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No evidence of top-down effects by ants on litter decomposition in a temperate grassland

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Abstract. Ants play multiple roles in ecosystems, but their ability to affect decomposition processes in temperate grasslands is relatively unknown. We investigated whether the suppression of ant populations influenced litter decomposition in grasslands via predation of some decomposers (e.g., mites and springtails) and/or microbial activity and composition. We performed two successful ant suppression treatments (seven weeks, 37% suppression, year 1, 10 weeks, 70% suppression, year 2) over the course of a 59-week experiment. We then assayed the effects of ant suppression using coarse- and fine-mesh litterbags and evaluated litter chemistry, microbial and arthropod communities, and microbial enzyme activity. Ant suppression efforts reduced ant abundance and altered ant, arthropod decomposer, and non-ant predator community composition. However, ant suppression did not affect decomposer arthropod abundance, litter mass loss, microbial composition, or enzyme activity in litterbags. Litterbag mesh size did not alter microbial composition, perhaps due to a failure to exclude decomposers, as mites and springtails were more or equally abundant in fine-mesh bags. Nevertheless, mesh size did change litter chemistry, suggesting that mesh size-mediated microenvironments affect decomposition environment regardless of invertebrate exclusion. Coarse-mesh litterbags had higher concentrations of microbial sugars, lignin, and N and lower concentrations of litter C and crystalline cellulose than fine-mesh litterbags. Litterbag mesh size may alter decomposition processes irrespective of invertebrate abundance. We found no evidence that ant predation was an important driver of decomposer populations or decomposition in these systems, and we suspect redundancy at the top of the detrital food web dilutes the role of ants.

Key words: *Lasius neoniger*; litter chemistry; litterbags; microarthropods; soil organic matter; trophic interactions.

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Introduction

Biodiversity within an ecosystem can be important for maintaining ecosystem processes. However, habitat loss, global changes in temperature and precipitation, and catastrophic weather events threaten biodiversity globally (Hoekstra et al. 2005, IPCC 2019). Losing

individual species can also diminish functional diversity, which can in turn result in a loss of important ecosystem processes such as pollination and decomposition (Potts et al. 2010, Parr et al. 2016). Given the continued threats to biodiversity, it is increasingly important to document how individual species may alter ecosystem processes.

Ants are dominant and ubiquitous components of terrestrial ecosystems that exert a disproportionate effect on ecosystem processes relative to their size (Del Toro et al. 2012). As consumers and ecosystem engineers, ants can redistribute food resources within a habitat and concentrate these resources within nest structures (Cammeraat and Risch 2008, Frouz et al. 2008), which may alter the diversity and abundance of soil microbes and arthropods (Boulton and Amberman 2006). By altering soil conditions and associated bottom-up effects, ants can increase nutrient availability to plants (Frouz et al. 2008, Bierbaß et al. 2015). However, ants can also exert strong top-down control of invertebrates in many terrestrial systems (Sanders and Platner 2006, Parr et al. 2016, Wills and Landis 2018) with the potential to affect ecosystem processes such as decomposition, especially in northern temperate regions (Nemec 2014). Ecologists have described the effects of other invertebrate predators, notably spiders, on prey dynamics and decomposition (Hawlena et al. 2012, Schmitz et al. 2013), but similar investigations in ants have not been conducted.

In the north central USA, ants are predominantly ground nesting and generally opportunistic foragers that exploit many different food sources (Gregg 1946, Wheeler 1994, Coovert 2005, Ellison 2012). Ants adjust their diet seasonally depending on food availability and colony needs (Clark and King 2012, Caut et al. 2013). For example, ants may shift diet from predation (insect-based protein) to more plant-based food items throughout the season (Kajak et al. 1972, Kim et al. 2019). Ants' shifting trophic position amplifies the breadth of impact ants may have on ecosystems, as they may interact with many different soil functions (Briones 2014, Frouz 2018). As predators of microbial consumers, ants have the potential to indirectly stimulate or suppress microbial populations by suppressing arthropod consumers such as mites and springtails. In a deciduous forest, ant removal was shown to increase oribatid mites, but did not affect collembola (Zelikova et al. 2011). Excluding arthropod decomposers also has been shown to affect microbial processes in surface soil such as decomposition (Bradford et al. 2002, Soong et al. 2016). Plant litter decomposition is the first step toward grassland C accumulation; however, the soil fauna effect on decomposition may be positive, negative, or neutral, because fauna may transfer litter to a different part of the soil profile without altering total mineralization (Cotrufo et al. 2015, Frouz 2018).

Working in grasslands, Wills et al. (2019) showed that reduction in ant abundances decreased the suppression of sentinel insect prey (Lepidopteran eggs). We build on this study to evaluate the effects of ant suppression on soil arthropod and microbial communities and on litter decomposition and litter chemistry. Relatively little work explores the roles of ants as consumers in temperate grasslands, and particularly lacking are studies addressing the effect of ants on decomposers (Wills and Landis 2018). We addressed the questions: (1) How does ant suppression affect soil invertebrate and microbial communities? and (2) Does ant suppression affect the rate of litter mass loss in litterbags? In addition, we used coarse- and fine-mesh litterbags (1.7 mm and 50 μm) to include or exclude arthropod decomposer micro- and meso-fauna, groups that are expected to speed decomposition (Bradford et al. 2002). If ant abundance reduced arthropod decomposer populations, we would expect to observe a larger effect of ants on decomposition in coarse-mesh bags, where invertebrate decomposers act as shredders of plant material and create more surface area and opportunity for enzymatic decay. We surveyed arthropod decomposers, microbial community and abundance, extracellular enzyme activity (EEA), and litter chemistry to describe the decomposition environment under high and low ant population conditions and with and without arthropod decomposers (coarse- vs. fine-mesh).

Materials and Methods

Site description

We conducted this work at Brooklyn Natural Wildlife Area in Dane County, Wisconsin (owned by the Wisconsin Department of Natural

Resources, 42°52′3.08″ N, 89°29′18.22″ W) a prairie restored in 2004 on Gale silt loam (Soil Survey Staff and Natural Resources Conservation Service 2015). The site, plot design, and ant treatment were previously described in Wills et al (2019), though the present study occurred in 2015–2016 while previous research ended in 2015. Vegetation consisted of grasses (e.g., *Schizachyrium scoparium*, *Panicum virgatum*, and *Elymus canadensis*) and wildflowers (e.g., *Rudbeckia hirta*, *Solidago rigida*, and *Chamaecrista fasciculata*). In June 2015, we established eight 8 × 8 m plots in four pairs. One plot within each pair was randomly selected as the control and the other designated an ant poison treatment to suppress ant abundances. Plots within each pair were separated by 20 m and each pair separated by 30 m. The plots were considered independent because foraging of ant species in Wisconsin is generally limited to several meters (0–5 m) from the nest (Pudlo et al. 1980, Traniello and Levings 1986, Ness et al. 2004) and preliminary experiments at this site (2014) indicated that poison baits did not impact ant abundance >5 m from baits. Each plot was further subdivided into four 4 × 4 m quadrants with a sampling station established in the center of each quadrant (i.e., 2 m from any edge and 4 m from any other sampling station). At each station, we deployed baits (control or poison) and an ant pitfall trap (see Pitfall traps below) each separated by 0.5 m. The published foraging distances for temperate grassland ant species ranges from <0.5 to 10 m, and thus, the density of baits provided enough coverage within the experimental plots, while minimizing the effect of poison baits on control plots 20 m away (Pudlo et al. 1980, Traniello and Levings 1986, Ness et al. 2004).

