Fire affects root decomposition, soil food web structure and carbon flow in the tallgrass prairie

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Abstract

Root litter decomposition is a major component of carbon (C) cycling in grasslands, where it provides energy and nutrients for soil microbes and fauna. This is especially important in grasslands where fire is common and removes aboveground litter accumulation. In this study, we investigated whether fire affects root decomposition and C flow through the belowground food web. In a greenhouse experiment, we applied ^{13}C-enriched big bluestem (*Andropogon gerardii*) root litter to intact tallgrass prairie soil cores collected from annually burned (AB) and infrequently burned (IB) treatments at the Konza Prairie Long Term Ecological Research (LTER) site. Incorporation of ^{13}C into microbial phospholipid fatty acids and nematode trophic groups was measured on six occasions during a 180-day decomposition study to determine how C was translocated through the soil food web. Results showed significantly different soil communities between treatments and higher microbial abundance for IB. Root decomposition occurred rapidly and was significantly greater for AB. Microbes and their nematode consumers immediately assimilated root litter C in both treatments. Root litter C was preferentially incorporated in a few groups of microbes and nematodes, but depended on burn treatment: fungi, Gram-negative bacteria, Gram-positive bacteria, and fungivore nematodes for AB and only omnivore nematodes for IB. The overall microbial pool of root litter-derived C significantly increased over time but was not significantly different between burn treatments. The nematode pool of root litter-derived C also significantly increased over time, and was significantly higher for the AB treatment at 35 and 90 days after litter addition. In conclusion, the C flow from root litter to microbes to nematodes is not only measurable, but significant, indicating that higher nematode trophic levels are

critical components of C flow during root decomposition which, in turn, is significantly affected by fire. Not only does fire affect the soil community and root decomposition, but the lower microbial abundance, greater root turnover, and the increased incorporation of root litter C by microbes and nematodes for AB suggests that annual burning increases root litter-derived C flow through the soil food web of the tallgrass prairie.

1. Introduction

Soils contain an immense diversity of soil microorganisms and soil fauna, and are of key importance to terrestrial ecosystems nutrient cycling and carbon (C) storage (Wall et al., 2010;Wall, 2004;Bardgett, 2005;Smith et al., 2015). Understanding the roles of the soil food web in regulating belowground processes of decomposition, nutrient cycling, and C cycling is recognized as a hot topic of research in soil ecology (Bardgett and Cook, 1998;Holtkamp et al., 2011;Holtkamp et al., 2008;Carrillo et al., 2011;Osler and Sommerkorn, 2007;Bardgett et al., 2013;van der Putten et al., 2013). This is especially because we still lack a clear understanding of how soil fauna contribute to these ecosystem processes and the ecosystem services they provide (Nielsen et al., 2011;Carrillo et al., 2011;Brussaard, 1998;Bardgett and Cook, 1998;Smith et al., 2015). Within the soil fauna, nematodes, which can occur at densities of approximately 1 million to 10 million m^{-2} in grasslands (Bardgett et al., 1997;Yeates et al., 1997), are thought to play a fundamental yet poorly understood role in soil C dynamics (Staddon, 2004;Nielsen et al., 2011;Wall et al., 2008;Osler and Sommerkorn, 2007).

In tallgrass prairie ecosystems, fire is a historical disturbance that has ecosystem level effects on plant dynamics and other processes (Knapp et al., 1998). Frequent fires

66 can have large effects on plant productivity, plant community composition, and root
67 properties (Kitchen et al., 2009;Knapp et al., 1998), which can significantly alter
68 ecosystem processes such as litter decomposition and C cycling (Ojima et al.,
69 1994;Johnson and Matchett, 2001;Soong and Cotrufo, 2015). Litter decomposition is an
70 important component of belowground C cycling and root litter C provides a major energy
71 source for soil biota (Eisenhauer and Reich, 2012). Since fire removes aboveground
72 litter, and enhances root growth and belowground C allocation, root detrital input may
73 be an even more important energy source for decomposer food webs in frequently
74 burned grasslands (Seastedt et al., 1991;O'Lear et al., 1996). Furthermore, root
75 decomposition studies have been highlighted as crucial because root litter is a major
76 source of soil C (Rasse et al., 2005), contributing more than aboveground litter, and
77 very little research has been done on the topic (Schimel and Schaeffer, 2012).
78 The belowground effects of fire have additional impacts on soil biodiversity and
79 their functions. Burning causes changes in the soil surface energy budget by removing
80 plant litter accumulation (O'Lear et al., 1996;Knapp and Seastedt, 1986). This leads to
81 changes in soil conditions, such as nitrogen content, carbon content, temperature and
82 moisture, which could impact microbial and faunal activities or change detritivore
83 community composition. Microbial community compositional changes have been
84 reported as a result of fire: for example, fire alters microbial community composition by
85 reducing Gram-negative and Gram-positive bacteria (Docherty et al., 2011) and
86 increasing arbuscular mycorrhizae (Hamman et al., 2007). Also, fire initially impacts the
87 overall abundance of nematodes negatively (Whitford et al., 2014), but this rebounds
88 quickly and certain groups, such as colonizing bacterivore nematodes, respond

89 positively after fire (Jones et al., 2006;Todd, 1996). Such changes in soil community
90 composition have been shown to impact litter decomposition (Verhoef and Brussaard,
91 1990). While most litter decomposition is ultimately the product of soil fungal and
92 bacterial metabolic activities, soil fauna also play a role in litter decomposition by
93 influencing these microbial activities and altering litter chemical composition (Coleman
94 and Crossley, 1996;Verhoef and Brussaard, 1990;Petersen and Luxton, 1982;Xin et al.,
95 2012;Mamilov, 2000;Coleman and Hendrix, 2000;Carrillo et al., 2011;Swift et al.,
96 1979;Soong et al., submitted). However, little is known about how fire impacts both soil
97 microbial and faunal community function or if frequently burned grasslands' soil
98 communities are more specialized to decompose root litter than unburned soil
99 communities.
100 Addition of ^{13}C-enriched plant litter to soil allows tracing litter-derived C into soil
101 microbial and faunal groups during decomposition. This technique has been used to
102 study plant-C utilization by microbial communities in soils by examining ^{13}C
103 incorporation into microbial phospholipid fatty acids (PLFA; e.g., Denef et al.,
104 2009;Rubino et al., 2010;Kohl et al., 2015;Soong et al., submitted). Also, natural
105 abundances of ^{13}C and ^{15}N have been useful for studying structures of soil faunal
106 communities (e.g., collembolans, earthworms, enchytraeids, arthropods, gastropods,
107 and nematodes; Chahartaghi et al., 2005;Albers et al., 2006;Goncharov et al.,
108 2014;Crotty et al., 2014;Kudrin et al., 2015). Furthermore, C flow though soil faunal
109 trophic groups can be traced and quantified using ^{13}C in labeling experiments (Albers et
110 al., 2006;Pollierer et al., 2007;Elfstrand et al., 2008;Ostle et al., 2007;D'Annibale et al.,
111 2015;Gilbert et al., 2014). However, root turnover and aboveground litter inputs are the

112 main basis for soil faunal trophic groups in the chiefly detrital-based grassland soil food
113 webs (Ostle et al., 2007) and these previous studies often focus only on C from recent
114 photosynthate, ignore some of the most abundant soil fauna groups (e.g., nematodes),
115 and do not consider how disturbances, such as fire, might affect C pathways
116 belowground.

