

Insect mutualisms buffer warming effects on multiple trophic levels

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Abstract. Insect mutualisms can have disproportionately large impacts on local arthropod and plant communities and their responses to climatic change. The objective of this study was to determine if the presence of insect mutualisms affects host plant and herbivore responses to warming. Using open-top warming chambers at Harvard Forest, Massachusetts, USA, we manipulated temperature and presence of ants and *Chaitophorus populicola* aphids on *Populus tremuloides* host plants and monitored ant attendance and persistence of *C. populicola*, predator abundance, plant stress, and abundance of *Myzus persicae*, a pest aphid that colonized plants during the experiment. We found that, regardless of warming, *C. populicola* persistence was higher when tended by ants, and some ant species increased aphid persistence more than others. Warming had negligible direct but strong indirect effects on plant stress. Plant stress decreased with warming only when both ants and *C. populicola* aphids were present and engaged in mutualism. Plant stress was increased by warming-induced reductions in predator abundance and increases in *M. persicae* aphid abundance. Altogether, these findings suggest that insect mutualisms could buffer the effects of warming on specialist herbivores and plants, but when mutualisms are not intact, the direct effects of warming on predators and generalist herbivores yield strong indirect effects of warming on plants.

Key words: ant–aphid mutualisms; climate change; pest; plant–insect interactions; predation; species interactions; warming.

INTRODUCTION

Recent work suggests that species interactions can have larger impacts than, and/or interactive effects with, climatic changes on ecological communities (Rouified et al. 2010, Jochum et al. 2012, Shurin et al. 2012). The responses of plants to climate change are shaped by their interactions with other plants (e.g., Suttle et al. 2007), herbivorous and mutualist insects (e.g., DeLucia et al. 2012), and even herbivore predators (e.g., Barton et al. 2009). However, the multi-trophic impacts of insect–insect mutualisms on plants and arthropod communities under warming remain unexplored.

Ant–aphid mutualisms are widespread and affect competitive, predator–prey, and plant–herbivore interactions (Savage and Peterson 2007, Styrsky and Eubanks 2007, Sanders and van Veen 2010). The presence of ant–aphid mutualisms can yield reductions in arthropod abundance and species richness (Wimp and Whitham 2001, Styrsky and Eubanks 2007). In some cases, host plants benefit from the presence of ant–hemipteran mutualisms, especially if they are under

heavy pressure from other herbivores (Styrsky and Eubanks 2007, Moreira et al. 2012).

Ant abundance and composition are being altered by warming (Pelini et al. 2011, Diamond et al. 2012), but research regarding the responses of their aphid partners and predation rates to warming has been ambiguous. Several studies found that aphid fecundity or density increases with temperature (Strathdee et al. 1995, Holopainen and Kainulainen 2004). Others, however, found decreased or unchanged density (Roy et al. 2004, Adler et al. 2007), reduced adult survival (Ma et al. 2004), or increased time and resources dedicated to avoiding heat stress (Ma and Ma 2012). Some studies found that predation rates increase with temperature (Jalali et al. 2009, Simonsen et al. 2009), whereas others have found that attack rates for some predators peak near ambient temperatures (Khan and Khan 2010) or only increase with temperature when the prey are highly mobile (Vucic-Pestic et al. 2011).

The objective of this study was to identify the direct, indirect, and potential interactive effects of warming and insect mutualisms on multiple trophic levels. In a fully crossed design, we manipulated the presence of ants and *Chaitophorus populicola*, an aphid that specializes on *Populus* plants and is tended by ants, and warming (0–5.5°C) using potted *P. tremuloides* in open-top warming chambers at Harvard Forest, Massachusetts, USA. We monitored ant attendance of *C. populicola*, predator

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abundance, *C. populicola* persistence, *Myzus persicae* (a pest aphid that colonized the host plants during the experiment) abundance, and *P. tremuloides* stress. In addition to expecting direct aphid, predator, and host plant responses to warming, we expected warming to have strong indirect effects on aphids and host plants mediated through predator responses to warming and presence of *C. populicola*-tending ants. Ultimately, we predicted that these indirect effects would yield significant interactions between warming and ant-*C. populicola* treatments on host plants. We modeled the direct and indirect effects of warming and ant presence on predators, both aphid species, and the host plant. Finally, to test if plant responses to warming are shaped differently by the presence of ant-aphid mutualisms, we determined if there were significant interactions between warming and ant-*C. populicola* presence combinations on host plant stress.

