Consumption of mycorrhizal and saprophytic fungi by Collembola in grassland soils

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Abstract

Although soil-dwelling Collembola can influence plant growth and nutrient cycling, their specific role in soil food webs is poorly understood. Soil-free microcosm studies suggest that Collembola are primarily fungivores where they feed preferentially on saprophytic fungi (SF) over other fungal types. We directly assessed collembolan consumption of arbuscular mycorrhizal fungi (AMF) and SF using plant–soil mesocosms and natural abundance stable carbon isotope techniques. Mycorrhizal \textit{Andropogon gerardii} (C\textsubscript{4} grass) seedlings were placed in pots containing Collembola and soil from a C\textsubscript{3} plant dominated site, while mycorrhizal \textit{Pascopyrum smithii} (C\textsubscript{3} grass) seedlings were placed in pots with Collembola and soil collected at a C\textsubscript{4} plant dominated site. After 6 weeks, collembolans assimilated carbon derived from C\textsubscript{3} and C\textsubscript{4} sources in both \textit{A. gerardii} and \textit{P. smithii} treatments. Comparing Collembola isotope values in AMF vs. AMF-suppressed treatments, our data show that both AMF and SF were consumed in these experimental soil environments.

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Keywords: Arbuscular mycorrhizal fungi; Saprophytic fungi; Collembola; Grassland; Natural abundance stable isotopes; \textsuperscript{\delta}13C; \textit{Andropogon gerardii}; \textit{Pascopyrum smithii}

1. Introduction

Soil microarthropods can play important roles in ecosystem processes, such as nutrient cycling (Rooney et al., 2006; Scheu and Setala, 2002). Edaphic Collembola have been shown to affect nitrogen (N) and phosphorus (P) content of plants (Harris and Boerner, 1990), as well as rates of carbon (C) cycling in soils (Johnson et al., 2005). The heterogeneous and opaque nature of soil environments, however, limits our ability to assess the mechanisms by which soil microarthropods affect ecosystem dynamics. Understanding the feeding habits of soil microarthropods, such as Collembola, in the soil environment is an important step in identifying such mechanisms. We directly examine collembolan consumption of plant symbiotic arbuscular mycorrhizal fungi (AMF) and decomposer saprophytic fungi (SF) in a reciprocal mesocosm experiment using natural abundance stable C isotope techniques.

Saprophytic fungi are ubiquitous in soils and are the primary component of the fungal pathway of soil food webs (Coleman et al., 1983; Rooney et al., 2006). They tend to decompose more recalcitrant forms of plant residue and soil organic matter than bacteria, and by balancing the faster nutrient turnover rates of labile organic matter through bacterial pathways, SF are important in ecosystem N and P dynamics (Coleman et al., 1983; Wall and Moore, 1999). An increased abundance of SF-feeding soil fauna has been associated with decreased rates of decomposition (Hedlund and Sjogren Ohrn, 2000; Petersen and Luxton, 1982).

The symbiosis formed between AMF and plant roots is important to the productivity of many terrestrial ecosystems (Smith and Read, 2002). This mycorrhizal symbiosis is characterized by fungal uptake of soil P and N, largely unattainable by plants which are exchanged for plant-derived C (Smith and Read, 2002). In grassland ecosystems AMF colonize the roots of most plant species (Smith and Read, 2002; Wilson and Hartnett, 1997, 1998). While productivity of the dominant warm-season grass species...
responds positively to AMF (Wilson and Hartnett, 1998), other species, including some forbs and sub-dominant grasses, may be negatively affected by the symbiosis (Wilson and Hartnett, 1997). With these varied relationships between different plant species and AMF colonization, feeding on AMF by soil Collembola may result in complex effects on plant performance and community structure (Gange, 2000; Harris and Boerner, 1990; Partsch et al., 2006).

