost on reproductive success? *Ecol. Entomol.* 41, 201–210. doi: 10.1111/een.12292

Sgolastra, F., Bosch, J., Molowny-Horas, R., Maini, S., and Kemp, W. P. (2010). Effect of temperature regime on diapause intensity in an adult-wintering

Hymenopteran with obligate diapause. *J. Insect Physiol.* 56, 185–194. doi: 10.1016/j.jinsphys.2009.10.001

Sgolastra, F., Kemp, W. P., Buckner, J. S., Pitts-Singer, T. L., and Maini, S. (2011). The long summer: pre-wintering temperatures affect metabolic expenditure and winter survival in a solitary bee. *J. Insect Physiol.* 57, 1651–1659. doi: 10.1016/j.jinsphys.2011.08.017

Sheffield, C. S., Ratti, C., Packer, L., and Griswold, T. (2011). Leafcutter and mason bees of the genus Megachile Latreille (Hymenoptera: Megachilidae) in Canada and Alaska. *Can. J. Arthropod Identif.* 18:29. doi: 10.3752/cjai.2011.18

Sherry, R., Zhou, X., Gu, S., Arnone, J. A., and Schimel, D. S. (2007). Divergence of reproductive phenology under climate warming. *Proc. Natl. Acad. Sci. U.S.A.* 104, 198–202. doi: 10.1073/pnas.0605642104

Sparks, T. H., and Menzel, A. (2002). Observed changes in seasons: an overview. *Int. J. Climatol.* 22, 1715–1725. doi: 10.1002/joc.821

Stephen, W. P., Bohart, G. W., and Torchio, P. F. (1969). "The biology and external morphology of bees with a synopsis of the genera of northwestern America," in *The Biology and External Morphology of Bees with a Synopsis of the Genera of Northwestern America* (Corvallis, OR: The Agricultural Research Station; Oregon State University), 140.

Stephen, W. P., and Torchio, P. F. (1961). Biological notes on the leafcutter bee *Megachile* (*Eutricharaea*) *rotundata* (Fabricius) (Hymenoptera: Megachilidae). Pan-Pac. *Entomology* 37, 89–93.

Torchio, P. F. (1985). Field experiments with the pollinator species, *Osmia lignaria* Propinqua Cresson, in apple orchards 5 (1979–1980), methods of introducing bees, nesting success, seed counts, fruit yields (Hymenoptera, Megachilidae). *J. Kans. Entomol. Soc.* 58, 448–464.

Torchio, P. F. (1989). In-nest biologies and development of immature stages of 3 *Osmia* species (Hymenoptera, Megachilidae). *Ann. Entomol. Soc. Am.* 82, 599–615. doi: 10.1093/aesa/82.5.599

Torchio, P. F., and Tepedino, V. J. (1980). Sex-ratio, body size and seasonality in a solitary bee, *Osmia lignaria* Propinqua Cresson (Hymenoptera, Megachilidae). *Evolution* 34, 993–1003. doi: 10.1111/j.1558-5646.1980.tb04037.x

Torchio, P. F., and Tepedino, V. J. (1982). Parsivoltinism in three species of *Osmia* bees. *Psyche* 89, 221–238. doi: 10.1155/1982/60540

Tylianakis, J. M., Didham, R. K., Bascompte, J., and Wardle, D. A. (2008). Global change and species interactions in terrestrial ecosystems. *Ecol. Lett.* 11, 1351–1363. doi: 10.1111/j.1461-0248.2008.01250.x

Vanbergen, A. J., Baude, M., Biesmeijer, J. C., Britton, N. F., Brown, M. J. F., Brown, M., et al. (2013). Threats to an ecosystem service: pressures on pollinators. *Front. Ecol. Environ.* 11, 251–259. doi: 10.1890/120126

Waters, J. S., and Harrison, J. F. (2012). *Insect Metabolic Rates*. Wiley-Blackwell.

Wcislo, W. T., and Cane, J. H. (1996). Floral resource utilization by solitary bees (Hymenoptera: Apoidea) and exploitation of their stored foods by natural enemies. *Annu. Rev. Entomol.* 41, 257–286. doi: 10.1146/annurev.en.41.010196.001353

Wickham, H. (2009). *ggplot2: Elegant Graphics for Data Analysis.* New York, NY: Springer.

Yocum, G. D., Rinehart, J. P., and Kemp, W. P. (2012). Duration and frequency of a high temperature pulse affect survival of emergence-ready *Megachile rotundata* (Hymenoptera: Megachilidae) during low-temperature incubation. *J. Econ. Entomol.* 105, 14–19. doi: 10.1603/EC11210

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

REVIEW
published: 20 September 2019
doi: 10.3389/fevo.2019.00348

Insect Hybridization and Climate Change

Erica L. Larson[1*†], **Robin M. Tinghitella**[1†] **and Scott A. Taylor**[2†]

[1] Department of Biological Sciences, University of Denver, Denver, CO, United States, [2] Department of Ecology and Evolution, University of Colorado Boulder, Boulder, CO, United States

Contemporary global change is altering ecosystems at an unprecedented pace and scale. This critical period is a crisis for biodiversity, but the perturbations caused by global environmental change also offer new opportunities to study the evolution of species boundaries—their persistence, formation, or collapse—over rapid evolutionary timescales. Increasing temperature means and fluctuations have the potential to alter gene flow between species through changes in species distributions, interactions, life history, and temperature-dependent behavior. This is particularly true for insects, whose geographic ranges, behaviors, and life history traits are temperature dependent. Here, we review the potential for climate change to influence gene flow and species boundaries between closely related insect species. We focus on studies that have tracked changes in climate and insect distributions and/or have evaluated temperature dependent reproductive barriers between species.

Keywords: speciation, hybrid zones, temporal isolation, range expansions, mate choice

OPEN ACCESS

Edited by:
Lora A. Richards,
University of Nevada, Reno,
United States

Reviewed by:
Clement Fisher Kent,
York University, Canada
Josh Jahner,
University of Nevada, Reno,
United States

Correspondence:
Erica L. Larson
erica.larson@du.edu

†ORCID:
Erica L. Larson
orcid.org/0000-0003-3006-645X
Robin M. Tinghitella
orcid.org/0000-0002-0049-5539
Scott A. Taylor
orcid.org/0000-0001-9580-9125

Specialty section:
This article was submitted to
Population and Evolutionary
Dynamics,
a section of the journal
Frontiers in Ecology and Evolution

Received: *24 April 2019*
Accepted: *02 September 2019*
Published: *20 September 2019*

Citation:
Larson EL, Tinghitella RM and
Taylor SA (2019) Insect Hybridization
and Climate Change.
Front. Ecol. Evol. 7:348.
doi: 10.3389/fevo.2019.00348

> *"It is the mode of life as a whole that is challenged by the environment"*
> -(Masaki, 1961)

Our understanding of the nature of species boundaries—the delineation of species through reproductive barriers—comes largely from studies of range expansions and secondary contact during the Quaternary ice ages (∼2.4 Mya, Hewitt, 2011; Canestrelli et al., 2016). The hybrid zones formed by these range expansions are windows on evolutionary process, places where we can link phenotypes that maintain species boundaries with their underlying genotype and the processes that drove their divergence (Hewitt, 1988; Harrison, 1990). Contemporary global change is altering species' habitat at an unprecedented pace and scale. This critical period is a crisis for biodiversity (Dirzo et al., 2014; Wiens, 2016), including insects (Sánchez-Bayo and Wyckhuys, 2019). These perturbations also offer a new opportunity to study the evolution of species boundaries—their persistence, formation, or collapse—over rapid evolutionary timescales (Brennan et al., 2015; Taylor et al., 2015; Grabenstein and Taylor, 2018).

Geographic variation within species is key to understanding at what level and how consistently species boundaries will respond to climate change (Rowe et al., 2014). Although we often think of "species interactions" as constants, they clearly vary, because species themselves are variable, as are the environmental contexts of their interactions (Harrison, 1985; Larson et al., 2014; Mandeville et al., 2015, 2017). If species are adapted to local climate, then they will respond differently to climate change across their range. This is true for all aspects of climate change (e.g., temperature, humidity, and precipitation), but in particular, latitudinal variation in temperature is changing rapidly and can play a significant role in reproductive timing and behavior in many organisms (Senner et al., 2017).

Temperature changes may differentially affect reproduction and life-history traits of populations at different latitudes and/or elevations (Cohen et al., 2018), leading to varied consequences for populations occupying different regions of geographically expansive ranges. For example, populations at lower latitudes may have narrower thermal tolerances and be more affected by changing temperatures (Janzen, 1967; Addo-Bediako et al., 2000).

Insects may be particularly susceptible to anthropogenic temperature change because their ranges, life history traits (e.g., lifecycle, development time), and behaviors (e.g., song) are often temperature dependent (Tauber and Tauber, 1981; Masaki, 1983; Doherty, 1985). Increasing temperature means and fluctuations have the potential to alter gene flow between insect species through changes in distributions, interactions, life history, and temperature-dependent behavior. Here we review the potential for climate change to influence gene flow and species boundaries between insect species. We focus on studies that have tracked changes in climate and insect distributions and/or have evaluated temperature dependent reproductive barriers between species. Our goal is to highlight the exciting field-based experiments that are possible as insects respond to changing climate, and the opportunities to understand the origin and maintenance of biodiversity in insect hybrid zones.

SEASONAL LIFE CYCLE VARIATION IN INSECTS

Seasonality shapes nearly all aspects of an insect's life history. Development, activity, and reproduction are all synchronized to seasonal fluctuations in light, temperature, moisture, and resource availability (Masaki, 1961; Tauber and Tauber, 1981). Often, insects withstand seasonal extremes via diapause—a period of low metabolic activity and developmental arrest triggered by daylight and/or temperature. In species with large ranges, seasonal adaptation is complicated by latitudinal and elevational transitions in season length, temperature and photoperiod (i.e., changes in day length). For some insects, diapause is obligatory and the developmental timing of diapause is fixed throughout their range (e.g., Berner et al., 2004). Other insects can respond to the length of the growing season by changing the onset (initiation) and close (termination) of diapause (e.g., Dingle et al., 1990; Dingle and Mousseau, 1994). In some cases, changes in the timing and duration of diapause determines the number of generations per year (voltinism). Across the range of a single species, populations can shift from one generation at higher latitudes or elevations to two or more generations per year at lower latitudes or elevations (including non-diapausing populations) (Levy et al., 2014). In the extreme, diapausing twice in a single life cycle is common in northern or high elevation species, likely because insects in these habitats need two seasons to complete development (Pickford, 1953).

To accommodate shifts in the length of growing season or the number of generations per year, some insects can adjust their rate of development. This appears to be more common in insects with long development times relative to the growing season (i.e., species with many nymphal stages, see Kivelä et al., 2011). For instance, the Emma field cricket, *Telogryllus emma*, is distributed across Japan and develops more quickly in northern populations than in southern populations (Masaki, 1967). The rate of development may in turn affect body size—shorter growing seasons can select for more rapid development and smaller body size (Parsons and Joern, 2013). In insects, this phenomenon has been called the Converse of Bergman's rule (Masaki, 1967; Mousseau, 1997). However, there is not a simple correlation between body size and latitude. As species shift from continuous development at lower latitudes they can transition to two generations a year (bivoltinism) before reaching one generation a year (univoltinism) at higher latitudes. Fitting two generations in per year in mid-latitudes may come at a cost to body size (**Figure 1**). As a result, insects often have a concomitant shifting distribution in body size and life cycle across latitudinal and elevational gradients, known as a "sawtooth" pattern (Masaki, 1978a).

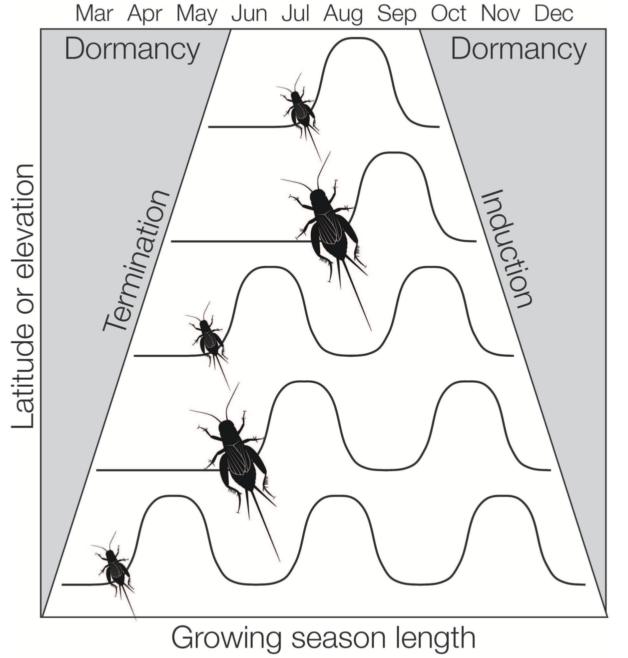


FIGURE 1 | How season length can affect the number of generations per year (voltinism) and body size in insects. As latitude or elevation increases, changes in the termination and initiation of diapause can shift to accommodate decreasing season length. Likewise, insects may develop faster and/or reach smaller adult body sizes to accommodate shorter growing seasons. Body size can also decrease in the south as species accommodate more generations per year. This figure is modified from Levy et al. (2014).

The shift in seasonal phenologies in insects can be so striking that Masaki (1978b) proposed the term "climatic speciation" to explain the parapatric distributions of populations with differing seasonal phenologies across latitudes. Indeed, temporal isolation due to local seasonal adaptation has been documented in many species [**Table 1**, see also references in the very thorough review by Taylor and Friesen (2017)], though perhaps climatic speciation is not as widespread as Masaki envisioned. Species that are

TABLE 1 | Examples of species or subspecies temporally isolated through seasonal phenology.

Species/subspecies	Common name	Estimated divergence	Nature of temporal isolation	Evidence of moving hybrid zone, range expansion or host shift?	References
Allonemobius socius and *A. fasciatus*	Ground crickets	6,000–60,000 ya	Latitudinal variation in voltinism	Hybrid zone is moving northwards	Howard, 1986; Tanaka, 1991; Britch et al., 2001
Gryllus firmus and *G. pennsylvanicus*	Field crickets	200,000 ya	Time in egg to adult development, latitudinal variation in *G. firmus* development time	Hybrid zone may be expanding, but possible correlations with climate have not been studied	Harrison, 1985; Larson et al., 2013
Inurois punctigera early and late-winter adults	Japanese winter geometrid moths	Mean F_{ST} 0.382 (mtDNA), 0.027 (AFLP)[a]	Seasonal adult emergence	Dispersal to harsher winter climates is predicted to lead to repeated shifts to early and later winter adults.	Yamamoto and Sota, 2009, 2012
Magicicada spp. 17- and 13-year life cycles	Periodical cicadas	≥0.5 Mya	Seasonal adult emergence. Species are isolated by differing life cycles as well as broods that emerge in different years.	Life cycle switching is believed to be due to selection pressure from changing climate.	Marshall and Cooley, 2000; Simon et al., 2000; Cooley et al., 2001; Sota et al., 2013; Koyama et al., 2016
Melanoplus devastator and *M. sanguinipes*	Grasshoppers	Mean F_{ST} 0.5	Development time and reproductive diapause		Orr, 1996
Neophasia menapia Early and late adults	Pine white butterfly	G_{ST} 0.057 (goat mountain), 0.031 (mendocino pass)[b]	Seasonal adult emergence		Bell et al., 2017
Ostrinia nubilalis E and Z pheromone strains	European corn borer moth	$F_{ST} = 0.05$	Latitudinal variation in voltinism	Introduced in early 20th century, subsequent range expansion	Dopman et al., 2010; Dopman, 2011; Levy et al., 2014; Kozak et al., 2017
Papilio glaucus and *P. canadensis*	Swallowtail butterflies	0.5–0.6 mya	Seasonal adaptation (diapause and growth)		Ording et al., 2010; Scriber, 2011; Scriber et al., 2014; Ryan et al., 2018
Polyommatus agestis and *P. artaxeres*	Brown argus butterfly	NA	Latitudinal variation in voltinism	The southern species is expanding northward, likely displacing the northern species hybrid zone is expanding	Mallet et al., 2010
Rhagoletis pomonella apple and haw races	Apple maggot fly	∼160 ya	Seasonal adult emergence, corresponding to host phenology		Feder and Filchak, 1999; Filchak et al., 2000; Ragland et al., 2012
Thaumetopoea pityocampa winter and summer larval strains	Pine processionary moth	560 generations	Seasonal diapause timing	The southern summer strain initially spread along the Portugal coast, but species distribution models predicted that the summer strain range will decrease with climate change.	Pimentel et al., 2006; Santos et al., 2007, 2011; Burban et al., 2016; Godefroid et al., 2016; Leblois et al., 2017

[a] *Calculated from Yamamoto and Sota (2012).*

[b] *G_{ST} between early and late flight adults within a site were similar to G_{ST} between geographically isolated populations.*

distributed across large latitudinal or elevational gradients often have different seasonal lifecycles, but still maintain sufficient gene flow to be considered a single species (Amari et al., 2018). However, climate change may dramatically alter interactions among species or parapatrically distributed populations. For species to persist in the face of climate change, populations must synchronize their phenology with the environmental conditions and with other interacting populations (Senner et al., 2017).

Insects are responding to climate change by altering the seasonal timing of adult emergence (Diamond et al., 2011; Maurer et al., 2018). For example, over the past 50 years, Rocky Mountain grasshoppers at high elevation have delayed development, while low elevation populations have advanced development (Buckley et al., 2015). Insects are also responding by increasing the number of generations they have per year (Tobin et al., 2008; Altermatt, 2010). Even the simplest changes in insect life cycles can affect temporal isolation between sympatric taxa (Taylor and Friesen, 2017). Species may adjust the timing of diapause or the number of generations per year so that reproductive activity among populations or species are no longer synchronized (Santos et al., 2007, 2011) or there is less temporal isolation between closely related species (Dopman et al.,

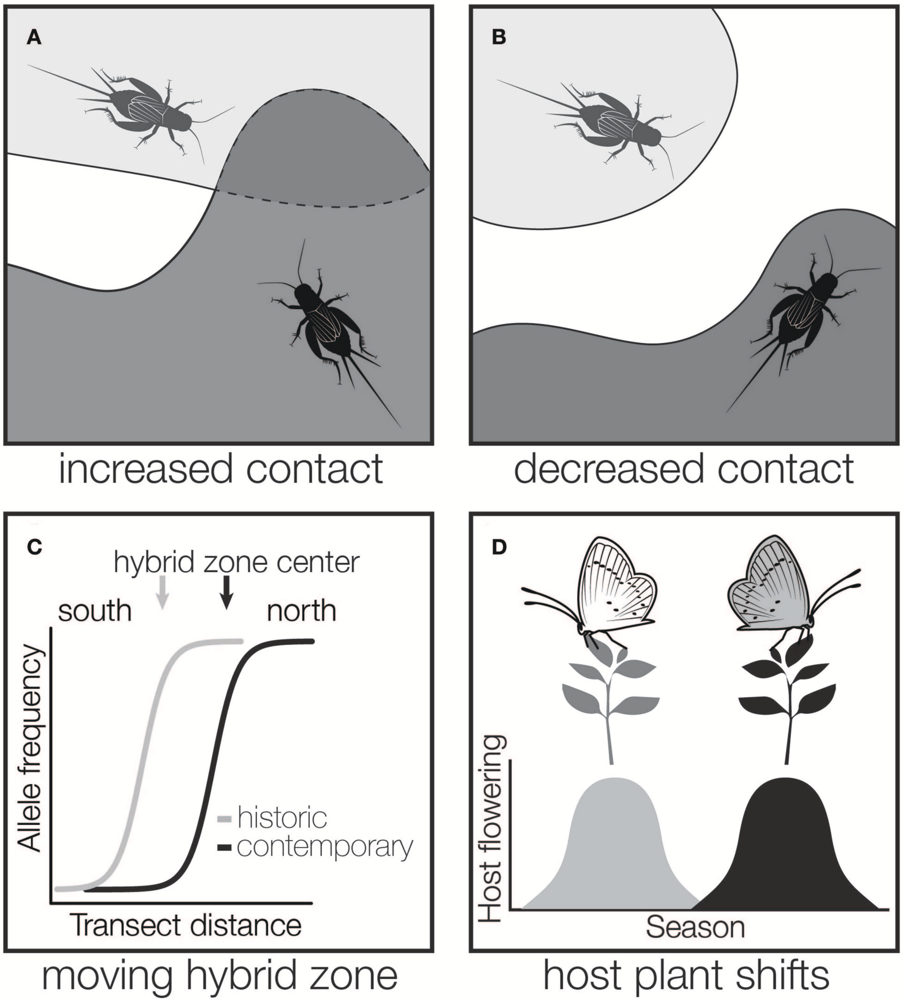


FIGURE 2 | Possible outcomes of insect range changes on the potential for hybridization. As insect ranges change due to climate, we can expect that some species that were previously allopatric will have new areas of contact **(A)**. Likewise, ranges may shrink for some species or populations, potentially decreasing hybridization or gene flow **(B)**. Species that already interact in hybrid zones may experience new species interactions (e.g., changing species densities, new environmental contexts) as hybrid zones shift with changing species ranges **(C)**. As species occupy new habitat, there is the potential for herbivorous insects to shift to new host species, leading to increased or decreased contact with other species **(D)**.

2010). Shifts in the timing of adult emergence will change the context of species interactions: reproductive barriers may collapse, or climate change could lead to losses of population connectivity (Grabenstein and Taylor, 2018). Although we have focused on temperature, all aspects of climate change will affect insect seasonal phenology, particularly precipitation (which also influences diapause timing). Changes in seasonal life cycle are also intimately tied with changes in species distributions and host plant shifts.

RANGE EXPANSIONS WITH CHANGING CLIMATE

As global climate rapidly changes, insects are modifying their distributions. Using historic and contemporary surveys, coupled with climate data, studies have documented both northward and elevational range expansions (Parmesan et al., 1999; Nufio et al., 2010; Breed et al., 2013; Menéndez et al., 2013; Ryan et al., 2018) or changes to insect migration routes (Sparks et al., 2007). So far, we lack the ability to predict which species will undergo range expansions, which appears to be species dependent (Hellmann et al., 2008; Menéndez et al., 2013). However, it is clear that species' responses to climate change are leading to new (Wellenreuther et al., 2010) or shifting (Ryan et al., 2018) overlap between species (**Figure 2A**). Climate change will also lead to range contractions (Merrill et al., 2008), and the greater potential for loss of population connectivity and isolation (**Figure 2B**).

Beyond the generation of new regions of hybridization, species that currently interact in hybrid zones may experience changing species interactions as hybrid zones move in response to climate change (**Figure 2C**). While hybrid zone movement is likely common (Buggs, 2007), and in fact expected for hybrid zones that are associated with environmental factors [e.g., mosaic hybrid zones (Harrison, 1986), clinal hybrid zones (Endler, 1977)], there are few long term studies of shifts in insect hybrid zones, and fewer still that link movement to environmental variation (Dasmahapatra et al., 2002). As a result, how modified species interactions in insect hybrid zones might be altered by climate change generally remains unclear. Still, moving hybrid zones are excellent opportunities to study the nature of species interactions and a concerted effort should be made to detect moving hybrid zones and consistently sample them through time. The few long-term studies of moving insect hybrid zones have provided some of our most robust observations of climate-related introgression: more discoveries will be made if we pay closer attention to moving insect hybrid zones.

For example, the Michigan portion of the hybrid zone between two North American tiger swallowtail butterflies in the genus *Papilio* (*P. glaucus* and *P. canadensis*) has shifted northward in response to climate change over the last ∼30 years (Scriber, 2011; Scriber et al., 2014; Ryan et al., 2018). By tracking the hybrid zone through time, Scriber and colleagues have learned much about interspecific gene flow, ecological divergence, incipient speciation, and hybrid zone movement. Importantly, researchers have been able to document introgression of species-specific alleles in response to climate change. This includes alleles related to the ability of *Papilio* larvae to detoxify host plants, which have introgressed northwards 200 km over the past 15 years due to strong selection on novel detoxification phenotypes produced within the hybrid zone (Scriber, 2011; Scriber et al., 2014). Alleles related to diapause control have also introgressed northwards in the *Papillo* hybrid zone, highlighting how range expansions can correspond to changes in seasonal phenologies (Crozier and Dwyer, 2006).

The link between range expansions and seasonal adaptation is not always intuitive. The mosaic hybrid zone between the ground crickets *Allonemobius fasciatus* (northern species) and *A. socius* (Howard and Waring, 1991) was shown to have shifted northward over a 14-year period (Britch et al., 2001). This shift is due, at least in part, to year to year variation in population growth rate of either species. Hybrid zones are often at the edge of a species' range, so even slight perturbations to the environment may have a major impact on the relative fitness of parental species or hybrids where populations overlap. However, the northward expansion of *A. socius* may be constrained by the *A. socius* bivoltine lifecycle (Fedorka et al., 2012). Moving hybrid zones are a complex interaction between population level dynamics, and the potential for local adaptation (i.e., standing genetic variation or phenotypic plasticity).

Both the *Papillo* and *Allonemobius* hybrid zones have been studied extensively in one region of a fairly large area of contact. The geographic consistency of the patterns that have been documented is unknown. Replicating these studies across different areas of contact will give us greater insight into how local adaptation and population structure influence changing species contact zones. In general, we should aim to study geographically expansive hybrid zones that can provide new insight into the consistency of reproductive barriers across different climatic gradients (see section "Ways Forward").

INTERACTIONS BETWEEN SEASONALITY AND HOST PLANT SHIFTS

Climate change is predicted to alter herbivorous insect and host plant interactions. The availability and quality of host plants can be altered by changing temperatures and precipitation. Host plant populations may decline, forcing insects to shift to novel hosts (Parmesan, 2006), or the stress of increasing temperatures and drought may expose plants to novel pests, facilitating the spread of some herbivores (Jamieson et al., 2012). Changing seasonal phenologies for insects or their hosts can cause insects to become desynchronized with their host plant, possibly forcing them on to a new host. Host plant shifts (and associated range expansions) can lead to increased or decreased contact between species or involve changes in seasonality (Forbes et al., 2017; **Figure 2D**). An excellent example of this is the apple maggot fly, *Rhagoletis pomonella*, which underwent a host shift from North American native hawthorn fruit to introduced apple trees in the 1860s (Bush, 1969). Apple host races of *R. pomonella* have advanced their adult emergence by ∼3 weeks to accommodate the earlier availability of apple fruit, causing temporal isolation between apple and hawthorn host races (Feder and Filchak, 1999; Filchak et al., 2000). This means that apple host races enter diapause earlier in the fall while temperatures are still relatively warm. Metabolic rates in insects increase with external temperature, even during diapause, and the apple host races must maintain higher energy stores to survive longer pre-winter diapause (Ragland et al., 2012). The shift from haw to apple may have been preceded by prior host shifts in the *R. pomonella* species complex, secondary contact, and gene flow among allopatric populations as *R. pomonella* species expanded their ranges from isolated southern populations in the US and Mexico (∼1.5 mya). As *R. pomonella* species moved north, the timing of their diapause likely shifting with increasing latitudes and novel host species (e.g., snowberries, blueberries, sparkleberries, flowering dogwood, and finally apple; Feder et al., 2003, 2005; Xie et al., 2008; Mattsson et al., 2015).

COUPLING AND MISMATCH OF SIGNALS AND CHOOSER PREFERENCES

Global climate change poses largely unknown threats to insect reproduction (Dell et al., 2011; Narins and Meenderink, 2014; Gibert et al., 2016). Individuals mate within critical temperature ranges, and the signals that some insects use to communicate are thermally sensitive (Dolbear, 1897; Edmunds, 1963; Doherty, 1985; Pires and Hoy, 1992; Conrad et al., 2017). Acoustic communication is the primary sexual signal for many insects and insect songs can be used in long-distance mate location, courtship, and male-male competition contexts. Several characteristics of songs (e.g., frequency, pulse length, interpulse length), but most notably, the pulse rate, are temperature-dependent; the pulse rate of cricket songs, for instance, quite famously increases linearly with temperature in multiple species (Walker, 1962; Walker and Cade, 2003). The temperature-dependent aspects of signals are often the very traits that are used to identify appropriate mates (Beckers and Schul, 2008).

Sexual signals are typically coupled with chooser (often females) preferences for those signals within species, ensuring that males attract conspecific females and females respond to conspecific males. Temperature coupling in reproductive traits (correspondence between changes in male signals and female preferences with temperature) has been demonstrated in several insects in response to temperatures experienced during development (Grace and Shaw, 2004) or as adults (Doherty and Hoy, 1985; Pires and Hoy, 1992). But, signal-preference mismatches are likely to stem from climate change because chooser preferences are not consistently thermally sensitive (Greenfield and Medlock, 2007; Deutsch et al., 2008). For instance, the mean carrier frequency of the courtship songs of *Drosophila montana* is temperature-dependent, but there is no temperature coupling of female preferences for carrier frequency (Ritchie et al., 2001). If the increased mean temperatures and temperature fluctuations characteristic of climate change shift male signals but not female preferences, or signals and preferences shift in different ways or to different degrees, we may find signal-preference mismatches with a variety of reproductive consequences (**Figure 3**). These consequences include mating with other species if altered temperatures increase the overlap between female preferences and heterospecific signals (Jang and Gerhardt, 2006) by changing characteristics of preference functions like peak preference, strength of preference, or tolerance (sensu Rodríguez et al., 2012). Increased temperatures may also reduce females' threshold for mating, increasing the frequency of mating and perhaps the likelihood of mismating (Kindle et al., 2006).

Cricket songs provide a concrete example. Divergence in song and mate preferences has played a key role in diversification of crickets (Otte, 1992; Mendelson and Shaw, 2005). Cricket songs consist of repeated pulses of sound organized into groups of chirps or trills. Males produce a conspicuous long-distance calling song to attract females from afar and, once in close proximity, switch to a lower intensity courtship song that is required before females will mount to receive the sperm-containing packet. Female crickets show the strongest preferences for temporal features of the calling and courtship songs (Gabel et al., 2016), and both songs contribute to pre-copulatory isolation between species (Fitzpatrick and Gray, 2001; Jang and Gerhardt, 2006; Maroja et al., 2014; Gray et al., 2016; Hennig et al., 2016). Closely related Hawaiian swordtail crickets (*Laupala* sp.), for instance, are ecologically and morphologically indistinguishable, but differ primarily in the pulse rate of their courtship song, which is thought to be reproductively isolating (Mendelson and Shaw, 2002, 2005). If increased temperatures shift female preferences directionally so that they encompass pulse rate characteristics of closely related species that co-occur geographically, we should find a signal-preference mismatch within species and increased mismating between species (**Figure 3**).

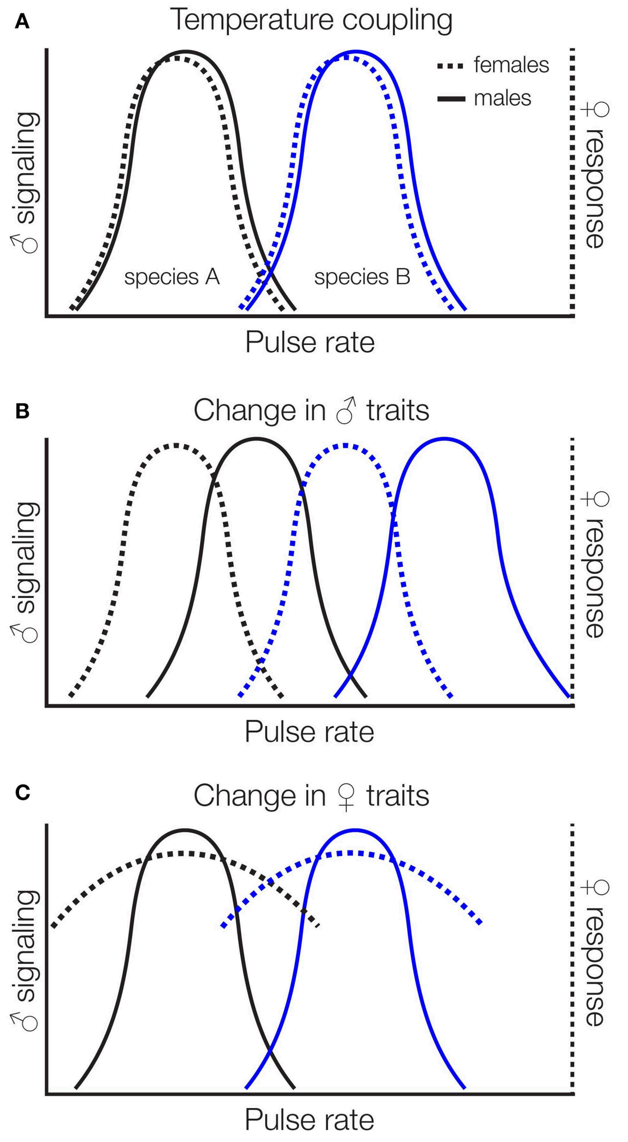


FIGURE 3 | Potential impacts of climate change on temperature dependent signals and preferences. Signals and chooser preferences are typically coupled **(A)**. If increased mean temperatures, for instance, shift signals but not chooser preferences **(B)**, or chooser preferences but not signals **(C)**, or signals and preferences shift differentially (not shown), we might expect mismatches that lead to heterospecific mating. In **(C)** we show one possible shift in chooser preferences (more "relaxed" preference functions with high relative chooser responsiveness that encompasses more signal values), but shifts in peak preference and other characteristics are also possible.

Finally, behavioral ecologists have recently emphasized the importance of distinguishing between choosers' preferences and the mating decisions they make (Rosenthal, 2017). Preferences are not always reflected in mating decisions, and both are environmentally dependent. Even in the absence of changes in preference functions (the response of choosers to variation in signalers traits), if shifts in climate alter individuals' motivation to mate, sexual receptivity, or choices (including through changes in species interactions and changes in range or climate described above), there will be consequences for gene flow and the boundaries between closely related insect species.

WAYS FORWARD

The long-term permanence of species boundaries in the face of rapid change has been difficult to study, in part because interactions between closely related species are not constants. We have highlighted ways that reproductive barriers between species can be temperature sensitive. Future studies should conduct manipulative experiments to determine the temperature dependence of reproductive traits such as development time, host plant associations, and sexual signals. We should expand our taxonomic framework for studying the impacts of climate change on species boundaries. Much of the existing work has focused on a few well-studied groups (e.g., *Lepidoptera* and *Orthoptera*, see **Table 1**), which are poor generalizations for the diversity of insects. Perhaps most importantly, we should account for variation within species and the environmental context of their interactions (Larson et al., 2014; Mandeville et al., 2017). We lack geographically comprehensive field-based studies that account for variation within species, ecological context, and environmental changes. To understand how species will respond to climate change, we need to study geographic variation in the maintenance of species boundaries. This will involve studying species interactions across broad latitudinal or elevation transects. Patterns across these scales will be complicated by abiotic gradients, such as temperature, but also diet, predators, parasites, and competitors will determine the nature of species interactions (Masaki, 1961). The hardest aspect may simply be sampling populations across large continental scales.

Natural history collections are an obvious way forward for documenting changes in insect phenology and ranges (Kharouba et al., 2019). Collections provide a record of the recent past (Holmes et al., 2016), and can be a baseline for tracking insect's changing phenologies and geographic locations. Natural history collections have been successfully used to pinpoint the past timing of adult emergence (Nufio et al., 2010; Maurer et al., 2018), and to track changes in species ranges (Ryan et al., 2018). Collections and survey data (see citizen science data below) are limited to telling us that an event happened, but not the exact timing of the event in most cases. However, statistical methods can provide fairly accurate estimates of sparsely sampled populations (Pearse et al., 2017). Coupled with burgeoning methods for sequencing and analyzing historic DNA (Bi et al., 2013; Jones and Good, 2015; Ali et al., 2016), collections can be used to contrast historic and contemporary gene flow. Not only should we utilize natural history collections as much as possible, but we should make a concerted effort to add to collections through specimens, photo documentation and preservation of quality tissue samples for genomic analyses. These collections are particularly important in hybrid zones, where temporal datasets will allow future scientists to directly observe the impacts of climate change on species boundaries (Taylor et al., 2015).

Much of what we know about the effects of climate change on insects are based on insect pests with long-term monitoring (Boggs, 2016). In the future we should expand the taxonomic diversity of climate change studies and use creative ways to collect data through citizen science projects with the public or in the classroom (Silvertown, 2009; Dickinson et al., 2012; Breed et al., 2013). Early citizen science projects often focused on charismatic vertebrates (e.g., eBird Sullivan et al., 2009), and initially the path forward for citizen science of less popular organisms, like insects, seemed less tractable. Now applications like iNaturalist, insect tracking websites (Berenbaum, 2012; Prudic et al., 2017), and public Bioblitzes are increasing natural history awareness and making citizen science data about insect distributions more accessible (Basset and Lamarre, 2019).

We are facing the devastating loss of uncharacterized biodiversity of insects (Habel et al., 2019; Sánchez-Bayo and Wyckhuys, 2019). While this is an unprecedented crises, these perturbations are also field-based experiments that provide an opportunity to understand the origin and maintenance of biodiversity (Taylor et al., 2015). Hybrid zones have provided many insights into the nature of species boundaries (Harrison and Larson, 2014; Gompert et al., 2017), but extant hybrid zones have formed and shifted over glacial scales. Climate change is an opportunity to understand how changing environments lead to new species, through the disruption of gene flow, the collapse of species through the breakdown of barriers, or the maintenance of species boundaries—all at a pace that is observable in our lifetime.

AUTHOR CONTRIBUTIONS

EL, RT, and ST contributed to the preparation of the manuscript.

FUNDING

RT and ST were supported by grants from the National Science Foundation (RT: IOS-1846520 and ST IOS-1754898).

ACKNOWLEDGMENTS

Discussions with Genevieve Kozak, Cesar Nufio, Kasey Fowler-Finn, and Ryan Bracewell helped clarify our thinking, as did feedback from the University of Denver Ecology and Evolution group.

REFERENCES

Addo-Bediako, A., Chown, S. L., and Gaston, K. J. (2000). Thermal tolerance, climatic variability and latitude. *Proc. Biol. Sci.* 267, 739–745. doi: 10.1098/rspb.2000.1065

Ali, O. A., O'Rourke, S. M., Amish, S. J., Meek, M. H., Luikart, G., Jeffres, C., et al. (2016). RAD capture (Rapture): flexible and efficient sequence-based genotyping. *Genetics* 202, 389–400. doi: 10.1534/genetics.115.183665

Altermatt, F. (2010). Climatic warming increases voltinism in European butterflies and moths. *Proc. Biol. Sci.* 277, 1281–1287. doi: 10.1098/rspb.2009.1910

Amari, H., Zebsa, R., Lazli, A., Bensouilah, S., Mellal, M. K., Mahdjoub, H., et al. (2018). Differential elevational cline in the phenology and demography of two temporally isolated populations of a damselfly: Not two but one taxon? *Ecol. Entomol.* 44, 93–104. doi: 10.1111/een.12680

Basset, Y., and Lamarre, G. P. A. (2019). Toward a world that values insects. *Science* 364, 1230–1231. doi: 10.1126/science.aaw7071

Beckers, O. M., and Schul, J. (2008). Developmental plasticity of mating calls enables acoustic communication in diverse environments. *Proc. Biol. Sci.* 275, 1243–1248. doi: 10.1098/rspb.2007.1765

Bell, K. L., Hamm, C. A., Shapiro, A. M., and Nice, C. C. (2017). Sympatric, temporally isolated populations of the pine white butterfly *Neophasia menapia*, are morphologically and genetically differentiated. *PLoS ONE* 12:e0176989. doi: 10.1371/journal.pone.0176989

Berenbaum, M. R. (2012). BeeSpotter at the University of Illinois. *Am. Entomol.* 58, 25–28. doi: 10.1093/ae/58.1.0025

Berner, D., Körner, C., and Blanckenhorn, W. U. (2004). Grasshopper populations across 2000 m of altitude: is there life history adaptation? *Ecography* 27, 733–740. doi: 10.1111/j.0906-7590.2005.04012.x

Bi, K., Linderoth, T., Vanderpool, D., Good, J. M., Nielsen, R., and Moritz, C. (2013). Unlocking the vault: next-generation museum population genomics. *Mol. Ecol.* 22, 6018–6032. doi: 10.1111/mec.12516

Boggs, C. L. (2016). The fingerprints of global climate change on insect populations. *Curr. Opin. Insect Sci.* 17, 69–73. doi: 10.1016/j.cois.2016.07.004

Breed, G. A., Stichter, S., and Crone, E. E. (2013). Climate-driven changes in northeastern US butterfly communities. *Nature Clim. Change* 3, 142–145. doi: 10.1038/nclimate1663

Brennan, A. C., Woodward, G., Seehausen, O., Muñoz-Fuentes, V., Moritz, C., Guelmami, A., et al. (2015). Hybridization due to changing species distributions: adding problems or solutions to conservation of biodiversity during global change? *Evol. Ecol. Res.* 16, 475–491. Available online at: http://evolutionary-ecology.com/abstracts/v16/2905.html

Britch, S. C., Cain, M. L., and Howard, D. J. (2001). Spatio-temporal dynamics of the *Allonemobius fasciatus- A. socius* mosaic hybrid zone: a 14-year perspective. *Mol. Ecol.* 10, 627–638. doi: 10.1046/j.1365-294x.2001.01215.x

Buckley, L. B., Nufio, C. R., Kirk, E. M., and Kingsolver, J. G. (2015). Elevational differences in developmental plasticity determine phenological responses of grasshoppers to recent climate warming. *Proc. Biol. Sci.* 282, 20150441–20150446. doi: 10.1098/rspb.2015.0441

Buggs, R. J. A. (2007). Empirical study of hybrid zone movement. *Heredity* 99, 301–312. doi: 10.1038/sj.hdy.6800997

Burban, C., Gautier, M., Leblois, R., Landes, J., Santos, H., Paiva, M.-R., et al. (2016). Evidence for low-level hybridization between two allochronic populations of the pine processionary moth, *Thaumetopoea pityocampa* (*Lepidoptera*: *Notodontidae*). *Biol. J. Linn. Soc.* 119, 311–328. doi: 10.1111/bij.12829

Bush, G. L. (1969). Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (*Diptera*, *Tephritidae*). *Evolution* 23, 237–251. doi: 10.1111/j.1558-5646.1969.tb03508.x

Canestrelli, D., Porretta, D., Lowe, W. H., Bisconti, R., Carere, C., and Nascetti, G. (2016). The tangled evolutionary legacies of range expansion and hybridization. *Trends Ecol. Evol.* 31, 677–688. doi: 10.1016/j.tree.2016.06.010

Cohen, J. M., Lajeunesse, M. J., and Rohr, J. R. (2018). A global synthesis of animal phenological responses to climate change. *Nature Clim. Change* 8, 1–8. doi: 10.1038/s41558-018-0067-3

Conrad, T., Stöcker, C., and Ayasse, M. (2017). The effect of temperature on male mating signals and female choice in the red mason bee, *Osmia bicornis* (L.). *Ecol. Evol.* 7, 8966–8975. doi: 10.1002/ece3.3331

Cooley, J. R., Simon, C., Marshall, D. C., Slon, K., and Ehrhardt, C. (2001). Allochronic speciation, secondary contact, and reproductive character displacement in periodical cicadas (*Hemiptera*: *Magicicada* spp.): genetic, morphological, and behavioural evidence. *Mol. Ecol.* 10, 661–671. doi: 10.1046/j.1365-294x.2001.01210.x

Crozier, L., and Dwyer, G. (2006). Combining population-dynamic and ecophysiological models to predict climate-induced insect range shifts. *Am. Nat.* 167, 853–866. doi: 10.1086/504848

Dasmahapatra, K. K., Blum, M. J., Aiello, A., Hackwel, S., Davies, N., Bermingham, E. P., et al. (2002). Inferences from a rapidly moving hybrid zone. *Evolution* 56, 741–753. doi: 10.1111/j.0014-3820.2002.tb01385.x

Dell, A. I., Pawar, S., and Van Savage, M. (2011). Systematic variation in the temperature dependence of physiological and ecological traits. *Proc. Natl. Acad. Sci. U.S.A.* 108, 10591–10596. doi: 10.1073/pnas.1015178108

Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., et al. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl. Acad. Sci. U.S.A.* 105, 6668–6672. doi: 10.1073/pnas.0709472105

Diamond, S. E., Frame, A. M., Martin, R. A., and Buckley, L. B. (2011). Species' traits predict phenological responses to climate change in butterflies. *Ecology* 92, 1005–1012. doi: 10.1890/10-1594.1

Dickinson, J. L., Shirk, J., Bonter, D., Bonney, R., Crain, R. L., Martin, J., et al. (2012). The current state of citizen science as a tool for ecological research and public engagement. *Front. Ecol. Environ.* 10, 291–297. doi: 10.1890/110236

Dingle, H., and Mousseau, T. A. (1994). Geographic variation in embryonic development time and stage of diapause in a grasshopper. *Oecologia* 97, 179–185. doi: 10.1007/BF00323147

Dingle, H., Mousseau, T. A., and Scott, S. M. (1990). Altitudinal variation in life cycle syndromes of California populations of the grasshopper, *Melanoplus sanguinipes* (F.). *Oecologia* 84, 199–206. doi: 10.1007/BF00318272

Dirzo, R., Young, H. S., Galetti, M., Ceballos, G., Isaac, N. J. B., and Collen, B. (2014). Defaunation in the Anthropocene. *Science* 345, 401–406. doi: 10.1126/science.1251817

Doherty, J., and Hoy, R. (1985). The auditory behavior of crickets: some views of genetic coupling, song recognition, and predator detection. *Q. Rev. Biol.* 60, 457–472. doi: 10.1086/414566

Doherty, J. A. (1985). Temperature coupling and "trade-off" phenomena in the acoustic communication system of the cricket, *Gryllus bimaculatus* De Geer (*Gryllidae*). *J. Exp. Biol.* 114, 17–35.

Dolbear, A. (1897). The cricket as a thermometer. *Am. Nat.* 31, 970–971. doi: 10.1086/276739

Dopman, E. B. (2011). Genetic hitchhiking associated with life history divergence and colonization of North America in the European corn borer moth. *Genetica* 139, 565–573. doi: 10.1007/s10709-010-9514-4

Dopman, E. B., Robbins, P. S., and Seaman, A. (2010). Components of reproductive isolation between North American pheromone strains of the European corn borer. *Evolution* 64, 881–902. doi: 10.1111/j.1558-5646.2009.00883.x

Edmunds, L. JR (1963). The relation between temperature and flashing intervals in adult male fireflies, *Photinus pyralis*. *Ann. Entomol. Soc. Am.* 56, 716–718. doi: 10.1093/aesa/56.5.716

Endler, J. A. (1977). *Geographic Variation, Speciation, and Clines*. Princeton, NJ: Princeton University Press.

Feder, J. L., Berlocher, S. H., Roethele, J. B., Dambroski, H., Smith, J. J., Perry, W. L., et al. (2003). Allopatric genetic origins for sympatric host-plant shifts and race formation in *Rhagoletis*. *Proc. Natl. Acad. Sci. U.S.A.* 100, 10314–10319. doi: 10.1073/pnas.1730757100

Feder, J. L., and Filchak, K. E. (1999). It's about time: the evidence for host plant-mediated selection in the apple maggot fly, *Rhagoletis pomonella*, and its implications for fitness trade-offs in phytophagous insects. *Entomol. Exp. Appl.* 91, 211–225. doi: 10.1046/j.1570-7458.1999.00486.x

Feder, J. L., Xie, X., Rull, J., Velez, S., Forbes, A., Leung, B., et al. (2005). Mayr, Dobzhansky, and Bush and the complexities of sympatric speciation in *Rhagoletis*. *Proc. Natl. Acad. Sci. U.S.A.* 102, 6573–6580. doi: 10.1073/pnas.0502099102

Fedorka, K. M., Winterhalter, W. E., Shaw, K. L., Brogan, W. R., and Mousseau, T. A. (2012). The role of gene flow asymmetry along an environmental gradient in constraining local adaptation and range expansion. *J. Evol. Biol.* 25, 1676–1685. doi: 10.1111/j.1420-9101.2012.02552.x

Filchak, K. E., Roethele, J. B., and Feder, J. L. (2000). Natural selection and sympatric divergence in the apple maggot *Rhagoletis pomonella*. *Nature* 407, 739–742. doi: 10.1038/35037578

Fitzpatrick, M. J., and Gray, D. A. (2001). Divergence between the courtship songs of the field crickets *Gryllus texensis* and *Gryllus rubens* (*Orthoptera*, *Gryllidae*). *Ethology* 107, 1075–1085. doi: 10.1046/j.1439-0310.2001.00730.x

Forbes, A. A., Devine, S. N., Hippee, A. C., Tvedte, E. S., Ward, A. K. G., Widmayer, H. A., et al. (2017). Revisiting the particular role of host shifts in initiating insect speciation. *Evolution* 71, 1126–1137. doi: 10.1111/evo.13164

Gabel, E., Gray, D. A., and Hennig, R. M. (2016). How females of chirping and trilling field crickets integrate the 'what' and 'where' of male acoustic signals during decision making. *J. Comp. Physiol. A* 202, 823–837. doi: 10.1007/s00359-016-1124-x

Gibert, J. P., Chelini, M.-C., Rosenthal, M. F., and DeLong, J. P. (2016). Crossing regimes of temperature dependence in animal movement. *Glob. Change Biol.* 22, 1722–1736. doi: 10.1111/gcb.13245

Godefroid, M., Rocha, S., Santos, H., Paiva, M. R., Burban, C., Kerdelhué, C., et al. (2016). Climate constrains range expansion of an allochronic population of the pine processionary moth. *Diversity Distrib.* 22, 1288–1300. doi: 10.1111/ddi.12494

Gompert, Z., Mandeville, E. G., and Buerkle, C. A. (2017). Analysis of population genomic data from hybrid zones. *Annu. Rev. Ecol. Evol. Syst.* 48, 207–229. doi: 10.1146/annurev-ecolsys-110316-022652

Grabenstein, K. C., and Taylor, S. A. (2018). Breaking barriers: causes, consequences, and experimental utility of human-mediated hybridization. *Trends Ecol. Evol.* 33, 198–212. doi: 10.1016/j.tree.2017.12.008

Grace, J. L., and Shaw, K. L. (2004). Effects of developmental environmental environment on signal-preference coupling in a Hawaiian cricket. *Evolution* 58, 1627–1633. doi: 10.1111/j.0014-3820.2004.tb01744.x

Gray, D. A., Gutierrez, N. J., and Chen, T. L. (2016). Species divergence in field crickets: genetics, song, ecomorphology, and pre-and postzygotic isolation. *Biol. J. Linn. Soc.* 117, 192–205. doi: 10.1111/bij.12668

Greenfield, M. D., and Medlock, C. (2007). Temperature coupling as an emergent property: parallel thermal effects on male song and female response do not contribute to species recognition in an acoustic moth. *Evolution* 61, 1590–1599. doi: 10.1111/j.1558-5646.2007.00140.x

Habel, J. C., Samways, M. J., and Schmitt, T. (2019). Mitigating the precipitous decline of terrestrial European insects: requirements for a new strategy. *Biodiversity Conserv.* 28, 1–18. doi: 10.1007/s10531-019-01741-8

Harrison, R. G. (1985). Barriers to gene exchange between closely related cricket species. II. Life cycle variation and temporal isolation. *Evolution* 39, 244–259. doi: 10.1111/j.1558-5646.1985.tb05664.x

Harrison, R. G. (1986). Pattern and process in a narrow hybrid zone. *Heredity* 56, 337–349. doi: 10.1038/hdy.1986.55

Harrison, R. G. (1990). Hybrid zones: windows on evolutionary process. *Oxford Surveys Evol. Biol.* 7, 69–128.

