

Contrasting ectomycorrhizal fungal communities on the roots of co-occurring oaks (*Quercus* spp.) in a California woodland

Melissa H. Morris¹, Matthew E. Smith², David M. Rizzo², Marcel Rejmánek³ and Caroline S. Bledsoe¹

¹Department of Land, Air and Water Resources, ²Department of Plant Pathology, and ³Section of Evolution and Ecology, University of California, Davis, CA 95616, USA

Summary

Author for correspondence:
Melissa Morris
Tel: +1 530 752 4131
Fax: +1 530 752 1552
Email: mhmorris@ucdavis.edu

Received: 17 September 2007
Accepted: 19 November 2007

- Plant host species is considered an important factor influencing ectomycorrhizal (EM) communities. To gain insights into the role of host species in structuring EM communities, EM communities on sympatric oak (*Quercus*) species were compared in the Sierra Nevada foothills of California.
- Using molecular methods (polymerase chain reaction, cloning, restriction fragment length polymorphism and DNA sequencing), EM fungi on roots of deciduous *Quercus douglasii* and evergreen *Quercus wislizeni* trees were identified from 64 soil cores.
- The total EM species richness was 140, of which 40 taxa were detected on both oak hosts. Greater diversity and frequency of EM fungi with epigeous fruiting habit were found on *Q. wislizeni*, while taxa in the Ascomycota were more frequent and diverse on *Q. douglasii*.
- Using ordination, it was determined that both soil extractable phosphorus and oak host species explained a significant proportion of the variation in EM species distribution. These results indicate that plant host species can be an important factor influencing EM fungal community composition, even within congeneric trees.

Key words: Ascomycota, ectomycorrhizal fungal communities, internal transcribed spacer DNA sequence, oak woodland, *Quercus douglasii* (blue oak), *Quercus wislizeni* (interior live oak).

New Phytologist (2008) doi: 10.1111/j.1469-8137.2007.02348.x

© The Authors (2008). Journal compilation © *New Phytologist* (2008)

Introduction

Oaks (*Quercus* spp.) are ecologically and economically important woody plants that are widely distributed from temperate deciduous forests to the montane tropics (Nixon, 1993). In California, oak woodlands cover c. 10% of the state and harbor more diversity of flora and fauna than any other habitat in California (Barbour & Minnich, 2000; Standiford, 2002). Encircling the foothills of California's Central Valley, blue oak woodlands support key ecosystem functions, such as providing wildlife habitat, maintaining water quality and facilitating nutrient cycling (Standiford, 2002; Dahlgren

et al., 2003). Although oaks are critically dependent on ectomycorrhizal (EM) fungi to increase root absorptive area and to enhance nutrient acquisition (Smith & Read, 1997), there are relatively few studies on belowground EM communities in oak ecosystems (Avis *et al.*, 2003; Valentine *et al.*, 2004; Richard *et al.*, 2005; Walker *et al.*, 2005; Smith *et al.*, 2007).

In arid and semiarid ecosystems, the concentration of resources beneath individual trees or shrubs can result in the formation of 'resource islands' or 'islands of fertility' (Padien & Lajtha, 1992; Belsky *et al.*, 1993). Increased concentrations of nutrients, organic matter and soil microbial biomass under

trees or shrubs in arid and semiarid environments indicate that individual long-lived plants can have a significant effect on soil resources under their canopies (Belsky *et al.*, 1993; Schlesinger *et al.*, 1996; Burke *et al.*, 1998). Blue oak woodlands are a mosaic of scattered individual trees or clusters of trees surrounded by grassland (Allen-Diaz *et al.*, 1999). The dominant overstory tree, winter-deciduous blue oak (*Quercus douglasii*), often occurs in association with evergreen interior live oak (*Quercus wislizeni*) (Nixon, 2002). Within this savanna–woodland mosaic, isolated trees can influence the environment under their canopies, creating islands of enhanced soil fertility, and potential EM fungal habitat, as compared with adjacent grasslands (Holland, 1973; Callaway *et al.*, 1991; Frost & Edinger, 1991; Dahlgren *et al.*, 1997).

As the level of sophistication in sampling and identifying EM fungi has increased over time, it has become clear that plant host species may strongly influence the associated EM community, both above- and belowground (Molina *et al.*, 1992; Newton & Haigh, 1998; Wardle, 2002; Ishida *et al.*, 2007). Most previous studies that have examined host effects on EM root communities have considered phylogenetically distant hosts (e.g. from different genera or families) (Horton & Bruns, 1998; Horton *et al.*, 1999; Cullings *et al.*, 2000; Kennedy *et al.*, 2003; Richard *et al.*, 2005; Nara, 2006). Studies of congeneric hosts have not found substantial differences in EM communities, suggesting that related plants tend to host similar EM communities (Walker *et al.*, 2005; Ishida *et al.*, 2007). However, the effects of host tree on EM communities may be a result of indirect factors such as differences in leaf litter, carbon production or modification of soil properties (Conn & Dighton, 2000; Dickie *et al.*, 2006). The majority of research on EM communities has been conducted in closed canopy forests (Horton & Bruns, 2001) where host canopies, root systems, and litterfall routinely overlap, diffusing host influences on EM communities. Because of the scattered distribution of trees, oak woodlands are an excellent system in which to compare EM communities on congeneric hosts.

We hypothesized that there might be differences in the belowground EM communities on co-occurring *Q. douglasii* and *Q. wislizeni* for two reasons. First, we observed differences in fruiting patterns of EM fungi under these two oak hosts. We frequently found a diverse array of epigeous EM fruiting bodies under *Q. wislizeni*, but epigeous species were less frequent and diverse under *Q. douglasii* (M. E. Smith & M. H. Morris, pers obs). Secondly, ecological and functional differences between deciduous *Q. douglasii* and evergreen *Q. wislizeni* could influence EM communities. Rates of photosynthesis, foliar nutrient content, litter decomposition and carbon production often vary between evergreen and deciduous species (Monk, 1966; Chabot & Hicks, 1982). Litter layer depth and herbaceous vegetation also differ markedly between the understories of *Q. wislizeni* and *Q. douglasii* (Frost & Edinger, 1991; Downie & Taskey, 1997; Rejmánek *et al.*, 2005).

This study investigated belowground EM communities on root tips of two co-occurring *Quercus* species in a blue oak woodland. DNA sequences from root tips were compared to a fruiting body sequence database of taxa collected from the site over a 4-yr period (Smith *et al.*, 2007). We predicted that there would be a greater frequency of epigeous EM species on the roots of *Q. wislizeni* than on *Q. douglasii* based on observations of fruiting patterns and the differences in ecology between the two *Quercus* hosts. The objectives of our study were (1) to document diversity and structure of EM communities on two *Quercus* hosts in a blue oak woodland; (2) to compare EM communities on *Q. douglasii* and *Q. wislizeni*; and (3) to investigate the influence of abiotic soil characteristics on the distribution of EM fungi.