Collembola are a widely distributed and abundant group of soil microarthropods with densities of up to 57,000 individuals/m² in grassland soils (Gange and Bower, 1997). Although they can employ diverse feeding strategies, edaphic species are considered to be primarily fungivorous (Bardgett et al., 1993; Hopkin, 1997; Rusek, 1998). The presence of Collembola has been associated with increased growth and respiration of AMF (Thimm and Larink, 1995), as well as increased plant growth and nutrient concentrations (Harris and Boerner, 1990; Larsen and Jakobsen, 1996). Although detailed mechanisms for these responses are unknown, moderate collembolan grazing may stimulate growth of AMF (Bakonyi et al., 2002; Lussenhop, 1996; Lussenhop and BassiriRad, 2005) or enhance plant available N and P through fecal deposition or by stimulating SF-mediated decomposition (Gange, 2000; McGonigle, 1994; Rusek, 1998). Additionally, collembolan grazing has also been found to decrease rates of soil C cycling by severing AMF hyphal networks (Partsch et al., 2000; McGonigle, 1994; Rusek, 1998). Additionally, collembolan grazing has also been found to decrease rates of soil C cycling by severing AMF hyphal networks (Partsch et al., 2000; McGonigle, 1994; Rusek, 1998). Additionally, collembolan grazing has also been found to decrease rates of soil C cycling by severing AMF hyphal networks (Partsch et al., 2000; McGonigle, 1994; Rusek, 1998).

Elucidating collembolan feeding habits in soil habitats will provide a more mechanistic understanding of their role in soil food webs and nutrient dynamics (Partsch et al., 2006; Scheu et al., 1999). It is unclear whether Collembola preferentially consume AMF or SF (Finlay, 1985; Harris and Boerner, 1990; Jorgensen et al., 2003; Klironomos et al., 1999; Moore et al., 1985; Scheu and Folger, 2004; Thimm and Larink, 1995), although soil-free laboratory microcosm experiments conclude they have a general preference for SF (Jorgensen et al., 2003; Klironomos and Kendrick, 1996; Klironomos et al., 1999; Schreiner and Bethlenfalvy, 2003; Tiunov and Scheu, 2005; Verhoef et al., 1988). Given the complexity of the soil environment, however, actual consumption of fungal resources may not reflect collembolan preferences per se. For example, the ability of most soil microarthropods, including Collembola, to disburse to resources and forage throughout the soil matrix is constrained by structural and architectural characteristics of the soil, including pore spaces (Larsen et al., 2004; Schrader and Lingnau, 1997), which may result in more opportunistic rather than selective feeding patterns.

The primary objective of our study is to evaluate Collembola feeding habits on AMF and SF in complex plant-soil systems. We conducted an experiment with Collembola in mesocosms containing (1) non-sterile C₄ soils and mycorrhizal C₃ plants and (2) non-sterile C₃ soils and mycorrhizal C₄ plants. Because natural variation in stable C isotope concentrations (δ¹³C) of organic matter derived from C₃ vs. C₄ photosynthetic pathways is generally maintained through trophic levels, analysis of Collembola body tissue δ¹³C allows us to assess C assimilated from AMF (δ¹³C similar to plants) and SF (δ¹³C similar to soil organic matter) (Fry, 2006). By including reciprocal plant-soil treatments, we are able to assess feeding habits in the presence of obligate (Andropogon gerardii) and facultative (Pascopyrum smithii) mycotrophic grasses. We expect Collembola will consume AMF to some extent in both treatments, although consumption of AMF is likely to be greater in mesocosms with the obligate mycotroph since AMF are likely to be most abundant in that treatment. We also assess the population response of Collembola to the presence of AMF in these systems. Because of increased resource diversity, we hypothesize that Collembola populations will be greatest when both fungal types are present. This study is the first to assess Collembola feeding habits in soil-based mesocosms.

2. Methods and materials

An experiment with reciprocal treatments (Fig. 1) that manipulated the δ¹³C signatures of plants, Collembola, soil, and both fungal types was conducted during the summer 2005 at Kansas State University, Manhattan, KS, USA, using soils and Collembola collected at Konza Prairie Biological Station (KPBS). In each trial, the δ¹³C signature of SF and AMF were different from one another (representative of C₃ vs. C₄ substrates used by the fungi) so that we could determine relative consumption by Collembola using their δ¹³C values (Fry, 2006).