117 This project was designed to trace C from decomposing root litter into components
118 of the soil food web over time for annually (AB) and infrequently burned (IB) prairie
119 soils. Our conceptual approach included the production of a ^{13}C-enriched tallgrass (big
120 bluestem, *Andropogon gerardii*) root litter, its incubation in intact AB and IB prairie soil
121 cores in a greenhouse, and quantifying the incorporation of root litter C within the soil
122 food web over time. We hypothesized that: 1) The soil community would be less
123 abundant and less diverse in the AB treatment due to the disturbance of fire, which
124 removes surface organic inputs, increases soil temperatures, and decreases soil
125 moisture, 2) root litter mass loss would be greater and occur faster for AB, and 3) root
126 litter would be a more important C source for microorganisms and nematodes from AB
127 prairie, which would thus incorporate root litter-derived C more quickly and in greater
128 amounts than those from IB prairie.
129

130 **2. Materials and Methods**

131 **2.1 Site description and soil collection**

132 Soil samples were taken from historically unplowed tallgrass prairie at the Konza Prairie
133 Long Term Ecological Research (LTER) station in eastern Kansas, United States
134 (39°05'N, 96°35'W). Average monthly temperatures range from -2.7° C in January to

26.6° C in July, with 835mm of total annual precipitation on average. Following a similar sampling design of a concurrent field study by Soong and Cotrufo (2015), we used soils from two fire treatment areas at Konza Prairie LTER: annual spring burn and 20-year burn. Each treatment area is approximately 60 hectares and has silty-clay textured Argiustoll soils. The two treatment areas are in close proximity to one another with minimal geological and edaphic differences. The annual spring burn treatment area (labeled SpB by the Konza Prairie LTER) was burned yearly each spring since 1972, and was burned prior to soil collection on 26 April 2011. The annual spring burn treatment area had soil pH 6.2. The 20-year burn treatment area (labeled 20B by the Konza Prairie LTER) was last burned by an unprescribed wildfire on 5 April 1991; previously, a prescribed burn occurred on 3 May 1975. The 20-year burn treatment had soil pH 6.1. For specific soil characterization data for these sites including %C, %N, pyrogenic organic C content and bulk density see Soong and Cotrufo (2015). Soil from the annual spring burn treatment area will be referred to as annually burned (AB) and the 20-year burn as infrequently burned (IB) for the remainder of this paper.

Soil cores (10cm deep x 10cm diameter) were extracted from upland soil of the two fire treatment areas at KPBS on 14 June 2011. Sampling was spread out within each of these areas to capture site variability. Specifically, cores were taken every 3m in a 24m x 18m grid for a total of 48 soil cores from each treatment area. For both treatment areas, soil cores were taken beneath the dominant grass, *Andropogon gerardii.* These soil cores were extracted by driving PVC collars (10cm diameter) in to a depth of 10cm soil, and carefully digging out the collars while preserving soil core structure. The soil cores, or mesocosms, intact in PVC collars, were packed into sterile

158 plastic bags in the field, kept in coolers with ice packs, and transported to greenhouses
159 at Colorado State University (CSU), Fort Collins, CO, USA for the decomposition
160 experiment. Every effort was made to minimize disturbance to the soil.
161 Field temperature and moisture were measured at time of soil collection for both
162 AB and IB soils. Soil temperature was recorded in the field and daily during the
163 greenhouse incubation using a temperature probe coupled to a PP system (PP-system,
164 SRC-1). Initial soil moisture was determined by gravimetric water content (GWC) by
165 subtracting the oven-dry weight of soil (105°C) from the field moist weight. All soil pots
166 were weighed and %GWC was estimated based on initial field levels. Soil moisture was
167 maintained daily at 20% GWC by weighing the cores every other day and adding
168 deionized water as needed to bring up soil moisture levels.
169

170 **2.2 Production of ^{13}C-enriched root litter**

171 Prior to experiment setup, *Andropogon gerardii* was grown from rhizomes in soil-free
172 potting mix for one growing season in a continuous labeling chamber at 4 atom% ^{13}C-
173 CO_2 atmosphere, fertilized weekly for 21 weeks with a ^{15}N-KNO_3 solution (7 atom%)
174 (Soong et al., 2014). After the growing season, plants were harvested and roots were
175 separated from shoots. Roots were then washed, air-dried and a sub-sample analyzed
176 for %C, %N, and ^{13}C and ^{15}N enrichment by an Elemental Analyzer (EA; Carlo Erba NA
177 1500) connected to a continuous flow Isotope Ratio Mass Spectrometer (IRMS; VG
178 Isochrom, Isoprime Inc., Manchester, UK). The root litter had a C and N concentration
179 of 44.37% and 1.49%, respectively, and an isotopic enrichment of δ^{13}C 1882.37‰ (3.12
180 atom %) and δ^{15}N 12147.21‰ (4.61 atom %).

181

182 **2.3 Decomposition experiment**

183 Our experimental design consisted of two burn treatments and two litter treatments in a
184 fully factorial design (2 burn treatment x 2 litter treatment x 6 harvests x 4 replicates =
185 96). Soil cores from AB and IB treatments were incubated inside the PVC collars with
186 either of two different litter treatments: control (no litter) or litter addition (^{13}C-enriched
187 root litter). A total of 48 nylon litterbags (8cm x 8cm, 1mm mesh size) were prepared,
188 each containing approximately 1.5g of the air-dried ^{13}C-enriched root litter and buried in
189 the soil (24 AB and 24 IB) for the litter addition treatment. Subsamples of root litter were
190 dried in an oven at 70°C for oven-dry mass correction. To minimize disturbance to the
191 soil, each soil core was carefully removed from the PVC collar, sliced in half horizontally
192 (Sanaullah et al., 2010), a litterbag was placed in the center, and the two halves of the
193 core were restored together into the PVC collar. The remaining cores were sliced in half
194 then put back together, with no litterbag added, and established as control treatments.
195 All PVC collars were established on top of sand to allow for drainage and were
196 contained individually in pots to prevent cross contamination. The experiment was
197 conducted in a greenhouse at the Colorado State University Plant Growth Facility.

198 To assess decomposition and biotic community changes over time, 6 destructive
199 harvests occurred over 180 days, i.e., at 3, 10, 21, 35, 90, and 180 days. At each
200 harvest date, four replicates of each of the four treatments were harvested for analyses
201 of soil, root litter, and biota. Specifically, the litterbag was carefully removed from the
202 soil and set aside, each soil core was removed from the collar, placed into a sterile
203 plastic bag and well-mixed to homogenize soil. Each homogenized soil sample was

sub-sampled for PLFA analysis and nematode extraction. The roots were retrieved from the litterbag before drying in an oven at 45°C for 5 days. Mass loss was assessed by subtracting the remaining mass of roots (oven-dried) from the initial mass of roots (oven-dry mass corrected). All litter samples were then analyzed for %C and ^{13}C as described above for the initial litter material. Only C dynamics are discussed in this study.