To suppress the ant populations, we mixed an insecticide, fipronil (Termidor SC, BASF Corporation, Research Triangle Park, North Carolina, USA), into a honey bait and a peanut butter bait with a concentration of 0.0095% (w/v). Fipronil is slow acting (allowing for transfer between individuals) and effective at reducing survival of both queens and workers (Hooper-Bui and Rust 2000). Fipronil also readily mixes with sugar or protein-based attractants. In the ant poison treatment plots, poison baits were deployed as a single point source at the center of a sampling station (two baits per 4 × 4 m). Control plots received both types of baits (honey and a peanut butter bait) of equal volume containing no insecticide. The baits were set out in 20-mL scintillation vials fitted with 0.5-cm^2 hardware cloth covers to exclude other predaceous arthropods larger than ants (Wills et al. 2019). Baits were replaced each week from 10 June to 22 July 2015 and from 13 May to 15 July 2016.

Pitfall traps

To sample ground-dwelling arthropods, we used pitfall traps (100-mL specimen cups with 5 cm diameter opening) filled with a 50:50 mixture of propylene glycol and water to preserve insects. Pitfall traps were placed in the four corners of each plot for 48 h and collected weekly for the duration of fipronil poison treatments (7 weeks in 2015, 10 weeks in 2016). We avoided sampling during periods of heavy rainfall to avoid overflow. From pitfall samples, all ants (Formicidae) were identified to species. Non-ant ground-dwelling arthropods were also recorded to either subclass, order, or family, including beetles (Carabidae, Staphylinidae); spiders (Lycosidae, Linyphiidae, Salticidae, and Thormisidae); crickets (Gryllidae); harvestmen (Opiliones); earwigs (Dermaptera); slugs (Gastropoda); isopods (Isopoda); millipedes (Diplopoda); springtails (Collembola); and mites (Acari). The beetles, spiders, crickets, harvestmen, earwigs, and slugs were considered predators because they have been observed consuming sentinel egg prey (Grieshop et al. 2012). We considered isopods, millipedes, springtails, and mites as decomposers. Mites are generalist consumers of bacteria, fungi, and dead plant material found in soil and litter (Schneider-Carsten and Maraun 2004, Wehner et al. 2016). Springtails were included because ants are known predators of springtails (Reznikova and Panteleeva 2001) and because springtails are important decomposers in grasslands (Bonkowski and Roy 2012).

Litterbags

Litterbags were constructed with nylon mesh of two sizes (1.7 mm coarse, and 50 μm fine) and polyester thread. In the coarse-mesh litterbags, 1.7-mm mesh was used only for the upper surface and 50-μm mesh was used for the lower surface, to reduce mass loss through the bottom of the bag. The coarse-mesh bags were expected to exclude only macro-fauna, for example, earthworms, slugs, insect larvae, and allow access to

mites, springtails, and isopods (Bradford et al. 2002). In fine-mesh litterbags, the entire bag was made of 50-μm mesh. All litterbags were 10 × 10 cm and filled with 6.0 g oven-dried switchgrass (*P. virgatum*) stalks and leaves harvested in Madison, Wisconsin in March 2015, dried at 60°C, and cut into 2- to 5-cm pieces. All bags were placed 2 m from the central bait station, alternating between fine and coarse-mesh sizes. Bags were placed in contact with the soil, pinned with landscaping staples, and covered by the surrounding vegetation. A total of 32 bags were laid out in each plot (2 mesh sizes × 4 collection time points × 4 bags per time point). Litterbags were deployed on 16 June 2015, one week after the start of the fipronil poison treatments. Bags were collected at 4 time points: 8 weeks (after 7 weeks of 2015 fipronil poison treatment), 22 weeks (prior to winter freeze), 51 weeks (after 2 weeks of 2016 fipronil poison treatment), and 59 weeks (after 10 weeks of 2016 fipronil poison treatment). Note that only the 8-week pickup and the 59-week pickup represent time points after intensive ant suppression. The 22- and 51-week time points allowed us to investigate whether any early effects of ant suppression on decomposition process persisted after ants had the opportunity to re-colonize the ant suppression plots. In addition, the 51-week time point represents the beginning of the second ant suppression period, establishing a new baseline for interpretation of the data collected at the end.

At each collection time point, four bags of each mesh size were collected from each plot. Two of the bags were used for microarthropod extraction and mass loss determination, and two were used to determine EEA, lipid biomarkers, and plant cellulose, polysaccharides, and lignin. Using a 2.5-cm soil probe, soil immediately below each litterbag was sampled to 5 cm depth at the same time. Samples were transported on ice to the UW-Madison in coolers. Soil was gently scraped off of litterbags before they were dried at 60°C and weighed. Mass loss (dry weight basis) was corrected for soil contamination after heating 0.5-g subsamples of ground litter in a muffle furnace to 370°C for 1 h and 450°C for 4 h. Soil contamination increased from 6% of total mass at 8 weeks, to 10% at 59 weeks. Litter and soil samples were weighed field-moist and dried at 60°C to estimate moisture at pickup time points.

Microarthropod extraction

Berlese funnels (Macfadyen 1953) were used to extract microarthropods from soil and litterbags into ethanol. Litter or soil (approximately 50 mL volume) was placed onto 50-μm mesh below a light bulb in a 10°C refrigerator for one week. Very few organisms (0 or <3) were collected from soil samples, so these data are not presented. In litter samples, mites, springtails, isopods, spiders, ants, dipteran larvae, millipedes, and beetles were identified under a 10–40× dissecting microscope, but only mites and springtails were common enough to be included in the analysis.

Extracellular enzyme activity

All EEA analyses were carried out within 24 h of collection of litterbags from the field, as freezing and refrigerating samples can affect EEA (Wallenius et al. 2010). A 0.5-g litter or 1-g soil subsample were analyzed for activity of α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosidase, β-D-cellobiosidase, L-Leucine-7-amidomethylcoumarin, phosphatase, and β-xylosidase (AG, BG, NAG, CELL, LAP, PHOS, and XYL, respectively) using a well-established procedure modified from Saiya-Cork et al. (2002) and Bell et al. (2013). Enzyme-specific fluorescent-labeled substrate was mixed with a slurry of sample and buffer and incubated to allow enzymes to encounter the substrate. Enzyme activity for all enzymes was summed at each time point. The reaction took place in microplate wells (n = 16) and fluorescence was measured using a microplate fluorometer with 365-nm excitation and 450-nm emission filters, using standard curves (developed separately for each sample) to calculate enzyme activity.

Lipid biomarker analysis

Lipid analysis was carried out on freeze-dried samples as described in Oates et al. (2017). Ground litter samples from litterbags or soil (from below litterbags) were extracted with a single-phase solution of phosphate buffer: $CHCl_3$:CH_3OH (0.8:1:2). The extracted lipids were saponified with NaOH–CH_3OH, converted to fatty acid methyl esters with CH_3OH–HCl, and subsequently analyzed with a Hewlett-Packard 6890 gas chromatograph (Agilent Tech, Santa Clara, California, USA) using a 25 m × 0.2 mm × 0.33 μm Agilent J&W Ultra-2 (5% phenyl)-methylpolysiloxane capillary

column (Agilent) and flame ionization detector. Fatty acid methyl esters were identified using MIDI's Sherlock software and their EUKARY and TSBA40 databases (Microbial ID, Newark, Delaware, USA). Single indicator lipids were used to evaluate the relative abundance of actinobacteria (10Me16:0, 10Me18:0), gram-negative (16:1ω7c), gram-positive (i15:0), arbuscular mycorrhizal fungi (AMF, 16:1ω5c), and other fungi (18:2ω6,9c; Zelles 1997, 1999, Olsson 1999, Ratledge 2008, Frostegård et al. 2011).

