TESTING THE KEYSTONE MUTUALISM HYPOTHESIS IN A FOREST FOOD WEB: PATTERN, PROCESS, AND ECOLOGICAL IMPACT

By

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Dedication

This work is dedicated to my mentors, teachers, and professors. They taught me that while ecological research is a scientific enterprise, the appreciation of nature is a spiritual endeavor. I have learned the most from scientists who have made it their life’s work to share the beauty of biology with others.

“There are men charged with the duty of examining the construction of the plants, animals and soils which are instruments of the great orchestra… the construction of instruments is the domain of science, while the detection of harmony is the domain of poets.”

Aldo Leopold, Sand County Almanac 1949
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Thesis Abstract

Mutualisms are increasingly appreciated for their contribution to the structure and dynamics of ecological communities. Food-for-protection mutualisms involving ants and sap-feeding insects are a widespread example of a positive interaction with significant impacts on populations and communities. Sap-feeders provide carbohydrate-rich honeydew to mutualistic ants, and this food source restructures other trophic interactions involving ants. Ants engaged in mutualism are more likely to prey on herbivores and reduce overall herbivory on host plants. Despite the widespread importance of ant-sap-feeder mutualisms in many terrestrial ecosystems, a mechanistic understanding of how sap-feeding insects alter ant trophic ecology remains obscure. The carbohydrate-rich food made available by sap-feeding insects has the potential to modify ant behavior, populations and community structure in the presence of so-called sap-feeders. In this thesis, I study the mechanisms of this keystone mutualism in a forest food web to (a) examine patterns of trophic interactions arising from ant-sap-feeder mutualisms (Chapter 1), (b) determine if and how this mutualism changes ant abundance and community structure so as to strengthen predatory effects of ants on chewing herbivores (Chapter 2) (c) determine if and how this mutualism changes ant behavioral responses to prey and competitors (Chapter 3), and (d) evaluate the consequences of these mechanisms for forest tree growth (Chapter 4). In sum, this thesis demonstrates that ant-sap-feeder mutualisms modify tri-trophic food webs in Connecticut forests, that changes in ant community structure and behavior are the primary mechanisms for this variation, and that this interaction has a significant impact on forest productivity over multiple growing seasons.
Introduction: Ecological variation in ant-sap-feeder mutualisms and ant predatory effects

The ecological impacts of populations in communities are often determined by trophic interactions. Food web theory seeks to determine the role of consumer-resource relationships in structuring ecosystems, and many core concepts in ecology trace their origins to natural history observations of predator-prey or plant-herbivore interactions (Estes et al. 2016). Employing manipulations of consumers in the field has been an effective tool to understand the importance of species since experimental removal of *Pisaster* starfish from intertidal food webs (Paine 1966, Kareiva and Levin 2002). The concept of “keystone species” clearly identifies the fundamental role of species traits, not just population size or relative abundance, in determining the ecological impact of consumers in food webs (Paine 1966).

Due to their ubiquity and ease of observation, ants (Formicidae:Hymenoptera) have been an important taxonomic group in the development of food web theory in their own right. In *Animal Ecology* (1927), Charles Elton’s first examples of food chains described predatory woodpeckers feeding on ant nests in Malvern Hills, England. In the same chapter, Elton framed his conception of niches in the context of trophic interactions, using sap-sucking aphids on *Pinus* as an example of an insect which specialized on a single food resource. Furthermore, he stated that some ants in this food chain also specialize on consuming these aphid’s excretions. Later work has described interactions between ants and sap-feeding insects (like aphids) as a widespread mutualism in which there is a reciprocal benefit for both populations: ants receive excretions called “honeydew” and, in turn, ant workers attack any natural enemies that threaten sap-feeding herbivores (Buckley 1987). While actively feeding from host plants, sap-feeders
are susceptible to predators and parasites; to protect their mutualists ants aggressively attack ladybird beetles or parasitoid wasps (Stadler and Dixon 2008). For ants, honeydew can be a significant proportion of colony diet (Lach et al. 2010): carbohydrate-rich food sources fuel ant forager activity and supplement the carbohydrate demands of colony growth (Shik et al. 2014). Ant-sap-feeder mutualisms are both widespread in terrestrial ecosystems and have many participant species (Stadler and Dixon 2008). Many ant species engage in mutualism facultatively with sap-feeders (Davidson et al. 2004), and sap-feeding is a common herbivore feeding strategy in the insect order Hemiptera, including the families Aphidae, Membracidae and Coccidae (Styrsky and Eubanks 2007).

The impacts of ants on food webs are considerable owing to their high abundance, omnivory, and the frequency with which ants engage in mutualistic interactions (Holldobler and Wilson 1991). Ant-sap-feeder mutualistic interactions have been examined across a wide range of ecosystems in particular due to their apparent importance to arthropod food webs (reviewed in Styrsky and Eubanks, 2007). In some communities, ant-sap-feeder mutualisms can dramatically restructure arthropod communities and even impact host-plant performance (Kaplan and Eubanks 2005). For example, when ants and aphids were present on Populus trees, ants dramatically reduced the numbers of caterpillars, spiders, and non-mutualistic ants (Wimp and Whitham 2001). Styrsky and Eubanks (2007) argued that when ant-sap-feeder mutualisms fundamentally alter communities, the mutualism is functioning as a “keystone interaction.” Keystone interactions function similar to keystone species, which exert large impacts on communities with cascading indirect consequences; this terminology echoes Paine’s observations of keystone species with disproportionately large impacts on food webs.
(Paine 1969). In the same way that specific predatory traits of *Pisaster* provided the mechanisms for its “keystone”-like properties in its community (Power et al. 1996), the experiments in this thesis examine the mechanistic basis by which ant-sap-feeder interactions have strong effects on food webs. I refer to this assemblage of predictions regarding the disproportionate impacts of ant-sap-feeder interactions as the “keystone mutualism hypothesis.” Importantly, despite the wide range of research on the “keystone mutualism hypothesis” relatively little work has examined the specific traits of ant communities that have strong ecological impacts in the presence of sap-feeders.

Multiple reviews, meta-analyses, and books have addressed this mutualism and its ecological impact (Styrsky and Eubanks 2007, Stadler and Dixon 2008, Lach et al. 2010, Rosumek et al. 2010, Zhang et al. 2013). Four important points from these efforts are crucial to this thesis:

(1) Determining the ecological impact of keystone ant-sap-feeder mutualisms requires addressing mechanisms of variation in a tri-trophic framework. Variation in plant traits modify the outcome of ant-sap-feeder interactions (Mooney et al. 2010), thus plant variation is a central component contributing to the cascading effects of this mutualism on other herbivores and the host plant itself.

(2) Ant-sap-feeder interactions are widespread in terrestrial environments, including temperate, tropical, and boreal forests, as well as agricultural ecosystems (Styrsky and Eubanks 2007). Where this mutualism occurs, the indirect effects can ripple through the rest of the herbivore and predator community, potentially driving trophic cascades which impact host-plant growth and fitness (Wimp and Whitham 2001, Grinath et al. 2012).
(3) Carbohydrates provided by sap-feeders can alter ant ecology at multiple levels of biological organization, including behavior (McGlynn and Para 2016), population size (Kaplan and Eubanks 2005), and community structure (Davidson 1998). Integrating these three scales will be necessary to determine mechanisms underlying keystone mutualisms.

(4) Ant-sap-feeder mutualisms can be either beneficial or detrimental for host plants depending on context of the herbivore community. For example, the cascading, indirect effects of ant-sap-feeder mutualisms on host plants are positive if ant predation of other herbivores, such as leaf-chewing caterpillars, offsets the costs of sap-feeding (Styrsky and Eubanks 2010), and negative if ant predation does not offset the cost of sap-feeding (Grinath et al. 2012).

In this dissertation I examine the ecological impact of keystone mutualisms on arthropod communities and deciduous trees in the northeastern coastal forest ecoregion of the U.S.A. Ant genera such as Camponotus and Formica engage in mutualism with sap-feeding Hemiptera, are numerically abundant (Ellison et al. 2012), and act as important predators foraging on foliage in this ecosystem. Caterpillars are dominant herbivores that consume deciduous foliage in spring and summer (larval Lepidoptera in this system are primarily in the family Geometridae and superfamily Noctuoidea). Sap-feeders in this study system are treehoppers (Membracidae) and scale insects (Coccidae). Host plants are the deciduous trees Acer rubrum, Betula lenta, Carya spp., Fagus grandifolia, Hamamelis virginiana, Prunus serotina, Quercus alba and Querucus rubra, which are dominant tree and shrub species in this ecoregion (Olson et al. 2001).

In the first chapter, I test two hypotheses that explain variation in the top-down effects of ants on caterpillars and host plants. First, the density-dependent predation
hypothesis states that the strength of ant predation is determined by the abundance of caterpillars on host plants. Second, in the keystone mutualism hypothesis, sap-feeders may strengthen the predatory effects of ants, leading to stronger predation on caterpillars and reduced herbivory on host plants. This large-scale ecological field experiment examines variation in ant effects among eight tree species, and provides support for the keystone mutualism hypothesis for three host-plant species.

In the second chapter, I test three hypotheses to explain community-level mechanisms underlying the ecological consequences of ant-sap-feeder mutualisms. First, the predator recruitment hypothesis argues that sap-feeders increase the local density of predatory ants, increasing ant predatory effects on caterpillars. Second, the predator richness hypothesis holds that sap-feeders increase the number of species of ants found on host plants, increasing the number of predator species that prey on caterpillars, leading to overall stronger top-down effects of the entire ant community. Finally, in the predator body size hypothesis, sap-feeders increase the proportional abundance of large-bodied ant species, which can capture the widest size range of caterpillars, thus increasing the overall strength of predatory effects. Through selective manipulation of the ant community, this field experiment gave evidence for the predator body size hypothesis as a mechanism driving stronger top-down effects of ants in the presence of sap-feeders.

In the third chapter, I examine the relationship between ant-sap-feeder mutualisms and ant predatory behavioral traits by testing four hypotheses. First, the superior protector hypothesis predicts that the ant species that dominates sap-feeder interactions is more aggressive towards caterpillar prey or competitors. Second, the aggressive defense hypothesis predicts that sap-feeders induce increased aggression towards prey and
competitors. Third, the intensified competition hypothesis proposes that induced aggression specifically increases ant competitive interactions. Fourth, the prey acquisition hypothesis predicts that sap-feeders specifically increase ant opportunistic predation of herbivores (and not competition). Through a series of behavioral assays examining the relative roles of induced behaviors and interspecific variation in behaviors, experiments supported the superior protector hypothesis, revealing that carpenter ants were more aggressive than other members of the ant community. While aggression was not induced by sap-feeders, the increased representation of aggressive ant species shown in Chapters 1 and 2 suggests that the behavioral traits of carpenter ants drive the stronger top-down effects of ants in the presence of sap-feeders.

In the fourth chapter, I test alternative hypotheses for the indirect effects of ant-sap-feeder mutualisms on the growth of *Carya* saplings over multiple years. The ant-instigated trophic cascade hypothesis states that ant predation of herbivores cascades down to host plants, increasing the growth of saplings even in the absence of sap-feeders. Alternatively, the cascading keystone mutualism hypothesis predicts that ant predation would only cascade down to host plants in the presence of sap-feeders. In support of the cascading keystone mutualism hypothesis, we observed significantly higher growth rates for *Carya* saplings if ants were present and sap-feeders were observed on the tree in at least one year over the course of the experiment. If host trees were never occupied by sap-feeders, ant predation had no positive or negative impact on the growth of saplings. This result demonstrates that ant-sap-feeder mutualisms have significant consequences for forest tree growth and forest productivity.
References Cited


Multiple interaction types determine the impact of ant predation of caterpillars in a forest community

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Abstract. Direct and indirect effects of predators are highly variable in complex communities, and understanding the sources of this variation is a research priority in community ecology. Recent evidence indicates that herbivore community structure is a primary determinant of predation strength and its cascading impacts on plants. In this study, we use variation in herbivore community structure among plant species to experimentally test two hypotheses in a temperate forest food web. First, variation in the strength of predator effects, such as ant predation of caterpillars, is predicted to be density dependent, exhibiting stronger effects when prey abundance is high (density-dependent predation hypothesis). Second, mutualistic interactions between ants and sap-feeding herbivores are expected to increase the abundance of predatory ants, strengthening predation effects on herbivores with cascading effects on host plants (keystone mutualism hypothesis). Using a large-scale predator exclusion experiment across eight dominant tree species, we tracked changes in insect density on 862 plants across two years, recording 2,322 ants, 1,062 sap-feeders, 5,322 caterpillars, and quantifying herbivory on 199,338 leaves. In this experiment, density-dependent predation did not explain variation in the direct or indirect effects of ants on caterpillars and herbivory. In partial support of the keystone mutualism hypothesis, sap-feeders strengthened top-down effects of ants on caterpillars under some conditions. However, stronger ant predation of caterpillars did not lead to measurable trophic cascades on trees occupied by sap-feeders. Instead, the presence of sap-feeders was associated with increased per capita feeding damage by caterpillars, and this bottom-up effect attenuated the indirect effects of ants on host plants. These findings demonstrate that examining the multi-trophic impacts of mutualisms and predation in the context of the broader community can reveal patterns otherwise masked by compensatory interactions.

Key words: ants; bottom-up effects; caterpillars; food webs; herbivory; indirect interactions; mutualism; predation; sap-feeders; top-down effects; trophic cascades; trophic interactions.

INTRODUCTION

Understanding sources of variation in the strength of top-down effects is a central issue in food web ecology (Terborgh and Estes 2010). In their seminal paper, Price et al. (1980) made a strong case that carnivore-herbivore interactions cannot be well understood without consideration of the plants on which they occur. Even though this call for a tri-trophic perspective has been followed in subsequent ecological research (e.g., Tscharntke and Hawkins 2002, Ohgushi et al. 2012, Mooney et al. 2012), relatively few studies have investigated the strength of predator effects in the context of complex food webs rather than food chains based on a single plant species or a set of closely related plant species (Singer et al. 2012). Notably, the top-down effects of predators can vary among plants due to variation in herbivore communities on those plants (e.g., Wimp and Whitham 2001, Mooney and Agrawal 2008, Abdala-Roberts et al. 2014, Singer et al. 2014) because herbivores can vary in both abundance and the types of interactions with which they engage other trophic levels. In this broader context of herbivore and plant community variation, predicting the consequences of predatory effects in complex food webs has proven difficult because additional interaction types can generate opposing effects to antagonistic interactions (Spiesman and Inouye 2015) and bottom-up effects can attenuate the effects of predation (Gruner 2004). For example, mutualists have the potential to act as third parties, mediating the effects of predation and herbivory and altering the impacts of these antagonistic interactions on community structure (Melian et al. 2009, Palmer et al. 2015).

Ant predation of caterpillars is a common antagonistic interaction in terrestrial food webs. Ants are abundant omnivores that frequently attack and prey on insect herbivores (Lach et al. 2010), and caterpillars are predominant insect herbivores in many plant communities (Futuyma and Gould 1979, Schowalter et al. 1986, Novotny et al. 2002, Summerville et al. 2003). Here we consider predatory effects of ants broadly to encompass both their consumptive and non-consumptive effects on prey behavior (Preisser et al. 2005). Ant predatory effects have sizable impacts on the overall abundance of...
leaf-chewing herbivores on host plants, including caterpillars (Montllor and Bernays 1993, Karhu and Neuvonen 1998, Piñol et al. 2010), but ant suppression of these herbivores frequently varies among host-plant species or host-plant genotypes (Karhu 1998, Sipura 2002, Sam et al. 2015). In some cases, the predatory effects of ants reduce leaf-chewing damage or other types of herbivory, providing widespread examples of trophic cascades in terrestrial ecosystems, especially when ants are recruited in plant defense (Rosumek et al. 2009, Romero and Koricheva 2011, Moreira et al. 2012).

