

There is high potential for the formation of common mycorrhizal networks between understorey and canopy trees in a mixed evergreen forest

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Summary

1 The patterns of ectomycorrhizal (ECM) host specificity between understorey and canopy trees were investigated in three mixed evergreen forest stands in northern coastal California. ECM root tips from the dominant canopy (*Pseudotsuga menziesii*) and understorey (*Lithocarpus densiflora*) trees were sampled from 18 soil cores (six per stand) and identified using molecular techniques (PCR, RFLP, and DNA sequencing of the rDNA ITS region).

2 We found 56 ECM taxa; 17 on both hosts, 27 solely on *Pseudotsuga* and 12 on *Lithocarpus*. There were no significant differences in ECM taxon richness or diversity across stands, although ECM taxon richness was significantly higher on *Pseudotsuga* than *Lithocarpus*. ECM taxa similarity across stands was low.

3 Multiple-host ECM taxa had significantly higher abundance than single-host ECM taxa and 13 of the 17 multiple-host ECM taxa were present on both hosts within at least one core. Twelve of the 14 cores had at least one ECM taxon that was present on both hosts, although the specific taxon varied between cores and stands. In addition, shared ECM taxa often had unequal relative abundances on the two hosts.

4 Taken together, our results suggest that there is high potential for common mycorrhizal networks to form between *Lithocarpus* understoreys and *Pseudotsuga* canopies in mixed evergreen forests.

Key-words: common mycorrhizal networks, fungal host specificity, *Lithocarpus densiflora*, mixed evergreen forest, *Pseudotsuga menziesii*

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Introduction

Several studies have documented that ectomycorrhizal (ECM) hyphae can physically connect plants (Trappe & Fogel 1977; Brownlee *et al.* 1983; Finlay & Read 1986a; Finlay & Read 1986b), and thereby create ECM networks within or between plant species. These networks may have significant ecological implications for plant interactions by providing access to larger nutrient pools (Newman 1988), mediating competition (Perry *et al.* 1989), and allowing for resources to be transferred between linked individuals (Finlay & Read 1986a; Finlay & Read 1986b; Arnebrant *et al.* 1993; Simard *et al.* 1997).

Molina *et al.* (1992) identified a range of host specificity among ECM fungi and concluded that, overall,

host specificity was moderate to low as required if they are to form networks between plants. This conclusion was based largely on laboratory and glasshouse studies and on observations of fruiting behaviour (Grand 1968; Molina & Trappe 1982a; Molina & Trappe 1982b). Massicotte *et al.* (1999), for example, found that conifer and hardwood species shared many of the same ECM morphotypes when grown together in pots. However, the ecological significance of these results is unclear because of the wider range of niches potentially present in natural soil environments (Harley & Smith 1983).

Recent field studies indicate that ECM host specificity between canopy trees is low (Horton & Bruns 1998; Cullings *et al.* 2000). There also appears to be low ECM host specificity between understorey and canopy plants (Visser 1995; Jonsson *et al.* 1999). Visser (1995) documented that many of the ECM fungi associated with *Pinus banksiana*, the canopy dominant tree, were also present on *Arctostaphylos uva-ursi*, a common understorey plant in mature *P. banksiana* woodlands.

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Jonsson *et al.* (1999) found the dominant fungal species in *Pinus sylvestris* forests made up 92% and 73% of the *P. sylvestris* seedling and mature *P. sylvestris* tree mycorrhizas, respectively. Although both studies examined host specificity between understorey and canopy plants, it is still not clear whether understorey species with the potential to develop into canopy species can be linked into ECM networks with a different canopy species. Interspecific connections between the forest understorey and canopy may be important for maintaining forest communities following disturbance (Perry *et al.* 1992) and for long-term forest structure (Newberry *et al.* 2000).

Determining the level of host specificity in ECM assemblages is an essential first step towards investigating the ecological role of ECM networks. If there is a high potential for ECM networks, further research can focus on how actual ECM connections influence interspecific interactions between hosts. In this study, we sampled the ECM assemblages associated with a *Pseudotsuga menziesii* canopy and *Lithocarpus densiflora* understorey in three adjacent stands. Our objectives were (i) to document the ECM assemblages in mixed evergreen forest stands and examine their structure, diversity, and similarity, and (ii) to determine the potential for common ECM networks between understorey and canopy tree species.

Materials and methods

SITE CHARACTERISTICS

The study was conducted along Bolinas ridge in the Marin Municipal Water District watershed on Mount Tamalpais (37°54' N, 122°37' W) in southern Marin County, California, USA. The 9000-ha watershed encompasses a wide variety of vegetation types, including grassland, chaparral and mixed evergreen forest (Parker 1991). The mixed evergreen forest canopy is dominated by *P. menziesii* (hereafter referred to as *Pseudotsuga*), with *L. densiflora* (hereafter referred to as *Lithocarpus*), *Quercus agrifolia*, *Quercus chrysolepis*, *Arbutus menziesii*, *Umbellularia californica* and *Sequoia sempervirens* also present (Horton *et al.* 1999). The understorey is dominated by *Lithocarpus* and *Q. agrifolia*, with very few *Toxicodendron diversifolia* and *Rosa* spp. present. *Pseudotsuga* juveniles are absent from the understorey of the closed canopy forest. The study area is characterized by a Mediterranean climate with seasonal summer drought and average annual precipitation of 1250 mm (Dunne & Parker 1999). Soils in the study area are a composite of the Centesima loam and Barnabe very gravelly loam series, which are derived from weathered sandstone or shale (USDA 1990). Floral nomenclature follows Hickman (1993).