Litter chemistry

Chemical analyses of litter were carried out in the DOE-Great Lakes Bioenergy Research Center's Cell Wall Facility for sugars according to Santoro et al. (2010) and lignin monomers according to Albersheim et al. (1967) and Selvendran and O'Neill (2006). After oven-drying, subsamples were ground to a fine powder and analyzed for percent C and N by dry combustion using a Flash EA 1112 CN Automatic Elemental Analyzer (Thermo Finnigan, Milan, Italy).

Statistical analysis

Arthropod counts from the four pitfall traps within a plot were pooled weekly. We used Simpson's Diversity index (1-*D*) to estimate diversity within different insect taxonomic groups for each plot by week. We analyzed abundance and diversity of ants, decomposers, and non-ant predators using repeated measures ANOVA (PROC MIXED, SAS v9.4; SAS Institute, Cary, North Carolina, USA). Treatment and week were treated as fixed effects and plot as a random effect using an auto-regressive covariance matrix. All abundance data were $\log_{10}(x + 1)$ transformed to normalize data residuals. Because years differed significantly in ant and non-ant ground-dwelling arthropod abundance (see *Results*), we analyzed each year separately. We used a redundancy analysis (RDA) and permutation test to visualize and assess treatment effects using the *vegan* package in R (R Core Team 2016, v3.4.0). Taxa with fewer than three observations were excluded (Kindt and Coe 2005), and the abundances of remaining groups were $\log_{10}(x + 1)$ transformed.

All litterbag data were analyzed with a three-way ANOVA for a split-split plot randomized complete block design with ant (poison or control) as the whole-plot treatment, mesh (coarse or fine) as the split-plot treatment, and time point (8, 22, 51, 59 weeks) as the split-split plot treatment within each of four blocks. This is equivalent to a repeated measures analysis with a compound symmetry correlation structure. Analyses were conducted using PROC MIXED (SAS v9.4; SAS Institute 2004). Enzyme data and PLFA guild data were $\ln(x)$ transformed to normalize data residuals as necessary. Post hoc testing among treatments at $\alpha = 0.05$ was conducted by comparing least squares means using PROC LSMEANS in SAS with the Tukey-Kramer adjustment for multiple comparisons. When interactions with time point occurred, treatments were compared at each time point. Microbial communities were visualized using non-metric multidimensional scaling on distance matrices constructed using the Bray-Curtis method within the vegan package in R (R Core Team 2016). A PERMANOVA test was conducted using adonis to evaluate the effects of ant treatment, mesh size, and time point on microbial community composition.

Results

Invertebrate responses

We found a significant decrease in ant abundance in the ant treatment plots relative to control plots in both 2015 (37% reduction, $F_{1,18.2} = 15.68$, $P < 0.001$) and 2016 (70% reduction, $F_{1,21.6} = 56.43$, $P < 0.001$; Fig. 1, Table 1). In 2015, we collected 1496 ants in control plots and 929 in poison plots, and in 2016, we collected 574 ants in control and 172 ants in poison plots. We were relatively more effective in 2016 at suppressing ant abundances likely because we started baiting earlier in the season, before peak ant activity. Notably, there was a large drop off in ant abundance week 1 to week 2 in 2015. One trap in a control plot in week 1 was moved because it was too close to a nest, trapping 244 ants in week 1, and it is not uncommon for ants to shift nests in response to physical disturbance (McGlynn 2012), in this case from replacing the pitfall. The 2015 poisoning reduced the populations of numerically dominant species including *Lasius neoniger* (Emery) and *Formica montana* (Wheeler), which are known to prevent the establishment of less dominant ant species in grasslands (Table 1; Moranz et al. 2013). In 2016, other

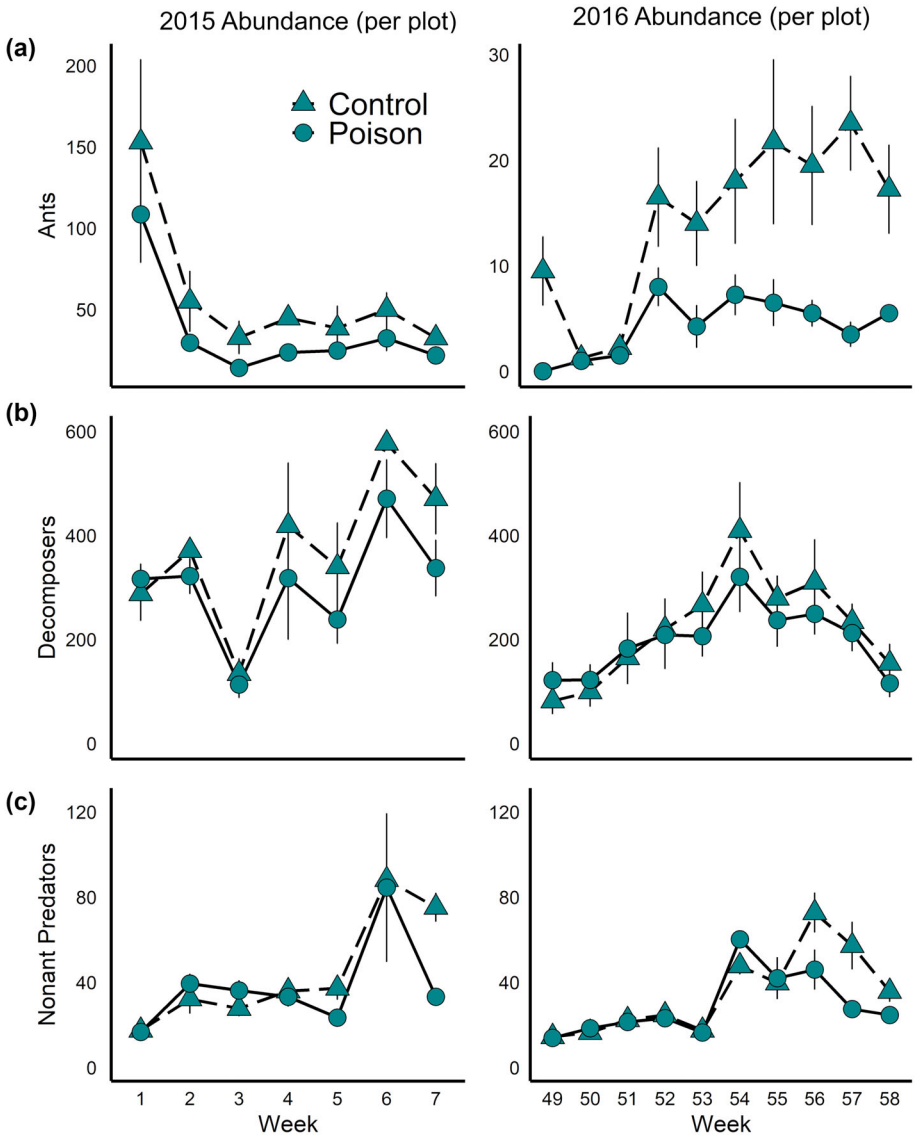

Fig. 1. Mean (± standard error, $n = 4$) total abundance and diversity of (a) ants, (b) decomposers, and (c) non-ant predators by week per plot from pitfalls collected between 10 June to 22 July 2015 and 13 May to 15 July 2016.

species like the less competitive *Prenolepis impairis* (Thomas Say) were more common, increasing ant diversity (Appendix S1: Fig. S1). The RDA showed an effect of ant treatment on ant community composition in both 2015 ($P = 0.001$) and 2016 ($P = 0.001$, Appendix S1: Fig. S2). In 2015, multiple ant species appeared to be affected by the poison, but only *L. neoniger* was consistently reduced by the poison treatment and contributed to the difference among arthropod decomposer communities across both years (Appendix S1: Fig. S2a).