![Fig. 1. Experimental design of Andropogon gerardii and Pascopyrum smithii trials in relation to C₄ and C₃-labeled Collembola and soil organic matter (SOM) components, as well as application of the fungicide Benomyl (AMF-suppressed pots). Arbuscular mycorrhizal fungi (AMF) colonize and receive carbon from plant roots, while saprophytic fungi (SF) use SOM as a carbon source.](Image)
2.1. *Pascopyrum smithii* trial

*P. smithii* (Rydb.) (western wheatgrass), a facultative mycotroph, is a native C₃ grass. *P. smithii* seeds were sown in C₃ soil collected at KPBS and grown for 4 weeks prior to initiation of the experiment to allow AMF colonization of roots. Three replicate plants were harvested before the experiment began to ascertain the degree of AMF colonization. Roots were stained with Trypan blue following the method of Koske and Gemma (1989) and scored for percent AMF colonization using the magnified griddeline intersect method developed by McConigle et al. (1990). In addition to indicating the strength of the relationship between AMF and the plant, percent AMF root colonization is also positively correlated with the biomass of hyphae in soil (Hart and Reader, 2002). Average *P. smithii* colonization was 16% in 4-week-old seedlings.

Soil was collected at KPBS from beneath a native C₄ plant community dominated by *A. gerardii*. The soil contained 20 μg/g available P (Bray I), 32 μg/g NH₄-N, and 3 μg/g NO₃-N as determined by the Kansas State University Soil Testing Laboratory. Collembola (Onychiuridae and Isotomidae) were elutriated from a portion of the C₄ soil so their C isotope signature would initially be similar to that of the C₄ soil. One *P. smithii* seedling was transplanted into each of 16 plastic pots (6 cm diameter × 25 cm deep) containing 530 g (dry weight) non-sterile C₄ soil. At the same time, six to ten of the C₄-labeled Collembola (equivalent to 2123–3539 individuals/m²) were placed in each C₄-labeled soil pot approximately 2 cm below the soil surface. Every attempt was made to add 10 individuals per pot, however individuals were occasionally lost during the transfer process. Benomyl (methyl-1-(butylcarbamoyl)-2-benzimidazole carbamate) was applied to half (8) of the pots at a rate of 8.3 g/m³ every 2 weeks to suppress AMF root colonization. This is a fungicide previously used to successfully suppress AMF in greenhouse mesocosms with no phytotoxic effects on either of the grass species used in our current study (Wilson and Hartnett, 1997).

Four pots each of the mycorrhizal and fungicide treatments were destructively harvested approximately 3 and 6 weeks after initiation of the experiment, sufficient time for collembolan reproduction and turnover of body tissue C (Briones et al., 1999; Chamberlain et al., 2004). Aboveground plant biomass was clipped, dried at 60 °C for 48-h, and weighed. Roots were removed from the soil, stained, and scored for AMF colonization using the methods described above. The soil was left in the pots which were wrapped in foil and stored at 4 °C for less than 48-h prior to elutriation of live Collembola from the soil. Soil, plant, and Collembola were analyzed for ¹³C signature using a Sercon GSL prep unit fitted to a Europa 20–20 continuous flow mass spectrometer (Sercon, Crewe, UK). Values for ¹³C are reported in δ¹³C notation and referenced against the Vienna Pee Dee Belemnite (VPDB) standard (‰ or 1.12372% ¹³C). Because the soil in each pot was elutriated, we analyzed eight replicate subsamples of bulk soil to estimate the soil δ¹³C value.

2.2. *Andropogon gerardii* trial

*A. gerardii* Vitman (big bluestem) is a native C₄ grass and an obligate mycotroph (Wilson and Hartnett, 1998). *A. gerardii* seeds were sown in native C₄ grassland soil and allowed to grow for 4 weeks to allow AMF colonization of *A. gerardii* roots. AMF colonization was scored using the methods described above on three replicate plants prior to initiation of the experiment. Average *A. gerardii* colonization was 24% in 4-week-old seedlings.

Soil was collected at KPBS from an area that had supported predominantly C₃ vegetation for at least 12 of the past 15 years. The soil contained 21 μg/g available P (Bray I), 20 μg/g NH₄-N, and 0.6 μg/g NO₃-N as determined by the Kansas State University Soil Testing Laboratory. Collembola (Onychiuridae and Isotomidae) were elutriated from a portion of the C₃ soil so their C isotope signature would initially be similar to that of the C₃ soil.

