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Crop residue and Collembola interact to determine the growth of mycorrhizal pea plants

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Abstract The effects of collembolan grazing on arbuscular mycorrhizal (AM) fungi and plant growth were studied in a controlled experiment utilizing a mix of AM fungi and the dominant collembolan species (*Isotoma* sp.) indigenous to the experimental soil. Collembolan (+/– Col) effects were examined in the presence and absence of crop residue (+/– Litter) incorporated into the experimental soil. Significant interactions between collembolans and crop residue occurred for mycorrhizal colonization of roots and plant growth. In the absence of crop residue, collembolans reduced root length colonized by AM fungi, total plant dry mass and seed pod yield. However, in the presence of crop residue, collembolans had no effect on root colonization by AM fungi, and increased total plant mass and pod yield. Crop residue increased root colonization by AM fungi, numbers of bacteria and saprophytic fungi (colony forming units), small- (<5 μm) and large- (>5 μm) diameter hyphal lengths in soil, and the final population of collembolans in soil. Collembolans reduced both small- and large-diameter hyphae in soil and the number of saprophytic fungi (colony forming units, p =0.052). Feeding preference experiments conducted in vitro showed that *Isotoma* sp. preferred to graze on mycorrhizal roots over nonmycorrhizal roots when given no other food choice. However, when crop residue was added as a food choice, *Isotoma* sp. showed a clear feeding preference for crop residue. We conclude that collembolan grazing on mycorrhizae can be detrimental to plant growth when other fungal food sources are limited, but grazing on mycorrhizal fungi does not occur when ample organic matter and associated saprophytic fungi are present in soils.

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Introduction

Interactions between plant roots and soil are complex and involve many organisms comprising the soil food web. Arbuscular mycorrhizal (AM) fungi, which function both within roots and in the soil matrix, are a vital component of the soil biota since they act as a link between plant and soil (Bethlenfalvay 1992; Smith and Read 1997). Plant responses to AM fungi vary greatly in the field as they depend on biotic and abiotic characteristics of the soil and environmental factors. This often, unpredictable variation may be limiting the utilization of these fungi as a biological resource in agriculture. A lack of plant growth enhancement by AM fungi, which may be observed in the field, has been attributed to antagonism with other soil microbes (Wilson et al. 1988) or soil fauna (McGonigle 1988). Such organisms are severely depleted in most mycorrhizal experiments conducted in sterilized soils. A definition of management practices that can realize the potential benefits of AM fungi in agriculture depends on our understanding of the interactions between roots, AM fungi and the soil biota. Of particular relevance to mycorrhizal effectiveness are fungivorous soil animals, since they may consume the primary nutrient- and water-uptake organ of the mycorrhiza: the AM soil mycelium.

Springtails (Collembola) and mites are the most numerous arthropods in temperate agricultural soils (Beare et al. 1992; Hendrix et al. 1986). Collembolans comprise the majority of the total fungivorous faunal biomass in field plots established at the Vegetable Research Farm (VRF), Oregon State University (Schreiner and Moldenke 1998, unpublished data). Both field (Finlay 1985; McGonigle and Fitter 1988) and pot (Harris and Boerner 1990; Warnock et al. 1982) studies have shown that collembolans can regulate plant benefit derived from AM symbiosis. Low densities of collembolans may be beneficial to mycorrhiza formation and

nutrient uptake in plants, perhaps due to stimulation of hyphal growth rates (Finlay 1985) or by releasing nutrients from dead mycelia (Harris and Boerner 1990). High collembolan densities can reduce plant response to AM fungi through the disruption of the AM soil mycelium and the loss of nutrient uptake by AM soil hyphae (Finlay 1985; Harris and Boerner 1990; Warnock et al. 1982). It is unclear from most studies, however, if high collembolan densities would also depress AM fungi when other soil fungi are present as an alternative food source.

The manipulation of saprophytic fungi in experimental systems with AM plants has shown that non-AM fungi may indeed be preferred by collembolans and hence reduce grazing pressure on AM fungi. Klironomos and Kendrick (1996) have found that five of six fungivorous arthropod species preferred to graze on conidial fungi rather than on AM fungi in pots. These authors (Klironomos and Kendrick 1995) also found that the addition of forest litter to the soil surface in pots increased AM root colonization and nutrient uptake in maple seedlings when collembolans were present. Therefore, collembolans may benefit the mycorrhiza in forest ecosystems by preferentially grazing on saprophytic fungi associated with the litter layer and releasing nutrients for uptake by AM fungi. It is well-known that soil arthropods accelerate the decomposition and mineralization of plant residues in both forest and agricultural systems (Beare et al. 1992; Ineson et al. 1982; Seastedt 1984). What is not understood is whether collembolans would also benefit plant growth in an agricultural system where plant residues are incorporated into the soil and the processes of decomposition by non-AM fungi and nutrient uptake by AM fungi are not spatially separated. In other words, what are the impacts of collembolan grazing when the detritusphere (Beare et al. 1995) and the mycorrhizosphere (Linderman 1988) overlap?

