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Spatial structure and richness of ectomycorrhizal fungi colonizing bioassay seedlings from resistant propagules in a Sierra Nevada forest: comparisons using two hosts that exhibit different seedling establishment patterns

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Abstract: In this study we analyzed the spatial structure of ectomycorrhizal fungi present in the soils as resistant propagules (e.g. spores or sclerotia) in a mixed-conifer forest in the Sierra Nevada, California. Soils were collected under old-growth *Abies spp.* stands across approximately 1 km and bioassayed with seedlings of hosts that establish best in stronger light (*Pinus jeffreyi*) or that are shade-tolerant (*Abies concolor*). Ectomycorrhizal fungi colonizing the roots were characterized with molecular techniques (ITS-RFLP and DNA sequence analysis). *Wilcoxina*, five *Rhizopogon* species and *Cenococcum* were the most frequent of 17 detected species. No spatial structure was detected in the resistant propagule community as a whole, but *P. jeffreyi* seedlings had higher species richness and associated with seven *Rhizopogon* species that were not detected on *A. concolor* seedlings. We drew two conclusions from comparisons between this study and a prior study of the ectomycorrhizal community on mature trees in the same forest: (i) the resistant propagule community was considerably simpler and more homogeneous than the active resident community across the forest and (ii) *Cenococcum* and *Wilcoxina* species are abundant in both communities.

Key words: *Abies concolor*, *Pinus jeffreyi*, sclerotia, Sierra Nevada, spore

INTRODUCTION

Studies of ectomycorrhizal (ECM) fungi suggest that some species are more common colonizers of roots early in forest succession (both primary and secondary succession) while others tend to be more abundant later as the forest matures (Fleming et al 1986). Because early successional dominants do persist in the late successional setting (Visser 1995), and because their ability to dominate appears to be context dependent with some species (Newton 1992, Keizer and Arnolds 1994), the specific terminology referring to these classes of fungi is not agreed upon. However terminology and successional patterns aside, it is clear that a fundamental difference in colonization strategy was identified by these and more recent studies: the prominent early successional fungi are more effective at colonizing roots by spores and propagules that are resident in the soil (collectively referred to as the resistant propagule community or RPC, Taylor and Bruns 1999) compared to the prominent late successional fungi that instead are presumed to colonize primarily by the extension of mycelium from other colonized roots (Fleming 1984, Simard et al 1997, Baar et al 1999, Taylor and Bruns 1999).

We know that members of the RPC are the most abundant colonizers of seedlings after stand-replacing fire in coastal California *Pinus muricata* forests (Baar et al 1999) and that ECM fungal colonization of roots increases seedling survival and performance in dry conditions (Parke et al 1983, Horton et al 1999). However the benefits that a plant host receives from a mycorrhizal fungus may vary both by fungal and host species (Perry et al 1989, van der Heijden and Kuyper 2003) and not all hosts are equally receptive to a given RPC species (Massicotte et al 1999). Differences in how the RPC is structured across space and time for different hosts therefore could affect seedling establishment patterns.

Studies (Jonsson et al 1999, Kennedy et al 2003) continue to demonstrate that the ECM fungi found on roots of overstory hosts in mature or old forests commonly act as the primary source of inoculum for seedlings. ECM fungal communities in similarly aged forests are generally species rich and their composition can change substantially within a few meters or less (Jonsson et al 2000, Peter et al 2001, Tedersoo et al 2003, Lilleskov et al 2004). The inoculum provided to seedlings in this setting therefore is likely to be similarly structured, as appears to be the case in Walker et al (2005). In contrast RPC fungi detected on field and bioassay seedlings appear to be much less

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diverse (Baar et al 1999, Massicotte et al 1999, Taylor and Bruns 1999) and in the case of species in the genus *Rhizopogon* are a ubiquitous source of inoculum in California pine forests (Kjøller and Bruns 2003).

The goals of our study were (i) to determine the composition and spatial structure of the RPC and (ii) to compare the composition and spatial structure of the RPC to that of the resident ECM root community that is found on the roots of mature trees in the same forest. Based on a recent study of *Rhizopogon* spore banks across five pine forests in California (Kjøller and Bruns 2003) we predicted that the RPC will be consistently dominated by a few species and will not have large differences in species composition across scales of 25–1000 m. However there were three important differences between our study and that of Kjøller and Bruns: (i) we did not limit our sampling to a single genus; (ii) we used two different bioassay hosts letting us account for any species-level differences between either the hosts or the fungi; and (iii) we sampled within an old growth forest that has not been logged or experienced a fire disturbance in more than 90 y. The absence of fire might highlight differences between the mature forest colonizer and the RPC because the latter may decline or change in composition in the absence of disturbance. On the other hand small-scale disturbances such as windthrow, disease, mammal activity or the longevity of propagules may let the RPC persist.

Other studies also have addressed the comparative structure of the RPC and the ECM community but have differed in significant ways. For example trenching studies have provided a good view into both communities but have assessed the mature root community indirectly through the ECM colonizing planted seedlings and based comparisons of these communities on ECM morphotyping (Fleming 1984, Simard et al 1997). A molecular-based comparison (Taylor and Bruns 1999) suggested the RPC was more homogenous than ECM mature root communities because more species were detected frequently across plots. However their study was based on one transect (25 m) of sampling, which limits our ability to generalize about this pattern.

