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Protozoan communities around conifer roots colonized by ectomycorrhizal fungi

Abstract Protozoan communities around roots with different types of ectomycorrhizae were distinct. These protozoan communities differed both qualitatively and quantitatively with the host (*Pinus ponderosa, Pseudotsuga menziesii, Picea sitchensis, Tsuga heterophylla* and *Abies grandis*) and the ectomycorrhizal fungal species. Based on the species identified and the numbers of individuals of each species, six communities of protozoa were found associated with specific ectomycorrhizae. Previous researchers have shown that mycorrhizal colonization of roots alters the amounts and types of exudates produced by roots, which in turn alters the bacterial community present. Most likely, mycorrhizal colonization of roots influences the protozoan community around roots by controlling the bacterial community. However, the protozoan community may in turn influence the successional dynamics of ectomycorrhizal fungi on different host root systems by a variety of mechanisms. These mechanisms could include: (1) preying upon individuals and perhaps removing particular species of bacteria from the mycorrhizosphere; and (2) controlling nitrogen mineralization in the rhizosphere. Further work needs to be performed to determine the interaction between these quadrate (plant-bacteria-fungi-protozoa) associations.

Key words Protozoa · Ectomycorrhizal fungi Douglas fir · Microbial ecology · Biodiversity

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Introduction

The role of protozoa in influencing or controlling rhizosphere processes is marginally understood. However, information can be found: (1) showing that some amoebae can be used as biocontrol agents, limiting the incidence of certain fungal root pathogens (Chakraborty and Old 1982); (2) indicating that flagellates and amoebae have a role in nitrogen mineralization dynamics in the short grass prairie (Elliott and Coleman 1977; Coleman 1985; Ingham et al. 1986); (3) illustrating the role of amoebae in releasing N for plants in agricultural systems in the Netherlands (Kuikman et al. 1990); (4) describing the use of protozoa as indicators of pollutants in Europe (Foissner 1986).

Protozoa feed mainly on bacteria, although, as indicated above, there is also evidence that certain amoebae feed on fungi (Lousier and Bamforth 1990). Some groups of flagellates may be "osmotrophs", that is, they can use dissolved organic carbon compounds and thus may compete with bacteria for food resources. Protozoa occur in higher numbers in the rhizosphere than in nonrhizosphere soil (Elliott and Coleman 1977; Bryant et al. 1982; Bamforth 1985; Clarholm 1985; Foissner 1986; Kuikman et al. 1990). Protozoa are attracted to and reproduce more rapidly in the rhizosphere because of the high numbers of bacteria present (Bryant et al. 1982; Clarholm 1985).

At least in crop plants, bacterial numbers and the composition of the rhizosphere bacterial community depend heavily on the amount and the type of exudates produced by roots (summarized by Paul and Clark 1990). In those few grasses, trees, and shrubs examined, mycorrhizal colonization of roots alters the amounts and types of exudates produced by the roots (reviewed by Ingham and Molina 1991). Therefore, mycorrhizal colonization of roots should alter the bacterial communities and thereby the protozoan community around roots.

As ectomycorrhizal succession proceeds (Marks and Foster 1967; Mason et al. 1983; Fleming et al. 1984; Last

et al. 1987; Blasius and Oberwinkler 1989), the protozoan community should also change. This assumes that there is grazing preference by, or outright selection of, protozoan species based on the bacterial community present, an idea often discussed but not studied in situ. Conversely, the protozoan community that develops, based on the initial rhizosphere bacterial community, may influence the further successional dynamics of both the bacterial community and ectomycorrhizal fungal colonization.

The objectives of the present greenhouse study were to determine: (1) whether protozoa occurred around roots colonized by different ectomycorrhizal fungi; (2) whether distinct protozoan communities could be identified; (3) whether protozoan communities differed with different plant species, even when the mycorrhizal fungus colonizing the different host species was the same; (4) whether distinct associations of protozoa with ectomycorrhizal fungi could be identified. The conifer seedlings used in this study were grown as part of a larger study of the pattern of specificity of several *Rhizopogon* and allied fungal species (Massicotte et al. 1994).

Materials and methods

Seedling preparation and growth conditions

Seeds of grand fir [*Abies grandis* (Dougl.) Lindl.], sitka spruce [*Picea sitchensis* (Bongard) Carriere], ponderosa pine (*Pinus ponderosa* Laws.), Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] and western hemlock [*Tsuga heterophylla* (Raf.) Sarg.] were soaked overnight in water, dried on paper towels, cold stratified at 4° C for 37 days, and then transferred to containers (Massicotte et al. 1994). Only 60-ml monoculture container (16.5 cm long, 2.5 cm diameter) were examined, except for one sample (*Rhizopogon subcaerulescens* on *Pseudotsuga menziesii*) which originated from a dual-seedling container (with pine) with a cacpacity of 160 ml. Containers were filled to 2.5 cm from the top with a 1:1 mixture of peat and vermiculite. These substrates were not sterilized for two reasons: (1) autoclaving results in the release of materials toxic to seed germination; (2) the plants were to be grown in a nonsterile greenhouse.

