SOIL ORGANISMS **94** (1) · April 2022 pp. 41–53

Experimental warming weakens positive plant diversity effects on pitfall trap sampled ant diversity

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Received 21 January 2022 | Accepted 28 March 2022
Published online at www.soil-organisms.de 1 April 2022 | Printed version 15 April 2022
DOI 10.25674/so94iss1id177

Abstract

Ants are important components of many terrestrial ecosystems because of their high abundance, their central position in food webs, and because they can strongly influence ecosystem properties such as soil aeration, nutrient cycling, and plant community composition. Moreover, ants are also known to respond strongly to changes in environmental and biological conditions. In particular, two major anthropogenic environmental impacts – climate change and the loss of primary producers – may have interactive effects on ant communities. To examine this potential interaction, we quantified pitfall trap sampled ant diversity and activity across a fully factorial experiment manipulating temperature and grassland plant species richness at the Cedar Creek Ecosystem Science Reserve in Minnesota, USA. Consistent with previous arthropod studies, we found a significant increase in sampled ant diversity in plots with higher sown plant species richness, such that plots with the largest number of plant species also had the highest sampled ant diversity. However, the strength of this relationship declined significantly in experimentally warmed subplots, especially when considered for higher aggregated spatial scales of samples. Taken together, these results suggest that the positive effects of plant diversity on sampled ant diversity may be partially undermined under warmer conditions.

Keywords Ant | climate change | community ecology | diversity loss | experimental warming

1. Introduction

Because ants are dominant ecological components of many terrestrial systems, understanding how environmental changes influence ant communities is important for predicting effects on ecosystems at large (Wilson 1987). Ants strongly influence arthropod community structure through predation and competition (Human & Gordon 1997), and are adept ecosystem engineers (Folgarait 1998). Consequently, they contribute to a wide range of ecosystem functions and services (Del Toro et al. 2012), and, for example, can significantly alter how nutrients cycle in ecosystems (Wagner et al. 1997), the structure and aeration of soils (de Bruyn 1999), and the kinds of plant species that can colonize a given region (Boyd 2001).

Environmental changes also influence ant communities. Like all organisms, ant fitness and performance vary as a function of temperature, and conditions that are too cold or too hot can lead to steep declines in activity and survival (Huey et al. 2012). Especially well-studied among ants are the concepts of critical thermal maxima

ISSN 1864-6417 (print) · **ISSN** 2509-9523 (online)

and minima, which, respectively, describe temperatures above or below which individuals become incapacitated and eventually die (Kay & Whitford 1978, Cerda et al. 1998). Although decreases in foraging and nest survival often occur well before critical thermal limits are reached, these metrics are popular because they can be measured consistently in laboratory settings, and can be reasonably good indicators of overall temperature tolerance (Kay & Whitford 1978). Importantly, critical thermal limits vary greatly among ant species – for example, even within a single region, critical thermal maxima can differ by almost 10°C (Oberg et al. 2011). These interspecific differences in temperature tolerance are thought to be primary determinants of ant community composition (Diamond et al. 2011, Bujan et al. 2020), leading to partitioning of geographic regions and microenvironments based on thermal constraints (Korzukhin et al. 2001). Indeed, a long-term study of 85 ant species in Central Europe found that along with soil moisture, thermal maximum was the most influential factor in determining niche segregation among species (Seifert 2017).

There is currently mixed evidence regarding how ant communities are likely to respond to temperature increases caused by climate change. It has been suggested that ant diversity may increase as a result of warming in cooler sites, whereas in warmer sites, warming could lead to ant diversity declines (Del Toro et al. 2015). Along natural temperature gradients, ant diversity has generally been found to increase as a function of mean annual temperature (Del Toro 2013, Diamond et al. 2016). One potential explanation for this trend would be that more ant species are limited by lower thermal tolerances than by upper tolerances, although the overall pattern is also confounded with elevation and latitudinal diversity gradients. For example, in a study of over 130 North American ant species, Bujan et al. (2020) found that the temperature ranges that species inhabited were significantly related to their critical thermal minima, but not their maxima. Nevertheless, there is also evidence that extreme warming, or temperature increases in already warm sites, can have significant detrimental effects on many ant species and on ant diversity. For example, when measured across large temperature gradients, a 'hump-shaped' relationship can emerge, with ant diversity initially increasing with temperature, but then declining at higher temperatures (Seifert 2017). Similarly, in a survey tracking changes in ant communities across North America over 20 years, Kaspari et al. (2019) found that small temperature increases ($< +1$°C) were associated with slightly higher overall nest abundance, but nest abundance declined steeply in regions with larger temperature increases. These results also accord with those from a warming experiment replicated across two forested sites in the eastern United States, where heating treatments had no detectable effect on ant community diversity in the cooler northern site, whereas it led to a small but significant decline in ant diversity at the warmer southern site (Pelini et al. 2014). Furthermore, in both sites, warming led to decreases in overall stability of ant community (Diamond et al. 2016).

To better understand these context dependent effects of warming on ant communities, in this paper we test how ant diversity and activity respond to experimental warming along a gradient of increasing plant species richness. Our study is embedded within an existing long-term experiment testing the effects of warming on grassland plant communities (the Biodiversity and Climate experiment, or 'BAC', experiment number 249) at the Cedar Creek Long Term Ecological Research site near Bethel, MN, USA. In particular, BAC includes a fully factorial combination of experimental warming treatments and an experimental plant diversity gradient.

We focus on the interactive effects of experimental warming and plant diversity manipulations for two reasons. First, the impacts of climate change extend well beyond direct effects of warming, and include effects on the diversity, composition, and productivity of primary producers (Parmesan & Hanley 2015). Accounting for these indirect effects is important, since both plant diversity and plant biomass directly impact insect communities (Kaspari et al. 2000, Haddad et al. 2009, Scherber et al. 2010, Schuldt et al. 2019), in particular by enhancing the structural and functional diversity of resources, thereby supporting a higher abundance and diversity of insects (Eisenhauer et al. 2013, Schuldt et al. 2019). Proxies of primary productivity have also been shown to be positively related to ant diversity (Gibb et al. 2019), and previous studies at Cedar Creek have identified positive effects of plant diversity on aboveground plant biomass production (Tilman et al. 1997) (Fig. 1B), and positive effects of plant biomass on insect abundance and diversity (Haddad et al. 2001).

Second, plant biodiversity – including plant species richness and community structure – modulates abiotic and biotic conditions, including temperature (e.g. Thakur et al. 2017), and therefore has the potential to alter the effects of warming on ant communities. In particular, increased aboveground biomass, which is positively correlated with plant biodiversity in most grassland experiments (Cardinale 2012), shades the soil surface and increases evapotranspiration, which both lower the temperature (Whittington et al. 2013, Cowles et al. 2016) (Fig. 1A,C–D), and increase relative humidity under the canopy (Wright et al. 2014) (Fig. 1B). Previously published results from BAC also show that increases in sown plant diversity caused significant reductions in temperatures both above and belowground, and that heating treatments caused significantly less warming in plots sown with more plant species (S1A–B in Cowles et al. 2016; Fig. 1 and Figs S1–3 in this paper). While these results from experimental systems may not necessarily reflect the full range of conditions observed across natural environments, positive relationships between plant biodiversity and standing biomass – and thus presumably also buffering effects on temperature and humidity – have been observed across a very wide range of natural grasslands (Grace et al. 2016). Because ant communities are known to be sensitive to changes in soil temperature and moisture (Seifert 2017), it therefore seems possible that these interactive effects of climate change, and buffering effects of vegetation, could be influential on sampled ant diversity across many different natural systems.

