THESIS

FIRE MANAGEMENT EFFECTS ON CARBON FLOW FROM ROOT LITTER TO THE SOIL COMMUNITY IN A TALLGRASS PRAIRIE

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Elizabeth Ashley Shaw

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Master's Committee:

Advisor: Diana H. Wall

M. Francesca Cotrufo
Eugene F. Kelly

ABSTRACT

FIRE MANAGEMENT EFFECTS ON CARBON FLOW FROM ROOT LITTER TO THE SOIL COMMUNITY IN A TALLGRASS PRAIRIE

Belowground litter decomposition is a major component of carbon cycling in grasslands, where it provides energy and nutrients for soil microbes and fauna. Fire, a historically frequent disturbance and a common management tool, removes above ground biomass and litter accumulation making belowground root litter of greater importance to decomposer food webs. While many studies use biomass measures of soil faunal groups to estimate changes in soil food web structure and energy flow, little is known about the flow of C from root litter to soil microbial and nematode communities in grasslands and if biomass measures can indicate this flow of C at a fine scale.

Our greenhouse experiment first investigated how C from *Andropogon gerardii* (big bluestem) root litter was allocated into different soil microbial and nematode groups in frequently burned (FB) and infrequently burned (IB) tallgrass prairie soil. Incorporation of ^{13}C into microbial fatty acids and nematode communities was determined on six occasions during decomposition in order to examine whether different groups of microorganisms and fauna were specialized on the root-litter derived C. Results showed that FB and IB soils supported microbial communities of differing community composition and abundance. IB had, generally, higher microbial abundance, more strongly dominated by bacteria than FB soil. Compound-specific stable isotope ratio analysis showed that root litter-C was more quickly incorporated into FB soil microbes. By the end of the experiment, all microbial groups were more highly ^{13}C enriched in FB

soils than in IB soils, with the exception of gram-negative bacteria for which there was no significant difference between the two soils. For nematodes, there was no significant difference in abundances; however, fungivore nematodes only incorporated root litter-C in FB soil while bacterivores, omnivores and predators derived at least some C from root litter in both treatments. Despite lower abundance of microbes in FB soil, total root litter mass loss did not differ between FB and IB soil, indicating higher microbial activity in FB soil. Our results reveal that FB prairie soil food webs are more closely coupled to root litter decomposition, where root litter is of increased importance as a C and nutrient source due to the frequent removal of standing biomass and shoot litter by fire.

In the second part of our greenhouse experiment, we compared soil energy channel biomass measures with C flow into the soil food web. By coupling the energy channel biomass measurement approach with our decomposition study (using stable isotope enrichment to trace the flow of C into nematode trophic groups), we compared the quantified C flow to nematode energy channel biomass measures during decomposition of ^{13}C-labeled big bluestem root litter. We hypothesized that biomass measures for nematode bacterial and fungal energy channels would indicate the proportion of root litter derived C incorporated into each nematode energy channel. Nematode biomasses and δ^{13}C values were assessed initially (day 0) and after 180 days of incubation. Results showed the nematode bacterial energy channel dominated over the nematode fungal energy channel in both FB and IB grasslands. Yet, FB grassland soil had significantly higher nematode bacterial energy channel biomass than IB at time 0. In both soils, the nematode bacterial energy channel biomass increased significantly after the addition of root litter and there were no differences in the

nematode bacterial channel biomass between the two soils at the final harvest (180 days). There were no differences between FB and IB soil's nematode fungal energy channel biomass at either day 0 or 180 days. ^{13}C analysis of nematodes confirmed our hypothesis, as more root litter-C was concentrated in the dominant nematode bacterial energy channel in both FB and IB grassland soils. However, the IB soil's nematode bacterial energy channel had incorporated significantly more root litter derived C than the FB soil, despite no differences in these energy channel biomasses at the final harvest. The FB soil food web showed the opposite effect for the nematode fungal energy channel. These results indicate that while energy channel biomass measurements of nematodes give a broad overview of C flow, ^{13}C decomposition tracer studies are more precise, and provide exact measures of C flow through soil food webs for ecosystem research.

Overall, our results highlight the general view that plant litter is an important C-source in grasslands and further show that root litter-C is incorporated differently in frequently and infrequently burned soil food webs. We show that frequently burned soil food webs may be more specialized to decompose grass root litter. Our results indicate the C flow within soil food webs in differing burn management areas, and show differences between the frequently and infrequently burned tallgrass prairie.

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1. INTRODUCTION

Soils and their biodiversity play a vital role in the carbon (C) cycle as soil organic matter (SOM) holds two-thirds of the earth's terrestrial C and because soils are the primary location for SOM decomposition (IPCC 2000, 2007, Nielsen et al. 2011). Soil biota are diverse and perform a suite of ecosystem processes, including the transformation of SOM into recalcitrant soil organic C (SOC) and CO_2 during decomposition; thus, understanding how soil biota contribute to the balance and maintenance of different soil C pools is important (Fierer and Lennon 2011). While some studies indicate soil biota overall are important to C processes (Nielsen et al. 2011), there is need for more information about the role that soil faunal groups (e.g., those of nematodes and microarthropods, the most abundant soil faunal groups) play in soil C dynamics during decomposition.

While soil microbes complete much of decomposition and respire the majority of CO_2 that effluxes from soils, biotic interactions across microbial and faunal groups can alter soil C processes (Nielsen et al. 2011). Several studies explicitly show that multi-trophic interactions with soil fauna, including collembolans, mites, enchytraeids, isopods and earthworms, and soil microorganisms, alter C cycling and increase decomposition by stimulating rates of litter mass loss and C mineralization (Seastedt and Crossley 1984, Ingham et al. 1985, Wall et al. 2008, Nielsen et al. 2011). However, precise quantification of energy fluxes through the food web's energy channels is challenging, especially for soil faunal contributions (Pollierer et al. 2009).

Nematodes have several traits that make them helpful for evaluating carbon processes in ecosystems. Soil nematodes span a minimum of five trophic groups in

soils (Yeates et al. 1993), contribute to both bacterial and fungal energy channels (Hunt et al. 1987), and act as drivers of the decomposer soil food web (Moore and Deruiter 1991). By occupying the second or third levels in the soil food web, they integrate the physical, chemical, and biological traits associated with their resources (Nannipieri et al. 1990) and, because of this, are useful ecological indicators (Freckman 1988).

Land management practices impact soil fauna, such as nematodes, by altering trophic group and species composition, abundance and biomass (Freckman and Ettema 1993, Bardgett et al. 1996, Bossio et al. 1998, Ferris et al. 2001, Reed et al. 2009). In the tallgrass prairie, fire is a management strategy used to promote grass growth (Knapp et al. 1998) and has significant impact on aboveground primary productivity, plant community composition, and plant physiology (Knapp et al. 1998, Kitchen et al. 2009). Besides aboveground changes, frequent fire in the tallgrass prairie also impacts belowground ecosystem processes. Burning impacts decomposition substrates and rates, alters abiotic soil conditions, and impacts the soil biotic community (Knapp and Seastedt 1986, Ojima et al. 1994, O'Lear et al. 1996, Rice et al. 1998, Johnson and Matchett 2001). There is substantial evidence that soil disturbance in many ecosystems impacts decomposition rates and soil food web trophic structure (O'Lear et al. 1996, Neher et al. 2003, Neher et al. 2005, Reed et al. 2009), but it is unclear how long-term burning practices in the tallgrass prairie affects soil food web trophic compartmentalization and C flow belowground.

Previously, stable isotopic methods have been used to examine the incorporation of different C resources into soil animals and to explore trophic structure relationships in decomposer communities of agricultural fields (Albers et al. 2006). The potential for

using stable isotopes to study belowground trophic structure and C and N dynamics had been little explored until Eggers and Jones (2000) pointed out its usefulness. Since, most studies have focused on few taxa or certain guilds (Hishi et al. 2007), ignoring very abundant soil faunal groups such as nematodes, and few studies have explored the flow/cycle of C through the belowground food web (Ostle et al. 2007, Pollierer et al. 2007, Elfstrand et al. 2008).

These observations motivated the following study, in which the flow of C from decomposing root litter was traced into microbial and nematode groups and the effect different burning land management practices on the soil food web's C processes were investigated using a stable isotope approach. Decomposition and soil biology were studied in order to learn more about fundamental C processes in grasslands. We asked the following questions:

1) Are soil food webs from a frequently burned tallgrass prairie more closely coupled to root litter decomposition than soil food webs from infrequently burned tallgrass prairie?
2) How does C flow through the soil nematode food web during decomposition and does this change over time of decomposition?
3) Do common grassland management techniques (i.e. annual burning) impact C flow belowground?
4) Can the stable isotope approach be used to better quantify belowground energy channels vs. traditional biomass approaches?

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2. SOIL FOOD WEBS FROM FREQUENTLY BURNED PRAIRIE ARE CLOSELY COUPLED TO ROOT LITTER DECOMPOSITION

2.1 INTRODUCTION

Soils are exceptionally intricate ecosystems, contain an immense diversity of soil microorganisms and soil fauna, and are of key importance to terrestrial ecosystems in terms of nutrient cycling and carbon (C) storage (Wall 2004, Bardgett 2005, Wall et al. 2010). There is strong interest in understanding the roles of the soil food web in regulating belowground processes of decomposition, nutrient cycling, and C cycling (Bardgett and Cook 1998, Osler and Sommerkorn 2007, Holtkamp et al. 2008, Carrillo et al. 2011, Holtkamp et al. 2011), especially how soil fauna contribute to these ecosystem processes (Bardgett and Cook 1998, Brussaard 1998, Carrillo et al. 2011, Nielsen et al. 2011). Yet, more information on the role that abundant soil faunal groups, (for example, 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), play in soil C dynamics (Staddon 2004, Osler and Sommerkorn 2007, Wall et al. 2008, Nielsen et al. 2011) is needed.

Land management and land use practices affect soil and soil biota by altering trophic group and species composition, abundance and biomass (Freckman and Ettema 1993, Bardgett et al. 1996, Bossio et al. 1998, Ferris et al. 2001, Reed et al. 2009). In the tallgrass prairie (i.e. Konza prairie, Kansas), frequent burning is a management strategy used to promote growth of warm season grasses (by maintaining the

aboveground grass productivity and limiting the encroachment of woody species), usually for livestock grazing (Knapp et al. 1998). Frequent fires can have large effects on plant productivity, plant community composition, and root properties (Knapp et al. 1998, Kitchen et al. 2009) and frequent burn of grasslands impacts belowground biological activities and ecosystem processes as well as aboveground (Ojima et al. 1994, Johnson and Matchett 2001).

The belowground effects of fire may have additional impacts on soil biodiversity and their functions. Burning causes changes in the soil surface energy budget by removing plant litter accumulation (Knapp and Seastedt 1986, O'Lear et al. 1996). This may alter soil conditions, such as temperature and moisture, impacting microbial and soil faunal activity rates or changing detritivore species composition. Indirect effects of frequent fire, such as changes in the soil microbial community (Rice et al. 1998) as well as the increased organic inputs belowground (root growth and exudates, (Ojima et al. 1994), may be responsible for differences in decomposition rates between above and belowground litter (O'Lear et al. 1996).

Litter decomposition is an important component of belowground C cycling and root litter-C provides a major energy source for soil biota in grasslands (Eisenhauer and Reich 2012). Fire’s removal of aboveground litter and influence on augmented root growth, allocation of C belowground, and root detritus inputs may make belowground litter of increased importance to decomposer food webs (Seastedt et al. 1991, O'Lear et al. 1996). While most litter decomposition is ultimately the product of the metabolic activities of soil fungi and bacteria, soil fauna also play a role in litter decomposition by impacting microbial activities and changing litter chemical composition (Petersen and

Luxton 1982, Verhoef and Brussaard 1990, Coleman and Crossley 1996, Xin et al. 2012). For example, soil nematodes contribute to decomposition processes through the stimulation of microbial activities (Coleman and Crossley 1996, Coleman and Hendrix 2000, Mamilov 2000, Carrillo et al. 2011). Changes in soil community composition impacts litter decomposition (Verhoef and Brussaard 1990) and nutrient turnover (Carrillo et al. 2011). However, little is known about how fire management of grasslands impacts soil community composition, if these soil communities are more specialized to decompose root litter, and how root litter-C is fractionated through the soil food web.

Addition of ^{13}C-enriched plant litter with a dissimilar isotopic signature can be traced during decomposition as an isotopic fingerprint into microbial and faunal groups. This technique has been used to study microbial communities in soil ecosystems, examining assimilation of isotopically labelled plant substrates into microbial phospholipid fatty acids (PLFA) (Treonis et al. 2004, McMahon et al. 2005, Denef et al. 2009). In addition, food web structures of some soil faunal communities (e.g., collembola, earthworms, enchytraieds, microarthropods, gastropods, diplopods, and chilopods) have been studied (Chahartaghi et al. 2005, Albers et al. 2006) and C flow though soil faunal trophic groups (e.g. collembola, earthworms, enchytraieds, microarthropods, gastropods, diplopods, and chilopods) has been traced and quantified using ^{13}C (Albers et al. 2006, Ostle et al. 2007, Pollierer et al. 2007, Elfstrand et al. 2008). However, root turnover and aboveground litter inputs are the main basis for soil faunal trophic groups in the chiefly detrital-based grassland soil food webs (Ostle et al. 2007) and these studies often focus only on C from recent photosynthate, ignore some

of the most abundant soil fauna groups (i.e. nematodes), and do not consider how differing land management tools, such as fire, might affect C flow belowground.

This project was designed to trace C from decomposing root litter into components of the soil food web over time in burned and unburned prairie soil. Our conceptual approach included the production of a ^{13}C-enriched dominant tallgrass prairie grass (Big Bluestem, *Andropogon gerardii*) root litter, its incubation in intact frequently and infrequently burned prairie soil cores in a greenhouse, and quantifying the contribution of root litter-C to the soil food web over time. We hypothesized that frequently burned prairie soil will support a belowground community that is more specialized in the decomposition of root litter. Specifically, we hypothesize that:

1. Frequently burned prairie soil will support a different community composition of microorganisms and nematodes than infrequently burned prairie.
2. Microorganisms and nematodes from frequently burned prairie will incorporate C from root litter more quickly than those from infrequently burned prairie.
3. Over time, microorganisms and nematodes from frequently burned prairie soil will be most highly enriched in ^{13}C from root litter.
4. Total root litter derived C incorporated into the frequently burned soil food web will be higher than in the infrequently burned soil food web.

2.2 METHODS

2.2.1 Site description and soil collection

The soil samples were taken from the top 10cm of a “frequently burned” and an “infrequently burned” silty clay loam Argiustoll under historically unplowed tallgrass prairie at the Konza Prairie Biological Station (KPBS) in eastern Kansas, United States (39°05'N, 96°35'W)[1]. The site is part of the Long Term Ecological Research (LTER) network. Average monthly temperatures range from -2.7° C in January to 26.6° C in July, with 835mm of total annual precipitation on average[1].