Materials and Methods

Study area

The research was conducted in a blue oak woodland located in the Koch Natural Area at the Sierra Foothill Research and Extension Center (SFREC) in Yuba County, California, USA (39°14'N, 121°18'W). Within the protected natural area, we selected two adjacent sites approx. 250 m apart. Before 1960 the area experienced cattle grazing and frequent fires, but little disturbance has occurred since that time (J. M. Connor, unpublished). The dominant overstory tree, *Quercus douglasii* Hook. & Arn, and the subdominant *Quercus wislizeni* A. DC. co-occur in a mosaic ranging from open savanna to dense woodland. In the study area, tree architecture differs between the two oaks; *Q. wislizeni* is shrubby in stature with multiple stems while *Q. douglasii* is arboreal. Individual *Pinus sabiniana* Douglas trees are scattered throughout the landscape. Common shrubs include the non-EM *Rhamnus tomentella* Benth. ssp. *tomentella* and *Toxicodendron diversilobum* Torrey & Gray. Assorted exotic annual grasses and forbs form arbuscular mycorrhizas and dominate the understory (He *et al.*, 2006).

The Mediterranean climate is characterized by cool, wet winters (January–March) and hot, dry summers (June–September). Mean annual precipitation is 75 cm (range 23 to 132 cm) and mean annual temperature is 17.8°C (range 10–43°C). At the study area, elevation ranges from 400 to 600 m. Soils are derived from basic metavolcanic rocks and are fine-loamy, mixed, thermic, Mollic Haploxeralfs (Alfisol) with pH ranging from 5.2 to 7.1 (Huang, 1997).

Site 1

At site 1, we selected four pairs of *Q. douglasii* and *Q. wislizeni* trees scattered over a 25-ha area. Trees were estimated to be ≥ 50 yr old and were selected based on distance from each other and distance from other EM host species. Several studies suggest that oak roots in blue oak woodlands are concentrated beneath the tree canopy (Jackson *et al.*, 1990; Callaway *et al.*,

1991; Huang, 1997). In order to reduce the possibility that roots from other EM species were present within the sampling areas, the distance separating individual *Q. douglasii* and *Q. wislizeni* trees was 20–50 m. All *Q. wislizeni* trees had multiple stems (mean = 5.3 stems per tree) while all *Q. douglasii* trees had a single main stem that branched above c. 1.5 m. The average diameter at breast height (DBH; diameter at 1.5 m height) for *Q. douglasii* and *Q. wislizeni* was 47 cm and 43 cm, respectively. For trees with multiple basal stems, DBH was calculated as $2 \times \sqrt{\text{sum of the basal area of each stem}/3.14}$. For each tree, four soil cores were collected on 20 April 2004 in cardinal directions at approximately half canopy.

Site 2

At site 2, sampling was the same as at site 1 except that *Q. douglasii* soil cores were collected from four 16×16 m plots within a stand of 41 *Q. douglasii* trees (mean DBH = 30 cm; mean stems per tree = 1.3). Sampling was modified at site 2 because the distribution of *Q. douglasii* trees necessitated that cores be collected from plots around a cluster of trees and not from around individual isolated trees. A portion of the data presented here is a subset of the data collected by Smith *et al.* (2007): 16 cores from a single sampling date. Sampling of *Q. wislizeni* trees was the same as at site 1; a total of 16 soil cores were collected from four *Q. wislizeni* trees (mean DBH = 48 cm; mean stems per tree = 4.5) in cardinal directions at approximately half canopy. The four *Q. wislizeni* trees were between 25 and 140 m from the edge of the *Q. douglasii* plot and trees were estimated to be ≥ 50 yr old. Soil cores from site 2 were collected on 3 May 2004.

Sampling of ectomycorrhizal fine roots

Rocky soils made soil coring difficult, so we collected a standard volume of soil (900 cm^3) to an approximate depth of 10–12 cm. We sampled to this depth because the majority of oak fine-root (< 0.5 mm) biomass is located at depths of 0–20 cm and decreases below 20 cm (Millikin & Bledsoe, 1999). Soil samples were taken to the laboratory, stored at 4°C and processed for EM root tips within 2 wk. Fine roots were removed from soil samples and hand-washed over a 0.12-mm sieve. From each sample, healthy EM root tips were severed from roots and placed in a Petri dish with water. We randomly selected 100 EM root tips from the collection of tips in the Petri dish by picking tips and systematically swirling the Petri dish to redistribute roots tips between selections. We considered root tips to be colonized by EM fungi based on absence of root hairs and presence of a fungal mantle when examined under a dissecting microscope. EM roots were cleaned of debris with deionized water and freeze-dried for DNA analysis.

Soil analyses

Samples were collected and litter depth was measured in late April and early May, when soils were moist and relatively easy to penetrate. After EM root tips had been removed, soils were analyzed for pH (saturated paste in H_2O and 0.5 M CaCl_2), soil water content (gravimetric), extractable phosphorus (P) (Bray-P) and total carbon (C) and nitrogen (N) (Carlo Erba C/N analyzer; combustion method). Measurements of soil N, P and C were performed by the Division of Agricultural and Natural Resources Laboratory, University of California, Davis.

DNA extraction and PCR

From each sample of 100 pooled EM root tips, DNA was extracted by a modified cetyltrimethylammonium bromide (CTAB) method (Gardes & Bruns, 1993) followed by purification with the UltraClean soil DNA kit (MO BIO Laboratories, Carlsbad, CA, USA). The internal transcribed spacer (ITS) region and part of the 28S large subunit were amplified with primers ITS1F and LR3 (Gardes & Bruns, 1993; Hopple & Vilgalys, 1994). Amplifications were performed in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Foster City, CA, USA) with initial denaturation at 94°C for 5 min, followed by 20 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 4 min and final extension at 72°C for 7 min. Amplifications consisted of 15- μl reactions containing 1 Unit Platinum Taq High Fidelity (Invitrogen, Carlsbad, CA, USA), 1 \times polymerase chain reaction (PCR) buffer, 2 mM MgSO_4 , 0.2 μM dNTP, 0.38 μM primers and 2–3 μl of DNA template.

