

29 March 2016

Dr. Sharon Billings, Handling Topical Editor SOIL,

Dear Dr. Billings,

Thank you very much for your helpful comments with those of the referees on our manuscript (**soil-2015-52**), which we submitted for consideration of publication in *SOIL*. All of the comments and reviews received were very constructive, and we appreciate the opportunity to address them in a revision.

As per the instructions, we provide below a detailed point-by-point response to the referees' comments, with explanations of the changes we made and their locations in the text.

We again thank the referees and the Editor for the time dedicated to our manuscript and for their constructive comments. We believe our manuscript has improved with the changes we made, and hope that this new version will be suitable for publication in *SOIL*.

Yours sincerely,
E. Ashley Shaw
Colorado State University
Department of Biology

Editor:

Thank you for your conscientious responses to the reviewers' comments. I concur with Ref #2 that by changing some simple phrases in your work, you can expand the reach of the study to fire effects writ large in prairie systems, not just at Konza. I do not advocate wild speculation, but by making the suggested wording/phrasing changes you can indicate the broader relevance of your work. Please submit a revised version of the manuscript, reflecting your responses.

- **Thank you, we have worked to remove terminology surrounding fire as a management practice and have replaced these phrases with fire in a more general way. We agree that this has improved the broader relevance of the manuscript.**

Anonymous Referee #1

The present study has been conducted within the Konza prairie long-term ecological research program and focuses on the impacts of burning management on carbon fluxes from decomposing roots into parts of the soil food web, i.e. microorganisms and nematodes. The general topic is of broad interest since burning is a common management practice in tallgrass prairies and its effects on belowground carbon dynamics are not yet well understood. The study has been well designed and hypotheses are reasonable and formulated clearly. The main message/result of the study is that soil microbial- and nematode communities differ between annually and infrequently burned soils, with these differences modifying root decomposition and carbon fluxes. The authors suggest that soil communities of annually burned soils are better adapted to decompose root litter than those of infrequently burned soils, since root litter plays a larger role in the former soils. Generally, this study provides new and interesting insights into root carbon dynamics. Specifically, carbon fluxes from roots into microorganisms and nematodes, and how these differ between annually burned and infrequently burned sites is interesting and deserves publication. However, the manuscript has to be improved to be more concise. Also, there are some statistical issues that need to be corrected, see comments below.

Specific comments:

p. 926, line 14: "microbial community composition"

- **Corrected**

p. 927, line 5 - 11: "stable isotopes" is not very precise, better would be something like "natural abundances of ^{15}N and ^{13}C "; also you should make clear when you are talking about labeling experiments in the next sentence

- **We changed the sentence to clarify that trophic structure has been studied using ^{13}C and ^{15}N . We changed the following sentence (line 9) to specify labeling experiments.**

p. 931, line 21: The authors select the PLFAs 20:4n6 and 20:5n3 to represent protozoa (no reference provided). However, these fatty acids also occur in nematodes (Chen et al. 2001, Chamberlain et al. 2005), this should be taken into account.

- **Previous work has used the PLFAs 20:4n6 and 20:5n3 to represent protozoa (Gomez et al 2014; Ringelberg et al 1997); we do agree with referee #1 that these PLFAs occur in other eukaryotes, including nematodes.**
- **Due to this overlap and the minor role of these data in the manuscript, we will removed the protozoa (20:4n6 and 20:5n3) results from the revised manuscript. These data were a minor part of the results and discussion, and removing them did not affect our conclusions.**

p. 933, lines 16, 17: for analysis of variance, time, soil and litter addition were treated as categorical variables. This is not correct since time is not an independent variable. Instead, the authors should use a repeated measures GLM to separate "within" and "between" effects.

- **Our statistical methods were based on previous soil community analyses and were done in consultation with a statistician at Colorado State University. Our study was not a true repeated measures design, as we were not taking samples from the same plot or pot repeatedly over time, but instead destructively sampled individual pots of soil at each time**

point. We considered carefully the referee's suggestion and have even already performed the statistics as suggested by the referee. A repeated measures GLM does not change the overall statistical results, and the manuscript conclusions would not change if we were to use the repeated measures GLM instead.

- Because the experimental design was not a repeated measures design, we did not change the statistics.

p. 935, lines 23-25: regarding Fig. 5, to it looks like the sentence "Higher trophic levels: : : , this increased by the final harvest." is only true for IB and not for AB.

- We will clarify the sentence by writing, "Higher trophic levels (omnivore and predator nematodes) began to have root litter C incorporated into their biomass by 21 (IB, Fig. 5a) and 35 (AB, Fig. 5b) days. This amount increased by the final harvest with IB omnivore and predator nematodes having greater root litter C incorporated than AB by the final harvest (Fig. 5).

p. 937, line 18: change ": : impacts to organic matter decomposition: : :" to ": : impacts on organic matter decomposition: : :"

- corrected

P. 937, lines 22-24: Reformulate this sentence to state your actual results and then help the reader by shortly stating your second hypothesis.

- We changed the sentence structure to "Root litter mass loss was greater for the AB treatment, confirming our second hypothesis that decomposition would be greater for the AB treatment."

p. 937, 938: the paragraph on "Effects of burning management on root decomposition and root-C dynamics" should be written more concisely; it should become clearer which conclusions can be drawn from the present study, e.g. p. 938, lines 3-7: these two sentences "Other studies have compared: : :" and "These studies have shown: : :" should be combined;

- We combined the two sentences as follows, "Other studies have compared belowground decomposition in areas of contrasting burning treatments and have found that wood decomposed significantly faster..."

p. 938, line 7 "such differences" do you mean the differences you observed, or the differences observed by Reed et al. and O'Lear et al., or both? Make more clear when you are discussing your own results and put the emphasis on that. Similarly, p. 938, lines 10-14, how does this last sentence relate to your study, do you propose that N-scavenging may play a role in your AB soils?

- On p. 938, line 7 we replaced, "Such differences in decomposition between burning treatments" with, "Faster decomposition in annually burned prairie soil"
- On p. 938, lines 10-14, we are proposing that N-scavenging could play a role and we improved the transition to this sentence by changing it to read "Relative to unburned prairie soil, the soil conditions of frequently burned areas are often N-limited, causing microbes to scavenge for N before beginning decomposition"

p. 939, line 20-22: may be a matter of taste, but I would rephrase this to "per gram of soil, nematodes can hold as much as half of root litter derived-C as microbes do"

- We rewrote this sentence as suggested

Table 1: I would suggest to use the same abbreviations as in the text for your treatments (AB and IB), and not Freq. burn and Infreq. Burn

- Corrected

Fig. 3, caption: it is not clear what you mean by ": : significantly higher abundance of a particular trophic group between burn treatments: : :", I would suggest to write ": : significantly higher abundance of a particular trophic group in the respective burn treatment: : :"

- We remade this figure at the suggestion of Referee #2 and rewrote the caption to correspond to the new figure

Fig. 4, caption: ": : : , and root litter derived carbon incorporated in nematodes (c) are reported."

