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Below-ground ectomycorrhizal community structure in a recently burned bishop pine forest

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Summary

1 The effects of wildfire ash on ectomycorrhizal (EM) associations were investigated by sampling bishop pine (*Pinus muricata,* D. Don) seedlings from control and ash-removed plots 1.5 years after a severe fire in a northern Californian *P. muricata* forest. The below-ground community composition of EM at the site was characterized using molecular techniques (PCR-RFLP and nucleotide sequencing).

2 A total of 30 fungal taxa were observed, many of which differed in their distribution between treatment and control seedlings. However, most of the taxa that were distinctive to either treatment or control seedlings occurred only once across the site, precluding statistical detection of potential ash effects on EM community composition. There were no significant effects of ash removal on plot-level mycorrhizal community richness or diversity, and there were no distinct treatment-related clusters in a principal components analysis.

3 Analysis of the combined data indicated that numbers of fungal taxa per seedling, numbers of successive root depth increments colonized by the same taxon, and distances to neighbouring seedlings colonized by the same taxon, were randomly distributed across the site for the majority of mycorrhizal fungi. These distributional patterns suggest that the post-fire mycorrhizal community structure on *P. muricata* arose primarily from successful colonization by randomly distributed point-source fungal inocula within the upper mineral soil layer of the forest floor.

4 By comparison with pre-fire studies from similar *P. muricata* sites nearby, our data indicate that severe wildfire disturbance resulted in marked changes in mycorrhizal community composition, and a sharp increase in the relative biomass of ascomycetous fungi.

Key-words: mycorrhizas, fungi, diversity, *P. muricata*, fire, ash, spatial distribution

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Introduction

Nutrient uptake by most plants in natural ecosystems is strongly influenced by root associations with mycorrhizal fungi (Smith & Read 1997). In the infertile soil conditions that prevail in temperate and boreal forests, most nutrient acquisition is mediated by a sheath of ectomycorrhizal (EM) fungal tissue that envelopes tree root tips and connects to a hyphal network emanating out into the soil (Marschner 1995). For pines, the association is obligate; normal growth does not occur unless the roots are colonized by some EM fungi (Read 1998). However, individual EM fungal taxa can vary greatly in their ability to mobilize and transfer nutrients from the soil to plants, particularly with respect to organic nitrogen sources (Read 1991; Smith & Read 1997). Since a wide range of EM taxa are typically found in association with single tree species under natural conditions (Molina *et al.* 1992; Bruns 1995), the effect of differences in ecophysiological capabilities between particular mycorrhizal taxa on overall nutrient cycling and plant production within a forest stand depends on the proportion of total plants colonized by each taxon. For these reasons, the composition and distribution of fungal taxa within a mycorrhizal community (i.e. mycorrhizal community structure) is of interest to plant ecologists.

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Several studies have described EM species richness and composition primarily on the basis of above-ground sporocarp distributions (e.g. Bills *et al.* 1986; Dahlberg & Stenlid 1990; Deacon & Fleming 1992; Tyler 1994; Visser 1995; Baar & Kuyper 1998). However, it is the below-ground community structure of mycorrhizal associations that directly influences plant nutrient acquisition. Recent evidence suggests that there may be little correlation between above- and below-ground components of EM communities (Gardes & Bruns 1996; Dahlberg *et al.* 1997; Pritsch *et al.* 1997; Gehring *et al.* 1998; Jonsson *et al.* 1999a,b). Furthermore, these latter studies have focused on characterizing below-ground EM community composition in terms of abundances of different fungal taxa relative to the total number of mycorrhizal tips or total mycorrhizal biomass. Here, we adopt a functional perspective, presenting mycorrhizal abundances relative to number of colonized host plants to describe the below-ground community structure of mycorrhizal associations on *Pinus muricata* (D. Don) seedlings 1.5 years after an intense wildfire within a *P. muricata* forest. Since *P. muricata* was the only major EM host at the site, these data allow us to evaluate the potential for individual mycorrhizal taxa to influence nutrient cycling and plant production in a recently burned forest stand.