One *A. gerardii* seedling, six to ten C₃-labeled Collembola (2123–3539 individuals/m²), and C₃ soil were placed in each of 16 pots as in the *P. smithii* trial. Benomyl was applied as described above for the AMF-suppressed treatment. Half of the pots from each treatment were harvested after approximately 3 weeks. The remaining pots were harvested after 6 weeks. All pots were sampled as described for the *P. smithii* trial. There was insufficient root material to assess AMF colonization in one *A. gerardii* pot treated with fungicide at each harvest time.

2.3. Data analysis

The experiment was a complete factorial with two time intervals (3 and 6 weeks) and two fungicide levels (+ and −) with four replications. Two-way analysis of variance (SAS v.9.1, Cary, NC) was used to assess plant biomass, mycorrhizal root colonization, and Collembola δ¹³C and density responses. Data for each plant–soil trial were analyzed separately because we did not have a factorial design of plant and soil C isotope combinations (i.e. C₃ plant in C₃ soil, C₄ plant in C₄ soil). Collembola C isotope analysis was performed only on individuals from the families Onychiuridae and Isotomidae which are the same groups added to the pots at the beginning of the experiment. Individuals from these two families elutriated from the same pots were pooled to ensure adequate biomass for mass spectroscopy analysis. Individuals from other families (Sminthiridae, Hypogasturidae, and Entomobryidae) were rare.

3. Results

3.1. *Pascopyrum smithii* trial

There was no difference in *P. smithii* biomass between mycorrhizal and AMF-suppressed pots after 3 weeks, but
mycorrhizal plants had significantly higher biomass than plants from AMF-suppressed pots after 6 weeks (Table 1(A), Fig. 2A). Plants treated with fungicide had significantly lower mycorrhizal root colonization than those that did not receive fungicide (Table 1(A), Fig. 2C). The mean δ¹³C signature of *P. smithii* was −30.80 ± 0.36‰; there was no difference in the C isotope values between mycorrhizal and AMF-suppressed pots (Table 1(A)). The C isotope value of the C₄ soil was −13.17 ± 0.14‰.

In the *P. smithii* trial, mycorrhizal treatment did not have a significant influence on the density of Collembola, although density did increase significantly from 3 to 6 weeks (Table 1(A), Fig. 3A). There was no difference in Collembola δ¹³C between AMF-suppressed and mycorrhizal pots after 3 weeks; after 6 weeks Collembola in the mycorrhizal pots had significantly lower δ¹³C values than the 6-week AMF-suppressed or either treatment after 3 weeks (Table 1(A), Fig. 4A). One of the 6-week AMF-suppressed pots contained only one live collembolan and did not provide enough biomass for C isotope analysis.

### 3.2. *Andropogon gerardii* trial

One mycorrhizal *A. gerardii* plant at 3 weeks was more than 25 times larger than any of the other replicates, and was removed as an outlier from further analysis. Above-ground biomass of *A. gerardii* showed a significant increase from 3 to 6 weeks, and mycorrhizal plants were significantly larger than those in which mycorrhizae had been suppressed after 6 weeks (Table 1(B), Fig. 2B). Fungicide significantly reduced AMF root colonization of plants significantly lower mycorrhizal root colonization than those that did not receive fungicide (Table 1(A), Fig. 2C). The mean δ¹³C signature of *P. smithii* was −30.80 ± 0.36‰; there was no difference in the C isotope values between mycorrhizal and AMF-suppressed pots (Table 1(A)). The C isotope value of the C₄ soil was −13.17 ± 0.14‰.