Jointly, these aspects of ant and grassland plant ecology in temperate regions lead us to several hypotheses and predictions. First, in the absence of experimental heating, we expect to find a positive effect of sown plant species richness on pitfall trap sampled ant diversity, consistent with previous experimental results. Second, we propose two alternate hypotheses relating to direct effects of experimental heating on ants: if more ant species are constrained by their upper thermal limits at our site, then we expect to sample higher ant diversity in control subplots than in heated subplots; alternatively, if more ant species are constrained by lower thermal limits, then we expect to sample higher ant diversity in heated subplots. Finally, given that higher plant diversity and biomass reduce soil and surface temperatures in our system, we predict that effects of heat stress on ants will be reduced in plots sown with higher plant diversity, whereas any effects of cold stress will be magnified.

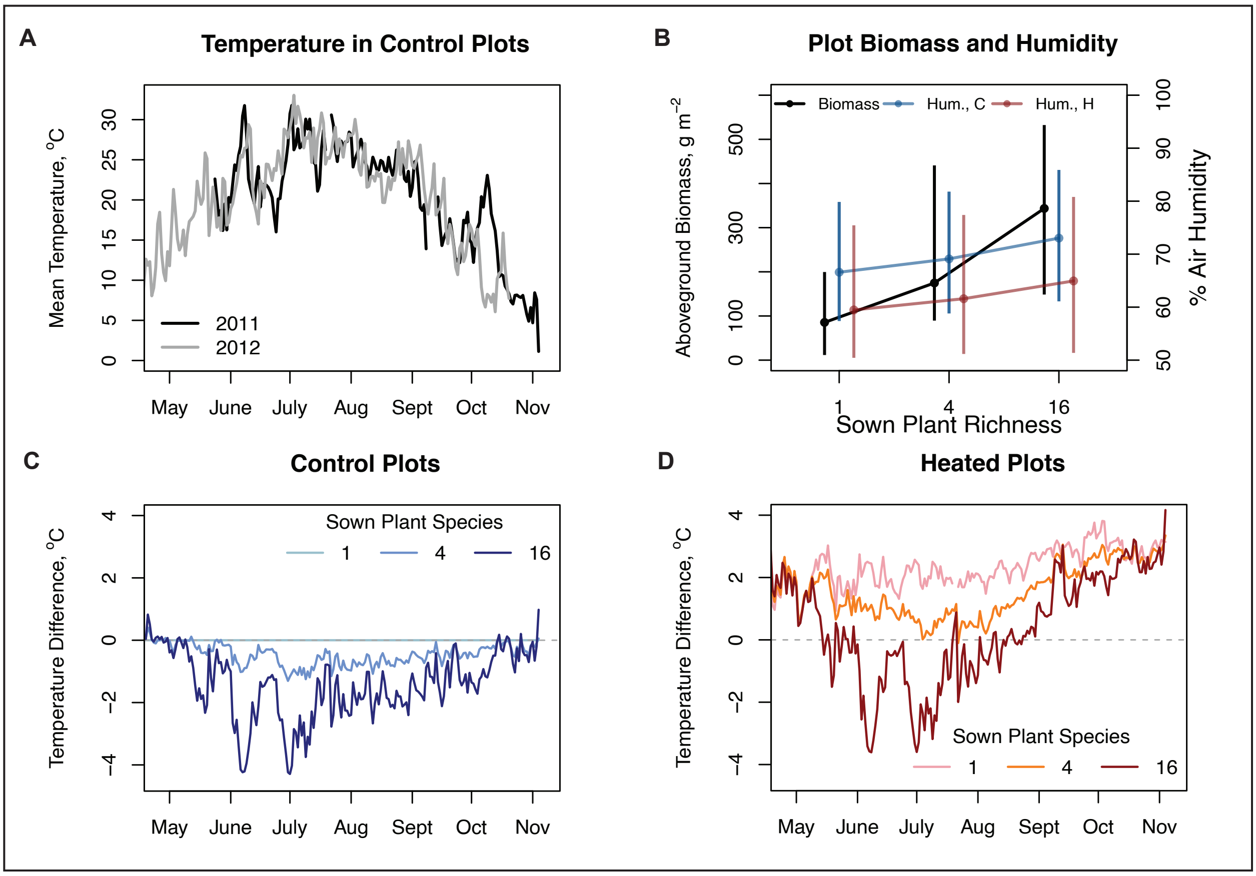

Figure 1. Environmental conditions in experimental subplots. (**A**) Mean daily temperatures measured 1 cm below the soil surface in control monoculture subplots for 2011 and 2012. (**B**) Mean subplot aboveground biomass for each sown plant species richness treatment averaged across control (**C**) and heated (H) treatments, as well as mean subplot-level relative humidity, measured 10 cm above the soil surface. Points and intervals show mean ± one standard deviation. (**C–D**) Difference between mean daily soil temperature measured in control monoculture subplots vs. mean daily soil temperature measured across each sown plant richness treatment in (**C**) control subplots or (**D**) heated subplots. Negative values indicate that conditions are cooler than the average temperature of control monocultures; positive values indicate conditions are warmer than in control monocultures. For daily average temperature trends and maximum/minimum daily temperature, see Figs S1–3 in the supplement.

2. Materials and methods

2.1 Experimental design

We used pitfall traps to sample ant communities in August and September over two years (2011 and 2012) within the BAC experiment at Cedar Creek. BAC was initiated in 2009, when a warming component was added to an existing plant biodiversity experiment (‘Big Bio’, experiment number 120). The 9 m × 9 m plots were seeded with 1, 4, or 16 perennial grassland species in 1994 and hand-weeded to maintain species composition (Tilman et al. 1997). A subset of 32 plots was chosen for experimental warming treatments, including 14 1-species, 9 4-species, and 9 16-species plots. Within each plot in BAC, 1200-watt electric infrared heating lamps were installed over 2.5 m × 3 m subplots, raising soil temperature by approximately 3°C at a dept of 1 cm below the soil surface (n.b. BAC includes both ‘high’ and ‘low’ heating treatments – only ‘high’ treatments were measured for this study). Heated vs. control subplots were assigned randomly within each plot, and the plots are distributed haphazardly across the full extent of the ca. 7 ha Big Bio experiment to avoid spatial confounding factors. Heaters are run continuously (i.e. 24 hours/day) every year from March to November.

Mean soil temperature was measured in each subplot using Thermochron iButton loggers – we report results for measurements taken at 1 cm below the soil surface, as these were the depths that were most consistently measured over time. The loggers measured hourly mean, maximum, and minimum temperatures, and were placed in the center of the plot, within approximately 20 cm of the pitfall trap locations where ants were sampled (see Ant sampling, below). Relative humidity was measured using Hygrochron iButton loggers, located 10 cm above the soil surface. These loggers were attached to the top of wooden stakes, and placed under a white plastic roof to shelter them from direct sunlight and rainfall. Average daily soil temperatures in the plots 1 cm below the soil surface varied from ca. 25–35°C in August, and ca. 10–25°C in September. However, maximum daily temperatures regularly exceeded 40°C, and often exceeded 50°C, especially in 2012, which was a particularly hot and dry year in central Minnesota (Fig. S2–3). In general, heating led to larger and more significant increases in nighttime temperatures than daytime temperatures (Fig. S1), and more significant increases in minimum temperatures than maximum temperatures (Fig. S2–3).