SAMPLING

In spring 2001, three forest stands were selected for sampling. Each stand was approximately 1000 m² in

area and separated by at least 75 m from the other sampled stands. In each stand, all *Lithocarpus* individuals less than 1 m in height and 1 cm in stem diameter at ground level were tagged. *Lithocarpus* individuals located at least 10 m away from any non-*Pseudotsuga* canopy tree and at least 5 m away from any other marked *Lithocarpus* individuals in the stand were flagged ($n = c. 20$ per stand) and six of these in each stand were randomly selected for sampling. Preliminary samples showed that roots of *Lithocarpus* did not extend further than 30 cm from the plant stem, whereas *Pseudotsuga* roots were ubiquitously distributed in the soil (P. Kennedy and A. Izzo, unpublished data). Soil cores (10 cm diameter \times 30 cm depth) were therefore taken directly over the selected *Lithocarpus* individuals to ensure sampling a sufficient quantity of *Lithocarpus* ECM root tips. Soil cores were bagged and stored at 4 °C for a maximum of 72 h before being processed.

For ECM root tip sampling, each core was placed in a large tray and thoroughly homogenized. Large clusters of roots were carefully cut into approximately 1-cm pieces and then re-mixed back into the core. A randomly selected subsample (400–1200 cm³) was removed, gently washed over a 0.355-mm sieve, and placed under a dissecting microscope for ECM root tip sorting. Due to the small size of *Lithocarpus* root tips, ECM biomass estimates post-lyophilization were not feasible. *Pseudotsuga* ECM root tip length and biomass were highly correlated ($r^2 = 0.88$, $n = 248$, $P < 0.05$) and because the proportions and shapes of *Pseudotsuga* and *Lithocarpus* ECM root tips were similar, we used root tip length as our measure of ECM abundance for both hosts. From preliminary tests of morphotype accumulation, we had determined that sampling root lengths of 75 mm for *Lithocarpus* and 350 mm for *Pseudotsuga* were sufficient to sample the dominant ECM morphotypes in a core (i.e. all those morphotypes that had 25% or greater abundance). Therefore, to ensure that all the dominant ECM morphotypes were sampled, we examined approximately 100 and 400 mm of *Lithocarpus* and *Pseudotsuga* ECM root tips per core, respectively. The hosts were easily distinguishable based on size differences, with *Lithocarpus* ECM root tips being much smaller than those of *Pseudotsuga*. ECM root tips were sorted by host and coarse morphotype (distinguished by colour, texture, and the presence or absence of emanating hyphae), and the total length of each morphotype was then quantified. We separated ECM morphotypes to the maximum extent possible, knowing that the molecular analyses would allow us to regroup any ECM taxon that had been mistakenly separated.

MOLECULAR IDENTIFICATION OF ECM FUNGI AND PLANT HOSTS

From the 18 cores, we separated 442 ECM samples (248 from *Pseudotsuga* and 194 from *Lithocarpus*). DNA was extracted from one root tip of each ECM

morphotype from each core. Due to their smaller size, fresh *Lithocarpus* ECM root tips were pulverized for 30 seconds with a 3-mm glass bead in a mini-bead beater (Biospec) and suspended in 1000 μ L CTAB/PVPP buffer (2% CTAB, 1% PVPP, 0.1 M Tris pH 8.0, 1.4 M NaCl, 0.02 M EDTA). Following a 60-minute incubation at 65 °C, samples were vortexed with 600 μ L chloroform : isoamyl alcohol (24 : 1) and centrifuged (13 000 g) for 5 minutes. Total nucleic acids were precipitated from the aqueous phase by adding 0.6 \times volumes of isopropanol followed by a 20-minute centrifugation (13 000 g). The resulting pellet was washed with 70% ethanol, dried at room temperature, and then resuspended in 50 μ L sterile TE buffer (pH 8). *Pseudotsuga* ECM DNA was extracted in the same way but after the 65 °C incubation, we used a Qiagen DNeasy kit (Qiagen Inc., Valencia, CA, USA) following manufacturer's instructions. The internally transcribed spacer regions of the rDNA were amplified using the primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990). PCR was performed in a PTC-100 thermal cycler (MJ Research Inc., Waltham, MA, USA) in conditions previously described (Gardes & Bruns 1993). We used fluorescent labelling to ensure detection of all bands in subsequent reactions. Fluorescent deoxynucleotides (Applied Biosystems) were added to a final concentration of 0.5–2.0 μ M in the PCR reaction.