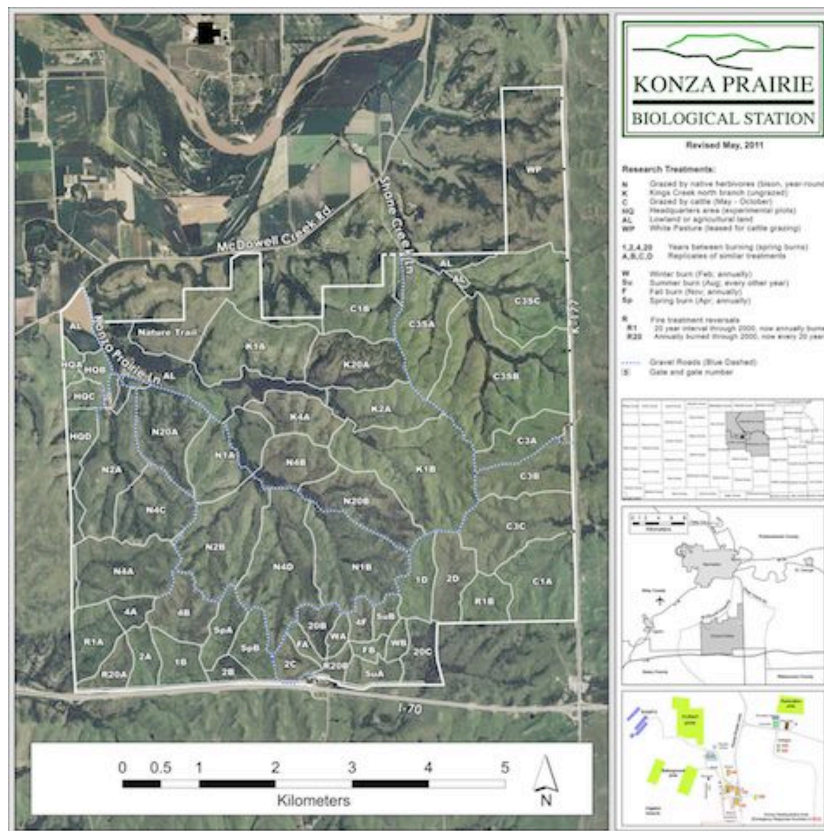

Figure 1. Annotated map of the Konza Prairie Biological Station, Eastern KS, USA.[1]

[1] Konza site information: http://kpbs.konza.ksu.edu/location.html

A total of 120 soil cores (10cm deep x 10cm diameter) were extracted from upland soil of two fire treatment areas at KPBS on 14 June 2011. "Frequently burned" soil (60 soil cores, 10cm deep x 10cm diameter) was taken from an annual spring burn (SpB, Fig. 1) watershed with soil characteristics: pH: 6.2, available P: 5.4 ppm, total C: 4.1%, and total N: 0.32% [2]. The annual spring burn watershed was burned yearly each spring since 1972, and was burned prior to soil collection on 26 April 2011. "Infrequently burned" soil (60 soil cores, 10cm deep x 10cm diameter) was taken from a 20-year burn (20B, Fig. 1) watershed with the following soil characteristics: pH: 6.1, available P: 3.7 ppm, total C: 5.5 % dry weight, and total N: 0.48% dry weight[2]. The 20-year burn watershed was last burned by wildfire (unprescribed) on 5 April 1991; previously, a prescribed burn occurred on 3 May 1975. Soil from the annual spring burn watershed will be referred to as frequently burned (FB) and the 20-year burn as infrequently burned (IB) for the remainder of this paper.

The soil cores were placed intact into PVC collars (10cm deep x 10cm diameter) in the field, packed into sterile plastic bags, kept in coolers with ice packs, and transported to greenhouses at Colorado State University (CSU), Fort Collins, CO, USA for the decomposition experiment. Every effort was made to minimize disturbance to the soil and thus, preserve the integrity of the soil biotic community.

Field temperature and moisture were measured at time of soil collection for both FB and IB soils to determine appropriate greenhouse parameters. Soil temperature was recorded in the field and daily during the greenhouse incubation using a temperature probe coupled to a PP system (PP-system, SRC-1). Initial soil moisture was determined

[2] Konza LTER database (NSC01): http://www.konza.ksu.edu/knz/pages/data/knzdata.aspx

by gravimetric water content (GWC) by subtracting the oven-dry weight of soil (105°C) from the wet weight. All soil pots were weighed and %GWC was estimated based on initial field levels. Soil moisture was maintained daily at 20% GWC, based on levels observed in the field.

2.2.2 Production of ^{13}C-enriched root litter

Prior to experiment setup, *Andropogon gerardii* was grown for one growing season from rhizomes in a closed continuous labeling chamber, located inside the CSU greenhouse, of ^{13}C-CO_2 atmosphere (4 atom%) maintained at a concentration of 360-400 ppm, in C-free soil media fertilized weekly for 21 weeks with a ^{15}N-KNO_3 solution (7 atom%) (Soong et al. Submitted). After the growing season, plants were harvested and roots were separated from shoots. Roots were then washed, air-dried and a sub-sample analysed for %C, %N, and ^{13}C and ^{15}N enrichment by an Elemental Analyser (Carlo Erba NA 1500) connected to a continuous flow Isotope Ratio Mass Spectrometer (VG Isochrom, Isoprime Inc., Manchester, UK). The root litter had a C and N concentration of 44.37% and 1.49%, respectively, and an isotopic enrichment of $\delta^{13}C$ 1882.37‰ and $\delta^{15}N$ 12147.21‰.

2.2.3 Decomposition experiment

The FB and IB soil samples were incubated in the PVC collars with two different litter treatments (no litter/control or litter addition), in a fully factorial design. A total of 60 nylon litterbags (8cm x 8cm, 1mm mesh size) were prepared, each containing approximately 1.5g of the air-dried labeled root litter, and one was buried in each of 30

FB and 30 IB soil collars. The masses of all litterbags were adjusted to the oven-dry mass. To minimize disturbance to the soil, each soil core was carefully removed from the PVC collar, sliced in half horizontally (Sanaullah et al. 2010), a litterbag was placed in the center, and the two halves of the soil core were restored to the PVC collar. The remaining FB and IB soil cores were sliced in half then put back together, with no bag added, and established as "control" treatments. All PVC collars were established on top of sand to allow for drainage and were held in individual compartments in gardening trays to preventing cross contamination. The experiment was maintained in the greenhouse.

To assess decomposition and biotic community changes over time, 6 destructive harvests occurred over 180 days, i.e., at 3 days, 10 days, 21 days, 35 days, 90 days, and 180 days. At each harvest date, 3 replicates of each of the 4 treatments were randomly selected for harvest. Then, the litterbag was carefully removed from the soil and set aside, soil cores were removed from collars, placed into sterile plastic bags and well-mixed to homogenize soil. The homogenized soil core was considered "bulk soil". Bulk soil was sub-sampled for PLFA analysis and sieved (2mm), any remaining plant material was carefully removed with forceps. Additionally, a 100g subsample of bulk soil was taken for nematode extraction.

The litterbag was then carefully rinsed with deionized water (soil + water was collected as "detritusphere soil" for nematode analysis, see below 2*.2.5 Nematode Community*). The washed roots were then dried in an oven at 45°C for 5 days and mass loss assessed by subtracting the mass of harvested roots (oven-dried) from the initial mass of roots (oven-dry mass corrected).

2.2.4 Microbial community

Microbial community structure was assessed by Phospholipid Fatty Acid (PLFA) analysis (For complete details on PLFA procedure, see *Appendix 1*). The PLFA extraction and identification methods for compound-specific $\delta^{13}C$ analysis were based on previous studies (Bossio and Scow 1995, Denef et al. 2007). For all treatments, approximately 6g soil subsamples from the bulk soil were extracted in duplicate at each harvest for PLFAs and 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 (For details on the specific PLFA methods, see Appendix 1). PLFA identifications were cross-referenced with a standard mixture of 37 fatty acid methyl esters (FAME) (37 Component FAME Mix, # 47885, Supelco Inc.).

The fatty acids i15:0, a15:0, i16:0, a17:0, i17:0 were chosen to represent gram-positive bacterial PLFAs and cy17:0, cis16:1ω9, 18:1ω11, and cy19:0 were chosen to represent gram-negative bacterial PLFAs. The fatty acids cis18:1ω9 and cis18:2ω9,12 were used as indicators of saprotrophic fungi (SAP). The fatty acids 10Me-16:0, 10Me-17:0, and 10Me-18:0 were used as indicators of actinomycetes. The ratio of fungal:bacterial PLFAs was used as a representative of the true ratio of fungal:bacterial biomass in soil (Bardgett et al. 1996).

For each harvest, PLFAs from the control treatments were used to calculate the carbon-13 enrichment of the PLFAs from the labeled root litter addition treatments. Carbon-13 enrichment (Δδ13C) was calculated by subtracting the control natural abundance PLFA $\delta^{13}C$ values from the labeled root-litter PLFA $\delta^{13}C$ values.

2.2.5 Nematode community

For both FB and IB treatments, soil nematodes were extracted from both 'bulk' soil and 'detritusphere' soil by the Baermann funnel method in deionized water (Hooper 1970). For bulk soil, a subsample of 100g of soil was taken and placed onto the Baermann funnels for nematode extraction. To analyze soil mixed with detritus, litterbags and root litter were rinsed with deionized water, and the solution of rinse-water + soil was collected onto separate Baermann funnels per each litter addition sample. Typically, this amounted to approximately 2g of soil. For bulk and detritusphere soil samples, an aliquot of 20mL of water + nematodes was extracted daily from each Baermann funnel for 3 days and collected into a beaker for a total volume of 60mL. Fresh deionized water (20mL) was replaced in the funnel each day.

After the extraction the total volume of each sample was reduced to 5mL and nematodes were counted and identified in five different trophic groups, according to Yeates et al. (1993) by using an inverted microscope (Olympus CKX 41, 200X magnification). After counting all samples, nematodes were picked using a micropipette and sorted to bacterivore, fungivore, plant parasite, omnivore, and predator trophic group under the inverted microscope (Olympus CKX41, 200X magnification)

For elemental and isotopic analysis 75 individuals from each trophic group were 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 tin capsule (8x5mm, Elemental Microanalysis BN/170056). The tin capsules containing the different nematode trophic groups were desiccated for 3 days and then prepared for analysis.

The tin capsules containing nematode samples were sent to Kansas State University where they were analyzed for C and N composition as well as isotopic values of ^{13}C and ^{15}N using a CE-1110 elemental analyzer (EA) via Conflo II interface for sample combustion and separation. The EA was coupled to a ThermoFinnigan Delta Plus mass spectrometer for isotopic analysis. A 3m gas chromatography column was packed with poraplot Q to separate N_2 and CO_2.

For each harvest, nematodes from the control treatments were used to calculate the carbon-13 enrichment of the nematodes from the labeled root litter addition treatments. Carbon-13 enrichment (Δδ13C) was calculated by subtracting the control nematode $\delta^{13}C$ values (natural abundance) from the labeled root-litter nematode $\delta^{13}C$ values.

2.2.6 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} and $R_{standard}$ are $^{13}C/^{12}C$ ratios of analyte CO_2 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:

$$f_r = (\delta_{BioL} - \delta_{BioC})/(\delta_{rL} - \delta_C)$$

δ_{BioL} and δ_{BioC} refer to the $\delta^{13}C$ signature of a biotic group in the litter-addition and control plots, respectively, and δ_{rL} and δ_{C} to the $\delta^{13}C$ signature of the root litter and control soil-C, respectively.

The effects of time, soil, 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.

2.3 RESULTS

2.3.1 Total root litter decomposition

At each successive harvest, slightly more root litter mass was lost in the FB soil, but this was not significantly different from the mass loss in IB soil (Fig. 2). Decomposition occurred rapidly (>30% mass loss) in the first 10 days of the experiment, and progressed slowly for the remainder of the experiment. By 180 days, the percent of root litter mass remaining in FB and IB soils was 53.00±4.31 and 57.91±2.20 respectively (Fig. 2).

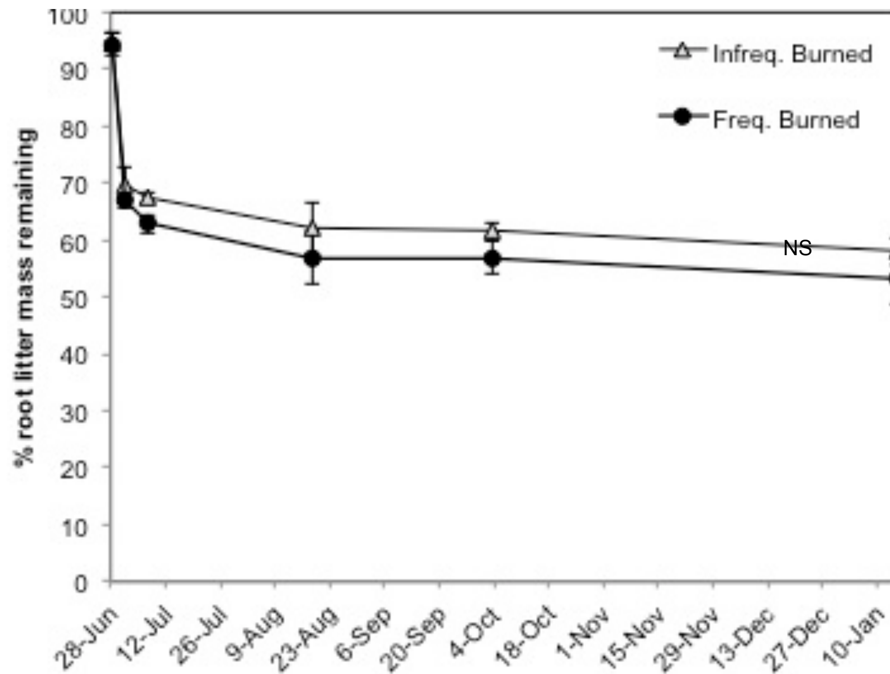

Figure 2. Percent of root litter mass remaining (with standard error bars) during decomposition. NS indicates no significant effect (n=3).

2.3.2 Effect of burning on root decomposition and microbial community

The microbial community responded significantly to root litter input and changed over time of decomposition. IB soil had significantly more total PLFA after litter addition for all harvests. FB soil had a significantly higher fungal:bacterial (F:B) PLFA ratio than IB soil at time 0, indicating stronger bacterial-dominance in the IB soil, but this difference diminished 3 days after litter addition (Table 1). The bacterial communities of FB and IB soil also differed (Table 1). The IB soil contained a more gram-positive (G+) dominant community than FB, indicated by a significantly higher gram-positive:gram-negative (G+:G-) bacterial ratio than the FB soil at time 0 (Table 1).

Table 1. Ratios of PLFA abundance per soil after litter addition. F:B PLFA indicates the ratio of fungal to bacterial biomarkers while Gram+:Gram- PLFA indicates the ratio of gram-positive bacterial to gram-negative bacterial biomarkers. Bold font indicates a significant difference (P<0.05) between soil habitats at an individual time point (n=3).