- corrected

Fig. 5: please provide reference to the abbreviations in the legend, as in figs. 1-3.

- **corrected**

Technical comments:

p. 939, line 20: replace "Setala" by "Setälä"

references: check for mutated vowels such as ä, ö, ü; e.g. it's Körner, C. and not Korner, C.

- **corrected**

Fig. 4, legend: use "AB" instead of "FB"

- **corrected**

Anonymous Referee #2

This is a nicely designed study to assess the effects of contrasting fire treatments on the decomposition of ¹³C-labeled root litter and subsequent movement through the soil microbial and nematode communities. The topic is interesting and the approach is relatively novel. However, I found the results and conclusions somewhat hard to follow, due primarily to some confusing text and some inconsistencies in the stated results and accompanying figures. Some of these issues should be relatively easy to address, while other may require some additional analysis or redrawing of figures to clarify. I don't think the manuscript is ready for publication in its current form, but a revised version may be. I provide both general and specific comments below. Although it is true that fire is "managed" today in most areas of tallgrass prairie, fire was historically an important natural factor driving ecological processes in these grasslands. Naturally occurring fires are also important in many other productive grasslands globally. As a result, I question why the authors appear to discuss fire only in the context of a management practice in this manuscript (beginning with the title "Burning management in the tallgrass prairie: : :", and continuing throughout the manuscript). Wouldn't the study have broader appeal by referring to the effects of fire per se, whether the fire is prescribed or naturally occurring? For example, line 23 could be altered to read "This is especially important in grasslands where fire is common and removes aboveground litter: : :" rather than "This is especially important in grasslands where fire is a common management practice and removes aboveground litter: : :". Likewise the words "management practice" could be removed from line 45, and elsewhere, without altering the meaning of the sentence and making the results more relevant to grassland fires in general. If you then want to note that fire, as a management practice, can affect soils and soil biota, you could do that with the text as written on page 4 (lines 66-70). I expect that many of the effects of prescribed and natural fires are similar (both remove aboveground detritus, both alter the soil microclimate, etc.) One might argue that some effects of prescribed and natural fires could vary, based on timing, intensity, etc., but that could be brought up in the Discussion, if the authors feel that is relevant.

- **We agree that the study has a broader appeal when discussed in terms of fire in general (rather than only as a management practice). In this revised manuscript, we have carefully reworded our introduction, discussion, title, and section titles to reflect this more general view.**

The use of ¹³C-labeled plant root litter to follow detrital C through both microbial pools and consumers (nematodes) is novel, and a valuable approach for assessing how fire alters soil food web and associated C flux. In that regard, this paper contributes some novel data and insights.

- **We appreciate this comment.**

Line 46 – stating that fire affects the soil community and root decomposition in "Konza Prairie LTER soils" seems too limiting and site-specific. Why not broaden this to tallgrass prairies soils, or something similar?

- **We agree and have broadened the abstract, introduction, and discussion to**

processes in tallgrass prairie soils.

Lines 126-127 – *Could the authors be more specific with respect to hypothesized differences in soil and nematode communities between the contrasting fire treatments. The hypothesis that will be “different” is OK, but are there more specific predictions that could be made based on what is known about effects of fire of organic matter inputs in these grasslands, changes in soil microclimate, etc.?*

- **We have revised this hypothesis as follows, “(1) The soil community would be less abundant and less diverse in the AB treatment due to the disturbance of fire, which removes surface organic inputs, increases soil temperatures, and decreases soil moisture.”**

Line 136 – *remove extra “the” before Long-Term Ecological Research site: : :*

- **corrected**

Lines 275-276 – *There appears to be different notations used in the formula (fR) and in the corresponding text (fr). Fix this so that capitalization is consistent.*

- **corrected**

I found portions of the Results section to be confusing. I think this is because some of the conclusions drawn in the text are not apparent in the figures that are referenced to support them. In addition, I had trouble interpreting some of the figures/figure legends.

- **We have carefully revised the results for clarity, referring the reader to figures when appropriate and adding in specific p-values when appropriate.**
- **We have also revised the figures and legends as suggested by referee #2 (see comments below), which has helped to improve the manuscript.**

Some specific examples follow:

Line 310 – *Authors state that PLFA abundance was significantly lower for the AB than for IB treatment and refer the reader to Fig. 2. However, Fig 2. does not explicitly include comparisons of either individual PLFA groups or total PLFA for all groups among fire treatments (i.e., panel A vs. panel B). Do the authors mean that PLFA averaged across all functional groups was significantly lower for AB than for IB treatment? If so, that should be explicitly stated in the text. Same comment applies to reference about bacterivores being more abundant in AB and plant parasitic nematodes being more abundant in IB (lines 314-315). This is not readily apparent in Figure 3.*

- **p. 934, line 10: We changed the sentence to say, “The total average PLFA abundance for AB was significantly lower than IB (P<0.05).”**
- **p. 934, lines 14-15: We removed the reference to Fig. 3 and added the appropriate statistical values.**

Note: “abundance” in line 314 should be “abundant”.

- **corrected**

There are also some issues/inconsistencies in the figures and figure legends. For example, the caption for Fig 2 indicates that data are based on n=3, but that’s not consistent with statements in the Methods that there were 4 replicates per treatment/harvest date (see lines 187 and 203). Why were only 3 replicates used in Fig. 2?

- **Clarifications for sample number were made in the methods and in each figure legend.**

The legend in Fig. 3 uses lower case ‘a’ and ‘b’, but should be upper case to be consistent with the figure labeling and with other figure legends.

- **corrected**

The x-axis in Fig. 4 is in units of months, while other figures with a temporal scale are in units of days. In addition, because the time between collection intervals is not evenly spaced, the figures that have a temporal x-axis should have those points scaled/spaced to reflect the actual time between collection intervals (i.e., in Fig. 5, the interval between 3 and 10 days is presented as

the same as the interval between 90 and 180 days, resulting in very misleading temporal patterns of C incorporation into the biota). This may or may not affect the authors' discussion of temporal dynamics of litter C movement.