2.4 Microbial community

Microbial community structure was assessed by Phospholipid Fatty Acid (PLFA) analysis. We ran three out of the four replicates (chosen at random) for PLFA analysis due to the expense and time required to run these analyses. Soil sub-samples for PLFA analysis were sieved to 2mm, with any visibly remaining plant material carefully removed with forceps. The PLFA extraction, quantification and $\delta^{13}C$ analysis methods were based on previous studies (Bossio and Scow, 1995;Denef et al., 2007;Gomez et al., 2014). For all treatments, approximately 6g soil subsamples from the bulk soil were lyophilized and extracted in duplicate using a modified Bligh-Dyer method (Gomez et al., 2014) at each harvest. Fatty acid methyl ester (FAME) derivatives were analyzed by capillary gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) (GC-C/TC DeltaPLUSXP Thermo Scientific) via a GC/C III interface. PLFA identifications were based on the retention times of two standard mixtures, a Supelco FAME mix (47885-U: Supelco 37 component FAME mix, Sigma-Aldrich) and a bacterial acid methyl ester mix (47080-U: BAME mix, Sigma-Aldrich). Representative samples were analyzed by gas chromatography-mass spectrometry (GC-MS; Shimadzu QP-

2010SE) and spectral matching was completed using the NIST 2011 mass spectral library (Shimadzu) to identify PLFAs that are not available in standard mixtures,

A number of PLFAs were selected as biomarkers for different microbial groups to investigate the soil microbial community composition (Frostegård and Bååth, 1996;Zelles, 1999). The PLFAs i15:0, a15:0, i16:0, a17:0, i17:0 were selected to estimate the abundance of Gram-positive bacteria, and cy17:0, cis16:1n9, 18:1n11, and cy19:0 for Gram-negative bacteria. Fungal abundance was based on cis18:1n9 and cis18:2n9,12, and methylated PLFAs 10Me-16:0, 10Me-17:0, and 10Me-18:0 were used as indicators of actinobacteria.

The abundance of individual PLFAs was calculated (ng g^{-1} soil) and used as a proxy for microbial biomass. Changes in the microbial community composition were evaluated based on relative PLFA abundance data, which were calculated as in Gomez et al. (2014).

2.5 Nematode community

For both AB and IB treatments, soil nematodes were extracted from each soil sample by a modified Baermann funnel method in deionized water after Hooper (1970). A subsample of 100g of soil was placed onto the Baermann funnels and an aliquot of water and nematodes removed daily for 3 days.

Nematodes were counted, identified and sorted using an inverted microscope (Olympus CKX41, 200X magnification) into five different trophic groups (bacterivore, fungivore, plant parasite, omnivore, and predator), based on Yeates et al. (1993), and trophic groups sorted into separate microcentrifuge tubes (0.5mL). For elemental and

isotopic analysis 75 individuals from each trophic group were then handpicked using an eyelash (Superfine eyelash with handle, Ted Pella, Inc., Prod no. 113) under a dissecting microscope (Olympus SZX10, 30X magnification), and transferred to a pre-weighed tin capsule (8x5mm, Elemental Microanalysis BN/170056) containing 120μL of deionized water. The tin capsules containing the different nematode trophic groups were desiccated for 3 days, weighed again to obtain final sample weights, and then prepared for analysis. The tin capsules containing nematode samples were analyzed for %C and ^{13}C using a CE-1110 EA coupled via Conflo II interface to an IRMS (ThermoFinnigan Delta Plus).

The absolute abundance of individual nematode groups was calculated (number nematodes kg^{-1} dry soil). Changes in the nematode community composition were evaluated based on relative nematode abundance data, which were calculated by dividing the absolute abundance of a nematode group by the sum of the absolute abundance of all nematode groups.

2.6 Data analyses

The isotope ratios are reported in terms of $\delta^{13}C$ (‰) values (Brenna et al., 1997), i.e.:

$$\delta^{13}C\ (‰) = (R_{sample} - R_{standard})/(R_{standard}) \times 10^3$$

where R_{sample} is the $^{13}C/^{12}C$ ratio of the sample and $R_{standard}$ refers to the reference standard, Pee Dee Belemnite.

The proportion of root-litter carbon incorporated into nematode and microbial tissue (f_R) was calculated by a two-source mixing model with:

$$f_R = (\delta_{BioR} - \delta_{BioC})/(\delta_R - \delta_{BioC})$$

δ_{BioR} and δ_{BioC} refer to the $\delta^{13}C$ signature of a group in the root litter-addition and the corresponding control, respectively, and δ_R to the $\delta^{13}C$ signature of the initial root litter.

The amount of root-derived C incorporated into individual PLFAs and nematode groups was calculated by multiplying the *f*-value by the absolute PLFA or nematode concentration (per g soil) for each individual PLFA or nematode group. The relative incorporation within each microbial group was calculated:

$$\text{PLFA-C}_{\text{root-derived/group}} = (\Sigma\text{PLFA-C}_{\text{group}} * 100) / \Sigma\text{PLFA-C}_{\text{root-derived all}}$$

The effects of time, soil burning treatment, and litter addition on microbial PLFA abundance, nematode densities, and microbial and nematode incorporation of root litter derived ^{13}C were analyzed by Analysis of Variance (ANOVA) methods using a generalization of the general linear model (GLM) in the Proc Mixed procedure. Statistical analyses were completed with SAS 9.3 (SAS Institute Inc., Cary, North Carolina). Data were analyzed using a three factor model, where y= time + soil + litter addition. Time, soil, and litter addition were treated as categorical variables. Data were tested to meet assumptions of normality and residuals were log transformed to achieve normality if necessary. Significance was accepted at a level of probability (P) of < 0.05.

A distance-based redundancy analysis (dbRDA) was used to evaluate differences in microbial and nematode community composition among fire and litter treatments. The dbRDA is a multivariate approach that is widely accepted and used for ecological studies to evaluate multispecies responses to several factors (Legendre and Anderson, 1999). For our dbRDAs, PLFA and nematode relative abundance data (mol% of each identified PLFA or nematode group) were used in two dbRDA models. A

296 distance matrix was calculated for each community using the Bray-Curtis measure to
297 model the species matrix. A principal coordinate analysis was performed on the
298 distance matrix and the resulting eigenvalues were applied to a redundancy analysis.
299 Ordination plots were drawn with ellipsoids (representing a 95% confidence interval)
300 around the multivariate community groups. The dbRDA and subsequent drawing of
301 ordination plots were performed using R (R Core Team, Vienna, Austria).
302
303 **3. Results**
304 **3.1. Effects of fire and root litter addition on the soil community**
305 Burn treatment had a significant effect on the soil community. The dbRDA revealed that
306 AB and IB community compositions of microbes and nematodes were significantly
307 different (Fig. 1A and Fig. 1B, respectively). For microbes, the differences in community
308 composition were driven by biomarkers for fungi (cis-C18:1n9, cis18:2n9,12) Gram-
309 negative bacteria (cy19:0), and Gram-positive bacteria (a17:0) (Fig. 1A). The total
310 average PLFA abundance for AB was significantly lower than IB treatment ($P<0.05$).
311 Specifically, there were lower proportions of PLFA biomarkers for Gram-positive
312 bacteria and fungi for AB (Fig. 2). Total nematode abundance did not differ between the
313 AB and IB treatment ($P=0.39$), but community structure was significantly different (Fig.
314 1B). While the differences for the AB soil were driven by fungivores and plant parasitic
315 nematodes, the IB soil community was influenced by omnivore and predator nematodes
316 (Fig. 1B).
317 With the addition of root litter to the soil, microbial and nematode communities
318 were changed (Fig. 1). The dbRDA revealed that the microbial community structure of

AB changed significantly with the addition of root litter, while IB did not (Fig 1A). Also, the AB and IB microbial communities became slightly more similar with root litter addition, yet these were still significantly different (Fig. 1A). As for abundance, 180 days after litter addition, there were no significant differences in abundance for any functional group for the IB or AB treatment relative to the control (Fig. 2).

