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A molecular survey of ectomycorrhizal hyphae in a California *Quercus–Pinus* woodland

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Abstract Ectomycorrhizal (ECM) hyphal communities have not been well characterized. Furthermore, there have been few studies where the ECM hyphal community is compared to fungi detected as sporocarps or ECM-colonized root tips. We investigated fungi present as hyphae in a well-studied California *Quercus–Pinus* woodland. Hyphal species present were compared to those found as sporocarps and ECM root tips at the same site. Hyphae were extracted from root-restrictive nylon mesh in-growth bags buried in the soil near mature *Quercus douglasii*, *Quercus wislizeni*, and *Pinus sabiniana*. Taxa were identified using PCR, cloning, and DNA sequencing of internal transcribed spacer and 28s rDNA. Among the 33 species detected, rhizomorph-forming ECM fungi dominated the hyphal community, especially species of Thelephoraceae and Boletales. Most fungi in soils near *Quercus* spp. and *P. sabiniana* were ECM basidiomycetes, but we detected two ECM ascomycetes and three non-mycorrhizal fungi. Many ECM species present as hyphae were also previously detected at this site as sporocarps (18%) or on ECM root tips (58%). However, the hyphal community was mostly dominated by different taxa than either the sporocarp or ECM root communities.

Keywords Boletales · Ectomycorrhizal fungi · Hyphae · Oak woodlands · Rhizomorphs · Thelephoraceae

Introduction

Ectomycorrhizal (ECM) fungal community studies have primarily sampled sporocarps or ECM root tips. In contrast, within the extensive literature on ECM fungi there are far fewer studies of ECM hyphae (Bastias et al. 2006; Genney et al. 2006; Kjøller 2006; Koide et al. 2005b; Korkama et al. 2007; Landeweert et al. 2005; Parrent and Vilgalys 2007; Peintner et al. 2007), and these fungal species pools have only recently been examined in the field (Anderson and Cairney 2007). Despite the lack of data on hyphal communities, we know that fungal hyphae are ubiquitous in soil and ecologically important. ECM hyphae (i.e., extra-radical mycelia) constitute a substantial proportion of microbial biomass (Högberg and Högberg 2002; Wallander et al. 2001) and are involved in several important processes: (a) nutrient uptake from (Smith and Read 2002), (b) mineral weathering (van Breemen et al. 2000), (c) litter decomposition (Leake et al. 2002), (d) host carbon storage (Simard et al. 2002), and CO_2 respiration (Heinemeyer et al. 2007).

There have been relatively few hyphal studies because ECM hyphae are challenging to study in the field. Hyphae are microscopic (ca 1–10 μm in diameter) and occupy opaque soil and leaf substrates; multiple hyphal species often grow intertwined with one another and cannot be

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morphologically distinguished (Leake et al. 2004). The development and use of in situ root-restrictive "in-growth" mesh bags have facilitated studies of ECM hyphal communities (Wallander 2006; Wallander et al. 2001) because in-growth bags allow easy extraction of hyphae for molecular analysis (Bastias et al. 2006; Landeweert et al. 2005).

Two recent studies have qualitatively compared ECM hyphal communities from mesh in-growth bags to the ECM communities colonizing roots. They found contrasting results; above- and below-ground communities were congruent in one study (Korkama et al. 2007), but dissimilar in the other (Kjøller 2006). Previous studies that compared fungi of ECM sporocarps and root tips generally report disparities between the above- and below-ground ECM communities (Dahlberg and Nylund 1997; Erland and Taylor 2002; Gardes and Bruns 1996). Sampling biases or molecular discrepancies such as internal transcribed spacer (ITS) variation may account for some of the discrepancies in species composition among ECM communities characterized by sporocarps, root tips, and hyphae (Horton 2002; Izzo et al. 2005; Kõljalg et al. 2000; Smith et al. 2007b). The few studies that compared ECM hyphal communities from bulk soil (as opposed to in-growth bags) with root tip and sporocarp communities found that the communities are usually different, but all three communities share similarly high levels of diversity (Landeweert et al. 2005; Peintner et al. 2007; Porter et al. 2008). However, in many ecosystems, ECM species dominant in one of the three communities (sporocarps, root tips, or hyphae) are often a small component of the other two communities (Horton and Bruns 2001; Porter et al. 2008; Smith et al. 2007a).