Each PCR sample was then digested for fluorescent restriction fragment length polymorphism (RFLP) using *Hinf*1 and *Alu*1. The restriction digests were analysed on acrylamide gels using an ABI 377 Genetic Analyser and Genescan 3.1 software (Applied Biosystems). Samples whose RFLP fragment sizes matched within \pm three base pairs were considered to be the same RFLP type. RFLP patterns can sometimes be misleading due to co-occurrence and coamplification of multiple ECM fungi on a single root tip, which produce combined RFLP patterns. While many background bands are often not visible in agarose gel analyses, fluorescent analyses are much more sensitive to these bands and therefore more prone to generating errant RFLP patterns. Sequencing each unique RFLP type allowed us to detect and resolve all of these discrepancies and to identify and correct for heterozygosity in the ITS region. Single-pass sequencing of the ITS1/5.8S/ITS2 region of the rDNA was performed on an ABI3100 Genetic Analyser and analysed with Sequence Analysis 3.4.1 software (Applied Biosystems Foster City, CA, USA). Of the 168 unique RFLP types, 48 were revealed to be artifactual due to coamplification. For those 48 RFLP types, we either analysed a new sample of the same type or excluded the type from the final analyses if there were no other samples available. RFLP types with identical sequences were grouped into one ECM taxon. All taxa known to be root endophytes were excluded. The mycorrhizal status of *Phialophora* is still unclear; however, it has been shown to benefit plants under certain conditions (Jumpponen 2001), and was therefore included in the final analyses. RFLP

analysis can fail to distinguish closely related taxa (Karen *et al.* 1997) and thus might overestimate the potential for ECM networks. To assess the validity of our RFLP groupings, we sequenced five dominant RFLP types at least twice. In all cases, our RFLP types only grouped ECM taxa with the same ITS sequence.

ITS sequences were initially grouped based on similar BLAST (Altschul *et al.* 1990) affinities, aligned using ClustalX (Thompson *et al.* 1997), and then manually adjusted. ECM taxon names were designated based conservatively upon the taxonomic level supported from the BLAST results. For example, a RFLP type sequence whose BLAST score sequence grouped closely with both *Thelephora* and *Tomentella* sequences would be considered in the family Thelephoraceae. Multiple RFLP types in the same genus of family were given individual numbers to signify genetic differences at roughly the species level.

While the size differences between *Lithocarpus* and *Pseudotsuga* ECM root tips were almost always obvious under the dissecting scope, we did occasionally observe ECM size ranges that slightly overlapped. Because host misidentifications would lead us to overestimate the potential for mycorrhizal networks, we used molecular methods to confirm host identity on a subset of 40 samples. The trnL intron region of the host cpDNA was amplified using the trnL_c and trnL_d primers (Taberlet *et al.* 1991) with the same PCR conditions as above. The trnL intron was digested with *Alu*1 according to manufacturer's conditions. RFLP patterns were visualized by agarose gel electrophoresis and ethidium bromide staining. All samples were found to match the host they were assigned to using ECM morphological characters alone.

STATISTICAL ANALYSES

The rate of successful amplification in 14 cores was $81 \pm 6\%$ (mean \pm SE) for *Pseudotsuga* ECM samples and $56 \pm 6\%$ for *Lithocarpus* ECM samples, but in four cores less than 25% of the ECM samples from one of the two hosts amplified. Data on the poorly amplified host (*Lithocarpus* in three cores and *Pseudotsuga* in one core) were removed from all analyses. Rarefaction (Krebs 1999) was used to estimate ECM taxon richness and diversity for each core. This method corrects for differences in the length of a host's ECM root tips that were examined per core. Samples were rarefied to a size of 76 mm of ECM root tip per core, which was the minimum length of either host species root tips examined from each core. ECM taxon diversity was calculated using Simpson's index of diversity (Krebs 1999). To test whether ECM taxon richness or diversity differed between stands and hosts, we conducted two two-way mixed model ANOVAs, with stand as a random factor and host a fixed factor. Prior to analysis, ECM taxa richness and diversity were tested for homoscedasticity using Cochran's *C*-test and determined to be homogeneous.

To assess if multiple-host ECM taxa occurred with higher frequency or in greater abundance across all 18 cores than single-host ECM taxa, we conducted two Mann–Whitney *U*-tests. Because we could not ascertain if taxa found only once were truly single-host, these analyses were limited to only those ECM taxa that were encountered at least twice. Multiple-host taxa were defined as those found on both host species and single-host taxa as those found on only one of the two host species. In these analyses, all single-host ECM taxa on *Pseudotsuga* and *Lithocarpus* were combined for comparisons with multiple-host ECM taxa.

The similarity of the ECM composition among the three stands was compared using the Sorenson's coefficient (Krebs 1999). Sorenson values were calculated for all ECM within a stand and separately for ECM on each of the hosts. To test whether the proportion of ECM taxa shared within each core was higher on *Lithocarpus* than on *Pseudotsuga*, we used a paired two-tailed *t*-test. For this analysis, we defined the proportion of ECM taxa shared as the proportion of the total ECM length in each core that belonged to ECM taxa that were found on both hosts within that core. Variances in the proportion shared between hosts were homogeneous as determined by an *F*-test.