- **We agree and have corrected temporal axes to be accurately spaced (e.g. Fig 4 and 5).**

Lines 324-328 – This section of the Results refers to changes in the nematode community driven by the addition of litter, and the time since litter addition. The statement about temporal changes following litter addition reference Fig. 3. However, it appears that Fig. 3 includes only data from the litter addition treatment! How can we know then that the temporal changes are due to the litter addition, and not just changes in the community over the course of the 180-day incubation? In order to demonstrate that the changes in nematode are a response litter addition, you would need to compare the temporal dynamics of nematodes in the litter-addition vs. the non-addition soil cores. Why was that not done here? In fact, I don't see any data from the non-addition cores in any of the figures, except Fig. 1. It seems to me that comparisons of changes in microbial and nematode communities over time in soil cores with and without litter additions would be a key part of this story, especially if the authors wish to attribute temporal changes to the addition of litter. Can the authors add these data, where appropriate?

- **We agree with the reviewer and have added the appropriate control data to figures 2 and 3. We have edited the results and discussion to reflect these changes.**

Line 422 – The Johnson and Matchett reference seems out of place here. I don't think that reference deals at all with the effects of pyrogenic material. In fact, there are other ways that burning can promote N limitation besides adding pyrogenic OM, such as by increasing inputs of detritus with a wider C:N ratio. There are many references to support that in tallgrass prairie.

- **We have edited this sentence and replaced this reference with Blair 1997 and Ojima 1994 when discussing N-limitation in frequently burned prairies.**

Fig. 1 – It is difficult to read the small font used to highlight groups with top 'species' scores on these graphs. In addition, the groups are not really 'species' right? Might be better to refer to them as functional groups or something similar?

- **We have increased the font size on Figure 1**
- **Although we do not want to call these functional groups, because the microbial unit is 'biomarkers' and the nematode unit is 'trophic groups,' we do agree that calling these "species" could be confusing.**
- **we changed our caption text, removing the word "species." This reads, "Groups with top scores are plotted along with ellipsoids..."**

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

3

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22 **Abstract**

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

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

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55 1. Introduction

56 Soils contain an immense diversity of soil microorganisms and soil fauna, and are of key
57 importance to terrestrial ecosystems nutrient cycling and carbon (C) storage (Wall et al.,
58 2010;Wall, 2004;Bardgett, 2005;Smith et al., 2015). Understanding the roles of the soil
59 food web in regulating belowground processes of decomposition, nutrient cycling, and C
60 cycling is recognized as a hot topic of research in soil ecology (Bardgett and Cook,
61 1998;Holtkamp et al., 2011;Holtkamp et al., 2008;Carrillo et al., 2011;Osler and
62 Sommerkorn, 2007;Bardgett et al., 2013;van der Putten et al., 2013). This is especially
63 because we still lack a clear understanding of how soil fauna contribute to these
64 ecosystem processes and the ecosystem services they provide (Nielsen et al.,
65 2011;Carrillo et al., 2011;Brussaard, 1998;Bardgett and Cook, 1998;Smith et al., 2015).
66 Within the soil fauna, nematodes, which can occur at densities of approximately 1
67 million to 10 million m⁻² in grasslands (Bardgett et al., 1997;Yeates et al., 1997), are
68 thought to play a fundamental yet poorly understood role in soil C dynamics (Staddon,
69 2004;Nielsen et al., 2011;Wall et al., 2008;Osler and Sommerkorn, 2007).

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

87 can have large effects on plant productivity, plant community composition, and root
88 properties (Kitchen et al., 2009;Knapp et al., 1998), which can significantly alter
89 ecosystem processes such as litter decomposition and C cycling (Ojima et al.,
90 1994;Johnson and Matchett, 2001;Soong and Cotrufo, 2015). Litter decomposition is an
91 important component of belowground C cycling and root litter C provides a major energy
92 source for soil biota (Eisenhauer and Reich, 2012). Since fire removes aboveground
93 litter, and enhances root growth and belowground C allocation, root detrital input may
94 be an even more important energy source for decomposer food webs in frequently
95 burned grasslands (Seastedt et al., 1991;O'Lear et al., 1996). Furthermore, root
96 decomposition studies have been highlighted as crucial because root litter is a major
97 source of soil C (Rasse et al., 2005), contributing more than aboveground litter, and
98 very little research has been done on the topic (Schimel and Schaeffer, 2012).

99 The belowground effects of fire have additional impacts on soil biodiversity and
100 their functions. Burning causes changes in the soil surface energy budget by removing
101 plant litter accumulation (O'Lear et al., 1996;Knapp and Seastedt, 1986). This leads to
102 changes in soil conditions, such as nitrogen content, carbon content, temperature and
103 moisture, which could impact microbial and faunal activities or change detritivore
104 community composition. Microbial community compositional changes have been
105 reported as a result of fire: for example, fire alters microbial community composition by
106 reducing Gram-negative and Gram-positive bacteria (Docherty et al., 2011) and
107 increasing arbuscular mycorrhizae (Hamman et al., 2007). Also, fire initially impacts the
108 overall abundance of nematodes negatively (Whitford et al., 2014), but this rebounds
109 quickly and certain groups, such as colonizing bacterivore nematodes, respond

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112 positively after fire (Jones et al., 2006; Todd, 1996). Such changes in soil community
113 composition have been shown to impact litter decomposition (Verhoef and Brussaard,
114 1990). While most litter decomposition is ultimately the product of soil fungal and
115 bacterial metabolic activities, soil fauna also play a role in litter decomposition by
116 influencing these microbial activities and altering litter chemical composition (Coleman
117 and Crossley, 1996; Verhoef and Brussaard, 1990; Petersen and Luxton, 1982; Xin et al.,
118 2012; Mamilov, 2000; Coleman and Hendrix, 2000; Carrillo et al., 2011; Swift et al.,
119 1979; Soong et al., submitted). However, little is known about how fire impacts both soil
120 microbial and faunal community function or if frequently burned grasslands' soil
121 communities are more specialized to decompose root litter than unburned soil
122 communities.