Two to three seeds were dropped into each container and covered with a thin layer of white quartz sand (8 grade) to minimize splash during watering. Most seeds germinated within 10 days, except for *Abies grandis* and *Tsuga heterophylla,* which germinated over a 1-month period. Antisplash devices were not used between fungal treatments because of space limitations in the greenhouse and because of a desire not to limit bacterial and protozoan community inoculum. If unique protozoan communities occurred in systems where cross-inoculation was not limited, then these mycorrhizae-bacterial-protozoan interactions would clearly have selected for conditions that favored only specific community compositions. All mycorrhizae growing on the root systems of these plants were visually assessed and separated, such that "fungal escape" did not obviate the purpose of this experiment.

Seedlings were grown in the greenhouse using a combination of sunlight and artificial light (280 μmol s^{-1} m^{-2}) provided by sodium-vapor lamps. Air temperature fluctuated between 21° C and 32° C. Seedlings were watered at least twice weekly with tap water. Each seedling was fertilized monthly with 5 ml of Peter's fertilizer (N–P–K/473–449–426 μg ml^{-1} plus trace elements) applied at half strength. This amounted to 11.9 mg N, 11.3 mg P and 10.7 mg K applied per seedling over the length of the experiment.

The sources of the protozoan community in this experiment were the potting soil, the air circulation system, tap water, fertilizer applications, and the initial mycorrhizal inocula applied to each pot of soil. All pots had equal likelihood of receiving all sources of protozoa, except for differences that might have occurred in the bacterial and protozoan communities inoculated with each sporocarp suspension. However, there is no evidence to indicate that protozoa are present in sporocarps or sporocarp suspensions.

As the seedlings grew in the greenhouse, the pots were open to cross-inoculation. *Thelephora terrestris,* a common greenhouse contaminant, was not purposely inoculated into pots at the beginning of the experiment, but was found on all tree species at the end of the experiment. Thus, it is quite likely that all pots received a common microbial and protozoan inoculum during the course of the experiment.

Inoculation

Hypogeous sporocarps of fungi were collected over 6 months from different habitats in the Pacific Northwest. Sporocarps were identified, put in tap water in 20-ml glass jars and stored at 4° C until use. A voucher for each fungus was deposited with the Oregon State University Herbarium (Department of Botany and Plant Pathology, Oregon State University, Corvallis, Ore.).

Each sporocarp was gently brushed to remove soil and organic matter, cut into pieces (1–3 cm) and blended at high speed for 5 min in 200 ml of distilled water in a Waring blender. A spore count was performed using a hemocytometer. Dilutions were made in distilled water to deliver the appropriate number of spores (Table 1) in two separate inoculations. No attempt was made to standardize the inoculum concentration (number of spores/ml) between the different fungi used. For each inoculation, 10 ml of spore slurry was deposited at the top of the soil surface in each container at 18 weeks and again at 21 weeks after seeding.

Ectomycorrhizae assessment

Inoculated seedlings (14 months old) were carefully removed from the support matrix (peat-vermiculite), and the roots washed using tap water. Cleaned roots were examined for ectomycorrhizae. Descriptions of the mycorrhizal characteristics included color, hyphal diameter, hyphal abundance, fungal mantle and rhizomorph morphology and are reported elsewhere (Massicotte et al. 1992). Presence and abundance of greenhouse contaminants such as *Thelephora terrestris* and *Mycelium radicis atrovirens* (MRA) were noted. Their key characteristics were found to be in agreement with descriptions published previously by Danielson (1991) and Danielson and Visser (1989). In several cases, mycorrhizae of these contaminant species were examined for the protozoan community associated with them. Roots not colonized by mycorrhizae were selected as control roots.

Root excision and protozoa examination

Four clusters (about 25–50 root tips within the four clusters) of each ectomycorrhizal type were removed with forceps and placed in separate sterile wells containing 1 ml sterile water, i.e., one cluster per well for a total of four replicates per mycorrhizal type tested. The four clusters were selected from two to three seedlings. Host and mycorrhizal species combinations are given in Table 2. The weight of the dry root mass originally placed in the well was determined by drying and weighing roots after incubation and identification of species.

The liquid in each well was examined periodically for protozoa using a dissecting microscope (day 0–40) after excision of root clusters. In these examinations, the plates containing the wells were not opened, nor was the root material disturbed. In addi-

Table 1 Acronym, collection number (Oregon State University, Herbarium), and inoculation dose for ectomycorrhizal fungi used in this study

Acronym	Species	Collection number	Number of spores/ seedling $\times 10^6$
Ra	*Rhizopogon arctostaphylii* Smith	T11385	31.8
Re1	*R. ellenae* Smith	T11283	16.6
Re2	*R. ellenae* Smith	T11375	9.0
Rf	*R. flavofibrillosus* Smith	T11182	18.7
Ro1	*R. occidentalis* Zeller & Dodge	T11381	10.3
Ro2	*R. occidentalis* Zeller & Dodge	T11320	25.6
Rr1	*R. rubescens* (Tul.) Tul.	T11378	1.8
Rr2	*R. rubescens* (Tul.) Tul.	T11355	37.4
Rp2	*R. parksii* Smith	T11323	17.3
Rp1	*R. parksii* Smith	T11227	34.1
Rsm	*R. smithii* Hosford	T11347	5.2
Rs2	*R. subcaerulescens* Smith	T11228	15.2
Rs1	*R. subcaerulescens* Smith	T11566	9.2
Rt	*R. truncatus* Linder	T11176	4.4
Rvu	*R. vulgaris* (Vitt.) M. Lange	T11568	29.1
Rv	*R. vinicolor* Smith	T11229	18.7
Tc	*Truncocolumella citrina* (Zeller) Singer & Smith	T11274	5.5
MRA	*Mycelium radicis atrovirens* (Greenhouse contaminant)		
Thel	*Thelephora terrestris* (Greenhouse contaminant)		