In this study, we investigate the predatory effects of ants on caterpillars across eight tree species in a temperate forest community. The comparison of tri-trophic interactions across coexisting tree species provides a unique perspective on the relative importance of alternative ecological mechanisms within the local community (Singer et al. 2012). We focus on two attributes of herbivore communities known from this system and hypothesized to influence ant–caterpillar–plant interactions.

The density-dependent predation hypothesis predicts that ant predation strength will increase with prey densities. We investigated density-dependent predation of caterpillars by ants in two distinct dimensions: (1) variation at the scale of habitat patches containing all eight tree species (i.e., among spatial blocks) (2) variation across tree species independent of spatial context (i.e., total caterpillar abundance and ant predatory effects for each tree species). We expected ant predation to be density dependent in these dimensions because ants are generalist predators (Lach et al. 2010) and ant predation is reported to be density-dependent for other prey (Sakata 1995, Harmon and Andow 2007). Likewise, strong ant predatory effects have been observed on host plants experiencing herbivore outbreaks of eruptive caterpillars (Fowler and Macgarvin 1985), suggesting that, at locations were caterpillar density was high, the effects of predatory ants would be higher as well. We also expected ant predation to vary across tree species because bird predation of caterpillars was density dependent across the same set of tree species included in our experiment (Singer et al. 2012).

In addition to density-dependent predation, we evaluate the mediating effects of mutualisms on ant predation of caterpillars. Mutualistic interactions with sap-feeders are known to mediate the predatory effects of ants (reviewed in Styrsky and Eubanks 2007, Rosumek et al. 2009, Zhang 2012). Ants frequently engage in a food-for-protection mutualism with the sap-feeding herbivore guild in which ants receive a sugar-rich excretion (“honeydew”) from sap-feeding Hemiptera in return for protection from natural enemies (Stadler 2008, Lach et al. 2010). Stronger suppression of non-mutualistic herbivores (i.e., caterpillars) occurs when ants recruit to sap-feeders to consume honeydew, increasing the density of foraging ants on host plants (e.g., Kaplan and Eubanks 2005, Grinath et al. 2012). If ant predatory effects are sufficiently strengthened, ant–sap-feeder mutualisms can reduce leaf-chewing herbivore abundance (Styrsky and Eubanks 2007) and individual plants harboring sap-feeders may experience increases in fitness (Messina 1981, Punttila et al. 2004, Styrsky and Eubanks 2010). We term this mechanism the “keystone mutualism hypothesis” after the proposal that ant–sap-feeder mutualisms can be a keystone interaction in food webs (Styrsky and Eubanks 2007). Ants actively engaged in mutualisms can dramatically suppress herbivore abundance and herbivory, compensating for the direct cost of sap-feeding when this suppression provides a net indirect benefit to host plants (Styrsky and Eubanks 2007, Zhang 2012). This interaction can have significant consequences for both plant and insect communities. For example, ant–sap-feeder mutualisms protected Betula pubescens trees from complete defoliation and mortality caused by outbreaks the autumal moth Episrita autumnata (Punttila et al. 2004). Consequences like these attribute “keystone-like” properties to ant–sap-feeder mutualisms because classic cases of keystone species generate trophic cascades by suppressing dominant herbivores (Paine 1969, Estes and Duggins 1995). Following the keystone mutualism hypothesis, we predict that the mutualism with sap-feeders will drive stronger predatory effects of ants, leading to increased suppression of the dominant herbivore guild (caterpillars) and trophic cascades via reduced leaf damage from chewing herbivores. We specifically address the predictions of the keystone mutualism hypothesis regarding herbivore abundance and trophic cascades, acknowledging that the keystone analogy may not hold with respect to maintaining community stability or diversity (Paine 1966) and disproportionate impacts on food webs relative to abundance of the keystone species (Power et al. 1996). Importantly, while recent studies have demonstrated the ecological importance of this mutualism at the scale of insect communities (e.g., Grinath et al. 2012), it is still unclear how other ecological processes may be acting simultaneously to determine the consequences of ant predation for herbivores and plants (Styrsky and Eubanks 2007).

In summary, we sought to determine the strength of predatory effects in a forest food web that are mediated by variation in herbivore communities at the scale of both habitat patches and tree species. We specifically addressed three key questions regarding variation in tri-trophic interactions among ants, sap-feeders, caterpillars, and their host plants. (1) How does the strength of predatory effects by ants vary within and among tree species in the same community? (2) To what degree is this variation explained by density-dependent predation or keystone mutualisms? (3) What ecological mechanisms determine the strength of trophic cascades from ants to plant damage by chewing herbivores?

**Materials and Methods**

**Study system**

Field experiments took place in Middlesex County, Connecticut, USA during spring and summer of 2011.
and 2012. Eight tree species that are common to the northeastern coastal forest ecoregion (Olson et al. 2001) were used in the experiments: *Acer rubrum* (red maple), *Betula lenta* (black birch), *Carya* spp. (hickories in the Euca ry subgroup: *Carya ovata*, *Carya tomentosa*, and *Carya glabra*), *Fagus grandifolia* (American beech), *Hamamelis virginiana* (witch hazel), *Prunus serotina* (black cherry), *Quercus alba* (white oak), and *Quercus rubra* (red oak). In spring and early summer, this plant community hosts a diverse assemblage of herbivores and predators, including over 100 species of macrolepidopteran caterpillars (Singer et al. 2012), sap-feeding Hemiptera in the families Membracidae (Wallace 2008) and Coccidae, and 20 species of predatory ants (Ellison et al. 2012; R. E. Clark, unpublished data). Two carpenter ant species (*Camponotus pennsylvanicus* and *C. chromaoides*) and *Formica neogagates* are the most abundant arboreal-foraging ant species typically interacting with sap-feeders and caterpillars (Weseloh 2000; R. E. Clark, personal observations). *Camponotus* and *Formica* are opportunist predators of caterpillars on woody vegetation (Tilman 1978, Fowler and Macgarvin 1985, Ito and Higashi 1991, Sanders and Pang 1992, Riihimaki et al. 2006) and these local members of these genera are demonstrated mutualists of sap-feeding insects (Morales 2011).

### Predatory ant exclusion experiments

To determine ant effects on caterpillars, ants were excluded from experimental tree branches or branch-sized saplings with a sticky barrier (Tanglefoot, Contech Enterprises, Victoria, British Columbia, Canada) applied to the base of branches. Branches with the ant-exclusion treatment (−ants) were paired with control branches (+ants) in close spatial proximity (~1–15 m) on two neighboring trees or on different branches on the same individual tree. Treatments were set up over a three-week period in spring of each year (5 May–29 May 2011 and 7 May–24 May 2012). In each year, we delimited six distinct blocks (separated by at least 100 m) in each of three forest sites (separated by ~10 km) in Middlesex County, Connecticut, USA: Cockaponset State Forest, in the town of Haddam; Millers Pond State Park, in the town of Durham; and Hurd State Park, in the town of East Hampton. This design yielded 36 total spatial blocks (2 × 6 × 3), each encompassing a habitat patch with each of the eight tree species and their shared ant community (R. E. Clark and E. R. Johnson, unpublished data). In 2011, each spatial block contained one replicate of ant exclusion and control treatments per host plant species (8 × 2), and in 2012 each spatial block contained two replicates of ant exclusions and controls for all host-plant species (8 × 4). The experiment included 288 branches/saplings in 2011 and 576 branches/saplings in 2012. Two branches in 2011 were unintentionally not sampled, resulting in 862 total branches/saplings.

### Sap-feeder community

Field experiments relied on the presence and absence of ant–sap-feeder mutualisms as they occurred naturally. The presence of mutualistic interactions on host plants was also determined if we observed membracid (treehopper) or coccid (scale insect) individuals on experimental branches during field collections of insects, after ant-exclusion treatments were erected (see below). 216 branches were occupied by sap-feeders during the course of this experiment (25.1% of branches), of which 11 were occupied by scale insects. We included branches with scale insects because they co-occurred with treehoppers in multiple instances, and we observed the same ant species engaged in mutualism with scale insects as well. Sap-feeder presence varied significantly among host-plant species (likelihood ratio test, $P < 0.001$; Appendix S1: Fig. S1). When present, treehoppers and scale insects occurred in small, low-density groups (2.36 ± 0.165 [mean ± SE]) and had a single generation in the 9-week duration of this experiment. Hence, there was no opportunity for sap-feeder population growth and density-dependent changes in benefits from mutualistic ants during each growing season, unlike aphids and their interactions with mutualistic ants (Kaplan and Eubanks 2005). Indeed, the ant-exclusion treatment had no impact on the presence (likelihood ratio test, $P = 0.789$; Appendix S1: Fig. S1) or abundance of sap-feeders (rank-sum test, $P = 0.126$).

### Field collections of insects

We sampled all experimental branches for caterpillars, ants, and sap-feeders over two successive 3-week periods in both years (6 June–8 July 2011 and 30 May–4 July 2012), such that each branch was sampled twice, 3 and 6 weeks after set-up. Insects were sampled by branch beating, which involved vigorously shaking branches dislodge ants, caterpillars, scale insects, and treehoppers that would then fall onto sheets held below. To reduce model complexity associated with repeated measures, insect counts were pooled across these two sampling events for all statistical analyses. Ants and caterpillars were identified to species, and sap-feeding Hemiptera were identified to family. In total, we sampled 2,322 ants, 1,062 sap-feeders, and 5,322 caterpillars.

### Leaf area and leaf damage measurements

The leaf area of each individual branch was estimated following Singer et al. (2012). We counted the number of leaves for each experimental branch in July 2011 and July 2012 at the conclusion of arthropod sampling. The total leaf area of each branch was calculated as the average area of a leaf of a given tree species, multiplied by the number of leaves on that branch. Average leaf area values were taken for each tree species used in the same forests sites as Singer et al. (2012). This continuous estimate of leaf area was used as an offset in models to statistically...
control for the effect of variation in host-plant branch size on insect abundance (Kotze et al. 2012). Hence, although statistical models (described below) use insect abundance as a response variable, insect density was modeled through our analyses.

We estimated herbivory by caterpillars on each experimental branch over the course of the 2012 season by quantifying the leaf area lost to chewing herbivores at the end of the experiment in mid-July. The following describes our methodology (technique modified from Barber and Marquis 2009). For each experimental branch, we visually classified the damage level of all leaves on each branch into the following six categories: (1) no leaf area lost, (2) 1–20% leaf area lost, (3) 20–40% leaf area lost, (4) 40–60% leaf area lost, (5) 60–80% area lost, and (6) 80–100% leaf area lost. For analysis, we assumed the estimated proportion of damage on each leaf to be the median of each category (0, 0.1, 0.3, 0.5, 0.7, 0.9). On all branches, we counted remnant petioles in category 6 since these likely indicated a leaf that had been completely consumed (80–100%) by chewing herbivores. To estimate leaf damage on a given experimental branch with these data, we used the following formula:

\[ A \times \sum_{i=1}^{n} (C_i \times M_i) \]

where \( A \) is the known average area of a single leaf for the host-plant species, \( C_i \) is the number of leaves in the \( i \)th damage category, \( M_i \) is the median proportion of leaf area lost in the \( i \)th damage category, and \( n \) is the number of damage categories (\( n = 6 \)). We assayed 199 338 leaves across 576 branches by this method.

**Statistical analyses**

We used generalized linear mixed models (GLMM) to determine ant-exclusion effects on caterpillar abundance, ant abundance, and leaf area lost to herbivory. GLMMs were run in R 3.1.3 (R Core Development Team 2016) using the lme4 package (Bates et al. 2016). Caterpillar abundance models were fitted to the Poisson distribution, which is appropriate for ecological count data (Sileshi 2006), and total ant abundance models were fitted to a negative binomial distribution due to a high proportion of zero values (Long 1997). Significance of fixed effects was estimated using Type II sum of squares (following Langsrud 2003) from Wald \( \chi^2 \) likelihood-ratio tests using the car package in R (Fox et al. 2015). Post-hoc tests, predicted marginal means, and standard errors for fixed effects were calculated using the LSMEANS package in R (Lenth 2016). Branch leaf area was log-transformed and used as an offset in all models, and GLMMs were tested for overdispersion using the BLMECO package in R, using \( \phi < 0.7 \) and \( \phi > 1.4 \) as thresholds for concern about overdispersion (Korner-Nievergelt et al. 2015).

To test the keystone mutualism hypothesis, we first constructed a GLMM utilizing a complete data set to determine the combined effects of ants, host-plant species, and sap-feeders on caterpillar abundance. Fixed effects included ant-exclusion treatment, host-plant species, sap-feeder presence and their factorial interaction terms. Site and year were treated as fixed effects due to few levels (Bolker et al. 2009) and spatial block (\( n = 36 \)) was treated as a random effect. To test the effect of sap-feeder presence on caterpillar per capita effects on leaf damage, we compared the leaf damage divided by the number of caterpillars between presence and absence of sap-feeders (ant-exclusion branches only) using Wilcoxon/Kruskal–Wallis tests (JMP 11 Pro; SAS Institute, Cary, North Carolina, USA). We also tested if sap-feeder presence increased the abundance of each ant species (\( C. chromaoides, C. pennisylvanicus \) and \( F. neogegates \)) in three separate Wilcoxon/Kruskal–Wallis tests.

To test the density-dependent predation hypothesis at the habitat patch scale, we used a subset of the 2012 data as an index of local caterpillar abundance in each spatial block. This approach was used because branch-level caterpillar density would otherwise be included as both predictor and response variables, creating a spurious correlation. We restricted this analysis to the 2012 data because all spatial blocks in this year contained an additional replicate that could be used to estimate caterpillar population density. Reference branches were always sampled in close proximity on the same host-plant species, on the same day, in the same spatial block. Caterpillar population density index and caterpillar abundance on experimental branches were positively correlated (Appendix S1: Table S4, \( P = 0.039 \)), giving confidence that this index represents effects of local factors at the habitat patch scale that contribute to caterpillar abundance on experimental host plants subjected to a shared predatory ant community. For analysis, we constructed a GLMM with caterpillar abundance in 2012 as a response variable, and predictor variables were host-plant species, sap-feeder presence, caterpillar population density index, and the interaction terms for host-plant species by sap-feeder presence and ant treatment by caterpillar population density index. A significant ant treatment by caterpillar population density index interaction term would provide support for the density-dependent predation hypothesis at this scale since it demonstrates that the ant treatment effect is mediated by caterpillar abundance.

In follow-up tests of density-dependent predation, we used nonparametric regression (Spearman’s \( \rho \) test, JMP 11 Pro) to evaluate the correlation between the average ant treatment effect (difference between −ant and +ant treatment for the tree species) and caterpillar population density index. First, for all tree species, we regressed the average ant treatment effect against the average caterpillar population density index (\( n = 8 \)). Second, since we observed one host-plant species experiencing ant effects in the absence of sap-feeders (\( P. serotina, \) Fig. 2), we ran a follow-up test to determine if density-dependent
predation was operating for this tree species at the scale of spatial blocks (n = 18).

We used structural equation modeling (confirmatory path analysis, sensu Shipley 2009) to evaluate a putative, community-level food web model inferred from univariate analyses. We employed piecewise structural equation modeling in R with the piecewiseSEM package (Lefcheck 2015). Confirmatory path analysis requires a priori knowledge of hypothetical causal relationships (e.g., caterpillars cause leaf damage), and evaluates a proposed path model based on data collected on the proposed interactions. In this case, we tested direct interactions represented by linear equations based on experimental predictor and response variables from our GLMMs. A proposed path model was rejected if it found any missing pathways with a significant effect on path model structure (Shipley 2009). If a model was rejected, connections were iteratively added until model acceptance was met. Once a model was accepted, single step changes in a path model (i.e., removal/addition of an interaction or altering the target of an interaction) were evaluated using the Akaike information criterion, corrected for sample size (AICc), to determine if these changes improved overall model fit (Lefcheck 2015). Finally, to evaluate the magnitude ant-instigated trophic cascades, we multiplied the path coefficient (β) for each direct effect in the casual chain: ant exclusion effect on caterpillars and caterpillar density effects on leaf damage (following Lefcheck 2015).