Previous studies in BAC have shown that plots sown with higher plant species richness have significantly lower temperatures. In particular, average summer temperatures 1 cm below the soil surface are roughly 2°C lower in plots sown with 16 species than in monocultures, and temperature increases caused by the heating treatments are about 50 % smaller in the more diverse plots (Fig. S1b in Cowles et al. 2016). More detailed information on the experimental design and plant community responses are available in Whittington et al. (2013) and Cowles et al. (2016). Temperature measurement data are available through Tilman (2018b), and plant aboveground biomass data are available through Tilman (2018c).

For reference, some details about the experimental site are summarized below: Cedar Creek is located in central Minnesota (45.40°N, 93.20°W; ca. 275 m above sea level), near the ecotone between the eastern deciduous forest and midwestern tallgrass prairie regions. Mean annual precipitation is around 77 cm, of which roughly 60 % falls between April and August. The site is characterized by sandy, well-draining soils, with relatively low nitrogen and organic content. Average summer high temperatures are around 27°C, with average winter daily low temperatures around –14°C; however, temperature extremes in the region are very large, and regularly exceed ±40°C (Clark et al. 2019). Plant communities in BAC are dominated by herbaceous grassland plant species, with average vegetation field heights ranging from ~0.5 m to 1.5 m depending on species composition (heights are typically greater in plots that include the tallgrass prairie species *Andropogon gerardii* and *Sorghastrum nutans*) (Sullivan et al. 2018). In accordance with historical fire regimes, plots are burned annually, which prevents the buildup of leaf litter over time.

2.2 Ant sampling

In August and September 2011 and 2012, we sampled ant diversity in all heated and control subplots in BAC. We utilized pairs of pitfall traps 5.1 cm in diameter, installed 10 cm apart in the center of each subplot. Each trap was held in a PVC ‘sleeve’ permanently installed in the ground, and filled with approximately 25 ml of a mixture of 300:2:1 tap water to dish soap to table salt by volume. We left traps open for 48-hours during each sampling event, collecting them on August 6 and September 11 in 2011, and August 19 and September 23 in 2012. None of the 48-hour periods included significant precipitation, and all sampling occurred before the first frost of the year. For all results discussed below, samples were pooled across years by subplot prior to analyses, resulting in a total of 128 sampling events (i.e. 32 plots, two subplots per plot, two measurements per year). We pooled data in this manner because preliminary analyses revealed that sample sizes were too small to detect within-subplot differences in ant communities. All specimens were identified to species using taxonomic keys (Coovert 2005, Fisher & Cover 2007, Ellison et al. 2012) and museum collections at Cedar Creek and the Harvard Museum of Comparative Zoology. All data are available through Tilman (2018a). Note that because destructive sampling was not permitted in BAC because it would interfere with other ongoing experiments, we were not able to quantify ant nest abundance, as this would have required digging up plots to sift through surface soil and litter. Nevertheless, since all plots in our study included spatially proximal warmed and non-warmed treatments, this contrast provides strong statistical evidence for changes in sampled ant diversity as a function of warming treatment (though see the Caveats section in the discussion for some notes on potential confounding effects of this spatial proximity, especially related to foraging behavior and movement of ants between subplots).

2.3 Statistical analyses

All data manipulation and analyses were conducted in R, version 4.0.2 (R. Development Core Team 2019). Full source code and data for all analyses are available in the supplement via www.soil-organisms.org, and online at github.com/adamtclark/BAC_ant_analysis. Following 'best practices' for analyzing ant diversity, we conduct our analyses on 'incidence' data (Pelini et al. 2014). Incidence – also called 'species density', e.g. sensu Gotelli & Colwell (2001) – summarizes the number of times that a species is present in a sampling event, and is generally considered preferable to metrics based on numbers of individual ants sampled, as individual counts can be confounded with nest size and location (Gotelli & Colwell 2001, Ellison et al. 2007, Gotelli et al. 2011). To account for differences in the relative incidence of species, and to prevent rare species from disproportionately impacting our results, we report ant diversity in terms of exponentiated Shannon diversity (however, results for richness were similar; see Fig. S5) (Jost 2007).

Although our experimental design is balanced with respect to heating treatments, the number of plots within each sown plant richness treatment varied. We therefore use rarefied estimates of ant diversity for all subsequent analyses. Rarefaction controls for differences in sample sizes to generate incidence data for our subsequent analyses (which would otherwise bias diversity metrics) by re-sampling data to estimate the expected diversity in each sampling group given constant sampling effort (Gotelli & Colwell 2001, Colwell et al. 2012). Rarefaction also shows how diversity varies across different scales of organization – i.e. for sample-level ('alpha') vs. treatment-level ('gamma') diversity (May et al. 2017, Leibold & Chase 2018). To rarefy our diversity estimates, we randomly sampled plots (without replacement) to generate samples ranging from a single plot, up to the maximum number of plots within each sown plant richness class (i.e. 14 monocultures vs. 9 4- and 16-species plots). We repeated the procedure 10,000 times (i.e. simple, uniform bootstrapping without replacement) to produce a distribution of ant diversity estimates for each level of sampling intensity.

For sampling intensity level, we then tested for effects of treatments on ant diversity using ordinary least squares linear regression. Each regression was fitted to rarefied exponentiated Shannon ant diversity, as a fully interactive function of sub-plot warming treatment (a binary variable, indicating heated or not heated), sampling month (a categorical variable, indicating either August or September), and plot-level sown plant species richness (a continuous variable), i.e. of the form 'ant diversity~heating*month*plant richness' in R's 'lm' notation. For easier interpretation of the resulting regression, we standardized sown plant richness by dividing raw values by the mean plant richness sown across all treatments, such that the 'y-intercept' of the regression corresponded to conditions at intermediate plant diversity levels (a hypothetical plot seeded with 7 plant species). Visual inspection of residual plots and normal quantile-quantile plots revealed no deviations from the standard assumptions required for OLS.

To test for differences in pitfall trap sampled ant diversity, we used multiple least squares regression, using the `lm` function in `R`. To control for pseudoreplication and to generate meaningful confidence intervals for analyses, we fit a separate regression for each of the 10,000 bootstrapped iterations from the rarefaction analyses. Because each aggregated observation was independent and balanced across plots and treatments, additional statistical controls, e.g. via a mixed effects model, were not necessary. We then used the resulting distribution of regression coefficients to quantify uncertainty in parameter estimates. In sum, this process resulted in a series of estimates of the effects of heating, sown plant richness, and sampling month on ant diversity, with separate estimates for each level of sampling intensity ranging from one plot up to a maximum of 9 plots (n.b. we did not conduct regressions for sampling intensities of >9 plots, as this would have required us to exclude data from the 4- and 16-species plots).

Finally, to test for differences in sampled ant activity across treatments, we fit linear mixed effects models using the `lmer` function in `R`, with a random intercept varying by plot, nested within year (Bates et al. 2015, R. Development Core Team 2019). Ant activity refers to the total number of individuals collected in pitfall traps, summed across all species. Note that unlike activity estimated at the level of individual species, which as discussed above can be biased for a number of reasons (Pelini et al. 2014), summed activity observed across all species can be a useful indicator of differences in overall abundance and foraging rate (Gibb et al. 2019). However, ant activity is not necessarily indicative of the frequency or size of ant nests, as activity can be influenced by many factors, including the proximity of nests to traps, and ant foraging behavior.