Table 2 Mycorrhizal types found and examined on roots of each host species

Host	Fungal associate
Pinus ponderosa	Ro1 *Rhizopogon occidentalis*
	Rsm *Rhizopogon smithii*
	Rvu *Rhizopogon vulgaris*
	Ro2 *Rhizopogon occidentalis*
	Rr1 *Rhizopogon rubescens*
	Rr2 *Rhizopogon rubescens*
	Rt *Rhizopogon truncatus*
	Rf *Rhizopogon flavofibrillosus*
	Ra *Rhizopogon arctostaphyli*
	Rs2 *Rhizopogon subcaerulescens*
	Rs1 *Rhizopogon subcaerulescens*
	Re1 *Rhizopogon ellenae*
	Re2 *Rhizopogon ellenae*
	MRA *Mycelium radicis atrovirens*
	Thelephora terrestris
	None (control roots)
Pseudotsuga menziesii	Rv *Rhizopogon vinicolor*
	Rs2 *Rhizopogon subcaerulescens*[a]
	Rp1 *Rhizopogon parksii*
	Rp2 *Rhizopogon parksii*
	Thelephora terrestris
	Tc *Truncocolumella citrina*
	MRA *Mycelium radicis atrovirens*
	None (control roots)
Tsuga heterophylla	MRA *Mycelium radicis atrovirens*
	Thelephora terrestris
	None (control roots)
Picea sitchensis	*Thelephora terrestris*
	None (control roots)
Abies grandis	*Thelephora terrestris*
	None (control roots)

[a] Originated from dual-culture container grown with pine

tion, approximately every 2 weeks, a drop of liquid was removed from each well using a sterile Pasteur pipette, placed on a microscope slide, covered with a coverslip, and examined at $\times 400$ total magnification using an Olympus differential interference contrast microscope (Foissner 1986).

Numbers of each species present were estimated by counting the individuals of each species observed at each examination date in each well containing roots. The number of each species of protozoa counted was assigned a category (0, <10, 10–50, 50–100, 100–500, >500). The relative density of protozoa originally present around the root system was approximated (1) as 0, if no individuals of that species was observed at any time; (2) as less than 10, if one to ten individuals were seen at sometime during the incubation period; (3) as 10–50, if numbers reached several hundred or more during the 40-day incubation period. These numbers are relative and serve as a general comparative measure, assuming that flagellates, amoebae and ciliates have similar growth rates and that the bacterial species on which they feed were presented in adequate supply to allow maximum populations to be attained. This information must be used with some caution since it is not absolute but is better than no information at all. These relative number ranges were the same between the four root systems examined for each tree and ectomycorrhizal species.

Results

Weights of the root clusters were minimal, ranging from 0.002 to 0.004 mg root per well. Descriptions and photographs of the mycorrhizal types excised and examined for protozoa in this experiment are described in Massicotte et al. (1994).

Different protozoan communities occurred around roots colonized by different mycorrhizal fungi (Tables 3–7). Within root clusters and nonmycorrhizal roots of the host *Pinus ponderosa,* six protozoan communities were delineated (Table 8). All six groups included representatives of all four major types of protozoa: ciliates, testate and naked amoebae, and flagellates. The species

Table 3 Approximate numbers of each protozoan group present in the mycorrhizosphere of ectomycorrhizae on *Pinus ponderosa* roots. For species abbreviations, see Tables 1 and 2

