ELSEVIER

Consumption of mycorrhizal and saprophytic fungi by Collembola in grassland soils

Jayne L. Jonas[a,*], Gail W.T. Wilson[a], Paul M. White[b], Anthony Joern[a]

[a]*Division of Biology, Kansas State University, 104 Ackert Hall, Manhattan, KS 66506, USA*
[b]*Department of Agronomy, Kansas State University, Manhattan, KS 66506, USA*

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 *Andropogon gerardii* (C_4 grass) seedlings were placed in pots containing Collembola and soil from a C_3 plant dominated site, while mycorrhizal *Pascopyrum smithii* (C_3 grass) seedlings were placed in pots with Collembola and soil collected at a C_4 plant dominated site. After 6 weeks, collembolans assimilated carbon derived from C_3 and C_4 sources in both *A. gerardii* and *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.
© 2007 Elsevier Ltd. All rights reserved.

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 phosphorous (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

*Corresponding author. Tel.: +1 785 532 7053; fax: +1 785 532 6653.
E-mail address: jjonas@ksu.edu (J.L. Jonas).

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/m^2 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 (Johnson et al., 2005).

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; Kaiser and Lussenhop, 1991; Klironomos and Ursic, 1998; Klironomos and Moutoglis, 1999; 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 Bethlenfalvay, 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 C_4 soils and mycorrhizal C_3 plants and (2) non-sterile C_3 soils

and mycorrhizal C_4 plants. Because natural variation in stable C isotope concentrations ($\delta^{13}C$) of organic matter derived from C_3 vs. C_4 photosynthetic pathways is generally maintained through trophic levels, analysis of Collembola body tissue $\delta^{13}C$ allows us to assess C assimilated from AMF ($\delta^{13}C$ similar to plants) and SF ($\delta^{13}C$ 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 Collembolan feeding habits in soil-based mesocosms.

2. Methods and materials

An experiment with reciprocal treatments (Fig. 1) that manipulated the $\delta^{13}C$ 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 $\delta^{13}C$ signature of SF and AMF were different from one another (representative of C_3 vs. C_4 substrates used by the fungi) so that we could determine relative consumption by Collembola using their $\delta^{13}C$ values (Fry, 2006).

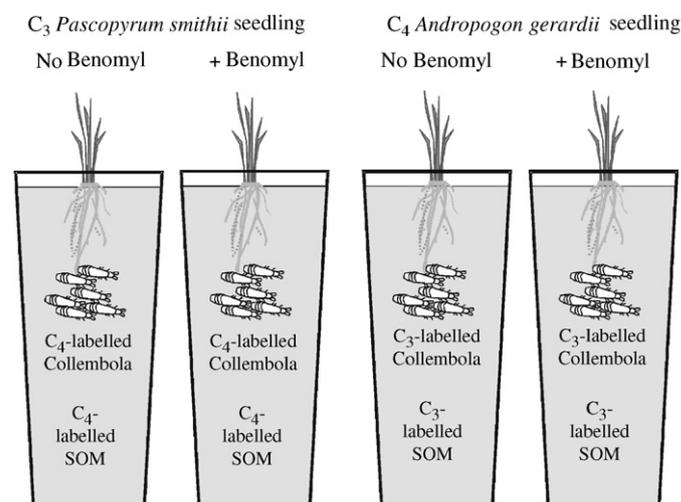Fig. 1. Experimental design of *Andropogon gerardii* and *Pascopyrum smithii* trials in relation to C_3- and C_4-labeled Collembola and soil organic matter (SOM) components, as well as application of the fungicide Benomyl (AMF-suppressed pots). Arbuscular mycorrhizal fungi (AMF) colonize and receive carbon from plant roots, while saprophytic fungi (SF) use SOM as a carbon source.

2.1. Pascopyrum smithii trial

P. smithii (Rydb.) (western wheatgrass), a facultative mycotroph, is a native C_3 grass. *P. smithii* seeds were sown in C_3 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 gridline intersect method developed by McGonigle 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 C_4 plant community dominated by *A. gerardii*. The soil contained $20 \mu g/g$ available P (Bray I), $32 \mu g/g$ NH_4-N, and $3 \mu g/g$ NO_3-N as determined by the Kansas State University Soil Testing Laboratory. Collembola (Onychiuridae and Isotomidae) were elutriated from a portion of the C_4 soil so their C isotope signature would initially be similar to that of the C_4 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 C_4 soil. At the same time, six to ten of the C_4-labeled Collembola (equivalent to 2123–3539 individuals/m^2) were placed in each C_4-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/m^3 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 ^{13}C signature using a Sercon GSL prep unit fitted to a Europa 20-20 continuous flow mass spectrometer (Sercon, Crewe, UK). Values for ^{13}C are reported in $\delta^{13}C$ notation and referenced against the Vienna Pee Dee Belemnite (VPDB) standard (0‰ or 1.12372% ^{13}C). Because the soil in each pot was elutriated, we analyzed eight replicate subsamples of bulk soil to estimate the soil $\delta^{13}C$ value.