3. Results

We collected roughly 18,000 ants, including three subfamilies (Formicinae, Myrmicinae, and Ponerinae), nine genera, and 15 species or species groups (Table 1). Most species were in the genus *Formica*, whereas most individuals were either *Monomorium emarginatum* or *Solenopsis molesta*.

Raw rarefaction results showed substantial differences among treatments in terms of sample-standardized pitfall trap sampled ant diversity (Fig. 2). At the scale of a single plot (i.e. treatment-level ‘alpha’ ant diversity), mean Shannon diversity from pitfall trap samples ranged from five to seven species equivalents (i.e. exponentiated Shannon diversity, e^H) in August, and about four to five in September. At the scale of the largest total number of samples in each plant diversity-by-heating treatment class (i.e. treatment-level ‘gamma’ ant diversity), mean exponentiated Shannon diversity of ants collected in the pitfall traps ranged from roughly seven to nine species equivalents regardless of sampling month. Importantly, results from monoculture plots suggest that ant diversity saturates around a sampling intensity of nine plots, suggesting that sample sizes available for plots sown with higher plant richness were sufficiently large to characterize the resident ant community.

Regressions revealed no significant differences in overall mean sampled ant diversity (i.e. regression intercept), regardless of sampling intensity or heating treatment (Fig. 3A,B), suggesting that warming had no

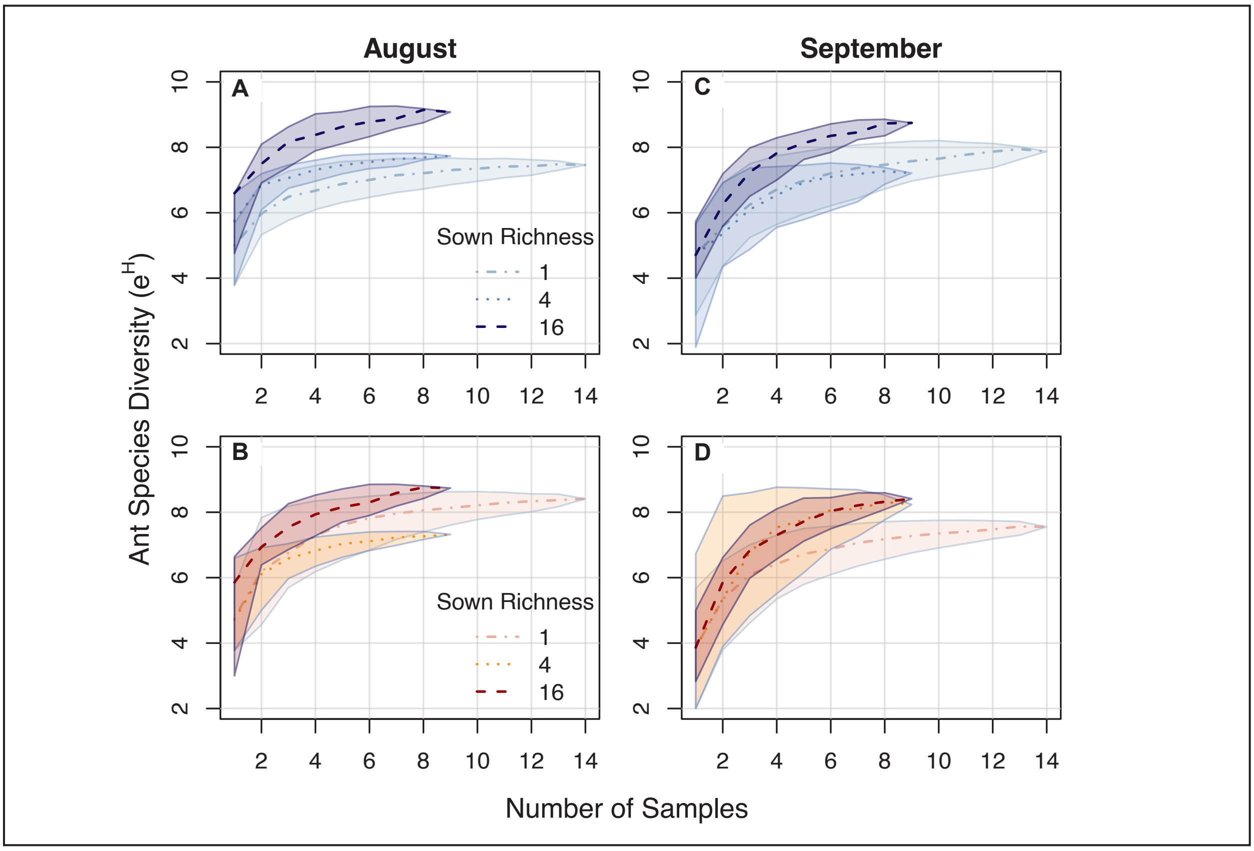

Figure 2. Rarefaction curves for ant species diversity measured from pitfall trap samples, as a function of the number of samples, measured as exponentiated Shannon diversity. Each sample represents a single subplot in a single month. Lines and shaded regions show mean ± one standard deviation, based on 10,000 random iterations, sampled without replacement. Left column shows results for August, right column shows September. Panels (**A**,**C**) show results in control subplots and (**B**,**D**) show results for heated subplots, with results separated by the sown plant species richness treatments. To see the same results with heated and control subplots paired together within panels, see Fig. S4a in the supplement.

Table 1. Ant community composition and incidence by heat treatment, out of a total of 128 occurrences (32 plots, two subplots per plot, sampled in each of two months). Species names are derived from group-specific keys and taxonomic revisions (Coovert 2005, Trager et al. 2007, LaPolla et al. 2013), and from Bolton (2006).

Ant Species	**Number of Occurrences**		
	Total	Control Subplots	Heated Subplots
FORMICINAE			
Camponotus noveboracensis (Fitch), 1855	1	1	0
Formica lasioides Emery, 1893	14	8	6
Formica montana Wheeler, 1910	26	11	15
Formica neogagates Viereck, 1903	15	7	8
Formica obscuripes Forel, 1886	21	11	10
Formica pallidefulva Latreille, 1802	18	10	8
Formica subsericea Say, 1836	5	1	4
Formica vinculans Wheeler, 1913	43	24	19
Lasius neoniger Emery, 1893	119	62	57
Nylanderia parvula (Mayr), 1870	19	11	8
MYRMICINAE			
Monomorium emarginatum DuBois, 1986	69	38	31
Myrmica americana Weber, 1939	78	43	35
Pheidole pilifera-group (Roger), 1863	70	33	37
Solenopsis molesta (Say), 1836	107	55	52
PONERINAE			
Ponera pennsylvanica Buckley, 1866	3	3	0

detectable effect on pitfall trap samples in plots sown with intermediate levels of plant species richness. However, positive effects of sown plant species richness on sampled ant diversity were detected for both control and heated subplots (Fig. 3C,D; slope is significantly positive at $p < 0.05$ for scales of 4 or more samples from control subplots in August, 6 or more samples for control subplots in September, and for 8 or more samples in heated subplots for both August and September). Moreover, the positive effect of plant richness on sampled ant diversity was significantly weaker in heated subplots, especially in the summer months and for larger numbers of samples (at treatment-level 'gamma' scale of 9 plots, $p < 0.004$ for August; $p < 0.057$ for September).

For ant activity, there was high variability across treatments and months, with a significant decline in activity in the fall relative to the summer (Fig. 4). Activity was somewhat higher in heated than in control subplots, although the differences were generally small and rarely significant.