Protozoan species	*Rhizopogon* species									MRA	Thel	Control roots
	Ro	Rsm	Rvu	Rf	Rr	Rt	Ra	Rs	Re			
Flagellates												
Bodo sp 1	10–50	0	<10	10–50	10–50	10–50	10–50	10–50	10–50	0	10–50	10–50
sp 2	<10	10–50	10–50	10–50	0	0	0	10–50	<10	0	10–50	10–50
sp 3	<10	10–50	10–50	10–50	10–50	10–50	10–50	10–50	10–50	10–50	0	10–50
Rhynchomonas	0	<10	<10	10–50	<10	10–50	10–50	<10	0	<10	10–50	0
Cercomonas	<10	<10	<10	<10	0	0	0	<10	<10	0	0	10–50
Testate amoebae												
Nebela collaris	<10	<10	10–50	10–50	10–50	10–50	10–50	10–50	10–50	0	0	10–50
Nebela galeata	<10	10–50	0	0	0	10–50	10–50	<10	10–50	0	0	0
Euglypha denticulata	<10	0	0	0	<10	0	0	0	0	0	0	0
Valkanovia sp.	<10	10–50	<10	<10	0	10–50	10–50	<10	<10	0	0	0
Difflugia lucida	<10	0	0	0	0	0	0	0	0	10–50	0	0
Trinema enchelys	<10	0	0	0	0	10–50	0	10–50	10–50	0	0	0
Trinema complanatum	0	<10	10–50	0	10–50	0	0	0	0	0	10–50	0
Corythionopsis sudzuki	0	0	0	0	0	10–50	0	0	10–50	0	0	0
Arcella sp.	0	0	0	0	0	0	0	<10	0	0	0	0
Naked amoebae												
Mayorella sp.	<10	0	<10	0	0	0	0	<10	<10	0	0	0
Echinamoebae sp.	0	<10	<10	<10	0	10–50	0	<10	10–50	0	10–50	0
Amoeba proteus	10–50	0	<10	<10	<10	10–50	0	10–50	10–50	0	<10	10–50
Metachaos sp.	10–50	0	0	0	0	0	0	0	0	0	0	0
Thecamoeba sp.	0	10–50	0	<10	0	0	0	10–50	0	0	0	0
Hyalodiscus sp.	0	0	0	0	0	0	0	<10	0	0	0	0
Vexillifera sp.	0	0	0	10–50	0	10–50	10–50	0	0	0	0	0
Polychaos sp.	10–50	0	<10	0	0	10–50	0	<10	<10	0	0	0
Pessonella sp.	0	0	<10	0	0	0	0	<10	0	<10	0	0
Ciliates												
Leptopharynx costatus	10–50	10–50	10–50	10–50	0	10–50	10–50	<10	10–50	0	0	0
Cylcidium glaucoma	10–50	10–50	10–50	10–50	<10	10–50	0	10–50	10–50	0	0	0
Woodruffia sp.	<10	0	0	0	0	0	0	0	0	0	0	0
Blepharisma japonicum	<10	10–50	10–50	0	<10	0	10–50	10–50	10–50	0	10–50	10–50
Colpoda sp.	10–50	0	10–50	0	10–50	10–50	10–50	10–50	10–50	0	0	10–50
Microthorax pusillus	<10	0	10–50	0	0	0	0	0	0	0	0	0
Pseudoblepharisma sp.	0	0	0	0	0	0	0	10–50	<10	0	0	0
Urostyla sp.	0	0	0	0	0	0	10–50	10–50	10–50	0	0	10–50
Rotifers	10–50	0	0	0	10–50	10–50	10–50	10–50	10–50	10–50	0	0
Insect larvae	<10	0	10–50	0	10–50	0	10–50	10–50	10–50	0	0	0
Nematodes	<10	<10	<10	0	10–50	10–50	10–50	<10	<10	0	0	0

present in the six groups were distinct based on either (1) species present or (2) the number of individuals present.

Pinus ponderosa

On *Pinus ponderosa,* protozoan community group 1 was always associated with *Rhizopogon subcaerulescens, R. flavofibrillosus, R. truncatus* and *R. vulgaris* (Table 8). This community consisted of all five species of flagellates, at least several species of testate amoebae, usually including *Nebela* or *Trinema,* 4–5 species of naked amoebae, with *Amoeba proteus* and *Echinamoebae* usually present, and a large diversity of ciliates.

Protozoan community group 2 (Table 8) was associated with *Rhizopogon occidentalis, R. ellenae,* and *R. smithii* and consisted of a slightly less diverse group of flagellates, with *Rhynchomonas* usually not present, a slightly less diverse group of testate amoebae with *Nebela galeata* usually missing, a very low diversity of naked amoebae, but a higher diversity of ciliates than in group 1.

Protozoan community group 3 (Table 8) was associated with *Rhizopogon rubescens* and *R. arctostaphylii.* Flagellate diversity was low, with only two species of *Bodo* and *Rhynchomonas* present, a low diversity of testate amoebae, no naked amoebae in the community and a low diversity of ciliates.

The protozoan communities associated with *Thelephora* (group 4) and with MRA (group 5) both had extremely low diversity. Only one representative each of naked and testate amoebae and ciliates occurred in group 4, while in group 5 two naked amoebae species were observed, but only a few individuals after several weeks of incubation.

Table 4 Approximate numbers of each protozoan group present in the mycorrhizosphere of ectomycorrhizae on *Pseudotsuga menziesii* roots. For species abbreviations, see Tables 1 and 2