RESULTS

The ant-exclusion treatment reduced ant abundance by 74% (Appendix S1: Table S1, P < 0.001). The presence of sap-feeders marginally increased total ant abundance (Appendix S1: Table S1, P = 0.060) and sap-feeders did not increase the abundance of the numerically dominant ant species, F. neogagates (signed-rank test, P = 0.192; Fig. 1). In dramatic contrast, sap-feeders increased the abundance of foraging carpenter ants (Camponotus spp.) on plants: C. chromaoides by 677% (signed-rank test, P < 0.001), and C. pennsylvanicus by 330% (signed-rank test, P = 0.004).

A three-way statistical interaction among ant-exclusion treatment, host-plant species, and the presence of sap-feeders (Appendix S1: Table S2, P < 0.001) demonstrated that ants reduced caterpillar abundance, but the strength of ant predation was contingent on sap-feeders and host-plant species (Fig. 2). In the absence of sap-feeders, ant exclusion significantly increased caterpillar abundance on P. serotina. In the presence of sap-feeders, ant exclusion increased caterpillar abundance on A. rubrum, H. virginiana, and Quercus alba (Fig. 2). Ant predatory effects on caterpillars were not detectable on B. lenta, Carya spp., F. grandifolia, and Q. rubra. On Carya spp., when ants were excluded from branches, caterpillar abundance was increased in the presence of sap-feeders (Fig. 2, Tukey HSD).

Ant suppression of caterpillars did not reduce herbivory (leaf damage to plants; Fig. 3; Appendix S1: Table S3, P = 0.343) and the effect of ant treatment on leaf damage was not increased by the presence of sap-feeders (Appendix S1: Table S3, P = 0.537) or modified by host-plant species (Appendix S1: Table S3, P = 0.816). Therefore, even though ant predation significantly reduced the abundance of caterpillars, we did not detect cascading indirect effects of ants on plant damage in this GLMM. The prediction of the keystone mutualism hypothesis that ant-sap-feeder mutualism reduces leaf-chewing herbivore damage was therefore not supported. Instead, there was a marginally significant positive association between sap-feeder presence and leaf damage (Appendix S1: Table S3, P = 0.61). Further analysis demonstrated that tree branches with sap-feeders had increased per capita (caterpillar) leaf damage (Appendix S1: Fig. S2, rank-sum test, P = 0.002). Thus, the indirect effect of sap-feeders on leaf damage was mediated by an average feeding increase of 34.5% by individual caterpillars in association with sap-feeders.

As evidence against the density-dependent predation hypothesis, ant predation of caterpillars did not increase with caterpillar density at the scale of spatial blocks or across host-plant species. Caterpillar population density index did not increase the magnitude of ant predation of caterpillars as modeled by the ant exclusion × caterpillar population density index interaction term (Appendix S1: Table S4, P = 0.110). Even though we observed an effect of ant predation in the absence of sap-feeders on P. serotina (Fig. 2), the strength of ant predation on this host-plant species was not correlated with caterpillar
population density index (Spearman’s \( \rho \), \( P = 0.968 \)). Similarly, there was no correlation between caterpillar population density index and the strength of ant predation across all eight host-plant species (Spearman’s \( \rho \), \( P = 0.530 \)).

For confirmatory path analysis, the proposed path model came directly from interpretations of univariate analyses on carpenter ant abundance, caterpillar abundance, and leaf herbivory. We hypothesized that the carpenter ants (\( C. \) chromaoides and \( C. \) pennsylvanicus) were driving predatory effects on caterpillars since these ant species were directly affected by sap-feeder presence (Fig. 1). Because these ant species both recruit to sap-feeders (Fig. 1), we included total carpenter ant abundance in the path analysis instead of total ant abundance.

The accepted path model (Fig. 3) supported interpretations of univariate analyses, with several key exceptions. We added a single path (ant-exclusion effect on leaf damage) to improve model fit (\( \Delta \text{AIC}_c = 3.2 \)). The path analysis confirms that ant predation (measured by ant exclusion) had a strong negative effect on caterpillar abundance, and caterpillar abundance predicted leaf herbivory. Therefore, the path model shows evidence for a causal chain resulting in a trophic cascade. However, sap-feeder presence also had a positive effect on leaf damage (Fig. 3), demonstrating that host plants harboring sap-feeders also experienced increased herbivory from leaf-chewers, such as caterpillars. This result matches outcomes from the GLMM analysis in which there was a marginally significant effect of sap-feeder presence on leaf herbivory (Appendix S1: Table S3, \( P = 0.064 \)). The path analysis thus illustrates that there are two opposing effects on host plants: ant-instigated trophic cascades and increases in herbivory by leaf-chewers associated with sap-feeders (Fig. 3). Indeed, this interaction chain is supported in the path analysis (Fig. 3) when we find the path coefficient for this chain of direct effects (following Lefcheck 2015). Consistent with a trophic cascade, there is an indirect effect of ants on host plant leaf damage via
MUTUALISMS MEDIATE ANT PREDATORY EFFECTS

changes to caterpillar density ($\beta = 0.0116 \times 0.0019 = 0.0002$). However, there is still a positive effect of sap-feeder presence on leaf damage ($\beta = 0.309$). Finally, host-plant species also determined variation in caterpillar abundance ($P < 0.001$, $\beta = -1.528$, host-plant effects not shown in Fig. 3) and carpenter ant abundance ($P < 0.001$, $\beta = 0.971$, host-plant effects not shown in Fig. 3). The increase in carpenter ant abundance did not reduce caterpillar abundance, and thus changes in the abundance of these predatory ants did not appear to drive the effects of ants on caterpillars.

DISCUSSION

The broad scope of the community studied here reveals a food web with top-down, horizontal, and bottom-up effects influencing both caterpillar abundance and leaf herbivory via multiple interaction types including predation, mutualism, and facilitation. While there were variable effects of ant predation on caterpillars among and within host-plant species, this variation could not be strictly attributed to variation in the presence of sap-feeders in the herbivore community. Ant predatory effects were consistent with the keystone mutualism hypothesis on three host-plant species, but not the others. This pattern is consistent with the literature reviewed by Styrsky and Eubanks (2007), but the ant-community mechanisms favoring strong top-down effects from ant–sap-feeder mutualisms remain enigmatic. Although we observed increases in carpenter ant abundance associated with sap-feeders, these increases did not appear to determine the strength of ant predation of caterpillars.

Density-dependent predation failed to explain variation in the predatory effects of ants at the scale of spatial blocks and host-plant species. This finding contrasts with Singer et al. (2012), showing that bird predation of caterpillars was density-dependent across tree species. However, parallel work in another carnivore guild found no evidence for density-dependent parasitism by tachinid and hymenopteran parasitoids for this plant–caterpillar community (Farkas and Singer 2013). While density-dependent predation failed to explain variation in ant effects at the spatial scales we anticipated, we did detect ant predation of caterpillars on $P. serotina$ in the absence of sap-feeders. This finding is relevant to density-dependent predation since this tree species has a relatively high abundance of caterpillars compared to other host-plant species in this system (Singer et al. 2012), suggesting that $P. serotina$ is the only host-plant species with high enough caterpillar densities for ant effects to occur in the absence of sap-feeders. Despite this observation, density-dependent predation did not explain variation in ant predation within this tree species. Other traits of $Prunus serotina$, such as architecture or secondary chemistry may uniquely affect ant–caterpillar interactions in this community. Past work has demonstrated that $P. serotina$ facultatively expresses extrafloral nectaries (Tilman 1978; R. E. Clark, personal observations), providing one potential mechanism for strong ant predation effects on this host-plant species. However, this explanation seems doubtful in our case because expression of extrafloral nectaries occurs briefly immediately following budbreak (Tilman 1978; R. E. Clark, personal observations) and we observed extrafloral nectaries on a single occasion for the 108 experimental $P. serotina$ branches/saplings observed during experimental setup. Despite this low frequency of extra-floral nectaries on $P. serotina$, we cannot rule out that ant-extrafloral nectary interactions occurred at other times in the growing season or at other times of the day or night. It is also possible that the effects of extrafloral nectaries might alter foraging ant community structure, such as increasing ant colony density or size near $Prunus serotina$ plants, thus increasing the density of foraging ants.

The increased per capita feeding damage by and abundance of caterpillars in association with sap-feeders and suggests two possible bottom-up mechanisms that
altered caterpillar abundance and feeding behavior. First, sap-feeders in this system may be non-random in their selection of trees, with sap-feeders preferentially feeding and living on high-quality hosts. These host plants may be local patches of high-quality food for caterpillars as well, with caterpillars preferentially feeding on these same host plants. In temperate forests, such as the one we studied, sunny microenvironments might support increased shoot growth associated with higher abundances of multiple herbivore guilds (Barber and Marquis 2009). There is some evidence for this pattern in deciduous forest communities that would explain the correlation between sap-feeders and caterpillars. For example, Bailey and Whitham (2002) observed sap-feeders and leaf-mining caterpillars were more abundant on host trees exhibiting recent, nutrient-rich growth following ecological disturbance. A second possible mechanism is facilitation of caterpillars by sap-feeders. Such facilitation has been observed in other systems, but has rarely been considered in analyses of ant-sap-feeder interactions, as pointed out by Styrsky and Eubanks (2007). For example, in greenhouse experiments with cotton plants, caterpillar survivorship was higher when sap-feeding aphids were present, but ants were excluded (Styrsky and Eubanks 2010). Likewise, caterpillars feeding on tomato plants that have been previously attacked by aphids exhibited higher growth rates and survival (Rodriguez-Saona et al. 2005). Because of a growing number of examples like these in other plant-herbivore networks, it is increasingly appreciated that herbivores can indirectly impact the growth of each other, in particular, through induced plant responses (Ohgushi 2005, Ohgushi et al. 2012). One mechanistic hypothesis underlying indirect interactions between herbivores is induced susceptibility. In this mechanism, sap-feeders activate defensive pathways mediated by salicylic acid, inhibiting a plant’s jasmonic-acid-mediated induced response to chewing herbivores (e.g. Stout et al. 1997, Thaler et al. 2010). As a result of these changes to plant defensive chemistry, chewing herbivores, such as caterpillars, experience increased performance on host plants occupied by other feeding guilds (Ali and Agrawal 2014). Our results have two reciprocal implications: first that strong ant effects in the presence of sap-feeders are attenuated by plant responses to herbivores, and second, that increased ant predation compensates for increased leaf herbivory by caterpillars in the presence of sap-feeders.

We propose that host-plant mediated indirect interactions between herbivore guilds may be an underappreciated additional impact of ant-sap-feeder mutualisms on arthropod food webs, especially in forest communities. These bottom-up effects also act in opposition to trophic cascades from the ant-sap-feeder mutualism, even on plant species in which the mutualism suppresses caterpillars. Our mixed support for ant-instigated trophic cascades contrasts with other studies on ant-sap-feeder mutualisms, mostly from agroecosystems, which show positive effects of ants on plants through trophic cascades when ants are engaged in mutualism (Styrsky and Eubanks 2007). In non-agricultural ecosystems, the indirect benefit plants gain from this mutualism varies significantly. For example, oaks that harbored sap-feeders experienced reduced seed predation from acorn weevils, demonstrating a strong link between plant fitness and ant-sap-feeder mutualisms (Ito and Higashi 1991). In contrast, Grinath et al. (2012) found that while sap-feeding insects strengthened ant predation on leaf beetles, leading to reduced herbivory, their host plants experienced reduced reproduction overall. These conflicting cases indicate that while ant-sap-feeder mutualisms have the potential to generate trophic cascades, whether these effects are realized in natural communities depends on factors not yet entirely understood. We suggest that the bottom-up effects, such as higher host-plant quality associated with sap-feeders, may attenuate ant trophic cascades even if strong predatory effects on herbivores are observed. Additionally, if leaf-chewing herbivores are facilitated by sap-feeders, ant predatory effects may be playing a compensatory role, resulting in a relatively neutral outcome for host plants. In the context of entire communities, the loss of mutualisms might release previously attenuated interactions and their potentially large ecological impacts (Palmer et al. 2015). Given that mutualisms are found in most food webs, compensation and attenuation of predatory effects can be important and underappreciated components of these positive interactions.

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Cascading effects of mutualism are driven by changes in predator body-size distribution

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Abstract

1. Community ecology has historically pooled species into functional groups (i.e. feeding guilds) to understand how feeding interactions structure communities. However, the strength of trophic interactions is often determined by the species composition and interactions within these guilds. This is further complicated in diverse predator communities since the trophic effects of a guild cannot be easily predicted by summing the effects of each species. As a result, understanding interactions within predator communities, and how shifts in predator community structure mediate changes in top-down effect strength, are important questions in community ecology.

2. To address predator community attributes and the consequences for food webs, we utilized a diverse predatory ant community which simultaneously engages in predation of herbivorous caterpillars and food-for-protection mutualisms with sap-feeding Hemiptera. We examined three proposed mechanisms whereby mutualism alters ant community structure, strengthening ant predation of caterpillars and indirect effects on plants: 1) increased abundance of predatory ants on host plants would strengthen predatory effects on caterpillars; 2) recruitment of additional ant species to host plants would increase predatory ant species richness and strengthen predator effects; 3) larger predators would become more prevalent in these communities through the recruitment of larger-bodied ant species, driving stronger predatory effects.
3. We experimentally manipulated ant and sap-feeder communities on maple (*Acer*) and hickory (*Carya*) trees in northeastern U.S. deciduous forests, and examined the impacts of these manipulations on macrolepidopteran caterpillars and leaf herbivory. We found that the total abundance or species richness in the ant community was not altered by the mutualistic sap-feeders. Instead, sap-feeders shifted ant community composition, favoring large-bodied ant species, strengthening suppression of caterpillars and reducing leaf herbivory.

4. We demonstrate that mutualistic interactions mediate ant trophic ecology via changes in ant community composition. More broadly, we show that predator traits, like body size, drove changes in top-down effect strength, even though other community attributes like predator abundance and diversity were not changed. We suggest that shifts in the community composition of predators with high functional redundancy has cascading indirect effects in food webs when considering the body-size variation of both predators and prey.

**Key-words** Predator-prey interactions, multiple predator effects, ants, caterpillars, treehoppers, herbivory, food-for-protection mutualisms, food web interactions, community structure

**Introduction**

The structure of predator communities plays a critical role in determining the ecological consequences of carnivory in food webs (Weis 2015; Duffy *et al.* 2007).
Pooling multiple predators into functional groups has historically helped to simplify trophic networks to more easily analyze consumer-resource interactions in natural communities (Simberloff and Dyan 1991; Schmitz 2007; Rudolf 2012). However, it is increasingly appreciated that dynamics within predator communities themselves mediate their consumptive effects in food webs and non-consumptive effects on prey behavior (e.g. Beckerman et al. 1997; Schmitz 2009; Davenport and Chalcraft 2013).

Consequential predator community dynamics range from relatively simple changes, such as increases in local predator abundance, to more complex shifts, such as changes in predator species richness or body-size distribution. Changes in these community attributes alter top-down effect strength, including direct prey suppression and indirect effects on lower trophic levels (Ives et al. 2005; Schmitz 2007). In terrestrial ecosystems in particular, dynamics within predator communities are important for predicting their direct effects on herbivores and their indirect effects on plants (Terborgh & Estes 2010; Wielgloss et al. 2013).