4. Discussion

Our results demonstrate interactive negative effects of plant diversity loss and warming on pitfall trap sampled ant diversity in our experimental grassland system. These differences were clearest in summer months and at the highest level of aggregation of samples within each treatment (i.e. treatment-level 'gamma' diversity, based on samples of 9 plots), for which we detected a loss of about 0.05 species equivalence units per plant species in heated vs. control subplots (Fig. 3C). In the context of our dataset, this effect would indicate that heating causes the loss of roughly one common ant species (or several rare ant species) from pitfall trap samples in monocultures relative to plots sown with 16 plant species. Effects were weaker and less significant in the fall months, when temperatures were generally cooler, as well as for lower levels of sample aggregation, such that the smallest and least statistically clear differences occurred at the scale of individual subplots.

This pattern is consistent with the loss of rare ant species in the warmest plots (i.e. heated monocultures). The positive effect of sown plant richness on sampled ant diversity that we measured in heated subplots was both less significant for plot-level ‘alpha’ diversity than for treatment-level ‘gamma’ diversity, and the overall effect size was smaller. This smaller overall effect would suggest that common ant species, which are disproportionately represented at the plot-level, were less strongly impacted by warming than rarer ant species (either in terms of their presence, or in terms of their foraging behavior). Our sample size is too small to statistically test for effects on individual ant species, but this explanation accords with results for two rare ant species, *Camponotus noveboracensis* and *Ponera pennsylvanica*, which were entirely absent from heated subplots (Table 1). Previous results from Harvard Forest, Massachusetts (Oberg et al. 2011), and Duke Forest, North Carolina (Ellison & Dunn 2021), indicate critical thermal limits of around 40°C for these species (both slightly below the average critical thermal limits for ant species reported at those sites). Although thermal tolerances cannot be reliably extrapolated across sites, maximum temperatures at our site did exceed this threshold more frequently in heated subplots than in control subplots (Fig. S2), potentially indicating that these two species were excluded from samples due to heat stress.

A problem with this explanation is that at the ‘gamma’ level, overall sampled ant diversity was actually higher in heated monocultures than in control monocultures (for both exponentiated Shannon and richness; see Fig. S4A–B, and Fig. S6). There are at least two possible explanations for why heated monocultures would have higher sampled ant diversity than controls. First, heating might have benefited ant species that are near their thermal minimum (Bujan et al. 2020). However, this explanation is not consistent with the strong increase in sampled ant diversity that we observed in plots sown with high plant species richness, as these were also by far the coolest plots in the experiment (Fig. 1B–C, S1–S3).

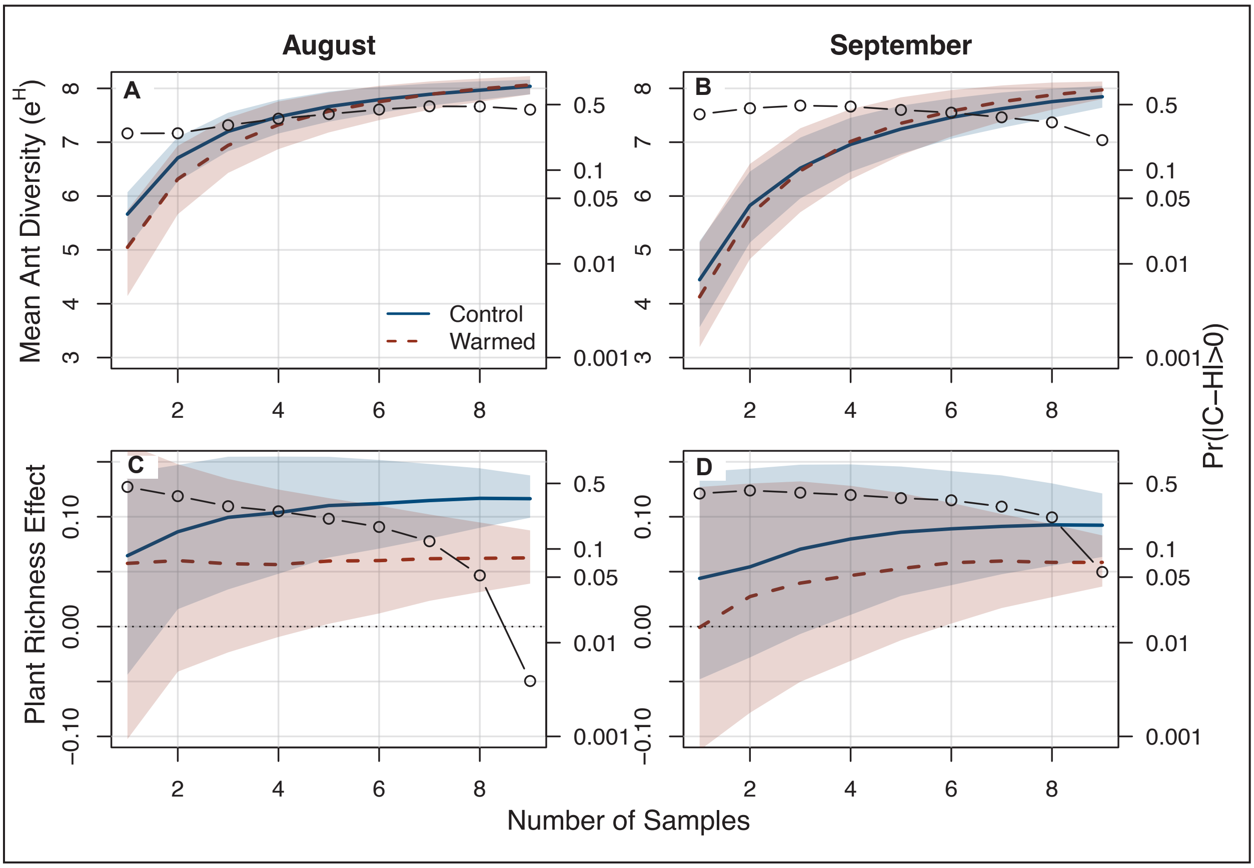

Figure 3. Results from regressions of rarefied ant diversity measured from pitfall trap samples, conducted across sampling intensities and measured as exponentiated Shannon diversity. Left column shows results for August, right column for September. Panels (**A**,**B**) show model intercept, and panels (**C**,**D**) show slope relative to plant species richness, indicating the effect of sown plant species richness on ant diversity. Red and blue lines and shaded regions show mean ± one standard deviation of parameter estimates for control and heated subplots, respectively. Black lines and points show p-values corresponding to tests of the hypothesis that the parameter value for control subplots differs significantly from that for heated subplots. Results for ant species richness, which are very similar to those for Shannon diversity, are presented in Fig. S5. For plots of the underlying ant diversity vs. sown plant species richness data used for the regression in this figure, see Figs S6A–B.

An alternative explanation is that warming led to higher overall ant activity, and thus higher detection of more species in pitfall trap samples (Kaspari et al. 2000, Gibb et al. 2019), in heated monocultures. This explanation is consistent with results from our activity analyses, which showed higher average ant activity in heated vs. control subplots, although differences were only significant in September (Fig. 4).

One remaining complication is that while increased ant activity might be responsible for higher sampled ant diversity in heated monocultures, it does not explain why differences disappear at higher sown plant richness levels. Differences in ant activity between control and heated subplots did not change significantly with plant diversity, and if anything, activity differences were slightly larger in plots sown with high plant richness (Fig. 4). If our results were driven primarily by differences in ant activity (i.e. the number of individuals sampled), we therefore would expect to find even higher sampled ant diversity in these heated subplots relative to controls (Gibb et al. 2019); however, this was not the case (Fig. S4).