The purpose of this study was to investigate whether crop residue incorporation in soil would influence the interactions between collembolans, mycorrhizal fungi and plant growth in a similar manner as was found when forest litter was added to the surface of soil. We suspected that collembolan grazing would not be as selective between non-AM and AM fungi whenever crop residues were incorporated into soil because both types of fungal hyphae would proliferate within the same microsites in soil. To avoid potential artifacts that may occur between soil organisms that do not co-exist within a given soil ecosystem, all organisms used in our study were isolated from the experimental soil at the VRF. Food choice trials for the collembolan species used in the pot study were also conducted in vitro to confirm our findings.

Materials and methods

Experimental design and statistics

The experiment was a 2×2 factorial design, with crop residue and collembolans as factors. Ten replications were included in each treatment for a total of 40 experimental units (potted plant-soil systems). Presence or absence of a factor within the treatments will be annotated as: +Litter or –Litter, and +Col or –Col. Response variables were analyzed by multi-factor analysis of variance (ANOVA). Variables that violated the assumption of homogeneity of variance (Bartlett's test at 95% confidence) were log-transformed prior to analysis. Log transformation resulted in homogenous variances for the affected variables. Feeding preference studies conducted in vitro were analyzed by single factor ANOVA. Mean contrasts were made using Tukey's post hoc test at 95% confidence.

Biological materials and soil

The growth medium was a mix of coarse sand:field soil (2:1) with good drainage properties, a condition essential to collembolan survival in pots (Schreiner 1998, unpublished data). The field soil (Chehalis series, mesic Cumulic Ultic Haploxeroll, pH 6.4) was collected from the VRF, Oregon State University, near Corvallis, Oregon. It contained (mg kg^{-1}): NO_3^--N, 10.1; NH_4^--N, 4.2; P ($NaHCO_3$-extractable), 62; P (total), 1,000; K, 340; Ca, 2,700; Mg, 550; SO_4^{2-}-S 2.0; B, 0.4; Cu, 4.0; Fe, 120; Mn, 21; Zn, 8.0 and had 3% organic matter. The soil mixture was autoclaved (121°C) for 1 h at 25% gravimetric moisture content and stored for 4 weeks at 25°C prior to use. The biological components (AM fungi, soil microbes) and crop residue (+/– Litter) were incorporated into the soil in bulk prior to filling pots. Collembolans were added to individual pots (+ Col treatment) after filling them halfway with soil mix, just prior to planting (see below).

The AM-fungal inoculum consisted of a mixture of five fungi isolated from the VRF: *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe INVAM OR211, *G. caledonium* (Nicol. & Gerd.) Trappe & Gerd. INVAM OR211, *G. aggregatum* (Schenck & Smith) Koske INVAM OR212, *G. clariodeum* Schenk & Smith INVAM OR214, and *Acaulospora trappei* Ames & Linderman INVAM OR213. Each of the fungi was isolated by hand-picking spores and culturing with *Sorghum bicolor* L. in a low-P soil described elsewhere (Bethlenfalvay et al. 1994). The AM-fungal inoculum was prepared by mixing whole-soil inoculum (containing spores, hyphae and colonized root fragments) from each of the five isolates in a large volume of tap water. The resulting suspension was decanted 3 times over a 45-μm sieve. The majority of root fragments were then floated off the final suspension after letting it stand for 30 min. Remaining spores and hyphae were mixed into the experimental soil. All pots received the equivalent of 2.5 g whole-soil inoculum from each species of AM fungi.

A microbial extract obtained from unsterilized field soil was also mixed into the experimental soil for all treatments to restore microbes native to the soil. This suspension also provided *Rhizobium* inoculum for nodule formation (Schreiner 1996, unpublished data). The microbial suspension was prepared by wet-sieving 200 g field soil through a 38-μm sieve. Protozoa were removed (confirmed by microscopic examination) from the microbial suspension by filtration (8 μm, Millipore, Bedford, Mass.), and the volume was adjusted to 500 ml. All pots received the equivalent of 10 ml extract.