2.2. Andropogon gerardii trial

A. gerardii Vitman (big bluestem) is a native C_4 grass and an obligate mycotroph (Wilson and Hartnett, 1998). *A. gerardii* seeds were sown in native C_4 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 C_3 vegetation for at least 12 of the past 15 years. The soil contained $21 \mu g/g$ available P (Bray I), $20 \mu g/g$ NH_4-N, and $0.6 \mu g/g$ NO_3-N as determined by the Kansas State University Soil Testing Laboratory. Collembola (Onychiurdiae and Isotomidae) were elutriated from a portion of the C_3 soil so their C isotope signature would initially be similar to that of the C_3 soil.

One *A. gerardii* seedling, six to ten C_3-labeled Collembola (2123–3539 individuals/m^2), and C_3 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 $\delta^{13}C$ 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. C_3 plant in C_3 soil, C_4 plant in C_4 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 (Sminthuridae, 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

Table 1
Results of analysis of variance tests for significant effects of time (T), fungicide application (F), and the interaction between time and fungicide ($T \times F$) in (A) *Pascoyrum smithii* and (B) *Andropogon geradii* treatments

Response variable	d.f. (n, d)	Factors		
		T	F	$T \times F$
(A) *Pascopyrum smithii*				
Plant biomass	1, 12	**7.78****	**8.61****	**5.68****
AMF root colonization	1, 10	0.15	**105.00*****	0.02
Plant $\delta^{13}C$	1, 10	3.13	0.11	0.01
Collembola density	1, 12	**7.33****	0.16	0.06
Collembola $\delta^{13}C$	1, 11	**4.02***	0.76	**3.25***
(B) *Andropogon gerardii*				
Plant biomass	1, 12	1.28	2.23	0.10
AMF root colonization	1, 10	0.69	**72.35*****	0.13
Plant $\delta^{13}C$	1, 12	0.35	**8.00****	0.92
Collembola density	1, 12	**15.85*****	**7.66****	0.32
Collembola $\delta^{13}C$	1, 10	0.25	2.03	ND

F-statistics are reported with all significant statistics ($p < 0.10$) in bold. There was no replication in stable carbon isotope ($\delta^{13}C$) 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)

  * $p < 0.10$.
  ** $p < 0.05$.
  *** $p < 0.01$.

significantly lower mycorrhizal root colonization than those that did not receive fungicide (Table 1(A), Fig. 2C). The mean $\delta^{13}C$ 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 C_4 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 $\delta^{13}C$ between AMF-suppressed and mycorrhizal pots after 3 weeks; after 6 weeks Collembola in the mycorrhizal pots had significantly lower $\delta^{13}C$ 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 mycorrhzial *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. Aboveground 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
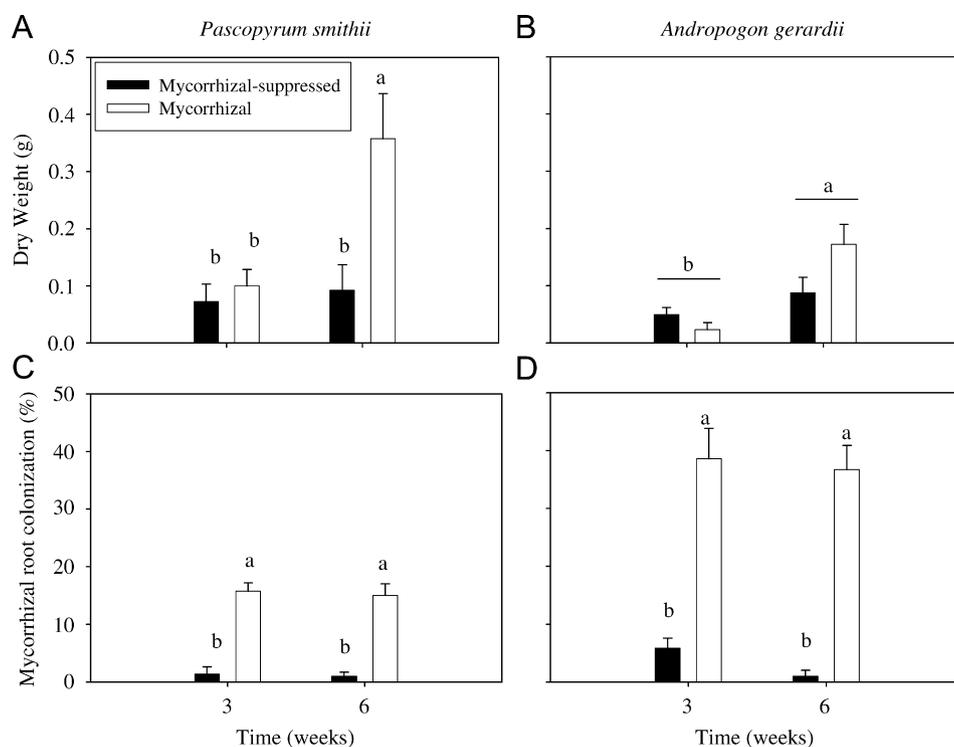