In predator communities, a single keystone species can strengthen top-down effects of the entire community (Menge et al. 1994; Hooper et al. 2005). Often the predatory trait responsible for a disproportionate effect of a predator is relatively large body size in comparison to the rest of the predator guild (Woodward & Hildrew 2002; Rouabah et al. 2014). A relationship between body-size variation and community-wide predator effects has been demonstrated in various taxa that range widely in body-size scale, from ladybird beetles (Dixon 2007) to large mammalian carnivores (Sinclair et al. 2003; Ripple, Rooney & Beschta 2012). Large predators often consume more individual
prey per capita than smaller predators in the same community, while also depredating multiple prey species of a wider range of body size in the same community (Emmerson & Raffaelli 2004). As a result, the relationship between predator and prey body size is an important predictor for determining the indirect effects of predation as well, especially for trophic cascades (Marquis 2010). The pivotal role of predator body size on trophic cascades is seen in ecological communities that have experienced extirpation of large predators wherein large herbivores have been released from top-down control and plant communities have undergone dramatic change (Estes et al. 2011).

Ants have sizable impacts on arthropod food webs since they are both numerically abundant and species-rich in terrestrial ecosystems (Hölldobler & Wilson 1991; Bluthgen et al. 2000). Arboreal-foraging ants are opportunistic predators (Skinner & Whittaker 1981; Lach, Parr & Abbott 2010), and they are some of the most common invertebrate predators of caterpillars (Montllor & Bernays 1993). In temperate forests, caterpillars are some of the most abundant foliage-feeding herbivores (Marshall and Cooper 2004), which collectively consume a large volume of plant biomass on woody plants (Kozlov et al. 2015). During periods of high foraging activity, ants can have marked impacts on caterpillar population densities in forest canopies (Hölldobler & Wilson 1991). In multiple case studies, ant predation clearly suppressed dominant herbivores on a single host-plant species, increasing plant growth and fitness (Messina 1989; Ito & Higashi 1991).

The trophic interactions of ants are often structured by mutualisms with phloem-feeding Hemiptera (Styrsky & Eubanks, 2007). While ants are often opportunistic
predators of herbivores, many ant species engage in a food-for-protection mutualism with the sap-feeding guild (Dixon 2007). On the one hand, this mutualism protects Hemiptera from natural enemies and facilitates sap-feeder herbivory. On the other hand, by recruiting ants to engage in mutualism, sap-feeders increase the number of predators on host plants, strengthening top-down effects on other herbivore guilds. Ant-sap-feeder mutualisms therefore benefit plants when it reduces other types of herbivory, as described in the keystone interaction hypothesis (Styrsky and Eubanks, 2007). However, the opposing and context-specific indirect effects on plants prevent general predictions about how this mutualism alters top-down effects on herbivores and plants. Because this mutualism often structures arboreal-foraging ant communities (reviewed in Rico-Grey & Oliveira 2007), looking directly at ant community attributes may reveal mechanisms driving the cascading effects of this mutualism in complex food webs.

In this study we use experimental manipulations (i.e. selective exclusions) and the presence of mutualistic interactions to understand how shifts in community attributes like predator abundance, species richness, and relative body size determine the strength of predator effects in food webs. In this study, we use ants as a focal predator group because ant communities can be manipulated on host plants, they exhibit a range of body sizes between species, and they are dominant arthropod predators in terrestrial food webs. Because there is often considerable variation in foraging ant communities on forest trees (Rico-Grey & Oliveira 2007), we hypothesized that the composition of predator species mediates top-down effects on herbivores and plants (Schmitz 2007; Snyder et al. 2006).
We examine three attributes of community structure that may determine the top-down effects of ant-sap-feeder mutualisms. First, in the predator recruitment hypothesis, ant recruitment to sap-feeders increases the local abundance of ants (Helms, Hayden & Vinson 2010). Recruitment of ants leads to more frequent predation of herbivores, including caterpillars (Styrsky & Eubanks 2010), thus strengthening the local top-down effects of ants. These predictions are supported by research in ant-plant mutualistic interactions, in which the benefits myrmecophytic plants gain from ants are driven by changes in total ant abundance (Dátillo et al. 2014). Second, in the predator richness hypothesis, sap-feeders recruit additional species of ants to host plants, increasing total predator diversity, which should increase total ant predation effects on caterpillars according to the general biodiversity-ecosystem functioning hypothesis (Ives et al. 2005; Straub & Snyder 2006, McCoy, Stier & Osenberg 2012). It is predicted that sap-feeders will increase ant diversity because honeydew rewards could recruit ant species to locations where they typically would not be well represented numerically (Davidson 1998). In past work in this system, we have observed that sap-feeders recruit carpenter ants and other ant species that are otherwise rarely observed foraging on plant foliage in the lower canopy (Clark et al. 2016). By increasing predator diversity, mutualist-recruited ants lead to reduced leaf area loss on trees (Nahas, Gonzaga, & Kleber 2012). Third, the predator body size hypothesis proposes that the presence of mutualistic sap-feeders will increase the numerical abundance of large-bodied ant species. Skewing the predator distribution toward larger body sizes is expected to result in a predator community that consumes more prey and larger prey. With fewer and/or smaller caterpillars remaining on
plants, there may be reduced herbivory on host plants where larger predatory ants are present.

To be supported as a mechanism underlying cascading effects of ant-sap-feeder mutualisms, any of these hypotheses would require the following evidence, which is assessed here. The ant community attribute (increased abundance, species richness, or shifted body size distribution) 1) must occur as the result of sap-feeders on host plants, and should be associated with 2) stronger predatory effects on caterpillars and 3) reduced leaf damage from chewing herbivores.

Materials and Methods

Study system. Field experiments took place in mixed deciduous forests located in Middlesex and New Haven Counties in Connecticut, USA during spring and summer of 2013, 2014 and 2015. Field sites were located in six spatially separated areas, including Cockaponset State Forest, in the town of Haddam; Higby Mountain Preserve, Middlefield; Hurd State Park, East Hampton; Lamentation Mountain State Park, Meriden; Mattabesett Trail by Asylum Reservoir no. 2, Middletown; Millers Pond State Park, Durham.

Arthropod communities on host trees. Two different field experiments utilized arthropod communities found on the tree species Acer rubrum (L.) and Carya spp., respectively. These tree species harbor diverse assemblages of caterpillars (Singer et al. 2012), and arboreal-foraging ants that are predators of caterpillars and mutualists of sap-feeding Hemiptera (Clark et al. 2016). Experiments on hickory trees include the three
local species of the Eucarya clade, *C. glabra* (Mill.), *C. ovata* (Mill.), and *C. tomentosa* (Sarg.), which host a shared caterpillar community (Singer et al. 2012). *Formica* and *Camponotus* ants comprise the majority of the ant community (Table S2), and these genera are common predators of caterpillars (Crider et al. 2012, Karhu and Neuvonen 1998, Altegrim 2005). *Formica neogagates* (Viereck) workers are numerically dominant ants with the average forager having a body length of 3.5 mm (Ellison et al. 2012). The local carpenter ant species, which we henceforth refer to as ‘large ants’, are *Camponotus chromaoides* (Bolton) and *Camponotus pennsylvanicus* (De Geer). These species have two worker sub-castes, with majors averaging 12.5 mm in length, 342% larger than *F. neogagates* workers. Minor workers in these species are typically 5.5 mm in length, 157% larger than *F. neogagates*. Both major and minor workers of these carpenter ants are behaviorally dominant over *F. neogagates*, and these two carpenter ant species prey on caterpillars and engage in mutualism with sap-feeders (R.E. Clark personal observations, Fig. S2).

**Ant community manipulation.** To determine the ecological impacts of ants on caterpillars and host plants, we manipulated ant communities at the level of tree branches and saplings. In both experiments, we used the three following treatments: a full ant exclusion (- ants), a selective exclusion of large ants (- large ants), and a control (+ ants). In the first treatment, all ant species were excluded from branches using a sticky barrier (Tanglefoot®, Contech Enterprises, Victoria BC, Canada). To erect this full ant exclusion, Tanglefoot resin was applied to 12-cm wide segments of plastic sheeting wrapped around branches and held on by 28-cm nylon cable ties (Commercial Electric®,
Home Depot USA, Atlanta GA). In the second treatment, the ant exclusion barrier was placed on branches, but ants were selectively excluded with a screen with 1.25 mm pores. We used ant specimens of the species *F. neogagates*, *C. chromoides* and *C. pennsylvanicus* to determine screen pore size. With chose a pore size that preserved specimens of *Formica neogagates* could be pushed through, but not *C. chromoides* or *C. pennsylvanicus*. In the field, this apparatus involved a PVC pipe fitted with a hose gasket and screen, and this treatment was then attached to the wire bridge using cable ties (Figure S1). This apparatus excludes larger ants from experimental branches because they cannot pass through the screen or Tanglefoot barrier. In experiment 1, control branches were those with no Tanglefoot (+ ants), whereas in experiment 2, we used a sham control consisting of the same Tanglefoot barrier as in the full ant exclusion with the addition of a wire bridge placed over it to allow any ant species to forage on host plants (+ ants). We included this sham control to account for any additional effects of the wire bridge used in the (- large ants) treatment.

**Insect collection.** In all experiments, we collected insects by beating tree branches vigorously over white collection sheets. We collected caterpillars to record their body length, measured with microcalipers to the nearest mm (head to anal plate). Ants were collected on beat sheets during caterpillar sampling. *Camponotus* and *Formica* ants were identified in the field, while all other ant genera were collected in ethanol to be identified under a dissecting microscope in the lab. In experiment 2, all sap-feeders removed from branches were stored in ethanol and identified to family. Any adult sap-feeders that were collected were identified to species (Table S3).
Experiment 1: Manipulation of the ant community on *A. rubrum*. This experiment determined if the predatory effects of larger ants could be isolated from the predatory effects of the remaining ant community, while avoiding any confounding effect of sap-feeder mutualisms on ant community structure. Ant exclusion treatments were applied in the week of 11-June 2013 on *A. rubrum* saplings and tree branches 1-2 meters from the ground. *Acer rubrum* has a relatively low frequency of sap-feeders, and the presence of sap-feeders significantly increases the abundance of carpenter ants on trees for up to three weeks (Clark *et al.* 2016).

The experimental design consisted of six spatial blocks that were located at two forest sites: Cockaponset State Forest and Millers Pond State Park. Treatments were set up in a factorial design: each of the six blocks contained eight full ant exclusions (- ants), eight large ant exclusions (- large ants), and eight controls (+ ants) for a total of 144 manipulated branches. At the time treatments were erected, all experimental branches had a sugar-bait (127 x 77mm paper card with a hole punch and smeared with grape jelly) placed on the terminal end of the branch tied with fishing line. We observed that these baits recruited ants for at least one week since ants were still feeding from them at the time caterpillars were collected. We used these baits to determine if barriers effectively excluded ants even when a resource-rich bait that is known to recruit ants (Agosti *et al.* 2000) was available on the branch. After one week, we returned to sample ants and caterpillars from these branches.

Experiment 2: Manipulation of ant and sap-feeder community on *Carya* spp. In 2014-2015, ants and sap-feeders were factorially manipulated on *Carya* spp. This
experiment had a 3 x 2 factorial design (- ants, - large ants, + ants x – sap-feeders, + sap-feeders) and was conducted over 2 field seasons. We set up the experiment across six spatially isolated sites, where 144 branches were manipulated in 2014, and 120 branches in 2015 (for replication at each site see Table S1). Treatments were initiated from 19-May to 29-May in 2014 and 18-May to 29-May in 2015 on host plants visibly harboring sap-feeding herbivores. For half of these experimental branches, we removed all sap-feeding herbivores. We visually scanned branches for sap-feeder nymphs and adults, and any observed treehoppers or scale insects were removed and placed in ethanol for identification. To control for variation in caterpillar abundance at the onset of the experiment, we first beat each branch to remove all caterpillars. We then used these caterpillars to stock all experimental branches with 3 caterpillars of similar length (ca. 1.5 cm), controlling for initial caterpillar abundance for all treatments. This caterpillar count reflected natural abundances observed both field seasons (mean caterpillar abundance per branch = 3.21 and SEM 0.161).

After all treatments were erected, we sampled caterpillars, ants and sap-feeders in the same order as set up (sampling started June 9 in 2014 and June 10 in 2015). For each spatial block, three weeks (ca. 21 days) passed between the initiation of treatments and the sampling of insects. After this arthropod sampling period, we measured leaf area and herbivory (described below).

Leaf area and leaf herbivory assay. In experiment 2, leaf damage was estimated to quantify the amount of leaf area consumed by chewing herbivores over the course of each season. After the completion of arthropod sampling in July 7 2014 and July 13 2015, we
counted all leaves on each experimental branch. For each experimental branch, we visually assigned all leaves to the following six categories: ‘no damage’, ‘1-20% leaf area lost’, ‘20-40% leaf area lost’, ‘40-60% leaf area lost’, ‘60-80% area lost’ and ‘80-100% leaf area lost’ (method modified from Barber and Marquis 2009). On all branches, we counted remnant petioles in the 80-100% category since these indicated leaves that were completely consumed by chewing herbivores. To estimate total leaf damage of each branch, we first calculated the product of the number of leaves in each damage category, the respective median of each of these categories (0.1, 0.3, 0.5, 0.7, 0.9), and a known average of *Carya* spp. leaves. We used the average leaf area for *Carya* spp. from Singer *et al.* (2012), in which the total area of individual leaves was directly measured from trees in a subset of the sites studied here.

**Statistical analyses.** Analyses were run in R 3.2.4 (R Core development team 2016), employing a series of six generalized linear mixed models (GLMM) in the lme4 package (Bates *et al.* 2015) on the following dependent variables: 1) total ant abundance 2) large ant (carpenter ant) abundance, 3) small ant (*Formica* and others) abundance, 4) caterpillar abundance, 5) caterpillar body size, and 6) leaf area lost (herbivory on host plants). In addition to these linear models, we used Kruskal-Wallis rank sum tests to evaluate the effects of sap-feeders on ant species richness (Pohlert 2016). For linear models involving insect abundance, we included log-transformed branch leaf area as an offset variable to account for sampling effort (Kotze, O’Hara & Lehmävirta 2012). For the leaf damage GLMM, we modeled the total number of leaves on each branch as a covariate. In all models, ant exclusion, sap-feeder manipulation, ant exclusion by sap-
feeder manipulation interaction, and year were fixed effects, while site and year were included in all models as random effects. Ant abundance models were fitted to the negative binomial distribution due to a high proportion of zeroes, and caterpillar abundance models were fitted to the Poisson distribution (Bolker et al. 2007). Significance values were calculated using Wald $\chi^2$ tests in the car package (Fox et al. 2015) using type II sums of squares (following Langsrud 2003). Predicted marginal means and standard errors of the mean were estimated using the lsmeans package in R (Lenth et al. 2015).

Results

**Ant community manipulation on *Acer***. The ant exclusion treatment (−ants) effectively excluded ants, reducing total ant abundance by 91.4% compared to controls (Fig. 1, GLMM $P < 0.001$). Likewise, the selective barrier was effective in specifically excluding large-bodied ants without altering the abundance of small-bodied ants. In this treatment, the abundance of large-bodied ants (−large ants) as measured by the abundance of *Camponotus* ants was reduced by 95% (Fig. 1b, GLMM $P < 0.001$), while the total ant abundance trended lower but was not statistically different from controls (Fig. 1, GLMM $P = 0.0762$). Conversely, the abundance of small ants was reduced by full exclusion by 94% (Fig. 1c, $P < 0.001$) and selective exclusion did not significantly reduce the abundance of small ants (Fig. 1c, GLMM $P = 0.58$).

Ant exclusion increased caterpillar abundance by 39.5% (Fig. 1d, GLMM $P = 0.022$), while selective exclusion of large ants generated an intermediate effect on
caterpillar abundance relative to full exclusion and no-exclusion treatments (Fig. 1d, Tukey HSD).

