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Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA,
IRVINE

The effects of climate change and biodiversity loss on mutualisms

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Ecology and Evolutionary Biology

by

Annika S. Nelson

Dissertation Committee:
Professor Kailen A. Mooney, Chair
Professor Diane R. Campbell
Professor Matthew E.S. Bracken

2019

DEDICATION

To

My parents, for fostering my love for science and the outdoors from a young age.

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- (6) Nelson, A.S., C.T. Symanski, M.J. Hecking*, and K.A. Mooney. 2019. Elevational cline in herbivore abundance driven by a monotonic increase in trophic-level sensitivity to aridity. *Journal of Animal Ecology*.
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- (4) Nelson, A.S., N. Carvajal Acosta, and K.A. Mooney. 2019. Plant chemical mediation of ant foraging. *Current Opinion in Insect Science* 32:98-103.
- (3) Nell, C.S., M.M. Meza-Lopez, J.R. Croy, A.S. Nelson, X. Moreira, J.D. Pratt, and K.A. Mooney. 2018. Relative effects of genetic variation sensu lato and sexual dimorphism on plant traits and associated arthropod communities. *Oecologia* 187:389-400.
- (2) Nelson, A.S., T. Scott, M. Barczyk, T.P. McGlynn, A. Avalos, E. Clifton, A. Das, A. Figueiredo, L. Figueroa, M. Janowiecki, S. Pahlke, J.D. Rana, and S. O'Donnell. 2018. Day/night upper thermal limits differ within *Ectatomma ruidum* ant colonies. *Insectes Sociaux* 65:183-189.
- (1) Mooney, E.H., J.S. Phillips, C.V. Tillberg, C. Sandrow, A.S. Nelson, and K.A. Mooney. 2016. Abiotic mediation of a mutualism drives herbivore abundance. *Ecology Letters* 19:37-44.

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ABSTRACT OF THE DISSERTATION

The effects of climate change and biodiversity loss on mutualisms

By

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Doctor of Philosophy in Ecology and Evolutionary Biology

University of California, Irvine, 2019

Professor Kailen A. Mooney, Chair

Mutualisms – interactions between two species that benefit them both – play a central role in structuring ecological communities and ecosystems. However, from an ecological perspective, mutualism strength (weakly to strongly beneficial) is highly variable across biotic and abiotic contexts. Global changes including climate change and biodiversity loss may be important drivers of variation in mutualisms. However, compared to other species interactions, we know little about the effects of global change on mutualisms or the cascading ecological consequences. To fill this gap, my dissertation assesses (1) the effects of climate change and biodiversity loss on ant-aphid mutualisms, (2) the mechanisms underlying such effects, and (3) the broader population-level consequences of variation in mutualisms for the species involved in the interaction. Ant-aphid mutualisms are experimentally tractable systems in which ants protect aphids against predators and parasitoids, and in return, aphids excrete a sugar-rich reward that ants consume. By experimentally manipulating aphid multi-trophic interactions along replicated climatic gradients, I found that aphids are more abundant at lower elevations where it is more arid (Chapter 1), and this is best explained by changes in interactions with natural enemies and mutualist ants (Chapters 1 and 2). Although the negative effects of natural enemies increase at

lower elevations, the positive effects of mutualist ants increase even more strongly, thus increasing aphid colony growth rates and abundance (Chapters 1 and 2). These results illustrate that multi-trophic interactions, and mutualisms in particular, are important drivers of species responses to changes in climate. In addition, with observational demographic studies, I investigated the effects of mutualist ant identity and diversity on aphid population dynamics. I found that aphids frequently associate with multiple ant species, both simultaneously and sequentially across their lifetimes. Aphids do not benefit from simultaneous mutualist ant diversity, likely because competing ants are ineffective mutualists. However, because ants differ in availability, aphids benefit from more consistent mutualist services with sequential ant diversity. In summary, by documenting the population-level effects of mutualisms and how these effects are mediated by climate change and biodiversity loss, my dissertation provides a window into the multi-trophic mechanisms by which global change structures ecological communities.

INTRODUCTION

Mutualisms occur in every ecosystem and play a central role in the evolution of life (Bronstein 2015). Mutualisms are believed to have been key to the origin of eukaryotic cells (Sagan 1967) and the spread of land plants (Pirozynski and Malloch 1975), and it is thought that every species participates in mutualisms, often with numerous species in multiple mutualism types (e.g., transportation, protection, and nutritional mutualisms) (Bronstein 2015). Because mutualisms can structure ecological communities by altering species abundances and distributions (Stachowicz 2001), they are often considered to be “keystone interactions” (Styrsky and Eubanks 2007). However, mutualisms vary across space and time and may break down in response to global change (Aizen et al. 2008, Tylianakis et al. 2008, Kiers et al. 2010), making it important to understand the factors that determine where and when they occur.

From an evolutionary perspective, mutualisms are predicted to be prone to cheating (with species taking benefits without reciprocating) and as a result be evolutionarily unstable (Sachs et al. 2004, Sachs and Simms 2006, Jones et al. 2015). Multiple mechanisms have been proposed to prevent cheating and promote mutualism persistence, including byproducts, partner fidelity feedback, and partner-choice sanctions models (Sachs et al. 2004). However, cheating and shifts to parasitism are increasingly being recognized as rare in nature (Frederickson 2013, Chamberlain et al. 2014), calling into question this long-standing assumption that mutualisms are evolutionarily unstable (Frederickson 2017).

From an ecological perspective, mutualisms are highly context-dependent, with their effects varying in magnitude (strong to weak) across space and time (Bronstein 1994, Chamberlain et al. 2014). This variation is often driven by changes in abiotic factors, such as temperature, precipitation, or nutrients. For example, in more fertile soils, plants may invest less

in mutualisms with mycorrhizal fungi because the nutrients that they provide are no longer needed (Hoeksema et al. 2010). Although not comprehensively studied, mutualisms are also proposed to be stronger towards the tropics and at lower elevations where the climate is warmer and more stable (Chamberlain and Holland 2009, Schemske et al. 2009). Moreover, mutualisms frequently vary across biotic contexts, such as with changes in the abundance or traits of the mutualist species or of third-party species (i.e., a third species that alters the interaction between the two focal mutualists) (Chamberlain et al. 2014). For example, plants do not receive protection by ants against herbivores when herbivores are absent (Chamberlain and Holland 2009). As a result, traits important for maintaining the mutualism (e.g., extrafloral nectaries) may be lost in the absence of herbivores, causing the mutualism to break down (Palmer et al. 2008).

Despite the large potential for variation in mutualisms, the extent of such variation and its broader ecological and evolutionary consequences are not well understood. Few studies have adequately assessed how mutualisms affect the fitness and population dynamics of the partners involved in the interaction. By definition, mutualists enhance the fitness of their partners and may do so through effects on multiple demographic vital rates (i.e., survival, growth, and reproduction) (Ohm and Miller 2014, Ford et al. 2015). However, mutualists can positively affect some vital rates while negatively affecting others (Bronstein 2001), and certain vital rates may influence population dynamics more strongly than others (Caswell 2001). Thus, evaluating the effects of mutualists on the fitness and population growth of their partners requires quantifying the effects on all three vital rates, although such comprehensive assessments are rare (but see Palmer et al. 2010, Bruna et al. 2014, Ohm and Miller 2014, Ford et al. 2015).

Moreover, in order to understand the extent of variation in mutualisms, it is essential to assess the outcomes of mutualisms in a multi-species context. Most mutualisms consist of many

interacting species that differ in the benefits they provide (Waser et al. 1996, Stanton 2003, Batstone et al. 2018). Species often interact with multiple mutualists simultaneously (Stachowicz and Whitlatch 2005, Schluter and Foster 2012), across different environmental conditions (Silverstein et al. 2012, Fitzpatrick et al. 2014), or sequentially across their ontogeny (Palmer et al. 2010). Nonetheless, few studies have compared the individual and interactive effects of multiple mutualist partners (Blüthgen and Klein 2011).

Summary

Mutualisms are ecologically and evolutionarily important but also highly variable across biotic and abiotic contexts. Because species differ not only in their qualities as mutualists, but also in their competitive abilities and responses to environmental conditions, predicting the outcomes of mutualisms in multi-species communities and across environmental gradients remains challenging. In particular, we lack information about the relative importance of multiple mechanisms that drive variation in mutualisms and whether such mechanisms interact. Moreover, it is unclear whether there are any general fitness effects of associating with multiple mutualist species or how competition among mutualists mediates such effects. Tackling such problems is imperative for predicting how mutualisms and their broader consequences will vary, particularly in response to climate change and biodiversity loss.

My dissertation seeks to evaluate the mechanisms underlying variation in mutualisms and the broader population-level consequences of such variation. To do so, I investigated the following questions:

1. How do mutualisms vary across multiple biotic and abiotic contexts?
2. Which mechanisms drive variation in mutualisms, and what is the relative importance of such effects?

3. How do mutualisms alter population dynamics and species abundances?

To address these questions, I used ant protection mutualisms as an experimentally tractable system. In these interactions ants protect myrmecophilous plants or insects (e.g., hemipterans) against natural enemies (i.e., herbivores or predators and parasitoids, respectively) in exchange for sugar-rich resources (i.e., extrafloral nectar or honeydew, respectively) (Way 1963, Stadler and Dixon 2005). For Chapter 1 I investigated the mechanisms driving variation in the abundance of an aphid along an elevational gradient in aridity. I found that aphids are more abundant at lower elevations (more arid sites) because although the negative effects of natural enemies increase, the positive effects of mutualist ants increase with aridity even more strongly. In Chapter 2 I evaluated why the strength of ant protection increases at lower elevations. I found that individual ant colonies forage at a faster tempo and natural enemies are more abundant at lower elevations, causing aphids to receive stronger benefits from ants. For Chapter 3 I compared the individual and combined effects of three mutualist ant species on aphid demography. I found that aphids do not benefit from associating with multiple ant species simultaneously, likely because competition among ants limits the benefits that they provide to aphids. In contrast, aphids benefit from sequential mutualist ant diversity because the highest quality mutualist occurs the least frequently. Altogether, my dissertation enhances our understanding of the complex multi-trophic mechanisms by which global change structures ecological communities.

CHAPTER 1

Elevational cline in herbivore abundance driven by a monotonic increase in trophic-level sensitivity to aridity

ABSTRACT

The abiotic environment drives species abundances and distributions both directly and indirectly through effects on multi-trophic species interactions. However, few studies have documented the individual and combined consequences of these direct and indirect effects. We studied an ant-tended aphid along an elevational gradient, where lower elevations were more arid. Hypotheses of stronger species interactions at lower elevations and a greater sensitivity of higher trophic levels to climate led us to predict increased top-down control of aphids by natural enemies (third trophic level) but even stronger protection from mutualist ants (fourth trophic level) with increasing aridity. As a result, we predicted that mutualism strength and aphid abundance would increase with aridity. We documented patterns of aphid abundance and tested for both the direct and multi-trophic indirect effects of aridity on aphid performance. To do so, we used both observational and manipulative methods across two years in replicate low and high elevation valleys, where summer temperatures decreased by 3.7°C and precipitation increased by 27 mm/mo from low to high elevations. Aphid colonies were 75% larger in the most (vs. least) arid sites, and this was best explained by changes in interactions with predators and ants. Aphids were unaffected by the direct effects of the abiotic environment or its indirect effects via host plant quality. In contrast, natural enemy effects increased with aridity; under ant exclusion, natural enemies had no effect on aphids in the least arid sites but depressed colony growth by 252% in the most arid sites. Ant activity also increased with aridity, with ants discovering more aphid colonies and experimental baits and allocating more foragers per aphid, although there was

no effect of aridity on ant abundance or community composition. Correspondingly, the mutualist services provided by ants increased with aridity; ants provided no benefits to aphids in the least arid sites but doubled colony growth in the most arid sites. In summary, an elevational cline in herbivore abundance was driven by a monotonic increase in trophic level sensitivity to aridity. These findings illustrate that predicting species responses to climate change will require a multi-trophic perspective.

INTRODUCTION

The abiotic environment is a key driver of species abundances and distributions. Nevertheless, our understanding of the precise mechanisms underlying species responses to the abiotic environment is often limited (Abdala-Roberts and Mooney 2013). While the abiotic environment can affect species abundances and distributions through direct physiological effects, it can also do so indirectly by altering interactions with other species at multiple trophic levels (Parmesan 2006, Gilman et al. 2010, Mooney et al. 2016). Thus, understanding how climate affects species interactions is essential for predicting changes in community structure under future climate change (Tylianakis et al. 2008, Gilman et al. 2010). However, because experimental manipulations of multi-trophic interactions along climatic gradients are rare, the exact mechanisms driving variation in species abundances and distributions are not well understood (Walther 2010, Mooney et al. 2016, Moreira et al. 2018).

Species interactions have classically been proposed to be stronger at lower latitudes and elevations where the climate is warmer and less seasonal (Coley and Barone 1996, Schemske et al. 2009, Moreira et al. 2018), but recent studies have questioned the generality of this paradigm (Moles et al. 2011, Zhang et al. 2015, Moles and Ollerton 2016). Such discrepancies may arise in

part because most tests for gradients in species interactions fail to adequately incorporate the ecological complexity in which the interactions are embedded (Moreira et al. 2018). Climate may alter interactions between two focal species through direct physiological effects on one or both of the species involved in the interaction (Gilman et al. 2010). Additionally, biotic interactions may vary if climate affects how one or both focal species interact with other species in the community, either at lower (e.g., resources) or higher (e.g., natural enemies) trophic levels (Gilman et al. 2010, Mooney et al. 2016, Moreira et al. 2018).

Evidence suggests that higher trophic levels are more sensitive to abiotic change and thus may be particularly important drivers of variation in species interactions (Nelson et al. 2019b). The increased sensitivity of higher trophic levels is likely due to their relatively smaller population sizes and greater metabolic demands (Petchey et al. 1999, Voigt et al. 2003, Vasseur and McCann 2005). Such sensitivity of higher trophic levels may alter interactions with species at lower trophic levels, ultimately affecting their abundances and distributions (Barton et al. 2009). For example, Barton et al. (2009) found that warming increased the top-down control of herbivores by predators, which indirectly affected plant biomass. Because higher trophic levels are often more sensitive to abiotic change and have strong fitness effects on species at lower trophic levels (Vidal and Murphy 2018), a multi-trophic perspective is necessary for understanding the mechanisms by which climate alters species abundances and distributions.

Ant protection mutualisms are experimentally tractable multi-trophic interactions that are ideal for testing for trophic gradients in sensitivity to abiotic change and the resulting effects on species abundances and distributions. In these interactions, myrmecophilous plants and insects (e.g., aphids) provide ants with carbohydrate-rich resources in exchange for protection against herbivores (Chamberlain and Holland 2009) or predators and parasitoids (Stadler and Dixon

2005), respectively. By doing so, ants can alter the abundance, distribution, and evolution of their mutualist partners (Olmstead and Wood 1990, Abdala-Roberts et al. 2012, Pellissier et al. 2012). Some evidence suggests that ant protection mutualisms are stronger at lower elevations and latitudes (Koptur 1985, Olmstead and Wood 1990, Chamberlain and Holland 2009), and this pattern may be driven by changes in plant quality (first trophic level) or the traits, abundances, and community composition of herbivores (second trophic level), natural enemies (third trophic level), or mutualist ants (fourth trophic level) (Petry et al. 2012, Chamberlain et al. 2014, Staab et al. 2015, Mooney et al. 2016). Thus, ant protection mutualisms provide opportunity to test for the multi-trophic mechanisms underlying gradients in species interactions and species abundances and distributions. Along these lines, one past study has tested for elevational variation in ant foraging behaviors and ant-aphid interactions. Nelson et al. (2019b) found that colonies of a single ant species (*Formica podzolica*) in more arid sites were more active and provided stronger tending and protection to aphids (*Aphis varians*) on potted plants (*Chamerion angustifolium*) placed adjacent to ant mounds. However, the study did not assess whether aridity affected aphids directly or indirectly through effects on host plant quality or top-down control by natural enemies, nor did it conduct surveys of naturally occurring aphids to determine the net effects of these dynamics on wild aphid abundance.

In this study, we assessed the multi-trophic mechanisms by which changes in aridity affect the abundance of the ant-tended aphid *Pterocomma beulahense* (Hemiptera: Aphididae) feeding on *Populus tremuloides* (Salicaceae; quaking aspen). To do so, we conducted observational and manipulative studies in replicate low and high elevation valleys, with study sites spanning approximately 630 m in elevation. As elevation increased, mean summer temperature decreased by 3.7°C and summer precipitation increased by 27 mm/mo, thus

constituting a gradient in aridity. We investigated the extent to which aridity affects aphid abundance (i) directly and indirectly by mediating host plant quality, (ii) by mediating top-down control by natural enemies, and (iii) by mediating protection provided by ants due to changes in ant abundance, taxonomic composition, or activity. Based on hypotheses and past evidence for stronger species interactions at lower elevations and a greater sensitivity of higher trophic levels to abiotic change, we predicted stronger negative effects of natural enemies (third trophic level) but even stronger positive effects of mutualist ants (fourth trophic level) with increasing aridity. As a result, we predicted that aphid abundance would increase with aridity. Accordingly, this study documents the individual and combined direct and multi-trophic indirect effects of climatic variation on species abundance.

MATERIALS AND METHODS

Study sites

This research was conducted near the Rocky Mountain Biological Laboratory (RMBL) in Gothic, CO (38.96°N, -106.99°W), in June-August 2012 and 2015. We worked in plots separated by at least 100 m in three “high elevation” valleys (East River, Slate River, and Washington Gulch), ranging in elevation from 2880-3196 m (2999 m \pm 85 SD), and in three “low elevation” valleys (Cement Creek, Spring Creek, and Taylor River), ranging in elevation from 2567-2758 m (2681 m \pm 71 SD) (Fig. 1.1; site coordinates are in Appendix 1A). We worked within separate valleys to provide relatively isolated populations and thus true replication of elevation and its abiotic correlates. Valleys within an elevational category (high vs. low) were 3 km or more apart, while high vs. low elevation valleys were 17 km or more apart.

Our sites spanned a regional aridity gradient (Petry et al. 2016) such as those often observed in mountain ecosystems (McCain 2007). From low to high elevations in 2012-2015, mean summer (May – September) temperature decreased by 3.7°C (12.6°C to 8.9°C) and monthly precipitation increased by 27 mm/mo (49 to 76 mm/mo) (PRISM Climate Group Model, <www.prism.oregonstate.edu>). Thus, while multiple covarying factors along this elevational gradient prevent us from disentangling the effects of individual abiotic factors (Körner 2007), collectively these differences constitute a gradient in aridity (Petry et al. 2016) (Appendix 1B).

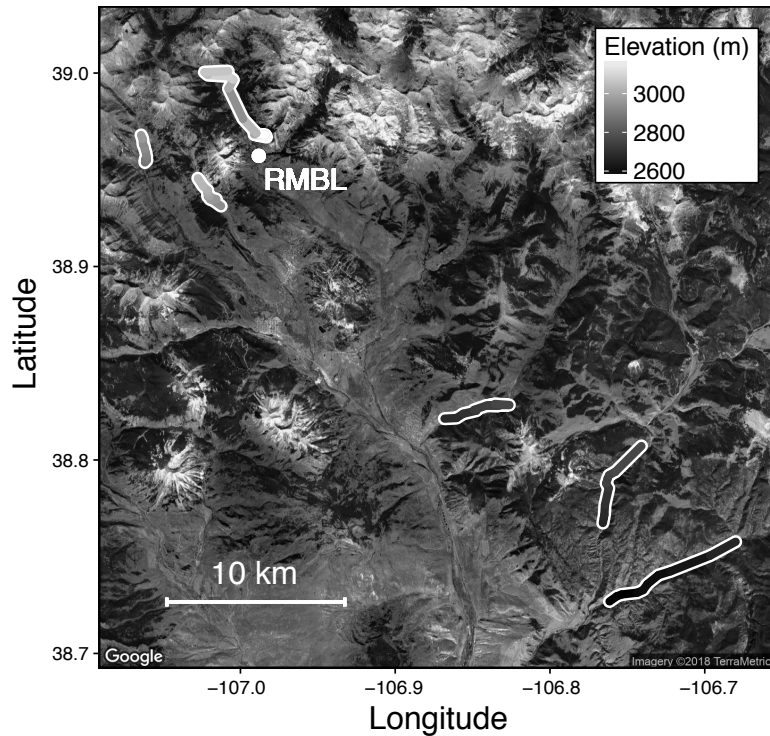


Figure 1.1. The distribution of plots in the three high (top left) and low elevation valleys (bottom right), with elevation (m) indicated by color. Because multiple climate variables co-vary along this gradient, we used principal component analysis to create a linear combination of climatic variables. The first principal component (PC1), serving as an effective proxy for aridity, decreases with elevation. With decreases in PC1, mean summer temperature decreases by 3.7°C

(12.6°C to 8.9°C; $R^2 = 0.98$), and mean monthly summer precipitation increases by 27 mm/mo (49 to 76 mm/mo; $R^2 = 0.90$).

Natural history

Pterocomma beulahense (Aphididae) is an ant-tended aphid that, like all aphids, is viviparous and reproduces clonally during the summer, resulting in short generation times and rapid population growth (Dixon 1985). *Pterocomma beulahense* colonizes stems of quaking aspen (Salicaceae: *Populus tremuloides*), which typically occurs between elevations of 2,200 m and 3,300 m in Colorado (Bretfeld et al. 2016). Our sites thus spanned approximately 60% of aspen's elevational range in the region, reaching elevations close to the upper limit. In the region surrounding RMBL, *Pt. beulahense* is tended by multiple ant taxa including *Camponotus* spp., *Formica fusca* and *Formica rufa* species groups (subfamily Formicinae), *Myrmica* spp. (subfamily Myrmicinae), and *Tapinoma sessile* (subfamily Dolichoderinae) (Mooney et al. 2016). To confirm the identity of the aphids, we collected voucher specimens from each valley in July 2018 and compared them to specimens at the C. P. Gillette Museum of Arthropod Diversity at Colorado State University using the “Aphids on the World’s Plants” online identification guide (Blackman and Eastop 2018). Specimens were slide-mounted in PVA Mounting Medium (Bioquip Products, Inc., Rancho Dominguez, CA, USA) and deposited in the RMBL collection. Ants were identified to taxonomic group with reference to the RMBL collection.

Aspen canopy surveys

To evaluate whether aphid abundance and multi-trophic interactions varied with aridity, we surveyed for ants and aphids in three plots within each valley in 2012. Plots were located on

the periphery of mature aspen stands to include only small saplings with accessible canopies. To consist of similar canopy areas, plots ranged in size from 27-81 m² based on ramet density. We searched canopies at a consistent rate, yielding comparable total sampling efforts (29 min ± 8 SD per plot). Individual ramets within plots were searched at different rates due to differences in canopy areas. For each ramet, we recorded the number and size of aphid colonies as well as the number and taxonomic identity of ants tending each colony and roaming elsewhere within the canopy. Sampling alternated between low and high elevation valleys so that sampling order and aridity were not confounded.

Trophic level manipulations

To determine how aridity altered interactions between *Pt. beulahense* and their resources, natural enemies, and mutualist ants, we experimentally manipulated aphid multi-trophic interactions in 2015. In 10-13 plots per valley (overall n = 63), we established three experimental *Pt. beulahense* colonies on branch terminuses (branch length = 59.4 ± 1.3 cm) on separate ramets (diameter at breast height = 2.4 ± 0.1 cm) spaced approximately 3 m apart. Colonies were initiated with 10 unwinged aphids that were locally collected (within each valley) and protected in mesh bags. When the experiment started (July 14 to 22; 3-8 days after colony establishment), we added additional aphids to colonies with fewer than eight aphids, resulting in initial sizes of 8-83 aphids (16.7 ± 9.7 SD).

Colonies were randomly assigned, with one replicate per plot, to one of three treatments: (a) ‘two trophic levels’ (plants and aphids; ants and natural enemies excluded), (b) ‘three trophic levels’ (plants, aphids, and natural enemies; ants excluded), or (c) ‘four trophic levels’ (plants, aphids, natural enemies, and ants). We placed electrical tape coated with Tanglefoot insect

barrier (Scotts-Miracle Gro Corporation, Marysville, OH, USA) around the branch below each aphid colony. For the three and four trophic levels treatments (colonies with natural enemies), we cut a slit in the mesh bag. For the four trophic levels treatment (colonies with ants), we secured a wire to the branch as a “bridge” for the ants to cross over the barrier (thus controlling for the presence of tape and Tanglefoot).

We visually surveyed each aphid colony for 20 s on at least four occasions before the experiment ended between August 11 and 13 (23-30 days after treatment establishment), recording the number and identity of aphids, natural enemies, and ants. Colonies where ants breached exclusions were removed from all analyses, resulting in the following sample sizes: 54, 40, and 63 colonies of two, three, and four trophic levels, respectively. Natural enemies present included parasitoid wasps (Hymenoptera: Braconidae), hover fly larvae (Diptera: Syrphidae), *Lygus* bugs (Hemiptera: Miridae), spiders (Araneae), and mites (Acari). Although many studies use insect barriers to experimentally manipulate multi-trophic interactions (e.g., Mooney et al. 2016), these methods may unintentionally exclude some ground-foraging predators such as spiders and mites. However, such bias presumably did not depend on aridity, and the most common natural enemies were either winged (e.g., parasitoid wasps) or oviposited by winged adults on or near aphid colonies (e.g., hover fly larvae). Furthermore, it is possible but unlikely that the ants preyed on aphids, since ants were never observed preying on aphids during the experiment or the 40 collective months of fieldwork performed by the authors on these interactions at these sites.

