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Soil microarthropods support ecosystem productivity and soil C accrual: evidence from a litter decomposition study in the tallgrass prairie

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Abstract

Soil fauna have been found to accelerate litter decomposition rates across many ecosystems, but little is known about their impact on soil organic matter formation during decomposition and their influence on ecosystem carbon and nitrogen cycling during this process. In a three-year litterbag-free decomposition study, we suppressed microarthropod abundance by 38% and tracked the fate of $^{13}$C- and $^{15}$N-labeled litter into different soil organic matter fractions and the microbial community. Microarthropod suppression slowed litter mass loss and decreased litter carbon input into the soil and soil microbes during the first 18 months of decomposition. The microarthropod suppression did not alter the total amount of carbon and nitrogen incorporated in the soil after complete surface litter mass loss. However, lower early-stage microbial carbon uptake due to lower early-stage litter inputs to the soil, as well as a significant decrease in the C:N ratio of litter-derived organic matter inputs to the mineral soil fractions, made less nitrogen available for plant uptake in the microarthropod suppression treatment. Thus, the acceleration of early-stage, more labile litter inputs to the soil altered the timing and availability of carbon and nitrogen inputs to the soil. A simulation of these effects on the tallgrass prairie ecosystem using the DayCent model predicts lower net primary productivity and lower total soil C and N mineralization when soil microarthropods are less abundant. Our results highlight the importance of soil microarthropods for ecosystem functioning through their role in transforming decomposing litter organic matter into soil organic matter and the feedback of this process to ecosystem productivity and soil C sequestration.

Keywords: Biogeochemistry; litter decomposition; phospholipid fatty acids (PLFA); soil microarthropods; soil organic matter; stable isotopes
1. Introduction

With ever-increasing atmospheric CO$_2$ concentrations, understanding the mechanisms controlling how much carbon (C) is stored in the soil from accumulation of plant inputs and how much nitrogen (N) is released during litter decomposition remain some of the most pressing issues in terrestrial ecosystem science (Todd-Brown et al., 2014). Litter decomposition is one of the main processes contributing to the formation of soil organic matter (SOM). However, traditional litter decomposition studies only monitor litter mass loss and do not track the fate of decomposing litter C and N in the soil (Cotrufo et al., 2009). While much is known about how climate and litter chemistry affect plant litter decomposition rates (Couteaux et al., 1995; Gholz et al., 2000), recent studies have highlighted the need to explicitly acknowledge the role of decomposers, both microbes (Grandy and Neff, 2008; Wieder et al., 2013) and soil fauna (de Vries et al., 2013; Garcia-Palacios et al., 2013), to improve our understanding of ecosystem C and N dynamics. Soil fauna influence processes such as litter decomposition rates (Wall et al., 2008) and soil N cycling (Carrillo et al., 2011). However, the underlying mechanisms and their influence on soil organic matter formation and C sequestration during litter decomposition are not well known (Garcia-Palacios et al., 2013).

Soil fauna have significant impacts on litter decomposition rates in some ecosystems, which calls for their inclusion in decomposition models to better represent unexplained variation across regions (Wall et al., 2008; Garcia-Palacios et al., 2013). Microarthropods can accelerate litter decomposition by increasing litter surface to volume ratios, thus allowing microbes to better access litter by physically moving litter into the soil (Chamberlain et al., 2006).

Additionally, microarthropods graze on microbial colonies and stimulate microbial activity while dispersing microbes and feces throughout the litter and into the soil (Petersen and Luxton, 1982;
Seastedt, 1984). Microbial transformation of fresh litter inputs in the soil is now thought to be the main precursor to long-term stabilization of SOM on soil minerals (Grandy and Neff, 2008; Miltner et al., 2009; Cotrufo et al., 2015). Therefore the role of soil microarthropods in either directly increasing litter inputs to the soil or stimulating microbial litter decomposition could have important implications for the contribution of decomposing litter to the formation of persistent SOM and its C:N balance.