Cloning and restriction digest screening

PCR products from four replicate reactions were pooled and cloned using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocols. For each sample, 48 transformed *Escherichia coli* colonies were randomly selected and used as templates for PCR amplification with ITS1F and LR3. Analyzing 48 clones from each sample allowed us to efficiently detect the common species in each sample. In a previous study with bulk soil, Landeweert *et al.* (2003) determined that analyzing 30 clones per soil sample was likely to be sufficient for detecting the most common EM species. Plasmids were amplified as described above except for minor modifications; the initial denaturation time was increased to 10 min, the extension time was decreased to 2 min, the number of cycles was increased to 25, the reaction volume was increased to 30 μl and recombinant Taq polymerase was used (Invitrogen). PCR products were digested with restriction enzymes *Hinf*I and *Alu*I. Fragments were run simultaneously on 1.5% agarose gels using SYBR Green I (Applied Biosystems) and scored visually as described

by Smith *et al.* (2007). Restriction fragment length polymorphism (RFLP) patterns were not compared across cores.

Sequencing and identification of EM root tips

From each sample, one to six representative clones for each unique RFLP pattern were sequenced with ITS1F using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) on an ABI 3730xl capillary sequencer (Applied Biosystems) at the College of Agriculture and Environmental Sciences Genomics Facility, University of California, Davis. When sequences from a single RFLP pattern resulted in multiple taxa, all clones from that RFLP type were sequenced. Sequences were edited using SEQUENCHER v4.2 (Gene Codes, Ann Arbor, MI, USA) and examined by BLAST searches against GenBank (National Center for Biotechnology Information) and a database of sequences from fruiting bodies collected at the site (Smith *et al.*, 2007).

Names were assigned to EM root sequences using the methodology of Smith *et al.* (2007). Briefly, EM root sequences matching fruiting bodies are designated by genus and species epithet. In cases where species identity was unclear, a genus name is followed by a Sierra Research Center (src) number. For species that did not match fruiting bodies, names are based on a combination of BLAST searching, sequence alignment, and advice from taxonomic experts (see Smith *et al.*, 2007). When ITS sequences were insufficient for phylogenetic placement, we sequenced part of the 28S large subunit using LR3 and performed a similar analysis as described above.

ITS sequences with $\geq 97\%$ identity were grouped together. This cut-off level is based on error rates generated by PCR, cloning, sequencing and interspecific variability within ITS and has been employed in other studies using ITS for EM species identification from roots and soil (Izzo *et al.*, 2005; O'Brien *et al.*, 2005; Walker *et al.*, 2005). We used a traditional species concept for assigning names to sequences because almost half of the EM root tips were identified based on matches to fruiting bodies from the site.

Generation of chimeric sequences is a potential problem when amplifying mixed-template environmental samples (Qiu *et al.*, 2001; Jumpponen, 2003). We checked for chimeras using both alignments and the Recombination Detection Program (RDP; Martin & Rybicki, 2000) as described by Smith *et al.* (2007). Six sequences were identified as possible chimeras and removed from the data set.

Statistical analyses

Data from four soil cores from each tree or plot were combined for statistical analyses. EM taxa were categorized into four fruiting habits: epigeous (aboveground fruiting), hypogeous (belowground fruiting), resupinate (crust-like) and unknown/asexual. We calculated the relative frequency

for each group (e.g. epigeous, hypogeous or resupinate) as the number of occurrences of species from each group divided by the number of occurrences of all taxa on a per tree/plot basis. Differences in the relative frequency of Ascomycota taxa and the relative frequency of EM taxa with epigeous, hypogeous and resupinate fruiting habits on *Q. douglasii* and *Q. wislizeni* were assessed using analysis of variance (ANOVA) with terms for oak species, site and the interaction (oak \times site). The primary overall significance level was controlled at $P < 0.05$ and pairwise comparisons between oaks were controlled at the $P < 0.0125$ level using Bonferroni correction. To improve homogeneity of variances, proportions were arcsine transformed. We calculated first- and second-order jackknife estimates of species richness using PC-ORD 4.0 (MjM Software Design, Gleneden Beach, OR, USA).

Differences in means for the seven soil variables from under the canopies of *Q. douglasii* and *Q. wislizeni* were also analyzed using ANOVA with terms for oak species, site and the interaction (oak \times site). The primary overall significance level was controlled at $P < 0.05$ and pairwise comparisons between oaks were controlled at the $P < 0.007$ level using Bonferroni correction. All ANOVA analyses were conducted in SAS (SAS Institute, Cary, NC, USA).

Relationships between EM community composition and environmental variables were analyzed with multivariate procedures (CANOCO v4.5; Microcomputer Power, Ithaca, NY, USA) using the number of times each unique RFLP type was detected per tree as an approximate measure of abundance. The number of unique RFLP types is an estimation of abundance because this measure may be influenced by PCR and cloning bias. Site was used as a covariable; oak species were treated as nominal environmental variables. Two types of ordination methods that can be used with species composition and environmental data are canonical correspondence analysis (CCA) and redundancy analysis (RDA). These ordination methods are based either on a linear (RDA) or unimodal (CCA) response of species to environmental gradients. In deciding whether to use linear or unimodal ordination methods, beta diversity in community composition along the ordination axes (as measured by gradient length) should be taken into account (Lepš & Šmilauer, 2003). If the longest gradient length is < 3.0 then linear method is most appropriate; if the longest gradient is > 4.0 then unimodal methods should be used (Lepš & Šmilauer, 2003). In this study the gradient length was 3.85. Therefore, we used both CCA and RDA. For RDA analyses, data were $\log + 1$ transformed. Environmental variables (soil pH, total N, total C, extractable P, soil moisture and litter depth) were tested for their contribution to the variation in the EM species data (Monte Carlo permutation test). In order to determine the influence of rare species on the ordination analysis we conducted CCA and RDA both with and without singletons. To quantify the proportional contribution of the significant

environmental variables, we conducted variance partitioning as described in Lepš & Šmilauer (2003).

Results

Ectomycorrhizal fungal diversity

Based on sampling of 6400 root tips, RFLP analysis of 3072 clones and sequencing of 1788 clones, total EM species richness on fine roots of *Q. douglasii* and *Q. wislizeni* was 140 taxa (Supplementary Material, Table S1). We identified 57 taxa (41%) based on sequence matches to fruiting bodies (sclerotia for *Cenococcum*) collected from the study area and surrounding woodlands. One species (*Amanita phalloides*) was identified based on > 99% similarity of the ITS region to sequences in GenBank. The remaining 82 taxa were identified to the level of genus (40), family (34) or order (eight). Sequence information from the 28s region was used for taxonomic placement of 57 taxa; all others were determined by ITS alone. Two root-associated fungi in the Helotiales (*Cadophora* sp. 1 and cf. *Lophiostoma* sp. 1) were also detected in five soil cores. Because these two species could not be unambiguously confirmed as EM symbionts, they were excluded from analyses.