MATERIALS AND METHODS

Site description.—This study was conducted at the Teakettle Experimental Forest, Sierra National Forest (SNF), California (36°58′N, 119°2′W) on the southwestern slope of the Sierra Nevada. Extensive details about this forest are described by North et al (2002). The elevation at this site is approximately 2100 m. The forest canopy is dominated by *Abies concolor* (white fir) and *Abies magnifica* (red fir) and also includes *Pinus jeffreyi* (Jeffrey pine), *Pinus lambertiana* (sugar pine) and *Calocedrus decurrens* (incense cedar). Of these we chose *P. jeffreyi* and *A. concolor* as ECM hosts that exhibit different seedling establishment patterns. *P. jeffreyi* more commonly establishes best in stronger light while *A. concolor* seedlings are more shade tolerant and commonly establish in dim light (Gray et al 2005). This difference also has been measured between *Pinus* spp. and *Abies* spp. in another similar forest (McDonald 1976). The climate in this region is characterized by a summer drought that presents a strong barrier to seedling establishment (Gray et al 2005). Mean annual precipitation is 125 cm, most of which comes in the form of snow Nov–May. The average monthly rainfall May–Nov is less than 2 cm. Air temperatures averages 15.5 C in the summer and 0.7 C in the winter.

Preparation of bioassays.—Soils were collected from 54 plots across Teakettle Experimental Forest. The plots (1 m^2) were located across six 200 m^2 compartments that were established as part of a large-scale study on forest management to represent the typical plant and site composition in this forest (North 2002b). The distance between the most distant plots was approximately 1500–1600 m. Soil was collected from four compartments (US3, UN3, UC3, BN2) in May 2001 and from two (BC1, BS2) in Aug 2001 that had been thinned within 6 wk. All compartments ultimately were included in the final analyses because the species composition of the thinned compartments collectively did not contain any obvious differences from the unthinned compartments. The organic soil layer generally would be the most affected by a fire and therefore we sampled only the mineral layer. The organic layer (5.6 cm thick on average in the closed canopy of this forest, North et al 2000) was scraped aside to expose the mineral layer before collection. We used a shovel to collect two samples of mineral soil (ca. 500 g each) 1 m apart at each plot and then pooled them. Nine plots were located within each compartment and on average these plots were 25–50 m apart (FIG. 1). In the case of the UN3 compartment soils were collected around previously described coring points where the ectomycorrhizal root community had been characterized (Izzo et al 2005a). Soils were sieved through a 2 mm screen leaving small root tips, root fragments and hyphae in addition to spores. Each of these therefore could act as an inoculum source, although hyphal-based inoculation should be less likely without it being connected to a host and given the length of time the soils sat before the experiment (4–7 mo). The prepared soils were stored at 4 C until the bioassays were begun in Dec 2001.

In Dec 2001 the soils were mixed roughly 1:1 (mass) with coarse sand (sterilized at 250 C 20 min wet cycle followed by a 250 C 20 min dry cycle) to improve drainage. All soil sieving and mixing with coarse sand was done in a ventilation hood to reduce outside contamination. The hood and sieve both were washed with soapy water between plot samples to minimize cross-contamination. The bioassay pots (2.5 cm wide × 16 cm deep RLC-4 Super ''Stubby'' Cell