**Effects of mutualism on ant community on *Carya***. We observed seven species of sap-feeders (six Membracidae and one Coccidae) actively engaged in mutualism with ants. In proximity to these sap-feeders we observed twelve species of ants foraging on branches. This level of ant species richness is typical for this highly facultative mutualism (Dixon 2007), in which multiple ant and sap-feeder species are found together on individual host plants. Our manipulation of the sap-feeder community and its effects on the ant community supported the body-size shift hypothesis: removal of sap-feeders on *Carya* branches reduced the abundance of large-bodied ants by 68.9% (Fig. 2b, Table 1, \( P < 0.001 \)), but did not change the abundance of small-bodied ants (Fig. 2c, Table 2, \( P = 0.864 \)). In contrast, we found no support for the predator recruitment hypothesis. When we removed sap-feeders from branches, the total abundance of predatory ants did not change (Fig. 2a, GLMM, \( P = 0.1663 \)). Similarly, we found no support for the predator richness hypothesis through manipulation of sap-feeders. Ant species richness was not significantly different when sap-feeders were present or removed (Fig. 2b, Kruskal-Wallace Rank-Sum Test, \( P = 0.3284 \)). Instead, *Camponotus* ants become better represented in the ant community (Fig 2b). We quantified this change in ant community structure using Pielou's measure of species evenness. In the presence of sap-feeders we observed an evenness index of 0.724, while the ant community without sap-feeders had lower evenness index of 0.594, a pattern that can be attributed to decreased representation of a dominant species in this community (Hallet *et al.* 2014).
Predatory effects of ants on caterpillars and leaf damage on *Carya*. Selectively excluding large-bodied ants resulted in increased caterpillar abundance by 26.3% (Fig. 3a, Table 3, \( P = 0.0014 \)), further supporting the predictions of the body-size shift hypothesis. Following this pattern, the presence of large ants reduced leaf damage by 12.6% (Fig. 3b, Tukey HSD). Finally, ant exclusion had a negative effect on the average body length of caterpillars found on host plants occupied by sap-feeders (ant treatment by sap-feeder treatment interaction, \( P = 0.016 \)), fitting the prediction that larger predatory ants can capture larger caterpillars. Caterpillar body size was 18% lower in the presence of large-bodied ants and sap-feeders, but not when large ants were excluded (Fig. 4, Tukey HSD).

Discussion

In this study, large-bodied predatory ants were responsible for strengthened top-down effects on caterpillars and plants, and the abundance of these large-bodied ants depended on sap-feeding mutualists. Despite the recruitment of large-bodied ants to host plants by sap-feeders, we did not observe changes to ant species richness or total predatory ant abundance. Our findings corroborate studies in which honeydew or other mutualistic resources alter the abundance of one or more ant species by shifting ant community composition (Davidson *et al.* 1998). Furthermore, these changes to ant community composition occurred in response to multiple species of sap-feeding Hemiptera (Table S2 and S3), suggesting that there could be multiple mutualistic effects occurring (Afkhami, Rudgers & Stachowicz 2014). Food resources provided by this sap-
feeder community generated a sterotypical shift to a community with greater numerical representation of large-bodied *Camponotus*. We therefore attribute the cascading effects to a shift in the body-size distribution of this predator guild, parallel to similar work involving other taxa (e.g. Griffen *et al.* 2015).

Our lack of evidence for the predator recruitment hypothesis contrasts with a range of ecological studies in which a single ant species exhibits a strong numerical response to mutualistic Hemiptera, increasing total ant abundance on host plants (Grover *et al.* 2009, Kaplan and Eubanks 2005). Furthermore, observations from agricultural ecosystems reveal that ant predatory effects are often primarily driven by a single numerically dominant species in that community (e.g. Mestre *et al.* 2016). In fact, our experiments on *Carya* suggest that the numerically dominant species in this community has negligible effects on caterpillar abundance when large-bodied ants are excluded. Our results are more reflective of work with keystone predators in which the predatory effects compared to other members of the community cannot be attributed to abundance alone (Menge *et al.* 1994; Hooper *et al.* 2005). This suggests ant communities occurring on host trees without sap-feeders do not have sufficiently strong top-down effects to indirectly benefit host trees, but not because ant abundance is lower on host plants without sap-feeders, but instead because the dominant ant species has relatively weak effects compared to larger-bodied predatory ants.

That manipulation of sap-feeders did not alter the species richness of predatory ants was somewhat surprising because food resources provided by plants and Hemiptera often increase local ant diversity (Bluthgen *et al.* 2000). In contrast, we observed
increased representation *Camponotus*, which are typically rare in the lower canopy in the absence of sap-feeders (Clark et al. 2016), instead of a change in species richness. This led to a more even ant community because there was no change in the abundance of *Formica neogagates*, which became less proportionally abundant with respect to other ant species (*sensu* Hallet et al. 2014). This finding corroborates observations of increasing complementarity in more even predator communities with ants (e.g. Wielgloss et al. 2013). Irrespective of the role of evenness and complementarity, richness was a poor predictor of predator effects, echoing observations that predator biomass is often a reliable predictor of consumptive of top-down effects on herbivores and herbivore grazing (Lefcheck and Duffy 2015).

Variation in predator arthropod community structure can mediate effects on herbivores and indirect effects on plant productivity (Snyder et al. 2006). For example, top-down effects of an entire predatory arthropod community were driven by the presence of a single ladybird beetle species that suppressed aphids and improved plant performance (Long & Finke 2014). However, it is unclear if the ultimate consequences for herbivory were the result of ants reducing the total abundance of caterpillars, or consuming large caterpillars which consume significantly more leaf biomass. In either scenario, large-bodied ants have sufficient impacts on caterpillar communities in this system to reduce total amount of leaf area consumed by chewing herbivores. This is similar to research in ant-plant mutualistic interactions in which functional traits of predatory ants, including body size, are a strong determinant of how effective ants are as plant mutualists (Rico-Grey & Oliveria 2007). We similarly observed an indirect
interaction that cascades down to host plants as a function of predatory ant traits. However, since this is a generalized response of the ant community (e.g. recruitment of *Camponotus* could occur on any host plant), this interaction has the potential to restructure herbivory patterns in forested ecosystems where sap-feeders are abundant.

In sum, different size classes of predators drove functional differences in top-down effects. Size classes considered in other ecological studies cover a wide range, from broad taxonomic groupings, such as comparing vertebrate and arthropod predators (Mooney 2007, Piñol *et al.* 2010), to two families of similar-sized predators (Nahas, Gonzaga, & Kleber 2012), or size classes that are structured by the age of predators of the same species (Rudolf, 2006). The consequences of relatively subtle changes in ant community structure driven by changes in body size distribution seem to contrast with predictions from models of predator impacts based on other functional traits. For example, work on arthropod predators, such as spiders, suggests that there should be high functional redundancy among predator species with similar hunting strategies (Sokol-Hessner & Schmitz, 2002). For multi-predator effects of ant communities, there should be considerable functional redundancy because these predator species have similar foraging domains and hunting modes (*sensu* Schmitz, 2007). We observed that large bodied ants were able to capture caterpillars later in development that might otherwise escape predation (Soomdat *et al.* 2014). Since the largest ant species can capture a larger range of prey (i.e. larger caterpillars), this sufficiently strengthens top-down effects and reduces herbivory. This suggests that even in predator communities with apparent functional redundancy in foraging domain and hunting mode, the pairing of body-size
trait-changes in the predator community with relevant traits of prey communities (e.g. caterpillar prey community on deciduous trees) determines if stronger predator effects on prey and host plants are actually realized.

Acknowledgements

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References


Table and Figures

Table 1: Analysis of Deviance on large ant abundance in 2014 and 2015 experiments on Carya.

<table>
<thead>
<tr>
<th>Factor</th>
<th>$X^2$</th>
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<th>$P$</th>
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<td>Sap-feeder Treatment (S)</td>
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<tr>
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<td>3.445</td>
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<td>A*S</td>
<td>0.789</td>
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<td>0.6739</td>
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Table 2: Analysis of Deviance on small ant abundance in 2014 and 2015 experiments on Carya.

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<tr>
<td>A*S</td>
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Table 3: Analysis of Deviance on total caterpillar abundance in 2014 and 2015 experiments on *Carya*.

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<td>Year</td>
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<td>0.3428</td>
</tr>
<tr>
<td>A*S</td>
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<td>0.7929</td>
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Table 4: Analysis of Deviance on total leaf area lost in 2014 and 2015 experiments on *Carya*.

<table>
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<tr>
<td>Sap-feeder Treatment (S)</td>
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<td>A*S</td>
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<tr>
<td>Year</td>
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<td>1</td>
<td>0.2270</td>
</tr>
<tr>
<td>Leaf Count</td>
<td>238.4</td>
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Figure 1: Abundance of large ants, small ants, and caterpillars on *Acer rubrum* (experiment 1). Points and error bars represent estimated mean and SEM. Points not connected by the same letter are significantly different (Tukey HSD).
Figure 2: Effect of sap-feeder manipulation on total ant abundance, large ant abundance, small ant abundance, and ant species richness on the host plant *Carya* spp. (experiment 2). Points and error bars represent estimated mean and SEM. A reduction in large ant abundance was the only attribute of the ant community altered by the removal of sap-feeders (*P* < 0.001).
Figure 3: Effect of ant exclusion on large ant abundance, small ant abundance, Caterpillar abundance and leaf herbivory on *Carya* spp. (experiment 2). Points and error bars represent estimated mean and SEM. Points not connected by the same letter are significantly different (Tukey HSD).
Figure 4: Effect of ant exclusion on changes to caterpillar body size distribution when sap-feeders were present or removed. Points and error bars represent estimated mean and SEM. Asterisks indicate significant difference between means for each treatment group (Tukey HSD).
Supporting Information
Table S1: Sites used for Experiment 2 and the number of manipulated branches at each location.

<table>
<thead>
<tr>
<th>Site</th>
<th>Manipulations in 2014</th>
<th>Manipulations in 2015</th>
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<tbody>
<tr>
<td>Asylum Reservoir</td>
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<td>6</td>
</tr>
<tr>
<td>Cockaponset State Forest</td>
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<td>36</td>
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<tr>
<td>Higby Mountain Preserve</td>
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<td>30</td>
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<td>Hurd State Park</td>
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<td>Millers Pond State Park</td>
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<td>12</td>
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<tr>
<td>Lamentation Mountain Park</td>
<td>24</td>
<td>18</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>144</strong></td>
<td><strong>120</strong></td>
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Table S2: Ant species found on *Carya* branches and saplings Experiment 2. Observed tending column refers to any observed behavior in which ants guarded Hemiptera or fed on honeydew.

<table>
<thead>
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<th>Ant species</th>
<th>Observed Tending</th>
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<tbody>
<tr>
<td><em>Camponotus americanus</em></td>
<td>Yes</td>
</tr>
<tr>
<td><em>Camponotus chromaiodes</em></td>
<td>Yes</td>
</tr>
<tr>
<td><em>Camponotus nearcticus</em></td>
<td>No</td>
</tr>
<tr>
<td><em>Camponotus pennsylvancus</em></td>
<td>Yes</td>
</tr>
<tr>
<td><em>Crematogaster lineolata</em></td>
<td>Yes</td>
</tr>
<tr>
<td><em>Dolichoderus plagiatius</em></td>
<td>No</td>
</tr>
<tr>
<td><em>Formica neogagates</em></td>
<td>Yes</td>
</tr>
<tr>
<td><em>Formica subsericea</em></td>
<td>Yes</td>
</tr>
<tr>
<td><em>Formica subanescens</em></td>
<td>Yes</td>
</tr>
<tr>
<td><em>Lasius neoniger</em></td>
<td>Yes</td>
</tr>
<tr>
<td><em>Tapinoma sessile</em></td>
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<tr>
<td><em>Temnothorax longispinosus</em></td>
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<tr>
<td><em>Temnothorax curvispinosus</em></td>
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Table S3: Sap-feeding Hemiptera species found on *Carya* branches and saplings in Experiment 2.

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
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<tbody>
<tr>
<td><em>Eulecanium caryae</em></td>
<td>Coccidae</td>
</tr>
<tr>
<td><em>Carynota mera</em></td>
<td>Membracidae</td>
</tr>
<tr>
<td><em>Heliconia excelsa</em></td>
<td>Membracidae</td>
</tr>
<tr>
<td><em>Microcentrus caryae</em></td>
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<tr>
<td><em>Telomona decorata</em></td>
<td>Membracidae</td>
</tr>
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<tr>
<td><em>Telomona unicolor</em></td>
<td>Membracidae</td>
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Supplemental Figures

Figure S1: Photograph of large ant (carpenter ant) exclusion treatment on *Carya* (R.E. Clark).
Figure S2: Photograph of *C. chromaooides* major worker capturing a caterpillar on *Carya* (R.E. Clark).
Behavioral differences between ant species determine the ecological consequences of a multispecies mutualism

Robert E. Clark* and Michael S. Singer
Wesleyan University Department of Biology

Abstract
Mutualistic interactions between ants and sap-feeding insects are widespread in terrestrial ecosystems. However, it is difficult to predict the ecological impacts of this facultative mutualism since multiple species of ants often tend sap-feeders in a shared environment. This study examines behavioral variation between two treehopper-tending ant species, explicitly testing interspecific variation in behavioral traits relevant to competition and predation, as well as intraspecific variation in induced behaviors. We employed a series of field experiments that manipulated ant diet with baits and utilized ants actively tending treehoppers for two ant dominant ant species, Camponotus chromaoides and Formica neogagates. We recorded 494 interactions between these ants and competitors, ladybird beetles and caterpillar prey. C. chromaoides, exhibited behavioral dominance over F. neogagates, and C. chromaoides was more likely to attack ladybird beetles, competing ants, and caterpillars. However, we observed no evidence that food rewards provided by sap-feeders induced changes in ant behavior for either species. This result supports other work in multispecies mutualisms in which one species dominates the interaction and drives the indirect effects of mutualisms in food webs, while also revealing the
importance of considering behavioral trait variation to predict the impacts of food-for-protection mutualisms.

Key Words

Mutualism, multi-species mutualism, ants, behavior, food webs, sap-feeders, caterpillars

Introduction

While the importance of mutualisms for food webs are well established (Bronstein 2015), “multispecies mutualisms” make it difficult to predict the ecological consequences of positive interactions (Palmer et al. 2010, Chamberlain et al. 2014). In natural communities, facultative mutualisms often involve multiple partners, with each partnership representing a novel combination of mutualist traits (Bascompte and Jordano 2007, Pringle and Gordan 2013). Different species pairs can mediate the impacts of mutualisms if some pairs have stronger ecological effects than others (e.g. Stanton 2013, Afkhami et al. 2014). For example, protection mutualisms are often facultative with multiple species of “protector” (Mooney and Mandal 2010), or “protectee” (Yoo et al. 2013): Protectees gain biotic defenses against natural enemies, weakening top-down control and increasing population densities (e.g. Chamberlain and Holland 2009), while protectors receive food sources from mutualists that often modify protector behavioral interactions with other species (Styrsky and Eubanks 2010, Palmer et al. 2015). Because protection mutualisms involve feeding interactions and are highly facultative, they provide an opportunity to understand how variation in partnerships can mediate the ecological impacts of mutualisms in food webs.
Ant food-for-protection mutualisms exemplify multispecies mutualisms since hosts can engage multiple ant species over time and space (Pringle and Gordon 2013). Ant communities exhibit considerable variation in behavioral traits, including aggression towards competitors, defense of territories against other ants, and foraging strategies for capturing prey (Sanders et al. 2007, Lach et al. 2010, Stuble et al. 2013). Additionally, following participation in protection, individual workers may exhibit changes in behavior relevant to competition and/or trophic interactions. For example, food subsidies provided to ants can modify the behavior of individual workers after feeding (Wilder and Eubanks 2010). These induced behaviors include increased aggression towards potential competitors for mutualists (Cheng et al. 2016), increased aggression towards workers from competing ant colonies (Dejean et al. 1997, Davidson 1998), and more frequent predation events (Ness et al. 2009, Rudolf and Palmer 2013). Notably, both behavioral trait differences between ant species and induced behavioral changes of individual ant workers cause similar behaviors: defense of mutualists against natural enemies, intensified competition between ants, and increased likelihood of predation of other herbivores. The goal of the proposed work is to disentangle the different responses of ant species to sap-feeders when multiple ant species act as participants.