Ant forager abundance and resource consumption

We sampled the ant community in all plots in both 2012 (18 plots) and 2015 (63 plots) using pitfall traps consisting of 50 mL plastic centrifuge tubes filled with soapy water and placed flush with the ground surface. Replicate traps in plots were separated by 3 m and placed at least 1 m away from ant mounds. We retrieved traps after 24-120 h (depending on ant accumulation rates) and counted the number of ants in each taxonomic group (*Camponotus* spp., *F. fusca* species group, *F. rufa* species group, *Myrmica* spp., and *T. sessile*). Although we did not identify all ants to the species level, we identified ants to the level of taxonomic variation (genus or species group) likely to be most relevant to aphid performance, as has been done in other studies (Mooney et al., 2016). In 2012 we sampled ants once between July 2 and August 14, with 8-16 traps per plot on a grid (depending on plot size). In 2015 we sampled ants once between June 24-27 and once between July 21-30, both times with two traps per plot. Any differences in ant abundance detected using this method could be the result of differences in ant colony number, size, or activity. All three of these factors may affect ant tending of aphids, and distinguishing between them was beyond the scope of this study.

In 2015 we also tested for the effects of aridity on ant consumption of artificial baits, consisting of 10 mL of a 20% solution by mass of forest honey (produced by bees foraging on hemipteran honeydew [Langnese Forest Honey, Bargtheide, Germany]) in 15 mL plastic centrifuge tubes fitted with cotton wicks. We paired baits with water controls and deployed them on the ground for 48-72 h on the same dates as the pitfall traps, separated from the traps by 3 m. To determine bait consumption rates (mg/h), we weighed baits before and after they were deployed and corrected for evaporative water loss (ants were never observed collecting water).

Statistical analysis

General approach

We tested for the effects of elevational changes in aridity on aphid abundance and multi-trophic interactions. To do so, we used principal component analysis (PCA) to create a linear combination of temperature and precipitation variables estimated for each site (Appendix 1B). The first principal component (PC1) accounted for 86.8% of the multivariate co-variation in the climate data and was positively correlated with all temperature variables but negatively correlated with precipitation. PC1 thus serves as an effective proxy for aridity (with aridity increasing along PC1) and was used as a continuous predictor variable (hereafter termed “aridity”) in all statistical analyses.

All analyses were based on testing for the fixed effect of aridity while accounting for the random effect of valley. Some analyses included the additional fixed effects of trophic level manipulations and covariates (Appendix 1C). We included the random effect of plot nested within valley for responses where plots were repeatedly sampled (over time or from multiple trees within a plot) and the random effect of tree nested within plot for responses measured at the level of the individual aphid colony. For univariate responses measured with count data (aphids, ants, and natural enemies), we used hurdle models, which consist of a generalized linear mixed model (GLMM) with a binomial distribution evaluating the probability of observing a zero value (i.e., presence vs. absence) and a GLMM with a truncated-at-zero distribution for all non-zero count data (i.e., abundance when present) (Zuur et al. 2009). For univariate responses measured with binary data (ant discovery of baits), we used GLMMs with binomial distributions. For univariate responses measured with continuous data (aphid colony growth rates and ant consumption of experimental baits), we used linear mixed models (LMMs). Finally, to test

whether dissimilarity in ant taxonomic composition in pitfall traps increased with differences in aridity, we used Mantel tests. Analyses were conducted in R 3.3.2 (R Core Team 2016) (further described in Appendix 1C).

Aspen canopy surveys

We tested for the effects of aridity on aphid abundance in terms of whether aphids were present (“aphid presence”) and, where present, the number of colonies per tree (“aphid colony number”) and the number of aphids per colony (“aphid colony size”) (Appendix 1C). Since one tree contained an unusually high number of colonies (37 vs. 3.1 ± 0.28 colonies), and three colonies on other trees were unusually large (>170 vs. 13.8 ± 0.77 aphids), we removed these outliers from the analyses (although with their inclusion, results were qualitatively similar). We assessed ant mutualistic services by testing for the effects of aridity and aphid colony size on whether ants were present (“tending ant presence”) and, where present, for the effects of aridity, colony size, and their interaction on the number of ants (“tending ant number”) (Appendix 1C). The test for a main effect of aridity assessed whether there was an overall difference in ant tending while controlling for aphid abundance, whereas the aridity x colony size interaction tested whether aridity altered the tending rate (i.e., number of ants per aphid). To assess ant foraging activity in the absence of aphid rewards, we assessed whether ants were present (“roaming ant presence”) and the number of ants per tree where present (“roaming ant number”) in the subset of trees lacking aphids (Appendix 1C). For all tests of aphid and roaming ant abundance, the time spent searching each tree (“search time”) was included as a covariate.

Trophic level manipulations

We assessed the direct effects of aridity as well as its effects on host plant, natural enemy, and ant effects on aphid colony per capita growth rate (“aphid colony growth”) (Appendix 1C). Aphid colony growth was calculated as $r = \ln(N_t/N_0)/t$, where N_0 is the initial population size at time $t = 0$, and N_t is the population size at the end of the experiment at time t , ranging from 20-25 days. We added 1 to all aphid counts to include aphid colonies that went extinct (so that $N_t = 1$). We tested for the effects of aridity, trophic level treatment, and their interaction. Because we found a significant interaction, we additionally tested for the effects of aridity directly and through changes in host plant quality by assessing aridity effects on aphid colony growth in the two trophic level treatment only. We also tested whether aridity mediated natural enemy and ant effects in separate models comparing the two vs. three trophic level treatments and the three vs. four trophic level treatments, respectively.

We further tested for aridity effects on whether natural enemies were ever present at an aphid colony (“natural enemy presence”) and, where present, the total number per colony (“natural enemy number”) (Appendix 1C). We also tested the effects of aridity on ant mutualistic services, assessing whether a colony was ever ant tended (“tending ant presence”) and, where present, the number of ants (“tending ant number”) (Appendix 1C). Analyses of natural enemy and tending ant presence included aphid colony size and aridity as fixed effects, and analyses of natural enemy and tending ant numbers also included the colony size x aridity interaction. The test for a main effect of aridity assessed overall differences in natural enemy and tending ant numbers while controlling for colony size, whereas the colony size x aridity interaction tested whether aridity altered recruitment rates (i.e., natural enemies or ants per aphid). Analyses of natural enemy presence and number also included trophic level treatment (three vs. four trophic

levels) to account for the effects of ants as well as the number of times a colony was observed to account for variation in sampling effort.

Ant forager abundance and resource use

We tested for the effects of aridity on ant forager abundance and taxonomic composition in pitfall traps (in 2012 and 2015 separately), calculating ant abundance as the number of ants collected per trap per day (“per trap-day”, *ln*-transformed for analyses of total abundance) (Appendix 1C). We also tested for the effects of aridity on ant discovery (“bait discovery”) and consumption (“bait consumption”) of experimental baits (Appendix 1C). We did not directly observe bait discovery, but bait consumption rates were bimodal, with one distribution centered at 0 mg/h and the other at a positive value. The former distribution presumably represents baits that were not discovered by ants. Since the data were continuous, rather than using a hurdle model, we categorized each bait as discovered vs. not discovered by fitting a Gaussian mixture model using the ‘normalmixEM()’ function in the ‘mixtools’ package (Benaglia et al. 2009), assigning each observation to one of the two distributions based on posterior probabilities (>50%). We then tested for the effects of aridity on bait discovery and consumption, calculated as the mass lost from baits averaged across both sampling dates.

Data deposition

Data available from the Dryad Digital Repository:
<<https://doi.org/10.5061/dryad.j6kn128>> (Nelson et al. 2019d).

RESULTS

Aspen canopy surveys

Aphid colony size significantly increased with aridity (GLMM; aridity, $Z = 2.72$, $P = 0.007$; search time $Z = -1.59$, $P = 0.111$), but there were no significant effects of aridity on aphid presence (GLMM; aridity, $Z = -0.39$, $P = 0.696$; search time, $Z = 2.07$, $P = 0.038$) or colony number per tree (GLMM; aridity, $Z = 0.31$, $P = 0.760$; search time, $Z = 1.58$, $P = 0.115$) (Fig. 1.2). There was no effect of aridity on roaming ant presence (GLMM; aridity, $Z = 0.26$, $P = 0.792$; search time, $Z = 3.47$, $P < 0.001$) (Fig. 1.3A) or roaming ant number (GLMM; aridity, $Z = 0.06$, $P = 0.949$; search time, $Z = 2.61$, $P = 0.009$). Tending ant presence was marginally significantly more frequent with increasing aridity ($Z = 1.83$, $P = 0.067$) (Fig. 1.3D) and significantly more frequent at larger aphid colonies (GLMM; $Z = 3.15$, $P = 0.002$). However, although tending ant number significantly increased with aphid colony size (GLMM; $Z = 6.56$, $P < 0.001$), there was no significant aridity x aphid number interaction ($Z = 0.90$, $P = 0.368$) or main effect of aridity ($Z = 0.35$, $P = 0.725$).

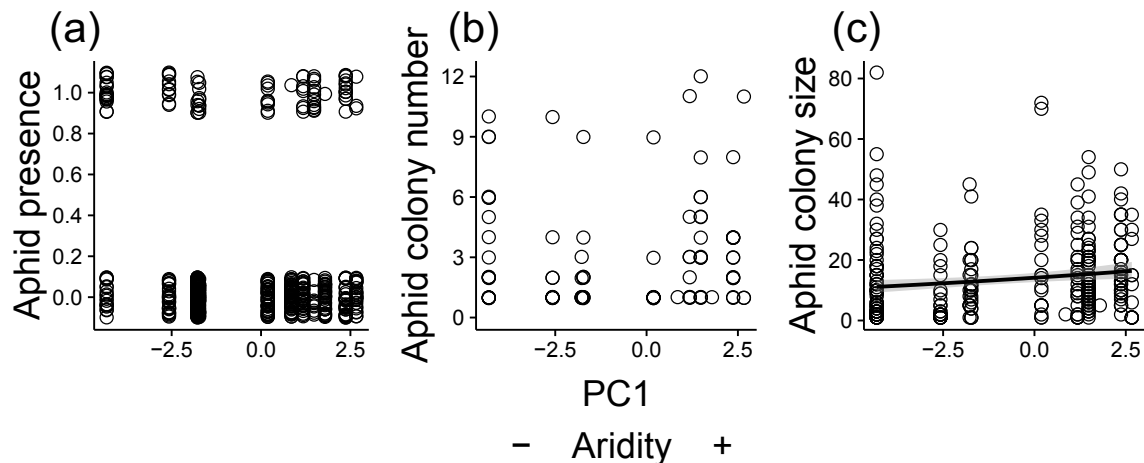


Figure 1.2. The relationship between PC1 (positively correlated with aridity; Appendix 1B) and the abundance of naturally occurring aphids, measured as (a) the presence (vs. absence) of aphids in a tree (“aphid presence”), and where present, (b) the number of aphid colonies per tree (“aphid

colony number”) and (c) the number of aphids per colony (“aphid colony size”). Points in panel (a) are vertically jittered to minimize overplotting.

Trophic level manipulations

Aphid colony growth depended on the aridity x trophic level treatment interaction (LMM; $F = 6.15$, $P = 0.003$) and the main effect of trophic level treatment ($F = 26.64$, $P < 0.001$), but the main effect of aridity was not significant ($F = 0.29$, $P = 0.615$) (Fig. 1.4). For the two trophic levels treatment only (testing for the direct effects of aridity and host plant effects), aphid colony growth did not differ with aridity (LMM; $F = 2.03$, $P = 0.208$). For the two vs. three trophic levels only (testing for natural enemy effects), there was a significant aridity x trophic level treatment interaction (LMM; $F = 13.25$, $P = 0.001$), with predators having stronger effects with increasing aridity. The main effect of trophic level treatment was significant ($F = 61.15$, $P < 0.001$), but the main effect of aridity was not ($F = 1.72$, $P = 0.246$). Similarly, for the three vs. four trophic levels only (testing for ant effects), there was a significant aridity x trophic level treatment interaction (LMM; $F = 6.22$, $P = 0.016$), with ants providing stronger benefits with increasing aridity. The main effect of trophic level treatment was significant ($F = 8.04$, $P = 0.006$), but the main effect of aridity was not ($F = 1.92$, $P = 0.231$).

Natural enemy presence did not depend on aridity (GLMM; $Z = 0.87$, $P = 0.386$), aphid colony size ($Z = -1.69$, $P = 0.091$), or trophic level treatment ($Z = 0.35$, $P = 0.723$) after accounting for sampling effort ($Z = 2.06$, $P = 0.040$). Similarly, natural enemy number where present did not depend on the aridity x colony size interaction (GLMM; $Z = -0.33$, $P = 0.744$) or the main effects of aridity (GLMM; $Z = 0.70$, $P = 0.485$), aphid colony size ($Z = -1.43$, $P = 0.153$), trophic level treatment ($Z = 0.46$, $P = 0.646$), or sampling effort ($Z = -0.34$, $P = 0.732$). In contrast, tending ant presence was significantly more frequent with increasing aridity (GLMM; Z

= 2.79, $P = 0.005$) (Fig. 1.3C) and at larger aphid colonies ($Z = 3.12$, $P = 0.002$). Tending ant number where present depended on the aridity x aphid colony size interaction (GLMM; $Z = 2.02$, $P = 0.043$), where per capita tending rates increased with aridity. There were also significant main effects of aridity ($Z = -2.72$, $P = 0.006$) and colony size ($Z = 3.60$, $P < 0.001$).

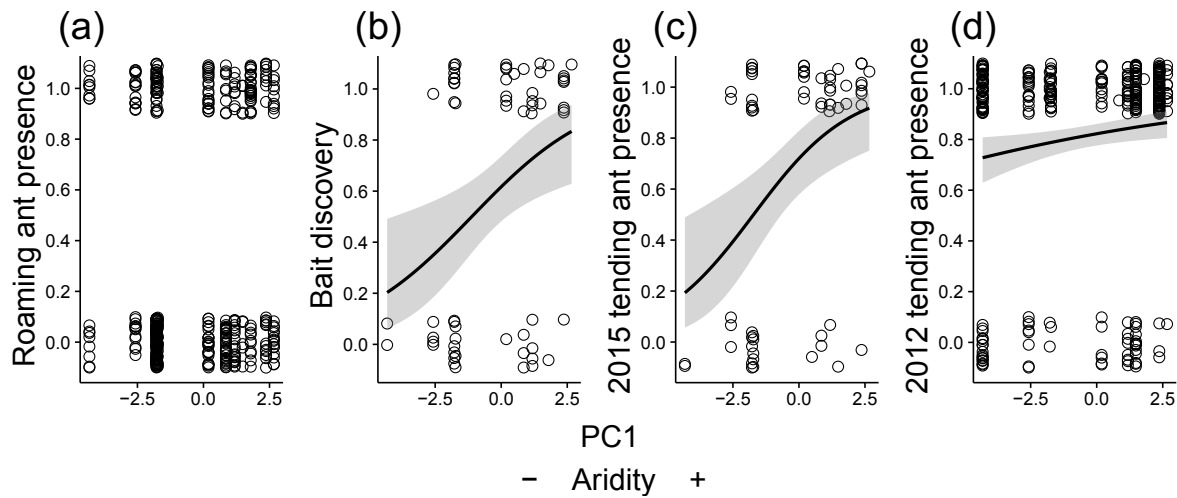


Figure 1.3. The relationship between PC1 (positively correlated with aridity; Appendix 1B) and (a) the presence (vs. absence) of ants in trees without aphids (“roaming ant presence”), (b) whether experimental baits were discovered (vs. undiscovered) by ants (“bait discovery”), and (c) the presence (vs. absence) of tending ants (“tending ant presence”) at experimentally established (2015) and (d) naturally occurring aphid colonies (2012). Points in all panels are vertically jittered to minimize overplotting.

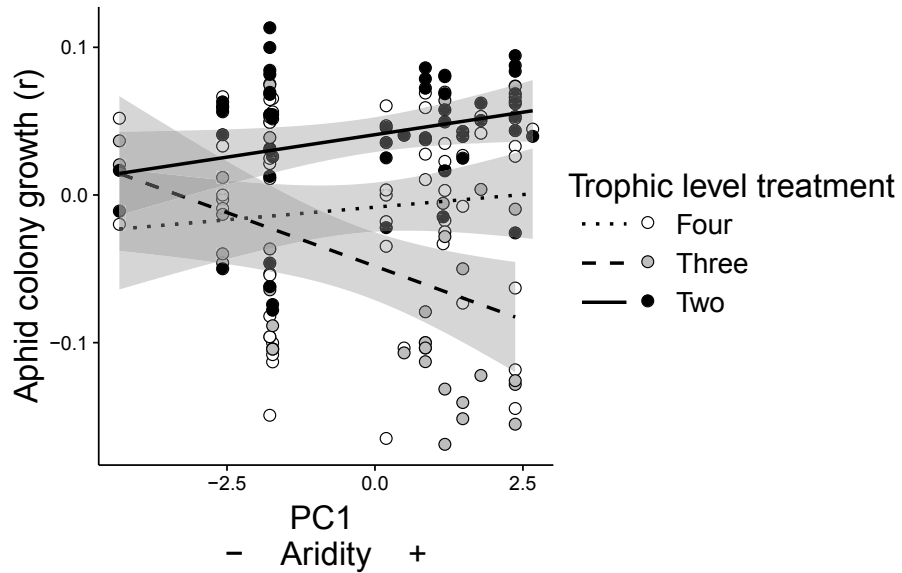


Figure 1.4. The relationship between PC1 (positively correlated with aridity; Appendix 1B) and aphid colony per capita growth (r) (“aphid colony growth”) in treatments of two, three, and four trophic levels.

Ant forager abundance and resource use

Ant frequency in pitfall traps did not vary with aridity, since all traps contained at least one ant. Ant abundance also did not depend on aridity in 2012 or 2015 (LMMs; $F = 0.15$, $P = 0.704$ and $F = 0.66$, $P = 0.431$, respectively), and neither did ant taxonomic composition (Mantel tests; $r = 0.08$, $P = 0.221$ and $r = -0.04$, $P = 0.738$, respectively) (Fig. 1.5). Ant bait discovery significantly increased with aridity (GLMM; $Z = 2.66$, $P = 0.008$) (Fig. 1.3B), although for those discovered, bait consumption did not depend on aridity (LMM; $F = 0.43$, $P = 0.524$).

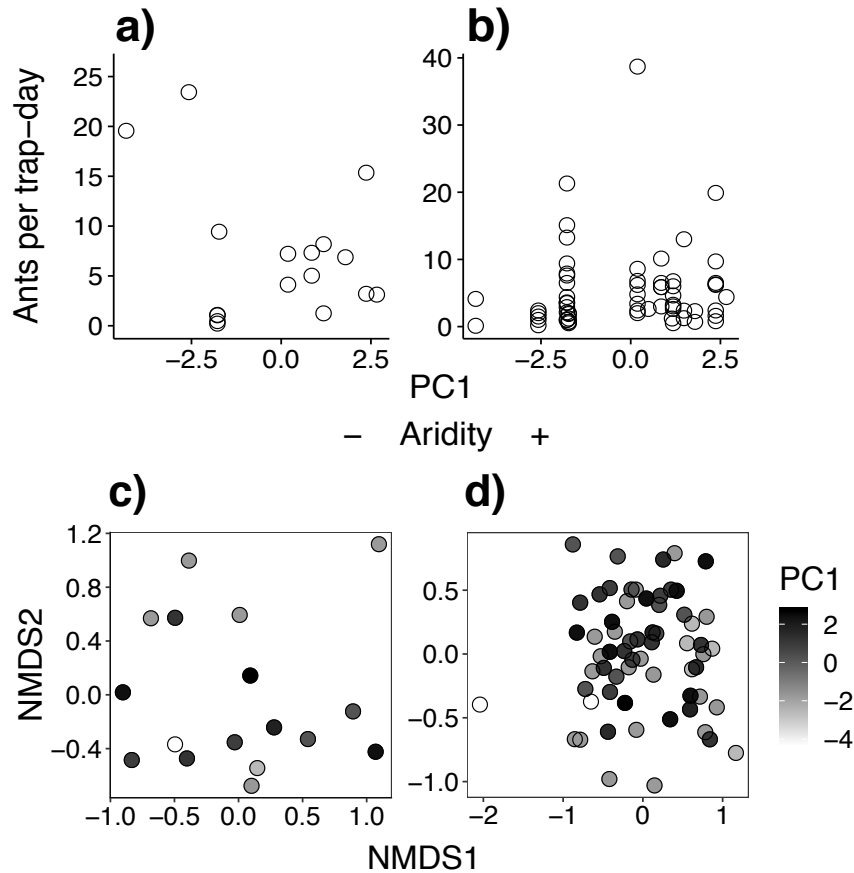


Figure 1.5. The relationship between PC1 (positively correlated with aridity; Appendix 1B) and the number of ants collected per trap per day within a site (“ants per trap-day”) in (a) 2012 and (b) 2015. NMDS plots of ant taxonomic composition (ants per trap-day; $\ln + 1$ transformed) in (c) 2012 and (d) 2015, with PC1 values indicated by color.

DISCUSSION

We have demonstrated that aphid abundance increases with aridity along an elevational gradient, and evidence suggests that this is due to higher trophic levels being more sensitive to abiotic change. We detected no effects of elevational changes in aridity on aphid performance either indirectly, through changes in host plant quality (first trophic level), or directly (second trophic level). However, the strength of natural enemy effects (third trophic level) increased with

aridity, with natural enemies having no effects at low aridity but suppressing aphid colony growth by 252% in the most arid sites. The strength of mutualist ant effects (fourth trophic level) also increased from the least to most arid sites, with ants discovering more aphid colonies and artificial baits, allocating more foragers per aphid, and as a result doubling aphid colony growth and causing aphid colonies to be 75% larger. Thus, increased aridity was associated with strong predator but even stronger ant mutualism effects, increasing the strength of the ant-aphid mutualism. In summary, this elevational cline in herbivore abundance was driven by a monotonic increase in trophic level sensitivity to aridity.

There is contrasting evidence for how abiotic variation affects sap-feeding herbivores directly and through changes in host plant quality. In this study, we did not find evidence for such effects. Likewise, in another study in the same region, there was no evidence for the direct effects of the abiotic environment or of changes in plant quality on the performance of the aphid *Aphis helianthi* feeding on *Ligusticum porteri* (a perennial herb) (Mooney et al. 2016). However, in other systems temperature and moisture have been shown to directly increase aphid colony growth rates and densities (Pons and Tatchell 1995, Barton and Ives 2014). Other studies have also found evidence for elevational changes in host plant quality, both in terms of intra-specific variation (Pellissier et al. 2014) and inter-specific variation (Wimp and Whitham 2001), and aspen defensive chemistry and herbivory are known to be mediated by extreme climatic events (e.g., heat or frost events; St. Clair et al. 2009, Lindroth and St. Clair 2013) and nutrient availability (Donaldson et al. 2006). In this study, it is possible that if plant drought stress increased with aridity, the negative effects of drought would have offset any positive direct effects of temperature on aphid performance. In addition, if our study had spanned a larger aridity gradient (with a mean monthly summer temperature and precipitation ranging greater than

3.7°C and 27 mm/mo, respectively), we may have found stronger effects. For example, Barton and Ives (2014) found evidence for direct effects upon experimentally elevating temperatures by 4.87°C, and Pellissier et al. (2014) found elevational variation in plant quality when temperatures ranged by 8°C. Nonetheless, our results suggest that other factors may be more important for driving differences in herbivore abundance along environmental gradients.

While we did not detect differences in natural enemy abundance, the top-down control of aphids by natural enemies increased with aridity. In other work along the same elevational gradient, we found that natural enemy abundance and top-down control of aphids (*A. varians* feeding on *C. angustifolium*) were greater at low elevations (Nelson et al. 2019b). Predation and parasitism by invertebrates is often stronger at lower elevations (Straw et al. 2009, Sam et al. 2015, Roslin et al. 2017), and experimental warming has been found to directly affect the abundance of predators of other aphids (*Chaitophorus populicola*) feeding on aspen (Marquis et al. 2014). It is possible that natural enemy abundance also increased with aridity in our system, but we may not have detected such patterns because many flying natural enemies (e.g., parasitoid wasps) spend only brief amounts of time at aphid colonies. Nonetheless, we have shown that aridity can influence the top-down control of aphids by natural enemies, although stronger natural enemy effects do not explain why aphid abundance increased with aridity.

Surprisingly, elevational changes in aridity did not mediate the effects of ants on aphids through changes in ant abundance or taxonomic composition. Ants are known to generally be more abundant and diverse at lower elevations (Lessard et al. 2007, Sanders et al. 2007, Machac et al. 2011), including near RMBL (Menke et al. 2014). In addition, other studies have found the effects of ants as mutualists and predators to be stronger at lower elevations and latitudes, where ants were more abundant or had different species compositions (Koptur 1985, Olmstead and

Wood 1990, Zelikova et al. 2008, Pellissier et al. 2012, Sam et al. 2015, Plowman et al. 2017).

While all ant taxa in this study are ground-dwelling, and pitfall trapping is a commonly accepted method for sampling ground-active ants (Lach et al. 2010), it is possible that there were differences in the ant community that we were unable to detect. For example, ant taxa may spend different amounts of time foraging in plant canopies than on the ground. However, the lack of evidence for changes in ant forager abundance or taxonomic composition suggests that additional mechanisms may also drive variation in ant protection mutualisms across environmental gradients.

Changes in aphid abundance and performance were best explained by aridity effects on mutualist ant activity. In 2012 tending ant presence increased marginally significantly with aridity, and in 2015 tending ant presence and number per aphid both increased significantly with aridity. These contrasting results (with aridity increasing ant tending in 2015 but not 2012) are likely due to differences in the nature of the studies. In 2015 we observed the performance of experimentally established aphid colonies over time, but the naturally occurring colonies that we observed in 2012 were those that had established and persisted (rather than gone extinct), and these colonies were primarily ant tended across all sites. Due to increased ant tending, aphid colonies grew faster in more arid sites. As a result, naturally occurring colonies were 75% larger in the most (vs. least) arid sites, although colony presence and number did not vary. It is surprising that aphid colony abundance did not also increase with aridity, since larger colonies often produce more winged aphids that disperse to establish additional colonies (Müller et al. 2001). However, enhanced colony growth rates and sizes may not ultimately result in increased colony number if successful establishment is low or if ant tending limits dispersal by reducing the production of winged aphids (Oliver et al. 2007, Tegelaar and Leimar 2014).

