The mixed effects of experimental ant removal on seedling distribution, belowground invertebrates, and soil nutrients

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Abstract. Ants are ubiquitous members of most forest communities, where they disperse seeds, prey on other species, and influence the flow of nutrients. Their effects are often described as substantial, but few studies to date have simultaneously examined how the presence of ants affects both above and belowground processes. In this study, we experimentally reduced ant abundance in a suite of deciduous forest plots in northern Georgia, USA to assess the effects of ants on the spatial distribution of a common understory plant species, *Hexastlylis arifolia*, the structure of soil mesofaunal communities, and soil nitrogen dynamics. Over the course of several years, the removal of ants led to significant spatial aggregation of *H. arifolia* seedlings near the parent plant, most likely due to the absence of the keystone seed dispersal species, *Aphaenogaster rudis*. Seedling emergence was higher in ant removal plots, but seedling aggregation did not affect first or second year seedling mortality. Ammonium concentrations were 10× higher in ant removal plots relative to control plots where ants were present in the first year of the study, but this increase disappeared in the second and third years of the study. The effects of ant removal on the soil mesofauna were mixed: removal of ants apparently did not affect the abundance of Collembola, but the abundance of oribatid mites was significantly higher in ant removal plots by year two of the study. Taken together, these results provide some of the first experimental evidence of the diverse direct and indirect effects of ants on both above and belowground processes in forest ecosystems and demonstrate the potential consequences of losing an important seed dispersing ant species for the plants they disperse.

Key words: ant removal; *Aphaenogaster rudis*; ecosystem processes; *Hexastylis arifolia*; multi-species interactions; seed dispersal; soil nutrient dynamics.

Citation: Zelikova, T. J., N. J. Sanders, and R. R. Dunn. 2011. The mixed effects of experimental ant removal on seedling distribution, belowground invertebrates, and soil nutrients. Ecosphere 2(5):art63. doi:10.1890/ES11-00073.1

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INTRODUCTION

Ants are ubiquitous in most ecosystems, and are often described as ecosystem engineers (Jones et al. 1994, Folgarait et al. 2002, Sanders and Frank van Veen 2011) and keystone species (Gove et al. 2007). Indeed, there is some evidence that ants play key roles in shaping plant population dynamics (Kalisz et al. 1999, Rodri-guez-Cabal et al. 2009), altering arthropod community structure both above and belowground (Wimp and Whitham 2001, Moya-Laraño and Wise 2007, Wardle et al. 2011, Sanders and Frank van Veen 2011), or modulating ecosystem processes (Folgarait 1998). However, surprisingly few studies have experimentally removed ants from an intact understory community to assess simultaneously their long-term impacts on plant

population structure, the structure of below-ground arthropod communities, and soil nutrient dynamics (but see Wardle et al. 2011). Where ants have been removed, the effort has tended to focus on behaviorally dominant species (see Schmitz et al. 2000, Gibb and Hochuli 2004, Wardle et al. 2011), which may not necessarily be the species with the greatest potential community effects. No study to date has examined the cascading effects of removing seed dispersing ants on the plants they disperse and on below-ground processes. In this study, we report on a three-year experiment aimed at uncovering the effects of ants on these patterns and processes in a deciduous forest in the southeastern USA.

Ants disperse upwards of 50% of the total herbaceous flora in some eastern deciduous forests in the U.S. (Handel et al. 1981, Gaddy 1986). Seed dispersal by ants (i.e., myrmeco-chory) is particularly important in that it involves hundreds of ant species and more than ten thousand plant species across many terrestrial ecosystems (Beattie and Hughes 2002, Rico-Gray and Oliveira 2007, Lengyel et al. 2009). The seed dispersal patterns that ants generate shape the initial spatial distribution of plants within populations and define the context for future ecological, demographic, and genetic interactions among emerging seedlings (Kalisz et al. 1999).

Though ant-seed dispersal mutualisms are generally considered to be diffuse, with multiple ant species interacting with seeds and dispersing them opportunistically, evidence is building that these interactions can depend disproportionately on one or a small suite of seed-dispersing ants (Giladi 2006, Gove et al. 2007, Zelikova et al. 2008, Ness et al. 2009). In southeastern temperate forests, *Aphaenogaster rudis* disperse the majority of ant-dispersed seeds and are considered to be keystone mutualists for myrmecochorous plants (Giladi 2004, Ness et al. 2009, Zelikova et al. 2008, Warren et al. 2010). Indeed, in one recent study (Mitchell et al. 2002), the single best correlate of myrmecochore abundance was the abundance of *A. rudis*, suggesting that these ants have a strong effect on myrmecochore fitness. While many aspects of the influence of *A. rudis* on seeds and seedlings have been considered, to our knowledge, no study has tracked the fate of seeds in the absence of *A. rudis*.

In addition to their effects on seeds, ants in general and *A. rudis* in particular have the potential to influence soil invertebrate communities and soil properties. Ants prey upon soil- and litter-dwelling invertebrates, and, at least in come cases, affect the abundance of particular soil taxa and the composition of soil communities (Wagner et al. 1997, Laakso 1999, Laakso and Setala 2000, Lenoir et al. 2003, Wilson 2005, Moya-Lorano and Wise 2007, Wardle et al. 2011, Sanders and Frank van Veen 2011). Ants may indirectly influence soil nutrients by suppressing populations of microbe-feeding mites and Collembolans. Changes in community composition and the abundance of key mesofaunal species can indirectly influence soil nutrient dynamics (Seastedt 1984, Seastedt and Coleman 1984, Coleman et al. 2004), most likely slowing rates of decomposition and nutrient flux. Ants can also accelerate some processes related to soil nutrients by aerating soil, shifting soil profiles, and altering soil chemical properties by translocating soil material during nest building and accumulating excreta and food refuse in or near the nest (Culver and Beattie 1983, De Bruyn and Conacher 1990, Wagner et al. 1997).