	F:B PLFA		Gram+:Gram- PLFA	
	FB	IB	FB	IB
Time (days)				
0	**0.26 (0.01)**	**0.17 (0.001)**	**1.38 (0.21)**	**1.81 (0.05)**
3	**0.20 (0.03)**	**0.16 (0.02)**	2.11 (0.24)	2.12 (0.11)
10	0.18 (0.01)	0.17 (0.02)	1.93 (0.07)	1.88 (0.13)
21	0.19 (0.03)	0.18 (0.02)	2.11 (0.13)	2.24 (0.07)
35	0.15 (0.02)	0.13 (0.01)	**1.96 (0.13)**	**2.30 (0.13)**
90	0.16 (0.02)	0.14 (0.01)	**1.75 (0.13)**	**2.19 (0.07)**
180	0.18 (0.01)	0.16 (0.01)	**1.76 (0.13)**	**2.07 (0.07)**

The microbial communities of FB and IB became more similar over time after root litter addition, as it did the F:B ratio became similar (Table 1). In the FB soils, the F:B ratio decreased indicating a growth of bacteria and/or a loss of fungi after litter addition. The IB soil F:B ratio remained approximately the same over the 180-day incubation. Both the FB and the IB soil habitats experienced a growth of gram-positive bacteria (Table 2) following root litter addition, increasing the gram-positive:gram-negative bacterial ratios for both soils (Table 1) and making this ratio more similar. By 35 days, gram-positive:gram-negative bacterial ratios in IB soil were again significantly higher than those of the FB soil.

The microbial community composition in FB soils had a stronger response to litter addition (i.e., change in mol% from the initial, day 0 mol%) than the IB community (Table 2). The microbial community of FB soil had a significant decrease in fungal (cis18:1ω9 and cis18:2ω9,12), actinomycetes (10Me-17:0 and 10Me-18:0), and gram-negative (cy17:0, cis16:1ω9, 18:1ω11, and cy19:0) abundance after root litter addition (Table 2a).

Table 2. Mean relative abundance, mol PLFA-C%, and (standard deviation) of individual biomarker PLFAs in a) frequently burned soil or b) infrequently burned soil for litter addition pots (n=3). For each PLFA, different lower case letters indicate significant mean differences in mol% between time points and bold font indicates a significantdifference in mol% from the Day 0 (initial) mol% (P < 0.05, LSMeans post-hoc test, n=3). ND indicates that a particular biomarker was not detected in a soil sample.

Community	PLFA	0 day	3 day	10 day	21 day	35 day	90 day	180 day
a) Freq. Burned								
Fungi	cis18:1ω9,	7.71 (0.87)a	5.33 (0.40)b	5.33 (0.21)bc	7.57 (1.47)a	3.87 (0.21)d	4.27 (0.47)de	4.93 (0.21)ce
	cis18:2ω9,12	5.16 (0.44)a	2.23 (1.20)b	1.17 (0.06)c	2.30 (0.44)b	0.67 (0.11)c	0.63 (0.57)c	0.90 (0.10)c
Gram-neg	cy17:0,	2.70 (0.16)a	1.90 (0.10)b	2.27 (0.15)b	2.07 (0.38)b	2.07 (0.15)b	2.13 (0.06)b	2.17 (0.32)b
	cis16:1ω9,	3.32 (0.28)a	4.20 (0.36)b	4.33 (0.15)b	3.90 (0.56)ab	3.23 (0.67)a	3.60 (0.44)a	3.90 (0.17)ab
	18:1ω11	6.36 (0.40)a	4.77 (0.60)bc	5.30 (0.56)ab	4.53 (2.45)bd	3.47 (0.47)cd	3.60 (0.17)d	4.50 (0.40)bd
	cy19:0	8.57 (0.10)a	5.57 (0.46)b	6.20 (0.44)bc	6.90 (0.44)c	8.20 (0.72)a	8.40 (0.53)a	6.67 (0.32)c
Gram-pos	i15:0	9.76 (2.19)a	11.77 (0.35)b	11.07 (1.18)ab	11.30 (0.96)ab	10.67 (1.17)ab	10.23 (1.53)ab	10.37 (0.70)ab
	a15:0	7.28 (1.34)a	11.60 (0.40)b	11.30 (1.01)b	11.13 (1.15)b	9.60 (0.46)c	9.07 (0.76)c	8.50 (0.30)ac
	i16:0	5.27 (0.16)a	5.60 (0.60)ab	5.57 (0.72)ab	5.27 (0.55)a	6.37 (0.61)c	5.77 (0.06)abc	6.13 (0.15)bc
	a17:0	3.60 (0.66)a	3.00 (0.17)b	3.27 (0.47)ab	2.87 (0.21)bc	3.00 (0.26)b	2.90 (0.10)bc	2.47 (0.15)c
	i17:0	2.91 (0.51)a	2.60 (0.17)ab	2.80 (0.44)a	2.37 (0.21)bc	2.40 (0.10)bc	2.37 (0.23)bc	2.10 (0.10)c
Actinomycetes	10me16:0	5.88 (1.33)ab	5.77 (0.25)ab	5.97 (0.21)ab	5.27 (0.51)a	6.13 (0.25)b	7.07 (0.80)c	5.70 (0.36)ab
	10me17:0	2.84 (0.58)a	2.06 (0.06)bc	1.93 (0.58)c	1.83 (0.06)c	2.50 (0.17)ab	2.77 (0.21)a	2.60 (0.20)a
	10me18:0	2.30 (0.18)a	1.50 (0.10)b	1.47 (0.05)b	1.50 (0.10)b	1.93 (0.12)c	2.10 (0.17)ac	2.03 (0.06)c
Protozoa	20:4ω6	ND	ND	ND	ND	ND	0.13 (0.23)	0.17 (0.29)
	20:5ω3	ND	ND	ND	ND	ND	ND	ND
Non-specific	C15:0	1.88 (0.18)a	2.73(0.06)bc	2.23 (0.49)ab	2.10 (0.46)a	2.63 (0.29)bc	2.97 (0.60)c	2.37 (0.58)ab
	C16:0	12.08 (1.43)ad	10.07(0.21)b	10.87 (0.21)b	10.57 (0.65)b	13.97 (0.65)c	12.80 (2.23)d	15.17 (0.87)e
	C18:0	2.49 (0.06)a	2.10 (0.10)a	2.10 (0.17)a	2.40 (0.10)a	2.27 (0.12)a	1.93 (0.64)a	2.20 (0.10)a
b) Infreq. Burned								
Fungi	cis18:1ω9,	4.69 (0.07)ab	4.17 (0.58)ab	4.60 (0.52)ab	4.80 (0.69)a	3.27 (0.15)c	3.43 (0.15)c	3.87 (0.15)bc
	cis18:2ω9,12	2.19 (0.38)ab	1.90 (0.46)a	2.03 (0.35)ac	2.70 (0.36)b	0.83 (0.29)c	0.97 (0.12)c	1.40 (0.30)c
Gram-neg	cy17:0,	1.80 (0.06)ab	1.50 (0.10)a	1.73 (0.06)ab	1.53 (0.06)a	1.93 (0.12)b	1.90 (0.26)b	1.90 (0.20)b
	cis16:1ω9,	4.84 (0.08)a	4.97 (0.21)a	4.83 (0.06)a	5.23 (0.15)a	3.00 (0.20)b	3.67 (0.15)c	3.40 (0.35)bc
	18:1ω11	7.03 (0.08)a	6.03 (0.40)abc	6.43 (0.51)ab	5.87 (1.10)bc	4.70 (0.40)d	4.47 (0.06)d	5.30 (0.17)c
	cy19:0	6.61 (0.16)ac	5.73 (0.12)ab	6.03 (0.31)ab	5.47 (0.49)bc	7.00 (0.10)c	6.93 (0.55)c	6.23 (0.72)ac
Gram-pos	i15:0	13.70 (0.53)ab	13.50 (0.62)ab	13.60 (1.21)ab	14.60 (0.36)a	14.33 (0.06)ab	13.17 (0.78)a	12.83 (0.59)b
	a15:0	12.57 (0.47)a	14.57 (1.27)b	12.67 (0.95)a	14.90 (0.72)b	11.57 (0.46)a	11.77 (0.12)a	10.17 (0.31)c
	i16:0	5.36 (0.65)ab	5.87 (0.81)ab	5.23 (0.21)a	5.63 (0.49)ab	5.53 (0.06)ab	5.87 (0.15)ab	6.00 (0.26)b
	a17:0	2.73 ($4e^{-4}$)a	2.50 (0.20)a	2.70 (0.36)a	2.43 (0.21)a	2.80 (0.01)a	2.67 (0.06)a	2.47 (0.15)a
	i17:0	2.29 (0.02)a	2.13 (0.21)a	2.33 (0.42)a	2.07 (0.15)a	2.40 (0.10)a	2.17 (0.15)a	2.17 (0.15)a
Actinomycetes	10me16:0	5.37 (0.35)a	5.03 (0.46)a	5.43 (0.91)a	4.83 (0.70)a	6.60 (0.20)bc	6.73 (0.32)c	5.77 (0.12)ab
	10me17:0	2.04 (0.12)a	2.23 (0.06)ab	2.23 (0.12)ab	2.00 (0.40)a	2.93 (0.06)c	2.70 (0.36)c	2.63 (0.23)bc
	10me18:0	1.76 (0.15)ab	1.57 (0.06)a	1.70 (0.10)ad	1.57 (0.15)a	2.00 (0.01)bc	2.07 (0.31)c	1.90 (0.17)c
Protozoa	20:4ω6	ND	ND	ND	ND	0.40 (0.10)a	0.47 (0.06)a	0.47 (0.06)a
	20:5ω3	ND	ND	ND	ND	0.50 (0.01)a	0.53 (0.06)a	0.53 (0.06)a
Non-specific	C15:0	2.35 (0.22)ab	2.83 (0.42)a	2.40 (0.26)ab	2.57 (0.29)ab	2.50 (0.26)ab	2.73 (0.67)a	2.03 (0.12)b
	C16:0	9.62 (0.08)ab	8.87 (0.64)a	9.37 (0.21)a	9.53 (0.55)a	11.07 (0.12)c	10.87 (1.11)b	12.90 (0.35)c
	C18:0	1.93 (0.10)a	1.67 (0.06)a	1.87 (0.15)a	1.57 (0.15)a	1.93 (0.06)a	1.57 (0.32)a	1.83 (0.15)a

Additionally, the FB soil had an immediate growth of gram-positive bacteria (significant increase in i15:0 and a15:0) at 3 days after litter addition (Table 2a). Over time, several gram-negative bacterial biomarkers (cy19:0 and cis16:1ω9) as well as the 10Me-16:0 biomarker for actinomycetes recovered in abundance while non-specific biomarkers also increased in abundance in FB soils (Table 2a). The IB soil community had a more delayed response to the root litter addition, with most significant changes occurring after 21 days (Table 2b). Similar to the FB soil community, the IB soil community decreased in fungal biomarker abundance (after 21 days, Table 2b) and had an immediate growth in gram-positive bacteria (a15:0, Table 2b).

Interestingly, at 35 days, biomarkers for protozoa, including 20:4ω6 and 20:5ω3 (which had not been detected previously), were detected in FB and in IB soils (Table 2a and 2b). However the mol% of protozoa biomarkers (20:4ω6 and 20:5ω3) was only significant in the IB soil (Table 2b). While the 20:4ω6 biomarker was present in the FB soil, it was not detected in every replicate and mol% of this biomarker was not found to be significant. The 20:5ω3 biomarker was not detected at all in the FB soil.

2.3.3 Effect of burning on root decomposition and soil nematode community

There were no statistically significant differences between nematode abundance in FB and IB soil throughout this experiment. Total nematodes decreased in both treatments immediately following root litter addition, but recovered populations by 10 days and continued to increase over time (Fig. 3).

The initial fungivore:bacterivore nematode (F:B nematode) ratio in IB bulk soil was significantly higher than the FB soil (Table 3). After root litter addition, both FB and

IB soils decreased in the F:B nematode ratio - indicating a loss of fungivores and/or an increase in bacterivores.

Table 3. Ratios of nematode abundance per soil after litter addition. F:B indicates the ratio of fungivore to bacterivore nematodes per kg of dry soil in both the bulk soil and the soil within the litterbag. Bold font indicates a significant difference (P<0.05) between soil habitats at an individual time point (n=3).

	F:B Nematodes (Bulk Soil)		F:B Nematodes (Litter Bag)	
	FB	IB	FB	IB
Time (days)				
0	**0.13 (0.05)a**	**0.27 (0.07)b**	-	-
3	0.10 (0.03)	0.18 (0.09)	**0.18 (0.05)a**	**0.03 (0.03)b**
10	**0.15 (0.10)a**	**0.03 (0.01)b**	0.03 (0.02)	0.01 (0.01)
21	**0.31 (0.15)a**	**0.03 (0.01)b**	0.12 (0.11)	0.04 (0.01)
35	0.04 (0.05)	0.01 (0.01)	**0.22 (0.03)a**	**0.06 (0.03)b**
90	0.16 (0.13)	0.06 (0.03)	**0.21 (0.15)a**	**0.04 (0.03)b**
180	**0.27 (0.07)a**	**0.15 (0.07)b**	0.26 (0.09)	0.21 (0.08)

Over time, the F:B nematode ratio decreased in the IB bulk soil, while in the FB bulk soil, the F:B nematode ratio fluctuated (Table 3). By the final harvest at 180 days, the FB soil had a significantly higher F:B nematode ratio (0.27±07) than the IB (0.15±07). This was the reverse of the day 0 harvest (Table 3).

The F:B nematode ratio of the soil from the litterbags appears to fluctuate in significance with the F:B nematode ratio of the bulk soil. In other words, at 10, 21, and 180 days, when the bulk soil had a significant difference between the FB and IB samples, the litterbag samples do not and vice-versa (Table 3). Initially, F:B nematode ratios within the litterbags is fairly low (0.18±0.05 and 0.03±0.03 for FB and IB, respectively) and grows for both soils by the final harvest (0.26±0.09 and 0.21±0.08 for FB and IB, respectively).