Our results suggest that the increased benefits with aridity of ant tending were due to intraspecific increases in ant activity. Despite no change in ant taxonomic composition, ant tending of aphids increased with aridity. In other work along the same elevational gradient (discussed in more detail below), we found that *Formica podzolica* colonies (the species that tended 44% and 72% of the ant-tended aphid colonies in this study in 2012 and 2015, respectively) are more active and as a result provide greater benefits to the aphid *A. varians* feeding on *C. angustifolium* at low elevations (Nelson et al. 2019b). This finding thus provides a direct indication that intraspecific variation in ant activity can drive differences in aphid performance along aridity gradients. Such changes in ant activity may be driven by temperature (Fitzpatrick et al. 2014, Sagata and Gibb 2016), water availability (Kaspari and Weiser 2000, Frizzi et al. 2016), the availability of other sugar-rich resources (e.g., extrafloral nectar; Pemberton 1998), or ant metabolic and nutritional requirements. While water may be limiting and pose desiccation risk to ants under arid conditions (Kaspari and Weiser 2000), such effects were unlikely in this study, since ant activity increased with aridity. Instead, it is more likely that ant activity increased in direct response to temperature, with ants being cold limited at higher elevations. Stable isotope analyses have shown that along the same elevational gradient, *F. podzolica* ants consume more carbohydrates versus proteins, possibly indicating that sugar-rich resources are more available or that ants have a faster metabolism in warmer sites (Nelson et al. 2019b).

The results from this study complement and extend the generality of the results from a recent study (Nelson et al. 2019b) of another aphid-plant system along the same aridity gradient. Studying the ant *Formica podzolica* alone, Nelson et al. (2019b) showed that ant tending and mutualist effects on aphids (*Aphis varians*) were stronger at more arid, lower elevation sites,

while experimentally controlling for variation in most other factors. The study manipulated ant access to aphids feeding on potted plants (*Chamerion angustifolium*) placed adjacent to *F. podzolica* mounds, assessing ant tending of aphids, diet (through stable isotopes), and effects on aphid performance. At the same time, plant genetic variation and environmental effects on plant quality were controlled for by consistently watering potted greenhouse-grown plants sourced from the same seeds. In addition, ant colonies were selected to be of uniform size, and variance due to the process of aphid discovery and competitive interactions with other ants was eliminated by placing potted plants immediately adjacent to ant colonies. Accordingly, Nelson et al. (2019b) explicitly demonstrated that with increasing aridity, *F. podzolica* interest in aphid tending and mutualist effects on aphid performance increased. Furthermore, although natural enemy access to aphids was not manipulated, they were more abundant at lower elevations. Thus, Nelson et al. (2019b) complements the current study in demonstrating that aridity increases ant propensity to tend aphids, and that the ant-mediated positive effects of aridity on aphids superseded the parallel negative effects of increasing natural enemy abundance. Together, these two studies provide strong evidence for a progressive sensitivity of trophic levels to climate.

Because ants are dominant species in many terrestrial ecosystems, variation in ant activity may have widespread cascading effects. As we have shown here, ants are important drivers of aphid abundance in temperate regions. Other work has shown that myrmecophilous (vs. non-myrmecophilous) treehopper (Olmstead and Wood 1990), lycaenid (Pellissier et al. 2012), and extrafloral nectary-bearing plant (Pemberton 1998) species are also generally less common in colder environments associated with decreased ant attendance, likely because ants are less abundant or active. Although aphids become less common towards the tropics, such

effects are likely not due to decreased ant tending but rather the difficulty of locating suitable host plants with increased plant diversity (Dixon et al. 1987).

In summary, this study provides evidence that changes in species abundance along environmental gradients can be driven by a trophic gradient in sensitivity to abiotic change. While elevational changes in aridity did not affect aphids directly or indirectly through changes in host plant quality (second trophic level), natural enemy (third trophic level) and mutualist ant effects (fourth trophic level) both increased with aridity. These results are consistent with two other studies of ant-aphid systems in the same region (*Aphis helianthi* feeding on *Ligusticum porteri* [Mooney et al. 2016] and *Aphis varians* feeding on *Chamerion angustifolium* [Nelson et al. 2019b]) that show a progressive sensitivity of higher trophic levels to temperature, with effects on higher (natural enemies and ants) but not lower (host plant quality or aphids directly) trophic levels. Such trophic gradients in sensitivity to abiotic change may be widespread and have also been observed, for example, in aquatic systems (Kishi et al. 2005). Overall, our work contributes to a growing body of literature showing that in order to predict the effects of climate change on community structure, we must assess the effects on multiple, and particularly higher, trophic levels.

SUPPLEMENTAL MATERIALS

Appendix 1A

Table S1.1 Location of each research plot. Elevation is listed both as categorical (high vs. low) and continuous (in meters).

Plot	Valley	Latitude	Longitude	Elevation	Elevation (m)
ER1	ER	38.96807	-106.98393	High	3196
ER2	ER	38.96695	-106.98317	High	3183
ER3	ER	38.96797	-106.98970	High	3014
ER4	ER	38.96887	-106.98972	High	3032
ER5	ER	38.96829	-106.99116	High	2990
ER6	ER	38.99199	-107.00769	High	3006
ER7	ER	38.99646	-107.00458	High	3075
ER8	ER	38.99677	-107.00489	High	3086
ER9	ER	38.99830	-107.00787	High	3097
ER10	ER	39.00100	-107.00794	High	3175
ER11	ER	38.97629	-106.99828	High	2941
ER12	ER	38.99896	-107.02102	High	3049
ER13	ER	39.00043	-107.02326	High	3067
SR1	SR	38.95667	-107.06063	High	2902
SR2	SR	38.95857	-107.06102	High	2912
SR3	SR	38.96198	-107.06210	High	2930
SR4	SR	38.96301	-107.06290	High	2926
SR5	SR	38.96796	-107.06419	High	2970
SR6	SR	38.96599	-107.06319	High	2965
SR7	SR	38.96065	-107.06177	High	2928
SR8	SR	38.95521	-107.06159	High	2880
SR9	SR	38.95498	-107.06043	High	2886
SR10	SR	38.95377	-107.06170	High	2865
WG1	WG	38.93125	-107.01250	High	2934
WG2	WG	38.93469	-107.01949	High	2964
WG3	WG	38.93636	-107.01980	High	2985
WG4	WG	38.93776	-107.02129	High	2997
WG5	WG	38.93937	-107.02110	High	3019
WG6	WG	38.94153	-107.02412	High	3033
WG7	WG	38.94563	-107.02750	High	3060
WG8	WG	38.93614	-107.02103	High	2968
WG9	WG	38.93429	-107.01783	High	2962
WG10	WG	38.93484	-107.01565	High	2956
CC1	CC	38.82136	-106.86814	Low	2675
CC2	CC	38.82170	-106.86037	Low	2678
CC3	CC	38.82391	-106.85602	Low	2696
CC4	CC	38.82592	-106.84899	Low	2721

CC5	CC	38.82550	-106.84750	Low	2708
CC6	CC	38.82806	-106.84128	Low	2727
CC7	CC	38.82830	-106.83979	Low	2733
CC8	CC	38.82898	-106.83567	Low	2750
CC9	CC	38.82806	-106.83308	Low	2746
CC10	CC	38.82840	-106.82630	Low	2753
SC1	SC	38.76741	-106.76583	Low	2662
SC2	SC	38.77000	-106.76522	Low	2669
SC3	SC	38.77173	-106.76484	Low	2673
SC4	SC	38.77680	-106.76448	Low	2696
SC5	SC	38.78542	-106.76203	Low	2715
SC6	SC	38.78786	-106.76370	Low	2740
SC7	SC	38.79035	-106.76278	Low	2729
SC8	SC	38.79221	-106.76175	Low	2744
SC9	SC	38.79388	-106.75779	Low	2757
SC10	SC	38.80720	-106.74093	Low	2858
TR1	TR	38.72688	-106.76152	Low	2571
TR2	TR	38.72955	-106.75683	Low	2572
TR3	TR	38.73048	-106.75371	Low	2567
TR4	TR	38.73144	-106.74355	Low	2582
TR5	TR	38.73273	-106.73985	Low	2593
TR6	TR	38.73591	-106.73697	Low	2590
TR7	TR	38.74052	-106.72987	Low	2592
TR8	TR	38.74249	-106.72199	Low	2609
TR10	TR	38.75773	-106.68004	Low	2669

Appendix 1B

We used principal component analysis (PCA) to create a linear combination of temperature and precipitation variables estimated for our sites using the PRISM Climate Group Model. Because of the relatively low resolution of the PRISM Climate Model, we were only able to ascribe the 10-13 replicate sites within each valley between two and four separate estimates of climatic variables, resulting in pseudo-replicated analyses. Nonetheless, analyses based on climatic variables provide insight into the mechanisms driving elevational patterns. Here, we report on the correlations between elevation and all climate variables (Fig. S1.1), the results from the PCA (Fig. S1.2), and the correlations between the first principal component (PC1) and all climate variables (Fig. S1.3).

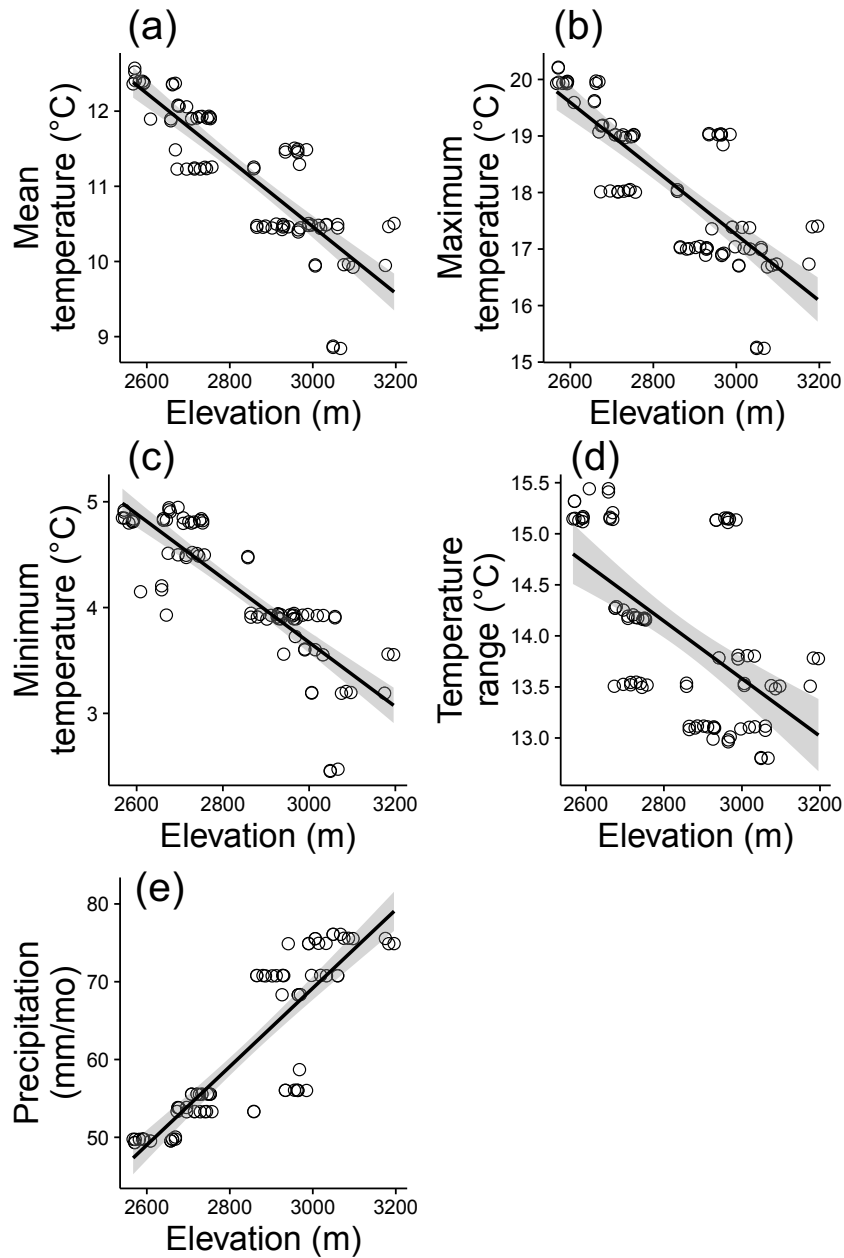


Figure S1.1. Relationship between elevation (m) and monthly summer temperature (a) mean, (b) maximum, (c) minimum, and (d) range as well as (e) monthly summer precipitation. Elevation was significantly positively correlated with mean temperature ($F = 153.0$, $P < 0.001$, $R^2 = 0.72$), maximum temperature ($F = 100.8$, $P < 0.001$, $R^2 = 0.62$), minimum temperature ($F = 162.6$, $P < 0.001$, $R^2 = 0.73$), and temperature range ($F = 27.2$, $P < 0.001$, $R^2 = 0.31$) and negatively correlated with precipitation ($F = 202.7$, $P < 0.001$, $R^2 = 0.77$).

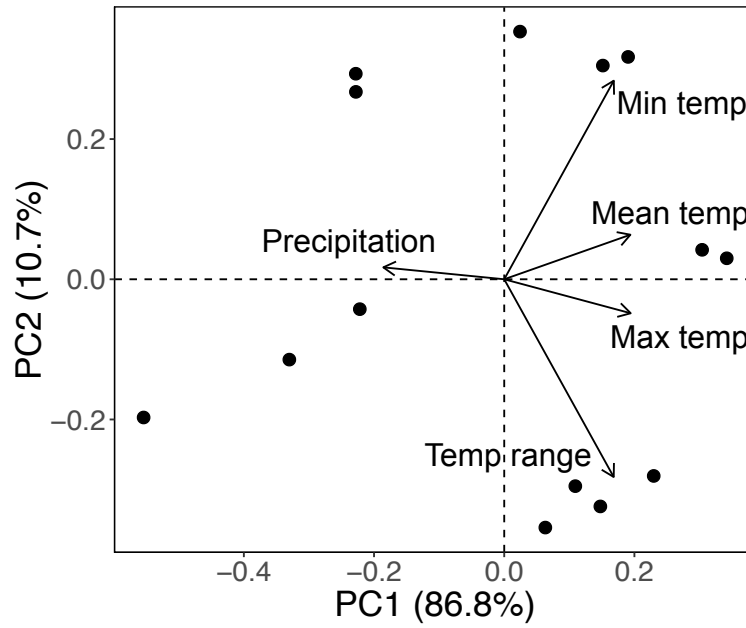


Figure S1.2. Biplot of principal components 1 versus 2. Dots indicate individual sites, and arrows indicate climate variable vectors. Axis labels indicate the percentages of variance explained.

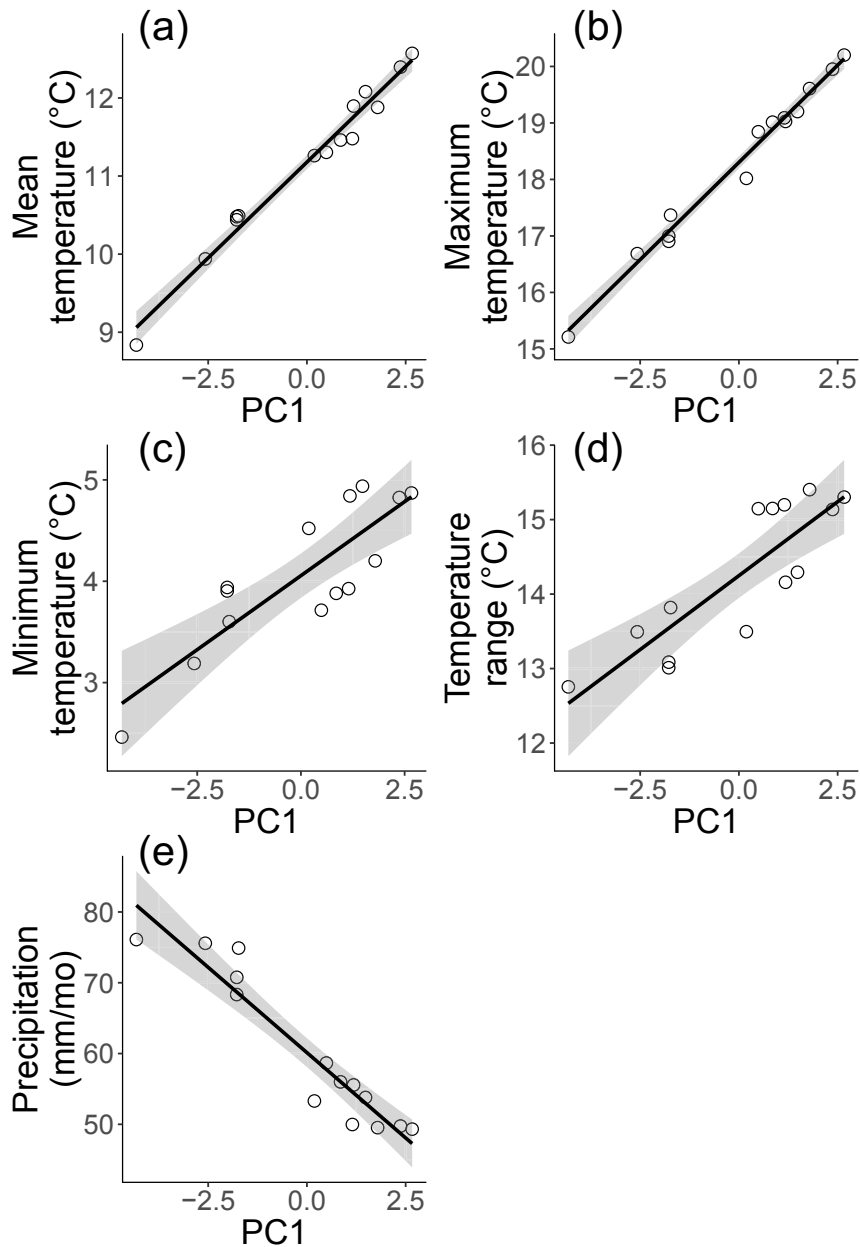


Figure S1.3. Relationship between PC1 and monthly summer temperature (a) mean, (b) maximum, (c) minimum, and (d) range as well as (e) monthly summer precipitation. PC1 is significantly positively correlated with mean ($F = 582.7$, $P < 0.001$, $R^2 = 0.98$), maximum ($F = 770.8$, $P < 0.001$, $R^2 = 0.98$), minimum ($F = 33.9$, $P < 0.001$, $R^2 = 0.74$), and range ($F = 33.7$, $P < 0.001$, $R^2 = 0.74$) in temperature but negatively correlated with precipitation ($F = 107.3$, $P < 0.001$, $R^2 = 0.90$). Thus, PC1 serves as a proxy for aridity.

Appendix 1C

All statistical analyses were conducted in R 3.3.2 (R Core Team 2016). For GLMMs we used the ‘`glmmadmb()`’ function in the ‘`glmmADMB`’ package (Fournier et al. 2012) and conducted Wald Z-tests using the ‘`summary()`’ function (Bolker et al. 2009). We fit the truncated-at-zero count models with three possible distributions: zero-altered Poisson, zero-altered negative binomial, and zero-altered negative binomial with a quasi-Poisson scale parameter. We report the results of the best-fitting models, determined by comparing AIC values using the ‘`AICtab()`’ function in the ‘`bbmle`’ package. For LMMs we used the ‘`lmer()`’ function in the ‘`lme4`’ package (Bates et al. 2015) and conducted F tests with Kenward-Roger’s approximations and type III sums of squares using the ‘`Anova()`’ function in the ‘`car`’ package (Fox and Weisberg 2010). When an interaction term was not statistically significant, it was removed from the model to evaluate the significance of other terms. For Mantel tests we used the ‘`mantel()`’ function with 999 permutations in the ‘`vegan`’ package (Oksanen et al. 2016). We plotted ordinations using non-metric multi-dimensional scaling (NMDS) along two dimensions using the ‘`metaMDS()`’ function in the ‘`vegan`’ package (Oksanen et al. 2016).

Table S1.2. Summary of statistical analyses of data from the (a) aspen canopy surveys, (b) trophic level manipulations, and (c) evaluation of ant abundance and resource use. Included are the response variable names, the description of how the response variable was calculated, the type of model and its distribution, and all fixed and random effects. Distributions used for generalized linear mixed models (GLMMs) include binomial, zero-altered Poisson (ZAP), zero-altered negative binomial (ZANB), and zero-altered negative binomial with a quasi-Poisson scale parameter (ZANB1).

a. Aspen canopy surveys

<i>Variable</i>	<i>Description</i>	<i>Model</i>	<i>Fixed effects</i>	<i>Random effects</i>
Aphid presence	Aphid presence (vs. absence) in a tree	GLMM, binomial	Aridity, Search time	Valley, Plot
Aphid colony number	Number of colonies when present in a tree	GLMM, ZANB	Aridity, Search time	Valley, Plot
Aphid colony size	Number of aphids per colony	GLMM, ZANB	Aridity, Search time	Valley, Plot, Tree
Tending ant presence	Ant presence (vs. absence) at an aphid colony	GLMM, binomial	Aridity, Aphid colony size	Valley, Plot, Tree
Tending ant number	Number of ants when present at an aphid colony	GLMM, ZANB1	Aridity, Aphid colony size, Aridity x Aphid colony size	Valley, Plot, Tree
Roaming ant presence	Ant presence (vs. absence) in an aphid-free tree	GLMM, binomial	Aridity, Search time	Valley, Plot
Roaming ant number	Number of ants when present in an aphid-free tree	GLMM, ZANB1	Aridity, Search time	Valley, Plot

b. Trophic level manipulations

<i>Variable</i>	<i>Description</i>	<i>Model</i>	<i>Fixed effects</i>	<i>Random effects</i>
Aphid colony growth	Aphid colony per capita growth rate*	LMM	Aridity, Trophic level treatment, Aridity x Trophic level treatment	Valley, Plot
Natural enemy presence	Natural enemy presence (vs. absence) at an aphid colony across all repeated observations	GLMM, binomial	Aridity, Mean aphid colony size, Trophic level treatment, Sampling effort	Valley, Plot
Natural enemy number	Total number of natural enemies when present at an aphid colony across all repeated observations	GLMM, ZANB1	Aridity, Mean aphid colony size, Aridity x Mean aphid colony size, Trophic level treatment, Sampling effort	Valley, Plot
Tending ant presence	Tending ant presence (vs. absence) at an aphid colony across all repeated observations	GLMM, binomial	Aridity, Mean aphid colony size	Valley
Tending ant number	Number of tending ants when present at the time when an aphid colony was at its largest size	GLMM, ZANB1	Aridity, Maximum aphid colony size, Aridity x Maximum aphid colony size	Valley

* First, we tested for an aridity x trophic level treatment interaction (across all trophic levels).

Because we found a significant interaction, we used two additional models to separately test whether aridity mediated natural enemy effects (two vs. three trophic levels only) and ant effects (three vs. four trophic levels only). All three models included the same fixed and random effects.

c. Ant abundance and resource use

<i>Variable</i>	<i>Description</i>	<i>Model</i>	<i>Fixed effects</i>	<i>Random effects</i>
Ant abundance	Number of ants collected per trap-day (<i>ln</i> -transformed)*	LMM	Aridity	Valley
Ant taxonomic composition	Number of ants collected per trap-day in each taxonomic group*	Mantel test	Aridity	None
Ant bait discovery	Ant bait discovery (vs. not discovered), determined based on a mixture model*	GLMM, binomial	Aridity	Valley
Ant bait consumption	Ant bait consumption when discovered, determined based on a mixture model*	LMM	Aridity	Valley

* We used separate models for 2012 and 2015. Because sampling occurred on multiple dates in 2015, the 2015 model also initially included the fixed effect of sampling date and the random effect of plot. However, because date was insignificant, these two effects were removed from the model, and the number of ants per trap-day was averaged across dates.

CHAPTER 2

Progressive sensitivity of trophic levels to warming underlies an elevational gradient in ant-aphid mutualism strength

ABSTRACT

Although species interactions are often proposed to be stronger at lower latitudes and elevations, few studies have evaluated the mechanisms driving such patterns. In this study, we assessed whether, and by which mechanisms, abiotic changes associated with elevation altered the outcome of an ant–aphid protection mutualism. To do so, we characterized the multi-trophic interactions among the ant *Formica podzolica*, the aphid *Aphis varians*, and aphid natural enemies occurring on the plant *Chamerion angustifolium* within replicate low and high elevation valleys. Low (versus high) elevation sites had longer summers (snowmelt 13 days earlier) and were on average 1.1°C warmer and 41% drier throughout the year. At low elevations, individual ant colonies consumed approximately double the volume of carbohydrate baits, likely due to a higher foraging tempo, and possibly due to a greater demand for sugar- versus protein-rich resources (as indicated by stable isotope analysis). Wild aphid colonies at low elevations were visited by 1.4-fold more natural enemies (controlling for variation in aphid abundance), while experimental aphid colonies on potted plants were tended 52% more frequently by ants. As a result, ants increased aphid colony survival by 66% at low elevations but had no detectable effect at high elevations; at low (versus high) elevations aphid colonies without ants had lower survival, demonstrating stronger predator effects, while aphid colonies with ants had higher survival, demonstrating even stronger ant benefits. Analyses for the effects of mean summer temperature yielded qualitatively identical results to those based on elevation. Collectively, these findings support predictions for a greater sensitivity of higher trophic levels to warming and

demonstrate how species interactions can vary across environmental gradients due to simultaneous changes in species traits and abundances across multiple trophic levels.

INTRODUCTION

Species interactions, from antagonisms to mutualisms, have been proposed to be stronger at lower elevations and towards the tropics, where temperatures are warmer and the climate is less seasonal (Schemske et al. 2009, Moreira et al. 2018). However, recent studies have questioned the generality of this pattern (Moles et al. 2011, Moles and Ollerton 2016), and we lack a clear mechanistic framework for predicting variation in species interactions along environmental gradients (Moreira et al. 2018). Because interspecific interactions are key determinants of species abundances and distributions, changes in these interactions can have widespread ecological and evolutionary effects (Aslan et al. 2013). Thus, understanding the factors that cause variation in species interactions is increasingly important for predicting how communities will be affected by climate change (Tylianakis et al. 2008, Gilman et al. 2010).