The use of dual $^{13}$C- and $^{15}$N-labeled leaf litter in decomposition studies is a powerful method for identifying the amount, location and transformation of litter inputs to the soil (e.g. (Bird et al., 2003; Fahey et al., 2011; Soong and Cotrufo, 2014) and soil food web (Ruf et al., 2006; Pollierer et al., 2007), but to our knowledge has not been used to examine the role of soil fauna in litter to soil C and N transformation processes in situ. Litter-derived $^{13}$C and $^{15}$N can be traced into primary SOM fractions separated by density and size (Christensen, 2001) to examine the relative stabilization, transformation and approximate mean residence time of fresh litter inputs to the soil (Trumbore, 1993; von Lützow et al., 2008). Additionally, microbial phospholipid fatty acid (PLFA) $^{13}$C can be used to examine microbial community uptake of decomposing litter C and microbial contribution to litter-derived C stabilization over time (Moore-Kucera and Dick, 2008), while $^{15}$N can be quantified in plant roots to trace the complete recycling of N from litter decomposition to new plant productivity (Zeller et al., 2000). By incubating $^{13}$C- and $^{15}$N-enriched Andropogon gerardii leaf litter on the soil surface for three years in a tallgrass prairie in conjunction with a microarthropod suppression treatment using naphthalene (Cotrufo et al., 2014), we were able to investigate the effects of soil microarthropods on the litter decomposition process throughout its complete mass loss and on the fate of litter C and N in the soil.
In this study, we investigate whether the acceleration of litter mass loss by microarthropods alters C and N sequestration in SOM either directly or indirectly through their influence on the microbial community. We hypothesize that soil microarthropods accelerate litter mass loss by promoting fragmentation and the incorporation of litter fragments into the soil (Luxton, 1972; Yang et al., 2012). Additionally, we hypothesize that soil microarthropods stimulate microbial turnover through top-down predation and thus have trophic cascading effects on the transformation of litter into SOM.

2. Materials and Methods

2.1 Isotopically labeled litter production and analyses

Uniformly 3.3804 atom % $^{13}$C and 3.9917 atom % $^{15}$N labeled *A. gerardii* Kaw was grown in a $^{13}$C and $^{15}$N continuous labeling chamber as described in Soong et al. (2014). Upon harvest after 22 weeks of growth in the chamber, the above-ground plant material was air-dried, cut into 20 cm lengths and mixed. Three replicate sub-samples of the initial litter were analyzed for water content by drying in an oven at 65 °C and for %C, %N, $\delta^{13}$C and $\delta^{15}$N on an elemental analyzer connected to an isotope ratio mass spectrometer (EA-IRMS, Carlo Erba NA 1500 coupled to a VG Isochrom continuous flow IRMS, Isoprime inc.) and for % hemicellulose, % cellulose and % acid un-hydrolysable residue (AUR) using the neutral detergent fiber (NDF) and acid detergent fiber (ADF) methods (Van Soest et al., 1991). The litter was comprised of 44.3% C, 1.47% N, 29% cellulose, 25% hemicellulose and 3.9% AUR.

2.2 Experimental site and design
The experiment was conducted at the Konza Prairie long-term ecological research station in Kansas, USA (39°05’W, 96°35’N; local site name R20B). This is a tallgrass prairie dominated by *A. gerardii*. The climate is temperate-continental with average annual precipitation of 835 mm and a mean annual temperature of 12.8 °C. The soil is a silty clay Mollisol (8% sand, 46% silt, 46% clay) and the site is topographically situated on a foot slope position (Knapp et al., 1998). Four replicate soil cores were taken within the experimental area and divided into the 0-2, 2-5, 5-10 and 10-20 cm soil layers for determination of soil bulk density. We found no evidence of carbonates in these soils (measured using pressure transducer following acid addition).

The soil microarthropod suppression (MS) treatment began in June 2010. At that time we installed 80 20-cm diameter PVC collars to a depth of 5 cm. We removed the native litter layer from within the collars and applied 4 ml of glyphosate (Roundup®) to deter plant growth within the collars. In the laboratory we determined the native litter dry weight, which was on average 18.4 g per collar surface area. The soil MS treatment consisted of monthly additions to the soil surface of 477 g/m² of naphthalene to repel microarthropods. Naphthalene (C₁₀H₈) is a chemical additive used to suppress soil microarthropod abundances in field decomposition studies, which was shown to have no effect on soil nematodes and no overall effect on PLFA abundances although a small amount of naphthalene may be used by some bacterial groups at the site (Cotrufo et al., 2014).

The litter decomposition experiment began on September 29, 2010, when 18.4 g dry-mass equivalent of $^{13}$C (δ$^{13}$C = 2113‰) and $^{15}$N (δ$^{15}$N = 10,309‰) labeled air-dried *A. gerardii* leaf litter was added to the PVC collars, and lasted for three years. Coarse plastic netting was used to cover the collars to prevent loss of the labeled litter or input of external litter. Five destructive soil and litter harvests occurred on May 1, 2011 (7 months), October 8, 2011 (12
months), April 13, 2012 (18 months), September 29, 2012 (24 months) and September 25, 2013 (36 months).