Harrison, R. G., and Larson, E. L. (2014). Hybridization, introgression, and the nature of species boundaries. *J. Hered.* 105, 795–809. doi: 10.1093/jhered/esu033

Hellmann, J. J., Pelini, S. L., Prior, K. M., and Dzurisin, J. D. K. (2008). The response of two butterfly species to climatic variation at the edge of their range and the implications for poleward range shifts. *Oecologia* 157, 583–592. doi: 10.1007/s00442-008-1112-0

Hennig, R. M., Blankers, T., and Gray, D. A. (2016). Divergence in male cricket song and female preference functions in three allopatric sister species. *J. Comp. Physiol. A* 202, 347–360. doi: 10.1007/s00359-016-1083-2

Hewitt, G. M. (1988). Hybrid zones-natural laboratories for evolutionary studies. *Trends Ecol. Evol.* 3, 158–167. doi: 10.1016/0169-5347(88)90033-X

Hewitt, G. M. (2011). Quaternary phylogeography: the roots of hybrid zones. *Genetica* 139, 617–638. doi: 10.1007/s10709-011-9547-3

Holmes, M. W., Hammond, T. T., Wogan, G. O. U., Walsh, R. E., LaBarbera, K., Wommack, E. A., et al. (2016). Natural history collections as windows on evolutionary processes. *Mol. Ecol.* 25, 864–881. doi: 10.1111/mec.13529

Howard, D. J. (1986). A zone of overlap and hybridization between two ground cricket species. *Evolution* 40, 34–43. doi: 10.1111/j.1558-5646.1986.tb05715.x

Howard, D. J., and Waring, G. L. (1991). Topographic diversity, zone width, and the strength of reproductive isolation in a zone of overlap and hybridization. *Evolution* 45, 1120–1135. doi: 10.1111/j.1558-5646.1991.tb04379.x

Jamieson, M. A., Trowbridge, A. M., Raffa, K. F., and Lindroth, R. L. (2012). Consequences of climate warming and altered precipitation patterns for plant-insect and multitrophic interactions. *Plant Physiol.* 160, 1719–1727. doi: 10.1104/pp.112.206524

Jang, Y., and Gerhardt, H. C. (2006). Divergence in the calling songs between sympatric and allopatric populations of the southern wood cricket *Gryllus fultoni* (*Orthoptera*: *Gryllidae*). *J. Evol. Biol.* 19, 459–472. doi: 10.1111/j.1420-9101.2005.01014.x

Janzen, D. H. (1967). Why mountain passes are higher in the tropics? *Am. Nat.* 101, 233–249. doi: 10.1086/282487

Jones, M. R., and Good, J. M. (2015). Targeted capture in evolutionary and ecological genomics. *Mol. Ecol.* 25, 185–202. doi: 10.1111/mec.13304

Kharouba, H. M., Lewthwaite, J. M. M., Guralnick, R., Kerr, J. T., and Vellend, M. (2019). Using insect natural history collections to study global change impacts: challenges and opportunities. *Phil. Trans. R. Soc. B* 374, 20170405–20170410. doi: 10.1098/rstb.2017.0405

Kindle, T. K., Johnson, K. M., Ivy, T. M., Weddle, C. B., and Sakaluk, S. K. (2006). Female mating frequency increases with temperature in two cricket species, *Gryllodes sigillatus* and *Acheta domesticus* (*Orthoptera*: *Gryllidae*). *Can. J. Zool.* 84, 1345–1350. doi: 10.1139/z06-127

Kivelä, S. M., Välimäki, P., Carrasco, D., Mäenpää, M. I., and Oksanen, J. (2011). Latitudinal insect body size clines revisited: a critical evaluation of the saw-tooth model. *J. Anim. Ecol.* 80, 1184–1195. doi: 10.1111/j.1365-2656.2011.01864.x

Koyama, T., Ito, H., Fujisawa, T., Ikeda, H., Kakishima, S., Cooley, J. R., et al. (2016). Genomic divergence and lack of introgressive hybridization between two 13-year periodical cicadas support life cycle switching in the face of climate change. *Mol. Ecol.* 25, 5543–5556. doi: 10.1111/mec.13858

Kozak, G. M., Wadsworth, C. B., Kahne, S. C., Bogdanowicz, S. M., Harrison, R. G., Coates, B. S., et al. (2017). A combination of sexual and ecological divergence contributes to rearrangement spread during initial stages of speciation. *Mol. Ecol.* 26, 2331–2347. doi: 10.1111/mec.14036

Larson, E. L., Guilherme Becker, C., Bondra, E. R., and Harrison, R. G. (2013). Structure of a mosaic hybrid zone between the field crickets *Gryllus firmus* and *G. pennsylvanicus*. *Ecol. Evol.* 3, 985–1002. doi: 10.1002/ece3.514

Larson, E. L., White, T. A., Ross, C. L., and Harrison, R. G. (2014). Gene flow and the maintenance of species boundaries. *Mol. Ecol.* 23, 1668–1678. doi: 10.1111/mec.12601

Leblois, R., Gautier, M., Rohfritsch, A., Foucaud, J., Burban, C., Galan, M., et al. (2017). Deciphering the demographic history of allochronic differentiation in the pine processionary moth *Thaumetopoea pityocampa*. *Mol. Ecol.* 27, 264–278. doi: 10.1111/mec.14411

Levy, R. C., Kozak, G. M., Wadsworth, C. B., Coates, B. S., and Dopman, E. B. (2014). Explaining the sawtooth: latitudinal periodicity in a circadian gene correlates with shifts in generation number. *J. Evol. Biol.* 28, 40–53. doi: 10.1111/jeb.12562

Mallet, J., Wynne, I. R., and Thomas, C. D. (2010). Hybridisation and climate change: brown argus butterflies in Britain (*Polyommatus* subgenus Aricia). *Insect Conserv. Divers.* 4, 192–199. doi: 10.1111/j.1752-4598.2010.00122.x

Mandeville, E. G., Parchman, T. L., McDonald, D. B., and Buerkle, C. A. (2015). Highly variable reproductive isolation among pairs of *Catostomus* species. *Mol. Ecol.* 24, 1856–1872. doi: 10.1111/mec.13118

Mandeville, E. G., Parchman, T. L., Thompson, K. G., Compton, R. I., Gelwicks, K. R., Song, S. J., et al. (2017). Inconsistent reproductive isolation revealed by interactions between *Catostomus* fish species. *Evol. Lett.* 1, 255–268. doi: 10.1002/evl3.29

Maroja, L. S., McKenzie, Z. M., Hart, E., Jing, J., Larson, E. L., and Richardson, D. P. (2014). Barriers to gene exchange in hybridizing field crickets: the role of male courtship effort and cuticular hydrocarbons. *BMC Evol. Biol.* 14, 65. doi: 10.1186/1471-2148-14-65

Marshall, D. C., and Cooley, J. R. (2000). Reproductive character displacement and speciation in periodical cicadas, with description of a new species, 13-year *Magicicada neotredecim*. *Evolution* 54, 1313–1325. doi: 10.1111/j.0014-3820.2000.tb00564.x

Masaki, S. (1961). Geographic variation of diapause in insects. *Bull. Fac. Agric. Hirosaki Univ.* 7, 66–98.

Masaki, S. (1967). Geographic variation and climatic adaptation in a field cricket (*Orthoptera*: *Gryllidae*). *Evolution* 21, 725. doi: 10.1111/j.1558-5646.1967.tb03430.x

Masaki, S. (1978a). Climatic adaptation and species status in the lawn ground cricket II. Body size. *Oecologia* 35, 343–356. doi: 10.1007/BF00345141

Masaki, S. (1978b). "Seasonal and latitudinal adaptations in the life cycles of crickets," in *Evolution of Insect Migration and Diapause*, ed H. Dingle (New York, NY: Springer), 72–100.

Masaki, S. (1983). Climatic speciation in Japanese ground crickets. *GeoJournal* 7, 483–490. doi: 10.1007/BF00218520

Mattsson, M., Hood, G. R., Feder, J. L., and Ruedas, L. A. (2015). Rapid and repeatable shifts in life-history timing of *Rhagoletis pomonella* (*Diptera*:

Tephritidae) following colonization of novel host plants in the Pacific Northwestern United States. *Ecol. Evol.* 5, 5823–5837. doi: 10.1002/ece3.1826

Maurer, J. A., Shepard, J. H., Crabo, L. G., Hammond, P. C., Zack, R. S., and Peterson, M. A. (2018). Phenological responses of 215 moth species to interannual climate variation in the Pacific Northwest from 1895 through 2013. *PLoS ONE* 13:e0202850. doi: 10.1371/journal.pone.0202850

Mendelson, T. C., and Shaw, K. L. (2002). Genetic and behavioral components of the cryptic species boundary between *Laupala cerasina* and *L. kohalensis* (*Orthoptera*: *Gryllidae*). *Genetica* 116, 301–310. doi: 10.1023/A:1021244812270

Mendelson, T. C., and Shaw, K. L. (2005). Sexual behaviour: rapid speciation in an arthropod. *Nature* 433, 375–376. doi: 10.1038/433375a

Menéndez, R., González-Megías, A., Jay-Robert, P., and Marquéz-Ferrando, R. (2013). Climate change and elevational range shifts: evidence from dung beetles in two European mountain ranges. *Glob. Ecol. Biogeogr.* 23, 646–657. doi: 10.1111/geb.12142

Merrill, R. M., Gutiérrez, D., Lewis, O. T., Gutiérrez, J., Díez, S. B., and Wilson, R. J. (2008). Combined effects of climate and biotic interactions on the elevational range of a phytophagous insect. *J. Anim. Ecol.* 77, 145–155. doi: 10.1111/j.1365-2656.2007.01303.x

Mousseau, T. A. (1997). Ectotherms follow the converse to Bergmann's rule. *Evolution* 51:630. doi: 10.1111/j.1558-5646.1997.tb02453.x

Narins, P. M., and Meenderink, S. W. F. (2014). Climate change and frog calls: long-term correlations along a tropical altitudinal gradient. *Proc. Biol. Sci.* 281:20140401. doi: 10.1098/rspb.2014.0401

Nufio, C. R., McGuire, C. R., Bowers, M. D., and Guralnick, R. P. (2010). Grasshopper community response to climatic change: variation along an elevational gradient. *PLoS ONE* 5:e12977. doi: 10.1371/journal.pone.0012977

Ording, G. J., Mercader, R. J., Aardema, M. L., and Scriber, J. M. (2010). Allochronic isolation and incipient hybrid speciation in tiger swallowtail butterflies. *Oecologia* 162, 523–531. doi: 10.1007/s00442-009-1493-8

Orr, M. R. (1996). Life-history adaptation and reproduction isolation in a grasshopper hybrid zone. *Evolution* 50, 704–716. doi: 10.1111/j.1558-5646.1996.tb03880.x

Otte, D. (1992). Evolution of cricket songs. *J. Orthoptera Res.* 1, 25–49. doi: 10.2307/3503559

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annu. Rev. Ecol. Evol. Syst.* 37, 637–669. doi: 10.1146/annurev.ecolsys.37.091305.110100

Parmesan, C., Ryrholm, N., Stefanescu, C., Hill, J. K., Thomas, C. D., Descimon, H., et al. (1999). Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature* 399, 579–583. doi: 10.1038/21181

Parsons, S. M. A., and Joern, A. (2013). Life history traits associated with body size covary along a latitudinal gradient in a generalist grasshopper. *Oecologia* 174, 379–391. doi: 10.1007/s00442-013-2785-6

Pearse, W. D., Davis, C. C., Inouye, D. W., Primack, R. B., and Davies, T. J. (2017). A statistical estimator for determining the limits of contemporary and historic phenology. *Nat. Ecol. Evol.* 1, 1876–1882. doi: 10.1038/s41559-017-0350-0

Pickford, R. (1953). A two-year life-cycle in grasshoppers (*Orthoptera*: *Acrididae*) overwintering as eggs and nymphs. *Can. Entomol.* 85, 9–14. doi: 10.4039/Ent859-1

Pimentel, C., Calvão, T., Santos, M., Ferreira, C., Neves, M., and Nilsson, J. Å. (2006). Establishment and expansion of a *Thaumetopoea pityocampa* (Den. & Schiff.) (*Lep. Notodontidae*) population with a shifted life cycle in a production pine forest, Central-Coastal Portugal. *Forest Ecol. Manag.* 233, 108–115. doi: 10.1016/j.foreco.2006.06.005

Pires, A., and Hoy, R. R. (1992). Temperature coupling in cricket acoustic communication. *J. Comp. Physiol. A* 171, 79–92. doi: 10.1007/BF00195963

Prudic, K., McFarland, K., Oliver, J., Hutchinson, R., Long, E., Kerr, J., et al. (2017). eButterfly: leveraging massive online citizen science for butterfly conservation. *Insects* 8, 53–12. doi: 10.3390/insects8020053

Ragland, G. J., Sim, S. B., Goudarzi, S., Feder, J. L., and Hahn, D. A. (2012). Environmental interactions during host race formation: host fruit environment moderates a seasonal shift in phenology in host races of *Rhagoletis pomonella*. *Funct. Ecol.* 26, 921–931. doi: 10.1111/j.1365-2435.2012.01992.x

Ritchie, M. G., Saarikettu, M., Livingstone, S., and Hoikkala, A. (2001). Characterization of female preference functions for *Drosophila montana* courtship song and a test of the temperature coupling hypothesis. *Evolution* 55, 721–727. doi: 10.1554/0014-3820(2001)055[0721:COFPFF]2.0.CO;2

Rodríguez, R. L., Hallett, A. C., Kilmer, J. T., and Fowler-Finn, K. D. (2012). Curves as traits: genetic and environmental variation in mate preference functions. *J. Evol. Biol.* 26, 434–442. doi: 10.1111/jeb.12061

Rosenthal, G. G. (2017). *Mate Choice: The Evolution of Sexual Decision Making From Microbes to Humans*. Princeton, NJ: Princeton University Press.

Rowe, K. C., Rowe, K. M. C., Tingley, M. W., Koo, M. S., Patton, J. L., Conroy, C. J., et al. (2014). Spatially heterogeneous impact of climate change on small mammals of montane California. *Proc. Biol. Sci.* 282:20141857. doi: 10.1098/rspb.2014.1857

Ryan, S. F., Deines, J. M., Scriber, J. M., Pfrender, M. E., Jones, S. E., Emrich, S. J., et al. (2018). Climate-mediated hybrid zone movement revealed with genomics, museum collection, and simulation modeling. *Proc. Natl. Acad. Sci. U.S.A.* 16:201714950. doi: 10.1073/pnas.1714950115

Sánchez-Bayo, F., and Wyckhuys, K. A. G. (2019). Worldwide decline of the entomofauna: a review of its drivers. *Biol. Conserv.* 232, 8–27. doi: 10.1016/j.biocon.2019.01.020

Santos, H., Burban, C., Rousselet, J., Rossi, J. P., Branco, M., and Kerdelhu,é, C. (2011). Incipient allochronic speciation in the pine processionary moth (*Thaumetopoea pityocampa*, *Lepidoptera*, *Notodontidae*). *J. Evol. Biol.* 24, 146–158. doi: 10.1111/j.1420-9101.2010.02147.x

Santos, H., Rousselet, J., Magnoux, E., Paiva, M.-R., Branco, M., and Kerdelhué, C. (2007). Genetic isolation through time: allochronic differentiation of a phenologically atypical population of the pine processionary moth. *Proc. Biol. Sci.* 274, 935–941. doi: 10.1098/rspb.2006.3767

Scriber, J., Elliot, B., Maher, E., McGuire, M., and Niblack, M. (2014). Adaptations to "thermal time" constraints in *Papilio*: latitudinal and local size clines differ in response to regional climate change. *Insects* 5, 199–226. doi: 10.3390/insects5010199

Scriber, J. M. (2011). Impacts of climate warming on hybrid zone movement: Geographically diffuse and biologically porous "species borders." *Insect Sci.* 18, 121–159. doi: 10.1111/j.1744-7917.2010.01367.x

Senner, N. R., Stager, M., and Cheviron, Z. (2017). Spatial and temporal heterogeneity in climate change limits species' dispersal capabilities and adaptive potential. *Ecography* 41, 1428–1440. doi: 10.1111/ecog.03234

Silvertown, J. (2009). A new dawn for citizen science. *Trends Ecol. Evol.* 24, 467–471. doi: 10.1016/j.tree.2009.03.017

Simon, C., Tang, J., Dalwadi, S., Staley, G., Deniega, J., and Unnasch, T. R. (2000). Genetic evidence for assortative mating between 13-year cicadas and sympatric "17-year cicadas with 13-year life cycles" provides support for allochronic speciation. *Evolution* 54, 1326–1336. doi: 10.1111/j.0014-3820.2000.tb00565.x

Sota, T., Yamamoto, S., Cooley, J. R., Hill, K. B. R., Simon, C., and Yoshimura, J. (2013). Independent divergence of 13- and 17-y life cycles among three periodical cicada lineages. *Proc. Natl. Acad. Sci. U.S.A.* 110, 6919–6924. doi: 10.1073/pnas.1220060110

Sparks, T. H., Dennis, R. L. H., Croxton, P. J., and Cade, M. (2007). Increased migration of *Lepidoptera* linked to climate change. *Eur. J. Entomol.* 104, 139–143. doi: 10.14411/eje.2007.019

Sullivan, B. L., Wood, C. L., Iliff, M. J., Bonney, R. E., Fink, D., and Kelling, S. (2009). eBird: A citizen-based bird observation network in the biological sciences. *Biol. Conserv.* 142, 2282–2292. doi: 10.1016/j.biocon.2009.05.006

Tanaka, S. (1991). Genetic compatibility and geographic profile of two closely related species of *Allonemobius* (*Gryllidae*: *Orthoptera*). *Ann. Entomol. Soc. Am.* 84, 29–36. doi: 10.1093/aesa/84.1.29

Tauber, C. A., and Tauber, M. J. (1981). Insect seasonal cycles: genetics and evolution. *Annu. Rev. Ecol. Syst.* 12, 281–308. doi: 10.1146/annurev.es.12.110181.001433

Taylor, R. S., and Friesen, V. L. (2017). The role of allochrony in speciation. *Mol. Ecol.* 26, 3330–3342. doi: 10.1111/mec.14126

Taylor, S. A., Larson, E. L., and Harrison, R. G. (2015). Hybrid zones: windows on climate change. *Trends Ecol. Evol.* 30, 398–406. doi: 10.1016/j.tree.2015.04.010

Tobin, P. C., Nagarkatti, S., Loeb, G., and Saunders, M. C. (2008). Historical and projected interactions between climate change and insect voltinism in a multivoltine species. *Glob. Change Biol.* 14, 951–957. doi: 10.1111/j.1365-2486.2008.01561.x

Walker, S. E., and Cade, W. H. (2003). The effects of temperature and age on calling song in a field cricket with a complex calling song, *Teleogryllus oceanicus* (*Orthoptera*: *Gryllidae*). *Can. J. Zool.* 81, 1414–1420. doi: 10.1139/z03-106

Walker, T. J. (1962). Factors responsible for intraspecific variation in the calling songs of crickets. *Evolution* 16, 407–428. doi: 10.1111/j.1558-5646.1962.tb03234.x

Wellenreuther, M., Tynkkynen, K., and Svensson, E. I. (2010). Simulating range expansion: male species recognition and loss of premating isolation in damselflies. *Evolution* 64, 242–252. doi: 10.1111/j.1558-5646.2009.00815.x

Wiens, J. J. (2016). Climate-related local extinctions are already widespread among plant and animal species. *PLoS Biol.* 14:e2001104. doi: 10.1371/journal.pbio.2001104

Xie, X., Michel, A. P., Schwarz, D., Rull, J., Velez, S., Forbes, A. A., et al. (2008). Radiation and divergence in the *Rhagoletis Pomonella* species complex: inferences from DNA sequence data. *J. Evol. Biol.* 21, 900–913. doi: 10.1111/j.1420-9101.2008.01507.x

Yamamoto, S., and Sota, T. (2009). Incipient allochronic speciation by climatic disruption of the reproductive period. *Proc. Biol. Sci.* 276, 2711–2719. doi: 10.1098/rspb.2009.0349

Yamamoto, S., and Sota, T. (2012). Parallel allochronic divergence in a winter moth due to disruption of reproductive period by winter harshness. *Mol. Ecol.* 21, 174–183. doi: 10.1111/j.1365-294X.2011.05371.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 26 September 2019
doi: 10.3389/fevo.2019.00361

Patterns of Thermal Sensitivity and Sex-Specificity of Courtship Behavior Differs Between Two Sympatric Species of *Enchenopa* Treehopper

Anthony Macchiano, Daniel A. Sasson, Noah T. Leith and Kasey D. Fowler-Finn*

Department of Biology, Saint Louis University, St. Louis, MO, United States

Predicting how insects will react to future thermal conditions requires understanding how temperature currently affects insect behavior, from performance traits to those involved in mating and reproduction. Many reproductive behaviors are thermally-sensitive, but little is known how temperature affects the behaviors used to find mates and coordinate mating. Here, we investigate how temperature influences courtship activity in two sympatric species of *Enchenopa* treehoppers (Hemiptera: Membracidae). *Enchenopa* use substrate-borne vibrational signals exchanged in male-female duets to facilitate pair formation prior to mating. In a controlled laboratory setting, we assessed the likelihood of males and females to produce courtship signals across a range of ecologically relevant temperatures. We found that changes in courtship activity across temperatures differed between the two species. We also found sex differences within species: in one species males were more likely to signal at higher temperatures, while in the other species females were more likely to signal at higher temperatures. Our results suggest that sex-specific responses to temperature may constrain mating to narrower ranges of temperatures. Furthermore, sympatric species may respond differently to changes in thermal variation despite sharing similar climactic history.

Keywords: plasticity, vibrational communication, sex-specific responses, sympatry, mating behavior

Edited by:
Shannon Murphy,
University of Denver, United States

Reviewed by:
Erin Brandt,
University of California, Berkeley, United States
Laurel Symes,
Cornell University, United States

****Correspondence:***
Anthony Macchiano
anthony.macchiano@slu.edu

Specialty section:
This article was submitted to Population and Evolutionary Dynamics,
a section of the journal Frontiers in Ecology and Evolution

Received: *27 May 2019*
Accepted: *11 September 2019*
Published: *26 September 2019*

Citation:
Macchiano A, Sasson DA, Leith NT and Fowler-Finn KD (2019) Patterns of Thermal Sensitivity and Sex-Specificity of Courtship Behavior Differs Between Two Sympatric Species of Enchenopa Treehopper. Front. Ecol. Evol. 7:361. doi: 10.3389/fevo.2019.00361

INTRODUCTION

Insect biomass is rapidly declining worldwide in the wake of global warming (Hallmann et al., 2017). Due to the crucial role that insects play in nearly every ecological setting, these declines could have far-reaching implications for ecosystem functioning (Losey and Vaughan, 2006; Gallai et al., 2009; Ollerton et al., 2011). Rising temperatures pose a threat to insects because of the thermal sensitivity of a wide range of physiological, morphological, and behavioral traits (Kingsolver and Huey, 2008; Gibert et al., 2016; Abram et al., 2017). Many studies have focused on the thermal sensitivity of traits that are related to organismal performance, such as growth rates, heat shock, or flight performance (Deutsch et al., 2008; Frazier et al., 2008; King and MacRae, 2015). Additionally, some studies have investigated the effects of temperature on various aspects of reproduction, with a focus on life history traits like the timing and length of mating seasons and average clutch sizes, or the location of breeding sites (Fielding et al., 1999; García-Barros, 2000; Guarneri et al., 2003; Braschler and Hill, 2007; Katsuki and Miyatake, 2009). However, temperature variation can also affect activity levels related to courtship and the coordination of mating. Because courtship activity precedes all other aspects of reproduction (Eberhard, 1994; Ejima and Griffith, 2007), thermal constraints on these behaviors may profoundly impact overall patterns of reproduction in insect populations. Furthermore, given that temperature-related breakdowns in mating may quickly lead to insect population declines (Høye et al., 2013; Chinellato et al., 2014), the thermal sensitivity of behaviors related to the coordination of mating should be examined in the context of global warming.

Increased temperatures can generate breakdowns in the coordination of mating if daily temperatures shift such that the thermal window of mating for an insect is no longer common in the environment. Mating activity is often highest at intermediate temperatures, but physiological constraints limit activity as temperatures deviate from this optimal range (Huey and Stevenson, 1979; Deutsch et al., 2008; Kingsolver and Huey, 2008; Angilletta, 2009). Shifts in temperature due to global warming could limit the availability of temperatures within the range of high reproductive activity and lead to reduced opportunities for mating. Additionally, sex-specific thermal effects may also generate breakdowns in sexual communication in the wake of global warming. While males and females within a species are often assumed to react similarly to environmental stressors like temperature (Shreve et al., 2004), sex-specific thermal responses in courtship activity could arise through sex differences in body size and thermoregulatory behavior during reproduction (Brown and Weatherhead, 2000; Matzkin et al., 2009; Darnell et al., 2013; Baudier et al., 2015; Foley et al., 2019). If reproductive activity levels across temperatures are sex-specific, shifts in temperatures could accentuate mismatches in male and female activity levels and reduce overlap when both sexes are actively seeking mates.

In this study, we examine how temperature affects courtship activity in light of the challenges that global warming poses to the coordination of mating. We examined patterns of courtship activity of males and females in two sympatric species of *Enchenopa* treehoppers (Hemiptera: Membracidae) across a range of temperatures. We then placed these activity patterns within the context of contemporary warming to assess the potential for temperature-related breakdowns in the coordination of mating. *Enchenopa* treehoppers are ∼1/2 cm sap-feeding insects that are highly host-plant-specific (Wood and Guttman, 1983; Wood, 1993; Rodríguez et al., 2004; Cocroft et al., 2008, 2010). They spend their entire lives, including coordinating mating, on their species-specific host plants. The complex is hypothesized to have diversified through shifts in host plant usage (Lin and Wood, 2002) and concurrent changes in sexual selection via female preferences on male signal traits (Rodríguez et al., 2004; Rodríguez and Cocroft, 2006). Multiple species can be found living in the same area (Wood, 1980), but are readily distinguished based on their use of host plant and courtship signal characteristics (Rodríguez and Cocroft, 2006; Cocroft et al., 2008). To find mates, sexually mature males will fly from stem to stem on their host plant and produce courtship advertisement signals in the form of plant-borne vibrations when they land (Hunt, 1994; Cocroft et al., 2008). If a female is receptive and finds a male signal attractive, she will respond with her own sex-specific vibrational courtship signals, which initiates an alternating signaling duet; this duet aids in mate localization and the initiation of pair formation prior to mating (Rodríguez et al., 2004; Rodríguez and Cocroft, 2006).

Enchenopa treehoppers are well-suited for studies of the effects of thermal variation on reproductive-related behaviors. The nature of the thermal environment for one of the species in the current study has been previously characterized (Jocson et al., 2019), and we know that temperature affects the expression of male advertisement signals and female mate preferences (Jocson et al., 2019). However, the likelihood of engaging in reproductive-related behaviors across temperatures is currently unknown. Aside from live male-female interactions, we are not aware of any studies that tested both males and females to determine how temperature affected each sex. The *Enchenopa* duetting system provides a mechanism with which to do so using vibrational playbacks to assay courtship activity without the other sex needing to be present (Rodríguez et al., 2004, 2012; Rodríguez and Cocroft, 2006).

Here, we focused on two sympatric species of *Enchenopa* treehopper (Hemiptera: Membracidae). One species lives on the host plant *Ptelea trifoliata* (Rutaceae) and the other on the host plant *Viburnum prunifolium* (Caprifoliaceae). Both host plants occur sympatrically at our study site. We expect the temperature where activity levels are highest and the range corresponding to high behavioral activity to reflect the thermal variation experienced by an organism in its historical habitat (Angilletta et al., 2002; Clusella-Trullas et al., 2011). While the biogeographic history of the two species under study is not known, they have overlapping distributions throughout their range (Wood, 1980), suggesting a somewhat shared thermal regime. Thus, we expect some similarity in behavioral responses to temperature variation. However, the scale of the thermal environment is important to consider (Logan et al., 2013) and thermal microclimates can vary between plant hosts (Suggitt et al., 2011; Pincebourde and Woods, 2012). Divergent selection from microclimates could result in different thermal optima and tolerances (Kellermann et al., 2012). Specifically for the two host plant species in our study, *P. trifoliata* is found primarily in semi-shaded areas and *V. prunifolium* tolerates full and partial sun. Thus, while we expected some similarity in activity patterns across temperatures due to the sympatry and close phylogenetic relatedness of the two *Enchenopa* species (Lin and Wood, 2002), it is not unreasonable to expect that the species on *V. prunifolium* may be more active at hotter temperatures as compared to the species on *P. trifoliata*.

In addition to species-specific responses to temperature, we might predict that males and females respond differently to temperature due to underlying sex-specific physiology (Brown and Weatherhead, 2000; Matzkin et al., 2009; Darnell et al., 2013; Baudier et al., 2015; Foley et al., 2019). However, courtship activity results from a complex interaction between physiology and behavioral decisions, and we currently lack a complete framework for making an informed prediction about sex-specific responses to temperature. The larger sex of a species—females in the case of *Enchenopa* (Hamilton and Cocroft, 2009)—is likely to have a higher thermal tolerance (Baudier et al., 2015; Foley et al., 2019). However, relative size does not always predict thermal preference. For example, males of some *Drosophila* species prefer hotter temperatures than females despite being smaller, but this is not always the case (Dillon et al., 2009). Furthermore, the costs of courtship can be higher at hotter temperatures (Teal, 1959), and these costs may be borne more by the typically more actively-courting males. Finally, even when thermal tolerance, thermal preference, and the costs of behaving at thermal extremes is known, these factors may not generate a straightforward prediction about when courtship occurs. For example, male jumping spiders are most active at low temperatures in the field while mating rates are greatest at high temperatures in a laboratory setting (Brandt et al., 2018) and male fiddler crabs court at the edge of their thermal limits (Allen and Levinton, 2014).

Here, we used vibrational playbacks to test the likelihood of male and female *Enchenopa* to produce courtship signals across a range of temperatures (18–36°C). This range reflects common temperatures experienced by *Enchenopa* treehoppers during the mating season for the species living on *P. trifoliata* (Jocson et al., 2019). Using a function-valued approach—treating the entire curve as the trait of interest (Meyer and Kirkpatrick, 2005; Kingsolver and Huey, 2008; Stinchcombe and Kirkpatrick, 2012; Hadjipantelis et al., 2013)—we compared the male versus female thermal courtship activity curves within species, and within-sex courtship activity curves across species. We found sex-specific responses to temperature, suggesting that changes in thermal regime could accentuate mismatches between males and females in optimal temperature for actively seeking mates. We also found species-specific responses to temperature, suggesting that warming could differentially affect the breakdown of mate coordination in two species with a shared historical temperature regime.

MATERIALS AND METHODS

Study Organisms

We specifically studied the two species of treehoppers in the *Enchenopa binotata* species complex that live on *P. trifoliata* and *V. prunifolium*. Many species in the *E. binotata* species complex have yet to be formally described and share a common genus and species name (Hamilton and Cocroft, 2009). Thus, we refer to our study species using the name of their host plants: *E. binotata* "Ptelea" and *E. binotata* "V. prunifolium." The two species are morphologically similar but easily distinguished by the mean signal frequency of the male advertisement signal (Wood and Guttman, 1983; Lin and Wood, 2002; Rodríguez et al., 2004; Cocroft et al., 2008, 2010). *Enchenopa binotata* "Ptelea" has a mean signal frequency of 350 Hz and *E. binotata* "V. prunifolium" has a mean signal frequency of 285 (Rodríguez and Cocroft, 2006) at a standardized 25°C. The data we present here includes an entirely new data set for *E. binotata* "V. prunifolium," and data set from *E. binotata* "Ptelea" collected for a separate study conducted in the same year (Leith et al., in review) but significantly expanded for our current study.

In May 2018, we collected *E. binotata* nymphs living on *V. prunifolium* and *P. trifoliata* host plants from Stephens Lake Park (38.927133,−92.320419) in Columbia, Missouri as 2–3rd instars. We brought the nymphs back to a greenhouse on the campus of Saint Louis University in St. Louis, MO where we reared them on potted and netted host plant exemplars in groups of ∼20. Upon the last molt to adulthood, males and females were separated to control for sexual/signaling experience (Fowler-Finn and Rodríguez, 2012). When the insects reached sexual maturity (about 2 weeks after the molt to adulthood for males and about 6 weeks after the molt to adulthood for females), we started our experimental trials testing the temperatures at which individuals were reproductively active. We conducted the experimental trials between June and July 2018.

General Approach

To determine the level of courtship activity of males and females across temperatures, we took advantage of the duetting system of *E. binotata* treehoppers: males signal to advertise to females and females respond (Rodríguez et al., 2004, 2012; Rodríguez and Cocroft, 2006). Reproductively receptive males readily signal when placed on a host plant or in response to a recording of a live duetting pair; similarly, females that are reproductively receptive will readily respond to recordings of live males (Rodríguez et al., 2004, 2012; Rodríguez and Cocroft, 2006). Thus, we used vibrational playbacks to test male and female receptivity. By repeating these vibrational playbacks across a range of temperatures in a controlled laboratory setting, we quantified variation in courtship activity across a range of temperatures. See below for details.

Vibrational Playback and Recording Set Up

We used the programs Audacity (v. 2.1.1; http://audacity.sourforge.net/) and MatLab (v.8.3 2014) to play vibrational primers from WAV files that were recorded from a live male-female duet at 25°C (for male primers) and a live male signaling at each specified temperature (for female primers, see below) from the populations tested. The recordings were selected based on representing an average signal type at the recording temperature. The WAV files were passed first through a Roland Duo-Capture USB interface (Model No. UA-11-MK2) and then transmitted to the plant through linear resonant actuators (LRA coin type Z-axis Model G0832012) affixed to the stem with beeswax, and played back at ∼0.2 mm/s. We monitored and recorded vibrational signals produced by the insects using accelerometers (Vibra Metrics Model No. 9002A with signal conditioner and power supply Model P5000) connected to Roland Duo-Capture Ex USB interfaces (Model No. UA-22) and PreSonus Audiobox USB interfaces.

To isolate the testing set up from background vibrations, the testing incubator rested on either 1-inch steel plates or 2-inch concrete slabs that were floated on a heavy table with rubber casters using partially-inflated bicycle inner tubes. The experimental host plant was further isolated from the testing incubator with sorbothane pads.

Experimental Trials

We measured the likelihood of males and females to engage in courtship activity across seven target temperatures (18, 21, 24, 27, 30, 33, 36°C) using the above vibrational playback protocol.

The selected temperatures span the range at which *E. binotata* has been found active during the mating season in the field (Jocson et al., 2019) with the upper limit corresponding to the point past which mortality starts occurring in the laboratory. We randomly assigned each individual to one of the seven testing temperatures and acclimated them in an incubator set to their assigned testing temperatures for at least 20 min prior to testing their courtship activity (following: Greenfield and Medlock, 2007). We then transferred the individual to the testing plant in the testing incubator set to their assigned temperature and allowed them to adjust to the plant for 2 min before playing back species- and sex-specific vibrational primers to the individual. Our methods follow well-established protocol for determining if male and female *E. binotata* are receptive (Rodríguez et al., 2004, 2012; Rodríguez and Cocroft, 2006; Cocroft et al., 2008).

For males, we played a species-specific male-female duet primer (a male advertisement signal followed by a female response signal) twice in quick succession, every 2 min during a 10 min trial. If the male responded to the primer at any point during the trial, he was marked as reproductively active. For females, we played three species-specific male signal bouts, with each bout consisting of three (*E. binotata* "V. prunifolium") or six (*E. binotata* "Ptelea") signals to match the mean signals per bout for the species (Hunt, 1994; Rodríguez et al., 2004; Rodríguez and Cocroft, 2006). Each signal bout was separated from the next by 15 s of silence. To reduce the likelihood of confounding variation among females in preference for male signals either due to temperature (Jocson et al., 2019) or individual variation (Fowler-Finn et al., 2017) with whether or a not a female was receptive at a given temperature, we varied the fundamental frequency of the three signal bouts. The first signal bout consisted of a recorded male signaling at the mean frequency of the species range (recorded at 25°C: 285 Hz for *E. binotata* "V. prunifolium," 350 Hz for *E. binotata* "Ptelea"; Rodríguez and Cocroft, 2006). The second primer we played to a female was a male signaling at the specific testing temperature (with the exception that signals recorded at 21°C were used for tests at 18°C in both species because we were unable to record males signaling at 18°C prior to the start of the experiment; and a signal recorded at 33°C was used for tests at 36°C in *E. binotata* "Ptelea" because we were unable to record males signaling at 36°C; see results). The third primer consisted of a male signal recorded at 21°C for females tested below 27°C, or recorded at 36°C (33°C for *E. binotata* "Ptelea") for females tested at or above 27°C. After all three signal bouts were played in a random order, we waited 2 min and repeated the process of playing all three primers in a newly randomized order. If a female produced a vibrational response to any of the primers during the trial, we considered her reproductively receptive. Our design ensured that females not only heard the mean preferred signal frequency, but they also heard a range of other signal frequencies to help account for any variation in female preference across individuals. This aspect was important because females tend to be less active than males and also more discriminating (Rodríguez et al., 2012). Thus, our design minimized the likelihood of falsely classifying a female as unreceptive.

We tested individuals one to three times, never testing them at the same temperature more than once. At the conclusion of the experiment, we had 113 trials for *E. binotata* "V. prunifolium: (males $N = 44$, females $N = 69$) and 153 trials for *E. binotata* "Ptelea" (males $N = 69$, females $N = 84$).

We used the function-valued approach to compare courtship activity patterns across a range of temperatures between the two species, and between the sexes within each species. Function-valued traits use mathematical functions to describe responses to continuous environmental variation (Meyer and Kirkpatrick, 2005; Kingsolver and Huey, 2008; Stinchcombe and Kirkpatrick, 2012; Hadjipantelis et al., 2013). Here, the response is the likelihood of producing a courtship signal/response and the environmental gradient is temperature. The curves we derive from the raw data illustrate the likelihood of exhibiting courtship activity across temperature—herein "thermal courtship activity curve."

Thermal Variation in Courtship Behavior Within and Across Species—Statistical Analyses

First, we tested for the effects of temperature on male and female courtship activity for each species independently by running nominal logistic regressions for each group (male and female of *E. binotata* "V. prunifolium" and male and female *E. binotata* "Ptelea") separately. The dependent variable was whether an individual produced a vibrational signal in response to the vibrational playbacks. The independent variables were temperature and a temperature × temperature interaction term. The temperature term tests for a linear response of courtship activity to temperature (e.g., increased activity as temperature increases). The temperature × temperature interaction specifically tests the quadratic response of courtship activity to temperature because many thermally-sensitive traits have quadratic shapes (i.e., they peak at intermediate temperatures).

Next we compared variation in courtship activity across temperature for the following pairings: (i) male vs. female *E. binotata* "V. prunifolium," (ii) male vs. female *E. binotata* "Ptelea," (iii) male *E. binotata* "V. prunifolium," vs. male *E. binotata* "Ptelea," and (iv) female *E. binotata* "V. prunifolium," vs. female *E. binotata* "Ptelea." We used nominal logistic regressions with the dependent variable in each model being whether an individual produced a signal in response to the vibrational playback (as above). To compare courtship activity curves across sexes within species the independent variables were: temperature, sex, a temperature × temperature interaction, a temperature × sex interaction, and a temperature × temperature × sex interaction term. The temperature × temperature × sex interaction term was the key variable that tested whether the shape of the function varied between the sexes, which would indicate that courtship activity of the two sexes was affected by temperature in different ways. To compare courtship activity curves within sex across species, we used the same independent variables, substituting species for sex. We ran all statistical analyses in JMP Pro (14.1.0).

Describing Thermal Variation in Courtship Behavior—Qualitative Analyses

We next qualitatively compared the curves to each other using the following methodology. To visualize thermal courtship activity curves, we generated cubic splines in R (with "mgcv" package) using the open-source program PFunc (Kilmer et al., 2017; see **Box 1**). Cubic spline regressions are non-parametric curves which illustrate changes in the expression of a trait without making assumptions of the shape of the curve (Kilmer et al., 2017). We generated thermal courtship activity curves independently for male and female *E. binotata* "V. prunifolium," and compared these to each other, as well as to thermal courtship activity curves for male and female *E. binotata* "Ptelea." From each thermal courtship activity curve, we extracted the thermal activity peak, thermal activity breadth, and thermal activity window using the PFunc program (**Box 1**). Moreover, we qualitatively assessed variation among groups in these three parameters of the thermal courtship activity curves using the values extracted by the PFunc program.

Historical and Contemporary Weather Data

Typical approaches for predicting responses to global warming involve assessing current trait expression and projecting forward given estimated thermal changes (Hoffmann and Sgrò, 2011). We use a variant of this approach by mapping thermal courtship activity curves onto ranges of variation in historical and contemporary temperature data. The immense amount of weather data available at the study site we use for the two species allows us to visualize how temperature has changed over recent time at this locale and enables us to make predictions regarding how these species may cope with rising temperatures in the future. We obtained historical (1975–1978) and contemporary (2015–2018) daily low and daily high temperatures from June 1 through July 31—the primary mating season for *E. binotata*—recorded from Columbia Regional Airport weather station in Columbia, MO (38.8169°, −92.2183°), accessible via the National Oceanic and Atmospheric Administration online database (www.ncdc.noaa.gov).

RESULTS

Thermal Effects on Courtship Activity

Courtship activity for males and females of both species showed both linear and quadratic responses to temperature (showing a peak at an intermediate temperature), except for female *E. binotata* "Ptelea" females, which showed only a linear response to temperature and thus were most active at the hottest testing temperatures (**Table 1**, **Figure 1**).

Comparison of Thermal Effects on Courtship Activity Within Species

Male and female *E. binotata* "V. prunifolium" thermal courtship activity curves were statistically similar, including the change in activity across temperatures (lack of statistically significant temperature × sex and temperature × temperature × sex interaction terms; **Table 2**) and overall courtship activity levels (lack of a significant sex term; **Table 2**, **Figure 2**). In contrast, the thermal courtship activity curves for male and female *E. binotata* "Ptelea" differed in both overall shape (significant temperature × temperature × sex term; **Table 2**, **Figure 2**) and overall activity level (significant sex term due to higher male courtship activity; **Table 2**, **Figure 2**).

Comparison of Thermal Effects on Courtship Activity Across Species

For both males and females, the species differed in both the overall courtship activity levels (significant species term; **Table 3**), as well as the overall shape of the thermal courtship activity curve (significant temperature × temperature × species term, although this result is marginally not significant for males; $p = 0.06$; **Table 3**).

Qualitative Analyses of Thermal Courtship Activity Curves

Male and female thermal courtship activity curves were more similar to each other in *E. binotata* "V. prunifolium" than in *E. binotata* "Ptelea." We found that thermal activity peak was 4.4°C higher in males vs. females in *E. binotata* "V. prunifolium," while thermal activity window was barely 1°C wider in females, yet thermal activity breadth was 3°C wider in males than females (**Table 4**). Interestingly the thermal activity peak in *E. binotata* "Ptelea" was 7.9°C higher in females vs. males, and female thermal activity window was 1.3°C wider than in males. However, thermal activity breadth was 4.5°C wider in males vs. females (**Table 4**).

Historical and Contemporary Weather Data

The historical and contemporary temperature ranges differ: the average daily minimum temperature increased significantly from 17.99 to 19.94°C (F ratio $= 42.6551$, Prob $> F = <0.0001$) and the average daily high temperature also increased significantly from 30.08 to 30.78°C (F ratio $= 4.5387$, Prob $> F = 0.0336$). When comparing thermal courtship activity curves to contemporary thermal conditions during the mating season, we found that the thermal activity breadth of all the thermal courtship activity curves except *E. binotata* "Ptelea" females either completely or nearly overlap with intermediate daily temperatures (24–27°C) during the active mating season (**Figure 2**). We also found that the thermal activity peak for *E. binotata* "V. prunifolium" males aligns with the contemporary mean daily high temperature (∼32°C), with females exhibiting courtship activity rates of >60% (**Figure 2**). In contrast, while male *E. binotata* "Ptelea" are still active at the contemporary mean daily high, females exhibit closer to ∼40% activity levels at this temperature (**Figure 2**).

DISCUSSION

We assessed two potential ways in which temperature-related breakdowns in the coordination of mating could occur in two sympatric species of *E. binotata* treehopper: reduced availability of optimal temperature ranges for actively finding mates and the accentuation of mismatches in activity between sexes. To do so, we quantified patterns of thermal sensitivity in male and female

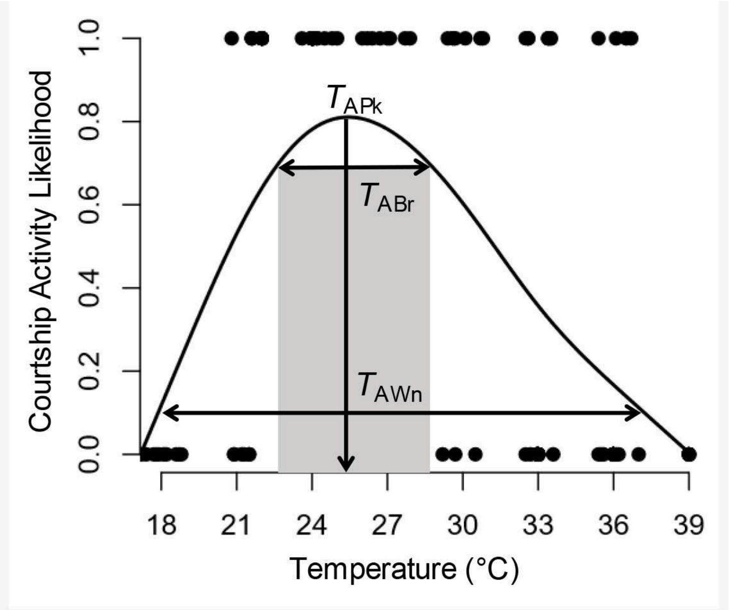
BOX 1 | Key definitions of terminology used to describe courtship activity curves and illustration of how these activity curves are derived from raw data and are measured.

The following definitions can be applied to any curve describing how activity levels of any behavior vary across any environmental variable. Here, we refer to courtship activity across temperature.

Thermal Activity Peak (T_{APk}): The temperature at which activity is highest.

Thermal Activity Breadth (T_{ABr}): The temperature range at which the likelihood of activity is > 90% of the maximum activity level.

Thermal Activity Window (T_{AWn}): The temperature range at which the likelihood of activity is 10%.



Individual data points represents the result of each test of a single individual and whether they produced a courtship signal or not at the testing temperature. The curve represents the likelihood of courtship activity occurring across environmental temperatures and is derived using a cubic spline regression. Cubic spline regressions make no assumptions about the shape of a curve other than that it is smooth (Schluter, 1988). To generate the curve, raw data (court yes or no) are input into the Pfunc module executed with a Python GUI (Kilmer et al., 2017). PFunc describes and analyzes functions by executing an R script that fits data using the gam function in the *mgcv* package (Wood, 2006). The script outputs measurements to users, which are then visualized through the interfacing Python GUI. The most recent version of the PFunc program can be downloaded from https://github.com/joccalor/pfunc/releases/latest).

TABLE 1 | The effects of temperature (linear and quadratic) on the likelihood of male signal production and female responses to male signals in *E. binotata* "Ptelea" and "V. prunifolium."

	L-R χ^2	*df*	*p*
Likelihood of male "V. prunifolium" signal (*N* = 44)			
Temperature	7.7	1	**0.0055**
Temperature × temperature	6.5	1	**0.0106**
Likelihood of female "V. prunifolium" response (*N* = 69)			
Temperature	5.4	1.2	**0.0204**
Temperature × temperature	18.0	1.2	**<0.0001**
Likelihood of male "Ptelea" signal (*N* = 69)			
Temperature	17.4	1.2	**<0.0001**
Temperature × temperature	37.3	1.2	**<0.0001**
Likelihood of female "Ptelea" response (*N* = 84)			
Temperature	11.0	1.2	**0.0009**
Temperature × temperature	0.2	1.2	0.6752

L-R χ^2 *denotes likelihood-ratio chi-squared value. Statistically significant terms indicated in boldface.*

courtship activity rates and then compared these activity patterns with contemporary temperatures. We found that male and female *E. binotata* "V. prunifolium" differ in optimal temperature for courtship activity, but overall show similar changes in courtship activity across temperatures. Thus, *E. binotata* "V. prunifolium" shows high overlap between the sexes in activity levels at the mean daily temperature range during the mating season. In contrast, male and female *E. binotata* "Ptelea" not only differed in optimal temperatures for courtship activity, but also exhibited strikingly different thermal courtship activity curves. In fact, male and female *E. binotata* "Ptelea" overlap in activity in a narrower range of temperatures than the other species and at temperatures lower than mean daily temperatures.

If we extrapolate mating rates from courtship activity curves—mating rates correspond more closely with male activity curves in *E. binotata* "Ptelea" (Leith et al., in review)—we predict that *E. binotata* "Ptelea" will experience greater disruption of the coordination of mating with global warming. Male *E. binotata* "Ptelea" peak in courtship activity at a temperature lower than

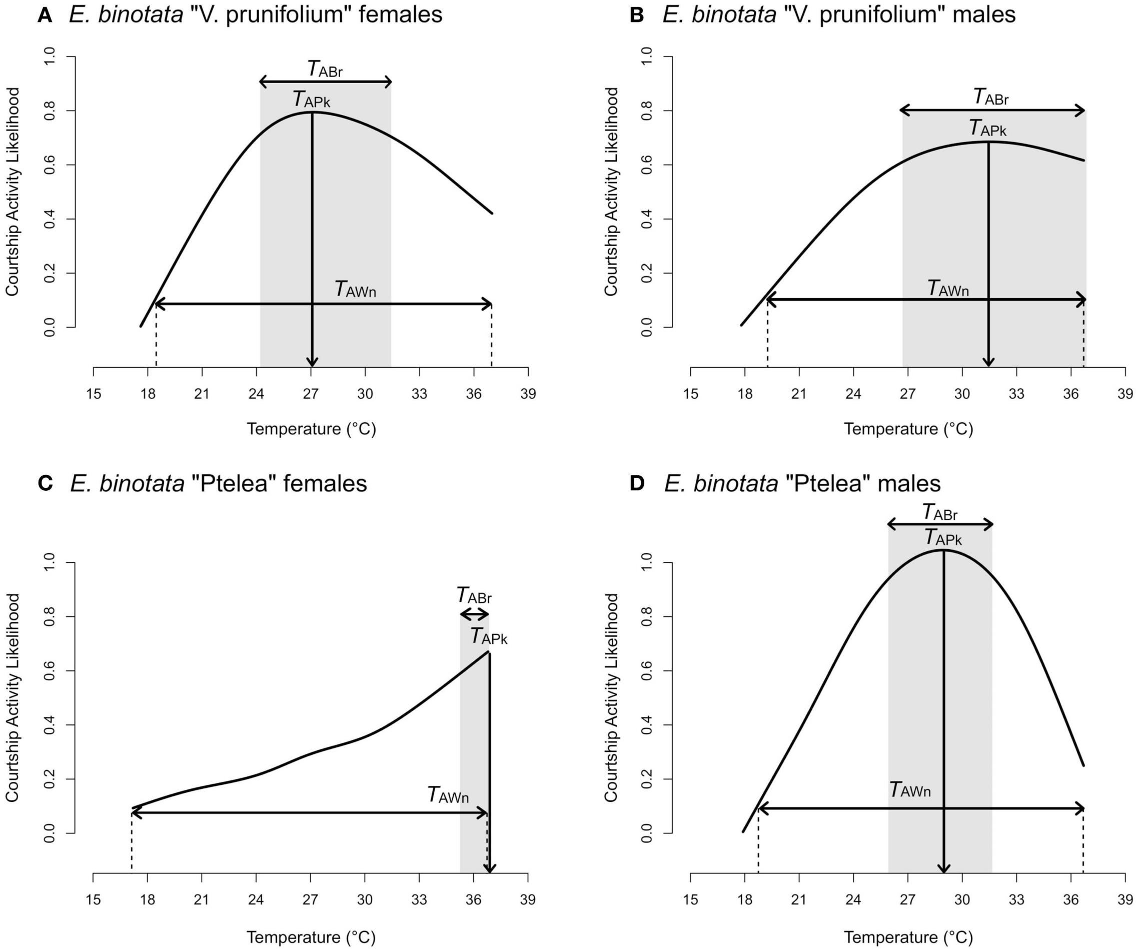


FIGURE 1 | Courtship activity curves of **(A)** *E. binotata* "V. prunifolium" females, **(B)** *E. binotata* "V. prunifolium" males, **(C)** *E. binotata* "Ptelea" females, and **(D)** *E. binotata* "Ptelea" males. "T_{APk}" represents the thermal activity peak; the shaded region and "T_{ABr}" represents the thermal activity breadth; the vertical dashed lines and "T_{AWn}" represent the thermal activity window.

contemporary mean daily high temperatures and nearly 8°C lower than females. Furthermore, *E. binotata* "Ptelea" appear quite restricted in the range at which both males and females are actively signaling, with males only being minimally active at temperatures for which females are most active. On the other hand, *E. binotata* "V. prunifolium" appears to be more of a thermal generalist, with higher courtship activity across a broad range of temperatures; furthermore, while the male thermal activity peak was 4.4°C higher than females, male activity patterns were broad and overlapped substantially with that of females. Given the extent to which daily temperatures at our study site have risen the past 40 years, windows for mating may be greatly reduced for *E. binotata* "Ptelea" unless they can adapt to a broader and hotter range of temperatures (Hoffmann and Sgrò, 2011; Sinclair et al., 2012; Austin and Moehring, 2013). Adaptation to local conditions will be particularly important for species like those in the *E. binotata* complex. These insects have limited mobility and patchy habitats, reducing the likelihood of dispersal to more suitable thermal habitats, which is a common response to global warming (Sinervo et al., 2010; Sunday et al., 2012).