Tests for gradients in species interactions typically measure the strength of a focal interaction over space (Schemske et al. 2009, Moreira et al. 2018), but this approach does not yield an understanding of the mechanisms underlying such patterns (Moreira et al. 2018). Pairwise interactions should vary if the abiotic environment directly affects the traits or abundances of one or both interacting species. If species respond in parallel, pairwise interactions may remain unchanged. However, evidence suggests that species often respond heterogeneously to changes in the abiotic environment (Visser and Both 2005, Tylianakis et al. 2008, Both et al. 2009). In particular, changes in abiotic conditions may have stronger effects on higher than lower trophic levels, possibly due to organisms at higher trophic levels having relatively greater

metabolic requirements or smaller population sizes (Petchey et al. 1999, Voigt et al. 2003, Vasseur and McCann 2005). As a result, trophic gradients in sensitivity to warming may be commonplace (Kishi et al. 2005, Barton et al. 2009), although the effect of warming on predator physiology (positive versus negative) is often variable (Tylianakis et al. 2008). In addition, gradients in pairwise interactions may be driven not only by environmental effects on the focal species, but also by variation in the multi-trophic food webs within which the interactions are embedded (Walther 2010, Mooney et al. 2016). Accordingly, a mechanistic understanding of gradients in species interactions requires documenting variation not only in interaction outcomes, but also in the abundances and traits of the interacting species, and the biotic contexts within which their interactions occurs (Gilman et al. 2010, Mooney et al. 2016).

Ant protection mutualisms are experimentally tractable multi-trophic interactions and, as such, are ideal systems for investigating the mechanisms driving clinal variation in species interactions. In these mutualisms, ants consume resources produced by myrmecophilous plants or insects (i.e., food bodies and extrafloral nectar or honeydew, respectively) and in exchange provide protection against natural enemies (i.e., herbivores or predators and parasitoids, respectively) (Way 1963, Janzen 1966). By doing so, ants alter the abundance, distribution, and evolution of their mutualist partners (Olmstead and Wood 1990, Abdala-Roberts et al. 2012, Pellissier et al. 2012) and can also influence associated plant and arthropod communities (Styrsky and Eubanks 2007, Schuldt et al. 2017). There is some evidence that ant protection mutualisms are stronger at lower elevations and latitudes (Koptur 1985, Olmstead and Wood 1990, Chamberlain and Holland 2009, Plowman et al. 2017). This pattern may be driven by the effects of the abiotic environment on the traits, abundances, and community composition not only of ants and their myrmecophilous partners, but also of their resources and natural enemies

(Petry et al. 2012, Chamberlain et al. 2014, Staab et al. 2015, Mooney et al. 2016). Because ant protection mutualisms constitute multi-trophic interactions (involving mutualist plants or herbivores, natural enemies and ants), they thus provide the opportunity to test the specific prediction for stronger elevational effects on higher trophic levels.

In this study, we evaluated whether elevation altered the performance of an ant-tended aphid by mediating the activity of mutualist ants and the abundance of natural enemies. Based on the hypotheses for stronger species interactions at lower elevations and for a trophic gradient in sensitivity to warming (Voigt et al. 2003, Schemske et al. 2009, Moreira et al. 2018), we made three predictions: first, the top-down control by natural enemies (third trophic level) would increase at low elevations; second, the protection provided by mutualist ants (fourth trophic level) would also increase at low elevations; third, if ants (fourth trophic level) respond more to elevation than predators (third trophic level), the mutualistic services provided by ants to aphids – protection from predators – would increase at low elevations. To test these predictions, we assessed the interactions between the ant *Formica podzolica* (Hymenoptera: Formicidae) and the aphid *Aphis varians* (Hemiptera: Aphididae) feeding on fireweed *Chamerion angustifolium* within replicate low and high elevation valleys. In doing so, we provide a novel test for the multi-trophic basis of elevational gradients in species interactions.

MATERIALS AND METHODS

Study sites

We conducted multiple complementary studies across four years (June-August in 2009, 2010, 2012 and 2015) near the Rocky Mountain Biological Laboratory (RMBL) in Gothic, CO (38°96'N, -106°99'W). In each year we sampled from the same ten sites (or a subset of these

sites) in each of three “low elevation” valleys (Cement Creek, Spring Creek, and Taylor River), with sites ranging in elevation from 2,544 to 2,748 m ($2,660 \text{ m} \pm 69 \text{ SD}$), and three “high elevation” valleys (East River, Slate River, and Washington Gulch), with sites ranging in elevation from 2,873 to 3,327 m ($2,987 \text{ m} \pm 108 \text{ SD}$) ($n = 30$ low and 30 high elevation sites, 60 total; Fig. 2.1). Each valley was at least 3 km apart, with 17 km (Euclidean distances) separating the low and high elevation valleys. Along a linear transect in each valley, neighboring sites were separated by a minimum of 30 m, with the most distant sites separated by up to 2,500 m.

Although we did not collect climate data within our sites, we used the PRISM Climate Group Model (<www.prism.oregonstate.edu>) to estimate climatic variables for each site. Based on the data available, we estimated that the low elevation sites in 2009–2015 were 1.1°C warmer ($3.5 \pm 0.3^\circ\text{C}$ versus $2.4 \pm 0.2^\circ\text{C}$, respectively [mean annual temperature \pm SD]) and 41% drier ($576 \pm 72 \text{ mm/year}$ versus $981 \pm 117 \text{ mm/year}$, respectively [mean annual precipitation \pm SD]) as the result of a regional north–south aridity gradient (Petry et al. 2016). For the summer months (May–September) in particular, the low elevation sites were estimated to have been 1.6°C warmer ($11.8 \pm 0.4^\circ\text{C}$ versus $10.2 \pm 0.4^\circ\text{C}$, respectively [mean monthly temperature \pm SD]), 29% drier ($45 \pm 3 \text{ mm month}^{-1}$ versus $63 \pm 6 \text{ mm month}^{-1}$, respectively [mean monthly precipitation \pm SD]), and had a longer growing season (snowmelt 13 days earlier at low [$131 \pm 3 \text{ day of year}$] versus high [$144 \pm 4 \text{ day of year}$] elevation sites as estimated from a regional regression; Petry et al. 2016).

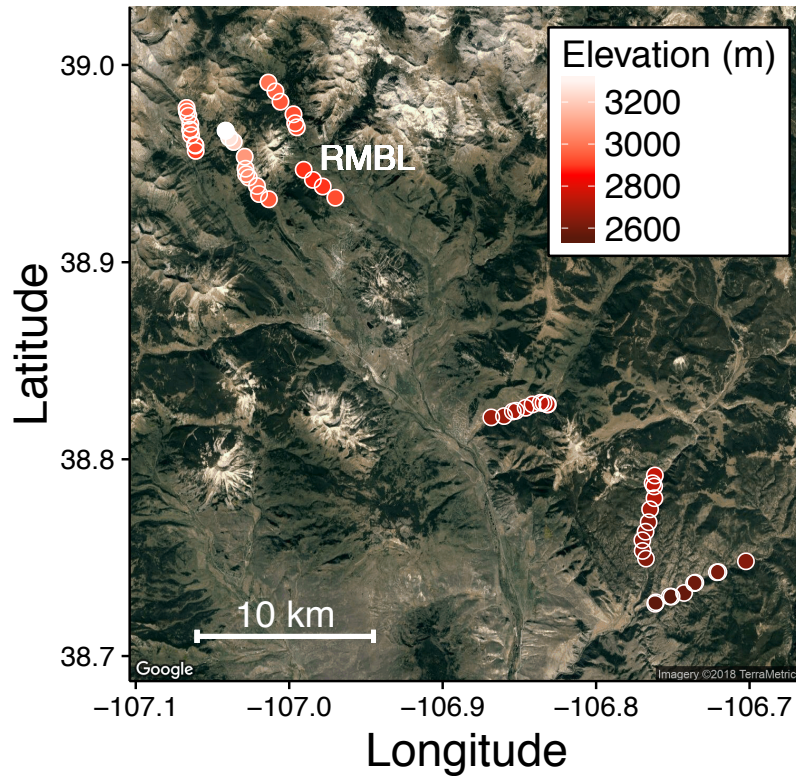


Figure 2.1. The distribution of sites in the three high (top left) and three low elevation valleys (bottom right), with elevation indicated by color. Low elevation sites were 1.1°C warmer ($3.5 \pm 0.3^{\circ}\text{C}$ versus $2.4 \pm 0.2^{\circ}\text{C}$, respectively [mean annual temperature \pm SD]), 41% drier (576 ± 72 mm year $^{-1}$ versus 981 ± 117 mm year $^{-1}$, respectively [mean annual precipitation \pm SD]), and had a longer growing season (snowmelt 13 days earlier at low [131 ± 3 day of year] versus high [144 ± 4 day of year]).

Natural history

The ant *Formica podzolica* (Hymenoptera: Formicidae) forms colonies typically consisting of 5,000–40,000 workers and one or multiple queens, with colonies sometimes occupying multiple separate nests (Deslippe and Savolainen 1995, DeHeer and Herbers 2004).

Formica podzolica commonly forages in plant canopies, where it preys on many arthropods and

also consumes honeydew produced by aphids (Mooney and Tillberg 2005). One such aphid, *Aphis varians* (Hemiptera: Aphididae), feeds on fireweed (Onagraceae: *Chamerion angustifolium*), which is an herbaceous perennial plant that hosts four aphid species (*Aphis helianthi*, *A. salicariae*, *A. varians* and *Macrosiphum valerianae*) in the Rocky Mountains in central Colorado (Addicott 1978). Of these aphids, *A. varians* is the most abundant in this region and reaches peak abundances in mid-July (Addicott 1978). *Aphis varians* is also the aphid that is most frequently tended by ants (including *F. podzolica* and less frequently *Camponotus* spp., ants in the *F. rufa* species group, and *Tapinoma sessile*) (Addicott 1978).

Analytical approaches

Our tests for elevation effects were focused on a categorical (high versus low) classification for several reasons. Because climatic differences along this elevational gradient (Fig. 2.1) are enhanced by the regional north–south aridity gradient (Petry et al. 2016), the effects of elevation are much greater among valleys (high versus low) than within valleys, making elevation a poor proxy for climatic differences. While the PRISM Climate Group Model provides estimates of climatic conditions within our study area, the relatively low resolution of the model means that the 10 replicate sites within each valley can only be ascribed between two and four separate estimates of climatic conditions, resulting in a pseudo-replicated analysis. Nevertheless, we provide supplemental statistical analyses (presented in Appendix 2B) using mean summer temperature (estimated from the PRISM Climate Group Model for May–September in 2009–2015) as a continuous predictor variable. Because analyses of temperature are pseudo-replicated, we emphasize our categorical analyses of elevation.

Variables assessed

Ant abundance and colony activity

To test for elevational differences in ant abundance and activity, we collected *F. podzolica* ants in pitfall traps in 2012 and 2015. Pitfall traps consisted of 50 ml plastic centrifuge tubes with 2.75 cm diameters that were filled with soapy water and placed flush with the ground surface. In 2015 each site contained two traps and was sampled twice (between 24 and 27 June and between 21 and 30 July) (full summary of response variables and sampling methods in Appendix 2A Table S2.1). In 2012 a subset of the sites (three sites distributed throughout each valley; n = 9 low and 9 high elevation sites, for a total of 18) was sampled once between 2 July and 14 August, with 8–16 traps on a grid (depending on site size) (Supplementary material Appendix 2A Table S2.1). Within a site traps were separated by 3 m and placed at least 1 m away from nearby ant mounds. After deploying traps for 24–120 h (depending on ant accumulation rates), we counted the total number of *F. podzolica* in all traps within each site. To assess whether ant abundance in pitfall traps differed with elevation, we used separate linear mixed models (LMMs) for 2012 and 2015, which both included the number of *F. podzolica* collected per pitfall trap per day within a site (rates used to account for differences in sampling time; cube-root transformed to improve the normality of residuals) as the response variable. Elevation was included as a fixed effect, and valley nested within elevation was included as a random effect (full summary of statistical analyses in Appendix 2A Table S2.2). Any elevational differences in ant abundance could have been due to differences in ant colony density, colony size or forager activity.

To test for intraspecific differences in ant colony activity, we examined one focal *F. podzolica* ant nest within each site in 2010 (n = 30 low and 30 high elevation nests, for a total of

60) (Appendix 2A Table S2.1). Nest mounds were selected to be of approximately the same size, based on the surface area of the ground covered. For each nest mound we measured the longest and shortest diameters, which we used to calculate the area of the mound as the area of an ellipse. Nest mound surface area has previously been found to be positively correlated with worker number in *Formica* colonies (Liautard et al. 2003). To confirm that the nest mounds we selected were of approximately the same size, we tested for elevational differences in ant nest mound area (ln-transformed to improve normality), with elevation as a fixed effect and valley nested within elevation as a random effect (Appendix 2A Table S2.2). Because two high-elevation ant colonies were unusually large (area >1 m² versus 0.28 ± 0.13 m²), we excluded them from this and all subsequent analyses, which did not qualitatively affect the result of this analysis. We evaluated ant activity by counting the number of ants on the mound surface on 7–9 separate occasions from 28 July to 24 August, likely providing a combined measure of the activity of foragers and of ants engaged in other activities (e.g., defense or nest construction) (Appendix 2A Table S2.1). We used a LMM to test for elevational differences in ant activity on the mound surface, with the mean number of ants observed on the mound across all dates (ln-transformed) as the response variable, elevation as a fixed effect, and valley nested within elevation as a random effect (Appendix 2A Table S2.2).

Ant diet as indicated by stable isotope analysis

We assessed whether ant diet varied with elevation by analyzing ant nitrogen and carbon stable isotopes. Elevational differences in resource availability or ant colony nutritional requirements could cause differences in ant diet measured using stable isotopes, which in turn could explain differences in ant interactions with aphids. To measure ant diet using stable isotopes, we collected 1–3 adults and pupae from a subset of the same focal ant colonies (4–5

mounds distributed throughout each valley; n = 28 total mounds) in August 2010 (Appendix 2A Table S2.1). Adult foraging ants were collected as they departed the mound surface (i.e., not returning foragers), and past work with this species has confirmed that only returning (not departing) foragers had full gasters (Mooney and Tillberg 2005). Thus, we used complete ants (without discharging their gasters) for all stable isotope analyses. The ants were dried at 60°C for 72 h before being ground to a fine powder with a bug grinding mill. Approximately 1 mg of this powder was packed into 5 × 9 mm tins for elemental analysis and mass spectrometry at the UC Irvine Stable Isotope Ratio and Mass Spectrometry Facility.

We measured the heavy: light isotopic ratios of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$). Because nitrogen isotopic values become enriched at higher trophic levels, $\delta^{15}\text{N}$ can be used to determine ant trophic position and whether ant diets are primarily based on plant-based carbohydrates or arthropod prey (Mooney and Tillberg 2005, Tillberg et al. 2006). Although carbon isotopes show little fractionation with trophic level, they can differ among primary producers (e.g., between C3 versus C4 plants) and thus indicate whether ants consumed resources based on different food webs (Blüthgen et al. 2003, Tillberg et al. 2006). We also measured ant C and N dry weight concentrations ('percent C' and 'percent N'), which were used to calculate C:N ratios to assess the relative contribution of carbohydrate- and protein-based resources to ant diets. We predicted that if higher temperatures at low elevations increased ant activity, foragers might consume additional carbohydrates relative to proteins (lower $\delta^{15}\text{N}$ and higher C:N) as 'fuel' (Davidson 1997). Similarly, if the composition of sugar-rich resources in ant diets (e.g., aphid honeydew, floral nectar, or extrafloral nectar) varies with elevation, we predicted that there would be differences in ant trophic position or $\delta^{13}\text{C}$ (Blüthgen et al. 2003).

To test for elevational differences in ant stable isotopes, we constructed separate LMMs with the $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, C:N ratio, percent C, and percent N as response variables. All models included elevation as a fixed effect and valley nested within elevation as a random effect. The models also included ant life stage (i.e., adult or pupa) as a fixed effect and ant colony nested within valley as a random effect (since adults and pupae were collected from the same nest mounds) (Appendix 2A Table S2.2).

Ant carbohydrate consumption as indicated by recruitment to baits

To further assess ant colony activity and diet, we examined ant consumption of carbohydrates from sets of artificial baits placed on the edge of each of the same focal nest mounds ($n = 30$ low and 30 high elevation mounds, for a total of 60) on three separate occasions (28 July, 11 and 17 August 2010) (Appendix 2A Table S2.1). Each set consisted of three baits containing 8 ml of 10% , 20% and 30% honey solutions in 15 ml plastic centrifuge tubes, which were plugged with cotton wicks, as well as an identical water-filled tube (0% honey). These baits were deployed for approximately 24 h (range 20 – 27 h). When baits were collected, we recorded the number of ants feeding in each tube ('forager abundance'). To determine bait consumption rates (mg h^{-1}), we weighed baits before and after they were deployed to calculate the mass lost per hour. We corrected these consumption rates for evaporative water loss, measured from the water control (ants were not observed collecting water); if evaporation rates exceeded consumption rates (producing a negative adjusted consumption rate), we presumed that the actual consumption rate was zero. While the potential sample size in this design for each elevation and bait concentration was 90 (10 sites \times 3 valleys per elevation \times 3 sampling dates = 90), in some cases consumption rates or ant counts were missing, resulting in the following realized sample sizes: 76 , 85 and 85 baits consisting of 10% , 20% and 30% honey (respectively)

at low elevations, and 84, 83 and 84 baits consisting of 10%, 20% and 30% honey (respectively) at high elevations. Ant forager abundance in the baits was small relative to the number of ants in the colony, as indicated by the fact that the number of ants observed in baits was 8.5% and 4.2% of the number of ants observed on the mound surface at low and high elevations, respectively. In addition, because baits were placed immediately adjacent to the nest, and nests were selected to be similar in size, we assume that any differences in forager abundance in the baits were due solely to differences in ant foraging decisions and did not reflect variation in forager availability.

We evaluated whether ant colony bait consumption rate, forager abundance in baits, and bait consumption per observed ant depended on elevation and sugar concentration. LMMs were used for both bait consumption rate and bait consumption rate per ant. We used a generalized linear mixed model (GLMM; for count data with non-normally distributed residuals) to assess forager abundance in the baits (observed once each time the baits were collected). The GLMM was fit with three possible distributions (Poisson, negative binomial, and negative binomial with a quasi-Poisson scale parameter), and we report results from the best-fitting model (negative binomial with a quasi-Poisson scale parameter), which was selected by comparing AIC values. To calculate bait consumption rate per ant, we divided consumption rates by forager abundances in the baits (with 1 added to all ant counts to include cases when ants were not observed). We $\ln + 1$ transformed both the bait consumption rate and bait consumption rate per ant to improve the normality of residuals. All models included the main and interactive effects of elevation and sugar concentration, as well as the random effect of valley nested within elevation. Since we collected data at each mound on three separate dates throughout the season, we conducted a repeated measures analysis by including date as a fixed effect and mound nested within valley as a random effect (Appendix 2A Table S2.2). In all models a significant main effect of elevation

would indicate an overall difference in ant feeding from baits, controlling for differences in sugar concentration. A significant elevation \times sugar concentration interaction would indicate that elevation altered the strength of ant responses to changes in resource quality.

Effect of ants on aphids

To assess whether elevation altered aphid performance and the effects of ants on aphid performance, we evaluated the ant tending, survival, and growth of aphid colonies on potted fireweed plants placed adjacent to the same focal *F. podzolica* ant nest mounds in each site (Appendix 2A Table S2.1). Plants were grown from seed collected from within 1 km of the RMBL, thus controlling for any elevational effects on plant quality. The seeds were germinated in early May 2010, and seedlings were grown individually in 125 ml pots in a greenhouse at the University of California at Irvine. In mid-June the plants were transported to the RMBL and transplanted into 2 l pots with locally collected soil. Plants were watered every other day and fertilized once per week. On 8 August 2010, we added ten unwinged aphids (*Aphis varians*) to each plant, with aphids sourced from a single colony from the valley in which the plant was to be deployed. At this time, plants were flowering and averaged 23 ± 1.6 cm in height. Aphids were placed among the flower buds, which is the location where they naturally feed. On 11 August we transported the potted plants and aphids to the ant nest mounds. We placed pairs of plants adjacent to each of the same focal nest mounds ($n = 30$ low and 30 high elevation plant pairs, for a total of 60) and randomly assigned one replicate per pair to either ant exclusion (with pots coated with fluon) or ant access treatments, with natural enemies allowed access to aphids in both treatments (Appendix 2A Table S2.1). Plants were watered every other day during the trial.

On 4–5 separate occasions from 11 to 25 August, we counted the aphids and ants on each

plant. When data collection began, aphid colonies ranged in size from 6 to 67 aphids (27 ± 12 SD). We excluded from analysis the aphid colonies where ants were observed to have breached the exclusions, resulting in the following sample sizes (out of 30 colonies for each elevation and treatment): 13 and 15 colonies with ants excluded at low and high elevations (respectively), and 30 and 28 colonies open to ants at low and high elevations (respectively). By tracking aphid performance under ant exclusion at low versus high elevations, we evaluated the combined direct and indirect effects of elevation through altered top-down control by natural enemies (while controlling for plant quality). Comparing the performance of aphid colonies under ant exclusion versus ant access at low versus high elevations allowed us to evaluate whether elevation mediated the effects of tending ants on aphids.

To test whether elevation affected ant tending of aphids, we evaluated whether the presence and number of ants at aphid colonies differed with elevation. To assess ant presence (versus absence) for aphid colonies in the ant access treatment, we used a GLMM with a binomial distribution. To evaluate ant number (for aphid colonies where ants were present; $n = 29$ and 18 at low and high elevations, respectively), we used a LMM. Ant number was calculated as the mean number of ants observed across all dates and was ln-transformed to improve the normality of residuals. Both models included the main effect of elevation, the number of aphids (averaged across all observations) as a covariate, and valley nested within elevation as a random effect (Appendix 2A Table S2.2). The model for ant number also included the elevation \times aphid number interaction. A significant main effect of elevation would indicate an overall difference in ant tending while controlling for aphid abundance, and a significant elevation \times aphid number interaction would indicate that elevation altered the ant per capita tending rate of aphids.

Furthermore, we evaluated the effects of elevation and ant tending treatment on aphid colony survival and growth. To assess survival (versus extinction), we used a GLMM with a binomial distribution, and to evaluate per capita growth rates (for surviving aphid colonies only; $n = 23$ and 6 with ants present and excluded [respectively] at low elevations and $n = 19$ and 10 with ants present and excluded [respectively] at high elevations), we used a LMM. Per capita growth rates were calculated as $r = [\ln(Nt/N0)]/t$, where $N0$ is the initial population size at time $t = 0$ and Nt is the population size at the final observation, time $t = 13-14$ days. Both models included the main and interactive effects of elevation and ant tending treatment and the random effects of valley nested within elevation and ant mound nested within valley (Appendix 2A Table S2.2).

Natural enemy abundance

To additionally measure whether elevation mediated the top-down control of aphids by natural enemies, we evaluated the abundance of natural enemies at aphid colonies that were naturally occurring. In 2009 in one of the low (Spring Creek) and one of the high elevation valleys (East River Valley), we randomly selected two plants within each of 15 blocks spanning a distance of approximately 1 km. On six separate occasions from 4 to 15 August we counted the number of aphids, and on plants where aphids were present ($n = 29$ and $n = 25$ plants at low and high elevations, respectively), we counted the number of natural enemies on each plant (Appendix 2A Table S2.1). The natural enemies observed included hover fly larvae (Diptera: Syrphidae), ladybird beetles (Coleoptera: Coccinellidae), parasitoid wasps (Hymenoptera: Braconidae), predatory mites (Acari), and spiders (Araneae).

We evaluated whether the abundance of natural enemies differed between the low and high elevation valleys using a LMM. The mean number of natural enemies per plant across all

observations ($\ln + 1$ transformed to improve normality of residuals) was the response variable. The model included valley as a fixed effect, the mean number of aphids as a covariate, and block nested within valley as a random effect (Appendix 2A Table S2.2). While we use these analyses to provide information about whether natural enemy abundance potentially varies with elevation, our data are based on limited sampling, and it is possible that any observed differences would reflect valley-specific rather than elevational differences.

Data analysis procedures

We conducted all statistical analyses in R 3.3.2 (R Core Team 2016). To construct the LMMs, we used the ‘lmer()’ function in the ‘lme4’ package (Bates et al. 2015). For the GLMMs we used the ‘glmmadmb()’ function in the ‘glmmADMB’ package (Fournier et al. 2012). We calculated and compared AIC values using the ‘AICtab()’ function in the ‘bbmle’ package. Wald χ^2 tests with type III sums of squares were used to test for the significance of fixed effects in all models using the ‘Anova()’ function in the ‘car’ package (Fox and Weisberg 2010). In all models where interaction terms were not significant, we removed them to test for the significance of the main effects. We used the ‘lsmeans()’ function in the ‘lsmeans’ package to calculate least-squares means (LS-means) and conduct Tukey pairwise comparisons (Lenth 2016).

Data deposition

Data available from the Dryad Digital Repository:
<<http://dx.doi.org/10.5061/dryad.kf2553j>> (Nelson et al. 2018).

RESULTS

Ant abundance and colony activity

Formica podzolica abundance in pitfall traps did not differ with elevation in 2012 or 2015 (LMMs: $\chi^2 = 0.006$, $P = 0.939$ and $\chi^2 = 0.254$, $P = 0.615$, respectively) (Fig. S2.5). Moreover, although the focal ant mounds were larger (19%) on average at high elevations, this difference was not statistically significant (LMM: $\chi^2 = 0.642$, $P = 0.423$). Similarly, the number of ants on the surface of the ant mounds was greater at high elevations, though not significantly so (45%; LMM: $\chi^2 = 3.194$, $P = 0.074$).