The experiment consisted of a split-split-plot fully randomized complete block design with 4 replicate blocks. Within each replicate block, 5 whole plots (4 m²) were randomly assigned to one of the five sampling dates. Each whole plot was split in half by naphthalene treatment (split-plots) so that in one half three subplots were treated with naphthalene (MS) while three subplots were not (Control). Each subplot consisted of two PVC collars, one with the labeled *A. gerardii* litter addition and one that was left as bare soil for use in the isotope mixing model as described below. For each PVC collar, soil was sampled at different depths (split-split plots). The experiment is thus treated as a split-split plot on a randomized complete block design, with sampling times assigned to whole plots, naphthalene treatment assigned to subplots and depth segment assigned to sub-subplots.

2.3 *Litter and soil sampling*

At each harvest date, we collected soil and litter samples from each collar. First, the litter was collected and stored in plastic bags. Then an intact soil core (6 cm diameter) was collected to 5 cm depth for microarthropod extraction and quantification. We excavated the remaining soil within the collar by incremental depths (0-2, 2-5, 5-10 and 10-20 cm) and the soil from each layer was stored separately in plastic bags at 4°C until they were processed within two weeks of collection. During the October 8, 2011, September 29, 2012 and September 25, 2013 sampling we additionally collected roots from *A. gerardii* plants growing immediately outside collars.

2.4 *Microarthropod extraction and quantification*
We extracted microarthropods from the 0-5 cm intact cores using Tullgren funnels (48 h dark followed by 48 h low, 48 h medium and 48 h full light/heat intensity) (Crossley and Blair, 1991). Microarthropods were enumerated and sorted by groups into separate microcentrifuge tubes (0.5 mL) by handpicking individuals under a dissecting microscope (Olympus SZX10, 30X magnification). The following groups were distinguished: oribatid mites (Acari: Oribatida), predatory mites (Acari: Mesostigmata and predatory Prostigmata) and springtails (Collembola). All microarthropods (maximum 100) for each group were then transferred to a pre-weighed tin capsule (8x5 mm, Elemental Microanalysis BN/170056) containing 120 µL of deionized water. The tin capsules containing the microarthropod groups were dried for 3 days, weighed for final sample weights and analyzed for %C, %N, $^{13}$C and $^{15}$N using a CE-1110 EA coupled via Conflo II interface to an IRMS (ThermoFinnigan Delta Plus). Gravimetric soil moisture was calculated by oven-drying 50 g of fresh soil at 105 °C for 48 h. Soil microarthropod numbers were subsequently converted to individuals per kg dry soil.

2.5 Bulk soil and litter analyses

At each harvest, the remaining litter was picked clean of any non- *A. gerardii* leaves, roots and soil, then weighed at field moisture. A subsample of the litter was oven-dried at 65 °C for analysis of gravimetric water content and another subsample was combusted at 660 °C in a muffle oven to determine ash content for the determination of ash-corrected litter dry mass. The bulk soil was sieved to 2 mm and a subsample was analyzed for gravimetric water content by mass loss after drying at 105 °C. The root samples were rinsed of all residual soil and oven-dried at 65 °C. All oven-dry litter, soil and root samples were then pulverized and analyzed for %C, %N, $\delta^{13}$C and $\delta^{15}$N on an EA-IRMS.
2.6 Soil organic matter fractionation

To separate the uncomplexed SOM and the primary organo-mineral complexes (Christensen, 1992), we employed a physical SOM fractionation scheme as described in Soong and Cotrufo (2015). Briefly, after dispersion, soil from the 0-2 and 2-5 cm soil layers from both the bare-soil and labeled-litter collars was fractionated by density into a light fraction (LF <1.85 g cm\(^{-3}\)) and a heavy fraction. The latter was separated by size into sand-sized (>53 µm), silt-sized (>2 µm) and clay-sized (<2 µm) fractions. All fractions were oven-dried at 105 °C prior to weighing and analysis of %C, %N, δ\(^{13}\)C and δ\(^{15}\)N on an EA-IRMS (see Appendix S1, Supplementary Table 1 for more information on the SOM fraction distribution).

2.7 PLFA extractions and \(^{13}\)C-PLFA measurements

A subsample of the 2 mm-sieved bulk soil from the 0-2 and 2-5 cm depth layers collected from the bare-soil and enriched-litter collars was picked clean of all visible roots, frozen (-20°C) and lyophilized for 48 h prior to phospholipid fatty acid (PLFA) extraction. We extracted PLFAs on these samples using conventional methods (Bligh and Dyer, 1959; Denef et al., 2007) as described in detail by Gomez et al. (2014).