Divergence in the thermal response of the two species we studied could involve many underlying ecological and physiological mechanisms (Kleynhans et al., 2014). The two species could vary in historical patterns of environmental conditions (Sih et al., 2011; Tuomainen and Candolin, 2011; Foster, 2013). For example, species that have experienced historically high fluctuations in temperature will show patterns of being more temperature generalists (Kingsolver, 2009). While we do not know how the thermal microclimates of the two host plant species compare, it is possible that *V. prunifolium* plants experience greater thermal variation, shaping a more generalist response. Other environmental and/or demographic properties could also shape adaptation to temperature variation in addition to selection from historical thermal regimes (Foster, 2013; Tuff et al., 2016) that may account for the differences between females of the two species. For example, the two treehopper species may differ in the temperatures at which they are most active at to reduce competition or avoid predators (Greenfield, 2002; Bailey, 2003) on their respective host plants. Regardless of mechanism, our results indicate that host plant species and other ecological interactions could potentially influence the vulnerability of insect species to thermal change.

As we predicted based on host plant microhabitat, *E. binotata* "V. prunifolium" males were active at hotter temperatures than *E. binotata* "Ptelea" males. However, females did not follow the same pattern. *E. binotata* "V. prunifolium" females peaked at the same temperature at which *E. binotata* "Ptelea" female leveled off in their activity levels. Furthermore, *E. binotata* "Ptelea" females maintained high courtship activity through the highest testing temperature 36°C. This high testing temperature

TABLE 2 | Comparisons between the effects of temperature on activity level of males and females within *E. binotata* "V. prunifolium" and "Ptelea."

	L-R χ^2	*df*	*p*
"V. prunifolium" Male vs. Female activity (*N* = 113)			
Temperature	13.1	1.5	**0.0003**
Sex	1.1	1.5	0.3046
Temperature × temperature	20.8	1.5	**<0.0001**
Temperature × sex	0.7	1.5	0.3917
Temperature × temperature × sex	0.1	1.5	0.7090
"Ptelea" Male vs. Female activity (*N* = 153)			
Temperature	20.5	1.5	**<0.0001**
Sex	42.1	1.5	**<0.0001**
Temperature × temperature	19.3	1.5	**<0.0001**
Temperature × sex	0.6	1.5	0.4494
Temperature × temperature × sex	23.1	1.5	**<0.0001**

L-R χ^2 *denotes likelihood-ratio chi-squared value. Statistically significant terms indicated in boldface.*

TABLE 3 | Comparisons between the effects of temperature on activity level of males and females between *E. binotata* "V. prunifolium" and *E. binotata* "Ptelea."

	L-R χ^2	*df*	*p*
Female activity between species (*N* = 153)			
Temperature	13.4	1.5	**0.0002**
Species	20.6	1.5	**<0.0001**
Temperature × temperature	7.4	1.5	**0.0064**
Temperature × species	0.3	1.5	0.6059
Temperature × temperature × species	10.5	1.5	**0.0012**
Male activity between species (*N* = 113)			
Temperature	22.7	1.5	**<0.0001**
Species	8.9	1.5	**0.0028**
Temperature × temperature	34.9	1.5	**<0.0001**
Temperature × species	0.0	1.5	0.8726
Temperature × temperature × species	3.5	1.5	0.0614

Statistically significant terms indicated in boldface.

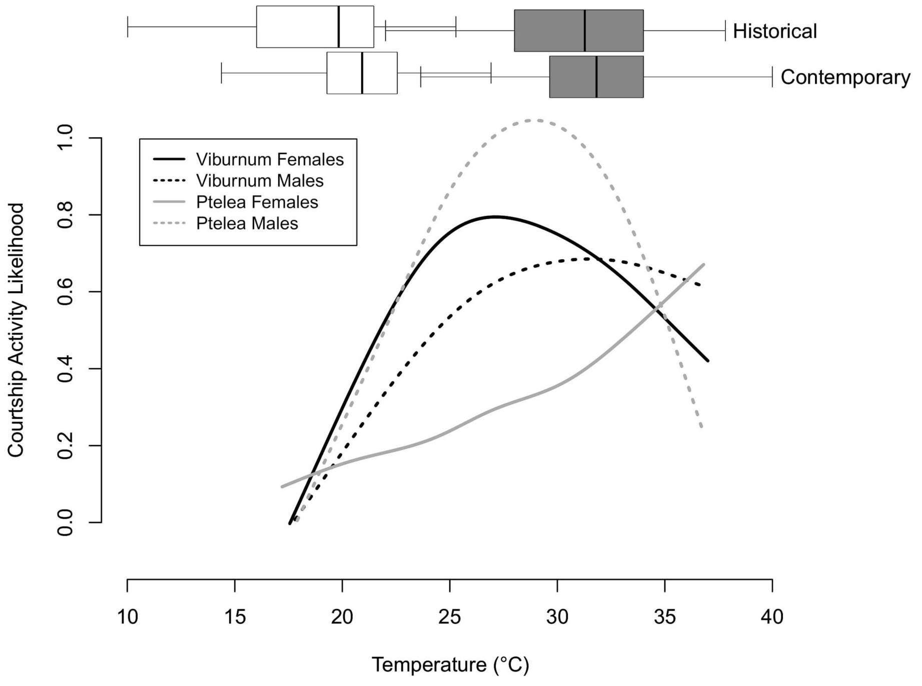


FIGURE 2 | Each courtship activity curve represented in **Figures 1A–D**, featuring historical and contemporary daily high (shaded) and daily low temperatures (white) from June 1 through July 31 between 1975 and 1978 (historical), and June 1 through July 31 between 2015 and 2018 (contemporary). Temperature box plots are not affiliated with the Y-axis.

TABLE 4 | Thermal activity peak, thermal activity window, and thermal activity breadth per grouping.

	Thermal activity peak (°C)	Thermal activity window (°C)	Thermal activity breadth (°C)
"V. prunifolium" males	31.5	19–36.7	26.8–36.7
"V. prunifolium" females	27.1	18.4–37.0	24.2–31.1
"Ptelea" males	28.9	18.7–36.7	25.9–31.7
"Ptelea" females	36.8	17.5–36.8	35.5–36.8

is also the point past which we start to see mortality in the laboratory (Jocson et al., 2019). It is notable that the *E. binotata* "Ptelea" female thermal courtship activity curve diverges from a typical performance curve. We do not have an explanation for why the patterns of sex-specific responses to temperature differ between species, or why *E. binotata* "Ptelea" females show such a distinct curve. However, more generally, potential causes of sex-specific thermal responses include differences between males and females in body size, physiological/metabolic costs of activity at hotter temperatures, or thermal by-products of sexual signaling (Block, 1994; Beaupre and Duvall, 1998; Cullum, 1998; Brown and Weatherhead, 2000; Kingsolver and Huey, 2008).

Differences among sexes in thermal courtship activity curves may affect mate coordination if sex-specific responses to temperature result in different daily activity patterns for males and females. Alternatively, sex-specific thermal courtship activity curves may have little effect on the coordination of mating if males and females utilize different thermal niches. For example, in crickets, males preferentially signal from warmer locations (Hedrick et al., 2002). In the host plant *P. trifoliata*, temperatures can vary over 5°C on a plant at a single point in time (Jocson et al., 2019), though how males and females utilize these temperatures is not yet known. Finally, it is also possible that patterns of activity in one sex may not predict the likelihood of mating occurring (e.g., Brandt et al., 2018). However, we found in *E. binotata* "Ptelea" that mating rates peak at the temperatures at which males are most likely to engage in courtship behavior (Leith et al., in review).

Sex-specific thermal courtship activity curves may also affect the mate selection process. In fiddler crabs, males court at very hot temperatures (Allen and Levinton, 2014), but also experience greater physiological stress at hotter temperatures when courting (Darnell et al., 2013). If only those males best able to withstand the stress can court at hot temperatures, females active at hotter temperatures may select the highest quality males (i.e., the handicap hypothesis; Cotton et al., 2004). However, for *E. binotata* "Ptelea," variability in male courtship signals and female mate preferences are more variable at hotter temperatures (Jocson et al., 2019), suggesting activity at hotter temperatures may result in a reduced ability to discriminate high quality mates. Further studies on the effects of temperature on mating-related behaviors will be necessary to generate a broader framework for understanding how temperature affects mate coordination and mate selection processes.

The results of our study predict that species occupying similar habitats may experience divergent effects of global warming on the coordination of mating. We found both species-specific and sex-specific patterns of courtship activity across temperatures. While we focused on patterns of courtship activity, shifts in temperature outside of contemporary ranges could impact several aspects of reproduction, ranging from mate selection and mating duration to egg viability (Huey and Stevenson, 1979; Shreve et al., 2004; Katsuki and Miyatake, 2009; Conrad et al., 2017; Jocson et al., 2019). A better understanding of how animals alter courtship and other reproductive behaviors across adverse thermal conditions can provide critical insight as to whether certain populations are at increased risk of decline. In particular, clear sex differences in responses to temperature indicate that understanding the effects of temperature on reproduction for a broad range of taxa may require testing sex-specific effects of thermal variation. It is too early to determine the long-term effects on insect populations due to temperature-related breakdowns in the coordination of mating. However, the consequences of global warming for sexual communication and the coordination of mating deserves more attention and we hope this study inspires more research into mating under variable thermal conditions.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

AM and KF-F designed the study. AM, NL, and DS collected data. AM, NL, and KF-F processed and analyzed the data. AM and KF-F were the primary authors with significant contributions from NL and DS.

FUNDING

Funding was provided by NSF IOS-1656818 to KF-F.

ACKNOWLEDGMENTS

We would like to thank E. Miller, C. Ballman, and S. Pidalta for helping with data collection, and M. T. Gonzales for generating the MATLAB script for playbacks. We would also like to thank M. FowlerFinn for design and construction of custom testing incubators and temperature sensors. Finally, we would like to thank two reviewers and S. Murphy for thoughtful feedback on the manuscript.

REFERENCES

Abram, P. K., Boivin, G., Moiroux, J., and Brodeur, J. (2017). Behavioural effects of temperature on ectothermic animals: unifying thermal physiology and behavioural plasticity. *Biol. Rev.* 92, 1859–1876. doi: 10.1111/brv.12312

Allen, B. J., and Levinton, J. S. (2014). Sexual selection and the physiological consequences of habitat choice by a fiddler crab. *Oecologia* 176, 25–34. doi: 10.1007/s00442-014-3002-y

Angilletta, M. J. (2009). *Thermal Adaptation: A Theoretical and Empirical Synthesis.* Oxford University Press. Available online at: https://www.oxfordscholarship.com/view/10.1093/acprof:oso/9780198570875.001.1/acprof-9780198570875 (accessed August 13, 2019).

Angilletta, M. J., Niewiarowski, P. H., and Navas, C. A. (2002). The evolution of thermal physiology in ectotherms. *J. Therm. Biol.* 27, 249–268. doi: 10.1016/S0306-4565(01)00094-8

Austin, C. J., and Moehring, A. J. (2013). Optimal temperature range of a plastic species, *Drosophila simulans*. *J. Anim. Ecol.* 82, 663–672. doi: 10.1111/1365-2656.12041

Bailey, W. J. (2003). Insect duets: underlying mechanisms and their evolution. *Physiol. Entomol.* 28, 157–174. doi: 10.1046/j.1365-3032.2003.00337.x

Baudier, K. M., Mudd, A. E., Erickson, S. C., and O'Donnell, S. (2015). Microhabitat and body size effects on heat tolerance: implications for responses to climate change (army ants: *Formicidae*, *Ecitoninae*). *J. Anim. Ecol.* 84, 1322–1330. doi: 10.1111/1365-2656.12388

Beaupre, S. J., and Duvall, D. J. (1998). Integrative Biology of RattlesnakesContributions to biology and evolution. *Bioscience* 48, 531–538. doi: 10.2307/1313315

Block, B. A. (1994). Thermogenesis in muscle. *Annu. Rev. Physiol.* 56, 535–577. doi: 10.1146/annurev.ph.56.030194.002535

Brandt, E. E., Kelley, J. P., and Elias, D. O. (2018). Temperature alters multimodal signaling and mating success in an ectotherm. *Behav. Ecol. Sociobiol.* 72:191. doi: 10.1007/s00265-018-2620-5

Braschler, B., and Hill, J. K. (2007). Role of larval host plants in the climate-driven range expansion of the butterfly Polygonia c-album. *J. Anim. Ecol.* 76, 415–423. doi: 10.1111/j.1365-2656.2007.01217.x

Brown, G. P., and Weatherhead, P. J. (2000). Thermal ecology and sexual size Dimorphism in Northern Water Snakes, Nerodia Sipedon. *Ecol. Monogr.* 70, 311–330. doi: 10.1890/0012-9615(2000)070[0311:TEASSD]2.0.CO;2

Chinellato, F., Battisti, A., Finozzi, V., and Faccoli, M. (2014). Better today but worse tomorrow: how warm summers affect breeding performance of a Scots pine pest. *Agrochimica* 58, 133–145. doi: 10.1400/226737

Clusella-Trullas, S., Blackburn, T. M., and Chown, S. L. (2011). Climatic predictors of temperature performance curve parameters in ectotherms imply complex responses to climate change. *Am. Nat.* 177, 738–751. doi: 10.1086/660021

Cocroft, R. B., RodrÍGuez, R. L., and Hunt, R. E. (2008). *Host Shifts, the Evolution of Communication, and Speciation in the Enchenopa binotata Species Complex of Treehoppers*. University of California Press. Available online at: http://california.universitypressscholarship.com/view/10.1525/california/9780520251328.001.0001/upso-9780520251328-chapter-7 (accessed April 18, 2019).

Cocroft, R. B., Rodríguez, R. L., and Hunt, R. E. (2010). Host shifts and signal divergence: mating signals covary with host use in a complex of specialized plant-feeding insects. *Biol. J. Linn. Soc.* 99, 60–72. doi: 10.1111/j.1095-8312.2009.01345.x

Conrad, T., Stöcker, C., and Ayasse, M. (2017). The effect of temperature on male mating signals and female choice in the red mason bee, *Osmia bicornis* (L.). *Ecol. Evol.* 7, 8966–8975. doi: 10.1002/ece3.3331

Cotton, S., Fowler, K., and Pomiankowski, A. (2004). Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proc. Biol. Sci.* 271, 771–783. doi: 10.1098/rspb.2004.2688

Cullum, A. J. (1998). Sexual dimorphism in physiological performance of whiptail lizards (Genus *Cnemidophorus*). *Physiol. Zool.* 71, 541–552. doi: 10.1086/515961

Darnell, M. Z., Fowler, K. K., and Munguia, P. (2013). Sex-specific thermal constraints on fiddler crab behavior. *Behav. Ecol.* 24, 997–1003. doi: 10.1093/beheco/art006

Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., et al. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl. Acad. Sci. U.S.A.* 105, 6668–6672. doi: 10.1073/pnas.0709472105

Dillon, M. E., Wang, G., Garrity, P. A., and Huey, R. B. (2009). Review: thermal preference in Drosophila. *J. Therm. Biol.* 34, 109–119. doi: 10.1016/j.jtherbio.2008.11.007

Eberhard, W. (1994). Evidence for widespread courtship during copulation in 131 species of insects and spiders, and implications for cryptic female choice. *Evolution* 48, 711–733. doi: 10.1111/j.1558-5646.1994.tb01356.x

Ejima, A., and Griffith, L. C. (2007). Measurement of courtship behavior in *Drosophila melanogaster*. *Cold Spring Harb. Protoc.* 2007:pdb.prot4847. doi: 10.1101/pdb.prot4847

Fielding, C. A., Whittaker, J. B., Butterfield, J. E. L., and Coulson, J. C. (1999). Predicting responses to climate change: the effect of altitude and latitude on the phenology of the Spittlebug Neophilaenus lineatus. *Funct. Ecol.* 13, 65–73. doi: 10.1046/j.1365-2435.1999.00009.x

Foley, H. B., Sun, P. Y., Ramirez, R., So, B. K., Venkataraman, Y. R., Nixon, E. N., et al. (2019). Sex-specific stress tolerance, proteolysis, and lifespan in the invertebrate Tigriopus californicus. *Exp. Gerontol.* 119, 146–156. doi: 10.1016/j.exger.2019.02.006

Foster, S. A. (2013). Evolutionary insights from behavioural geography: plasticity, evolution, and responses to rapid environmental change. *Evol. Ecol. Res.* 15, 705–731.

Fowler-Finn, K. D., Cruz, D. C., and Rodríguez, R. L. (2017). Local population density and group composition influence the signal-preference relationship in Enchenopa treehoppers (Hemiptera: *Membracidae*). *J. Evol. Biol.* 30, 13–25. doi: 10.1111/jeb.12994

Fowler-Finn, K. D., and Rodríguez, R. L. (2012). Experience-mediated plasticity in mate preferences: mating assurance in a variable environment. *Evolution* 66, 459–468. doi: 10.1111/j.1558-5646.2011.01446.x

Frazier, M. R., Harrison, J. F., Kirkton, S. D., and Roberts, S. P. (2008). Cold rearing improves cold-flight performance in Drosophila via changes in wing morphology. *J. Exp. Biol.* 211, 2116–2122. doi: 10.1242/jeb.019422

Gallai, N., Salles, J.-M., Settele, J., and Vaissière, B. E. (2009). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecol. Econom.* 68, 810–821. doi: 10.1016/j.ecolecon.2008.06.014

García-Barros, E. (2000). Body size, egg size, and their interspecific relationships with ecological and life history traits in butterflies (Lepidoptera: *Papilionoidea*, Hesperioidea). *Biol. J. Linn. Soc.* 70, 251–284. doi: 10.1111/j.1095-8312.2000.tb00210.x

Gibert, J. P., Chelini, M.-C., Rosenthal, M. F., and DeLong, J. P. (2016). Crossing regimes of temperature dependence in animal movement. *Glob. Change Biol.* 22, 1722–1736. doi: 10.1111/gcb.13245

Greenfield, M. D. (2002). *Signalers and Receivers: Mechanisms and Evolution of Arthropod Communication*. Oxford, NY: Oxford University Press.

Greenfield, M. D., and Medlock, C. (2007). Temperature coupling as an emergent property: parallel thermal effects on male song and female response do not contribute to species recognition in an acoustic moth. *Evolution* 61, 1590–1599. doi: 10.1111/j.1558-5646.2007.00140.x

Guarneri, A. A., Lazzari, C., Xavier, A. A. P., Diotaiuti, L., and Lorenzo, M. G. (2003). The effect of temperature on the behaviour and development of *Triatoma brasiliensis*. *Physiol. Entomol.* 28, 185–191. doi: 10.1046/j.1365-3032.2003.00330.x

Hadjipantelis, P. Z., Jones, N., John, M., Springate, D., and Knight, C. G. (2013). Function-valued traits in evolution. *J. R. Soc. Interface* 10:20121032. doi: 10.1098/rsif.2012.1032

Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., et al. (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS ONE* 12:e0185809. doi: 10.1371/journal.pone.0185809

Hamilton, K. G. A., and Cocroft, R. B. (2009). Establishing the identity of existing names in the North American *Enchenopa binotata* species complex of treehoppers (Hemiptera: *Membracidae*). *Entomol. News* 120, 554–565. doi: 10.3157/021.120.0513

Hedrick, A., Perez, D., Lichti, N., and Yew, J. (2002). Temperature preferences of male field crickets (*Gryllus integer*) alter their mating calls. *J. Comp. Physiol. A* 188, 799–805. doi: 10.1007/s00359-002-0368-9

Hoffmann, A. A., and Sgrò, C. M. (2011). Climate change and evolutionary adaptation. *Nature* 470, 479–485. doi: 10.1038/nature09670

Høye, T. T., Post, E., Schmidt, N. M., Trøjelsgaard, K., and Forchhammer, M. C. (2013). Shorter flowering seasons and declining abundance of flower visitors in a warmer Arctic. *Nat. Clim. Change* 3, 759–763. doi: 10.1038/nclimate1909

Huey, R. B., and Stevenson, R. D. (1979). Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. *Integr. Comp. Biol.* 19, 357–366. doi: 10.1093/icb/19.1.357

Hunt, R. E. (1994). Vibrational signals associated with mating behavior in the treehopper, *Enchenopa binotata* Say (Hemiptera: Homoptera: Membracidae). *J. N. Y. Entomol. Soc.* 102, 266–270.

Jocson, D. I., Smeester, M., Leith, N. T., Macchiano, A., and Fowler-Finn, K. D. (2019). Temperature coupling of mate attraction signals and female mate preferences in four populations of Enchenopa treehoppers (Hemiptera: Membracidae). *J. Evol. Biol.* doi: 10.1111/jeb.13506. [Epub ahead of print].

Katsuki, M., and Miyatake, T. (2009). Effects of temperature on mating duration, sperm transfer and remating frequency in *Callosobruchus chinensis*. *J. Insect Physiol.* 55, 113–116. doi: 10.1016/j.jinsphys.2008.10.012

Kellermann, V., Overgaard, J., Hoffmann, A. A., Fløjgaard, C., Svenning, J.-C., and Loeschcke, V. (2012). Upper thermal limits of Drosophila are linked to species distributions and strongly constrained phylogenetically. *Proc. Natl. Acad. Sci. U.S.A.* 109, 16228–16233. doi: 10.1073/pnas.1207553109

Kilmer, J. T., Fowler-Finn, K. D., Gray, D. A., Höbel, G., Rebar, D., Reichert, M. S., et al. (2017). Describing mate preference functions and other function-valued traits. *J. Evol. Biol.* 30, 1658–1673. doi: 10.1111/jeb.13122

King, A. M., and MacRae, T. H. (2015). Insect heat shock proteins during stress and Diapause. *Annu. Rev. Entomol.* 60, 59–75. doi: 10.1146/annurev-ento-011613-162107

Kingsolver, J. G. (2009). The well-temperatured biologist: (American Society of Naturalists Presidential Address). *Am. Nat.* 174, 755–768. doi: 10.1086/648310

Kingsolver, J. G., and Huey, R. B. (2008). Size, temperature, and fitness: three rules. *Evol. Ecol. Res.* 10, 251–268.

Kleynhans, E., Conlong, D. E., and Terblanche, J. S. (2014). Direct and indirect effects of development temperature on adult water balance traits of *Eldana saccharina* (Lepidoptera: Pyralidae). *J. Insect Physiol.* 68, 69–75. doi: 10.1016/j.jinsphys.2014.06.018

Lin, C. P., and Wood, T. K. (2002). Molecular phylogeny of the North American *Enchenopa binotata* (Homoptera: Membracidae) species complex. *Ann. Entomol. Soc. Am.* 95, 162–171. doi: 10.1603/0013-8746(2002)095[0162:MPOTNA]2.0.CO;2

Logan, M. L., Huynh, R. K., Precious, R. A., and Calsbeek, R. G. (2013). The impact of climate change measured at relevant spatial scales: new hope for tropical lizards. *Glob. Change Biol.* 19, 3093–3102. doi: 10.1111/gcb.12253

Losey, J. E., and Vaughan, M. (2006). The economic value of ecological services provided by insects. *Bioscience* 56:311. doi: 10.1641/0006-3568(2006)56[311:TEVOES]2.0.CO;2

Matzkin, L. M., Watts, T. D., and Markow, T. A. (2009). Evolution of stress resistance in Drosophila: interspecific variation in tolerance to desiccation and starvation. *Funct. Ecol.* 23, 521–527. doi: 10.1111/j.1365-2435.2008.01533.x

Meyer, K., and Kirkpatrick, M. (2005). Restricted maximum likelihood estimation of genetic principal components and smoothed covariance matrices. *Genet. Sel. Evol.* 37, 1–30. doi: 10.1186/1297-9686-37-1-1

Ollerton, J., Winfree, R., and Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos* 120, 321–326. doi: 10.1111/j.1600-0706.2010.18644.x

Pincebourde, S., and Woods, H. A. (2012). Climate uncertainty on leaf surfaces: the biophysics of leaf microclimates and their consequences for leaf-dwelling organisms. *Funct. Ecol.* 26, 844–853. doi: 10.1111/j.1365-2435.2012.02013.x

Rodríguez, R. L., and Cocroft, R. B. (2006). Divergence in female duetting signals in the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). *Ethology* 112, 1231–1238. doi: 10.1111/j.1439-0310.2006.01285.x

Rodríguez, R. L., Haen, C., Cocroft, R. B., and Fowler-Finn, K. D. (2012). Males adjust signaling effort based on female mate-preference cues. *Behav. Ecol.* 23, 1218–1225. doi: 10.1093/beheco/ars105

Rodríguez, R. L., Sullivan, L. E., and Cocroft, R. B. (2004). Vibrational communication and reproductive isolation in the Enchenopa Binotata species complex of treehoppers (hemiptera: Membracidae). *Evolution* 58, 571–578. doi: 10.1111/j.0014-3820.2004.tb01679.x

Schluter, D. (1988). Estimating the form of natural selection on a quantitative trait. *Evolution* 42, 849–861. doi: 10.1111/j.1558-5646.1988.tb02507.x

Shreve, S. M., Kelty, J. D., and Lee R. E. Jr. (2004). Preservation of reproductive behaviors during modest cooling: rapid cold-hardening fine-tunes organismal response. *J. Exp. Biol.* 207, 1797–1802. doi: 10.1242/jeb.00951

Sih, A., Ferrari, M. C. O., and Harris, D. J. (2011). Evolution and behavioural responses to human-induced rapid environmental change. *Evol. Appl.* 4, 367–387. doi: 10.1111/j.1752-4571.2010.00166.x

Sinclair, B. J., Williams, C. M., and Terblanche, J. S. (2012). Variation in thermal performance among insect populations. *Physiol. Biochem. Zool.* 85, 594–606. doi: 10.1086/665388

Sinervo, B., Méndez-de-la-Cruz, F., Miles, D. B., Heulin, B., Bastiaans, E., Cruz, M. V.-S., et al. (2010). Erosion of lizard diversity by climate change and altered thermal niches. *Science* 328, 894–899. doi: 10.1126/science.1184695

Stinchcombe, J. R., and Kirkpatrick, M. (2012). Genetics and evolution of function-valued traits: understanding environmentally responsive phenotypes. *Trends Ecol. Evol.* 27, 637–647. doi: 10.1016/j.tree.2012.07.002

Suggitt, A. J., Gillingham, P. K., Hill, J. K., Huntley, B., Kunin, W. E., Roy, D. B., et al. (2011). Habitat microclimates drive fine-scale variation in extreme temperatures. *Oikos* 120, 1–8. doi: 10.1111/j.1600-0706.2010.18270.x

Sunday, J. M., Bates, A. E., and Dulvy, N. K. (2012). Thermal tolerance and the global redistribution of animals. *Nat. Clim. Change* 2, 686–690. doi: 10.1038/nclimate1539

Teal, J. M. (1959). Respiration of crabs in Georgia salt marshes and its relation to their ecology. *Physiol. Zool.* 32, 1–14. doi: 10.1086/physzool.32.1.30152287

Tuff, K. T., Tuff, T., and Davies, K. F. (2016). A framework for integrating thermal biology into fragmentation research. *Ecol. Lett.* 19, 361–374. doi: 10.1111/ele.12579

Tuomainen, U., and Candolin, U. (2011). Behavioural responses to human-induced environmental change. *Biol. Rev. Camb. Philos. Soc.* 86, 640–657. doi: 10.1111/j.1469-185X.2010.00164.x

Wood, S. N. (2006). *Generalized Additive Models: An Introduction With R*. Boca Raton, FL: Chapman and Hall/CRC Available at: https://www.crcpress.com/Generalized-Additive-Models-An-Introduction-with-R-Second-Edition/Wood/p/book/9781498728331 (accessed August 13, 2019).

Wood, T. K. (1980). Divergence in the *Enchenopa binotata* say complex (Homoptera: membracidae) effected by host plant adaptation. *Evolution* 34, 147–160. doi: 10.1111/j.1558-5646.1980.tb04800.x

Wood, T. K. (1993). Diversity in the new World Membracidae. *Annu. Rev. Entomol.* 38, 409–433. doi: 10.1146/annurev.en.38.010193.002205

Wood, T. K., and Guttman, S. I. (1983). *Enchenopa binotata* complex: sympatric speciation? *Science* 220, 310–312. doi: 10.1126/science.220.4594.310

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 26 September 2019
doi: 10.3389/fevo.2019.00350

Impacts of Nutrient Subsidies on Salt Marsh Arthropod Food Webs: A Latitudinal Survey

Gina M. Wimp[1]*, *Danny Lewis*[1] *and Shannon M. Murphy*[2]

[1] *Department of Biological Sciences, Georgetown University, Washington, DC, United States,* [2] *Department of Biological Sciences, University of Denver, Denver, CO, United States*

Anthropogenic nutrient inputs into native ecosystems cause fluctuations in resources that normally limit plant growth, which has important consequences for associated foodwebs. Such inputs from agricultural and urban habitats into nearby natural systems are increasing globally and can be highly variable. Despite the global increase in anthropogenically-derived nutrient inputs into native ecosystems, the consequences of variation in subsidy amount on native plants and their associated foodwebs are poorly known. Salt marshes represent an ideal system to address the differential impacts of nutrient inputs on ecosystem and community dynamics because human development and other anthropogenic activities lead to recurrent introductions of nutrients into these natural systems. Previously, we have found in manipulative experiments that arthropod abundance increases in response to nutrient enrichment, with predators being the trophic group most strongly affected. We conducted a survey of Atlantic coastal *Spartina* marshes to test whether such local responses are indicative of responses at a landscape level. We examined the most abundant arthropod species associated with *Spartina* coastal marshes that receive variable amounts of anthropogenic nitrogen, and tested how this response varied across different arthropod functional groups (herbivores, epigeic feeders, and predators). Similar to what we found at a local scale, nutrient subsidies alter the trophic structure of the arthropod assemblage by changing the relative abundances of various feeding groups. Variable responses among predators to nitrogen density could be partly explained by diet breadth (e.g., generalists vs. specialists). Herbivores had a negative response to increasing plant nitrogen density; specialist predators tracked their herbivore prey and thus also responded negatively to nitrogen density. However, generalists were not negatively affected by nitrogen density and indeed some generalist predators responded positively to nitrogen density. Thus, the overall predator-to-herbivore ratio was also positively associated with nitrogen density. Our research helps us to understand how long-term nutrient enrichment of native ecosystems by human activities affects arthropod assemblages and foodweb dynamics.

Keywords: eutrophication, food web structure, latitudinal gradient, nitrogen, nutrient subsidies, salt marsh

Edited by:
Qinfeng Guo,
United States Forest Service (USDA),
United States

Reviewed by:
Florencia Botto,
Institute of Marine and Coastal
Research (IIMyC), Argentina
Qiang He,
Duke University, United States

***Correspondence:**
Gina M. Wimp
gmw22@georgetown.edu

Specialty section:
This article was submitted to
Population and Evolutionary
Dynamics,
a section of the journal
Frontiers in Ecology and Evolution

Received: 26 April 2019
Accepted: 02 September 2019
Published: 26 September 2019

Citation:
Wimp GM, Lewis D and Murphy SM (2019) Impacts of Nutrient Subsidies on Salt Marsh Arthropod Food Webs: A Latitudinal Survey. Front. Ecol. Evol. 7:350. doi: 10.3389/fevo.2019.00350

INTRODUCTION

Natural and anthropogenic inputs of nutrients (e.g., nitrogen) into native ecosystems often promote fluctuations in the availability of resources that normally limit plant growth (Robinson, 1994; Inouye and Tilman, 1995; Siemann, 1998; Haddad et al., 2000). Such inputs from agricultural and urban habitats into nearby natural systems are increasing globally and can be highly variable (Vitousek et al., 1997; Tilman, 1999; Boyer et al., 2002; Mayer et al., 2002; Valiela and Bowen, 2002; Valiela and Cole, 2002). Nutrient inputs promote changes in primary productivity and plant diversity that in turn can have important consequences for associated food-webs (Polis and Hurd, 1996; Polis et al., 1997a,b, 1998; Huxel and McCann, 1998; Haddad et al., 2000; Holmgren et al., 2001). Inputs of limiting nutrients (e.g., nitrogen) often concomitantly affect plant species richness, plant species composition, primary productivity, and plant tissue quality (C:N content) and determining their independent effects on the associated arthropod assemblage can be experimentally daunting (Kirchner, 1977; Siemann, 1998; Haddad et al., 2000). By working in natural plant monocultures (e.g., cordgrass-dominated coastal wetlands) the task is somewhat simplified because the cascading effects of nutrient subsidies on consumers will be driven largely by changes in primary productivity and plant nutrition (see Denno et al., 2002).

Despite the global increase in anthropogenically-derived nutrient inputs into native ecosystems, such as salt marshes, the consequences of variation in nutrient subsidies on native plants and their associated foodwebs are poorly known. Many researchers have tested the effects of nutrient subsidies on salt marshes in manipulative experiments (e.g., Valiela et al., 1978; Gratton and Denno, 2003; Wimp et al., 2010; Deegan et al., 2012; Murphy et al., 2012). For example, we have found that arthropod abundance increases in response to experimental nutrient enrichment, with predators being the trophic group most strongly affected (Wimp et al., 2010; Murphy et al., 2012). However, whether these results are consistent with observations from un-manipulated habitats across large geographic areas is unknown. Additionally, most of the studies that have examined the impacts of nitrogen inputs on salt marsh ecosystems have only been conducted using a small range of nitrogen input levels (often just a single level). In reality, salt marshes experience a wide range of nitrogen input levels, and higher levels of nitrogen can negatively affect plant producers. For example, excess nitrogen in the soil can lead to higher soil salinity, with negative effects on the plant. Excess nitrogen can also lead to a decrease in the root:shoot ratio, which can cause the plants to lodge or fall over because they are top-heavy (Deegan et al., 2012). Recently Wigand et al. (2018) demonstrated that in nutrient-enriched tidal creeks, soil shear strength in *Spartina* plots was significantly lower than in unmanipulated reference creeks and that these decreases in soil strength likely cause channel bank failures when *Spartina* plants collapse into tidal creeks. Such negative effects on plants may in turn impact herbivores. Finally, while our previous data demonstrates a positive effect of nutrient enrichment on higher trophic levels (Wimp et al., 2010; Murphy et al., 2012), such positive effects may describe a narrow set of circumstances. For instance, it is possible that the relationship between nutrient enrichment and higher trophic level consumers is only positive until a threshold is reached, and additional inputs of nitrogen will have no further effect. Additionally, the positive relationship may also only be possible when recruitment from nearby un-manipulated habitat is possible, which would mean that this pattern would only occur in experimental settings (e.g., manipulated plots nested within un-manipulated habitat) and not in marshes that are affected by widespread anthropogenic disturbance.

While bottom-up impacts of nitrogen addition may affect herbivore density and herbivory (Bertness et al., 2008; He and Silliman, 2015, 2016), top-down effects from predators may also limit herbivore populations. Previous studies have found that the impacts of nutrient addition on arthropod biomass and diversity are stronger for higher trophic levels (Wimp et al., 2010; Murphy et al., 2012). Thus, the positive response of herbivores to nutrient enrichment may eventually be checked by higher trophic level predators and parasites. It is unclear how higher trophic level predators and parasites may respond to nutrient enrichment across a wide range of nutrient enrichment levels, or within a larger spatial context. Because many arthropod predators gain not only additional prey, but also increasingly complex plant structure with higher levels of nutrient enrichment, this may diminish intraguild predation and cannibalism (Langellotto and Denno, 2006). Thus, the combined effects of additional prey resources and reduced competition/intraguild predation/cannibalism among predators may lead to a sustained positive response of predators/parasites to nutrient enrichment.

Increasing nitrogen inputs from anthropogenic sources are a common problem across a wide array of ecosystems, but one ecosystem that is particularly threatened by nitrogen inputs is coastal salt marshes where urbanization and agriculture are increasing nutrient runoff and nitrogen availability (Bertness et al., 2002, 2004). Land development and agriculture are jeopardizing coastal wetlands at an alarming rate, and one of the major threats is nitrogen runoff from neighboring anthropogenic sources, which alters vegetation dynamics, promotes the incursion of invasive species, and increases nitrogen availability (Bertness et al., 2002, 2004). An estimated 50% of the variation in nitrogen availability in *Spartina* marshes is explained by shoreline development such as housing developments and agriculture (Bertness et al., 2002). Because *Spartina* is nitrogen-limited, particularly in high-marsh habitats, nitrogen subsidies (natural and anthropogenic) result in dramatic increases in biomass, plant nitrogen content, plant architecture, and detritus (Mendelssohn, 1979a,b; Bertness et al., 2002; Denno et al., 2002; Gratton and Denno, 2003). The nitrogen-sensitive assemblage of arthropods associated with *Spartina* marshes (Denno et al., 2003; Huberty and Denno, 2006a,b) provides an ideal opportunity to study the food-web consequences of nitrogen subsidies at a large spatial scale. We compared the arthropod assemblage among Atlantic coastal *Spartina* marshes receiving variable amounts of anthropogenic nitrogen and at varying distances from potential upland N sources. We selected study marshes so that we could compare nearby marshes at similar latitudes that had different levels of nutrient inputs due to human development and agriculture. We predicted that if our results from our manipulated plot-experiments (Wimp et al., 2010; Murphy et al., 2012) accurately represent ecosystem-wide responses at greater geographic scales, then arthropod density should be greater in productive marshes that receive elevated subsidies of allochthonous nitrogen compared to more pristine marshes, and that the response should be stronger for higher trophic levels.

METHODS

Study System

The perennial cordgrass *Spartina alterniflora* (now reclassified as *Sporobolus alterniflorus*, but hereafter referred to as *Spartina*) dominates the vegetation of Atlantic coastal marshes where it grows in the intertidal zone (Redfield, 1972; Bertness, 1991). Many marshes are characterized by large, pure expanses of *Spartina* that directly abut upland habitats, either natural upland vegetation or agricultural and urban habitats (Warren and Niering, 1991; Bertness et al., 2002, 2004). Most nitrogen that is delivered to coastal waters and wetlands in the US derives from non-point sources, such as agriculture and atmospheric deposition (Howarth et al., 1996); in the Mid-Atlantic region agriculture is the dominant source of nitrogen to natural systems (Boyer et al., 2002). As terrestrially-derived nitrate flows downstream from anthropogenic sources, about one quarter of the nitrogen is intercepted by coastal wetlands (e.g., *Spartina* marshes) before it is able to reach open waters (Valiela and Cole, 2002). Nitrogen that is retained in the marsh is incorporated into plant biomass, denitrified, or buried in marsh sediments (Valiela and Teal, 1979; Denno et al., 2002; Valiela and Cole, 2002).

Spartina is a foundation species that serves as the only host plant for a variety of insect herbivores in several feeding guilds including sap-feeders, free-living folivores, stem borers, and leaf miners (Denno, 1977; Stiling and Strong, 1983; Denno et al., 2003, 2005). We were able to capitalize on a wealth of life-history, mesocosm feeding trials, and stable isotope information in this exhaustively studied system in order to categorize the functional roles of the arthropod species found in *Spartina* (e.g., Dobel et al., 1990; Denno et al., 2002, 2003; Finke and Denno, 2002, 2003, 2004, 2005, 2006; Gratton and Denno, 2003; Huberty and Denno, 2006a; Langellotto and Denno, 2006; Lewis and Denno, 2009; Wimp et al., 2010, 2013, 2019). Furthermore, previous studies have established the relative degree of habitat and feeding specialization for the dominant species (Denno, 1976, 1977, 1980; Wimp et al., 2013) and trophic interactions among the dominant species (Döbel and Denno, 1994; Finke and Denno, 2002; Denno et al., 2003, 2004, 2005; Ferrenberg and Denno, 2003). Sap-feeders are numerically dominant while free-living folivores, borers, and miners are less common (Denno et al., 2005). Of the sap-feeders, planthoppers (*Prokelisia dolus* and *P. marginata*) are the most abundant (Denno et al., 2000). Many herbivores show remarkable increases on N-subsidized *Spartina* due to enhanced colonization, fecundity and survival (Vince et al., 1981; Denno et al., 2002, 2003). Certain herbivores respond exclusively to increases in plant nitrogen content (N-sensitive "specialists" like *P. marginata*), whereas other species respond specifically to increases in plant biomass (several sap-feeders) (Huberty and Denno, 2006a,b). Overall, herbivores on *Spartina* are very responsive to nitrogen-induced changes in plant quality and biomass.

Spartina also has a rich assemblage of associated detritivores and algivores (collectively described as "epigeic feeders") from a variety of feeding guilds such as grazers, shredders, algal, and fungal feeders (Wimp et al., 2013). Nitrogen-loading promotes increased abundance and diversity of detritivores in *Spartina* and other systems, both as a consequence of increased detrital biomass and quality (%N) (Settle et al., 1996; Halaj and Wise, 2002).

Spartina also hosts a diversity of natural enemies for herbivores and epigeic prey, including invertebrate predators and parasitoids, but predators are a far more important source of mortality than parasitoids on mid-Atlantic marshes (Döbel and Denno, 1994). The predator assemblage includes both generalist predators (web-building and hunting spiders) and specialist predators (e.g., the mirid *Tytthus vagus*) (Döbel and Denno, 1994; Finke and Denno, 2002; Denno et al., 2003). Top carnivores are voracious intraguild predators and include hunting spiders (*Pardosa littoralis* and *Hogna modesta*) and katydids (*Conocephalus spartinae*) that feed on herbivores, detritivores, specialist predators as well as each other (Finke and Denno, 2003; Denno et al., 2004; Matsumura et al., 2004). Hunting spiders, such as *Pardosa*, inflict high mortality on herbivores, an effect that can cascade to basal resources such as biomass and tiller production (Finke and Denno, 2002; Denno et al., 2005). However, intraguild predation reduces the overall effectiveness of the predator complex in suppressing herbivore populations and thus dampens trophic cascades (Denno et al., 2003, 2004; Finke and Denno, 2004).

Survey Design and Methods

We selected 14 marshes along the mid-Atlantic coast between Maine and Virginia (**Figure 1**; **Supplement 1**). We chose these marshes such that they abutted large expanses of native upland vegetation (<5 km away) or were bordered by development or agriculture that would lead to an increase in nitrogen inputs (see Bertness et al., 2002). Initially, we chose marshes that spanned a gradient of minimally to heavily impacted based on proximity to upland development and rate of tidal flushing. Thus, according to these criteria, Virginia Coastal Reserve (VA), Great Bay Marsh (NJ), and Plum Island LTER (MA) were considered minimally impacted; Delaware Seashore State Park (DE), Caumsett State Historic Park (NY), Foxhill Salt Marsh (RI), Colt State Park (RI), Fogland Nature Preserve (RI), and Awcomin Marsh (NH) were considered moderately impacted; and Horseshoe Cove (NJ), Little Creek Wildlife Area (DE), Jamaica Bay Wildlife Refuge (NY), Urban Forestry Center (NH), and Fore River Sanctuary (ME) were considered heavily impacted marshes. However, we found that these criteria did not adequately capture nitrogen loading into each marsh and were highly subjective. We therefore used nitrogen density as a measure of nitrogen loading into each marsh since it represents a non-subjective measure and also captures variation in the way that marshes in different locations respond to nutrient addition. Specifically, in a previous study (Murphy et al., 2012) we found that *Spartina* growing in one marsh responded to nutrient manipulation with an

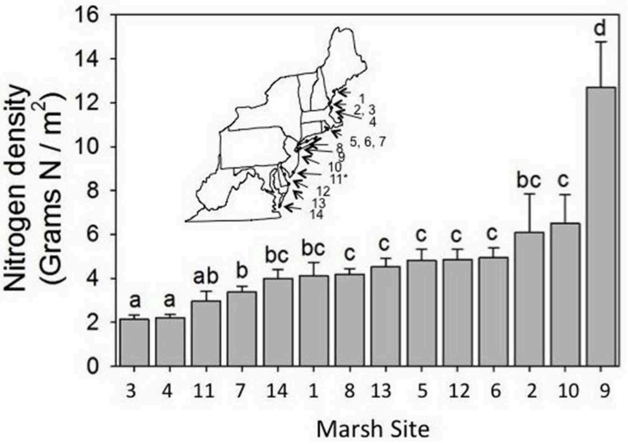


FIGURE 1 | Mean nitrogen density varied across marshes [$F_{(13,\ 136)} = 12.73$, $P < 0.0001$]. Inset shows approximate locations of the study marshes, which are numbered from north to south. We found no trend in nitrogen density with latitude [$F_{(1,\ 14.6)} = 2.62$, $P = 0.15$]. Specific information about marsh study locations and sample size can be found in **Supplement 1**. Error bars show standard errors of the means. Asterisk denotes site of our plot-level manipulations in NJ, and letters indicate significant differences.

increase in plant percent nitrogen, while *Spartina* growing in a different marsh responded with an increase in biomass (Murphy et al., 2012). Because nitrogen density captures both measures simultaneously (percent nitrogen and biomass), it allows us to make comparisons across different marshes. In each marsh we aimed to establish 4 square plots (10 m^2 each and separated by 50 m) along 3 replicated transects (separated by 100 m) running seaward from the upland, for a total of 12 plots per marsh. However, some marshes were too small to accommodate 12 plots and so these marshes had fewer plots (please see **Supplement 1** for exact sample sizes); there were 151 plots overall across 14 marshes. We conducted the survey between August 13 and 28, 2012 and started surveying marshes in the south and worked our way north to help control for phenology so that we sampled each site during peak biomass. In each plot we sampled: (1) aboveground and belowground biomass of *Spartina*, (2) biomass of detritus, (3) N-content of *Spartina*, and (4) the density of all arthropods.

Plant Samples

We measured plant biomass and height using 0.047 m^2 quadrats (Denno et al., 2002) by sorting the quadrat samples into live and dead plant material and measuring the height of living culms. For the live plant material, we washed it with deionized water, dried it in a drying oven at 60°C for 3 days, and then weighed it. To measure the N-content of *Spartina*, we subsampled plant snips (5–10 *Spartina* culms per plot), ground them in a Mixer Mill, and sent our samples to the Cornell Stable Isotope Laboratory for percent elemental analysis using an elemental analyzer-stable isotope ratio mass spectrometer system Thermo Delta V Advantage IRMS and Carlo Erba NC2500 EA systems.

We used the nitrogen content of *Spartina* leaves to estimate the level of enrichment experienced by a marsh. For each sample location, we multiplied live leaf biomass per square meter by percent nitrogen in those leaves to get nitrogen density, the number of grams of nitrogen in live *Spartina* leaves in a square meter of marsh. Nitrogen density reflects average nutrient input to the marsh over time and is independent of culm length or density. Thus, we were able to measure nitrogen density as a continuous variable in order to examine the relationship between nitrogen inputs and arthropod responses.

We sampled belowground biomass using a soil corer 8 cm in diameter and 16 cm long, but not all soil cores come out of the ground with that much material so we measured each core and controlled for volume in all root biomass measurements; we took one soil core sample per plot. We separated *Spartina* roots from soil using a sledge hammer and a high-power hose to loosen the dense, rhizomatous root mass enough to extract the soil. We then dried the roots in an oven set at 60°C for 72 h and then weighed them.

Arthropod Samples

We collected arthropods using a D-vac suction sampler with a 21 cm aperture, which was placed in 5 different locations within the plot for 3 s periods (following methods described in Murphy et al., 2012; Wimp et al., 2013). We collected arthropods during low tides so that we could place the D-vac head on the ground to effectively capture the epigeic assemblage. Previous studies have demonstrated that the D-vac suction sampler can effectively sample ground-dwelling arthropods in *S. alterniflora*, where it can remove 97% of the spiders in a collection area (Dobel et al., 1990). We immediately placed collected arthropods into closed containers with ethyl acetate, and transferred the samples into 75% ethanol. In the laboratory, we counted arthropods in each sample and converted these counts to number per square meter for each focal species.

Statistical Analyses

After calculating nitrogen density for each marsh survey site, we examined normality and equality of variance assumptions and our data met both assumptions. We used a One-Way ANOVA to test for differences in nitrogen density across the 14 marsh survey sites, followed by a Tukey's *post-hoc* test to examine significant differences among individual marsh sites. When we examined correlations among variables, live %N, root biomass, culm density, total herbivore density, total predator density, total epigeic prey density, and total spider density met normality and equality of variance assumptions. However, live biomass, thatch biomass, culm length, *Tytthus* density, and *Grammonota* density did not meet equality of variance and normality assumptions, and were square root transformed. *Pardosa* density required a log transformation to meet assumptions. Additionally, when we examined the relationship between nitrogen density and plant or arthropod assemblage variables, the uncertainty in our predictor variable (nitrogen density) was similar to the uncertainty in our response, so we could not use regression analysis, and we instead used correlation analysis.

RESULTS

We found variation in nitrogen density across sites [$F_{(13,\ 136)} = 12.73$, $P < 0.0001$; **Figure 1**], which allowed us to study how variation in nitrogen density affected salt marsh communities. Notably, we did not find a relationship between nitrogen density and latitude [$F_{(1,\ 14.6)} = 2.62$, $P = 0.15$], and so our results are not simply due to latitudinal gradients. Nitrogen density was greatest in Jamaica Bay, which is a salt marsh located near the JFK airport runway for New York City, and was lowest at Awcomin Marsh (NH) and the Plum Island LTER site (MA).

Live *Spartina* biomass is a component of nitrogen density, so we expected and found that the two variables were positively correlated ($\rho = +0.952$, $P < 0.0001$; **Figure 2**). This correlation remains significant even when the site with the highest nitrogen density (Site 9, Jamaica Bay, NY) is removed ($\rho = +0.902$, $P < 0.0001$). However, percent nitrogen of the plants was not correlated with nitrogen density ($\rho = +0.238$, $P = 0.41$; **Supplement 2**). We found a significant correlation between nitrogen density and *Spartina* culm length ($\rho = +0.763$, $P = 0.0015$; **Figure 2**), and this relationship remains significant when Site 9 is removed. Although culm length increased with nitrogen density, culm density decreased ($\rho = -0.681$, $P = 0.0073$), such that there were fewer culms per unit area. However, nitrogen density was not correlated with dead *Spartina* biomass (thatch) ($\rho = +0.252$, $P = 0.39$), or with *Spartina* root biomass ($\rho = -0.393$, $P = 0.16$); notably, when we remove the site with the highest nitrogen density (Site 9, Jamaica Bay, NY) the root biomass result becomes significant ($\rho = -0.583$, $P = 0.037$). Nitrogen density was also correlated with a significant decrease in root/leaf biomass ($\rho = -0.892$, $P < 0.0001$; **Figure 2**), and this correlation remains significant when we remove the site with the highest nitrogen density ($\rho = -0.883$, $P < 0.0001$).

When we examined the impacts of *Spartina* nitrogen density on higher trophic levels, we found that nitrogen density had a negative relationship with total herbivore density ($\rho = -0.610$, $P = 0.021$; **Figure 3**, see **Supplement 1** for a list of species found in most marshes). This response was likely driven by plant biomass; total herbivore density was negatively related to live biomass ($\rho = -0.584$, $P = 0.0283$), and was not correlated with live *Spartina* percent nitrogen ($\rho = +0.001$, $P = 0.99$), culm density ($\rho = +0.255$, $P = 0.38$), or culm length ($\rho = -0.389$, $P = 0.17$). However, nitrogen density had no relationship with the total density of epigeic feeders ($\rho = +0.342$, $P = 0.23$; **Figure 3**) or predator density ($\rho = +0.128$, $P = 0.66$; **Figure 3**). Notably, increasing nitrogen density led to an increase in the overall predator to herbivore ratio ($\rho = +0.624$, $P = 0.017$; **Figure 3**).