Fig. 2. Biomass (dry weight (g) $\pm$ 1 S.E.) of (A) *Pascopyrum smithii* and (B) *Andropogon gerardii* and percent root colonization by mycorrhizal fungi of (C) *P. smithii* and (D) *A. gerardii* 3 and 6 weeks after initiation of the experiments. Plants were either treated with a fungicide (AMF-suppressed) 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.
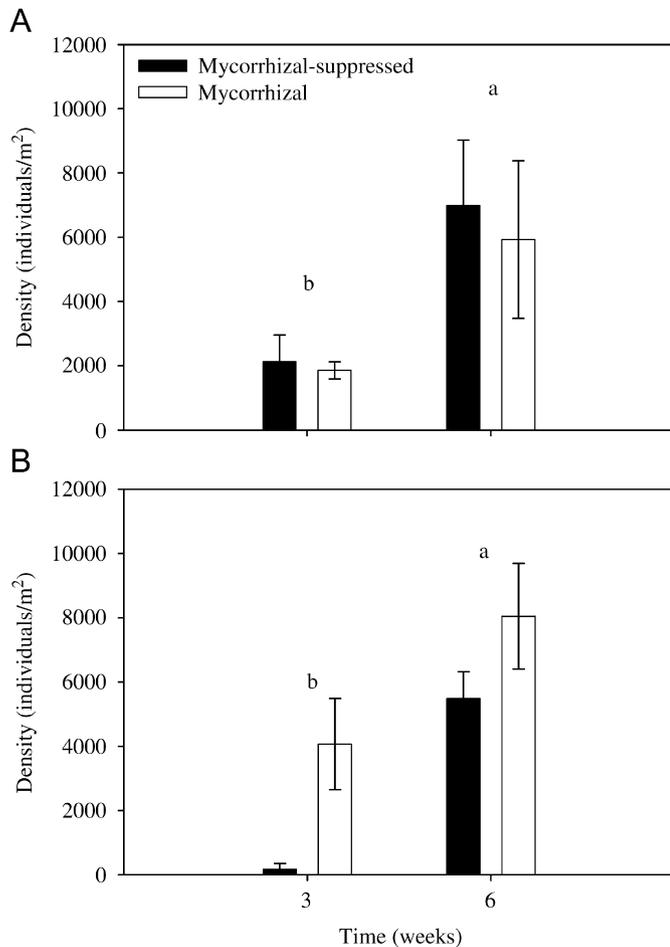
Fig. 3. Density (individuals/m^2 $\pm$ S.E.) of Collembolans after 3 or 6 weeks in mesocosms with (A) *Pascopyrum smithii* in C$_4$ soil or (B) *Andropogon gerardii* in C$_3$ soil. Plants were either treated with a fungicide (AMF-suppressed) or allowed to maintain a mycorrhizal association. Bars with different letters indicate significant differences ($p < 0.10$).