In low-elevation woodlands of California's Sierra Nevada, *Quercus douglasii* is the dominant ECM host but occurs over a wide geographic area with two other ECM hosts, *Q. wislizeni* and *Pinus sabiniana*. Three recent studies have thoroughly documented the diversity of ECM fungi as sporocarps and on ECM roots of *Q. douglasii*, *Q. wislizeni*, and *P. sabiniana* at a single woodland site (Morris et al. 2008; Smith et al. 2009, 2007a). These studies found that the *Quercus* spp. hosted a high proportion of Ascomycota whereas the *Pinus* ECM community was clearly dominated by Basidiomycota (Smith et al. 2009). All three ECM host communities had a high prevalence of ECM taxa with cryptic sporocarps (e.g., hypogeous and resupinate fungi). This well-documented research area offers an excellent opportunity to compare the dominant ECM fungal species in each community (e.g., sporocarps vs. ECM roots vs. hyphae). Since ECM fungi in each of these communities may be different, a comparison of these three species pools should provide a robust estimate of the entire ECM community (Anderson and Cairney 2007). Characterizing ECM community diversity is important because ECM species composition may affect host function and reproductive success via nutrient transport to the host, host drought tolerance, and seedling establishment (Courty et al. 2005; van Hees et al. 2005).

ECM hyphal communities have not been compared with both root tip and sporocarp communities from the same site. As a part of a larger study examining the ECM community in California woodlands, we harvested soil hyphae beneath mature trees of two *Quercus* spp. and *P. sabiniana* and compared the ECM hyphal species found with the ECM fungi detected on root tips and as sporocarps at the same site (Morris et al. 2008; Smith et al. 2009, 2007a). Based on previous sporocarp and root tip studies where differences in dominant species were found between communities, we hypothesized that the ECM hyphal community would also have different dominant species than the root tip and sporocarp communities. To test this hypothesis, we inserted root-restrictive, sand-filled in-growth bags in the upper soil horizon near three tree species. After 1 year, the in-growth bags were recovered, and molecular methods were used to characterize the extracted fungal hyphae.

Materials and methods

Study site

The study was conducted in the Koch Natural Area at the University of California Sierra Foothills Research and Extension Center (SFREC), in Browns Valley, CA, USA. The regional climate is Mediterranean, with cool wet winters and hot dry summers. Mean annual air temperature and precipitation are 15°C and 73 cm, respectively (Dahlgren and Singer 1994). Soils at the SFREC are fine-loamy, mixed, superactive Ultic Haploxeralfs and fine, mixed, superactive Typic Rhodoxeralfs (Dahlgren and Singer 1994, USDA NRCS http://websoilsurvey.nrcs.usda.gov/, October 13, 2008). The dominant ECM tree is *Q. douglasii* Hook & Arn. (blue oak), but it frequently co-occurs with *Quercus wislizeni* A. DC. (interior live oak) and *P. sabiniana* Douglas ex D. Don (foothill pine). At the Koch site, we selected *Q. douglasii*, *Q. wislizeni*, and *P. sabiniana* trees in a section that had been ungrazed for ≥45 years. *Q. wislizeni* and *P. sabiniana* grow intermixed, whereas *Q. douglasii* is found primarily in monodominant stands with a few *P. sabiniana* saplings. However, none of the *Q. douglasii* sampled for the study was within 30 m of the *P. sabiniana* saplings. In total the area spanned approximately 400 ha. No other ECM hosts were present at the site. A map of the hyphal sampling locations as well as the ECM root tip and sporocarp study sites (Morris et al. 2008; Smith et al. 2009, 2007a) is shown in Supplementary Data Figure S1.

Experimental design

In order to examine the species of ECM hyphae in soils at the site, in-growth bags (5 cm circumference × 10 cm length) were constructed by sealing the edges of 25 μm nylon mesh into a root-restrictive cylinder that allowed hyphal access. Each in-growth bag contained 300 g of sand (water leached, autoclaved medium-course) amended with a P-K fertilizer (0-10-10, 100 mg P/kg sand) to ensure that hyphae that encountered the bags would proliferate and provide us with adequate biomass for molecular analysis at the end of 1 year. No carbon source was added. We selected four trees of *Q. douglasii*, *Q. wislizeni*, and *P. sabiniana*, and at each tree we inserted two hyphal in-growth bags at canopy edge to a depth of 10 cm from the top of the mineral soil. Several extra in-growth bags were installed to check for hyphal growth throughout the year and to test molecular methods. The minimum distance between individual trees of the same species was 15 m. Trees were located at least 3 m from the nearest ECM host.