The relationship between nitrogen density and higher trophic levels varied according to predator group. Densities of the specialist predator *Tytthus vagus* declined marginally with increasing nitrogen density, similar to their planthopper prey ($\rho = -0.500$, $P = 0.069$; **Figure 4**), and were correlated with the densities of planthopper nymphs ($\rho = +0.431$, $P = 0.0220$). Densities of the web-building spider *Grammonota trivittata* ($\rho = +0.526$, $P = 0.053$) marginally increased and densities of the hunting spider *Pardosa littoralis* ($\rho = -0.278$, $P = 0.34$)

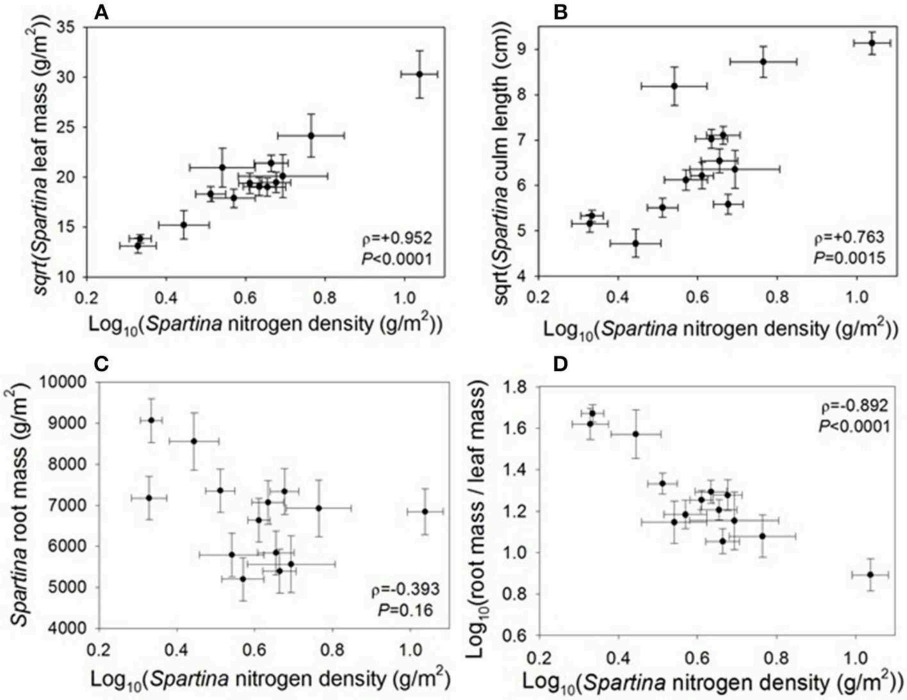


FIGURE 2 | Correlations between *Spartina* nitrogen density and *Spartina* **(A)** leaf biomass, **(B)** culm length, **(C)** root biomass when we remove the site with the highest nitrogen density the root biomass result becomes significant ($\rho = -0.583$, $P = 0.037$), and **(D)** root-to-shoot ratio.

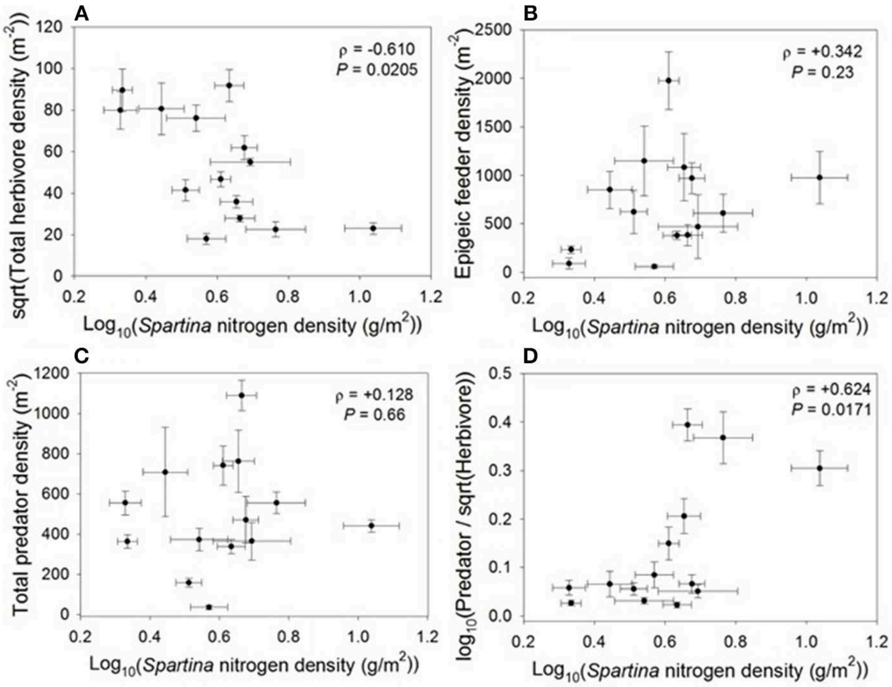


FIGURE 3 | Correlations between Spartina nitrogen density and **(A)** total herbivore density, **(B)** total epigeic feeder density, **(C)** total predator density, and **(D)** predator-to-herbivore ratio.

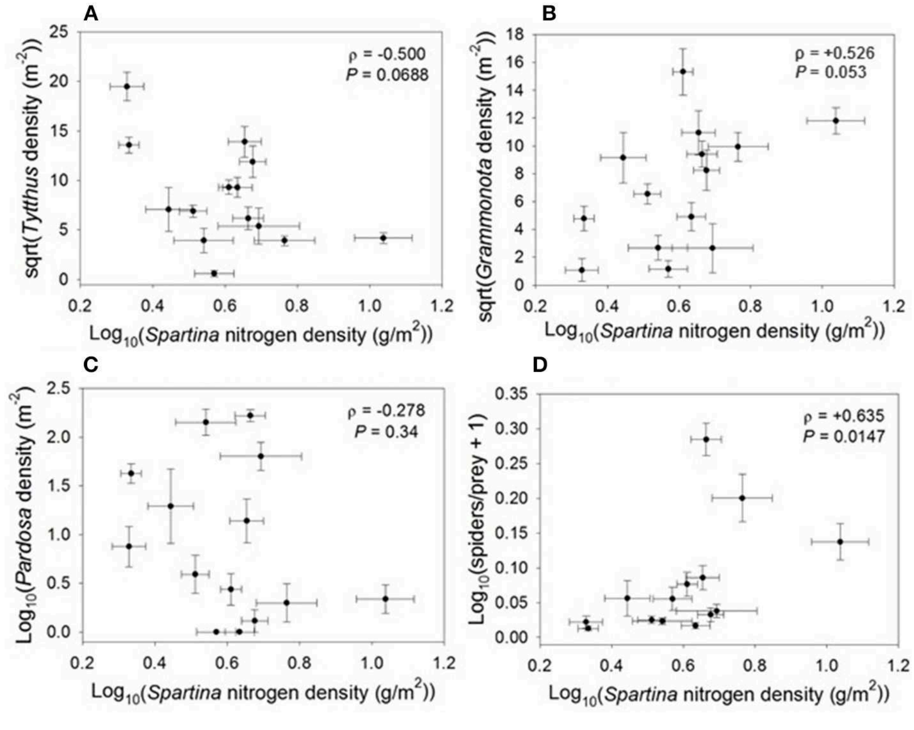


FIGURE 4 | Correlations between *Spartina* nitrogen density and **(A)** *Tytthus* density, **(B)** *Grammonota* density, **(C)** *Pardosa* density, and **(D)** spiders-to-prey ratio.

had no relationship with nitrogen density. Indeed, the only *Spartina* variable that was correlated with densities of the hunting spider *Pardosa littoralis* was thatch biomass ($\rho = +0.323$, $P = 0.03$); *Pardosa* is an intraguild predator and cannibalistic, and thatch provides a refuge from intraguild predation/cannibalism (Langellotto and Denno, 2006). Additionally, the spider/prey ratio increased with an increase in *Spartina* nitrogen density ($\rho = +0.635$, $P = 0.015$; **Figure 4**). Thus, *Spartina* nitrogen density had a negative relationship with specialist predators such as *Tytthus vagus*, but a positive relationship with generalist spiders as a group.

DISCUSSION

Many ecological field studies focus on local scales and study plot-level responses to anthropogenic disturbances, such as nutrient enrichment. However, if results from these plot-level experiments do not scale up to reflect responses observed across larger geographic gradients, then their value to scientific advancement is questionable. Importantly, we found that results from this study, in which we sampled marshes from 8 states along the eastern seaboard, agree with our previous research conducted at a local scale (Wimp et al., 2010; Murphy et al., 2012), thus validating the value of plot-level experiments. Such scalability is particularly important when we are considering substantial drivers of global change, such as nutrient enrichment, which can impact terrestrial, freshwater, and marine ecosystems. We found that while nutrient subsidies generally have positive effects on aboveground plant biomass, they also decrease the root:shoot ratio. Additionally, we found that the impacts of nutrient subsidies on consumers differs according to trophic level. Some generalist predator responses and the predator: herbivore ratio had a positive relationship with nutrient subsidies, but herbivores and specialist predators had no relationship or a negative relationship with nutrient subsidies, similar to our previous results from studies at a local scale (Wimp et al., 2010; Murphy et al., 2012). Notably, because we have previously conducted experimental studies at multiple sites, and used methods in which we added nutrients as either a 1-year pulse or a multi-year press (Murphy et al., 2012), we can use our experimental treatments to explain patterns at a larger, geographical scale.

In numerous small-plot or entire-marsh manipulative experiments, researchers have found a positive correlation between nutrient enrichment and aboveground biomass (e.g., Valiela et al., 1978; Denno et al., 2002; Gratton and Denno, 2003; Pennings et al., 2005; Deegan et al., 2007, 2012; Wimp et al., 2010; Murphy et al., 2012). Our results from salt marshes sampled across a much larger geographic gradient show the same pattern as the results from these local studies. We found that marshes with greater nitrogen density produced *Spartina* plants with greater aboveground biomass, measured as both leaf mass and plant height. However, while aboveground biomass increased with nutrient enrichment, belowground biomass decreased with increasing nitrogen density such that marshes that experienced high nutrient enrichment had plants with a significantly lower root:shoot ratio compared to plants located in marshes with lower levels of nutrient enrichment. Previous work by Deegan et al. (2012) showed that the root:shoot ratio decreased with increasing nutrient enrichment in an ecosystem-wide manipulative experiment. Deegan et al. (2012) suggested that increasing nutrient availability enabled plants to reduce their root biomass while increasing above ground biomass. However, these top-heavy plants were more likely to topple into creeks because they lacked the root architecture to stabilize the creek banks; thus the decrease in the root:shoot ratio may lead to marsh loss and increased coastal erosion. Notably, our results from un-manipulated marshes across a wider geographic gradient support the findings of Deegan et al. (2012) as we found a significant negative correlation between root:shoot ratio and nutrient density across 14 marshes.

Herbivore declines in response to increasing nitrogen density across our sites may seem puzzling at first, but these results actually agree with manipulative experiments that we have conducted at a local scale. Previously, we have found that patterns of plant allocation to plant quantity (biomass) and quality (percent nitrogen) differ according to site (Murphy et al., 2012). In this study, we found an increase in *Spartina* aboveground biomass with an increase in nitrogen density, but we did not find any relationship between nitrogen density and percent nitrogen. Thus, the plants with the highest percent nitrogen content were not necessarily the plants with the greatest biomass. Our previous experimental results found that when plant allocation to biomass is greater than plant allocation to percent nitrogen, changes in herbivore abundance are minimal for a multi-year press, and negligible for a single-year pulse (Murphy et al., 2012). This pattern could arise for a number of reasons. First, if herbivores have to process greater amounts of plant material to obtain the nitrogen they need, this could negatively impact their growth and development. Second, bottom-up effects related to plant defense and palatability are known to affect herbivores (Vidal and Murphy, 2018). *Spartina* plants from northern marshes are more palatable to herbivores than plants from southern marshes, but plants in southern marshes receive more herbivore damage, which may be why they are more heavily defended (Pennings et al., 2001; Pennings and Silliman, 2005). We did not measure palatability as part of our study, and so are unable to determine whether herbivore densities correlate with plant defense and nutrient density. Third, an increase in live biomass would lead to a greater number of sites for web attachment for spiders, and hiding locations for predators that are intraguild predators and cannibals (Langellotto and Denno, 2006). Thus, increased structural complexity could lead to greater top-down pressure. In support of this last explanation, we have consistently found that higher trophic level predators are more strongly affected by nutrient addition relative to herbivores in manipulative experiments (Wimp et al., 2010; Murphy et al., 2012).

While the predator:herbivore ratio had a positive relationship with nitrogen density, such responses were not consistent across predator groups. Variable responses among predators to nitrogen density could be partly explained by diet breadth (e.g., generalists vs. specialists). Herbivores had a negative response to increasing plant nitrogen density; specialist predators tracked their herbivore prey and thus also responded negatively to nitrogen density. However, generalists were not negatively affected by nitrogen density and indeed some generalist predators responded positively to nitrogen density. Thus, the overall predator:herbivore ratio was also positively associated with nitrogen density. Additionally, differences in predator responses to nutrient addition also explain why we found an increase the predator:herbivore ratio, but not overall predator density, with an increase in nitrogen density. Indeed, predators exhibited every possible response to an increase in nitrogen density; taxa had either positive or negative relationships with nitrogen density, or demonstrated no significant response. Understanding the mechanism behind this response is simplified for specialist relative to generalist predators. For instance, the specialist predator *Tytthus vagus* feeds only on planthopper herbivore eggs, so densities of this predator declined with an increase in nitrogen density, similar to their prey. However, for the two most common generalist predators (the hunting spider, *Pardosa littoralis* and the web-building spider, *Grammonota trivittata*), responses are driven by both prey and structural resources (Wimp et al., 2019) and their ability to feed on prey from different food webs. Even though herbivore density declines with nitrogen density, *Pardosa* and *Grammonota* are multi-channel omnivores that can feed on a combination of prey from the live plant and epigeic food webs (Wimp et al., 2013; Murphy et al., in review). Since epigeic prey densities had no relationship with nitrogen density, these generalist predators could use alternative prey from the epigeic food web when herbivores were not available. This may explain why *Pardosa* densities were not affected by a change in nitrogen density. However, the marginally positive response of *Grammonota* to an increase in nitrogen density is more likely to be driven by structural resources. *Grammonota* is a web-building spider that requires adequate scaffolding for web attachment, and taller *Spartina* plants would provide such a resource. Because spiders were either positively affected by nitrogen density or exhibited no response, when compared to the negative response of herbivores, the overall spider:herbivore ratio was positive.

Our research helps us to understand how long-term nutrient enrichment of native ecosystems from anthropogenic sources affects the arthropod assemblage and foodweb dynamics. We found that while above-ground biomass increased with nutrient density, the ratio of belowground roots to aboveground shoots decreased significantly with nutrient enrichment, which may lead to marsh loss as suggested by Deegan et al. (2012). Further, we found that herbivore abundance was significantly lower in marshes that experienced high levels of nutrient enrichment. Recently there have been reports of an insect apocalypse (Hallmann et al., 2017; Lister and Garcia, 2018; Sanchez-Bayo and Wyckhuys, 2019; but see Thomas et al., 2019) and our results suggest that nutrient enrichment may be just one of many possible mechanisms, as also suggested by Sanchez-Bayo and Wyckhuys (2019). While the negative effects of nitrogen enrichment on aquatic systems are obvious due to the creation of dead zones, the impacts of nitrogen enrichment on terrestrial systems are more subtle, but nonetheless important. Not every trophic level, or functional group, responds to fertilization in a similar manner, and positive responses to fertilization are not common, even among herbivores where such responses might be anticipated or even expected. Such divergent responses to fertilization can lead to altered trophic structure and ultimately affect ecosystem processes.

DATA AVAILABILITY

The datasets for this study can be found in Dryad using doi: 10.5061/dryad.j4d7r61.

AUTHOR CONTRIBUTIONS

DL performed the statistical analyses. GW and SM wrote the manuscript. All authors conceived the ideas, designed methodology, collected the data, contributed critically to subsequent drafts, and gave final approval for publication.

FUNDING

This research was supported by the National Science Foundation (NSF-DEB 1026067 to GW; NSF-DEB 1026000 to SM).

ACKNOWLEDGMENTS

We thank A. Adams, C. Ademawagun, A. Ayotte, E. Barnes, K. Beins, M. Brabson, L. Buttrick, L. Cepero, N. Chaudhuri, G. Connor, K. Grenis, C. Hallagan, K. Hauri, M. Hayes, R. Huang, K. Hoffman, E. Kuras, M. Lynch, H. Maness, J. McCarty, M. Monahan, G. Ngo, E. Noyes, D. Olney, K. Parnigoni, E. Phillips, E. Powell, L. Preudhomme, B. Rojewski, A. Styer, M. Uelk, J. Wade, S. Wu, and M. Zeigler for help in the field or processing the samples in the lab. We thank the following site managers for facilitating our research and permits at each site: Ken Able at the Rutgers University Marine Station; Katie Brown, Kara Wooldrik, and Jaime Parker at Fore River Sanctuary; Jim Raynes at Conservation Commission of the Town of Rye for Awcomin Marsh; A.J. Dupere at the Urban Forestry Center; Scott Ruhren at Foxhill Salt Marsh; George Frame with the National Parks Service Gateway National Recreation Area for permits at the Jamaica Bay Unit and the Sandy Hook Unit; Anne Giblin at Plum Island LTER; Cheryl Wiitala at Fogland Nature Preserve; Melissa Slaughter at Colt State Park; Katherine Thomas and Leonard Krauss at Caumsett State Historic Park; Gary Kreamer and Ken Reynolds at Little Creek Wildlife area; Amanda Deschenes and Chris Bennett at Delaware Seashore State Park; and Alex Wilke at Virginia Coast Reserve.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00350/full#supplementary-material

REFERENCES

Bertness, M. D. (1991). Zonation of *Spartina patens* and *Spartina alterniflora* in a New England salt marsh. *Ecology* 72, 138–148. doi: 10.2307/1938909

Bertness, M. D., Crain, C., Holdredge, C., and Sala, N. (2008). Eutrophication and consumer control of New England salt marsh primary productivity. *Conservat. Biol.* 22, 131–139. doi: 10.1111/j.1523-1739.2007.00801.x

Bertness, M. D., Ewanchuk, P. J., and Silliman, B. R. (2002). Anthropogenic modification of New England salt marsh landscapes. *Proc. Natl. Acad. Sci. U.S.A.* 99, 1395–1398. doi: 10.1073/pnas.022447299

Bertness, M. D., Silliman, B. R., and Jefferies, R. (2004). Salt marshes under siege. *Am. Sci.* 92, 54–61. doi: 10.1511/2004.1.54

Boyer, E. W., Goodale, C. L., Jaworski, N. A., and Howarth, R. W. (2002). Anthropogenic nitrogen sources and relationships to riverine nitrogen export in the northeastern U.S.A. *Biogeochemistry* 57/58, 137–169. doi: 10.1023/A:1015709302073

Deegan, L. A., Bowen, J. L., Drake, D., Fleeger, J. W., Friedrichs, C. T., Galvan, K. A., et al. (2007). Susceptibility of salt marshes to nutrient enrichment and predator removal. *Ecol. Appl.* 17, S42–S63. doi: 10.1890/06-0452.1

Deegan, L. A., Johnson, D. S., Warren, R. S., Peterson, B. J., Fleeger, J. W., Fagherazzi, S., et al. (2012). Coastal eutrophication as a driver of salt marsh loss. *Nature* 490, 388–392. doi: 10.1038/nature11533

Denno, R. F. (1976). Ecological significance of wing-polymorphism in Fulgoroidea which inhabit tidal salt marshes. *Ecol. Entomol.* 1, 257–266. doi: 10.1111/j.1365-2311.1976.tb01230.x

Denno, R. F. (1977). Comparison of the assemblages of sap-feeding insects (Homoptera-Hemiptera) inhabiting two structurally different salt marsh grasses in the genus *Spartina*. *Environ. Entomol.* 6, 359–372. doi: 10.1093/ee/6.3.359

Denno, R. F. (1980). Ecotope differentiation in a guild of sap-feeding insects on the salt marsh grass, *Spartina patens*. *Ecology* 61, 702–714. doi: 10.2307/1937435

Denno, R. F., Gratton, C., Dobel, H., and Finke, D. L. (2003). Predation risk affects relative strength of top-down and bottom-up impacts on insect herbivores. *Ecology* 84, 1032–1044. doi: 10.1890/0012-9658(2003)084[1032:PRARSO]2.0.CO;2

Denno, R. F., Gratton, C., Peterson, M. A., Langellotto, G. A., Finke, D. L., and Huberty, A. (2002). Bottom-up forces mediate natural-enemy impact in a phytophagous insect community. *Ecology* 83, 1443–1458. doi: 10.1890/0012-9658(2002)083[1443:BUFMNE]2.0.CO;2

Denno, R. F., Lewis, D., and Gratton, C. (2005). Spatial variation in the relative strength of top-down and bottom-up forces: causes and consequences for phytophagous insect populations. *Ann. Zool. Fennici* 42, 295–311.

Denno, R. F., Mitter, M. S., Langellotto, G. A., Gratton, C., and Finke, D. L. (2004). Interactions between a hunting spider and a web-builder: consequences of intraguild predation and cannibalism for prey suppression. *Ecol. Entomol.* 29, 566–577. doi: 10.1111/j.0307-6946.2004.00628.x

Denno, R. F., Peterson, M. A., Gratton, C., Cheng, J., Langellotto, G. A., Huberty, A., et al. (2000). Feeding-induced changes in plant quality mediate interspecific competition between sap-feeding herbivores. *Ecology* 81, 1814–1827. doi: 10.1890/0012-9658(2000)081[1814:FICIPQ]2.0.CO;2

Döbel, H. G., and Denno, R. F. (1994). "Predator planthopper interactions," in *Planthoppers: Their Ecology and Management*, eds R. F. Denno and T. J. Perfect (New York, NY: Chapman and Hall), 325–399.

Dobel, H. G., Denno, R. F., and Coddington, J. A. (1990). Spider (*Aranae*) community structure in an intertidal salt marsh: effects of vegetation structure and tidal flooding. *Environ. Entomol.* 19, 1356–1370. doi: 10.1093/ee/19.5.1356

Ferrenberg, S. M., and Denno, R. F. (2003). Competition as a factor underlying the abundance of an uncommon phytophagous insect, the salt marsh planthopper *Delphacodes penedetecta*. *Ecol. Entomol.* 28, 58–66. doi: 10.1046/j.1365-2311.2003.00479.x

Finke, D. L., and Denno, R. F. (2002). Intraguild predation diminished in complex-structured vegetation: implications for prey suppression. *Ecology* 83, 643–652. doi: 10.1890/0012-9658(2002)083[0643:IPDICS]2.0.CO;2

Finke, D. L., and Denno, R. F. (2003). Intraguild predation relaxes natural enemy impacts on herbivore populations. *Ecol. Entomol.* 28, 67–73. doi: 10.1046/j.1365-2311.2003.00475.x

Finke, D. L., and Denno, R. F. (2004). Predator diversity dampens trophic cascades. *Nature* 429, 407–410. doi: 10.1038/nature02554

Finke, D. L., and Denno, R. F. (2005). Predator diversity and the functioning of ecosystems: the role of intraguild predation in dampening trophic cascades. *Ecol. Lett.* 8, 1299–1306. doi: 10.1111/j.1461-0248.2005.00832.x

Finke, D. L., and Denno, R. F. (2006). Spatial refuge from intraguild predation: implications for prey suppression and trophic cascades. *Oecologia* 149, 265–275. doi: 10.1007/s00442-006-0443-y

Gratton, C., and Denno, R. F. (2003). Inter-year carryover effects of a nutrient pulse on *Spartina* plants, herbivores, and natural enemies. *Ecology* 84, 2692–2707. doi: 10.1890/02-0666

Haddad, N. M., Haarstad, J., and Tilman, D. (2000). The effects of long-term nitrogen loading on grassland insect communities. *Oecologia* 124, 73–84. doi: 10.1007/s004420050026

Halaj, J., and Wise, D. H. (2002). Impact of a detrital subsidy on trophic cascades in a terrestrial grazing food web. *Ecology* 83, 3141–3151. doi: 10.1890/0012-9658(2002)083[3141:IOADSO]2.0.CO;2

Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., et al. (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS ONE* 12:e0185809. doi: 10.1371/journal.pone.0185809

He, Q., and Silliman, B. R. (2015). Biogeographic consequences of nutrient enrichment for plant–herbivore interactions in coastal wetlands. *Ecol. Lett.* 18, 462–471. doi: 10.1111/ele.12429

He, Q., and Silliman, B. R. (2016). Consumer control as a common driver of coastal vegetation worldwide. *Ecol. Monogr.* 86, 278–294 doi: 10.1002/ecm.1221

Holmgren, M., Scheffer, M., Ezcurra, E., Guitierrez, J. R., and Mohren, G. M. J. (2001). El Nino effects on the dynamics of terrestrial ecosystems. *Trends Ecol. Evol.* 16, 89–94. doi: 10.1016/S0169-5347(00)02052-8

Howarth, R. W., Billen, G., Swaney, D., Townsend, A., Jaworski, N., Lajtha, K., et al. (1996). Regional nitrogen budgets and riverine N & P fluxes for the drainages to the North Atlantic Ocean: natural and human influences. *Biogeochemistry* 35, 75–139. doi: 10.1007/BF02179825

Huberty, A., and Denno, R. F. (2006a). Consequences of nitrogen and phosphorus limitation for the performance of two planthoppers with divergent life-history strategies. *Oecologia* 149, 444–455. doi: 10.1007/s00442-006-0462-8

Huberty, A., and Denno, R. F. (2006b). Trade-off in investment between dispersal and ingestion capability in phytophagous insects and its ecological implications. *Oecologia* 148, 226–234. doi: 10.1007/s00442-006-0371-x

Huxel, G. R., and McCann, K. (1998). Food web stability: the influence of trophic flows across habitats. *Am. Nat.* 152, 460–469. doi: 10.1086/286182

Inouye, R., and Tilman, D. (1995). Convergence and divergence of old-field vegetation after 11 years of nitrogen addition. *Ecology* 76, 1872–1887. doi: 10.2307/1940720

Kirchner, T. B. (1977). The effects of resource enrichment on the diversity of plants and arthropods in a shortgrass prairie. *Ecology* 58, 1334–1344. doi: 10.2307/1935085

Langellotto, G. A., and Denno, R. F. (2006). Refuge from cannibalism in complex-structured habitats: implications for the accumulation of invertebrate predators. *Ecol. Entomol.* 31, 575–581. doi: 10.1111/j.1365-2311.2006.00816.x

Lewis, D., and Denno, R. F. (2009). A seasonal shift in habitat suitability enhances an annual predator subsidy. *J. Anim. Ecol.* 78, 752–760. doi: 10.1111/j.1365-2656.2009.01550.x

Lister, B. C., and Garcia, A. (2018). Climate-driven declines in arthropod abundance restructure a rainforest food web. *Proc. Natl. Acad. Sci. U.S.A.* 115, E10397–E10406. doi: 10.1073/pnas.1722477115

Matsumura, M., Trafelet-Smith, G. M., Gratton, C., Finke, D. L., Fagan, W. F., and Denno, R. F. (2004). Does intraguild predation enhance predator performance? A stoichiometric perspective. *Ecology* 89, 2601–2615. doi: 10.1890/03-0629

Mayer, B., Boyer, E. W., Goodale, C. L., Jaworski, N. A., van Breemen, N., Howarth, R. W., et al. (2002). Sources of nitrate in rivers draining sixteen watersheds in the northeastern U.S.: isotopic constraints. *Biogeochemistry* 57/58, 171–197. doi: 10.1007/978-94-017-3405-9_5

Mendelssohn, I. A. (1979a). The influence of nitrogen level, form, and application method on the growth response of *Spartina alterniflora* in North Carolina. *Estuaries* 2, 106–112. doi: 10.2307/1351634

Mendelssohn, I. A. (1979b). Nitrogen metabolism in the height forms of *Spartina alterniflora* in North Carolina. *Ecology* 60, 574–584. doi: 10.2307/1936078

Murphy, S. M., Wimp, G. M., Lewis, D., and Denno, R. F. (2012). Nutrient presses and pulses differentially impact plants, herbivores, detritivores and their natural enemies. *PLoS ONE* 7:e43929. doi: 10.1371/journal.pone.0043929

Pennings, S. C., Clark, C. M., Cleland, E. E., Collins, S. L., Gough, L., Gross, K. L., et al. (2005). Do individual plant species show predictable resopnses to nitrogen addition across multiple experiments? *Oikos* 110, 547–555. doi: 10.1111/j.0030-1299.2005.13792.x

Pennings, S. C., and Silliman, B. R. (2005). Linking biogeography and community ecology: latitudinal variation in plant-herbivore interaction strength. *Ecology* 86, 2310–2319. doi: 10.1890/04-1022

Pennings, S. C., Siska, E. L., and Bertness, M. D. (2001). Latitudinal differences in plant palatability in Atlantic coast salt marshes. *Ecology* 82, 1344–1359. doi: 10.1890/0012-9658(2001)082[1344:LDIPPI]2.0.CO;2

Polis, G. A., Anderson, W. B., and Holt, R. D. (1997a). Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. *Annu. Rev. Ecol. Syst.* 28, 289–316. doi: 10.1146/annurev.ecolsys.28.1.289

Polis, G. A., and Hurd, S. D. (1996). Linking marine and terrestrial food webs: allochthonous input from the ocean supports high secondary productivity on small islands and coastal land communities. *Am. Nat.* 147, 396–423. doi: 10.1086/285858

Polis, G. A., Hurd, S. D., Jackson, C. T., and Sanchez-Pinero, F. (1997b). El Nino effects on the dynamics and control of an island ecosystem in the Gulf of California. *Ecology* 78, 1884–1897. doi: 10.1890/0012-9658(1997)078[1884:ENOEOT]2.0.CO;2

Polis, G. A., Hurd, S. D., Jackson, C. T., and Sanchez-Pinero, F. (1998). Multifactor population limitation: variable spatial and temporal control of spiders on Gulf of California islands. *Ecology* 79, 490–502. doi: 10.1890/0012-9658(1998)079[0490:MPLVSA]2.0.CO;2

Redfield, A. C. (1972). Development of a New England salt marsh. *Ecol. Monogr.* 42, 201–237. doi: 10.2307/1942263

Robinson, D. (1994). The responses of plants and their roots to non-uniform supplies of nutrients. *New Phytol.* 127, 635–647. doi: 10.1111/j.1469-8137.1994.tb02969.x

Sanchez-Bayo, F., and Wyckhuys, K. A. G. (2019). Worldwide decline of the entomofauna: a review of its drivers. *Biol. Conserv.* 232, 8–27. doi: 10.1016/j.biocon.2019.01.020

Settle, W. H., Ariawan, H., Astuti, E. T., Cahyana, W., Hakim, A. L., Hindayana, D., et al. (1996). Managing tropical rice pests through conservation of generalist natural enemies and alternative prey. *Ecology* 77, 1975–1988. doi: 10.2307/2265694

Siemann, E. (1998). Experimental tests of effects of plant productivity and diversity on grassland arthropod diversity. *Ecology* 79, 2057–2070. doi: 10.1890/0012-9658(1998)079[2057:ETOEOP]2.0.CO;2

Stiling, P., and Strong, D. R. (1983). Weak competition among *Spartina* stem borers, by means of murder. *Ecology* 64, 770–778. doi: 10.2307/1937200

Thomas, C. D., Jones, T. H., and Hartley, S. E. (2019). "Insectageddon": a call for more robust data and rigorous analyses. *Glob. Chang. Biol.* 25, 1891–1892. doi: 10.1111/gcb.14608

Tilman, D. (1999). Global environmental impacts of agricultural expansion: the need for sustainable and efficient practices. *Proc. Natl. Acad. Sci.U.S.A.* 96, 5995–6000. doi: 10.1073/pnas.96.11.5995

Valiela, I., and Bowen, J. L. (2002). Nitrogen sources to watersheds and estuaries: role of land cover mosaics and losses within watersheds. *Environ. Pollut.* 118, 239–248. doi: 10.1016/S0269-7491(01)00316-5

Valiela, I., and Cole, M. L. (2002). Comparative evidence that salt marshes and mangroves may protect seagrass meadows from land-derived nitrogen loads. *Ecosystems* 5, 92–102. doi: 10.1007/s10021-001-0058-4

Valiela, I., and Teal, J. M. (1979). The nitrogen budget of a salt marsh ecosystem. *Nature* 280, 652–656. doi: 10.1038/280652a0

Valiela, I., Teal, J. M., and Deuser, W. G. (1978). The nature of growth forms in the salt marsh grass *Spartina alterniflora*. *Am. Nat.* 112, 461–470. doi: 10.1086/283290

Vidal, M. C., and Murphy, S. M. (2018). Bottom-up vs. top-down effects on terrestrial insect herbivores: a meta-analysis. *Ecol. Lett.* 21, 138–150. doi: 10.1111/ele.12874

Vince, S. W., Valiela, I., and Teal, J. M. (1981). An experimental study of the structure of herbivorous insect communities in a salt marsh. *Ecology* 62, 1662–1678. doi: 10.2307/1941520

Vitousek, P. M., Mooney, H. A., Lubchenco, J., and Melillo, J. M. (1997). Human domination of Earth's ecosystems. *Science* 277, 494–499. doi: 10.1126/science.277.5325.494

Warren, R. S., and Niering, W. A. (1991). Vegetation change on a northeast tidal marsh: interaction of sea-level rise and marsh accretion. *Ecology* 74, 96–103. doi: 10.2307/1939504

Wigand, C., Watson, E. B., Martin, R., Johnson, D. S., Warren, R. S., Hanson, A., et al. (2018). Discontinuities in soil strength contribute to destabilization of nutrient-enriched creeks. *Ecosphere* 9:e02329. doi: 10.1002/ecs2.2329

Wimp, G. M., Murphy, S. M., Finke, D. L., Huberty, A. F., and Denno, R. F. (2010). Increased primary production shifts the structure and composition of a terrestrial arthropod community. *Ecology* 91, 3303–3311. doi: 10.1890/09-1291.1

Wimp, G. M., Murphy, S. M., Lewis, D., Douglas, M. R., Ambikapathi, R., Van-Tull, L., et al. (2013). Predator hunting mode influences patterns of prey use from grazing and epigeic food webs. *Oecologia* 171, 505–515. doi: 10.1007/s00442-012-2435-4

Wimp, G. M., Ries, L., Lewis, D., and Murphy, S. M. (2019). Habitat edge responses of generalist predators are predicted by prey and structural resources. *Ecology* 100:e02662. doi: 10.1002/ecy.2662

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

REVIEW
published: 15 October 2019
doi: 10.3389/fevo.2019.00375

A Potential Role for Phenotypic Plasticity in Invasions and Declines of Social Insects

Fabio Manfredini[1,2]*, **Marina Arbetman**[3]* and **Amy L. Toth**[4]*

[1] Department of Biological Sciences, Ecology and Evolutionary Biology, Royal Holloway, University of London, Egham, United Kingdom, [2] School of Biological Sciences, University of Aberdeen, Aberdeen, United Kingdom, [3] Grupo de Ecología de la Polinización (EcoPol), INIBIOMA (Comahue–CONICET), San Carlos de Bariloche, Argentina, [4] Department of Ecology, Evolution, and Organismal Biology, and Department of Entomology, Iowa State University, Ames, IA, United States

Eusociality, a form of animal social organization involving sterile and reproductive castes, is a rare, but highly ecologically successful form of life. There are striking examples of eusocial species with populations that are ecologically dominant in their native ranges, as well as remarkably successful globally as invasive species; prominent examples include fire ants and yellowjacket wasps. At the same time, there have been startling population declines in other social insects, notably bumble bees. Here, we explore the possible role of phenotypic plasticity in invasion biology and declines of social insect species. This topic is of particular interest, because social insects exhibit extreme behavioral, developmental, physiological, and morphological plasticity. It has been suggested that this plasticity may contribute to ecological dominance in some species, but could be a liability or cost to others. In this review, we explore the relationship between phenotypic plasticity, invasion biology, and vulnerability to global change in social insects. By considering plasticity at three levels– molecular, individual, and colony—we suggest ways in which considerations of phenotypic plasticity may help in managing social insect populations.

Keywords: social insect, phenotypic plasticity, species conservation, invasive species, global change

OPEN ACCESS

Edited by:
Shannon Murphy,
University of Denver, United States

Reviewed by:
Adam R. Smith,
George Washington University, United States
Brock Harpur,
Purdue University, United States

***Correspondence:**
Fabio Manfredini
fmanfredini79@gmail.com
Marina Arbetman
marbetman@comahue-conicet.gob.ar
Amy L. Toth
amytoth@iastate.edu

Specialty section:
This article was submitted to Population and Evolutionary Dynamics, a section of the journal Frontiers in Ecology and Evolution

Received: *18 June 2019*
Accepted: *17 September 2019*
Published: *15 October 2019*

Citation:
Manfredini F, Arbetman M and Toth AL (2019) A Potential Role for Phenotypic Plasticity in Invasions and Declines of Social Insects. Front. Ecol. Evol. 7:375. doi: 10.3389/fevo.2019.00375

INTRODUCTION

Phenotypic Plasticity in Social Insects

Phenotypic plasticity is defined as the ability of an individual organism to respond to the environment by producing alternative phenotypes based on the same genotype (Baldwin, 1896). Phenotypic plasticity is of great interest in ecology and evolution because it allows an organism to actively adjust its phenotype in response to environmental conditions; thus it is a major mechanism of ecological adaptation (Via et al., 1995; Lande, 2015; Beaman et al., 2016; Colautti et al., 2017). In addition, phenotypic plasticity has been suggested to be an important driver of evolutionary change; animals with more flexibility in how they respond to the environment may have greater potential to enter and survive in novel and/or changing habitats (Baldwin, 1896; West-Eberhard, 1989).

Eusocial insects show some of the most striking known examples of phenotypic plasticity known in the animal world, on both the individual and colony levels (Kennedy et al., 2017). These animals are defined by the presence of distinct castes within colonies—individuals that are specialized for specific types of activity, the most prominent being the division of labor between reproductive "royal" castes (e.g., queens) and non-reproductive castes (e.g., workers, Wilson, 1971).

In many species, reproductive caste differences are independent of genotype (Schwander et al., 2010)—e.g., in many eusocial bees, ants, and wasps, any egg has the potential to develop into a queen or a worker. As adults, there is further phenotypic plasticity, with specialized subcastes of individuals involved in different behavioral and physiological activities (e.g., foraging, nursing brood). One key aspect of colony level plasticity is the ability to alter division of labor (e.g., ratio of foragers and nurses) according to colony nutritional needs, colony demography, and environmental conditions (Robinson, 1992; Gordon, 1996; Traniello and Rosengaus, 1997). In some species, there is further phenotypic plasticity in the form of specialized forms of foraging preference (e.g., pollen vs. nectar foraging in honey bees), dominance status (e.g., in *Polistes* workers), and task-related behaviors including learning and memory abilities (Reeve and Nonacs, 1992; Robinson, 1992; O'Donnell et al., 2004). Thus, there is no doubt that social insects are champions of phenotypic plasticity.

Eusocial insects represent some of the most important known invasive species in the world. For example, in a list of the Top 100 most invasive species, 41% of invasive invertebrates are social insects (Lowe et al., 2000). This figure suggests a large overrepresentation of social insect as successful invaders, given the fact that only 2% of insect species are estimated to be eusocial. Although the aforementioned list is somewhat anecdotal, it is a well-established fact that some social insect species are spectacularly successful invasive species (Evans, 2010; Lach and Hooper-Bui, 2010). Are these two phenomena related? That is, is the extreme phenotypic plasticity exhibited by social insects one of the secrets to their worldwide success, and their ability to invade new environments and dominate ecosystems? Studies from other organisms suggest that indeed, the extent of phenotypic plasticity exhibited by a species can contribute to its ability to become an invasive species (Richards et al., 2006; Wilson, 2012).

On the other hand, there have been dramatic declines in insect populations worldwide over the past several decades (Sánchez-Bayo and Wyckhuys, 2019). Prominent examples of insect decline include, notably, several species of bees, which are highly valued as pollinators (Potts et al., 2010). Some of these are social bees, the best studied examples being various species of bumble bees. If plasticity is related to invasion success and the ability of social insect species to survive in novel environments, then why are other social insects in decline and so prominently of conservation concern? Could variation in the extent of phenotypic plasticity, specifically, a relative lack of plasticity in some social insect species, be related to species decline? And, is colony or individual level plasticity playing a role, or both? Evolutionary modeling studies (Chevin and Lande, 2010; Chevin et al., 2010) and studies in non-social insect species (reviewed in the sections that follow) have provided ample evidence that the extent of phenotypic plasticity can play a role in species extinction and robustness to environmental change.

The goal of this article is to explore the potential relationships between phenotypic plasticity, eusocial insect invasions, and eusocial insect declines. Specifically, we explore the hypothesis

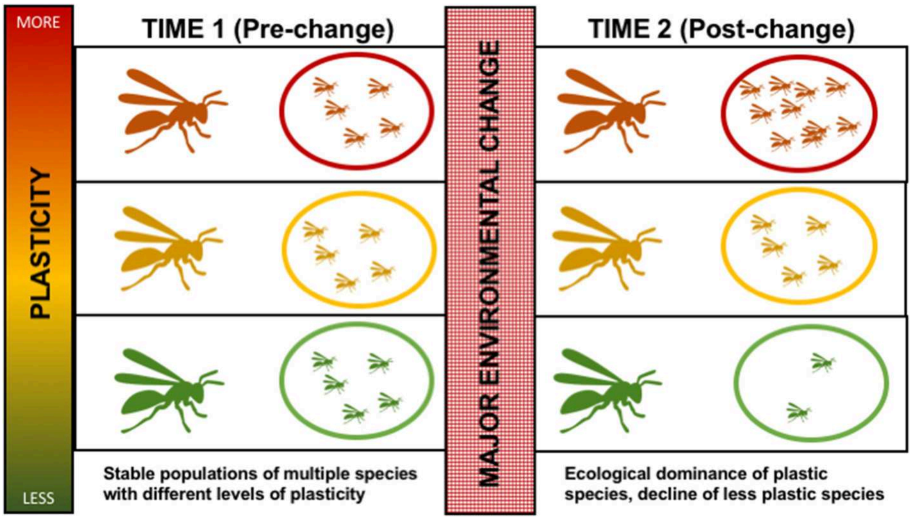


FIGURE 1 | Schematic representing how phenotypic plasticity may contribute to species declines or species invasions in the face of a major environmental change. Three hypothetical species are represented with variation in their level of phenotypic plasticity (overall plasticity, or related to specific traits) (see text). These hypothetical species vary in their level of phenotypic plasticity at both individual (insect drawing) and colony (nest drawing) levels, with green representing lowest plasticity, rust representing highest plasticity, and yellow representing intermediate plasticity. At time 1, all three species are able to coexist with stable populations (ovals), each taking advantage of different ecological niches with the less plastic species being successful despite lacking plasticity due to being able to specialize on certain aspects of its environment. A major environmental change (red stippled box in center) occurs, such as major habitat loss, climatic shift, arrival in a new exotic region, or stress from disease, toxins, or new competitors. At time 2, the major environmental change has served as a filter, with a higher probability of more plastic species (rust) prospering and increasing their populations (rust oval) with this change due to their ability to rapidly adapt to change and/or enter new niches. At the same time, the less plastic and more specialized species (green) is less likely to fit this new environment or withstand a large change and experiences population declines (green oval).

that drastic environmental change, such as current global climate and anthropogenic disturbance, will favor more phenotypically plastic social insect species, fueling their invasions, while harming less plastic species, contributing to their declines (**Figure 1**). A related hypothesis is that social insects themselves, due to their inherent plasticity because of their eusocial lifestyle, are buffered from environmental change. Because direct comparative studies of the population status and plasticity of social and non-social species are lacking, this review focuses on addressing the former hypothesis, with the goal of elucidating a path forward to answer the latter. Previous reviews have discussed traits related to social insect invasions (especially in ants, see Holway et al., 2002) and declines (especially in bees, see Williams et al., 2010). However, to our knowledge, there has been no review to synthesize this information in a more general framework, despite the fact that species traits and robustness to global change has been broadly explored outside of social insects (Jiguet et al., 2007; Pyšek et al., 2012). Here, we first briefly review the literature related to phenotypic plasticity in the context of organisms outside social insects, identifying traits that are potentially phenotypically plastic in other systems that have been proposed to contribute to invasive potential. Similarly, we review the literature related to life history traits associated with declines of species outside of social insects, and assess these traits in the context of potential relationships to phenotypic plasticity. We then review evidence from selected social insect taxa that represent both invasive and declining species, and examine whether this information supports/refutes the hypothesis that phenotypic plasticity contributes to population stability of social insects in the face of environmental change.

Phenotypic Plasticity in Various Traits as Facilitators of Biological Invasions

Before considering the potential role of phenotypic plasticity in any context, it is important to define phenotypic plasticity and clarify what makes it distinct from other processes that might produce phenotypic variation within a species. For example, inter-individual variation can also be the result of standing genetic variation in a trait (e.g., the result of balancing selection in which there is no single trait value that optimizes fitness), as well as stochastic, developmental variation that is not based on adaptive differences in gene expression or genotype (Gianoli and Valladares, 2011). In addition, variation in size and age among individuals within a population can be confounding factors when analyzing the extent of phenotypic plasticity in a population of organisms. Thus, how do we separate out "real" phenotypic plasticity from other sources of phenotypic variation? In many cases, whether a trait is truly the result of phenotypic plasticity is not known, especially if the species has not been extensively studied. However, one aspect that might be useful to discriminate phenotypic plasticity from other mechanisms is the timescale of the phenomenon that we observe. By definition, a plastic trait must experience variation within a short timespan (though this might be different from organism to organism), hence complex traits that have undergone change over a long period of time are not plastic. Also, plasticity might be a transient trait that is displayed by a species in a specific moment in history—for example immediately after settling into a new range—and then disappears when it is no longer needed. For the purposes of this study, we followed the principle that the study of phenotypic plasticity in an ecological context goes beyond addressing the question of whether plasticity exists (or not) in each specific case—because most traits are plastic to a certain level (Gianoli and Valladares, 2011).

Previous studies have provided some support for the idea that phenotypic plasticity plays a role in the success of invasive organisms. In a meta-analysis of plants, Davidson et al. (2011) found that invasive plants display higher phenotypic plasticity than their non-invasive counterparts—though this does not always correlate with increased fitness. However, Palacio-López and Gianoli (2011) did not find any difference in the extent of phenotypic plasticity between invasive and non-invasive plants. They hypothesize that the success in invasion might be linked instead to a better ability to adapt to specific ecological niches, but acknowledge that plasticity should be investigated very early in the process of invasion when adaptation has not occurred yet. Although no large-scale meta-analyses have been conducted on invasion potential and phenotypic plasticity in animals, below, we provide a brief review of *potentially* plastic traits that have been proposed to be associated with invasiveness in animals, highlighting some specific examples that lead to hypotheses about invasive traits in social insects.

Morphology and Physiology

There is a large literature on plasticity in morphology (external and internal) and physiology as related to invasiveness in animals. Invasive species are often released from natural competitors and enemies (see **Box 1**) that are absent in the new range (Liu and Stiling, 2006). It has been hypothesized that this release could be followed by a reduction of competition effort or defense against enemies, accompanied by an increased investment into reproduction (Blossey and Notzold, 1995). The ability to move resources from one physiological compartment to another (possibly a form of phenotypic plasticity) is thought to give invasive species an advantage compared to new competitors that they might encounter in the invasive range. Plastic morphological traits in invasive organisms are well-documented, such as the appearance of head spines in invasive *Daphnia* in North America when predator pressure is high (Engel and Tollrian, 2009), or adaptive adjustments in body size in brown anoles after invading two Floridian islands (Campbell and Echternacht, 2003). In considering social insects, body size plasticity among workers is a well-known aspect usually linked to division of labor in various species of ants and bumble bees (i.e., workers of different size undertaking different size-specific tasks, see for example Wilson, 1978). Thus, one might predict that invasive populations of social insects might display a wider range of body size plasticity (either within a colony or at the population level) compared to non-invasive populations—or an invasive species overall might display wider body size plasticity compared to other species that are non-invasive.

BOX 1 | The role of natural enemies in biological invasions.

An intriguing hypothesis often invoked to explain the evolutionary ecology of biological invasions is the "Enemy Release Hypothesis" (ERH). It postulates that invasive species might leave behind their natural enemies (predators, competitors, parasites, and pathogens) when establishing in a new range (reviewed in Liu and Stiling, 2006). This gives them an advantage with respect to sympatric species in the new range, as they can redirect resources originally allocated for fighting natural enemies toward different physiological compartments, for example growth and reproduction ("Evolution of Increased Competitive Ability" or EICA, Blossey and Notzold, 1995). EICA is often described as a natural consequence of the ERH. However, it is not fully clear whether specific conditions are needed for the EICA to be triggered and, in particular, whether it requires a certain amount of plasticity in the physiology of the organism. This could be a key feature that differentiates between successful and failed invaders.

The ERH has been documented for several species of social insects in relation to parasites and pathogens: for example, fire ants have been released from *Wolbachia*, two microsporidia and one RNA virus after invading South-East Asia and Oceania (Yang et al., 2010), Argentine ants have lost *Wolbachia* in seven out of eight regions where they have been introduced (Reuter et al., 2005), and paper wasps have left behind two insect parasitoids after invasion into North America (Cervo et al., 2000). However, there are also remarkable cases that refute the ERH: for example, many common pathogens have been detected in bumble bees that were introduced to South America (Arbetman et al., 2013), and a study detected high microsporidian infection rates in yellowjackets that invaded New Zealand (Lester et al., 2014). Furthermore, the ERH is just one side of the coin, as there are instances where invasive species appear more susceptible to new enemies than native sympatric species (often referred to as the "Increased Susceptibility Hypothesis" or "Exotic Prey Naïveté" hypothesis," Li et al., 2011). This has been documented for paper wasps in South Africa, where invasive *Polistes dominula* experience more parasite pressure than native sympatric *Polistes marginalis* (Roets et al., 2019), and for Asian hornets that invaded southern France, infected by conopid flies and mermithin nematodes (Villemant et al., 2015) that represent "new enemies."

In this complex scenario, plasticity might be a key element that underpins the ability of successful invaders to "forget" old enemies, when they are no longer around, while maintaining high levels of defense against new enemies that might be encountered. When dealing with parasites and pathogens, social insects display the potential for high levels of plasticity in their defense responses. They can choose, for example, between response that are at the individual or group levels (known as "social immunity," Cremer et al., 2007), physiological or behavioral, constitutive, or induced (Schmid-Hempel and Ebert, 2003), and they can also respond as immatures or adults. One pioneer study in this field has shown how invasive paper wasps in the USA have reduced their defense against new general pathogens (at both the individual and group levels) while maintaining a strong behavioral group response against an old parasitic enemy that was lost after invasion (Manfredini et al., 2013). This exemplifies how plasticity in immunocompetence might mediate the successful invasion of a social insect.

Acclimatization

Plasticity in response to temperature variation is undoubtedly one of the key aspects to consider in how species are able to survive and compete in novel or rapidly changing environments. For example, Trinidadian guppies have extensive plasticity in modulating their reproductive activity according to daily variation of temperature and light (Reeve et al., 2014). Because social insects possess various different castes with different body sizes and physiologies, it has been proposed that thermal plasticity could be an advantage in social insects, i.e., that a single colony may possess the ability to send out "thermal explorers" into an environment that would be more thermally limiting for a monomorphic species (Baudier and O'Donnell, 2017).

Dispersal Ability

There is no doubt about the fact that one key ingredient for a successful invasion is the ability of a given species to spread beyond the native range. A series of elegant studies on invasive cane toads in Australia has shown how the geographic spread of this species varies along a gradient of dispersal abilities from the range-core to the invasion-front, showing incredible plasticity (Rollins et al., 2015). In social insects, plasticity during dispersal may be essential at several points in the colony cycle including queen dispersal during colony founding, male dispersal during mating season, or in mature colonies, potential for the production of sexuals (e.g., production of one vs. multiple broods of sexual, dispersing individuals).

Behavioral Traits

Behavior encompasses a complex set of traits that are extremely important during biological invasions, as behavior is often the first line of response to environmental challenges. Aggression, exploratory behavior and plasticity in sexual/reproductive behavior are often important traits associated with successful invasions. For example, virile crayfish shifted towards more aggressive behavior in their invasive range compared to their native range (Glon et al., 2018). Dark-eyed junco songbirds changed their sexual behavior after introduction into Southern California, accompanied by changes in testosterone levels (Atwell et al., 2014). Social insects are well-known to show wide inter- and intraspecies variation in aggression, exploratory behavior, and reproductive strategies (e.g., single vs. multi-queen colony organization, Bengston and Jandt, 2014). Some of these behavioral traits have also been proposed to be related to invasion success in social insects, in particular high levels of aggression (Human and Gordon, 1999) and plasticity in queen number (Ingram, 2002).

Genome Structure and Function

There are many forms of genomic plasticity, spanning from complex mechanisms that involve large portions of the genome and produce changes over evolutionary time (e.g., structural polymorphisms, copy-number variations, and chromosome rearrangements Leitch and Leitch, 2008), to forms of plasticity that involve the functioning of specific genomic elements and produce variation at the phenotypic level in a much shorter amount of time (e.g., the life span of an individual or few generations): these include, for example, epistatic interactions between genes in the context of gene networks, interactions between transcription factors and regulatory elements, and epigenetic modifications. Some changes in genome structure can occur incredibly quickly and might be important in invasions. For example, autopolyploidy caused rapid speciation of the marbled crayfish from the slough crayfish and clonality mediated the incredible success of marbled crayfish as an invasive species in Madagascar (Gutekunst et al., 2018). Hybridization instead is thought to be one of the key factors at the basis of big-headed carps invasion into the Mississippi river and Lake Balaton (Cooke, 2016). Some social insects display examples of structural polymorphisms in their genomes that are associated with key social traits possibly linked to successful invasions, notably, the fire ant supergene associated with the presence of multiple queens in the colony, or "polygyny" (Wang et al., 2013). However, a direct correlation between the supergene and fire ant invasions has not been established, and a similar feature has also been detected in another ant that is not invasive *(Formica selysi*, Purcell et al., 2014).