Ant diet as indicated by stable isotope analysis

Elevation had no effect on ant diet assessed using stable isotopes, although some stable isotopes differed with ant life stage. We detected no effects of elevation on ant $\delta^{15}\text{N}$ (LMM: $\chi^2 = 1.197$, $P = 0.274$), C:N ratio (LMM: $\chi^2 = 2.435$, $P = 0.119$), $\delta^{13}\text{C}$ (LMM: $\chi^2 = 0.308$, $P = 0.579$), percent C (LMM: $\chi^2 = 0.295$, $P = 0.587$), or percent N (LMM: $\chi^2 = 0.709$, $P = 0.400$) (Fig. 2.2 and Fig. S2.6). For adult ants (versus pupae), the $\delta^{15}\text{N}$ was 16% greater (LMM: $\chi^2 = 26.826$, $P < 0.001$) and the $\delta^{13}\text{C}$ was 1% greater (LMM: $\chi^2 = 4.637$, $P = 0.031$), whereas the adult (versus pupa) C:N ratio was 25% lower (LMM: $\chi^2 = 56.393$, $P < 0.001$) due to a 39% increase in percent N for adults (LMM: $\chi^2 = 55.723$, $P < 0.001$) but no change in percent C (LMM: $\chi^2 = 1.442$, $P = 0.230$) (Fig. 2.2 and Fig. S2.6).

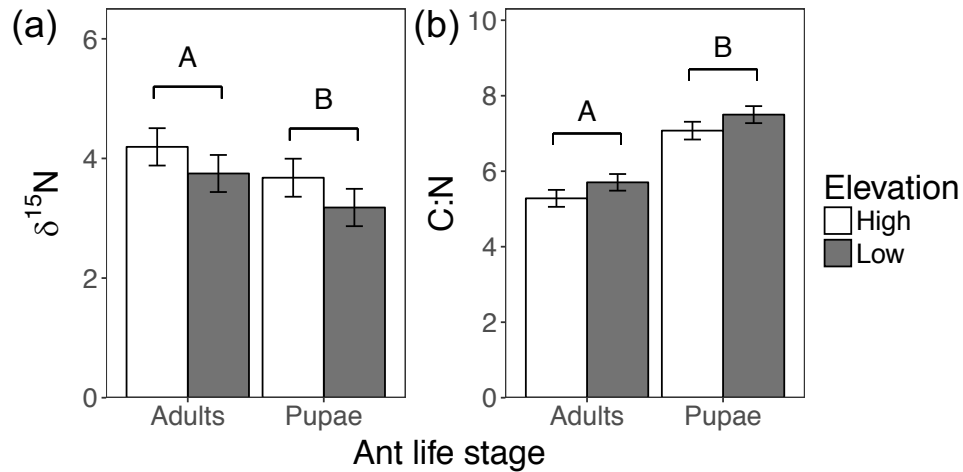


Figure 2.2. The mean (LS-means \pm SE) (a) $\delta^{15}\text{N}$ and (b) C:N ratio of adult ants and pupae collected from ant mounds at both low and high elevations. Letters indicate significant differences among groups. For both the $\delta^{15}\text{N}$ and C:N ratio, there was no significant effect of elevation.

Ant carbohydrate consumption as indicated by recruitment to baits

Ant colony bait consumption rates depended on the elevation x sugar concentration interaction (LMM: $\chi^2 = 10.748$, $P = 0.005$), where consumption rates increased more rapidly with sugar concentration at low elevation mounds (Fig. 2.3a). There were also significant main effects of elevation (2.3- fold greater at low elevations; LMM: $\chi^2 = 4.852$, $P = 0.028$), sugar concentration (LMM: $\chi^2 = 60.573$, $P < 0.001$) (Fig. 2.3a), and date (LMM: $\chi^2 = 68.024$, $P < 0.001$). However, forager abundance in the baits did not depend on an elevation x sugar concentration interaction (GLMM: $\chi^2 = 1.072$, $P = 0.585$), the main effect of elevation (GLMM: $\chi^2 = 2.072$, $P = 0.150$), or date (GLMM: $\chi^2 = 2.138$, $P = 0.343$), although forager abundance significantly increased with the sugar concentration (GLMM: $\chi^2 = 60.592$, $P < 0.001$) (Fig. 2.3b). Thus, similar to bait consumption rates, consumption rates per observed ant depended on

the elevation x sugar concentration interaction (LMM: $\chi^2 = 9.315$, $P = 0.009$) as well as the main effects of elevation (1.9-fold increase; LMM: $\chi^2 = 4.818$, $P = 0.028$), sugar concentration (LMM: $\chi^2 = 37.010$, $P < 0.001$), and date (LMM: $\chi^2 = 63.656$, $P < 0.001$) (Fig. 2.3c). When we corrected for multiple comparisons using the Bonferroni method, all results were qualitatively identical.

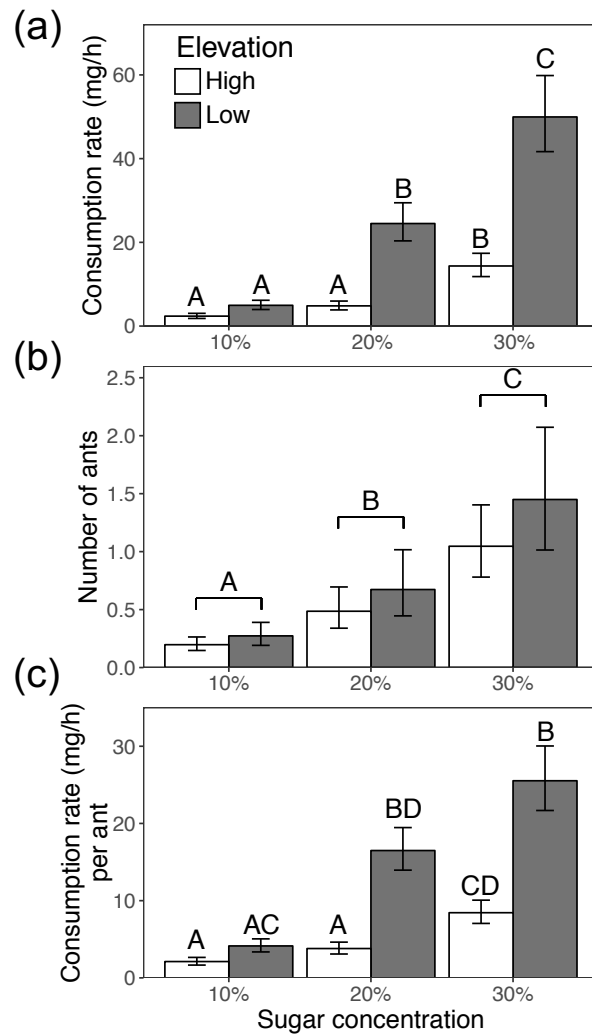


Figure 2.3. The mean (back-transformed LS-means \pm SE) (a) bait consumption rates (mg/h), (b) number of ants observed feeding in the baits, and (c) consumption rates per observed ant (mg h⁻¹ ant⁻¹) at low and high elevations (with replicates averaged across three dates). Letters indicate significant differences among groups.

Effect of ants on aphids

Similar to ant bait consumption, the proportion of aphid colonies tended by ants was 52% greater at low elevations (binomial GLMM: $\chi^2 = 7.030$, $P = 0.008$) but did not depend on the number of aphids in a colony (binomial GLMM: $\chi^2 = 2.114$, $P = 0.146$) (Fig. 2.4a). For aphid colonies that were ant tended, the number of tending ants increased with the number of aphids (LMM: $\chi^2 = 17.270$, $P < 0.001$) but did not depend on the elevation \times aphid number interaction (LMM: $\chi^2 = 1.983$, $P = 0.159$) or the main effect of elevation (LMM: $\chi^2 = 1.567$, $P = 0.211$) (Fig. 2.4b).

Aphid colony survival depended on the elevation \times ant treatment interaction (binomial GLMM: $\chi^2 = 7.370$, $P = 0.007$), where ants increased survival by 66% at low elevations (GLMM: $\chi^2 = 8.473$, $P = 0.004$) but had no detectable effect at high elevations (GLMM: $\chi^2 = 0.003$, $P = 0.960$) (Fig. 2.5a). There was also a significant main effect of elevation (binomial GLMM: $\chi^2 = 3.955$, $P = 0.047$), but the main effect of ant treatment was not statistically significant (binomial GLMM: $\chi^2 = 0.002$, $P = 0.963$) (Fig. 2.5a). For the aphid colonies that survived, ants doubled their per capita growth (LMM: $\chi^2 = 4.538$, $P = 0.033$) (Fig. 2.5b). However, per capita growth rates did not depend on an elevation \times ant treatment interaction (LMM: $\chi^2 = 0.078$, $P = 0.780$) or the main effect of elevation (LMM: $\chi^2 = 0.922$, $P = 0.337$) (Fig. 2.5b).

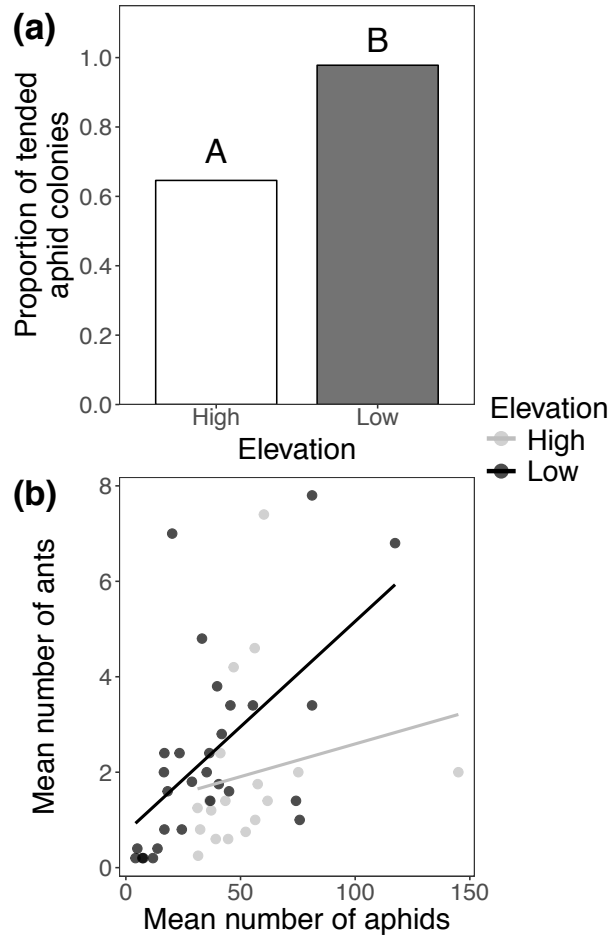


Figure 2.4. (a) The proportion of aphid colonies that were ant tended (the asterisk indicates a significant effect) and, where ants were present, (b) the relationship between the mean number of ants and the mean number of aphids per colony at low and high elevations. While separate trend lines are shown for low versus high elevations in panel (b), neither the aphid number x elevation interaction nor the main effect of elevation were statistically significant.

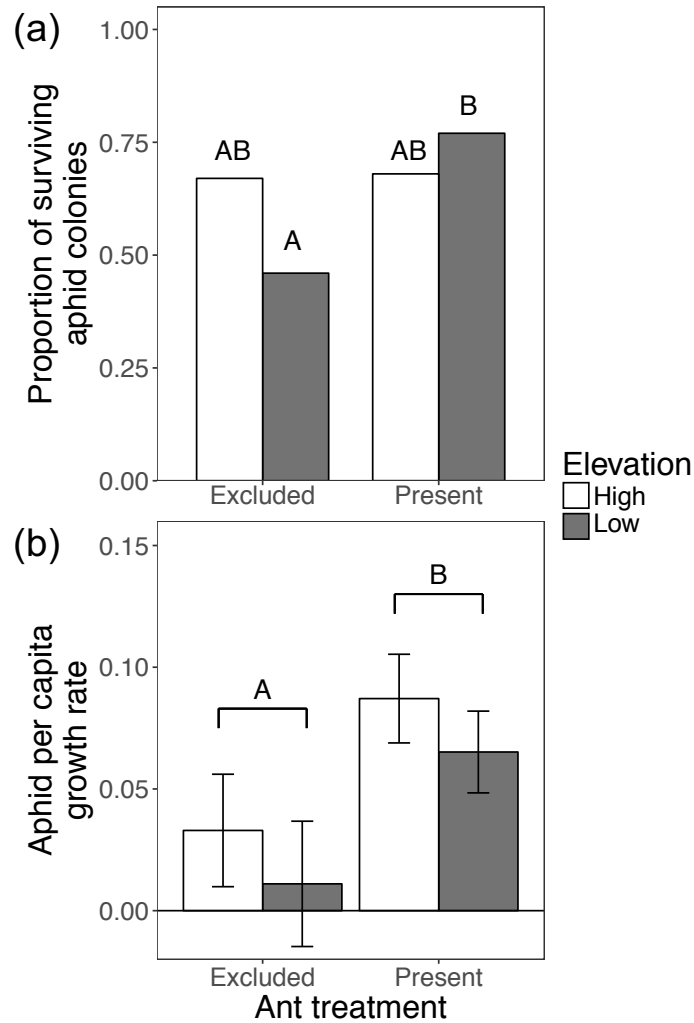


Figure 2.5. At low and high elevations where ants were excluded or allowed access ('present'), (a) the proportion of aphid colonies that survived during the experiment and (b) the mean (back-transformed LS-means \pm SE) per capita growth rates of the aphid colonies that survived. Letters indicate significant differences among groups.

Natural enemy abundance

Aphid natural enemies were 1.4-fold more abundant at the low elevation site (LMM: $\chi^2 = 9.627$, $P = 0.002$), but natural enemy abundance did not depend on the mean number of aphids per plant (LMM: $\chi^2 = 0.746$, $P = 0.388$) (Fig. S2.7).

Contrasting analytical approach

Supplemental statistical analyses (presented in Appendix 2B) were conducted based upon mean summer temperature (in place of analyses based upon elevation as high versus low). Although these analyses are pseudo-replicated, with the 10 replicates within a valley having only two to four estimates of mean summer temperature, they nonetheless provide some mechanistic insight into the factors driving elevational differences. With the exception of analyses of ant stable isotopes, the outcomes of these two approaches were qualitatively identical, with increasing temperature having the same effects as low (versus high) elevation (Appendix 2B). In the stable isotope analysis, we found that the C:N ratio (but no other measure of stable isotopes) significantly increased with mean summer temperature (LMM: $\chi^2 = 3.936$, $P = 0.047$) (Fig. S2.1).

DISCUSSION

Low (versus high) elevations had stronger predator effects and even stronger ant tending effects, which together resulted in a stronger ant–aphid mutualism. These results are consistent with predictions for stronger species interactions at lower elevations and for the progressive sensitivity of trophic levels to temperature. Low elevation sites were associated with a higher natural enemy abundance, and in the absence of ants, lower aphid colony survival, supporting the prediction for stronger predator effects under warmer conditions. At the same time, low elevation sites were associated with ants consuming more carbohydrates and tending aphids more frequently, and in the presence of ants, higher aphid colony survival. These findings are thus consistent with the prediction for even stronger effects of ants (versus natural enemies) under warmer conditions (Voigt et al. 2003, Mooney et al. 2016). As a result of aphid colonies at low

elevations having both lower survival without ants as well as higher survival with ants, ants increased aphid colony survival by 66% at low elevations but had no effect at high elevations. Furthermore, our supplementary analyses suggest that these effects were driven by increases in mean summer temperature. Accordingly, our findings support the notion that elevational gradients in multi-trophic interactions are driven by the progressive sensitivity of trophic levels to warming (Kishi et al. 2005, Barton et al. 2009).

Consistent with other studies of elevational gradients, we found a higher abundance of natural enemies at low elevations. Natural enemy abundance and diversity is often found to vary along environmental gradients (Hodkinson 2005) and frequently declines with increasing elevation and latitude (Straw et al. 2009, Sam et al. 2015, Moreira et al. 2018). Similar to our study, Straw et al. (2009) found that invertebrate predators of aphids were most abundant at low elevation sites, possibly causing aphids to be less abundant at low (versus mid) elevations. Importantly, such variation in predator effects may also cascade down to affect lower trophic levels. For example, Barton et al. (2009) found that warming increased the strength of the indirect effects of predators on terrestrial plant biomass. Likewise, Kishi et al. (2005) found that temperature altered predator foraging activity, resulting in cascading effects on lower trophic levels in an aquatic system.

Surprisingly, changes in abiotic conditions associated with elevation did not affect *Formica podzolica* ant abundance, as assessed using pitfall traps. In general, ants are known to be more abundant and diverse at lower elevations (Lessard et al. 2007, Sanders et al. 2007, Machac et al. 2011), and such patterns have been found in other sites near the RMBL (Menke et al. 2014). Moreover, previous studies have found ant mutualisms and the effects of ants as predators to be stronger at lower elevations and latitudes as the result of increases in ant

abundance or changes in ant species composition (Koptur 1985, Olmstead and Wood 1990, Zelikova et al. 2008, Pellissier et al. 2012, Sam et al. 2015, Plowman et al. 2017), including along even relatively short elevational gradients like that studied here (Binkenstein et al. 2017).

In contrast, elevation mediated ant demand for carbohydrate-rich resources, and as a result, ants tended aphids more frequently at low elevations. Stable isotope analysis of ants has previously demonstrated variation in ant diet among colonies of the same species within a single population (Mooney and Tillberg 2005, Tillberg et al. 2006) and along elevational gradients (Fiedler et al. 2007). While we did not detect an effect of elevation on ant stable isotopes, ant C:N ratios increased with mean summer temperatures (associated with low elevations), suggesting increased consumption of carbohydrates versus proteins. In addition, ants consumed more carbohydrate baits (per colony and per forager) and tended aphids more frequently at low elevations. Collectively, these results suggest that ants have a faster metabolism at low elevations where the climate is warmer, causing them to forage at a faster ‘tempo’ (sensu Davidson 1997). This conclusion is consistent with previous observations of variation in ant nutrient use along environmental gradients (Kaspari et al. 2008, Peters et al. 2014). However, further manipulative experiments are needed to determine the exact physiological and abiotic mechanisms underlying these changes in ant resource consumption. Possible factors include the direct effects of temperature, the availability of water or other carbohydrate resources, growing season length, and ant metabolic and nutritional demand (Cros et al. 1997, Cassill and Tschinkel 1999, Grover et al. 2007, Dussutour and Simpson 2009, Petry et al. 2012, Frizzi et al. 2016).

We did not investigate whether abiotic variation between low and high elevation sites mediates aphid performance directly or indirectly through changes in plant quality, but past studies suggest that such dynamics may not be important (Barton et al. 2009). For example,

Mooney et al. (2016) found that variation in the abundance and performance of the aphid *Aphis helianthi* between sunny meadow and shaded understory environments was not due to variation in the direct effect of temperature or in host plant quality (*Ligusticum porteri*), but rather was mediated entirely by variation in the mutualist services provided by ants. Similarly, Nelson et al. 2019c found that variation in the abundance and performance of the aphid *Pterocomma beulahense* between low and high elevation sites in the same region was not due to variation in the direct effect of temperature or host plant quality (*Populus tremuloides*), but was mediated by changes in the activity of mutualist ants. These past studies, in combination with the results provided here, thus suggest that the most significant consequences of variation in the abiotic environment for herbivore performance may be through effects on higher, and not lower trophic levels.

Because ants are dominant members of most terrestrial communities, elevational variation in ant activity can have widespread ecological consequences. In our study sites, *F. podzolica* engages in protection mutualisms with other honeydew-producing hemipterans (e.g., the aphids *A. helianthi* and *A. salicariae* on fireweed as well as hemipterans on other host plants) and extrafloral nectar-producing plants (e.g., *Helianthella quinquenervis*) (Addicott 1978, Inouye and Taylor 1979). Nelson et al. (2019c) found that because ants at low elevations were more active and tended the aphid *Pt. beulahense* more frequently, aphid abundance was greater at low elevations. It is likely that differences in ant activity shape the distribution and abundance of many such mutualist species along this elevational gradient. Because ant protection mutualisms are considered to be ‘keystone interactions’ that have widespread effects on community structure (Styrsky and Eubanks 2007), variation in ant protection may also affect communities of associated species along environmental gradients. Moreover, because ants serve important roles

as predators, nutrient recyclers (Griffiths et al. 2018), and seed dispersers (Giladi 2006), it is likely that variation in ant activity along abiotic gradients affects multiple ecosystem processes.

In summary, the results of this study are consistent with the prediction for a trophic gradient in sensitivity to abiotic change (Petchey et al. 1999, Voigt et al. 2003, Vasseur and McCann 2005) that in turn results in a gradient in interaction strength. These findings are consistent with the studies of two other ant-aphid systems in the same region (*Aphis helianthi* on the host plant *Ligusticum porteri*, Mooney et al. 2016, and *Pterocomma beulahense* on the host plant *Populus tremuloides*, Nelson et al. 2019c) that show evidence for temperature effects on higher trophic levels (predators and ants) but not on plant quality or aphid performance. Thus, this work highlights that in order to predict the consequences of climate change across entire food webs, it is important to understand the causes and consequences of trophic gradients in sensitivity to abiotic change.

SUPPLEMENTAL MATERIALS

Appendix 2A

Here, we provide an overview of the variables measured (Table S2.1) and the statistical analyses used to analyze those variables (Table S2.2).

Table S2.1. Summary of the variables measured, with a description of how they were measured, the year(s) in which they were measured, the number of sampling dates, and the sampling design and sample size.

<i>Variable</i>	<i>Description</i>	<i>Year</i>	<i>Number of sampling dates</i>	<i>Sampling design and sample size</i>
Ant abundance	Number of ants per trap per day within a site	2012, 2015	2 in 2015; 1 in 2012	2 pitfall traps in all 60 sites in 2015; 8-16 traps in 18 sites in 2012 (a subset of the 60 sites; 3 per valley)
Ant mound area	Surface area of the ground covered	2010	1	Focal ant mounds in all 60 sites
Ant mound activity	Mean number of ants on the mound surface across all observations	2010	7-9 (differed among valleys)	Focal ant mounds in all 60 sites
Ant diet	Ant $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, C:N ratio, percent C, and percent N	2010	1	1-3 adults and pupae from ant mounds in 28 sites (a subset of the 60 sites; 4-5 per valley)
Bait consumption rate	Mass lost per hour	2010	3	3 bait concentrations per bait set at ant mounds in all 60 sites
Ant abundance in baits	Number of ants per bait	2010	3	3 bait concentrations per bait set at ant mounds in all 60 sites

Bait consumption per ant	Mass lost per ant per hour	2010	3	3 bait concentrations per bait set at ant mounds in all 60 sites
Tending ant presence	Presence (vs. absence) of tending ants	2010	4-5 (differed among valleys)	Aphid colonies on potted plants placed adjacent to ant mounds in all 60 sites; ant access treatment
Tending ant number	Mean number of tending ants (when present) across all dates	2010	4-5 (differed among valleys)	Aphid colonies on potted plants placed adjacent to ant mounds in all 60 sites; ant access treatment
Aphid colony survival	Survival (vs. extinction) throughout the experiment	2010	1 (survival assessed on the last date)	Aphid colonies on potted plants placed adjacent to ant mounds in all 60 sites; ant access and exclusion treatments
Aphid colony growth rate	Per capita growth rate, calculated as $r = [\ln(N_t/N_0)]/t$	2010	2 (growth assessed by comparing colony sizes on the first and last dates)	Aphid colonies on potted plants placed adjacent to ant mounds in all 60 sites; ant access and exclusion treatments
Natural enemy abundance	Mean number of natural enemies per plant across all dates	2009	6	2 plants in each of 15 blocks in one of the low and one of the high elevation valleys

Table S2.2. Summary of the statistical analyses conducted for each response variable. We used linear mixed models (LMMs) and generalized linear mixed models (GLMMs) to analyze the data. For response variables that were transformed to improve the normality of the residuals, the type of transformation is indicated. All fixed and random effects included in the models are listed.

<i>Response variable</i>	<i>Statistical model</i>	<i>Data transformation</i>	<i>Fixed effects</i>	<i>Random effects</i>
Ant abundance	LMM	Cube-root	Elevation	Valley
Ant mound area	LMM	\ln	Elevation	Valley
Ant mound activity	LMM	\ln	Elevation	Valley
Ant $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, C:N ratio, percent C, and percent N	LMMs (separate for each response variable)	None	Elevation, ant life stage	Valley, ant mound
Bait consumption rate	LMM	$\ln + 1$	Elevation, sugar concentration, Elevation x sugar concentration, Date	Valley, ant mound
Ant abundance in baits	GLMM (negative binomial distribution with a quasi-Poisson scale parameter)	None	Elevation, sugar concentration, Elevation x sugar concentration, Date	Valley, ant mound
Bait consumption per ant	LMM	$\ln + 1$	Elevation, sugar concentration, Elevation x sugar concentration, Date	Valley, ant mound
Tending ant presence	GLMM (binomial distribution)	None	Elevation, mean aphid number	Valley

Tending ant number	LMM	ln	Elevation, mean aphid number, elevation x mean aphid number	Valley
Aphid colony survival	GLMM (binomial distribution)	None	Elevation, ant tending treatment, elevation x ant tending treatment	Valley, ant mound
Aphid colony growth rate	LMM	None	Elevation, ant tending treatment, elevation x ant tending treatment	Valley, ant mound
Natural enemy abundance	LMM	$ln + 1$	Valley, mean number of aphids	Block

Appendix 2B

To test whether variation in temperature drives variation in the ant-aphid mutualism along this elevational gradient, we repeated all statistical analyses (described in the Methods and Appendix 1) treating mean summer temperature (estimated from the PRISM Climate Group Model for May-September in 2009-2015) as a continuous predictor variable in place of elevation (high vs. low) as a categorical predictor variable. Due to the relatively low resolution of the PRISM Climate Group Model, the 10 replicate sites within each valley can only be ascribed between two and four separate estimates of climatic conditions, resulting in a pseudo-replicated analysis. Nevertheless, these analyses provide some mechanistic insight into the factors driving elevational differences. Results from all statistical analyses were qualitatively similar to our tests where elevation was classified as categorical (high vs. low), with the exception of ant stable isotope analyses. Here, we present a summary of the key results from these analyses.

