

## ORIGINAL PAPER

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**Palatability of microfungi to soil arthropods in relation to the functioning of arbuscular mycorrhizae**

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**Abstract** We investigated the feeding preferences of six species of mites and collembolans for three fungi commonly associated with roots of *Acer saccharum* (*Glomus macrocarpum*, *Alternaria alternata* and *Trichoderma harzianum*), from a maple-forest soil in southern Ontario, Canada. Experiments were also conducted *in vitro* to determine animal feeding responses to (1) increasing quantities of hyphal biomass, (2) the presence of root vs. litter fungal substrates, and (3) hyphae of different widths of *Glomus macrocarpum*. The results indicate that arthropods prefer to graze in the litter region rather than in the deeper soil layers. Under ideal moisture/temperature conditions, animals are forced to the lower regions by interspecific interactions. They prefer to graze on hyphae of conidial fungi rather than on those of arbuscular mycorrhizal fungi. When arbuscular mycorrhizal fungal hyphae are grazed, there is a clear preference for the narrower hyphae, which are those further away from the root. The thicker hyphal segments, commonly found connecting “absorptive hyphal fans” to roots, were less preferred. These data are not consistent with the hypothesis that microarthropods are detrimental to arbuscular mycorrhizal associations, and suggest that Glomalean fungi may have evolved mechanisms to deter grazing by microarthropods.

**Key words** Acari · *Acer saccharum* · Collembola · Soil fungi · Arbuscular mycorrhizae · Hyphal grazing · *Glomus macrocarpum*

**Introduction**

Soil arthropods are integral components of the complex below-ground food web (Coleman 1985) and have impor-

tant effects on fungal population and community dynamics. Fungi are very abundant in the soil (Kendrick and Burges 1962; Christensen 1969; Morrall 1974; Gochenaur 1978; Bissett and Parkinson 1979; Widden 1979; Domsch et al. 1980), and represent a considerable potential food resource for the soil fauna. The saprobic fungi have been fairly well studied in this respect. However, very little attention has been paid to the arbuscular mycorrhizal fungi (Fitter and Sanders 1992). This is surprising, considering that (1) arbuscular mycorrhizal fungi are associated with the roots of an estimated 300000 plant species (Harley and Smith 1983), (2) these fungi make up a large proportion of the fungal biomass in soils (Read 1992), and (3) plant roots are a major source of energy for below-ground systems, and a substantial proportion of net primary production is diverted to mycorrhizal fungi (Finlay and Söderström 1992).

Animal-arbuscular mycorrhizal fungus interactions can be very important because disturbance of the mycorrhizal hyphal network by soil fauna through grazing can reduce the efficiency of the mutualistic association, mainly by reducing the transport of mineral nutrients to roots (Fitter and Sanders 1992). The temporal and spatial occurrences of arthropods and arbuscular mycorrhizal fungi in soils are similar (McGonigle and Fitter 1988; Klironomos and Kendrick 1995), so interactions between the two are to be expected. Also, analysis of the maple-forest soil on which the present study is based has shown a positive correlation between peak arthropod abundances and peak mycorrhization (Klironomos et al. 1993; Klironomos and Kendrick 1995). Using biotrons, Lussenhop (1993) showed that animals are more abundant in the rhizosphere than in the surrounding soil, and are frequently found travelling along root surfaces.

Gut content analyses on animals from dual pot cultures (in which arbuscular mycorrhizal fungi were the only major mycological component of the soil) have revealed the presence of arbuscular mycorrhizal fungal hyphae (Warnock et al. 1982). Grazing of arbuscular mycorrhizal fungi by collembola was observed by Moore et al. (1985). They showed that although both arbuscular

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mycorrhizal fungal mycelium and spores were used as food sources by the microarthropods, the hyphae were severed rather than entirely ingested.

Preliminary experiments have shown that grazing can eliminate any possible benefits derived from the mycorrhizal symbiosis. In dual pot culture, Warnock et al. (1982) and Finlay (1985) showed that the growth of *Allium porrum* was increased by mycorrhizal colonization and subsequently reduced by the collembola *Folsomia candida* and *Onychiurus ambulans*, respectively. A similar phenomenon was reported by Harris and Boerner (1990), who added *F. candida* to pots containing mycorrhizal *Gernaicum robertianum*. The authors suggested that this was due to grazing of the external hyphae of arbuscular mycorrhizal fungi, which rendered the colonization ineffective. Finlay (1985) and Harris and Boerner (1990) found that the animals had different effects at different population densities. In the field, a reduction in indigenous collembolan densities, produced by application of the insecticide chlorfenvinphos, was associated with an increased shoot mass and shoot P content in *Trifolium pratense* (Finlay 1985). A similar effect was reported by McGonigle and Fitter (1988) with the grass *Holcus lanatus*, again suggesting that collembolans reduce the benefits of mycorrhizal colonization.

Laboratory studies of the interactions between arbuscular mycorrhizal fungi and arthropods have been very simple, consisting usually of the addition of one animal species to a dual pot culture. Plant roots in soils, however, interact with many fungi at any one time, members belonging to mycorrhizal, pathogenic, saprobic, and parasitic groups. Also, the application of biocides to field soils can affect other non-target organisms in the rhizosphere, a phenomenon which is difficult to quantify.