METHODS

Study organisms.—*Chaitophorus populicola* is a specialist aphid found throughout North America that feeds on poplar and cottonwood trees of the *Populus* genus. It typically feeds on terminal shoots and is tended by ants (Richards 1972). *Myzus persicae* is found worldwide and feeds on a wide range of host plants. It is a common agricultural pest due to its ability to transmit plant viruses (Capinera 2001). *Populus tremuloides*, commonly known as trembling aspen, is a fast-growing pioneer species that is broadly distributed throughout North America (Burns and Honkala 1990).

Experimental design.—We transplanted 36 *P. tremuloides* saplings (47–103 cm tall) from a ~100-m² area at Harvard Forest, Massachusetts, USA into 40 cm diameter pots in nearby open-top warming chambers. These ~22-m³ warming chambers use continuous forced air delivery to increase air temperature within them by 0–5.5°C (see Pelini et al. [2011] for a full description). Potted aspens were placed on the ground inside of the warming chambers, where they were exposed to ambient or 1.5°, 2.5°, 3.5°, 4.5°, or 5.5°C above ambient air conditions from June through August. Each chamber contained four potted aspens, each being one of four different ant-*C. populicola* combinations: A, aphids present, ants absent; B, ants present, aphids absent; C, ants and aphids present (intact mutualism); D, ants and aphids absent. We excluded ants from the treatments A and D by spreading a 6-cm band of Tanglefoot insect barrier (The Tanglefoot Company, Grand Rapids, Michigan, USA) directly onto *P. tremuloides* trunks. *Chaitophorus populicola* aphids were collected from our aspen collection site and added to (30 per aspen) the treatments A and C at the beginning of the experiment. During our semiweekly visual inspections of the plants, ants were observed tending *C. populicola* on all C treatment trees but were almost never present on B treatment trees. No ants were observed on A or D treatment (ant exclusion) trees. During the course of the

experiment the pest aphid *M. persicae* colonized leaves on many of our study aspens indiscriminately across treatments, but ants were rarely observed interacting with them. We watered the aspens throughout the study period as necessary.

Data collection.—During semiweekly visual surveys, we recorded the number of *C. populicola*, type and number of predators (e.g., harvestmen, beetles, and spiders), and species and number of ants tending *C. populicola*. Using these data, we calculated *C. populicola* population persistence time (i.e., the number of days an aphid colony persisted on a single plant before dropping off to avoid heat or predators [see Ma and Ma 2012]) and total predator abundance (cumulative for all predators over the course of the experiment). For *M. persicae*, we compared total leaf coverage of each plant at the end of the experiment using a 0–5 scale (0 being no *M. persicae* presence and 5 being near-complete leaf coverage). At the end of the experiment, we assessed plant performance using an Opti-Sciences OS-30P Chlorophyll Fluorometer (Opti-Sciences, Hudson, New Hampshire, USA) to assess plant stress by taking two dark adapted F_v/F_m (variable fluorescence divided by maximum fluorescence) values per leaf on three leaves per plant. F_v/F_m is a widely used metric of plant stress that represents the maximum potential efficiency of photosystem II under current stress levels. F_v/F_m values decrease with increasing plant stress.

Data analysis.—We used partial least squares path modeling analysis to evaluate the main effects of and interactions between warming, ant tending, and predator abundance on *C. populicola* persistence and *M. persicae* abundance as well as the impact of the aphid species' persistence and abundance on overall plant stress. We also used a linear mixed model to examine the importance of ant species (random effect), warming (fixed effect), predator abundance (fixed effect), and their interactions on *C. populicola* persistence. Finally, to determine if plant performance differed in the presence of ant-aphid mutualisms vs. only one or neither of the mutualistic partners and their interactions with warming, we used ANCOVA followed by post hoc linear models for each ant-aphid treatment to model F_v/F_m as a function of ant-aphid combination, warming (covariate), and the interaction between them. All analyses were implemented in R version 2.15.2 (R Development Core Team 2012).

RESULTS

Ants were observed attacking harvestmen, beetle, and spider predators of *C. populicola*, and ant presence was the dominant factor (see Table 1 for path coefficients) driving increases in *C. populicola* persistence (Fig. 1). The species of ant tending a particular *C. populicola* colony did not change over the course of the experiment. *Chaitophorus populicola* colonies tended by *Formica subsericea* ants, the most common attendants in this study, had lower persistence than those tended by the

TABLE 1. Standardized loadings coefficients for direct, indirect, and total relationships between model variables.