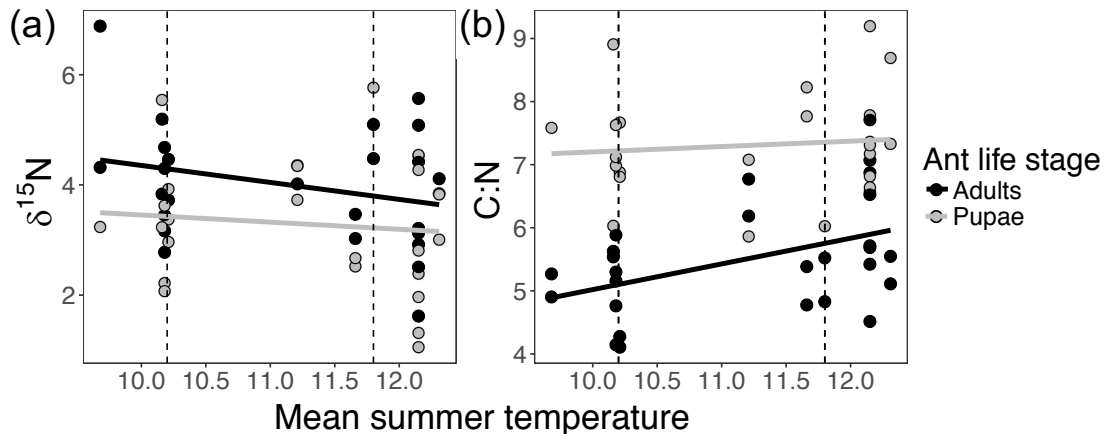


Figure S2.1. The (a) $\delta^{15}\text{N}$ and (b) C:N ratio of adult ants and pupae, based on mean summer temperature. Dashed vertical lines indicate the mean summer temperatures of the low ($10.2 \pm 0.4^\circ\text{C}$) and high ($11.8 \pm 0.4^\circ\text{C}$) elevation sites. We detected no significant effect of temperature on ant $\delta^{15}\text{N}$ (LMM: $\chi^2 = 1.542$, $P = 0.214$), although adult (vs. pupae) $\delta^{15}\text{N}$ was significantly

greater (LMM: $\chi^2 = 26.762$, $P < 0.001$). In contrast, the C:N ratio significantly increased with temperature (LMM: $\chi^2 = 3.936$, $P = 0.047$) and was lower for adults (vs. pupae) (LMM: $\chi^2 = 55.856$, $P < 0.001$).

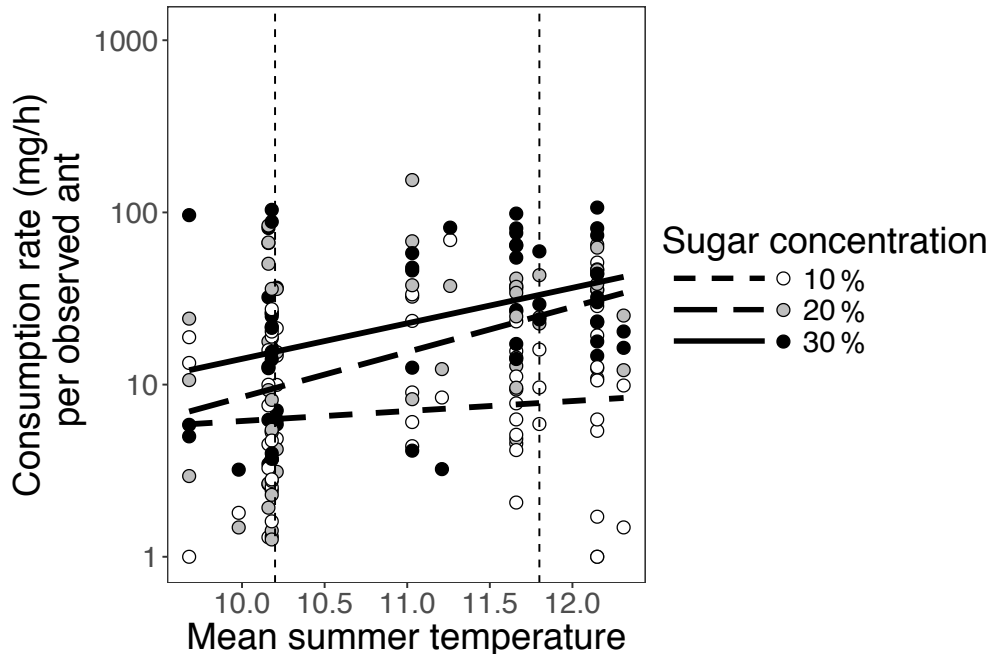


Figure S2.2. The consumption rates per observed ant (mg/h/ant) of three bait concentrations (10%, 20%, and 30% sugar; replicates averaged across dates), based on mean summer temperature. Dashed vertical lines indicate the mean summer temperatures of the low ($10.2 \pm 0.4^\circ\text{C}$) and high ($11.8 \pm 0.4^\circ\text{C}$) elevation sites. Sugar consumption per ant depended on the temperature x sugar concentration interaction (LMM: $\chi^2 = 8.652$, $P = 0.013$) as well as the main effect of date (LMM: $\chi^2 = 63.058$, $P < 0.001$). The main effects of temperature (LMM: $\chi^2 = 3.293$, $P = 0.070$) and sugar concentration (LMM: $\chi^2 = 5.627$, $P = 0.060$) were both marginally significant.

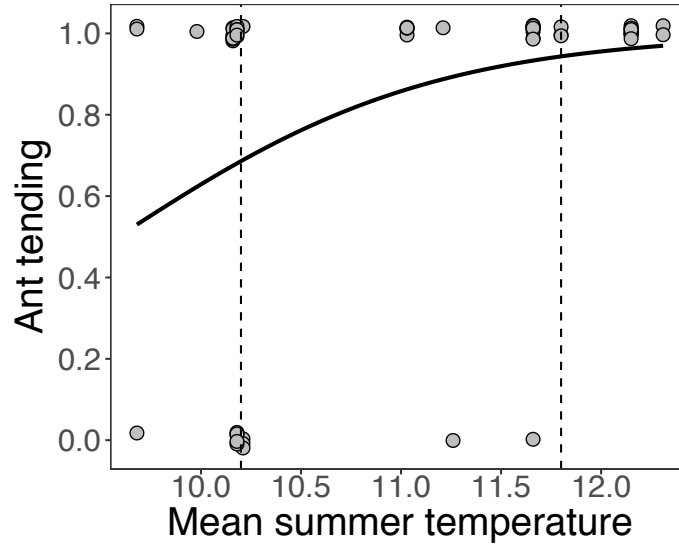


Figure S2.3. The probability of aphid colonies being tended by ants, based on mean summer temperature. Points are vertically jittered to minimize overplotting, and dashed vertical lines indicate the mean summer temperatures of the low ($10.2 \pm 0.4^\circ\text{C}$) and high ($11.8 \pm 0.4^\circ\text{C}$) elevation sites. The likelihood of ant tending significantly increased with temperature (binomial GLMM: $\chi^2 = 4.409$, $P = 0.036$) but did not depend on the number of aphids in a colony (binomial GLMM: $\chi^2 = 1.478$, $P = 0.224$).

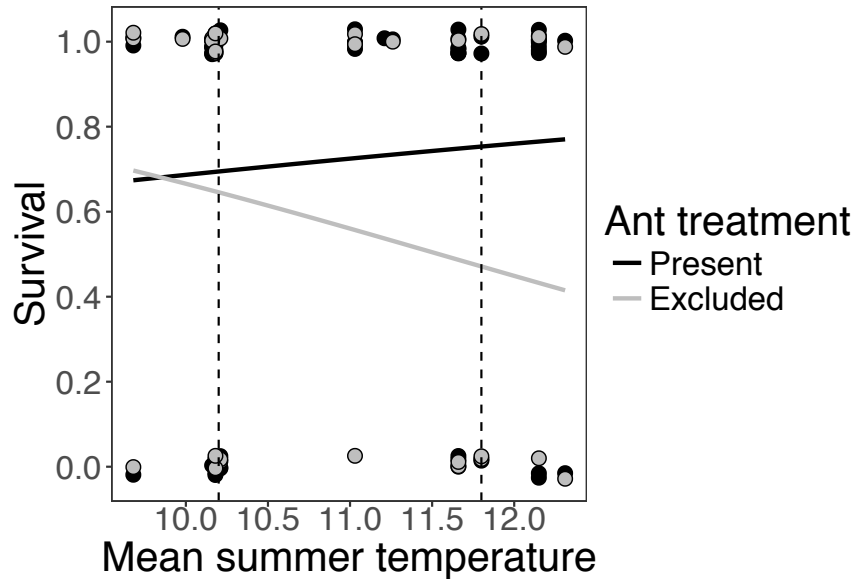


Figure S2.4. The probability of aphid colony survival when ants were excluded or allowed access (“present”), based on mean summer temperature. Points are vertically jittered to minimize overplotting, and dashed vertical lines indicate the mean summer temperatures of the low ($10.2 \pm 0.4^{\circ}\text{C}$) and high ($11.8 \pm 0.4^{\circ}\text{C}$) elevation sites. Aphid colony survival depended on the temperature x ant treatment interaction (binomial GLMM: $\chi^2 = 4.280$, $P = 0.039$), where aphids received greater benefits of ant protection at warmer temperatures. The main effect of ant treatment was also significant (binomial GLMM: $\chi^2 = 3.920$, $P = 0.048$), but the main effect of temperature was not (binomial GLMM: $\chi^2 = 2.201$, $P = 0.138$).

Appendix 2C

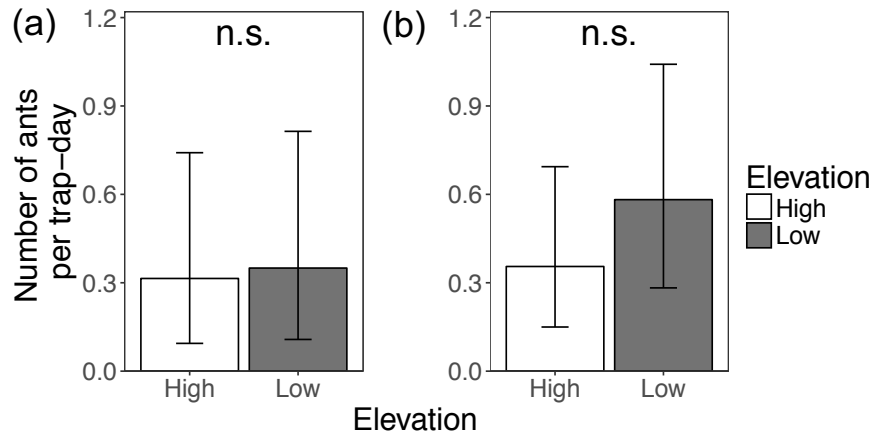


Figure S2.5. The mean (back-transformed LS-means \pm SE) number of *F. podzolica* ants collected per pitfall trap per day at low and high elevations in (a) 2012 and (b) 2015.

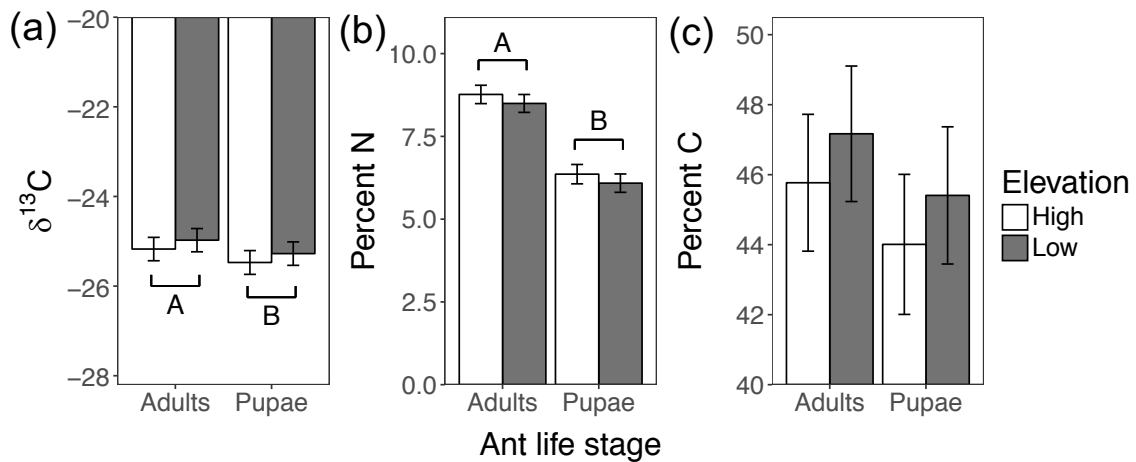


Figure S2.6. The mean (LS-means \pm SE) (a) $\delta^{13}\text{C}$, (b) percent N, and (c) percent C of adult ants and pupae collected from ant mounds at both low and high elevation sites. Letters indicate significant differences between ant life stages. Percent C did not differ with ant life stage, and there were no significant effects of elevation on $\delta^{13}\text{C}$, percent N, or percent C.

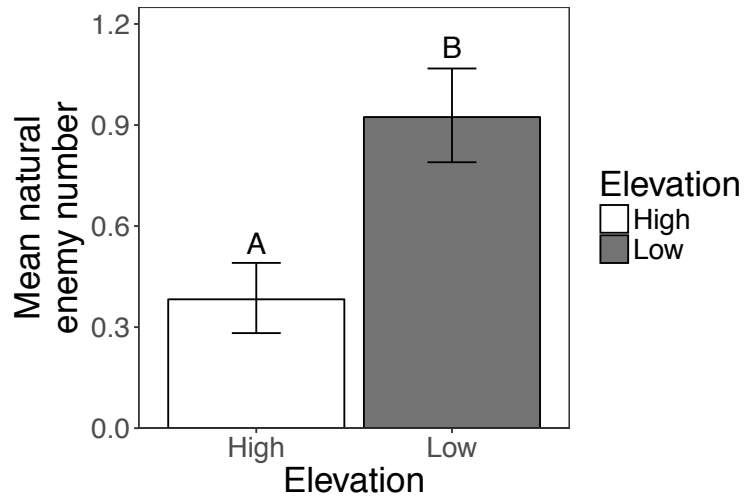


Figure S2.7. The mean (LS-means \pm SE) number of natural enemies observed across six observations at naturally occurring aphid colonies within one high (East River Valley) and one low elevation valley (Spring Creek). Letters indicate significant differences between groups.

CHAPTER 3

Sequential but not simultaneous mutualist diversity increases partner fitness

ABSTRACT

Most mutualisms involve many interacting species, but we know little about how mutualist identity and diversity affect partner fitness and population dynamics. When species associate with multiple mutualists simultaneously (“simultaneous diversity”), they may benefit if partners provide complementary benefits. In addition, when species associate with multiple mutualists serially throughout their lifetimes (“sequential diversity”), they may benefit not only if partners provide complementary benefits but also if they differ in their relative rankings or availability over space or time. Here, we tested whether populations of the aphid *Aphis asclepiadis* benefit from both simultaneous and sequential mutualist ant diversity. To do so, we parameterized demographic models with three years of field-collected data, which we used to compare estimates of aphid population growth rates (λ) under varying levels of ant diversity. To test for simultaneous diversity effects, we compared estimates of λ for aphid colonies that were constantly untended, tended individually by each of three ant species, and tended by multiple species simultaneously. To test for sequential diversity effects, we compared estimates of λ for aphid colonies in full and reduced ant community models, incorporating probabilities of ant arrival and departure in addition to effects on vital rates when present. Models showed that each ant individually enhanced aphid colony survival and growth but decreased alate production, resulting in positive net effects on aphid colony λ . However, ants largely did not provide complementary benefits, and λ was predicted to be lower with simultaneous ant diversity than for aphids tended by any individual species. Nonetheless, the most effective ant mutualist was predicted to occur less frequently than the other species, except at the largest aphid colonies.

Thus, due to this trade-off in mutualist quality and availability, aphids benefitted from sequential mutualist ant diversity. In all, this study shows that variation not only in mutualist quality but also in mutualist availability can determine the population-level outcomes of mutualisms. Moreover, even if simultaneous mutualist diversity is not beneficial, associating with multiple mutualists sequentially can have important benefits for lifetime fitness.

INTRODUCTION

Mutualisms play a key role in structuring ecological communities and maintaining biodiversity (Stachowicz 2001, Bracken et al. 2007, Aslan et al. 2013, Bronstein 2015). However, because natural selection is expected to favor cheaters that take benefits without reciprocating, the persistence of mutualisms is a central paradox in biology (West et al. 2007, Jones et al. 2015). To prevent cheating, mutualisms have often been predicted to evolve into more specialized interactions in which species limit their associations to the most cooperative partners (Frederickson 2013). Nevertheless, most mutualisms consist of networks of interacting species, and specialists tend to interact with highly generalist species (i.e., networks are nested with asymmetric specialization) (Bascompte 2009). Organisms often interact with multiple mutualist partners simultaneously (Stachowicz and Whitlatch 2005, Schluter and Foster 2012), across different environmental conditions or geographic locations (Silverstein et al. 2012, Fitzpatrick et al. 2014), or sequentially across ontogeny (Palmer et al. 2010).

One possible explanation for such widespread generalization in mutualisms is that species may benefit from mutualist partner diversity (Batstone et al. 2018). In the biodiversity-ecosystem functioning literature, the presence of different plant functional groups has been shown to increase ecosystem functioning through complementarity or facilitation among species

(Cardinale et al. 2007). Analogously, if different mutualists provide complementary benefits or facilitate each other, species may receive greater benefits from associating with multiple partners (Stachowicz and Whitlatch 2005, Fontaine et al. 2005, McCutcheon and Moran 2007, Blüthgen and Klein 2011, Fründ et al. 2013).

The extent to which species benefit from mutualist partner diversity is not well understood but may depend on whether partners overlap in time. In many mutualisms, species associate with multiple partners at the same time (“simultaneous diversity”), such as when pollinators visit co-flowering plants (Mitchell et al. 2009) or when multiple mycorrhizae colonize the same host plant (Werner and Kiers 2015b). Species also often serially associate with multiple mutualist partners (“sequential diversity”), either across short (e.g., during different times of day) (Stone et al. 1996, Fitzpatrick et al. 2014) or longer time scales (e.g., across seasons or entire lifetimes) (Waser and Real 1979, Palmer et al. 2010). Both simultaneous and sequential mutualist diversity may be advantageous if partners perform complementary functions or facilitate each other (Waser and Real 1979, Stachowicz and Whitlatch 2005, Ogilvie and Thomson 2016). For example, Stachowicz and Whitlatch (2005) found that a red alga benefits associating with two gastropod species because they perform complementary functions by removing different suites of invertebrates that would otherwise foul its surface. However, when multiple mutualists occur simultaneously, competition for their shared mutualist could reduce the benefits that they would otherwise provide or receive, such as when co-flowering plants compete for pollination (Waser 1978, Palmer et al. 2003, Stanton 2003, Mitchell et al. 2009, Ashman and Arceo-Gómez 2013). As a result, selection may favor divergence in mutualist overlap so that competition is minimized, and it is possible that sequential mutualist diversity may generally be more beneficial than simultaneous diversity (Waser 1978).

Species can benefit from sequential mutualist diversity not only if mutualists perform complementary functions or facilitate each other, but also if their relative rankings or abundances vary across time. For example, Palmer et al. (2010) found that mutualist ant rankings change across *Vachellia* (formerly *Acacia*) *drepanolobium* ontogeny, and associating with different ant partners over time thus enhances plant lifetime fitness. Additionally, sequentially associating with multiple partners may allow species to receive benefits more reliably across contexts (Batstone et al. 2018). For example, when heat-sensitive algal endosymbionts are lost during coral bleaching, corals may uptake more heat-tolerant algae that allow them to continue to receive mutualist services (Silverstein et al. 2012). However, competition among partners and priority effects can alter the consequences of sequential mutualist diversity by determining how frequently and in what order associations with different partners occur (Palmer et al. 2003, Stanton 2003, Werner and Kiers 2015a). If the strongest competitors are poor mutualists (e.g., Bennett and Bever 2009), competition may limit associations with high quality mutualists, causing the mutualism to break down. Alternatively, if the strongest competitors are effective mutualists, species may benefit from turnover among mutualist partners driven by competition (Palmer et al. 2000, 2010).

To our knowledge, no previous study has compared the fitness effects of simultaneous versus sequential mutualist diversity on partner lifetime fitness and population dynamics. Because species differ not only in mutualist quality but also in abundance and competitive ability, predicting the consequences of mutualist partner diversity remains challenging. By definition, mutualists enhance the fitness of their partners and may do so through positive effects on one or more demographic vital rates (i.e., survival, growth, and reproduction) (Ohm and Miller 2014, Ford et al. 2015). However, mutualists can positively affect some vital rates while

negatively affecting others (Bronstein 2001), and certain vital rates may influence population dynamics more strongly than others (Caswell 2001). Thus, we cannot infer how mutualists affect partner fitness from only measuring effects on single vital rates. Rather, demographic models that integrate information about all three vital rates to project their effects on population dynamics are needed to determine the overall effects of mutualists on partner fitness. Such studies are rare (but see Price et al. 2008, Palmer et al. 2010, Geib and Galen 2012, Ohm and Miller 2014, Bruna et al. 2014, Ford et al. 2015) but necessary for understanding the consequences of mutualist diversity.

We investigated the effects of mutualist ant (Formicidae) identity and diversity on the population dynamics of the aphid *Aphis asclepiadis* (Aphididae) feeding on the host plant *Ligusticum porteri* (Apiaceae). Specifically, we used demographic models of aphid populations parameterized with field-collected data to test for the effects of ant mutualist quality and availability on aphid populations, as well as to test the fitness effects of simultaneously versus sequentially associating with up to three mutualist ant species. While all three ant species can co-occur and tend aphids within the same site as well as during the same times of day and season, individual aphid colonies differ in the diversity of ants that associate with them. Here, we considered ant diversity to be simultaneous when an individual aphid colony was tended by two or three ant species at the same time, and we considered ant diversity to be sequential when an individual aphid colony was only tended by one ant species at a single time but tended by multiple ant species throughout its lifetime. We hypothesized that if ants performed complementary functions, aphids would benefit from both simultaneous and sequential ant diversity. However, we hypothesized that if ants competed for aphids, ants would be ineffective mutualists and aphids would thus benefit from sequential but not simultaneous ant diversity.

MATERIALS AND METHODS

Study site and natural history

In July 2017-2019, we worked in an open, subalpine meadow (38.926°N, 106.963°W) near the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado. This meadow is on a northeast-facing slope approximately 2930 m above sea level. It is dominated by herbaceous perennial plants, including *Ligusticum porteri* (Apiaceae; “osha”), which is widely distributed throughout the Rocky Mountain region (Terrell and Fennell 2009). Across the three study years, there were large differences in snowmelt date (ranging from May 5 in 2018 to June 6 in 2019) and summer temperature and precipitation (climate further described in Appendix 3A).

Aphis asclepiadis (Aphididae; formerly *A. helianthi*) is an ant-tended aphid that colonizes the leaves and flowering stalks of *L. porteri* (Mooney et al. 2016) as well as other host plants in nearby sites (Addicott 1978). *Aphis asclepiadis*, like all aphids, gives live birth and reproduces asexually during the summer, resulting in short generation times and rapid colony growth (Dixon 1985). While most *A. asclepiadis* aphids are unwinged, *A. asclepiadis* exhibits a wing polyphenism; winged aphids (“alates”) are produced in response to stressful environmental conditions (e.g., crowding, poor host plant quality, or high predation risk), allowing clones to disperse to establish new colonies (Müller et al. 2001, Braendle et al. 2006). Thus, aphid colonies start small (with a single alate) and grow larger over time.

Near the RMBL, *A. asclepiadis* commonly associates with the mutualist ants *Formica podzolica*, *Formica rufa* group (subfamily Formicinae), and *Tapinoma sessile* (subfamily Dolichoderinae) (Mooney et al. 2016). In this mutualism, ants protect the aphid against natural enemies in exchange for a sugar-rich liquid reward (“honeydew”) that the aphid excretes (Way 1963, Stadler and Dixon 2005). Ant protection mutualisms are ideal for investigating the effects

of associating with multiple mutualist partners, since they often involve several ant species that vary considerably in mutualist quality, abundance, and competitive ability (Chamberlain and Holland 2009). The ants in our study site differ in abundance and also follow a dominance-discovery trade-off, with *F. rufa* being the most aggressive ant but less abundant and slower to discover resources than *F. podzolica* and *T. sessile* (Nelson and Mooney, unpublished data). Previous work in this region has shown ant-aphid mutualisms to vary with the abiotic environment (Mooney et al. 2016, Nelson et al. 2019b, 2019c), ant diet (Petry et al. 2012), and host plant sex (Mooney et al. 2012) and chemical cues (Nelson et al. 2019a, 2019e).

Demographic data collection

We collected observational demographic data to assess the individual and combined effects of *F. podzolica*, *F. rufa*, and *T. sessile* on aphid colony vital rates and population dynamics. Because aphids live in colonies that consist of genetically identical individuals, the true “individual” could be considered the genotype. Although including the genetic structure of aphid populations was infeasible in this study, we estimated ant effects on aphid lineages by measuring the effects of ants on “vital rates” of aphid colonies. Briefly, we measured aphid colony vital rates by marking and repeatedly censusing *L. porteri* host plants for aphids. All aphids on an individual host plant were considered to be part of the same colony. Aphid colony survival and growth were evaluated based on the presence and change in the number of aphids, respectively, and fecundity was evaluated based on the number of alates that dispersed to establish new colonies.