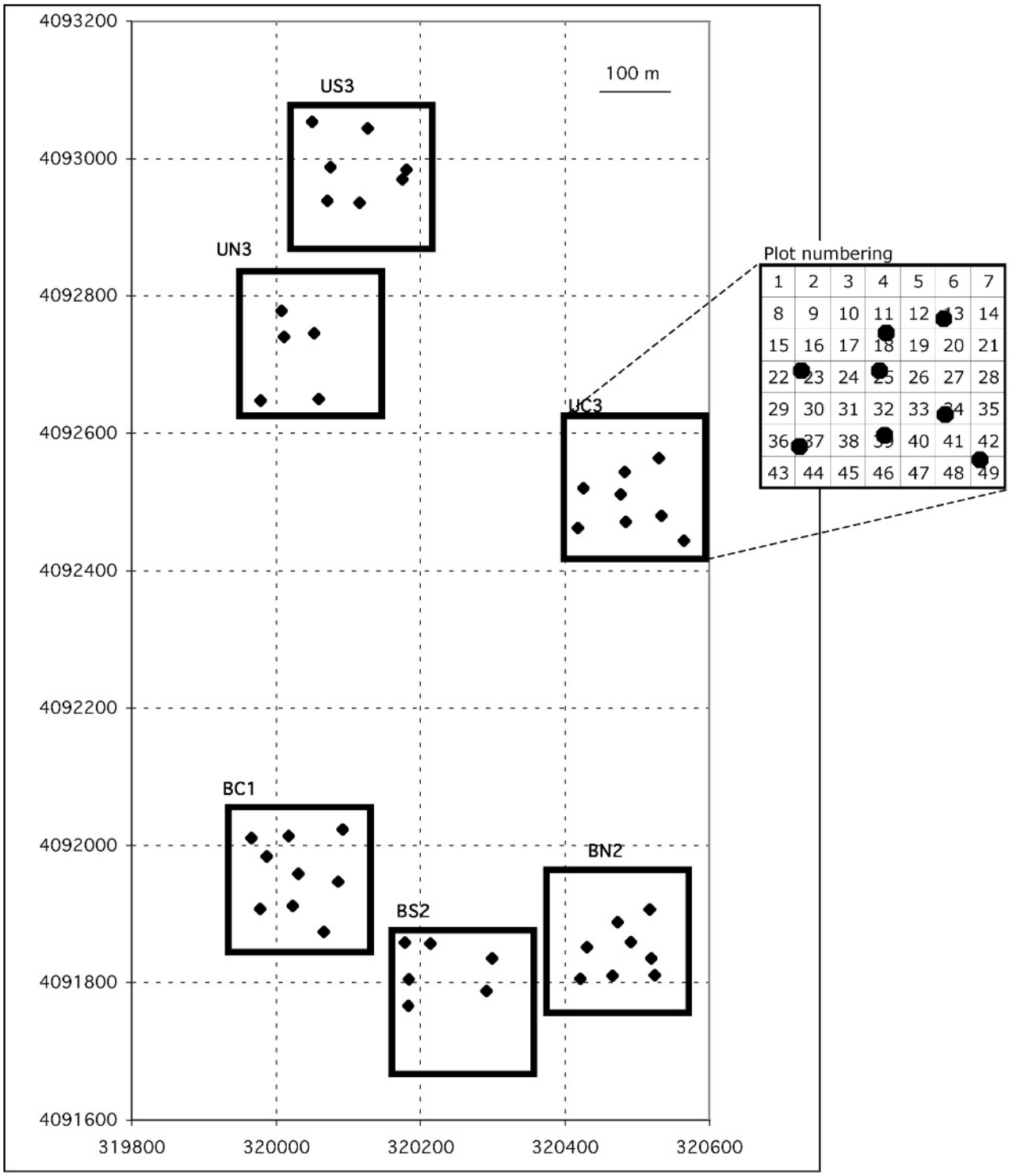

FIG. 1. Spatial arrangement of all plots across Teakettle Experimental Forest. X-Y values are in UTM coordinates. Filled circles indicate the location of a plot. Plots that were not analyzed due to insufficient seed germination are not shown here. Inlay shows the numbering of plots (2 m transect) within the six compartments (4 ha each) as established in North (2002b). Actual sampling points were within 15 m of numbered grid points.

Cone-tainers, Steuwe & Sons Inc., Corvalis, Oregon) were soaked in 10% bleach for 30 min then soaked in distilled water 30 min. Polyester fill (purchased from JoAnn Fabrics) was added to the bottom of pots to contain the soil.

Seeds from Jeffrey pine and white fir collected in the Sierra Nevada were obtained from Placerville Nursery (Camino, California). Seeds were surface sterilized in hydrogen peroxide for 10 min, soaked in running water for 48 h and stratified at 4 C for 2 (pine) or 3 (fir) mo before planting. A total of eight pots per plot (432 pots total) were set up, half of which were assayed with fir and the other half with pine. A single germinated seed was placed on the soil surface of each pot. A total of 10 control seeds were planted in a 50:50 mix of soil (extra from the plot collections) and coarse sand that both had been sterilized as described above. We avoided growing seedlings in the greenhouse to minimize contamination by thelephoroid fungi. Seedlings instead were grown under a lab bench fitted with grow lights. Pots were grouped by plots and the entire rack was rotated randomly every 2 wk to control for any environmental variation within the growing area. Seedlings were watered twice a week with approximately 10 mL tap water. A number of precautionary measures were used to safeguard against cross-contamination including adding a layer of coarse sand to the top of each pot, leaving 5 cm between samples from different plots and washing collecting trays underneath the seedlings after every watering to remove any soil. As a result of these efforts no contamination was observed. The seedlings were grown 14 h in the light (75 $\mu mol/m^2/s$) and 10 h in the dark with a constant temperature of 22–23 C.

Characterization of the seedling ectomycorrhizal community.—Pine seedlings were harvested after 8 mo. The fir seedling root systems were much less developed than the pine root system and therefore were grown 5 additional mo. At harvest each seedling was removed from its pot and rinsed under tap water to remove soil from the root. Roots of each seedling were analyzed under a dissecting microscope for 15 min. Roots were considered to be ectomycorrhizal if they (i) had no root hairs, showed swelling and had evidence of hyphae present or (ii) if a hyphal sheath clearly was present.

Because of the large area that was included in this study, as well as the high diversity of *Rhizopogon* species shown to exist in a single hectare in a nearby forest (North 2002a), we took an aggressive molecular approach to ensure that we were not underestimating RPC species richness. One representative of each unique morphotype from each seedling was chosen for molecular analysis. Total genomic nucleic acids were isolated from the root tips with a modification of the QIAGEN DNeasy kit protocol (QIAGEN Inc., Valencia, California) described by Izzo et al (2005a) except that fresh root tips were used immediately after collection. PCR was performed in a PTC-100 thermalcycler (MJ Research Inc., Waltham, Massachusetts) in conditions described by Gardes and Bruns (1993) with 5 μL of the isolated nucleic acids in a 50 μL reaction and an annealing temperature of 51 C. The internal transcribed spacer (ITS) region was amplified with the ITS1F (Gardes and Bruns 1993) and ITS4 (White et al 1990) primer pair that targets both ascomycetes and basidiomycetes. PCR product was digested enzymatically with HinfI and AluI (New England Biolabs, Waltham, Massachusetts), analyzed by agarose gel electrophoresis and the resulting fragments sized with the program GelReader (NCBI). We sequenced the ITS1/5.8S/ITS2 region of multiple representatives of each unique RFLP type across all samples. Sequences that were different by 4% or less were considered to be the same species, with most intraspecific differences being 2% or less. Each sequence type was compared to the GenBank database with BLAST searches (Altschul et al 1997) and named according to the closest apparent taxonomic level. Sequences from RPC fungi were compared directly to those obtained on roots in this forest (Izzo et al 2005a). More detailed phylogenetic analysis was performed on *Rhizopogon* species by comparing them to the sequence types identified by Kjøller and Bruns (2003) using distance analysis.

Species richness estimates.—Species accumulation curves were constructed to estimate our success in measuring richness of the RPC within compartments and across all plots. The average number of species seen at different sampling intensities was based on 100 random samplings of all plots pooled either within a compartment or across the entire forest. These samplings were done without replacement and were calculated in EstimateS (Colwell 2000, EstimateS: statistical estimation of species richness and shared species from samples. Version 6.0b1. User's guide and application. http://viceroy.eeb.ucon.edu/estimates). Because we did not have estimates on true propagule abundance at a plot, we used the Chao2 estimate for species richness. This is a nonparametric index based on the accumulation of new species (Chao 1987). A similar analysis was performed across all sites with Rhizopogon species only to allow comparisons to another spore bank study done in this region (Kjøller and Bruns 2003).