Neither AB nor IB nematode community composition was significantly changed with the addition of root litter, but there was a general shift in the community (Fig. 1B) and total abundance of nematodes differed significantly through time (Fig. 3). The shift in the litter-addition communities was largely driven by bacterivore nematodes (Fig. 1B), and the abundance of bacterivore nematodes significantly increased with root litter addition for both treatments ($P=0.033$) through time. Additionally, for the litter addition treatment there were some differences between burn treatment such as: fungivore nematodes were significantly more abundant for AB than IB at 90 days ($P=0.032$); conversely, omnivore nematodes were significantly more abundant for IB at 180 days ($P=0.047$). There were no significant differences in abundance of plant parasitic or predator nematodes between AB and IB after litter addition.

3.2 Effects of fire on root decomposition and root-C dynamics

Significantly more root litter mass was lost for the AB treatment ($P=0.028$). Decomposition occurred rapidly (>30% mass loss) in the first 10 days and progressed slowly for the remainder of the experiment. By day 180, the percent of root litter mass remaining for the AB and IB treatment was 53.0±2.3% and 57.9±2.2%, respectively, and

likewise, more root litter C was lost from the AB treatment (P=0.03). Both time and burn treatment had significant effects on the root litter C pool dynamics (Fig. 4A).

3.3 Effects of fire on soil community utilization of root-C

Soil biota (both microbial PLFA biomarkers and nematodes) assimilated root litter ^{13}C for both AB and IB. All microbial groups and the microbivore nematode groups utilized root litter C immediately after root litter addition and throughout the experiment for both treatments. However, this C was translocated differently through the soil communities for AB and IB treatments (Fig. 5). Plant parasitic nematodes did not have a significant amount of root litter C incorporated into their biomass in either treatment. Higher trophic levels (omnivore and predator nematodes) began to have root litter C incorporated into their biomass by 21 (IB, Fig. 5A) and 35 (AB, Fig. 5B) days. This amount increased by the final harvest with IB omnivore and predator nematodes having greater root litter C incorporated than AB by the final harvest (Fig. 5).

The microbial biomarkers assimilation of root litter C increased significantly over time for both treatments (Fig. 4B). Despite higher total PLFA concentration in the infrequent burn treatment, the microbial pool of root litter C was not different between treatments. While there was generally more root litter derived C in the PLFAs initially (days 3, 10, 21) for IB and a lag in root litter C uptake for AB (Fig. 4B), the effect of burn treatment and the interaction of burn treatment and time was not significant for this pool of C. Also, the flow of C through the different groups of the microbial community was similar for each burn treatment (Fig. 5). In general, Gram-negative bacteria dominated the C uptake initially (days 3 to 21) and this shifted to Gram-positive dominance by 35 days

for both burn treatments (Fig. 5). Fungal use of root litter C differed slightly for the burn treatments, with fungi from the AB treatment increasing in root litter C over time (Figs 5C and 5D).

The nematodes' assimilation of root litter C also increased significantly over time for both treatments (Fig. 4C). While the burn treatment alone was not significant, the interaction of time and burn treatment was highly significant for the nematode C pool. At day 35 and 90, the nematode root litter-derived C pool was significantly higher for AB than the IB treatment (Fig. 4C). The flow of C through the nematode community also differed somewhat (Figs 5A and 5B). For both treatments bacteria and, correspondingly, bacterivore nematodes played a dominant role in root litter C utilization for both AB and IB soils (Fig. 5). Bacterivore nematodes dominated the nematode community in abundance and incorporated the greatest amount of root litter C overall; however, the other trophic groups differed between burning treatment. For the IB treatment, omnivore and predator nematodes utilized a significant portion of root litter C by 35 days after litter addition, but not for AB. For the AB treatment, fungivore nematodes significantly incorporated root litter C from day 3, but not for the IB treatment.

When we looked at the proportions of root litter C incorporated into individual group's biomass, there were differences between burn treatments. Overall, fungivore nematodes, saprotrophic fungi (cis-18:2n9,12), Gram-negative bacteria (18:1n11), and Gram-positive bacteria (a17:0 and i16:0) incorporated significantly more root litter C for the AB treatment than the IB treatment (Table 1). Only omnivore nematodes incorporated more root litter C for the IB treatment (Table 1).

4. Discussion

4.1. Effects of fire on the soil community

Burning has significant impacts on the belowground community including soil microbes and soil nematodes. We found that both soil microbial and nematode community structure differed with long-term burn treatments (Fig. 1), with the AB treatment also showing reduced microbial biomass (via PLFA methods). These findings support our first hypothesis, that different burn treatments would house different soil communities, and confirmed previous observations. In particular, Todd (1996) showed that bacterivore nematodes respond positively to frequent fire while predator nematodes do not. Jones et al. (2006) later corroborated that study via molecular methods. Additionally, fire has been shown to reduce overall microbial biomass and specifically affects Gram-negative and Gram-positive bacteria and fungi (Docherty et al., 2011;Ajwa et al., 1999). Such differences in the soil communities have implications for ecosystem function, such as impacts on organic matter decomposition (Verhoef and Brussaard, 1990).

4.2. Effects of fire on root decomposition and root-C dynamics

Root litter mass loss was greater for the AB treatment, confirming our second hypothesis that decomposition would be greater for the AB treatment. These results were in agreement with the observed higher aboveground litter respiration in the AB as compared to the IB site (Soong and Cotrufo, 2015). Yet, in a root decomposition study by Reed et al. (2009) there were no significant main effects of burning on root decomposition; however, low precipitation may have masked the effects of burning on

410 decomposition for that study. Other studies have compared belowground decomposition
411 in areas of contrasting burning treatments and have found that wood decomposed
412 significantly faster in annually burned tallgrass prairie compared to unburned prairie
413 (Reed et al., 2005;O'Lear et al., 1996). Faster decomposition in annually burned prairie
414 soil could be due to the indirect effects of burning on the soil community composition or
415 to the direct effects on soil conditions (i.e., heat, moisture, nutrients), which would
416 impact decomposition processes (O'Lear et al., 1996). For example, relative to
417 unburned tallgrass prairie soils the soil conditions of frequently burned areas are often
418 N-limited (Blair, 1997;Ojima et al., 1994), causing microbes to scavenge for N before
419 beginning decomposition (Soong and Cotrufo, 2015;Craine et al., 2007). N-mining by
420 microbes in N-limited areas has been shown to increase decomposition rates in other
421 areas (Craine et al., 2007).
422
423 **4.3 Effects of fire on soil community utilization of root-C**

424 Corroborating part of our third hypothesis, we found that, overall, a significantly higher
425 amount of ^{13}C was incorporated into the total soil community for AB, indicating greater
426 utilization of root litter C in this frequently burned soil. In particular, fungivore nematodes
427 and specific biomarkers for fungi, Gram-negative bacteria, and Gram-positive bacteria
428 had a significantly higher proportion of their biomass composed of root litter C,
429 suggesting that root litter C was a more important C source for the AB soil food web.
430 Additionally, despite significantly lower microbial abundance for the AB treatment, there
431 was no difference in the amount of root litter derived C in the total microbial pool
432 between AB and IB treatment. In this way, our study offers some support for the

433 hypothesis that decomposition is strongly affected by decomposer community
434 composition instead of the abundance (Wickings et al., 2012). In other words, distinct
435 decomposer communities (such as the significantly different AB and IB communities)
436 could have differing metabolic or functional capabilities. Perhaps the AB community
437 incorporates a greater proportion of the root litter C into biomass because those biota
438 are predisposed to take advantage of this C source due to the recurrent impacts of fire.
439 This may also indicate different mechanisms such as higher microbial turnover or
440 increased microbial grazing by nematodes during decomposition of roots for the AB
441 treatment.