The biomarker PLFAs analyzed within this dataset included: 18:1\(\omega9\)c and 18:2\(\omega6,9\)c (indicative of saprophytic fungi), 16:1\(\omega5\) (indicative of arbuscular mycorrhizal fungi; AMF), i15:0, a15:0, i16:0, i17:0 and a17:0 (indicative of Gram-positive bacteria), cy17:0, cy19:0, 16:1\(\omega7\)c and 18:1\(\omega7\)c (indicative of Gram-negative bacteria), 14:0, 15:0 and 18:0 (non-specific bacterial markers) and 10Me PLFAs (indicative of Actinobacteria) (Kroppenstedt, 1985; Olsson et al., 1995; Zelles, 1997). \(^{13}\)C values were corrected using working standards (12:0 and 19:0).
calibrated on an EA-IRMS. Fatty acid methyl esters (FAME) concentrations and $^{13}$C signatures were measured by capillary gas chromatography-combustion-isotope ratio mass spectrometry (GC-c-IRMS) (Trace GC Ultra, GC Isolink and DeltaV IRMS, Thermo Scientific). Details on the instrument parameters can be found in Gomez et al. (2014). PLFAs were identified and quantified using an external 37 FAME and bacterial acid methyl ester (BAME) mix (Sigma Aldrich, St Louis, MO, USA). To identify those fatty acids not available in commercial standard mixes, several samples were analyzed by GC-MS (Shimadzu QP-2010SE) and spectral matching using the NIST 2011 mass spectral library (Shimadzu). To obtain $\delta^{13}$C values of the PLFAs, measured $\delta^{13}$C FAME values were corrected individually for the addition of the methyl group during transesterification by simple mass balance (Denef et al., 2007). The abundance of individual PLFAs was calculated in absolute C amounts (PLFA-C, ng g$^{-1}$ soil) based on the PLFA-C concentrations in the liquid extracts and used as a proxy for microbial biomass.

2.8 Root analysis

Twelve, 24 and 36 months after the start of the study, we collected roots of *A. gerardii* grasses growing immediately outside all of the litter-treated collars as well non-labeled background *A. gerardii* roots from immediately outside of the four replicate block area (to avoid any potential isotope contamination). Roots were oven-dried (65 °C), pulverized and analyzed for $\delta^{15}$N on an EA-IRMS to determine any MS treatment effect on N uptake of the decomposing litter by nearby plants.

2.9 *DayCent* simulation of microarthropod suppression
We parameterized the ecosystem model DayCent (Parton et al., 1998) based on conditions at our site, including a standard tallgrass prairie land management practice of burning every four years, as a base run of the control treatment in our experiment. In order to simulate the observed effects of microarthropods on the biogeochemistry of the system, we ran the same model with a 20% reduced surface litter decay rate and reduced C:N ratio of surface-active and slow SOM pools by 30%, approximately corresponding to the measured reductions of our MS treatment on litter and soil C and N processing (see following results). The simulation of the control and MS treatments began in year 1900 and were applied through year 2100, with daily mean temperature and precipitation data informing the model from 1984 through 2013 ([climby.lternet.edu; Konza Prairie dataset](climby.lternet.edu)).

2.10 Data analysis

We tested the effect of the MS treatment on microarthropod abundances, $\delta^{13}$C and $\delta^{15}$N of the microarthropods, litter mass remaining, C and N remaining at each sampling time point, PLFAs and root $\delta^{15}$N from the litter-added plots using a general linear mixed model. Microbial community analyses of fungi:bacteria ratio were calculated by summing the PLFA-C from all of the biomarkers from each group from the litter-added plots and analyzed using the same general linear mixed model. The model included MS treatment and time of sampling as fixed effects and block and block $\times$ sampling time as random effects.

The litter contribution to the soil, soil physical fractions and PLFA-C was assessed for the litter-added plots as compared to the bare soil plots within each subplot. The isotopic mixing model was applied as follows:

$$f_{\text{litter}} = \frac{\delta_S - \delta_B}{\delta_{\text{litter}} - \delta_B}$$
where $f_{\text{litter}}$ is the litter-derived C (or N) fraction of bulk soil, SOM or PLFA sample, $\delta_s$ and $\delta_B$ is the $\delta^{13}$C (or $\delta^{15}$N) of the specific bulk soil, SOM or PLFA sample from the litter-treatment collar ($\delta_s$) and the bare soil ($\delta_B$), and $\delta_{\text{litter}}$ is the $\delta^{13}$C (or $\delta^{15}$N) of the initial litter. The amount of litter-derived C and N in these pools were obtained by multiplying the $f_{\text{litter}}$ values to corresponding C (or N) pool sizes. Litter-derived C and N pools in the SOM fractions and litter-derived PLFA-C pools were calculated for the 0-5 cm soil depth summing the respective 0-2 and 2-5 cm pool sizes.