Protozoan species	*Rhizopogon* sp.			MRA	Tc	Thel	Control roots
	Rv	Rs	Rp				
Flagellates							
Bodo sp 1	10–50	10–50	10–50	10–50	10–50	<10	0
sp 2	10–50	10–50	10–50	0	0	<10	0
sp 3	10–50	10–50	10–50	0	10–50	10–50	10–50
Rhynchomonas	10–50	10–50	10–50	0	10–50	10–50	10–50
Cercomonas	0	<10	10–50	0	0	0	<10
Testate amoebae							
Nebela collaris	10–50	10–50	10–50	0	10–50	10–50	10–50
Nebela galeata	0	10–50	<10	0	10–50	0	0
Euglypha denticulata	0	0	0	0	0	<10	<10
Valkanovia sp.	10–50	0	0	0	0	0	10–50
Difflugia lucida	10–50	0	0	0	0	0	0
Trinema enchelys	10–50	10–50	10–50	0	0	0	0
Trinema complanatum	0	0	0	0	0	0	0
Corythionopsis sudzuki	0	0	0	0	0	0	0
Arcella sp.	0	0	0	<10	0	0	0
Naked amoebae							
Mayorella sp.	0	<10	<10	0	0	0	0
Echinamoebae sp.	<10	10–50	10–50	0	10–50	0	10–50
Amoeba proteus	<10	10–50	10–50	0	<10	<10	0
Metachaos sp.	0	0	0	0	0	0	0
Thecamoeba sp.	10–50	10–50	0	0	0	0	0
Hyalodiscus sp.	0	0	0	0	0	0	0
Vexillifera sp.	10–50	0	10–50	0	10–50	0	0
Polychaos sp.	<10	0	0	0	0	0	0
Pessonella sp.	0	0	0	0	<10	0	0
Ciliates							
Leptopharynx costatus	10–50	0	10–50	0	10–50	0	10–50
Cylcidium glaucoma	<10	0	<10	0	10–50	0	0
Woodruffia sp.	10–50	0	<10	0	0	0	0
Blepharisma japonicum	10–50	0	10–50	0	0	0	0
Colpoda sp.	10–50	10–50	10–50	0	10–50	0	0
Microthorax pusillus	10–50	10–50	0	0	10–50	0	0
Pseudoblepharisma sp.	0	0	10–50	0	0	<10	0
Urostyla sp.	0	10–50	<10	<10	0	<10	0
Rotifers	<10	10–50	0	0	<10	0	0
Insect larvae	10–50	10–50	10–50	0	10–50	0	0
Nematodes	<10	0	<10	0	0	0	0

Protozoan communities associated with control roots, i.e., roots of *Pinus ponderosa* with no mycorrhizal fungi, had greater diversity than MRA or *Thelephora,* but less diversity than protozoan communities around mycorrhizae of *Rhizopogon* (Table 3).

Pseudotsuga menziesii and *Picea sitchensis*

For those mycorrhizal species found on both *Pseudotsuga menziesii* and *Picea* roots, similar protozoan communities were found (Table 9). The species of *Rhizopogon* colonizing Douglas fir were different to those on ponderosa pine, but very similar groups of protozoa were found around *Rhizopogon parksii,* as well as around *Truncocolumella* on Douglas fir, and associated with *R. subcaerulescens, R. flavofibrillosus, R. truncatus* and *R. vulgaris* on ponderosa pine (Table 8). Therefore, this protozoan community was labeled as group 1, even though it was associated with different mycorrhizal species on different hosts, and flagellate diversity was slightly lower on Douglas fir than in ponderosa pine.

Protozoan community group 2 occurred with *Rhizopogon vinicolor* on *Pseudotsuga.* Protozoan community group 3 was not observed on *Pseudotsuga* or *Picea,* while protozoan communities around *Thelephora* and MRA on these hosts were the same as on *Pinus* (Table 9).

MRA occurred on four host species, *Pinus, Peudotsuga, Picea* and *Tsuga.* Regardless of host, the protozoan community around this mycorrhizal type (group 5) had extremely low diversity, lower than around control roots, except in *Tsuga. Thelephora* occurred on all five host species, with notable variation in the diversity of the protozoan community group that occurred around it, based on host species colonized. In *Pinus, Pseudotsuga* and *Picea,* protozoan community group 5 was usually less diverse, or nearly the same as the community around control roots (group 6). The protozoan

Table 5 Approximate numbers of each protozoan group present in the mycorrhizosphere of ectomycorrhizae on *Tsuga heterophylla* roots. For species abbreviations, see Tables 1 and 2

Protozoan species	MRA	Thel	Control roots
Flagellates			
Bodo sp 1	0	10–50	10–50
sp 2	0	10–50	0
sp 3	10–50	10–50	0
Rhynchomonas	10–50	10–50	0
Cercomonas	0	0	0
Testate amoebae			
Nebela collaris	0	10–50	10–50
Nebela galeata	0	10–50	10–50
Euglypha denticulata	0	0	0
Valkanovia sp.	0	0	0
Difflugia lucida	10–50	0	0
Trinema enchelys	10–50	10–50	0
Trinema complanatum	0	0	0
Corythionopsis sudzuki	0	0	0
Naked amoebae			
Echinamoebae sp.	10–50	10–50	0
Amoeba proteus	0	10–50	0
Metachaos sp.	0	0	0
Thecamoeba sp.	0	<10	0
Hyalodiscus sp.	0	0	0
Vexillifera sp.	0	10–50	0
Polychaos sp.	0	0	0
Pessonella sp.	<10	0	0
Ciliates			
Leptopharynx costatus	0	10–50	0
Cylcidium glaucoma	0	0	10–50
Woodruffia sp.	0	0	0
Blepharisma japonicum	0	0	0
Colpoda sp.	0	10–50	0
Microthorax pusillus	0	0	0
Pseudoblepharisma sp.	0	0	0
Urostyla sp.	0	0	0
Rotifers	10–50	0	10–50
Insect larvae	0	10–50	0
Nematodes	0	0	0

community associated with non-colonized, or control, roots was the same on *Pseudotsuga, Picea* and *Pinus,* suggesting that the exudates from non-colonized roots of all three tree species are similar, at least from a bacterial/protozoan community point-of-view. However, in *Tsuga* and *Abies,* the protozoan community around *Thelephora* was more diverse than around control roots. Clearly, *Thelephora* had markedly different effects on different hosts, whereas MRA and *Rhizopogon subcaerulescens* (the later only on Douglas fir and pine) had consistent effects across different host species.