Since soil microarthropods have been previously shown to be selective feeders (Visser and Whittaker 1977; Addison and Parkinson 1978; Booth and Anderson 1979; Parkinson et al. 1979; Takeda and Ichimura 1983; Newell 1984a, b; Moore et al. 1987; Shaw 1988; Klironomos et al. 1992), and since the palatability to soil arthropods of arbuscular mycorrhizal fungi compared to non-mycorrhizal fungi has apparently never been investigated, a different approach to studying this interaction was taken. The present study was designed to answer the following questions: (1) Do soil arthropods respond to increasing hyphal biomass? (2) Do arthropods express any preference for arbuscular mycorrhizal fungal hyphae compared to those of conidial fungi (hyphomycetes)? (3) Are the feeding preferences of arthropods consistent with the hypothesis that these animals are detrimental to the mutualistic association? (4) How important are interactions between the fungus and its substrate in determining feeding preferences? (5) When only arbuscular mycorrhizal fungal hyphae are available, which portion of the extraradical hyphal network is most likely to be grazed, the thick hyphal fragments near the root (Friese and Allen 1991) or the thinner hyphal fragments further away from the root?

## Materials and methods

### Plant

Seedlings of *Acer saccharum* Marsh. used in these experiments were grown from seed under sterile conditions. The seeds were supplied by the Forestry Canada seed bank, Chalk River, Ontario, Canada (seedlot 8830273). Seed coats were removed, the seed was allowed to soak overnight in distilled water, and then sterilized in 35% hydrogen peroxide for 30 min. After being rinsed in sterile distilled water, the seeds were placed individually in 1% agar slants. Test-tubes were placed in the dark at 4 °C, and checked for germination and contamination every 30 days. Most seeds germinated after 3 months. When radicles had extended, the tubes were removed from the cold and dark. As the primary leaves were emerging, and when good lateral roots were present, the seedlings were planted in 100-mm (4-inch) pots filled with Turface (an expanded clay potting medium).

### Fungi

The fungi used in this study were isolated from a maple-forest soil in southern Ontario, Canada (Brundrett and Kendrick 1988; Klironomos and Kendrick 1995). For arbuscular mycorrhizal fungal isolation, whole soil inoculum was used in trap cultures (Morton 1990), and one isolate of *Glomus macrocarpum* Tulasne & Tulasne was successfully maintained in dual pot culture with the host *A. saccharum*. Conidial fungi were isolated by plating root fragments of *A. saccharum* on 2% malt-extract agar (Klironomos and Kendrick 1995). Two fungal isolates, a darkly pigmented *Alternaria alternata* (Fr.: Fr.) Keissl. and a lightly pigmented *Trichoderma harzianum* Rifai were used. Cultures were maintained at 5 °C on 1% malt-extract agar slants and subcultured periodically.

### Animals

The six microarthropods used in this study were also extracted from the maple-forest soil in southern Ontario, Canada, using a canister-type soil arthropod extractor (Lussenhop 1971). Three mite species [*Lasiobelba rigida* (Ewing), *Ceratozetes gracilis* (Michael), and *Nothrus anaunensis* (Can. & Franz.)], and three springtail species [*Folsomia candida* Willem, *Folsomia penicula* Bagnall, and *Tullbergia clavata* Mills] were used in these experiments. These animals (except *F. candida*) could not be cultured successfully and so had to be extracted and sorted for each experiment. Only adult animals were used, since young animals rarely move from their release site, with or without food (Johnson and Wellington 1983).

### Test 1: Preference assay on hyphal biomass

Three sets of experiments, each with a different fungal isolate, were conducted using 85 × 10-mm Petri dishes filled with a 25:1 plaster of paris/charcoal mixture. Each Petri-dish contained a 3 × 3 grid of 0.5-cm root fragments containing one of three levels of fungal biomass. At each food station, three fragments were placed together. Three Latin square designs (Moore et al. 1987) were used for each experiment, each replicated four times, resulting in 12 squares/experiment and 36 treatment replications.

For the experiment on the mycorrhizal fungus *G. macrocarpum*, individual maple seedlings were removed from a 6-month-old dual pot culture and their roots placed initially in dilute Calgon solution (sodium hexametaphosphate) for 24 h to help disperse the Turface medium away from the mycorrhizae (Moutoglou et al. 1995). The plants were then transferred to distilled water using three different

time treatments (1, 7, and 14 days). After 1 day the roots had almost no external hyphae; after 7 days, a few hyphae were present; after 14 days, hyphae and spores of *G. macrocarpum* were abundant. These roots were thus used in three fungal biomass treatments. Sometimes, hyphae were present which did not belong to arbuscular mycorrhizal fungi, and these root segments were not used.

