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How ant nests increase soil biota richness and abundance: a field experiment

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Abstract. Although many studies have shown that ant nests tend to increase soil nutrient concentrations, only a few have examined ant impact on soil biota. To date, no one has examined the mechanism behind this complex 'ant effect.' In this study, we employed a 2×2 complete factorial design (water × food) in the field to mimic the effects of harvester ant nests (*Messor andrei*) on soil. We hypothesized that, in the absence of ants, addition of moisture and food (seeds and insects) would interact to produce conditions found in ant nests. Our results indicated that the addition of food to the soil (regardless of water addition) best mimicked the conditions found inside *M. andrei* nests. Both food-treated and ant-nest soils supported higher numbers of bacteria, nematodes, miscellaneous eukaryotes, and microarthropods compared to the other soil treatments. Microbial richness was also highest in ant and food-treated samples. Moreover, the ant effect in our experiment occurred in just two months. Because ants are a widespread, abundant group with many long-lived species, they could substantially influence soil properties and belowground food webs and may have important restoration/conservation implications for terrestrial communities.

Introduction

Few studies have examined factors affecting species richness for terrestrial microbiota, and soil biota diversity has largely gone unstudied for multiple subgroups (e.g., Boag and Yeates 1998). Major factors affecting the diversity and/or abundance of belowground biota include soil nutrients, moisture, and temperature (Campbell and Biederbeck 1976), physical soil disturbances (Doran 1987), and interactions among fauna (Beare et al. 1992; Wagner et al. 1997; Laakso and Setälä 1998). Ants (Hymenoptera: Formicidae) are a major structuring force in many terrestrial communities worldwide and have various functional roles, such as scavenging, predation, granivory, and omnivory. Because ants belong to a number of guilds and interact with many different taxa ranging from plants to insects to vertebrates, they play a prominent role in structuring diversity and abundance of other taxa in many communities, such as soil biota (Whitford 1996; Folgarait 1998).

Most ants nest in the soil and may affect soil biota via numerous pathways. For instance, ant activity and respiration increase moisture and temperature in

the surrounding soil (Cole 1994; Whitford 1996). Ground-nesting ants increase soil nutrients by carrying aboveground, nutrient-rich material several centimeters belowground (Friese and Allen 1993; Folgarait 1998). Ants also build belowground galleries and tunnels, thereby disturbing and creating new soil structure (Cole 1994). Finally, ants directly interact with soil biota through predation and commensalism (Laakso and Setälä 1997, 1998).

Although belowground and aboveground communities are tightly linked through plants, earthworms, and insect larvae (e.g., Strong et al. 1996; Whitford 1996; Mikola et al. 2001; Preisser 2003; Zak et al. 2003), the two systems are spatially distinct, and ants may be critical in moving aboveground resources to belowground consumers. Unlike other soil-nesters (e.g., termites), most ants are omnivorous and exploit a variety of materials such as seeds, plant tissue, and insect carcasses (Friese and Allen 1993; Whitford 1996). This is especially true for the so-called granivorous ants, which, in addition to seeds, consume an assortment of resources, such as soft- and hard-bodied insects and bird and mammal feces (e.g., MacKay 1981; Hölldobler and Wilson 1990). Ants in the genus *Messor* are major insect granivores and are widespread in arid and semi-arid regions throughout the world (Whitford 1996). Individual colonies of *Messor* are long-lived and can thrive for up to 10 years in a single location (Hölldobler and Wilson 1990, but see also Brown 1999). Harvester ant nests can be one or more meters deep (e.g., MacKay 1981), and an average *Messor andrei* nest is approximately 60 cm wide on the surface (pers. observ.).

We recently reported that *M. andrei* increases abundance and richness of multiple soil taxa and concentrates N, P, and organic matter (OM) in their nests (Boulton et al. 2003). Other researchers have observed similar trends for other ant species (Wagner et al. 1997; Laakso and Setälä 1998; Folgarait 1998). However, we lack experimental evidence for the mechanism behind these 'ant effects,' which could be due to any number of factors, such as ant predation, food storage, excretion/elimination, soil structure, and other ant behaviors or nest qualities. Of all these factors, food and moisture additions to soil are the most reasonable to test experimentally. It would be difficult to mimic ant-nest structure or to add/subtract ant nests (the latter would involve an insecticide, which could damage the soil food web as well). Thus, in this study, we attempted to mimic the effects of *M. andrei* nests on soil and its biota by employing a 2×2 complete factorial design (water $\times$ food). We hypothesized that, in the absence of ants, moisture, and food additions would interact to produce the 'ant effect' by mimicking many of the qualities found in ant nests.