Tsuga, Abies and *Picea* roots did not form mycorrhizae with *Rhizopogon* in these pot culture systems. *Pinus ponderosa* had the greatest diversity of protozoan species present in the rhizosphere communities, followed by *Pseudotsuga menziesii.* The amoebae species *Trinema complanatum, Corythionopsis sudzuki, Metachaos,* and *Hyalodiscus* were never found in association with *Pseudotsuga* roots.

On the first sample date (day 0), no protozoa were observed, since roots were removed from the potting medium and washed several times in sterile medium using sterile conditions. By day 7, flagellates were observed, and by day 10, ciliates and testate amoebae were observed. By day 30, some species of protozoa had disappeared completely, while the testate amoebae were present in high numbers. By day 40, no new species were seen and in most cases, significant decreases in numbers had occurred.

In general, if a protozoan species was observed in one root group, it was also observed on the other three replicates root groups, with numbers of individuals in the same range. For this reason, no statistical analysis was performed on the information in Tables 3–7, since the values are expressed as the average range for each protozoan species around the four root clusters of the same tree species and mycorrhizal type.

Table 6 Approximate numbers of each protozoan group present in the mycorrhizosphere of ectomycorrhizae on *Picea* roots. For species abbreviations, see Tables 1 and 2

Protozoan species	MRA	Thel	Control roots
Flagellates			
Bodo sp 1	10–50	10–50	0
sp 2	10–50	10–50	10–50
sp 3	0	0	10–50
Rhynchomonas	0	10–50	0
Cercomonas	0	0	0
Testate amoebae			
Nebela collaris	0	10–50	10–50
Nebula galeata	0	0	0
Euglypha denticulata	0	0	0
Valkanovia sp.	0	0	0
Difflugia lucida	0	0	0
Trinema enchelys	0	0	0
Trinema complanatum	0	0	0
Corythionopsis sudzuki	0	0	0
Naked amoebae			
Echinamoebae sp.	0	0	10–50
Amoeba proteus	10–50	10–50	0
Metachaos sp.	0	0	0
Thecamoeba sp.	0	0	0
Hyalodiscus sp.	0	0	0
Vexillifera sp.	0	0	0
Polychaos sp.	0	0	0
Ciliates			
Leptopharynx costatus	0	10–50	10–50
Cylcidium glaucoma	0	0	0
Woodruffia sp.	0	0	10–50
Blepharisma japonicum	0	0	0
Colpoda sp.	0	0	10–50
Microthorax pusillus	0	0	0
Pseudoblepharisma sp.	0	0	0
Urostyla sp.	0	0	0
Rotifers	0	0	0
Insect larvae	0	0	10–50
Nematodes	0	0	0

Discussion

In this study, we assessed relative numbers and community structure of protozoa around roots of different host plants colonized by different mycorrhizal fungi and roots not colonized by mycorrhizal fungi. It is not possible to compare these data with previous work because there have been no previous investigations of protozoan communities present around or on portions of roots colonized by ectomycorrhizal fungi. However, the rhizosphere of grasses, which are colonized by endomycorrhizal fungi, in general has a higher number of flagellates and amoebae than in nonrhizosphere soil (Darbyshire and Greaves 1967; Bamford 1985; Foissner 1986).

The objectives of this study were to determine: (1) whether protozoa occurred around roots colonized by different ectomycorrhizal fungi; (2) whether unique protozoan communities could be identified; (3) whether protozoan communities differed with different plant species, even when the mycorrhizal fungus colonizing the different host species was the same; (4) whether unique associations of protozoa with ectomycorrhizal fungi could be identified.

Table 7 Approximate numbers of each protozoan group present in the mycorrhizosphere of ectomycorrhizae on *Abies* roots. For species abbreviations, see Tables 1 and 2

Protozoan species	Thel	Control roots
Flagellates		
Bodo sp 1	10–50	10–50
sp 2	10–50	10–50
sp 3	0	10–50
sp 4	10–50	0
Rhynchomonas	10–50	0
Testate amoebae		
Nebela collaris	10–50	0
Nebela galeata	10–50	0
Euglypha denticulata	0	0
Valkanovia sp.	10–50	0
Difflugia lucida	0	0
Trinema enchelys	0	10–50
Naked amoebae		
Mayorella sp.	0	10–50
Echinamoebae sp.	10–50	10–50
Amoeba proteus	0	10–50
Metachaos sp.	0	0
Thecamoeba sp.	0	0
Hyalodiscus sp.	10–50	0
Ciliates		
Leptopharynx costatus	10–50	0
Cylcidium glaucoma	10–50	0
Woodruffia sp.	0	10–50
Blepharisma japonicum	10–50	0
Colpoda sp.	10–50	10–50
Microthorax pusillus	0	0
Pseudoblepharisma sp.	10–50	0
Rotifers	0	0
Insect larvae	0	10–50
Nematodes	0	0

Table 8 Protozoan communities associated with mycorrhizal types on *Pinus ponderosa* roots