Fig. 4. Stable carbon isotope signature (δ^{13}C VPDB ‰ $\pm$ 1 S.E.) of edaphic Collembola grown in mesocosms with (A) *Pascopyrum smithii* in C$_4$ soil or (B) *Andropogon gerardii* in C$_3$ soil for 3 or 6 weeks. Mesocosms were either treated with a fungicide (AMF-suppressed) or allowed to maintain a mycorrhizal association. Shaded areas represent 95% confidence intervals of plant and soil carbon isotope signatures (δ^{13}C ‰). The dashed line in panel B represents the estimated signature of saprophytic fungi in the C$_3$ soil (Kohzu et al., 1999; Trudell et al., 2004). Bars with different letters indicate significant differences ($p < 0.10$).

(Table 1(B), Fig. 2D). Overall, the *A. gerardii* δ^{13}C signature was -11.38 ± 0.18‰, although it was significantly less negative in the mycorrhizal (-10.94 ± 0.23‰) than AMF-suppressed pots (-11.82 ± 0.20‰) (Table 1(B)). The C$_3$ signature of the bulk soil used in this experiment was -20.12 ± 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 1(B), Fig. 3B). Because three of the four experimental pots that had been treated with fungicide did not contain live Collembola (density = 0 individuals/m^2) after 3 weeks, we could not test for an interaction between time and fungicide treatment on Collembola δ^{13}C values. There were no significant differences in the δ^{13}C signature of Collembola based on either time or mycorrhizal treatment (Table 1(B), Fig. 4B).

4. Discussion

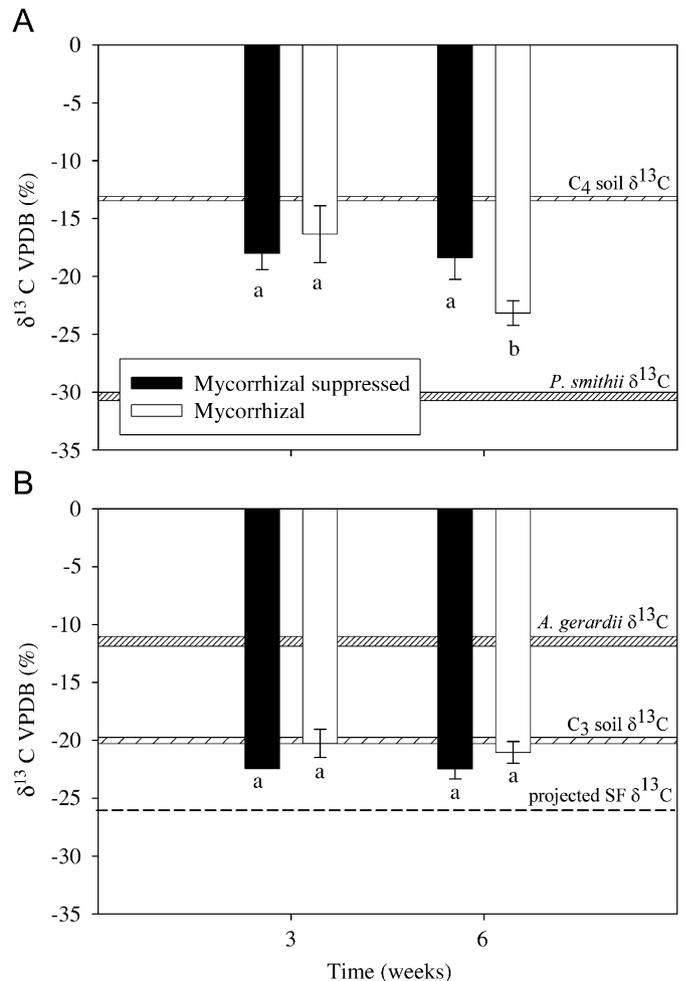Our approach differs from previous Collembola feeding studies which have relied on indirect methods such as fecal