Relationships	Direct effects	Indirect effects	Total effects
Warming, predators	-0.55	-0.01	-0.56
Warming, ant-tended aphid	-0.06	-0.07	-0.13
Warming, pest aphid	0.55	0.07	0.62
Warming, host plant	-0.04	-0.42	-0.46
Ant presence, predators	0.16	0.00	0.16
Ant presence, ant-tended aphid	0.75	0.01	0.76
Ant presence, pest aphid	-0.16	0.03	-0.12
Ant Presence, host plant	0.00	0.15	0.15
Predators, ant-tended aphid	0.08	0.00	0.08
Predators, pest aphid	-0.14	0.01	-0.13
Predators, host plant	0.00	0.09	0.09
Ant-tended aphid, pest aphid	0.07	0.00	0.07
Ant-tended aphid, host plant	0.10	-0.05	0.05
Pest aphid, host plant	-0.65	0.00	-0.65

Note: The ant-tended aphid is *Chaitophorus populicola*, the pest aphid is *Myzus persicae*, and the host plant is *Populus tremuloides*.

other two ant species, *Camponotus pennsylvanicus* and *Lasius umbratus* ($F_{2,6} = 16$, $P = 0.004$; Fig. 2), but warming, predator abundance, and their interactions with each other and species of ant tending had no significant effects on *C. populicola* persistence.

Ant presence decreased *M. persicae* abundance, indirectly increasing host plant efficiency (F_v/F_m , which decreases with increasing plant stress; see Table 1 for path coefficients). However, warming was the dominant factor driving reductions in predator and increases in *M. persicae* abundance. Consequently, warming indirectly reduced host plant F_v/F_m (Table 1, Fig. 1).

Variation in host plant F_v/F_m was associated with differences in ant-aphid combinations on plants ($F_{3,24} = 5.8$, $P = 0.004$), warming ($F_{1,24} = 3.3$, $P = 0.062$), and their interaction ($F_{3,24} = 2.4$, $P = 0.058$). More specifically, F_v/F_m increased with warming when ant-aphid mutualisms were intact, i.e., ants and *C. populicola* were present ($P < 0.001$) but decreased when ants were present but *C. populicola* were not ($P < 0.001$; Fig. 3). Plants that lacked ants and *C. populicola* showed more variability in their performance and had lower F_v/F_m

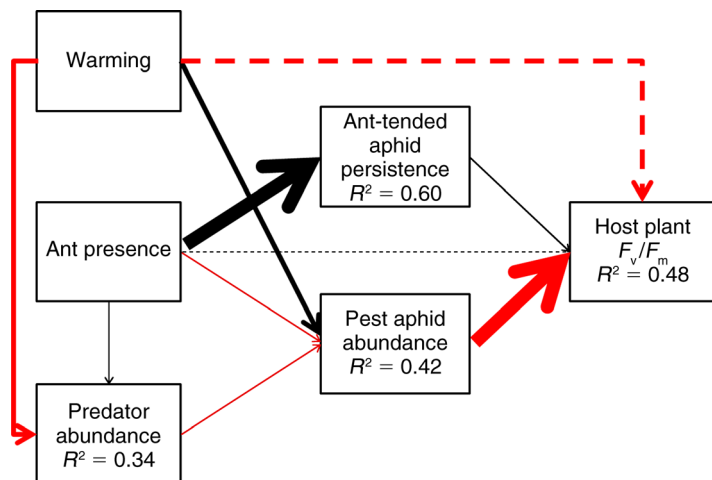
values than the other ant-*C. populicola* combinations (Fig. 3).

DISCUSSION

Warming had strong direct effects on predator abundance, which decreased with warming, and *Myzus persicae* pest aphid abundance, which increased with warming. However, species engaged in mutualistic associations (ant-tended *Chaitophorus populicola* aphids) were neither directly nor indirectly affected by warming. Warming had negligible direct but strong indirect effects on plant stress. Plant stress decreased with warming only when both ants and *C. populicola* aphids were present and engaged in mutualism. Plant stress was increased by warming-induced reductions in predator abundance and increases in *M. persicae* aphid abundance.