As a purely phenomenological explanation, it appears that the pattern we observe is driven by negative effects of heating on the otherwise strong positive effect of sown plant richness on sampled ant diversity. Specifically, while pitfall trap samples from control subplots gain roughly 2 exponentiated Shannon ant species equivalents in plots sown with 16 plant species relative to monocultures, samples in heated subplots gain only one ant species equivalent (Fig. 3). Were this pattern to hold at higher levels of plant diversity, we would expect these declines to intensify. Indeed, in an additional subset of plots in BAC sown with 32 plant species, which were meant to more closely mimic the diversity of local natural grasslands, we applied the same ant sampling methods as described above, and found a net loss of 2 ant species in heated subplot pitfall trap samples relative to controls (again including *P. pennsylvanica*). While we did not formally analyze these results because BAC included only 6 32-species plots, and because these plots were not weeded, which makes them difficult to compare to the rest of the experiment, it is worth noting that this difference in sampled ant diversity almost exactly matches our extrapolated expectation of one ant species equivalent lost per 16 sown plant species in heated plots vs. controls.

A possible mechanistic explanation for our results is that they are driven by a mixture of processes. First, in monocultures, sampled ant diversity was relatively low, and increased activity caused by heating treatments could have led to increased observed incidence of some ant species simply because more ant individuals crossed through the sampled area per unit time (Gibb et al. 2019). Second, overall ant diversity increased with sown plant species richness, potentially due to increased primary productivity, higher habitat complexity, or a higher abundance and diversity of food items (Eisenhauer et al. 2013, Schuldt et al. 2019). Third, a subset of ant species were excluded from samples in heated subplots, potentially because temperatures either directly exceeded their critical thermal limits, or due to chronic exposure to less acute heat stress (Kay & Whitford 1978). Finally, in plots sown with higher plant richness, overall temperatures were lower, and effects of heating on soil temperatures were less acute (Cowles et al. 2016), thus leading to a reduced negative effect of heating sampled ant diversity in plots with higher plant diversity.

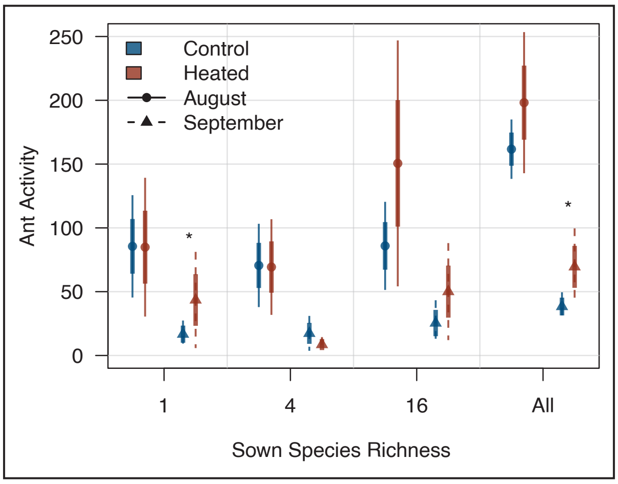

Figure 4. Average total number of ant individuals per sample (i.e. 'activity') as a function of sown plant species richness treatment, warming treatment, and sampling month. 'All' shows results pooled across sown plant species richness treatments. Points, thick intervals, and thin intervals show mean ± one and two standard deviations, respectively. Asterisks indicate cases with significant differences between activity in control vs. heated subplots at $p < 0.05$. In all cases, average activity is significantly higher in August than in September at $p < 0.001$. For a similar summary of plot-level ant richness and Shannon diversity, see Fig. S7.

This combination of processes is also consistent with results from several other studies of warming effects on ants. For example, Kaspari et al.'s 20-year observational study of North American ants likewise suggests that while moderate warming can lead to increases in observed ant diversity, more intense warming is associated with ant diversity declines (Kaspari et al. 2019). Interestingly, Kaspari et al. found similar results for both species incidence and overall colony abundance, suggesting that increased ant diversity associated with moderate warming is not solely driven by changes in activity. Similarly, in their studies of warming effects in northern vs. southern North American sites, Pelini et al. (2014) reported relatively modest declines in ant diversity with warming, and only at their warmer southern site. Interestingly, a common feature across all of these experiments, including ours, is that observed changes in ant diversity were relatively small compared to those observed across larger geographically driven temperature gradients (Del Toro 2013, Pelini et al. 2014). This limited effect of warming on ants in experimental systems is potentially indicative of the limited spatial extent of experimental heating treatments, which can only encompass a few square meters, thereby allowing ants to enter and leave the warmed region in response to temperature stress. Our results are therefore likely conservative estimates of the effects expected from warming at larger spatial and temporal scales.

Because warming treatments similar to the one used in BAC have reported long-term temperature increases of 1–2°C as deep as a meter below the soil surface (Bell et al. 2010), it is likely that our warming treatments influenced both surface foraging and nest abundance, reducing species ability to adapt through behavioral change (e.g. by moving nests or foraging activity to deeper in the soil). Many ants relocate their nests frequently (McGlynn et al. 2004), and any process that changes the rate and relative probability with which ant species replace one another at sites can eventually lead to local exclusion of some species (Clark et al. 2011, Diamond et al. 2016). It is therefore possible that over time, the effects that we document here may intensify even at the limited spatial scale of our experimental plots. Similarly, there are almost certainly rare ant species that we failed to detect in our study. Other studies have attempted to address detection errors by applying diversity indices or extrapolating out rarefaction curves, which can provide a rough indication for the number of unobserved species in a sampled region (Pelini et al. 2014). Again, these methods would be highly inaccurate given our small sample sizes, which is why we do not apply them here (Haegeman et al. 2013). Nevertheless, given that the loss of rare species appears to drive the negative effects of heating on ant diversity that we observe at high levels of sown plant species richness, better accounting for these rare ant species would likely reveal even stronger heating effects than we report here.

4.1 Caveats

It is tempting to directly extrapolate our results for pitfall trap sampled ant diversity as an estimate of overall changes in ‘true’ ant diversity at our site (e.g. including the size, density, and presence or absence of nests). However, we stress that this kind of comparison is difficult. In particular, because heated subplots in our study were relatively small, and always immediately proximate to non-heated regions, it is possible that sampled ant diversity in our heated pitfall trap samples simply indicates a change in foraging behavior – e.g. due to ants avoiding the heated plots when temperatures exceeded their thermal maxima, and/or entering the plots when temperatures dropped below thermal minima. Thus, depending on the ant species and the local temperature, these spatial effects could have led to either increases or decreases in sampled ant diversity relative to the actual diversity of ant species at our site. Nevertheless, given that our results accord with those from other larger-scale experiments, and from natural systems, there is at least some reason to believe that our findings could be indicative of future trends, e.g. when larger-scale warming due to climate change will reduce possibilities to avoid heat stress by changing foraging locations or behaviors.

4.2 Conclusions

Our results show that experimental warming decreases the positive effects of plant species richness on pitfall trap sampled ant diversity. Our results therefore suggest that although warming and loss of plant diversity both impact ants, positive effects of plant richness on ant diversity may be partially undermined under warmer conditions. These results therefore have implications both for basic understanding of ant biology and community ecology, and for potential efforts to mitigate the effects of climate change on local ant communities.