In terms of genome function, changes in the expression of several key genes after invasion were reported for cane toads in Australia (Rollins et al., 2015) and goby fish in the Great Lakes (Wellband and Heath, 2017). Epigenetic mechanisms have also been suggested to be relevant in facilitating phenotypic changes associated with invasion; for example in house sparrows increased variation in genome-wide DNA methylation was detected in invasive populations in Kenya (Liebl et al., 2013), and in invasive ascidians and corals, methylation changes quickly occurred in response to environmental challenges (Putnam et al., 2016; Huang et al., 2017). Epigenetic mechanisms are particularly relevant for the success of biological invasions as they can produce new variation (and therefore adaptation) even in the lack of genetic diversity (Hawes et al., 2018), and even more so when combined with plasticity in other regulators of genetic activity, such as non-coding RNAs or transposable elements (Stapley et al., 2015; Marin et al., 2019). Epigenetic mechanisms have also been a topic of substantial interest in social insects, where they have been demonstrated to be relevant to caste plasticity, worker division of labor, and learning and memory in social insects (Glastad et al., 2019). In the context of social insects we predict that epigenetic modifications may facilitate phenotypic plasticity in the case of invasion potential.

Limited Phenotypic Plasticity in the Context of Species Declines

The Earth is experiencing massive species declines worldwide, which according to the IPBS are "*already at least tens to hundreds of times higher than… averaged over the past 10 million years*" (IPBES et al., 2019). Thus, understanding major factors contributing to species declines is key for conservation purposes. Although there might not be a single or simple reason for the decline of any one species, some general causes, and or correlations with decline have pervaded the conservation literature (Didham et al., 2007). In general, the survival and/or persistence of species are thought to result from a combination of both extrinsic (e.g., environmental change) and intrinsic (e.g., species characteristics) factors (but see Fisher et al., 2003). Most research related to decline focuses on the extrinsic factors affecting species, including habitat (land/sea) use change, direct exploitation of species by humans, climate change, pollution, and negative impacts of invasive species. Although there has been some work trying to integrate intrinsic traits with extrinsic pressures (Murray et al., 2010), there is less understanding about the importance of plasticity of species' intrinsic traits. Understanding the true causes of decline of a species is often a complex situation, as a single extrinsic factor affects very many different intrinsic traits. For example, "climate change" can challenge a species' physiology, acclimatization, dispersal ability, and also behavioral traits. Here we summarize and give examples of specific traits associated with decline in different organisms, and explore whether phenotypic plasticity could be related to such declines.

Morphology and Physiology

Phenotypic plasticity can allow an organism to rapidly respond to a dynamic and temporally variable environment (e.g., climate change, habitat change use), whereas the lack of a plastic response has the potential to push a species to reduce its area of occupancy or result in a decrease in the number of individuals (i.e., species decline). It has been proposed that large-bodied species are more prone to decline than small-bodied species; for example, large-bodied carabid beetles have declined in Belgium more than smaller ones (Kotze and O'hara, 2003). In amphibians, a group of prime conservation concern, it has been reported that *Rana sylvatica* have a plastic response to desiccation, that results in decreases in post-metamorphic immune function. This physiological plasticity may thus result in a tradeoff with immune system functions, potentially contributing to increased sensitivity of amphibians to disease and decline (Gervasi and Foufopoulos, 2008). A recent study found that wild bees decreased in body size in the absence of native floral resource availability and that bees with particularly small or large body were most susceptible, suggesting that body size could plastically buffer bees from dearth and habitat disturbance (Grab et al., 2019).

Acclimatization

The ability of species to adapt to changing environments, particularly shifts in temperature, is one of the main challenges in a warming world. Some species show superior abilities to adapt to temperature changes, while others are adversely affected. For example, it has been shown that in an endangered turtle (*Podocnemis lewyana*), environmental sex determination (a form of phenotypic plasticity) is sensitive to temperature stability during the egg incubation period (Gómez-Saldarriaga et al., 2016). Another study demonstrated that declining species of springtails were more sensitive to desiccation under warmer temperatures as compared to invasive springtails (Chown et al., 2007). In the case of social insects, among bumble bees in the genus *Bombus*, some declining species may also be suffering from rising global temperatures, as evidenced by shrinking distributions in warmer areas along with a failure to move closer to polar regions (Kerr et al., 2015).

Dispersal Ability

The ability to disperse is an important challenge for declining species, particularly in situations of population fragmentation. If species have limited dispersal abilities or cannot plastically adjust their dispersal strategies in response to habitat loss, they may be unable to recolonize habitat fragments and become more susceptible to local extinctions (Kokko and López-Sepulcre, 2006). For example, dispersal ability may be associated with declines in large-bodied carabid beetles, which have poorer dispersal abilities as compared to smaller, more mobile species (Kotze and O'hara, 2003). Limited dispersal of sexuals (reproductive males and females) from their natal colonies has also been suggested to be one of the factors responsible for the rarity of wood ants in a fragmented landscape (Gyllenstrand and Seppä, 2003).

Behavioral Traits

Behavioral traits, as dynamic responses to environmental stimuli, are by their very nature plastic. However, species can vary greatly in the extent of behavioral flexibility of different traits, including numerous traits relevant to coping with major environmental change; e.g., cognitive capacity, foraging preferences, exploratory behavior, and territoriality. Host behavior can also influence the transmission of novel and/or damaging pathogens; e.g., in Australian rainforest frogs in the genus *Litoria*, species differences in contact frequency (with contaminated frogs and water) were associated with differences in chytridiomycosis infection rate, and these reflected their conservation status (Rowley and Alford, 2007). In the eusocial bee *Plebeia droryana*, behavioral emergence from diapause occurs at a specific temperature (suggesting a lack of plasticity). This trait has been suggested to be a potential liability, as climate patterns producing milder winters or sudden extreme colds could prevent normal colony initiation in this species of conservation concern (Dos Santos et al., 2015).

CASE STUDIES: INVADING AND DECLINING SOCIAL INSECT SPECIES

Below, we highlight prominent case studies covering various species and genera of social insects related to population invasions and declines. The purpose of this is not to provide a comprehensive review of the literature, but rather to highlight some of the most prominent, well-known declining and invading species and consider what is known about the traits contributing to each of these phenomena in light of phenotypic plasticity. We focus on hymenopteran social insects (ants, wasps, and bees) because this allows us to most coherently explore shared life history traits associated with these phenomena. Although termites are eusocial insects with numerous highly invasive species found throughout the world (Evans et al., 2013), in this review we do not provide specific examples of termites because they are taxonomically distant from Hymenoptera and also because there is less known about the ecology of termite invasions as much of this research has occurred in urban environments (Buczkowski and Bertelsmeier, 2017).

Ants

Ants are among the most successful invaders. There are 5 ants in the list of the world's top 100 invasive species (Lowe et al., 2000), and among these *Solenopsis invicta* (fire ants) and *Linepithema humile* (Argentine ants) are the two best studied examples. Some traits that *S. invicta* and *L. humile* have in common (also shared by other top ant invaders) have been linked to their successful invasions (Holway et al., 2002): first, the South American origin, where a rich ant biodiversity promotes high competition for resources and recurrent floods create a permanently unsettled environment; second, a highly omnivorous diet; and third, a flexible social structure, that can range from individual colonies to multi/unicoloniality. On the flip side, there are also examples of critically endangered ants, such as red wood ants in Northern Europe and dinosaurs ants in Australia (Lach et al., 2010). However, well-documented examples of declining ant species are few. This could be a consequence of a lack of research on the topic, due to the predominating view of ants as problematic invasive organisms, or the fact that ants are in fact less likely to experience declines because the group possesses shared life history traits that allow them to better cope with environmental changes.

Fire Ants

Ants in the genus *Solenopsis* are commonly referred to as "fire ants" and they are all native to the Americas. Several fire ants like *S. invicta*, *Solenopsis geminata*, and *Solenopsis richteri* have become invasive in different parts of the world at different times in history (Ascunce et al., 2011; Gotzek et al., 2015), while others have remained localized to their native range (e.g., *Solenopsis xyloni*). Therefore, this is an interesting cluster of closely related ants that offers a unique opportunity to investigate specific traits associated with invasiveness. The best characterized invasive fire ant is *S. invicta*, commonly known as the "red imported fire ant". *S. invicta* first became invasive in the USA, where it is currently outcompeting the earlier invaders *S. geminata* and *S. richteri*, and subsequently invaded in many other parts of the world. In the USA, *S. invicta* displays better acclimatization than *S. geminata* to extremes in temperature, relative humidity and light levels (Wuellner and Saunders, 2003), and it also shows more tolerance than *S. richteri* to heat and desiccation (Chen et al., 2014). A series of studies also revealed that *S. invicta* better responds to habitat disturbance than *S. geminata* (Plowes et al., 2007; LeBrun et al., 2012; Axen et al., 2014) and displays higher potential of dispersal than *S. geminata*, being able to engage in mating flights earlier in the season (Tschinkel, 2006) and to produce larger colonies on average (Trible et al., 2018). Finally, *S. invicta* outcompetes *S. geminata* at food sites (Morrison, 2000), experiences lower mortality during interspecific aggressive interactions (Lai et al., 2015), and quickly responds to the loss of the functional queen with dealation among female sexuals and onset of egg-laying (Vargo and Porter, 1993), a mechanism of developmental plasticity that *S. geminata* lacks.

Argentine Ants

Linepithema humile, commonly known as the "Argentine ant," shares with *S. invicta* the ability to acclimatize to a wide range of conditions of temperature, humidity, and disturbance (Di Girolamo and Fox, 2006; Suarez et al., 2008). In particular, *L. humile* is extremely flexible and opportunistic when choosing a nesting site and can easily relocate (Heller and Gordon, 2006): this trait is a consequence of its nomadic nature that evolved in the native range as a response to repeated flooding events

(Suarez et al., 2008). In common with *S. invicta*, Argentine ants have also a great potential to disperse (Aron, 2001; Abril et al., 2013; Abril and Gómez, 2014) and the ability to outcompete other ants at food sites (Rowles and O'Dowd, 2007). Most interestingly, during interspecific interactions *L. humile* adopt a typically plastic behavior called "the bourgeois strategy," whereby the extent of the aggression displayed is proportional to the size of the propagule (Carpintero and Reyes-López, 2008; Sagata and Lester, 2009). A unique plastic trait that has been observed in Argentine ants so far is the shift in diet that followed invasion. Shik and Silverman (2013) documented a shift from protein-rich to carbohydrate-rich diet mediated by the mutualistic interactions with honeydew-producing aphids, while Hu et al. (2017) reported a shift from nitrogen-rich to nitrogen-poor (sugar-rich) diet. Hence, in this scenario, plasticity emerged by the combination of an omnivorous diet with the ability to modify sugar/protein ratios according to new colony needs.

Declining Ants

Research on endangered ants, despite being limited, has identified few common traits across species that appear to be linked to reduced plasticity. Habitat disturbance has serious negative consequence on red wood ants in the genus *Formica*. Deforestation has the most drastic impact, not only because it reduces wood availability that these ants rely on for nesting and feeding (Stockan et al., 2010), but also because wood ants are extremely sensitive to conditions of light, shading, vegetation, tree cover and increased human pressure, that usually increases with deforestation (Sorvari and Hakkarainen, 2007; Dekoninck et al., 2010). Limited dispersal of reproductive individuals is another factor potentially associated with decline among endangered ants. The dinosaur ant *Nothomyrmecia macrops* relies exclusively on solitary founding to start a new colony (Sanetra and Crozier, 2002), and both dinosaur and wood ants produce female sexuals that display restricted dispersal ranges (Gyllenstrand and Seppä, 2003; Sanetra and Crozier, 2003).

Wasps

Unlike ants, which are all eusocial, species of wasps in the family Vespidae span the full range of behaviors from solitary, to small colony "primitively eusocial" species, to species with moderate size populations, to highly social species with perennial nests containing up to 1 million of individuals (Jandt and Toth, 2015). One of the top 100 invasive species is a yellowjacket wasp (*Vespula vulgaris,* discussed below), and nearly all of the invasive wasps are temperate species with small to moderately large colony sizes and annual colony cycles (Lester et al., 2013). As with ants, there are nearly no data on species or population declines in social wasps; thus it is inconclusive whether this group is truly lacking in declining species or that the group as a whole is less susceptible to environmental change.

Paper Wasps

Polistes paper wasps are a successful and cosmopolitan genus of primitively eusocial wasps (Ross and Matthews, 1991). They are well-known as model organisms for the study of sociality, valued for their phenotypic plasticity in the context of their social biology (Jandt et al., 2014). Most members of the genus *Polistes* are characterized by high levels of physiological and behavioral plasticity in terms of social caste and dominance status. For example, the typical female *Polistes* larva has multiple layers of plasticity: (1) like many social insect species, any egg has the potential to develop into a queen or worker and caste bias is determined pre-imaginally via differential nutrition (Hunt, 2007), (2) more uniquely, there are no external morphological differences between queens and workers and all females are totipotent with respect to whether they act as workers or queens, depending on social context, and (3) adults engage in dominance contests which serve to structure a social hierarchy with the most dominant wasp being main egg-layer on the nest.

There are over 300 members of the genus found worldwide, yet one species, *Polistes dominula*, native to Europe, is notable in that it has successfully invaded many different geographic regions around the globe, including much of the United States and Canada in North America, Patagonia in South America (Sackmann et al., 2003), and South Africa (Roets et al., 2019). What can explain its success as an invader (compared to other species of *Polistes*), and are any of these traits related to phenotypic plasticity? To begin to explore this question, it is useful to compare traits within the genus *Polistes*, and to date the best information we have is on *P. dominula* in the context of its well-studied invasion in North America.

In the invasive range of *P. dominula* in the United States, multiple native species of *Polistes* are present across different parts of the range. In particular, in the Northern and Eastern United States, *Polistes fuscatus* and *Polistes metricus* are two native congeners that appear to have been displaced and/or outcompeted by *P. dominula*. Comparing the nesting and social biology of *P. dominula* with the two native species, the invasive species has a more diverse diet (Cervo et al., 2000), the option to reuse nests (Giovanetti et al., 1996), shorter brood development time leading to larger nests containing more individuals (Cervo et al., 2000), a large range of number of foundresses (especially a higher upper limit, whereas *P. fuscatus* and *P. metricus* are more often single-founded and/or with a smaller number of co-foundresses; Liebert et al., 2006), more variable nest sites and architectures (Gamboa et al., 2002), more variable nesting locations (e.g., ability to colonize new habitats Cervo et al., 2000), and a larger thermal tolerance range during flight (Weiner et al., 2011). In addition, *P. dominula* has also invaded in South Africa, likely outcompeting the native *Polistes marginalis*. There are likely to be some common features with the North American situation, e.g., higher colony productivity and broader seasonal activity patterns in *P. dominula* related to the South African invasion (Roets et al., 2019).

Part of the reason that this species may have such flexibility in its habits is that it is one of the most abundant and widespread Old World social wasps (Cervo et al., 2000; Höcherl and Tautz, 2015); thus the species possesses characteristics allowing it to thrive in a wide variety of habitats in its native range. This adaptability may be the result of genetic diversity and/or phenotypic plasticity. Because genetic diversity in the invasive range in the USA is fairly high (Johnson and Starks, 2004), it is not yet obvious whether the aforementioned variable traits that may be related to the invasive potential of *P. dominula* are attributable to phenotypic plasticity vs. genetic diversity.

Yellowjacket Wasps

Vespula wasps, commonly known as yellowjackets, are native to the Northern hemisphere and are characterized by (typically) annual colonies founded by single queens, morphological differences between queens and workers, and moderate to large colony sizes (Ross and Matthews, 1991). These wasps have an age-based division of labor among workers and possess complex communication systems related to foraging and recruitment to food sources (Overmyer and Jeanne, 1998; Hurd et al., 2007). They have a highly generalized diet consisting of insect and small vertebrate prey of various types, carrion, nectar from flowers, and rotting/fermenting fruit (Archer, 2012). The genus *Vespula* includes 23 species, with three that are well-known invasive species that have successfully established on several continents (Lester and Beggs, 2019). The German yellowjacket (V*espula germanica*) is native to much of the Northern Old World (Europe, Asia, and Africa) and invasive in North and South America, Australia, and New Zealand. The common wasp (*Vespula vulgaris*) is native and widespread in Eurasia, and invasive in Hawaii, Oceania, and South America. The western yellowjacket (*Vespula pensylvanica*) has a Nearctic native range, and has become invasive in Hawaii. Because multiple *Vespula* species have become important invaders, below, we provide an overview and discuss traits and examples from all three species.

Several studies have investigated possible factors and traits contributing to the great success of *Vespula* sp. as worldwide invaders, indicating that plasticity in key life history traits might have fueled their invasiveness. A common trait of invasive *Vespula* species is that they have rapidly (suggesting plasticity) shifted from an annual colony cycle to the formation of perennial colonies that last for multiple years, likely due to their ability to persist in the milder climates that characterize some areas of their invaded ranges. This has been reported for different invasive *Vespula* species: *V. vulgaris* in Tasmania (Spradbery, 1973), *V. germanica* in New Zealand (Thomas, 1960), and *V. pensylvanica* in Hawaii (Wilson et al., 2009). In all these areas, invasive wasps have become able to produce massive colonies that are significantly larger than what is observed in the native range, denoting a great amount of plasticity in the social structure of the colony.

Interestingly, invasive *V. pensylvanica* in Hawaii demonstrate the concomitant occurrence of several plastic life history traits that may have mediated its successful invasion: a shift from annual to perennial colony structure, wide diet breadth, and wide variation in individual longevity (Wilson et al., 2009). However, another study reported that *V. pensylvanica* displays a certain level of plasticity in queen number and duration of colony cycle also in its native range in California (Visscher and Vetter, 2003): therefore it remains to be tested whether plasticity in this case was a pre-existing condition that facilitated invasion or rapidly evolved in invasive populations of these wasps.

Another major factor potentially contributing to the invasion success of *Vespula* wasps is their behavioral and cognitive plasticity, especially in terms of foraging behavior (Lester and Beggs, 2019). *Vespula* wasps are also known for their ability to navigate new routes and recruit nest mates to new food sources, likely enhanced by their excellent learning and memory abilities (D'adamo and Lozada, 2003). These wasps are highly generalist foragers and their flexible diet has enabled them to take advantage of a huge variety of food sources in the invasive range, often dominating, and restructuring local ecosystems (Beggs and Rees, 1999). A striking example has been the ability of *V. vulgaris* to aggressively monopolize honeydew resources of the *Nothofagus* forest in New Zealand (Harris et al., 1991; Beggs, 2001). The honeydew provides opportunities for massive carbohydrate intake, which has been linked to the fast growth and competitive advantage of *V. vulgaris* colonies, which strikingly resembles what has been observed in invasive Argentine ants (see above).

Finally, two additional (related) traits that show plasticity and might have mediated wasp invasions are spring emergence time and thermal tolerance. For example, spring emergence occurs earlier in *V. vulgaris* than in *V. germanica*, with the former dominating at the extreme southern and northern ranges of their distribution. Plasticity in time of emergence and nesting strategy may provide an advantage for *V. vulgaris* in the context of nest site competition with sympatric congeneric species in colder regions. *V. vulgaris* also display a larger range of temperature and habitat tolerance, and are found at significantly higher latitudes than congener *V. germanica* (Lester and Beggs, 2019). Milder temperatures have been associated with *Vespula* wasps extending their distribution northward in Finland (Sorvari, 2013).

Bees

Unlike ants and wasps which are often viewed as pest species, social bees are generally considered beneficial insects due to their pollination services and, in the case of honey bees, honey production. They have been the major subjects of a large body of recent research into population declines and losses of managed colonies. By some estimates, at least half of species in certain bee groups (e.g., bumble bees) are in decline (e.g., Wood et al., 2019), and rates of honey bee colony losses have been historically high in many areas of the world (Ratnieks and Carreck, 2010). At the same time, there are some notable examples of successful exotic and invasive social bees. Thus, bees span the gamut from invasive to declining, sometimes with examples of both within the same genus or even species. This provides a unique opportunity to examine the traits of species associated with winning or losing in the face of global change.

Bumble Bees

Species in the bumble bee genus *Bombus* are considered a key group for both wild plant and crop pollination. These bees have an annual colony cycle, with morphologically differentiated queens and workers, and moderate colony sizes (Goulson, 2003). Like all bees, they have a diet comprised entirely of pollen and nectar, but many species of bumble bees are fairly generalist in foraging on a wide variety of different flower species. The genus comprises about 260 species of primitively eusocial insects (Cameron et al., 2007) spanning from invasive (*B. hortorum, B. hypnorum, B. impatiens, B.*

lucorum, B. ruderatus, B. subterraneus, and B. terrestris) to "Critically Endangered" species (*B. affinis, B. franklini, B. rubriventris, B. suckleyi, and B. variabilis*) based on global IUCN categorization (IUCN Red List of Threatened Species, 2016), and including possible extinctions (e.g., *B. cullumanus*) (Williams et al., 2013).

Bombus terrestris is an important managed pollinator that has been introduced for crop pollination in many parts of the world; despite introductions of other *Bombus* species, *B. terrestris* has been the most successful worldwide invader. This European species has become invasive in New Zealand (Macfarlane and Gurr, 1995), Japan (Matsumura et al., 2004), Tasmania (Hingston, 2006), Chile (Ruz and Herrera, 2001), and Argentina (Torretta et al., 2006). This wide range shows the ability of this species to survive and reproduce in a wide range of climates, geographies, and living among different congeners (both native and introduced). Because the speed and spread of the invasions is among the fastest recorded worldwide (e.g., Morales et al., 2013), and genetic diversity of some introduced populations is known (Schmid-Hempel et al., 2007), it might be possible to test whether the invasion success of this species is due to phenotypic plasticity.

Several traits have been suggested to be related to invasions in *B. terrestris* and represent interesting candidates to consider in the context of phenotypic plasticity. One trait that may provide an advantage to *B. terrestris* over native sympatric congeners is that its queens emerge earlier in the spring (Dafni et al., 2010), suggesting possible thermal adaptations. Early emergence (and long cycles of activity in general) have also been documented in introduced *B. terrestris* populations in Japan and Argentina (Inoue et al., 2008). In the Patagonia region of South America, *B. terrestris* also show an extended period of activity (early in the spring and late in the fall) compared to two bumble bee species that it has apparently displaced in this region, the introduced *Bombus ruderatus* and the native *Bombus dahlbomii* (Arbetman, 2017). Earlier emergence than other species may provide *B. terrestris* with the opportunity to take sole advantage of the earliest flowering resources as well as occupy optimal nesting sites before other species. Although to date there is no definitive evidence that spring emergence time is a plastic trait, this possibility deserves to be directly tested, given the possibility that this trait appears to provide an advantage over native species.

Diet breadth is another plastic trait that might be linked to successful invasion in bumble bees. In terms of its foraging behavior, *B. terrestris* is able to utilize a wide variety of native and non-native plants (Ruz and Herrera, 2001), readily foraging on multiple different crops, and is also known to "nectar rob" at high rates to access flowers with long corollas (Ishii et al., 2008). In New Zealand, the less successful introduced species *B. ruderatus* uses a narrower range of flowers and is more restricted to areas with warmer, drier climates as compared to *B. terrestris* (Macfarlane and Gurr, 1995). *B. hortorum*, another New Zealand introduced species, has not adapted well to areas with regular early summer droughts and a limited availability of flowers (Macfarlane and Gurr, 1995 and references therein). The high worker number in *B. terrestris* colonies, compared to other native and introduced species, may also allow this species to effectively outcompete other congenerics in terms of exploiting available foraging resources (Ings et al., 2006).

Numerous bumble bees have been well-documented to be in decline, by some estimates up to 50% of native *Bombus* species in some regions of the world (Wood et al., 2019). It seams that between 1/3 to 1/2 of Bombus species are declining (Arbetman et al., 2017; Wood et al., 2019). Several hypotheses have been put forward to understand the factors and life history traits associated with decline in certain bumble bee species, some of which may relate to phenotypic plasticity. For example, in the US, declining species show a narrower range of plants used (Wood et al., 2019) suggesting a less flexible diet may make some bumble bee species more prone to decline in the face of habitat loss. A European study suggested that declining species were unable to shift floral plant species usage through decades of land use change, whereas stable species were (Kleijn et al., 2008). Some bumble bee species have been documented to be narrowing their geographical areas of occupancy. This may be related to climate change and could happen as a result of higher thermal sensitivity or lower thermal tolerance. These patterns have been observed in those bumble bee species that are cold-adapted and originated in temperate regions of the globe (Kerr et al., 2015). Declining species may also have narrower seasonal activity compared to stable or invading species, again suggesting there may be a connection to thermal tolerance and/or thermal activity ranges (Arbetman, 2017). Another important factor in declines of native *Bombus* species has likely been pathogen and parasite spillover from commercially produced and introduced bees (Meeus et al., 2011; Arbetman et al., 2013). It is not known whether this relates simply to naïveté to specific pathogens, or to limited immune system investment in declining species. There also appears to be a phylogenetic component to patterns of *Bombus* decline, with some subgenera of *Bombus* more prone to decline, especially species with small geographic ranges (Arbetman et al., 2017). This suggests that narrow habitat requirements could make some bumble bee species more susceptible to decline.

Honey Bees

The honey bee *Apis mellifera* is perhaps the best studied single species of social insects; as a semi-domesticated pollinator, honey producer, and fascinating behavioral model system. *A. mellifera* are highly social bees with a perennial life cycle, strongly differentiated reproductive castes, a highly-structured worker division of labor based on age polyethism. *A. mellifera* has a vast native geographical distribution across large parts of Africa, Europe, and the Middle East. Interestingly, because of its value for pollination and honey production, this species has been introduced (and is managed by humans) in nearly every country, all over the world. While in the native range there are multiple subspecies that vary in behavior, colony structure, and morphology, the introduced varieties of *A. mellifera* have been mostly restricted to fewer subspecies or mixed race bees with desirable traits such as gentleness and high honey production. While many honey bee colonies are managed by humans, in many areas of its invasive and native range, wild honey bee colonies can be found.

As a semi-domesticated, managed species that has been introduced repeatedly in many, many places across the globe, perhaps the most surprising observation about *A. mellifera* is the fact that it has not become highly invasive in many parts of its introduced range. On the contrary, in many regions, including parts of its native range (in Europe) and some areas of its introduced range (North America), *A. mellifera* colonies have sustained high mortality and beekeepers are struggling to keep bees alive (Neumann and Carreck, 2010), in the face of multiple, interacting environmental stressors such as disease, poor nutrition, and pesticides (Smith et al., 2013). Most of these are mixes of European subspecies, especially *A. mellifera ligustica* and *A. mellifera*. An exception to this pattern has occurred with the so-called "killer" or Africanized bees (AHB), which are genetic mixes between *A. mellifera scutellata*, a subspecies native to Africa, and European races of *A. mellifera*. These bees appear to be more resistant to some of the stressors that are challenging European honey bees (EHB); in fact, the population status of the AHB has not been documented to decline, instead, these Africanized honey bees have undergone a rapid expansion out of Brazil (where they were originally accidentally released) and become well-established across large parts of South, Central, and North America, where they appear to be continuing to spread (Lin et al., 2018).

Thus, in *A. mellifera* we have different genetic backgrounds of bees, one in decline and one that is more invasive, sometimes living side by side in the same environment. This provides a unique opportunity to dissect the traits that may differentiate winners and losers in the face of an environmental change. Differences in traits between AHB and EHB that have been cited include: less honey storage in AHB vs. EHB (Winston et al., 1983), higher propensity to abscond and initiate a new nest in AHB vs. EHB (Winston et al., 1979), and higher resistance to *Varroa* mites in AHB vs.

	INVASIVE ANTS		**INVASIVE WASPS**		**INVASIVE BEES**		**DECLINING**	
	S. invicta	***L. humile***	***P. dominula***	***Vespula sp.***	***B. terrestris***	***A. mellifera***	**ants**	**bees**
Acclimatization	[10] [53]	[13] [42]	[32] [48]	[27] [40]	[3] [11]			[3] [23]
Disturbance	[5] [26] [31]						[12] [39]	
Dispersal	[44] [45]	[1] [2] [4]	[28]		[11]	[50]	[17] [36] [37]	
Behaviour	[30]	[8] [34] [35]		[27]				
Diet		[20] [38]	[9]	[6] [7] [18] [49]	[11] [22]	[51]		[24] [52]
Nesting		[19]	[9] [15] [16]		[11] [21]		[41]	
Morphology			[9] [32]	[43] [47] [49]	[11]			
Physiology	[46]		[29]			[33]		
Mortality	[25]			[49]				
Genetics		[14]						

[1] Abril and Gómez, 2014; [2] Abril et al., 2013; [3] Arbetman, 2017; [4] Aron, 2001; [5] Axen et al., 2014; [6] Beggs 2001; [7] Beggs and Rees 1999; [8] Carpintero and Reyes-López, 2008; [9] Cervo et al., 2000; [10] Chen et al., 2014; [11] Dafni et al., 2010; [12] Dekoninck et al., 2010; [13] Di Girolamo and Fox, 2006; [14] Felden et al., 2019; [15] Gamboa et al., 2002; [16] Giovanetti et al., 1996; [17] Gyllenstrand and Seppä, 2003; [18] Harris 1991; [19] Heller and Gordon, 2006; [20] Hu et al. 2017; [21] Inoue et al., 2008; [22] Ishii et al., 2008; [23] Kerr et al., 2015; [24] Kleijn et al., 2008; [25] Lai et al., 2015; [26] LeBrun et al., 2012; [27] Lester and Beggs, 2019; [28] Liebert et al. 2006; [29] Manfredini et al., 2013; [30] Morrison, 2000; [31] Plowes et al., 2007; [32] Roets et al., 2019; [33] Rosenkranz, 1999; [34] Rowles and O'Dowd, 2007; [35] Sagata and Lester, 2009; [36] Sanetra and Crozier, 2002; [37] Sanetra and Crozier, 2003; [38] Shik et al. 2013; [39] Sorvari and Hakkarainen, 2007; [40] Sorvari, 2013; [41] Stockan et al., 2010; [42] Suarez et al., 2008; [43] Thomas 1960; [44] Trible et al., 2018; [45] Tschinkel 2006; [46] Vargo and Porter, 1993; [47] Visscher and Vetter, 2003; [48] Weiner et al., 2011; [49] Wilson et al. 2009; [50] Winston et al., 1979; [51] Winston et al., 1983; [52] Wood et al., 2019; [53] Wuellner and Saunders, 2003

FIGURE 2 | Summary of research studies (to date) that have investigated traits that show the potential for plasticity (or lack thereof) in association with invasions or declines of various taxa of social insects. The traits reported in the figure are the same as discussed in the main text (with the relevant references). Individual traits have been classified and clumped together according to broadly defined macro-categories in line with what reported for other organisms (sections Phenotypic plasticity in various traits as facilitators of biological invasions and Limited phenotypic plasticity in the context of species declines). Some macro-categories include traits that are highly relevant/specific for social insects: "Dispersal" includes range of mating flights, colony founding strategy, time of queens emergence from diapause and time of release of sexuals; "Behavior" includes intraspecific competition for food resources; "Morphology" includes colony size; "Mortality" refers to individual mortality of single insects. Pictures shown above are (from left to right): the fire ant *Solenopsis invicta*, the paper wasp *Polistes dominula*, the Africanized honey bee *Apis mellifera*, and the bumble bee *Bombus dahlbomii* (Photo credits: Wikimedia Commons, except bumble bee by Amy Toth).

EHB due to more efficient hygienic behavior (Rosenkranz, 1999). It is possible that some of these traits may relate to phenotypic plasticity, but studies have not directly addressed how plastic or fixed these traits are in AHB compared to EHB.

On the contrary, in a different environment, e.g., China, *A, mellifera ligustica* has become an invasive species, outcompeting, and displacing native *Apis cerana* honey bees (Ji et al., 2003). Also, in the Bonin Islands off of Japan, *A. mellifera* (subspecies not specified), has also become invasive with likely negative impacts on biodiversity (Kato et al., 1999). Stable feral populations of *A. mellifera* have also established in Australia (Oldroyd et al., 1997), and managed colonies have been more resilient than in other parts of the world, which may be partially due to lack of *Varroa* mites (Neumann and Carreck, 2010). These differences in the status of *A. mellifera ligustica* across different regions of the world demonstrate the important role of the environmental conditions in determining whether a species becomes invasive. This could relate to specific forms of phenotypic plasticity being advantageous in certain environments.

DISCUSSION

Synthesis and Implications for Population Management

This review synthesizes recurrent traits that have been linked to successful invasions and declines in social insects across multiple species of ants, bees, and wasps (summarized in **Figure 2**). Some of these traits exhibit evidence of high levels of plasticity in invasive social insects. These include flexible dispersal and colony founding strategies, broad diet breadth, and rapid colony growth in response to high resource availability, and flexible preferences for nesting sites. Other traits linked to invasiveness in social insects, but that have not been directly investigated in relation to plasticity include thermal tolerance, reproductive potential, and genomic plasticity and/or structure [but see Felden et al. (2019)]. However, these traits have been shown to be plastic in other invasive organisms (reviewed above) and therefore have the potential to also be important in invasive social insects. On the flip side, related to declines of social insects, we uncovered evidence for a small group of recurring traits in declining ants and bumble bees for which a lack of plasticity might be linked to the decline of some species, including specialized diets and restricted habitat and nesting requirements (**Figure 2**). Below, we discuss some of the most relevant plasticity-related traits in the context of population management strategies; i.e., mitigation strategies to reduce invasive species and conserve declining species.

Dispersal Strategies and Foraging Opportunities

Our review strongly suggests a recurring pattern in which invasive social insects display a high dispersal range and mode, while declining social insects are often limited in the capacity to disperse and in their repertoire of modalities with which to do so. This observation suggests the following strategies may be effective:

- Actions to target **invasive social insects** in temperate climates should be done early in the spring season, as these organisms often emerge earlier than native sympatric competitors. Targeting queens that emerge from winter diapause or newly mated queens after mating flights would be more effective than targeting whole mature colonies later in the season. Although targeted species may respond by delaying dispersal/colony founding (due to plasticity), this strategy at least has the potential to reduce their success and place them on more of an "even playing field" with native species.
- **Endangered social insects** need great support at the dispersal stage, because colony founding is a critical moment in their life cycle with a high rate of failure and mortality. To support dispersing queens, actions should be taken to provide foraging and nest site options during the time period when new queens emerge/found a new colony (and not sooner, in the case of competition with earlier emerging invasive species). This might involve managing the landscape to introduce flowering/seed producing plants that bloom during critical periods of the life cycle of threatened species, providing nest boxes and/or habitats adequate for nesting, and providing appropriately placed nesting sites for species with limited ranges of dispersal.

Nesting Sites and Disturbance

This review strongly suggests that the ability to find and secure nesting sites may be an important "filter" separating invasive and declining social insects. Plasticity in nesting strategies becomes particularly relevant in association with anthropogenic habitat disturbance, which in general results in limiting nesting possibilities for declining species while opening new possibilities for more flexible invasive species.

- **Invasive social insects** often utilize human-altered or disturbed environments heavily for nesting substrates (e.g., *P. dominula* paper wasps nesting on buildings). We suggest sudden, large scale disruption of natural habitats are likely to favor invasive species. We suggest that smaller scale and more patchy patterns of change, leaving some native habitat refuges in place within the matrix of a rapidly developing area, may both reduce the spread of invaders and buffer against damages created to the native community in the area. If large scale disturbance is unavoidable, it becomes important to compensate with measures that create required habitat niches (e.g., nest boxes) for native species most likely to be affected by loss of nesting habitat.
- In addition, more research is needed to identify the key nesting needs (microhabitat, substrate, size) of **endangered social insects**, if possible, pinpointing what makes them distinct from invasive competitors. For example, a successful strategy in insect conservation has been accomplished with the creation of the "bee hotels" for solitary bees and wasps.

In reality, many/most modern ecosystems are already composed of invasive and endangered species coexisting at the same time. At the same time, new invaders are moving into more and more regions across the globe, and are often not reported or noticed until substantial populations have established. In general, we suggest that preserving and boosting populations of declining species should be a general priority, as a solid and diversified native community can help prevent the establishment/spread of invasive species.

Knowledge Gaps

There is no doubt that exploring the role of phenotypic plasticity in invasions and declines of social insects is a fascinating area of research. It is also evident that we have just started to uncover the wide range of forms that this association can take, and more work needs to be done in the future. First of all, a main priority is to understand where plasticity (or the lack thereof) originates. For example, for successful invaders, is plasticity in key traits that fostered invasiveness a pre-existing condition that was widespread among native populations? Or did plastic traits evolved after invasion only in those populations that became invasive? This was one of three fundamental issues on the nature of phenotypic plasticity identified by Kennedy et al. (2017). Addressing this question will require comparing in a rigorous way (e.g., common garden experiments) the extent of plasticity (i.e., amplitude of the range of responses) for the trait of interest in populations of conspecifics from the native and invasive ranges. We foresee that, though this approach might be relatively easy to perform for temperate species (e.g., many wasps and bees), the logistic will be sometimes challenging for tropical or subtropical species like many invasive ants, whose native range is in most cases thick forest or remote and frequently flooded areas in South America and South East Asia. Complementary to this approach, the study of failed invasions (**Box 2**) will be fundamental to test whether failure to invade is linked to lack of plasticity—which can be consequently used to refute the hypothesis of plasticity as a species-level pre-condition for invasiveness.

On the other hand, if it is true that some social insects are lacking of adequate levels of phenotypic plasticity and therefore fail to cope with environmental changes, the obvious question is how did these species survive until now? Probably lower plasticity might be advantageous in more stable environments, due to the fact that maintaining plasticity when it is not needed might be costly (reviewed in Auld et al., 2009). We need to address this compelling question in social insects, and keep in mind that there are two ways of quantifying costs in these organisms: at the individual level (as a proxy for energy expenditure), and at the colony level (which instead reflects real fitness costs, as the colony is the real reproductive unit in social insects).

Finally, we suggest two additional aspects should be prioritized in the investigation of phenotypic plasticity in invasive and endangered social insects: social structure and genetics. These traits are particularly relevant for social insects and are tightly interconnected. We have reported here that social structure can change after invasion: for example, transitions to unicoloniality and polygyny in ants, or from annual to perennial colonies in wasps. All these phenomena suggest pre-exisiting plasticity in social structures of these species, but in most cases we lack evidence of such flexibility in native populations of these invaders. Understanding how major transitions in social structure occur relates to fundamental questions of sociobiology, for they must accommodate substantial changes in the "rules" of the nest, such as multiple queens coexisting together instead of one, or the elimination of colony boundaries and the loss of aggressive behavior toward neighboring conspecifics. How is plasticity at this complex behavioral level achieved? Is it encoded at the genetic level and, if so, does it relate to the unique haplodiploid reproductive system that is such

BOX 2 | What do we know about failed invasions?

The separation between invasive and non-invasive species is far from being clear-cut and this dichotomy is often used to simplify nomenclature. There are species that might be invasive in some parts of the world while remaining stable somewhere else (e.g., *B. terrestris*), and other species that might be at a pre-invasion stage (or lag-phase, Chapple et al., 2012) and therefore are not yet referred to as invasive. Particularly intriguing are those cases of "failed invasions," when species that are usually described as successful invaders fail to establish in an area where they have been introduced. Failed invasions represent a unique opportunity to understand the underpinnings of biological invasions as they often highlight the key components (e.g., phenotypic plasticity) that are missing for a successful invasion.

Two types of failure have been identified (Zenni and Nuñez, 2013): (a) failure to naturalize, whereby an alien species is unable to survive, reproduce or grow to a minimum size in the new range; and (b) failure to invade after naturalization, which correspond to inability to spread. Unsurprisingly option (a) is more difficult to observe, in particular with reference to unintentional introductions. As a matter of fact, taxonomic, and geographic biases have been reported to explain our limited knowledge on failed invasions: for example, reports on plants and insects are underrepresented compared to vertebrates, and Asia and Africa have significantly fewer records compared to the Western world. In terms of mechanisms that could be at the basis of failed invasions, five categories have been identified: (1) abiotic resistance, including both macroclimatic, and local-scale factors; (2) biotic resistance, such as competition with or predation by resident species; (3) genetic constraints, that might selectively affect only a subset of populations within a species; (4) propagule pressure, linked to the size of the initial settlements; and (5) mutualist release, which acts exactly opposite to the "enemy release" described in **Box 1**.

A few examples of failed invasions have been reported among social insects. Notably, four ant species (three of which are worldwide successful invaders) failed to invade New Zealand: the yellow crazy ant, the red fire ant, the Papuan thief ant and the electric ant. Analogously, seven species of bumble bees were introduced in New Zealand, but only *B. terrestris* managed to establish and spread successfully all over the region. The lack of invasion is striking, considering that New Zealand has been highly permeable to the invasion of other social insects such as yellowjacket wasps. Interestingly, a subspecies of *B. terrestris*, the Sardinian bumble bee (*B. terrestris sassaricus*), failed to establish in southern France, despite the fact that it had been introduced intentionally for crop pollination (Ings et al., 2010). Studies of failed invasions, especially using contrasts between closely related species or the same species in different regions, have great potential for helping us to better understand the environmental and phenotypic drivers of invasiveness.

a key trait of all social insects? An important step in this direction has been achieved with the discovery of the Gp-9 region in fire ants, a large portion of a chromosome tightly linked to social structure (Wang et al., 2013). One of the two variants of this region is associated with polygyny, and polygyny is the social form that appears to facilitate invasiveness. We suggest, moving forward, that the study of phenotypic plasticity and its genetic basis in the context of social insect invasions can help understanding major evolutionary transitions in social organization.

AUTHOR CONTRIBUTIONS

AT, FM, and MA developed the idea for the review and wrote the paper.

FUNDING

This work was supported by a Fulbright Science and Technology Scholarship to AT and National Geographic Species Recovery Grant Number NGS-57001R-19 to MA and AT.

REFERENCES

Abril, S., Diaz, M., Enríquez, M. L., and Gómez, C. (2013). More and bigger queens: a clue to the invasive success of the Argentine ant (Hymenoptera: Formicidae) in natural habitats. *Myrmecol. News* 18, 19–24.

Abril, S., and Gómez, C. (2014). Strength in numbers: large and permanent colonies have higher queen oviposition rates in the invasive Argentine ant (*Linepithema humile*, Mayr). *J. Insect. Physiol.* 62, 21–25. doi: 10.1016/j.jinsphys.2014.01.004

Arbetman, M. P. (2017). *La declinación de Abejorros (bombus) y sus Potenciales Causas, a Distintas Escalas Espaciales* (Ph.D. thesis). Universidad Nacional del Comahue, Argentina. Available online at: http://rdi.uncoma.edu.ar:8080/handle/123456789/183

Arbetman, M. P., Gleiser, G., Morales, C. L., Williams, P., and Aizen, M. A. (2017). Global decline of bumblebees is phylogenetically structured and inversely related to species range size and pathogen incidence. *Proc. R. Soc. B Biol. Sci.* 284:20170204. doi: 10.1098/rspb.2017.0204

Arbetman, M. P., Meeus, I., Morales, C. L., Aizen, M. A., and Smagghe, G. (2013). Alien parasite hitchhikes to Patagonia on invasive bumblebee. *Biol. Invasions* 15, 489–494. doi: 10.1007/s10530-012-0311-0

Archer, M. E. (2012). *Vespine Wasps of the World*: *Behaviour, Ecology & Taxonomy of the Vespinae.* Siri Scientific Press.

Aron, S. (2001). Reproductive strategy: an essential component in the success of incipient colonies of the invasive Argentine ant. *Insectes Soc.* 48, 25–27. doi: 10.1007/PL00001740

Ascunce, M. S., Yang, C.-C., Oakey, J., Calcaterra, L., Wu, W.-J., Shih, C.-J., et al. (2011). Global invasion history of the fire ant Solenopsis invicta. *Science* 331, 1066–1068. doi: 10.1126/science.1198734

Atwell, J. W., Cardoso, G. C., Whittaker, D. J., Price, T. D., and Ketterson, E. D. (2014). Hormonal, behavioral, and life-history traits exhibit correlated shifts in relation to population establishment in a novel environment. *Am. Nat.* 184, E147–E160. doi: 10.1086/678398

Auld, J. R., Agrawal, A. A., and Relyea, R. A. (2009). Re-evaluating the costs and limits of adaptive phenotypic plasticity. *Proc. R. Soc. B Biol. Sci.* 277, 503–511. doi: 10.1098/rspb.2009.1355

Axen, H. J., Wildermuth, A., and Helmscahan, S. (2014). Environmental filtering of foraging strategies mediates patterns of coexistence in the fire ants *Solenopsis geminata* and *Solenopsis xyloni*, and their interspecific hybrids. *Ecol. Entomol.* 39, 290–299. doi: 10.1111/een.12100

Baldwin, J. M. (1896). A new factor in evolution. *Am. Nat.* 30, 441–451. doi: 10.1086/276408

Baudier, K. M., and O'Donnell, S. (2017). Weak links: how colonies counter the social costs of individual variation in thermal physiology. *Curr. Opin. Insect. Sci.* 22, 85–91. doi: 10.1016/j.cois.2017.06.004

Beaman, J. E., White, C. R., and Seebacher, F. (2016). Evolution of plasticity: mechanistic link between development and reversible acclimation. *Trends Ecol. Evol.* 31, 237–249. doi: 10.1016/j.tree.2016.01.004

Beggs, J. (2001). The ecological consequences of social wasps (Vespula spp.) invading an ecosystem that has an abundant carbohydrate resource. *Biol. Conserv.* 99, 17–28. doi: 10.1016/S0006-3207(00)00185-3

Beggs, J. R., and Rees, J. S. (1999). Restructuring of Lepidoptera communities by introduced Vespula wasps in a New Zealand beech forest. *Oecologia* 119, 565–571. doi: 10.1007/s004420050820

Bengston, S. E., and Jandt, J. M. (2014). The development of collective personality: the ontogenetic drivers of behavioral variation across groups. *Front. Ecol. Evol.* 2:81. doi: 10.3389/fevo.2014.00081

Blossey, B., and Notzold, R. (1995). Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. *J. Ecol.* 83, 887–889. doi: 10.2307/2261425

Buczkowski, G., and Bertelsmeier, C. (2017). Invasive termites in a changing climate: a global perspective. *Ecol. Evol.* 7, 974–985. doi: 10.1002/ece3.2674

Cameron, S. A., Hines, H. M., and Williams, P. H. (2007). A comprehensive phylogeny of the bumble bees (*Bombus*). *Biol. J. Linn. Soc.* 91, 161–188. doi: 10.1111/j.1095-8312.2007.00784.x

Campbell, T. S., and Echternacht, A. C. (2003). Introduced species as moving targets: changes in body sizes of introduced lizards following experimental introductions and historical invasions. *Biol. Invasions* 5, 193–212. doi: 10.1023/A:1026172314139

Carpintero, S., and Reyes-López, J. (2008). The role of competitive dominance in the invasive ability of the Argentine ant (Linepithema humile). *Biol. Invasions* 10, 25–35. doi: 10.1007/s10530-007-9103-3

Cervo, R., Zacchi, F., and Turillazzi, S. (2000). Polistes dominulus (Hymenoptera, Vespidae) invading North America: some hypotheses for its rapid spread. *Insect. Soc.* 47, 155–157. doi: 10.1007/PL00001694

Chapple, D. G., Simmonds, S. M., and Wong, B. B. (2012). Can behavioral and personality traits influence the success of unintentional species introductions? *Trends Ecol. Evol.* 27, 57–64. doi: 10.1016/j.tree.2011.09.010

Chen, J., Rashid, T., and Feng, G. (2014). A comparative study between *Solenopsis invicta* and *Solenopsis richteri* on tolerance to heat and desiccation stresses. *PLoS ONE* 9:e96842. doi: 10.1371/journal.pone.0096842

Chevin, L.-M., Lande, R., and Mace, G. M. (2010). Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS Biol.* 8:e1000357. doi: 10.1371/journal.pbio.1000357

Chevin, L. M., and Lande, R. (2010). When do adaptive plasticity and genetic evolution prevent extinction of a density-regulated population? *Evol. Int. J. Org. Evol.* 64, 1143–1150. doi: 10.1111/j.1558-5646.2009.00875.x

Chown, S. L., Slabber, S., McGeoch, M., Janion, C., and Leinaas, H. P. (2007). Phenotypic plasticity mediates climate change responses among invasive and indigenous arthropods. *Proc. R. Soc. B Biol. Sci.* 274, 2531–2537. doi: 10.1098/rspb.2007.0772

Colautti, R. I., Alexander, J. M., Dlugosch, K. M., Keller, S. R., and Sultan, S. E. (2017). Invasions and extinctions through the looking glass of evolutionary ecology. *Philos. Trans. R. Soc. B Biol. Sci.* 372:20160031. doi: 10.1098/rstb.2016.0031

Cooke, S. L. (2016). Anticipating the spread and ecological effects of invasive bigheaded carps (Hypophthalmichthys spp.) in North America: a review of modeling and other predictive studies. *Biol. Invasions* 18, 315–344. doi: 10.1007/s10530-015-1028-7

Cremer, S., Armitage, S. A. O., and Schmid-Hempel, P. (2007). Social immunity. *Curr. Biol.* 17, R693–R702. doi: 10.1016/j.cub.2007.06.008

D'adamo, P., and Lozada, M. (2003). The importance of location and visual cues during foraging in the German wasp (Vespula germanica F.)(Hymenoptera: Vespidae). *N. Zeal. J. Zool.* 30, 171–174. doi: 10.1080/03014223.2003.9518336

Dafni, A., Kevan, P., Gross, C. L., and Goka, K. (2010). Bombus terrestris, pollinator, invasive and pest: an assessment of problems associated with its widespread introductions for commercial purposes. *Appl. Entomol. Zool.* 45, 101–113. doi: 10.1303/aez.2010.101

Davidson, A. M., Jennions, M., and Nicotra, A. B. (2011). Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecol. Lett.* 14, 419–431. doi: 10.1111/j.1461-0248.2011.01596.x

Dekoninck, W., Hendrickx, F., Grootaert, P., and Maelfait, J.-P. (2010). Present conservation status of red wood ants in north-western Belgium: worse than previously, but not a lost cause. *Eur. J. Entomol.* 107, 209–218. doi: 10.14411/eje.2010.028

Di Girolamo, L. A., and Fox, L. R. (2006). The influence of abiotic factors and temporal variation on local invasion patterns of the Argentine ant (Linepithema humile). *Biol. Invasions* 8, 125–135. doi: 10.1007/s10530-004-1572-z

Didham, R. K., Tylianakis, J. M., Gemmell, N. J., Rand, T. A., and Ewers, R. M. (2007). Interactive effects of habitat modification and species invasion on native species decline. *Trends Ecol. Evol.* 22, 489–496. doi: 10.1016/j.tree.2007.07.001

Dos Santos, C. F., Nunes-Silva, P., and Blochtein, B. (2015). Temperature rise and its influence on the cessation of diapause in *Plebeia droryana*, a eusocial bee (Hymenoptera: Apidae). *Ann. Entomol. Soc. Am.* 109, 29–34. doi: 10.1093/aesa/sav096

Engel, K., and Tollrian, R. (2009). Inducible defences as key adaptations for the successful invasion of Daphnia lumholtzi in North America? *Proc. R. Soc. Biol. Sci.* 276, 1865–1873. doi: 10.1098/rspb.2008.1861

Evans, T. A. (2010). "Invasive termites," in *Biology of Termites: A Modern Synthesis*, eds D. Bignell, Y. Roisin, N. Lo (Dordrecht: Springer), 519–562. doi: 10.1007/978-90-481-3977-4_19

Evans, T. A., Forschler, B. T., and Grace, J. K. (2013). Biology of invasive termites: a worldwide review. *Annu. Rev. Entomol.* 58, 455–474. doi: 10.1146/annurev-ento-120811-153554

Felden, A., Paris, C., Chapple, D. G., Suarez, A. V., Tsutsui, N. D., Lester, P. J., et al. (2019). Native and introduced Argentine ant populations are characterised by distinct transcriptomic signatures associated with behaviour and immunity. *NeoBiota* 49, 105–126. doi: 10.3897/neobiota.49.36086

Fisher, D. O., Blomberg, S. P., and Owens, I. P. (2003). Extrinsic versus intrinsic factors in the decline and extinction of Australian marsupials. *Proc. R. Soc. London. Ser. B Biol. Sci.* 270, 1801–1808. doi: 10.1098/rspb.2003.2447

Gamboa, G. J., Greig, E. I., and Thom, M. C. (2002). The comparative biology of two sympatric paper wasps, the native *Polistes fuscatus* and the invasive *Polistes dominulus* (Hymenoptera, Vespidae). *Insect. Soc.* 49, 45–49. doi: 10.1007/s00040-002-8278-y

Gervasi, S. S., and Foufopoulos, J. (2008). Costs of plasticity: responses to desiccation decrease post-metamorphic immune function in a pond-breeding amphibian. *Funct. Ecol.* 22, 100–108. doi: 10.1111/j.1365-2435.2007.01340.x

Gianoli, E., and Valladares, F. (2011). Studying phenotypic plasticity: the advantages of a broad approach. *Biol. J. Linn. Soc.* 105, 1–7. doi: 10.1111/j.1095-8312.2011.01793.x

Giovanetti, M., Cervo, R., and Turillazzi, S. (1996). "Comb reutilization in *Polistes dominulus* (Hymenoptera: Vespidae)," in *Proceedings of the VI Conference of the Italian Section of the International Union for the Study of Social Insects*, Vol. 1. eds F. Le Moli, A. Mori, and D. Grasso (Insect Soc. Life), 101–105.