counts (Klironomos and Kendrick, 1996; Klironomos and Ursic, 1998; Klironomos et al., 1999), the location of individuals relative to different food sources (Hedlund and Sjogren 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 Bethlenfalvay, 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 $\delta^{13}C$ 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 C_4 signal in the AMF-suppressed pots may be due to either AMF grazing or root herbivory. The C_4 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 ^{13}C isotope (Fry, 2006). Additionally, the C isotope value of the soil was higher than anticipated; it was −20‰, whereas a value more similar to C_3 vegetation (−27‰ to −30‰) was expected. The field from which the C_3 soil was collected had been planted with C_3 vegetation for only 12 of the last 15 years. Portions of older C_4-C in the stable C pool mixed with more recent C_3-C could have resulted in the higher than expected soil $\delta^{13}C$ signature. Additionally, carbonates (~ +8‰ $\delta^{13}C$) are present in KPBS soils and would also lead to a less negative $\delta^{13}C$ signature than expected. Accounting for these issues, the isotope value of SF was likely closer to −25‰ based on the C signature of C_3 vegetation (e.g. *P. smithii* $\delta^{13}C = −30.8‰$) 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 Benomyl 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/m^2/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 enchytraied 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 $2.9–13.1 \times 10^3$ individuals/m^2 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 C_3 and C_4-derived C compounds. For instance, Chamberlain et al. (2004) found that when fed diets of the same yeast species grown on different C sources (C_3 vs. C_4), 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* ($38 \pm 3.1\%$) and *P. smithii* ($15 \pm 1.1\%$) 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 (Tiunov and Scheu, 2005) and plant–fungal (Larsen and Jakobsen, 1996) interactions with consequent implications for plant performance and nutrient cycling (Harris and Boerner, 1990).

References

Bakonyi, G., Posta, K., Kiss, I., Fabian, M., Nagy, P., Nosek, J.N., 2002. Density-dependent regulation of arbuscular mycorrhiza by collembola. Soil Biology & Biochemistry 34, 661–664.

Bardgett, R.D., Frankland, J.C., Whittaker, J.B., 1993. The effects of agricultural management on the soil biota of some upland grasslands. Agriculture, Ecosystems and Environment 45, 25–45.

Bardgett, R.D., Bowman, W.D., Kaufmann, R., Schmidt, S.K., 2005. A temporal approach to linking aboveground and belowground ecology. Trends in Ecology and Evolution 20, 634–641.

Briones, M.J.I., Ineson, P., Sleep, D., 1999. Use of $\delta^{13}C$ to determine food selection in collembolan species. Soil Biology & Biochemistry 31, 937–940.

Chamberlain, P.M., Bull, I.D., Black, H.I.J., Ineson, P., Evershed, R.P., 2004. Lipid content and carbon assimilation in Collembola: implications for the use of compound-specific carbon isotope analysis in animal dietary studies. Oecologia 139, 325–335.

Coleman, D.C., Reid, C.P.P., Cole, C.V., 1983. Biological strategies of nutrient cycling in soil systems. Advances in Ecological Research 13, 1–55.

Finlay, R.D., 1985. Interactions between soil micro-arthropods and endomycorrhizal associations of higher plants. In: Fitter, A.H. (Ed.), Ecological Interactions in Soil. Blackwell Scientific, Oxford, pp. 319–331.

Fry, B., 2006. Stable Isotope Ecology. Springer, New York.

Gange, A.C., 2000. Arbuscular mycorrhizal fungi, Collembola and plant growth. Trends in Ecology and Evolution 15, 369–372.

Gange, A.C., Bower, E., 1997. Interactions between insects and mycorrhizal fungi. In: Gange, A.C., Brown, V.K. (Eds.), Multitrophic Interactions in Terrestrial Systems. Blackwell Science, Oxford.

Harris, K.K., Boerner, R.E.J., 1990. Effects of belowground grazing by collembola on growth, mycorrhizal infection, and P uptake of *Geranium robertianum*. Plant and Soil 129, 203–210.

Hart, M., Reader, R.J., 2002. Does percent root length colonization and soil hyphal length reflect the extent of colonization for all AMF? Mycorrhiza 12, 297–301.

Hedlund, K., Sjogren Ohrn, M., 2000. Tritrophic interactions in a soil community enhance decomposition rates. Oikos 88, 585–591.

Hopkin, S.P., 1997. Biology of the Springtails. Oxford University Press, Oxford.

Jakobsen, I., Rosedahl, L., 1990. Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. New Phytologist 115, 77–83.

Johnson, D., Krsek, M., Wellington, E.M.H., Sctott, A.W., Cole, L., Bardgett, R.D., Read, D.J., Leake, J.R., 2005. Soil invertebrates disrupt carbon flow through fungal networks. Science 309, 1047.

Jonsson, L.M., Nilsson, M.-C., Wardle, D.A., Zackrisson, O., 2001. Context dependent effects of ectomycorrhizal species richness on tree seedling productivity. Oikos 93, 353–364.

Jorgensen, H.B., Elmholt, S., Petersen, H., 2003. Collembolan dietary specialisation on soil grown fungi. Biology and Fertility of Soils 39, 9–15.