In this study, we consider the influence of ground-foraging ant species on plant population dynamics, soil mesofauna community dynamics, and soil nutrient concentrations. We experimentally address the net effects of ants on both plants and soil processes by testing three inter-related hypotheses, namely that removal of ants will (1) alter seed germination rates and the spatial distribution and survival of *Hexastylis arifolia* seedlings, (2) cause a shift in the composition of the soil mesofauna community, specifically by reducing the abundance and diversity of microbe-feeding Acari and Collembola, and (3) alter soil nitrogen concentrations.

MATERIALS AND METHODS

Site and study species description

We carried out this study at Whitehall Experimental Forest in Athens, Georgia, USA (33°52′ N latitude, 150–240 m elevation) from April 2005 until August 2008. This site is characterized by a mixture of mature oak-hickory deciduous forest, pine plantations, and mixed pine-hardwood patches of differing age. We focused our study on a common understory herbaceous perennial,

Hexastylis arifolia (Michx. Aristolochiaceae), commonly called little brown jug. The distribution of *H. arifolia* ranges throughout the southeastern United States. It is a long-lived ant-dispersed plant that occurs in mature deciduous forests (Gonzalez 1972) and is abundant at the study site. The age of first reproduction for *H. arifolia* is 7–10 years (Gonzalez 1972) and each mature fruit has an average of 19–22 seeds (Gonzalez 1972, Giladi 2004). Flowers tend to be close to the ground and generally develop in early spring and mature by the end of May (Gonzalez 1972). The mature fruit begins to decompose, allowing ground-foraging ants access to the seeds; at Whitehall Experimental Forest, most of the seed dispersal by ants occurs in late May through early June (Giladi 2004). Ants disperse seeds, on average, <1 m away from the maternal plant (Giladi 2004). There is no evidence of a seed bank for *H. arifolia,* and seeds germinate within a year of reproduction, producing a distinct seedling with two cotyledons.

Eight ant species disperse *H. arifolia* seeds at this site. However, *A. rudis* ants disperse the majority (~70%) of *H. arifolia* seeds (Giladi 2004). *A. rudis* ants nest in soil, in matted leaf litter, under rocks, and in bark and leaves (Talbot 1951). Colony size averages 600–700 individuals (Talbot 1951, Heithaus et al. 2005). At Whitehall Experimental Forest, *A. rudis* nest site density is on average 0.5 nests/m^2 (Giladi 2004).

Experimental design

In April 2005, we located 40 flowering *H. arifolia* individuals and randomly assigned each to one of two treatments: ant removal and control. We then established 20 2 × 2 m ant removal plots and 20 2 × 2 m control plots. We placed each ant exclosure around one reproductive *H. arifolia* individual before the seeds had ripened and removed ants with a combination of placing a physical barrier—a 20 cm high sheet metal wall, trenched 5 cm into the ground—with Tanglefoot (the Tanglefoot Company, MI) application around the top of the exclosure walls, and broadcast application of AMDRO (0.036% hydramethylnon, Amdro, Inc., GA), an ant-specific pesticide that degrades quickly (Meer et al. 1982) and has minimal effects on other arthropods (Apperson et al. 1994). AMDRO was broadcast at a rate of 56.5 g plot^{-1} year^{-1}. Both Tanglefoot and

AMDRO were applied in early May each year and re-applied in June to maintain the ant removal plots. Control plots contained one reproductive *H. arifolia* plant, but no physical barrier and no addition of AMDRO or Tanglefoot. In 2005, we assessed the presence of ants in our plots using tuna baiting prior to the application of AMDRO and immediately after. *A. rudis* ants were present at tuna baits before the addition of AMDRO and no *A. rudis* ants were found at tuna baits after AMDRO application. We continued monitoring the ant community with a combination of pitfall trapping and leaf litter sampling. We placed a single 60 mL pitfall trap in each plot annually during the summer to check that we successfully reduced ant abundance in ant removal plots and that these plots remained *A. rudis*-free throughout the course of the experiment. Additionally, we used mini-Winklers to sample 0.25 m^2 of leaf litter in each plot in 2005 and 2006. Litter was sifted before being suspended in Winkler sacks in the lab at room temperature for 48 hours. All invertebrates were collected into 70% ethanol and ants were sorted and identified to species. In 2005, we successfully reduced overall ant abundance and removed *A. rudis* ants from plots; low levels of *A. rudis* abundance were maintained in the ant removal plots throughout the experiment. Table 1 lists the ant species collected in the ant removal and control plots throughout the experiment.

In 2006, we added 15 2 × 2 m experimental control plots to ensure that the presence of the barrier itself did not affect seedling germination and survival. The experimental control plots had the physical barrier in place, but no Tanglefoot or AMDRO application. We carried out a separate field experiment in 2006 to determine if AMDRO addition into ant removal plots significantly altered soil nitrogen. In May 2006, we collected soil from sites adjacent to the experimental plots, homogenized the soil, and added it into 30 1-gallon nursery pots, placing leaf litter on top and one resin bag at the bottom. We added AMDRO into 15 pots proportionally to the amount added into ant removal plots per field season, while the other 15 pots served as controls. We terminated the experiment in August 2006, extracted all resin with 2 M KCl, and analyzed the extract for nitrate and ammonium at Colorado State University Soils testing laboratory. The addition of

Table 1. Ant species collected in pitfall traps (2005–2007) and leaf litter (2005–2006). First number indicates species occurrence (number of traps where the species was collected) and the number inside the parentheses indicates the total number of ant workers collected.