For the experiments on conidial fungi, 5-ml suspensions containing a total of  $1 \times 10^6$  conidia of either *A. alternata* or *T. harzianum* were added to roots of 6-month old maple seedlings in 100-mm (4-inch) Turface-filled pots, using a syringe. The fungi were incubated for 1, 7, or 21 days before the fragments were added to the dishes. After 1 day, the root fragments were free of external hyphae; after 7 days, a moderate amount of hyphae was present; after 21 days, the roots were completely covered by hyphae. Also, by the 21st day, *T. harzianum* was heavily sporulating whereas *A. alternata* was just beginning to form conidia. The six arthropod species were tested in separate sets of experiments. Ten individuals were added to each dish and 48 h later, a fecal count was performed within a 0.5-cm radius around each root fragment.

#### Test 2: Preference assay on three microfungi

The conidial fungi were prepared as described above, in test 1, and were allowed to incubate for 7 days. Fragments with moderate fungal growth were used. For *G. macrocarpum*, roots were removed from the pot culture and fragments with abundant extraradical hyphae were used. Root fragments from all three fungal treatments were placed in plaster of paris/charcoal-filled Petri dishes using a Latin square design. The overall set-up differed from that of test 1 in that  $4 \times 4$  grids were used, with four Latin square designs, each replicated four times, resulting in 16 squares per experiment and 64 treatment replications. The six arthropod species were tested in separate experiments. Ten individuals were added to each dish and 48 h later, a fecal count was performed within a 0.5-cm radius around each root fragment.

#### Test 3: Microcosm experiment

This experiment was used to study the vertical distribution of soil animals in relation to their feeding behaviour. Maple seedlings were grown in Turface-filled 100-mm (4-inch) pots with cheesecloths added, as shown in Fig. 1, to separate the different layers. Four treatments were applied to the pots: (1) inoculation of arbuscular mycorrhizal fungi to roots, (2) inoculation of arbuscular mycorrhizal and conidial fungi to roots, (3) inoculation of arbuscular mycorrhizal fungi to roots and conidial fungi to litter, and (4) inoculation of arbuscular mycorrhizal and conidial fungi to roots and conidial fungi to litter. Controls were not inoculated with fungi. Each treatment was replicated 70 times. With the first 60 replicates,

10 replicates were used for each of the six animal species with 30 individuals added to each pot (each animal species was added to separate microcosms). For the last 10 replicates, five individuals of each species were added to each pot, again for a total of 30 individuals (six animal species were added to the same microcosm). After 48 h, the cheesecloth layers were separated, and the animals were extracted using the canister-type soil arthropod extractor (Lussenhop 1971). The number of animals found in each layer was recorded.

For the arbuscular mycorrhizal inoculation of roots, 5 g root inoculum of *G. macrocarpum* was placed in root region 1 (Fig. 1) as maple seedlings were planted, with 5 g non-mycorrhizal root being added to the control pots.

For the conidial fungus inoculation of roots, a 5-ml water suspension containing  $1 \times 10^6$  conidia of each of the two conidial fungi was added to root region 1 (Fig. 1) with a syringe. This was done 5 months after the arbuscular mycorrhizal inoculation. A water control was added to other pots. The animals were added 7 days after inoculation of the roots with the two hyphomycetes.

For the conidial fungus inoculation of litter, maple-leaf litter was sorted to separate all unwanted debris and 2-g portions were placed in 125-ml Erlenmeyer flasks along with 25 ml distilled water. The flasks were autoclaved for 2 min, left overnight to cool, and inoculated with pure cultures of the microfungi. Using a 5-mm diameter cork borer, two plugs of each fungus were removed from the edge of an actively growing culture, and placed aseptically into the flasks which were kept at 25 °C for 2 weeks. After adding the animals, 5 g litter was placed on top.

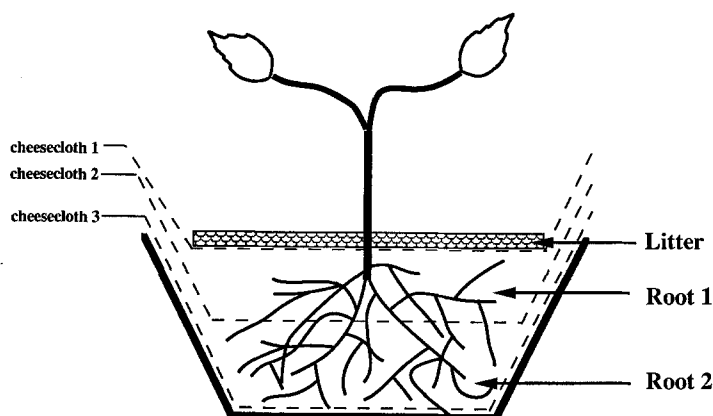
#### Test 4: Preference assay on arbuscular mycorrhizal hyphae of different widths

Hyphal fragments were extracted from *G. macrocarpum/A. saccharum* dual pot culture using the Bardgett (1991) method. The fragments were separated into two width categories,  $>10 \mu\text{m}$  and  $<5 \mu\text{m}$ . Two sets of 30 fragments from each width category were placed equidistantly around the perimeter of the plaster of paris/charcoal-filled dishes, alternating each food type. Five replicates were performed. Ten animals were added to the centre of each dish. Forty-eight hours later, a fecal count was performed at a 0.5-cm radius around the hyphal fragments. The six arthropod species were tested in separate experiments. In all four experiments, the animals were starved for 1 week before testing. The dishes were kept in the dark at room temperature.