Spatial tests.—Of the 54 plots only those in which at least three or four seedlings successfully germinated for either host (45 plots for a total of 202 seedlings) were included in the final analyses. To test for spatial structure across the forest Mantel tests were employed with the program R4.0 (Casgrain and Legendre 2001). Community similarity was calculated with Sorenson's index of similarity (Magurran 1988) using the formula $2a/(2a+b+c)$ where a is the number of species shared, b is the number of species unique to plot 1 and c is the number of species unique to plot 2.

The Sorenson similarity index of the RPC between plots was compared to the Sorenson index calculated from the active ECM root community data obtained from a study focusing on the UN3 compartment (Izzo et al 2005a). To make tests across the RPC and root communities comparable (i) a subset of the *P. jeffreyi* plots (n = 17) were chosen that were within 50 m of the *A. concolor* plots and (ii) only distances up to 250 m between plots—the maximum distance across the root study—were considered. The mature root community collected across 3 y in Izzo et al (2005a) was summarized for each of those plots (n = 9) and

the Sorenson's similarity index calculated as above. After determining that there was no spatial structure between plots the community similarities were compared with a nonparametric test (Kruskal-Wallis).

RESULTS

Spore bank composition.—The germination rates for *P. jeffreyi* were much higher, resulting in 40 sites (149 seedlings) that were included in the analysis compared to 17 sites (53 seedlings) for *A. concolor*. No ECM colonization was detected in the sterile controls. Overall ECM colonization of roots per seedling across all plot samples was similar for both *P. jeffreyi* and *A. concolor* (data not shown). In all 1005 root tips were selected for molecular analysis. We were successful in typing 903 of the roots by RFLP analysis and subsequent DNA sequencing. The remaining samples were mixed or did not amplify and were excluded from the analysis. Seventeen species-level taxa (TABLE I) were identified with DNA sequence analysis. With the exception of Basidiomycota14, all sequence types had close matches to named sequences already submitted to GenBank. Ascomycota11 matched an unidentified postdisturbance ectomycorrhizal fungus but was not a close match to any named samples. Phialophora1 and Phialophora2 differed only in the presence of an insert and were considered to be the same species. *P. jeffreyi* seedlings had 2.4 ± 1.0 (mean ± stdev) species per seedling (range 1–6) and 4.2 ± 1.6 species (range 2–9) per plot. *A. concolor* had generally fewer species with 1.5 ± 0.6 species per seedling (range 1–3) and 2.2 ± 1.0 species per plot (range 1–4). The most frequent RPC fungi belonged to three genera—*Wilcoxina, Rhizopogon* (five species) and *Cenococcum* (FIG. 2). Wilcoxina1 and *R. roseolus* were the most common on all of the *P. jeffreyi* seedlings from a plot and each were found in roughly 90% of the plots. A total of seven *Rhizopogon* species were found overall. *A. concolor* seedlings were dominated by Wilcoxina1 and *C. geophilum* and did not yield any unique RPC species. There were 12 total plots for which we had data for both host species letting us directly compare the fungi colonizing each host (FIG. 2). Across these sites *P. jeffreyi* associated with all the species that *A. concolor* did and in addition was colonized by multiple *Rhizopogon* species. However in five of the overlapping plots *C. geophilum* colonized *A. concolor*—indicating it was present in those soils—but was not detected on *P. jeffreyi*.

TABLE I. Species-groups identified in this study. Study types are based on a database of sequences obtained from Sierra National Forest (SNF) and are used for cross-referencing in these studies. These types are named based on the results of BLAST searches of the ITS1 sequence (shown below). *Rhizopogon* species were analyzed more thoroughly and identified by phylogenetic analysis based on the alignment and nomenclature used in Kjøller and Bruns (2003) with the complete ITS1/5.8S/ITS2 sequence. Hosts on which the fungal types were detected are noted as P (*P. jeffreyi*) and A (*A. concolor*). Asterisks indicate types that also were detected on roots in Izzo et al (2005a)

SNF type name	Host	bp	Best match (Accession #)	% Similarity/bp	Accession #
Ascomycota11	P/A	160	unnamed post-fire ECM isolate (AJ410863)	93%/154	AY587740
Basidiomycota14*	P	135	*Amphinema sp.* O48 (AJ534707)	98%/58	AY587741
*Cenococcum geophilum**	P/A	141	*Cenococcum geophilum* (AY112935)	98%/141	AY587742
Helotiales1*	P	169	Ericoid mycorrhizal sp. (AF072296)	90%/154	AY587743
Phialophora1	P/A	164	*Phialophora finlandia* (AF486119)	99%/164	AY587744
Phialophora2	P/A	510	*Phialophora finlandia* (AF486119)	96%/510	AY587745
R. arctostaphyli gr	P	216	*Rhizopogon arctostaphyli* (AF224478)	100%/216	AY587746
R. ellenae gr	P	232	*Rhizopogon idahoensis* (AF377123)	100%/231	AY587747
R. occidentalis gr	P	319	*Rhizopogon occidentalis* (AF058305)	95%/319	AY587748
R. olivaceotinctus gr	P	273	*Rhizopogon olivaceotinctus* (AJ515509)	98%/273	AY587749
R. roseolus gr	P	223	*Rhizopogon roseolus* (AF058315)	99%/223	AY587750
R. salebrosus gr*	P	223	*Rhizopogon salebrosus* AHS69299 (AF377157)	99%/223	AY587751
R. versicolores gr	P	306	*Rhizopogon smithii* (AF158017)	98%/306	AY587752
Sebacinoid1*	P	200	Uncultured sebacinaceous fungus (AY187617)	85%/199	AY587753
Suillus1	P	209	*Suillus pseudobrevipes* (L54107)	99%/209	AY587754
Wilcoxina1*	P/A	189	*Wilcoxina rehmii* AF266708)	100%/189	AY587755
Wilcoxina4	P/A	189	*Wilcoxina mikolae* (AY219841)	93%/180	AY587756

Species richness estimates.—Species accumulation curves indicate that we adequately sampled the RPC for *A. concolor* across the forest but not for *P. jeffreyi* (FIG. 3). After sampling only five plots the most frequent RPC species generally were seen for each host. However, while species accumulation for *A. concolor* plateaued, that for *P. jeffreyi* continued to increase linearly. The total species richness expected across 17 plots (the maximum number of plots for fir) was estimated to be 5.5 species for the fir seedlings and 16.6 for the pine seedlings based on the Chao2 estimator. The genus *Rhizopogon* was calculated to have 8.6 species present as RPC species across the sampling area (Chao2 estimator).