| | Pitfall trap | | | | | | Leaf litter | |
| | 2005 | | 2006 | | 2007 | | 2005 | |
Ant species	Control	Ant removal	Control	Ant removal	Control	Ant removal	Control	Ant removal
Aphaenogaster rudis	13(17)	1(1)	13(28)	3(3)	11(19)	1(1)	10(65)	
Aphaenogaster fulva							2(11)	
Amblyopone pallipes							1(1)	
Camponotus americanus			1(1)					
Camponotus chromaiodes			1(1)					
Camponotus nearcticus		1(1)						1(1)
Crematogaster minutissima			2(2)				1(3)	1(2)
Formica fusca subsericea	1(1)		1(42)					
Hypoponera opacior								1(4)
Myrmicinae Americana			1(1)				2(2)	1(2)
Paratrechina parvula		3(3)	5(5)	3(3)			11(155)	5(18)
Pheidole sp. 1	2(4)		1(1)		1(3)			
Pheidole sp. 2	4(8)							
Pheidole sp. 3	1(1)							
Pheidole sp. 4	1(1)							
Pheidole dentata			6(42)	2(3)	12(53)	10(53)	1(1)	
Ponera pennsylvanica			1(1)				6(8)	4(6)
Prenolepis imparis			7(45)	1(2)	3(3)		1(38)	
Solenopsis aurea			1(1)					
Solenopsis geminata	1(4)							
Solenopsis molesta	1(1)		1(1)		1(2)			
Tapinoma sessile		1(1)						
Temnothorax curvispinosus		1(1)	5(40)					
Total no. species	8	5	14	3	5	2	9	6
Total no. workers	37	7	211	11	80	54	284	33

AMDRO into pots did not significantly affect soil nitrogen; neither NO_3 (t = −0.254, df = 1, p = 0.80) nor NH_4 (t = −0.1009, df = 1, p = 0.92) differed between the AMDRO addition and control pots.

Effects of ant removal on Hexastylis arifolia

To examine whether the removal of ants affected the spatial distribution of *Hexastylis arifolia* seedlings, we located and individually tagged all first-year seedlings in each plot in April 2006 and continued monitoring seedling germination, establishment, and survival annually through August 2008. We measured the radial distance of each seedling from the nearest adult plant that was in flower the previous year and also gave each seedling an x, y coordinate within a 1-m² frame around the closest reproductive plant.

Using each seedling's x, y coordinates, we calculated the radial distance of each *Hexastylis arifolia* seedling from its maternal plant and averaged across seedlings to get a mean radial distance per reproductive plant. To determine the degree of seedling clumping, we calculated nearest neighbor distances between seedlings, using the x, y coordinates for each seedling. Each individual reproductive plant was treated as an independent replicate, with plants averaged across plots and plots nested within treatments. To examine the effects of ant removal on measures of *H. arifolia* population dynamics, we first examined if the response variables mean radial *H. arifolia* seedling distance away from maternal plant, seedling emergence in 2006–2008, and first year seedling mortality were inter-correlated within treatments. To determine the effect of ant removal on seedling mortality, we used a single-factor ANOVA with ant removal as the main effect in the model. Seedling mortality data were log-transformed to meet assumptions of normal distribution of error terms.

Effects on ant removal on soil mesofauna and soil nutrients

To examine how the removal of ants affected soil nutrient dynamics, we used mixed ion resin-bags to monitor soil NO_3^- and NH_4^+. Resin bags

were made with mixed bed ion exchange resin (JT Baker) and knee-high panty hose. We placed two resin bags at 10-cm depth 1 m apart in the middle of each plot and replaced them twice per growing season, with an over-winter set left from November until April of each year. We sampled the soil mesofauna community with a combination of pitfall traps and leaf-litter sampling. Pitfall traps consisted of 60 mL cups buried flush with the ground and filled with soapy water. We collected leaf litter from one 0.25 m^2 quadrat within each plot and suspended the fine litter in Winkler sacks in the lab for 48 hours at room temperature. We identified all extracted ants, collembolans, and Acari, identifying ants to species, Collembola to family, and Acari to suborders Oribatei, Mesostigmata, and Prostigmata. We relied on the expertise of Ernest Bernard and his lab members at the University of Tennessee to aid in identification of the collembolans and Acari. In 2007, we present only pitfall trap results and present pitfall trap results with leaf litter results for 2005 and 2006. We did not collect soil invertebrates in 2008.

Leaf litter collections and pitfall trapping differ in how effectively they sample ants, Acari, and Collembola; leaf litter and pitfall traps efficiently sample ants and mites, but pitfall traps more effectively sample Collembola. As a result, we initially analyzed leaf litter and pitfall trap results separately, but saw no differences in trends between the two methods, combining them for final analyses and reporting the combined results. The number of ant workers collected is treated as ant abundance and ant occupancy is calculated as the number of plots where the ant species was collected divided by the total number of plots sampled and reported as a percentage. To determine the effects of ant removal on Acari and Collembola abundance, we analyzed the data in two ways. We used a repeated-measures MANOVA to examine the effect of treatment over time on soil NH_4^+ and NO_3^- concentrations and the abundance of Acari and Collembola, examining the effects on Acari orders and Collembola family, as well as pooling across all orders within Acari and across feeding guilds (Phycophages/herbivores, primary decomposers, and secondary decomposers) within Collembola (Chahartaghi et al. 2005). Because our experimental design is not perfectly balanced

and we initiated an experimental control treatment one year after the start of the experiment, we split the repeated-measures MANOVA analyses into two parts, using a repeated-measures MANOVA to examine ant removal and control plots from 2006–2008 and running a separate ANOVA to examine the effects of all three treatments on the response variables in 2007 and 2008, including "time" as a covariate and testing for the interaction between "year" and treatment. All reported values, unless otherwise noted, are means ± standard errors. We used JMP 8.0 (SAS Institute) statistical software for all analyses.