Overall, the presence of tending ants was the most important driver of performance in *C. populicola*. As found in other studies (e.g., Wimp and Whitham 2001, Stewart-Jones et al. 2007, Herbert and Horn 2008), ant-tended colonies survived longer, likely because ants protected the aphids from predators. In contrast to the

FIG. 1. Path analysis model. Each box indicates a measured predictor or response variable. Arrows indicate the hypothesized cause and effect relationship between two variables, and line width is scaled by the standardized loading coefficients for each connection for those with coefficients ≥ 0.10 . Red lines indicate negative relationships, and black lines indicate positive relationships. Dashed lines indicate relationships for which indirect effects were stronger than direct effects. Coefficients of determination (R^2) are reported in boxes with response variables. The ant-tended aphid is *Chaitophorus populicola*, the pest aphid is *Myzus persicae*, and the host plant is *Populus tremuloides*. F_v/F_m is variable fluorescence divided by maximum fluorescence.



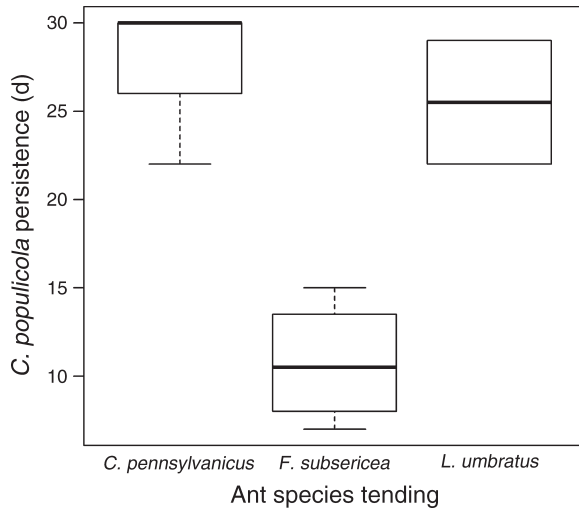


FIG. 2. *Chaitophorus populicola* persistence (mean \pm SE) as a function of species of ant-tending (*Camponotus pennsylvanicus*, *Formica subsericea*, and *Lasius umbratus*). Box plots show the median (center line in box), 25% quartiles (top and bottom of box), and the interquartile range (whiskers).

strong relationship between warming and abundance of predators and the pest aphid *M. persicae*, warming had a negligible impact on *C. populicola* persistence. *Chaitophorus populicola* colonies tended by *F. subsericea* did not persist as long as those tended by *C. pennsylvanicus* or *L. alienus*, implying that *F. subsericea* was less effective at protecting the aphids from predators than the other two species. These interspecific differences in the impacts of ant tenders on aphid are likely to be magnified under warming, particularly because *Camponotus* spp. in the region studied here are declining (S. L. Pelini, unpublished data).

For plants, the direct effects of warming were negligible; rather, plant responses to warming were mediated indirectly through *C. populicola*–ant mutualisms and *M. persicae* responses to warming and its consequent reduction in predation. These top-down cascading effects resulting from warming impacts on higher trophic groups have been demonstrated in a variety of aquatic and terrestrial systems (e.g., Barton et al. 2009, Jochum et al. 2012, Shurin et al. 2012). Altogether, these findings suggest that insect mutualisms could buffer the effects of warming on specialist herbivores and plants, but, when mutualisms are not intact, the direct effects of warming on predators and generalist herbivores yield strong indirect effects of warming on plants.

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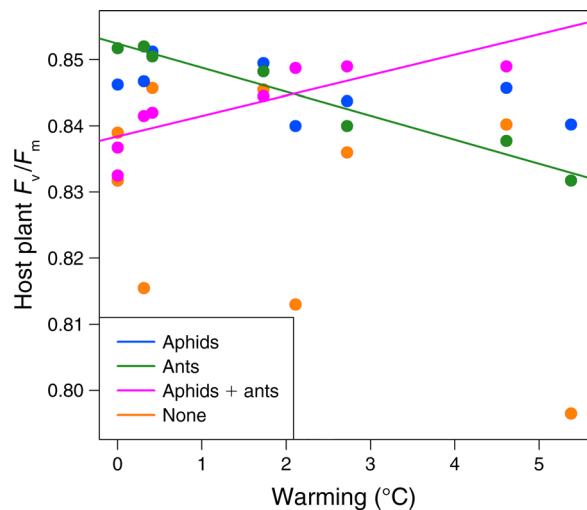


FIG. 3. Interaction plot of host plant (*Populus tremuloides*) performance (F_v/F_m) with different ant–aphid (*C. populicola*) combinations at different levels of warming. Lines are best-fit regression lines from the statistically significant relationships ($P \leq 0.05$).

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