442 We also hypothesized that root-C would be incorporated more quickly for AB. Yet
443 despite the overall greater amount of root-C incorporation by AB, microbes and
444 nematodes both immediately incorporated root-C for both treatments (Fig. 4B and 4C).
445 There was a slight lag in microbial uptake of root litter C for AB, but not for IB (Fig. 4B).
446 This lag could correspond to the time microbes needed to scavenge N in the N-limited
447 AB soil before commencing root decomposition (Manzoni et al., 2012). Yet through
448 time, evidence exists for greater cycling of root litter C to the microbivore nematodes of
449 the AB food web. The root litter derived nematode-C pool was significantly greater in the
450 AB treatment at 35 and 90 days after root addition. This accumulation of C in
451 nematodes indicates a greater or faster flow of root litter C from the microbes to their
452 nematode consumers. Others have suggested that most energy from detritus flows to
453 microbes and only a negligible amount of energy flows to the higher trophic levels of the
454 soil food web (Setala, 2005). Our study opposes this view, as we show that per gram of
455 soil, nematodes can hold as much as half of root litter derived-C as microbes do (Fig.

4B and 4C).

5. Conclusions

Our results provide evidence that frequent fire affects decomposition processes and adds a temporal dynamic of C flow through the soil food web. We have shown that decomposing roots are an important C-source for microbes and nematodes in this tallgrass prairie soil. ^{13}C originating from root litter was traced into different nematode trophic groups, indicating that they had utilized root-derived C by feeding on bacteria, fungi, protists, other nematodes, or other soil organisms. Our study shows that not only does fire affect the soil community composition and root litter mass loss, but the lower microbial abundance, greater root turnover, and the increased incorporation of root litter C by fungi, Gram-negative bacteria, Gram-positive bacteria, and fungivore nematodes for AB indicates greater root litter-derived C flow through the soil food web for AB. Until now, nematodes' contribution to root litter decomposition was inconclusive, but we have shown that nematodes incorporate a significant amount of root litter C across trophic levels and this differs by fire treatment. Thus, both microbial and higher nematode trophic levels are critical components of C flow during root decomposition, which, in turn, is significantly affected by fire.

Acknowledgements

This project was funded by the National Science Foundation under grant no. 0918482. We are grateful to the Konza Prairie LTER site for making this research possible. We thank the Wall Lab, especially K. Ivanovich and E. Bernier, for assistance with work in

the field and laboratory. We thank the staff of the Colorado State University's EcoCore Analytical Facility (http://ecocore.nrel.colostate.edu/) and Kansas State University's Stable Isotope Mass Spectrometry Laboratory (http://www.k-state.edu/simsl/SIMSL_Home.html) for their support with analyses.

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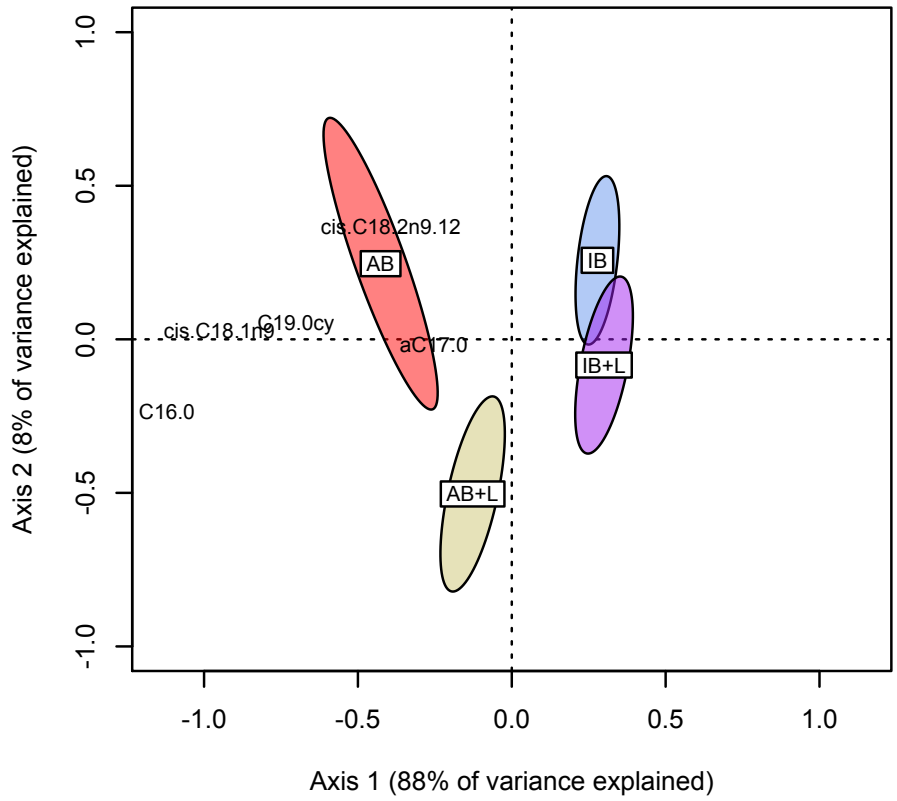
711

712 Table 1. Overall mean relative contribution (f) of root litter C to PLFA-C and nematode-C
713 with (standard errors), n=18. The relative contribution of root litter C was calculated only
714 for the PLFA biomarkers and nematode trophic groups from root litter addition samples
715 that were significantly different in $d^{13}C$ from the control. **Bold font** indicates a
716 significantly higher f-value for a burn treatment.
717