After 1 year in the ground (April 2004 to April 2005), in-growth bags were harvested, transported to the laboratory on ice, lyophilized, and stored at −20°C. To extract hyphae from the sand, each in-growth bag was thawed and emptied into a 500-mL wide-mouth container. Containers were shaken by hand for 15 s, causing the hyphae to form large clusters. Hyphae were extracted by repeatedly passing each sample through a 2 × 2 mm screen. Hyphae were examined under a dissecting microscope, and penetrating herbaceous roots were removed with forceps. Hyphae were weighed and stored at −20°C.

Molecular techniques

DNA was extracted from 30 mg subsamples of hyphae (Lee et al. 1988) using an Ultra Clean Soil DNA Isolation Kit (Mo Bio Laboratories, Solana Beach, CA, USA). ITS and partial 28s rDNA were amplified using PCR primers ITS1-F and LR3 (Gardes and Bruns 1993; Hopple and Vilgays 1994). PCR reactions consisted of 10× PCR Gold Buffer (Applied Biosystems, Foster City, CA, USA), 25 mM $MgCl_2$, 10 mM dNTPs, 5 μM of both primers, 100× BSA (New England Biolabs, Ipswich, MA, USA), 5 u/μL AmpliTaq Gold DNA Polymerase (Applied Biosystems), and 3 μL of DNA extract for a total volume of 50 μL per reaction. PCR products were screened using 1.5% agarose gels stained in SYBR green (Applied Biosystems). Successful amplicons were cloned using the TOPO TA Cloning Kit for Sequencing (Invitrogen, Carlsbad, CA, USA). Clones were grown overnight on plates with Luria–Bertani media. A total of 48 positive clones were selected, and of those we randomly chose 18 clones, which were sent for sequencing with ITS1-F by the UC Davis CAES Genomics Facility. The number of sequenced clones was based on preliminary runs that detected ca. three fungal taxa per in-growth bag suggesting that random sequencing of 18 clones should detect the most common species. Four ECM clones were also sequenced with the primer LR3 to further confirm their phylogenetic placement.

Identification of fungal taxa

ITS sequences were initially edited with BioEdit (Hall 1999) and Sequencher v 4.1 (Gene Codes Inc., Ann Arbor, MI, USA). BLAST searches were used to compare hyphal sequences to GenBank and a database of ECM roots and sporocarps collected at SFREC (Morris et al. 2008; Smith et al. 2009, 2007a). For sequences from speciose ECM groups (e.g., Thelephoraceae, *Inocybe*), we compiled alignments and manually examined sequence matches using Mesquite (Maddison and Maddison 2006) and ClustalX (Thompson 2003). Fungal taxa were identified and named as in Smith et al. (2007a). Microscopic observations of hyphal morphology suggested that multiple fungal species were present within each sample, introducing the possibility of chimeras (Gonzalez et al. 2005). To detect chimeras, all sequences that did not match existing SFREC sequences were examined using BLAST searches, sequence alignments, and the program RDP (Martin and Rybicki 2000) as in Smith et al. (2007a). Three chimeras were removed from the analyses.

Community analyses

We used data from 24 of the 25 experimental in-growth bags for community analyses (24 from the experimental design and one extra bag). Relative frequencies for a given tree species were calculated as the number of occurrences of each fungal taxon per in-growth bag divided by the total number of occurrences.

Results

Hyphal taxa from in-growth bags

After 1 year, half of the in-growth bags had a few small holes (ca. 1–3 mm in diameter) created by herbaceous roots. No ECM roots were present, so punctured in-growth bags were included in analyses. When extracting the hyphae from the in-growth bags, we encountered rhizomorphs, thick-walled septate hyphae, and thick-walled septate hyphae with clamp connections. On average there were 2.8 total taxa per bag and 2.4 ECM taxa per bag. The most diverse bag had six taxa per bag

and the least diverse had one taxon per bag. Molecular analysis revealed 33 fungal taxa from 25 in-growth bags (eight *Q. douglasii*, eight *Q. wislizeni*, eight *P. sabiniana*, and one extra bag; Table 1). Of the 32 taxa used for analysis, 27 (84.4%) were Basidiomycota and three (9.4%) were Ascomycota. Chytridiomycota and Zygomycota were each represented by one species (3.1%). There were 29 (90.6%) ECM taxa and three (9.4%) non-mycorrhizal (NM) taxa. Among the ECM taxa, 93% were Basidiomycota.