We also tested whether the relative abundance of the multihost ECM taxa differed on the two hosts. For these analyses, we compared the relative abundances of each multiple-host ECM taxon found in at least two cores on each host using paired two-tailed *t*-tests. We hypothesized there would be no difference in the relative abundance on the two hosts. The relative abundances were arcsine transformed to improve homogeneity. All statistics were considered significant at $P = 0.05$.

Results

COMMUNITY STRUCTURE

A total of 56 ECM taxa were found in the three stands (Table 1). Of these, 27 were on *Pseudotsuga*, 12 were on *Lithocarpus*, and 17 were found on both hosts. Mean ECM richness and diversity was not significantly different between stands (richness: $F_{2,25} = 0.353$, $P = 0.7059$; diversity: $F_{2,25} = 0.514$, $P = 0.604$). However, *Pseudotsuga* had significantly higher ECM richness than *Lithocarpus* ($F_{1,2} = 840.443$, $P = 0.001$) (Fig. 1). ECM diversity did not differ significantly between the two hosts ($F_{1,2} = 2.635$, $P = 0.246$) (Fig. 1b) and there was no significant interaction between host and stand (richness: $F_{2,25} = 0.052$, $P = 0.951$; diversity: $F_{2,25} = 0.329$, $P = 0.722$).

The similarity of the ECM assemblages among the three stands was relatively low (Table 2). When analysing the whole ECM assemblage (including taxa on both *Pseudotsuga* and *Lithocarpus*), the average pairwise coefficient of similarity was 0.35 ± 0.03 (mean \pm SE) between stands. When the stands were analysed by each host separately, the stands had lower average pair-

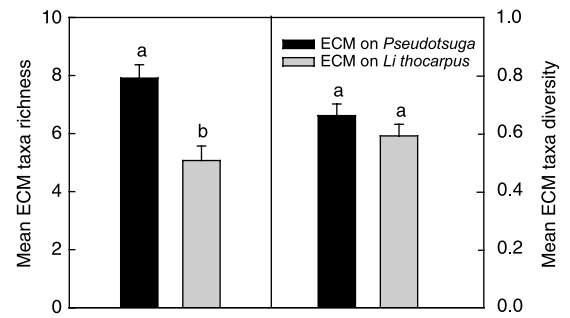


Fig. 1 Mean ECM taxa richness and diversity on *Pseudotsuga menziesii* and *Lithocarpus densiflora*. Different letters indicate a significant difference ($P < 0.05$).

wise similarity (*Pseudotsuga* 0.32 ± 0.04 , *Lithocarpus* 0.20 ± 0.11). As some ECM taxa were present on both hosts but not in the same stand, values calculated with both hosts combined were slightly higher than values calculated for each host separately. There was no clear relationship between the distance that separated stands and their similarity in ECM composition. For example, stands D and H were consistently more similar in ECM composition than stands D and T, although stands D and T were much closer.

HOST SPECIFICITY

Multiple-host ECM taxa represented 62% of the total abundance (i.e. ECM root tip length), while single-host ECM taxa on *Pseudotsuga* and *Lithocarpus* represented 27% and 11%, respectively. Multiple-host ECM taxa had significantly higher abundance than single-host ECM taxa ($U = 58$, $P = 0.005$), but the frequency of occurrence was not significantly different ($U = 41.5$, $P = 0.27$). The majority of ECM taxa had low abundance and occurred in only one core (Fig. 2).

There was variation in the abundance of multiple-host ECM taxa on the two hosts (Fig. 3). Of the 17 multihost taxa, 11 had greater abundance on *Lithocarpus* (e.g. *Russula* 5) and six on *Pseudotsuga* (e.g. Agaricales 7), but none of the differences were significant (in all paired tests, $P > 0.05$).

Thirteen of the 17 multiple-host ECM fungi were present on both hosts within at least one core, suggesting that the majority of the multiple-host ECM taxa had the potential to form networks between the two hosts (Table 1). In addition, 12 of the 14 cores (86%) had at least one taxon that was present on both hosts (Table 3). However, the proportion of shared ECM taxa varied among cores and stands. The average proportion of shared ECM taxa was somewhat higher on *Lithocarpus* (0.29) than *Pseudotsuga* (0.22), but not significantly ($t = 0.88$, d.f. = 13, $P = 0.39$). The identity of shared ECM taxa also varied between cores. For example, *Russula* 5 and Agaricales 7 were shared in multiple cores, while other taxa such as *Thelephora* 1 were shared at only one core.