5. Acknowledgements:

We thank Stefan Cover for assistance in identifying our ant specimens, Jim Krueger, Troy Mielke, Colleen Satyshur, and Kally Worm for providing supplies and assistance in collecting and processing samples, Elizabeth Borer for providing a recipe for our pitfall trap collection liquid, and Peter Wragg for organizing collection and databasing of the temperature data. We are also grateful to the Chase lab group at iDiv for helpful suggestions on our statistical methods. This work was supported by grants from the US National Science Foundation Long-Term Ecological Research Program (LTER) to DT including DEB-0620652 and DEB-1234162. ATC was partially funded by an NSF Graduate Research Fellowship, grant number 00006595. ATC is an associate member of the Climate Change Graz Field of Excellence at University of Graz. N.E. acknowledges support by the German Centre for Integrative Biodiversity Research Halle–Jena–Leipzig, funded by the German Research Foundation (FZT 118).

6. References

Bates, D., M. Mächler, B. Bolker & S. Walker (2015): Fitting Linear Mixed-Effects Models Using lme4. – Journal of Statistical Software **67**.

Bell, J. E., R. Sherry & Y. Luo (2010): Changes in soil water dynamics due to variation in precipitation and temperature. – An ecohydrological analysis in a tallgrass prairie: Water Resources Research **46**.

Bolton, B. (2006): Bolton's catalogue of ants of the world, 1758-2005. – Cambridge, Mass., Harvard University Press.

Boyd, R. S. (2001): Ecological benefits of myrmecochory for the endangered chaparral shrub Fremontodendron decumbens (Sterculiaceae). – American Journal of Botany **88**: 234–241.

de Bruyn, L. A. L. (1999): Ants as bioindicators of soil function in rural environments. – Agriculture Ecosystems & Environment **74**: 425–441.

Bujan, J., K. A. Roeder, K. Beurs, M. D. Weiser & M. Kaspari (2020): Thermal diversity of North American ant communities: Cold tolerance but not heat tolerance tracks ecosystem temperature. – Global Ecology and Biogeography **29**: 1486–1494 [http://doi.org/10.1111/geb.13121].

Cardinale, B. (2012): Impacts of Biodiversity Loss. – Science **336**: 552–553 [http://doi.org/10.1126/science.1222102].

Cerda, X., J. Retana & S. Cros (1998): Critical thermal limits in Mediterranean ant species: trade-off between mortality risk and foraging performance. – Functional Ecology **12**: 45–55.

Clark, A. T., J. M. H. Knops & D. Tilman (2019): Contingent factors explain average divergence in functional composition over 88 years of old field succession. – Journal of Ecology **107**: 545–558 [http://doi.org/10.1111/1365-2745.13070].

Clark, A. T., J. J. Rykken & B. D. Farrell (2011): The Effects of Biogeography on Ant Diversity and Activity on the Boston Harbor Islands, Massachusetts, USA. – Plos One **6**: 11.

Colwell, R. K., A. Chao, N. J. Gotelli, S.-Y. Lin, C. X. Mao, R. L. Chazdon & J. T. Longino (2012): Models and estimators linking individual-based and sample-based rarefaction, extrapolation and comparison of assemblages. – Journal of Plant Ecology **5**: 3–21 [http://doi.org/10.1093/jpe/rtr044].

Coovert, G. A. (2005): The ants of Ohio (Hymenoptera: Formicidae). – Bulletin of the Ohio Biological Survey **15**: 1–196.

Cowles, J. M., P. D. Wragg, A. J. Wright, J. S. Powers & D. Tilman (2016): Shifting grassland plant community structure drives positive interactive effects of warming and diversity on aboveground net primary productivity. – Global Change Biology **22**: 741–749 [http://doi.org/10.1111/gcb.13111].

Del Toro, I. (2013): Diversity of Eastern North American Ant Communities along Environmental Gradients. – PLoS ONE **8**: e67973 [http://doi.org/10.1371/journal.pone.0067973].

Del Toro, I., R. R. Ribbons & S. L. Pelini (2012): The little things that run the world revisited : a review of ant-mediated ecosystem services and disservices (Hymenoptera: Formicidae). – Myrmecological News **17**: 133–146.

Del Toro, I., R. R. Silva & A. M. Ellison (2015): Predicted impacts of climatic change on ant functional diversity and distributions in eastern North American forests. – Diversity and Distributions **21**: 781–791 [http://doi.org/10.1111/ddi.12331].

Diamond, M., D. Sorger, J. Hulcr, S.L. Pelini, D.T. Israel, C. Hirsch, E. Oberg & R.R. Dunn (2011): Who likes it hot? A global analysis of the climatic, ecological, and evolutionary determinants of warming tolerance in ants. – Conservation Biology.

Diamond, L. M. Nichols, S. L. Pelini, C. A. Penick, G. W. Barber, S. H. Cahan, R. R. Dunn, A. M. Ellison, N. J. Sanders & N. J. Gotelli (2016): Climatic warming destabilizes forest ant communities. – Science Advances **2**: e1600842 [http://doi.org/10.1126/sciadv.1600842].

Eisenhauer, N., T. Dobies, S. Cesarz, S. E. Hobbie, R. J. Meyer, K. Worm & P. B. Reich (2013): Plant diversity effects on soil food webs are stronger than those of elevated CO2 and N deposition in a long-term grassland experiment. – Proceedings of the National Academy of Sciences **110**: 6889–6894 [http://doi.org/10.1073/pnas.1217382110].

Ellison, A. & R. Dunn (2021): (Ants) Under Climate Change at Harvard Forest and Duke Forest 2009-2015. – Environmental Data Initiative [http://doi.org/10.6073/PASTA/4F6E15DCFD1ABFA62F2C582F39804730].

Ellison, A. M., N. J. Gotelli, E. J. Farnsworth & G. D. Alpert (2012): A field guide to the ants of New England. – New Haven [Conn.], Yale University Press: 398 p.

Ellison, A. M., S. Record, A. Arguello & N. J. Gotelli (2007): Rapid inventory of the ant assemblage in a temperate hardwood forest: species composition and sampling methods. – Environmental Entomology **36**: 766–775.

Fisher, B. & S. Cover (2007): Ants of North America: a guide to the genera. – Berkeley, CA, University of California Press.

Folgarait, P. J. (1998): Ant biodiversity and its relationship to ecosystem functioning: a review. – Biodiversity and Conservation **7**: 1221–1244.

Gibb, H., B. F. Grossman, C. R. Dickman, O. Decker & G. M. Wardle (2019): Long-term responses of desert ant assemblages to climate. – Journal of Animal Ecology **88**: 1549–1563 [http://doi.org/10.1111/1365-2656.13052].

Gotelli, N. J. & R. K. Colwell (2001): Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. – Ecology Letters **4**: 379–391.

Gotelli, N. J., A. M. Ellison, R. R. Dunn & N. J. Sanders (2011): Counting ants (Hymenoptera: Formicidae): biodiversity sampling and statistical analysis for myrmecologists. – Myrmecological News **15**: 13–19.

Grace, J. B., T. M.Anderson, E. W. Seabloom, E.T. Borer, P. B. Adler, W. S. Harpole, Y. Hautier, H. Hillebrand, E. M. Lind, M. Pärt & et al. (2016): Integrative modelling reveals mechanisms linking productivity and plant species richness. – Nature **529**: 390–393 [http://doi.org/10.1038/nature16524].