123 Addition of ¹³C-enriched plant litter to soil allows tracing litter-derived C into soil
124 microbial and faunal groups during decomposition. This technique has been used to
125 study plant-C utilization by microbial communities in soils by examining ¹³C
126 incorporation into microbial phospholipid fatty acids (PLFA; e.g., Deneff et al.,
127 2009; Rubino et al., 2010; Kohl et al., 2015; Soong et al., submitted). Also, natural
128 abundances of ¹³C and ¹⁵N have been useful for studying structures of soil faunal
129 communities (e.g., collembolans, earthworms, enchytraeids, arthropods, gastropods,
130 and nematodes; Chahartaghi et al., 2005; Albers et al., 2006; Goncharov et al.,
131 2014; Crotty et al., 2014; Kudrin et al., 2015). Furthermore, C flow through soil faunal
132 trophic groups can be traced and quantified using ¹³C in labeling experiments (Albers et
133 al., 2006; Pollierer et al., 2007; Elfstrand et al., 2008; Ostle et al., 2007; D'Annibale et al.,
134 2015; Gilbert et al., 2014). However, root turnover and aboveground litter inputs are the

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

142 This project was designed to trace C from decomposing root litter into components
143 of the soil food web over time for annually (AB) and infrequently burned (IB) prairie
144 soils. Our conceptual approach included the production of a ¹³C-enriched tallgrass (big
145 bluestem, *Andropogon gerardii*) root litter, its incubation in intact AB and IB prairie soil
146 cores in a greenhouse, and quantifying the incorporation of root litter C within the soil

147 food web over time. We hypothesized that: 1) The soil community would be less
148 abundant and less diverse in the AB treatment due to the disturbance of fire, which
149 removes surface organic inputs, increases soil temperatures, and decreases soil
150 moisture, 2) root litter mass loss would be greater and occur faster for AB, and 3) root
151 litter would be a more important C source for microorganisms and nematodes from AB
152 prairie, which would thus incorporate root litter-derived C more quickly and in greater
153 amounts than those from IB prairie.

154 2. Materials and Methods

155 2.1 Site description and soil collection

156 Soil samples were taken from historically unplowed tallgrass prairie at the Konza Prairie
157 Long Term Ecological Research (LTER) station in eastern Kansas, United States
158 (39°05'N, 96°35'W). Average monthly temperatures range from -2.7° C in January to
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171 26.6° C in July, with 835mm of total annual precipitation on average. Following a similar
172 sampling design of a concurrent field study by Soong and Cotrufo (2015), we used soils
173 from two fire treatment areas at Konza Prairie LTER: annual spring burn and 20-year
174 burn. Each treatment area is approximately 60 hectares and has silty-clay textured
175 Argiustoll soils. The two treatment areas are in close proximity to one another with
176 minimal geological and edaphic differences. The annual spring burn treatment area
177 (labeled SpB by the Konza Prairie LTER) was burned yearly each spring since 1972,
178 and was burned prior to soil collection on 26 April 2011. The annual spring burn
179 treatment area had soil pH 6.2. The 20-year burn treatment area (labeled 20B by the
180 Konza Prairie LTER) was last burned by an unprescribed wildfire on 5 April 1991;
181 previously, a prescribed burn occurred on 3 May 1975. The 20-year burn treatment had
182 soil pH 6.1. For specific soil characterization data for these sites including %C, %N,
183 pyrogenic organic C content and bulk density see Soong and Cotrufo (2015). Soil from
184 the annual spring burn treatment area will be referred to as annually burned (AB) and
185 the 20-year burn as infrequently burned (IB) for the remainder of this paper.

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

194 plastic bags in the field, kept in coolers with ice packs, and transported to greenhouses
195 at Colorado State University (CSU), Fort Collins, CO, USA for the decomposition
196 experiment. Every effort was made to minimize disturbance to the soil.

197 Field temperature and moisture were measured at time of soil collection for both
198 AB and IB soils. Soil temperature was recorded in the field and daily during the
199 greenhouse incubation using a temperature probe coupled to a PP system (PP-system,
200 SRC-1). Initial soil moisture was determined by gravimetric water content (GWC) by
201 subtracting the oven-dry weight of soil (105°C) from the field moist weight. All soil pots
202 were weighed and %GWC was estimated based on initial field levels. Soil moisture was
203 maintained daily at 20% GWC by weighing the cores every other day and adding
204 deionized water as needed to bring up soil moisture levels.

205

206 **2.2 Production of ¹³C-enriched root litter**

207 Prior to experiment setup, *Andropogon gerardii* was grown from rhizomes in soil-free
208 potting mix for one growing season in a continuous labeling chamber at 4 atom% ¹³C-
209 CO₂ atmosphere, fertilized weekly for 21 weeks with a ¹⁵N-KNO₃ solution (7 atom%)
210 (Soong et al., 2014). After the growing season, plants were harvested and roots were
211 separated from shoots. Roots were then washed, air-dried and a sub-sample analyzed
212 for %C, %N, and ¹³C and ¹⁵N enrichment by an Elemental Analyzer (EA; Carlo Erba NA
213 1500) connected to a continuous flow Isotope Ratio Mass Spectrometer (IRMS; VG
214 Isochrom, Isoprime Inc., Manchester, UK). The root litter had a C and N concentration
215 of 44.37% and 1.49%, respectively, and an isotopic enrichment of δ¹³C 1882.37‰ (3.12
216 atom %) and δ¹⁵N 12147.21‰ (4.61 atom %).

217

218 **2.3 Decomposition experiment**

219 Our experimental design consisted of two burn treatments and two litter treatments in a
220 fully factorial design (2 burn treatment x 2 litter treatment x 6 harvests x 4 replicates =
221 96). Soil cores from AB and IB treatments were incubated inside the PVC collars with
222 either of two different litter treatments: control (no litter) or litter addition (¹³C-enriched
223 root litter). A total of 48 nylon litterbags (8cm x 8cm, 1mm mesh size) were prepared,
224 each containing approximately 1.5g of the air-dried ¹³C-enriched root litter and buried in
225 the soil (24 AB and 24 IB) for the litter addition treatment. Subsamples of root litter were
226 dried in an oven at 70°C for oven-dry mass correction. To minimize disturbance to the
227 soil, each soil core was carefully removed from the PVC collar, sliced in half horizontally
228 (Sanauallah et al., 2010), a litterbag was placed in the center, and the two halves of the
229 core were restored together into the PVC collar. The remaining cores were sliced in half
230 then put back together, with no litterbag added, and established as control treatments.
231 All PVC collars were established on top of sand to allow for drainage and were
232 contained individually in pots to prevent cross contamination. The experiment was
233 conducted in a greenhouse at the Colorado State University Plant Growth Facility.