Ant-sap-feeder mutualisms have been described as a “keystone interaction” due to their disproportionately large effects on arthropod food webs (Styrsky and Eubanks 2007). Phloem-feeding Hemiptera produce sugary excretions that are consumed by ant workers, providing carbohydrate resources that support ant worker activity and colony growth (Shik and Silverman 2013, Shik et al. 2014, Wills et al. 2015). Ant workers recruit to sap-feeders and remain in close proximity to feed on honeydew and protect sap-feeders from
natural enemies (Stadler and Dixon, 2008). The consequences of this mutualism fall into three general groups: First, a key benefit for sap-feeders is protection from natural enemies, including common arthropod predators or parasitoids (Stadler and Dixon, 2008). Ants in proximity to sap-feeders attack ladybird beetles and other natural enemies of sap-feeders (Philips and Willis 2005), reducing overall abundance of predators on host plants (Wimp and Whitham 2001). Second, ants are more likely to defend host plants or territory containing sap-feeders from competitors, intensifying ant competition, leading to increased territorial disputes between ant colonies, ultimately resulting in altered ant community dynamics (Wilder et al. 2013). Third, carbohydrate rewards provide an energy subsidy to ant workers, facilitating increased predatory aggression towards other herbivores through a variety of physiological mechanisms (Ness et al. 2009, McGlynn and Para 2016). Here we specifically examine multiple ant species respond to sap-feeders via these three categories of behavioral responses in ants. Four hypotheses were tested in behavioral experiments with two species of ants.

In the superior protector hypothesis, one ant species engaged in mutualism will exhibit higher aggression compared to the rest of the community. We propose this hypothesis because invasive or behaviorally dominant ant species often monopolize mutualistic interactions in ant communities (Grover et al 2007), and these same ant species are responsible for intensified competition (Wilder et al. 2013) and increased predation (Kaplan and Eubanks 2004). This hypothesis therefore predicts that ant species that exhibit behavioral dominance over other members of the ant community should also be more likely to attack competitors or prey.
Alternatively, the aggressive defense hypothesis (*sensu* McGlynn and Para 2016) states that ant workers recently provided carbohydrates from mutualists will exhibit increased aggression towards natural enemies that potentially threaten sap-feeders (McGlynn and Para 2016). Under these conditions, ants would be more likely to attack or other predatory arthropods. This hypothesis predicts that ant workers that have recently consumed carbohydrates from mutualists would exhibit a higher rate of attack behaviors towards natural enemies like ladybird beetles. In the intensified competition hypothesis, it is predicted that individual ant workers would be more likely to aggressively attack competing ants. This occurs since carbohydrates are important food recourses for colonies, ant competitive interactions occur more frequently in the presence mutualists (Grover et al. 2007, Kay et al. 2010). This prediction includes increased aggression towards either intraspecific ant competitors from different colonies or interspecific competing ant species that also tend sap-feeders. In the prey acquisition hypothesis (*sensu* Mglynn and Para 2016), ants are more likely to engage in opportunistic predation of herbivores or other potential prey. This occurs because sap-feeder food rewards are carbohydrate rich and protein-poor, and ants will capture protein-rich prey as a compensatory response (Ness et al. 2009, Christensen et al. 2010, McGlynn and Para 2016). This hypothesis predicts that individual ants that have recently engaged in mutualism with sap-feeder will show increased frequency of attempted predation on non-sap-feeding herbivores.
Table 1. Hypotheses, predictions for ant behaviors displayed and experiments where these predictions are evaluated.

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Ant behavioral responses</th>
<th>Comparison</th>
<th>Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superior protector</td>
<td>Dominant ant species more likely to attack interactors (e.g. competitors and prey)</td>
<td>Interspecific variation between two ant species</td>
<td>2,3</td>
</tr>
<tr>
<td>Aggressive defense</td>
<td>After consuming carbohydrates from mutualists, ants more likely to attack predators of sap-feeders</td>
<td>Induced behavioral variation</td>
<td>1,2,3</td>
</tr>
<tr>
<td>Intensified competition</td>
<td>After consuming carbohydrates from mutualists, ants more likely to attack competing ants</td>
<td>Induced behavioral variation</td>
<td>2,3</td>
</tr>
<tr>
<td>Prey acquisition</td>
<td>After consuming carbohydrates from mutualists, ants more likely to attack potential prey</td>
<td>Induced behavioral variation</td>
<td>1,2,3</td>
</tr>
</tbody>
</table>

Methods

Study System and sites

We conducted field experiments in mixed deciduous forests in Middlesex County, Connecticut, USA. Additional research in this system has demonstrates that ant-sap-feeder interactions on mediate ant predatory effects on caterpillars (Clark et al. 2016). This community is comprised of the two *Camponotus chromaoides* and *Formica neogagates*, two abundant ant species in upland mixed deciduous forests in New England, USA (Ellison et al. 2012). Further work has demonstrated that exclusion of *Camponotus* from trees weakens top-down control of caterpillars (Clark and Singer, *in review*). *C. chromaoides* and *F. neogagates* both actively tend sap-feeders on host trees in a shared habitat (Clark et al. 2016, Fig. S5) and therefore fit the criteria of multispecies mutualism. These observations, as well as evidence that *C. chromaoides* recruits to sap-feeders in past work (Clark et al. 2016) establishes this ant species as the dominant mutualist (i.e.
superior protector species) in this community, and is therefore the species of focus for the superior protector hypothesis (e.g. we predict that \textit{C. chromaoides} will be more aggressive than \textit{F. neogagates}).

Three experiments examined the relationship between ant behavior and food resources provided by mutualistic sap-feeders or baits with field-collected ants. Behavioral assays took place in the field in Summer 2014 and 2015 at four locations: Cockaponset State Forest, in the town of Haddam; Mattabesett Trail by Reed’s Gap, Durham; Mattabesett Trail by Asylum Reservoir no. 2, Middletown; and Millers Pond State Park, Durham.

Experiment 1 examined the response of \textit{Formica} to prey and competitors alone with workers collected from the tree species \textit{Acer rubrum, Betula lenta, Carya spp., Fagus grandifolia, Hamamelis virginiana, Prunus serotina} and \textit{Quercus rubra}. In experiment 2 and 3, \textit{Carya} spp. trees were used since these species are the primary host of sap-feeders used in Experiment 3.

\textbf{Ant behavior at honeydew proxy baits}

Experiments 1 and 2 evaluated the prediction that ant aggressive behaviors would be more frequent for ants that had recently consuming carbohydrates from mutualists. Experiments used ants that have recently fed at sucrose baits that approximate treehopper honeydew. “Honeydew proxy baits” were 15mL plastic test tubes filled to 50% volume and plugged with a cotton ball. Baits were filled with 10% sucrose solution of table sugar (sucrose) and distilled water. Sucrose elicits recruitment responses from ants (Detrain et al. 2010) and is used in comparable studies of ant behavior in which ants were provided carbohydrates \textit{via} baits (Petry et al. 2012). In the field, baits were mounted onto branches
with plastic “zip” ties, after which we waited at least 3 hours for ant recruitment (Fig S2). Ant recruitment to these baits mirrored ant responses to treehoppers, with *Camponotus* and *Formica* feeding at baits within a short period of time, followed by *Camponotus* displacing *Formica* at contested baits (unpublished data).

More specifically, experiment 1 tested the intensified competition hypothesis regarding interspecific competition between *C. chromaoides* and *F. neogagates* and the prey acquisition hypothesis for *F. neogagates*. In the field, we examined predatory behavior of *F. neogagates* and competition between *F. neogagates* and *C. chromaooides*. In this experiment, interspecific competitors were collected from different individual host plants in proximity to baited plants (< 10m) and used immediately in competition assays. Observations took place 17-June to 10-July 2014 in Miller’s Pond State Park.

Experiment 2 examined the behavior of both *Formica* and *Camponotus* towards ladybird beetles, intraspecific competitor ants, and caterpillar prey. This design provided tests for all four hypotheses in a framework that determines the relative importance of intraspecific induced behavior (e.g. behavioral shifts in *F. neogagates* or *C. chromaooides* after consuming carbohydrates) from interspecific differences (e.g. *C. chromaooides* being more likely to attack prey than *F. neogagates* overall). For the ladybird beetle assay, we used *Harmonia axyridis*, that were purchased from Bug Sales Biological Supply (Amazon.com, Inc., Seattle, WA) and kept in an insectarium in the lab. *H. axyridis* is an exotic species that is now a broadly distributed predator of sap-feeders in the eastern U.S., and has been used in other studies examining ant defense of sap-feeders (Barton and Ives 2014). For intraspecific competitors, we used a technique employing collected colony fragments of *F. neogagates* and *C. chromaooides*; colony fragments were kept the lab
insectarium for < 2 weeks, after which new colony fragments were collected as needed at field locations not being used for behavioral experiments (e.g. Pohl et al. 2016). For predation assays, ants were presented with lesser wax moth caterpillars, Achroia grisella, which were purchased from DBD Pet Biological Supply (Amazon.com, Inc., Seattle, WA) and kept in small food containers in the lab. We used A. grisella as a palatable prey for ants since ants have been reported to opportunistically feed on this species (Hood et al. 2003). This species lacks defense traits found in other tree-feeding caterpillar species that exhibit behavioral and morphological defenses to deter predatory invertebrates (e.g. Gentry and Dyer 2002). Ant rejection of native prey items therefore would confound the results of this experiment if rejection was attributed to caterpillar defensive traits, not ant behavioral response to sap-feeders. Observations took place 22-June to 6-July 2015 at Millers Pond, Cockaponset State Forest, and Asylum Reservoir no. 2.

Ant behavior at sap-feeding treehoppers

Experiment 3 examined induced behavior caused by ants recently engaged in mutualism with sap-feeders and behavioral differences between ant species. We therefore examined all hypotheses for Camponotus and Formica using sap-feeders as a source of carbohydrates, similar to experiment 2. We visually searched for sap-feeders on Carya spp. trees in May and early June 2015. Host tree with Carynota mera treehoppers were flagged and used as a source of ants in behavioral experiments on the same day. While other treehoppers and members of the sap-feeder guild were observed in this system C. mera treehopper nymphs are the most abundant species found in spring (Clark and Singer in review). We completed assays with ants tending sap-feeders in addition to baits to account for other mechanisms by which treehoppers may influence ant behavior in two
ways. First, while honeydew primarily contains plant sugars and water, there are additional sugar compounds in honeydew which elicit behavioral responses from ants (Detrain et al. 2010). Second, treehoppers utilize acoustic communication for mate recognition and alarms, and ants respond to these auditory cues while tending treehoppers (Morales et al. 2008). We observed both ant species tending *C. mera* nymphs (Fig. S4). Experiments took place on 3-June to 19-June 2015 at all four field sites.

**Scoring ant behavior**

To score ant behavioral aggression towards competitors and prey, we used a modified version of ant aggression assays employed in studies of nest-mate recognition between worker ants (Roulston et al. 2003) and ant competition assays (Buczkowski and Bennet 2007). Behavioral assays examined one-one-one interactions of ants in fluon-lined containers with an individual ladybird beetle, competing ant, or waxworm caterpillar. Ant workers were collected directly from host plants in the field, where a container was held under foliage, and the host plant was gently tapped causing the ants to drop. Attack behaviors was recorded once ants were placed into assay containers (Fig. S5). Attack behavior included biting of the cuticle, antennae, or mandibles, or tackling other insects (following Suarez et al. 2002). If ants encountered an interactor, but did not show any aggressive behaviors other than touching with antennae, then the trial was recorded as absence of attack behavior. Once encounters occurred, the time was recorded, the assay was ended and ants were removed from the container. Worker ants were only used once, and then released at the conclusion of daily field experiments to prevent resampling the same individual ants. Trials were timed for 600 seconds in experiment 1 and 300 seconds in experiments 2 and 3. If the ants did not move or did not encounter the
interactor within this time, the trial was ended. Trials that timed out in this manner were excluded from final analyses because no interaction occurred.

Experiments 2 and 3, the intensified competition hypothesis and prey acquisition hypothesis were tested by comparing attack behaviors of Formica against competing Camponotus ants and waxworm caterpillars. Both experiments had a complete factorial design on Carya spp. trees (2 ant species x 3 interactors x 2 treatments). There were 12 observations per replicate: Six C. chromaoides ants and six F. neogagates ants. For each of these species, three worker ants in proximity to baits or sap-feeders were collected from host plants with baits and three were collected from the forest floor as a control. Ants would be transported in small tubes and behavioral assays took place in containers with soil on the bottom. These control ants were collected at least 5m from any baited tree, but no more than 25m from where experimental ants were collected. For Experiment 2, we ran 50 factorial replicates of behavioral trials (300 trials total). For experiment 3, we ran 15 factorial replicates (90 trials total). We recorded 104 behavioral interactions with F. neogagates in this experiment.

Statistical analysis

Analyses were run in R 3.2.4 (R Core development team 2016). To model the binary response of ants to competitors and prey (attack behaviors present or absent), analyses used binomial generalized linear mixed models (GLMMs) in the lme4 package in R (Bates et al. 2015). In experiment 1 host plant species was used as a random effect. In experiment 2 and 3, we used spatiotemporal blocks (time and site in which the study was completed) to account for site-level variation. Since baits in close proximity resample ants from a shared colony territory, we used individual groups of baits (on trees within
5m of each other) as a random effect in a repeated measure design to account for potentially resampling ants from the same colony on trees or the forest floor. Fixed effects in these models were ant species, interactor (ladybird beetle, waxworm caterpillar, intraspecific competitor), and the presence of treehoppers or honeydew proxy bait. To calculate statistical significance of fixed effects, we employed Wald $\chi^2$ tests in the car package (Fox and Weisberg 2011) using type II sums of squares (following Langsrud 2003). Parameter estimates reported from analyses (predicted marginal means of binomial outcome and standard error) were generated using the lsmeans package in R (Lenth 2016).

Results

Ant behavior at honeydew proxy baits

We did not observe evidence for induced behaviors for either ant species. In experiment 1, contrary to predictions of the prey acquisition hypothesis, ants collected in proximity to baits were not more likely to attack caterpillars compared to ants collected from control host plants (Binomial GLMM, $P = 0.64$, $N = 26$). Contrary to predictions of the intensified competition hypothesis, we observed that *F. neogagates* avoided *C. chromaoides* and did not attack competitors in all competition trials (Fig. S1, $N = 26$, no statistical test was run because all outcomes were the same). This result suggests that regardless of proximity to sap-feeders, *F. neogagates* is behaviorally subdominant to *C. chromaoides*. Natural history observations during this experiment corroborated this result: often *F. neogagates* workers that approached any baits monopolized by *C. chromaoides*, fled from a competitive encounter and drop from host plants.
In support of the protector trait hypothesis, we observed that *C. chromaoides* ants were more likely to attack prey or competitors than *F. neogagates* (Fig. 1, Binomial GLMM, \( P = 0.0047 \)). In 21% of trials, *C. chromaoides* attacked prey or competitors, compared to 4% of trials with *F. neogagates*. *C. chromaoides* were more likely to attack competing *C. chromaoides* compared to ladybird beetles and caterpillars (Fig. 2), indicating their aggressive stance is likely targeted towards intraspecific competition with other carpenter ant colonies. Not only was *F. neogagates* likelihood of attacking prey or competitors low, there was no significant variation in *F. neogagates* attack between competing ants, ladybird beetles, or caterpillars (Fig. 3). In experiment 2 we observed no evidence for any of the induced behavior hypotheses. Ants were not more likely to attack prey or competitors if they had recently been collected near baits (Fig. S2, Binomial GLMM, \( P = 0.9563 \)), and this result did not differ between either *C. chromaoides* or *F. neogagates*.