Methods

Study site

Our field experiment was carried out from April–June 2001 in northern California at the McLaughlin Natural Reserve (Napa, Lake, and Yolo

Counties). McLaughlin has a high percentage of serpentine soil, which is characteristically high in magnesium and other heavy metals and low in calcium (UC NRS 2000). The Reserve has a Mediterranean climate – hot, dry summers and cool, wet winters. Summer air temperatures can be as high as 40 °C, while winter temperatures can fall below freezing. Mean annual precipitation is 75 cm. The Reserve lies within the California Floristic Province and supports serpentine mixed chaparral, cypress chaparral, and grasslands (UC NRS 2000). Ten *M. andrei* nests, located on serpentine grassland, were selected for the experiment. These nests were at least 5 m from one another.

Mimicking food and water inputs of ants

The most important component of our field experiment (described in detail below) was the food (seeds and insects) and water additions to the soil. In order to approximate the amount of food added to the soil by *M. andrei* on a daily basis, we collected foragers returning to these same study nests during spring 2000 in order to describe the variety and types of food returned to their nests. The analysis of food items carried by these workers was used to determine what should be added as experimental food. Because we could not imitate ant-nest structure (which includes compartments for larvae, food storage, etc.), we mixed our food additions with non-ant soil (hereafter referred to as 'implant soil'). Most items (83%) returned to the nest by workers were seed material (mean diameter of seeds 1.8 ± 5.9 mm), 13% were insect carcasses or parts, and 4% were leaf material or unidentifiable. There was roughly a 1:6 insect:-seed ratio in our samples, which we mimicked using commercial poppy seeds and crickets. However, the weight of all insects returned to nest (2.9 ± 4.3 g) often equaled the weight of all seeds returned to the nest (3.7 ± 1.8 g) per day. The average weight of all items returned per day to *M. andrei* nest was 6.9 ± 3.1 g. Based on these weight results, we added 8 g of seeds and 6 g of insects to each implant core. Because we were not able to add food continuously to these cores (like the ants do on a daily basis), we doubled the weight of our seeds and insects. Our implant cores were in place for 60 d, but they had a small volume compared to the volume of an entire *M. andrei* nest. For this reason, we did not multiply the original seed and insect weights by 60, which would have mimicked the daily input of *M. andrei* ants over 60 d.

Poppy seeds were selected for our food additions based on their diameter (~1 mm), which approximated the mean diameter of seeds collected from *M. andrei* foragers returning to the nest. The native California poppy, *Eschsholtzia californica*, also occurs throughout McLaughlin (UC NRS 2000) and is a possible seed source for *M. andrei* in nature. Because many of the seeds and insect carcasses were often damaged or carried back to the nest in parts, experimental food was masticated in a food processor after being microwaved for 3 min – both processes prevented the commercial seeds from germinating in

the field. Crickets were used for the insect additions and were frozen for 24 h before being homogenized in a food processor.

The amount of water added to the water and food + water cores was based on saturation of the core (i.e., 50% volumetric water content). In several pilot cores, approximately 8 ml of water was sufficient to saturate a core. Even-though ant nests are rarely fully saturated, saturation was selected for these treatments for two reasons. First, cores were visited weekly, so our water additions had to last longer than 1 or 2 days. Second, implant soil lacked the structure of non-implant soil due to removal and mixing procedures. Unstructured soils do not hold water as well as normal soil (Brady and Weil 1996), which further necessitated over-watering. However, our homogenized, structureless soil implants resembled ant-nest soils because ant nests also contain unstructured soil due to ant tunneling.