Partially degraded crop residue (crop residue) was collected from the surface of field plots at the VRF, 2 weeks after mowing a *S. bicolor* cover crop. It was air-dried, shredded, sieved (4 mm), autoclaved (1 h), and mixed into the experimental soil (8 g pot^{-1}) for each + Litter pot. The sterilized crop residue contained 15.6 mg N and 2.28 mg P per gram of dry mass, and had a C/N ratio of 28.8 and C/P ratio of 197 (C content 45%). Residue was sterilized to ensure that fauna and microflora associated with this material were eliminated.

Collembolans were isolated from field plots and the dominant species (*Isotoma* sp.) was reared on baker's yeast in vials containing moist plaster and charcoal (Moldenke 1994). Each pot inoculated with collembolans (+ Col) received 50±5 adult individuals (equivalent to 3.5×10^3 collembolans m^{-2}) collected under a dissecting microscope and transferred to small vials containing tap water. The water in the vials was poured onto the soil surface of half-filled pots, carrying the collembolans with it. In this way the animals could be transferred without any evident effects on their activities. The pots were then gently filled to the top with the appropriate soil (+/− Litter) according to the experimental design. Pea (*Pisum sativum* L., cv. Little Marvel) seeds, previously grown from a single seed to minimize genotypic variation, were surface sterilized (10% bleach, a trace of soap, 10 min), rinsed, allowed to imbibe (8 h), and sown into pots (750 g soil).

Escape and movement of collembolans from pot to pot was minimized by fitting each pot with two layers of window screen (1 mm) on the pot bottoms. Pot tops were covered with Styrofoam circles and overlayed with four layers of cheesecloth, held in place with a rubber band. The shoot of the pea plants grew through a small hole cut in the cheesecloth.

Growth conditions, harvest, and biological assays

Plants were grown in a greenhouse from 23 December 1996 to 14 February 1997, in Corvallis, Oregon. Supplemental lighting was provided by 1,000-W, phosphor-coated metal halide lamps (General Electric) for a 14-h photoperiod providing 500 µmol m^{-2} s^{-1} PAR at soil level in addition to natural sunlight. Soils were moistened slowly (30 ml aliquots) until water appeared in saucers placed below the pots. Pots were checked 2 to 3 times every day and moistened only when the saucers were dry. Soluble plant nutrients that leached through the soil and into the saucers were returned to the soil surface during the next watering cycle. No fertilizers were used.

Pea pods were harvested when fully expanded (53 days), oven dried at 70°C for 3 days, and weighed. The remaining vegetative shoots were cut at soil level, oven dried and weighed. All pots were brought to field capacity after harvest and allowed to drain overnight to equalize soil moisture contents. A soil core (2-cm diameter, ~20 g fresh weight soil) was removed from each pot the following day, and stored at 4°C in plastic bags. Five cores from each treatment (all odd numbered pots) were used to estimate microbial numbers (bacteria and fungi) and soil hyphal lengths. Each soil core was suspended in 200 ml phosphate-buffered saline solution (Zuberer 1994) in sterilized blender cups and blended on high speed for 30 s in a Waring 2-speed blender.

Total bacteria and fungi were estimated by the dilution plate count method (one-tenth strength, tryptic soy agar for bacteria; Zuberer 1994; rose bengal/streptomycin agar for fungi; Martin 1950). Serial dilutions were prepared and microbes were cultured in duplicate plates using the pour-plate method, which minimized interference caused by *Bacillus mucoides* growth on other microbes. Bacteria were counted after 2- and 5-days, and fungi after 3-days incubation at 20°C. Qualitative differences in the saprophytic fungi isolated from different treatments were assessed by comparing morphotypes on dilution plates. The four most abundant types, based on colony size, color, and fruiting structures (if present), were recorded for each treatment. Those morphotypes that could not be identified to genus (lack of fruiting structures) on rose-bengal medium were sub-cultured on half-strength potato-dextrose agar for up to 4 weeks to aid in identification.

Soil hyphal lengths for both small-diameter fungal hyphae (<5 µm, primarily representing saprophytic fungi—see later) and large-diameter hyphae (>5 µm and nonseptate, primarily representing AM fungi) were determined using separate assays. A 10-ml aliquot of the blended soil suspension was diluted with 100 ml tap water and sieved on a 38-µm sieve to remove clay that interferes with microscopic examination. Material remaining on the sieve, including hyphal fragments, was transferred to a large beaker with water and appropriate dilutions were used to estimate either small- or large-diameter hyphae.

Small-diameter hyphal lengths were determined in a Howard mold-counting chamber (Hausser Scientific, Philadelphia, Pa.) at ×150 (phase-contrast microscopy) using an eyepiece micrometer. The total hyphal length within one-fifth of the chamber volume was recorded. Only those hyphae of less than 5 µm diameter were recorded. Hyphae that appeared to be arbuscular mycorrhizal hyphae (nonseptate, random branching pattern, and random wall thickness) were excluded. Hyphal lengths were recorded in duplicate for each individual soil sample.