**Ant behavior at sap-feeding treehoppers**

Results from this experiment matched outcomes from experiment 2: *C. chromaoides* exhibited attack behaviors towards prey or competitors in 14% of trials, which was significantly higher compared to 4% of trials for *F. neogagates* (Fig. 3, Binomial GLMM, \( P = 0.0146 \)). Additionally, for both ant species, there was still no support for any of the induced behavior hypotheses. Ants taken in proximity to sap-feeders were not more likely to attack intraspecific competitors, ladybird beetles, or caterpillars compared to control ants (Fig. S3, \( P = 0.999 \)).
Discussion

This study demonstrates ecologically significant behavioral differences between two species of ants that engage in protection mutualisms with sap-feeders. Of the two ant species examined, *C. chromaoides* exhibited behavioral dominance over *F. neogagates*, and this ant species was more likely to engage in intraspecific competition, predation of caterpillars, and defense of mutualists via attacks on ladybird beetles. These behavioral traits were not induced by carbohydrates provided from mutualists: *C. chromaoides* ants exhibited an increased suite of aggressive behavior independent of the presence of sap-feeders or a honeydew proxy. This provides evidence for the protector trait hypothesis with *C. chromaoides*, but we did not find evidence for aggressive defense, intensified competition, or prey acquisition hypotheses for either ant species examined.

Similar support for the superior protector hypothesis is seen other work in which behaviorally aggressive invasive ants recruit to sap-feeders and defend mutualistic sap-feeders. Fire ants *Solenopsis invicta* quickly recruit to cotton plants in large numbers by cotton aphids, and these aggressive ants quickly suppressed any arthropod that would attempt to forage on these plants (Kaplan and Eubanks 2005). Argentine ants *Linipethema humile* also recruit to scale insects and aphids, excluding other native ant species from access to this mutualistic interaction (Wilder et al. 2013), and attacking other arthropods (Grover et al. 2007). In both cases aggressive behavioral traits are not necessarily induced by sap-feeders, but are instead constitutive behavioral traits found in a competitively dominant invasive species. Consequently, in these systems changes to food web dynamics on hosts plants occupied by ants and sap-feeders occur through recruiting ants with these behavioral traits, rather than requiring induction of predatory or
competitive traits due to honeydew. Our result suggests that this mechanism may operate in non-invaded ant communities, and the arthropod food webs those ants engage in as well. In our study we observed a less dramatic scenario than those illustrated with invasive ants, since *C. chromaoides* is not an overwhelmingly competitively dominant species in these ant communities. However, this ant species still consistently elicited comparatively higher attack rates of competitors and prey.

In other research on multi-species protection mutualisms, often the most abundant protector species exhibits behavioral traits responsible for mutualistic services (Palmer et al. 2010), and these species determine the indirect effects of mutualisms on herbivores and/or plants. In our case, an abundant mutualistic ant species (*F. neogagates*), did not exhibit behavioral traits that would drive indirect effects *via* protection mutualisms, such as increased aggression towards prey. However, there are multiple ant species engaged in this mutualism, including one (*C. chromaoides*), and sap-feeders are effectively recruiting this behavioral aggressive species to host plants. In locations where this ant species is found, sap-feeders therefore can drive strong indirect effects *via* the traits of their protector species. This implies that sap-feeders sample from a diversity in protector species in multispecies mutualisms, and this could be an important component in determining the ecological significance of multiple species mutualisms in natural communities where ant community composition varies from location to location. Mutualisms with many may produce to a “sampling effect” (e.g. Tilman 1999) where it is more likely than an ant species is recruited that has aggressive behavioral traits. Further work examining ant-sap-feeder interactions in communities with many species of protector may help elucidate this mechanism.
A number of studies demonstrate ant behavioral changes in response to nutritional composition of mutualist-provided food resources (Ness et al. 2009, McGlynn and Para 2016) but we did not observe increased predatory behavior in either species. However, these cases systems utilized a single ant species engaged in mutualistic interactions (McGlynn and Para 2016), or behavior was modified by extralfloral nectaries provided by plants, not honeydew provided by sap-feeders. For example, *Crematogaster opuntiae* exhibited increased preference for protein-rich prey after consuming C-rich extralfloral nectary and increased consumption of protein baits and caterpillars (Ness et al. 2009). Other research provides mixed support for hypotheses similar to aggressive defense, intensified competition or prey acquisition. Furthermore, some work has shown that food resources can also decrease ant predatory behaviors: *Solenopsis invicta* given carbohydrate foods consumed less lipid and protein-rich prey compared to ants without access to carbohydrates (Wilder and Eubanks 2010), and it is suggested that ants are partially satiated by food rewards and therefore less likely to search out food resources (Stenberg et al. 2011).

Understanding the context-dependence of species interactions and demonstrating the impacts of mutualisms on food web dynamics is an important goal in population and community ecology (Agrawal et al. 2007). A growing number of studies have addressed this gap *via* experiments with multispecies protection mutualisms between ants and sap-feeders (reviewed in Ness et al. 2010), examining variation in ant protectors (Mooney and Mundal 2010) and sap-feeders (Yoo et al. 2013). This interaction provides opportunities to examine theoretical predictions regarding context-dependency of species interactions since there is considerable diversity in ant participants for sap-feeder
mutualisms, with 41% of ant genera contain species which tend sap-feeders (Oliver et al. 2008). Moreover, ant sap-feeder mutualisms are widespread in terrestrial ecosystems, making the case that this is an ecologically important interaction in many communities (Rosumek et al. 2010, Zhang 2012), but the majority of studies lack insight into behavioral mechanisms (reviewed in Styrsky and Eubanks 2007). Relevant to this, our study demonstrated that ant species traits are effective predictors of variation in the consequences of this mutualism, while induced behavioral changes appeared to be less important compared to variation between ant species. We propose that our work, in addition to other studies in system like these, makes the argument for a trait-based framework for ant-sap-feeder interactions that considers interspecific and intraspecific variation in ant behavior relevant to predation and competition.
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References


Figures

Fig. 1. Proportion of encounters where ants exhibited aggressive behaviors towards for both ant species in experiment 2. *C. chromaooides* was significantly more likely to present attack behaviors compared to *F. neogagates* (Binomial GLMM for Ant Species, $P = 0.0047$). Bar height represents mean proportion across all three interactors (ladybird beetle, competing ants, caterpillar prey) and error bar represents SEM.
Fig. 2. Proportion of encounters where *C. chromaoides* exhibited aggressive behaviors towards when collected from baits. *C. chromaoides* aggression was highest towards intraspecific competitors (Binomial GLMM for Interactor $P = 0.0004$, Tukey HSD for *C. chromaoides*). Bar height represents mean proportion of attacks and error bar represents SEM.
Fig. 3. Proportion of encounters where *F. neogagates* exhibited aggressive behaviors towards when collected from baits. *F. neogagates* did not exhibit any significant variation in aggressiveness between intraspecific competitors, ladybird beetles or caterpillar prey (Binomial GLMM $P = 0.1399$). Bar height represents mean proportion of attacks and error bar represents SEM.
Fig. 4. Proportion of encounters where ants exhibited aggressive behaviors towards for both ant species in experiment 3. For ants collected near sap-feeders, *C. chromaoides* was significantly more likely than *F. neogagates* to attack prey or competitors (Binomial GLMM for Ant Species, $P = 0.0146$). Bar height represents mean proportion across all three interactors (ladybird beetle, competing ants, caterpillar prey) and error bar represents SEM.
Fig. S1: *F. neogagates* response to *C. chromaoides* competitors after feeding at baits. In behavioral trials, *F. neogagates* did not exhibit aggressive behaviors towards *C. chromaoides* in all encounters, regardless of the presence of carbohydrates. Bar height indicates count of interactions between *F. neogagates* and *C. chromaoides*.
Fig. S2. *C. chromaoides* workers feeding at honeydew proxy bait.

Fig. S3. *C. chromaoides* (left) and *F. neogagates* actively tending *C. mera* nymphs.
Fig S3: Examples of attack behaviors presented by *C. chromaoides* towards waxworms (left) and intraspecific competitors (right).
Evidence for the cascading effect of a keystone mutualism on hickory tree growth

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Abstract
Trophic cascades can increase plant growth when herbivores are suppressed by predators, but the strength of these effects are often context specific. In many ecosystems, ant predatory effects on herbivores have cascading effects on plant growth, the effects of ants on plants are often contingent on the presence of sap-feeder mutualists. While many studies have investigated ant-instigated trophic cascades and the importance of ant-sap-feeder mutualisms for plant growth, relatively few experimentally examine the putative benefits for woody plant growth over long time periods. In this study we excluded ants for three growing seasons and measured woody tissue growth on hickory saplings in a temperate forest. We monitored herbivore communities and herbivory (caterpillars, sap-feeding Hemiptera, and cerambycid beetles). This experiment tested whether ants have cascading indirect effects on host plants with and without sap-feeder presence. Positive indirect effects on host plants that depend on the presence of sap-feeders would constitute evidence for the cascading keystone mutualism hypothesis. Alternatively, positive effects of ants on plant growth without dependence on sap-feeders would support the ant-instigated trophic cascade hypothesis. In support of the cascading keystone mutualism hypothesis, the combination of ants and sap-feeders increased hickory sapling growth.
Without sap-feeders, ants did not have sufficiently strong top-down effects to change sapling growth. Interestingly, in the absence of ants, sap-feeders appeared to facilitate caterpillars, but overcompensating ant predation offset this effect. Ants also reduced the abundance of twig-girdles caused by cerambycid beetles. These two findings show that the mutualism between ants and sap-feeding herbivores benefits forest tree growth by suppressing multiple herbivore guilds of chewing herbivores. If these findings from arthropod communities on hickory apply to other forest tree species harboring ant-sap-feeder mutualisms (e.g., oaks), this mutualism could be a major driver of forest productivity.

**Key Words**
Trophic cascades, forest productivity, mutualisms, ants, caterpillars, ant-sap-feeder mutualisms, keystone interactions

**Introduction**

The mutualism between ants and sap-feeding herbivores can have such far-reaching impacts on food webs that they have been termed “keystone interactions” (Styrsky and Eubanks 2007). One such impact is the cascading indirect effect of ant-sap feeder mutualisms on host plants (Romero and Koricheva 2011). These trophic cascades occur when ants actively engaged in mutualisms suppress other herbivore guilds, indirectly benefiting the plant by reducing total herbivory (Punttila et al. 2004, Lach et al. 2010). However, in order for there to be a positive, indirect effect of ants engaged in mutualism with sap-feeders, ant predatory effects have to more than offset the cost of
Sap-feeding herbivory for host plants (Styrky and Eubanks 2007). Sap-feeding herbivores consume phloem sap and produce honeydew, which ants consume in return for protecting sap-feeders from natural enemies (Stadler and Dixon 2007). Consequently, ants facilitate direct negative effects of sap-feeders on their host plants, and in order for a net benefit to be received by plants, ants have to remove herbivores that cause more damage than do the sap-feeding mutualists (Kaplan and Eubanks 2010).

Studies in which ant-sap-feeder mutualisms benefit plant growth or reproduction are restricted to herbaceous plants or plants in agroecosystems (e.g. Messina 1981, Moreira and Del-Claro 2005, Styrsky and Eubanks 2010). Therefore, it is not clear to what degree ant-sap-feeder mutualisms may indirectly benefit or harm the growth of long-lived woody plants in natural communities. Studies with trees typically measure changes in total herbivory for non-sap-feeding herbivory (e.g. Karhu 1998), or loss of seeds to seed predators (Ito and Higashi 1991). Ant protection from natural enemies can facilitate population outbreaks of sap-feeding herbivores, dramatically increasing herbivory on host plants; this increase in herbivory by sap-feeders in outbreaks is unlikely to be offset by ant predation of other herbivores (Renault et al. 2005, Brightwell and Silverman 2010). For these reasons, a manipulative experiment that addresses the combined effects of ants, sap-feeders and the remainder of the tree-feeding herbivore community is necessary. Here we use such an experiment to test for a net positive effect of ant-sap-feeder mutualisms on long-lived woody plants in a natural forest community.

The proposed work focuses on ant-sap-feeder interactions on deciduous trees in temperate forests, utilizing the entire herbivore community that occurs on adult trees from spring to summer. Ant-sap-feeder mutualisms are common on hickories and oaks in
Northeastern Coastal forests of the U.S.A. (Wallace 2008, Clark et al. 2016). Past experiments examining ant-sap-feeder interactions on forest trees in this community show that ants reduce herbivory from leaf-chewing caterpillars (Clark et al. 2016), and that overall leaf-chewing herbivory is lowest when ants and sap-feeders are both present on host plants (Clark and Singer in prep. [Chapter 2]). Ant predation may cascade down to host plants since ants actively suppress the dominant herbivore guild in this community. Furthermore, that ant suppression of herbivores was intensified when sap-feeders were present suggests that keystone mutualisms may ultimately benefit host plants. Notably, the sap-feeders in this system occur in low densities and do not experience population outbreaks on individual trees (Clark et al. 2016). Given these observations, it is likely that ant predation of caterpillars may drive trophic cascades and host trees would experience higher growth when ants and sap-feeders are present. In this system, sap-feeders facilitated increased leaf-herbivory by caterpillars, which was consistent with induced susceptibility (Clark et al. 2016). Additionally, in some cases we observed that sap-feeders were correlated with increased abundance of caterpillars on host plants where ants were excluded (Clark and Singer, in review [Chapter 2]). In induced susceptibility, sap-feeding herbivores reduce a plant's chemical and physical defenses against chewing herbivores (Ali and Agrawal 2014). Sap-feeding triggers defensive responses in plants mediated by salicylic acid hormone pathway (Stout et al. 1998). This pathway reduces a plant's expression of the complementary jasmonic acid pathway and the resulting defensive products, which deter and defend against chewing herbivores (Thaler et al. 2012). Since we observed some evidence for induced
susceptibility as a mechanism, we therefore included this consideration in our examination of keystone mutualisms.

We examined three hypotheses. First, in the cascading keystone mutualism hypothesis, ants will benefit forest tree growth in the presence of sap-feeders because this mutualism increases the abundance of predatory *Camponotus* ants on trees (Clark et al. 2016), strengthening ant predation on other herbivores. Consequently, we predict that ants will only increase tree growth in the presence of sap-feeders, and ants will not benefit trees when sap-feeders are absent. A corollary to this hypothesis is that ant predation will have overcompensating benefits to plants if sap-feeding facilitates herbivory by other guilds through induced susceptibility (Ali and Agrawal 2014).

Second, in the herbivore facilitation hypothesis, ant-sap-feeder mutualisms decrease plant growth because ants are protecting sap-feeders from natural enemies. Additionally, due to induced susceptibility, the positive indirect effect on chewing herbivores, induced by sap-feeders, is not compensated by predatory ant effects. Finally, the ant-instigated trophic-cascade hypothesis predicts that ants will have a positive, indirect effect on forest tree growth even if sap-feeders are absent.