Experimental design

We manipulated soil moisture and food additions in soil 3 m away from each nest. Five soil cores were removed at each of the ten nest sites using a bulk density sampler (5 cm × 30 cm). We took one core from the nest center and four cores that were each 3 m from the nest and 1 m from each other. Each core was assigned to one of five treatments: ant nest (no additions), control (no additions), water-addition, food-addition, and food + water-addition cores (see Figure 1). We chose to space our 'satellite' cores 3 m from the nest because ant nests can influence the surrounding soil up to 1–2 m away from the nest center (e.g., Whitford and DiMarco 1995; Dean et al. 1997). Previous work in this system also supports these findings (Boulton et al. 2003), so placing our experimental cores 3 m from the nest center should have constituted non-ant areas.

After the soil core was removed from each site, the soil was mixed and all visible organic matter (e.g., adult ants, pupae, larvae, leaf or seed material) was

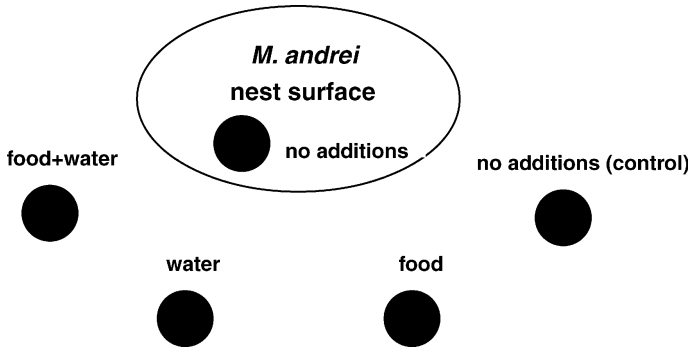

Figure 1. Diagram represents experimental design in the field. Black circles represent manipulated soil cores. Each of the four non-ant cores is 1-m from each other and 3 m from the ant-nest center.

removed and placed in a vial for later analysis. We then collected soil 100 m from our study site, removed visible organic matter, mixed, and implanted it into each of the five vacant cores described above (ant-nest core plus four treatment cores) for a total of 50 experimental cores (10 nest sites times 5 cores/nest site). The soil used from this adjacent site was also analyzed ($N = 3$) for chemistry and microbiota (as described below) to ensure that it was typical, non-ant soil. Hereafter, we use the term 'implant soil' to denote soil that was experimentally treated and used to replace original soil in and near each ant nest.

Water-addition cores at each site received 8 ml of tap water after the soil was implanted into the core. As described in the previous section, food-addition cores were mixed with masticated poppy seeds and homogenized cricket prior to implantation. Food + water cores were a combination of these two core treatments (i.e., mixed with seeds and crickets pre-implantation, as well as watered post-implantation). Soil temperature and moisture were monitored weekly for all implants. The water and food + water treatments received 8 ml of water whenever their volumetric soil content fell below the levels found in the adjacent ant nest. After 60 days, we retrieved each core with the bulk density sampler.

Soil attributes

Before sampling, soil temperature and volumetric water content was recorded at 20 cm depth. Water content was measured using the HydroSense soil probe (Campbell Scientific, Inc.). In the laboratory, 0.5 cm^3 of soil from the collected core was removed in order to measure its pH using pH indicator paper (LaMotte Soil pH Kit). In the laboratory, a sub-sample ($\sim$25 g) of each soil core was passed through a 2-mm mesh sieve, dried, and transported to the Division of Agriculture and Natural Resources (DANR) Analytical Lab at the University of California, Davis for analysis of soil N, P, and OM.

Soil biota

We quantified soil biota richness and abundance following methods described in detail in Boulton et al. (2003). For each soil core, abundance and richness was determined for bacteria, fungi, and other eukaryotes (e.g., ciliates) using phospholipid fatty acid (PLFA) analysis. Nematodes were extracted from soil ($\sim$10 g per sample) using Baermann funnels, and the entire suspension was examined at 140× magnification with a dissecting microscope. Each nematode was identified to feeding guild via mouthpart morphology (Yeates et al. 1993; Bongers and Bongers 1998; Jaffee et al. 1998), which is as effective as high-resolution taxonomy in characterizing food web structure (Parmelee et al. 1995). Finally, microarthropods were extracted from soil ($\sim$30 g per sample) using Tullgren funnels and were identified as mites, collembolans, or miscellaneous microarthropods (e.g., proturans, larvae, or unidentifiable) under 120× magnification.