Results

Effects of ant removal on Hexastylis arifolia

We successfully removed most ants from ant removal plots in the first year of the study. The mean number of workers of all species combined in control plots was more than 8× greater than in ant removal plots (control n = 19, mean = 16.95 ± 5.24 SE, ant removal n = 20, mean = 2 ± 0.17 SE; Table 1). Additionally, workers of the reported keystone mutualist, A. rudis, made up 2% of all collected ants in the ant removal plots. In contrast, A. rudis was the most common and abundant ant found in control plots in 2005, occupying 68% of control plots and making up 22.8% of all collected ants.

Overall, H. arifolia seedlings in ant removal plots were more than 7× closer to their maternal plants (8.03 cm ± 0.79 SE) than in control plots (46.88 cm ± 4.57, experimental control = 55.48 ± 5.75 SE; $F_{2,36} = 11.69$, p < 0.0001; Fig. 1A). As a consequence of this pattern and the greater density of seedlings in ant removal plots, seedlings in ant removal plots (mean nearest neighbor distances = 11.32 cm ± 1.76 SE) were also significantly more aggregated than in control plots (mean nearest neighbor distance = 51.14 cm ± 10.8 SE; $F_{1,40} = 10.41$, p < 0.0025; Fig. 1B). Seedling emergence did not differ between treatments in 2006 ($F_{1,37} = 0.006$, p = 0.98; Fig. 2A), but within control plots, seedling emergence was positively correlated with NH_4^+ concentrations in 2005 ($R^2 = 0.21$, p = 0.05). In 2007, seedling emergence was more than 2× higher in the ant removal plots (8.5 ± 2.54 SE) than in the control plots (3.84 ± 1.24 SE) and more than 4×
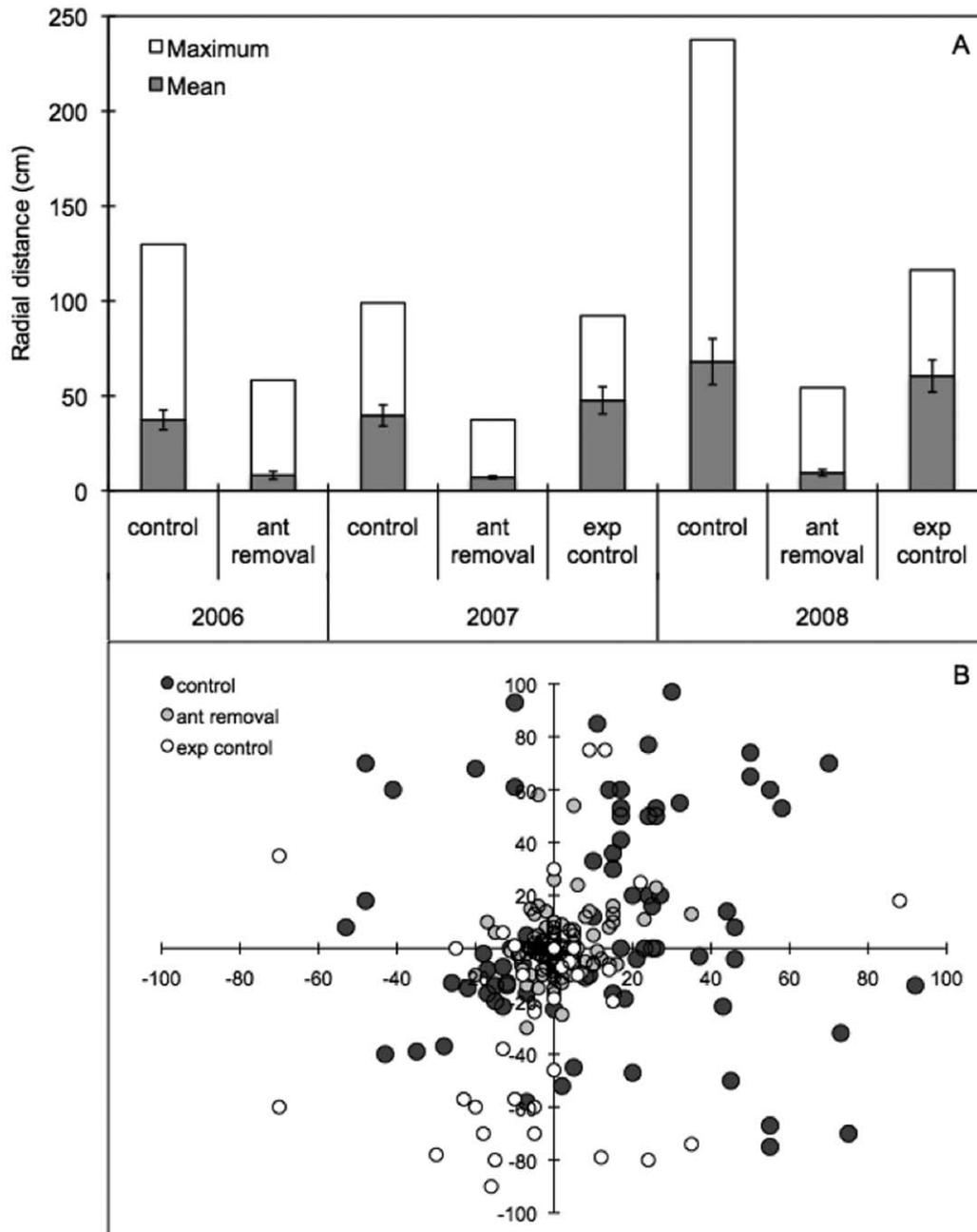
Fig. 1. (A) Mean and maximum radial distance of first year seedlings to the maternal plant. Mean calculated per plant and pooled across plants within plot. Error bars represent ± 1 SE. Sample size: control N = 19, ant removal N = 20, experimental control N = 15. (B) Spatial distribution of seedlings within 1 m^2 around the maternal plant. Each dot represents the radial distance from each seedling to maternal plant.