Fire is probably the most important natural disturbance factor influencing EM community dynamics and succession in coniferous forests (Dahlberg & Stenlid 1995). It can affect the EM community in at least three ways. First, depending on fire intensity, the organic layer containing most EM biomass may be eliminated, soils can be partially sterilized by heat, and host carbon sources may be disrupted. Second, indirect effects of fire such as the deposition of ash during wildfire can alter soil pH and the availability of nitrogen, phosphates and alkaline earth metals (Raison 1979; Grogan *et al.* 2000), potentially altering the community of functioning EM fungi (Erland & Söderström 1991; Sagara 1992; Bååth *et al.* 1995). Third, for serotinous-coned tree species such as *P. muricata,* fire initiates production of a new generation of host seedlings for EM colonization.

Early post-fire studies at Point Reyes, California, USA, (Horton *et al.* 1998; Baar *et al.* 1999) reported substantial variation in EM community composition on *P. muricata* seedlings both within and between sites. Natural patchiness in fire intensity and/or ash deposition may contribute to heterogeneity in mycorrhizal community composition after wildfire. A manipulative experiment at Point Reyes has already indicated that surface ash deposits significantly increased soil pH and nitrogen availability in the first growing season after fire (Grogan *et al.* 2000). Here, we used the same plots to test the hypothesis that wildfire ash deposits can influence mycorrhizal community composition on establishing *P. muricata* seedlings in the second growing season after fire.

The structure of mycorrhizal communities after wildfire may also be influenced by effects of fire intensity on mycorrhizal inoculum potential (Perry *et al.* 1989). In mature forest stands, mycelial networks are thought to be the primary source of fungal inoculum (Deacon & Fleming 1992) leading to EM clones of 30–40 m diameter in some cases (Dahlberg & Stenlid 1990; Bonello *et al.* 1998). Low intensity forest fires that leave plant hosts and the organic layer containing most mycelia largely undamaged, do not substantially alter EM community composition (Jonsson *et al.* 1999b). By contrast, marked changes in EM community composition occur after intense wildfires (Visser 1995; Torres & Honrubia 1997). In a pre-fire study at Point Reyes, the EM community on mature *P. muricata* trees was markedly different to that present as resistant propagules in the soil (Taylor & Bruns 1999). Baar *et al.* (1999) demonstrated that the resistant propagule community largely survived the intense fire (as spores, sclerotia or mycorrhizal root tips), and that this community could have supplied inocula for most of the EM taxa observed on naturally regenerating *P. muricata* seedlings after the fire. Together, these results suggest that spores and sclerotia may replace mycelia as the dominant inoculum-type during mycorrhizal colonization after severe disturbance.

In addition to our experimental test of ash effects on EM, we analysed the spatial distribution of below–ground mycorrhizal associations across all recurring fungal taxa at the site to investigate if the *in situ* community structure was consistent with a change in relative importance of different inoculum-types during development of post-fire mycorrhizal composition on *P. muricata*. We hypothesized that if successful colonization from surviving mycelial networks was common, or if there was a spatially patchy distribution of fire-resistant propagules or post-fire dispersed spores, then seedling association with a particular mycorrhizal taxon would be related to proximity to adjacent seedlings colonized by the same taxon; thus, non-random spatial patterns of mycorrhizal colonization should occur. Alternatively, if mycelial survival was either non-existent or limited to small point-sources and the distributions of spore and sclerotial inocula were relatively homogeneous, then a random association between fungal species and spatial location of seedlings would be expected. We tested this hypothesis at the community level in order to discern the dominant inoculum-type during development of mycorrhizal community structure after severe wildfire disturbance.

Materials and methods

STUDY SITE

This research was conducted in a bishop pine (*P. muricata*) forest within the Point Reyes National Seashore in north North California (38° 03′ 39″ N, 122° 50′ 24″ W, altitude 210 m a.s.l.). The climate is mediterranean with cool, wet winters and warm, dry summers. Soil moisture levels are dominated by rainfall from November through May although coastal fog drip occurs throughout the year. *Pinus muricata* is a serotinous-coned species that occurs in small even-aged stands along the Californian coastal fog belt (Munz & Keck 1968). These stands tend to develop on ridge-tops surrounded by Northern Coastal Scrub communities dominated by *Baccharis pilularis* (Barbour & Major 1988). Before the introduction of fire management practices, wildfire was a regular perturbation of these ecosystems that occurred approximately every 40 years (Sugnet 1985). Cross-sections indicated that the trees at our site ranged from 32 to 38 years old.