Kaiser, P.A., Lussenhop, J., 1991. Collembolan effects on establishment of vesicular–arbuscular mycorrhizae in soybean (*Glycine max*). Soil Biology and Biochemistry 23, 307–308.

Klironomos, J.N., Kendrick, B., 1996. Palatability of microfungi to soil arthropods in relation to the functioning of arbuscular mycorrhizae. Biology and Fertility of Soils 21, 43–52.

Klironomos, J.N., Moutoglis, P., 1999. Colonization of non-mycorrhizal plants by mycorrhizal neighbours as influenced by the collembolan, *Folsomia candida*. Biology and Fertility of Soils 29, 277–281.

Klironomos, J.N., Ursic, M., 1998. Density-dependent grazing on the extraradical hyphal network of the arbuscular mycorrhizal fungus, *Glomus intraradices*, by the collembolan, *Folsomia candida*. Biology and Fertility of Soils 26, 250–253.

Klironomos, J.N., Bednarczuk, E.M., Neville, J., 1999. Reproductive significance of feeding on saprobic and arbuscular mycorrhizal fungi by the collembolan, *Folsomia candida*. Functional Ecology 13, 756–761.

Koch, A.M., Croll, D., Sanders, I.R., 2006. Genetic variability in a population of arbuscular fungi causes variation in plant growth. Ecology Letters 9, 103–110.

Kohzu, A., Yoshioka, T., Ando, T., Takahashi, M., Koba, K., Wada, E., 1999. Natural ^{13}C and ^{15}N abundance of field-collected fungi and their ecological impact. New Phytologist 144, 323–330.

Koske, R.E., Gemma, J.N., 1989. A modified procedure for staining roots to detect VA-mycorrhizas. Mycological Research 92, 486–505.

Larsen, J., Jakobsen, I., 1996. Effects of a mycophagous Collembola on the symbioses between *Trifolium aubterraneum* and three arbuscular mycorrhizal fungi. New Phytologist 133, 295–302.

Larsen, T., Schjonning, P., Axelsen, J., 2004. The impact of soil compaction on euedaphic Collembola. Applied Soil Ecology 26, 273–281.

Lussenhop, J., 1996. Collembola as mediators of microbial symbiont effects upon soybean. Soil Biology & Biochemistry 28, 363–369.

Lussenhop, J., BassiriRad, H., 2005. Collembola effects on plant mass and nitrogen acquisition by ash seedlings (*Fraxinus pennsylvanica*). Soil Biology & Biochemistry 37, 645–650.

Martikainen, E., Haimi, J., Ahtianinen, J., 1998. Effects of dimethoate and Benomyl on soil organisms and soil processes—a microcosm study. Applied Soil Ecology 9, 381–387.

McGonigle, T.P., 1994. The significance of grazing on fungi in nutrient cycling. Canadian Journal of Botany 73, S1370–S1376.

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., Swan, J.A., 1990. A new method which gives an objective measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. New Phytologist 115, 495–501.

Milcu, A., Partsch, S., Langel, R., Scheu, S., 2006. The response of decomposers (earthworms, springtails and microorganisms) to variation in species and functional group diversity of plants. Oikos 112, 513–524.

Miller, R.M., Reinhardt, D.R., Jastrow, J.D., 1995. External hyphal production of vesicular–arbuscular mycorrhizal fungi in pasture and tallgrass prairie communities. Oecologia 103, 17–23.

Mitschunas, N., Eagner, M., Filser, J., 2006. Evidence for a positive influence of fungivorous soil invertebrates on the seed bank persistence of grassland species. Journal of Ecology 94, 791–800.

Moore, J.C., St. John, T.V., Coleman, D.C., 1985. Ingestion of vesicular–arbuscular mycorrhizal hyphae and spores by soil microarthropods. Ecology 66, 1979–1981.

Olsson, P.A., Johnson, N.C., 2005. Tracking carbon from the atmosphere to the rhizosphere. Ecology Letters 8, 1264–1270.

Partsch, S., Milcu, A., Scheu, S., 2006. Decomposers (Lumbricidae, Collembola) affect plant performance in model grasslands of different diversity. Ecology 87, 2548–2558.

Petersen, H., Luxton, M., 1982. A comparative analysis of soil fauna populations and their role in decomposition processes. Oikos 39, 287–388.