We did not observe a significant effect of ant treatment on the diversity or abundance of total non-ant predators in 2015 ($F_{1,7.99} = 1.31$, $P = 0.183$) or 2016 ($F_{1,24} = 4.05$, $P = 0.056$; Table 1, Appendix S1: Fig. S1) suggesting minimal non-target effects on other ground foraging predators. However, crickets and carabid beetles were more common in control than poison plots ($P < 0.005$,

Table 1. *P* values from the repeated measures ANOVA for (log-transformed) abundances of ants, decomposers, and non-ant predators (per plot) found in pitfall traps.

	2015			2016		
Source	Ant abundance	Decomposer abundance	Non-ant predator abundance	Ant abundance	Decomposer abundance	Non-ant predator abundance
Ant treatment (Treatment)	**0.0009**	0.2853	0.1830	**<0.0001**	0.8521	0.0555
Week	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Treatment × Week	0.9635	0.5874	**0.0016**	**0.0003**	0.3414	0.0617

Bold cells represent $P < 0.05$.

Table 2. Relative abundance (as percentages) of organisms collected in pitfall traps throughout the duration of the ant poison treatment in 2015 and 2016.

	2015		2016	
Organism	Control	Poison	Control	Poison
Ant species				
Lasius neoniger	88.81	65.68	84.84	70.35
Formica montana	6.27	22.22	4.18	21.51
Aphaenogaster rudis	0.80	0.39	1.57	2.33
Lasius alienus	0.00	0.59	0.35	1.16
Temnothorax ambiguus	0.12	0.49	1.05	0.00
Formica argenta	0.43	0.79	1.05	3.49
Prenolepis impairs	0.00	0.10	6.10	0.58
Solenopsis molesta	1.91	3.74	0.17	0.58
Myrmica nearctica	0.68	4.52	0.17	0.00
Crematogaster cerasi	0.18	0.10	0.17	0.00
Ponera pennsylvanica	0.00	0.29	0.35	0.00
Stenamma brevicorne	0.80	1.08	0.00	0.00
Total no. specimens	1626	1017	574	172
Non-ant predators				
Carabids	1.75	1.03	3.94	2.89
Staphylinids	2.86	4.21	1.93	2.89
Spiders	41.21	72.47	61.96	73.83
Harvestmen	0.95	1.40	1.29	2.38
Crickets	44.95	10.49	21.35	6.03
Slugs	8.27	10.39	9.53	11.98
Total no. specimens	1257	1068	1396	1177
Decomposers				
Isopods	0.22	0.06	0.11	0.03
Millipedes	0.05	0.07	0.20	0.15
Springtails	96.68	94.36	90.46	89.53
Mites	3.05	5.51	9.23	10.30
Total no. specimens	10,396	8460	8875	7905

crickets, $P < 0.05$, carabids, Table 2) suggesting species-specific responses to the ant treatment. A week × treatment interaction in 2015 in non-ant predators mainly stemmed from a large decline in non-ant predators in poison treatment plots in the last week of 2015 (Fig. 1, Table 1).

There was no significant effect of ant treatment on decomposer abundance ($F_{1,7.99} = 1.31$, $P = 0.285$) or 2016 ($F_{1,8.02} = 0.04$, $P = 0.852$; Table 1). Within pitfalls, week-to-week variation in decomposer abundance was greater than ant treatment differences (Fig. 1, Table 1). Within litterbags, mites and springtails both responded significantly to mesh size and time point, but not ant treatment (Fig. 2, Table 3). More mites were found in fine-mesh bags (Fig. 2b), and populations of both mites and springtails peaked at 22 weeks. For springtails (Fig. 2a), mesh × time point and mesh × ant × time point interactions suggest that there was no consistent effect of mesh or ant treatment on their abundances.

Litter decomposition and chemistry responses

Mass remaining in litterbags decreased significantly over time but was not affected by ant treatment or mesh size (Fig. 3a, Table 3). There was greater soil contamination of the litter in coarse-mesh bags (16% vs. 12% of total mass in coarse- vs. fine-mesh bags, data not shown, $P = 0.01$). Litter C concentration was greater in fine-mesh bags (Fig. 3b, Table 3). Litter N increased over time but did not differ by mesh size or ant treatment (Fig. 3c). Soil C and N concentrations were greater under coarse-mesh bags and increased over time (Fig. 3d, e). Litter moisture was highly variable over time, with a significant three-way interaction between poison, mesh, and time point ($P < 0.05$, Table 3). Only in control plots at 8 weeks were fine bags more moist than coarse bags (Table 3, data not shown).

Mesh size altered litter chemistry throughout the experiment, but ant treatment did not. Broadly, the hexose (rhamnose, mannose, fucose, and glucose) concentrations were greater in coarse-mesh litterbags and increased over time

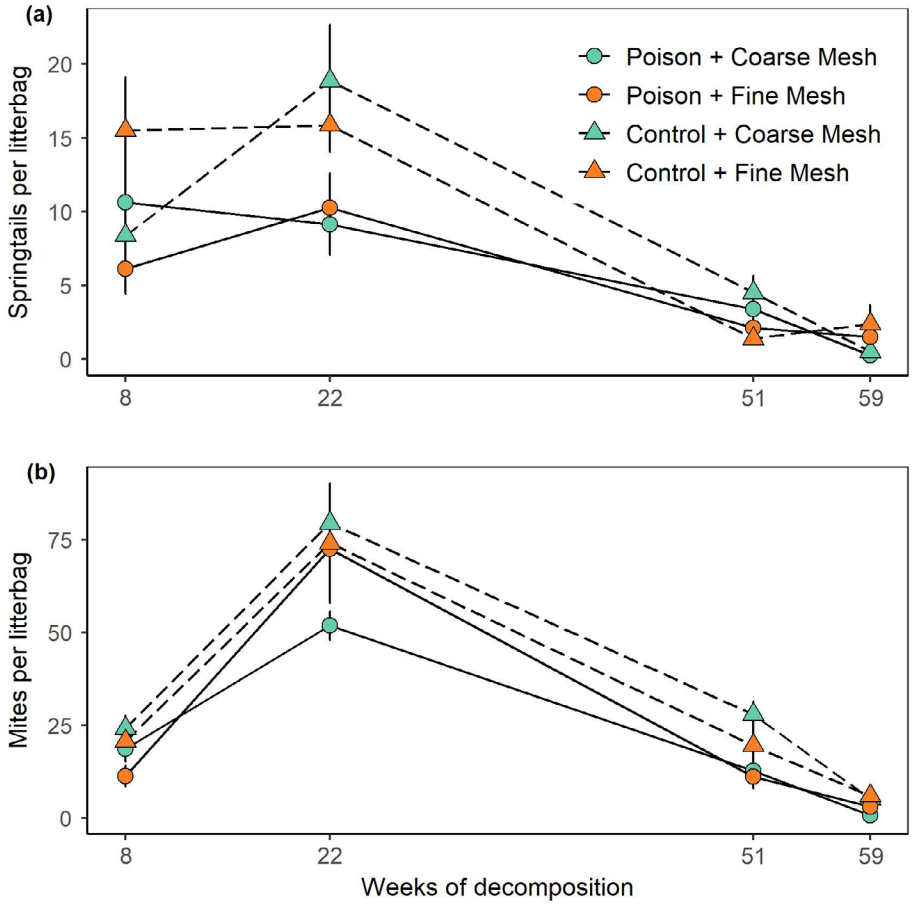

Fig. 2. Mean (± standard error) of springtails (a) and mites (b) extracted with Berlese funnels from litterbags over the course of decomposition. Ant poison treatment took place between weeks 1 and 7, and 49 and 58. See Table 3 for statistical comparisons among treatments and sample time points.

while all other polysaccharide concentrations decreased over time (Tables 4, 5). Common plant compounds including arabinose, xylose, and crystalline cellulose were preferentially depleted in coarse-mesh litterbags. Lignin concentrations also increased over time across mesh and ant treatments. The GM/AX ratio of (galactose + mannose)/(arabinose + xylose), considered an indicator of relative contributions of microbial sugars (Murayama 1984, Amelung et al. 2008), increased over time and was greater in coarse-mesh litterbags at 22 and 59 weeks.