higher than in experimental control plots (1.47 ± 0.86 SE; $F_{2,51}$ = 3.71, p = 0.03) but was not correlated with any other variable besides treat- ment. Similarly, in 2008, seedling emergence in ant removal plots was more than 2× higher (6.3 ± 2.04 SE) than in control plots (2.42 ± 0.63 SE)
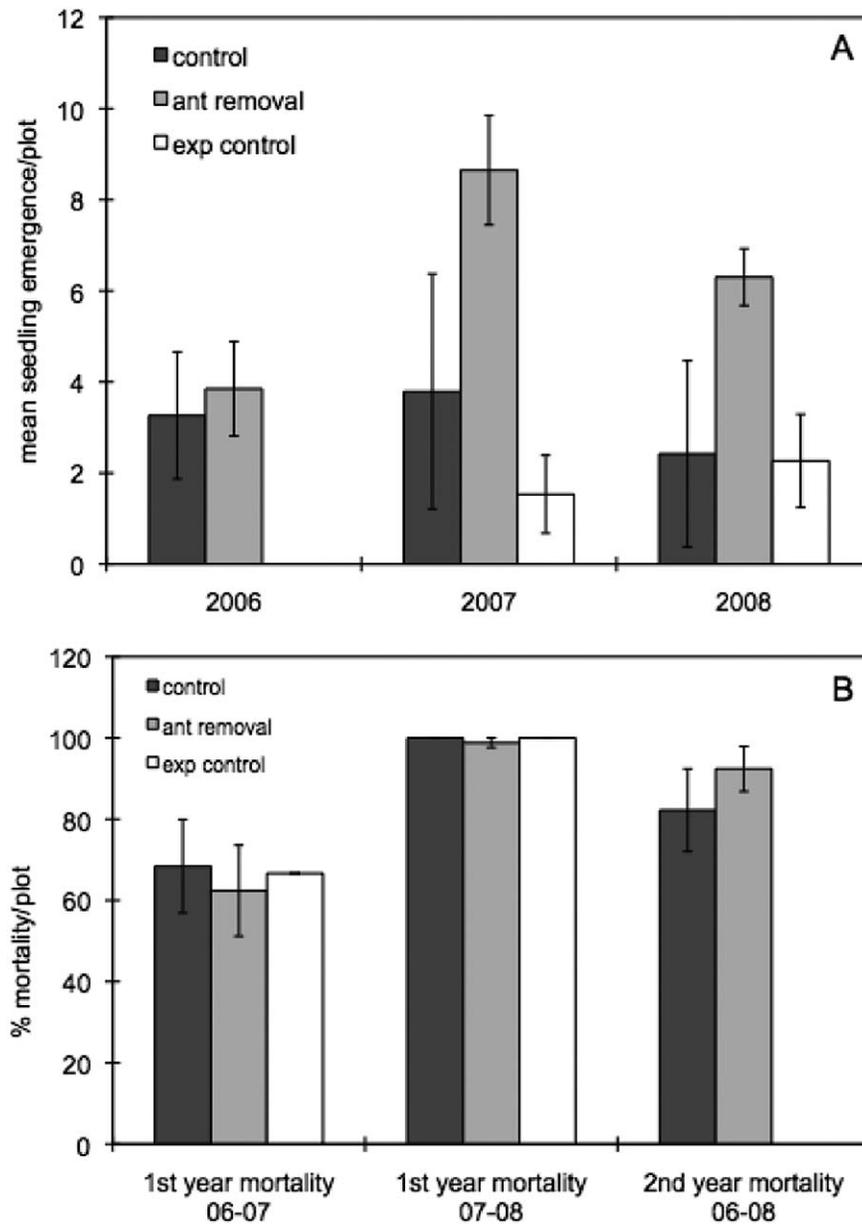
Fig. 2. (A) Mean number of emerged seedlings per plot ± 1 SE. (B) Percent seedling mortality, calculated as the number of marked seedlings that survived the first and second year post germination. Error bars represent ± 1SE.

and in experimental control plots (2.27 ± 1.02 SE; $F_{2,51} = 2.58$, p = 0.09).

Seedling mortality (log-transformed for analyses) was similar in control (68.27 ± 11.5% SE) and ant removal plots (62.38 ± 11.2% SE; t = 0.24, df = 17, p = 0.81; Fig. 2B) from 2006 to 2007 and from 2007 to 2008 (control = 100%, ant removal =

98.8 ± 1.19% SE; t = −0.96, df = 25, p = 0.35) and did not depend on seedling density or degree of seedling aggregation (measured as nearest neighbor distance). Within ant removal plots, seedling mortality between 2006–2007 was negatively correlated NH_4^+ concentrations in 2005 (R^2 = 0.39, p = 0.04). Second year seedling mortality is

the mortality of seedlings that germinated and survived the first year but not the second year. Second year mortality (log-transformed) was similar across treatments (control N = 19, mean percent mortality = 83.83 ± 10.16 SE, ant removal N = 20, mean percent mortality = 92.40 ± 5.55 SE; t = −0.18, df = 20, p = 0.86) and did not depend on seedling density or degree of seedling aggregation. However, the coefficients of variation for second year mortality differed between treatments (control CV = 36.91, ant removal CV = 20.79), indicating that second year mortality was more variable in control plots.