#### Statistical analyses

Designs for the Petri-dish tests were modifications of the replicated Latin square described by Moore et al. (1987) Each design consisted different rows and similar columns. Designs were considered fixed effects.

**Fig. 1** Set-up for test 3 (microcosm experiment). The cheesecloths were used to separate the potting medium into three vertical layers (litter, root 1, and root 2)



The statistical model used was:

$$y = \mu + d + t + (td) + e$$

where  $y$  is the fecal count,  $\mu$  is a constant,  $d$  is the design effect,  $t$  is the treatment effect,  $td$  is the treatment  $\times$  design interaction, and  $e$  is the residual error.

Response variables that departed from normality or with large variances were transformed with  $\ln(x+1)$ . The Tukey post-hoc test was used on significant treatment  $F$  ratios following analysis of variance. For the microcosm experiments, Pearson  $\chi^2$  goodness-of-fit tests to a 33:33:33 ratio were used to detect differences among the soil layers. The recorded data were pooled for all replicates. Cochran  $Q$ -tests were used on significant  $\chi^2$ . All statistics were performed using the SYSTAT software package (Wilkinson 1990).

**Table 1** Feeding response [mean  $\ln(\text{fecal count} + 1)$  10 animals $^{-1}$ ] by soil fauna to hyphal biomass of *Glomus macrocarpum*. Values followed by the same alphabetical letter are not significantly different at  $P < 0.05$  (Turkey mean separation test)

	<i>Lasiobelba rigida</i>	<i>Ceratozetes gracilis</i>	<i>Nothrus anaunensis</i>	<i>Folsomia candida</i>	<i>Folsomia penicula</i>	<i>Tullbergia clavata</i>
<b>Biomass</b>						
High	2.26a	1.89a	1.51a	4.17a	2.70a	2.39a
Low	2.05a	1.77a	1.46a	4.15a	2.34a	2.37a
Control	0.43b	0.46b	0.30b	0.17b	0.54b	0.12b
<b>Factors</b>						
$F_{\text{design}}$	1.16	0.27	0.32	1.72	0.30	1.25
$F_{\text{treatment}}$	16.03	9.25	7.09	434.98	23.65	59.39
$F_{\text{design} \times \text{treatment}}$	0.44	0.41	1.42	2.36	0.36	1.37
$P_{\text{design}}$	0.328	0.766	0.728	0.198	0.747	0.302
$P_{\text{treatment}}$	0.000	0.001	0.003	0.000	0.000	0.000
$P_{\text{design} \times \text{treatment}}$	0.779	0.801	0.255	0.078	0.836	0.272

**Table 2** Feeding response by soil fauna to hyphal biomass of *Alternaria alternata*. For further explanations see Table 1

	<i>Lasiobelba rigida</i>	<i>Ceratozetes gracilis</i>	<i>Nothrus anaunensis</i>	<i>Folsomia candida</i>	<i>Folsomia penicula</i>	<i>Tullbergia clavata</i>
<b>Biomass</b>						
High	3.23a	2.92a	1.60a	4.48a	3.01a	2.73a
Low	3.02a	2.73a	0.93ab	4.71a	3.02a	2.42a
Control	1.18b	1.17b	0.17b	0.86b	1.17b	0.81b
<b>Factors</b>						
$F_{\text{design}}$	4.20	2.59	0.40	0.92	0.39	0.42
$F_{\text{treatment}}$	39.51	28.41	8.64	156.90	16.98	21.46
$F_{\text{design} \times \text{treatment}}$	1.71	6.30	0.21	1.09	0.44	1.90
$P_{\text{design}}$	0.026	0.093	0.677	0.409	0.681	0.661
$P_{\text{treatment}}$	0.000	0.000	0.001	0.000	0.000	0.000
$P_{\text{design} \times \text{treatment}}$	0.177	0.001	0.929	0.383	0.778	0.140

**Table 3** Feeding response by soil fauna to hyphal biomass of *Trichoderma harzianum*. For explanations see Table 1

	<i>Lasiobelba rigida</i>	<i>Ceratozetes gracilis</i>	<i>Nothrus anaunensis</i>	<i>Folsomia candida</i>	<i>Folsomia penicula</i>	<i>Tullbergia clavata</i>
<b>Biomass</b>						
High	3.11a	2.79a	2.69a	4.20a	2.34a	2.72a
Low	2.75a	2.98a	1.19b	3.97a	0.51b	2.96a
Control	0.80b	0.49b	0.46c	1.21b	0.38b	0.21b
<b>Factors</b>						
$F_{\text{design}}$	0.56	0.90	2.83	1.87	2.86	0.80
$F_{\text{treatment}}$	48.70	52.47	34.95	49.76	35.58	83.32
$F_{\text{design} \times \text{treatment}}$	1.34	0.72	0.54	0.69	0.88	2.57
$P_{\text{design}}$	0.580	0.418	0.077	0.173	0.074	0.461
$P_{\text{treatment}}$	0.000	0.000	0.000	0.000	0.000	0.000
$P_{\text{design} \times \text{treatment}}$	0.282	0.585	0.707	0.607	0.489	0.061

## Results

In experiment 1, the microarthropods did not show a preference for high or low concentrations of fungal biomass (Tables 1–3). In the majority of tests, fecal counts around high and low fungal biomass concentrations did not differ significantly. The exceptions were *F. penicula* and *N. anaunensis* feeding on *T. harzianum*, where the numbers of fecal pellets were significantly higher near root fragments containing a high fungal biomass. Treatment effects were significant, but design effects and treatment  $\times$  design effect were not, the only exception being *L. rigida* feeding on *A. alternata*.