Hyphal species diversity

Bags near *P. sabiniana* and *Q. wislizeni* yielded 14 and 15 fungal taxa, respectively, while *Q. douglasii* had nine taxa. *Q. douglasii*, *Q. wislizeni*, and *P. sabiniana* trees each had two NM fungal associates. ECM taxa comprised the majority of fungal occurrences. The relative frequency of ECM vs. NM was similar near *Q. douglasii*, *Q. wislizeni*, and *P. sabiniana* (0.85, 0.83, and 0.89 respectively; Table 1).

Basidiomycota were dominant, while Ascomycota were found less frequently. Only two ECM ascomycetes were detected (Pyronemataceae sp. B and *Tricharina* sp. 2).

Fungal species overlapped among the three tree species. *Octaviania* sp. 1, Thelephoraceae2, and *Nectria* sp. 3 were found in bags near all three tree species (Table 2). Six taxa were found near two tree species. However, most fungal species were singletons (18 taxa, 56%); they were detected in one in-growth bag beneath one tree species (Fig. 1). Thelephorales and Boletales were the most frequently detected ECM orders. Most ECM species that we detected can form rhizomorphs (Fig. 1). BLAST results for fungi are presented in Table 2.

Hyphae vs. root tips vs. sporocarps

The ECM hyphal community from this study was compared to the ECM sporocarp and root tip communities previously detected with *Q. douglasii*, *Q. wislizeni*, and *P. sabiniana* (Morris et al. 2008; Smith et al. 2009, 2007a). Of the 33 taxa detected in the hyphal in-growth bags, 58% (19 taxa) were previously found on ECM roots, but only 18% of hyphal species (six taxa) were encountered as sporocarps (Table 3). Fifteen taxa were not previously detected at this site. Sampling intensity for each study can be found in Table S1.

Discussion

Ectomycorrhizal hyphae community inside of in-growth bags

Finding many ECM Basidiomycota and relatively few Ascomycota in the hyphal in-growth bag communities mirrors results in other recent hyphal studies in habitats with ECM plants, regardless of the ecosystem or whether bulk soil or in-growth bags are sampled (Koide et al. 2005a; Landeweert et al. 2003; Peintner et al. 2007). The number of ECM species per bag (2.4 species/bag) was concurrent with another hyphal in-growth bag study that reported 3.1 ECM species per bag (Kjøller 2006). We detected many of the same dominant phylogenetic groups that are frequently recovered from other hyphal studies (e.g., Thelephoraceae, Altheliales, and Boletales; Bastias et al. 2006; Kjøller 2006; Korkama et al. 2007; Parrent and Vilgalys 2007). Why do some fungal groups colonize in-

Table 1 Relative frequency of different fungal orders in hyphal in-growth bags near *Quercus douglasii*, *Q. wislizeni*, and *Pinus sabiniana* trees in a California woodland

Phylum	Order	Relative frequency			
		Q. doug.	*Q. wis.*	*P. sab.*	Total
Ectomycorrhizal					
Asco	Pezizales	0.000	0.045	0.071	0.043
Basidio	Agaricales	0.000	0.045	0.000	0.014
	Altheliales	0.000	0.000	0.036	0.014
	Boletales	0.350	0.182	0.179	0.229
	Russulales	0.000	0.045	0.036	0.029
	Sebacinales	0.000	0.045	0.000	0.014
	Thelephorales	0.500	0.500	0.536	0.514
Non-mycorrhizal					
Asco	Hypocreales	0.100	0.091	0.071	0.086
Chytrid	Spizellomycetales	0.050	0.000	0.000	0.014
Zygo	n/a	0.000	0.045	0.071	0.043
	Total	1.000	1.000	1.000	1.000

Asco Ascomycota, *Basidio* Basidiomycota, *Chytrid* Chytridiomycota, *Zygo* Zygomycota, *Q. doug. Quercus douglasii*, *Q. wiz. Q. wislizeni*, *P. sab. Pinus sabiniana*

Table 2 BLAST results and number of occurrences for hyphal taxa found near mature *Quercus* and *Pinus* trees