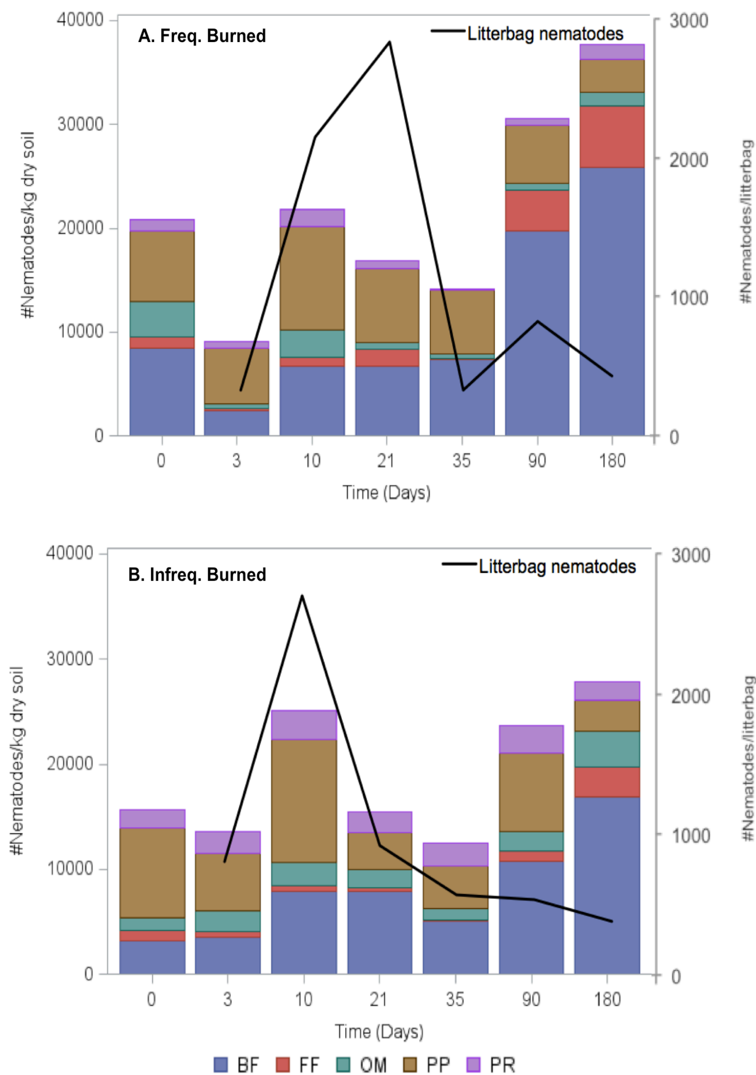

Figure 3. Change in nematode trophic group densities (#Nematodes/kg dry soil) and total number of nematodes (#Nematodes/litterbag) within litterbags over time of root litter decomposition for both A) Frequently Burned and B) Infrequently Burned soil samples. Day 0 indicates the initial densities of nematode trophic groups before the greenhouse incubation with root litter addition. For nematode trophic groups: BF=Bacterial Feeding, FF= Fungal Feeding, OM=Omnivore, PP= Plant Parasite, and PR=Predator.

After root litter addition, the trophic structure of the nematode community changed for both FB and IB soils compared to the control (Fig. 3). Particularly, the density of bacterivores increased after root litter addition. By 90 days, the density of bacterivores (6561±534 nematodes/kg dry soil) was more than double the initial density

(2847±730 nematodes/kg dry soil) in FB soils (Fig. 3A). The IB soil experienced similar changes. Bacterivores increased from initial densities of 1324±528 nematodes/kg dry soil to 4592±2324 nematodes/kg dry soil by 90 days in IB soil (Fig. 3B). Enlarged densities of bacterivores were also present in the final harvest for both soils (180 days, Fig. 3). Besides bacterivores, fungivores also increased in abundance by the final harvest. At 180 days, fungivore nematodes were at densities of 1462±411 and 909±247 nematodes/kg dry soil for FB and IB soils, respectively (Fig. 3).

Also notable are the densities of the higher trophic levels (omnivore and predator nematodes) in both soils (Fig. 3). Initially, IB soil had higher densities of predator nematodes (657±188 nematodes/kg dry soil) than FB soil (386±88 nematodes/kg dry soil); however these differences were not significantly different (P=0.51). The number of predator nematodes in both soils did not change greatly over time, however IB soil did continue to have higher densities of predators, although the difference between IB and FB were no longer significant (Fig. 3).

Furthermore, omnivore nematodes initially had higher densities in FB soil (1066±446 nematodes/kg dry soil) than IB soil (448±174 nematodes/kg dry soil) (Fig. 3). This difference was statistically significant (P=0.02). However, for FB soil, the density of omnivore nematodes decreased after root litter addition and did not recover by the final harvest (Fig. 3A). On the other hand, IB soil, which initially had a low density of omnivore nematodes, experienced a growth in omnivores (to 1016±384 nematodes/kg dry soil) by the final harvest (Fig. 3B). This was significantly higher than the abundance of omnivore nematodes in the FB soil (P=0.01) at 180 days (Fig. 3). In summary, the

difference in abundance of omnivore nematodes in FB and IB at day 180 was the reversal of the day 0 omnivore densities.

Responses of nematodes in litterbags after root litter addition were idiosyncratic. By 3 days, nematodes were already detected in litterbags and total nematodes in the litterbag increased to ~2000 nematodes/litterbag for both soils by 10 days (Fig. 3). The nematodes found within the litterbag extract were almost entirely bacterivores and fungivores (Appendix 5.2, Table 5).

2.3.4 Carbon allocation to soil community during root litter decomposition

The $\delta^{13}C$ values of the PLFAs and nematodes in control soils and in all soils at day 0, varied among individual groups and ranged between -14.2 ‰ and -23.0 ‰ for FB soil and between -19.0 ‰ and -26.5 ‰ for IB soil (Appendix 2, Table 6). In general, the natural abundance of ^{13}C was more depleted in IB soil than FB soil in the initial and control samples, but the differences were not found to be significant. The addition of ^{13}C-enriched roots resulted in a significant increase in the $\delta^{13}C$ values of most PLFA biomarkers and nematode trophic groups. $\delta^{13}C$ values of individual PLFAs and nematode groups differed greatly among soil habitats (FB and IB) over time of ^{13}C-labeled root decomposition.

The microbial community from the frequently burned treatment became significantly enriched ($P<0.05$) in ^{13}C (compared with the control treatment) faster than (i.e., at an earlier harvest occasion) the corresponding microbial groups from the infrequently burned treatment, with the exception of gram-negative biomarker cy17:0, gram-positive biomarkers a17:0 and i17:0, and actinomycete biomarker 10Me-18:0 (Fig.

4). These five biomarkers from IB were significantly enriched at the same harvest occasion as the corresponding biomarker for FB. No biomarkers from IB were enriched before corresponding biomarkers from FB. The first microbial groups to assimilate C from the root litter in FB soil were gram-negative and gram-positive bacteria (at 3-day harvest gram-negative biomarkers cis16:1ω9, 17:0cy, 18:1ω11 and gram-positive biomarkers a15:0 and i16:0 were significantly enriched, P<0.05) (Fig. 4a). For the IB soil, only gram-negative bacteria had assimilated ^{13}C from the root litter (significantly enriched biomarkers 17:0cy, 18:1ω11, P<0.05) by 3 days (Fig. 4a).

Saprotrophic fungi were actively assimilating root litter-C in FB soils well-before in IB soils. This was shown by significant ^{13}C enrichment in saprotrophic fungal groups (biomarker cis18:2ω9,12) relative to the control (P<0.05) in FB soil beginning at 10 days after litter addition (Fig. 4b). Significant ^{13}C enrichment (relative to the control) for fungi (cis18:2ω9,12) was not detected in IB soils until 90 days after litter addition (Fig. 4e). In addition to fungi, significant ^{13}C enrichment was detected in actinomycetes in FB soil before it was detected in IB soil (biomarker 10Me-17:0 at 21 days versus 35 days, respectively).

By the final harvest at 180 days after litter addition, all microbial groups were significantly enriched with ^{13}C (compared to the control, P<0.05) in both FB and IB soil. In addition, at the final harvest, all microbial groups were significantly more enriched in ^{13}C in the FB soil (compared to the IB soil, P<0.05), with the exception of gram-negative bacteria (biomarkers 17:0cy, cis16:1ω9, and 19:0cy) for which there was no statistical difference in $\delta^{13}C$ values between FB and IB.

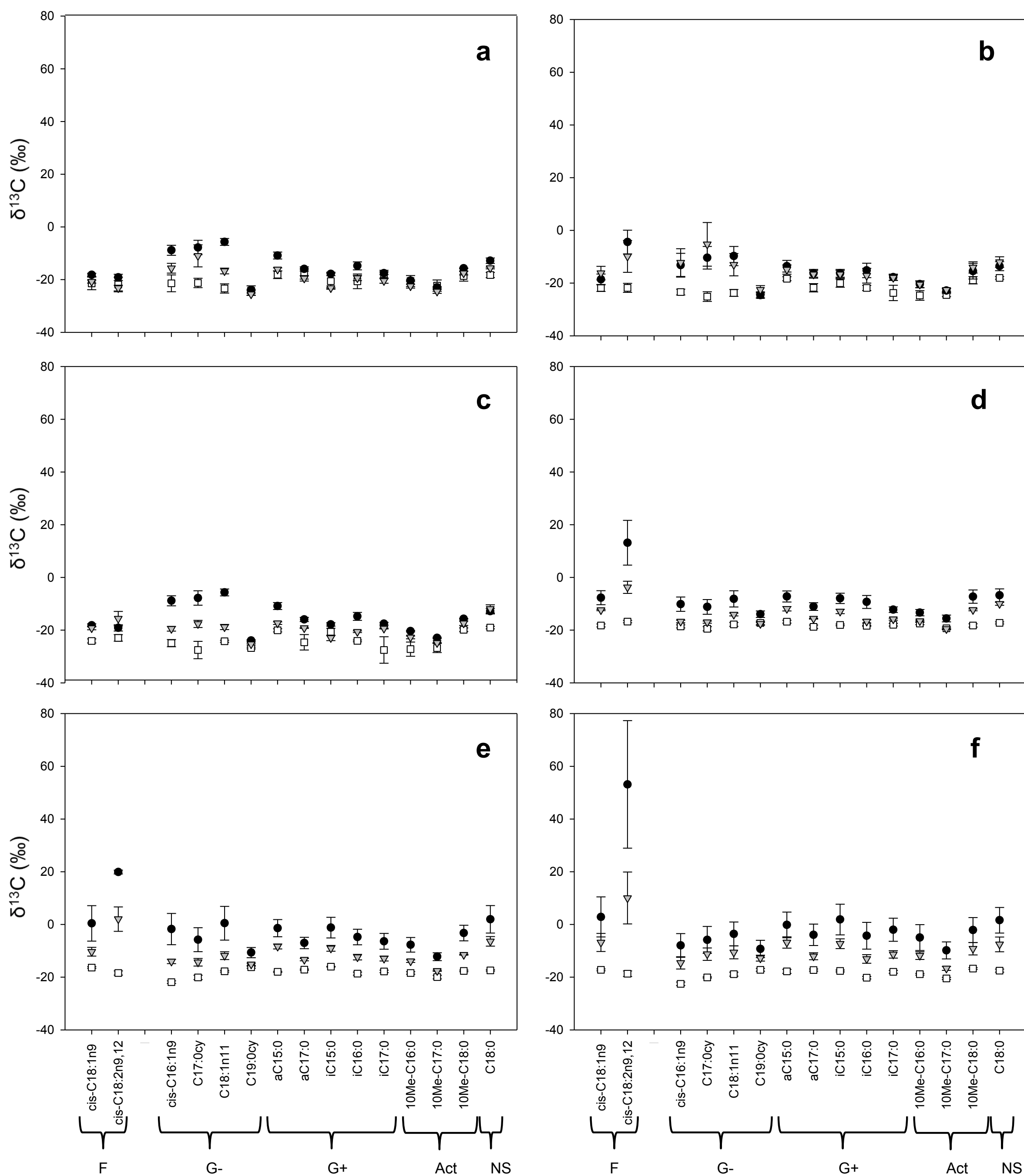

Figure 4. (a-f) δ^{13}C-values (‰) of PLFAs in soil. Treatments are frequently burned soil with ^{13}C-enriched root litter (●), infrequently burned soil with ^{13}C-enriched root litter (▼), and an unlabeled control of both burning treatments (□) on six harvest occasions after root litter addition at (a) 3 days, (b) 10 days, (c) 21 days, (d) 35 days, (e) 90 days, and (f) 180 days. Mean values of 3 replicates and standard error. F = fungi, G- = gram-negative bacteria, G+ = gram-positive bacteria, Act = actinomycetes, and NS = non-specific for all bacteria.

Protozoa, which were not detected in FB and IB soils until 35 days (Table 2) became significantly enriched in ^{13}C by 35 days and 90 days for IB and FB soils, respectively (data not shown). The protozoan biomarker, 20:4ω6, was the most highly enriched biomarker for both soils (FB, $\Delta\delta^{13}$C 73.4±15.3 ‰; and IB, $\Delta\delta^{13}$C 44.8±17.2 ‰) at 180 days (Appendix 2, Table 6).

Bacterial feeding nematodes had significantly higher δ^{13}C values compared to the control treatment for both FB and IB soils at all harvest occasions (Fig. 5). Bacterial feeding nematodes were significantly more enriched (P<0.05) in ^{13}C in in FB soils compared to IB soils at 21, 35, and 90 days after litter addition (Fig. 5c-e). Bacterial feeding nematodes were only more significantly enriched (P<0.05) in IB soils at 10 days after litter addition (Fig. 4b). At the other 2 harvest occasions (3 and 180 days) there was no significant difference between the δ^{13}C values of bacterial feeding nematodes in FB and IB soils, although both were enriched relative to the control (Fig. 5a and 5f). In addition, the δ^{13}C values of fungal feeding nematodes were only statistically different from the control values (P<0.05) in FB soils at 3, 10, 21, and 90 days (Fig. 5). Fungal feeding nematodes were not significantly enriched in ^{13}C at any harvest occasion for IB soils.