Glastad, K. M., Hunt, B. G., and Goodisman, M. A. D. (2019). Epigenetics in insects: genome regulation and the generation of phenotypic diversity. *Annu. Rev. Entomol.* 64, 185–203. doi: 10.1146/annurev-ento-011118-111914

Glon, M. G., Reisinger, L. S., and Pintor, L. M. (2018). Biogeographic differences between native and non-native populations of crayfish alter species coexistence and trophic interactions in mesocosms. *Biol. Invasions* 20, 3475–3490. doi: 10.1007/s10530-018-1788-y

Gómez-Saldarriaga, C., Valenzuela, N., and Ceballos, C. P. (2016). Effects of incubation temperature on sex determination in the endangered magdalena river turtle, *Podocnemis lewyana*. *Chelonian Conserv. Biol.* 15, 43–53. doi: 10.2744/CCB-1170.1

Gordon, D. M. (1996). The organization of work in social insect colonies. *Nature* 380, 121–124. doi: 10.1038/380121a0

Gotzek, D., Axen, H. J., Suarez, A. V., Helms Cahan, S., and Shoemaker, D. (2015). Global invasion history of the tropical fire ant: a stowaway on the first global trade routes. *Mol. Ecol.* 24, 374–388. doi: 10.1111/mec.13040

Goulson, D. (2003). *Bumblebees: Their Behaviour and Ecology*. Oxford: Oxford University Press.

Grab, H., Brokaw, J., Anderson, E., Gedlinske, L., Gibbs, J., Wilson, J., et al. (2019). Habitat enhancements rescue bee body size from the negative effects of landscape simplification. *J. Appl. Ecol.* 56, 2144–2154. doi: 10.1111/1365-2664.13456

Gutekunst, J., Andriantsoa, R., Falckenhayn, C., Hanna, K., Stein, W., Rasamy, J., et al. (2018). Clonal genome evolution and rapid invasive spread of the marbled crayfish. *Nat. Ecol. Evol.* 2, 567–573. doi: 10.1038/s41559-018-0467-9

Gyllenstrand, N., and Seppä, P. (2003). Conservation genetics of the wood ant, *Formica lugubris*, in a fragmented landscape. *Mol. Ecol.* 12, 2931–2940. doi: 10.1046/j.1365-294X.2003.01975.x

Harris, R. J., Thomas, C. D., and Moller, H. (1991). The influence of habitat use and foraging on the replacement of one introduced wasp species by another in New Zealand. *Ecol. Entomol.* 16, 441–448. doi: 10.1111/j.1365-2311.1991.tb00237.x

Hawes, N. A., Fidler, A. E., Tremblay, L. A., Pochon, X., Dunphy, B. J., and Smith, K. F. (2018). Understanding the role of DNA methylation in successful biological invasions: a review. *Biol. Invasions* 20, 2285–2300. doi: 10.1007/s10530-018-1703-6

Heller, N. E., and Gordon, D. M. (2006). Seasonal spatial dynamics and causes of nest movement in colonies of the invasive Argentine ant (*Linepithema humile*). *Ecol. Entomol.* 31, 499–510. doi: 10.1111/j.1365-2311.2006.00806.x

Hingston, A. B. (2006). Is the exotic bumblebee *Bombus terrestris* really invading Tasmanian native vegetation? *J. Insect. Conserv.* 10, 289–293. doi: 10.1007/s10841-006-6711-7

Höcherl, N., and Tautz, J. (2015). Nesting behavior of the paper wasp Polistes dominula in Central Europe—a flexible system for expanding into new areas. *Ecosphere* 6, 1–11. doi: 10.1890/ES15-00254.1

Holway, D. A., Lach, L., Suarez, A. V., Tsutsui, N. D., and Case, T. J. (2002). The causes and consequences of ant invasions. *Annu. Rev. Ecol. Syst.* 33, 181–233. doi: 10.1146/annurev.ecolsys.33.010802.150444

Hu, Y., Holway, D. A., Łukasik, P., Chau, L., Kay, A. D., LeBrun, E. G., et al. (2017). By their own devices: invasive Argentine ants have shifted diet without clear aid from symbiotic microbes. *Mol. Ecol.* 26, 1608–1630. doi: 10.1111/mec.13991

Huang, X., Li, S., Ni, P., Gao, Y., Jiang, B., Zhou, Z., et al. (2017). Rapid response to changing environments during biological invasions: DNA methylation perspectives. *Mol. Ecol.* 26, 6621–6633. doi: 10.1111/mec.14382

Human, K. G., and Gordon, D. M. (1999). Behavioral interactions of the invasive Argentine ant with native ant species. *Insect. Soc.* 46, 159–163. doi: 10.1007/s000400050127

Hunt, J. H. (2007). *The Evolution of Social Wasps*. Oxford: Oxford University Press.

Hurd, C. R., Jeanne, R. L., and Nordheim, E., V (2007). Temporal polyethism and worker specialization in the wasp, *Vespula germanica*. *J. Insect Sci.* 7, 1–13. doi: 10.1673/031.007.4301

Ingram, K. K. (2002). Plasticity in queen number and social structure in the invasive Argentine ant (*Linepithema humile*). *Evolution* 56, 2008–2016. doi: 10.1111/j.0014-3820.2002.tb00127.x

Ings, T. C., Ings, N. L., Chittka, L., and Rasmont, P. (2010). A failed invasion? Commercially introduced pollinators in Southern France. *Apidologie* 41, 1–13. doi: 10.1051/apido/2009044

Ings, T. C., Ward, N. L., and Chittka, L. (2006). Can commercially imported bumble bees out-compete their native conspecifics? *J. Appl. Ecol.* 43, 940–948. doi: 10.1111/j.1365-2664.2006.01199.x

Inoue, M. N., Yokoyama, J., and Washitani, I. (2008). Displacement of Japanese native bumblebees by the recently introduced Bombus terrestris (L.)(Hymenoptera: Apidae). *J. Insect Conserv.* 12, 135–146. doi: 10.1007/s10841-007-9071-z,

IPBES, E. S., Brondizio, J., Settele, S., and Díaz, H. T. N. (editors). (2019). *Global Assessment Report on Biodiversity and Ecosystem Services of the Intergovernmental Science- Policy Platform on Biodiversity and Ecosystem Services*.

Ishii, H. S., Kadoya, T., Kikuchi, R., Suda, S.-I. I., and Washitani, I. (2008). Habitat and flower resource partitioning by an exotic and three native bumble bees in central Hokkaido, Japan. *Biol. Conserv.* 141, 2597–2607. doi: 10.1016/j.biocon.2008.07.029

IUCN Red List of Threatened Species (2016). IUCN. Available online at: www.iucnredlist.org (accessed June 6, 2016).

Jandt, J. M., Bengston, S., Pinter-Wollman, N., Pruitt, J. N., Raine, N. E., Dornhaus, A., et al. (2014). Behavioural syndromes and social insects: personality at multiple levels. *Biol. Rev.* 89, 48–67. doi: 10.1111/brv.12042

Jandt, J. M., and Toth, A. L. (2015). "Physiological and genomic mechanisms of social organization in wasps (Family: Vespidae)," in *Advances in Insect Physiology*, eds A. Zayed and C. Kent (Elsevier), 95–130. doi: 10.1016/bs.aiip.2015.01.003

Ji, R., Xie, B., Yang, G., and Li, D. (2003). From introduced species to invasive species–a case study on the Italian bee Apis mellifera L. *Chinese J. Ecol.* 22, 70–73.

Jiguet, F., Gadot, A., Julliard, R., Newson, S. E., and Couvet, D. (2007). Climate envelope, life history traits and the resilience of birds facing global change. *Glob. Chang. Biol.* 13, 1672–1684. doi: 10.1111/j.1365-2486.2007.01386.x

Johnson, R. N., and Starks, P. T. (2004). A surprising level of genetic diversity in an invasive wasp: polistes dominulus in the northeastern United States. *Ann. Entomol. Soc. Am.* 97, 732–737. doi: 10.1603/0013-8746(2004)097[0732:ASLOGD]2.0.CO;2

Kato, M., Shibata, A., Yasui, T., and Nagamasu, H. (1999). Impact of introduced honeybees, Apis mellifera, upon native bee communities in the Bonin (Ogasawara) Islands. *Popul. Ecol.* 41, 217–228. doi: 10.1007/s101440050025

Kennedy, P., Baron, G., Qiu, B., Freitak, D., Helanterä, H., Hunt, E. R., et al. (2017). Deconstructing superorganisms and societies to address big questions in biology. *Trends Ecol. Evol.* 32, 861–872. doi: 10.1016/j.tree.2017.08.004

Kerr, J. T., Pindar, A., Galpern, P., Packer, L., Potts, S. G., Roberts, S. M., et al. (2015). Climate change impacts on bumblebees across continents. *Science* 349, 177–180. doi: 10.1126/science.aaa7031

Kleijn, D., Kleijn, D., Raemakers, I., and Raemakers, I. (2008). A retrospective analysis of pollen host plant use by stable and declining bumble bee species. *Ecology* 89, 1811–1823. doi: 10.1890/07-1275.1

Kokko, H., and López-Sepulcre, A. (2006). From individual dispersal to species ranges: perspectives for a changing world. *Science* 313, 789–791. doi: 10.1126/science.1128566

Kotze, D. J., and O'hara, R. B. (2003). Species decline—but why? Explanations of carabid beetle (Coleoptera, Carabidae) declines in Europe. *Oecologia* 135, 138–148. doi: 10.1007/s00442-002-1174-3

Lach, L., and Hooper-Bui, L. M. (2010). Consequences of ant invasions. *Ant Ecol.* 2010, 261–286. doi: 10.1093/acprof:oso/9780199544639.003.0015

Lach, L., Parr, C., and Abbott, K. (2010). *Ant Ecology*. Oxford: Oxford University Press.

Lai, L.-C., Hua, K.-H., and Wu, W.-J. (2015). Intraspecific and interspecific aggressive interactions between two species of fire ants, Solenopsis geminata and S. invicta (Hymenoptera: Formicidae), in Taiwan. *J. Asia. Pac. Entomol.* 18, 93–98. doi: 10.1016/j.aspen.2014.09.003

Lande, R. (2015). Evolution of phenotypic plasticity in colonizing species. *Mol. Ecol.* 24, 2038–2045. doi: 10.1111/mec.13037

LeBrun, E. G., Plowes, R. M., and Gilbert, L. E. (2012). Imported fire ants near the edge of their range: disturbance and moisture determine prevalence and impact of an invasive social insect. *J. Anim. Ecol.* 81, 884–895. doi: 10.1111/j.1365-2656.2012.01954.x

Leitch, A. R., and Leitch, I. J. (2008). Genomic plasticity and the diversity of polyploid plants. *Science* 320, 481–483. doi: 10.1126/science.1153585

Lester, P. J., and Beggs, J. R. (2019). Invasion success and management strategies for social Vespula wasps. *Annu. Rev. Entomol.* 64, 51–71. doi: 10.1146/annurev-ento-011118-111812

Lester, P. J., Beggs, J. R., Brown, R. L., Edwards, E. D., Groenteman, R., Toft, R. J., et al. (2013). The outlook for control of New Zealand's most abundant, widespread and damaging invertebrate pests: social wasps. *NZ Sci. Rev.* 70, 56–62. Available online at: http://hdl.handle.net/2292/35313

Lester, P. J., Gruber, M. A. M., Brenton-Rule, E. C., Archer, M., Corley, J. C., Dvorák, L., et al. (2014). Determining the origin of invasions and demonstrating a lack of enemy release from microsporidian pathogens in common wasps (*Vespula vulgaris*). *Divers. Distrib.* 20, 964–974. doi: 10.1111/ddi.12223

Li, Y., Ke, Z., Wang, S., Smith, G. R., and Liu, X. (2011). An exotic species is the favorite prey of a native enemy. *PLoS ONE* 6:e24299. doi: 10.1371/journal.pone.0024299

Liebert, A. E., Gamboa, G. J., Stamp, N. E., Curtis, T. R., Monnet, K. M., Turillazzi, S., et al. (2006). Genetics, behavior and ecology of a paper wasp invasion: polistes dominulus in North America. *Ann Zoologici Fennici* 43, 595–624.

Liebl, A. L., Schrey, A. W., Richards, C. L., and Martin, L. B. (2013). Patterns of DNA methylation throughout a range expansion of an introduced songbird. *Integr. Comp. Biol.* 53:351–358. doi: 10.1093/icb/ict007

Lin, W., McBroome, J., Rehman, M., and Johnson, B. R. (2018). Africanized bees extend their distribution in California. *PLoS ONE* 13:e0190604. doi: 10.1371/journal.pone.0190604

Liu, H., and Stiling, P. (2006). Testing the enemy release hypothesis: a review and meta-analysis. *Biol. Invasions* 8, 1535–1545. doi: 10.1007/s10530-005-5845-y

Lowe, S., Browne, M., Boudjelas, S., and De Poorter, M. (2000). "100 of the world's worst invasive alien species a selection from the global invasive species database," in *Publ. by Invasive Species Spec. Gr. a Spec. Gr. Species Surviv. Comm. World Conserv. Union (IUCN)* (Hollands Printing Ltd.), 12p. First Publ. as Spec. lift-out Aliens 12.

Macfarlane, R. P., and Gurr, L. (1995). Distribution of bumble bees in New Zealand. *N. Zeal. Entomol.* 18, 26–36. doi: 10.1080/00779962.1995.9721999

Manfredini, F., Grozinger, C. M., and Beani, L. (2013). Examining the "evolution of increased competitive ability" hypothesis in response to parasites and pathogens in the invasive paper wasp *Polistes dominula*. *Naturwissenschaften* 100, 219–228. doi: 10.1007/s00114-013-1014-9

Marin, P., Genitoni, J., Barloy, D., Maury, S., Gibert, P., Ghalambor, C. K., et al. (2019). Biological invasion: the influence of the hidden side of the (Epi) genome. *Funct. Ecol.* doi: 10.1111/1365-2435.13317

Matsumura, C., Yokoyama, J., and Washitani, I. (2004). Invasion status and potential ecological impacts of an invasive alien bumblebee, *Bombus terrestris* L. (Hymenoptera: Apidae) naturalized in Southern Hokkaido, Japan. *Glob. Environ. Res. Ed.* 8, 51–66.

Meeus, I., Brown, M. J., De Graaf, D. C., and Smagghe, G. (2011). Effects of invasive parasites on bumble bee declines. *Conserv. Biol.* 25, 662–671. doi: 10.1111/j.1523-1739.2011.01707.x

Morales, C. L., Arbetman, M. P., Cameron, S. A., and Aizen, M. A. (2013). Rapid ecological replacement of a native bumble bee by invasive species. *Front. Ecol. Environ.* 11, 529–534. doi: 10.1890/120321

Morrison, L. W. (2000). Mechanisms of interspecific competition among an invasive and two native fire ants. *Oikos* 90, 238–252. doi: 10.1034/j.1600-0706.2000.900204.x

Murray, K. A., Rosauer, D., McCallum, H., and Skerratt, L. F. (2010). Integrating species traits with extrinsic threats: closing the gap between predicting and preventing species declines. *Proc. R. Soc. B Biol. Sci.* 278, 1515–1523. doi: 10.1098/rspb.2010.1872

Neumann, P., and Carreck, N. L. (2010). *Honey Bee Colony Losses. J. Apicult. Re.* 49, 1–6. doi: 10.3896/IBRA.1.49.1.01

O'Donnell, S., Donlan, N. A., and Jones, T. A. (2004). Mushroom body structural change is associated with division of labor in eusocial wasp workers (Polybia aequatorialis, Hymenoptera: Vespidae). *Neurosci. Lett.* 356, 159–162. doi: 10.1016/j.neulet.2003.11.053

Oldroyd, B. P., Thexton, E. G., Lawler, S. H., and Crozier, R. H. (1997). Population demography of Australian feral bees (*Apis mellifera*). *Oecologia* 111, 381–387. doi: 10.1007/s004420050249

Overmyer, S. L., and Jeanne, R. L. (1998). Recruitment to food by the German yellowjacket, *Vespula germanica*. *Behav. Ecol. Sociobiol.* 42, 17–21. doi: 10.1007/s002650050407

Palacio-López, K., and Gianoli, E. (2011). Invasive plants do not display greater phenotypic plasticity than their native or non-invasive counterparts: a meta-analysis. *Oikos* 120, 1393–1401. doi: 10.1111/j.1600-0706.2010.19114.x

Plowes, R. M., Dunn, J. G., and Gilbert, L. E. (2007). The urban fire ant paradox: native fire ants persist in an urban refuge while invasive fire ants dominate natural habitats. *Biol. Invasions* 9, 825–836. doi: 10.1007/s10530-006-9084-7

Potts, S. G., Roberts, S. P. M., Dean, R., Marris, G., Brown, M. A., Jones, R., et al. (2010). Declines of managed honey bees and beekeepers in Europe. *J. Apic. Res.* 49, 15–22. doi: 10.3896/IBRA.1.49.1.02

Purcell, J., Brelsford, A., Wurm, Y., Perrin, N., and Chapuisat, M. (2014). Convergent genetic architecture underlies social organization in ants. *Curr. Biol.* 24, 2728–2732. doi: 10.1016/j.cub.2014.09.071

Putnam, H. M., Davidson, J. M., and Gates, R. D. (2016). Ocean acidification influences host DNA methylation and phenotypic plasticity in environmentally susceptible corals. *Evol. Appl.* 9, 1165–1178. doi: 10.1111/eva.12408

Pyšek, P., Jarošík, V., Hulme, P. E., Pergl, J., Hejda, M., Schaffner, U., et al. (2012). A global assessment of invasive plant impacts on resident species, communities and ecosystems: the interaction of impact measures, invading species' traits and environment. *Glob. Chang. Biol.* 18, 1725–1737. doi: 10.1111/j.1365-2486.2011.02636.x

Ratnieks, F. L., and Carreck, N. L. (2010). Clarity on honey bee collapse? *Science* 327, 152–153. doi: 10.1126/science.1185563

Reeve, A. J., Ojanguren, A. F., Deacon, A. E., Shimadzu, H., Ramnarine, I. W., and Magurran, A. E. (2014). Interplay of temperature and light influences wild guppy (*Poecilia reticulata*) daily reproductive activity. *Biol. J. Linn. Soc.* 111, 511–520. doi: 10.1111/bij.12217

Reeve, H. K., and Nonacs, P. (1992). Social contracts in wasp societies. *Nature* 359, 823–825. doi: 10.1038/359823a0

Reuter, M., Pedersen, J. S., and Keller, L. (2005). Loss of Wolbachia infection during colonisation in the invasive Argentine ant *Linepithema humile*. *Heredity* 94, 364–369. doi: 10.1038/sj.hdy.6800601

Richards, C. L., Bossdorf, O., Muth, N. Z., Gurevitch, J., and Pigliucci, M. (2006). Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecol. Lett.* 9, 981–993. doi: 10.1111/j.1461-0248.2006.00950.x

Robinson, G. E. (1992). Regulation of division of labor in insect societies. *Annu. Rev. Entomol.* 37, 637–665. doi: 10.1146/annurev.en.37.010192.003225

Roets, F., Benadé, P. C., Samways, M. J., and Veldtman, R. (2019). Better colony performance, not natural enemy release, explains numerical dominance of the exotic *Polistes dominula* wasp over a native congener in South Africa. *Biol. Invasions* 21, 925–933. doi: 10.1007/s10530-018-1870-5

Rollins, L. A., Richardson, M. F., and Shine, R. (2015). A genetic perspective on rapid evolution in cane toads (*Rhinella marina*). *Mol. Ecol.* 24, 2264–2276. doi: 10.1111/mec.13184

Rosenkranz, P. (1999). Honey bee (Apis mellifera L.) tolerance to *Varroa jacobsoni* Oud. in South America. *Apidologie* 30, 159–172. doi: 10.1051/apido:19990206

Ross, K. G., and Matthews, R. W. (1991). *The Social Biology of Wasps*. Ithaca, NY: Cornell University Press.

Rowles, A. D., and O'Dowd, D. J. (2007). Interference competition by Argentine ants displaces native ants: implications for biotic resistance to invasion. *Biol. Invasions* 9, 73–85. doi: 10.1007/s10530-006-9009-5

Rowley, J. J., and Alford, R. A. (2007). Behaviour of Australian rainforest stream frogs may affect the transmission of chytridiomycosis. *Dis. Aquat. Organ.* 77, 1–9. doi: 10.3354/dao01830

Ruz, L., and Herrera, R. (2001). Preliminary observations on foraging activities of *Bombus dahlbomii* and *Bombus terrestris* (Hym: Apidae) on native and non native vegetation in Chile. *Acta Hortic* 561, 165–169. doi: 10.17660/ActaHortic.2001.561.24

Sackmann, P., Villacide, J. M., and Corley, J. (2003). Presencia de una nueva avispa social exótica, *Polistes dominulus* (Hymenoptera: Vespidae) en la *Patagonia argentina*. *Pan-Pac. Entomol.* 75, 58–59.

Sagata, K., and Lester, P. J. (2009). Behavioural plasticity associated with propagule size, resources, and the invasion success of the Argentine ant *Linepithema humile*. *J. Appl. Ecol.* 46, 19–27. doi: 10.1111/j.1365-2664.2008.01523.x

Sánchez-Bayo, F., and Wyckhuys, K. A. G. (2019). Worldwide decline of the entomofauna: a review of its drivers. *Biol. Conserv.* 232, 8–27. doi: 10.1016/j.biocon.2019.01.020

Sanetra, M., and Crozier, R. H. (2002). Daughters inherit colonies from mothers in the'living-fossil'ant Nothomyrmecia macrops. *Naturwissenschaften* 89, 71–74. doi: 10.1007/s00114-001-0288-5

Sanetra, M., and Crozier, R. H. (2003). Patterns of population subdivision and gene flow in the ant Nothomyrmecia macrops reflected in microsatellite and mitochondrial DNA markers. *Mol. Ecol.* 12, 2281–2295. doi: 10.1046/j.1365-294X.2003.01900.x

Schmid-Hempel, P., and Ebert, D. (2003). On the evolutionary ecology of specific immune defence. *Trends Ecol. Evol.* 18, 27–32. doi: 10.1016/S0169-5347(02)00013-7

Schmid-Hempel, P., Schmid-Hempel, R., Brunner, P. C., Seeman, O. D., and Allen, G. R. (2007). Invasion success of the bumblebee, Bombus terrestris, despite a drastic genetic bottleneck. *Heredity* 99, 414–422. doi: 10.1038/sj.hdy.6801017

Schwander, T., Lo, N., Beekman, M., Oldroyd, B. P., and Keller, L. (2010). Nature versus nurture in social insect caste differentiation. *Trends Ecol. Evol.* 25, 275–282. doi: 10.1016/j.tree.2009.12.001

Shik, J. Z., and Silverman, J. (2013). Towards a nutritional ecology of invasive establishment: aphid mutualists provide better fuel for incipient Argentine ant colonies than insect prey. *Biol. Invasions* 15, 829–836. doi: 10.1007/s10530-012-0330-x

Smith, K. M., Loh, E. H., Rostal, M. K., Zambrana-Torrelio, C. M., Mendiola, L., and Daszak, P. (2013). Pathogens, pests, and economics: drivers of honey bee colony declines and losses. *Ecohealth* 10, 434–445. doi: 10.1007/s10393-013-0870-2

Sorvari, J. (2013). Social wasp (Hymenoptera: Vespidae) beer trapping in Finland 2008-2012: a German surprise. *Entomol. Fenn.* 24, 156–164. doi: 10.33338/ef.8983

Sorvari, J., and Hakkarainen, H. (2007). Wood ants are wood ants: deforestation causes population declines in the polydomous wood ant *Formica aquilonia*. *Ecol. Entomol.* 32, 707–711. doi: 10.1111/j.1365-2311.2007.00921.x

Spradbery, J. P. (1973). *Wasps. An Account of the Biology and Natural History of Social and Solitary Wasps, With Particular Reference to Those of the British Isles.* Seattle: University of Washingon Press.

Stapley, J., Santure, A. W., and Dennis, S. R. (2015). Transposable elements as agents of rapid adaptation may explain the genetic paradox of invasive species. *Mol. Ecol.* 24, 2241–2252. doi: 10.1111/mec.13089

Stockan, J. A., Rao, S., and Pakeman, R. (2010). Nesting preferences of the threatened wood ant Formica exsecta (Hymenoptera: Formicidae); implications for conservation in Scotland. *J. insect Conserv.* 14, 269–276. doi: 10.1007/s10841-009-9255-9

Suarez, A. V., Holway, D. A., and Tsutsui, N. D. (2008). Genetics and behavior of a colonizing species: the invasive Argentine ant. *Am. Nat.* 172, S72–S84. doi: 10.1086/588638

Thomas, C. R. (1960). *The European wasp (Vespula germanica Fab.) in New Zealand.* Wellington, NZ: Department of Scientific and Industrial Research.

Torretta, J. P., Medan, D., and Abrahamovich, A. H. (2006). First record of the invasive bumblebee Bombus terrestris (L.) (Hymenoptera, Apidae) in Argentina. *Trans. Am. Entomol. Soc.* 132, 285–289. doi: 10.3157/0002-8320(2006)132[285:FROTIB]2.0.CO;2

Traniello, J. F. A., and Rosengaus, R. B. (1997). Ecology, evolution and division of labour in social insects. *Anim. Behav.* 53, 209–213. doi: 10.1006/anbe.1996.0289

Trible, W., Shoemaker, D. D., and Gotzek, D. (2018). Sociometry of *Solenopsis geminata* (Hymenoptera: Formicidae) reveals variation in colony-level phenotypes in fire ants. *Myrmecol. News* 26, 47–53. doi: 10.25849/myrmecol.news_026:047

Tschinkel, W. R. (2006). *The Fire Ants*. Cambridge, MA: Harvard University Press.

Vargo, E. L., and Porter, S. D. (1993). Reproduction by virgin queen fire ants in queenless colonies: comparative study of three taxa (*Solenopsis richteri*, hybridS. invicta/richteri, *S. geminata*) (Hymenoptera: Formicidae). *Insect. Soc.* 40, 283–293. doi: 10.1007/BF01242364

Via, S., Gomulkiewicz, R., De Jong, G., Scheiner, S. M., Schlichting, C. D., and Van Tienderen, P. H. (1995). Adaptive phenotypic plasticity: consensus and controversy. *Trends Ecol. Evol.* 10, 212–217. doi: 10.1016/S0169-5347(00)89061-8

Villemant, C., Zuccon, D., Rome, Q., Muller, F., Poinar G. O. Jr., and Justine, J.-L. (2015). Can parasites halt the invader? Mermithid nematodes parasitizing the yellow-legged Asian hornet in France. *PeerJ.* 3:e947. doi: 10.7717/peerj.947

Visscher, P. K., and Vetter, R. S. (2003). Annual and multi-year nests of the western yellowjacket, Vespula pensylvanica, in California. *Insect. Soc.* 50, 160–166. doi: 10.1007/s00040-003-0636-x

Wang, J., Wurm, Y., Nipitwattanaphon, M., Riba-Grognuz, O., Huang, Y.-C., Shoemaker, D., et al. (2013). A Y-like social chromosome causes alternative colony organization in fire ants. *Nature* 493, 664–668. doi: 10.1038/nature11832

Weiner, S. A., Noble, K., Upton, C. T., Woods, W. A., and Starks, P. T. (2011). A role for thermoregulation in the *Polistes dominulus* invasion: a comparison of the thermoregulatory abilities of the invasive wasp *P. dominulus* and the native wasp *P. fuscatus. Insect. Soc.* 58, 185–190. doi: 10.1007/s00040-010-0136-8

Wellband, K. W., and Heath, D. D. (2017). Plasticity in gene transcription explains the differential performance of two invasive fish species. *Evol. Appl.* 10, 563–576. doi: 10.1111/eva.12463

West-Eberhard, M. J. (1989). Phenotypic plasticity and the origins of diversity. *Annu. Rev. Ecol. Syst.* 20, 249–278. doi: 10.1146/annurev.es.20.110189.001341

Williams, N. M., Crone, E. E., T'ai, H. R., Minckley, R. L., Packer, L., and Potts, S. G. (2010). Ecological and life-history traits predict bee species responses to environmental disturbances. *Biol. Conserv.* 143, 2280–2291. doi: 10.1016/j.biocon.2010.03.024

Williams, P. H., Byvaltsev, A., Sheffield, C., and Rasmont, P. (2013). *Bombus cullumanus* - An extinct European bumblebee species? *Apidologie* 44, 121–132. doi: 10.1007/s13592-012-0161-x

Wilson, E. E., Mullen, L. M., and Holway, D. A. (2009). Life history plasticity magnifies the ecological effects of a social wasp invasion. *Proc. Natl. Acad. Sci. U.S.A.* 106, 12809–12813. doi: 10.1073/pnas.0902979106

Wilson, E. O. (1971). *The Insect Societies*. Cambridge, MA: Harvard University Press.

Wilson, E. O. (1978). Division of labor in fire ants based on physical castes (Hymenoptera: Formicidae: Solenopsis). *J. Kansas Entomol. Soc.* 51, 615–636.

Wilson, E. O. (2012). *The Social Conquest of Earth*. New York, NY: WW Norton & Company.

Winston, M. L., Otis, G. W., and Taylor O. R. Jr., (1979). Absconding behaviour of the Africanized honeybee in South America. *J. Apic. Res.* 18, 85–94. doi: 10.1080/00218839.1979.11099951

Winston, M. L., Taylor, O. R., and Otis, G. W. (1983). Some differences between temperate European and tropical African and South American honeybees. *Bee World* 64, 12–21. doi: 10.1080/0005772X.1983.11097902

Wood, T. J., Gibbs, J., Graham, K. K., and Isaacs, R. (2019). Narrow pollen diets are associated with declining Midwestern bumble bee species. *Ecology* 100:e02697. doi: 10.1002/ecy.2697

Wuellner, C. T., and Saunders, J. B. (2003). Circadian and circannual patterns of activity and territory shifts: comparing a native ant (Solenopsis geminata, Hymenoptera: Formicidae) with its exotic, invasive congener (*S. invicta)* and its parasitoids (Pseudacteon spp., Diptera: Phoridae) at a centra. *Ann. Entomol. Soc. Am.* 96, 54–60. doi: 10.1603/0013-8746(2003)096[0054:CACPOA]2.0.CO;2

Yang, C.-C., Yu, Y.-C., Valles, S. M., Oi, D. H., Chen, Y.-C., Shoemaker, D., et al. (2010). Loss of microbial (pathogen) infections associated with recent invasions of the red imported fire ant *Solenopsis invicta*. *Biol. Invasions* 12, 3307–3318. doi: 10.1007/s10530-010-9724-9

Zenni, R. D., and Nuñez, M. A. (2013). The elephant in the room: the role of failed invasions in understanding invasion biology. *Oikos* 122, 801–815. doi: 10.1111/j.1600-0706.2012.00254.x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 22 October 2019
doi: 10.3389/fevo.2019.00396

Declines and Resilience of Communities of Leaf Chewing Insects on Missouri Oaks Following Spring Frost and Summer Drought

Robert J. Marquis[1]*, **John T. Lill**[2], **Rebecca E. Forkner**[3], **Josiane Le Corff**[4], **John M. Landosky**[1] **and James B. Whitfield**[5]

[1] Department of Biology, University of Missouri-St. Louis, St. Louis, MO, United States, [2] Department of Biological Sciences, George Washington University, Washington, DC, United States, [3] Department of Biology, George Mason University, Fairfax, VA, United States, [4] Institut de Recherche en Horticulture et Semences, INRA-Université d'Angers, Beaucouzé, France, [5] Department of Entomology, University of Illinois at Urbana-Champaign, Urbana, IL, United States

OPEN ACCESS

Edited by:
Lora A. Richards,
University of Nevada, Reno,
United States

Reviewed by:
Ken Oyama,
National Autonomous University of
Mexico, Mexico
Bastien Castagneyrol,
INRA Centre
Bordeaux-Aquitaine, France

***Correspondence:**
Robert J. Marquis
robert_marquis@umsl.edu

Specialty section:
This article was submitted to
Population and Evolutionary
Dynamics,
a section of the journal
Frontiers in Ecology and Evolution

Received: 11 May 2019
Accepted: 04 October 2019
Published: 22 October 2019

Citation:
Marquis RJ, Lill JT, Forkner RE, Le Corff J, Landosky JM and Whitfield JB (2019) Declines and Resilience of Communities of Leaf Chewing Insects on Missouri Oaks Following Spring Frost and Summer Drought. Front. Ecol. Evol. 7:396. doi: 10.3389/fevo.2019.00396

Extreme weather events dramatically impact populations of individual insect species but the consequences of such events for entire insect communities are not well documented. We present evidence that mid spring frosts and summer drought negatively affect the community of insects found on Missouri oaks (*Quercus alba* and *Q. velutina*), amounting to a 23–186 fold decrease depending on the oak species, insect body size and feeding guild, and the specific weather event. Depending on the event, spring faunas required 1–5 years and summer faunas 1–4 years following spring frosts to reach pre-event levels. The impact of summer drought on leaf tying caterpillars also lasted over an extended period of time; it was 5 years before numbers of leaf ties reached pre-drought levels. Smaller-bodied species of leaf tying Lepidoptera took longer to recover than larger-bodied species following the drought. Overall, we found no evidence for a general decline in abundance, even a modest one, during the 20 years of study of faunas on oak trees in southeastern Missouri. However, the risk of mid-season frost damage to trees is expected to increase with predicted earlier onset of spring. Similarly, the effects of drought reported here are likely to increase with time, as the climate in the Midwest U.S. is only expected to become warmer and drier during the summer months. Understanding the impact of such weather events on insect communities influences our ability to predict how habitat and landscape management, or lack thereof, will influence future patterns of insect abundance and diversity.

Keywords: insect decline, climate change, insect populations, drought, spring frosts, *Quercus*, herbivorous insects

INTRODUCTION

Many foliage-feeding insect species, particularly those in temperate forests, undergo dramatic fluctuations in abundance across years (Barbosa and Schultz, 1987; Royama, 1997; Barbosa et al., 2012). Insects whose numbers reach sufficiently high levels that they cause noticeable defoliation are labeled as "pests" (Nothnagle and Schultz, 1987). Other species do not go through such eruptions and have been classified as non-outbreak species (Mason, 1987). Intrinsic characteristics of the insect species including body size, gregariousness in the larval stage, host range, and maternal effects have been used to categorize particular insect species with respect to the likelihood of going through an outbreak. Host plant characteristics, natural enemy impacts, and weather all have been suggested as factors extrinsic to the insect species that might lead to insect outbreaks (Hunter, 1991; Koricheva et al., 2012).

The opposite of outbreaks are, of course, population declines. Determining which factors drive declines of insect communities is necessary for predicting how insects will respond to current and future anthropogenic activities. The fact that some species of insects go through periodic increases and then decreases apparently in the absence of human influence makes it difficult to distinguish these population cycles from actual declines due to anthropogenic effects. For example, in our study system of Lepidoptera larvae on *Quercus* in Missouri, USA, fluctuating abundances from year to year are linked to the time of year when a particular insect species is feeding. Spring-feeding Lepidoptera are more variable in their population dynamics than summer-feeding species because of the greater unpredictability of the timing of spring foliage availability than that of summer foliage (Forkner et al., 2008). Based on studies for which there is a minimum 10 years of continuous sampling for multiple species, there is increasing evidence that some insect populations are declining worldwide (Hallmann et al., 2017; Sanchez-Bayo and Wyckhuys, 2019). Climate change as it directly affects insect survivorship has been suggested as the cause of insect declines in the wet tropical forest of Puerto Rico (Lister and Garcia, 2018).

The impacts of local weather events on insect survival may have both short term and long term effects. Hurricanes can increase the number of leaf-feeding Lepidoptera as result of the ensuing new leaf flush (Torres, 1992), and spring frosts contribute to the population dynamics of some Lepidoptera (LeRoux et al., 1963). Drought has been proposed to influence forest insect populations (Mattson and Haack, 1987), although support is mixed (Martinet and Allen, 1987; Kolb et al., 2016). Outbreaks of the variable oakleaf caterpillar (*Lochmeus manteo*) were associated with multiple year droughts in the Missouri Ozarks in 1970 (Law and Gott, 1987) and again in 1980 (Gass, 1971; Law and Gott, 1987). Similarly, years with warmer-than-average summer temperatures in Canada were associated with population outbreaks of fall webworm (*Hyphantria cunea*: Morris, 1964). Even within the same species and during the same drought period, one population of the checkerspot butterfly responded by increasing while all others declined (Ehrlich et al., 1980). Declines in species richness of multiple adult moth species in Indiana oak-hickory forests were associated with the severe 2012 drought in the Midwest, USA (Summerville and Marquis, 2016). Perhaps even more threatening is the possibility of multiple events (e.g., spring frosts and summer droughts) occurring in the same year.

Long term data are important with regard to questions of impacts of climate and human management on insect populations (Summerville and Marquis, 2016; Sanchez-Bayo and Wyckhuys, 2019). Such data are necessary because they give evidence for general trends of increases or declines. In addition, the longer the data set, the more likely it is to capture infrequent climatic events, which may have persistent effects, and to estimate recovery times following such events. Importantly, they help us distinguish the impact of local weather events, which may be short term, from long term trends, that might indicate the influence of extensive habitat modification and/or climate change.

We focus here are on two types of climatic events, spring frosts and summer droughts. Both were linked to immediate declines in insect numbers for leaf-feeding insects on oaks (*Quercus*) in Missouri, USA. Depending on the event, these declines in numbers persisted for a number of years afterward. Spring frosts can directly affect insects, by disrupting metabolism and killing cells through the formation of ice crystals (Bale, 1996; Sinclair et al., 2013), and indirectly affect them by killing the young leaves (Augspurger, 2013) on which the insects feed. Summer drought could also have direct and indirect effects. Exposure to desiccating humidity can kill insects (Willmer, 1980). This desiccation effect might by mitigated by body size (larger insects would be less vulnerable) (Le Lagadec et al., 1998; Kærsgaard et al., 2004) or by specific adaptations, such as body hairs or physiological mechanisms (Philip et al., 2008). Indirectly, drought can reduce water content of plant tissues upon which the insects feed (Mattson and Haack, 1987).

We used long term census data to determine the effects of mid spring frosts and summer droughts on the abundances of these insects. Specifically, we asked: (1) What is the impact at the community level on abundances following such events relative to pre-event abundances? (2) How long do communities require to return to pre-event levels? and (3) What climatic factors are linked to the observed declines? Here, the "community" consists of the leaf-chewing insects (mid spring frost study), or a subset of them (summer drought study), found on *Quercus* in Missouri, USA. In the case of the mid spring frost events, we hypothesized that abundances would decline in the year of the frost, and that the time to recovery would be greater for the mostly univoltine spring fauna than for the more commonly multivoltine summer fauna. The spring and summer fauna of leaf chewing insects on Missouri oaks are each themselves quite diverse (75+ species each) but compositionally distinct, sharing fewer than 5 species (Marquis et al., 2019). Following the 2012 drought, we hypothesized that recovery would require multiple years, and that large-bodied species would recover before small-bodied species because of the smaller surface area to volume ratio in the former. To illustrate the impacts of two mid spring frost events, we used data originally collected to estimate impacts of forest management on biodiversity and sustainability on forest productivity during the years 1991–2010 (Forkner et al., 2006). To explore the impact of a major drought in 2012, we used data collected sporadically on the abundances of leaf tying caterpillars prior to the drought year and then continuously through 2018 following the drought. We describe how local climate data are associated with the observed population events, and then discuss the results in light of predicted future changes in local weather, i.e., climate change, for our region. Although the time scale of the two studies was different and the studies occurred in different locations, the insects associated with the two oak species in the two locations are much the same (Marquis et al., 2019).

MATERIALS AND METHODS

Mid Spring Frosts

We sampled leaf-chewing insect abundance on *Quercus alba* and *Q. velutina* for the years 1991–2010 as part of the Missouri Ozark Forest Ecosystem Project (MOFEP), located in southeastern Missouri. The MOFEP experiment consisted of three treatments (control, uneven-aged management, and even-aged management) applied at the landscape scale, with three replicates ("plots") per treatment, each replicate ∼400 ha in size. We present data here only from control plots, which were not directly impacted by local forest management activities. See Brookshire et al. (1997) and Sheriff and He (1997) for more detailed descriptions of the experimental design. Methods and results based on insect sampling in the MOFEP are presented in detail in previous publications (Forkner and Marquis, 2004; Forkner et al., 2006; Summerville and Marquis, 2016). Specifically for each of the three control treatment plots, we sampled 1–6 stands depending on the year. All sampled plants of each stand were on the same slope, and no more than 25 m apart. For each stand, we sampled a minimum 5 plants and up to 12 plants per tree species per stand, to reach a sample size of ∼3,000 *Q. alba* leaves and ∼1,200 *Q. velutina* leaves per stand. Plants sampled were either saplings or trees with low-hanging branches. We sampled all leaves on saplings or all leaves of marked branches of larger trees. We do not know the possible effect of plant size on the interactions described here. Initial studies and species accumulation curve analyses indicated that this was sufficient sampling to estimate abundance and diversity of leaf-chewing oak insects in these areas (Marquis and Le Corff, 1997). For the years 1991–2008, we systematically sampled four times during the growing season (early May, late June, late July, and late August-early September) to account for seasonal turnover in the identity of species present. For 2009–2010, we sampled only the spring fauna, in May, because of funding constraints. Number of stands sampled per plot in each year were the following: 1991 (1), 1992–2008 (6), 2009–2010 (2). We inspected the top and bottom of each leaf of each marked branch or tree, and all twigs, branches, and trunks associated with entire saplings, or the marked branches of larger trees, recording the number and identity of all encountered leaf chewing insect species. Once per year, the number of leaves/tree that were censused was recorded allowing computation of insect densities (caterpillars/m^2 foliage). Sampled insects were left undisturbed on the trees unless it was necessary to rear them in the laboratory for identification. Sampling was prohibited in 1996 (**Figure 1**), the first year of timber harvest in MOFEP.

Summer Drought

At Cuivre River State Park (CRSP) in east central Missouri, we have been studying the impact of lepidopteran larvae that silk together neighboring leaves, leaf blade to leaf blade, to form a leaf tie, on the resulting secondary inhabitants of these leaf ties (Lill and Marquis, 2003; Lill, 2004; Marquis and Lill, 2010; Reinhardt, 2011; Wang et al., 2012; Baer and Marquis, 2014). There are 15 species of Lepidoptera at CRSP that construct leaf ties. The vast majority of leaf ties are constructed in the summer, after leaves have hardened, often persisting after the original tie maker has completed its larval life stage.

In 2012, a major drought occurred throughout the Midwestern U.S. This drought was associated with back-to-back La Niña events in 2010–2011 and then again in 2011–2012 (Rippey, 2015). We first noticed almost a complete absence of leaf ties on oaks in the St. Louis region in June-August 2013. Thus, we began sampling in 2013 at CRSP, continuing to 2018. We counted the number of leaf ties in August to October (depending on the year) that had accumulated through the summer on 15–30 understory *Q. alba* saplings, 1.5–3 m tall. Sample size varied depending on the design of the experiment taking place in a given year. We sampled where our pre-drought studies had taken place, i.e., in closed canopy forest north of the Assistant's Road and in the North Woods Wild Area of CRSP, both of which had not been subjected to prescribed burning. We recorded the lepidopteran inhabitants of all of these late season ties. In addition, we haphazardly sampled leaf ties through each of the summer months from 2013 to 2018 (75–100 h per summer), recording inhabitants, with the goal of specifically determining when species recorded in leaf ties (pre-drought) first re-appeared subsequent to the drought. We also recorded sightings of Limacodidae slug caterpillars, who we thought might be particularly vulnerable to the drought because of their relatively unsclerotized ventral surface (Epstein, 1996). A series of short term studies conducted at CRSP prior to 2012 provide estimates of leaf tie abundance and leaf tying caterpillar identity pre-drought (Lill and Marquis, 2003; Lill, 2004; Marquis and Lill, 2010; Reinhardt, 2011). Specifically, we had estimates of leaf tie abundance on *Q. alba* for 8 of the 16 years from 1996 to 2012: 1996 (Lill, 2004), 1997 (Marquis and Lill, 2010), 1999–2000 (Lill and Marquis, 2003), 2005–2006 (Marquis, unpublished), 2009 (Reinhardt, 2011), 2010 (Wang et al., unpublished). To estimate the effect of body size on the year of first appearance of a species in sampling following the 2012 drought, we measured the length of 10 adult voucher specimens per species, reared from larvae collected on *Quercus* in Missouri (in the collection of R.J. Marquis at University Missouri-St. Louis).

Climate Data

Temperature and precipitation data were extracted from the local weather stations closest to the study sites for the study years (National Centers for Environmental Information: https://www.ncei.noaa.gov/). For the years 1991–2002, we used spring temperature data (February 1 to May 31) principally from the Summersville weather station (border of Shannon and Texas Counties, MO, 37.14002°N, −91.62797°W, 376.7 m elevation), supplemented with data from the weather station in Van Buren, MO, Carter County (36.975°N, −91.0186°W, 304.8 m elevation) when data were missing from Summersville. For the years 2003–2010, we used temperature data from the Big Spring weather station in Carter County (southern boundary of MOFEP, 36.975°N, −91.0181°W, 304.6 m elevation). Data for the same years from the Carr Creek weather station in Shannon County at the northern boundary of MOFEP (37.1806°N, −91.1181°W 426.7 m elevation), an alternative source, were highly correlated (Pearson) with those of the same years from Big Spring (maximum daily temperature for

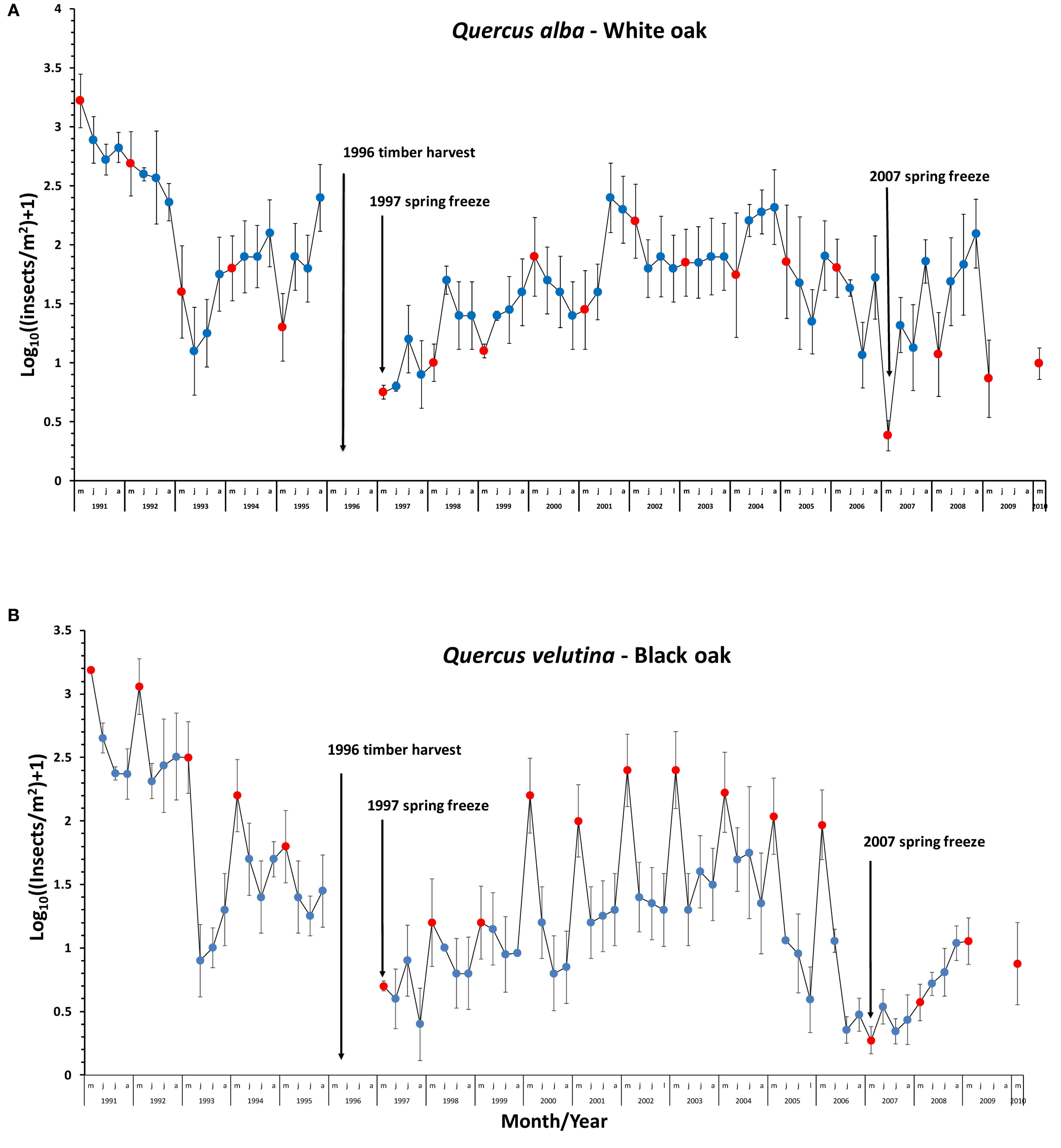


FIGURE 1 | Effect of mid spring freezes (indicated by vertical black arrows, 1997 and 2007) on mean abundance of leaf chewing insects on **(A)** *Quercus alba* and **(B)** *Q. velutina* in Missouri Ozark Forest Ecosystem Project (MOFEP) control plots, southeastern Missouri. Red dots and blue dots indicate values for the spring (May) and summer (June-August) communities, respectively. Timber harvest in MOFEP occurred in 1996, during which data collection was prohibited. Values for each point are means of means (site/stand) for one (1991), six (1992–2008), or two stands (2009–2010) for each of three control sites in the MOFEP. Error bars are 95% confidence intervals for each point. Note that data are presented on a log$_{10}$ scale. No sampling occurred September through April, when there are few insects, and/or no leaves on trees (mid-October to March or later).

February 1 to May 31 2007 $r = 0.995$, $P < 0.0001$, minimum daily temperature for February 1-May 31 2007 $r = 0.9751$, $P < 0.0001$).

Temperature and precipitation data were extracted for the years 1996–2018 from Troy, MO, the nearest data station to CRSP (Lincoln County, 38.95°N, −91°W, 170.7 m elevation).

Long term (1895–2019) temperature and precipitation data used to represent local climate at CRSP were extracted from various weather stations across Lincoln County (https://www.ncei.noaa.gov/).

Statistical Analysis

To estimate the time to recovery after the two spring freezes and the 2012 drought, we calculated the 95% confidence intervals for each census. We concluded that when the confidence interval for the insect abundance in a particular post-event year did not overlap with the 95% confidence interval for the most immediate pre-event year, recovery to "normal" had yet to take place. For the leaf tying caterpillar data set, body size (average adult body length measured on 10 reared adults) was correlated with the number of years passing before a species was recorded post-drought.

We followed the lead of Augspurger (2013) in seeking weather variables linked to declines in insect abundance for the MOFEP over the 1991–2010 period (see also Story and Congalton, 1986). Augspurger (2013) found that the magnitude of leaf freeze occurring in Trelease Woods, ∼480 km to the northeast of MOFEP, was most strongly linked to the number of days >20°C in March, in combination with the number of days experiencing <0°C in April. We tested the predictive reliability of various one-factor error matrices, and one two-factor error matrix, extracting measures from the climate data sets available for the years 1991–2010. The data set included the number of growing degree days (GDD, the mean of daily maximum and minimum temperature minus 10°C, summed over all days from February 1 to the day in April with the lowest minimum temperature), the number of warm days in March, and the number of cold days in April. The complete data set and the annual pattern of spring temperatures for 1991–2010 are given in **Table S1**.

A similar analysis was not feasible for the drought-associated decline in insect abundance at CRSP, with only one event. We were able, however, to compare precipitation and temperature data for 2012 with past and future data (years 1895–2018) to estimate how frequently such an event might have occurred previously. All analyses were conducted in SAS version 9.4.