Spatial structure.—The similarity of the RPC species composition found between plots did not change significantly with distance across the forest for either *A. concolor* seedlings (Mantel test, $P = 0.31$) or *P. jeffreyi* (Mantel test, $P = 0.32$). Treating the *Phialophora* types as separate species did not affect this result. One relatively frequent species on pine seedlings—*R. salebrosus*—might have limitations in its distribution because it was found strictly in the UC and US compartments. However it never was detected on more than one seedling and therefore this pattern might be due strictly to limitations of our sampling. *Rhizopogon* species in the RPC were diverse at small scales with many instances of four or five different species being detected in a plot (1 m^2).

Comparison of the RPC to ECM root community.—The similarity of the RPC on *A. concolor* seedlings across plots was significantly higher than the RPC on *P. jeffreyi* seedlings (Kruskal-Wallis, $P < 0.0001$). The composition of the RPC across plots was more homogeneous for both host species than the ectomycorrhizal fungal community on roots of mature trees at the same scale (FIG. 4).

Six RPC species also were identified as being active on the roots of mature trees described by Izzo et al (2005a). Two of these species, *C. geophilum* and Wilcoxina1, were detected on the mature roots in a high percentage of soil cores but colonized a low percentage of roots in these cores. However both species ranked high in abundance at the forest scale because no other species were as widespread on mature roots. For example *Cenococcum geophilum* ranked 31st of all species in its average relative abundance within cores (0.21), but had the 21st highest average abundance within plots (0.12) and the 2nd highest across all plots (0.10). Wilcoxina1 showed similar patterns with ranks of 15th (0.29 average relative abundance), 9th (0.21) and 3rd (0.07) within cores, plots and across all plots respectively. A number of RPC taxa were not detected on the roots of mature trees. Of these, two species—*Rhizopogon olivaceotinctus* and *Rhizopogon occidentalis*—were relatively frequent and occurred respectively in 43% and 28% of the plots.

DISCUSSION

Composition and spatial structure of RPC.—The RPC detected in our study is dominated by relatively few taxa that are distributed evenly across the forest (FIG. 2). As a result we measured no difference in the similarity of the RPC between plots across the spatial scales (25–150 m). A study of the ECM mature root community in this forest similarly showed no spatial structure at similar scales (Izzo et al 2005a). However because different fungi tended to dominate each plot in the mature root community, the similarity between plots was much lower compared to the RPC (FIG. 4). The RPC therefore represents a much more homogeneous and predictable inoculum source for seedlings compared to the highly diverse and patchy field root community in this forest. Assuming an overall benefit of ECM inoculation this predictability might help buffer the stochastic elements of seedling establishment such as dispersal, predation, microsite suitability and the timing or scale of a disturbance.

Host-specific differences were detected in the RPC in our assays that suggest ways that shifts from a mature ECM root community to the RPC could favor certain hosts and warrant further testing. The most obvious difference was that *P. jeffreyi* associated with more species than *A. concolor* primarily due to its ability to associate with seven *Rhizopogon* species in addition to all of the fungi colonizing *A. concolor*. Any benefits that *Rhizopogon* provides to *P. jeffreyi* seedlings, such as quick access to nutrients through its prolific growth of exploration hyphae (Agerer 2001) or by simply increasing the inoculum load in the RPC, could play a role in the success of *P. jeffreyi* seen in some postdisturbance settings (e.g. Bock and Bock 1977). Some of the specificity of *Rhizopogon* toward *P. jeffreyi* that we observed might be overestimated due to the fact that our seedlings were grown in monoculture. While many species of *Rhizopogon* do exhibit some degree of host specificity toward *Pinus* or *Pseudotsuga* both in the field (Molina et al 1992) and on bioassay seedlings (Massicotte et al 1999), Massicotte et al (1994) found that some *Rhizopogon* species would inoculate *A. grandis* if grown in dual culture with *P. ponderosa*.