Data analysis

Data were analyzed using one-way MANOVA with soil treatment as the categorical, independent variable (i.e., ant, control, food, water, and food + water). We used Tukey's test for *a posteriori* comparisons to explore the significant differences across dependent variables due to soil treatment (Sokal and Rohlf 1995). For example, a significant difference between ant soil and another soil category indicated dissimilarity, while no significant difference between ant soils and a given soil category suggested that the two soils were similar to each other for that particular response variable. In order to meet parametric assumptions and to use standard units across dependent variables, all data were transformed into their standard normal deviates, $(Y_i - \mu)/\sigma$. When non-transformed means are reported for a factor, they are followed by their standard deviation. We used the statistical package SPSS (version 10.0.7) for all above analyses. Principal component analysis (PCA) was run using PC-ORD, version 4.0.

Results

Pre-treatment soils

The implant soil was typical of non-ant soil found at this site in that N, P, and OM were significantly reduced and soil taxa were less abundant compared to ant soil. The four non-ant, pre-treatment soil cores ($N = 40$) taken near each nest did not significantly differ from one another in chemical or biological properties. One-way analysis of variance tests on the non-ant, pre-treatment soils yielded insignificant differences for all abiotic and biotic dependent variables (for all tests, df $= 39$, $p > 0.05$). In comparisons between ant and non-ant soil, there were significantly more bacteria, fungi, nematodes, miscellaneous eukaryotes, PLFAs, and microarthropods and higher concentrations of N, P, and OM in ant-nest cores than in non-ant soils (data not shown).

Post-treatment soils

In general, ant, food, and food + water soils resembled each other with higher concentrations of N, P, and OM and with more types and abundance of bacteria, miscellaneous eukaryotes, nematodes, and microarthropods (Table 1). Fungal abundance and soil moisture were the only two dependent variables that did not show this trend. The MANOVA analysis also indicated a significant multivariate effect of soil treatment on the dependent variables ($F_{52} = 7.2$, $p = 0.0001$; Table 2).

The *a posteriori* comparisons consistently revealed significant differences between ant-nest samples and control and water-addition cores, while ant,

Table 1. Results of one-way MANOVA comparing the five soil categories post-treatment: ant, control, water-addition, food-addition, food + water-addition.

Effect	Hypothesis df		Error df			*F*	*p*
Intercept	13.0		33.0			178.5	0.0001
Treatment	52.0		129.9			7.2	0.0001
Variable	Ant-soil core	Control core	Food + water-addition core	Food-addition core	Water-addition core	*F*	*p*
Temperature (°C)	26.1 ± 2.2	28.4 ± 1.2	28.4 ± 1.8	28.1 ± 1.7	28.8 ± 1.1	5.1	0.002
Moisture (%)	5.7 ± 3.3	5.7 ± 9.5	4.8 ± 9.2	5.3 ± 1.5	6.4 ± 1.8	1	0.444
pH	6.6 ± 0.4	7.0 ± 0.0	7.5 ± 0.4	7.1 ± 0.2	7.0 ± 0.0	11.5	0.0001
Nitrogen (%)	0.4 ± 0.1	0.1 ± 0.0	0.4 ± 0.1	0.3 ± 0.1	0.1 ± 0.0	66.3	0.0001
Phosphorous (%)	26.2 ± 28.4	3.5 ± 0.7	63.5 ± 47.1	42.6 ± 46.7	2.8 ± 0.8	45	0.0001
Organic matter (ppm)	4.1 ± 0.9	2.1 ± 0.2	4.6 ± 0.6	4.2 ± 0.5	2.0 ± 0.2	47.9	0.0001
Bacteria abundance	99.8 ± 25.2	51.7 ± 9.3	131.0 ± 26.3	122.0 ± 36.4	52.4 ± 12.1	24.5	0.0001
Fungal abundance	9.5 ± 4.3	5.8 ± 2.3	1.6 ± 2.6	8.0 ± 21.0	6.0 ± 2.4	0.9	0.454
Misc. eukaryote abundance	3.7 ± 0.7	1.2 ± 0.8	3.8 ± 1.4	4.0 ± 2.9	1.2 ± 0.5	8.2	0.0001
PLFA richness	59.9 ± 5.3	44.4 ± 3.7	59.4 ± 6.0	58.2 ± 6.1	43.7 ± 3.9	21.2	0.0001
PLFA abundance	102.5 ± 24.9	46.2 ± 10.5	121.1 ± 21.6	114.4 ± 34.4	46.9 ± 13.0	25.4	0.0001
Nematode abundance	144.1 ± 111.0	80.0 ± 27.7	1577.2 ± 858.7	181.3 ± 98.3	60.0 ± 22.7	28.2	0.0001
Microarthropod abundance	18.7 ± 7.7	3.1 ± 5.4	11.7 ± 7.2	12.8 ± 11.2	3.5 ± 3.3	10.2	0.0001