Large-diameter hyphal lengths were estimated by a modified grid-line filtration method (Sylvia 1992). Dilutions of soil hyphal suspensions were filtered on a GN-6 membrane filter (pre-rinsed with 10% ethanol; Gelman Scientific, Ann Arbor, Mich.), stained with trypan-blue in acidified lacto-glycerol for 10 min, rinsed with tap water, and mounted on slides with a drop of glycerol. Hyphal crosses with grid-lines printed on the filters were determined at ×60 under bright-field microscopy. Only nonseptate hyphae with morphology similar to AM fungi (random branching pattern and random wall thickness) greater than 5 µm were counted.

Roots and collembolans were extracted from the soil remaining in each pot. Each pot was submerged in 10 l tap water in a 20-l container. The root system of each plant was separated from adhering soil under water and rinsed clean over the container. Root systems were spread out on glass plates and blotted dry. Root nodules on each plant were counted. Nodulation was classified on a scale from 0 to 4 (0, no nodules; 1, 1–20 small and/or 1–2 large nodules; 2, 21–40 small and/or 3–4 large nodules; 3, 41–60 small and/or 5–6 large nodules, 4, >60 small and/or 7 large nodules). After examining roots for nodules, the fresh weight was recorded and roots were cut into 2-cm segments and thoroughly mixed. A 1-g sample of roots was removed and stored in formaldehyde:acetic acid:ethanol (1%:5%:50%) for subsequent determination of root length and mycorrhizal colonization. The remaining roots were oven-dried and weighed. Total and AM-colonized root lengths were determined by the grid-line intersect method (Newman 1966) after clearing and staining roots with trypan blue (see Schreiner and Bethlenfalvay 1996).

Collembolans were collected from the soil:water slurry after removing the roots by decanting twice onto a 125-µm sieve. The arthropods were anesthetized on the sieve with 70% ethanol (spray bottle), and transferred to vials containing 70% ethanol. The final number of collembolans in each pot was counted under a dissecting microscope.

In vitro feeding preference trials

Two sets of experiments were conducted in vitro to identify the feeding preferences of *Isotoma* sp. using a food coloring method described by Thimm and Larink (1995). In the first experiment (repeated once), collembolans were given AM- or non-AM-colonized pea roots as food choices to test if they would preferentially be attracted to and consume mycorrhizal roots over uncolonized roots. Fifteen to twenty adult collembolans were transferred to clean jars with moist plaster/charcoal and starved for 2 days prior to introducing food choices. Roots collected from 8-week-old pot cultures of AM or non-AM plants (inoculated or not with the same fungi used in the pot experiment) were gently washed free from most of the adhering soil, cut into 1-cm fragments, and directly stained with different colored food dyes (McCormick, Hunt Valley, Md.). Microscopic examination of roots confirmed abundant AM hyphae and spores were attached to the AM roots while no hyphae were observed on non-AM roots. One-tenth of a gram of both AM and non-AM roots (stained different colors) were placed inside eight replicate jars. Roots were placed near the center of each jar 1 cm apart. Jars were incubated in a dark cabinet at room temperature. The number of collembolans having ingested either food source, distinguished by coloring of their gut contents, was recorded after 72 h (see Thimm and Larink 1995).

In the second experiment, crop residue colonized by saprophytic fungi was added as a third food choice in addition to AM and non-AM roots to test if they would prefer to graze on litter and

associated microflora as opposed to either type of root. Each potential food source was stained with a different colored food dye. Roots used in this experiment were from the same plant species as the litter, *S. bicolor*. Roots were prepared as earlier from 7-week-old pot cultures inoculated or not with AM fungi. Crop residue was prepared by incubating sterilized *S. bicolor* leaf litter in moist, field soil for 2 weeks. Soil used for this purpose was first air-dried to depress resident fauna and allow for extensive fungal colonization of the litter. Crop residue was retrieved from the soil and gently washed prior to staining with food color. Microscopic examination of the crop residue confirmed the presence of copious quantities of hyphae and conidia of saprophytic fungi. In these + Litter experiments, collembolans that had ingested any of the three food choices was determined after 48 h. A separate experiment employing bakers yeast stained with different colors showed no feeding preference of the animals based on food color alone (ANOVA p value =0.865).