The higher nematode trophic groups, predators and omnivores, became significantly enriched in ^{13}C later than the lower trophic levels (bacterial feeders and fungal feeders for FB and bacterial feeders for IB). While bacterial feeders (FB and IB) and fungal feeders (FB only) were significantly enriched immediately following root litter addition (3 day harvest, Fig. 5a), omnivores and predators did not have δ^{13}C values significantly higher (P<0.05) than the control treatment until 35 days

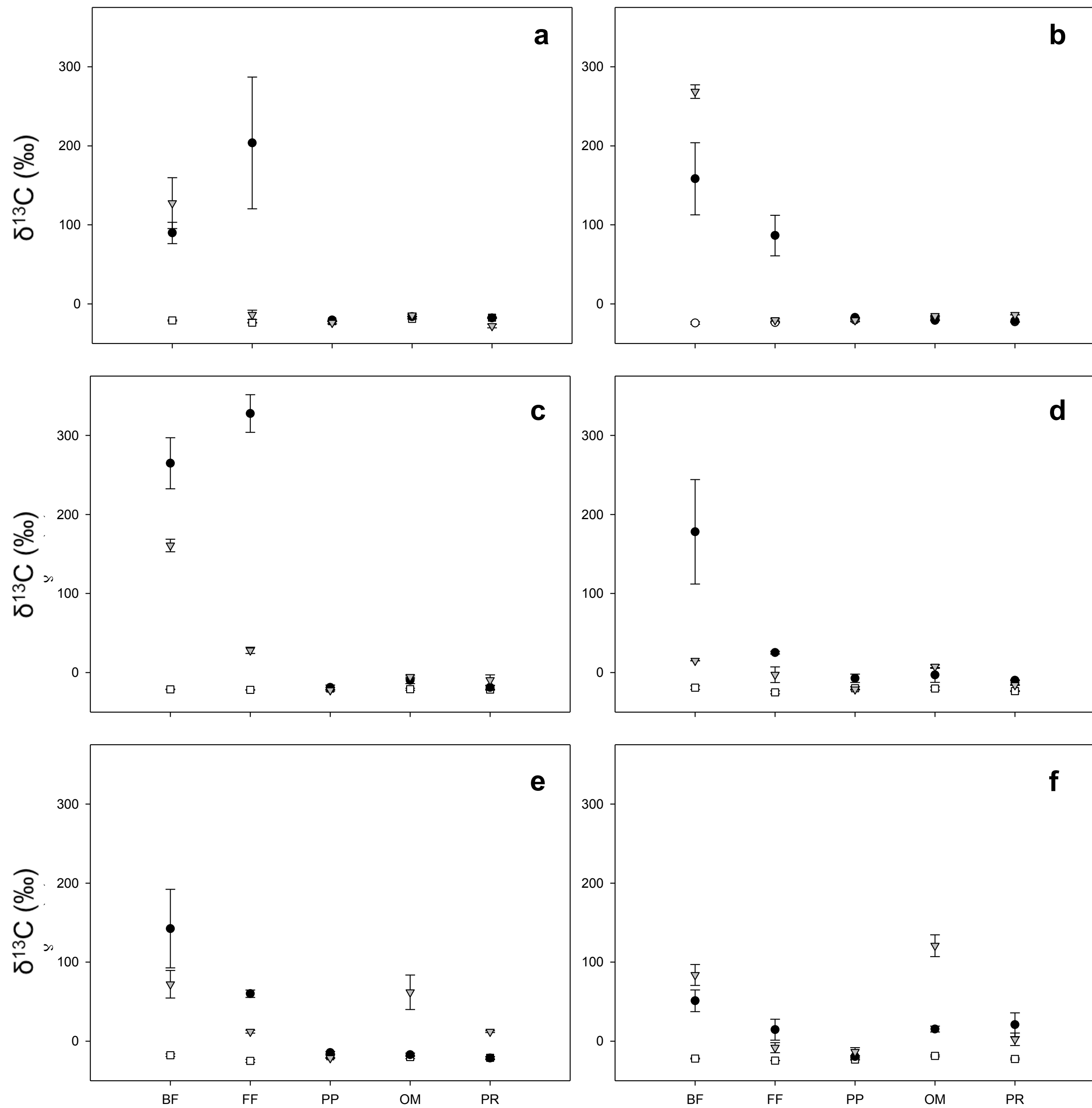

Figure 5. (a-f) δ^{13}C-values (‰) of nematodes in soil. Treatments are frequently burned soil with ^{13}C-enriched root litter (●), infrequently burned soil with ^{13}C-enriched root litter (▼), and an unlabeled control of both burning treatments (□) on six harvest occasions after root litter addition at (a) 3 days, (b) 10 days, (c) 21 days, (d) 35 days, (e) 90 days, and (f) 180 days. Mean values of 3 replicates and standard error. BF= bacterial feeding nematode, FF= fungal feeding nematode, PP= plant parasitic nematode, OM = omnivore nematode, and PR= predatory nematode.

(omnivores and predators, FB soil and omnivores only, IB soil), 90 days (omnivores and predators, IB soil only), and 180 days (omnivores and predators, FB and IB soils) (Fig. 5d and 5e). Omnivore and predator nematodes from IB soils were significantly higher ($P<0.001$) in $\delta^{13}C$ values than those from FB soils at 90 and 180 days (omnivores), and 90 days (predators). At the final harvest, the predator nematodes from FB soils were more highly enriched in ^{13}C ($P<0.01$) than those from IB soil (Fig. 5f).

Cumulative ^{13}C allocation to the total soil community at 180 days was 126.0±34.5 ng litter-C/g soil for FB soil communities and 128.2±32.0 ng litter-C/ g soil for IB soil communities (no significant difference). However, this C was allocated differently through the soil nematode communities in FB and IB soil. Root litter-C never made up more than 20% of the biomass of any group (Fig. 6). Root litter-C comprised >15% of the biomass of several trophic groups at 21 days including bacterivores and fungivores of the FB soil (Fig. 6A), and only bacterivores of the IB soil (Fig. 6B). Plant parasite nematodes did not have any root litter-C incorporated into their biomass in either FB or IB over time. The higher trophic levels began to have root litter-C incorporated into their biomass by 21 days, and this increased by the final harvest at 180 days (Fig. 6). In the IB soil, omnivore nematodes had the most root litter-C incorporated into their biomass at 180 days (Fig. 6B).

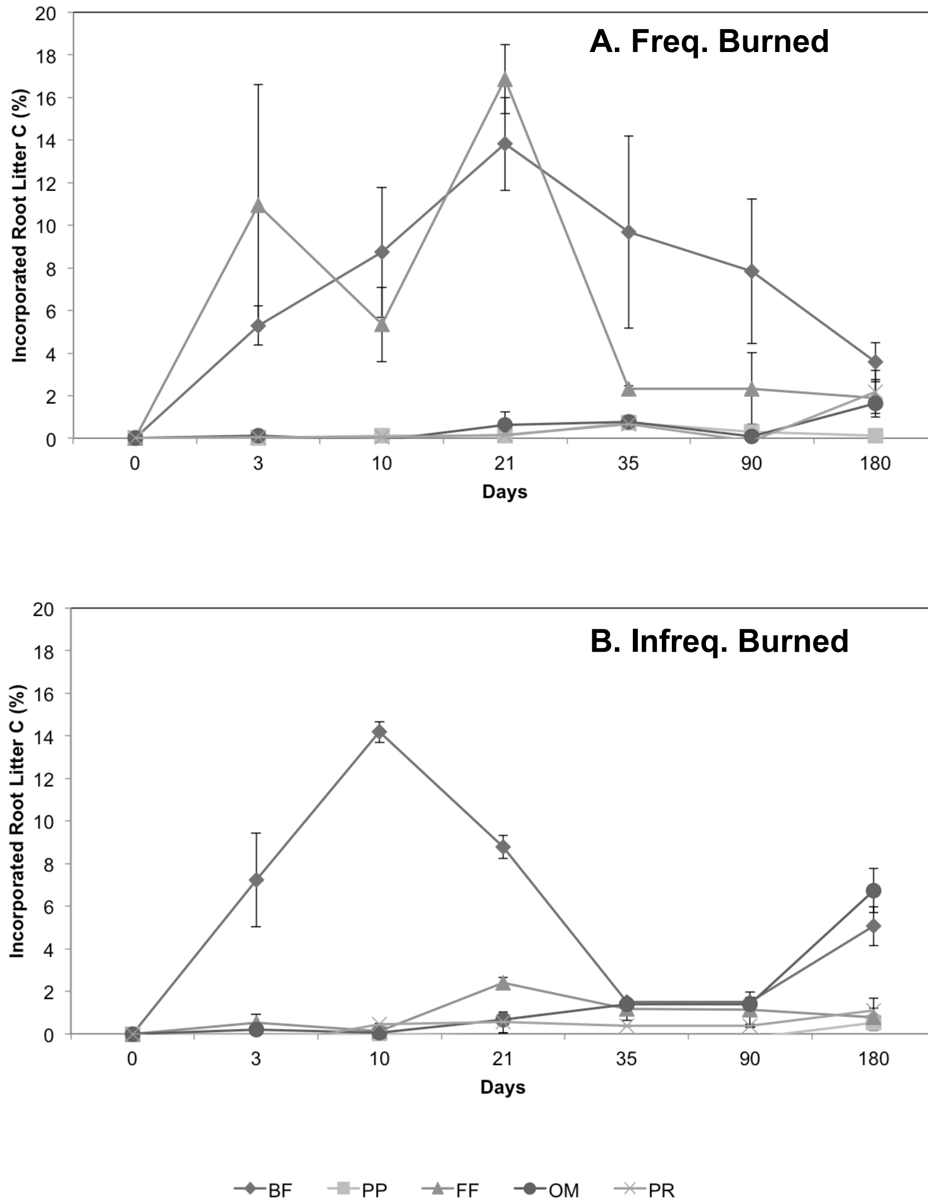

Figure 6. Percentages of root litter-C incorporated into the biomass-C of nematode trophic groups in frequently burned (A) and infrequently burned (B) over 180 days of decomposition.

2.4 DISCUSSION

2.4.1 Soil community composition

We hypothesized that the soil community of frequently burned and infrequently burned tallgrass prairie soils would be different in size and composition. We found this hypothesis to be true, as there was a significantly lower abundance of microbes in FB soil than IB soil at time 0 (measured as µg PLFA-C/ g soil, data not shown). In addition, the fungal to bacterial PLFA ratio (F:B) of FB soil was significantly greater than IB soil at time 0, while the gram-positive:gram-negative bacterial PLFA ratio (G+:G-) was significantly greater in the IB soil. The lower F:B and higher G+:G- in IB indicated an overall stronger bacterial-dominance with gram-positive bacteria being the most abundant there (G+:G- > 1). The FB community was also bacterial dominant (F:B < 1), but F:B was significantly higher than in IB and thus, more even than IB soil.

Nematode communities were also different in FB and IB soils. While there were no differences in total number of nematodes in FB and IB soils at time 0, there were significant differences in the nematode community composition. IB soil had a significantly higher fungivore:bacterivore nematode ratio than FB soil. While both FB and IB soils were bacterivore-dominant in the nematode community (fungivore:bacterivore nematode ratio < 1), the difference in the fungivore:bacterivore nematode ratio indicated a stronger dominance of bacterivore nematodes in FB soil, and a more even fungivore:bacterivore ratio in IB soil.

2.4.1 Root litter-C assimilating communities

$\delta^{13}C$ values of individual microbial and nematode groups differed greatly among soil habitats (FB and IB) over time of ^{13}C-labeled root decomposition, suggesting distinctive uptake rates and C-pathways of root litter-C by different microbial and, subsequently, nematode communities. We hypothesized that microorganisms and nematodes from the frequently burned tallgrass prairie soil would become enriched in ^{13}C from root litter more quickly than those from infrequently burned tallgrass prairie soil. For microorganisms, we found this hypothesis to be true. The microbial community from the FB soils became significantly enriched in ^{13}C (compared with the control treatment) faster than the microbial community from the IB soils. There were a few exceptions: gram-negative biomarker cy17:0, gram-positive biomarkers a17:0 and i17:0, and actinomycete biomarker 10Me-18:0 (Fig. 4). These four biomarkers were significantly enriched in ^{13}C at the same time in both IB and FB soils; no biomarkers from IB were enriched before corresponding biomarkers from FB.

Immediately following root litter addition, gram-negative and gram-positive bacteria were ^{13}C-enriched in FB soil, while only gram-negative bacteria were ^{13}C-enriched in IB soil. Gram-negative bacteria have been shown to quickly flourish after amending soil with plant material (Bossio et al. 1998, Peacock et al. 2001, Böhme et al. 2005, Elfstrand et al. 2008), which indicates that gram-negative bacteria are generalists and are competitive initially at the introduction of a C source. A study by Treonis et al. (2004) showed that gram-positive bacterial PLFA biomarkers had slower turnover times than gram-negative bacterial biomarkers. Thus, gram-negative bacteria may exhibit more rapid growth rates (Treonis et al. 2004), which would allow them to quickly colonize and

incorporate decaying plant material after additions (Elfstrand et al. 2008) in both FB and IB soils.

Interestingly, though relative abundances of fungi were lower in FB soils at time 0, significant ^{13}C enrichment of saprotrophic fungal groups in FB soil suggests that saprotrophic fungi were actively assimilating root litter-C well-before those groups in IB soil (10 days *vs*. 90 days after root litter addition). In other studies, biomarkers for saprotrophic fungi were among the most highly ^{13}C-enriched following pulse labeling of plants with ^{13}C-CO_2 (Butler et al. 2003, Treonis et al. 2004, Denef et al. 2009), indicating that these fungi can also quickly access and utilize C sources and may be closely tied to root-C. Significant ^{13}C enrichment in saprotrophic fungi in IB soils did not occur until 90 days after root litter addition, highlighting the perhaps more intimate relationship of saprotrophic fungi and plant root litter in FB soils.

In addition to fungi, significant ^{13}C enrichment was detected in actinomycetes in FB soil before it was detected in IB soil (21 days *vs*. 35 days, respectively). Actinomycetes are able to break down complex molecules (e.g. lignin), generating soluble carbohydrates, and are important agents of decomposition (Abdulla and El-Shatoury 2007). Possibly, the buildup of high-lignin root litter in on FB soil promotes the function of lignin-breaking actinomycetes groups.

Protozoa were not detected until 35 days in the IB soil and 90 days in the FB soil. At these time points, they were highly enriched in ^{13}C. Protozoa, primarily bacterial feeders, do not migrate toward decomposing substrates where new bacterial colonies may form (Griffiths and Caul 1993), and due to this, protozoa may have been delayed in accessing decomposer bacterial colonies. In addition, protozoa have shown feeding

preferences for bacteria without poisons, slimes, or pigmentation, and generally prefer gram-negative bacteria to gram-positive (Foster and Dormaar 1991). At the time protozoa become prolific in the IB and FB soils, the gram-negative PLFA abundance decreased (Table 2). All microbial groups had incorporated root litter derived C (as indicated by significant ^{13}C enrichment) in both FB and IB by the final harvest.

Overall, both soils indicated the same microbial succession of carbon-13 enrichment in microbial groups during decomposition, though the timing differed somewhat. In both soils, gram-negative and gram-positive bacteria were the first to become significantly enriched in ^{13}C, followed by saprotrophic fungi and actinomycetes, and finally by protozoa. Our data therefore suggest that in both FB and IB soils gram-negative bacteria and gram-positive bacteria are responsible for immediate processing of fresh root litter. While gram-negative bacteria and gram-positive bacteria remain prolific during decomposition, other groups become increasingly active decomposers of decaying root litter over time (as indicated by increasing carbon-13 enrichment). For example, saprotrophic fungi and actinomycetes become enriched in carbon-13 later in time in both soils, and may be responsible for processing of root litter-C later in decomposition.

All microbial groups were significantly more enriched in ^{13}C in the FB soil than in IB soil by the final harvest. This supports our hypothesis that FB soil would be more closely linked to root litter decomposition through higher ^{13}C enrichment of microbial groups. The one exception was gram-negative bacteria for which there was no statistical difference in $\delta^{13}C$ values between FB and IB. This is in agreement with other studies

that indicate that gram-negative bacteria is a generalist decomposer (Treonis et al. 2004, Elfstrand et al. 2008).

Microbial groups identified by PLFA biomarkers did not have as high carbon-13 enrichment as their nematode consumers. It is possible that the response of the microbial community to the litter input was muted by the grazing effect of the nematode consumers. This is likely, considering that bacterivore and fungivore nematodes were very highly enriched in ^{13}C immediately following root litter addition, despite the fact that their resources (such as fungi) were not highly enriched at that point. This contrasts with studies that have found that soil fauna, and particularly nematodes, have a positive effect on microbial activity during decomposition (Carrillo et al. 2011). The variability within the data could also reflect differences in soil (FB or IB) or differences in life cycle stages or turnover times within trophic groups, taxa, or species. Additionally, McMahon et al. (2005) showed an increase in PLFA abundance by 20-30% within the detritusphere, which was not sampled in this study for PLFAs. Microbial responses (abundance and carbon-13 enrichment) to litter are thought to be more distinctive in the detritusphere (McMahon et al. 2005) and it is possible that PLFA response to root litter addition was stronger in the area in close proximity to the substrate (i.e. litterbag).