Table 1 List of ectomycorrhizal (ECM) taxa sampled in three mixed evergreen forest stands on Mt Tamalpais, California, in 2001. The closest BLAST match represents the most accurate taxonomic match between our sample sequences and those in the NCBI database. The BLAST expected value represents the number of sequence matches expected by random chance (the smaller the value, the better the match between our sample sequences and those in the NCBI database)

ECM Taxa	GenBank Accession No.	Closest BLAST match	BLAST expected value	Number of hosts	Occurred in same core
Agaricales 1	AY310819	Cortinariaceae	$1.0 \times e^{-88}$	<i>Pseudotsuga</i>	No
Agaricales 2	AY310820	Cortinariaceae	$1.0 \times e^{-93}$	Both hosts	No
Agaricales 3	AY310821	Cortinariaceae	$1.0 \times e^{-90}$	<i>Lithocarpus</i>	No
Agaricales 4	AY310822	Cortinariaceae	$1.0 \times e^{-84}$	Both hosts	Yes
Agaricales 5	AY310823	Agaricales	$1.0 \times e^{-80}$	Both hosts	Yes
Agaricales 6	AY310824	Agaricales	$1.0 \times e^{-78}$	<i>Pseudotsuga</i>	No
Agaricales 7	AY310825	Tricholomaceae	$1.0 \times e^{-87}$	Both hosts	Yes
Agaricales 8	AY310826	Agaricales	$1.0 \times e^{-85}$	Both hosts	Yes
Agaricales 9	AY310827	Agaricales	$1.0 \times e^{-86}$	<i>Pseudotsuga</i>	No
Agaricales 10	AY310828	Agaricales	$1.0 \times e^{-86}$	<i>Pseudotsuga</i>	No
Agaricales 11	AY310829	Cortinariaceae	$1.0 \times e^{-89}$	Both hosts	No
Ascomycota 1	AY310830	Ascomycota	0	<i>Pseudotsuga</i>	No
Ascomycota 3	AY310831	Ascomycota	$1.0 \times e^{-09}$	<i>Pseudotsuga</i>	No
Ascomycota 4	AY310832	Ascomycota	$1.0 \times e^{-78}$	<i>Pseudotsuga</i>	No
Ascomycota 5	AY310833	Ascomycota	$1.0 \times e^{-65}$	Both hosts	Yes
Ascomycota 6	AY310834	Ascomycota	$1.0 \times e^{-82}$	Both hosts	Yes
<i>Athelia</i>	AY310835	<i>Athelia</i>	$1.0 \times e^{-93}$	<i>Pseudotsuga</i>	No
Basidiomycota 1	AY310836	Basidiomycota	$1.0 \times e^{-87}$	Both hosts	No
Basidiomycota 2	AY310837	<i>Sebacina</i>	0	<i>Pseudotsuga</i>	No
Basidiomycota 3	AY310838	Basidiomycota	$1.0 \times e^{-81}$	Both hosts	Yes
<i>Cenococcum</i>	AY310839	<i>Cenococcum</i>	0	<i>Lithocarpus</i>	No
<i>Clavulina</i>	AY310840	<i>Clavulina</i>	0	<i>Pseudotsuga</i>	No
Corticaceae	AY310841	Atheliaceae	$1.0 \times e^{-130}$	<i>Lithocarpus</i>	No
<i>Hymenogaster</i>	AY310842	<i>Hymenogaster</i>	0	<i>Lithocarpus</i>	No
<i>Lactarius</i> 1	AY310843	<i>Lactarius</i>	0	<i>Pseudotsuga</i>	No
<i>Lactarius</i> 2	AY310844	<i>Lactarius</i>	0	<i>Pseudotsuga</i>	No
<i>Macowanites</i>	AY310845	<i>Macowanites</i>	0	<i>Lithocarpus</i>	No
<i>Otidea</i> 2	AY310846	<i>Otidea</i>	0	<i>Pseudotsuga</i>	No
<i>Phialophora</i> 1	AY310847	<i>Phialophora</i>	0	<i>Pseudotsuga</i>	No
<i>Phialophora</i> 2	AY310848	<i>Phialophora</i>	0	Both hosts	No
<i>Rhizopogon</i> 1	AY310849	<i>Rhizopogon</i>	0	<i>Pseudotsuga</i>	No
<i>Rhizopogon</i> 2	AY310850	<i>Rhizopogon</i>	0	<i>Pseudotsuga</i>	No
<i>Russula</i> 1	AY310851	<i>Russula</i>	$1.0 \times e^{-111}$	<i>Pseudotsuga</i>	No
<i>Russula</i> 2	AY310852	<i>Russula</i>	$1.0 \times e^{-137}$	Both hosts	Yes
<i>Russula</i> 3	AY310853	<i>Russula</i>	$1.0 \times e^{-175}$	Both hosts	Yes
<i>Russula</i> 4	AY310854	<i>Russula</i>	0	<i>Pseudotsuga</i>	No
<i>Russula</i> 5	AY310855	<i>Russula</i>	0	Both hosts	Yes
<i>Russula</i> 6	AY310856	<i>Russula</i>	$1.0 \times e^{-33}$	<i>Pseudotsuga</i>	No
<i>Sebacina</i> 1	AY310857	<i>Sebacina</i>	$1.0 \times e^{-96}$	Both hosts	Yes
<i>Sebacina</i> 2	AY310858	<i>Sebacina</i>	0	<i>Pseudotsuga</i>	No
Thelephoroid 1	AY310859	<i>Thelephora</i>	0	Both hosts	Yes
Thelephoroid 2	AY310860	<i>Thelephora</i>	0	<i>Lithocarpus</i>	No
Thelephoroid 3	AY310861	<i>Tomentella</i>	0	<i>Pseudotsuga</i>	No
Thelephoroid 4	AY310862	<i>Tomentella</i>	0	<i>Lithocarpus</i>	No
Thelephoroid 5	AY310863	<i>Tomentella</i>	0	<i>Pseudotsuga</i>	No
Thelephoroid 6	AY310864	<i>Tomentella</i>	0	<i>Lithocarpus</i>	No
Thelephoroid 7	AY310865	<i>Tomentella</i>	0	<i>Lithocarpus</i>	No
Thelephoroid 8	AY310866	<i>Tomentella</i>	0	Both hosts	Yes
Thelephoroid 9	AY310867	<i>Tomentella</i>	0	<i>Pseudotsuga</i>	No
Thelephoroid 10	AY310868	<i>Tomentella</i>	0	<i>Pseudotsuga</i>	No
Thelephoroid 11	AY310869	<i>Tomentella</i>	$1.0 \times e^{-69}$	<i>Pseudotsuga</i>	No
Thelephoroid 12	AY314981	<i>Tomentella</i>	0	<i>Lithocarpus</i>	No
<i>Tricholoma</i>	AY310870	<i>Tricholoma</i>	0	<i>Pseudotsuga</i>	No
<i>Tuber</i> 1	AY310871	<i>Tuber</i>	$1.0 \times e^{-125}$	<i>Pseudotsuga</i>	No
<i>Tuber</i> 2	AY310872	<i>Tuber</i>	$1.0 \times e^{-167}$	<i>Lithocarpus</i>	No
<i>Wilcoxina</i>	AY310873	<i>Wilcoxina</i>	0	<i>Lithocarpus</i>	No
<i>Xerocomus</i>	AY310874	<i>Xerocomus</i>	0	<i>Pseudotsuga</i>	No