**Table 4** Feeding response by soil fauna to hyphae of three fungi. For further explanations see Table 1

	<i>Lasiobelba rigida</i>	<i>Ceratozetes gracilis</i>	<i>Nothrus anaunensis</i>	<i>Folsomia candida</i>	<i>Folsomia penicula</i>	<i>Tullbergia clavata</i>
<b>Fungus</b>						
<i>Glomus macrocarpum</i>	0.47b	0.46b	0.00b	1.47c	0.71b	0.65c
<i>Alternaria alternata</i>	1.90a	1.17a	1.50a	4.38a	3.01a	3.20a
<i>Trichoderma harzianum</i>	1.84a	1.47a	1.60a	2.97b	0.67b	1.84b
Sterile root	0.38b	0.30b	0.00b	0.57d	0.64b	1.68b
<b>Factors</b>						
$F_{\text{design}}$	0.655	1.466	2.451	0.707	0.848	0.967
$F_{\text{food}}$	27.570	11.438	70.826	71.661	37.721	38.515
$F_{\text{design} \times \text{food}}$	0.639	0.763	0.892	1.832	0.547	0.460
$P_{\text{design}}$	0.584	0.236	0.075	0.553	0.475	0.416
$P_{\text{food}}$	0.000	0.000	0.000	0.000	0.000	0.000
$P_{\text{design} \times \text{food}}$	0.758	0.651	0.540	0.087	0.833	0.894

All the microarthropods exhibited food preferences when the three microfungi were made available in the same dish (Table 4). The three mite species all preferred the conidial fungi (*A. alternata* and *T. harzianum*) equally over the arbuscular mycorrhizal fungus (*G. macrocarpum*). In fact, *G. macrocarpum* was not preferred over the sterile root control. The three collembolan species showed a more variable preference. *A. alternata* was always the preferred choice, with *T. harzianum* usually the second choice. *F. candida* preferred *G. macrocarpum* over the sterile root control. *F. candida* showed no significant preference among *T. harzianum*, *G. macrocarpum*, and the sterile root control. *T. clavata* preferred the sterile root control over *G. macrocarpum*.

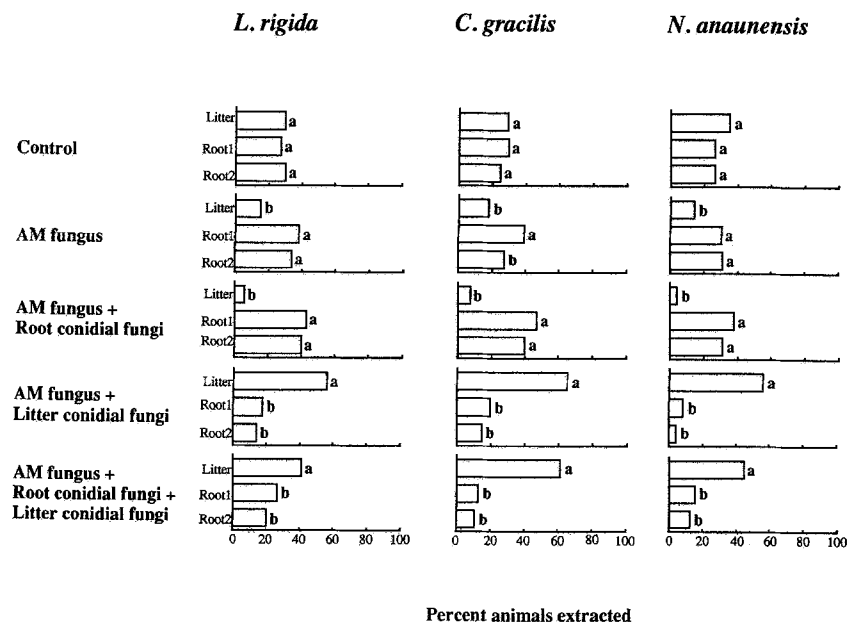
These preferences, however, were also substrate-dependent. The microcosm study showed that all the animals preferred to graze in the litter region rather than the rhizosphere (Figs. 2, 3), and that the animals will travel between soil layers to feed. When fungi were found only

in the root region, all the animals were also found in that region. When fungi were allowed to colonize both regions, then the animals were found in the root region at significantly lower frequencies.