We did however, examine the nematodes within the litterbags and found that nematodes were not only active in the soil, but were also found within the litterbags containing the labeled roots. Bacterial feeding nematodes are known to migrate toward decomposing plant material and are thought to be attracted to metabolites of bacterial colonies (Griffiths and Caul 1993). Fungal feeding nematodes have also been found to

migrate toward fungi (Griffiths and Caul 1993). In our study, the nematodes found within the litterbags were predominantly bacterivores and fungivores (Appendix 2, Table 5).

For nematodes, with the exception of fungivore nematodes in FB soils and predator nematodes in IB soils, we found that timing of enrichment was very similar for FB and IB soils. Fungivore nematodes were enriched by 3 days after litter addition in FB soil, but never were significantly enriched in IB soil. Also, predator nematodes became enriched earlier in IB soils (21 days), but did not become significantly enriched in ^{13}C in FB soils until 35 days after root litter addition. Bacterivore nematodes and omnivore nematodes were enriched in ^{13}C at 3 days and 35 days after root litter addition in both soils, respectively.

The decomposing roots appear to be an important C-source for nematodes in the tallgrass prairie soil. The ^{13}C originating from the root litter could be traced into different nematode trophic groups, indicating that they had assimilated ^{13}C by feeding on bacteria, fungi, protozoa, other nematodes, or other soil organisms. Bacterial feeding nematodes were most clearly linked root litter material in IB soil, while in FB soil both fungal feeding nematodes and bacterial feeding nematodes were significantly enriched. Using a mixing model approach, we determined that the fungivore nematodes of the FB soil were strongly linked to the decomposing root litter, with nearly 20% of their biomass-C being comprised of root litter-C by 21 days. In both FB and IB soil, bacterivores were also strongly linked to root litter, with >15% of their biomass as root litter-C by 21 days. Over time, as predator and omnivore nematodes began to incorporate root litter-C into biomass-C, the amount of root litter-C in the lower nematode trophic levels was reduced.

2.4.3 Effect of burning on root decomposition and total C in the soil food web

Frequent burning impacts soil microorganisms and soil fauna (Seastedt 1984, Seastedt and Crossley 1984, Seastedt 1988). Due to this, we expected total decomposition and total root litter derived C incorporated into the FB soil food web to be higher than in the IB soil food web. This hypothesis was not true. Total decomposition (assessed by %root litter mass remaining) did not differ between the two soils. In addition, the cumulative ^{13}C allocation to the different soil food webs was not different (126.0±34.5 and 128.2±32.0 ng root litter-C/ g soil for FB and IB soil communities, respectively). This contrasts with the results of previous studies. For example, O'Lear et al. (1996) found that prescribed burning as a management practice impacts decomposition rates. Buried wood had significantly higher decay rates in annually burned tallgrass prairie (Konza prairie, Kansas) than unburned tallgrass prairie (O'Lear et al. 1996). In addition, a single fire had less impact on decomposition rates than long-term annual burning (O'Lear et al. 1996), suggesting that differences are due to long-term changes caused by consistently burning. Yet, despite significantly lower abundances of microorganisms (as indicated by PLFA analysis), FB soil's food web decomposed as much root litter and accumulated as much root litter derived-C as the IB food web. We suggest that this may be due to a higher efficiency of soil microorganisms in the FB soil that are more specialized to decomposing root litter.

Our results underline the general view that soil decomposer food webs are based on C derived from litter. In the tallgrass prairie, most of the nutrients are belowground, with the majority bound as inorganic or insoluble organic forms (Seastedt and Ramundo 1990). In aboveground foliage decay, little N release occurs during the first few years of

decomposition, but in belowground root decomposition, N is mineralized at rate of about 25% per year (Seastedt 1988). Comparing rates of decomposition, Seastedt et al. (1991) found that aboveground foliage decomposition occurred at a rate of about 27% per year, while belowground root litter exceeded this at about 35% per year – perhaps due to the roots' proximity and close association with belowground decomposers. Our results highlight that root litter may be of increased importance as a C and nutrient source in frequently FB prairie, where standing biomass and shoot litter accumulation is removed often by fire.

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3. BIOMASS MEASURES OF NEMATODE SOIL ENERGY CHANNELS INDICATE GENERAL CARBON FLOW FOR DECOMPOSITION

3.1 INTRODUCTION

Soil food webs are intricate systems (Bardgett 2005). The trophic relationships of the soil food web are cryptic and difficult to study due to the small size of soil fauna, opaque habitat and complex mixture of organic material and soil particles (Cortois and de Deyn 2012). Nevertheless, understanding soil food web processes and their impact on decomposition and nutrient cycling is recognized as a research priority for a better understanding of ecosystem functioning (Albers et al. 2006, Holtkamp et al. 2008, Wall et al. 2008, Pollierer et al. 2009, de Vries et al. 2012).

Nematodes have several traits that make them helpful for evaluating ecosystem functioning (Freckman 1988). By being secondary or tertiary consumers in the soil food web, they integrate the physical, chemical, and biological attributes associated with their food sources (Nannipieri et al. 1990). Also, their life cycle, which lasts from days to years, is longer and more stable than the generation time of metabolically active microbial populations (Nannipieri et al. 1990). This makes nematodes more reliable over time for assessing food webs, because they do not simply shift with short-lived nutrient fluxes (Nannipieri et al. 1990). In addition, soil nematodes can be placed into a minimum of five trophic groups (Yeates et al. 1993), and they are primary drivers of the decomposer soil food web (Moore and Deruiter 1991)

Decomposer soil food webs are considered compartmentalized (Moore et al. 2005) with energy being processed at two rates: fast (bacterial channel) and slow (fungal channel) (Coleman et al. 1983, Moore and Hunt 1988). The bacterial and fungal energy channels process organic matter in independent ways. The bacterial energy channel processes labile organic matter and has been found to dominate in fertile and productive ecosystems (Swift et al. 1979, Coleman et al. 1983). The fungal energy channel processes more recalcitrant organic matter (i.e., litter containing high carbon to nitrogen (C:N) ratios and high abundance of lignin and structural compounds) and dominates in infertile and unproductive ecosystems (Swift et al. 1979). The contribution of each energy channel to the soil food web impacts stability, and food webs are more stable when energy is distributed equally between the two channels (Parton et al. 1987, Moore et al. 2005). However, the quantification of energy fluxes through the food web's energy channels is challenging, especially for soil faunal contributions (Pollierer et al. 2009).

Nematodes' trophic structure can indicate soil energy channels (Mikola and Setala 1999). Bacterivore and fungivore nematodes contribute only to the bacterial or fungal channels, respectively, while omnivore and predator nematodes derive energy from both primary sources (bacterial and fungal) and thus contribute to both energy channels (Hunt et al. 1987). For example, omnivorous nematodes are assumed to feed on bacteria, amoebae, flagellates and bacterivorous, fungivorous, and plant-parasitic nematodes (Hunt et al. 1987, Yeates et al. 1993, de Vries et al. 2012). Predaceous nematodes are assumed to feed on all other nematode groups (Yeates et al. 1993).

Many studies use biomass of soil trophic groups to estimate changes in the soil food web and its energy channels (Moore and Hunt 1988, Moore and de Ruiter 1997, Holtkamp et al. 2008, de Vries et al. 2012). The biomass of the different soil trophic levels can indicate bottom-up or top-down controls (Allison 2006) as well as soil food web structure in terms of its energy channels (Holtkamp et al. 2011). Energy channels have been described as a set of species consuming biomass that originates from the same chief energy resource (Moore et al. 1988). Each energy channel starts with a resource (e.g., plant roots, soil organic matter), continues through consumers of these resources, and ultimately terminates with a top predator (Moore and de Ruiter 1997). While bacterivore or fungivore nematode biomass can be quantified and contributes fully to its respective energy channel, for omnivore and predator nematodes, the biomass contribution to each energy channel has to be calculated using density dependent feeding preferences (Holtkamp et al. 2008, de Vries et al. 2012).

While energy channel measures have been used to estimate C flow through the soil food web, Moore et al. (2004) pointed out the necessity for new techniques to identify the exact amount of detritus consumed by soil food webs. Recently, stable isotopes (i.e., ^{13}C) have been used to quantify C flow though some soil faunal trophic groups, e.g. collembola, earthworms, enchytraieds, microarthropods, gastropods, diplopods, and chilopods (Albers et al. 2006, Ostle et al. 2007, Pollierer et al. 2007, Elfstrand et al. 2008). By adding ^{13}C-enriched plant litter to a system for decomposition, C can be traced and quantified over time as an isotopic fingerprint in soil biotic groups. This technique has been widely used to study microbial communities in soil

ecosystems, examining assimilation of isotopically labelled plant substrates into microbial groups (Treonis et al. 2004, McMahon et al. 2005, Denef et al. 2009).

In this decomposition study, we investigated the trophic compartmentalization of the nematode food web into energy channels and traced C fluxes to different nematode energy channels within the food web using stable C isotope enrichments. With the idea to study contrasting food-web structures, we compared the soil food web energy channels and C allocation in frequently burned (FB) and infrequently burned (IB) soils of the tallgrass prairie. We hypothesized that:

1) Energy channels for soil nematode food webs in the tallgrass prairie will differ based on long-term management practices (FB *vs* IB). Energy channel biomass of the soil nematode food web in the FB prairie will be bacterial dominated (due to litter layer removal and carbon inputs of annual fire) while energy channel biomass will be fungal dominated in the infrequently burned prairie (due to the accumulation of lignin and structurally complex litter over time).
2) Nematode bacterial and fungal energy channel biomass measurements provide a good indication of C flow from root litter into nematode energy channels (i.e., we expect more root litter derived C to accumulate in the nematode bacterial channel overall in FB soil due to the dominance of this energy channel in the FB tallgrass prairie, and vice versa for IB soil).

3.2 METHODS

3.2.1 Study site and soil collection

The soil samples were taken from the top 10cm of a frequently burned (FB) and an infrequently burned (IB) silty clay loam Argiustoll under historically unplowed tallgrass prairie at the Konza Praire Biological Station (KPBS) in eastern Kansas, USA (39°05'N, 96°35'W). Mean annual air temperatures range from -2.7° C in January to 26.6° C in July, with 835mm of total annual precipitation on average.[3] The plant community at the site is dominated by perennial C4 grasses: big bluestem (*Andropogon gerardii*), Indian grass (*Sorghastrum nutans*), little bluestem (*Schizachrium scoparium*), and switch grass (*Panicum virgatum*).[1]

Eighteen soil cores (10cm deep x 10cm diameter) were extracted at random from upland soil of two fire treatment areas at KPBS on 14 June 2011. Frequently burned soil (9 soil cores, 10cm deep x 10cm diameter) was taken from an annual spring burn watershed, termed SpB (Chapter 2, Fig. 1). Infrequently burned soil (9 soil cores, 10cm deep x 10cm diameter) was collected from a 20-year burn watershed, termed 20B (Chapter 2, Fig. 1). For soil characteristics of these sites, see Chapter 2.2.1 (Site description and soil collection).

The soil cores were placed in clean plastic (polyvinyl chloride, PVC) collars (10cm deep x 10cm diameter) in the field, preserving the integrity and structure of the soil cores. The soil collars were then packed into sterile plastic bags, placed into coolers with ice packs and kept cool, and transported to the Plant Growth Facility at Colorado

[3] Konza site information: http://kpbs.konza.ksu.edu/location.html

State University (CSU), Fort Collins, CO, USA for the decomposition experiment. Soil cores remained intact and disturbance was minimized to protect the soil biotic community.

Field soil moisture was measured at time of soil collection for both FB and IB soils by gravimetric water content (GWC). GWC was calculated by subtracting the oven-dry weight of soil (105°C) from the wet weight. All soil cores were weighed and %GWC was estimated based on initial field levels. Soil moisture was maintained daily at 20% GWC, based on levels observed in the field. Soil temperature was recorded in the field and daily during the greenhouse incubation using a temperature probe coupled to a PP system (PP-system, SRC-1).

3.2.2 ^{13}C root litter enrichment

The root litter enrichment was performed as in Chapter 2.2.2 (^{13}C root litter enrichment) (Soong et al. Submitted).

3.2.3 Decomposition experiment

FB and IB soil cores (3 replicates each, selected randomly) were set aside for initial "field" measurements (Time 0). The remaining FB and IB soil samples were incubated in PVC collars with two different litter treatments (no litter/control or litter addition). The treatments were randomized and had 3 replicates. For the litter addition treatment, approximately 1.5 grams of ^{13}C-labeled root litter were buried for both FB and IB soil. The masses of all litterbags were corrected to the oven-dry mass. To minimize disturbance to the soil, the soil core was carefully removed from PVC collar, sliced in

half horizontally (Sanaullah et al. 2010), a 1mm mesh bag containing the desiccated roots was placed in the center, and the two halves of the soil core were replaced in the PVC collar. The remaining 3 burned and 3 unburned soil cores were established as "control" treatments in PVC collars and no litter was added to the soil. To assure results were related to litter addition and not disturbance to the soil core, the control treatment cores were sliced in half, then put back together, but no litterbag was added. The experiment was maintained in the greenhouse for 180 days.

In summary, there were two soil types: FB, IB, each with two treatments: litter addition, and no litter addition (control). Field (Time 0) soil was also assessed to compare the natural field to the greenhouse experiment and the FB soil to IB soil for trophic groups, energy channels, and δ^{13}C values. To assess bacterial and fungal energy channel biomass changes and root litter C incorporation due to decomposition of root litter, all soil cores were destructively harvested after 180 days.

3.2.4 Nematode extraction and identification

For both FB and IB treatments, soil nematodes were extracted by the Baermann funnel method (in deionized water) for a total of 72 hours (Hooper 1970). The whole soil core was homogenized at each harvest and approximately 100g of soil was subsampled and placed onto the Baermann funnels for nematode extraction. Each day, an aliquot of 20mL of water + nematodes was taken into a beaker (for a total of 60mL) and fresh deionized water (20mL) was replaced in the funnel each day. Nematode samples were then reduced to 5mL for identification.

Under an inverted microscope (200X magnification) adult nematodes were identified and sorted (with micropipette) to trophic group (bacterivore, fungivore, plant parasite, omnivore, and predator) according to Yeates et al. (1993). Next, using dissecting microscope (30X magnification) and an eyelash (Superfine eyelash with handle, Ted Pella, Inc., Prod no. 113) to handpick nematodes, 75 individuals from each trophic group were transferred to a tin capsule (8x5mm, Elemental Microanalysis BN/170056) for elemental and isotopic analysis. The nematode samples in the tin capsules were desiccated for 3 days, and then prepared for analysis.

The tin capsules containing nematodes were sent to Kansas State University where they were analyzed for C and N composition as well as isotopic values of ^{13}C and ^{15}N using a CE-1110 elemental analyzer (EA) via Conflo II interface for sample combustion and separation. The EA was coupled to a ThermoFinnigan Delta Plus mass spectrometer for isotopic analysis. A 3m gas chromatography column was packed with poraplot Q to separate N_2 and CO_2.