EXPERIMENTAL DESIGN

A major wildfire burned 48 km^2 of the Point Reyes National Seashore during 3–7 October 1995. Fire intensity at the study site was particularly severe, killing all *P. muricata* trees, and consuming all canopy and understorey leaf material and the entire surface humic soil organic layer. On 23 October 1995, we established two plots 1–3 m apart in each of 7 blocks located randomly on clear patches of the forest floor along a broad transect covering an area approximately 20×50 m. Within each block, the plots (1.4×1.4 m) were bordered by wooden frames 15 cm high and randomly assigned to either treatment or control. We used a back-pack leaf-blower to remove the surface ash layer (including seed and charcoal material) from the treatment plots. Afterwards, all frames (treatment and control) were immediately covered with black PVC sheeting to prevent wind-dispersal of ash into or out of the plots. This sheeting was removed after consolidation of the surrounding surface ash layer by the first substantial rains in late November.

The serotinous nature of *P. muricata* cones and the intense heat of the fire resulted in an extensive *P. muricata* seed rain onto the forest floor in the days immediately after the burn. Since the blower treatment removed naturally dispersed *P. muricata* seeds in the ash-removed plots, we planted 100 locally collected *P. muricata* seeds in both treatment and control plots in late November. As a result, the density of *P. muricata* seedlings in the control plots was 1.75 times higher than in the ash-removed plots at the time of *P. muricata* seedling harvest. We assume that the surface blower treatment did not influence mycorrhizal inoculum potential because fungal spores and mycelium present on the surface of the mineral soil prior to the fire would have been burned. In addition, pre- and post-fire mycorrhizal bioassay studies of the mineral soil in *P. muricata* forest stands at Point Reyes (Baar *et al.* 1999; Taylor & Bruns 1999) have demonstrated that viable inocula of the dominant post-fire mycorrhizal fungi were abundant in the subsurface mineral soil layers both before and after the fire.

FIELD HARVEST

At the end of the first growing season (15 May 1996), all above-ground vegetation (except the *P. muricata* seedlings) was harvested from the central 1 m^2 of each plot. First year biomass and soil analyses results are reported in Grogan *et al.* (2000). In the following growing season, the plots were weeded occasionally to remove shoots of plant species other than *P. muricata*. On 31 March 1997, three *P. muricata* seedlings were selected randomly within a quadrant (0.25 m^2) that was randomly located in the central 1 m^2 of each plot. The relative positions of all selected seedlings were noted on a detailed site map before digging out as much of the seedling root system as possible to a depth of 40 cm. In addition, six soil cores (10 cm depth, 2 cm diameter) were randomly sampled from the remaining 0.75 m^2 of each experimental plot.

MYCORRHIZAL SAMPLING

EM (including ectendomycorrhizal, i.e. E-strain) root tips were removed from 5-cm depth increments of the root system, rinsed in water and sorted by morphotype using a stereo dissecting microscope. After removing all root tips, the remainder of each root system was divided into thick and fine root ($<$ 2 mm diameter) size classes. Mycorrhizal root tips were sorted into morphotypes on the basis of colour, mantle shape and surface texture, presence of cystidia, and EM branching pattern. Where the mycorrhizal status was ambiguous, especially when a well-developed mantle was not present, cross sections were made and examined under a compound microscope for the presence of a Hartig net (intercellular hyphae). Tips without root hairs and lacking an obvious sheath were assumed to be ectendomycorrhizal rather than non-mycorrhizal, and were separated on the basis of colour into additional categories for molecular analysis. Dark tips lacking turgidity were assumed dead. The seedlings were processed in random order to avoid sorting biases associated with the initial characterization of common morphotypes. We observed at least one mycorrhizal morphotype on each seedling. The morphotype samples from each depth increment of each seedling in each plot were lyophilized within 2 weeks of seedling harvest. Each sample was treated

separately in the subsequent molecular typing and pooled for data analysis only after the molecular typing indicated that the morphotypes were identical.