Haddad, N. M., G. M. Crutsinger, K. Gross, J. Haarstad, J. M. H. Knops & D. Tilman (2009): Plant species loss decreases arthropod diversity and shifts trophic structure. – Ecology letters **12**: 1029–1039.

Haddad, N. M., D. Tilman, J. Haarstad, M. Ritchie & J. M. H. Knops (2001): Contrasting effects of plant richness and composition on insect communities: A field experiment. – American Naturalist **158**: 17–35.

Haegeman, B., J. Hamelin, J. Moriarty, P. Neal, J. Dushoff & J. S. Weitz (2013): Robust estimation of microbial diversity in theory and in practice. – The ISME Journal **7**: 1092–1101 [http://doi.org/10.1038/ismej.2013.10].

Huey, R. B., M. R. Kearney, A. Krockenberger, J. A. M. Holtum, M. Jess & S. E. Williams (2012): Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. – Philosophical Transactions of the Royal Society B: Biological Sciences **367**: 1665–1679 [http://doi.org/10.1098/rstb.2012.0005].

Human, K. G. & D. M. Gordon (1997): Effects of Argentine ants on invertebrate biodiversity in northern California. – Conservation Biology **11**: 1242–1248.

Jost, L. (2007): Partitioning diversity into independent alpha and beta components. – Ecology **88**: 2427–2439.

Kaspari, M., L. Alonso & S. O'Donnell (2000): Three energy variables predict ant abundance at a geographical scale. – Proceedings of the Royal Society B: Biological Sciences **267**: 485–489.

Kaspari, M., J. Bujan, K. A. Roeder, K. Beurs & M. D. Weiser (2019): Species energy and Thermal Performance Theory predict 20-yr changes in ant community abundance and richness. – Ecology **100**: 12 [http://doi.org/10.1002/ecy.2888].

Kay, C. A. R. & W. G. Whitford (1978): Critical thermal limits of desert honey ants - Possible ecological implications. – Physiological Zoology **51**: 206–213.

Korzukhin, M. D., S. D. Porter, L. C. Thompson & S. Wiley (2001): Modeling Temperature-Dependent Range Limits for the Fire Ant *Solenopsis invicta* (Hymenoptera: Formicidae) in the United States. – Environmental Entomology **30**: 645–655 [http://doi.org/10.1603/0046-225X-30.4.645].

LaPolla, J., P. Hawkes & J. N. Fisher (2013): Taxonomic review of the ant genus Paratrechina, with a description of a new species from Africa. – Journal of Hymenoptera Research **35**: 71–82 [http://doi.org/10.3897/jhr.35.5628].

Leibold, M. A. & J. M. Chase (2018): Metacommunity Ecology, Volume 59. – Princeton University Press.

May, F., K. Gerstner, D. J. McGlinn, X. Xiao & J. M. Chase (2017): mobsim: An R package for the simulation and measurement of biodiversity across spatial scales. – Methods in Ecology and Evolution **9**: 1401–1408 [http://doi.org/10.1101/209502].

McGlynn, T. P., R. A. Carr, J. H. Carson & J. Buma (2004): Frequent nest relocation in the ant Aphaenogaster araneoides: resources, competition & natural enemies. – Oikos **106**: 611–621.

Oberg, E., I. Del Toro & S. Pelini (2011): Characterization of the thermal tolerances of forest ants of New England. – Insectes Sociaux.

Parmesan, C. & M. E. Hanley (2015): Plants and climate change: complexities and surprises. – Annals of Botany **116**: 849–864 [http://doi.org/10.1093/aob/mcv169].

Pelini, S. L., Diamond, L. M. Nichols, K. L. Stuble, A. M. Ellison, N. J. Sanders, R. R. Dunn & N. J. Gotelli (2014): Geographic differences in effects of experimental warming on ant species diversity and community composition. – Ecosphere **5**: art125 [http://doi.org/10.1890/ES14-00143.1].

R. Development Core Team (2019): R: a language and environment for statistical computing. – R Foundation for Statistical Computing.

Scherber, C., N. Eisenhauer, W. W. Weisser, B. Schmid, W. Voigt, M. Fischer, E.-D. Schulze, C. Roscher, A. Weigelt, E. Allan & et al. (2010): Bottom-up effects of plant diversity on multitrophic interactions in a biodiversity experiment. – Nature **468**: 553–556 [http://doi.org/10.1038/nature09492].

Schuldt, A., A. Ebeling, M. Kunz, M. Staab, C. Guimarães-Steinicke, D. Bachmann, N. Buchmann, W. Durka, A. Fichtner, F. Fornoff & et al. (2019): Multiple plant diversity components drive consumer communities across ecosystems. – Nature Communications **10**: 1460 [http://doi.org/10.1038/s41467-019-09448-8].

Seifert, B. (2017): The ecology of Central European non-arboreal ants – 37 years of a broad-spectrum analysis under permanent taxonomic control. – Soil Organisms 89: 1–67.

Sullivan, L. L., A. T. Clark, D. Tilman & A. K. Shaw (2018): Mechanistically derived dispersal kernels explain species-level patterns of recruitment and succession. – Ecology **99**: 2415–2420 [http://doi.org/10.1002/ecy.2498].

Thakur, M. P., D. Tilman, O. Purschke, M. Ciobanu, J. Cowles, F. Isbell, P. D. Wragg & N. Eisenhauer (2017): Climate warming promotes species diversity, but with greater taxonomic redundancy, in complex environments. – Science Advances **3**: e1700866 [http://doi.org/10.1126/sciadv.1700866].

Tilman, D. (2018a): BAC: Biodiversity and Climate. Ant responses to experimental warming and plant diversity. Environmental Data Initiative [http://doi.org/10.6073/PASTA/1991371638F8E2EFC783F6BDD04CF80C].

Tilman, D. (2018b): BAC: Biodiversity and Climate. Daily mean iButton soil and air temp, air relative humidity. – Environmental Data Initiative [http://doi.org/10.6073/PASTA/1333B6C2CDA080536479B918D8CAA483].

Tilman, D. (2018c): BAC: Biodiversity and Climate. Plant aboveground biomass data. Environmental Data Initiative [http://doi.org/10.6073/PASTA/214B327BC6FC0A240EC705B339E6DD83].

Tilman, D., J. Knops, D. Wedin, P. Reich, M. Ritchie & E. Siemann (1997): The influence of functional diversity and composition on ecosystem processes. – Science **277**: 1300–1302.

Trager, J. C., J. A. MacGown & M. D. Trager (2007): Revision of the Nearctic endemic Formica pallidefulva group, *in* Advances in ant systematics (Hymenoptera: Formicidae): homage to E. O. Wilson – 50 years of contributions. – Memoirs of the American Entomological Institute **80**: 610–636.

Wagner, D., M. J. F. Brown & D. M. Gordon (1997): Harvester ant nests, soil biota and soil chemistry. – Oecologia **112**: 232–236.

Whittington, H. R., D. Tilman & J. S. Powers (2013): Consequences of elevated temperatures on legume biomass and nitrogen cycling in a field warming and biodiversity experiment in a North American prairie. – Functional Plant Biology **40**: 1147.

Wilson, E. O. (1987): The little things that run the world. (The importance and conservation of invertebrates). – Conservation Biology **1**: 344–346.

Wright, A., S. A. Schnitzer & P. B. Reich (2014): Living close to your neighbors: the importance of both competition and facilitation in plant communities. – Ecology **95**: 2213–2223 [http://doi.org/10.1890/13-1855.1].