Taxon Name	Trophic status	Number of occurrences (bags)			DNA region	Highest informative BLAST result	Percent match
		Q. doug.	*Q. wiz.*	*P. sab.*			
Altheliaceae sp. A	EM	0	0	1	ITS and 28s	*Amphinema byssoides*	576/606 (95%)[a]
***Inocybe* sp. 10**	**EM**	**0**	**1**	**0**	**ITS**	***Inocybe* sp. KGP60**	**621/657 (94%)**
Lactarius xanthogalactus	EM	0	1	0	ITS	*Lactarius xanthogalactus*	685/687 (99%)
Melanogaster cf. *tuberiformis*	EM	1	3	0	ITS	*Melanogaster variegatus*	573/627 (91%)[b]
***Nectria* sp. 3**	**NM**	**2**	**2**	**2**	**ITS**	***Nectria mauritiicola***	**717/718 (99%)**
Octaviania sp. 1	EM	5	1	2	ITS	*Xerocomus cisalpinus*	471/500 (94%)[b]
Olpidiaceae1	**NM**	**0**	**1**	**2**	**ITS**	***Olpidium brassicae***	**259/272 (95%)**
***Pseudotomentella* sp. 1**	**EM**	**0**	**0**	**1**	**ITS**	***Pseudotomentella tristis***	**527/567 (92%)**
Pyronemataceae sp. B. (cf. *Pustularia*)	EM	0	0	1	ITS and 28s	*Pustularia patavina*	520/533 (97%)[a]
Rhizopogon arctostaphyli (src57)	EM	1	0	3	ITS	*Rhizopogon arctostaphyli*	659/660 (99%)
Russula cf. *amoenolens*	EM	0	0	1	ITS	*Russula amoenolens*	581/606 (95%)[b]
Sebacinales2	EM	0	1	0	ITS	Sebacinales	679/701 (96%)
Spizellomycetales1	**NM**	**1**	**0**	**0**	**ITS**	***Spizellomyces kniepii***	**203/212 (95%)**
Thelephoraceae2	EM	2	1	4	ITS	*Tomentella bryophila*	597/634 (94%)[b]
Thelephoraceae3	EM	0	1	0	ITS	*Tomentella stuposa*	595/621 (95%)[b]
Thelephoraceae6	EM	1	0	0	ITS	*Tomentella badia*	556/581 (95%)[b]
Thelephoraceae11	EM	1	0	0	ITS	*Tomentella ferruginea*	424/429 (98%)[b]
Thelephoraceae14	EM	2	0	0	ITS	*Tomentella atramentaria*	542/595 (91%)[b]
Thelephoraceae15	EM	0	2	0	ITS	*Tomentella badia*	595/619 (96%)[b]
Thelephoraceae29	**EM**	**0**	**0**	**2**	**ITS**	***Tomentella stuposa***	**539/554 (97%)**[b]
Thelephoraceae30	**EM**	**0**	**0**	**2**	**ITS**	***Tomentella badia***	**559/579 (96%)**[b]
Thelephoraceae31	**EM**	**0**	**1**	**1**	**ITS**	***Tomentella lilacinogrisea***	**527/560 (94%)**[b]
Thelephoraceae32	**EM**	**0**	**0**	**1**	**ITS**	***Tomentella lateritia***	**551/584 (94%)**[b]
Thelephoraceae33	**EM**	**0**	**1**	**0**	**ITS**	***Tomentella pilosa***	**544/562 (96%)**[b]
Thelephoraceae34	**EM**	**0**	**0**	**1**	**ITS**	***Tomentella sublilacina***	**531/583 (91%)**[b]
Thelephoraceae35	**EM**	**0**	**1**	**0**	**ITS**	***Tomentella ferruginea***	**539/564 (95%)**[b]
Tomentella badia[c]	EM				ITS	*Tomentella badia*	548/589 (93%)[b]
Tomentella cf. *atramentaria*	EM	1	0	0	ITS	*Tomentella atramentaria*	577/587 (98%)[b]
Tomentella sp. (src755)[d]	EM	0	1	0	ITS	*Tomentella botryoides*	544/583 (93%)[b]
Tomentella sp. (src820)[d]	EM	0	1	0	ITS	*Tomentella bryophila*	580/637 (91%)[b]
Tomentella sp. (src824)	EM	3	0	0	ITS	*Tomentella fuscocinerea*	435/467 (93%)[b]
Tomentella sp. (src834)	EM	0	2	3	ITS	*Tomentella badia*	555/582 (95%)[b]
Tricharina sp. 2	EM	0	1	1	ITS	*Geopora cooperi*	494/561 (88%)[e]

Taxa not previously found at UC Sierra Foothills Research and Extension Center are in bold

Agar Agaricales, *Alth* Altheliales, *Bol* Boletales, *Pez* Pezizales, *Rus* Russulales, *Seb* Sebacinales, *Thel* Thelephorales, *Hyp* Hypocreales, *Spiz* Spizellomycetales, *n/a* not available, *Q. doug. Quercus douglasii*, *Q. wiz. Q. wislizeni*, *P. sab. Pinus sabiniana*

[a] Identification based on 28s rDNA

[b] Identification based on UNITE database

[c] Taxa found in extra *P. sabiniana* bag and not used for relative frequency calculations