To collect vital rate data, we marked all *L. porteri* plants and censused them for aphids within one plot each summer (for three total plots). While plots were located within the same

meadow, they were selected in different locations each year based on where ants were observed to be actively tending aphids. Each plot (approximately 15 x 40 m area) included between 150 and 210 *L. porteri* plants. To supplement data collected from these plots, we also censused aphid colonies on between 90 and 140 additional *L. porteri* plants surrounding the plots (approximately 75 x 100 m area), focusing sampling effort on aphid colony states that were not well represented within the plot (e.g., smallest and largest aphid colonies). Because aphid colonies and ant partners can change rapidly over a short time period, we censused each plant for aphids at three-day intervals for up to seven observations ($N = 2,262$ observations of 347 plants from July 7-30, 2017; $N = 1,665$ observations of 284 plants from July 2-18, 2018; $N = 1,628$ observations of 243 plants from July 9-25, 2019). For each census, we counted the number of unwinged and winged aphids. We also assigned each aphid colony to an “ant tending state,” where colonies were considered to be untended if there were no ants on the plant, to be tended by one of the three ant species (*F. podzolica*, *F. rufa*, or *T. sessile*) if that ant was the only species present, or to be tended by multiple species simultaneously if there were two or three ant species present on the plant (due to low sample sizes, all combinations of two or three species were grouped together).

Demographic modeling and analysis

To test for the effects of mutualist ant identity and diversity on aphid colony demography, we built size-structured integral projection models (IPMs) (Easterling et al. 2000, Ellner et al. 2016) in R 3.6.1 (R Core Team 2019). The IPMs project aphid colony asymptotic “population” growth rates (λ ; i.e., changes in aphid colony abundance) under different ant tending states, based on ant-specific effects on aphid colony vital rates (dependent on colony size at time t), as well as estimates of the establishment rate and size distribution of new aphid

colonies. We chose to build the models based on three-day census intervals because ant turnover occurred frequently and would have not been adequately captured by longer intervals.

Nonetheless, from each IPM, we projected three-day λ (λ_3), which we used to then estimate season-long λ (30-day; $\lambda_{30} = \lambda_3^{10}$). For each estimate of λ_{30} , we calculated 95% bootstrap confidence intervals ($N = 1,000$ simulations), stratifying sampling by ant tending state and study year. Details about parameter estimation and IPM model structure are included in Appendix 3B. Here, we briefly summarize our main modeling approaches used to assess the effects of ant identity and diversity on aphid population growth.

IPMs were constructed following two main approaches. We built five “no transition” models to assess the performance of untended aphid colonies (one model) and colonies consistently tended by each ant species alone (three models) or by multiple species simultaneously (one model for all combinations of two or three ant species). These models did not allow for transitions among ant tending states and thus assessed aphid performance within the context of ants being consistently present or absent. In contrast, we also constructed “ant transition” models, in which aphid colonies were allowed to transition among ant species or between tended and untended states. In so doing, these models modified estimates of aphid colony vital rates to also reflect differences in the probability of ants arriving at and departing from aphid colonies, due to any potential differences in ant abundance in the environment, aphid colony discovery and abandonment rates, and competitive interactions among ants. A total of six “ant transition” models were constructed to assess the performance of aphid colonies with the potential to be tended by each ant species alone (three models), two ant species sequentially (three models for all pairwise combinations of species), and three ant species sequentially (one model).

To assess the effects of mutualist ant identity on aphid populations, we compared estimates of λ_{30} from IPMs of aphid colony populations that were untended with those that were tended by each ant species alone. Importantly, ant effects could depend both on mutualist quality (i.e., effects on vital rates) and availability (i.e., probability of arrival/departure). We evaluated ant mutualist quality by comparing estimates of λ_{30} from the “no transition” models of each ant species when present. To assess the effects of ant availability on aphid populations, we compared estimates of λ_{30} for each ant species from the “ant transition” models (testing for both quality and availability) relative to the “no transition” models (testing only for quality).

To assess the effects of mutualist ant diversity on aphid populations, we compared estimates of λ_{30} from IPMs of aphid colony populations that were tended by each of the individual ant species with those that were tended by multiple species. To understand the effects of simultaneous ant diversity within the context of consistent ant presence, we compared estimates of λ_{30} from the “no transition” model for multiple ant species with all “no transition” models for the single ant species. Because the effects of sequential ant diversity can only be understood within the context of transitions among ant states and thus inconsistent ant presence, we compared estimates of λ_{30} from “ant transition” models for all possible combinations of multiple ant species with those for each of the individual component ant species.

To parameterize the IPMs, we modeled aphid colony vital rates and ant tending state transitions (at time $t+3$ days based on aphid colony size and ant tending state at time t) by fitting a series of alternative possible generalized linear models (GLM) for survival, alate production, and ant transitions or Gaussian location-scale additive models (GAMLSS) for mean and variance in growth. We used Aikake’s information criterion (AIC) to select best-fitting models of each vital rate and ant transition probabilities. We compared models with different functional forms

for aphid colony size (calculated as the natural logarithm of the number of aphids at time t), with colony size as a linear or quadratic term in the GLMs or as a linear or smoothing term in the GAMLSSs. We also tested among models with different combinations of other fixed effects, including the main effect of ant tending state, the size x ant tending state interaction, and year as a covariate. Because all best-fit models included year, parameter estimates were averaged across years to construct IPMs to compare the effects of ants. GLMs were constructed using the ‘glm()’ function in the ‘stats’ package (R Core Team 2019) or the ‘glm.nb()’ function in the ‘MASS’ package (Venables and Ripley 2002), GAMLSSs were constructed using the ‘gam()’ function with the ‘gaulss()’ family in the ‘mgcv’ package (Wood 2011), and AIC values were calculated using the ‘AIC()’ function in the ‘stats’ package (R Core Team 2019).

Because we were unable to track alate dispersal, we estimated aphid colony recruitment rate as the number of newly established colonies observed during all censuses of the plots at time $t+3$ days divided by the number of alates produced by all aphid colonies in the plots during the previous censuses, at time t . The size distribution of new aphid colonies was estimated by fitting an exponential decay function for the observed sizes of all newly established colonies in the plots using the ‘fitdist()’ function in the ‘fitdistrplus’ package (Delignette-Muller and Dutang 2015). We used the same estimates of the recruitment rate and size distribution of new aphid colonies in all IPMs. By doing so, we assumed that ant tending state did not influence the probability of an alate successfully dispersing to establish a new colony. It is possible that ants could reduce the rate at which alates disperse from their parent colonies, since ants have previously been shown to limit the mobility of unwinged aphids (Oliver et al. 2007). Nonetheless, it was not possible to measure alate departure rates, and there is no reason to expect that the success of an alate after departure should be affected by its previous ant tending state. Thus, while over- or under-

estimating these parameters could have influenced our estimates of λ , the relative rankings of λ for populations of aphid colonies tended by the different ant species should likely be similar. Similar approaches for estimating recruitment have been commonly used in other studies (e.g., Ford et al. 2015), and here we found changes to aphid colony recruitment rates had proportionally weak effects on overall results from all IPMs (assessed in terms of kernel-level elasticities; Fig. S3.2).

RESULTS

In total, we conducted 5,555 censuses of 874 individual host plants, and aphids were present for 3,990 censuses (72%) across 786 (90%) individual plants. The median aphid colony size was 61 aphids, with observed sizes ranging from one to 3,017 aphids. Of the aphid colonies tracked across all seven observations within a summer ($N = 271$ colonies; includes only the colonies that survived across the entire 21-day study period), 271 (100%) were ant tended during at least one observation, indicating that ant tending likely plays an important role in aphid colony success. Of all aphid colonies ($N = 786$; includes all colonies, whether or not they were observed across the duration of the study period), 301 (38%) were tended by multiple ant species simultaneously during a total of 643 (12%) censuses. Although most aphid colonies were only tended by two ant species at a time (*F. podzolica* and *F. rufa*, $N = 149$ colonies over 643 censuses; *F. podzolica* and *T. sessile*, $N = 125$ colonies over 188 censuses; *F. rufa* and *T. sessile*, $N = 42$ colonies over 56 censuses), some were also tended simultaneously by all three ants ($N = 25$ colonies over 28 censuses). Moreover, 337 aphid colonies (43% of all colonies) were tended by multiple ant species sequentially. While most aphid colonies experienced only one or two transitions between ant species, some experienced up to six changes (colonies with one change,

$N = 143$; two changes, $N = 116$; three changes, $N = 53$; four changes, $N = 18$; five changes, $N = 5$; six changes, $N = 2$). In total, 174 (64%) aphid colonies that survived for the entire 21-day duration of the study (within a summer) were tended by multiple ant species during their lifetime, with 128 (74%) of them being tended by two ant species and 46 (26%) of them being tended by three.

Aphid colony vital rates and mutualist transition probabilities

Aphid colony size at time t (\ln -transformed) was a strong predictor of all vital rates (Fig. 3.1) and ant transition probabilities at time $t+3$ days (Fig. 3.2 and Fig. S3.3) (Appendix 3B). Relative to the best-fit models, Δ AIC values of null models that did not include size as a predictor variable were large. For all GLMs (survival, alate number, and ant transition models), quadratic functions of size provided the best model fits. In the best-fit GAMLSS, size was included as a smoothing predictor of both mean and variance in growth. Moreover, all best-fit models included the main effect of ant species at time t , with some models (variance in growth, alate number, and ant transition probabilities) also including a size x ant species interaction. The main effect of year (as a covariate) was retained in all best-fit models, and vital rate and ant transition model predictions were thus averaged across years.

In terms of ant effects on aphid colony vital rates, all ants had positive effects on survival and growth but negative effects on alate number (relative to untended colonies). Across all colony sizes, aphids tended by *F. rufa* at time t had higher vital rates (greater intercepts for survival and growth at time $t+3$ days, and a steeper slope for alate number at time t) than all other ant-tended colonies (Fig 3.1). Some vital rates were higher for aphid colonies tended by *F. podzolica* versus *T. sessile* (greater survival intercept), whereas others were higher for those

tended by *T. sessile* (greater growth intercept and alate number slope). Compared to all other ant-tended colonies, vital rates (survival and growth intercepts as well as alate number slope) were lowest for colonies tended simultaneously by multiple ant species.

In terms of ant availability, larger aphid colonies at time t were more likely to be ant-tended at time $t+3$ days (Fig. 3.2 and Fig. S3.3). While *T. sessile* was most likely to tend colonies that were small at time t , *F. podzolica* was most likely to tend medium-sized colonies, and *F. rufa* was most likely to tend large colonies (Fig. 3.2 and Fig. S3.3). Thus, although *F. rufa* was the highest quality mutualist (in terms of its effects on vital rates when present at time t), it was only predicted to tend aphid colonies more frequently than the other ant species when colonies exceeded sizes of approximately 875 aphids.

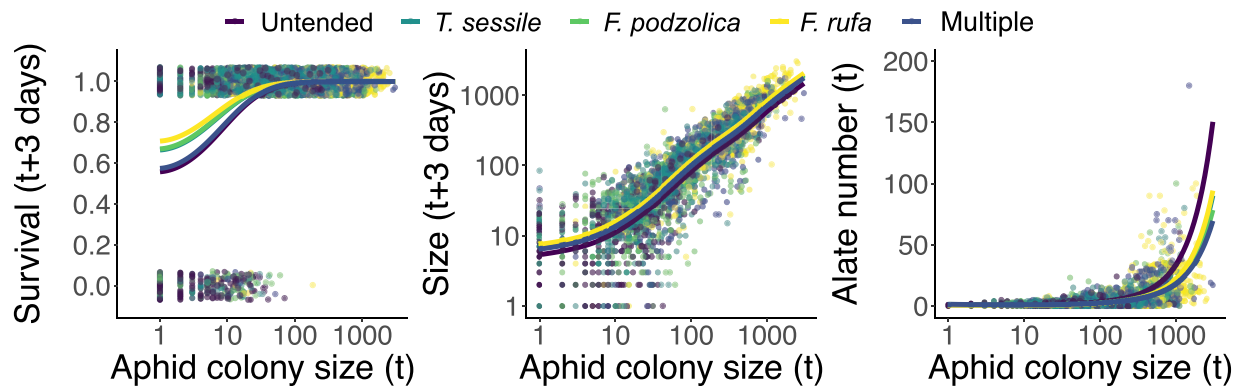


Figure 3.1. Predicted aphid colony vital rates from best-fit models based on colony size and ant tending state at time t .

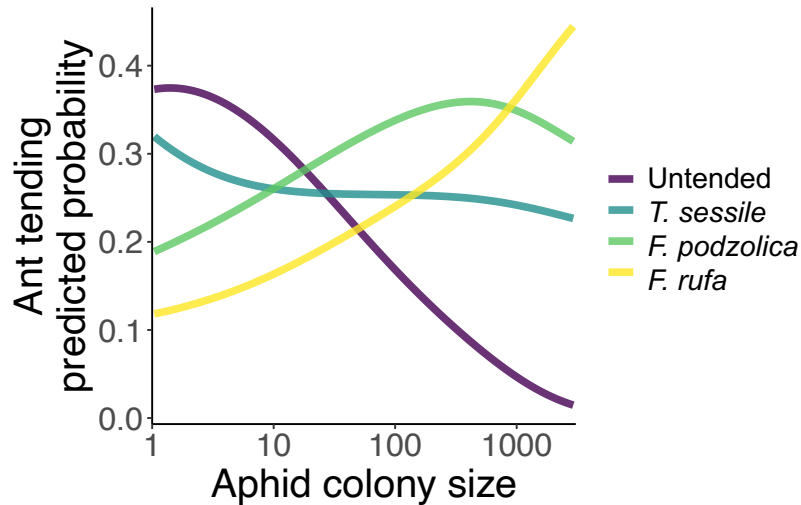


Figure 3.2. Best-fit predicted probabilities of tending by each ant species at time $t+3$ days based on ant transition probabilities and aphid colony size at time t .

Effects of ant mutualist quality and availability

Relative to aphid colonies that were consistently untended ($\lambda_{30} = 0.85$), each individual ant species had positive effects on season-long (30 days) asymptotic population growth rates, both in the “no transition” models testing for ant quality as mutualists as well as the “ant transition” models that incorporated differences in ant arrival and departure probabilities and thus tested for both ant quality and availability (Fig. 3.3). Because the “ant transition” models allowed for colonies to transition between ant tended and untended states, the positive effect of each ant on λ_{30} was less pronounced compared to from the “no transition” models where aphid colonies were consistently ant tended (Fig. 3.3). Interestingly, ant rank order in mutualist effectiveness reversed between the “no transition” and “ant transition” IPMs. In the “no transition” models based on ant effects on vital rates (assessing ant quality but not availability), aphid colonies tended by *F. rufa* had the highest population growth rate ($\lambda_{30} = 3.19$), followed by *F. podzolica* and *T. sessile*, which both had weaker positive effects that were similar in

magnitude ($\lambda_{30} = 2.23$ and 2.34 , respectively) (Fig. 3.3). However, mutualist quality traded off with ant availability ($F. podzolica > T. sessile > F. rufa$), where the highest quality partner ($F. rufa$) was predicted to occur at the lowest frequency at all but the largest aphid colonies (Fig. 3.2). As a result, the “ant transition” models (integrating both ant quality and availability) predicted a reversal in ant mutualist effectiveness, with aphid colonies tended by $F. podzolica$ having the highest population growth rate ($\lambda_{30} = 1.40$), followed by $T. sessile$ ($\lambda_{30} = 1.18$) and $F. rufa$ ($\lambda_{30} = 0.98$), respectively (Fig. 3.3).

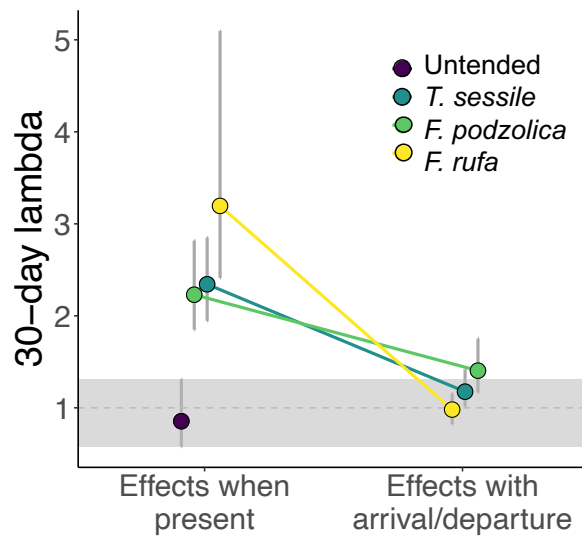


Figure 3.3. Season-long (30 days) asymptotic population growth rates (λ_{30}) and 95% bootstrap confidence intervals. Estimates are from IPMs for populations of aphid colonies that were untended or tended by each of three mutualist ant species alone, both from “no transition” IPMs that tested for ant effects when consistently present, as well as “ant transition” IPMs that also incorporated differences in ant arrival and departure probabilities and thus allowed for aphid colonies to switch between ant tended and untended states. The estimate of λ_{30} for aphid colonies that were consistently untended (confidence interval shown as the gray horizontal band) was used as a baseline to test for the effect of each ant species, both for “no transition” and “ant transition” model results.

Effects of simultaneous and sequential ant diversity

Comparisons between populations of aphid colonies tended consistently (from the “no transition” IPMs) by single versus multiple simultaneous ant species show that aphid colonies do not benefit from simultaneous ant diversity, with multiple species in combination being lower quality mutualists (Fig. 3.4). Although aphid colony population growth increased relative to untended colonies, those that were tended by multiple species simultaneously performed worse ($\lambda = 1.72$) than aphid colonies tended consistently by any of the three single ant species (Fig. 3.4). In contrast, comparisons between “ant transition” models testing for both mutualist quality and availability for all combinations of one, two, or three ant species show that aphid colonies benefit from sequential ant diversity (Fig. 3.5). Estimates of λ_{30} from models of each pairwise combination of ant species were similar to or greater than those from models including the two component ant species alone (both *F. rufa* and *T. sessile*, $\lambda_{30} = 1.20$; both *F. rufa* and *F. podzolica*, $\lambda_{30} = 1.41$; both *F. podzolica* and *T. sessile*, $\lambda_{30} = 1.64$) (Fig. 3.5). Out of all “ant transition” models, population growth rate was highest for aphid colonies allowed to be tended sequentially by all three ant species ($\lambda_{30} = 2.01$) (Fig. 3.5).

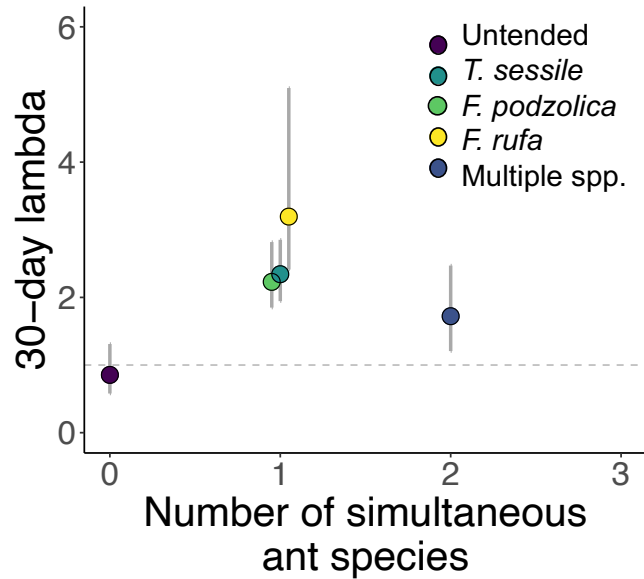


Figure 3.4. Season-long (30 days) asymptotic population growth rates (λ_{30}) and 95% bootstrap confidence intervals estimated from the “no transition” IPMs for populations of aphid colonies that were consistently untended, tended by each of three individual mutualist ant species, or tended simultaneously by multiple mutualist ant species. Because these models did not allow for transitions among ant states or from being tended to untended, they thus tested for mutualist ant quality (i.e., effects on vital rates) but not differences in availability (i.e., differences in arrival/departure probabilities). All combinations of two or three ant species were included in a single IPM testing for simultaneous diversity effects. Because most colonies were only tended simultaneously by two (versus three) ant species, the estimate of λ_{30} from the multi-species model is shown as being for two species.

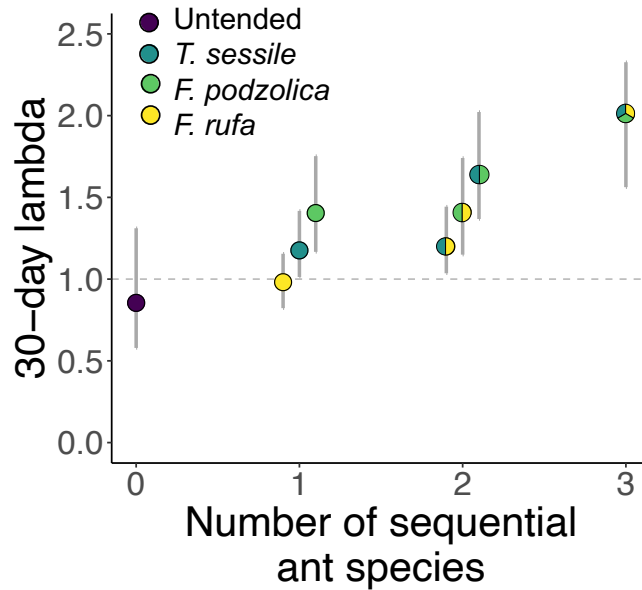


Figure 3.5. Season-long (30 days) asymptotic population growth rates (λ_{30}) and 95% bootstrap confidence intervals estimated from “ant transition” IPMs for populations of aphid colonies that were untended or tended by each ant species individually and all possible combinations of two and three ant species, while allowing for transitions among ant species as well as between tended and untended states. These models thus integrated ant mutualist quality (i.e., effects on vital rates) and availability (i.e., differences in arrival/departure probabilities) to assess overall mutualist effectiveness.

DISCUSSION

We found that aphids benefitted from sequential but not simultaneous mutualist diversity, demonstrating the importance of studying multi-species mutualisms across multiple scales. Each ant species was an effective mutualist in terms of its quality when present and increased aphid colony fitness through positive effects on survival and growth but not alate production. However, ants largely did not perform complementary functions, and out of all the consistently ant tended aphid populations, vital rates were lowest for colonies tended simultaneously by multiple ant

species. These decreases in vital rates translated to a net negative effect of simultaneous ant diversity on population growth relative to colonies consistently tended by any of the three ant species individually. However, because the highest quality mutualist when present (assessed in terms of effects on population growth when present), was predicted to tend aphid colonies the least frequently, aphids were predicted to benefit from sequential ant diversity. Thus, a species can benefit from associating with lower quality mutualists if they are more abundant than the highest quality partner. Overall, these findings for positive effects of mutualist diversity support the prediction that generalization in mutualisms may often result in increased partner fitness (Batstone et al. 2018).

To our knowledge, this is the first study to compare the demographic effects of simultaneous versus sequential mutualist diversity. In other demographic studies of ant protection mutualisms, sequential ant diversity was common but simultaneous ant diversity did not occur or was rare, likely because ants competed more strongly in these systems for sole occupancy of mutualist host plants (Palmer et al. 2010, Bruna et al. 2014). Nonetheless, both simultaneous and sequential mutualist diversity may have important effects for partner fitness in many mutualisms (e.g., plant-pollinator, legume-rhizobia, and plant-mycorrhizae mutualisms) (Waser and Real 1979, West et al. 2002, Kiers and van der Heijden 2006). While much research has focused on mutualist partner diversity in the context of cheating and stabilizing mechanisms (Simms and Taylor 2002, Kiers et al. 2011), few studies have assessed whether or when species benefit from mutualist partner diversity. Overall, our results match the expectation that sequential mutualist diversity can be beneficial if species perform complementary functions, are complementary in their availabilities, or facilitate each other (Waser and Real 1979, Palmer et al.

2010), but that simultaneous mutualist diversity may be detrimental due to heightened competition among mutualist species (Waser 1978).

We found evidence for negative diversity effects when multiple ant species partner simultaneously with aphids. Although λ_{30} was predicted to be greater than for untended colonies, aphids received the weakest benefits from ants when tended simultaneously by multiple species. These results suggest that the ants largely did not provide complementary benefits in terms of aphid colony vital rates, and that they instead may have had antagonistic effects. It is possible that antagonisms among ants weakened the benefits that they provided to aphids, with ants spending more energy deterring competitors rather than tending aphids. In line with this prediction, we found that fewer total ants recruit to aphid colonies when multiple ant species are present (Nelson and Mooney, unpublished data). Alternatively, it is also possible that lower aphid colony performance is not caused by but instead correlated with simultaneous ant diversity. If aphid colonies are unattractive (e.g., on low quality host plants or far from ant nests), ants may spend less energy tending them as well as less energy defending them against competing ant species. Thus, aphid colonies with lower vital rates may also be more likely to be simultaneously tended by multiple ant species. Nonetheless, experimental ant exclusion data indicate that ants drive differences in aphid colony vital rates, rather than only select colonies that already differ in quality due to other factors (Nelson et al., unpublished data).

In contrast to our results, other studies have found evidence for positive effects of simultaneous mutualist diversity. For example, Stachowicz and Whitlatch (2005) found that a red alga benefits from simultaneously associating with two gastropod species that consume different invertebrates that would otherwise cause fouling. Similarly, Piovia-Scott et al. (2017) showed that greater species richness of microbes on frog skin reduced the abundance of a fungal

pathogen. Such positive effects of simultaneous mutualist diversity are likely to occur when mutualist species perform complementary functions. Moreover, positive diversity effects may be more common when competition among mutualists is weak. For example, because the two gastropod species studied by Stachowicz and Whitlatch (2005) consumed different invertebrates associated with the red alga, they likely did not compete for the same mutualist-associated resources. However, the ants in this study likely performed similar functions (e.g., protected aphids against the same natural enemies) and consumed the same resources (i.e., honeydew) produced by their shared aphid partner. As a result, it is perhaps unsurprising that we found evidence for negative simultaneous diversity effects.