Results

Effects of crop residue and collembolans on soil biota

The addition of crop residue to our soils had a profound impact on most of the soil organisms assayed. The addition of crop residue increased the fecundity of collembolans, such that the final populations in + Litter soils was more than 4 times that of the – Litter soils (Table 1). The final population of collembolans in the – Litter treatment corresponded to field densities of roughly 2×10^4 individuals m^{-2}, which is within the range of field estimates for collembolans at the VRF (mean values for ten sampling times over 2 years ranged from 6×10^3–5.1×10^4 individuals m^{-2}). However, the final number of collembolans reached in our + Litter treatment was equivalent to 9×10^4 individuals m^{-2}, a value larger than our highest single sample estimate in the field of 7.6×10^4 collembolans m^{-2}.

Crop residue increased the numbers of saprophytic fungi and bacteria as estimated from plate counts. The increase in fungi on plates was more pronounced than the effect on bacteria. The diversity of fungal morphotypes found on plates was also increased by crop residue. The – Litter soils had 5–8 fungal morphotypes on plates, while the + Litter soils had 10–15 different saprophytic fungi. The – Litter soils were dominated by a fast-growing, yellow *Penicillium* sp., a fast-growing *Trichoderma sp*., a white-sterile fungus, and a slow-growing, green *Penicillium* sp. The latter three fungi and *Gilmaniella humicola* were most commonly found in + Litter soils. The fast-growing, yellow *Penicillium* sp. was rarely found on plates from the + Litter soils.

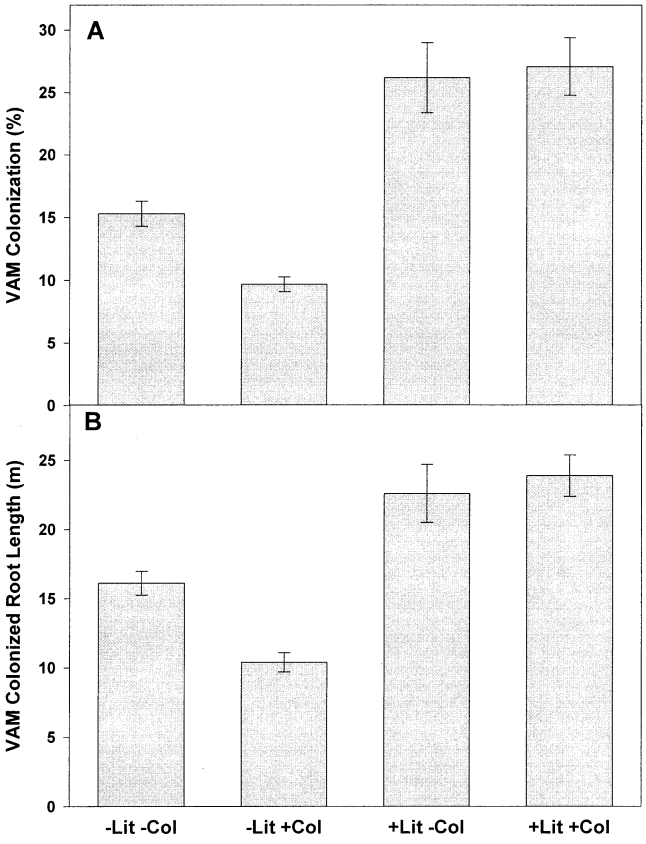

Fig. 1 Effects of crop residue and collembolans on (**A**) the percentage and (**B**) the total length of roots colonized by AM fungi in potted pea plants. Data represent means with standard errors (n =10). ANOVA significance values were: **A** percent colonization – Litter <0.001, Col =0.012, Litter × Col =0.003; **B** root length colonized – Litter <0.001, Col =0.014, Litter × Col =0.001

Crop residue addition significantly increased the colonization of pea roots by AM fungi (Fig. 1). Percentage of roots colonized by AM fungi was twofold greater

Table 1 Effects of crop residue (litter) and collembola on soil biota (means and standard errors) in potted pea plants at harvest (53 DAP—days after planting)

Treatments		Collembola [a]	Fungi g^{-1} soil (cfu $\times10^4$)	Bacteria g^{-1} soil (cfu $\times10^7$)	Nodulation Index [b]
– Litter	– Collembola	0	1.96 (0.8)	3.13 (0.7)	2.9 (0.2)
	+ Collembola	303 (45)	0.84 (0.4)	2.73 (0.6)	3.1 (0.2)
+ Litter	– Collembola	0.4 (0.3)	45.1 (11.2)	6.41 (0.8)	3.1 (0.2)
	+ Collembola	1,304 (167)	20.8 (4.7)	5.04 (0.5)	3.0 (0.2)
ANOVA sig. levels					
Litter		<0.001	<0.001	0.001	0.806
Collembola		<0.001	0.052	0.214	0.816
Litter × Col.		<0.001	0.744	0.485	0.456