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

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

246

247 **2.4 Microbial community**

248 Microbial community structure was assessed by Phospholipid Fatty Acid (PLFA)
249 analysis. We ran three out of the four replicates (chosen at random) for PLFA analysis
250 due to the expense and time required to run these analyses. Soil sub-samples for
251 PLFA analysis were sieved to 2mm, with any visibly remaining plant material carefully
252 removed with forceps. The PLFA extraction, quantification and δ¹³C analysis methods
253 were based on previous studies (Bossio and Scow, 1995;Denef et al., 2007;Gomez et
254 al., 2014). For all treatments, approximately 6g soil subsamples from the bulk soil were
255 lyophilized and extracted in duplicate using a modified Bligh-Dyer method (Gomez et
256 al., 2014) at each harvest. Fatty acid methyl ester (FAME) derivatives were analyzed by
257 capillary gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-
258 IRMS) (GC-C/TC DeltaPLUSXP Thermo Scientific) via a GC/C III interface. PLFA
259 identifications were based on the retention times of two standard mixtures, a Supelco
260 FAME mix (47885-U: Supelco 37 component FAME mix, Sigma-Aldrich) and a bacterial
261 acid methyl ester mix (47080-U: BAME mix, Sigma-Aldrich). Representative samples
262 were analyzed by gas chromatography-mass spectrometry (GC-MS; Shimadzu QP-

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263 2010SE) and spectral matching was completed using the NIST 2011 mass spectral
264 library (Shimadzu) to identify PLFAs that are not available in standard mixtures,

265 A number of PLFAs were selected as biomarkers for different microbial groups to
266 investigate the soil microbial community composition (Frostegård and Bååth,
267 1996;Zelles, 1999). The PLFAs i15:0, a15:0, i16:0, a17:0, i17:0 were selected to

268 estimate the abundance of ~~Gram~~-positive bacteria, and cy17:0, cis16:1n9, 18:1n11, and
269 cy19:0 for ~~Gram~~-negative bacteria. Fungal abundance was based on cis18:1n9 and
270 cis18:2n9,12, and methylated PLFAs 10Me-16:0, 10Me-17:0, and 10Me-18:0 were used
271 as indicators of actinobacteria.

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

276

277 2.5 Nematode community

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

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

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291 isotopic analysis 75 individuals from each trophic group were then handpicked using an
292 eyelash (Superfine eyelash with handle, Ted Pella, Inc., Prod no. 113) under a
293 dissecting microscope (Olympus SZX10, 30X magnification), and transferred to a pre-
294 weighed tin capsule (8x5mm, Elemental Microanalysis BN/170056) containing 120µL of
295 deionized water. The tin capsules containing the different nematode trophic groups
296 were desiccated for 3 days, weighed again to obtain final sample weights, and then
297 prepared for analysis. The tin capsules containing nematode samples were analyzed for
298 %C and ¹³C using a CE-1110 EA coupled via ConFlo II interface to an IRMS
299 (ThermoFinnigan Delta Plus).

300 The absolute abundance of individual nematode groups was calculated (number
301 nematodes kg⁻¹ dry soil). Changes in the nematode community composition were
302 evaluated based on relative nematode abundance data, which were calculated by
303 dividing the absolute abundance of a nematode group by the sum of the absolute
304 abundance of all nematode groups.

305

306 2.6 Data analyses

307 The isotope ratios are reported in terms of δ¹³C (‰) values (Brenna et al., 1997),

308 i.e.:

$$309 \delta^{13}\text{C} (\text{‰}) = (R_{\text{sample}} - R_{\text{standard}}) / (R_{\text{standard}}) \times 10^3$$

310 where R_{sample} is the ¹³C/¹²C ratio of the sample and R_{standard} refers to the reference

311 standard, Pee Dee Belemnite.

312 The proportion of root-litter carbon incorporated into nematode and microbial

313 tissue (f_R) was calculated by a two-source mixing model with:

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315
$$f_R = (\bar{\delta}_{\text{BioR}} - \bar{\delta}_{\text{BioC}}) / (\bar{\delta}_R - \bar{\delta}_{\text{BioC}})$$

316 $\bar{\delta}_{\text{BioR}}$ and $\bar{\delta}_{\text{BioC}}$ refer to the $\delta^{13}\text{C}$ signature of a group in the root litter-addition and the
317 corresponding control, respectively, and $\bar{\delta}_R$ to the $\delta^{13}\text{C}$ signature of the initial root litter.

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

322
$$\text{PLFA-C}_{\text{root-derived/group}} = (\sum \text{PLFA-C}_{\text{group}} * 100) / \sum \text{PLFA-C}_{\text{root-derived all}}$$

323 The effects of time, soil burning treatment, and litter addition on microbial PLFA
324 abundance, nematode densities, and microbial and nematode incorporation of root litter
325 derived ^{13}C were analyzed by Analysis of Variance (ANOVA) methods using a
326 generalization of the general linear model (GLM) in the Proc Mixed procedure.

327 Statistical analyses were completed with SAS 9.3 (SAS Institute Inc., Cary, North
328 Carolina). Data were analyzed using a three factor model, where $y = \text{time} + \text{soil} + \text{litter}$
329 addition. Time, soil, and litter addition were treated as categorical variables. Data were
330 tested to meet assumptions of normality and residuals were log transformed to achieve
331 normality if necessary. Significance was accepted at a level of probability (P) of < 0.05 .

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

338 distance matrix was calculated for each community using the Bray-Curtis measure to
339 model the species matrix. A principal coordinate analysis was performed on the
340 distance matrix and the resulting eigenvalues were applied to a redundancy analysis.
341 Ordination plots were drawn with ellipsoids (representing a 95% confidence interval)
342 around the multivariate community groups. The dbRDA and subsequent drawing of
343 ordination plots were performed using R (R Core Team, Vienna, Austria).

344

345 3. Results

346 3.1. Effects of fire and root litter addition on the soil community

347 Burn treatment had a significant effect on the soil community. The dbRDA revealed that

348 AB and IB community compositions of microbes and nematodes were significantly

349 different (Fig. 1A and Fig. 1B, respectively). For microbes, the differences in community

350 composition were driven by biomarkers for fungi (cis-C18:1n9, cis18:2n9,12) Gram-

351 negative bacteria (cy19:0), and Gram-positive bacteria (a17:0) (Fig. 1A). The total

352 average PLFA abundance for AB was significantly lower than IB treatment ($P < 0.05$).

353 Specifically, there were lower proportions of PLFA biomarkers for Gram-positive

354 bacteria and fungi for AB (Fig. 2). Total nematode abundance did not differ between the

355 AB and IB treatment ($P = 0.39$), but community structure was significantly different (Fig.

356 1B). While the differences for the AB soil were driven by fungivores and plant parasitic

357 nematodes, the IB soil community was influenced by omnivore and predator nematodes

358 (Fig. 1B).

359 With the addition of root litter to the soil, microbial and nematode communities

360 were changed (Fig. 1). The dbRDA revealed that the microbial community structure of

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

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

387 3.2 Effects of fire on root decomposition and root-C dynamics

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

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

421

422 3.3 Effects of fire on soil community utilization of root-C

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

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

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451 for both burn treatments (Fig. 5). Fungal use of root litter C differed slightly for the burn
452 treatments, with fungi from the AB treatment increasing in root litter C over time (Figs
453 5C and 5D).