Effects of ant removal on soil mesofauna

Ant removal did not affect the total abundance of mites or Collembola (MANOVA Wilks' $\lambda_{7,31}$ = 0.79, p = 0.33; Table 2). But both the total abundance of mite and Collembola varied among years (Acari, $F_{2,36}$ = 61.47, p < 0.0001; Collembola, $F_{2,36}$ = 68.68, p < 0.0001), due to an overall decrease in the abundance of Mesostigmatidae mites from 2005 to 2006 and 2007, and increases in the abundance of Prostigmata and Oribatei mites in 2007, Hypogastrurinae and Sminthuridae Collembola in 2006, and Entomobryidae Collembola in 2007. Grouping Collembola into feeding guilds, there were no differences between ant removal and control plots for any of the three feeding guilds across years. When considered alone, the mean number of mites per plot in ant removal plots was 2× greater than in control plots in 2006 ($F_{2,51}$ = 3.33, p = 0.04) but did not differ among treatments in 2005 and 2007. This effect was largely due to the increase in the abundance of Oribatei mites in ant removal plots in 2006 (Time × Treatment interaction, $F_{2,36}$ = 3.29, p = 0.045).

Effects of ant removal on soil nutrients

Total inorganic nitrogen concentrations in ant removal plots were more than 3× higher than in control plots in the first year of the ant removal treatment (June 2005, t = 3.18, df = 1, p = 0.004; October 2005, t = 5.0, df = 1, p < 0.0001). This result was largely driven by the large increase in NH_4, but not NO_3, in ant removal plots in 2005, where concentration of NH_4 was 11.8× higher than in control plots (Fig. 3 A, B). The difference in nitrogen concentrations between treatments disappeared in subsequent years.

DISCUSSION

The experimental removal of ants from a southeastern temperate forest resulted in the aggregation of seedlings around H. arifolia maternal plants in the ant removal plots, likely due to the exclusion of A. rudis, the most common and important seed dispersing ant species in many eastern deciduous forests (Ness et al. 2009, Warren et al. 2010, Zelikova et al. 2008). This result is consistent with the idea that ants (and A. rudis in particular) play a major role in seed dispersal in southwestern deciduous forests. While many studies report the importance of A. rudis and more generally, of seed-dispersing ants, it is often inferred from observed patterns in seed removal but not tested experimentally. Our findings fill an important gap in our knowledge of the spatial and demographic consequences of seed dispersal by ants, specifically shedding light on the critical early demographic stages.

If the function of elaiosomes and more generally, of myrmecochory, is to elicit dispersal of seeds away from maternal plants by ants, we should expect to find fitness benefits associated with dispersal away from parental and sibling competition. Seedling aggregation is expected to be associated with decreased plant fitness if competition or density-dependent processes are driving differential survival of offspring and structuring the spatial distribution of plants (Janzen 1970, Connell 1971). Unexpectedly, in our study, we found that increased aggregation was not associated with decreased seed germination or seedling mortality in the first three years. In fact, germination was higher where ants were removed and seedling mortality increased from the first to the second year of the study across all plots, regardless of treatment, and was 100% in some plots, suggesting that spatial aggregation of seedlings is not a main cause of mortality. In contrast, working at the same field site, Giladi (2004) also saw a high degree of seedling aggregation near fruiting adults and reported that H. arifolia seedling mortality was negatively affected by the presence of neighbors, though seedling mortality did not decrease with distance away from fruiting adults, suggesting interactions with neighboring seedlings, rather than adults, were more important (Giladi 2004).

Table 2. Mean number (with SE below) of Acari and Collembola individuals collected in pitfall traps and leaf litter in control (C) and ant removal (AR) plots between 2005–2007. There were no significant differences in soil mesofauna abundances between control and experimental control plots; therefore, these plots are combined. Missing cells indicate that we were unable to recover specimens from collected material.

| | Pitfall trap | | | | | | Leaf litter | | | |
| | 2005 | | 2006 | | 2007 | | 2005 | | 2006 | |
Soil mesofauna	C	AR	C	AR	C	AR	C	AR	C	AR
Acari										
Prostigmatidae			0.1	0.29	0.03	0.05	1	0.6	2.24	3.11
			(0.07)	(0.16)	(0.03)	(0.05)	(0.2)	(0.17)	(0.48)	(0.63)
Mesostigmatidae			1.29	1.5	0.06	0	5.79	12.05	0.24	0.84
			(0.24)	(0.36)	(0.04)		(0.91)	(2.65)	(0.18)	(0.34)
Oribatei			0.48	0.79	1.21	1.3	19.94	15.95	35.79	57.47
			(0.15)	(0.24)	(0.3)	(0.5)	(5.01)	(2.59)	(3.5)	(8.41)
Total Acari			1.86	2.57	1.29	1.35	26.74	28.9	38.27	61.42
			(0.2)	(0.57)	(0.3)	(0.51)	(5.32)	(4.84)	(3.61)	(8.77)
Collembola										
Entomobryidae	2.42	1.65	1.85	1.55	9.18	7.9	11.95	8.45		
	(0.58)	(0.53)	(0.28)	(0.22)	(0.89)	(0.91)	(2.72)	(1.82)		
Sminthuridae	0.42	0.15	2.26	2.15	2.21	2.75	2.42	2.7		
	(0.14)	(0.08)	(0.35)	(0.54)	(0.36)	(0.47)	(0.61)	(0.64)		
Hypogastruridae	0.16	0	2.47	6.95	0.12	0	0.21	0.05		
	(0.12)		(0.73)	(3.85)	(0.07)		(0.16)	(0.05)		
Tomoceridae	0.37	0.2	0.18	0.4	0.26	0.3	18.94	16.7		
	(0.23)	(0.09)	(0.09)	(0.13)	(0.09)	(0.11)	(3.16)	(2.94)		
Isotomidae	0	0	0.03	0	0	0	1.53	1		
			(0.03)				(0.47)	(0.31)		
Total Collembola	3.47	2	6.82	11.05	20.18	22.25	35.05	30.4		
	(0.81)	(0.61)	(0.89)	(3.97)	(1.77)	(1.78)	(6.03)	(4.82)		

While we saw no evidence that seedling aggregation negatively affected seedling survival in the first year, we did find a negative correlation between seedling mortality and soil ammonium concentrations one year after ant removal, suggesting that nutrient limitation may affect seedling mortality.