[a] Individuals per pot
[b] See Materials and methods for description

in + Litter than – Litter soils. Similarly, the length of roots colonized by AM fungi was significantly greater in + Litter soils. The main effect of litter on AM colonization of roots is noteworthy, despite the fact that there was a significant interaction between litter and collembolans, because the interaction was due to a collembolan response in – Litter soils and no response in + Litter soils. Crop residue had a similar effect on the external mycelium of the mycorrhiza, assessed by the length of large diameter hyphae in soil (Fig. 2). Crop residue addition resulted in roughly a threefold increase of large-diameter hyphae in the soil. Small-diameter hyphal lengths in soil that predominantly represented saprophytic fungi were also increased by crop residue (Fig. 2). Interactions between litter and collembolans were not significant for either measure of soil hyphae.

The main effects of collembolans in our experiment were not as broad as the effects of crop residue and were restricted to measures of fungi. Collembolans reduced small-diameter and large-diameter hyphal lengths in soil (Fig. 2), although the reduction in small-diameter hyphae was more pronounced. Collembolans also reduced the number of saprophytic fungi detected via plate counts (p =0.052). We consider this effect to be significant as fungal cfus were reduced by more than twofold in each of the respective crop residue treatments.

Significant interactions between crop residue and collembolan treatments on soil biota occurred for the final collembolan populations (Table 1) and for root colonization by AM fungi (Fig. 1). The interaction between crop residue and collembolan treatments on the final collembolan population was expected since half of the treatments had no collembolans. The interaction between crop residue and collembolans on AM colonization of roots was a result of collembolans having reduced mycorrhiza in the absence of crop residue while having no effect on mycorrhiza in the presence of crop residue (Fig. 1). This apparent reduction in grazing of mycorrhizal fungi by collembolans whenever crop residue was added to our soils is supported by linear regressions between the final number of collembolans per pot and root colonization by AM fungi. In the absence of crop residue, collembolan populations were negatively correlated to percent root colonization (r =–0.659, p =0.002), while in the presence of crop residue these data were not correlated (r =0.149, p =0.542).

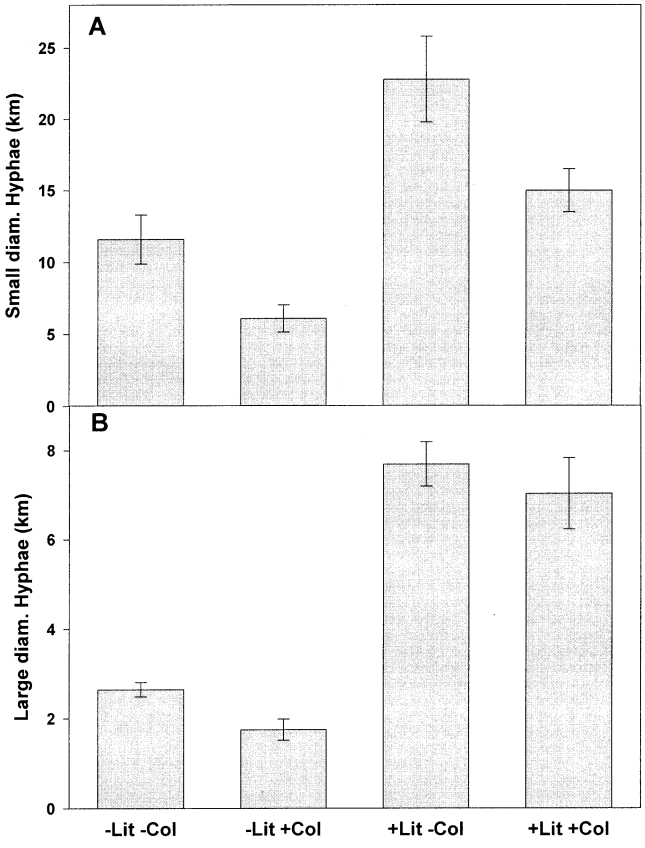

Fig. 2 Effects of crop residue and collembolans on (**A**) small-diameter (<5 μm) and (**B**) large-diameter (>5 μm) hyphal lengths in soil of potted pea plants. Data represent means with standard errors (n =10). ANOVA significance values were: (**A**) small-diameter hyphae – Litter <0.001, Col =0.006, Litter × Col =0.584; (**B**) large-diameter hyphae – Litter <0.001, Col =0.007, Litter × Col =0.181

Effects of crop residue and collembolans on pea plant growth

The main effects of crop residue and collembolan treatments on plant growth were generally not significant. Crop residue addition reduced stem and leaf dry mass of peas, but did not significantly affect other plant parameters (Table 2). While it was not possible to determine the cause for the depression of vegetative plant growth, we suspect that fungi or bacteria that proliferated in response