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

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

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480 **4. Discussion**

481 **4.1. Effects of fire on the soil community**

482 Burning has significant impacts on the belowground community including soil microbes
483 and soil nematodes. We found that both soil microbial and nematode community
484 structure differed with long-term burn treatments (Fig. 1), with the AB treatment also
485 showing reduced microbial biomass (via PLFA methods). These findings support our
486 first hypothesis, that different burn treatments would house different soil communities,
487 and confirmed previous observations. In particular, Todd (1996) showed that
488 bacterivore nematodes respond positively to frequent fire while predator nematodes do
489 not. Jones et al. (2006) later corroborated that study via molecular methods.
490 Additionally, fire has been shown to reduce overall microbial biomass and specifically
491 affects Gram-negative and Gram-positive bacteria and fungi (Docherty et al., 2011; Ajwa
492 et al., 1999). Such differences in the soil communities have implications for ecosystem
493 function, such as impacts on organic matter decomposition (Verhoef and Brussaard,
494 1990).

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496 **4.2. Effects of fire on root decomposition and root-C dynamics**

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

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519 decomposition for that study. Other studies have compared belowground decomposition
520 in areas of contrasting burning treatments, and have found that wood decomposed
521 significantly faster in annually burned tallgrass prairie compared to unburned prairie
522 (Reed et al., 2005;O'Lear et al., 1996). Faster decomposition in annually burned prairie
523 soil could be due to the indirect effects of burning on the soil community composition or
524 to the direct effects on soil conditions (i.e., heat, moisture, nutrients), which would
525 impact decomposition processes (O'Lear et al., 1996). For example, relative to
526 unburned tallgrass prairie soils, the soil conditions of frequently burned areas are often
527 N-limited (Blair, 1997;Ojima et al., 1994), causing microbes to scavenge for N before
528 beginning decomposition (Craine et al., 2007)(Soong and Cotrufo, 2015). N-mining by
529 microbes in N-limited areas has been shown to increase decomposition {Craine, 2007
530 #796}.

532 4.3 Effects of fire on soil community utilization of root-C

533 Corroborating part of our third hypothesis, we found that, overall, a significantly higher
534 amount of ¹³C was incorporated into the total soil community for AB, indicating greater
535 utilization of root litter C in this frequently burned soil. In particular, fungivore nematodes
536 and specific biomarkers for fungi, Gram-negative bacteria, and Gram-positive bacteria
537 had a significantly higher proportion of their biomass composed of root litter C,
538 suggesting that root litter C was a more important C source for the AB soil food web.
539 Additionally, despite significantly lower microbial abundance for the AB treatment, there
540 was no difference in the amount of root litter derived C in the total microbial pool
541 between AB and IB treatment. In this way, our study offers some support for the

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

572 We also hypothesized that root-C would be incorporated more quickly for AB. Yet
573 despite the overall greater amount of root-C incorporation by AB, microbes and
574 nematodes both immediately incorporated root-C for both treatments (Fig. 4B and 4C).
575 There was a slight lag in microbial uptake of root litter C for AB, but not for IB (Fig. 4B).
576 This lag could correspond to the time microbes needed to scavenge N in the N-limited
577 AB soil before commencing root decomposition (Manzoni et al., 2012). Yet through
578 time, evidence exists for greater cycling of root litter C to the higher trophic levels of the
579 AB food web. The root litter derived nematode-C pool was significantly greater in the AB
580 treatment at 35 and 90 days after root addition. This accumulation of C in nematodes
581 indicates a greater or faster flow of root litter C from the microbes to their nematode
582 consumers. Others have suggested that most energy from detritus flows to microbes
583 and only a negligible amount of energy flows to the higher trophic levels of the soil food
584 web (Setälä, 2005). Our study opposes this view, as we show that per gram of soil,
585 nematodes can hold as much as half of root litter derived-C as microbes do (Fig. 4B and

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610 4C).

611

612 5. Conclusions

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

628

629 Acknowledgements

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642

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870

871 Table 1. Overall mean relative contribution (f) of root litter C to PLFA-C and nematode-C
 872 with (standard errors), n=18. The relative contribution of root litter C was calculated only
 873 for the PLFA biomarkers and nematode trophic groups from root litter addition samples
 874 that were significantly different in d¹³C from the control. **Bold font** indicates a
 875 significantly higher f-value for a burn treatment.