Notes: Although non-transformed means and standard deviations are listed, all data were transformed to their standard normal deviates for the analysis in order to meet parametric assumptions. Upper part of table refers to multivariate tests, while the lower part lists the results from univariate *F* tests for each of the dependent variables. For all variables, the total degrees of freedom = 49. Abundance refers to number of individuals per sample (sample size defined in Methods).

Table 2. Results of *a posteriori* comparisons from MANOVA results, which examine the similarities and differences between ant cores and each experimental treatment.

Dependent variable	Ant vs. control	Ant vs. food + water	Ant vs. food	Ant vs. water
Temperature (°C)	−0.26*	−0.30*	−0.34*	−0.26*
Moisture (%)	0.00	0.08	0.03	−0.06
pH	−0.86*	−1.62*	−0.95*	−0.82*
Nitrogen (%)	0.59*	−0.14	−0.36	0.66*
Phosphorous (%)	1.36*	−0.75	−0.54	1.03*
Organic matter (ppm)	0.51*	−0.13	−0.05	0.56*
Bacteria abundance	0.65*	−0.56*	−0.39	0.65*
Fungal abundance	0.39	0.79	0.31	0.44
Misc. eukaryote abundance	1.44*	−0.65	−0.36	1.36*
PLFA richness	0.57*	0.33	0.09	0.81*
PLFA abundance	0.60*	−0.45	−0.42	0.64*
Nematode abundance	1.13*	−2.85*	−0.07	1.15*
Microarthropod abundance	1.45*	0.90*	0.08	1.50*

Notes: This is a partial listing of MANOVA unplanned comparisons for soil category using the Tukey test on mean differences. Abundance refers to number of individuals per sample (sample size defined in Methods). Mean differences are reported only for ant soil vs. all other soil types. The standard normal deviates were used for this analysis.
*$p < 0.05$.

food + water, and food cores generally similar (Table 2). This finding applied to N, P, and OM, miscellaneous eukaryotes, and PLFA richness and abundance. Exceptions to this result are as follows. Ant-nest cores were significantly different from all other soil treatments for soil temperature (cooler in ant nests vs. all other soil categories) and pH (more acidic in ant vs. non-ant soils). Soil moisture and fungal abundance did not differ across any of the five soil categories. The *a posteriori* results revealed that ant cores were similar to food-addition cores, but significantly different from food + water-addition cores for bacteria, nematode, and microarthropod abundance (Table 2).

Because there was collinearity among response variables, we performed a PCA (Tabachnick and Fidell 1996). The first two eigenvalues explained the majority of the variance in the data (58.8%). Axis one was composed of bacterial abundance, PLFA richness and abundance, and N, P, and OM. For axis two, nematode and fungal abundance and soil pH and temperature loaded high. The bivariate plot suggests two groups, similar to the MANOVA findings above: ant-nest soils group loosely with food- and food + water-addition soils, while the control and water-addition cores form a separate, tight cluster (Figure 2).

Food web characteristics in post-treatment soils

Based on our PLFA results, our post-treatment samples included all the major bacterial subgroups, such as methyl-, saturated-, unsaturated-, iso-, anteiso-, and branched-bacteria (Bossio and Scow 1998). One-way ANOVAs indicated