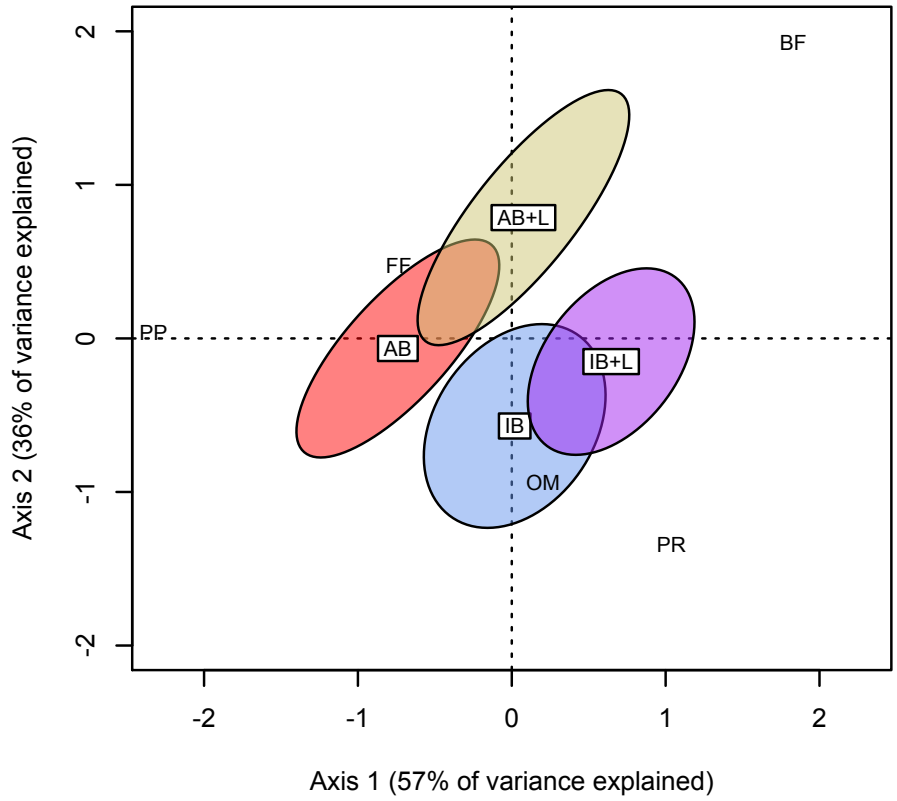
Functional Group		**AB Mean *f*-root litter x 100**	**IB Mean *f*-root litter x 100**
	PLFA Biomarker		
Fungi SAP	cis-C18:1n9	0.4 (0.14)	0.3 (0.05)
	cis-C18:2n9,12	**1.6 (0.37)**	1.1 (0.15)
Gram-	cis-C16:1n9	0.6 (0.11)	0.3 (0.07)
	C17:0cy	0.6 (0.09)	0.4 (0.10)
	C18:1n11	**0.7 (0.10)**	0.4 (0.06)
	C19:0cy	0.1 (0.06)	0.1 (0.03)
Gram+	aC15:0	0.4 (0.08)	0.3 (0.05)
	aC17:0	**0.3 (0.06)**	0.1 (0.03)
	iC15:0	0.3 (0.12)	0.2 (0.05)
	iC16:0	**0.4 (0.08**)	0.2 (0.05)
Actinobacteria	10Me-C16:0	0.3 (0.08)	0.1 (0.04)
	10Me-C17:0	0.2 (0.07)	0.1 (0.03)
	10Me-C18:0	0.3 (0.08)	0.3 (0.06)
Nematodes	Trophic Group		
	Bacterivore	8.2 (1.4)	6.4 (1.4)
	Fungivore	**7.5 (1.8)**	ns
	Omnivore	0.5 (0.2)	**1.7 (0.7)**
	Predator	0.5 (0.3)	0.4 (0.2)

718
719
720

A.

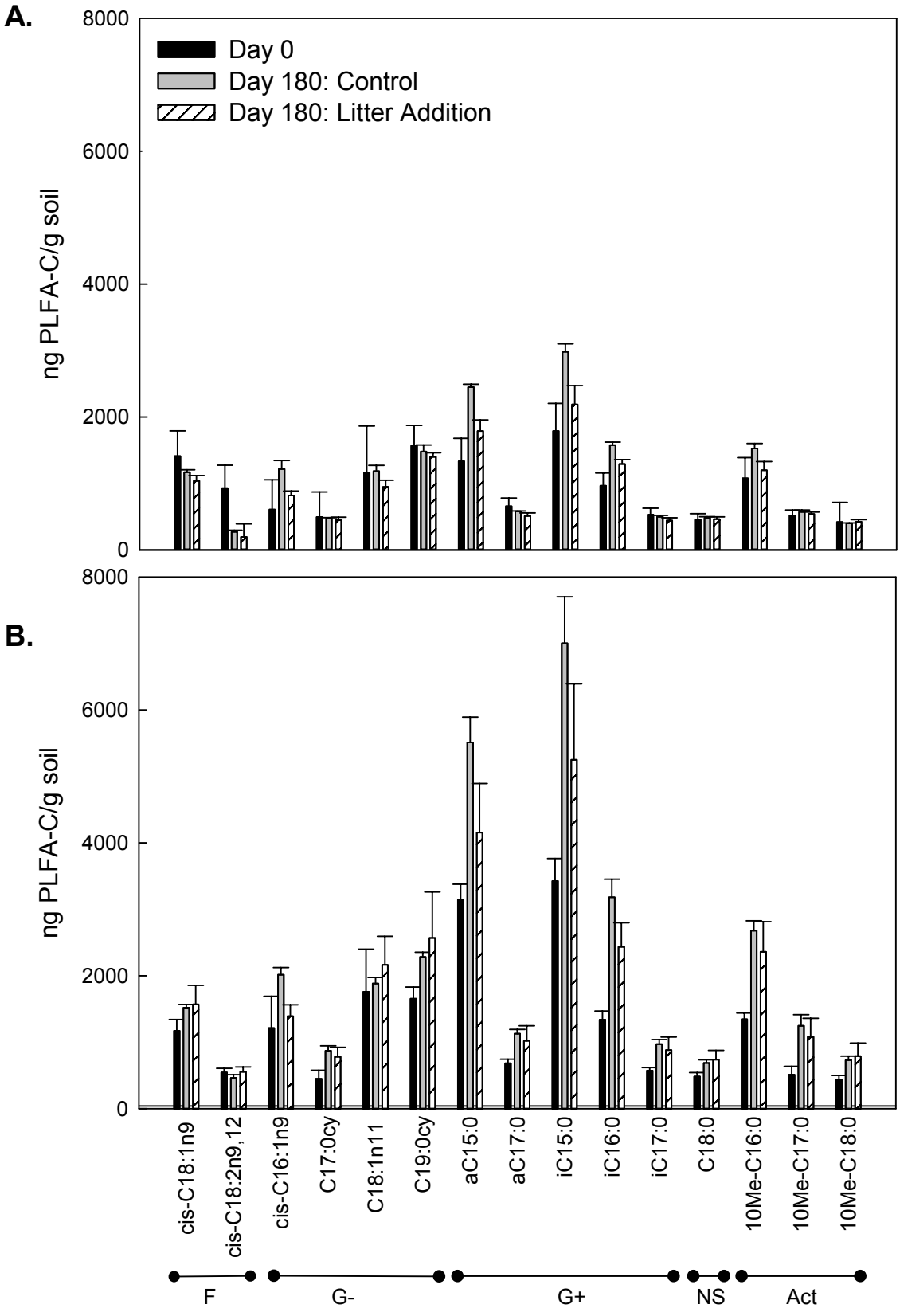

cis.C18.2n9.12
AB
IB
cis.C18.1n9
C19.0cy
aC17.0
IB+L
C16.0
AB+L
Axis 2 (8% of variance explained)
Axis 1 (88% of variance explained)
1.0
0.5
0.0
-0.5
-1.0

B.