Indeed, there is conflicting evidence in the scientific literature regarding the effects of seedling and adult aggregation on measures of fitness (Nathan and Muller-Landau 2000), with some studies reporting positive effects of aggregated spatial structure (Monstesinos et al. 2006, Stoll and Prati 2001, Tirado and Pugnaire 2003), while others negative effects (Anderson 1988, Boyd 2001, Kalisz et al. 1999, Packer and Clay 2000). In our study, we did not find evidence that increased aggregation significantly decreased seedling survival, but it may be that the effects of competition are greater as plants mature and shade each other. Because *H. arifolia* does not mature to reproductive stage for seven years, we were unable to directly examine the effects of aggregation on life-time demographic factors other than seedling survival, though work by

Giladi (2004) and Warren et al. (2010) indicate that an increase in local density decreases survival and growth of *H. arifolia* seedlings and adults. Our study was limited to three years and many of the factors that exert selective pressure on plant survival, such as density dependence and pathogen accumulation, take longer than 3 years to emerge. Thus, it is likely that we were unable to capture the effects of these longer-term processes. Finally, our study focused on one plant species and the intra-specific consequences of seedling aggregation. However, a large body of theoretical and experimental work highlights the importance of environmental and spatial heterogeneity for population and community dynamics (e.g., Chesson 2000, Seabloom et al. 2005). Examining the more long-term consequences of ant removal and seedling aggregation and incorporating these into the broader community context would clearly be exciting avenues of research (e.g., Wardle et al. 2011).

In addition to reducing the effects of density-dependent processes, the deposition of seeds into nutrient-rich microsites, either within ant nests or into refuse piles, is another potential benefit of
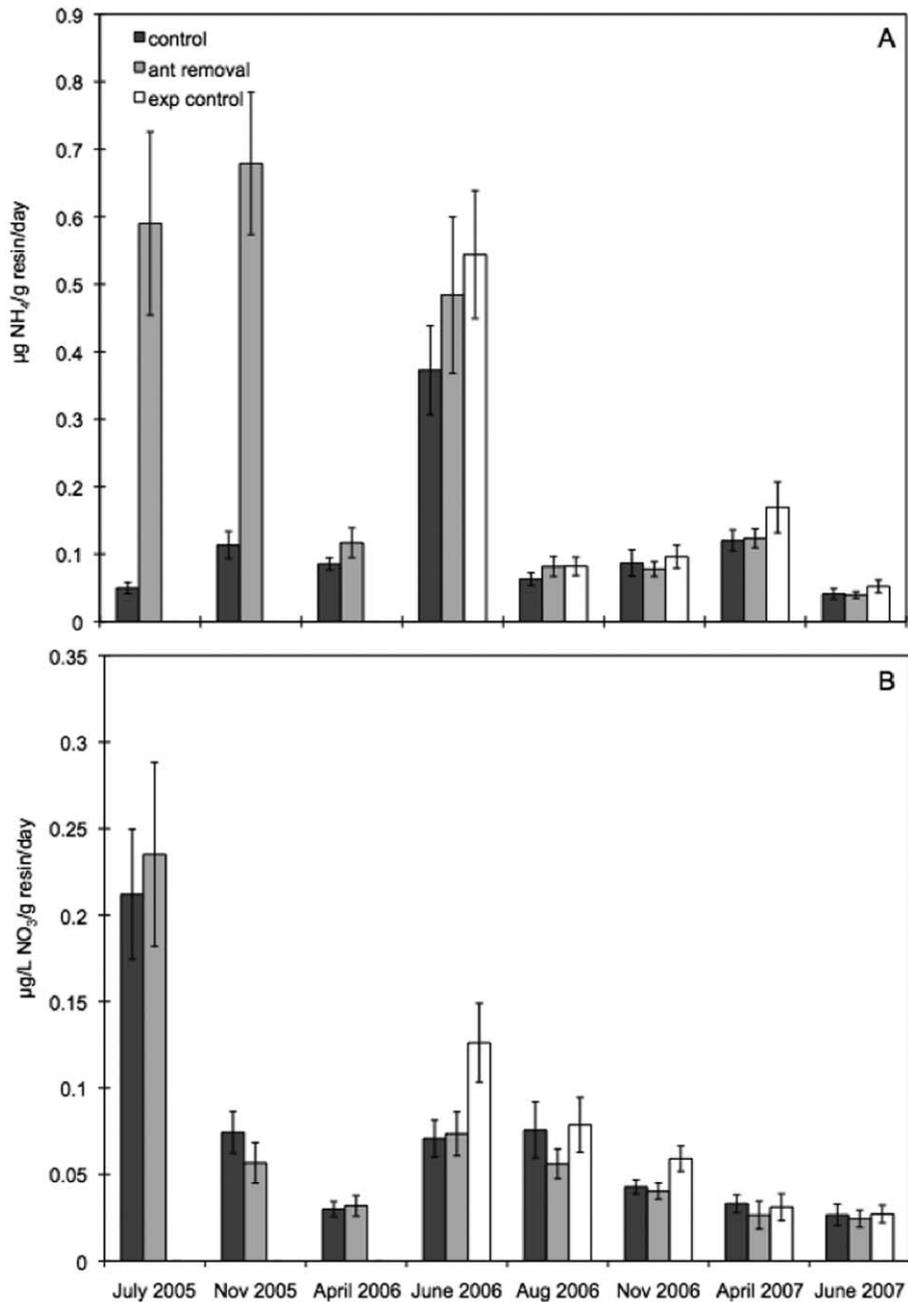
Fig. 3. (A) NH_4^+ and (B) NO_3^- concentrations by treatment and sample date, which denotes the date when the resin bags were set in the field. Error bars represent $\pm$ 1 SE.