Microbial activity, as assayed by EEA, microbial biomass, and composition, was not affected by ant treatment in litter or soil (Table 3). The total EEA for litter and soil, as well as CELL, PHOS, AG, BG, LAP, and XYL, declined sharply after 8 weeks (Fig. 4a, b). This trend was slightly different for NAG activity, which peaked at 22 weeks and declined in 2016 (data not shown). In addition, activities of CELL and BG in litter were correlated with litter cellulose and glucose concentrations across time points and treatments (Appendix S1: Fig. S3). In litter, total microbial lipid biomass increased over time (Fig. 4c, $P < 0.01$). Microbial composition significantly shifted over time (PERMANOVA $P = 0.001$), but ant and mesh size treatments did not influence microbial community (Fig. 5a). The soil lipid composition also changed significantly by time point only (PERMANOVA $P = 0.001$, Fig. 5b), and total lipid biomass in soil samples was lower at 51 weeks than previous pickups (Fig. 4d, $P < 0.01$).

Table 3. ANOVA *P* values for litter mass loss, litter fauna, litter and soil C and N, total microbial biomass, total extracellular enzyme activity (EEA).

Source	Mass remaining	Litter C	Litter N	Soil C	Soil N	Litter mites	Litter springtails	Litter moisture	Litter EEA	Soil EEA	Litter lipids	Soil lipids
Ant	0.611	0.232	0.892	0.0582	0.112	0.930	0.975	0.417	0.913	0.798	0.752	0.415
Mesh	0.636	**0.007**	0.757	**0.0548**	**0.0505**	**0.0498**	**0.0057**	0.331	0.237	0.999	0.718	0.780
Ant × mesh	0.536	0.175	0.585	0.0771	0.650	0.353	0.317	**0.0245**	0.139	0.634	0.493	0.736
Weeks	**<0.0001**	**<0.001**	**<0.0001**	**<0.0001**	**0.006**	**<0.0001**	**<0.001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.005**	**<0.0001**
Ant × weeks	0.322	0.240	0.227	0.612	0.551	0.37	0.466	0.233	0.279	0.532	0.744	0.224
Mesh × weeks	0.229	0.205	**0.0326**	0.246	0.978	0.589	**0.0346**	**0.0734**	**0.0557**	0.750	0.738	0.656
Ant × mesh × weeks	0.749	0.283	0.862	0.55	0.392	0.324	**0.0332**	**0.0473**	0.515	0.478	0.094	0.120

Bold cells represent $P < 0.05$.

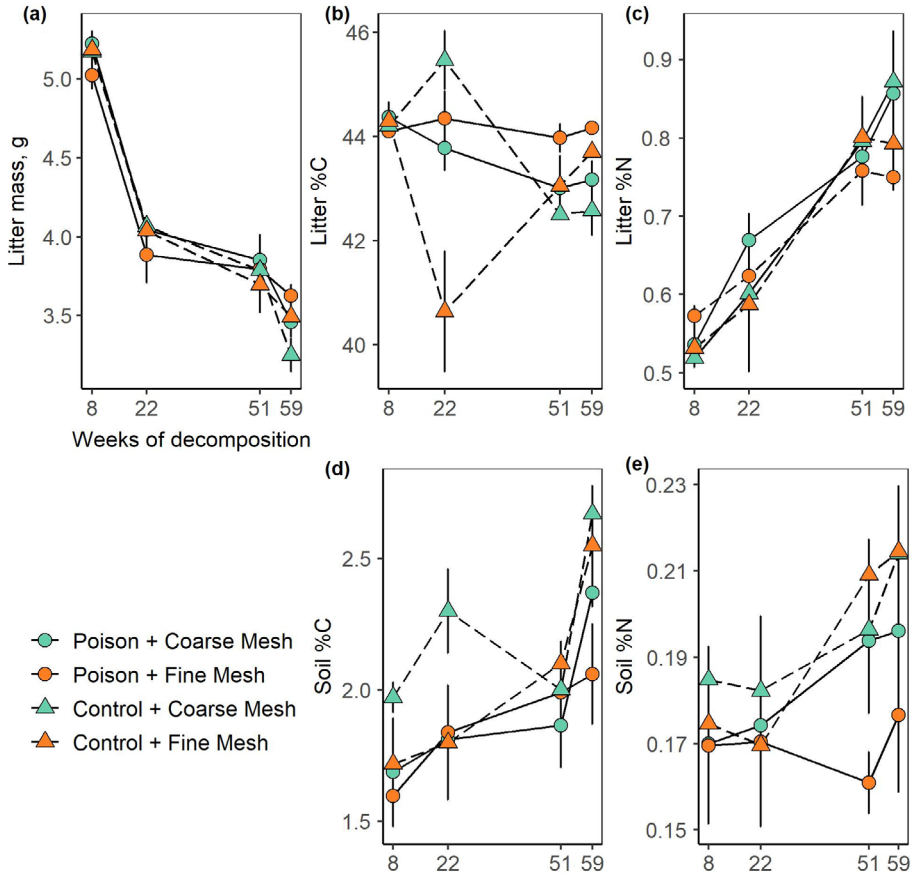

Fig. 3. Litter mass (a), and litter (b, c) and soil (d, e) C and N concentrations over the course of decomposition. Ant poison treatment took place between weeks 1 and 7, and 49 and 58. Soil was sampled 0–5 cm immediately below litterbags. See Table 3 for statistical comparisons among treatments and sample time points.

Some lipids can be used as indicators of specific microbial guilds, such as actinomycetes, AMF, non-AMF fungi, gram-negative (Gm−), and gram-positive bacteria (Gm+). In litter, all indicator lipid guilds increased over time, with the highest concentrations of lipids usually

Table 4. TableLitter concentrations (mg/g litter) of various compounds over the course of decomposition by weeks (8, 22, 51, 59) and mesh size (coarse, fine).