Table 2 Effects of crop residue and collembola on pea plant growth (means and standard errors) at harvest (53 DAP)

Treatments		Stem & leaf dry mass (g)	Pod dry mass (g)	Root dry mass (g)	Whole plant dry mass (g)	Root length (m)
– Litter	– Collembola	2.35 (0.12)	3.02 (0.09)	0.82 (0.05)	6.19 (0.21)	108 (7)
	+ Collembola	2.16 (0.12)	2.70 (0.16)	0.74 (0.04)	5.60 (0.24)	106 (5)
+ Litter	– Collembola	1.84 (0.11)	2.52 (0.14)	0.74 (0.08)	5.10 (0.26)	91 (7)
	+ Collembola	2.00 (0.14)	3.09 (0.17)	0.79 (0.07)	5.88 (0.33)	98 (9)
ANOVA sig. levels						
Litter		0.010	0.714	0.879	0.180	0.086
Collembola		0.874	0.412	0.862	0.733	0.632
Litter × Col.		0.174	0.004	0.248	0.014	0.484

Table 3 Feeding preferences of Collembola determined in vitro based on color of gut contents given different colored food sources. *Means followed by different letters* within each experiment are significantly different at 95% confidence (Tukey HSD)

Experiment	Material ingested	% Animals feeding
1. Roots only[a]	Non-AM roots	5.0 (2.6) b
	AM roots	95.0 (2.7) a
ANOVA sig. level		<0.001
2. Roots and litter[b]	Non-AM roots	8.3 (2.7) b
	AM roots	15.5 (5.6) b
	Crop residue	76.3 (7.6) a
ANOVA sig. level		<0.001

[a] Non-AM and AM colonized lettuce roots given as food choices
[b] Non-AM, AM-colonized sorghum roots, and sorghum crop residue given as food choices

to crop residue addition competed with roots for nutrients or resulted in enhanced activity of parasitic organisms. The collembolan treatment had no main effects on plant growth (Table 2).

Highly significant interactions between crop residue and collembolans occurred for both pod mass and total plant mass (Table 2). The impact of collembolans was completely opposite in the respective crop residue treatments. Collembolans reduced plant performance in the absence of litter, while they increased plant growth in the presence of litter.

Collembolan feeding preference trials

Food choice experiments conducted in vitro showed that collembolans preferred to graze on roots colonized by mycorrhizal fungi over non-mycorrhizal roots when no other food source was available (Table 3). *Isotoma* sp. preferred to graze on AM-colonized roots nearly 20 to 1 over non-AM roots. When collembolans were presented with three food choices (non-AM roots, AM roots, or partially degraded residue) in the second experiment they clearly preferred to graze on the crop residue over either type of roots (Table 3).

Discussion

The feeding preferences of fungivorous soil animals are of interest in agricultural systems because fungal grazing may alter the balance between mutualistic, pathogenic, and saprophytic fungi and impact plant growth and nutrient uptake. A proliferation of collembolans in soils may affect plant growth through a variety of mechanisms. Decreases in plant growth have been most often reported in mycorrhizal plants and can result from: (1) grazing on the external mycelium of AM fungi reducing nutrient uptake (Moore et al. 1985; Warnock et al. 1982), (2) grazing on roots directly by collembolans thus reducing nutrient uptake (Harris and Boerner 1990), or (3) grazing on saprophytic-fungal mycelium to the extent that mineralization of organic matter and nutrient release are reduced (Hanlon and Anderson 1979). Increases in plant growth may arise from: (1) grazing on pathogenic/parasitic fungi thus reducing disease incidence in plants (Curl et al. 1988), (2) grazing on saprophytic fungi (living or dead) releasing nutrients held in fungal biomass thus increasing plant nutrient uptake (Ineson et al. 1982; Seastedt 1984), or (3) a compensatory stimulation of fungal growth rates (in AM or saprophytic fungi) as a consequence of grazing leading to greater nutrient uptake by AM fungi or increased mineralization and nutrient release by saprophytic fungi (Finlay 1985; Hedlund et al. 1991). While any one of these possible mechanisms may be important in determining the outcome of grazing by collembolans on plant growth, the availability of different fungal food sources must have a large impact on which mechanism(s) operate in nature. For example, collembolans may eat a particular fungus in laboratory culture, but they may not do so in the field where other food choices are available.