Group 1	Associated with *Rhizopogon subcaerulescens, R. Flavofibrillosus, R. truncatus* and *R. vulgaris* Flagellates: all five species Testate amoebae: at least three different species, including *Nebela* or *Trinema* Naked amoebae: four to five different species including *Amoeba proteus* and *Echinamoebae* Ciliates: usually four to six species
Group 2	Associated with *Rhizopogon occidentalis, R. ellenae,* and *R. smithii* Flagellates: three to four species, *Rhynchomonas* usually missing from the community Testate amoebae: two to four species, *Nebela galeata* usually missing Naked amoebae: few species Ciliates: a high diversity of species
Group 3	Associated with *Rhizopogon rubescens* and *R. arctostaphylii* Flagellates: diversity low, usually only *Bodo* species 1 and 3, and *Rhynchomonas* Testate amoebae: very few species Naked amoebae: no naked amoebae Ciliate: diversity low
Group 4	Associated with *Thelephora* Flagellates: usually several species Testate amoebae: only single species Naked amoebae: only single species Ciliates: only single species
Group 5	Associated with MRA Flagellates: few species Testate amoebae: few to no species Naked amoebae: few to no species Ciliates: none
Group 6	Associated with control, noncolonized roots Flagellates: four to five flagellate species Testate amoebae: usually only *Nebela collaris* Naked amoebae: *Amoeba proteus* or *Echinamoeba* Ciliates: usually single species in low numbers

Table 9 Protozoan communities associated with mycorrhizae on *Pseudotsuga menziesii* and *Picea sitchensis. Tsuga heterophylla* and *Abies grandis* were not considered because too few replicates were taken

Group 1	*Rhizopogon parksii* and *Truncocolumella* (on Douglas fir only) supported a protozoan community similar to group 1 on *Pinus ponderosa*), except that flagellate diversity was lower
Group 2	*Rhizopogon vinicolor* and *R. subcaerulescens* (on Douglas fir only) instead of *R. occidentalis, R. ellenae,* and *R. smithii* as occurred on *Pinus ponderosa* (Table 4)
Group 3	Not observed on these hosts
Group 4	The *Thelephora* protozoan community on Douglas fir and *Picea* was similar to that seen on *Pinus ponderosa*
Group 5	The MRA protozoan community on Douglas fir and on *Picea* was similar to that seen on *Pinus ponderosa*
Group 6	The protozoan community around noncolonized roots was similar to that seen on *Pinus ponderosa*

Clear answers were obtained to the first two questions. Protozoa occur around roots colonized by ectomycorrhizal fungi in much higher numbers than nonmycorrhizal roots. Unique protozoa communities were identified and these communities were repeatedly obtained from several roots colonized with the same fungus. Even where protozoa could move throughout the entire rhizosphere and from pot to pot, either during watering or as hitchhikers on the surfaces of nematodes and microarthropods, which could easily travel between pots, these unique communities were maintained. Our hypothesis arising from these results is that differences in the mycorrhizosphere over the 14 months of the experiment directly influenced the species richness and diversity of the protozoan community present.

Objectives three and four were not as clearly answered, although it is apparent that different mycorrhizae supported different communities of protozoa. Communities were distinct in terms of both numbers and species composition. The same community composition was found for several mycorrhizal root pieces from different individuals of the same host plant species colonized by the same fungal species. However, different hosts colonized by the same mycorrhizal species also had the same protozoan community. In addition, there appeared to be groups of several species of mycorrhizae that supported similar protozoan communities.

This is the first report of this phenomen in the literature. Our study provides evidence that mycorrhizal fungi characteristically and repeatably alter the bacterial community around the roots of different host plants, thus influencing the composition of the protozoan community. Numbers of protozoa on control roots of *Pseudotsuga* and *Tsuga* were generally lower than on colonized roots (Tables 4–7). *Pseudotsuga* and *Tsuga* protozoan communities on control roots were comprised mainly of flagellates and *Nebela* (Tables 4, 5). Species composition was similar across these host species, suggesting that root exudate patterns and amounts were similar for these two species.

On *Picea* and *Pinus* (Tables 3, 6), both the number of each species of protozoa and the number of individuals present were very low on roots colonized by MRA and *Thelephora,* and on control roots. Flagellates, *Amoeba proteus* (although not in *Picea*), and several ciliate species comprised the community on control roots of these two species. This suggests that exudate production from these types of roots was low, and consequently the number of bacterial species and the protozoa utilizing these bacteria.

On noncolonized *Abies* roots (Table 7), the number of species present was greater than for other hosts, but the number of individuals of each species was low, much lower than around *Thelephora* mycorrhizae on this host species. Most of the *Rhizopogon* and *Truncocolumella* inoculated in this study are specific to certain tree host species, yet these mycorrhizal fungi harbored a greater diversity of protozoa, and thus perhaps bacteria, than the broader host range fungi such as MRA and *Thelephora.* One hypothesis based on these observations which needs further investigation is that a functional difference exists between restricted and broad host range mycorrhizal fungi with respect to their ability to maintain a higher diversity of protozoa.

Since mycorrhizal colonization of roots alters the amount and type of exudate produced, the bacterial community is altered (reviewed in Ingham and Molina 1991). Once protozoa respond to this change in bacterial species, predation of the bacteria around the roots, production of mineralized N and secondary metabolites by protozoa and surviving bacteria, probably influences the successional dynamics of ectomycorrhizal fungi and bacteria in the rhizosphere of these host root systems. This leads to questions about the magnitude and importance of protozoan predation on successional dynamics and process rates. While interactions of this type are complex and challenge the researcher to assess the ultimate effect of presence/absence of different species on plant growth and ecosystem function, there is no doubt that they are important. Future studies of ecosystem function need to incorporate this level of resolution.