Rillig, M.C., 2004. Arbuscular mycorrhizae and terrestrial ecosystem processes. Ecology Letters 7, 740–754.

Rooney, N., McCann, K., Gellner, G., Moore, J.C., 2006. Structural asymmetry and the stability of diverse food webs. Nature 442, 265–269.

Rusek, J., 1998. Biodiversity of Collembola and their functional role in the ecosystem. Biodiversity and Conservation 7, 1207–1219.

Sabatini, M.A., Innocenti, G., 2000. Soil-borne plant pathogenic fungi in realtion to some collembolan species under laboratory conditions. Mycological Research 104, 1197–1201.

Salamon, J., Schaefer, M., Alphei, J., Schmid, B., Scheu, S., 2004. Effects of plant diversity on Collebola in an experimental grassland ecosystem. Oikos 106, 51–60.

Scheu, S., Folger, M., 2004. Single and mixed diets in Collembola: effects on reproduction and stable isotope fractionation. Functional Ecology 18, 94–102.

Scheu, S., Setala, H., 2002. Multitrophic interactions in decomposer food-webs. In: Tscharntke, T., Hawkins, B.A. (Eds.), Multitrophic Level Interactions. Cambridge University Press, Cambridge, pp. 223–264.

Scheu, S., Theenhaus, A., Hefin Jones, T., 1999. Links between the detritivore and the herbivore system: effects of earthworms and Collembola on plant growth and aphid development. Oecologia 119, 541–551.

Schrader, S., Lingnau, M., 1997. Influence of soil tillage and soil compaction on microarthropods in agricultural land. Pedobiologia 41, 202–209.

Schreiner, R.P., Bethlenfalvay, G.J., 2003. Crop residue and Collembola interact to determine the growth of mycorrhizal pea plants. Biology and Fertility of Soils 39, 1–8.

Seastedt, T.R., 1984. Belowground macroarthropods of annually burned and unburned tallgrass prairie. American Midland Naturalist 111, 405–408.

Smith, S.E., Read, D.J., 2002. Mycorrhizal Symbiosis, second ed. Academic Press, New York.

Thimm, T., Larink, O., 1995. Grazing preferences of some collembola for endomycorrhizal fungi. Biology and Fertility of Soils 19, 266–268.

Tiunov, A.V., Scheu, S., 2005. Arbuscular mycorrhiza and Collembola interact in affecting community composition of saprotrophic microfungi. Oecologia 142, 636–642.

Tomlin, A.D., 1981. Effects on soil fauna of the fungicide, Benomyl, used to control earthworm populations around an airport. Protection Ecology 2, 325–330.

Trudell, S.A., Rygiewicz, P.T., Edmonds, R.L., 2004. Patterns of nitrogen and carbon stable isotope ratios in macrofungi, plants, and soils in two old-growth conifer forests. New Phytologist 164, 317–335.

van der Heijden, M.G.A., 2002. Arbuscular mycorrhizal fungi as determinant of plant species diversity: in search of underlying mechanisms and general principles. In: van der Heijden, M.G.A., Sanders, I. (Eds.), Ecological Studies. Vol. 157: Mycorrhizal Ecology. Springer, Berlin, Germany, pp. 243–265.

van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Weimken, A., Sanders, I.R., 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396, 69–72.

Verhoef, H.A., Prast, J.E., Verweij, R.A., 1988. Relative importance of fungi and algae in the diet and nitrogen nutrition of *Orchesella cincta*

(L.) and *Tomocerus minor* (Lubbock) (Collembola). Functional Ecology 2, 195–201.

Wall, D.H., Moore, J.C., 1999. Interactions underground. BioScience 49, 109–117.

Watkins, N.K., Fitter, A.H., Graves, J.D., Robinson, D., 1996. Quantification using stable carbon isotopes of carbon transfer between C-3 and C-4 plants linked by a common mycorrhizal network. Soil Biology & Biochemistry 28, 471–477.

Wilson, G.W.T., Hartnett, D.C., 1997. Effects of mycorrhizae on plant growth and dynamics in experimental tallgrass prairie microcosms. American Journal of Botany 84, 478–482.

Wilson, G.W.T., Hartnett, D.C., 1998. Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. American Journal of Botany 85, 1732–1738.

Zhu, Y.-G., Miller, R.M., 2003. Carbon cycling by arbuscular mycorrhizal fungi in soil–plant systems. Trends in Plant Science 8, 407–409.