[d] Found previously in an unpublished study by M. E. Smith and not used in percent overlap calculations for species groups

[e] This sequence was highly similar to *Tricharina* sp. 1 from Smith et al. (2007a), which was linked to *Tricharina* with 28s rDNA. Also, Geopora and *Tricharina* are closely related genera (Perry et al. 2007)

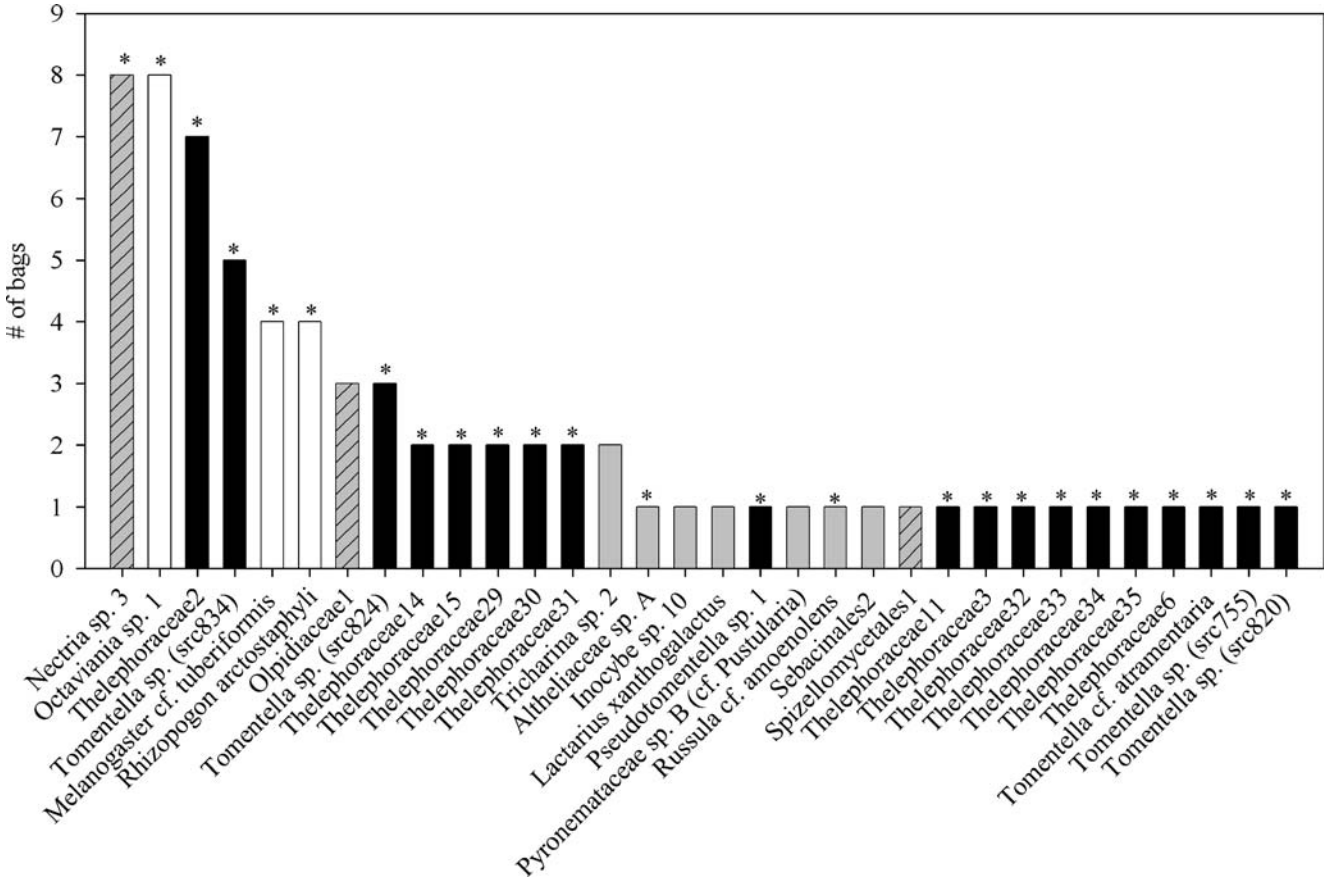

Fig. 1 Frequency of fungal taxa detected in hyphal in-growth growth bags. *Black bars* represent taxa in Thelephoraceae, *white bars* represent taxa in Boletales, and *gray bars* represent taxa from less frequently detected taxonomic groups. *Diagonal stripes* indicate non-mycorrhizal species; all others are ectomycorrhizal. *Asterisks* represent potential for a species to form rhizomorphs

growth bags, regardless of ecosystem, soil type, host, or other variables? The answer may relate to the strategies that ECM fungi use to proliferate in soil from the host root tip, in other words, foraging strategies of different phylogenetic groups (i.e., "exploration types"; Agerer 2001; Kjøller 2006).