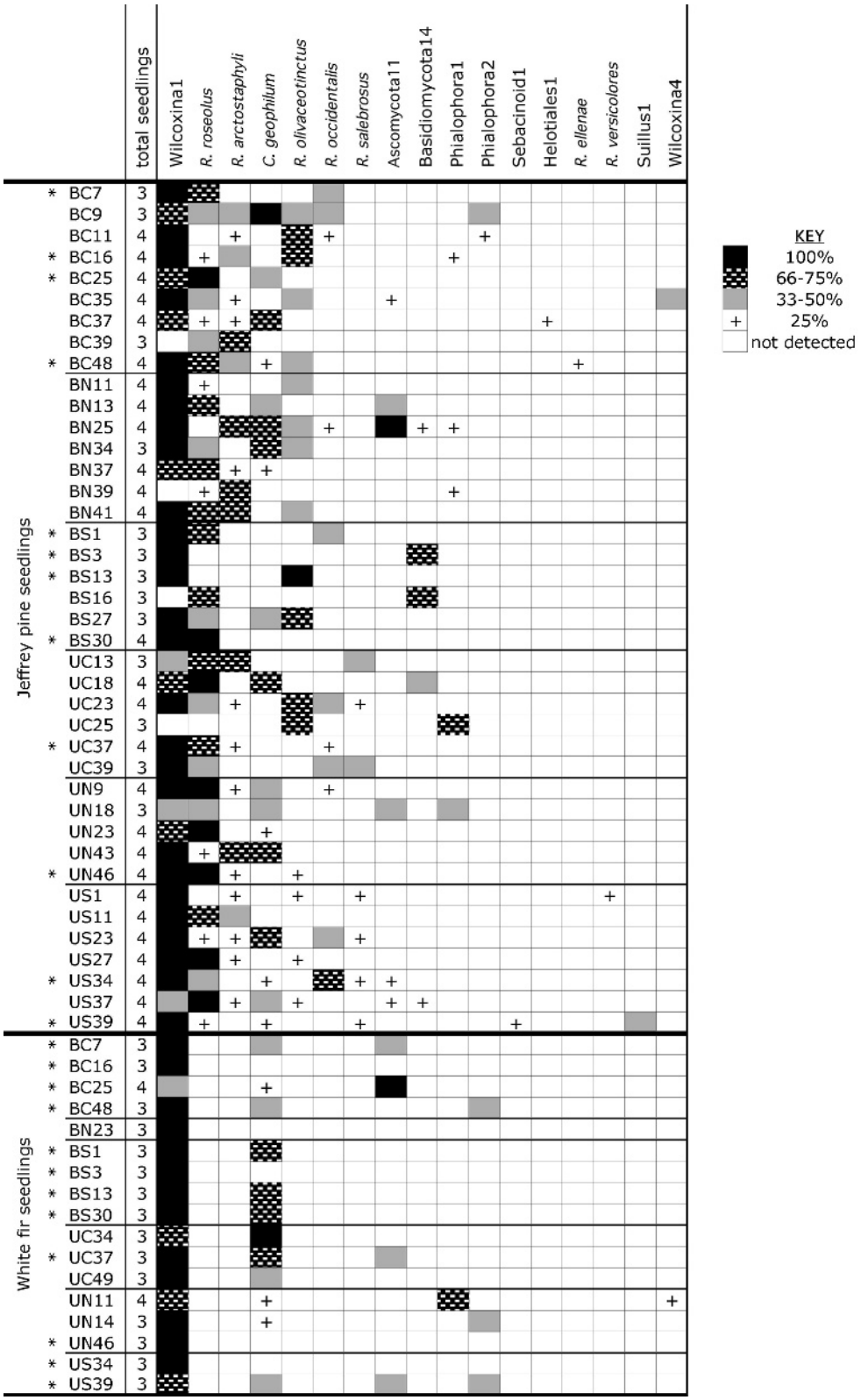

FIG. 2. Frequency of occurrence for RPC species on individual bioassay seedlings. Species are ordered from left to right based on decreasing frequency on *P. jeffreyi*. Asterisks indicate plots with data from both hosts.

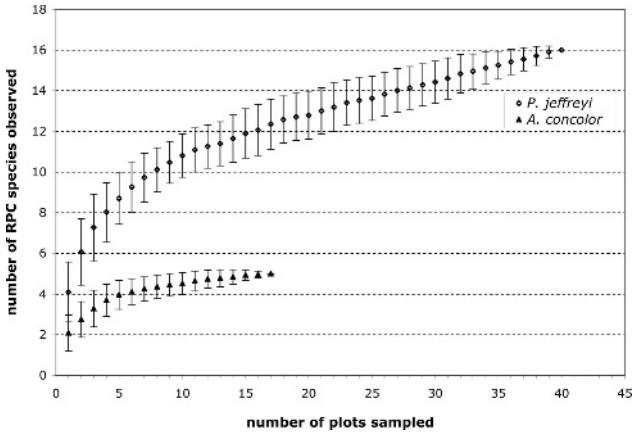

FIG. 3. Species accumulation curves for ectomycorrhizal species detected on different bioassay host types. Bars indicate standard error based on 100 subsamples with replacement.

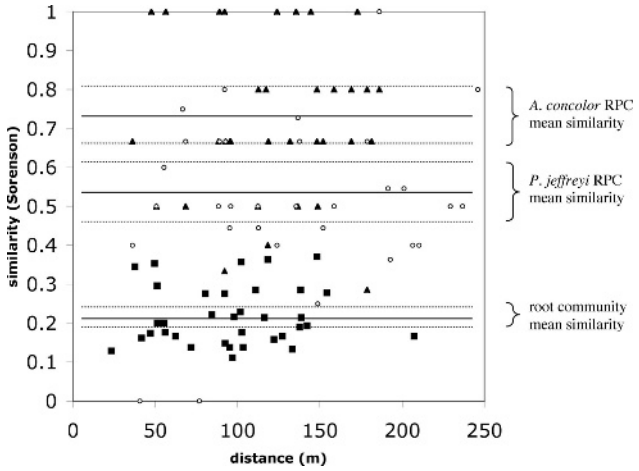

FIG. 4. Between-plot similarity across distance for different community types. Plot-plot Sorensons similarity index for ECM fungi detected in bioassays (σ = *A. concolor* RPC, O = *P. jeffreyi* RPC) and on field roots (ν = active root community from Izzo et al [2005a]). Solid lines indicate average similarity across all plots. Dashed lines indicate 95% confidence interval of the mean.