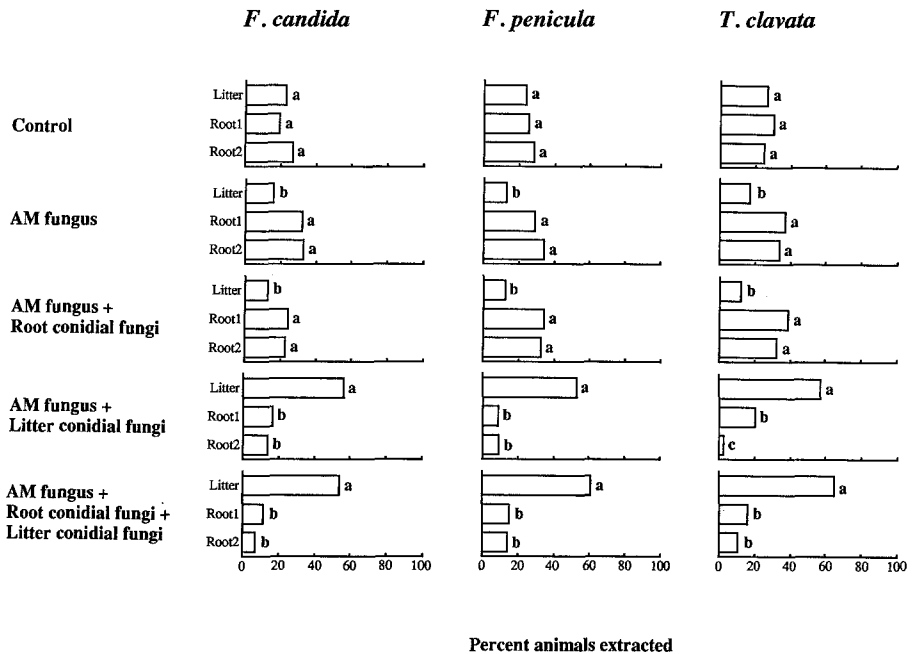
When all six animal species were placed in the same pot, however, their distributions differed (Figs. 4, 5). Although we have shown that all six animal species prefer to feed in the litter zone, in this experiment four animal species (*L. rigida*, *N. anaunensis*, *F. penicula*, and *T. clavata*) were apparently forced down to the lower layers. This is evidence that some form of interspecific interaction among the animals superseded their normal choice.

Four of the six animal species showed a preference for thin hyphae of *G. macrocarpum* (those narrower than 5  $\mu\text{m}$ ) over those that were thicker (Table 5). The other two animals (*C. gracilis* and *F. candida*) showed no preference.

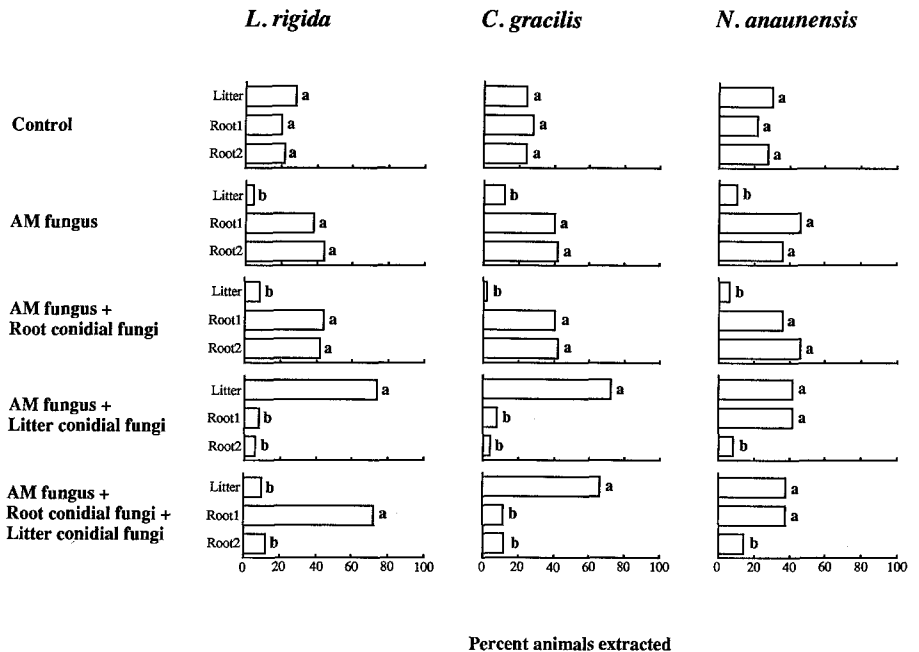
**Fig. 2** Vertical distribution of mites with the six arthropod species added to separate microcosms (test 3). The three vertical layers are described in Fig. 1. AM arbuscular mycorrhizal, *L. Lasiobelba*, *C. Ceratozetes*, *N. Nothrus*; bars followed by the same letter are not significantly different at  $P < 0.05$



**Fig. 3** Vertical distribution of collembolans with the six arthropod species added to separate microcosms (test 3). *F. Folsomia*, *T. Tullbergia*; for further explanations see Fig. 2



**Fig. 4** Vertical distribution of mites with all six arthropod species added to a single microcosm (test 3). For further explanations see Fig. 2