Ant	Lignin	Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose	Crystalline cellulose	GM/AX	Hemi-cellulose
8											
Coarse											
Poison	23.43^{AB}	1.34^{AB}	0.34^{A}	31.95^{E}	227.47^{C}	1.63^{A}	6.93^{A}	28.47^{C}	430.12^{D}	0.033^{A}	269.64^{D}
Control	23.23^{AB}	1.36^{AB}	0.36^{A}	32.41^{E}	224.49^{C}	1.69^{A}	6.88^{A}	30.46^{C}	427.98^{D}	0.033^{EA}	267.20^{D}
Fine											
Poison	22.89^{ABC}	1.40^{A}	0.37^{AB}	33.05^{DE}	224.93^{C}	1.63^{A}	7.15^{AB}	29.33^{BC}	430.43^{D}	0.034^{A}	268.52^{D}
Control	23.42^{ABC}	1.32^{A}	0.34^{AB}	32.36^{DE}	227.74^{D}	1.47^{A}	6.57^{AB}	30.22^{BC}	439.02^{D}	0.031^{A}	269.80^{D}
22											
Coarse											
Poison	24.72^{CD}	1.56^{ABCD}	0.40^{AB}	25.89^{A}	175.77^{A}	2.77^{BC}	6.85^{A}	25.53^{AB}	344.17^{BC}	0.048^{B}	213.25^{A}
Control	24.85^{CD}	1.56^{ABCD}	0.46^{AB}	25.62^{A}	171.26^{A}	2.98^{BC}	6.93^{A}	25.62^{AB}	336.92^{BC}	0.050^{B}	208.81^{A}
Fine											
Poison	24.75^{BC}	1.43^{AB}	0.35^{AB}	26.93^{A}	190.14^{AB}	2.21^{B}	6.70^{AB}	24.38^{A}	372.53^{C}	0.041^{B}	227.76^{AB}
Control	24.28^{BC}	1.52^{AB}	0.42^{AB}	27.35^{A}	182.46^{AB}	2.43^{B}	6.97^{AB}	24.19^{A}	365.37^{C}	0.045^{B}	221.14^{AB}
51											
Coarse											
Poison	24.32^{ABCD}	1.80^{CDE}	0.59^{BC}	27.07^{AB}	180.29^{AB}	3.64^{C}	6.62^{A}	23.99^{A}	345.36^{BC}	0.050^{B}	220.00^{ABC}
Control	23.65^{ABCD}	1.74^{CDE}	0.55^{BC}	27.05^{AB}	181.09^{AB}	3.13^{C}	6.36^{A}	24.50^{A}	354.16^{BC}	0.046^{B}	219.93^{ABC}
Fine											
Poison	24.25^{ABCD}	1.77^{CD}	0.46^{AB}	27.80^{AB}	184.77^{A}	2.79^{BC}	6.68^{AB}	22.37^{A}	359.98^{BC}	0.045^{B}	224.27^{AB}
Control	24.27^{ABCD}	1.71^{CD}	0.46^{AB}	27.83^{AB}	183.65^{A}	2.80^{BC}	6.65^{AB}	23.24^{A}	357.79^{BC}	0.045^{B}	223.10^{AB}
59											
Coarse											
Poison	25.16^{CD}	2.54^{F}	0.74^{D}	30.47^{BC}	187.43^{AB}	4.17^{D}	7.87^{B}	23.70^{A}	301.21^{A}	0.055^{C}	233.23^{BC}
Control	25.26^{CD}	2.54^{F}	0.75^{D}	28.90^{BC}	179.77^{AB}	4.29^{D}	7.61^{B}	23.45^{A}	291.46^{A}	0.058^{C}	223.85^{BC}
Fine											
Poison	25.61^{D}	2.11^{E}	0.59^{CD}	30.55^{CDE}	200.84^{B}	3.18^{CD}	7.05^{AB}	20.90^{A}	341.13^{B}	0.044^{BC}	244.32^{C}
Control	24.52^{D}	2.16^{E}	0.66^{CD}	31.25^{CDE}	196.15^{B}	3.39^{CD}	7.62^{AB}	21.78^{A}	326.22^{B}	0.048^{BC}	241.24^{C}

Notes: GM/AX is the ratio of (galactose + mannose)/(arabinose + xylose). Different letters following means represent differences among mesh × weeks interactions. Ant poison treatment took place between weeks 1 and 7, and 49 and 58.

Table 5. TableANOVA results (*P* values) for litter chemistry over the course of decomposition.

Source	Lignin	Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose	Crystalline cellulose	GM/AX	Hemi-cellulose
Ant	0.675	0.8155	0.3512	0.6701	0.4052	0.1957	0.5808	0.2774	0.3218	0.1734	0.5446
Mesh	0.3332	0.0506	0.5029	0.837	0.1692	0.1149	0.7371	0.5321	**0.0249**	0.3018	0.2348
Weeks	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Ant × mesh	0.2097	0.4526	0.056	0.2513	0.5732	0.239	**0.0441**	0.5297	0.4915	0.0899	0.8599
Ant × weeks	0.6711	0.7596	0.5088	0.9427	0.5545	0.173	0.3319	0.7359	0.5045	0.0888	0.7248
Mesh × weeks	0.7616	**0.0015**	0.1674	0.763	**0.0455**	**0.0084**	0.3152	0.3231	**0.0401**	**0.0062**	0.1028
Ant × mesh × weeks	0.2218	0.8425	0.7526	0.3037	0.8561	0.4515	0.1872	0.8713	0.8416	0.5954	0.9032

Notes: GM/AX is the ratio of (galactose + mannose)/(arabinose + xylose). Ant poison treatment took place between weeks 1 and 7, and 49 and 58.

Bold cells represent $P < 0.05$.

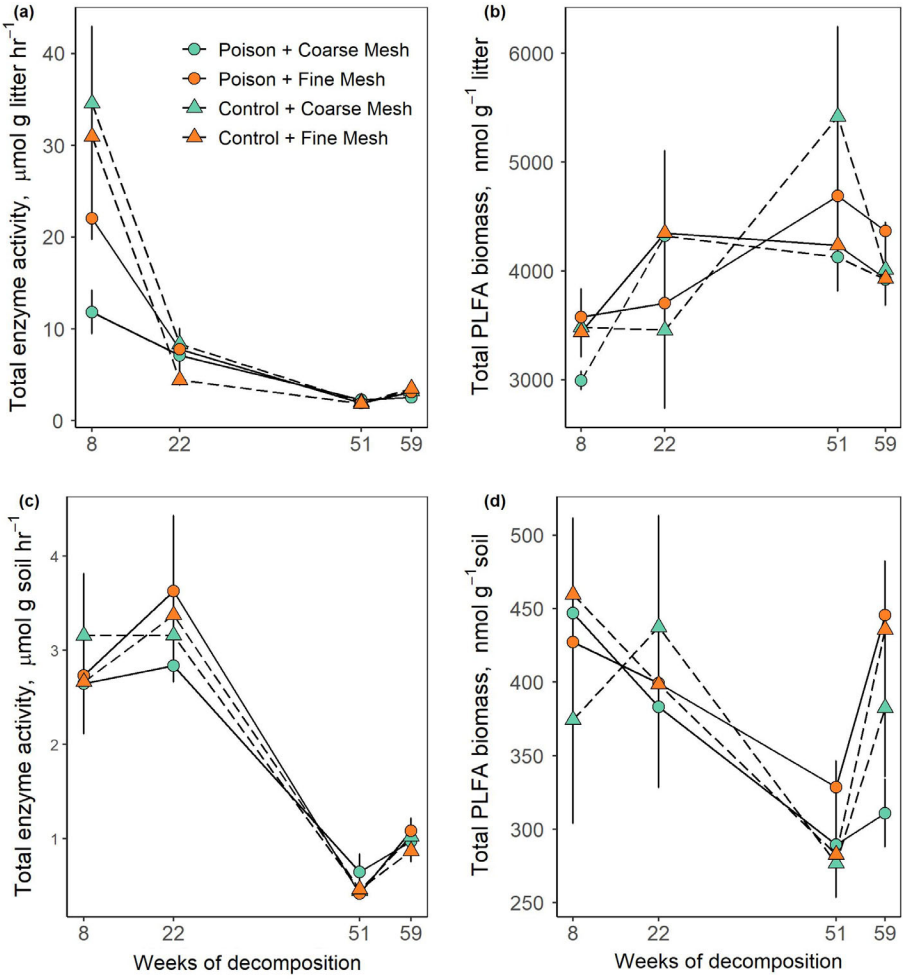

Fig. 4. Microbial activity in litter and adjacent soil over the course of decomposition. Summed extracellular enzyme activity in (a) litter, and (b) soil and total microbial biomass as measured by lipid extraction in (c) litter and (d) soil. Ant poison treatment took place between weeks 1 and 7, and 49 and 58. Soil was sampled to 5 cm immediately below litterbags. See Table 3 for statistical comparisons among treatments.

found at 51 or 59 weeks (Appendix S1: Table S1). The litter Gm+/Gm− ratio increased over time but the litter fungi:bacteria ratio did not significantly change over time. In soil, all guilds except actinomycetes changed by time point, with no consistent patterns across guilds (Appendix S1: Table S2). Soil non-AMF fungi were greater early in decomposition at 8 and 22 weeks, while soil AMF were relatively depleted only at 51 weeks. Both Gm+ and Gm− bacterial were significantly depleted at 51 weeks, and the soil fungi:bacteria ratio was significantly lower. The soil Gm+/Gm− ratio, often used to indicate microbial stress, increased over time. There was no effect of ant treatment or mesh on any microbial guilds in soil or litter (Appendix S1: Tables S1, S2).