Table 2 ECM taxa similarity among the three mixed evergreen forest stands. The similarity values (calculated as the coefficient of Sorenson) are reported in the upper halves of the matrices with the distances (m) among stands reported in the lower halves of the matrices

ECM taxa on both hosts				ECM taxa on <i>Pseudotsuga</i>				ECM taxa on <i>Lithocarpus</i>			
Stand	D	H	T	Stand	D	H	T	Stand	D	H	T
D	–	0.44	0.32	D	–	0.38	0.32	D	–	0.37	0.0
H	200	–	0.36	H	200	–	0.31	H	200	–	0.23
T	75	275	–	T	75	275	–	T	75	25	–

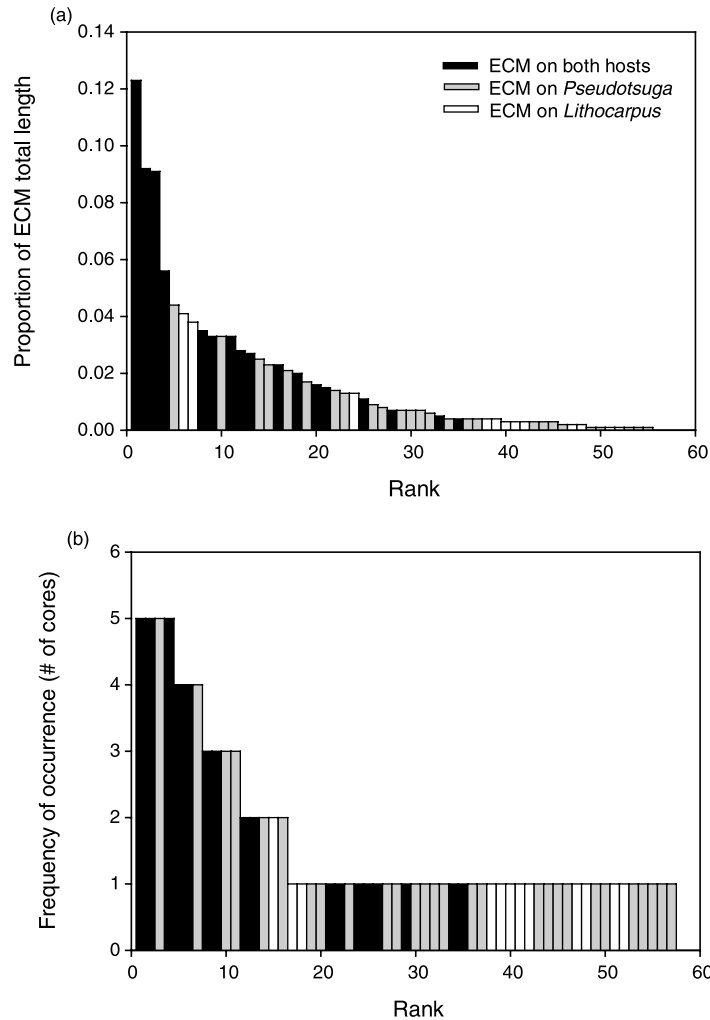


Fig. 2 Rank-abundance (a) and rank-occurrence (b) plots of multiple-host and single-host ECM taxa. Multiple-host taxa are defined as those taxa found on both host species and single-host taxa as those found on only one of the two host species. The ordering of ECM taxa with identical abundances or occurrences is represented randomly.