876

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

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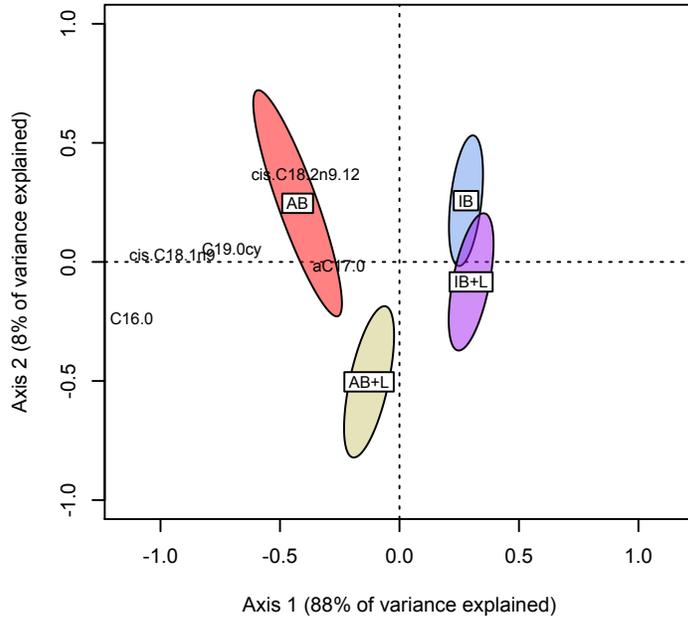
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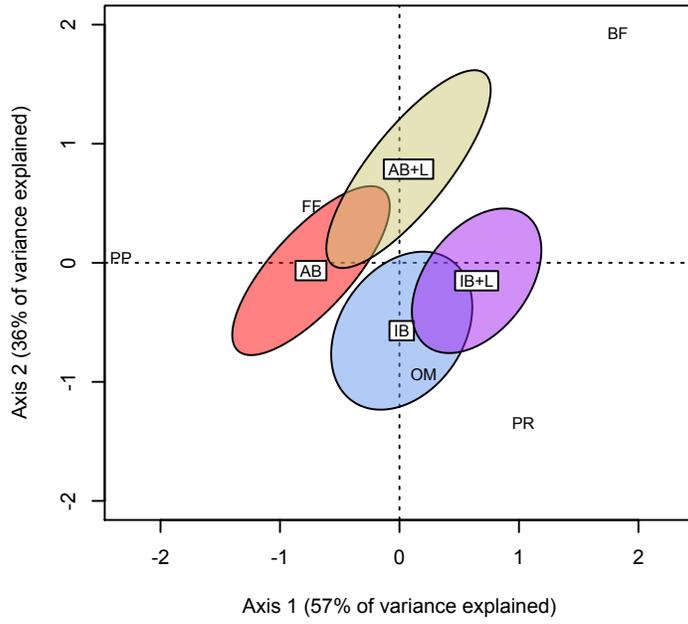
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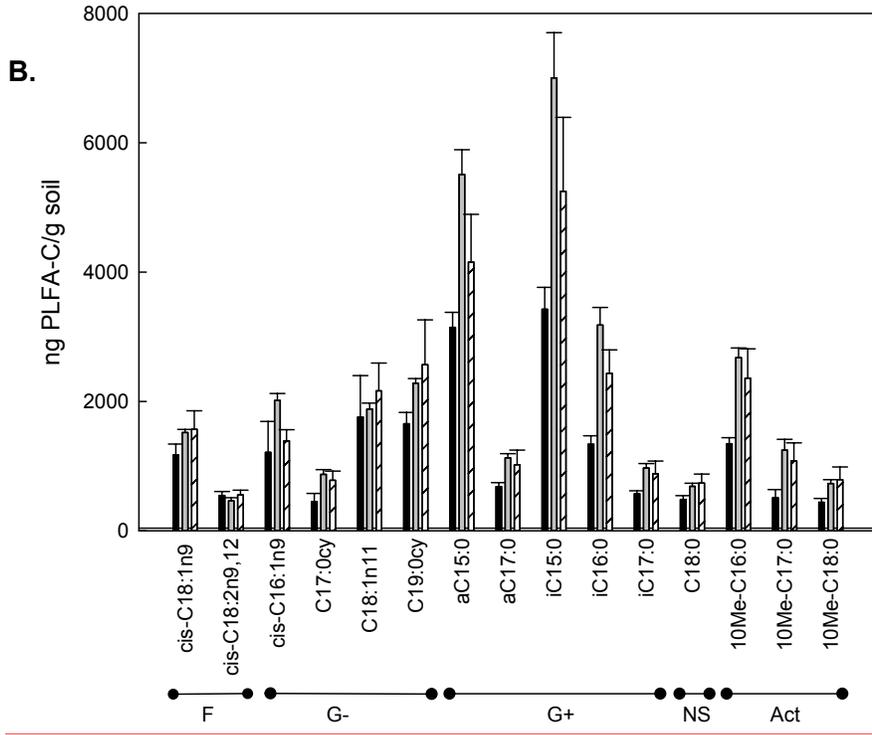
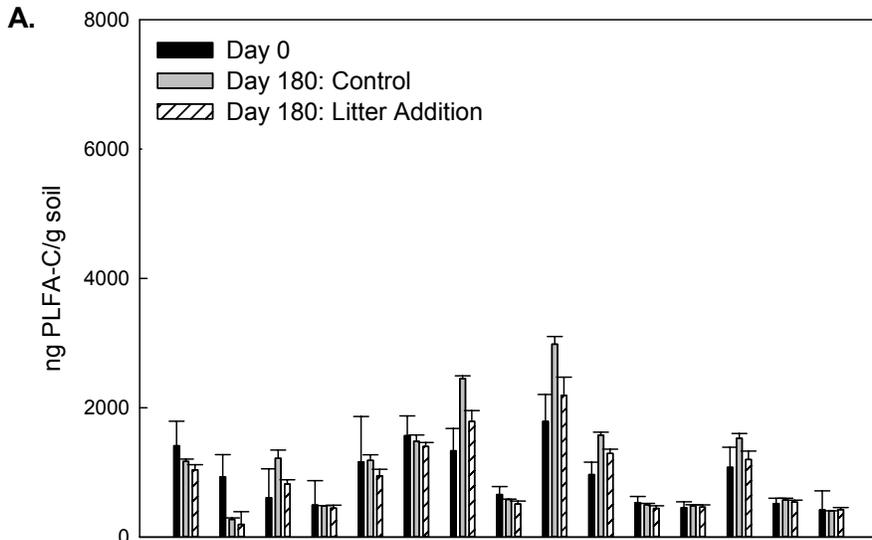
A.



B.



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



894

895 Figure 2. Abundances of PLFA biomarkers for the annual burn (A) and infrequent burn

896 (B) treatments with litter addition for the day 0 and final 180 day harvest. Data are

897 averages (n=3) with standard error bars. For PLFA groups: F=fungi, G+= Gram-positive

898 bacteria, G-= Gram-negative bacteria, NS= non-specific bacteria, Act=Actinobacteria.

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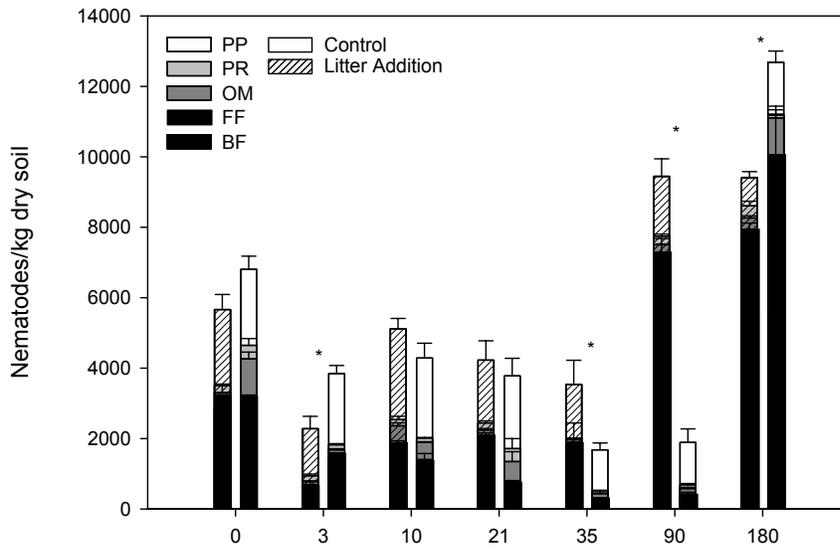
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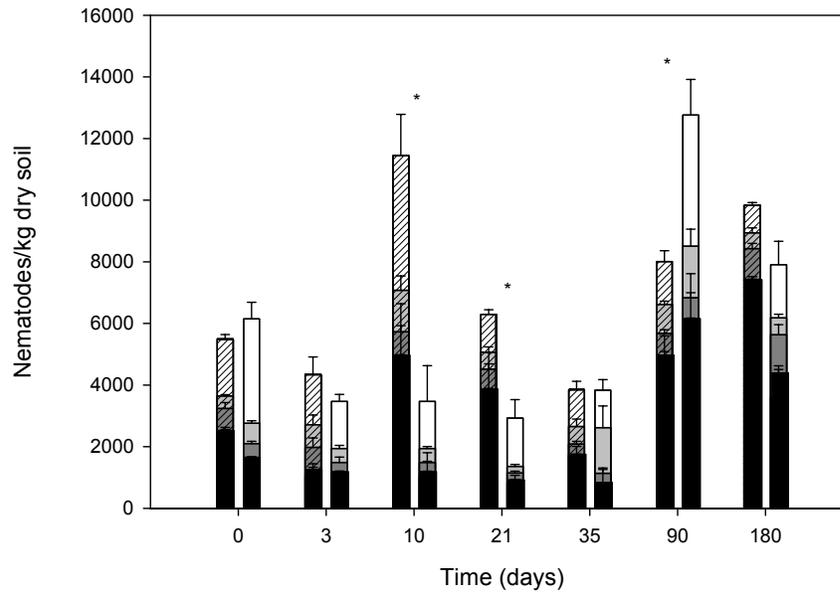
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A.