Discussion

No effects of ant suppression on decomposition and decomposers

We found no effect of ant reduction on decomposition rates or populations of arthropod and microbial decomposers, which was surprising given their ability to serve as both predators and

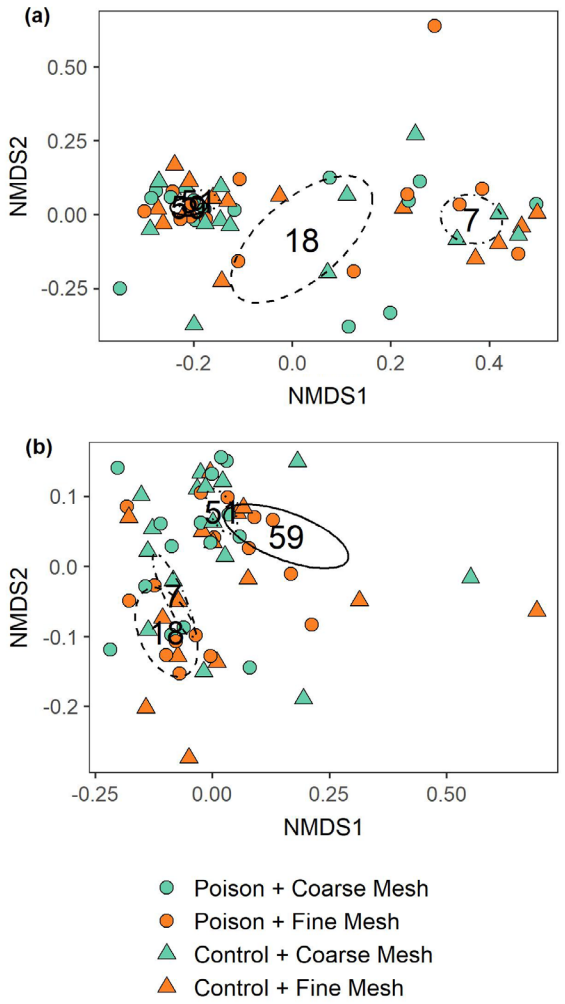

Fig. 5. Non-metric multidimensional scaling (NMDS) plots of lipid peaks in (a) litter (stress = 0.13) and (b) soil (stress = 0.14). There was no effect of ant treatment or mesh size, but there was a significant effect of time point in both soil and litter (PERMANOVA $P < 0.01$). Lines represent 95% confidence ellipses for the area covered by each literbag pickup time point, labeled by number of weeks of decomposition. Ant poison treatment took place between weeks 1 and 7, and 49 and 58. Soil was sampled to 5 cm immediately below litterbags.

herbivores. The lack of ant effects suggests that there may be complex or indirect interactions with other members of the food web, bringing functional redundancy to these diverse grasslands. Redundancy at the top of the detrital food web may limit the potential for a single-species-mediated trophic cascade on decomposition while other ecosystem features are more sensitive. For example, Schmitz (2009) found that predator type was correlated with plant community, plant C:N, and N mineralization, but not overall decomposition rate. Removing various ant species including *Aphaenogaster picea* (Wheeler 1908) and *Camponotus pennsylvanicus* (De Geer 1773) from a hemlock forest did not change decomposition of cellulose or lignin or total soil respiration (Kendrick et al. 2015). In contrast, Liu et al. (2014) found that increasing spider density decreased springtail abundance and slowed decomposition rate in fine-mesh bags without changing total microbial biomass. At our site, the most abundant ant species was *L. neoniger* (Table 2), and while often described as an omnivore (see Del Toro et al. 2015: Appendix S1), it is known to be a common predator in open grassy areas (Kirk 1981, López and Potter 2000, Wills et al. 2019). There is some evidence that *Lasius niger* consumes springtails (Reznikova and Panteleeva 2001), and broad evidence of predation by ants continues to grow (Grieshop et al. 2012, Nemec 2014). But in the case of *L. neoniger* and other ants at this site, other arthropod species may have effectively back-filled the predatory effects of *L. neoniger* as its abundance waned.

Previous work has shown that ants in grasslands are commonly observed removing sentinel prey items (Grieshop et al. 2012) and are likely significant predators of lepidopteran eggs at this site (Wills et al. 2019). However, it is possible that ant predation on decomposers is a relatively minor part of the overall flow of matter and energy in these grasslands because ants rely on multiple food sources (King 2016). For example, previous work at this site showed that ants are flexible in their trophic breadth and position (Kim et al. 2019). High rates of seed predation also suggest that ants have abundant resources at this site (Wenninger et al. 2016) and may be leaning more toward herbivory than predation. Therefore, the removal of ants may not have had any significant direct effects on decomposition because ants were not important consumers of decomposers at this site. Further research into ant and other arthropod diets would help future studies disentangle these complex interactions and their impacts on ecosystem processes.

Without acting as decomposers, ants can play an important role in decomposition by consuming or competing with predators that do consume decomposers. For example, Schuch et al. (2008) found that on both springtail and spiders (e.g., Linyphiidae) were more common on ant nests than further away, which was attributed to a change in soil condition which attracted springtail and spiders. However, from a methodological standpoint, detecting ant suppression effects in litterbags may be difficult because the mesh-confined litter presents a homogeneous and increasingly unappetizing resource over the course of decay. In our study, very few decomposers were found in litterbags after 22 weeks. Wickings et al. (2012) also reported that detritivore density was highest in the first year of a three-year litterbag study, suggesting that more decomposed litter in litterbags does not provide attractive food sources for mites and springtails. Previous studies have used litterbags and pitfalls to quantify the effects of ants on belowground arthropods (Zelikova et al. 2011) but may be insufficient to sample the breadth of decomposer communities. In our case, we were able to assay arthropod populations via pitfall traps, litter, and soil samples simultaneously. Combining soil and pitfall traps has been shown to uncover more species of springtail in diversity studies (Querner and Bruckner 2010), but we found very few springtail in our soil samples. Shallow and infrequent soil sampling probably limited our ability to detect effects of our treatments in soil-dwelling species of springtail and mites, but the agreement of litter sampling and pitfall trap data increases our confidence in that these decomposers did not respond to ant abundance manipulation.

We do acknowledge that our treatment design could have precluded detecting effects. In a similar study, Parr et al. (2016) that found ants affected both decomposition and herbivory in 100 × 100 m plots separated by 500 m. Other studies in temperate north temperate regions have used relatively small plots (2–4 m^2) to examine the effects of ants on decomposers (Sanders and Platner 2006, Schuch et al. 2008, Zelikova et al. 2011). While our plots were significantly smaller than 10,000 m^2, they are relatively larger than similar studies and reasonable considering the baiting design (point source poison baits with a physical exclusion barrier). While broadcast bait applications would have enabled us to expand our treatment plots, we would introduce greater potential for non-target effects of the poison baits. Plot size and distance between plots (20 m) were selected because the ants commonly found in Wisconsin have foraging ranges of <0.5–10 m (Pudlo et al. 1980, Traniello and Levings 1986, Ness et al. 2016). Thus, the distance separating the experimental plots minimizes the effect of poison baits on adjacent plots. Given the foraging behavior of ants in Wisconsin grasslands, we are confident the non-significant effect of ant treatment on decomposers is most likely a result of the nature (possibly none), strength (possibly weak), or, as discussed below, spatial variability of the interactions within the site.