As we know more about specific functions of protozoa and bacteria, such as protozoan preference for bacterial species, or the rate at which each species performs its particular function given certain environmental conditions, we may be able to infer functional differences in the benefits to a host plant of different ectomycorrhizal fungal species. Given information on specific function, more light may be shed on the dilemma of apparent niche redundancy of soil organisms.

In this greenhouse study, we demonstrated that: (1) distinct communities of protozoa can occur around different mycorrhizal species; (2) there can be similar protozoan communities on roots of different host species with the same mycorrhizal fungal colonist, at least in simplified greenhouse studies.

What is the extent and importance of these interactions in field situations? We hypothesize that the same relationship exists in field situations, although results from a greenhouse pot study inoculated with controlled mycorrhizal and, therefore, limited microbial populations should be extrapolated with caution to field situations.

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References

Bamforth SS (1985) The role of protozoa in litters and soils. J Protozool 32:404–409

Blasius D, Oberwinkler F (1989) Succession of mycorrhizae – a matter of tree age or stand age. Ann Sci For 46:758–761

Bryant RJ, Woods LE, Coleman DC, Fairbanks BC, McClellan JF, Cole CV (1982) Interactions of bacterial and amoebal populations in soil microcosms with fluctuating moisture content. Appl Environ Microbiol 43:747–752

Chakraborty S, Old KM (1982) Mycophagous soil amoeba: interactions with three plant pathogenic fungi. Soil Biol Biochem 14:247–255

Clarholm M (1985) Interactions of bacteria, protozoa and plants leading to mineralization of soil nitrogen. Soil Biol Biochem 17:181–187

Coleman DC (1985) Through a ped darkly: an ecological assessment of root-soil-microbial-faunal interactions. In: Fitter AH, Atkinson D, Read DJ, Usher MB (eds) Ecological interactions in soil. Blackwell, Cambridge, UK, pp 1–21

Danielson RM (1991) Temporal changes and effects of amendments on the occurrence of sheathing (ecto-)mycorrhizas of conifers growing in oil sands tailings and coal spoil. Agric Ecosyst Environ 35:261–281

Danielson RM, Visser S (1989) Host response to inoculation and behavior of introduced and indigenous ectomycorrhizal fungi of jack pine grown on oil-sands tailings. Can J For Res 19:1412–1421

Darbyshire JF, Greaves MP (1967) Protozoa and bacteria in the rhizosphere of *Sinapis alba* L., *Trifolium repens* L. and *Lolium perenne* L. Can J Microbiol 13:1057–1068

Elliott ET, Coleman DC (1977) Soil protozoan dynamics in a shortgrass prairie. Soil Biol Biochem 9:113–118

Fleming LV, Deacon JW, Last FT, Donaldson SJ (1984) Influence of propagating soil on the mycorrhizal succession of birch seedlings transplanted to a field site. Trans Br Mycol Soc 82:707–711

Foissner W (1986) Soil protozoa: fundamental problems, ecological significance, adaptations, indicators of environmental quality, guide to the literature. Prog Protistol 2:69–212

Ingham ER, Molina R (1991) Interactions of mycorrhizal fungi, rhizosphere organisms and plants. In: Barbosa P (ed) Microorganisms, plants and herbivores. Wiley, New York, pp 169–198

Ingham ER, Trofymow JA, Ames RN, Hunt HW, Morley CR, Moore JC, Coleman DC (1986) Trophic interactions and nitrogen cycling in a semiarid grassland soil. I. Seasonal dynamics of the soil foodweb. J Appl Ecol 23:608–615

Kuikman PJ, Van Elsas JD, Jassen AG, Burgers SLGE, Van Veen JA (1990) Population dynamics and activity of bacteria and protozoa in relation to their spatial distribution in soil. Soil Biol Biochem 22:1063–1073

Last FT, Dighton J, Mason PA (1987) Successions of sheathing mycorrhizal fungi. Trees 2:157–161

Lousier JD, Bamforth SS (1990) Soil protozoa. In: Dindal D (ed) Soil biology guide. Wiley, New York, pp 97–136

Marks GC, Foster RC (1967) Succession of mycorrhizal associations on individual roots of radiata pine. Aust For Res 31:194–201

Mason PA, Wilson J, Last FT (1983) The concept of succession in relation to the spread of sheathing mycorrhizal fungi on inoculated tree seedlings growing in unsterile soils. Plant Soil 71:247–256

Massicotte HB, Melville LH, Li CY, Peterson RL (1992) Structural aspects of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) tuberculate ectomycorrhizae. Trees 6:137–146

Massicotte HB, Molina R, Luoma DL, Smith JE (1994) Biology of the ectomycorrhizal genus *Rhizopogon*. II. Patterns of host-fungus specificity following spore inoculation of diverse hosts grown in mono- and dual-culture. New Phytol 126:677–690

Paul EA, Clark FE (1990) Soil microbiology and biochemistry. Academic Press, San Diego, Calif