The reversal of collembolan effects on mycorrhizal fungi and pea plant growth as a result of crop residue addition was the salient result of our study. Collembolans reduced plant growth in the absence of crop residue, but enhanced plant growth in the presence of crop residue. Our study supports the work by Klironomos and Kendrick (1995) who found maple seedling growth to be enhanced by fungivorous arthropods in the presence of litter, but inhibited in its absence. They attributed these effects to a shift in grazing from AM fungi to non-AM fungi that were introduced with and sustained by the litter. We have reached the same conclusion in our study and further show that selective collembolan grazing on litter-associated fungi can occur even when crop residue is incorporated into soil. The dominant collembolan species in our soil grazed on mycorrhizal fungi to the extent that plant growth was reduced only in our – Litter treatment presumably because other soil fungi were in limited quantity.

The fact that collembolans preferred to graze on saprophytic fungi over mycorrhizal fungi was clearly evident in our study. The negative impact of collembolans on root colonization by mycorrhizal fungi and the reduction in large diameter hyphae lengths that occurred in our – Litter soils did not occur when crop residue was added. The negative effect of collembolans on saprophytic fungi determined via plate counts and on small-diameter hyphae lengths in soil was the same in either – Litter or + Litter treatments. While we made every effort to distinguish saprophytic fungi and mycorrhizal fungi in our assays of small- and large-diameter hyphae respectively, there is no doubt that some of what we measured as small-diameter hyphae were mycorrhizal and that some large-diameter hyphae may have been saprophytic fungi. We believe that selective grazing on saprophytic fungi had occurred in spite of this problem with hyphal assays, because our root colonization and saprophytic fungal plate count data support our soil hyphae findings. In addition, the fact that collembolan populations were

fourfold higher in + Litter soils as compared to – Litter soils showed that litter and associated fungi supported greater collembolan production. Results from the in vitro food choice experiments support this strong preference for collembolan grazing on saprophytic fungi.

The impact of our treatments on plant growth was not as straightforward as their impact on soil fungi, even though the interaction between collembolans and crop residue on total plant mass and pod yield were consistent with selective grazing on decomposer fungi. Crop residue alone resulted in a significant decrease in stem and leaf mass of peas, but did not reduce other measures of plant growth. We interpreted these divergent effects on plant growth to be due to inhibition of vegetative growth during the early stages of our experiment in response to crop residue. This growth reduction was later overcome during the pod-filling stage. What caused this inhibition of plant growth is unknown. Mycorrhizal fungi may have caused a transient depression in plant growth due to their proliferation in response to crop residue. Root colonization by AM fungi and large-diameter soil hyphae (primarily representing AM hyphae) increased substantially in the presence of crop residue suggesting that the mycorrhiza competed with shoots for reduced carbon. Stimulation of root colonization by organic matter and hyphal proliferation by AM fungi in organic patches in soil are well known (Joner and Jakobsen 1995; St. John et al. 1983; Warner 1984). Reduced plant growth in our + Litter soils could also be explained by the presence of pathogenic or parasitic fungi that were enhanced by crop residue. If this was the case, then plants may have developed resistance as they aged or the causal organism(s) may have been displaced from the microbial community around roots as a consequence of microbial succession during decomposition or even by direct collembolan grazing. Therefore, the increase in pod yield that occurred in + Litter soils when collembolans were added could have been due to a reduction of plant parasitic fungi as collembolan grazing continued. The decrease in pod yield that occurred in – Litter soils, however, was most likely due to the grazing of mycorrhizal fungi by collembolans.

It has been suggested that grazing on mycorrhizal fungi may only occur at artificially high collembolan densities (Larink 1997). Our results show that grazing on mycorrhizal fungi can be significant at typical field densities of collembolans, such as we had in our – Litter soils. Indeed, we saw no evidence of grazing on mycorrhizal fungi by collembolans in our + Litter soils where collembolan populations were much higher than typical field populations. Our study suggests that collembolan population density is less important than is the density of other fungal food sources in determining whether collembolans graze on mycorrhizal fungi. Our results in + Litter soils further suggest that the density dependent effects of collembolans on mycorrhizal fungi that can result from inadvertent clipping of hyphae (as opposed to consumption) may not apply to all Collembola and mycorrhizae interactions (Klironomos and Ursic 1998).

In summary, our study confirmed that collembolan grazing on mycorrhizal fungi can be detrimental to host plant growth whenever other fungal food sources are limited. However, grazing on mycorrhizal fungi does not occur to an appreciable extent and plant growth is enhanced by collembolans when organic matter is added to soil. Collembolans appear to discriminate between mycorrhizal fungi and decomposer fungi, preferring to graze on the latter, even when the hyphae of these different types of fungi are intermingled in soil. Ensuring that plants derive a benefit from mycorrhizal fungi and fungivorous arthropods co-existing in agricultural soils is dependent on the supply of crop residues supporting saprophytic fungal growth.

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