The trophic effects of ants on decomposition may be limited to the immediate nest vicinity and our random placement of litterbags would have dampened this signal. *L. neoniger* is a ground-nesting, generalist ant species, common in open areas in the Midwest (Wodika et al. 2014), that builds diffuse polydomous nest structures with satellite nests (Traniello and Levings 1986). Ant nests alter various soil properties, though not in a consistent direction (Farji-Brener and Werenkraut 2017, Zhang et al. 2018). The nests of *Lasius flavus* and *L. niger* may have lower soil N and higher C mineralization rates (Frouz et al. 2003, Holec and Frouz 2006), higher soil N (Wu et al. 2010), or higher potential N mineralization but lower potential C mineralization (Bierbaß et al. 2015). Collembola were also denser near *L. niger* colonies than 2 m away (Schuch et al. 2008). *L. niger* is common to grasslands in Europe and closely related to *L. neoniger* from our study. In another grassland experiment, *L. niger* and *L. flavus* showed slower decomposition of cellulose filter paper in 0.1-mm mesh in nests than surrounding soil (0.5–2.5 m distance, Holec and Frouz 2006). Ant nests typically make up a small portion of the total surface area of the soil (1–11%, Lobry de Bruyn 1999), and we do not know how far their influence extends. Dissolved organic carbon under ant nests has been shown to be higher compared to samples taken just 30 cm away in a European deciduous forest (Stadler et al. 2006). A spatially explicit sampling design accounting for nest placement might more

fully assess the extent of ant impacts on decomposition and other soil properties in grasslands.

Mesh size moderated decomposing litter chemistry irrespective of food web community

By deploying coarse- and fine-mesh litterbags, we hoped to examine decomposition processes with and without arthropod decomposers in addition to microorganisms; however, we were not successful in excluding decomposers from fine-mesh litterbags. Since all litterbags contained mites and springtails, we conclude that fine-mesh bags did not successfully exclude decomposers, which may have entered the bags as juveniles or through holes made as bags were pinned to the soil surface. Differences in litter chemistry, however, reveal that the mesh size of litterbags affected decomposition processes regardless of decomposer abundance.

Our study confirms previous suspicions that decomposition differences found between fine- and coarse-litterbags, traditionally attributed to arthropod exclusion, should be interpreted with caution (Bradford et al. 2002, Kampichler and Bruckner 2009, Xie 2020). One meta-analysis found that soil fauna sped litterbag decomposition across global biomes, but the effect was only significant in a deciduous forest (Frouz et al. 2015). Fine-mesh bags in our study had, at different time points, elevated GM/AX, litter C, and hemi- and crystalline cellulose concentrations despite similar or slightly greater abundances of arthropods, and all these differences must have stemmed from other elements of the decomposition environment. For example, coarse-mesh bags may allow for increased litter loss or leaching (Kampichler and Bruckner 2009) and increased soil contamination (as we found), which may allow for greater microbial colonization in coarse-mesh litter bags. Slightly warmer temperatures and higher moisture were noted in fine-mesh bags in a boreal forest (Bokhorst and Wardle 2013), which ought to speed decomposition, but we did not find consistently higher moisture in fine-mesh bags. When moisture was manipulated in spruce litterbags of 45 and 1000 μm, Taylor et al. (2004) found that springtails and mites were not sensitive to moisture treatment. Taylor et al. (2004) also found that later in decomposition, fine-mesh bags had higher C mineralization and retained less N. Our study followed the same pattern, though the litter C was only numerically different between mesh sizes. Conceivably, decreased C leaching in fine-mesh bags may have mitigated warmer temperature and higher moisture, to produce no overall effect on C loss. Litter may be transformed during decomposition into different substances depending on the composition and metabolic capacities of the decomposer community (Wickings et al. 2012); and the environment of the litterbag may shape decomposition processes irrespective of arthropod or microbial decomposer community.

Evidence for SOM formation from decomposing litter

In just over one year, ~45% of senesced switchgrass was decomposed irrespective of ant abundance or mesh size treatment. Increasing GM/AX ratio and decreasing decomposers in all litterbags point to a transition from physical destruction by decomposers to microbial colonization. High rates of decomposition (3% litter mass lost/day during the first 8 weeks), and greater EEA at 8 weeks, indicate rapid transformation of litter C. At 59 weeks, low decomposition rates (0.1% litter mass lost/day during the last 8 weeks) despite increased GM/AX and litter microbial biomass across guilds suggested that the microbial populations were relying more on recycled C from microbial sources than plant C as a food source. Interestingly, we found a contrasting decrease in most microbial lipid indicators in soil samples over time, indicating that litter C leachate was not boosting microbial populations after a year of decomposition. This fits Cotrufo et al.'s (2015) conceptual model proposing that microbially derived SOM is formed early in decomposition by leaching of soluble products, while fragmentation dominates the pathway of SOM formation from litter later in decomposition. Our results suggest that after the first year of decomposition, the litterbags were leaching less microbially available substrate into the soil below, but soil C continued to increase, perhaps because of accumulating microbial necromass (Miltner et al. 2012, Kallenbach et al. 2015, Liang et al. 2017). Using isotopes, Wachendorf et al. (2020) were able to recover up to 11% of litter-derived C in soil 2 cm below litterbags after 200 d, while Cotrufo et al. (2015) found that

litter-derived soil C increased even after 1 yr of decomposition, so the higher soil C we observed below litterbags at 59 weeks could easily be due to C leachate. The temporal changes in indicator lipids likely arose from changes in available substrate. For example, increasing N concentration previously has been related to increases in the ratio of Gm+/Gm− indicator lipids (Zhou et al. 2017).

We found no evidence for arthropod decomposers effects on microbial abundance or litter C mineralization. Other research shows that that decomposers mediate microbial C processing, increasing litter-derived C in microbes in the soil (Soong et al. 2016), and predator abundance increased retention of glucose-derived C in microbes and microarthropds (Strickland et al. 2012). However, effects of soil fauna on N_2O production, another microbial-driven soil process, were mixed (Kuiper et al. 2013), and reducing decomposer food web functional complexity had no impact on ecosystem processing in a Scots pine nursery (Liiri et al. 2002). Despite increasing scientific interest in the interactions between soil fauna and soil processes, detecting these relationships remains difficult because of functional redundancy and wide variation among climates and ecosystems (Briones 2014, Frouz 2018).

Conclusions

Overall, arthropod and microbial abundance, microbial composition, and decomposition rates were not affected by ant reduction. Although ants are known to affect the detrital food web through several pathways (Wills and Landis 2018), we found no evidence of trophic cascades when ant abundances were reduced (e.g., Schmitz 2009, Strickland et al. 2013, Donald et al. 2018). The use of litterbags to evaluate decomposition may underestimate effects of ants, which may have stronger effects concentrated around nests. On the other hand, the differences in litter chemistry in mesh bags of different sizes suggest that alteration of microenvironments could occur as an artifact of litterbag treatments. Studying decomposition via isotopic tracers or CO_2 efflux may illuminate spatial patterns of ant predation effects. It may be that litterbag decomposition rate is not a property that is sensitive to removal or manipulation of a single species, because the pathways by which plant litter is mineralized into CO_2 are myriad.

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