**Methods**

**Study System**

Hickory (*Carya*) trees in northeastern U.S. deciduous forests host a diverse herbivore community including caterpillars and sap-feeding Hemiptera (Clark et al. 2016). Ants are numerically dominant omnivores in deciduous forests (Moya-Larano et al. 2007) and *Carya* harbor several species of predatory ants (e.g., *Camponotus* and
*Formica* spp., Clark et al. 2016), which prey on caterpillars and consume honeydew from sap-feeding Hemiptera (Weseloh et al. 1995). Two observable sources of damage to *Carya* trees are leaf-chewing caterpillars, which remove about 3.09 cm² leaf area per branch in late spring and early summer (Clark and Singer, in prep [Chapter 2]), and twig-girdling *Oncideres cingulata* beetles (Rice 1995), which damage and kill entire branches. These two sources of damage are important because defoliating herbivory reduces photosynthesis, negatively impacting overall shoot growth on trees (Piper et al. 2015) and twig-girdling damages shoots and removes terminal ends of shoots from saplings and mature trees (personal observations).

**Experimental Design**

To test our hypotheses, we employed an ant-exclusion experiment on hickory saplings for a three-year duration. Experiments took place in upland deciduous forests where mature *Carya* spp. (hickories in the subgenus Eucarya, including *C. glabra*, *C. ovata*, and *C. tomentosa*) trees also occupy the upper canopy level of the forest (Foster et al. 1998). The goal of this experiment was to measure the strength of ant trophic cascades on hickory shoot growth, record the contribution of sap-feeders to this indirect effect, and observe if ant predatory effects sufficiently offset the cost of sap-feeding for long-lived woody plants. In spring 2014, we selected 96 hickory trees in 6 forest locations in Middlesex and New Haven Counties in Connecticut, USA. We utilized 12 trees in Millers Pond State Park in Durham, 12 in Higby Mountain Park in Middlefield, 12 in Giuffrida Park in Meriden, 12 trees in Hurd State Park in East Hampton, 12 trees along the Mattabesset Trail in Middletown, and 36 trees in the Cockaponset State Forest in
Haddam. We manipulated ants on pairs of trees, choosing two saplings found in close proximity (2-20 m distance between pairs). All saplings were 2-4 m in height. We excluded ants (-ants) from one tree in each pair via a sticky barrier on a plastic collar: Tanglefoot (Contech enterprises, Vancouver, BC) was applied to a plastic sheet (10 x 20 cm) circumscribing the trunk (50 cm above the ground) and fastened tightly with plastic Zip-ties. For all experimental trees, we selected saplings that did not have any branches touching other trees so ants could only access saplings via the sapling trunk. In late winter of 2015 and 2016, Tanglefoot was reapplied to this barrier to maintain ant exclusion. Control trees (+ants) had sheet plastic affixed to the trunk with Zip-ties, and no Tanglefoot, to account for potential physical effects of this plastic collar on sapling growth.

Sap-feeder presence/absence on host trees was determined through visual scanning of shoots where treehoppers and scale insects feed in spring and summer (species reported in Clark and Singer, in review [Chapter 2]). These sap-feeding Hemipterans feed in small aggregations at the base of new shoots (hereafter “twigs”) and were therefore readily apparent when taking twig growth measurements (personal observations). Caterpillar abundance was determined through visual scanning of foliage, and recording total caterpillar counts on all trees within a 2-week period in May 2015 and 2016. Total counts of twig-girdles were made in August 2016 at the completion of the experiment.

Yearly growth of trees was determined by measuring the distances between sequential terminal bud-scale scars on twigs (Fig S1). Bud scale-scars indicate the location of previous years’ terminal buds, providing a visual record of seasonal primary
woody tissue growth on a twig (Raven et al. 1999). We used the distance between bud-scale scars as a measure of the amount of lateral twig growth in each growing season. To choose which twigs to measure, we used a random sub-sampling approach modified from Vickers et al. (2014), yielding 216 measurements from each experimental tree. To arrive at this number of measurements, we randomly selected six branches arising from the trunk of each tree and randomly selected six twigs arising from each branch (6 branches x 6 years x 6 twigs). On each twig, we recorded the bud-scale scar distance to estimate growth in the years 2016, 2015, 2014, 2013, 2012 and 2011 (Fig. S1). Distance between bud-scale scars was determined by using microcalipers in mm (Fig. S1). Five saplings died over the course of the three-year experiment. In total, we took 20,736 measurements of twig growth via recording bud-scale scar distances. In analyses, we pooled and averaged all twig growth measurements for each tree (89 trees) in each year (6 years). We then subtracted the sum of the mean twig growth per tree after the treatment was applied (2014-2016) from the sum of the mean twig growth per tree before the ant-exclusion treatment (2011-2013) to determine the effect of the experimental manipulation on twig growth. For sake of simplicity, in figures and discussion, we refer to the average distance between bud-scale scars for each experimental tree as “twig growth.”

To quantify local habitat quality for *Carya* saplings, we assayed twig growth of branches from nearby *Carya* trees (2-20 meters from experimental trees) during the same growing seasons as those assayed for experimental trees (i.e. phytometrics, Wheeler et al. 1991). We selected branches from the four *Carya* trees (saplings or adult trees with trunks < 25-cm diameter) closest to each experimental tree. Bud-scale distance measurements were taken with microcalipers following the same methodology used to
record twig growth in experimental trees. In statistical analyses, twig growth in these reference trees was used as a covariate in models. This covariate estimates habitat quality by quantifying variation in growth-limiting resources and abiotic conditions (following Ung et al. 2001), and high-quality sites are expected to have trees with more twig growth.

**Statistical Analyses**

We employed a series of generalized linear mixed models (GLMMs) to assess ant treatment, sap-feeder, and covariate effects on caterpillar abundance, herbivore damage, and twig growth on experimental trees. Models were run using lme4 package (Bates et al. 2015) in R 3.3.1 (R Core development team 2016). Caterpillar abundance (count data) was treated as a Poisson-distributed response variable, twig-girdle damage (presence/absence) as binomial, sap-feeder presence/absence as binomial, and twig growth (in mm) as normally distributed (following Bolker et al. 2009). Fixed effects in the twig growth model were ant treatment, sap-feeder presence, and mean phytometric tree growth associated with each experimental tree. The models for caterpillar abundance, twig-girdle damage, and sap-feeder presence, included ant treatment as a fixed effect. For the caterpillar model, we used pre-treatment twig growth as a covariate. Statistical significance for treatment effects was determined using Type II SS (Langsgrund 2003) calculated by the car package in R (Fox et al. 2015). Predicted marginal means, standard errors, and regression coefficients were calculated using the lsmeans package in R (Lenth et al. 2015).
Results

Ants significantly reduced caterpillar abundance, but this effect depended on the interaction between the ant-exclusion treatment and sap-feeder presence (Fig. 1, Table S1). Consistent with other work in this system, sap-feeder presence was associated with an increased abundance of caterpillars when ants were excluded (Fig. 1), but not when ants were present (Fig. 1). This suggests that ant predatory effects compensate for facilitation of caterpillars by sap-feeders (e.g., Clark et al. 2016). Ant exclusion significantly reduced the frequency of sap-feeder presence over the course of the experiment, suggesting that mutualistic services provided by ants facilitate sap-feeding herbivores on host plants (Table S2, $P = 0.017$). Host-plant growth (i.e. pre-treatment twig growth) and phytometric tree growth did not predict the local presence of sap-feeders (Table S2), suggesting that sap-feeder occupancy was not determined by host-plant quality effects arising from past growth history or local habitat quality. This result indicates that the dispersion of host-specific sap-feeders was more contingent on mutualistic services provided by ants than on host-plant stress (e.g., Huberty and Denno 2004) or host-plant vigor (Price 1991). Finally, girdled twigs were more abundant on trees with ants excluded (Table S3), demonstrating that ants can reduce herbivory from Oncideres beetles.

Twig growth of hickory saplings depended on the interaction between ant exclusion and the presence of sap-feeding insects (Fig. 3, Table S4). The change in average twig growth (pre- vs post-treatment) did not depend on ant exclusion in the absence of sap-feeders (Fig. 3, Table S4). When sap-feeders were present, however, the change in twig growth was negative when ants were excluded and positive when ants had
access to saplings (Fig. 3, Table S4). Cascading effects of the ant-sap-feeder mutualism were measureable on host-plant growth, as host trees with ants and sap-feeders experienced an average of 1.1 cm increased yearly growth per twig compared to trees with ants present but no sap-feeders ($P = 0.0044$, Fig. 3)

**Discussion**

In support of the cascading keystone mutualism hypothesis, ant predatory effects sufficiently offset the cost of sap-feeding herbivory and increased tree growth over the course of multiple growing seasons. The positive net effect of the ant-sap-feeder mutualism on tree growth presumably resulted from ant predatory effects on caterpillars; caterpillars are abundant on forest trees (Clark et al. 2016) and ants are active foraging predators that can exhibit strong suppression of relatively sedentary herbivores (Rosenheim et al. 2004). However, in the absence of sap-feeders, ants had no detectable effect on twig growth, contrary to the predictions of the ant-instigated trophic cascade hypothesis. Consequently, the indirect benefit ants provided to *Carya* growth was entirely contingent on the presence of sap-feeders. Although sap-feeders appeared to facilitate caterpillar herbivory, consistent with previous observations in this system (Clark et al. 2016, Clark and Singer, in prep. [Chapter 2]), the prediction of the herbivore facilitation hypothesis was contradicted by our finding that ant-sap-feeder mutualisms had a net positive effect on plant growth. Ant predation therefore overcompensated for sap-feeding herbivory and sap-feeder facilitation of other herbivores.

This study differs from other work by providing evidence that ant-sap-feeder mutualisms can benefit forest trees. Past studies have typically measured herbivory or
changes in growth over a single growing season (e.g. Sipura 2002, Perfecto and Vandermeer 2006, Zhang et al. 2015), whereas our observations demonstrate that ant-sap-feeder mutualisms may impact forest productivity over the course of multiple growing seasons. This experiment corroborates results in which ant-sap-feeder mutualisms result in net increases in plant growth (Messina 1981, Styrsky and Eubanks 2010), however, there are several studies on forest trees in which ant-sap-feeder mutualisms had net negative effects. For example, ant-tended coccids (scale insects) on Acer rubrum (red maple) had outbreaks that reduced plant growth and seed production (Brightwell and Silverman 2010). Comparatively, ant-sap-feeder mutualisms on Picea abies (Norway spruce) did not have a net positive effect since this species has few defoliating herbivores to offset the cost of sap-feeding (Kilpelainen et al. 2009). In both examples, ants engaged in mutualism likely imparted no net benefit to tree growth because sap-feeders were highly abundant relative to other herbivore guilds. This herbivore community structure contrasts with that on Carya, on which sap-feeders, such as coccids and membracids, are far less abundant than are chewing herbivores, such as caterpillars (Clark and Singer, in prep. [Chapter 2]).

Mutualistic ant-plants have increased growth when defended by ant bodyguards (Rico-Gray and Oliveira 2007). While the impacts of ant-plant mutualisms for plant productivity have been demonstrated in some systems (e.g., Letourneau 1998), the implications for ant-sap-feeder mutualisms on plant productivity are less clear (Styrsky and Eubanks 2007). Based on our evidence, we argue that forest tree productivity may benefit from ant-sap-feeder mutualisms in a similar manner as observed in ant-plant mutualisms. Notably, for ant-plants, like host plants with ant-sap-feeder mutualisms, the
benefit to plant growth is contingent on the costs of herbivory (Frederickson et al. 2012). Consequently, ant-sap-feeder mutualisms have the potential to increase productivity in temperate forests just as ant-plant protection mutualisms do. In this system hickory trees are dominant in upland forests in this ecoregion (Olson et al. 2001) and sap-feeders occur on half the branches of sampled trees (Clark et al. 2016). In temperate forests, outbreaking or invasive insects have large impacts on forest productivity (Coulson and Stephen 2006). By contrast, ecosystem ecologists generally argue that favorable abiotic conditions, not the presence of non-outbreaking herbivores, dominate patterns of primary productivity in terrestrial ecosystems (Polis 1999). From this perspective, the strong impact of ant-sap-feeder mutualisms on tree growth is unexpected, especially because outbreaking or invasive insects were not significant players in this system. Extrapolating from our results to the other dominant tree species (e.g., Quercus spp.) in this community, the occurrence of ant-sap-feeder mutualisms and abundance of chewing herbivores on these tree species suggests that ant-sap-feeder mutualisms could potentially determine primary productivity at the landscape level. In conclusion, this study strongly supports the cascading keystone mutualism hypothesis for Carya trees in Northeastern Coastal Forest, and further suggests that ant-sap-feeder mutualisms in temperate forests may be an underappreciated driver of plant primary production.
Fig 1. Mean caterpillar abundance (May 2015-2016) on experimental *Carya* saplings after ant-exclusion and control treatments were applied in May 2014. Bar height represents predicted marginal means for caterpillar abundance and error bars represent predicted standard error of the mean from GLMM.
Fig. 2 Effect of the ant-exclusion treatment on sap-feeder frequency on experimental Carya trees (2014-2016). Dots indicate the predicted marginal mean frequency of saplings occupied by sap-feeders and error bars indicate the predicted marginal standard error of the mean for treatment (- Ants) and control (+ Ants) trees.
Fig 3. Change in twig growth after ant exclusion showing interaction between ant treatment and sap-feeder presence. Values above zero indicate twig elongation increased in 2014-2016 (post-treatment years) compared to 2011-2013 (pre-treatment years). Lower values indicate reduced twig elongation in post-treatment years. Bar height indicates predicted means and error bars indicate predicted standard errors of the mean. $P$-values in figures are from planned contrasts evaluating the effect of ant exclusion in the absence or presence of sap-feeders.
Fig 4. Mean number of twig girdles per experimental Carya sapling. Dots indicate the predicted marginal mean abundance of twig girdles and error bars indicate the predicted marginal standard error of the mean for treatment (- Ants) and control (+ Ants) trees.
Acknowledgments

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Table S1: Treatment and covariate effects on caterpillar abundance (Poisson GLMM)

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<th>$\chi^2$</th>
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<tr>
<td>Ant treatment (A)</td>
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<td>0.677</td>
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<tr>
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Table S2. Treatment and covariate effects on sap-feeder presence (Binomial GLMM)

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Table S3. Treatment and covariate effects on frequency of twig girdles from *Oncideres cingulata* (Negative Binomial GLMM)

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Table S4: Treatment and covariate effects on average change in twig growth after treatments were applied (GLMM).

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<td>A x S</td>
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Figure S1: Schematic diagram of twig growth measurements.
Figure S2: Plot representing correlation between measured change in twig growth (mm) and reference tree twig growth used as indicators of habitat quality (phytometric tree growth). Each dot indicates a single experimental tree (96) matched to the twig growth of four reference trees in close spatial proximity in the field. Fitted line indicates estimated slope and the gray area indicates the estimated standard error from GLMM.
References


Conclusions

In Chapter 1, I used a large-scale ant-exclusion experiment to quantify the role of ant-sap-feeder mutualisms in driving variation in top-down effects of ants in a tri-trophic food web encompassing eight tree species and the caterpillars feeding on them. This chapter provides the first evidence that ant-sap-feeder mutualisms are conditionally important in determining community-wide variation in the strength of top-down effects.

In Chapter 2, experimental manipulation of ant communities in the field revealed how ant-sap-feeder mutualisms recruit larger-bodied carpenter ants, increasing predatory effects of the ant community on the caterpillar community. Research in other systems primarily invokes increased ant abundance as the primary mechanism driving stronger ant predation on host plants with sap-feeders. My study, however, shows that changes in ant community structure and the body-size traits of ants were responsible for keystone mutualism effects in this system. In Chapter 3, behavioral experiments with ants found that interspecific variation in ant predatory behaviors (rather than induced behavior changes within species) accounted for the ecological impact of ant-sap-feeder mutualisms. In Chapter 4, the experimental exclusion of ants from *Carya* trees showed positive effects of ant-sap-feeder mutualisms on shoot growth. This final result supports the original predictions of the keystone mutualism hypothesis, and provides the most definitive evidence to date that ant-sap-feeder mutualisms impact forest productivity in nature.