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### Table 1

Results of analysis of variance tests for significant effects of time (T), fungicide application (F), and the interaction between time and fungicide (T × F) in (A) *Pascoyrum smithii* and (B) *Andropogon gerardii* treatments.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>d.f. (n, d)</th>
<th>Factors</th>
<th>T</th>
<th>F</th>
<th>T × F</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) <em>Pascoyrum smithii</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant biomass</td>
<td>1, 12</td>
<td></td>
<td>7.78**</td>
<td>8.61**</td>
<td>5.68**</td>
</tr>
<tr>
<td>AMF root colonization</td>
<td>1, 10</td>
<td>0.15</td>
<td>105.00***</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Plant δ¹³C</td>
<td>1, 10</td>
<td>3.13</td>
<td>0.11</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Collembola density</td>
<td>1, 12</td>
<td>7.33**</td>
<td>0.16</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Collembola δ¹³C</td>
<td>1, 11</td>
<td>4.02*</td>
<td>0.76</td>
<td>3.25*</td>
<td></td>
</tr>
<tr>
<td>(B) <em>Andropogon gerardii</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant biomass</td>
<td>1, 12</td>
<td>1.28</td>
<td>2.23</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>AMF root colonization</td>
<td>1, 10</td>
<td>0.69</td>
<td>72.35***</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Plant δ¹³C</td>
<td>1, 12</td>
<td>0.35</td>
<td>8.00**</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Collembola density</td>
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<td>15.85***</td>
<td>7.66**</td>
<td>0.32</td>
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</tr>
<tr>
<td>Collembola δ¹³C</td>
<td>1, 10</td>
<td>0.25</td>
<td>2.03</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

F-statistics are reported with all significant statistics (*p* < 0.10) in bold. There was no replication in stable carbon isotope (δ¹³C) data for Collembola at 3 weeks in the *A. gerardii* treatment, therefore the interaction was not determined (ND). d.f. (n, d) = degrees of freedom (numerator, denominator)

**p < 0.10.

***p < 0.05.

****p < 0.01.

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Fig. 2. Biomass (dry weight (g) ± 1 S.E.) of (A) *Pascoyrum smithii* and (B) *Andropogon gerardii* and percent root colonization by mycorrhizal fungi of (C) *P. smithii* and (D) *A. gerardii* 3 and 6 weeks after initiation of the experiments. Plants were either treated with a fungicide (AMF-suppressed) or allowed to maintain a mycorrhizal association. Bars with different letters indicate significant differences (*p* < 0.10). Only time was significant in panel (B) *A. gerardii* biomass as indicated by the solid lines and letters above the 3 and 6 week bars.
A. gerardii signature was \(-11.38 \pm 0.18\%\), although it was significantly less negative in the mycorrhizal \((-10.94 \pm 0.23\%\)) than AMF-suppressed pots \((-11.82 \pm 0.20\%\)) (Table 1B). The C3 signature of the bulk soil used in this experiment was \(-20.12 \pm 0.29\%\).

In addition to being more abundant in the mycorrhizal A. gerardii pots than the AMF-suppressed pots, the density of collembolans was also significantly higher after 6 weeks than 3 weeks (Table 1B, Fig. 3B). Because three of the four experimental pots that had been treated with fungicide did not contain live Collembola (density = 0 individuals/m²) after 3 weeks, we could not test for an interaction between time and fungicide treatment on Collembola \(\delta^{13}C\) values. There were no significant differences in the \(\delta^{13}C\) signature of Collembola based on either time or mycorrhizal treatment (Table 1B, Fig. 4B).

4. Discussion

Our approach differs from previous Collembola feeding studies which have relied on indirect methods such as fecal counts (Klironomos and Kendrick, 1996; Klironomos and Ursic, 1998; Klironomos et al., 1999), the location of individuals relative to different food sources (Hedlund and Sjögren Ohrn, 2000; Jorgensen et al., 2003; Verhoef et al., 1988) or the presence of dark hyphae in the guts of collembolans (Bardgett et al., 2005; Schreiner and Bethlenfalvi, 2003). By assessing the stable C isotope signatures of collembolan body tissues, we found that they assimilated C from both AMF and SF; AMF was assimilated to a greater degree in the P. smithii trial than in the A. gerardii trial.

Because the C isotope signatures are generally maintained through trophic levels (Fry, 2006), the C isotope values of the plants were used as a surrogate for those of the AMF. Likewise, the isotope values of the bulk soil were used as a proxy for non-mycorrhizal fungi because SF were expected to be processing soil organic matter, which should...
have the same signature as the soil. Given the differences in the natural abundance stable isotope values of the soil and plants in our treatments, there were four potential outcomes in our experiment. First, if collembolans were feeding exclusively on root material their isotope values would reflect that of the plant and not differ between the AMF-suppressed and mycorrhizal pots. Secondly, if they fed only on AMF their isotope values would be similar to that of the plant in the mycorrhizal treatment. Thirdly, if they were feeding only on SF their δ13C signature would reflect that of the SF in the bulk soil. Finally, collembolan isotope values intermediate between the soil and plant values would indicate mixed-feeding on both AMF and SF.