RESULTS

Mid Spring Frosts: Leaf-Chewing Insects on *Quercus alba* and *Q. velutina* in Southeastern Missouri

There were three major declines in the May fauna (**Figure 1**, note log scale). In May 1993, abundances on *Q. alba* declined by 91.8% and by 72.2% on *Q. velutina*. The summer faunas (comparing August values) also declined for both species (*Q. alba*: 75.7%, *Q. velutina*: 91.8%). The spring fauna on *Q. alba* did not reach its former 1992 level until 2002, and the summer values by 1994 (based on overlapping 95% confidence intervals). The spring fauna on *Q. velutina* reached its former 1992 level by 2000, but by 2008 (the last year of our sampling), the summer fauna on *Q. velutina* still had not reached the high levels of 1991–1992 (**Figure 1B**). We saw no frost-killed leaves in our May 1993 sampling. There was only one April day in 1993 when the temperature was below 0°C (**Figure 2A**) (National Centers for Environmental Information: https://www.ncei.noaa.gov/).

The other two major declines during the 1991–2010 period occurred in 1997 and 2007 (**Figure 1**). Declines were 71.8–98% for the spring faunas (compared to the previous May values), and 0–97% for the summer faunas, depending on the year and the oak species. By 1998, the spring fauna on both oak species had

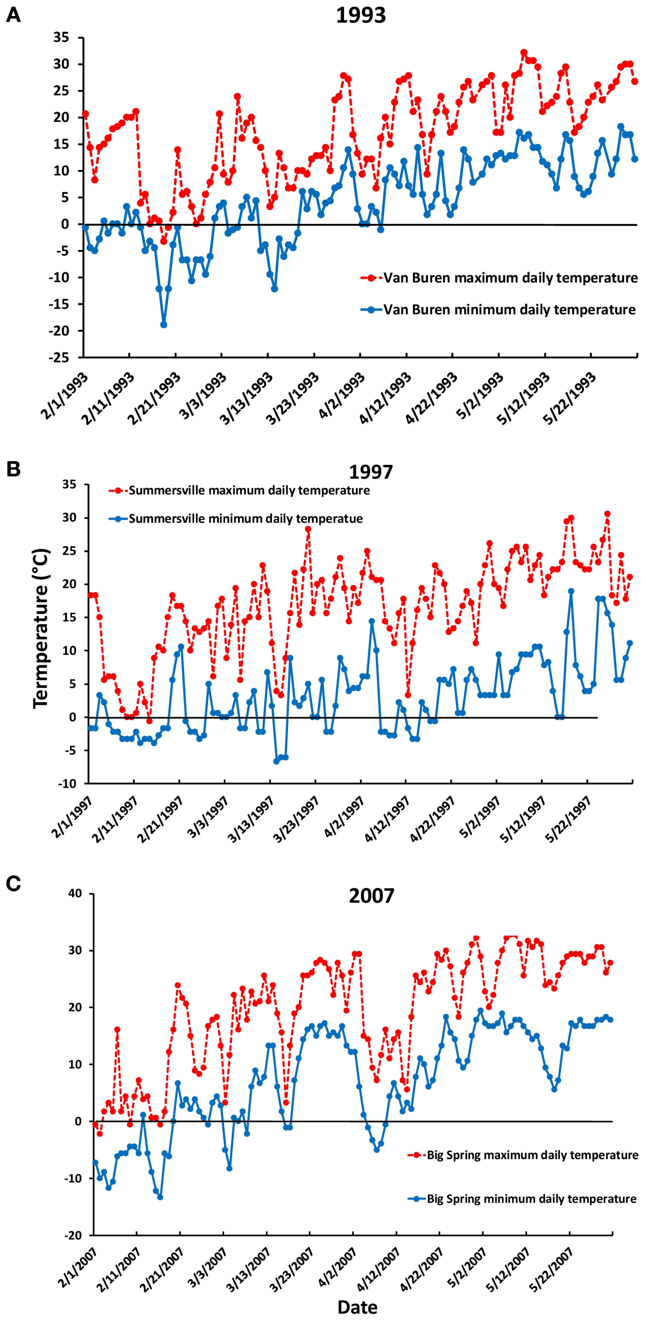


FIGURE 2 | Daily maximum and minimum temperatures for **(A)** 1993, Van Buren weather station, **(B)** 1997, Summersville weather station, **(C)** 2007, Big Spring weather station (https://www.ncei.noaa.gov). See text for station locations.

recovered following the 1997 decline. Following the 2007 decline, by 2010 (at the end of our sampling), the spring fauna still had not recovered to 2006 levels on either oak species. June and July levels on *Q. alba* in 1998 were similar to those in 1995, while August numbers did not reach 1995 levels until 2001. June and July numbers on *Q. velutina* recovered by 1999 and August values by 2001. Following the 2007 event, June numbers of insects on *Q. alba* and *Q. velutina* had recovered by 2008. July and August numbers did not decline in 1997 for either host species.

There were early April spring frosts (temperatures below 0°C) in both 1997 and 2007, lasting 7 days in 1997 and 5 days in 2007 (**Figures 2B,C**). Our observations are that these frosts killed young leaves over wide expanses of the MOFEP experimental region in both years (see also Gu et al., 2008). In MOFEP sampled stands, frozen leaves occurred over approximately 50 percent of the landscape in 1997, while 50–90% of all leaves in all marked stands were frozen in 2007. May sampling in 2007 was delayed by 1 week until new leaves appeared.

In addition to the 1997 and 2007 April freeze events, there were high daily maximum temperatures in March (9 days over 20°C in March of both years) (National Centers for Environmental Information: https://www.ncei.noaa.gov/). During the 20 years of this study, this combination of high March temperatures followed by an extended freeze of 5 days or more only occurred in 1997 and 2007. In contrast, in 1993, when insect numbers declined markedly on *Q. alba* compared to 1992, only 2 days in March were over 20°C, and there was only 1 day in April when the minimum temperature was $<0°$C ($-1.1°$C, **Figure 2A**). Accordingly, high temperatures in March and low temperatures in April together perfectly predicted that occurrence of leaf-killing frosts in 1997 and 2007, and never predicted such an event when it did not occur (**Table 1**). None of the single variable models tested proved as accurate (**Table 1**). One sample t-tests revealed that 9 days of temperatures $>20°$C was significantly greater than the mean value for the other years (mean ± s.e. = 7.00 ± 1.30, $t = -1.94$, $P = 0.0366$), and 5 days $<0°$C was significantly greater than the mean value for the other years excluding 1997 (mean ± s.e. = 1.47 ± 0.36) ($t = -9.72$, $P < 0.0001$).

TABLE 1 | Error matrix for parameter(s) used to predict number of years between 1991 and 2010 with observed frost damage to leaves.

Parameter	Frost leaf kills correctly predicted	Non-damage years incorrectly predicted
ONE PARAMETER		
GDD		
≥0	2	18
≥50	2	14
≥100	1	6
≥150	1	2
≥200	1	2
≥300	1	0
March Temperatures		
2d ≥15.6°C	2	18
2d ≥17.3°C	2	18
2d ≥21.1°C	2	15
2d ≥23.9°C	2	14
2d ≥26.7°C	2	11
2d ≥23.9°C + 2d >26.7°C	2	14
April Lowest Temperatures		
≤1.1°C	2	14
≤0°C	2	11
≤−1.7°C	2	5
≤−2.2°C	2	3
≤−4.4°C	1	2
TWO PARAMETERS		
≥1 d in March of ≥26.7°C and ≥4 d in April of ≤−2.2°C	2	0

GDD, growing degree days, calculated as the temperature (in °C) per day −10°C summed from February 1 to the coldest day in April. A perfect parameter(s) would correctly predict the years in which frost killed leaves occurred and included no years in which frost damaged leaves did not occur (see Augspurger, 2013).

Summer Drought: Leaf Tying Caterpillars on *Quercus alba* at Cuivre River State Park

The average density (± s.e.) of leaf ties at the end of the season on *Quercus alba* at CRSP prior to the 2012 drought was 5.6 ± 0.2 (N = 8 years) (**Figure 3**). During 2012, especially in the late summer (when the second generation of leaf tying caterpillars typically peaks; Lill, 2004), our casual observation was that tie abundance was not noticeably low but there were few or no living caterpillars in the ties in August and September. In 2013, there were very few ties (none in the area in which plants were censused). There were only three leaf ties on census plants in 2014. Subsequent to 2014, the density of ties steadily increased. The density of ties post-drought, however, did not reach the pre-2012 drought level average until 2017, a full 5 years after the drought year (**Figure 3**). Only two species of leaf tying caterpillars were encountered in 2013, and these were the two largest species of this guild at CRSP, *Psilocorsis reflexella* and *Antaeotricha schlaegeri* (both Depressariidae). Other species became sufficiently abundant to be recorded in censuses and casual sampling of leaf ties at staggered intervals: *Pseudotelphusa quercinigracella* (non-banded form) (Gelechiidae), *Psilocorsis cryptolechiella*, *Rectiostoma xanthobasis* (Depressariidae), *Pococera expandens* (Pyralidae) (first re-recorded in 2015), *Arogalea cristifasciella* (Gelechiidae) (first re-recorded in 2017), and *Chionodes fuscomaculella* (Gelechiidae) (first re-recorded in 2018). As of 2018, there were still five species known to occur at CRSP prior to the drought that had yet to be recorded in annual censuses and by casual sampling: *Antaeotricha ossella*, *A. humilis*, *Coleotechnitis quercivorella* (Gelechiidae), *Aristotelia* sp. (Gelechiidae), and *Pseudotelphusa quercinigracella* (banded form). Body size (adult forewing length) was negatively correlated with the number of years before the species was first recorded post-drought ($r = -0.797$, $P = 0.0178$, N = 8 species). In addition, often occurring at relative low numbers at our study sites (Lill et al., 2006), we have seen only five individuals of Limacodidae (slug caterpillars) during the years 2012–2018 [one *Isa textula* (2014) and four *Acharia stimulea*

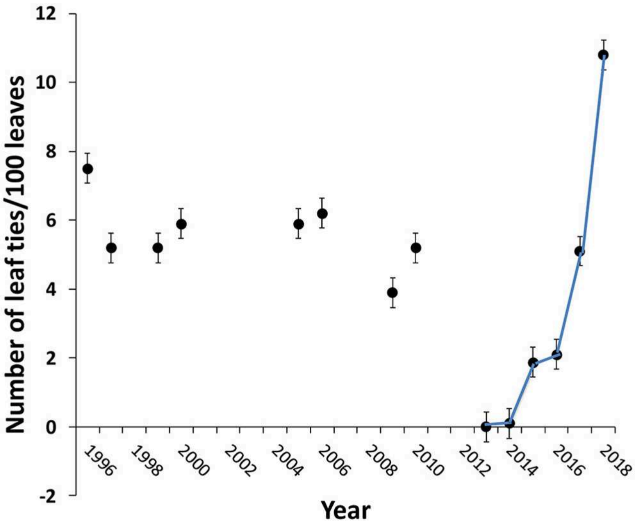


FIGURE 3 | Impact of 2012 drought on the number of leaf ties on *Quercus alba* at Cuivre River State Park, near Troy, MO. Data for years 2013–2018, this study. Error bars are 95% confidence intervals for each point. Data sources for years prior to 2012: 1996 (Lill, 2004), 1997 (Marquis and Lill, 2010), 1999–2000 (Lill and Marquis, 2003), 2005–6 (Marquis, unpublished), 2009 (Reinhardt, 2011), 2010 (Wang et al., unpublished). $N = 15$–30 saplings per year sampled between August and October, depending on the year.

(2018)] at CRSP (at a site that would typically host at least a dozen species in any given year).

The decline in abundance of insects at CRSP was associated with a major drought coupled with abnormally high temperatures (**Figure 4**). Rainfall on average was about 8% lower than the annual average throughout the Midwest (Rippey, 2015). But this lower rainfall was coupled with high summer temperatures not previously seen since 1936. Rainfall in Lincoln County for the months of May–August 2012 was 72% lower than the 1895–2018 average (158 vs. 410 mm), and daily temperature for those same months was 8% higher than 1895–2018 average (24.39°C vs. 22.42°C) (NCEI records).

This combination of low summer rainfall and high summer temperatures has only occurred five other times in Lincoln County, MO, between 1895 and 2018: 1913, 1914, 1930, 1934, and 1936. The fact that there was no decline in leaf-tying caterpillar abundance in 2018 at CRSP (**Figure 3**) despite the high May-August temperatures there (average 24.4°C) (**Figure 4**), strongly supports the hypothesis that both high temperatures and low rainfall are required to impact these insect communities. Rainfall during May-August in 2018 was only 19% lower than the 123 years average. The drought conditions of 2012 were alleviated by a return to normal rainfall in early 2013 and more moderate temperatures the following summer.

DISCUSSION

Our sampling reveals that insect abundance on Missouri oak trees varies dramatically over relatively short periods of time. From 1991 to 2007 (the highest and lowest sampled years, **Figure 1**) in southeastern Missouri, the range in numbers of insects per plant was 645-fold on *Quercus alba* and 890-fold on *Q. velutina*. Part of this variation was due to declines brought on by mid spring frosts that killed large numbers of young leaves. These frosts reduced both spring and summer feeding insects despite the re-flush of new leaves within a relatively short period of time. In addition, at a more local site in east central Missouri, leaf ties made by leaf tying Lepidoptera all but disappeared following an extreme summer drought the previous year (**Figure 3**). Subsequent to the spring freezes and summer drought, insect populations recovered, taking 1–5 years (or more following the 2007 freeze event) depending on the oak species and the weather event. The fact that these populations did recover and that initial declines were coincident with these weather events suggests that the declines were due to the weather events themselves and not to some unrecognized human-driven cause. Much of the southern half of Missouri is contiguous forest, the majority of which is mature second growth oak-hickory forest (Spencer et al., 1992). In addition, the study sites are contiguous with a portion of the Mark Twain National Forest, which contains 708 km^2 of forest. Ninety percent of the 2,600 ha of Cuivre River State Park is in forest cover, and although the park is surrounded by agricultural land, the study sites were about 2 km from the nearest park boundaries, making it unlikely that insect declines were due to the drift of sprayed insecticides from neighboring agricultural lands (Hallmann et al., 2017).

The immediate effect of April frost on the spring fauna was most likely a loss of food, as opposed to a direct effect of freezing on the insects themselves, although early instar larvae could be susceptible. At a location near St. Louis (the Tyson Research Center), a population of fall cankerworm, *Alsophila pometaria* (Geometridae), had been increasing over the years prior to 2007, but was decimated by the spring freeze. Caterpillars of *A. pometaria* were seen searching for food on twigs containing only frozen leaves (Marquis et al., 2019). The mechanism underlying recorded impacts of the spring freeze on the summer fauna is less clear. Perhaps overwintering pupae were sensitive to the abnormally low temperatures during a time when they were close to breaking diapause or quality of re-flush leaves was low (Rooke and Bergström, 2007). Whatever the cause, the more rapid recovery of the summer fauna (relative to the spring fauna) subsequent to the 2007 freezing event could be due in part to the much higher numbers of multivoltine species comprising the summer assemblage, as almost all of the spring fauna is univoltine.

One of the hypothesized advantages of building shelters by insects is protection against abiotic factors (Lill and Marquis, 2007). For example, low humidity has been experimentally shown to kill leaf shelter building caterpillars removed from their shelters (Hunter and Wilmer, 1989). If indeed leaf shelter construction routinely buffers desiccation prone inhabitants from stress, the high temperatures and low humidity associated with the extreme drought of 2012 may have surpassed their ability to do so. The loss of ties could have triggered declines in other species, given that the ties are secondarily colonized by an array of other arthropod species, including most of the other species of leaf tying caterpillars (e.g., Marquis and Lill, 2010). There is some evidence that leaf ties on *Q. alba* provide refuge for the Asiatic oak weevil, *Cyrtepistomus castaneus,* during years of

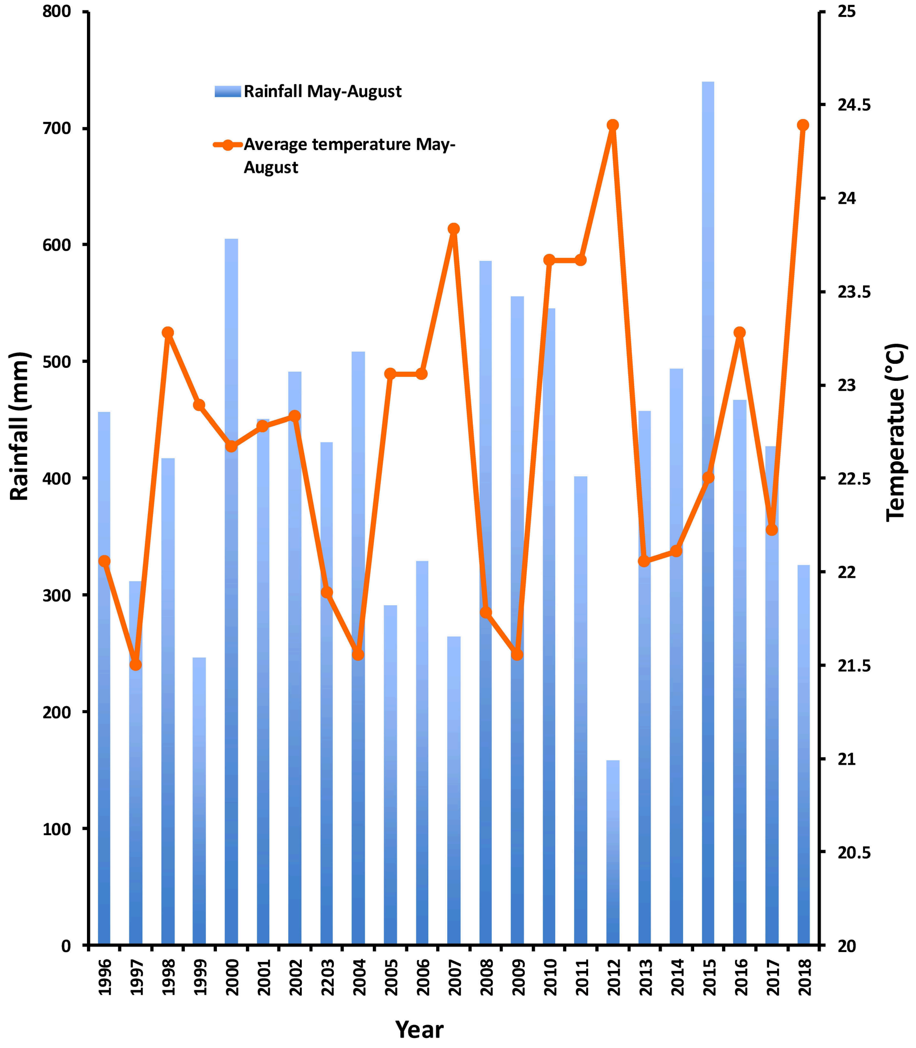


FIGURE 4 | Total rainfall and mean daily temperature for the months May-August over the years 1996–2018 for the Troy, MO, weather station (https://www.ncei.noaa.gov), the closest weather station to Cuivre River State Park (∼6.6 km).

low abundance (Lill and Marquis, 2003). The fact that smaller-bodied species were more heavily affected, and that Limacodidae, which generally do not secondarily occupy leaf ties, continued to be exceedingly low in abundance 5 years after the drought strongly suggests that temperature and low humidity together were lethal. We saw no evidence of high abundances of either *Lochmaeus manteo* or *Cecrita* (*Heterocampa*) *guttivitta* (both Notodontidae) subsequent to the 2012 drought, at least in the St. Louis region. Drought has been suggested to trigger outbreaks in both species (Law and Gott, 1987; Martinet and Allen, 1987, respectively). Perhaps multiple years of drought are required to trigger such outbreaks.

The high abundance of oak feeding insects seen in our 1991 sampling in MOFEP, and less so in 1992, may be an example of a coincidence of high populations of many spring feeding species. These spring feeding species are called the "leaf roller and looper complex" in numerous pest reports by the Missouri Department of Conservation produced over the period 1968–1980 (e.g., Gass, 1973). In 1991, there were extremely high numbers of Geometridae, particularly *Phigalia strigataria*, various Totricidae, including *Cenopis pettitana*, *Pseudexentera faracana,* and *P. spoliana*, and Gelechiidae, particularly *Chionodes adamas* and *C. pereyra* (Gelechiidae) (R. J. Marquis and J. Whitfield, unpublished data). Similar outbreaks of several species occurred in the St. Louis region in 2013, resulting in patches of nearly complete defoliation of canopy trees (R. J. Marquis, pers. obs.). Such events are also reported from Virginia (Asaro and Chamberlin, 2015). Law and Gott (1987) suggest that such outbreaks are triggered by extended droughts in previous years. A sharp decline in abundance in 1993 from the previous high levels in years 1991–1992 occurred for insects on both oak species, but particularly on *Q. alba* (**Figure 1**). Unlike the declines in 1997 and 2007, daily high temperatures in March 1993 only once went above 20°C, and only once below 0°C (**Figure 2A**). Instead of climatic factors, avian and arthropod predators and parasitoids might have played a role. Both predation of caterpillars by birds (Marquis and Whelan, 1994) and parasitism of caterpillars (Le Corff et al., 2000) can be high in the Missouri Ozarks.

Given the observed declines in insect numbers at many locations across the globe (Sanchez-Bayo and Wyckhuys, 2019), one might be tempted to make the same conclusion from our data (**Figure 1**). At least superficially, there seems to be an overall trend of a modest decreasing abundance from 1991 to 2010 on both *Q. alba* and *Q. velutina*. At least for these faunas on Missouri oaks, however, one must recognize that local weather events have dramatic effects on insect numbers. These weather events cause declines, which are then followed by rebounds. Continuous sampling for a few years, interspersed with large numbers of years in which no data were gathered (e.g., Lister and Garcia, 2018), could mask these important dynamics as evidenced by the current study. Moreover, the impacts of these local weather events must be understood in order that they might not be mistaken for the effects of humans on insects, perhaps via habitat destruction or climate change. Continuous data are very important. The low numbers in 1997 might be interpreted as a part of a general decline from 1991 to 1995, particularly for *Q. alba* (**Figure 1A**). Missing data from 1996 call into question whether numbers in 1997 were low due to the spring freeze in 1997 or were already low in 1996. Fortunately, our data captured a second freeze event in 2007, showing a dramatic decline in May 2007 numbers compared to May 2006 for both host plant species (**Figure 1**). Furthermore, temperatures did not go below 0°C in either April or May 1996 at any of the weather stations in the region.

The picture that emerges for our study sites in southeastern Missouri is that there is little evidence that overall abundances are declining during the years 1991–2010. In fact, there is a low likelihood for humans to have caused such changes in the southern half of Missouri, in the form of habitat destruction and pesticides, because forest cover is so continuous and widespread. Impacts of climate change are a possibility, but the data presented here show no overall decline. In fact, just the opposite seems to be the case. Our sampling in a chronosequence suggests that insect abundance and species richness are only increasing as these forests continue to age following widespread deforestation in the late 1800's and early 1900's (Jeffries et al., 2006).

Although we do not see evidence for a decline in abundance over the years studied, we suggest two cautionary notes regarding future climate impacts on herbivorous insect faunas in north temperate regions. First, evidence suggests that the likelihood of mid spring frosts has increased over the last 100+ years in nearby Illinois (Augspurger, 2013) and is only expected to increase in the future (Gu et al., 2008). In addition because of warmer winters, plants are likely to be more susceptible to these frosts when they do occur (Gu et al., 2008). Finally, different plant species appear to be differentially susceptible to the same frost event (e.g., oaks more than sugar maple) (Gu et al., 2008; Augspurger, 2013; Muffler et al., 2016). Thus, the faunas on oaks may be more susceptible than those found on other host plant species.

The second cautionary note is that climate in the Midwest of the U.S. is predicted to become warmer and drier in the summer months in the near future (Hayhoe et al., 2018). Based on our results from Cuivre River State Park, we predict that drought will decrease populations of insects both directly because of high temperatures, low humidity, and low host plant water content, and indirectly by disrupting positive interactions involving shelter building insects, and the secondary inhabitants of those shelters.

In light of these predictions for future climate change, our results suggest mid spring frosts and summer droughts of magnitudes high enough to negatively affect insect abundance on oaks in our region are likely to increase, ultimately leading to long term declines. In addition, insect declines associated with both mid spring frosts and summer droughts are likely to have bottom up effects on the natural enemies that simultaneously use those insects as a food resource and serve to control their populations in more typical years. The magnitude of such bottom up effects on the third trophic level is unknown at this time.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

RM and RF conducted the statistical analyses. RM wrote the original manuscript draft. JTL, RF, JL, JML, and JW edited subsequent drafts. All authors helped with data collection.

FUNDING

This work was supported by the Missouri Department of Conservation, USDA Grant No. 02DG 1124225 430, and National Science Foundation grant 6164397.

ACKNOWLEDGMENTS

We thank numerous undergrad and graduate students at the University of Missouri-St. Louis for help with insect sampling. In addition to the many students, we thank F. DiTirro, N. Greig, J. McGrath, and G. Wang who all helped with data collection. R. Jensen helped with logistics on the ground in MOFEP, and B. Schuette did the same at CRSP. Patrick Guinan provided information on the NCEI climate data base, and V. Redensek and S. Bahar provided many helpful suggestions regarding climate data analysis. The Missouri Department of Conservation, the USDA, and the National Science Foundation provided funding. The Missouri Department of Natural Resources provided access to Cuivre River State Park, and Washington University gave us access to the Tyson Research Center.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00396/full#supplementary-material

REFERENCES

Asaro, N., and Chamberlin, L. A. (2015). Outbreak history (1953-2014) of spring defoliators impacting oak-dominated forests in Virginia, with emphasis on gypsy moth (*Lymantria dispar* L.) and fall cankerworm (*Alsophila pometaria* Harris). *Amer. Entomol.* 61, 174–185. doi: 10.1093/ae/tmv043

Augspurger, C. K. (2013). Reconstructing patterns of temperature, phenology, and frost damage over 124 years: spring damage is increasing. *Ecology* 94, 41–50. doi: 10.1890/12-0200.1

Baer, C. S., and Marquis, R. J. (2014). Native leaf-tying caterpillars influence host plant use by the invasive Asiatic oak weevil through ecosystem engineering. *Ecology* 95, 1472–1478. doi: 10.1890/13-1689.1

Bale, J. S. (1996). Insect cold hardiness: a matter of life and death. *Eur. J. Ent.* 93, 369–382.

Barbosa, P., Letourneau, D. K., and Agrawal, A. A. (2012). *Insect Outbreaks Revisited*. Chichester, UK: J. Wiley-Blackwell.

Barbosa, P., and Schultz, J. C. (1987). *Insect Outbreaks*. San Diego, CA: Academic Press.

Brookshire, B. L., Jensen, R., and Dey, D. C. (1997). "The Missouri ozark forest ecosystem project: past, present, and future," in *Proceedings of the Missouri Ozark Forest Ecosystem Project Symposium: An Experimental Approach to Landscape Research, St. Louis, MO, General Technical Report NC-193*, eds B. L. Brookshire, and S. Shifley (Saint Paul, MN: USDA Forest Service; North Central Forest Experiment Station), 1–25.

Ehrlich, P. R., Murphy, D. D., Singer, M. C., Sherwood, C. B., White, R. R., and Brown, I. L. (1980). Extinction, reduction, stability and increase: the responses of checkerspot butterfly (*Euphydryas*) populations to the California drought. *Oecologia* 46, 101–105. doi: 10.1007/BF00346973

Epstein, M. E. (1996). *Revision and Phylogeny of the Limacodid-group Families, with Evolutionary Studies on Slug Caterpillars (Lepidoptera: Zygaenoidea)*. Smithsonian Contributions to Zoology 582. Washington, DC: Smithsonian Institution Press.

Forkner, R. E., and Marquis, R. J. (2004). Uneven-aged and even-aged logging alter foliar phenolics of oak trees remaining in forested habitat matrix. *For. Ecol. Manage.* 199, 21–37. doi: 10.1016/j.foreco.2004.03.044

Forkner, R. E., Marquis, R. J., Lill, J. T., and Le Corff, J. (2006). Impacts of forest management on oak-feeding caterpillar populations in the Missouri Ozarks. *Cons. Biol.* 20, 429–440. doi: 10.1111/j.1523-1739.2006.00346.x

Forkner, R. E., Marquis, R. J., Lill, J. T., and Le Corff, J. (2008). Timing is everything? Phenological synchrony and population variability in leaf-chewing herbivores of *Quercus*. *Ecol. Entomol.* 33, 276–285. doi: 10.1111/j.1365-2311.2007.00976.x

Gass, R. D. (1971). *Life History and Ecology of the Variable Oak Leaf Caterpillar, Heterocampa manteo (Dbldy.), in Missouri (Lepidoptera: Notodontidae)*. Jefferson City, MO: Missouri Department of Conservation.

Gass, R. D. (1973). *Missouri Forest Pest Report*. Jefferson City, MO: Missouri Department of Conservation.

Gu, L., Hanson, P. J., Post, W. M., Kaiser, D. P., Yang, B., Nemani, R., et al. (2008). The 2007 eastern U. S. spring freeze: increased cold damage in a warming world? *Bioscience* 58, 253–262. doi: 10.1641/B580311

Hallmann, C. A., Sorg, M., Jongejans, E., Siepe, H., Hofland, N., Schwan, H., et al. (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS ONE* 12:e0185809. doi: 10.1371/journal.pone.0185809

Hayhoe, K., Wuebbles, D. J., Easterling, D. R., Fahey, D. W., Doherty, S., Kossin, J., et al. (2018). "Our changing climate," in *Impacts, Risks, and Adaptation in the United States: Fourth National Climate Assessment, Vol II*, eds D. R. Reidmiller, C. W. Avery, D. R. Easterling, K. E. Kunkel, K. L. M. Lewis, T. K. Maycock, and B. C. Stewar (Washington, DC: U.S. Global Change Research Program), 72–144.

Hunter, A. F. (1991). Traits that distinguish outbreaking and nonoutbreaking Macrolepidoptera feeding on northern hardwood trees. *Oikos* 60, 275–282.

Hunter, M. D., and Wilmer, P. G. (1989). The potential for interspecific competition between two abundant defoliators on oak: leaf damage and habitat quality. *Ecol. Ent.* 12, 373–382. doi: 10.1111/j.1365-2311.1987.tb01018.x

Jeffries, J., Marquis, R. J., and Forkner, R. E. (2006). Community composition, species richness, and abundance of oak herbivore insects in a chronoseries of temperate forest. *Ecol. Appl.* 16, 901–912. doi: 10.1890/1051-0761(2006)016[0901:FAIOIH]2.0.CO;2

Kærsgaard, C. W., Holmstrup, M., Malte, H., and Bayley, M. (2004). The importance of cuticular permeability, osmolyte production and body size for the desiccation resistance of nine species of Collembola. *J. Insect Phys.* 50, 5–15. doi: 10.1016/j.jinsphys.2003.09.003

Kolb, T. E., Fettig, C. J., Ayres, M. P., Bentz, B. J., Hicke, J. A., Mathiasen, R., et al. (2016). Observed and anticipated impacts of drought on forest insects and diseases in the United States. *For. Ecol. Manag.* 380, 321–334. doi: 10.1016/j.foreco.2016.04.051

Koricheva, J., Klapwijk, M. J., and Bjorkman, C. (2012). "Life history traits and host plant use in defoliators and bark beetles: implications for population dynamics," in *Insect Outbreaks Revisited*, eds P. D. Barbosa, D. K. Letourneau, and A. A. Agrawal (Chichester, UK: J. Wiley-Blackwell), 177–196.

Law, J. R., and Gott, J. D. (1987). *Oak Mortality in the Missouri Ozarks*. Knoxville, TN: Sixth Central Hardwood Forest Conference.

Le Corff, J., Marquis, R. J., and Whitfield, J. B. (2000). Temporal and spatial variation in a parasitoid community associated with the herbivores that feed on Missouri *Quercus*. *Env. Entomol.* 24, 46–58. doi: 10.1603/0046-225X(2000)029[0181:TASVIA]2.0.CO;2

Le Lagadec, M. D., Chown, S. L., and Scholtz, C. H. (1998). Desiccation resistance and water balance in southern African keratin beetles (Coleoptera, Trogidae): the influence of body size and habitat. *J. Comp. Phys. B* 168, 112–122. doi: 10.1007/s003600050127

LeRoux, E. J., Paradis, R. O., and Hudon, M. (1963). Major mortality factors in the population dynamics of the eye-spotted bud moth, the pistol casebearer, the fruit-tree leaf roller, and the European corn borer in Quebec. *Mem. Entomol. Soc. Canada* 95, 67–92. doi: 10.4039/entm9532067-1

Lill, J. T. (2004). Seasonal dynamics of leaf-tying caterpillars on white oak. *J. Lep. Soc*. 58, 1–6.

Lill, J. T., and Marquis, R. J. (2003). Ecosystem engineering by caterpillars increases insect herbivore density on white oak. *Ecology* 84, 682–690. doi: 10.1890/0012-9658(2003)084[0682:EEBCII]2.0.CO;2

Lill, J. T., and Marquis, R. J. (2007). "Microhabitat manipulation: ecosystem engineering by shelter-building insects," in *Ecosystem Engineers: Concepts, Theory, and Applications in Ecology*, eds K. M. D. Cuddington, J. E. Byers, A. Hastings, and W. G. Wilson (San Diego, CA: Elsevier Press), 107–138.

Lill, J. T., Marquis, R. J., Forkner, R. E., Le Corff, J., Holmberg, N., and Barber, N. A. (2006). Leaf pubescence affects distribution and abundance of generalist slug caterpillars (Lepidoptera: Limacodidae). *Environ. Ent.* 35, 797–806. doi: 10.1603/0046-225X-35.3.797

Lister, B. C., and Garcia, A. (2018). Climate-driven declines in arthropod abundance restructure a rainforest food web. *Proc. Natl. Acad. Sci. U.S.A.* 115, E10397–E10406. doi: 10.1073/pnas.1722477115

Marquis, R. J., and Le Corff, J. (1997). "Estimating pre-treatment variation in the oak leaf-chewing insect fauna of the Missouri ozark forest ecosystem project," in *Proceedings of the Missouri Ozark Forest Ecosystem Project Symposium: An Experimental Approach to Landscape Research*, eds B. L. Brookshire, and S. R. Shifley (St. Paul, MN: USDA Forest Service, North Central Forest Experiment Station. General Technical Report NC-193), 332–346.

Marquis, R. J., and Lill, J. T. (2010). Impact of plant architecture versus leaf quality on attack by leaftying caterpillars on five oak species. *Oecologia* 163, 203–213. doi: 10.1007/s00442-009-1519-2

Marquis, R. J., Passoa, S. C., Lill, J. T., Whitfield, J. B., Le Corff, J., Forkner, R. E., et al. (2019). *An Introduction to the Immature Lepidopteran Fauna of Oaks in Missouri.* Morgantown, WV: USDA Forest Service, Forest Health Assessment and Applied Sciences Team, FHAAST-2018–T-2005.

Marquis, R. J., and Whelan, C. J. (1994). Insectivorous birds increase plant growth through their impact on herbivore communities of white oak. *Ecology* 75, 2007–2014. doi: 10.2307/1941605

Martinet, P. J., and Allen, D. C. (1987). Relationship between outbreaks of saddled prominent, *Heterocampa guttivitta* (Lepidoptera: Notodontidae), and drought. *Environ. Entomol.* 16: 246–249. doi: 10.1093/ee/16.1.246

Mason, R. R. (1987). "Nonoutbreak species of forest Lepidoptera," in *Insect Outbreaks*, eds P. Barbosa, and J. C. Schultz (San Diego, CA: Academic Press), 31–58.

Mattson, W. J., and Haack, R. A. (1987). The role of drought in outbreaks of plant-eating insects. *Bioscience* 37, 110–118. doi: 10.2307/1310365

Morris, R. F. (1964). The value of historical data in population research, with particular reference to *Hyphantria cunea* Drury. *Can. Ent.* 96, 356–368. doi: 10.4039/Ent96356-1

Muffler, L., Beierkuhnlein, C., Aas, G., Jentsch, A., Scheiger, A. H., Zohner, C., et al. (2016). Distribution ranges and spring phenology explain late frost sensitivity in 170 woody plants from the Northern Hemisphere. *Glob. Ecol. Biogeogr.* 25, 1061–1071. doi: 10.1111/geb.12466

Nothnagle, P. J., and Schultz, J. C. (1987). "What is a forest pest?," in *Insect Outbreaks*, eds P. Barbosa, and J. C. Schultz (San Diego, CA: Academic Press), 59–80.

Philip, B. N., Yi, S. X., Elnitsky, M. A., and Lee, R. E. (2008). Aquaporins play a role in desiccation and freeze tolerance in larvae of the goldenrod gall fly, *Eurosta solidaginis*. *J. Exp. Biol.* 211, 1114–1119. doi: 10.1242/jeb.016758

Reinhardt, J. R. (2011). *Ecosystem engineering and environmental context: the impact of leaftying caterpillars and foliage quality on the arthropod community associated with white oak (Quercus alba, L.)* (masters thesis). St. Louis, MO: University of Missouri-St. Louis.

Rippey, B. R. (2015). The, U. S. drought of 2012. *Weather Climate Extreme* 10, 57–64. doi: 10.1016/j.wace.2015.10.004

Rooke, T., and Bergström, R. (2007). Growth, chemical responses and herbivory after simulated leaf browsing in *Combretum apiculatum*. *Plant Ecol.* 189, 201–212. doi: 10.1007/s11258-006-9177-5

Royama, T. (1997). "Population dynamics of forest insects: are they governed by single or multiple factors?," in *Forests and Insects*, eds A. D. Watt, N. E. Stork, and M. D. Hunter (London: Chapman and Hall), 37–48.

Sanchez-Bayo, R., and Wyckhuys, K. A. G. (2019). Worldwide decline of the entomofauna: a review of its drivers. *Biol. Cons.* 232, 8–27. doi: 10.1016/j.biocon.2019.01.020

Sheriff, S. L., and He, Z. (1997). "The experimental design of the Missouri Ozark Forest Ecosystem Project," in *Proceedings of the Missouri Ozark Forest Ecosystem Project Symposium: An Experimental Approach to Landscape Research, St. Louis, MO, June 3–5. General Technical Report NC-193*, eds B. L. Brookshire, and S. Shifley (St. Paul, MN: USDA Forest Service, North Central Forest Experiment Station), 26–40.

Sinclair, B. J., Ferguson, L. V., Salehipour-shirazi, G., and MacMillan, H. A. (2013). Cross-tolerance and cross-talk in the cold: relating low temperatures to desiccation and immune stress in insects. *Integrat. Comp. Biol.* 53, 545–556. doi: 10.1093/icb/ict004

Spencer, J. S. Jr., Roussopoulos, S. M., and Massengale, R. A. (1992). *Missouri's Forest Resource, 1989: An Analysis*. St. Paul, MN: USDA Forest Service, North Central Forest Experiment Station, NC-139.

Story, M., and Congalton, R. G. (1986). Accuracy assessment: a user's perspective. *Photogr. Eng. Remote Sens.* 52, 397–399.

Summerville, K. S., and Marquis, R. J. (2016). Comparing the responses of larval and adult Lepidopteran communities to timber harvest using long-term, landscape-scale studies in oak-hickory forests. *For. Ecol. Manag.* 387, 64–72. doi: 10.1016/j.foreco.2016.08.050

Torres, J. A. (1992). Lepidoptera outbreaks in response to successional changes after the passage of Hurricane Hugo in Puerto Rico. *J. Trop. Ecol.* 8, 285–298. doi: 10.1017/S0266467400006544

Wang, H. G., Marquis, R. J., and Baer, C. S. (2012). Both host plant and ecosystem engineer identity influence leaf-tie impacts on the arthropod community of *Quercus*. *Ecology* 93, 2186–2197. doi: 10.1890/11-1838.1

Willmer, P. G. (1980). The effects of a fluctuating environment on the water relations of larval Lepidoptera. *Ecol. Ent.* 5, 271–292. doi: 10.1111/j.1365-2311.1980.tb01150.x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 26 November 2019
doi: 10.3389/fevo.2019.00452

Bottom-Up and Top-Down Effects of Forest Fragmentation Differ Between Dietary Generalist and Specialist Caterpillars

Riley M. Anderson[1]*, Nicole M. Dallar[1], Nikki L. Pirtel[2], Christian J. Connors[2], James Mickley[2], Robert Bagchi[2] and Michael S. Singer[1]

[1] Department of Biology, Wesleyan University, Middletown, CT, United States, [2] Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT, United States

OPEN ACCESS

Edited by:
Lora A. Richards,
University of Nevada, Reno,
United States

Reviewed by:
Freerk Molleman,
Adam Mickiewicz University, Poland
John Thomas Lill,
George Washington University,
United States

***Correspondence:**
Riley M. Anderson
rmanderson@wesleyan.edu

Specialty section:
This article was submitted to
Population and Evolutionary
Dynamics,
a section of the journal
Frontiers in Ecology and Evolution

Received: 28 February 2019
Accepted: 11 November 2019
Published: 26 November 2019

Citation:
Anderson RM, Dallar NM, Pirtel NL, Connors CJ, Mickley J, Bagchi R and Singer MS (2019) Bottom-Up and Top-Down Effects of Forest Fragmentation Differ Between Dietary Generalist and Specialist Caterpillars. Front. Ecol. Evol. 7:452. doi: 10.3389/fevo.2019.00452

Ecological interactions increasingly occur in the context of anthropogenic landscape alteration, such as landscape fragmentation, which engenders numerous changes to abiotic and biotic processes. Theory and empirical evidence suggest that species that are ecologically specialized or positioned at higher trophic levels are most sensitive to the effects of landscape fragmentation, yet the mechanisms underlying this sensitivity remain hypothetical. Here we present an initial test of the hypothesis that landscape fragmentation affects tri-trophic interactions more severely for dietary specialist than for generalist insect herbivores. We specifically tested a bottom-up hypothesis, that fragmentation reduces hostplant food quality to herbivores, with the most pronounced effects for dietary specialists. We also tested a top-down hypothesis, that fragmentation reduces parasitism of caterpillars, with the most pronounced effects on dietary specialists. We studied interactions among trees, caterpillars, and parasitoids in forest fragments (3–1013 ha) in Connecticut, U.S.A. in 2017 and 2018. To address the bottom-up hypothesis, we measured the growth performance of multiple dietary specialist and generalist caterpillar species fed leaves from red maple and witch hazel trees sampled from large or small forest patches. We assessed the top-down hypothesis by quantifying mortality from insect parasitoids of many of the same caterpillar species on these tree species sampled from a range of forest patch sizes. Fragmentation did not reduce herbivore growth performance for either dietary specialist or generalist caterpillars even though it accelerated desiccation rates of leaves of both tree species over the growing season. However, dietary specialization increased herbivore sensitivity to yearly variation in food quality, as the growth efficiency of specialist caterpillars varied between 2017 and 2018, while that of generalist caterpillars did not. As predicted, parasitism rates of dietary specialist caterpillars declined as forest fragment size declined, whereas parasitism of generalists was independent of fragment size. Therefore, interactions involving dietary specialist herbivores were most sensitive to top-down environmental variation. Assuming that dietary specialist caterpillars are primarily attacked by host-specific parasitoids, this top-down effect is consistent with the trophic theory of island biogeography, which predicts the sharpest declines of ecologically specialized predators and parasites in small habitat patches.

Keywords: habitat modification, herbivore performance, hostplant quality, Lepidoptera, parasitoid, tri-trophic interactions

INTRODUCTION

The anthropogenic fragmentation of landscapes has the potential to dramatically alter species interactions (Aizen et al., 2012; Martinson and Fagan, 2014; Morante-Filho et al., 2016). The mechanisms by which species interactions may be altered by landscape fragmentation are numerous (Bagchi et al., 2018), complex (Murphy et al., 2016), and often context-dependent (Martinson and Fagan, 2014; Pfeifer et al., 2017). Community responses to landscape fragmentation are variable (Fáveri et al., 2008) and may be associated with differences in species traits such as dispersal ability (Roland and Taylor, 1997; Öckinger et al., 2010), physiological tolerance to extreme or variable abiotic conditions (Fourcade et al., 2017), or the ability to consume multiple resource types when resources are limited or absent (i.e., broad diet breadth) (Öckinger et al., 2010). That is, landscape fragmentation may differentially affect species and their interactions, and the community differences may be attributed to specific traits of the interactors.

Two theoretical frameworks seek to explain how landscape fragmentation changes communities. The trophic theory of island biogeography offers the most general and clear predictions. This framework proposes that area and isolation effects will exclude species that are ecologically specialized in some dimension relative to their generalist counterparts in the same community (Holt, 2010; Gravel et al., 2011). Additionally, species that are positioned at higher trophic levels, or have large resource requirements may be especially sensitive to the effects of landscape fragmentation (Kruess and Tscharntke, 1994; Holt et al., 1999; Gravel et al., 2011). Although there are grounds for both theoretical predictions, empirical evidence is still sparse.

The edge resource model is an alternative theoretical perspective that focuses on species responses to resource availability along habitat edges rather than area and isolation effects (Ries and Sisk, 2004; Ries et al., 2004). This model suggests that because of altered or variable abiotic and biotic conditions at habitat edges, resources will be spatially patchy or reduced in quality. One general prediction from this hypothesis is that habitat specialist species will tend to have negative responses to edges, whereas habitat generalists will have neutral or positive responses (Ries et al., 2004; Murphy et al., 2016). Although there is some empirical evidence for this prediction, this model has received limited testing (Ries et al., 2004; Wimp et al., 2011; Murphy et al., 2016).

As a major component of many fragmented terrestrial ecosystems, insect herbivores provide an opportunity to test alternative hypotheses that predict how species with contrasting levels of ecological specialization respond to landscape fragmentation. As insect herbivore population sizes are shaped by both their resources and the abundance and composition of predator communities (Vidal and Murphy, 2018), it is necessary to consider how landscape fragmentation may alter both the bottom-up (resource driven) and top-down (predator-driven) mechanisms (Murphy et al., 2016; Bagchi et al., 2018).

Bottom-Up Mechanisms

Landscape fragmentation may alter the availability of hostplant species through neutral sampling processes (Connor and McCoy, 1979), or by island biogeographic processes (MacArthur and Wilson, 1967; Hanski, 1999). As the ability for herbivores to locate specific hosts decreases in fragmented landscapes (Brown and Crone, 2016), limited hostplant availability may force insect herbivores to use alternative or suboptimal hosts, including hosts of the same or different species (Fox and Morrow, 1981), which may alter herbivore performance (Gripenberg et al., 2010).

Even when host plants are not limiting, landscape fragmentation may directly alter plant-herbivore interactions by acting at the plant phenotypic level. However, the extent to which landscape fragmentation may modify intraspecific plant phenotypic variation in anti-herbivore resistance traits or overall palatability is poorly understood (Bagchi et al., 2018). One hypothesis is that landscape fragmentation may alter the abiotic and biotic drivers of phenotypic variation in plant traits that determine food quality for herbivores. In small habitat patches, plant inbreeding may occur more frequently as population sizes diminish (Honnay et al., 2005; Leimu et al., 2010). Inbred plants may have altered nutritional quality and defensive responses (Ridley et al., 2011; Kittelson et al., 2015). Therefore, plant inbreeding has the potential to alter herbivore performance (Hull-Sanders and Eubanks, 2005).

The edge effects associated with landscape fragmentation may directly alter abiotic conditions that mediate intraspecific hostplant quality. Landscape fragmentation often adds new edges and increases the proportion of edge-to-interior habitat, intensifying edge effects in small, heavily fragmented landscapes (Ries and Sisk, 2004; Briant et al., 2010; Tuff et al., 2016). Fragment edges are often warmer, drier, more climatically variable, and have higher photosynthetically active radiation (Chen et al., 1999; Ries and Sisk, 2004; Savilaakso et al., 2009; Tuff et al., 2016; Agosta et al., 2017). These altered abiotic conditions and increased variability have been shown to increase canopy desiccation (Briant et al., 2010) which may alter plant traits that contribute to food quality for insect herbivores (e.g., Fortin and Mauffette, 2001).

Experimentally manipulated abiotic conditions have been shown to alter plant traits with consequences for insect herbivore performance (Buse et al., 1998; Levesque et al., 2002; Barber and Marquis, 2011). Winter Moth (*Operophtera brumata*) caterpillars fed leaves from *Quercus robur* grown in elevated temperatures grew less efficiently than caterpillars fed leaves grown in ambient temperatures (Buse et al., 1998). Additionally, the leaves of the tropical plant, *Casearia nitida* (Salicaceae) were tougher, thicker, and drier in young forests where ambient conditions were hotter, drier, and more variable, relative to leaves from mature forests with cooler, less variable ambient conditions (Agosta et al., 2017). Moreover, when caterpillars of the saturniid moth, *Rothschildia lebeau*, were fed leaves from *Casearia nitida* grown in early successional tropical forests, their growth and development were reduced relative to caterpillars fed leaves from mature tropical forests (Agosta et al., 2017). These examples suggest that altered abiotic conditions may indirectly alter food quality for insect herbivores through changes in plant traits. However, increased temperature may also directly influence insect herbivore performance and consumption rates; no clear general patterns have emerged because insect herbivores vary widely in their responses (Lemoine et al., 2014). Despite the possible mechanisms described above, whether or how landscape fragmentation affects insect herbivore performance through changes in intraspecific hostplant quality is a neglected topic of research.

Top-Down Mechanisms

Predator and parasitoid populations are predicted to decline in fragmented landscapes as sensitivity to habitat patch size increases with trophic position (Holt et al., 1999; Gravel et al., 2011) and degree of habitat specialization (Gravel et al., 2011; Murphy et al., 2016). The loss of natural enemies in fragmented landscapes may release prey populations from top-down control (Kruess and Tscharntke, 1994; Anton et al., 2007; Schüepp et al., 2014). However, the extent to which insect herbivores are released from predation may depend on the enemy's traits such as dispersal and competitive ability, and diet breadth (Roland and Taylor, 1997; Cagnolo et al., 2009; Nieminen and van Nouhuys, 2017). Release from top-down control may also depend on the herbivore's diet breadth because existing evidence from insect communities suggests that specialist predators and natural enemies tend to target dietary specialist herbivore prey (Dyer and Gentry, 2002; Stireman and Singer, 2003; Zvereva and Kozlov, 2016), and generalist predators and natural enemies tend to target generalist herbivores (Dyer, 1995; Singer et al., 2014; Zvereva and Kozlov, 2016), although there are exceptions to this pattern. Specifically, ecologically specialized predators and parasitoids that are highly dependent on ecologically specialized prey will be most susceptible to local extinction in fragmented landscapes when their prey is either reduced in abundance or entirely absent (Holt, 1996; Wimp et al., 2011). Therefore, communities in fragmented landscapes are likely to be deficient in interactions between ecologically specialized insect herbivores and their specialized natural enemies (Bagchi et al., 2018). There are many examples of predator declines in fragmented landscapes (Kruess and Tscharntke, 1994; Anton et al., 2007; Schüepp et al., 2014; Pfeifer et al., 2017), but the degree to which prey species of contrasting diet breadth are released from top-down control in a fragmented context remains understudied.

Here we investigate bottom-up and top-down mechanisms by which forest fragmentation alters tri-trophic interactions of dietary specialist and generalist herbivores in the same order (Lepidoptera) and feeding guild (exophytic leaf chewers). Theory on bottom-up mechanisms suggests that forest fragmentation could affect intraspecific hostplant quality, with distinct effects on dietary specialist and generalist herbivores. Both the trophic theory of island biogeography and the edge resource model postulate that forest fragmentation will render its strongest negative effects on ecologically specialized predators and natural enemies (Gravel et al., 2011; Murphy et al., 2016). To test these predictions, we assess (1) the effect of forest fragmentation on intraspecific hostplant quality in terms of caterpillar performance (growth efficiency) and leaf water content (a measure of food quality) and (2) parasitism rates of caterpillars collected from forest fragments of varying sizes.

METHODS

Experimental Overview

We used a landscape-level experiment with manipulative and observational components to measure effects of forest fragmentation on intraspecific hostplant quality and caterpillar mortality due to parasitoids. Over the summers of 2017 and 2018, we collected caterpillars and leaves from temperate deciduous forest patches in Connecticut (U.S.A.) varying in size by two orders of magnitude. We compared leaf water content from plants grown in either small and heavily fragmented forests or large, continuous forests. Caterpillars were reared in laboratory environments to study their growth efficiency (performance in response to hostplant quality) on leaves collected from small and large forest patches. Additionally, laboratory-rearing also allowed us to quantify relative parasitism rates of caterpillars collected in forest patches of varying size.