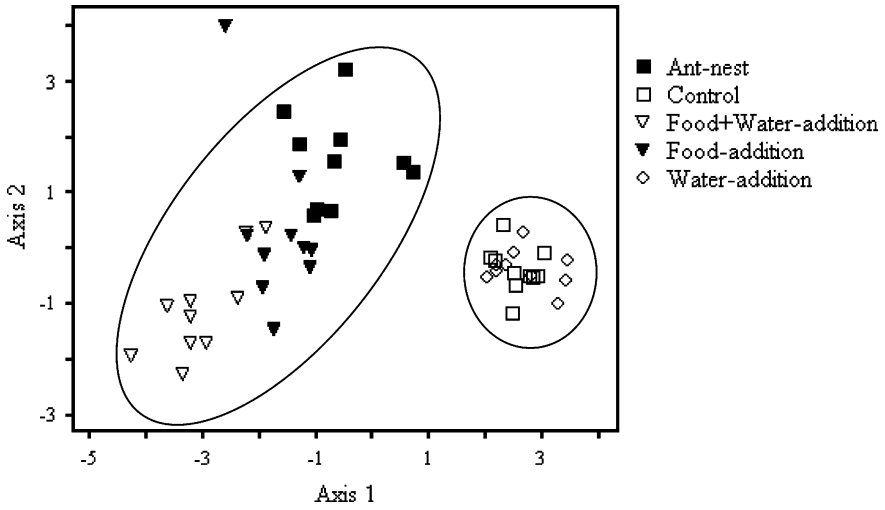

Figure 2. Scatter plot of factor scores for the 50 samples according to a PCA of 10 response variables.

that bacterial subgroups were significantly different across soil treatment: saturated $F_{49} = 11.8$, $p < 0.0001$; unsaturated $F_{49} = 5.6$, $p < 0.001$; iso $F_{49} = 29.4$, $p < 0.0001$; anteiso $F_{49} = 33.1$, $p < 0.0001$; methyl $F_{49} = 6.4$, $p < 0.0001$; and branched $F_{49} = 29.6$, $p < 0.0001$. In general, the food + water treatment had the most bacteria across bacterial subgroups, while ant and food cores resembled each other with the second highest amount; the control and water-addition cores had the fewest individuals across all bacterial subgroups.

Nematodes in all feeding groups were up to 10× more abundant in the food + water treatment compared to the other treatments. Ant and food soils were most similar to each other and had the next highest number of nematodes, while the control and water cores had the fewest nematodes. With the exception of plant parasites and predaceous nematodes, the number of individuals in each feeding guild was significantly different across treatments: bacterivores $F_{49} = 15.0$, $p < 0.0001$; fungivores $F_{49} = 25.9$, $p < 0.0001$; and omnivores $F_{43} = 3.6$, $p < 0.01$.

Fungivorous nematodes were numerically dominant across all soil treatments, accounting for 79% of all nematodes identified. Bacterivores were 14% of the remaining nematodes, omnivores were 3%, and predators and plant parasites were each $< 1\%$ (3% of the total could not be identified). Of the fungivores, 9% were *Hexatylina* spp., 9% were *Tylenchus* spp., and the vast majority (82%) were *Aphelenchoides* and *Aphelenchus* spp. Bacterivores consisted of individuals from the orders Rhabditida and Araeolaimida. Omnivores were from the order Dorylaimida, and the few predators identified were from the order Mononchida.

We obtained few microarthropods from our control and water-addition samples. Mites, collembolans, and miscellaneous microarthropods each were most abundant in ant, food, and food + water samples, with ant soils containing the overwhelming majority of all microarthropods. The abundance of these animals significantly differed across soil treatments: mites $F_{49} = 5.1$, $p < 0.01$; collembolans $F_{49} = 6.7$, $p < 0.0001$; miscellaneous microarthropods $F_{49} = 10.4$, $p < 0.0001$. Mites belonged to the Opilioacariformes and Acariformes groups, and collembolans were from the families Onychiuridae and Entomobryidae. The miscellaneous category included various proturans, mite and insect larvae, and unidentifiable arthropod specimens. We did not observe any beetles, earthworms, or other macro-invertebrates in any of our samples.

Ant impact on fresh soil

Ants affected implant soil in 2 months. Depauperate, nutrient-poor soils added to ant nests contained significantly more bacteria, nematodes, microarthropods, and other soil biota and had higher levels of N, P, and OM than controls (Table 1). When implant soil from ant nests was compared to ant soil from the pre-treatment samples, it had significantly more bacteria, nematodes, microarthropods, and other soil biota and had higher levels of P and OM (data not shown). The three exceptions to this trend were no change in soil nitrogen and higher soil moisture and pH in the pre-treatment nest-soils compared to the implant nest-soils.