MOLECULAR ANALYSES

DNA extraction, polymerase chain reaction (PCR) amplification and restriction fragment length polymorphism (RFLP) protocols followed Gardes & Bruns (1993). The internal transcribed spacer (ITS) region of the 18S nuclear ribosomal repeat was amplified from DNA isolated from one to three mycorrhizal tips using either the fungal-specific primer pair ITS-1F/ITS-4 or the basidiomycete-specific pair ITS-1F/ITS-4B (Gardes & Bruns 1993). Of 194 total samples, 31 failed to amplify perhaps because of relatively small size (most weighed less than 0.001 g) or because the fungi were absent or dead. These samples (representing 6% of the total mycorrhizal root tip biomass) were not included in the data analyses. Three restriction enzymes (*Alu*I, *Dpn*II and *Hinf*I) were used to characterize and match fungal ITS-RFLP patterns to previously identified taxa (Gardes & Bruns 1993). We checked sorting accuracy within each morphotype sample by extracting additional root tips from the 10 largest biomass samples. In all cases, identical RFLP patterns were obtained for original and repeat extractions. However, additional PCR amplifications during taxonomic identification (see below) indicated that four morphotype samples were a mixture of two different taxa. In these cases, we apportioned the sample biomass equally between the two taxa.

Taxonomic identifications for those morphotypes with RFLP patterns that did not have matches in our database were obtained by sequencing three different DNA regions: (i) both spacers of the ITS region of the nuclear ribosomal repeat and the intercalated 5.8S rRNA gene; (ii) an approximately 400 bp fragment of the mitochondrial large subunit rDNA; and (iii) an approximately 600 bp fragment at the 5′ end of the nuclear large subunit rDNA. The primer pairs used for amplification were ITS1-F/ITS-4, ML-5/ML-6 and ITS1-F/Tw-14, respectively. PCR products were sequenced using ITS-4, ITS-5, ML-5, ML-6, Ctb-6 and Tw-13 (Gardes & Bruns 1993; Bruns *et al.* 1998; Taylor & Bruns 1999). Cycle sequencing was done by the reaction termination method using fluorescence-labelled dideoxyribonucleotide. The sequence reaction and the processing of the reaction products for electrophoresis were performed following the instructions for the sequencing kit (ABI PRISM™ Dye Terminator Cycle Sequencing Core Kit, Perkin-Elmer Corporation). Electrophoresis and data collection were done on an ABI Model 377 DNA Sequencer (Perkin-Elmer Corporation). DNA Sequencing Analysis (version 2.12) and Sequence Navigator (version 1.0.1) were used for processing the raw data. Those few morphotypes that were not successfully identified from our molecular database have been labelled 'unknown' and numbered in accordance with previous publications from T.D. Brun's laboratory (Gardes & Bruns 1996; Horton & Bruns 1998; Horton *et al.* 1998; Baar *et al.* 1999; Taylor & Bruns 1999).

PLANT AND SOIL NUTRIENT ANALYSES

All plant material was dried at 65°C for 96 h before determining mass. Total N and P in plant leaf samples was determined by micro-Kjeldahl digestion/colourimetric analyses using the nitroferricyanide procedure for N (Lachat 1990a) and the phosphomolybdate procedure for P (Lachat 1990b). The soils from within each plot were pooled and sieved (2 mm) to remove stones and coarse roots prior to extraction (within 36 h of sampling). Moisture content was determined by drying subsamples at 65°C for 48 h. Soil pH was measured in the supernatant from a solution of 5 g fresh soil to 5 mL distilled H_2O that had been shaken and then allowed to settle for 10 min (McLean 1982). Soil ammonium and nitrate were measured by extracting *c.* 20 g of fresh soil in 50 mL 2 M KCl. The solutions were shaken for 1 h and then filtered (Whatman no. 1) before freezing. The filter paper was pre-leached with *c.* 30 mL of the extracting solution to remove any soluble inorganic N from the paper. Later, samples and blank controls were analysed colourimetrically for NH_4-N (Lachat 1990c).