In the *P. smithii* trial, our results provide strong evidence that Collembola consumed AMF in addition to SF. Using the two-pool isotope mixing model of Fry (2006), AMF constituted up to 57% of their diet, while SF ranged from 43% to 82% of their diet. It is important to note that although AMF was suppressed by the fungicide, it was not eliminated. Therefore, the C₄ signal in the AMF-suppressed pots may be due to either AMF grazing or root herbivory. The C₄ portion of collembolan tissues most likely resulted from AMF grazing however, since the Collembola isotope value was significantly more depleted (closer to the plant isotope value) in the mycorrhizal pots compared to the AMF-suppressed pots after 6 weeks (Fig. 4B).

In the *A. gerardii* trial, collembolan C isotope values were most similar to the bulk soil which should indicate feeding on SF only. However, the collembolan values were more depleted than those of the bulk soil and fractionation of C isotopes between trophic levels generally results in enrichment, not depletion, of the 13C isotope (Fry, 2006). Additionally, the C isotope value of the soil was higher than anticipated; it was −20‰, whereas a value more similar to C₃ vegetation (~−27‰ to −30‰) was expected. The field from which the C₃ soil was collected had been planted with C₃ vegetation for only 12 of the last 15 years. Portions of older C₄-C in the stable C pool mixed with more recent C₃-C could have resulted in the higher than expected soil δ¹³C signature. Additionally, carbonates (~+8‰ δ¹³C) are present in KPBS soils and would also lead to a less negative δ¹³C signature than expected.

Accounting for these issues, the isotope value of SF was likely closer to −25% on based on the C signature of C₃ vegetation (e.g. *P. smithii* δ¹³C = −30.8‰) and studies of C isotope fractionation in decomposer fungi (Kohzu et al., 1999; Trudell et al., 2004) (dashed line, Fig. 4A). Given this assumption and using the two-pool isotope mixing model of Fry (2006), the isotope values of Collembola in the *A. gerardii* treatment indicate that approximately 50–71% of their diet consisted of SF. Because Benomyd did not completely eliminate AMF in the fungicide treatment, we are unable to discern unambiguously whether the 29–50% of the plant-derived C assimilated by collembolans was the result of root herbivory or AMF grazing.

Although Gange and Bower (1997) reported a negative response of the collembolan *Folsomia candida* to Benomyl, we found no evidence of persistent negative effects of the fungicide in our experiments. In the *P. smithii* trial, there were no differences in collembolan densities between the AMF-suppressed and AMF pots. Although only one of the four replicate AMF-suppressed *A. gerardii* mesocosms harvested after 3 weeks contained live Collembola, they were abundant in all replicate AMF-suppressed pots (3200–6700 individuals/m²/pot) harvested after 6 weeks. Tomlin (1981) found that onychiurid and podurid collembolans, in addition to prostigmatid and cryptostigmatid mites, responded positively to Benomyl although both mesostigmatid mites and earthworms were negatively affected. In a study by Martikainen et al. (1998), Benomyl did not affect enchytraeids or nematods, nor did it alter microbial respiration or inorganic soil nitrogen.

Collembolan densities in both the *P. smithii* and *A. gerardii* trials reached the 2.9–13.1 × 10³ individuals/m² range reported for Kansas tallgrass prairie (Seastedt, 1984) by week 6. The significant increase in collembolan densities in both trials from week 3 to week 6 suggests that they were successfully reproducing and resource availability was not limiting population growth during that period. Further, the mycorrhizal *A. gerardii* treatment supported significantly larger collembolan densities than the AMF-suppressed pots. Kaiser and Lussenhop (1991) found a similar response to mycorrhizal vs. non-mycorrhizal treatments when Collembola were introduced to pots containing seedling *Glycine max*. The lack of a density response to the mycorrhizal treatment in the *P. smithii* trial may indicate Collembola–fungus–plant species-specific responses similar to those reported by Salamon et al. (2004) and Milcu et al. (2006).