We tested the effect of the MS treatment, sampling time and soil depth on the amount of litter-derived C and N incorporation (and C:N ratios) into the bulk soil, SOM fractions and PLFAs using a general linear mixed model including MS treatment, time of sampling, soil depth and all interactions as fixed effects and replicate block, block $\times$ sampling time and block $\times$ sampling time $\times$ MS treatment as random effects with standard variance components. We analyzed differences in the DayCent simulations of the control and MS scenarios over the years 1900-2100 using a paired t-test. Significance of all pairwise differences was determined using the Tukey-Kramer method for multiple comparisons. We checked for normality of the data and homogeneity of variances of the residuals and applied a log-transformation when necessary. We analyzed all general linear mixed models using SAS® software version 9.3. In all cases, we used type III tests of fixed effects.

3. Results

3.1 Microarthropod suppression

The abundance of total microarthropods, including oribatid mites, predatory mites and collembolans, was reduced from an average of 642 individuals kg$^{-1}$ dry soil (20,445 individuals
m−2) in the control to 395 individuals kg−1 dry soil (12,580 individuals m−2) in the MS treatment (F_{1,67}=8.40; p=0.0051) in the top 0-5 cm of the bare soil and litter-added plots. The reduction of oribatid mites (the most abundant group) was the main driver of this effect. The abundance of oribatid mites was reduced by 50% (F_{1,65}=10.16, p<0.001) in the MS treatment across all time harvests, while predatory mites were reduced by 34% (F_{1,65}=0.70, p=0.4055), and collembolans were not affected at all (1% reduced, F_{1,65}=1.36, p=0.2475). By averaging the δ^{13}C and δ^{15}N values of all three microarthropod groups within each sample, we found that over the course of our experiment the microarthropods in the MS treatment were significantly less enriched in both δ^{13}C (F_{1,12}=30.23; p=0.0001) and δ^{15}N (F_{1,12}=16.74, p=0.0015) as compared to the control (see Appendix S1; Supporting Information).

### 3.2 Microarthropod suppression effect on litter mass loss

After 36 months of decomposition in the field, the labeled *A. gerardii* litter had lost 98% of its initial mass (Fig. 1a). Sampling time (F_{4,30}=110.80; p<0.0001) and MS treatment (F_{1,30}=5.88, p=0.0215) had significant effects on the amount of litter mass remaining during the experiment. At 12 and 18 months the control treatment had significantly less mass remaining than the MS treatment. However, there was no difference in the mass remaining after 7, 24 or 36 months of incubation in the field (Fig. 1a).

By applying the isotope-mixing model to the litter ^{13}C and ^{15}N content we could examine the amount of endogenous and exogenous, e.g. coming from the environment (Zeller et al., 2000; Frey et al., 2003), C and N in the litter over time. We found no significant differences in exogenous C (Fig. 1b) and N (Fig. 1c) incorporated into the litter between the MS treatment and control and the ratio of endogenous C:N remaining in the litter was only different at the 12
month harvest (t=3.46; p=0.0005). Exogenous N was an important source of N during decomposition, accounting for approximately 30% of the litter N content from 6-24 months.

3.3 Microarthropod suppression effect on litter inputs belowground

Litter-derived C and N were recovered down to 20 cm in the soil, with the majority recovered in the top 5 cm (Fig. 2). As litter decomposition progressed over time, the total amount of litter-derived C in the soil increased (Fig. 2). After 98% mass loss at 36 months, we recovered 19% and 18% of the initial litter C in the soil down to 20 cm in the control and MS treatment, respectively, with no statistically significant difference between treatments (F_{1,6}=0.12; p=0.7417). However, over all five harvests and four depths, there was a small but significant MS treatment effect of decreasing litter-derived C in the soil (F_{1,105}=5.48; p=0.0174). There was no effect of MS treatment on litter-derived N incorporation into the soil (F_{1,96}=0.02; p=0.8950) and 55-59% of the initial litter N was incorporated into the soil at the end of the study. This resulted in 15% higher C:N ratios in the control than the MS bulk soils across all five harvests (F_{1,105}=5.47; p=0.0212).