Ectomycorrhizal communities on *Q. douglasii* and *Q. wislizeni* were similar in species richness and community structure at both sites (Supplementary Material, Table S2). A total of 88 EM taxa occurred on *Q. douglasii* roots, of which 40 (45%) were detected as fruiting bodies. A total of 92 EM taxa were found on *Q. wislizeni* roots, of which 44 (48%) were detected as fruiting bodies. Many species were rare (found in only one soil core). A total of 46 (52%) EM taxa were found in only one soil core from *Q. douglasii* and 44 (48%) taxa were found in only one soil core from *Q. wislizeni*. The mean number of species in a soil core was 6.5 and the range was 2 to 12. The mean number of species detected around a tree (four soil cores) was 19.6 and the range was 16 to 25.

Ectomycorrhizal community composition

Basidiomycota species richness was more than double that of Ascomycota (Basidiomycota = 100 taxa; Ascomycota = 40 taxa). Basidiomycota species richness was 54 taxa on *Q. douglasii* and 67 taxa on *Q. wislizeni*, while species richness of Ascomycota was 34 taxa on *Q. douglasii* and 25 taxa on *Q. wislizeni*. There was a greater relative frequency of Ascomycota on *Q. douglasii* than on *Q. wislizeni* (49% versus 31%, respectively; $n = 8$, $F = 10.8$, $P = 0.0066$) and site effects and the interaction were not significant. At the family level, the most frequently detected taxa on both oak species were in the Thelephoraceae (Table 1). Taxa in the Pyronemataceae, Tuberaceae and Cortinariaceae (mostly *Inocybe*) occurred frequently on roots of both oak hosts. By contrast, taxa in the Russulaceae were frequent and diverse on

Table 1 Relative frequency of ectomycorrhizal families from root tips of *Quercus douglasii* and *Quercus wislizeni* trees

	No. of genera ¹	Frequency (% of total)	
		<i>Q. douglasii</i>	<i>Q. wislizeni</i>
Basidiomycota			
Thelephoraceae	2	23.8	23.4
Cortinariaceae	2	11.9	9.8
Sebacinales ²	–	7.1	8.7
Other Basidiomycota ³	12	4.6	12.2
Russulaceae	3	3.7	14.5
Basidiomycota subtotal		51.1	68.6
Ascomycota			
Pyronemataceae	5	14.4	8.7
Cenococcum ⁴	1	12.4	6.2
Tuberaceae	1	13.0	8.8
Pezizaceae	4	7.5	4.3
Helvellaceae	1	1.6	3.1
Helotiales ⁵	1	0.0	0.4
Ascomycota subtotal		48.9	31.4

Relative frequency was calculated as the number of occurrences of species from each family divided by the total number of occurrences of all taxa. Within each phylum, taxa are ordered in decreasing frequency on *Q. douglasii*.

¹Number of genera is approximate because of taxonomic uncertainties.

²Sebacinales is at the level of order because of taxonomic uncertainties within this group and recent evidence indicating that Sebacinales constitutes a new order, Sebacinales (Weiss *et al.*, 2004).

³Other Basidiomycota include taxa from 12 genera in 10 families, altogether accounting for 5% (*Q. douglasii*) and 12% (*Q. wislizeni*) of the relative frequency. Genera in this category include: *Amanita*, *Boletus*, *Clavariadelphus*, *Clavulina*, *Entoloma*, *Gautieria*, *Hebeloma*, *Hygrophorus*, *Hysterangium*, *Laccaria*, *Melanogaster* and *Octavianina*.

⁴*Cenococcum* is listed at the genus level because it is an asexual ascomycete with unresolved classification.

⁵Helotiales is listed at the level of order because we were unable to identify taxa to family.

Q. wislizeni (relative frequency = 14.5%; species richness = 12) but less frequent and diverse on *Q. douglasii* (relative frequency = 3.7%; species richness = 4; Supplementary Material, Table S1). However, Kendall's rank correlation coefficient showed no significant difference between the frequencies of higher level taxonomic groups (families and orders) on *Q. douglasii* and *Q. wislizeni* (Kendall's $\tau = 0.29$, $P = 0.21$; Table 1).

Patterns of overall community structure were similar at the two sites; however, some individual taxa differed between sites. For example, *Laccaria* cf. *bicolor* was the dominant species on *Q. wislizeni* at site 1 (detected on all trees) but was detected on *Q. wislizeni* from only one core at site 2. Similarly, the second most frequent species on *Q. douglasii* at site 1, *Tuber californicum*, was not detected on *Q. douglasii* at site 2. Although some frequently

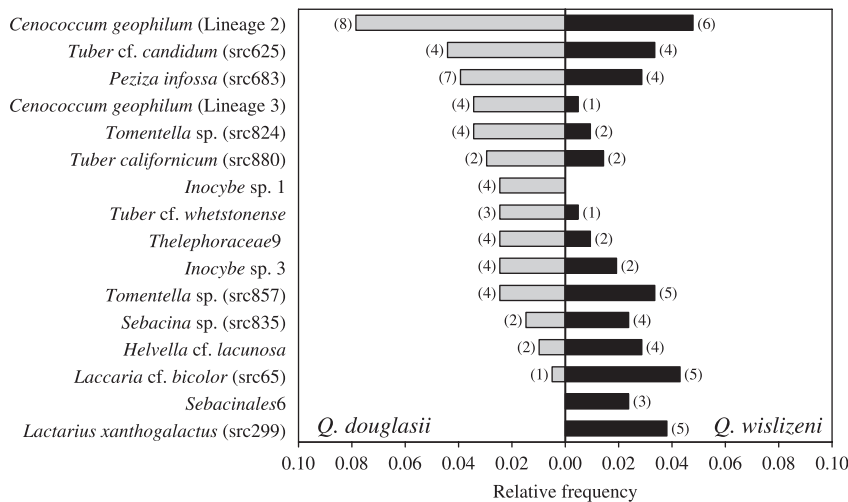


Fig. 1 Relative frequency of the most common ectomycorrhizal taxa (found in five or more soil cores) on roots of *Quercus douglasii* and *Quercus wislizeni*. Relative frequency was calculated as the number of occurrences of each species divided by the total number of occurrences of all taxa. The number of trees (or plots) on which each species occurred is shown in parentheses.

occurring taxa differed between sites, many of the common species were found on both *Q. douglasii* and *Q. wislizeni* (Fig. 1). For example, *Cenococcum geophilum* (lineage 2) (Douhan & Rizzo, 2005) and *Tuber cf. candidum* were detected frequently on both oak species. Overall, 40 taxa were found on both hosts and *Q. douglasii* and *Q. wislizeni* shared 13 out of the 16 most frequently occurring EM fungi (Fig. 1).