In this study, the Thelephoraceae and Boletales were the most diverse and frequently encountered groups. Both Thelephoraceae ("medium-distance smooth exploration types") and Boletales ("long-distance exploration types") form abundant extramatrical hyphae and differentiated rhizomorphs to explore distant soil resources from the host root tip (Agerer 2001, 2006). Although not as frequent or diverse as Thelephoraceae and Boletales, the rhizomorph-forming Altheliales were also detected, but only with *P. sabiniana*. Many species in these phylogenetic groups (Boletales, Thelephoraceae, and Altheliales) often produce sporocarps on decaying wood or litter away from soil (Binder and Hibbet 2006; Kõljalg et al. 2000; Larsson et al. 2004). Taxa in the Boletales also form large, long-lived genets (Hirose et al. 2004), sclerotia *in situ* (Smith and Pfister 2009), and at least some Boletales and Thelephoraceae are early successional, pioneer species (Ashkannejhad and Horton 2006; Peay et al. 2007; Taylor and Bruns 1999). These traits suggest that some species from these groups grow quickly over long distances and maintain some saprophytic ability, despite being mycorrhizal (Buée et al. 2007; Koide et al. 2008).

Non-mycorrhizal hyphal community inside of in-growth bags

Two of the three NM species, *Nectria* sp. (Ascomycota) and Olpidiaceae1 (Zygomycota), were detected in multiple in-growth bags. *Nectria* sp., a NM fungus that most likely forms rhizomorphs (Goos 1962; Went 1973), was frequently found inside in-growth bags near all three tree species. *Nectria* have been implicated as saprotrophs, endophytes, plant pathogens, and animal pathogens (asexual state: *Acremonium*) and display a very wide range of nutritional modes, so it is difficult to assess why *Nectria* sp. was so common in our in-growth bags. In a separate study, ITS sequences of the same *Nectria* sp. were routinely recovered from healthy ECM roots of naturally established *Q. douglasii* seedlings (M.E. Smith unpublished), suggesting

Table 3 Presence (+) and absence (−) of shared hyphal fungal taxa on root tips and sporocarps by hyphal in-growth bag tree host from UC SFREC

Hyphal taxa	ECM root tips			
	Sporocarp (Smith et al. 2007b)	*Q. doug.* (Smith et al. 2007b; Morris et al. 2008)	*Q. wis.* (Morris et al. 2008)	*P. sab.*(Smith et al. 2009)
Quercus douglasii				
Melanogaster cf. *tuberiformis*	++	−	+	−
Octaviania sp. 1	−	+	−	−
Rhizopogon arctostaphyli	++	−	−	++
Thelephoraceae2	−	+	+	−
Thelephoraceae6	−	+	+	−
Thelephoraceae11	−	+	−	−
Thelephoraceae14	−	−	+	−
Tomentella cf. *atramentaria*	+	+	−	−
Tomentella sp. (src824)	+	++	+	−
Quercus wislizeni				
Inocybe sp. 10	−	−	+	−
Lactarius xanthogalactus	++	−	++	−
Melanogaster cf. *tuberiformis*	++	−	+	−
Octaviania sp. 1	−	+	−	−
Sebacinales2	−	++	−	−
Thelephoraceae2	−	+	+	−
Thelephoraceae3	−	+	−	−
Thelephoraceae15	−	−	+	−
Tomentella sp. (src834)	+	−	+	+
Tricharina sp. 2				+
Pinus sabiniana				
Altheliaceae sp. A	−	−	−	+
Octaviania sp. 1	−	+	−	−
Pyronemataceae sp B.	−	−	−	++
Rhizopogon arctostaphyli	++	−	−	++
Russula cf. *amoenolens*	−	+	−	+
Thelephoraceae2	−	+	+	−
Tomentella sp. (src834)	+	−	+	+
Tricharina sp. 2				+

Root tip data are listed by host. Most frequently taxa are indicated by "+ +"

that this species may be a common root endophyte at this site. Similarly, Murat et al. (2005) frequently detected DNA sequences of Nectriaceae sp. from healthy ECM roots in an Italian natural truffle-ground, suggesting that Nectriaceae root endophytes may be widespread in Mediterranean habitats. In contrast to *Nectria* sp., Olpidiaceae1 (*Olpidium* sp.) is probably a root parasite (Campbell and Sim 1994; White et al. 2006). The frequency of Olpidiaceae1 within our in-growth bags is probably explained by the fact that they produce large numbers of motile spores that disperse through the soil and then persist as long-lived resting spores (Campbell 1985).