seed dispersal by ants (Culver and Beattie 1983, Giladi 2006, Ruhren and Dudash 1996). While we did not directly design our study to address this hypothesis, we can make predictions about nutrient-enrichment based on the measurements

we made. If nutrient enrichment were the case in our study, we would expect to find increased soil nitrogen concentrations in control plots, where ants were present. Instead, we found a temporary increase in soil ammonium concentrations in

ant removal plots relative to control plots and no persistent differences in nitrogen concentrations in the following two years. Furthermore, soil nitrogen did not change in control plots, providing evidence that *A. rudis* ants, and more generally, other ant species at our site do not accumulate soil nutrients in or around their nests. In fact, *A. rudis* colonies have high emigration rates, residing in a nest site for as little as 30 days (Smallwood 1982) with very local (less than one meter) nest relocations (Smallwood 1982). Such high emigration rates may mean that *A. rudis* does not nest in any one location long enough to accumulate nutrients in the soil; additionally, high emigration rates may contribute to a more diffuse accumulation of nutrients and overall heterogeneity of soil nutrients. Regardless of the mechanism, we found no evidence for soil nutrient-enrichment by ant nests in our study.

The increase in seedling emergence in ant removal plots was an unexpected result in light of studies that have shown that when ants handle seeds and remove elaiosomes, seed germination rates increase (Christian 2001, Horvitz 1981, Lobstein and Rockwood 1993, Ruhren and Dudash 1996). However, some studies report the opposite trend and have proposed several possible explanations for the decrease in germination success. In the process of handling seeds, ants may damage the seed coat (Christian and Stanton 2004, Zettler et al. 2001) and increase fungal infection. Ants may also deposit seeds into unfavorable germination sites (Rice and Westoby 1986, Christian and Stanton 2004) such as logs or move seeds into marginal habitat (Giladi 2004). The distribution of *H. arifolia* in the study area is patchy, with distinct boundaries between occupied and unoccupied habitats (Giladi 2004, Warren et al. 2010), suggesting that the distribution of suitable habitat is not continuous. However, ants, especially *A. rudis*, generally disperse seeds short distances (Giladi 2004, Zelikova et al. 2008). Therefore, it is less likely that the seeds are consistently being dispersed into marginal habitat. A final and more likely possibility in our study is that the detection probability for seedling differs between control and ant removal plots. Indeed, first year *H. arifolia* seedlings are inconspicuous, growing underneath the leaf litter. In ant removal plots, where seedlings are clumped around the mater-

nal plant, they are easy to locate; however, in controls, where seedlings are dispersed throughout the plots, we likely did not find all the seedlings that germinated. Additionally, we searched within a 1m^2 grid around the maternal plant, but seeds could have been dispersed out of the plot, further underestimating seedling emergence rates in control plots.

The overall effect of ant removal on belowground processes consisted of the increase in ammonium concentrations and the increase in the abundance of oribatid mites, but only for one year in both cases. It remains an open question why ammonium concentrations increased dramatically when ant colonies were killed, though one possibility is that the excess ammonium is derived from decomposing ant colony members. Another possible explanation is that trenching plots severed plant roots and their subsequent decomposition and mineralization could have led to a nitrogen pulse. However, we found no such pulse in experimental control plots, which were also trenched. Soil mesofauna responses to ant removal proved spatially variable and inconsistent across either treatments or years. While collembolan assemblages were largely unaffected by ant removal, the abundance of oribatid mites increased in ant removal plots one year after the treatment was applied, and overall mesostigmatid and prostigmatid mite abundance decreased regardless of treatment. Mites can contribute significantly to decomposition processes and nutrient turnover (Visser 1985, Siepel and Maaskamp 1994) and Oribatidae mites, which are the most numerous microarthropods in temperate soil ecosystems (Seastedt 1984), largely feed on microbial biomass (Siepel and Maaskamp 1994). One possible explanation for the increase in oribatid abundance is that removing ants released these mites from ant predation (Masuko 1994, Wilson 2005, Milton and Kaspari 2007, Moya-Laraño and Wise 2007), increasing microbial turnover in ant removal plots. We did not assess the effects of ant removal treatments on taxa such as earthworms, spiders, ground beetles, and salamanders. Therefore, we cannot rule out the possibility that taxa other than ants may have influenced soil mesofauna and soil nutrients, though likely not the spatial distribution of seedlings. In either case, the effect of ant removal on nutrient concentrations was short lived and

suggests that the general effect of ants in this community on trophic and nutrient dynamics of the litter and soil may be minimal.

Conclusions

In this system, ants reduce aggregation of *H. arifolia* seedlings, decrease seed germination and have mixed effects on soil nutrients and oribatid mites. It remains to be seen if the reduction in seedling aggregation by ants leads to long-term effects on *H. arifolia* population dynamics. In the intermediate term, we did not see such effects. Nor were there effects of *A. rudis*, or other ant species, on other soil invertebrates or on soil nutrient properties. While there was sometimes a modest increase in mite abundance where ants were absent, the effect was inconsistent. Similarly, the nutrients we measured were unaffected by ants after the first year of study. In short, over the time scales considered here, ants in this system, and the species *A. rudis* that probably handles the vast majority of myrmecochorous seeds in eastern North America, influence the spatial distribution of seedlings, with limited short-term effects on seedlings mortality and inconsistent effects belowground.

ACKNOWLEDGMENTS

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