Discussion

We successfully mimicked many aspects of ant nests and their influence on belowground chemistry and biota through food additions. A surprising result was that *M. andrei* 'manipulated' the depauperate soil placed in their nests during our 2-month experiment by significantly increasing soil nutrients and organismal abundance and richness. This suggests that ant effects on soil food webs and nutrients can occur quickly, which may have important implications for restoration work as discussed below. Because the food + water effect was much greater than the ant effect for some variables, the reduced particle size of our food via a food processor could have increased the rate of decomposition and subsequent soil changes, which would explain this discrepancy between ant and food + water soils.

Although we predicted that food and water would interact to mimic ant-nest effects, food additions alone explain most of the variation in soil biota and nutrients at this site during early summer months. Our results indicate that soils from the ant cores most resemble soils from the food + water and food cores. Our water additions were effective only in combination with the food

additions. This is further supported by the fact that the control implants (no additions) were most similar to the water-only treatment for the majority of the dependent variables. These results were consistently obtained for the sub-groupings within each taxon. Bacterial, nematode, and microarthropod sub-groups tended to be most similar between the ant, food + water and food cores, while the control and water soils had the lowest abundance for each of the sub-types. Although our moisture treatment was ineffective by itself, it is striking that the food + water treatment had the greatest abundance for the majority of the sub-groups.

There were visible fungal hyphae on top of the food and food + water cores when we retrieved them in the field; however, our MANOVA results indicate that fungi did not differ between treatments. Since large numbers of fungivorous nematodes, as obtained in our results, indicate high fungus production, absence of increased fungus biomass in those samples could be due to strong top–down effects of fungivores or to a failure in our PLFA analysis. PLFA markers are well developed for bacteria, but fungal markers are less well documented (White and Findlay 1988; Bossio and Scow 1995). Thus, our lack of findings for the fungi could be due to PLFA limitations and not to a real trend in nature.

Mites, collembolans and other microarthropods were significantly more abundant in ant soils than in all other cores, eventhough similarities were shared between the ant, food + water, and food treatments. There are two possible explanations for this result. First, there are many variables associated with ant nests that we could not mimic. For example, *M. andrei* probably affects the soil structure and/or behaves in ways that might facilitate colonization by these microarthropods. Second, because microarthropods were already present in greater numbers in pre-treatment ant soils than in non-ant soils, they probably colonized the ant cores more quickly than the treatment cores, which had a relatively depauperate microarthropod community before our experimental manipulation.

Our moisture treatment was ineffective, as evidenced by our MANOVA results. We may have overestimated the importance of soil moisture in this system, or we may have implemented the moisture treatment too early in the season when moisture was not a limiting factor. Had this experiment been carried out in the hottest summer months (July–September), perhaps we would have been able to capitalize on more dramatic soil moisture differences between ant and non-ant soil.

This research is the first mechanistic approach to the influence of ant nests on soil chemistry and biota. Our results align with previous studies showing that ants increase soil nutrients and the abundance of most soil taxa (Wagner et al. 1997; Laakso and Setälä 1998). Boulton et al. (2003) showed that *M. andrei* nests at this same site have higher concentrations of N, P, and OM and more abundant soil taxa. Although many studies have examined how ants affect soil chemistry, only a handful of studies have shown that ants positively affect soil food webs. Our results suggest that *M. andrei* exerts such a positive

effect primarily via the addition of food, mostly seeds. Moreover, this ant effect can occur quickly – in just 2 months based on our findings. Because ants are widespread and are the most abundant eusocial insect with many long-lived species, they could substantially influence soil and belowground food webs in a number of ecosystems.

The results we report here have important implications for conservation and restoration. In terms of conservation, countless studies have suggested a variety of factors that negatively or positively associate with biota richness and abundance, although few attempt to unravel the mechanism behind such a relationship. Our findings show the relationship between a given variable and biota richness/abundance and then examine experimentally how this effect occurs. From a restoration perspective, native ant species could be crucial in improving soil quality for re-establishing indigenous animals and plants. For this reason, researchers have attempted to protect and/or restore ants to various wooded areas in Europe and Canada (e.g., Pavon 1950, 1960; Bradley 1972). Our research focuses on one species of ant on serpentine soil, so future work should address how this ant effect varies by season, habitat, and ant species.

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