For each harvest, nematodes from the control cores were used to calculate the carbon-13 enrichment of the nematodes from the labeled root litter addition cores. Carbon-13 enrichment (Δδ13C) was calculated by subtracting the control nematode $\delta^{13}C$ values (natural abundance) from the labeled root-litter nematode $\delta^{13}C$ values.

3.2.5 Nematode energy channels

First, we measured the biomass of each nematode trophic group (bacterivore, fungivore, omnivore, and predator). For each of these trophic groups nematode biomass was calculated by:

$$1)\ B_{TG}=(M_{100}/100)*X_{total}$$

Where 1) B_{TG} is the biomass of a trophic group, M_{100} is the dry mass of 100 nematodes from a trophic group, and X_{TG} is total number of nematodes/kg dry soil for a trophic group. The moisture content of the total nematode biomass was assumed to be 75% and the carbon content to be 50% of the dry weight (Sohlenius and Sandor 1987). We then used the biomass measures of each nematode trophic group to calculate the nematode bacterial and fungal energy channel biomass.

To quantify the nematode bacterial and fungal energy channel biomass, we summed the biomass of all nematode trophic groups contributing to that channel. Bacterivore and fungivore nematodes contributed fully to the bacterial or fungal channels, respectively. For omnivore and predator nematodes, the biomass contribution to each energy channel was calculated using density dependent feeding preferences (Holtkamp et al. 2008, de Vries et al. 2012) because omnivorous and predaceous nematode groups derive energy from both primary sources (bacterial and fungal) and thus contributed to both energy channels. Each nematode energy channel was quantified for each sample by summing the biomass of groups within that level:

$$2)\ B_{BEC} = B_{BTG}+(D_o*B_{OTG})+(D_p*B_{PTG})$$

$$3)\ B_{FEC} = B_{FTG}+(D_o*B_{OTG})+(D_p*B_{PTG})$$

Where 1) B_{BEC} is the biomass of the nematode bacterial energy channel, B_{BTG} is the biomass of the bacterivore trophic group, B_{OTG} is the biomass of the omnivore trophic group, B_{PTG} is the biomass of the predator trophic group, and D is the density dependent feeding preference of omnivores (D_o) or predators (D_p) for the fungal energy channel. 2) B_{FEC} is the biomass of the nematode fungal energy channel, B_{FTG} is the

biomass of the fungivore trophic group, B_{OTG} is the biomass of the omnivore trophic group, B_{PTG} is the biomass of the predator trophic group, and D is the density dependent feeding preference of omnivores (D_o) or predators (D_p) for the fungal energy channel. We assumed that all individuals identified to each nematode trophic group had the same feeding preferences (Moore et al. 1988). We did not calculate biomass for the (living) root energy channel, as this was outside of our research aims.

Because the order of magnitude of biomass differs between trophic levels, the biomass of the nematode trophic groups was standardized by dividing the biomass of each trophic group by the overall mean of that group over all treatments. This standardization made it so that the contribution of a group to an energy channel was independent of its own biomass (Holtkamp et al. 2008). Calculations of fungal and bacterial channel biomass largely followed the description of (Holtkamp et al. 2008) with the exception of the functional group of omnivorous and predacious nematodes, for which feeding preferences were used from (Hunt et al. 1987).

3.2.6 Nematode C incorporation from root decomposition

The fraction of root litter-derived carbon in soil fauna body tissue (f_{RL}) was calculated by a two-source mixing model with:

$$4)\ f_{RL} = (\delta_{TG} - \delta_S)/(\delta_{RL} - \delta_S)$$

δ_{TG} refers to the ^{13}C signature of a nematode trophic group, and δ_S and δ_{RL} to the ^{13}C signature of the soil and root litter, respectively.

To assess the amount of root litter derived C in each nematode trophic group, the fraction of root litter derived C in soil fauna body tissue (f_{RL}) was multiplied by the biomass of each nematode trophic group.

3.2.7 Data analyses

Differences between biomass of energy channels and differences in total C-incorporation by trophic groups between burning management strategies were tested using a one-way ANOVA with Tukey post-hoc test. The assumption of normality was tested with the Shapiro–Wilk test and the assumption of homogeneity of variances between groups with the Levene's test. If these assumptions were not met, differences between treatments were tested with the non-parametric Kruskal–Wallis test.

3.3 RESULTS

3.3.1 Nematode energy channel biomass

Overall, the nematode bacterial energy channel was dominant over the nematode fungal energy channel in tallgrass prairie soil ($p<0.05$, Fig. 7). There were differences, however, in the degree of bacterial channel dominance (Time 0). The frequently burned (FB) soil was strongly nematode bacterial energy channel dominant (low nematode fungal to bacterial energy channel ratio), while the infrequently burned (IB) soil was nematode bacterial energy channel dominant, but had a significantly higher nematode fungal to bacterial energy channel ratio than the FB soil (Fig. 8C).

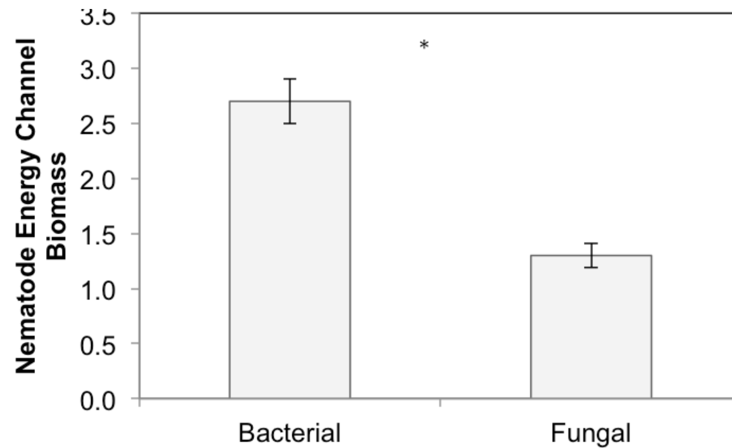

Figure 7. Standardized biomass of the nematode bacterial energy channel and nematode fungal energy channel over all treatments (mean ± standard error). Asterisks (*) denote significant differences between channel biomass at the p<0.05 level (n=6).

The average biomass of the nematode bacterial energy channel differed between FB and IB soil at time 0 ($p = 0.04$, Fig. 8A). The biomass of the bacterivore nematodes increased significantly for both soils in the root litter addition treatment in the decomposition study ($p<0.05$, Table 4), but the total nematode bacterial energy channel biomass only increased significantly for the IB soil in the litter addition treatment ($p=0.03$, Fig. 8A). These two results seem contradictory but the bacterial channel biomass incorporates the contribution of all other nematode trophic groups (in addition to bacterivore nematodes) to the bacterial energy channel.

The average nematode fungal energy channel biomass was not different in the field (Time 0) FB or IB soil, nor did it change over time of decomposition (Fig. 8B). In addition, besides bacterivores, the biomass of other nematode trophic groups did not change significantly in response to litter addition for either FB or IB soil (Table 4).

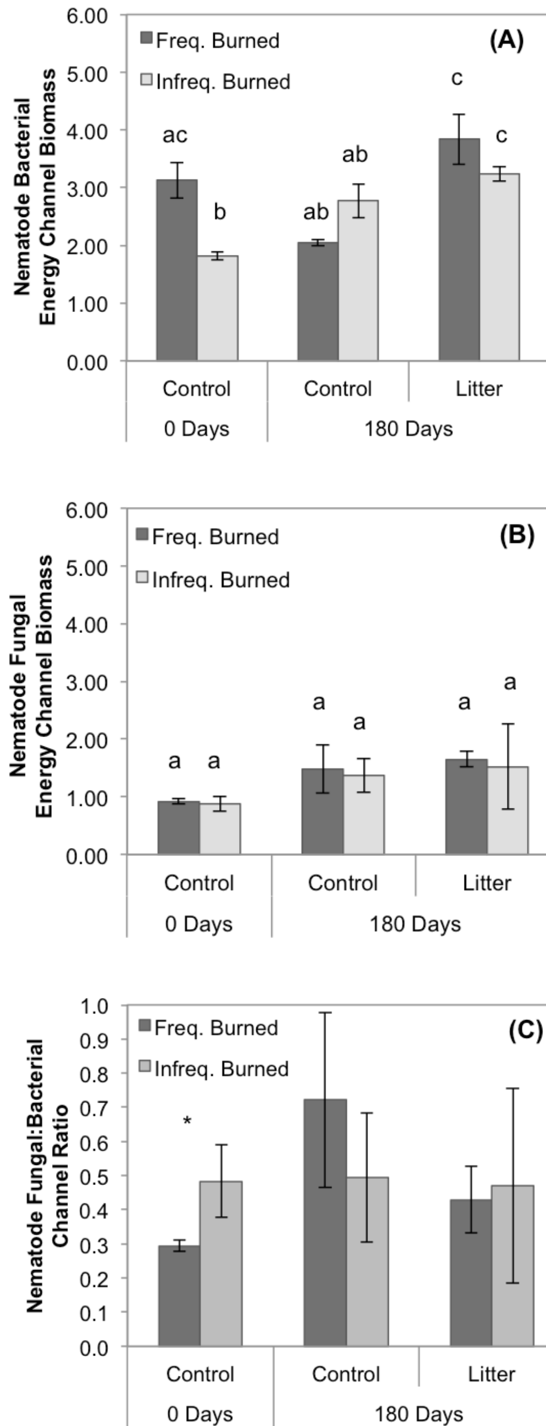

Figure 8. Standardized biomass of the A) nematode bacterial energy channel and B) nematode fungal energy channel for the time 0, control, and litter treatments for both frequently and infrequently burned soil (mean ± standard error). Different letters denote significant differences in energy channel biomass at the $p<0.05$ level (n=3). C) Ratio of the standardized biomass of the nematode fungal to bacterial energy channel. Asterisks (*) denote significant differences in fungal to bacterial energy channel ratios between frequently and infrequently burned soil at the $p<0.05$ level (n=3).

Table 4. Measured biomasses of nematode trophic groups (all in mg C/kg dry soil/10cm (depth)) for control and litter addition at 0 and 180 days (mean ± standard error) (n=3).

Treatment	Trophic Group	0 Days Control	180 Days Control	180 Days Litter Addition	Decomposition effect? [a] (p<0.05)
Freq. Burned	Bacterivore	**2.27 (0.08)a**	1.71 (0.58)a	**4.19 (1.97)b**	yes
	Fungivore	0.31 (0.01)a	0.68 (0.30)a	0.73 (0.06)a	no
	Omnivore	1.25 (0.38)a	0.68 (0.18)a	0.45 (0.01)a	no
	Plant Parasite	2.33 (0.69)a	0.50 (0.01)b	1.85 (1.32)a	no
	Predator	0.26 (0.03)a	0.18 (0.02)a	0.50 (0.18)a	no
Infreq. Burned	Bacterivore	**0.54 (0.19)a**	1.43 (0.32)ab	**2.26 (0.42)b**	yes
	Fungivore	0.33 (0.12)a	0.54 (0.28)a	0.62 (0.55)a	no
	Omnivore	0.36 (0.19)a	1.19 (0.22)a	0.82 (0.07)a	no
	Plant Parasite	2.10 (0.19)a	0.72 (0.42)a	1.32 (0.24)a	no
	Predator	0.49 (0.11)a	0.32 (0.14)a	0.51 (0.02)a	no

[a]Significant if there was a difference between averages of Litter Addition treatment and Field (Time 0) biomasses.
Different lower case letters denote significant differences (P<0.05) in trophic group biomass within a treatment over time.
Bold font indicates significant differences (P<0.05) in a trophic group biomass between the two treatments at one time.

3.3.2 Carbon flow into nematode energy channels

Bacterivorous nematodes incorporate more root litter derived C than any other nematode trophic group for both FB and IB soil (Fig. 9). In addition, fungivorous nematodes incorporated significantly more root litter derived C in the FB soil than in the unburned soil. Conversely, both bacterivorous and omnivorous nematodes accumulated significantly more root litter derived C in the IB soil than the FB soil.

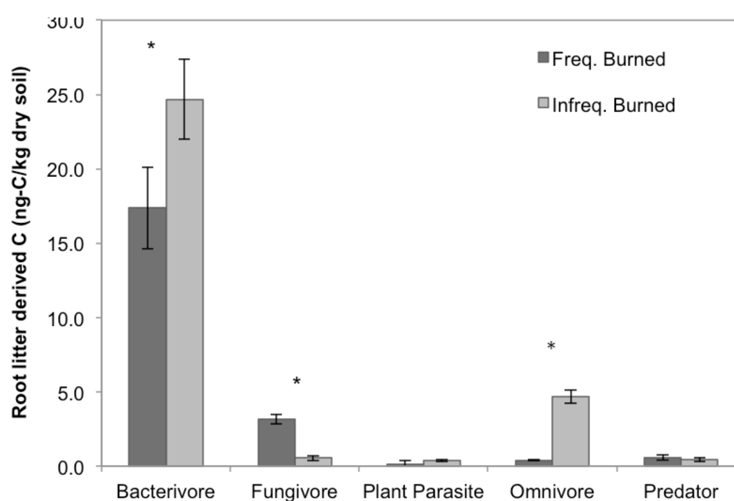

Figure 9. Total root litter derived C per nematode trophic group (all in ng nematode biomass-C/ kg dry soil). Asterisks (*) denote significant differences in amount of root litter derived C at the p<0.05 level (n=3).

Overall, the nematode bacterial energy channel incorporated significantly more root litter C than the fungal energy channel (Fig. 10). In comparing FB to IB, the bacterial energy channel from IB incorporated significantly more root litter derived C than the bacterial energy channel from FB soil. Conversely, there was significantly more C incorporated in the fungal energy channel of FB soil than the IB soil (Fig. 10).

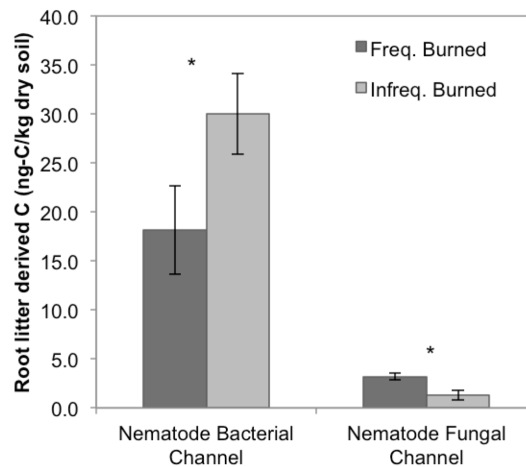

Figure 10. Total root litter derived C per nematode energy channel (all in ng biomass-C/ kg dry soil). Asterisks (*) indicate significant difference between root litter derived C in the frequently burned and infrequently burned soil energy channel ($p<0.05$ by one way ANOVA, LSmeans post-hoc test, n=3).