ECM hypha vs. sporocarp vs. root tip communities

Certain ECM species were notable because they were common in one or two of the three different fungal communities (hyphae vs. root tips vs. sporocarps), but were rare or absent from others (Morris et al. 2008; Smith et al. 2009, 2007a). For example, sporocarps of *Octaviania* were never detected in 4 years of sampling, and this species was rare on ECM roots (e.g., present in two out of 166 sampled root cores on three host plant species), but it was commonly detected in the hyphal bags. In contrast, sporocarps and hyphae of *Melanogaster* were frequently

detected, but this species was rare on roots. *Rhizopogon arctostaphyli* was commonly detected as sporocarps, hyphae, and on roots. Thelephoraceae was the only phylogenetic group that was prevalent in all three communities (hyphae vs. root tips vs. sporocarps) and on both *Quercus* and *Pinus*, although few individual species were found in all communities.

Ascomycota were not common as hyphae, but were diverse and prevalent on ECM root tips and as sporocarps under *Quercus* and, to a lesser degree, with *P. sabiniana* (Morris et al. 2008; Smith et al. 2009, 2007a). Several genera that were commonly detected on root tips or as sporocarps at this site were notably absent from the hyphal community: *Cenoccocum geophilum*, *Genea*, and *Tuber* (see also Douhan and Rizzo 2005). *Genea* has not yet been reported from a hyphal study, *Tuber* was reported in one in-growth bag study (Parrent and Vilgalys 2007), whereas *C. geophilum* has been reported in both in-growth bag (Korkama et al. 2007) and bulk soil hyphae studies (sampled without bags; Genney et al. 2006; Koide et al. 2005a). The hyphae of most ascomycetes grow as "short distance exploration types," which have abundant hyphae, but do not proliferate extensively into the soil from the host root tip (Agerer 2001). It is possible that our bags were not installed in close proximity to roots, and placement may explain low numbers of ascomycetes from in-growth bags in our study.

Published studies report small overlap between sporocarps and ECM root tip communities (22%; Peter et al. 2001), between root tips and bulk soil hyphae (20%; Peintner et al. 2007), and between sporocarps and bulk soil hyphae (7%; Porter et al. 2008). These studies concluded that ECM aboveground, root tip, and soil communities are distinct. Smith et al. (2007a) as well as Nieto and Carbone (2009) had 46% and 38% overlap, respectively, between sporocarp and ECM root tip taxa, suggesting that sporocarp sampling methods and sampling intensity may account for some of these observed patterns. Although sampling intensity differed in the hyphal study compared to the sporocarp and root tip studies, there was a high percentage of overlap between root tips and hyphal species. Low sporocarp sampling effort beneath *Pinus* (Smith et al. 2009) may partially account for the smaller overlap between ECM hyphae and sporocarps in this study. In particular, long-term and intensive sporocarp sampling is required to fully document fungal communities (Porter et al. 2008; Straatsma et al. 2001). However, since many of the documented hyphal species were Thelephoraceae, which produces cryptic resupinate sporocarps that are very difficult to find and identify (Kõljalg et al. 2000), it is not surprising that there was low overlap between the hyphal and sporocarp communities.

Conclusion

This is the first study to examine ECM hyphal communities in a Mediterranean woodland habitat and the first study where ECM hyphal communities have been directly compared to the ECM species found as sporocarps and on root tip from the same site. We detected a diverse assemblage of ECM species in hyphal in-growth bags and despite the intensive sampling from previous studies found several new ECM taxa. The dominant taxa in hyphal bags were rhizomorph-forming basidiomycetes particularly taxa in the Thelephorales and Boletales. Although many of these species had been previously detected at this site, the dominant ECM hyphal species were generally not abundant on ECM roots or as sporocarps. Despite the fact that Ascomycota were frequently detected on ECM roots and as sporocarps at this site, we only detected two ECM ascomycetes from our root in-growth bags. This finding suggests that hyphal bags selectively detect fungi that form rhizomorphs and explore the soil using long-distance and medium-distance exploration strategies. The high diversity of ECM species and the large number of singleton taxa indicate that more sampling is needed before definitive inferences about host associations can be made.

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