Differences in fruiting habit

Most EM taxa found on oak roots formed fruiting bodies that were epigeous (49) or resupinate (48). Less common fruiting types were hypogeous (30), asexual (3) and unknown (10). The roots of *Q. wislizeni* had a significantly higher relative frequency of taxa with epigeous fruiting than *Q. douglasii* ($n = 8$, $F = 21.41$, $P = 0.0006$; Fig. 2a,b); site effects ($n = 8$, $F = 0.55$, $P = 0.471$) and oak \times site interaction ($n = 8$, $F = 2.87$, $P = 0.116$) were not significant. Two epigeous taxa, *Laccaria cf. bicolor* and *Lactarius xanthogalactus*, were common on *Q. wislizeni* and infrequent or absent on *Q. douglasii* (Fig. 1). The species richness of epigeous fruiting types was 25 taxa on roots of *Q. douglasii* and 32 on roots of *Q. wislizeni*. The relative frequency of taxa with hypogeous fruiting on the two oak species differed between sites, as indicated by a significant oak \times site interaction ($n = 8$, $F = 13.41$, $P = 0.003$). Analysis of the interaction showed that *Q. douglasii* had a significantly higher relative frequency of hypogeous taxa at site 1 ($P = 0.0023$; Fig. 2a) but there were no significant differences between hypogeous taxa on oak species at site 2 ($P = 0.9716$; Fig. 2b). The relative frequency of taxa with resupinate fruiting did not differ significantly between *Q. douglasii* and *Q. wislizeni* ($n = 8$, $F = 0.09$, $P = 0.770$) and site effects ($n = 8$, $F = 0.12$, $P = 0.737$) and oak \times site interaction ($n = 8$, $F = 1.51$, $P = 0.243$) were not significant.

Soil characteristics

Soil samples from under the canopy of *Q. wislizeni* had a deeper litter layer than those from under the canopy of *Q. douglasii* ($n = 8$, $F = 35.5$, $P < 0.0001$; Supplementary Material, Table S3). There were no significant differences in total C, total N or extractable P in soil samples from *Q. douglasii* and *Q. wislizeni* (Supplementary Material, Table S3). Differences in soil moisture between the two oak species were not significant after Bonferroni correction ($n = 8$, $F = 6.05$, $P = 0.030$). However, there was a significant site effect; soil moisture was higher at site 1 than at site 2 ($n = 8$, $F = 34.75$, $P < 0.0001$). Soil pH measured in water was higher under *Q. douglasii* at site 1, but showed no pattern at site 2 (significant oak \times site interaction; $n = 8$, $F = 4.73$, $P = 0.050$).

Ordination

Direct gradient ordination of EM fungal communities using RDA showed that the conditional effect of extractable P (i.e. the effect in addition to the other variables included in the model) was highly significant ($P = 0.004$) in explaining the distribution of EM species (Fig. 3). Oak species was the second most significant variable ($P = 0.024$). Variance partitioning revealed that extractable P explained 11.3% and oak species 9.6% of the variation in the species data. The two variables together explained 20.9% of the variance, demonstrating that there is no shared effect of these two variables, which is consistent with the corresponding vector and centroids in Fig. 3. The oak \times extractable P interaction was not significant ($P = 0.12$), indicating that soil extractable P concentrations are essentially independent of oak species identity. Other environmental variables had no significant conditional effect. However, the independent effect of soil pH in CaCl_2 was almost significant ($P = 0.054$) and

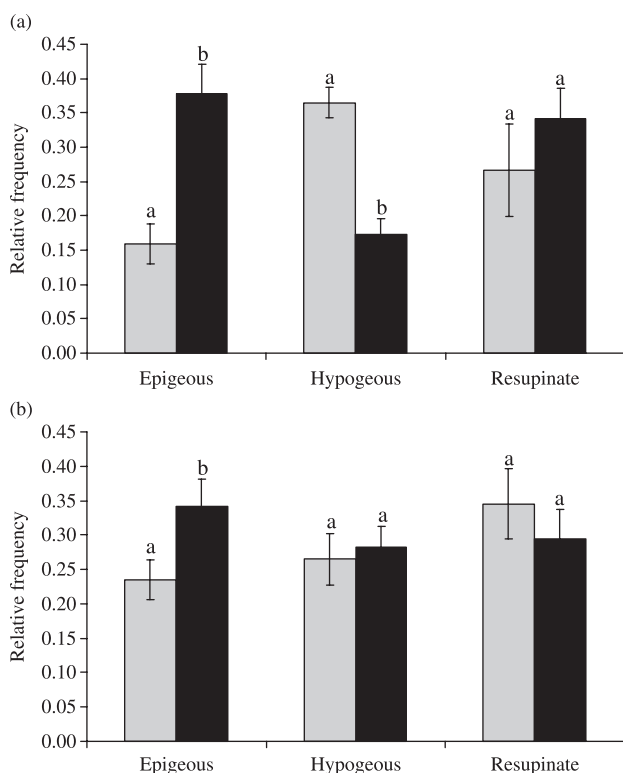


Fig. 2 Mean relative frequency of fruiting habits of ectomycorrhizal (EM) species occurring on *Quercus douglasii* (gray bars) and *Quercus wislizeni* (black bars) roots at (a) site 1 and (b) site 2. Relative frequency was calculated as the number of occurrences of species from each fruiting habit divided by the total number of occurrences of all taxa. Within fruiting categories, columns with different letters were significantly different ($P < 0.01$, Bonferroni correction). Error bars are \pm SEM.

this variable explained an additional 7.4% of the variance. Results were similar both with and without singletons and results from CCA were consistent with those from RDA (data not shown).