BF
AB+L
FF
PP
AB
IB+L
IB
OM
PR
Axis 2 (36% of variance explained)
Axis 1 (57% of variance explained)
2
1
0
-1
-2

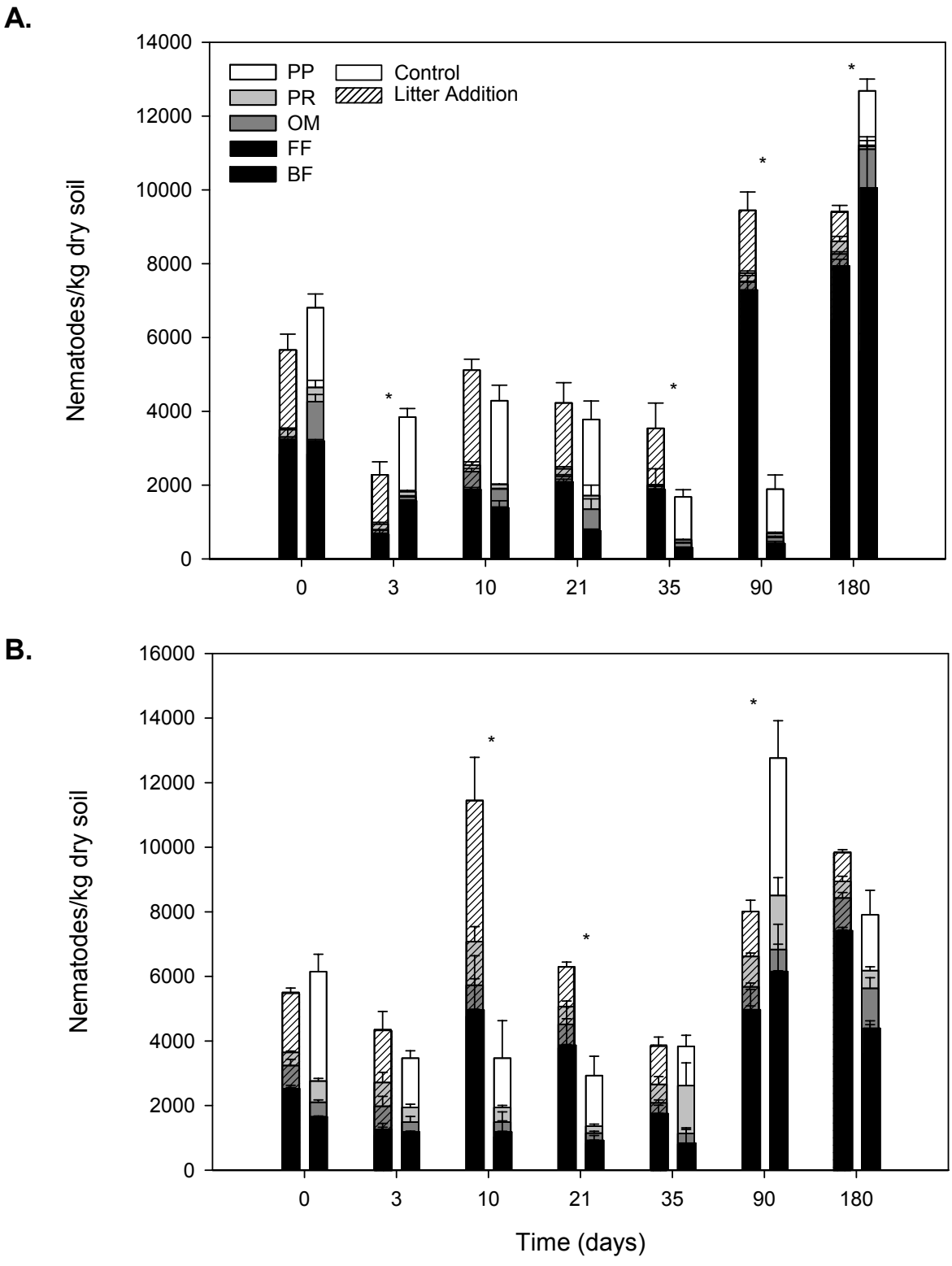
721

Figure 1. Community structure plots depicted from results of the distance-based redundancy analysis performed on relative abundance of PLFA biomarkers (A) and on nematode trophic groups (B); Groups with top species scores are plotted along with ellipsoids. Ellipsoids represent 95% confidence intervals. The first and second capscales are depicted by Axis 1 and Axis 2, respectively. Percentage of variance explained by each capscale is indicated. Treatments are indicated by: AB=annually burned, IB=infrequently burned, and +L=litter addition. For nematode trophic groups: BF=Bacterivore, FF= Fungivore, OM=Omnivore, PP= Plant Parasite, and PR=Predator.


A.
B.
Day 0
Day 180: Control
Day 180: Litter Addition
ng PLFA-C/g soil
8000
6000
4000
2000
0
cis-C18:1n9
cis-C18:2n9,12
cis-C16:1n9
C17:0cy
C18:1n11
C19:0cy
aC15:0
aC17:0
iC15:0
iC16:0
iC17:0
C18:0
10Me-C16:0
10Me-C17:0
10Me-C18:0
F
G-
G+
NS
Act

730

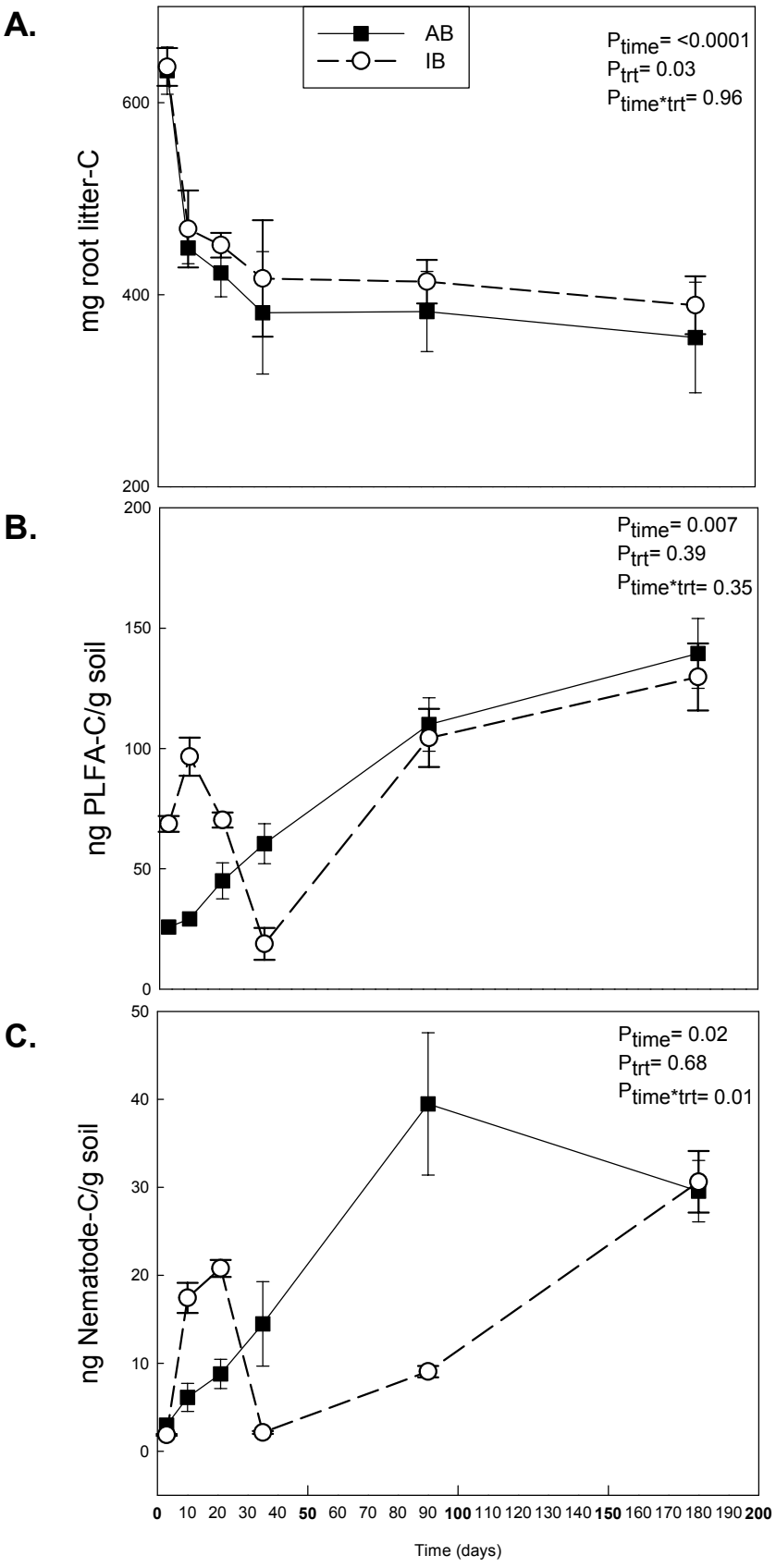
Figure 2. Abundances of PLFA biomarkers for the annual burn (A) and infrequent burn (B) treatments with litter addition for the day 0 and final 180 day harvest. Data are averages (n=3) with standard error bars. For PLFA groups: F=fungi, G+= Gram-positive bacteria, G-=Gram-negative bacteria, NS= non-specific bacteria, Act=Actinobacteria.