Comparison of the RPC to the root community.—By comparison of DNA sequences obtained from the RPC in this study and from a study of mature ECM roots in this same forest (Izzo et al 2005a) we were able to determine that roughly one-third of the RPC species were active on the roots of mature trees in this old-growth forest. Of course this remains a conservative estimate because, while we appear to have reasonably sampled the RPC community (FIG. 3), the sampling of the mature root community was much more limited, as it almost always is (Taylor 2002). Most notably two sclerotia-forming species (Wilcoxina1 and *C. geophilum*) were found to be the most frequent in both studies. Both of these species were only moderately abundant on the field roots in small soil cores, however they were the most frequent colonizers when compared across a full compartment (4 ha). Their frequency, coupled with the rarity of most mature forest species, made Wilcoxina1 and *C. geophilum* the second and third most abundant species at that 4 ha scale. The other RPC species that were detected on field roots were not as abundant in the mature forest. However, similar to Wilcoxina1 and *Cenococcum*, they increased their prominence on field roots relative to other species as the scale increased, which reflected the overall widespread distribution of the RPC fungi at Teakettle Experimental Forest.

While the idea that ruderal fungi can persist in a mature setting is not new (e.g. Visser 1995), it is notable that two of the dominant RPC species are exceptionally abundant on the roots in this old-growth forest, a pattern also present in a mature *Pinus muricata* forest (Taylor and Bruns 1999). The Taylor and Bruns study highlighted the differences seen between the RPC and the root community, however the three most common RPC taxa—*Tomentella sublilacina*, a *Phialophora*-like fungus and *Cenococcum geophilum*—respectively ranked first, fourth and eighth of the 17 species found on the field root community. A number of features of the RPC taxa could account for this pattern. The taxa in our study *Wilcoxina1* and *Cenococcum geophilum* generally are thought to be well adapted to disturbance, most likely due to their sclerotia (Miller et al 1994, Torres and Honrubia 1997). *Cenococcum* often is considered also to be resistant to drought or dry conditions (e.g. Pigott 1982, Coleman et al 1989). It is plausible therefore that a common event such as seasonal drying of the upper soil layers could shift the competitive environment in favor of these species. In addition drought or small mammal diggings may disrupt the mycelial network sufficiently to create openings for RPC competitors to maintain themselves locally. Even if such disturbances occur at a small scale, the frequent and well distributed RPC propagules in this forest would make the impact of numerous small-scale events become more obvious at larger scales, as we observed. On the other hand it cannot be ruled out that these taxa are actually relatively effective competitors under nondrought conditions, as might be suggested by the observation of common acropetal replacement of other ECM fungi by *Cenococcum* (Massicotte et al 1999).

Ecology of Rhizopogon *species.*—Our view of *Rhizopogon* composition in the RPC is similar to the

Sierra National Forest site of Kjøller and Bruns (2003) despite the differences in the altitudes (2100 m vs. 1400 m), host composition (mixed-conifer vs. *P. ponderosa*), stand fire history (old-growth stands vs. those with recent fire) and screening methodology used in each study (i.e. direct molecular analysis of roots vs. culturing). Both studies show that there are commonly four or five *Rhizopogon* species ready to inoculate *Pinus* hosts across just a few meters. Of these, *Rhizopogon arctostaphyli, R. salebrosus* and, to some extent, *R. olivaceotinctus*, and *R. occidentalis* are frequent across both studies and sites, suggesting that they are widespread throughout Sierra National Forest across both altitude and host compositional gradients. Generation of this pattern requires some combination of frequent fruiting, efficient dispersal and long-lived spores, all traits known to be exhibited by *Rhizopogon* species (Molina et al 1999). While all four have spores that are well distributed and viable, only *R. salebrosus* and *R. arctostaphyli* have been detected as sporocarps or on the mature roots in this region (North 2002a, Izzo et al 2005b). This pattern supports the idea that spore longevity plays a role in the maintenance of *R. olivaceotinctus* and *R. occidentalis*. Some species of *Rhizopogon* have spores that remain viable at least 2 y (Miller et al 1994), but if these species were last active after a large scale forest disturbance, their spores would need to be viable for decades. Because of the limitations presented when studying mature root communities and hypogeous sporocarps additional sampling in this forest is needed to rule out the possibility that these species are maintained at low levels.

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LITERATURE CITED

Agerer R. 2001. Exploration types of ectomycorrhizae: a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza 11:107–114.

Altschul SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST—a new generation of protein database search programs. Nucleic Acid Res 25:3389–3402.

Baar J, Horton TR, Kretzer AM, Bruns TD. 1999. Mycorrhizal colonization of *Pinus muricata* from resistant propagules after a stand-replacing wildfire. New Phytol 143:409–418.

Bock C, Bock J. 1977. Patterns of post-fire succession on the Donner Ridge Burns, Sierra Nevada. USDA Forest Service Gen. Tech. Rep. WO-3.

Casgrain P, Legendre P. 2001. The R package for multivariate and spatial analysis. Version 4.0. User's Manual University of Montreal: Montreal, Canada,

Chao A. 1987. Estimating the population size for capture-recapture data with unequal catchability. Biometrics 43:783–791.

Coleman M, Bledsoe C, Lopushinsky W. 1989. Pure culture response of ectomycorrhizal fungi to imposed water stress. Can J Bot 67:29–39.

Fleming LV. 1984. Effects of soil trenching and coring on the formation of ectomycorrhizas on birch seedlings grown around mature trees. New Phytol 98:143–154.

———, Deacon JW, Last FW. 1986. Ectomycorrhizal succession in a Scottish birch wood. In: Gianinazzi-Pearson V, Gianinazzi S, eds. Physiological and genetical aspects of Mycorrhizae. Paris: INRA. p 259–264.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes–application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118.

Gray A, Zald H, Kern R, North M. 2005. Stand conditions associated with tree regeneration in Sierran mixed-conifer forests. For Sci 51(3):198–210.

Horton TR, Bruns TD, Parker VT. 1999. Ectomycorrhizal fungi associated with *Arctostaphylos* contribute to *Pseudotsuga menziesii* establishment. Can J Bot 77:93–102.

Izzo A, Agbowo J, Bruns T. 2005a. Detection of plot-level changes in ectomycorrhizal communities across years in an old-growth mixed-conifer forest. New Phytol 166(2):619–630.