Discussion

Previous studies have suggested that closely related host species, such as trees from the same genus or family, support similar EM communities (Horton & Bruns, 1998; Cullings *et al.*, 2000; Ishida *et al.*, 2007). In this study, many of the most frequent EM species were found on both *Quercus* hosts; however, the EM communities on the two hosts differed in several notable ways. We found a greater diversity and frequency of taxa that produce epigeous sporocarps on the roots of *Q. wislizeni* as compared with *Q. douglasii*. This finding is consistent with observations of aboveground fruiting patterns; we observed greater fruiting of epigeous taxa under *Q. wislizeni* than under *Q. douglasii* (M. E. Smith & M. H. Morris, pers obs), suggesting that environmental conditions under *Q. wislizeni* may be more favorable for epigeous taxa. This pattern is exemplified by taxa in the family

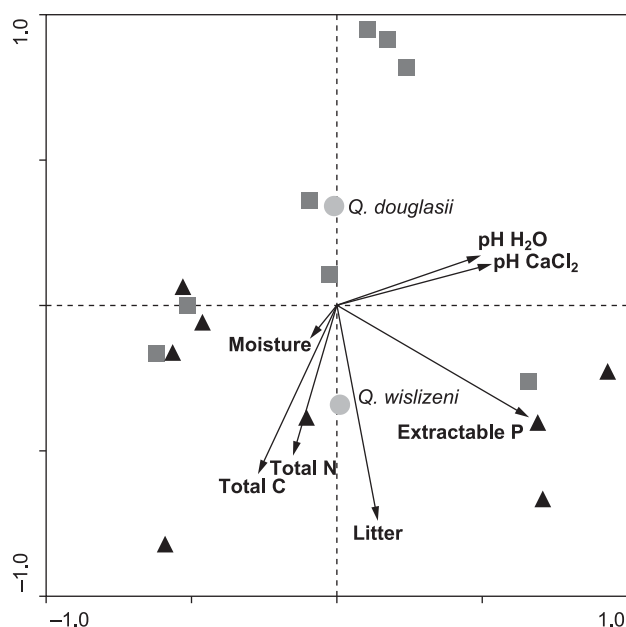


Fig. 3 Redundancy analysis of ectomycorrhizal (EM) samples from *Quercus douglasii* and *Quercus wislizeni* trees and soil variables. Extractable P ($P = 0.004$) and oak species ($P = 0.024$) were significant in accounting for variation in EM species composition. Squares, samples from *Q. douglasii* trees; triangles, samples from *Q. wislizeni* trees; circles, centroids of *Q. douglasii* and *Q. wislizeni* samples. Vectors represent strength and direction of particular environmental factors.

Russulaceae, which were primarily epigeous and tended to be more frequent on *Q. wislizeni* (relative frequency = 14.5%) than on *Q. douglasii* (relative frequency = 3.7%). Smith *et al.* (2007) also found a similarly low relative frequency (2.9%) of taxa in the Russulaceae on *Q. douglasii* after intensively sampling roots from 94 soil cores over a 2-yr period.

By contrast, Ascomycota were significantly more frequent on the roots of *Q. douglasii* as compared with those of *Q. wislizeni*. Taxa in the Ascomycota are increasingly recognized as an important component of EM communities (Vrålstad *et al.*, 2002; Dickie & Reich, 2005; Tedersoo *et al.*, 2006; Smith *et al.*, 2007), and this study demonstrates the prevalence of Ascomycota as EM fungal partners on both *Q. douglasii* and *Q. wislizeni*. We detected Ascomycota from a wide range of lineages (Pyronemataceae, Tuberales, Pezizaceae, and others) and the three most common species were Ascomycota (accounting for 14% of species occurrences). This evidence suggests that Ascomycota are not only diverse but may also play an important ecological role in this ecosystem.

Although we did not experimentally explore the reasons for the differences between the EM communities on the roots of the two *Quercus* species, these hosts differ in several important ways. Evergreen versus deciduous habit is one important difference between the two oak species. In addition, *Q. wislizeni* has a deeper litter layer, less herbaceous vegetation and fewer exotic species in the understory than *Q. douglasii*

(Frost & Edinger, 1991; Rejmánek *et al.*, 2005; this study). It is likely that these physiological and ecological differences between the two host species act in concert to influence their associated EM communities. For example, leaf litter can strongly influence EM communities (Baar & de Vries, 1995; Conn & Dighton, 2000; Dighton *et al.*, 2000; Cullings *et al.*, 2003) and increased litter accumulation under *Q. wislizeni*, but not *Q. douglasii*, may reduce soil evaporation (Williams *et al.*, 1990). Although soil moisture was not significantly different between oaks at the end of the rainy season, moisture differences are likely to be more pronounced at other times of the year. Not only the amount but also the composition of leaf litter has been shown to influence EM fungi (Koide *et al.*, 1998; Conn & Dighton, 2000). Evergreen and deciduous leaves are known to differ in chemical composition, with evergreen leaves having higher lignin concentrations than deciduous leaves (Aerts, 1995). Reduced lignin and litter accumulation in the canopy under *Q. douglasii* may favor Ascomycota taxa that are considered less efficient at decomposition of lignified organic matter (e.g. litter and wood) than Basidiomycota (Brundrett, 2002).

Of the eight environmental variables in the RDA ordination, species distributions were most strongly associated with extractable P and secondarily with oak host species. The importance of EM fungi in P uptake is well known (Smith & Read, 1997), but the role of P in influencing EM community composition is not well understood. Heterogeneity of extractable P may create niches for different species of fungi. Harrington & Mitchell (2005) found that soil organic matter and extractable P contributed significantly to explaining the distribution of EM types on *Dryas octopetala* in grass heaths. Independent of extractable P, the distribution of EM fungi was also influenced by oak host species, suggesting that both variables influence the EM communities, but it is unclear how these factors are related. Future research on the complex interactions among host plant, soil properties and soil biota is needed in order to better understand the direct and indirect effects of host species on EM communities. This research demonstrates significant differences in EM communities on two congeneric tree species, indicating that in this oak woodland phylogenetically similar plant hosts can create distinct ecological niches for EM fungi.

Acknowledgements

We thank the University of California Sierra Foothill Research and Extension Center for logistical support in the field, M. A. Perez-Perez for assistance with data collection and R. M. Davis for advice and suggestions. We are especially grateful to G. W. Douhan for his valuable contributions to this research. Three anonymous reviewers provided helpful comments on earlier drafts of this manuscript. This research was supported by a grant to C. S. Bledsoe from the National Science Foundation Biocomplexity program (DEB-9981711).