A.
B.
PP
PR
OM
FF
BF
Control
Litter Addition
Nematodes/kg dry soil
Time (days)
0
3
10
21
35
90
180
0
2000
4000
6000
8000
10000
12000
14000
16000
*

737

738

739 Figure 3. Change in nematode trophic group abundance (#Nematodes/kg dry soil) over
740 time for both A) annual burn and B) infrequent burn treatments with litter addition. Day 0
741 indicates the initial densities of nematode trophic groups before the greenhouse
742 incubation with root litter addition. Asterisks (*) indicate significantly different total
743 abundance of nematodes between litter treatments (n=4). For nematode trophic groups:
744 BF=Bacterivore, FF= Fungivore OM=Omnivore, PP= Plant Parasite, and PR=Predator.


A.
AB
IB
P_{time}= <0.0001
P_{trt}= 0.03
$P_{time*trt}$= 0.96
mg root litter-C
600
400
200
B.
P_{time}= 0.007
P_{trt}= 0.39
$P_{time*trt}$= 0.35
ng PLFA-C/g soil
200
150
100
50
0
C.
P_{time}= 0.02
P_{trt}= 0.68
$P_{time*trt}$= 0.01
ng Nematode-C/g soil
50
40
30
20
10
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200
Time (days)

745

746 Figure 4. Root litter C dynamics during incubation for the annual burn and infrequent
747 burn treatments. Data are averages with standard error bars. The root litter carbon (A),
748 root litter derived carbon incorporated in microbial phospholipid fatty acids (PLFA) (B),
749 and root litter derived carbon incorporated in nematodes (C) are reported.
750

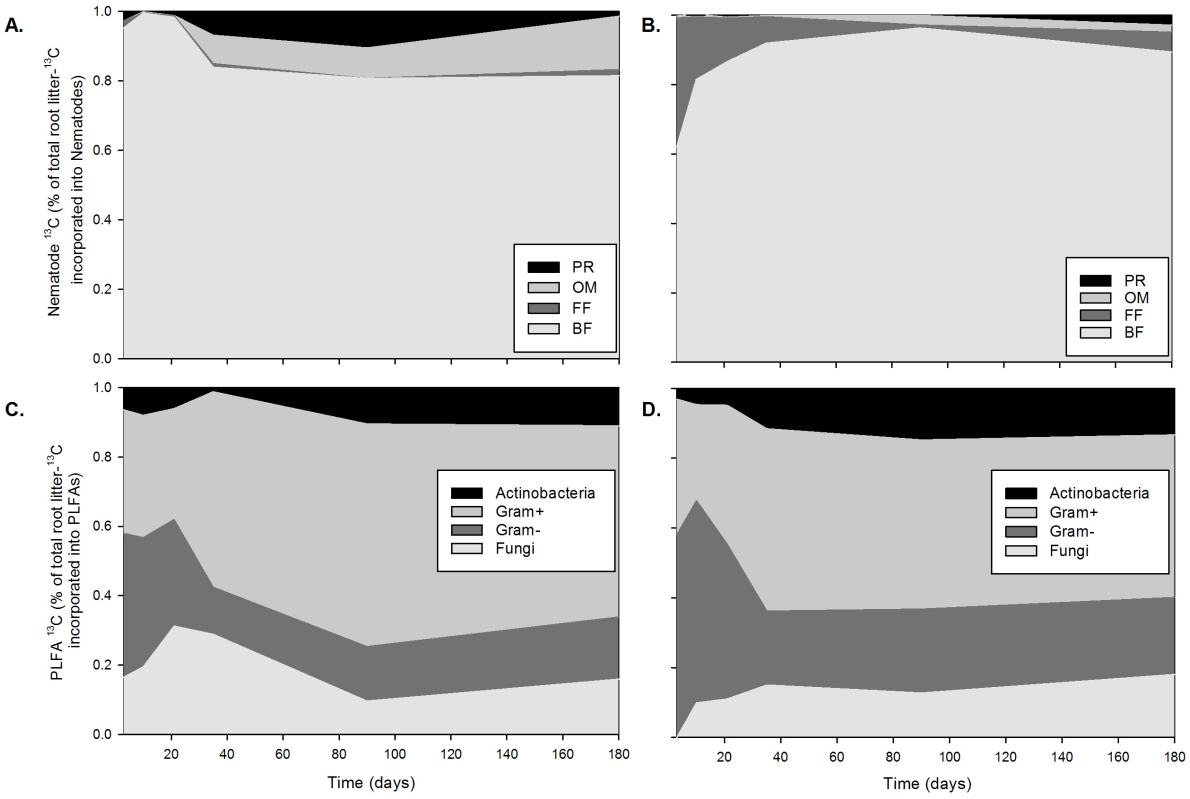


Figure 5. Root litter C incorporation into microbial PLFAs and nematode trophic groups. Panels (A) and (C) are infrequent burn treatment and (B) and (D) are annual burn treatment. Panels (A) and (B) show the percentage of total litter-derived C (^{13}C) incorporated into the total nematode signature quantified at each time point, and panels (C) and (D) show the percentage of total litter-derived C (^{13}C) incorporated into the total PLFA signature at each time point. For nematode trophic groups: BF=Bacterivore, FF= Fungivore OM=Omnivore, PP= Plant Parasite, and PR=Predator.