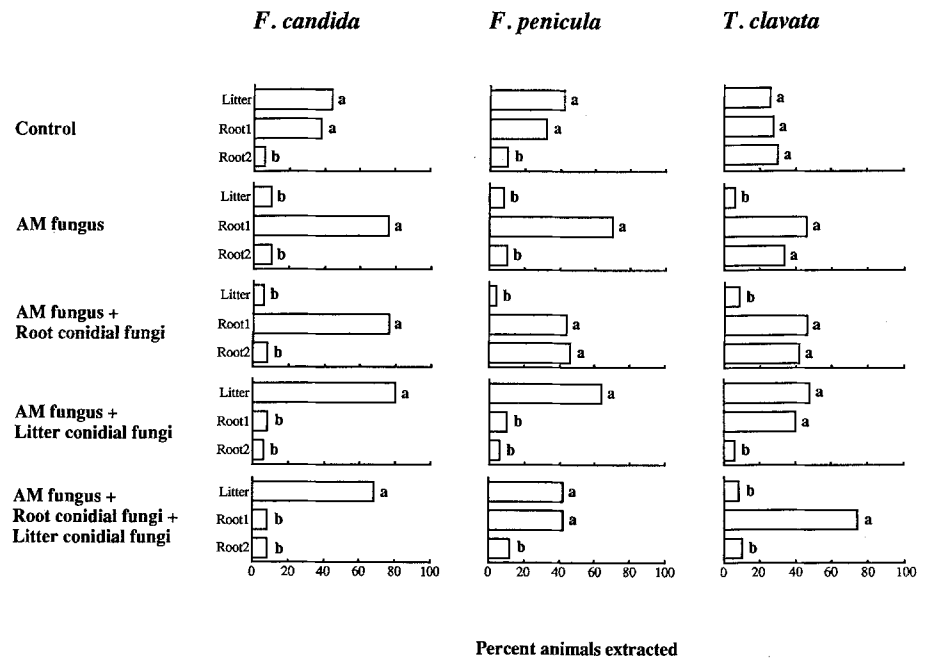


## Discussion

Our data clearly show that soil microarthropods are selective feeders. Conidial fungi, particularly *A. alternata*, were the preferred diet in these experiments. Although it is dangerous to generalize from simple feeding experiments in the laboratory to mycorrhizal functioning in the field, a survey of the literature suggests that these results form part of a general pattern.

A number of laboratory experiments have shown that soil arthropods can feed on arbuscular mycorrhizal fungi (Koske 1981; Warnock et al. 1982; Finlay 1985; Moore et al. 1985; Harris and Boerner 1990; Kaiser und Lussenhop 1991), and cause a decrease in nutrient uptake by grazing on the extraradical hyphal network. However, these studies failed to provide the animals with a choice of diet, since no common non-mycorrhizal root-associated fungi were included. We know of no food preference study in which arbuscular mycorrhizal fungi and non-mycorrhizal

**Fig. 5** Vertical distribution of collembolans with all six arthropod species added to a single microcosm (test 3). *F. Folsomia*, *T. Tullbergia*; for further explanations see Fig. 2



fungi were compared. All previous food preference assays involving soil arthropods have focused on decaying leaf-litter fungi in forest ecosystems, and no arbuscular mycorrhizal fungi were included (Dash and Cragg 1972; Addison 1977; Parkinson et al. 1979; Aitchison 1983; Klironomos et al. 1992). Like the present study, these experiments demonstrated that soil arthropods show a preference for dermatiaceous microfungi. When arbuscular mycorrhizal fungus hyphae were grazed, the thinner segments were selected. Fine, exploratory hyphae are essential to the maintenance of an enlarged depletion zone around the root, and destruction of these thinner hyphae must have serious effects on mycorrhizal function. However, fine hyphae are collectively attached to the root by a coarse hypha (Friese and Allen 1991), and simple mathematics suggest that disruption of a single thin hypha is less detrimental than disruption to a single thick hypha.

The exact reasons for the food preferences are unknown. Whether food choices are based on chemical attraction or repulsion, or whether members of the Glomales have much coarser, thick-walled hyphae is not clear and these aspects need to be examined in more detail. A comparison of the cytoplasmic chemical makeup of thin and thick vesicular-arbuscular mycorrhizal hyphae may help to explain this phenomenon. All these observations, therefore, lend support to the hypothesis

that preferential feeding on non-mycorrhizal fungi largely avoids catastrophic effects on the efficacy of arbuscular mycorrhizal associations, since arthropods that come in contact with roots are more apt to feed on non-mycorrhizal fungi.

The food preferences of soil microarthropods may be of adaptive significance to mycorrhizal functioning. Arbuscular mycorrhizal fungi are believed to have been involved in the transition of plants from water to land (Pyrozinski and Malloch 1975), and their survival has always been closely linked to that of plant roots. An intact extraradical network is important to the plant for nutrient uptake, and damage to this network can have strong implications for plant fitness. These fungi may, therefore, have adopted ways of avoiding intensive grazing by the soil fauna. This was indicated in the feeding tests (Table 4), not only by the strong animal food preference for conidial fungi over *G. macrocarpum*, but also by their showing no preference between sterile roots and roots colonized by *G. macrocarpum*. In fact, one animal (*T. clavata*) showed a preference for sterile rather than mycorrhizal roots. These data may reflect evolution by the fungi of strategies to deter grazers, much as plants have evolved both physical and chemical defenses to avoid being eaten (Gilbert 1971; Rhoades 1979).