References

- Aerts R. 1995. The advantages of being evergreen. *Trends in Ecology & Evolution* 10: 402–407.
- Allen-Diaz B, Bartolome JW, McClaran MP. 1999. California oak savanna. In: Anderson RC, Fralish JS, Baskin JM, eds. *Savannas, barrens, and rock outcrop plant communities of North America*. New York, NY, USA: Cambridge University Press, 322–339.
- Avis PG, McLaughlin DJ, Dentinger BC, Reich PB. 2003. Long-term increase in nitrogen supply alters above- and below-ground ectomycorrhizal communities and increases the dominance of *Russula* spp. in a temperate oak savanna. *New Phytologist* 160: 239–253.
- Baar J, de Vries FW. 1995. Effects of manipulation of litter and humus layers on ectomycorrhizal colonization potential in Scots pine stands of different age. *Mycorrhiza* 5: 267–272.
- Barbour MG, Minnich RA. 2000. California upland forests and woodlands. In: Barbour MG, Billings WD, eds. *North America terrestrial vegetation*. New York, NY, USA: Cambridge University Press, 161–202.
- Belsky AJ, Mwonga SM, Amundson RG, Duxbury JM, Ali AR. 1993. Comparative effects of isolated trees on their undercanopy environments in high- and low-rainfall savannas. *The Journal of Applied Ecology* 30: 143–155.
- Brundrett MC. 2002. Coevolution of roots and mycorrhizas of land plants. *New Phytologist* 154: 275–304.
- Burke IC, Lauenroth WK, Vinton MA, Hook PB, Kelly RH, Epstein HE, Aguiar MR, Robles MD, Aguilera MO, Murphy KL *et al.* 1998. Plant-soil interactions in temperate grasslands. *Biogeochemistry* 42: 121–143.
- Callaway RM, Nadkarni NM, Mahall BE. 1991. Facilitation and interference of *Quercus douglasii* on understory productivity in central California. *Ecology* 72: 1484–1499.
- Chabot BF, Hicks DJ. 1982. The ecology of leaf life spans. *Annual Review of Ecology and Systematics* 13: 229–259.
- Conn C, Dighton J. 2000. Litter quality influences on decomposition, ectomycorrhizal community structure and mycorrhizal root surface acid phosphatase activity. *Soil Biology and Biochemistry* 32: 489–496.
- Cullings KW, New MH, Makhija S, Parker VT. 2003. Effects of litter addition on ectomycorrhizal associates of a lodgepole pine (*Pinus contorta*) stand in Yellowstone National Park. *Applied and Environmental Microbiology* 69: 3772–3776.
- Cullings KW, Vogler DR, Parker VT, Finley SK. 2000. Ectomycorrhizal specificity patterns in a mixed *Pinus contorta* and *Picea engelmannii* forest in Yellowstone National Park. *Applied and Environmental Microbiology* 66: 4988–4991.
- Dahlgren RA, Horwath WR, Tate KW, Camping TJ. 2003. Blue oak enhance soil quality in California oak woodlands. *California Agriculture* 57: 42–47.
- Dahlgren RA, Singer MJ, Huang X. 1997. Oak tree and grazing impacts on soil properties and nutrients in a California oak woodland. *Biogeochemistry* 39: 45–64.
- Dickie IA, Oleksyn J, Reich PB, Karolewski P, Zytkowski R, Jagodzinski AM, Turzanska E. 2006. Soil modification by different tree species influences the extent of seedling ectomycorrhizal infection. *Mycorrhiza* 16: 73–79.
- Dickie IA, Reich PB. 2005. Ectomycorrhizal fungal communities at forest edges. *Journal of Ecology* 93: 244–255.
- Dighton J, Bonilla ASM, Jimenez-Nunez RA, Martinez N. 2000. Determinants of leaf litter patchiness in mixed species New Jersey pine barrens forest and its possible influence on soil and soil biota. *Biology and Fertility of Soils* 31: 288–293.
- Douhan GW, Rizzo DM. 2005. Phylogenetic divergence in a local population of the ectomycorrhizal fungus *Cenococcum geophilum*. *New Phytologist* 166: 263–271.