Discussion

Although the total number of single-host ECM taxa was higher than multiple-host ECM taxa across the three stands, multiple-host ECM taxa were significantly more abundant. Additionally, 78% of the multiple-host ECM taxa were found at least once on both hosts in the same core, which suggests that the potential for the *Lithocarpus* understorey to be connected by mycorrhizal networks to the *Pseudotsuga* canopy is high.

These potential connections appeared to be very common, although the shared ECM taxa varied between cores.

Our results agree with other studies that have reported that multiple-host taxa have greater abundance than single-host taxa (Horton & Bruns 1998; Horton *et al.* 1999; Cullings *et al.* 2000). We cannot, however, be certain that the ECM taxa that occurred on both hosts within the same core belonged to the same genotype, as the sizes of genets vary dramatically between species

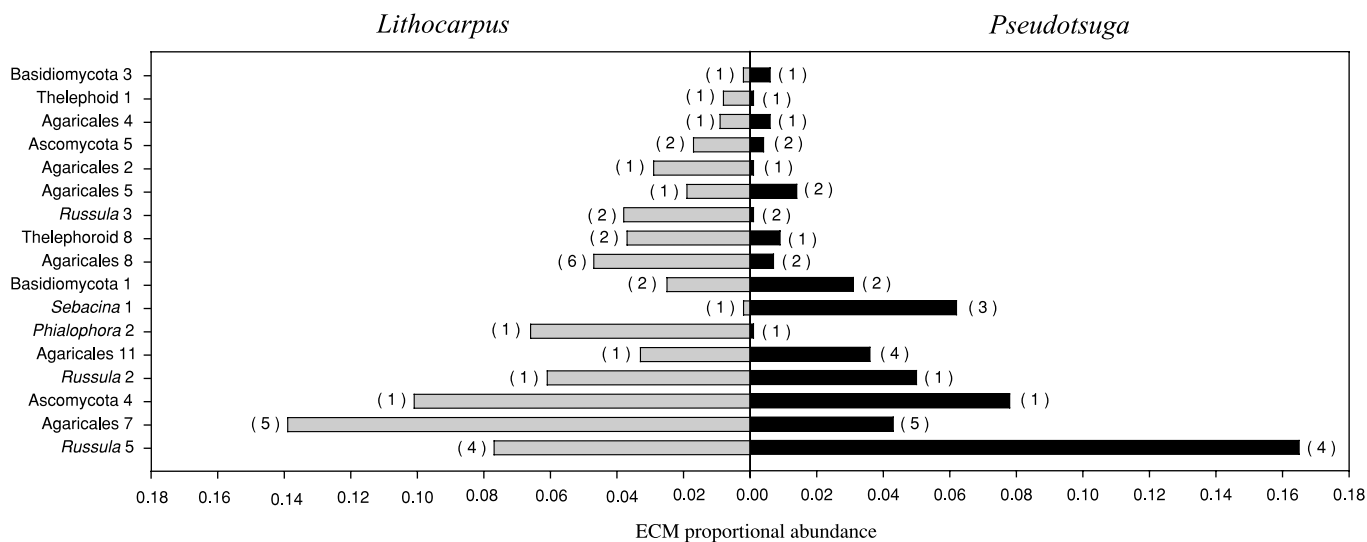


Fig. 3 Proportional abundance of multiple-host ECM taxa on *Lithocarpus densiflora* (grey bars) and *Pseudotsuga menziesii* (black bars). The proportions are relative to the total ECM length examined on each host. The number of cores in which the ECM taxa occurred is shown in parenthesis.

Table 3 Proportion of ECM taxa shared between hosts. The proportion shared is defined as the proportion of the total ECM length in each core that belonged to ECM taxa that were found on both hosts with that core (e.g. in core D1, Agaricales 8 occupied 0.09 of the total ECM length on *Pseudotsuga menziesii* and 0.16 of the total ECM length on *Lithocarpus densiflora*)

Stand	Core	Host		ECM taxa shared
		<i>Pseudotsuga</i> Proportion shared	<i>Lithocarpus</i> Proportion shared	
D	1	0.09	0.16	Agaricales 8
D	2	0.00	0.00	None
D	3	0.02	0.14	Thelephoroid 1
H	1	0.47	0.81	Ascomycota 4, Agaricales 7
H	2	0.45	0.03	<i>Sebacina</i> 1
H	3	0.82	0.17	<i>Russula</i> 5
H	4	0.27	0.18	<i>Russula</i> 5
H	5	0.04	0.70	Agaricales 7, Ascomycota 5
T	1	0.16	0.20	Agaricales 5
T	2	0.00	0.00	None
T	3	0.15	0.83	Agaricales 7
T	4	0.23	1.00	<i>Russula</i> 3, Agaricales 7, <i>Russula</i> 2
T	5	0.10	0.06	Thelephoroid 8
T	6	0.64	0.35	<i>Russula</i> 5