An examination of the SOM fractions in the 0-2 and 2-5 cm depths revealed a significant overall effect of the MS treatment of reducing litter-C incorporation into the LF (F_{1,45}=10.58; p=0.0022), sand-sized (F_{1,60}=11.63; p=0.0012), silt-sized (F_{1,45}=26.05; p<0.0001) and clay-sized fractions (F_{1,60}=14.66; p=0.0003; Fig. 3, 0-2 and 2-5 cm depths summed). The MS treatment had a significant interaction with time and was not significant at the final soil harvest (p>0.05) for litter-derived C in the SOM fractions (Fig. 3). There was no consistent effect of the MS treatment on litter N inputs to any of the measured SOM fractions (Fig. 3). Consequently, C:N ratios were significantly higher in the control LF (F_{1,45}=6.36; p=0.0153), sand-sized (F_{1,60}=7.79; p=0.0070),
silt-sized ($F_{1,60}=146.32; p=<0.0001$) and clay-sized ($F_{1,75}=86.17; p<0.0001$) SOM fractions than the MS fractions. It remained 25% higher in the silt- and clay-sized fractions even at the final harvest ($F_{1,12}=62.72$ (clay) and $F_{1,12}=106.89$ (silt); $p<0.0001$ (both); Fig. 4, 0-2 and 2-5 cm depths summed).

The roots of *A. gerardii* grasses growing immediately outside of the litter-treated collars showed a measurable uptake of litter-derived N compared to the background. At the 12-month harvest, $\delta^{15}$N enrichment of the roots adjacent to the control plots was significantly higher (average 49‰ $\delta^{15}$N, standard error= 13) than the MS plots (average 26‰ $\delta^{15}$N, standard error= 8; $F_{1,20.9}=4.44; p=0.0474$). At the 24 and 36 month harvests the two treatments did not differ and $\delta^{15}$N enrichment reached 81‰ (standard error= 19) at the 36-month harvest.

### 3.4 Microarthropod effect on microbial community and litter-derived C incorporation

There was no effect of MS treatment on the overall abundance of PLFAs in the litter-added plots ($F_{1,45}=0.27; p=0.6087$). However, the MS-treated plots had a lower amount of litter-C incorporation into all of the PLFAs than the control plots, which was statistically significant throughout the experiment ($F_{1,45}=28.42; p<0.0001$; Fig. 5a). Microbial incorporation of litter C into PLFAs decreased with depth from the 0-2 cm soil layer to the 2-5 cm soil layer ($F_{1,45}=28.42; p<0.0001$). All of the microbial groups identified had incorporated litter-derived C throughout the 36-month incubation, with decreasing amounts over time (Fig. 5a, Appendix S2). By the 36-month harvest, overall PLFA litter-derived C incorporation was low and the amount of litter-derived C in the PLFAs remained stable between the 24 and 36-month harvests (Fig. 5a).

The total abundance of fungal PLFA-C to bacterial PLFA-C (ratio=0.19) was not affected by the MS treatment. However, the MS treatment did have a slightly greater amount of litter-
derived C in the fungal PLFAs relative to the bacterial PLFAs (0.200 for MS and 0.178 for control; \( F_{1,60} = 5.90; \ p = 0.0182 \)).

We calculated the relative litter-derived C incorporation into microbial PLFAs by dividing the amount of litter-derived C found in the PLFAs by the total amount found in the bulk soil of the same layer at each sampling time (Fig. 5b). PLFA relative litter-derived C incorporation in the 0-2 cm soil depth was lower than the 2-5 cm depth (\( F_{1,45} = 43.97; \ p < 0.0001 \)), and decreased over time (\( F_{4,15} = 76.79; \ p < 0.0001 \)). The MS treatment did not differ significantly from the control treatment in relative microbial litter-derived C incorporation (\( F_{1,45} = 1.21; \ p = 0.2774 \)).