Collembolan patterns of feeding on AMF and SF differed between the *P. smithii* and *A. gerardii* treatments. Their diet consisted of less AMF when it was associated with the obligate mycotroph (*A. gerardii*), and more AMF when it was associated with the facultative mycotroph (*P. smithii*). This discrepancy may be due to nutritional or chemical differences between C₃ and C₄-derived C compounds. For instance, Chamberlain et al. (2004) found that when fed diets of the same yeast species grown on different C sources (C₃ vs. C₄), collembolan tissues had different fatty acid profiles. Or, because Collembola, AMF, and SF species were not standardized between the two experiments, the different outcomes may be due to interactions at the species-level (Jorgensen et al., 2003; Salamon et al., 2004) which we are not able to address further with this study.

Because we were primarily interested in the role of AMF relative to SF in collembolan diets and SF were found in greater abundance than AMF in an adjacent tallgrass prairie restoration experiment (P.M. White, unpublished data), we did not specifically analyze the fungal communities of the soils used in this experiment. Soil-dwelling Collembola may consume other fungal types (i.e., pathogenic fungi) that occur in soils, which may account for a portion of consumption we attribute to SF.
However, given that Collembola fed SF or ectomycorrhizal fungi (Scheu and Folger, 2004) began reproducing about a week earlier than those fed pathogenic fungi (Sabatini and Innocenti, 2000) and that pathogenic fungi species can lead to 100% mortality of some collembolan species (Sabatini and Innocenti, 2000), we would not expect pathogenic fungi to contribute significantly to their diets when alternative resources are available.

The mycorrhizal treatment was associated with increased aboveground biomass of both A. gerardii and P. smithii only at the 6-week harvest (when seedlings were 10 weeks old). At that time, AMF colonization of both A. gerardii (38 ± 3.1%) and P. smithii (15 ± 1.1%) was lower than the levels reported by Wilson and Hartnett (1998) (50.2% and 19.3%, respectively). Because our experiment was terminated when plants were still seedlings, the plants may not have had ample time for AMF colonization to reach levels consistent with those of perennial plants. This illustrates the importance of accounting for stage- or age-based interactions and organism reproduction rates when designing such multi-trophic level experiments or attempting to apply results at the field scale.

At the ecosystem scale, AM fungi are important in regulating C flux from plants to the soil (Olsson and Johnson, 2005; Rillig, 2004; Zhu and Miller, 2003). They can consume up to 20% of plant C (Jakobsen and Rosedahl, 1990; Watkins et al., 1996) and are often the largest contributor to soil microbial biomass (Miller et al., 1995). The importance of AM mycelial networks for belowground C flow highlights the need to investigate the extent of impact these abundant fungal-feeding invertebrates impose on these systems (Johnson et al., 2005). Our results reported here indicate collembolans should be viewed as selective polyphagous feeders that depend, at least in part, on fungi that are readily available to them. Selective feeding by fungal-feeding collembolans may affect fungal community composition and therefore the relative dominance of AM vs. SF taxa. Whether the effects of these shifts will be observed at the plant community level remain to be investigated. However, mycorrhizal fungal diversity and/or composition may impact ecosystem productivity at the species level (Jonsson et al., 2001; van der Heijden, 2002; van der Heijden et al., 1998) and even at the genetic level (Koch et al., 2006). Reductions in SF abundance due to fungivorous Collembola have resulted in increased longevity of seeds in soil seed banks, potentially influencing the dynamics of plant populations and the assembly of plant communities (Mitschunas et al., 2006). Further comprehensive multifactorial interaction studies that examine both above- and belowground community dynamics are needed before more specific conclusions can be drawn.

5. Conclusions

Using stable isotope techniques, this is the first study to demonstrate consumption of both saprophytic and mycorrhizal fungi by Collembola in soil-based mesocosms. Our results indicate that (1) grassland Collembola will consume both AMF and SF, and (2) collembolan densities can respond positively to the presence of AMF in the soil environment. Collembolan grazing on both saprophytic and mycorrhizal fungi may alter fungal–fungal (Tiu nov and Scheu, 2005) and plant–fungal (Larsen and Jakobsen, 1996) interactions with consequent implications for plant performance and nutrient cycling (Harris and Boerner, 1990).

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