**Table 5** Feeding response (mean fecal count 10 animals<sup>-1</sup>) by soil fauna to arbuscular mycorrhizal fungal hyphae of different widths. Data were analysed by Mann-Whitney *U*-test. For further explanations see Table 1

	<i>Lasiobelba rigida</i>	<i>Ceratozetes gracilis</i>	<i>Nothrus anaunensis</i>	<i>Folsomia candida</i>	<i>Folsomia penicula</i>	<i>Tullbergia clavata</i>
> 10 µm thick	0.4	0.6	0.1	4.8	0.2	0.2
< 5 µm thick	2.7	0.4	1.3	6.6	3.2	2.0
<i>U</i> statistic	20	56	28	42	24	25
<i>P</i> value	0.015	0.592	0.040	0.543	0.026	0.033

The activities of animals in feeding on non-mycorrhizal fungi may help arbuscular mycorrhizal fungi compete for resources, i. e., colonization of plant roots. The grazing of thin hyphae could be damaging to active hyphal tips, but could also help to separate arbuscular mycorrhizal fungal spores from the hyphal network. In a study on other fungi of the order Mucorales, moderate grazing of the hyphal tips stimulated hyphal branching and growth (Hedlund et al. 1991). Because the Glomales cannot be grown in monoculture (Williams 1992), no evidence of such a phenomenon has yet been shown with arbuscular mycorrhizal fungi. The use of soil rhizotrons and laboratory glass chambers may clarify some of the details of this interaction.

Whereas the animal response to different species of fungi was strong, very little response was detected to food abundance. This is contrary to the trends detected with the same animals in the field (Klironomos and Kendrick 1995), where increasing animal abundance was positively correlated with total hyphal length in the soil. However, since there was no way to separate different fungal groups, and since the total hyphal length was also positively correlated with fungal diversity, the animals may not have been responding to food abundance, but instead to fungal diversity or to an increased chance of encountering a preferred food. In cases where collembolans and mites have limited food, such preferences may not hold, according to optimal foraging theory (Smith 1992). There is no evidence of this in our field site however, since hyphal abundance was extremely high (Klironomos and Kendrick 1995).

Our data also show that the substrate on which the fungus is growing has an effect on the food preferences of the animals. Both the mites and collembolans preferred to graze on fungi colonizing litter rather than on the same fungi growing on roots. Bengtsson et al. (1988) have shown that fungi produce a number of volatile compounds which may be attractive or repellent to collembolans. These authors demonstrated the movement of two fungi on a ranking scale when a different substrate was used, a phenomenon also demonstrated by Klironomos et al. (1992), who compared collembolan food preferences among fungi grown on Norway spruce and balsam fir litter. This also emphasizes the need to replicate this set of experiments using roots from different plant species.

Under microcosm conditions, food preferences strongly affected the vertical distribution of the animals. Nevertheless, even with the clear preference for fungi on decaying litter, these same arthropods are quite abundant in the rhizosphere (Klironomos and Kendrick 1995). This is partly a result of fluctuations in abiotic variables, especially in moisture and temperature, which force some animals into lower soil layers (Klironomos and Kendrick 1995) and to interspecific competition among the arthropods, as seen in the microcosm experiments.

Soil fauna, by displaying a preference for darkly pigmented microfungi, can also act as an excellent dispersal agent for these fungi, helping them to reach the

lower layers of soil. The fungi are adapted to above-ground conditions, and it is thought that the dark pigments of these fungi protect them in the canopy from ultraviolet radiation (Pugh and Boddy 1988). Visser et al. (1987) isolated many species of fungi associated with the body and feces of the woodland collembolan *Onychiurus subtenuis*. Many of those fungi were darkly pigmented. As soil animals migrate to lower layers of the mineral soil (Klironomos and Kendrick 1995) they carry such fungi with them, resulting in their colonization of plant roots.

The field study (Klironomos and Kendrick 1995), not being a true "experiment", has not proved which of the arthropod interactions are real. We do not know whether any of them, especially interspecific interactions like competition, are sufficiently widespread to have an important effect on the dynamics of these coexisting arthropod species. It is generally accepted in ecological theory that the existence of ecological differences justifies the assumption that guilds are structured by competition among the members. It is interesting that in laboratory experiments, such as the ones reported here, interactions such as competition have been detected. This is significant, since approximately complete sets of experiments have rarely been carried out on all members of a proposed guild. The combination of field work (Klironomos and Kendrick 1995) and laboratory work (present study) has revealed some of the ecological interactions that determine zonation, competition being one of them.

In this paper we have shown that the soil microarthropod community displays fungal food preferences in vitro. These preferences depend on the substrate on which the fungus is grown, litter being preferred over roots. The evidence suggests that preferential grazing may be an important component in the functioning of arbuscular mycorrhizal associations. Our data show that the interactions among mycophagous microarthropods, the fungi, their substrates, and the functioning of arbuscular mycorrhiza are extremely complex. However, since this is the first report on fungal food preferences dealing with a combination of arbuscular mycorrhizal and non-arbuscular non-mycorrhizal fungi, more work needs to be performed to strengthen the hypothesis put forward here, ideally using other arbuscular mycorrhizal fungi and host species. These interactions deserve much more thorough investigation.

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