In contrast, aphids benefitted from sequential mutualist ant diversity. Similar studies of the demographic effects of sequential mutualist diversity have shown varying results (Palmer et al. 2010, Ohm and Miller 2014, Bruna et al. 2014). Bruna et al. (2014) found that when the plant *Maieta guianensis* switched mutualist ant partners, its populations were predicted to grow at a rate comparable to that of populations colonized exclusively by the poorer mutualist. In contrast, Ohm and Miller (2014) and Palmer et al. (2010) both found that partner switching helped plants avoid the costs of interacting with parasitic ants, and in some cases, led to positive diversity effects. Palmer et al. (2010) found that populations of *Vachellia* (formerly *Acacia*) *drepanolobium* trees sequentially colonized by a greater diversity of mutualist ant species outperform those colonized by any ant species individually. However, the mechanisms explaining such positive diversity effects differ between Palmer et al. (2010) and our study. The ants associated with *V. drepanolobium* perform contrasting functions (i.e., there are trade-offs between their effects on survival and reproduction) and thus enhance vital rates in complementary ways. However, in our system, the ants perform similar functions and did not

have complementary effects on aphid colony vital rates. Nonetheless, the most effective mutualist (*F. rufa*) was predicted to tend aphid colonies less frequently than the other two ant species unless the colonies were large. Thus, because aphid colonies were predicted to be tended more consistently by more ant species, ant diversity was predicted to increase aphid colony population growth. Our findings match the theoretical expectation that mutualists should benefit from increased partner diversity if the most effective partner is not the most abundant (Perfectti et al. 2009).

In summary, this study shows that even if multiple mutualist partners do not perform complementary functions, species can benefit from mutualist diversity due to differences in partner availability. These findings may point to broader generalities about the ecology of multi-species mutualisms. For example, it is possible that species may be more likely to benefit from sequential than simultaneous mutualist diversity, since there are many possible mechanisms by which sequential diversity may be beneficial and mutualists may be less likely to compete when diversity occurs sequentially. Although much work on mutualisms has focused on the mechanisms that prevent cheating and maintain mutualism stability, here we show that multi-species mutualisms are able to persist and be beneficial when partners trade-off in quality and availability. Thus, this study contributes to a growing body of literature (Palmer et al. 2010, Batstone et al. 2018) illustrating the importance of biological diversity in mutualisms.

SUPPLEMENTAL MATERIALS

Appendix 3A

Weather data were collected within the study site (temperature) or in nearby sites (precipitation, snowmelt date, and soil moisture). During each summer, we measured temperature every 30 min using two HOBO Pendant Temperature/Light data loggers (Onset Computer Corporation, Bourne, MA, USA; data from the two data loggers were averaged). Total daily precipitation and snowmelt date were measured at the Rocky Mountain Biological Laboratory (38.963°N, 106.993°W; data collected by billy barr, available from <http://www.gothicwx.org/>), and hourly soil moisture was measured at 25 cm depth at the Snodgrass Mountain weather station (38.929°N, 106.986°W). We averaged multiple measurements of weather variables from within each three-day census period in 2017, 2018, and 2019 and compared these three-day averages among years (Fig. S3.1).

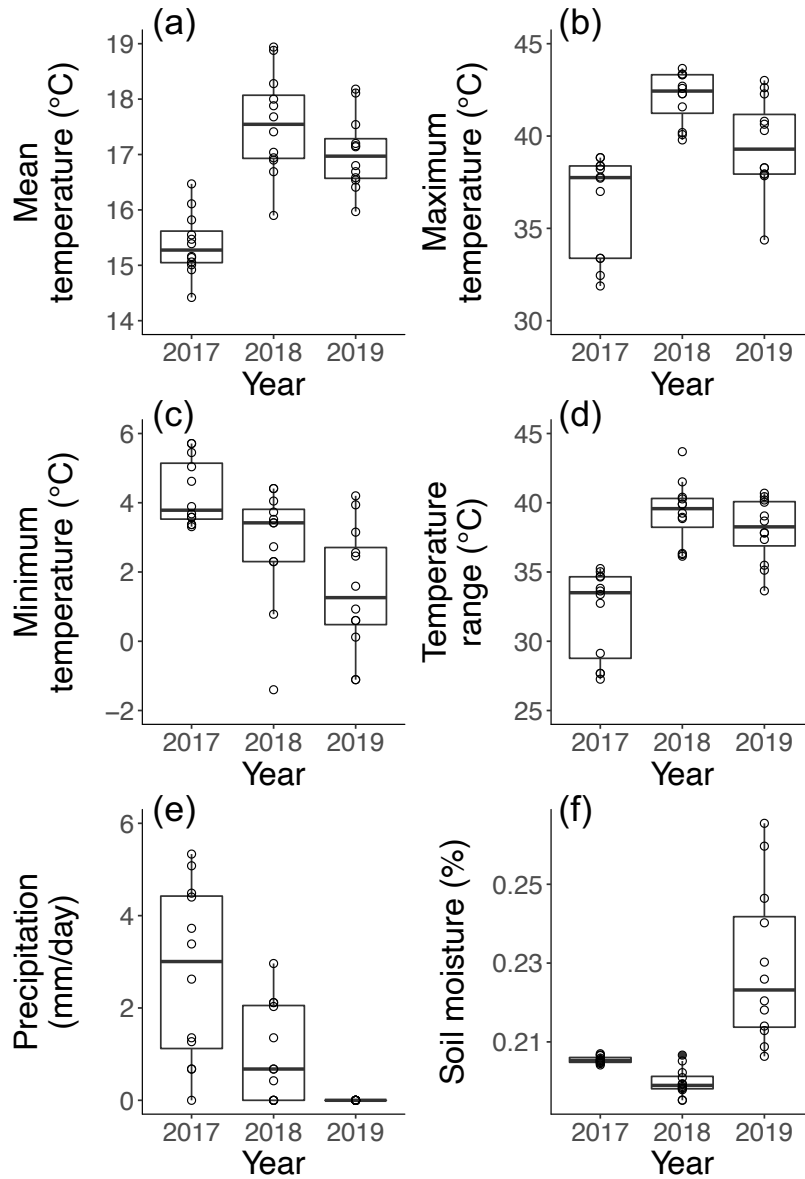


Figure S3.1. Across each three-day census period in 2017, 2018, and 2019, boxplots of the (a) mean, (b) maximum, (c) minimum, and (d) range in temperatures, as well as (e) daily precipitation and (f) percent soil moisture. Points show average values for each three-day census interval. There were significant differences across years in the mean ($F = 29.09$, $P < 0.001$), maximum ($F = 19.02$, $P < 0.001$), minimum ($F = 9.98$, $P < 0.001$), and range in temperature ($F = 26.30$, $P < 0.001$), as well as daily precipitation ($F = 14.84$, $P < 0.001$) and percent soil moisture ($F = 21.42$, $P < 0.001$).

Appendix 3B

Integral projection model structure

We constructed “no transition” and “ant transition” integral projection models (IPMs) to assess the effects of mutualist ant identity as well as simultaneous and sequential diversity on aphid colony population dynamics. IPMs are discrete-time population models that are structured by a continuous state variable (e.g., size at time t) (Easterling et al. 2000, Ellner et al. 2016). Our IPMs projected the asymptotic “population” growth rates (λ ; i.e., changes in aphid colony abundance) of aphid colonies tended by different mutualist ant species, based on ant-specific effects on aphid colony vital rates, dependent on colony size at time t . To assess the effects of mutualist ants when present (“no transition” models that did not incorporate changes in ant tending states), we constructed five separate IPMs that project aphid colonies in each ant tending state (untended, tended individually by each of the three ant species, and tended simultaneously by multiple ant species) forward one timestep (across the three-day interval to time $t+3$ days). These models followed the form:

$$n(y, t + 1) = \int_L^U K(x, y)n(x, t)dx$$

where $n(x, t)dx$ is the number of aphid colonies with sizes in the range $[x, x + dx]$ at time t . The IPM kernel function is $K(x, y)$, where $K(x, y) = P(x, y) + F(x, y)$. The function $P(x, y)$ represents the probability that an x -sized colony will survive, $S(x)$, and grow to size y at time $t+3$ days, $G(x, y)$, where $P(x, y) = S(x)G(x, y)$. The function $F(x, y)$ represents the per-capita number of y -sized recruits produced at time $t+3$ days by x -sized parent aphid colonies at time t , where $F(x, y) = f_n(x)p_r f_d(y)$. Here, $f_n(x)$ is the number of alates produced by an x -sized aphid colony, p_r is an estimated constant for the proportion of alates that successfully establish new aphid colonies, and $f_d(y)$ is the size distribution of new colonies. Size- and ant-dependent

vital rates were modeled with generalized linear models (survival and alate number) or Gaussian location-scale additive models (mean and variance in growth) (models described below in Tables S3.1-S3.3). In all IPMs, the lower integration limit (L) was set to the smallest observed aphid colony size (1 aphid), and the upper integration limit (U) was set to the maximum observed size across all ant tending states (3,017 aphids). To avoid eviction, models were constrained so that all individuals remained within the size range (Williams et al. 2012). We projected λ from each IPM by discretizing the kernels using the midpoint rule with 100 mesh points.

To evaluate the effects of ants both due to effects on vital rates (i.e., ant quality) as well as differences in arrival and departure at aphid colonies (i.e., ant availability), we built “ant transition” models as “mega-matrix” IPMs that summarize transitions among all possible aphid colony sizes and ant tending states under varying levels of ant diversity (Hunter and Caswell 2005, Metcalf et al. 2009). Submatrices of the mega-matrix consisted of the same discretized “no transition” IPM kernels for aphid colonies that were untended and tended consistently by each of the three ant species alone described above, weighted by the probabilities of transitioning among all ant tending states from time t to $t+3$ days. Ant transition probabilities depended on the main effects of starting aphid colony size and ant state at time t as well as their interaction in a multinomial logistic regression (ant transition statistical model described below in Table S3.4, shown in Fig. 3.2).

First, we constructed a “full ant community” mega-matrix, M_f , consisting of 4 x 4 submatrices following the form:

$$M_f = \begin{pmatrix} \mathbf{Q}_U \mathbf{T}_{U,U} & Q_{Fp} T_{Fp,U} & Q_{Ts} T_{Ts,U} & Q_{Fr} T_{Fr,U} \\ Q_U T_{U,Fp} & \mathbf{Q}_{Fp} \mathbf{T}_{Fp,Fp} & Q_{Ts} T_{Ts,Fp} & Q_{Fr} T_{Fr,Fp} \\ Q_U T_{U,Ts} & Q_{Fp} T_{Fp,Ts} & \mathbf{Q}_{Ts} \mathbf{T}_{Ts,Ts} & Q_{Fr} T_{Fr,Ts} \\ Q_U T_{U,Fr} & Q_{Fp} T_{Fp,Fr} & Q_{Ts} T_{Ts,Fr} & \mathbf{Q}_{Fr} \mathbf{T}_{Fr,Fr} \end{pmatrix}$$

where Q_i is the 100 x 100 discretized IPM kernel for aphid colonies tended by ant species i at time t , and $T_{i,j}$ is the 100 x 100 ant tending state transition matrix for the proportion of aphid colonies initially tended by ant species i that were predicted to switch to ant species j at time $t+3$ days. Subscripts indicate ant tending states as being untended (“ U ”) or tended by *F. podzolica* (“ Fp ”), *T. sessile* (“ Ts ”), or *F. rufa* (“ Fr ”). Submatrices along the diagonal (bolded) represent aphid colonies that remained in the same ant tending states after all size transitions, whereas all other submatrices represent colonies that underwent transitions in ant tending states. Newly established aphid colonies were always assigned to the “untended” starting ant state.

We tested for the effects of ant identity and sequential diversity by comparing the “full ant community” model with “reduced ant community” models in which we removed all possible combinations of one or two ant species from the full mega-matrix. These reduced community models included the discretized IPM kernels and ant transition matrices for aphid colonies tended by all of the remaining ant species. However, removing one or two ant species from the models required that we modify the ant transition matrices to adjust for the missing probabilities of transitioning to the removed ant states (e.g., so that the probabilities of transitioning from each ant tending x aphid colony size state to all remaining states still summed to 1). Following the approach used by Palmer et al. (2010), the probabilities of transitioning to any states involving the removed ants were reassigned so that the transition probabilities among all remaining ant tending states (including the untended state) increased in proportion to their originally estimated probabilities (so that all adjusted probabilities summed to 1). From each full and reduced mega-matrix, we projected λ and the frequencies of aphid colonies in each ant tending state (Fig. S3.4) to assess the effects of varying levels of ant identity and sequential diversity on aphid fitness and population structure.

Vital rate and ant transition models

To model aphid colony demographic vital rates and ant tending state transitions, we selected best-fit models among multiple possible generalized linear (GLMs) for survival (Table S3.1), alate production (Table S3.3), and ant transitions (Table S3.4) or Gaussian location-scale additive models (GAMLSS) for mean and variance in growth (Table S3.2). Here, we present the factors included in each model, the Aikake's information criterion (AIC) values of each model, and the difference between the AIC value of each model and the best-fit model. In all models, "size" and "ant" refer to the aphid colony size and ant tending state at time t .

Table S3.1 Results of aphid colony survival model selection.

Model type	Size	Independent variables:				AIC	Δ AIC
		Size ²	Ant	Year	Interactions		
GLM						2015.6	746.2
GLM	X					1389.1	119.7
GLM	X	X				1351.0	81.6
GLM	X		X			1365.4	96.0
GLM	X			X		1301.7	32.3
GLM	X		X	X		1296.1	26.7
GLM	X	X	X			1329.5	60.1
GLM	X	X		X		1273.6	4.2
GLM*	X	X	X	X		1269.4	0.0
GLM	X		X		Size x ant	1364.0	94.6
GLM	X	X	X		Size x ant	1335.3	65.9
GLM	X		X	X	Size x ant	1292.8	23.4
GLM	X	X	X	X	Size x ant	1274.3	4.9

* Best-supported model. Survival at time $t+3$ days was modeled as a binary logistic regression.

Table S3.2 Results of model selection for mean and variance in aphid colony growth.

Mean growth:

Model type	Independent variables:				AIC	Δ AIC
	Smooth predictors	Size	Ant	Year		
GAMLSS					11817.7	5039.6
GAMLSS		X			7449.9	671.8
GAMLSS	Size				7158.5	380.5
GAMLSS	Size		X		7097.3	319.2
GAMLSS	Size			X	6830.5	52.4
GAMLSS*	Size		X	X	6778.1	0
GAMLSS	Size x ant		X		7107.2	329.1
GAMLSS	Size x ant		X	X	6781.8	3.7

Variance in growth:

Model type	Independent variables:				AIC	Δ AIC
	Smooth predictors	Size	Ant	Year		
GAMLSS					6778.1	612.8
GAMLSS		X			6394.0	228.8
GAMLSS	Size				6351.4	186.1
GAMLSS	Size		X		6345.1	179.8
GAMLSS	Size			X	6191.3	26.0
GAMLSS	Size		X	X	6175.4	10.2
GAMLSS	Size x ant		X		6341.1	175.8
GAMLSS*	Size x ant		X	X	6165.3	0

* Best-supported models. Mean and variance in growth were modeled as a Gaussian location-scale additive model, with both the mean and variance at time $t+3$ days modeled as smooth functions of size at time t . Smooths were based on thin plate regression splines, with smoothing parameters selected using restricted maximum likelihood. The numbers of basis dimensions for mean ($k = 10$) and variance ($k = 16$) in growth were selected to be large enough so that the shape of the smooth did not change as k continued to increase.

Table S3.3 Results of aphid colony alate number model selection.

Model type	Independent variables:					AIC	Δ AIC
	Size	Size ²	Ant	Year	Interactions		
GLM						16852.2	2447.2
GLM	X					14954.9	550.0
GLM	X	X				14749.4	344.5
GLM	X		X			14943.0	538.0
GLM	X			X		14719.0	314.0
GLM	X		X	X		14700.9	296.0
GLM	X	X	X			14737.3	332.4
GLM	X	X		X		14408.4	3.5
GLM	X	X	X	X		14411.2	6.2
GLM	X		X		Size x ant	14902.9	497.9
GLM	X	X	X		Size x ant	14734.7	329.7
GLM	X		X	X	Size x ant	14619.7	214.7
GLM	X	X	X	X	Size x ant	14404.9	0

* Best-supported model. Alate number at time t was modeled as a negative binomial GLM.

Table S3.4 Results of ant tending state transition probabilities model selection.

Model type	Size	Independent variables:				AIC	Δ AIC
		Size ²	Ant	Year	Interactions		
GLM						6264.1	3543.0
GLM	X					5496.6	2775.5
GLM	X	X				5491.4	2770.3
GLM	X		X			2834.5	113.4
GLM	X			X		5205.6	2484.5
GLM	X		X	X		2765.0	43.9
GLM	X	X	X			2828.5	107.4
GLM	X	X		X		5188.5	2467.5
GLM	X	X	X	X		2750.5	29.4
GLM	X		X		Size x ant	2795.6	74.5
GLM	X	X	X		Size x ant	2798.3	77.2
GLM	X		X	X	Size x ant	2721.8	0.7
GLM	X	X	X	X	Size x ant	2721.1	0

* Best-supported model. The probabilities of transitioning among ant tending states (from time t to $t+3$ days) were modeled by a multinomial logistic regression.

Appendix 3C

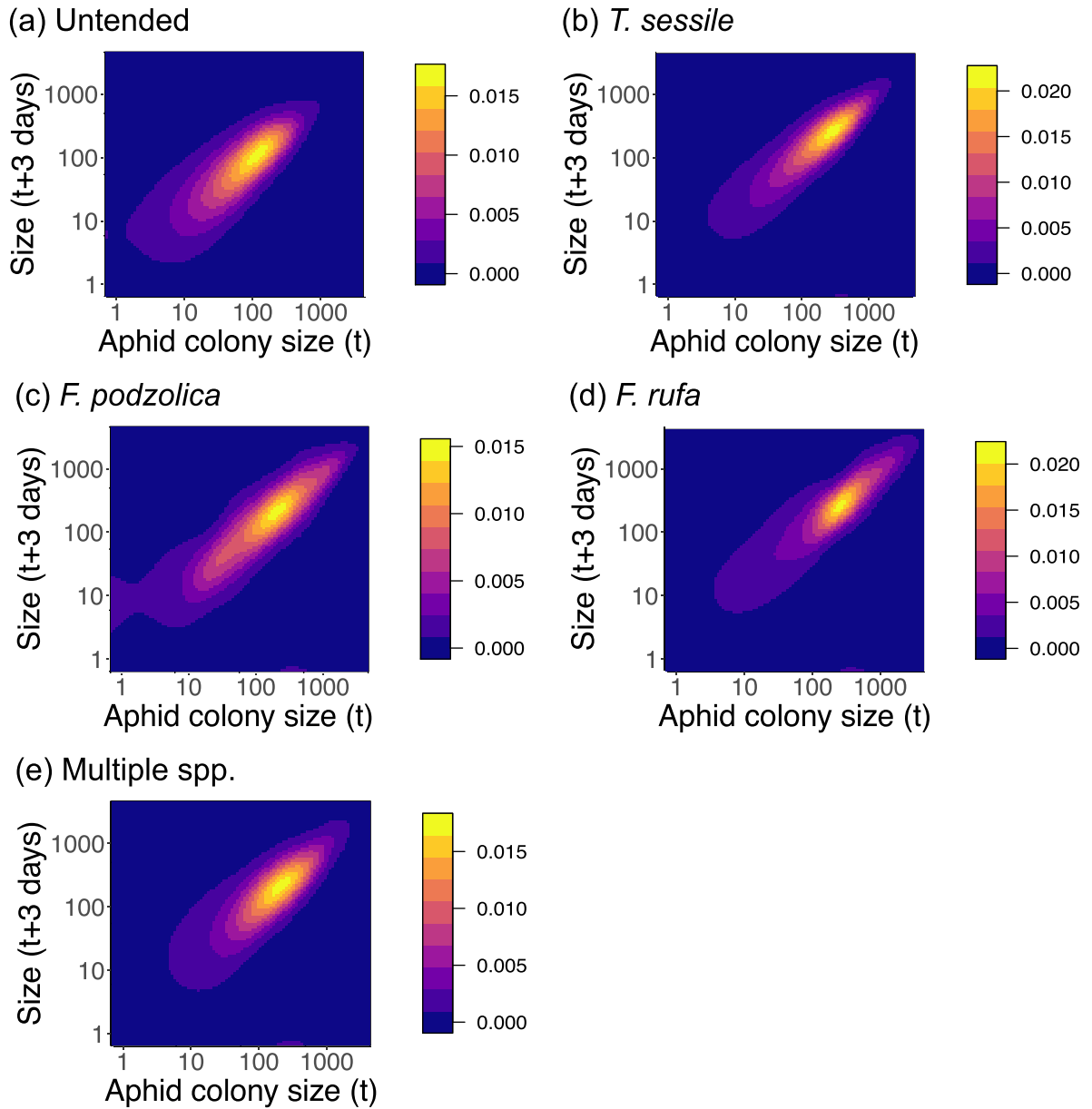


Figure S3.2. Elasticities (proportional sensitivity of λ) for the IPMs in which aphid colonies were consistently (a) untended or tended by (b) *T. sessile*, (c) *F. podzolica*, (d) *F. rufa*, or (e) multiple species simultaneously. Lighter colors indicate greater elasticities.

Appendix 3D

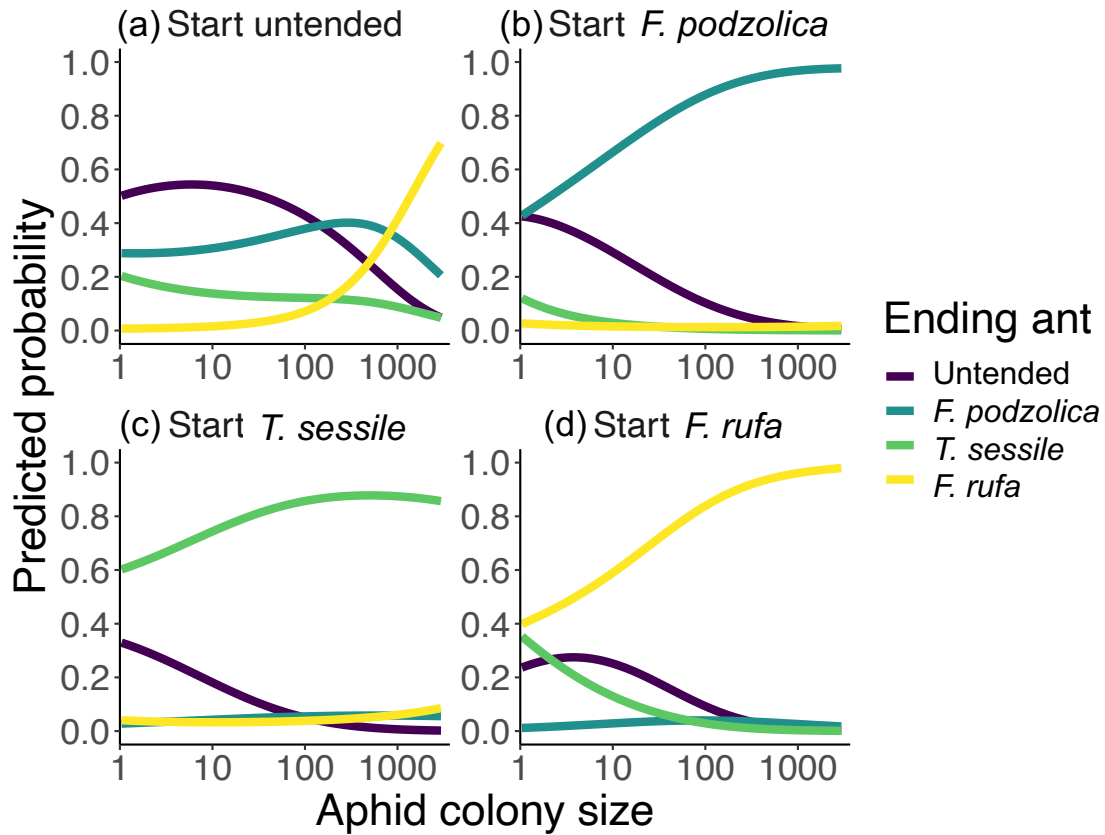


Figure S3.3. Best-fit probabilities of transitioning from each starting ant tending state (panels) to every ending ant tending state (colored lines) based on aphid colony size (averaged across years).

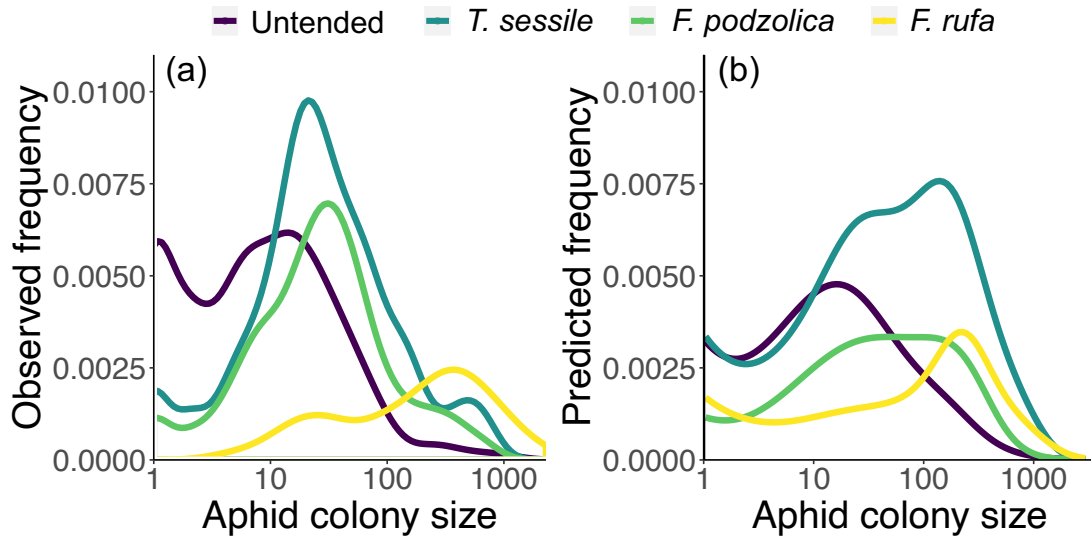


Figure S3.4. The (a) observed and (b) predicted frequencies of aphid colonies in each ant tending state across all sizes (averaged across years). Predicted frequencies are the stable size distributions from the full ant community model.

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