3.5 Ecosystem response to microarthropod suppression in DayCent

DayCent simulation of the role of microarthropods in increasing initial litter mass loss rates (Fig. 1) and increasing the C:N ratio of litter inputs to the surface soil active and slow SOM pools (Fig. 4) resulted in marked shifts in ecosystem C and N availability when projected over time (Fig. 6). Major inter-annual fluctuations are mainly driven by a four-year burn cycle and climatic events, but significant shifts of 12% reduced NPP (\( t_{400} = 4.91, \ p < 0.0001 \)), both above- and belowground, 21% reduced total N mineralization (\( t_{400} = 6.48, \ p < 0.0001 \)), 11% reduced total soil C after 200 years (\( t_{400} = 169.60, \ p < 0.0001 \)) and reduced C in the active (\( t_{400} = 3.38, \ p < 0.0008 \)) and slow surface (\( t_{400} = 3.09, \ p < 0.0021 \)) SOM pools, but no difference in surface litter mass (\( t_{400} = -0.18, \ p = 0.8579 \)) due to the MS treatment are projected (Fig. 6).

4. Discussion
During our three-year decomposition study, microarthropod abundance was reduced by 38% in the MS treatment. Our experiment confirms previous findings that the acceleration of litter mass loss by microarthropods is limited to the early stages of decomposition (Smith and Bradford, 2003; Garcia-Palacios et al., 2013).

Although we found the MS treatment to only affect the first 18 months of litter decomposition, this early phase may be the most critical to mineral-associated SOM formation and litter-derived N recycling (Cotrufo et al., 2015). The immediate contribution and persistence of litter-derived C and N in the silt and clay fractions during the initial winter period, prior to major contributions to the LF, indicates that leaching of dissolved organic matter (DOM) is a likely mechanism for the contribution of litter-derived OM to the mineral soil fractions (Kalbitz et al., 2005; Cotrufo et al., 2015; Soong et al., 2015). During the 12 to 18-month mid-phase of the experiment, we saw an influx of litter fragments in the LF, along with a prominent effect of the MS treatment on decreasing litter-derived C inputs to all of the SOM fractions and reducing the C:N ratio of litter-derived OM in the SOM fractions. It was during these early and mid-phases of decomposition that microbial PLFA incorporation of litter-derived C as well as relative microbial incorporation of the litter-derived C were both highest. This could be due to increasing proportional lignin content of the litter over time (Berg, 2000), which is associated with a lower substrate use efficiency (Lekkerkerk et al., 1990). The reduced C:N ratio of the litter-derived OM in the SOM fractions in the MS treatment (Fig. 4) indicated enhanced microbial transformation of the original litter material and N immobilization (Paul, 2014). Since microbial uptake and transformation of litter inputs are thought to be precursors to stabilized SOM formation (Wickland et al., 2007; Grandy and Neff, 2008), the role of microarthropods in increasing litter OM inputs to the soil during the early high substrate use efficiency period of litter decomposition.
may be important to the formation of mineral-associated SOM during decomposition. At the final 36-month soil harvest, we saw an influx of litter-derived LF to the soil for both the MS and control treatment. However, there was no MS treatment effect on this final stage of litter inputs to the soil when microbial substrate use efficiency was at its lowest. Our litterbag-free incubation method allowed us to capture this final physical input of litter fragments to the soil, which is often inhibited by litterbag studies (Cotrufo et al., 2010).

Microarthropods and other soil fauna play an important role in litter decomposition rates globally (Wall et al., 2008; Garcia-Palacios et al., 2013) and may be critical drivers of soil C and N cycling (de Vries et al., 2013), but the mechanisms underlying these patterns are not clear. Our MS treatment decreased the amount of $^{13}$C incorporation in microbial PLFAs without changing the proportion of litter-derived C in the soil taken up by the microbes, indicating that the microarthropods increased litter inputs to the soil but did not stimulate more rapid microbial uptake of litter-derived C. The decline in PLFA incorporation of $^{13}$C over time also suggests the importance of microbial litter-derived C incorporation during the earlier stages of decomposition. Furthermore, it was only during the earlier stages of decomposition, when litter quality was high, that we saw an MS effect on litter-derived N uptake by surrounding *A. gerardii* growing roots. In frequently disturbed ecosystems such as the tallgrass prairie, which is frequently burned (Collins and Wallace, 1990), these early-stage impacts of microarthropods on C and N uptake by soil microbes and plants could have a great impact on ecosystem productivity.

One of the most apparent and lasting effects of the MS treatment was on lowering the C:N ratio of litter-derived OM inputs to the silt and clay fractions of the soil. Fungi generally have a higher C:N ratio than bacteria (Paul, 2014). However, we did not see a shift in the fungi:bacteria ratio to indicate that this change in C:N ratio was caused by a top-down impact of
microarthropods on a shifting microbial community. Instead it appears that the microarthropods
themselves may have a direct role in inducing N mineralization, as previously reported (Carrillo
et al., 2011).