B.



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

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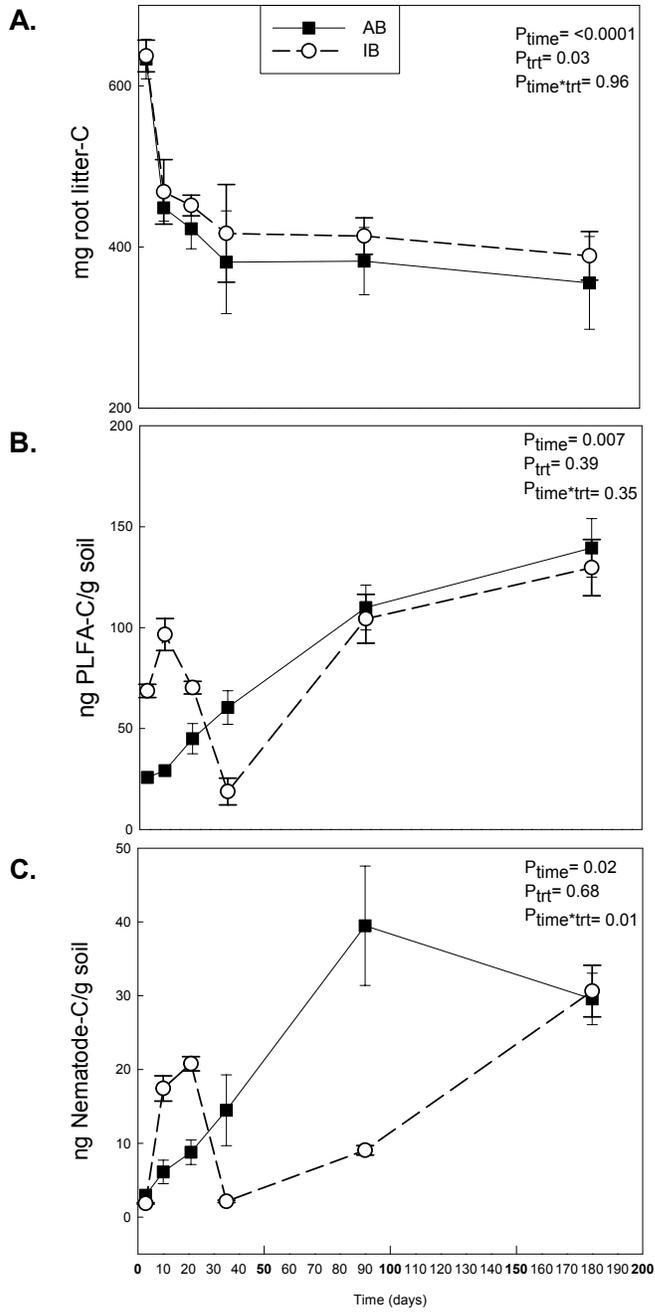
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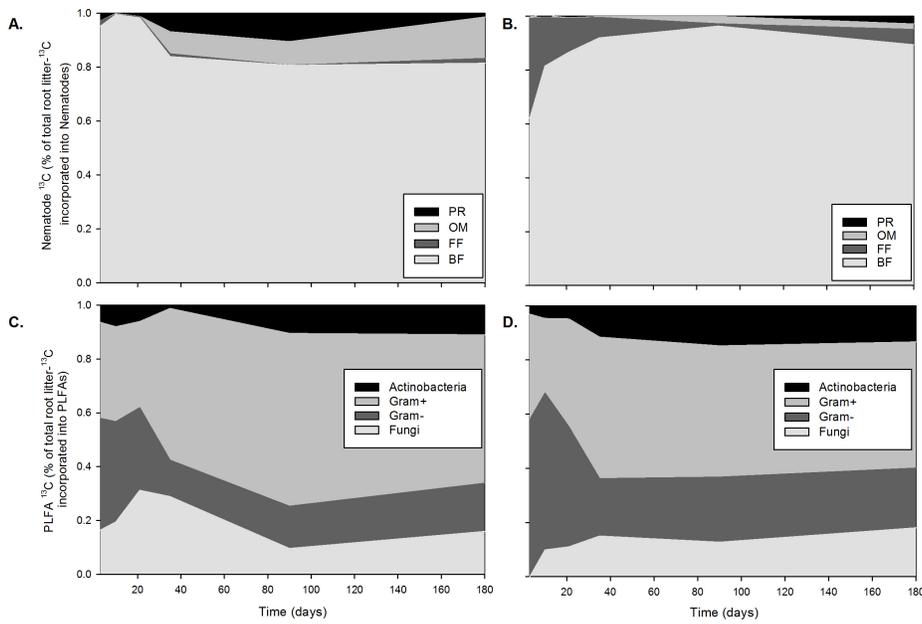
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921 Figure 4. Root litter C dynamics during incubation for the annual burn and infrequent
922 burn treatments. Data are averages with standard error bars. The root litter carbon (A),
923 root litter derived carbon incorporated in microbial phospholipid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



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930 Figure 5. Root litter C incorporation into microbial PLFAs and nematode trophic groups.

931 Panels (A) and (C) are infrequent burn treatment and (B) and (D) are annual burn

932 treatment. Panels (A) and (B) show the percentage of total litter-derived C (^{13}C)

933 incorporated into the total nematode signature quantified at each time point, and panels

934 (C) and (D) show the percentage of total litter-derived C (^{13}C) incorporated into the total

935 PLFA signature at each time point. For nematode trophic groups: BF=Bacterivore, FF=

936 Fungivore OM=Omnivore, PP= Plant Parasite, and PR=Predator.

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