- Downie DE, Taskey RD. 1997. Soil characteristics of blue oak and coast live oak ecosystems. In: Pillsbury NH, Verner J, Tietje WD, eds. *Proceedings of a symposium on oak woodlands: ecology, management, and urban interface issues, 19–22 March 1996, San Luis Obispo, CA*. General Technical Report PSW-GTR-160. Albany, CA, USA: Pacific Southwest Research Station, Forest Service, USDA, 65–74.
- Frost WF, Edinger SB. 1991. Effects of tree canopies on soil characteristics of annual rangeland. *Journal of Range Management* 44: 286–288.
- Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118.
- Harrington TJ, Mitchell DT. 2005. Ectomycorrhizas associated with a relict population of *Dryas octopetala* in the Burren, western Ireland. 1. Distribution of ectomycorrhizas in relation to vegetation and soil characteristics. *Mycorrhiza* 15: 425–433.
- He X, Bledsoe CS, Zasoski RJ, Southworth D, Horwath WR. 2006. Rapid nitrogen transfer from ectomycorrhizal pines to adjacent ectomycorrhizal and arbuscular mycorrhizal plants in a California oak woodland. *New Phytologist* 170: 143–151.
- Holland VL. 1973. A study of soil and vegetation under *Quercus douglasii* H. & A. compared to open grassland. PhD dissertation, Berkeley, CA, USA: University of California.
- Hopple J, Vilgalys R. 1994. Phylogenetic relationships among coprinoid taxa and allies based on data from restriction site mapping of nuclear rDNA. *Mycologia* 86: 96–107.
- Horton TR, Bruns TD. 1998. Multiple-host fungi are the most frequent and abundant ectomycorrhizal types in a mixed stand of Douglas fir (*Pseudotsuga menziesii*) and bishop pine (*Pinus muricata*). *New Phytologist* 139: 331–339.
- Horton TR, Bruns TD. 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. *Molecular Ecology* 10: 1855–1871.
- Horton TR, Bruns TD, Parker VT. 1999. Ectomycorrhizal fungi associated with *Arctostaphylos* contribute to *Pseudotsuga menziesii* establishment. *Canadian Journal of Botany* 77: 93–102.
- Huang X. 1997. Watershed hydrology, soil and biogeochemistry in an oak woodland annual grassland ecosystem in the Sierra foothills, California. PhD dissertation, Davis, CA, USA: University of California.
- Ishida TA, Nara K, Hogetsu. 2007. Host effects on ectomycorrhizal fungal communities: insights from eight host species in mixed conifer-broadleaf forests. *New Phytologist* 174: 430–440.
- Izzo A, Agbowo J, Bruns TD. 2005. Detection of plot-level changes in ectomycorrhizal communities across years in an old-growth mixed-conifer forest. *New Phytologist* 166: 619–630.
- Jackson LE, Strauss RB, Firestone MK, Bartolome JW. 1990. Influence of tree canopies on grassland productivity and nitrogen dynamics in deciduous oak savanna. *Agriculture, Ecosystems and Environment* 32: 89–105.
- Jumpponen A. 2003. Soil fungal community assembly in a primary successional glacier forefront ecosystem as inferred from rDNA sequence analysis. *New Phytologist* 158: 569–578.
- Kennedy PG, Izzo AD, Bruns TD. 2003. There is high potential for the formation of common mycorrhizal networks between understory and canopy trees in a mixed evergreen forest. *Journal of Ecology* 91: 1071–1080.
- Koide RT, Suomi L, Stevens CM, McCormick L. 1998. Interactions between needles of *Pinus resinosa* and ectomycorrhizal fungi. *New Phytologist* 140: 539–547.
- Landeweert R, Leeflang P, Kuypers TW, Hoffland E, Rosling A, Wernars K, Smit E. 2003. Molecular identification of ectomycorrhizal mycelium in soil horizons. *Applied and Environmental Microbiology* 69: 327–333.
- Lepš J, Šmilauer P. 2003. *Multivariate analysis of ecological data using CANOCO*. Cambridge, UK: Cambridge University Press.
- Martin D, Rybicki E. 2000. RDP: detection of recombination amongst aligned sequences. *Bioinformatics* 16: 562–563.
- Millikin CS, Bledsoe CS. 1999. Biomass and distribution of fine and coarse roots from blue oak (*Quercus douglasii*) trees in northern Sierra Nevada foothills of California. *Plant and Soil* 214: 27–38.
- Molina R, Massicotte H, Trappe JM. 1992. Specificity phenomena in mycorrhizal symbiosis: community-ecological consequences and practical implications. In: Allen MF, ed. *Mycorrhizal functioning*. New York, NY, USA: Chapman & Hall, 357–423.
- Monk CD. 1966. An ecological significance of evergreenness. *Ecology* 47: 504–505.
- Nara K. 2006. Pioneer dwarf willow may facilitate tree succession by providing late colonizers with compatible ectomycorrhizal fungi in a primary successional volcanic desert. *New Phytologist* 171: 187–198.
- Newton AC, Haigh JM. 1998. Diversity of ectomycorrhizal fungi in Britain: a test of the species–area relationship, and the role of host specificity. *New Phytologist* 138: 619–627.
- Nixon KC. 1993. The genus *Quercus* in Mexico. In: Ramamoorthy TP, Bye R, Lot A, Fa J, eds. *Biological diversity of Mexico: origins and distribution*. New York, NY, USA: Oxford University Press, 447–458.
- Nixon KC. 2002. The oak (*Quercus*) biodiversity of California and adjacent regions. In: Standiford RB, McCreary D, Purcell KL, eds. *Proceedings of the fifth symposium on oak woodlands: oaks in California's changing landscape, 22–25 October 2001, San Diego, CA*. General Technical Report PSW-GTR-184. Albany, CA, USA: Pacific Southwest Research Station, Forest Service, USDA, 3–20.
- O'Brien HE, Parrent JL, Jackson JA, Moncalvo, Vilgalys R. 2005. Fungal community analysis by large-scale sequencing of environmental samples. *Applied and Environmental Microbiology* 71: 5544–5550.
- Padien DJ, Lajtha K. 1992. Plant spatial pattern and nutrient distribution in pinyon-juniper woodlands along an elevational gradient in northern New Mexico. *International Journal of Plant Sciences* 153: 425–433.
- Qiu X, Wu L, Huang H, McDonel PE, Palumbo AV, Tiedje JM, Zhou J. 2001. Evaluation of PCR-generated chimeras, mutations, and heteroduplexes with 16S rRNA gene-based cloning. *Applied and Environmental Microbiology* 67: 880–887.
- Rejmánek M, Richardson DM, Pyšek P. 2005. Plant invasions and invasibility of plant communities. In: van der Maarel E, ed. *Vegetation ecology*. Malden, MA, USA: Blackwell Publishing, 332–355.
- Richard F, Millot S, Gardes M, Selosse M-A. 2005. Diversity and specificity of ectomycorrhizal fungi retrieved from an old-growth Mediterranean forest dominated by *Quercus ilex*. *New Phytologist* 166: 1011–1023.
- Schlesinger WH, Raikes JA, Hartley AE, Cross AF. 1996. On the spatial pattern of soil nutrients in desert ecosystems. *Ecology* 77: 364–374.
- Smith ME, Douhan GW, Rizzo DM. 2007. Ectomycorrhizal community structure in a xeric *Quercus* woodland based on rDNA sequence analysis of sporocarps and pooled roots. *New Phytologist* 174: 847–863.
- Smith SE, Read DJ. 1997. *Mycorrhizal symbiosis*, 2nd edn. London, UK: Academic Press.
- Standiford, R. 2002. California's oak woodlands. In: McShea WJ, Healy WM, eds. *Oak forest ecosystems: ecology and management for wildlife*. Baltimore, MD, USA: Johns Hopkins University Press, 280–303.
- Tederso L, Hansen K, Perry BA, Kjoller R. 2006. Molecular and morphological diversity of peizizalean ectomycorrhiza. *New Phytologist* 170: 581–596.
- Valentine LL, Fiedler TL, Hart AN, Petersen CA, Berninghausen HK, Southworth D. 2004. Diversity of ectomycorrhizas associated with *Quercus garryana* in southern Oregon. *Canadian Journal of Botany* 82: 123–135.
- Vrålstad T, Schumacher T, Taylor AFS. 2002. Mycorrhizal synthesis between fungal strains of the *Hymenoscyphus ericae* aggregate and potential ectomycorrhizal and ericoid hosts. *New Phytologist* 153: 143–152.

- Walker JF, Miller Jr. OK, Horton JL. 2005. Hyperdiversity of ectomycorrhizal fungus assemblages in mixed forests in the southern Appalachian Mountains. *Molecular Ecology* 14: 829–838.
- Wardle DA. 2002. *Communities and ecosystems: linking the aboveground and belowground components*. Princeton, NJ, USA: Princeton University Press.
- Weiss M, Selosse M-A, Rexer K-H, Urban A, Oberwinkler F. 2004. *Sebacinales*: a hitherto overlooked cosm of heterobasidiomycetes with a broad mycorrhizal potential. *Mycological Research* 9: 1003–1010.
- Williams CE, Lipscomb MV, Johnson WC, Nilsen ET. 1990. Influence of leaf litter and soil moisture regime on early establishment of *Pinus pungens*. *American Midland Naturalist* 124: 142–152.

Supplementary Material

The following supplementary material is available for this article online:

Table S1 Ectomycorrhizal taxa from roots of *Quercus douglasii* and *Quercus wislizeni* trees growing in a blue oak woodland in northern California

Table S2 Species richness of ectomycorrhizal fungal communities found on roots of *Quercus douglasii* and *Quercus wislizeni*

Table S3 Comparison of mean (\pm SE) characteristics of soils collected under canopies of *Quercus douglasii* and *Quercus wislizeni* ($n = 8$)

This material is available as part of the online article from:
<http://www.blackwell-synergy.com/doi/abs/10.1111/j.1469-8137.2007.02348.x>
(This link will take you to the article abstract).

Please note: Blackwell Publishing are not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than about missing material) should be directed to the journal at *New Phytologist* Central Office.