and locations (Dahlberg & Stenlid 1994; Gherbi *et al.* 1999; Gryta *et al.* 2000; Fiore-Donno & Martin 2001; Guidot *et al.* 2001; Redecker *et al.* 2001). However, in most cases, genets extend through spaces larger than a 10-cm diameter core, suggesting that, within each core, we sampled the same fungal genotype. However, there may be some overlap in the distribution of genets that could result in more than one genotype per core (Jany *et al.* 2002). Demonstrating that plants are actually connected by the ECM networks would require identification of the same fungal genotypes on both hosts within a core, which could be accomplished using taxon specific primers (Egger 1995). The number of hosts per ECM taxa may also have been underestimated due to the restricted area sampled. If additional samples were examined, some additional taxa, particu-

larly those that we found only once, would probably be encountered on both hosts. This means that we are likely to have underestimated the abundance of multiple-host ECM taxa.

We found significantly higher ECM taxa richness on *Pseudotsuga* than *Lithocarpus*, which may be due to a number of factors. The *Pseudotsuga* individuals examined were likely to be older than those of *Lithocarpus* and therefore have much larger and more developed root systems that could potentially harbour a greater number of ECM taxa. Because *Pseudotsuga* individuals were in the forest canopy, they have more fixed carbon than understorey *Lithocarpus* individuals and may allocate more below-ground, resulting in a more diverse ECM community. Alternatively, this difference may reflect intrinsic differences in the number of mycorrhizal

associations that can be formed by these two hosts. *Pseudotsuga* is reported to associate with over 2000 ECM species across its geographical range (Trappe 1977), but little is known about the diversity of mycorrhizal associations for *Lithocarpus*. However, in a glasshouse study, Massicotte *et al.* (1999) found 14 morphotypes on *Pseudotsuga* seedlings and 10 on *Lithocarpus* seedlings, with all 10 of the morphotypes on *Lithocarpus* also found on *Pseudotsuga*. Finally, the differences in ECM taxa richness may be the result of temporal variation in some factor that differs between hosts (e.g. fine root production), which could influence the number of ECM taxa associated with each host at the time of sampling.

The relative abundances of the multiple-host ECM taxa varied between hosts. Although significant host preferences were not detected when cores were combined (Fig. 3), on an individual core basis, the relative abundance of a given ECM taxon often varied considerably between the two hosts. Other studies have shown that ECM fungi may exhibit some degree of host preference (Molina *et al.* 1997; Cullings *et al.* 2001). While ECM fungi may interact with multiple hosts, they may not interact equally due to differential carbon input (Cullings *et al.* 2001), colonization susceptibility (Molina *et al.* 1997), and local soil environmental conditions (Gehring *et al.* 1998). It is possible that differential ECM abundances may affect the significance of ECM networks, as suggested by Finlay (1989), who found that differential ECM abundances affected the amounts of resources transferred between linked hosts.

We do not know if any materials are being transferred between the *Pseudotsuga* canopy and *Lithocarpus* understorey. Hogberg *et al.* (1999) found that up to 90% of the carbon in below-ground ECM networks was supplied by the canopy trees in a mixed species Swedish forest, but they did not determine if carbon moved into understorey plants. Simard *et al.* (1997) found that significant amounts of carbon were transferred from *Betula papyrifera* seedlings growing in full sun to experimentally shaded *Pseudotsuga* seedlings, resulting in a significant net carbon gain, which suggests that carbon may move preferentially to plants in shaded environments. Yet even if *Lithocarpus* individuals are not receiving carbon directly from the *Pseudotsuga* canopy, they may benefit from mycorrhizal networks because individuals that tap into mycorrhizal networks will have access to larger nutrient pools (Newman 1988). In addition, their ECM associates will be receiving the majority of their carbon from the canopy individuals (Hogberg *et al.* 1999), which will decrease the carbon cost of the symbiosis for understorey individuals. This lowered cost and enhanced nutrient availability would be very likely to aid establishment and persistence in light-limited forest understoreys. Recent evidence of such facilitation was put forth by Horton *et al.* (1999), who found that seedlings of *Pseudotsuga menziesii* shared many ECM fungi with mature *Arctostaphylos* spp. and showed that *Pseudot-*

suga seedlings successfully established only in areas with *Arctostaphylos* individuals.

Similarly high spatial heterogeneity has been observed in other studies and appears to be a general pattern of ECM assemblages (Dahlberg 2001; Horton & Bruns 2001). Despite the patchy distributions of individual taxa, we found that multiple-host ECM taxa were present in the majority of the cores. The widespread occurrence of multiple-host taxa would allow *Lithocarpus* individuals to tap into ECM networks throughout the forest understorey, but the composition of these networks varies due to the spatial heterogeneity of the community. Additionally, temporal variation in fungal species abundances could influence the occurrence and composition of ECM networks. Because there may be significant differences in the transfer of resources depending on the specific ECM taxa shared between hosts (Finlay 1989), studies that directly address the autecology of different ECM taxa will significantly enhance our understanding of functional aspects of mycorrhizal networks.

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