3.4 DISCUSSION

We hypothesized that nematode bacterial energy channel would be the dominant energy channel for FB tallgrass prairie soils, while the nematode fungal energy channel would be dominant for IB tallgrass prairie soils. Results showed that this hypothesis was true for FB soil and not true for IB soils, as the nematode bacterial energy channel was more dominant than the fungal energy channel in both FB and IB soils. In addition, ratios of fungal to bacterial nematode energy channel biomass revealed that nematode

energy channel biomass in the FB prairie had a significantly lower fungal to bacterial nematode energy channel ratio than the IB (Time 0), supporting our hypothesis that FB soil would be more strongly bacterial dominated than the IB soil. Other studies have suggested that litter layer removal and carbon inputs caused by frequent fire increase belowground production by plants (Johnson and Matchett 2001), thus stimulating root herbivores and detritivores (especially bacteria and bacterial feeders) in the soil community (Seastedt and Ramundo 1990, Todd 1996). In addition, the heat from fire can have a sterilizing effect on soil (Hart et al. 2005), and Pietikainen and Fritze (1995) found that fungi were more susceptible to this (<56% reduction in fungal biomass in prescribed burn areas), which may lead to a dominance of bacteria in FB areas.

The dominance of the nematode bacterial energy channel in IB soil in this study contrasts with findings of some earlier grassland soil food web studies. For example, Todd (1996) sampled unburned sites of the tallgrass prairie over time and concluded that the high proportion of fungivore to bacterivore nematodes indicated a fungal-dominant food web. In addition, results of other grassland studies have found a dominant fungal-based food web (Freckman et al. 1979, de Vries et al. 2012). However, factors such as the specific season of sampling (Bardgett et al. 1997), increased N deposition over time (Todd 1996), grazing-exclusions (Freckman et al. 1979), and root litter incorporation into the soil, versus placement on the soil surface (Holland and Coleman 1987), have been found to influence soil food webs and may promote a bacterial dominant energy channel. Previously, Hunt et al. (1987) noted the significance of bacterial dominance found in the shortgrass prairie, because the fungal channel is generally assumed to surpass or equal the bacterial channel in grasslands. In their

study, Hunt et al. (1987) measured the active fungal biomass (instead of the total fungal biomass as previous studies had done). Later and in accordance with Hunt et al., Dangi et al. (2010) found that in grassland soils, bacteria were more abundant, but microbial activity was dominated by fungi.

Ultimately, litter decomposition is the product of soil microbial metabolism, but soil fauna, such as nematodes, also contribute to litter decomposition by impacting microbial activities and changing litter chemical composition (Petersen and Luxton 1982, Verhoef and Brussaard 1990, Coleman and Crossley 1996, Xin et al. 2012). In addition, nematodes are a good indicator of food web activity in soil, due to their span of five trophic levels and relatively long life cycle (Yeates et al. 1993, Neher 2001). While nematodes account for around 10% of soil metabolism (Macfadyen 1963, Elkins and Whitford 1982), their significance in decomposition and carbon cycling processes should not be dismissed (Nielsen et al. 2011). Their disproportionate impact on N cycling (compared to their small biomass) has been noted (Freckman 1988); however, nematodes additionally affect decomposition and carbon cycling processes indirectly (Petersen and Luxton 1982, Freckman 1988), but might additionally be considered as a pool of soil-C. We found that nematode bacterivores acquired up to approximately 27 ng root litter derived-C/kg dry soil (data not shown), which was not surprising, considering that their primary food source, microbes, can incorporate 200-500 ng litter derived-C/kg soil (Elfstrand et al. 2008) during decomposition. Our findings indicate that nematodes acquire more C during root decomposition than other studies that have estimated nematode-C consumption (Sohlenius 1979). As secondary consumers of C,

nematodes may play an important role as both mediators of microbial-C dynamics and as a temporary carbon pool.

We expected that the quantification of nematode bacterial and fungal energy channel biomass would indicate the proportion of litter C incorporated into each nematode energy channel. Earlier studies suggested that measurements of soil food web channels could provide a useful tool in estimating decomposition pathways (Ferris et al. 2001, Ruess 2003, Culman et al. 2010) and we hypothesized that the flow of C would mirror the decomposition pathways depicted by the nematode energy channel measures. In general, our hypothesis was confirmed - the nematode bacterial energy channel (which dominated in biomass) accumulated more root litter derived C than the fungal channel for both FB and IB soils. However, surprisingly, the nematode bacterial channel accumulated more root litter C in the IB than the FB soil despite no significant differences in biomass for this channel between IB and FB soil at 180 days. Likewise, the nematode fungal energy channel incorporated significantly more root litter C in FB than IB soil despite no differences in fungal energy channel biomass (180 days). These differences could indicate a higher carbon use efficiency for the nematode bacterial channel in IB soil and nematode fungal channel in FB soil (de Vries et al. 2012). We found these results surprising, as we expected the biomass of an energy channel would indicate the proportion of C accumulated by that energy channel. Our results indicate that while nematode energy channel biomass measurements indicate C flow at a coarse scale, ^{13}C decomposition tracer studies are more precise for depicting finer differences in C flow within energy channels.

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4. CONCLUSIONS

To our knowledge this is the first study of carbon flow from decaying roots through a soil food web in tallgrass prairie soil using stable isotopes as a tool. In this study, there were indications of differences in the decomposer pathway in the two soil management strategies: frequently burned and infrequently burned. This was especially shown by the high ^{13}C enrichment of fungivore nematodes in only frequently burned soil, even though bacterivore nematodes played a large role in both soils. There was also indication of specialization by certain microbial groups in the two soils. Actinomyetes, fungi, and gram-positive bacteria utilized root litter-C earlier in the frequently burned soil, and only later did so in the infrequently burned soil. Gram-negative bacteria were highly ^{13}C enriched in both soils, indicating fast turnover and C-source generalization by this group.

Despite lower overall microbial abundance in frequently burned soil, there were no differences in total decomposition or the amount of C acquired from root litter by the soil food web from frequently burned soil. This showed frequently burned soil's higher efficiency in decomposing root litter. Our results illustrate that root litter-C is incorporated differently in frequently and infrequently burned soil, and frequently burned soil food webs may be more specialized to decompose root litter. We show the C flow within soil food webs in differing burn management areas, and show differences between the frequently and infrequently burned tallgrass prairie.

The nematode energy channel biomass measures for the tallgrass prairie soil, indicated, in general, the magnitude of C flow from decaying roots into the energy channels of the nematode food web. Both frequently and infrequently burned soils were

nematode bacterial energy channel dominant. ^{13}C analysis showed that the nematode fungal pathway incorporated more root litter-C in frequently burned soil and the bacterial pathway incorporated more root litter-C in infrequently burned soil (despite no differences biomass measures). Frequently burned soil may have a more efficient fungal pathway, while infrequently burned soil may have a more efficient bacterial pathway. These results indicate that while energy channel biomass measurements of nematodes give a broad indication of C flow, ^{13}C decomposition tracer studies are more precise for ecosystem research.

5. APPENDICES

5.1 DETAILS OF PHOSPHOLIPID FATTY ACID EXTRACTION AND DERIVATIZATION METHODS FOR MICROBIAL COMMUNITY

The PLFA extraction and derivatization methods for compound-specific $\delta^{13}C$ analysis were based on previous studies (Bossio and Scow 1995, Denef et al. 2007). For both burned and unburned treatments, approximately 6g soil samples were sieved (2mm), and any remaining fine roots were removed with forceps. Soil samples were extracted in duplicate at each harvest using chloroform/methanol/phosphate-buffer at a 1:2:1 ratio. During the chloroform phase, all lipids were recovered. By successive elution with chloroform, acetone, and methanol, the lipids were separated on silica gel columns. The polar lipid fraction, eluted with methanol, was then subjected to mild alkaline transesterification by methanolic KOH. This formed fatty acid methyl esters (FAME). Next, these 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.

Prior to GC-C-IRMS analysis, two internal standards (12:0 and 19:0) were added to the FAME extract. The fatty acids of each sample were then classified by their relative retention times vs. the 12:0 and 19:0 internal standards. The fatty acid identifications were then cross-referenced with a standard mixture of 37 FAMEs (37 Component FAME Mix, # 47885, Supelco Inc.).

Mass balance was used to correct the $\delta^{13}C$ values of the individual FAMEs

acquired from the GC-C-IRMS for the addition of the methyl group during transesterification:

$$\delta^{13}C_{PLFA} = \frac{[(N_{PLFA} + 1)\delta^{13}C_{FAME} - \delta^{13}C_{MeOH}]}{N_{PLFA}}$$

where N_{PLFA} is the quantity of C atoms of the PLFA component, $\delta^{13}C_{FAME}$ signifies the $\delta^{13}C$ value of the FAME after transesterification, and $\delta^{13}C_{MeOH}$ is the $\delta^{13}C$ value of the methanol used for transesterification.

5.2 ADDITIONAL TABLES

Table 5. Total number of nematodes/kg dry soil (standard error) for frequently burned and infrequently burned bulk soil and litterbags.

Trophic Group	0 day	3 day	10 day	21 day	35 day	90 day	180 day
a)Freq. Burn							
1. Soil							
Bacterivore	2847 (365)	602 (205)	1665 (126)	1668 (444)	1833 (710)	6561 (267)	6481 (1611)
Fungivore	350 (47)	67 (34)	232 (62)	421 (85)	47 (13)	1316 (482)	1462 (206)
Omnivore	1066 (223)	115 (17)	658 (99)	157 (45)	90 (32)	232 (23)	322 (73)
Predator	386 (44)	163 (52)	433 (107)	199 (68)	32 (16)	244 (80)	345 (153)
Plant Parasite	2160 (431)	1335 (409)	2479 (341)	1785 (632)	1540 (770)	1850 (677)	810 (188)
2. Litterbag							
Bacterivore		194 (35)	1754 (927)	2097 (462)	184 (9)	569 (183)	255 (90)
Fungivore		34 (9)	23 (4)	265 (141)	41 (5)	82 (6)	56 (14)
Omnivore		4 (2)	1 (1)	3 (3)	2 (1)	2 (1)	8 (4)
Predator		1 (1)	2 (2)	0 (0)	5 (2)	0 (0)	4 (4)
Plant Parasite		5 (1)	11 (5)	11 (4)	10 (3)	11 (3)	7 (4)
b)Infreq. Burn							
1. Soil							
Bacterivore	1324 (264)	1076 (429)	4760 (2171)	3781 (1467)	1719 (336)	4592 (1162)	6503 (1081)
Fungivore	328 (26)	185 (76)	203 (28)	83 (31)	27 (10)	377 (137)	910 (124)
Omnivore	448 (87)	715 (355)	762 (233)	651 (200)	336 (104)	705 (143)	1016 (192)
Predator	658 (94)	738 (363)	1350 (540)	554 (193)	570 (279)	946 (120)	510 (188)
Plant Parasite	3388 (626)	1637 (648)	4373 (1544)	1224 (172)	1189 (325)	2179 (408)	905 (92)
2. Litterbag							
Bacterivore		521 (178)	2170 (536)	605 (226)	309 (50)	290 (63)	141 (48)
Fungivore		13 (4)	7 (1)	26 (10)	16 (4)	8 (2)	24 (3)
Omnivore		3 (1)	0 (0)	3 (2)	3 (1)	0 (0)	3 (1)
Predator		1 (1)	1 (1)	2 (1)	1 (1)	0 (0)	1 (1)
Plant Parasite		12 (2)	6 (3)	8 (2)	8 (2)	0 (0)	3 (2)

Table 6. Day-0 $\delta^{13}C$ values (standard error) and ^{13}C enrichment, $\Delta\delta^{13}C$, (standard error) of microbes and nematodes over time after labeled root litter addition.

Community	0 days	3 days	10 days	21 days	35 days	90 days	180 days
Freq. Burn							
Microbes							
Fungi	-18.2 (1.2)	-0.9 (1.5)	13.4 (5.7)	9.7 (8.8)	35.7 (9.7)	54.6 (6.9)	91.7 (27.0)
Gram-	-21.1 (2.3)	38.2 (2.7)	26.5 (5.8)	44.5 (5.2)	24.2 (4.7)	54.6 (9.6)	51.4 (7.5)
Gram+	-18.8 (4.3)	14.9 (1.7)	10.3 (3.3)	21.3 (3.4)	25.6 (3.5)	57.4 (6.4)	70.7 (7.8)
Actinomycetes	-19.9 (4.8)	0.6 (0.3)	0.9 (2.9)	5.8 (3.3)	12.2 (3.3)	28.4 (5.3)	35.8 (7.0)
Protozoa	ND	ND	ND	ND	ND	48.2 (11.3)	73.4 (15.3)
Nematodes							
Bacterivore	-19.7 (3.7)	109.5 (33.2)	181.0 (29.5)	286.2 (78.0)	200.3 (61.7)	162.1 (21.8)	74.1 (33.0)
Fungivore	-23.0 (0.1)	226.7 (20.9)	110.8 (62.6)	349.5 (58.4)	48.3 (5.2)	83.0 (11.3)	39.1 (31.8)
Herbivore	-15.4 (1.0)	0.5 (1.0)	2.8 (3.3)	2.3 (2.0)	5.2 (6.3)	6.4 (5.9)	2.7 (1.9)
Omnivore	-14.2 (1.4)	2.7 (0.3)	-2.1 (1.3)	13.0 (9.9)	16.0 (22.5)	1.6 (5.2)	33.9 (10.0)
Predator	-18.7 (2.1)	0.9 (1.4)	0.7 (0.5)	3.3 (6.5)	13.7 (1.1)	-2.8 (4.8)	45.2 (36.2)
Infreq. Burn							
Microbes							
Fungi	-26.5 (2.1)	24.0 (1.4)	41.9 (7.5)	33.2 (2.9)	12.0 (3.8)	22.4 (5.1)	36.9 (11.9)
Gram-	-25.6 (1.6)	34.5 (3.2)	50.3 (5.9)	22.2 (1.6)	4.0 (2.1)	17.6 (1.1)	24.1 (4.4)
Gram+	-23.0 (2.2)	12.4 (1.3)	25.3 (3.3)	11.6 (0.7)	5.2 (1.9)	30.3 (1.6)	35.3 (4.5)
Actinomycetes	-25.1 (2.8)	10.7 (1.2)	18.0 (2.3)	10.2 (0.9)	-1.5 (2.7)	9.1 (1.8)	15.0 (3.8)
Protozoa	ND	ND	ND	ND	36.3 (2.6)	53.9 (10.5)	57.5 (11.6)
Nematodes							
Bacterivore	-22.1 (0.3)	149.7 (79.0)	294.4 (17.1)	182.0 (20.0)	30.9 (5.0)	72.1 (22.3)	104.7 (32.6)
Fungivore	-24.5 (0.1)	10.8 (14.4)	2.6 (3.0)	50.0 (8.7)	24.1 (4.2)	12.0 (17.1)	10.7 (13.0)
Herbivore	-22.9 (0.0)	-1.0	0.3	-2.8	-4.0	2.1	16.3
Omnivore	-19.0 (0.8)	4.5 (3.1)	1.5 (2.6)	13.7 (2.8)	28.7 (1.5)	62.1 (7.8)	139.6 (37.7)
Predator	-19.6 (2.9)	-11.9 (5.7)	9.7 (0.7)	11.4 (17.9)	7.7 (0.5)	12.1 (8.0)	23.1 (20.0)