Study Sites

Our study sites spanned a 3,500 km^2 area in central and eastern Connecticut, U.S.A., in which the landscape is composed of patches of Northeastern Coastal Forest (Olson et al., 2001) surrounded by a matrix of suburban and agricultural land (**Figure 1**). We took advantage of existing forest patches ranging in area from 2.93 to 1,013 ha (**Table 1**). Forest patches were selected in blocks, with each block containing 2–3 fragments of contrasting area so that each small forest fragment (<50 ha) was paired with a large forest fragment (>200 ha) and sometimes an additional medium forest fragment (50–200 ha). The inclusion of fragments of contrasting area within each block ensured that fragment sizes were spatially interspersed to avoid issues with spatial autocorrelation in the statistical analyses. Forest boundaries varied and included two-lane roads, major highways, powerline clearings, and residential areas. Many of the sites in the small area class are bordered by major edges such as roads, multi-lane highways, power-line clearings, residential development, or industrial or commercial facilities. Within each forest patch, we restricted our collecting to upland areas that were similar in forest composition, comprising primarily mature *Quercus alba* (white oak), *Quercus rubra* (red oak), *Acer*

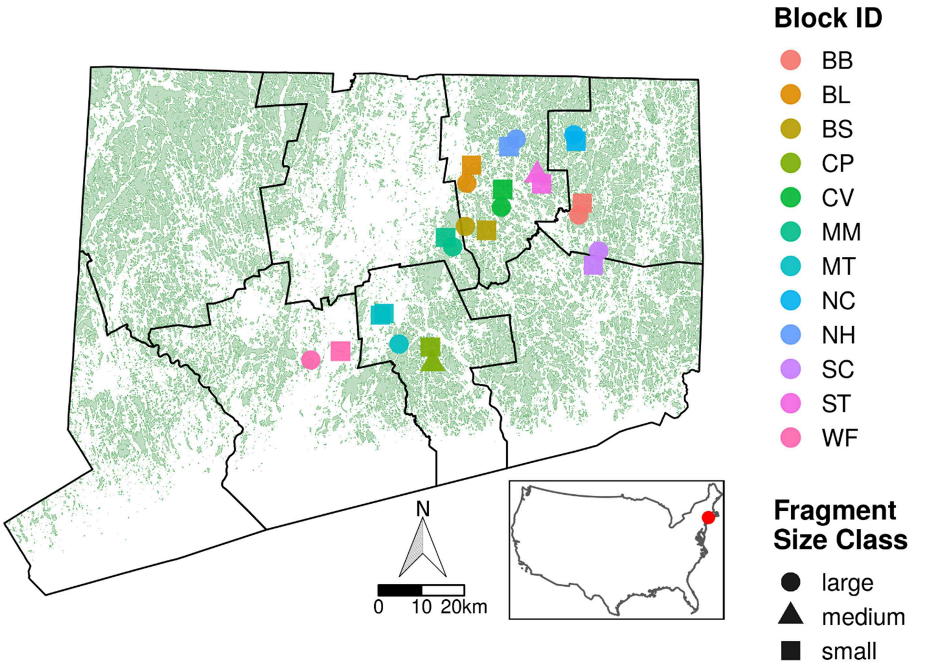


FIGURE 1 | Map of Connecticut with forest fragments in green, county boundaries in black, and inset showing the position of Connecticut within the United States. Superimposed on the map of Connecticut are our 25 study sites spanning a 3,500 km^2 area in east-central Connecticut. Experimental blocks are represented by different colors, and fragment area is indicated by shape. Large fragments are >201 ha, medium sites are 51–200 ha, and small sites are <50 ha. See **Table 1** for further site and block information.

rubrum (red maple), *Acer saccharum* (sugar maple), *Carya* spp. (hickories), and *Betula lenta* (black birch). Common woody understory species were *Hamamelis virginiana* (witch hazel), *Kalmia latifolia* (mountain laurel), *Ostrya virginiana* (American hophornbeam) and *Carpinus caroliniana* (American hornbeam). Plant communities were relatively homogeneous across sites. We did not collect data near patch edges, although both the small and large sites typically harbored more exotic plant species at the edge.

Plants

We studied growth performance and parasitism of caterpillars collected from *A. rubrum* and *H. virginiana* because these plant species were common in all forest sites and consistently harbored both dietary specialist and generalist caterpillars.

Caterpillars

Caterpillars of various larval stages were collected from *A. rubrum* and *H. virginiana* branches and saplings at 28 forest sites over the same 6-week period in June and early July of 2017 and 2018. In total, 882 caterpillars were collected, representing 49 species. We beat branches with a wooden dowel to shake caterpillars from branches onto a 1 m^2 canvas sheet (Wagner, 2005). All caterpillars >1 cm in length were collected and individually placed into 33 ml plastic vials with a leaf from the host plant on which they were found. Caterpillars were collected with equal sampling effort in each patch as part of a concurrent study investigating the strength of bird and ant predation in forest patches of varying size. The community composition of caterpillar species was similar across patches of varying size as the host plants used harbor a typical and consistent community of dietary specialist caterpillars that feed exclusively on either *A. rubrum* or *H. virginiana*, respectively, and dietary generalist caterpillars that feed on many of the hardwood species found in these forests. For caterpillar species with >5 individuals collected, 94% (16/17) of dietary generalist species and 100% (9/9) of dietary specialist species were found in both large and small patches.

To characterize the diet breadth of caterpillar species, we identified every caterpillar to the species level so that we could compile host plant records for each caterpillar species using our own data and published sources (Wagner et al., 2002, 2011; Wagner, 2005). Based on these hostplant records, we calculated pairwise phylogenetic distances between woody hosts of each caterpillar species using a large phylogeny of seed plants (Smith and Brown, 2018). The mean of these pairwise phylogenetic distances was then used as a quantitative measure of diet breadth for each caterpillar species (**Figure 2**). Based on this quantitative measure of diet breadth, the caterpillar community studied here appears to fit discretely into two groups (specialist or generalist). Caterpillars that entirely or predominantly consume plants from one taxonomic family were considered dietary specialists, whereas caterpillars that commonly consume plants from >1 plant family were considered dietary generalists (**Figure 2**). Therefore, we evaluate all analyses using the discrete diet breadth descriptors, "specialist" and "generalist" to make

TABLE 1 | Forest sites used varied in size by two orders of magnitude.

Block	Site name	Fragment category	Fragment size (ha)	Coordinates	Caterpillars collected for rearing
BB	Beaver Brook	Small	26.94257	41.766865N,−72.122551W	No
BB	Beaver Brook	Large	388.8101	41.742252N, −72.132301W	No
BL	Reservoir Rd	Small	12.82078	41.84557N, −72.429924W	No
BL	Valley Falls Park	Large	534.5746	41.808037N, −72.442978W	Yes
BS	Bishop Swamp	Small	10.96292	41.710239N, −72.388237W	No
BS	Gay City State Park	Large	532.4439	41.718437N, −72.446922W	No
CP	Hubbard Road	Small	2.973018	41.467499N, −72.544258W	Yes
CP	Cockaponset Forest	Large	206.4385	41.432442N, −72.538094W	Yes
CV	Lee Farm	Small	41.43562	41.7952N, −72.343071W	Yes
CV	Nathan Hale	Large	409.5277	41.758369N, −72.347W	No
MM	Goodale Hill	Small	99.03798	41.695019N, −72.5019W	Yes
MM	Meshomasic State Forest	Large	1013.98	41.675827N, −72.482056W	Yes
MT	Millers Pond	Large	416.2225	41.473549N, −72.631172W	Yes
MT	Wilbert Snow School	Small	11.70627	41.537201N, −72.673019W	Yes
MT	Wadsworth Falls State Park	Small	53.70021	41.533619N, −72.682961W	Yes
NC	Natchaug Summer	Small	56.02064	41.895432N, −72.13916W	No
NC	Natchaug Moon	Large	342.9047	41.908905N, −72.145622W	No
NH	Nye Holman State Forest	Small	6.224506	41.88443N, −72.326073W	No
NH	Nye Holman State Forest	Large	237.4603	41.900101N, −72.305763W	No
SC	Salt Rock State Forest	Small	28.89396	41.637264N, −72.091393W	No
SC	Mohegan State Forest	Large	659.6357	41.66748N, −72.076942W	No
ST	Whetton Woods Tract	Small	14.95767	41.806938N, −72.235321W	Yes
ST	Fenton Tract	Medium	194.7279	41.825401N, −72.247597W	No
WF	Choate Koller Env. Center	Small	8.640295	41.458382N, −72.793602W	Yes
WF	Sleeping Giant State Park	Large	419.1018	41.440399N, −72.876083W	Yes

Forest sites were classified as small (<50 ha), medium (50–200 ha), and large (>200 ha) fragments.

analyses and interpretation more straightforward. Small sample sizes of caterpillar species precluded comparative analyses at the caterpillar species level.

Experimental Methods

Herbivore Performance Assay of Hostplant Quality

To test the hypothesis that forest fragmentation may alter the intraspecific quality of host plants as food for caterpillars, we reared all field-collected caterpillars from both years under controlled conditions in a laboratory setting. We measured variation in food quality for each hostplant species as the variation in growth efficiency of these lab-reared caterpillars. Upon laboratory arrival, each caterpillar was placed individually in a 160 ml plastic cup with a lid and starved for 24 h prior to initial weighing to avoid weighing gut contents and to account for disparities in larval instar among caterpillars at the time of collection. After starvation, each caterpillar was weighed to the nearest 1 mg and was randomly assigned a diet treatment group of either leaves from small (<50 ha) or large (>200 ha) forests. Each caterpillar in the assay received leaves only from its randomly assigned treatment group and from the hostplant species on which it was collected. Each day, each caterpillar in the assay received new leaves from a batch of field-collected leaves (see below). Leaves used in the assay were stored for no more than 5 days. Five days of sealed storage at 4°C had no effect on leaf water content for either hostplant species (linear regression, $P_{storage.duration} > 0.05$, data not shown).

As a proxy for total food consumed, the fecal pellets of each caterpillar in the assay were collected daily and dried to constant mass at the end of the assay. Caterpillars completed the assay after pupating. Subsequently, pupae were sacrificed by overnight storage at −20°C, and then dried to constant mass. Many caterpillars brought to the laboratory for rearing either died from unknown causes ($N = 468$), pupated shortly after collection ($N = 25$), were parasitized ($N = 71$), or had incomplete data ($N = 31$) and were excluded from the assay. While none of the caterpillars that died from unknown causes had parasitoids emerge, it is possible that some of these individuals were parasitized. We did not dissect cadavers to confirm the cause of death. The proportion of caterpillars that died from unknown causes was similar among treatment groups ($\chi^2 = 0.01$, $df = 1$, $P = 0.91$). Similarly, whether caterpillars were collected from large or small forest fragments had little influence on unknown mortality ($\chi^2 = 1.71$, $df = 1$, $P = 0.19$).

Leaf Water Content

To test the hypothesis that forest fragmentation alters phenotypic variation in plant traits, we compared the water content of leaves of *A. rubrum* and *H. virginiana* collected from 21 forest sites ranging in size from 3 to 1,013 ha in the summer of 2018. Leaf water content is a reliable measure of food quality for folivores of temperate trees, as it often correlates positively with herbivore growth rate, leaf nitrogen, and overall palatability (Mattson and Scriber, 1987). Leaves were transported to the laboratory in sealed plastic bags kept in a cooler with ice. In the laboratory, leaves were stored in sealed plastic bags containing a damp paper towel and refrigerated at 4°C. Upon laboratory arrival, 10–13 randomly selected leaves from each host plant at each site were weighed to the nearest 1 mg. Leaves were then dried at 40°C for 1 week, or until stable dry masses were reached. Leaf water content was calculated as the ratio of water mass to fresh mass. Although leaves used for water content measurements were haphazardly selected, we intentionally avoided leaves that were not representative of the leaves that would be fed to caterpillars. That is, leaves with necrotic edges, browning, extreme size, or other anomalies were not used in either the leaf water content study or the performance assay.

Parasitoid Emergence

Throughout the performance assay, all caterpillars that had parasitoids emerge were removed from the assay and recorded. In 2018, we supplemented these data by separately rearing 189 caterpillars that were field-collected from 26 sites within the same fragmentation network used in the performance assay. Only caterpillars from *A. rubrum* and *H. virginiana* were collected. These supplemental caterpillars were individually reared in 160 ml plastic cups in similar laboratory conditions as the performance assay.

Data Analysis

All analyses were performed in R, version 3.5.1 (R Core Team., 2018).

Growth efficiency, quantified as pupal dry mass per unit dry fecal mass, was used as a measure of hostplant quality from the herbivores' perspective. Growth efficiency was quantified as the slope of the relationship between Log_{10} (dry pupal mass) and Log_{10} (dry fecal mass) (Singer, 2001). Growth efficiency is the amount of growth per unit plant material consumed, with fecal mass as a proxy for the amount of plant material consumed. Variation in this slope indicates variation in caterpillar growth efficiency and interactions between covariates (e.g., leaf origin and caterpillar diet breadth) and the relationship between dry fecal mass on dry pupal mass measure the covariates' effect on growth efficiency. We used a linear mixed-effects model (Bates et al., 2015) to analyze variation in Log_{10} (dry pupal mass) across individual caterpillars as a function of Log_{10} (dry fecal mass), leaf origin (small or large forest patches), the year the caterpillar was reared (2017 or 2018), hostplant species, and caterpillar diet breadth. To quantify how caterpillar growth efficiency varies between dietary generalists and specialists, we included a two-way interaction between diet breadth and Log_{10} (dry fecal mass). To determine whether this efficiency is modified by factors such as leaf origin and year, we also included each of these factors in a three-way interaction with diet breadth and Log_{10} (dry fecal mass). We allowed the intercept and effect of caterpillar species to vary among individuals as normally-distributed random effects (random intercept and slope model) to account for the variation among species in both Log_{10} (dry pupal mass) and growth efficiency. In preliminary analyses, the initial mass of the caterpillars was added as covariate. However, this term did not modify growth efficiency in any iteration of the model and was subsequently dropped from further analyses. The caterpillars in this experiment were fed leaves from small or large forest patches from multiple blocks. As a result, we cannot separate the variance associated with block from the error variance.

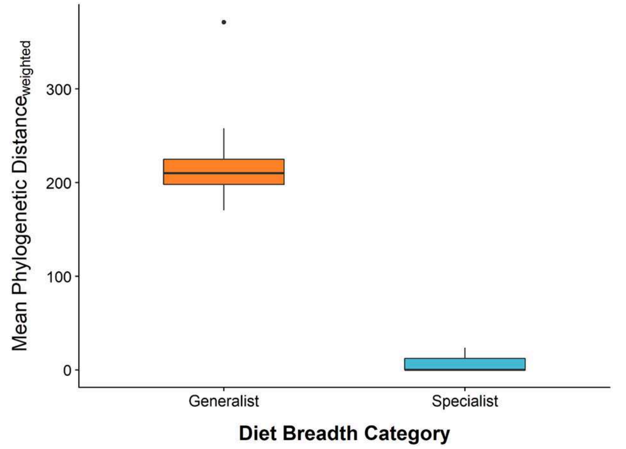


FIGURE 2 | A comparison of each caterpillar species' continuous measure of diet breadth (mean phylogenetic distance among hostplant species) and categorically defined specialist and generalist subsets of the caterpillar community. Horizontal bars represent the median, box edges represent the 1st and 3rd interquartile range, whisker tips represent the minimum and maximum, and points are outliers. Outliers represent caterpillar species with small sample sizes.

To quantify the effect of forest fragmentation on leaf water content we used a generalized linear mixed-effects model with a Beta error distribution (Brooks et al., 2017) to model leaf water content as a function of forest fragment area, hostplant species, collection date and their two- and three-way interactions. Separate intercepts for collection site were modeled as normally distributed random effects. As refrigerated storage did not alter leaf water content, we pooled measurements from leaves taken across 5 days of storage.

The probability of mortality from parasitoids was modeled as a function of forest fragment area, diet breadth, and their two-way interaction using a generalized linear mixed-effects model (Bates et al., 2015) assuming a binomial error distribution. Separate intercepts for block and collection site (nested within block) were modeled as normally distributed random effects. All records of parasitism were pooled across years and tree species to increase sample sizes. Preliminary analyses confirmed that neither days from collection to pupation nor collection date had any effect on the probability of parasitism. Consequently, these terms were not included in the final model. We confirmed the lack of spatial autocorrelation in the residuals by examining semi-variograms of the residuals (no trend observed) and computing Moran's I auto-correlation coefficient ($I = -0.03$, $P = 0.635$)

Estimating confidence intervals and making formal inferences from generalized linear mixed-models (GLMMs) is complicated by the absence of a standard method to calculate the denominator degrees of freedom associated with reference distributions. One flexible and robust approach for constructing confidence intervals (CIs) around the parameter estimates from GLMMs is parametric bootstrapping (Davison and Hinkley, 1997; Gelman and Hill, 2007). We simulated 1,000 sets of response data from the posterior distribution of the parameter estimates, refitted the model to each simulated data set and calculated the 2.5 and 97.5% quantiles of the parameter estimates across the refitted models to obtain their 95% confidence intervals. We estimated approximate, two-tailed *P*-values following the methodology of Bagchi et al. (2011). We present parameter estimates on the scale of the linear predictor with 95% CIs and approximate *P*-values.

RESULTS

Bottom-Up Effects

In total, 290 caterpillars completed the growth performance assay (dietary generalists: $N = 161$, 22 spp.; dietary specialists: $N = 129$, 8 spp.). Although there was substantial variation in final mass (dry pupal mass), dietary specialist caterpillars were smaller, on average, than generalists ($\beta_{\text{specialists}} = -0.308$, 95% CI = 0.529–0.098, $P = 0.008$). Dietary generalist caterpillars fed *H. virginiana* leaves were larger, on average, than those fed *A. rubrum* leaves ($\beta_{\text{host plant species}} = 0.149$, 95% CI = 0.076–0.223, $P < 0.001$). However, hostplant species did not modify growth efficiency in any two- or three-way interactions and these interaction terms were subsequently removed from further analyses.

As expected, dry pupal mass was strongly associated with dry fecal mass ($\beta_{\text{frass}} = 0.216$, 95% CI = 0.115–0.318, $P < 0.001$, **Figure 3**). This growth efficiency relationship was not dependent on leaf origin for either dietary specialist or generalist caterpillars ($\beta_{\text{frass : diet breadth : leaf origin}} = -0.009$, 95% CI = −0.121–0.100, $P = 0.860$; **Figure 3**), suggesting that intraspecific variation in hostplant quality is not associated with forest fragment area in this system.

Variation in growth efficiency between dietary generalist and specialist caterpillars was highly dependent on year. Generalists had approximately equal growth efficiencies across years ($\beta_{\text{frass : generalist : year}} = 0.049$, 95% CI = −0.061–0.155, $P = 0.408$, **Figure 4**), while specialists grew significantly more efficiently in 2018 ($\beta_{\text{frass : specialist : year}} = 0.282$, 95% CI = 0.130–0.446, $P < 0.001$, **Figure 4**). Notably, in 2017, dietary specialist caterpillars grew less efficiently than generalists while the opposite was true in 2018 (**Figure 4**).

Additionally, to confirm that our analytical approach of using all species in a single model had not masked species-specific results, we ran the same models described above (without the random effect terms) for each of the nine (4 dietary specialists and 5 dietary generalists) most abundant species individually and found results consistent with the model that includes all caterpillar species. That is, growth efficiency was not modified by the leaf origin treatment for any of the nine most abundant species ($P > 0.10_{\text{each species}}$). In individual analyses, year similarly modified the growth efficiency of dietary specialist caterpillars ($P < 0.05_{\text{each species}}$) but not dietary generalist caterpillars ($P > 0.10_{\text{each species}}$).

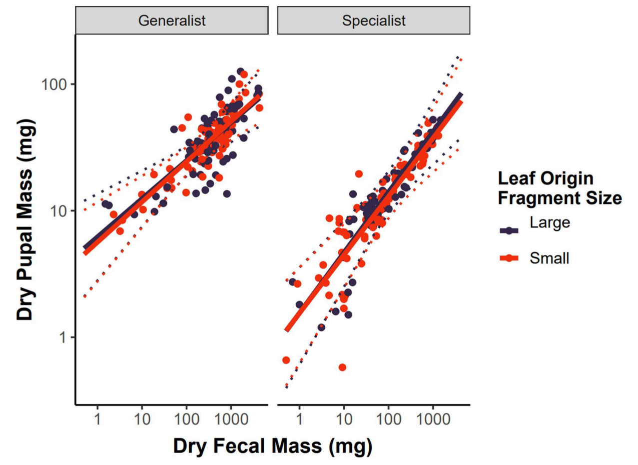


FIGURE 3 | Growth efficiency of dietary specialist and generalist subsets of the caterpillar community on leaves collected from small (<50 ha) or large (>200 ha) forest patches. We define growth efficiency as the relationship between dry fecal mass (a proxy for total food consumed) and dry pupal mass (finishing mass). Variation in the slope of these relationships would indicate variation in intraspecific hostplant quality. Solid lines represent the predicted values from LMMs, dashed lines represent 95% confidence intervals around the predictions, and points are the raw data reshaped to account for the random effects of caterpillar species.

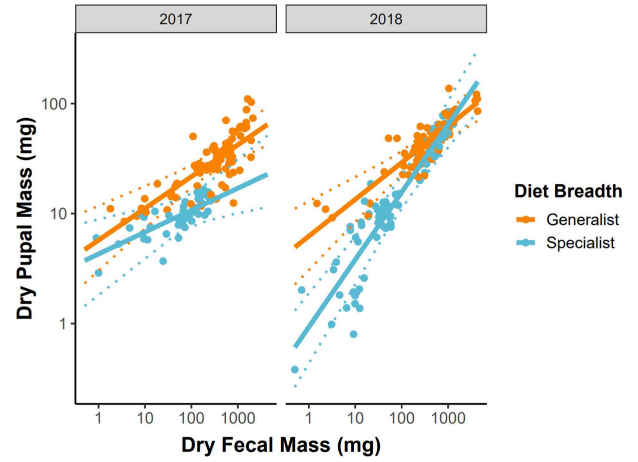


FIGURE 4 | Growth efficiency across years for dietary specialist and generalist subsets of the caterpillar community. Variation in the slope of these relationships indicates variation in growth efficiency between dietary specialist and generalist caterpillars. Solid lines represent the predicted values from LMMs, dashed lines represent 95% confidence intervals around the predictions, and points are the raw data reshaped to account for the random effects of caterpillar species.

In 2018, we quantified leaf water content from 1,077 leaves of *A. rubrum* and 1,063 leaves of *H. virginiana*. There was substantial variation in leaf water content among leaves within sites. *H. virginiana* leaves had consistently more water than *A. rubrum* leaves ($\beta_{\text{host plant species}} = 0.260$, 95% CI = 0.249–0.271,

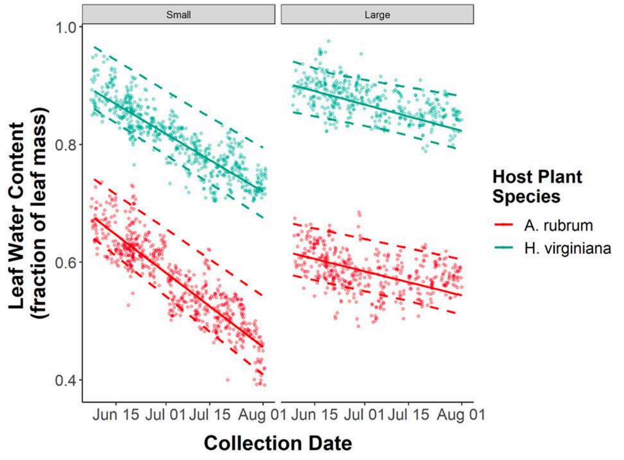


FIGURE 5 | Seasonal declines in leaf water content of *Acer rubrum* and *Hamamelis virginiana* trees in relation to forest patch size. Solid lines represent the predicted values from GLMMs, dashed lines represent 95% confidence intervals around the predictions, and points are the raw data.

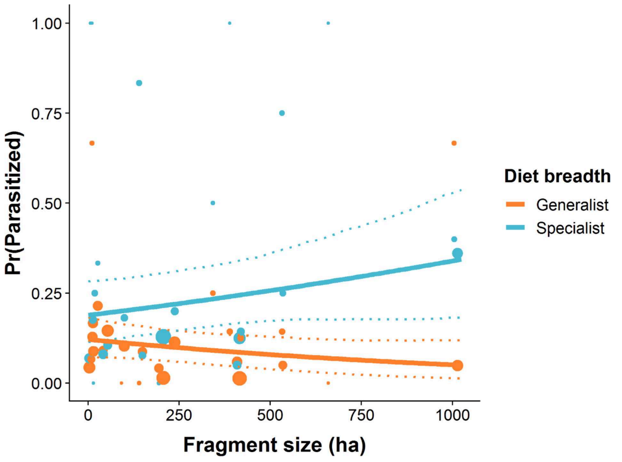


FIGURE 6 | The probability of parasitism for dietary specialist and generalist subsets of the caterpillar community in relation to forest patch size. Solid lines represent the predicted values from GLMMs, dashed lines represent 95% confidence intervals around the predictions, points are the raw data, and the size of the point represents the sample size.

$P < 0.001$, **Figure 5**). Consistent with the herbivore growth performance assay results, forest fragment area had no effect on leaf water content for *A. rubrum* ($\beta_{\text{fragment area : }A.\ rubrum} = 0.006$, 95% CI = −0.004–0.048, $P = 0.756$). However, for *H. virginiana* there was a slight increase in leaf water content with forest fragment area ($\beta_{\text{fragment area : }H.\ virginiana} = 0.025$, 95% CI = 0.013–0.036, $P < 0.001$). As expected, leaf water content declined throughout the summer ($\beta_{\text{collection date}} = -0.042$, 95% CI = −0.050–0.035, $P < 0.001$, **Figure 5**), with no noticeable difference between the slopes for *A. rubrum* and *H. virginiana* ($\beta_{\text{collection date : }H.\ virginiana} = 0.006$, 95% CI = −0.004–0.017, $P = 0.240$, **Figure 5**). Leaf water content declined more rapidly over time in smaller forest fragments ($\beta_{\text{collection date : fragment area}} = 0.024$, 95% CI = 0.016–0.031, $P < 0.001$, **Figure 5**), regardless of host plant species.

Top-Down Effects

Of the 1071 caterpillars collected (dietary specialists: $N = 401$, 13 spp.; dietary generalists: $N = 670$, 40 spp.), 12.0% (129) were parasitized. Parasitoids emerged from 71 dietary specialist caterpillars (17.7%) across 3 species and 58 generalist caterpillars (8.7%) across 17 species. Dietary specialist caterpillars had a higher probability of parasitism than did generalist caterpillars ($\beta_{\text{specialist}} = 0.9706$, 95% CI = 0.610–1.341, $P < 0.001$). Additionally, the probability of parasitism for specialist caterpillars increased with forest fragment area ($\beta_{\text{fragment area : specialist}} = 0.501$, 95% CI = 0.112–0.964, $P = 0.022$, **Figure 6**). In the largest fragments, dietary specialist caterpillars were 1.8 times as likely to be parasitized relative to those in the smallest fragments (**Figure 6**). The probability of parasitism for generalist caterpillars decreased slightly with increased forest fragment area ($\beta_{\text{fragment area : generalist}} = -0.273$, 95% CI = −0.673–0.036, $P = 0.088$, **Figure 6**).

DISCUSSION

Bottom-Up Mechanisms

The hypothesis that forest fragmentation alters intraspecific hostplant quality was not supported. Neither dietary specialist nor generalist caterpillars varied in growth efficiency when fed leaves from forests of contrasting area. As food quality is best measured by the growth response of the herbivore, these findings suggest that intraspecific hostplant quality is not affected by forest fragmentation, at least in this system and at these scales. To our knowledge, no other studies have directly measured insect herbivore performance in the context of area effects associated with forest fragmentation. Alternatively, edge effects in similar forest types have been shown to alter larval performance in polyphagous *Malacosoma disstria* (Lasiocampidae) caterpillars fed leaves from *Acer saccharum* (sugar maple) (Fortin and Mauffette, 2001). Compared to those fed leaves from forest interiors, *M. disstria* caterpillars fed leaves from forest edges were larger and had greater survival; these effects were attributed to higher levels of nitrogen, soluble sugars, and total phenolics in leaves grown at the forest edge despite having lower leaf water content (Fortin and Mauffette, 2001). Another explanation for the lack of an effect of forest fragment size on caterpillar growth performance is that lab-rearing conditions may attenuate differences in abiotic conditions that the herbivore experiences in field settings. For example, abiotic factors such as temperature may also mediate herbivore performance more directly. Although not tested here, higher temperature can increase consumption rates (Buse et al., 1998), which could enable herbivores to compensate for reduced food quality. However, whether and the degree to which temperature and food quality interact is difficult to predict because herbivores vary widely in their responses to increased temperature (Lemoine et al., 2014).

Although there was no effect of forest fragmentation on growth efficiency in our study, dietary generalist caterpillars grew larger on leaves of *H. virginiana*, which had a higher water content than *A. rubrum*. This result is consistent with previous evidence that leaves of *H. virginiana* offer higher food quality to dietary generalist caterpillars than do those of *A. rubrum* (Singer et al., 2012). That dietary generalist caterpillars found on both hostplant species grew larger on *H. virginiana* lends support for leaf water content contributing to food quality. Along with leaf nitrogen content, leaf water content has been shown to be highly predictive of herbivore growth performance (Scriber and Slansky, 1981), although the disparities may also be attributable to other differences in fiber or secondary metabolites (Mattson and Scriber, 1987).

Interestingly, the community of dietary specialist caterpillars grew much less efficiently in 2017, while the community of dietary generalist caterpillars grew with equivalent efficiency in both years. While forest fragment area cannot explain the decrease in growth efficiency of dietary specialist caterpillars, other landscape variables may be at play. In 2017, the forest region in this study experienced an outbreak of Gypsy moth (*Lymantria dispar*, a dietary generalist) caterpillars, which resulted in large-scale defoliation (Pasquarella et al., 2018). We hypothesize that the magnitude of this defoliation may have induced anti-herbivore resistance via increased mechanical defenses such as leaf toughness, which has been shown to reduce insect herbivore performance (Clissold et al., 2009). Induced mechanical defenses such as leaf thickness or toughness may disproportionately affect dietary specialist species that are often smaller (Davis et al., 2013, this study; **Figures 3**, **4**) and may not have the ability to efficiently chew tough leaves. Dietary generalists, however, often have to cope with substantial resource variation across many host plants (Michaud, 1990). Consequently, they may have superior physiological mechanisms that buffer them against year-to-year variation in host plant quality.

The effects of forest fragment area on leaf water content were nuanced. Consistent with other studies of forest fragmentation that have measured leaf water content (Fáveri et al., 2008; Rossetti et al., 2014), we found that leaf water content varied independently of fragment area for *A. rubrum*. There was a slight increase in water content in *H. virginiana* leaves from large fragments, although the magnitude of this effect was small and did not translate into better quality food for caterpillars. In our study, the rate at which leaf water content declined throughout the summer was dependent on fragment area with leaves from the small forest fragments showing the sharpest decline in water content throughout the summer (**Figure 4**). Leaf water content declined in the large forest fragments as well, but at a lower rate (**Figure 4**). To our knowledge, this is the first example of temporal dependence in the relationship between leaf water content and forest fragment area. Similar studies on leaf water content have found either no effect of area (Fáveri et al., 2008), only edge effects (Briant et al., 2010; Rossetti et al., 2014), or edge by area interactive effects (Briant et al., 2010).

It is possible that the herbivore performance assay did not reflect these temporal differences in leaf water content because the majority of caterpillars in our assay pupated by early July, before which the disparities in leaf water content between large and small fragments were small. As the majority of Lepidoptera in these forests complete their larval development in late spring or early summer (Wagner et al., 2002, 2011; Wagner, 2005), it is possible that the faster desiccation rate of leaf water in small forest fragments may only affect insect herbivores that are multivoltine or have prolonged development times that carry late into the summer.

Top-Down Mechanisms

Theoretical frameworks based on area and isolation effects and on edge effects predict that species' persistence in fragmented habitats is inversely related to degree of ecological specialization (Ries et al., 2004; Gravel et al., 2011). Trophic island biogeography theory (Holt et al., 1999; Gravel et al., 2011) additionally predicts that trophic rank will contribute to sensitivity to fragmentation. That is, ecologically specialized predators are most likely to become locally extinct in small habitat patches. Our results are consistent with these predictions, although they cannot distinguish among these alternative theoretical frameworks. That the probability of parasitism for dietary specialist caterpillars increased with forest fragment area while the probability of parasitism for generalist caterpillars varied independently of fragment area suggests to us that host-specific parasitoids were poorly represented in the communities of small forest fragments. Parasitoids that emerged from caterpillars in this study were not identified, thus we cannot conclude explicitly that the lower parasitism rates are due to a loss of host-specific parasitoids in small forest fragments. However, caterpillar-parasitoid interaction data from both tropical (Dyer and Gentry, 2002) and temperate (Stireman and Singer, 2003) communities suggest a widespread pattern of host-specific parasitoids attacking host-specific caterpillars. If the caterpillar-parasitoid interactions in the community studied here show this same pattern, the observed decline in parasitism of dietary specialist caterpillars in small forest patches would support the prediction that specialized parasitoids are especially sensitive to landscape fragmentation (Gravel et al., 2011; Murphy et al., 2016). A key test of both area/isolation and edge effects comes from studies of the leafminer-parasitoid network in Chaco-Serrano dry forest remnants of Argentina (Valladares et al., 2006; Cagnolo et al., 2009). Parasitism rates of leafminers declined with decreasing area of forest remnants, but increased along forest edges despite negative edge responses of parasitoid abundance (Valladares et al., 2006). It is important to note that the area/isolation effects were independent of edge effects on parasitism rate. Further study revealed that species richness of leaf-miner parasitoids declined in the smallest forest remnants with host-specific parasitoids showing sharper species-area relationships than their generalist counterparts (Valladares et al., 2012). Together, these results and others (e.g., Aizen et al., 2012) suggest that heavily fragmented landscapes are deficient in specialized interactions between parasitoids and hosts.

In conclusion, our results show evidence of both bottom-up and top-down effects of forest fragmentation on the plant-insect community. However, the bottom-up effect we studied, intraspecific variation in hostplant quality, did not support the hypothesis that leaves of trees in relatively fragmented forest were of such reduced food quality as to limit caterpillar growth performance. It is still possible that plant community changes wrought by forest fragmentation do have significant effects upon the insect herbivore community in this system, and we are actively investigating this possibility. By contrast, the top-down effect we studied, mortality of caterpillars due to parasitoids, was altered by landscape fragmentation in a manner that accorded with theoretical predictions (area/isolation effects, edge effects). Our results provide preliminary evidence that forest fragmentation can alter ecological communities by disrupting trophic interactions.

DATA AVAILABILITY STATEMENT

All data used in the analyses presented here can be found in the **Supplemental Material**. Please contact the corresponding author for access to R code.

AUTHOR CONTRIBUTIONS

RA, MS, RB, and CC conceived the ideas. MS and RB obtained funding. RA, ND, NP, and CC carried out the experiments. RA drafted the initial manuscript. MS, RB, and JM contributed sections and revisions. RA, RB, and JM performed statistical analyses and developed the figures.

FUNDING

This work was provided by the NSF (DEB: 1557086, REU: 1820555), Wesleyan University and the University of Connecticut.

ACKNOWLEDGMENTS

We thank David L. Wagner (U. Connecticut) for help with caterpillar identification and rearing; thanks to Adrianna Perez, Hanna Holcomb, Christopher White, Riley Hawkins-Hecock, Justin Zhao, Hooman Musavi, Courtney Marren, Caitlin Garvey, Daniel Charron, Samuel Erickson, and Ian Barron for their assistance with field and lab work.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00452/full#supplementary-material

REFERENCES

Agosta, S. J., Hulshof, C. M., and Staats, E. G. (2017). Organismal responses to habitat change: herbivore performance, climate and leaf traits in regenerating tropical dry forests. *J. Anim. Ecol.* 86, 590–604. doi: 10.1111/1365-2656.12647

Aizen, M. A., Sabatino, M., and Tylianakis, J. M. (2012). Specialization and rarity predict nonrandom loss of interactions from mutualist networks. *Science* 335, 1486–1489. doi: 10.1126/science.1215320

Anton, C., Zeisset, I., Musche, M., Durka, W., Boomsma, J. J., Settele, J. (2007). Population structure of a large blue butterfly and its specialist parasitoid in a fragmented landscape. *Mol. Ecol.* 16, 3828–38. doi: 10.1111/j.1365-294X.2007.03441.x

Bagchi, R., Brown, L. M., Elphick, C. S., Wagner, D. L., and Singer, M. S. (2018). Anthropogenic fragmentation of landscapes: mechanisms for eroding the specificity of plant–herbivore interactions. *Oecologia* 187, 521–533. doi: 10.1007/s00442-018-4115-5

Bagchi, R., Philipson, C. D., Slade, E. M., Hector, A., Phillips, S., Villanueva, J. F., et al. (2011). Impacts of logging on density-dependent predation of dipterocarp seeds in a south east Asian rainforest. *Philos. Trans. R Soc. Lond. B Biol. Sci.* 366, 3246–3255. doi: 10.1098/rstb.2011.0034

Barber, N. A., and Marquis, R. J. (2011). Light environment and the impacts of foliage quality on herbivorous insect attack and bird predation. *Oecologia* 166, 401–409. doi: 10.1007/s00442-010-1840-9

Bates, D., Machler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw*. 67, 1–48. doi: 10.18637/jss.v067.i01

Briant, G., Gond, V., and Laurance, S. G. W. (2010). Habitat fragmentation and the desiccation of forest canopies: a case study from eastern Amazonia. *Biol. Conserv*. 143, 2763–2769. doi: 10.1016/j.biocon.2010.07.024

Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., et al. (2017). GlmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J.* 9, 378–400. doi: 10.32614/RJ-2017-066

Brown, L. M., and Crone, E. E. (2016). Minimum area requirements for an at-risk butterfly based on movement and demography. *Conserv. Biol.* 30, 103–112. doi: 10.1111/cobi.12588

Buse, A., Good, J. E. G., Dury, S., and Perrins, C. M. (1998). Effects of elevated temperature and carbon dioxide on the nutritional quality of leaves of oak (*Quercus robur* L.) as food for the winter moth (*Operophtera brumata* L.). *Funct. Ecol.* 12, 742–749. doi: 10.1046/j.1365-2435.1998.00243.x

Cagnolo, L., Valladares, G., Salvo, A., Cabido, M., and Zak, M. (2009). Habitat fragmentation and species loss across three interacting trophic levels: effects of life-history and food-web traits. *Conserv. Biol*. 23, 1167–1175. doi: 10.1111/j.1523-1739.2009.01214.x

Chen, J., Saunders, S. C., Crow, T. J., Naiman, R. J., Kimberley, D., Mroz, G. D., et al. (1999). In forest microclimate and ecosystem ecology landscape the effects of different management regimes. *BioScience* 49, 288–297. doi: 10.2307/1313612

Clissold, F. J., Sanson, G. D., Read, J., and Simpson, S. J. (2009*). Gross vs.* net income: how plant toughness affects performance of an insect herbivore. *Ecology* 90, 3393–3405. doi: 10.1890/09-0130.1

Connor, E. F., and McCoy, E. D. (1979). The statistics and biology of the species-area relationship. *Am. Nat*. 113, 791–833. doi: 10.1086/283438

Davis, R. B., Ounap, E., Javoiš, J., Gerhold, P., and Tammaru, T. (2013). Degree of specialization is related to body size in herbivorous insects. *Evolution* 67, 583–589. doi: 10.1111/j.1558-5646.2012.01776.x

Davison, A. C., and Hinkley, D. V. (1997). *Bootstrap Methods and Their Application. 1st Edn.* Cambridge: Cambridge University Press. doi: 10.1017/CBO9780511802843

Dyer, L. (1995). Tasty generalists and nasty specialists? Antipredator mechanisms in tropical Lepidopteran larvae. *Ecology* 76, 1483–1496. doi: 10.2307/1938150

Dyer, L., and Gentry, G. (2002). On the conditional nature of neotropical caterpillar defenses against their natural enemies. *Ecology* 83, 3108–3119. doi: 10.1890/0012-9658(2002)083[3108:OTCNON]2.0.CO;2

Fáveri, S. B., Vasconcelos, H. L., and Dirzo, R. (2008). Effects of Amazonian forest fragmentation on the interaction between plants, insect herbivores, and their natural enemies. *J. Trop. Ecol*. 24, 57–64. doi: 10.1017/S0266467407004592

Fortin, M., and Mauffette, Y. (2001). Forest edge effects on the biological performance of the forest tent caterpillar (Lepidoptera: Lasiocampidae) in sugar maple stands. *Ecoscience* 8, 164–172. doi: 10.1080/11956860.2001.11682642

Fourcade, Y., Ranius, T., and Öckinger, E. (2017). Temperature drives abundance fluctuations, but spatial dynamics is constrained by landscape configuration:

implications for climate-driven range shift in a butterfly. *J. Anim. Ecol*. 86, 1339–1351. doi: 10.1111/1365-2656.12740

Fox, L. R., and Morrow, P. A. (1981). Specialization: species property or local phenomenon. *Science* 211, 887–893. doi: 10.1126/science.211.4485.887

Gelman, A., and Hill, J. (2007). *Data Analysis Using Regression and Multilevel/Hierarchical Models*. New York, NY: Cambridge University Press. doi: 10.1017/CBO9780511790942

Gravel, D., Massol, F., Canard, E., Mouillot, D., and Mouquet, N. (2011). Trophic theory of island biogeography. *Ecol. Lett*. 14, 1010–1016. doi: 10.1111/j.1461-0248.2011.01667.x

Gripenberg, S., Mayhew, P. J., Parnell, M., and Roslin, T. (2010). A meta-analysis of preference-performance relationships in phytophagous insects. *Ecol. Lett*. 13, 383–393. doi: 10.1111/j.1461-0248.2009.01433.x

Hanski, I. (1999). *Metapopulation Ecology*. Oxford: Oxford Univeristy Press.

Holt, R. D. (1996). "Food webs in space: an island biogeographic perspective," in *Food Webs, Integration of Patterns and Dynamics*, eds G. A. Polis and K. O. Winemiller (London: Chapman & Hall), 313–323. doi: 10.1007/978-1-4615-7007-3_30

Holt, R. D. (2010). "Toward a trophic theory of island biogeography," in *The Theory of Island Biogeography Revisted*, eds J. B. Losos and R. E. Ricklefs (Princeton, NJ: Princeton University Press), 143–185. doi: 10.1515/9781400831920.143

Holt, R. D., Lawton, J. H., Polis, G. A., and Martinez, N. D. (1999). Trophic rank and the species–area relationship. *Ecology* 80, 1495–1504. doi: 10.1890/0012-9658(1999)080[1495:TRATSA]2.0.CO;2

Honnay, O., Jacquemyn, H., Bossuyt, B., and Hermy, M. (2005). Forest fragmentation effects on patch occupancy and population viability of herbaceous plant species. *New Phytol*. 166, 723–736. doi: 10.1111/j.1469-8137.2005.01352.x

Hull-Sanders, H. M., and Eubanks, M. D. (2005). Plant defense theory provides insight into interactions involving inbred plants and insect herbivores. *Ecology* 86, 897–904. doi: 10.1890/04-0935

Kittelson, P. M., Wagenius, S., Nielsen, R., Qazi, S., Howe, M., Kiefer, G., et al. (2015). How functional traits, herbivory, and genetic diversity interact in echinacea: implications for fragmented populations. *Ecology* 96, 1877–1886. doi: 10.1890/14-1687.1

Kruess, A., and Tscharntke, T. (1994). Habitat fragmentation, species loss, and biological control. *Science* 264, 1581–1584. doi: 10.1126/science.264.5165.1581

Leimu, R., Vergeer, P., Angeloni, F., and Ouborg, N. J. (2010). Habitat fragmentation, climate change, and inbreeding in plants. *Ann. N. Y. Acad. Sci*. 1195, 84–98. doi: 10.1111/j.1749-6632.2010.05450.x

Lemoine, N. P., Burkepile, D. E., and Parker, J. D. (2014). Variable effects of temperature on insect herbivory. *PeerJ* 2:e376. doi: 10.7717/peerj.376

Levesque, K. R., Fortin, M., and Mauffette, Y. (2002). Temperature and food quality effects on growth, consumption and post-ingestive utilization efficiencies of the forest tent caterpillar Malacosoma disstria (Lepidoptera: Lasiocampidae). *Bull. Entomol. Res*. 92, 127–136. doi: 10.1079/BER2002153

MacArthur, R. H., and Wilson, E. O. (1967). *The Theory of Island Biogeography*. Princetown, NJ: Princeton University Press.

Martinson, H. M., and Fagan, W. F. (2014). Trophic disruption: a meta-analysis of how habitat fragmentation affects resource consumption in terrestrial arthropod systems. *Ecol. Lett*. 17, 1178–1189. doi: 10.1111/ele.12305

Mattson, W. J., and Scriber, J. M. (1987). "Nutritional ecology of insect folivores of woody plants: nitrogen, water, fiber, and mineral considerations," in *Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates*, eds F. Jr. Slansky and J. G. Rodrigues (New York, NY: John Wiley and Sons), 105–146.

Michaud, J. P. (1990). Conditions for the evolution of polyphagy in herbivorous insects. *Oikos* 57, 278–279. doi: 10.2307/3565951

Morante-Filho, J. C., Arroyo-Rodríguez, V., Lohbeck, M., Tscharntke, T., and Faria, D. (2016). Tropical forest loss and its multitrophic effects on insect herbivory. *Ecology* 97, 3315–3325. doi: 10.1002/ecy.1592

Murphy, S. M., Battocletti, A. H., Tinghitella, R. M., Wimp, G. M., and Ries, L. (2016). Complex community and evolutionary responses to habitat fragmentation and habitat edges: what can we learn from insect science? *Curr. Opin. Insect Sci*. 14, 61–65. doi: 10.1016/j.cois.2016.01.007

Nieminen, M., and van Nouhuys, S. (2017). The roles of trophic interactions, competition and landscape in determining metacommunity structure of a seed-feeding weevil and its parasitoids. *Ann. Zool. Fenn.* 54, 83–95. doi: 10.5735/086.054.0109

Öckinger, E., Schweiger, O., Crist, T. O., Debinski, D. M., Krauss, J., Kuussaari, M., et al. (2010). Life-history traits predict species responses to habitat area and isolation: a cross-continental synthesis. *Ecol. Lett*. 13, 969–979. doi: 10.1111/j.1461-0248.2010.01487.x

Olson, D. M., Dinerstein, E., Wikramanayake, E. D., Burgess, N. D., Powell, G. V. N., Underwood, E. C., et al. (2001). Terrestrial ecoregions of the world: a new map of life on earth. *BioScience* 51, 933–938. doi: 10.1641/0006-3568(2001)051[0933:TEOTWA]2.0.CO;2

Pasquarella, V. J., Elkinton, J. S., and Bradley, B. A. (2018). Extensive gypsy moth defoliation in southern New England characterized using Landsat satellite observations. *Biol. Invasions* 20, 3047–3053. doi: 10.1007/s10530-018-1778-0

Pfeifer, M., Lefebvre, V., Peres, C. A., Banks-Leite, C., Wearn, O. R., Marsh, C. J., et al. (2017). Creation of forest edges has a global impact on forest vertebrates. *Nature* 551, 187–191. doi: 10.1038/nature.24457

R Core Team. (2018). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. https://www.r-project.org/

Ridley, C. E., Hangelbroek, H. H., Wagenius, S., Stanton-Geddes, J., and Shaw, R. G. (2011). The effect of plant inbreeding and stoichiometry on interactions with herbivores in nature: *Echinacea angustifolia* and its specialist aphid. *PLoS ONE* 6:e24762. doi: 10.1371/journal.pone.0024762

Ries, L., Fletcher, R. J. Jr., Battin, J., and Sisk, T. (2004). Ecological responses to habitat edges: mechanisms, models, variability explained. *Ann. Rev. Ecol. Evol. Syst*. 35, 491–522. doi: 10.1146/annurev.ecolsys.35.112202.130148

Ries, L., and Sisk, T. (2004). A predictive model of edge effects. *Ecology* 85, 2917–2926. doi: 10.1890/03-8021

Roland, J., and Taylor, P. D. (1997). Insect parasitoid species respond to forest structure at different spatial scales. *Nature* 386, 710–713. doi: 10.1038/386710a0

Rossetti, M. R., González, E., Salvo, A., and Valladares, G. (2014). Not all in the same boat: trends and mechanisms in herbivory responses to forest fragmentation differ among insect guilds. *Arthropod-Plant Inte*. 8, 593–603. doi: 10.1007/s11829-014-9342-z

Savilaakso, S., Koivisto, J., Veteli, T. O., and Roininen, H. (2009). Microclimate and tree community linked to differences in Lepidopteran larval communities between forest fragments and continuous forest. *Divers. Distrib*. 15, 356–365. doi: 10.1111/j.1472-4642.2008.00542.x

Schüepp, C., Uzman, D., Herzog, F., and Entling, M. H. (2014). Habitat isolation affects plant-herbivore-enemy interactions on cherry trees. *Biol. Control* 71, 56–64. doi: 10.1016/j.biocontrol.2014.01.007

Scriber, J. M., and Slansky, F. Jr. (1981). The nutritional ecology of immature insects. *Ann. Rev. Entomol*. 26, 183–211. doi: 10.1146/annurev.en.26.010181.001151

Singer, M. S. (2001). Determinants of polyphagy by a woolly bear caterpillar: a test of the physiological efficiency hypothesis. *Oikos* 93, 194–204. doi: 10.1034/j.1600-0706.2001.930203.x

Singer, M. S., Farkas, T. E., Skorik, C. M., and Mooney, K. A. (2012). Tritrophic interactions at a community level: effects of host plant species quality on bird predation of caterpillars. *Am. Nat*. 179, 363–374. doi: 10.1086/664080

Singer, M. S., Lichter-Marck, I. H., Farkas, T. E., Aaron, E., Whitney, K. D., and Mooney, K. A. (2014). Herbivore diet breadth mediates the cascading effects of carnivores in food webs. *Proc. Nat. Acad. Sci. U. S.A.* 111, 9521–9526. doi: 10.1073/pnas.1401949111

Smith, S. A., and Brown, J. M. (2018). Constructing a broadly inclusive seed plant phylogeny. *Am. J. Bot*. 105, 302–314. doi: 10.1002/ajb2.1019

Stireman, J. O., and Singer, M. S. (2003). Determinants of host-parasitoid associations: insights from a natural Tachinid-Lepidopteran community. *Ecology* 84, 296–310. doi: 10.1890/0012-9658(2003)084[0296:DOPHAI]2.0.CO;2

Tuff, K. T., Tuff, T., Davies, K. F. (2016). A framework for integrating thermal biology into fragmentation research. *Ecol. Lett*. 19, 361–374. doi: 10.1111/ele.12579

Valladares, G., Cagnolo, L., Salvo, A. (2012). Forest fragmentation leads to food web contraction. *Oikos* 121, 299–305. doi: 10.1111/j.1600-0706.2011.19671.x

Valladares, G., Salvo, A., Cagnolo, L. (2006). Habitat fragmentation effects on trophic processes of insect-plant food webs. *Conserv. Biol*. 20, 212–217. doi: 10.1111/j.1523-1739.2006.00337.x

Vidal, M. C., and Murphy, S. M. (2018). Bottom-up vs. top-down effects on terrestrial insect herbivores: a meta-analysis. *Ecol. Lett.* 21, 138–150. doi: 10.1111/ele.12874

Wagner, D. L. (2005). *Caterpillars of Eastern North America*. Princeton, NJ: Princeton University Press.

Wagner, D. L., Ferguson, D., McCabe, T., Reardon, R. (2002). *Geometriod Caterpillars of Northeastern and Appalachian Forests. FHTET-2001*. Morgantown, WV: USFS Technology Transfer Bulletin. doi: 10.5962/bhl.title.150204

Wagner, D. L., Schweitzer, D. F., Sullivan, J. B., and Reardon, R. C. (2011). *Owlet Caterpillars of Eastern North America*. Princeton, NJ: Princeton University Press.

Wimp, G. M., Murphy, S. M., Lewis, D., and Ries, L. (2011). Do edge responses cascade up or down a multi-trophic food web? *Ecol. Lett*. 14, 863–870. doi: 10.1111/j.1461-0248.2011.01656.x

Zvereva, E. L., Kozlov, M. V. (2016). The costs and effectiveness of chemical defenses in herbivorous insects: a meta-analysis. *Ecol. Monographs* 86, 107–124. doi: 10.1890/15-0911.1

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read
for greatest visibility
and readership

FAST PUBLICATION
Around 90 days
from submission
to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative,
and constructive
peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers
acknowledged by name
on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data
and methods to enhance
research reproducibility

DIGITAL PUBLISHING
Articles designed
for optimal readership
across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics
track visibility across
digital media

EXTENSIVE PROMOTION
Marketing
and promotion
of impactful research

LOOP RESEARCH NETWORK
Our network
increases your
article's readership