A simple manipulation of the structural litter inputs to the soil and the C:N ratios of the
surface SOM active and slow pools in DayCent allowed us to examine the ecosystem-scale
impacts of a long-term microarthropod reduction. Although DayCent does not include
representations of soil biota, it has been shown to represent the biogeochemistry of the tallgrass
prairie ecosystem in Kansas well (Lu et al., 2001). Our manipulation of the litter and soil pools in
DayCent demonstrates how the biogeochemical impacts of microarthropods that we observed
could be integrated into existing ecosystem models to quantify their effects on ecosystem
processes. The simulation represents the results that we found when we reduced microarthropod
abundances by 38% in the top 0-5 cm of the soil, so it may underestimate the role of
microarthropods on the ecosystem. However, these modeling results suggest that the increased
early-stage litter decomposition C inputs to the soil and higher C:N ratio of litter-derived SOM
associated with greater microarthropod abundances have important long-term implications for
ecosystem processes.

4.1 Conclusions

The use of naphthalene in a three-year, litterbag-free study of $^{13}$C- and $^{15}$N-labeled leaf
litter decomposition allowed us to quantify the effects of microarthropod suppression on the fate
of litter-derived C and N in the soil over time. An equal fraction (18-19%) of litter-derived C
remained in the soil after complete surface litter mass loss under control and 38% reduced
microarthropod abundances. We observed an initial effect of the MS treatment on litter mass
loss, incorporation of litter-derived C into SOM and soil microbes, and litter-derived N into plant
roots. Although none of these treatment effects persisted until the end of the study, they may have disproportionally large effects in frequently disturbed ecosystems such as the tallgrass prairie where the litter layer is burned off every two to four years (Collins and Wallace, 1990). The major persistent effect of the MS treatment was a reduced C:N ratio of litter-derived SOM formation. A simulation of this impact using DayCent revealed how the effect of a reduction in microarthropod abundances could have cascading impacts on plant N availability, soil C storage and net primary productivity. These results demonstrate that soil fauna, such as microarthropods, play key roles in the functioning of ecosystems and deserve explicit consideration in both empirical studies and models of soil biogeochemical processes.

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Fig. 1. Litter mass remaining (a) during three years of decomposition in the field for the control (black symbols) and microarthropod-suppression (MS, white symbols) treatments of the $^{13}$C- and $^{15}$N-labeled *A. gerardii* litter. Carbon (b) and Nitrogen (c) endogenous mass losses (circles) and exogenous mass gains (triangles). Endogenous values are based on the $^{13}$C and $^{15}$N lost from the original litter, and exogenous values are calculated based on initial litter $^{13}$C and $^{15}$N dilution from exogenous soil inputs. Values are averages, with bars as standard error (n=4).

Fig. 2. Average litter-derived C recovered in the soil for the control (open) and microarthropod-suppression (MS, cross-hatched) treatment. Error bars are standard error (n=4).

Fig. 3. Litter-derived C (circles) and N (triangles) in 0-5 cm a) light fraction, b) sand-sized, c) silt-sized, and d) clay-sized soil organic matter fractions for the control (black symbols) and microarthropod-suppression (MS; white symbols) treatments. Scales are different between panels to highlight treatment effects. Values are averages, with error bars as standard error (n=4).

Fig. 4. Litter-derived C to N ratios in the a) light fraction, b) sand-sized, c) silt-sized, and d) clay-sized soil organic matter fractions in the 0-5 cm soil depth for the control (black symbols) and microarthropod-suppression (MS; white symbols) treatments. Values are averages, with error bars as standard error (n=4).

Fig. 5. a) PLFA incorporation of litter-derived C for the control (dark bars) and microarthropod-suppression (MS; white bars) treatment for the 0-2 cm depth (open bars) and 2-5 cm depth (cross-hatched bars). b) Relative microbial incorporation of litter-derived C calculated as the ratio of ng of litter-derived PLFA C per ng$^{-1}$ of litter-derived C in the soil for the control (black) and MS (white) in the 0-2 cm depth (circles) and 2-5cm depth (triangles). Values are averages, with error bars as standard error (n=4).

Fig. 6. DayCent simulations of the impacts of a 38% microarthropod suppression on decreasing early-stage litter mass loss rates by 20% and decreasing the C:N ratio of the active and slow surface soil organic matter pools by 30% on a) net primary productivity, b) surface structural litter mass, c) total nitrogen mineralization and d) soil organic matter total carbon. The control scenario is simulated by the black line and the microarthropod-suppression scenario is simulated by the grey line. In both simulations, burning occurs every four years.
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