———, Meyer M, Trappe J, North M, Bruns T. 2005b. Hypogeous ectomycorrhizal fungal species on roots and in small mammal diet in a mixed-conifer forest. For Sci 51(3):243–254.

Jonsson L, Anders D, Tor-Erik B. 2000. Spatiotemporal distribution of an ectomycorrhizal community in an oligotrophic Swedish *Picea abies* forest subjected to experimental nitrogen addition: above- and below-ground views. For Ecol Manage 132:143–156.

———, Dahlberg A, Nilsson MC, Kåren O, Zackrisson O. 1999. Continuity of ectomycorrhizal fungi in self-regenerating boreal *Pinus sylvestris* forests studied by comparing mycobiont diversity on seedlings and mature trees. New Phytol 142:151–162.

Keizer PJ, Arnolds E. 1994. Succession of ectomycorrhizal fungi in roadside verges planted with common oak (*Quercus robur* L.) in Drenthe, the Netherlands. Mycorrhiza 4:147–159.

Kennedy PG, Izzo AD, Bruns TD. 2003. There is high potential for the formation of common mycorrhizal networks between understorey and canopy trees in a mixed evergreen forest. J Ecol 91:1071–1080.

Kjøller R, Bruns TD. 2003. *Rhizopogon* spore bank communities within and among California pine forests. Mycologia 95:603–613.

Lilleskov E, Bruns T, Horton T, Taylor D, Grogan P. 2004. Detection of forest stand-level spatial structure in ectomycorrhizal fungal communities. FEMS Microbiol Ecol 49(2):319–332.

Magurran AE. 1988. Ecological diversity and its measurement. London: Croom Helm.

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

———, Molina R, Tackaberry LE, Smith JE, Amaranthus MP. 1999. Diversity and host specificity of ectomycorrhizal fungi retrieved from three adjacent forest sites by five host species. Can J Bot 77:1053–1076.

McDonald P. 1976. Forest regeneration and seedling growth from five major cutting methods in north-central California. USDA Forest Ser. Res. Pap. PSW-183.

Miller SL, Torres P, McClean TM. 1994. Persistence of basidiospores and sclerotia of ectomycorrhizal fungi and *Morchella* in soil. Mycologia 86:89–95.

Molina R, Massicotte H, Trappe J. 1992. Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. In: Allen MF, ed. Mycorrhizal functioning—an integrative plant-fungal process. London: Chapman & Hall. p 357–423.

———, Trappe JM, Grubisha LC, Spatafora JW. 1999. Chapter 5: Rhizopogon. In: Cairney J, Chambers S, eds. Ectomycorrhizal fungi: key genera in profile. Heidelbert: Springer-Verlag. p 129–161.

Newton AC. 1992. Toward a functional classification of ectomycorrhizal fungi. Mycorrhiza 2:75–79.

North M, Oakley B, Chen J, Erickson H, Gray A, Izzo A, Johnson D, Ma S, Marra J, Meyer M, Purcell K, Rambo T, Roath B, Rizzo D, Schowalter T. 2002. Vegetation and ecological characteristics of mixed-conifer and red-fir forests at the Teakettle Experimental Forest. USDA Forest Ser. Gen. Tech. Rep. PSW-GTR-186.

———. 2002a. Seasonality and abundance of truffles from oak woodlands to red fir forests. USDA Forest Ser. Gen. Tech Rep. PSW-GTR-183.

———. 2002b. The Teakettle experiment. USDA Forest Ser. Gen. Tech. Rep. PSW-GTR-183.

Parke JL, Linderman RG, Black CH. 1983. The role of ectomycorrhizas in drought tolerance of Douglas-fir seedlings. New Phytol 94:83–95.

Perry DA, Margolis H, Choquette C, Molina R, Trappe JM. 1989. Ectomycorrhizal mediation of competition between coniferous tree species. New Phytol 112:501–512.

Peter M, Ayer F, Egli S, Honegger R. 2001. Above- and below-ground community structure of ectomycorrhizal fungi in three Norway spruce (*Picea abies*) stands in Switzerland. Can J Bot 79:1134–1151.

Pigott C. 1982. Survival of mycorrhiza formed by *Cenococcum geophilum* Fr. in dry soils. New Phytol 92(4):513–517.

Simard SW, Perry DA, Smith JE, Molina R. 1997. Effects of soil trenching on occurrence of ectomycorrhizas on *Pseudotsuga menziesii* seedlings grown in mature forests of *Betula papyrifera* and *Pseudotsuga menziesii*. New Phytol 136:327–340.

Taylor AFS. 2002. Fungal diversity in ectomycorrhizal communities: sampling effort and species detection. Plant Soil 244:19–28.

Taylor DL, Bruns TD. 1999. Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: minimal overlap between the mature forest and resistant propagule communities. Mol Ecol 8:1837–1850.

Tedersoo L, Koljalg U, Hallenberg N, Larsson KH. 2003. Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. New Phytol 159:153–165.

Torres P, Honrubia M. 1997. Changes and effects of a natural fire on ectomycorrhizal inoculum potential of soil in a *Pinus halepensis* forest. For Ecol Manage 96:189–196.

van der Heijden EW, Kuyper TW. 2003. Ecological strategies of ectomycorrhizal fungi of *Salix repens*: root manipulation versus root replacement. Oikos 103:668–680.

Visser S. 1995. Ectomycorrhizal fungal succession in jack pine stands following wildfire. New Phytol 129:389–401.

Walker J, Miller O, Horton J. 2005. Hyperdiversity of ectomycorrhizal fungus assemblages on oak seedlings in mixed forests in the southern Appalachian Mountains. Mol Ecol 14:829–838.

White TJ, Bruns TD, Lee SB, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols—a guide to methods and applications. New York: Academic Press. p 315–322.