STATISTICAL ANALYSES

The three seedlings from each plot were pooled for statistical analyses of mycorrhizal community and plant parameters. We used the Shannon/Weaver index (Zar 1996) to calculate mycorrhizal diversity for each plot. Counts of the number of mycorrhizal taxa per plot were square-root transformed prior to statistical analysis. Soil pH values were converted to mean H^+ ion concentrations prior to data analysis. Variables were analysed for effects of treatment (two levels) and block (seven levels) using two-factor analyses of variance (ANOVAs). Block effects were not statistically significant in any of the analyses and are therefore not reported. The seedlings from one of the ash-removed plots were mistakenly discarded after harvest. Consequently, six replicates were used in the analysis of treatment effects on mycorrhizal community and plant parameters, and seven replicates were used in the analysis of soil parameters.

Frequency distributions were compared to the Poisson distribution using chi-square 'goodness of fit' tests (Zar 1996). Shortest distances between seedlings colonized by the same mycorrhizal fungus were calculated for each group of seedlings associated

with a recurring taxon. In order to do this in a systematic manner for all taxa on seedlings scattered across a broad transect (20 m × 50 m), we selected the colonized seedling closest to one end of the transect as the initial starting point for each taxon, calculated the shortest distance to a neighbouring seedling colonized by the same taxon, and then calculated the shortest distance from the latter seedling to an additional neighbouring seedling colonized by the same taxon, and so on across the site.

Results

A total of 30 different molecularly identified taxa were observed on *P. muricata* (Table 1), of which six were present only on seedlings from ash-removed plots and 13 were present only in control plots (Fig. 1). However, although fungal composition differed substantially with treatment (Fig. 1), most of the taxa that were distinctive to either ash-removed or control treatments occurred on single *P. muricata* seedlings. Even the most frequent taxa within each treatment (*Tuber* sp., Russuloid 3, *Wilcoxina* sp.) were present on only one-third of all seedlings sampled (Fig. 1). At the replicate plot level, neither mean richness (total number) nor diversity of mycorrhizal taxa from three randomly sampled seedlings were significantly affected by the treatment ($F_{1,12} = 2.19$, $P < 0.20$; $F_{1,12} = 3.83$, $P < 0.11$, respectively). Overall, our results indicate a post-fire mycorrhizal community consisting of a large num-

Table 1 Mycorrhizal fungal taxa on *P. muricata* identified by PCR-RFLP with restriction enzymes *Alu*I, *Dpn*II and *Hinf*I. Numbers in columns for each restriction enzyme are the DNA fragment sizes (in base pairs) after the PCR product was cut with that enzyme. The basidiomycetous fungi were amplified with primers ITS1-F and ITS-4B, except for a few taxa (labelled with an asterisk, *) that amplified only with primers ITS1-F and ITS-4. The ascomycetous fungi and unidentified fungi were amplified with the primers ITS1-F and ITS-4.

	Restriction enzyme		
Taxonomic identification	AluI	DpnII	HinfI
Basidiomycetous fungi			
Amanita gemmata ‡	405/254/106/93	382/238/131	354/236
Clavulina-like †	490/200/138/116	285/211	277/194/167/122
Hebeloma spp. ‡	340/257/214	560/258	398/356/145
Inocybe spp. †‡	580/215	560/264	344/210/175/120
Rhizopogon ochraceorubens ‡	453/345/106/87	343/293/257	355/254/164/140/77
Rhizopogon subcaerulescens ‡	471/334/87	295/240	252/133/75
Rhizopogon olivaceotinctus-like‡	545/264	298/247/230	258/174/158/135/105
Russula amoenolens ‡	335/253/191/90	385/124	321/252/205
Russuloid 3 †	530/269	273/222	384/180
Russuloid 4*†	540	220/170	340/320
Russuloid 5 †	540/275	390/264/235/138/65	371/120
Russuloid 6*†	450/225	240/227/156	370/305
Suillus pungens/*brevipes* †‡	671/97/83	235/221/133/76	284/238
Suilloid 2*†	480/285	245/175	245/230/137
Suilloid 3 †	490/200/138/116	285/211	277/194/167/122
Suilloid 4*†	483/110	264/180	338/270
Suilloid 5*†	570	420/200	320
Thelephoroid 8 †	548/195	555/278	344/230/195/124
Tomentella sublilacina ‡	458/111/86	374/227/196	366/223/126/107
Tomentella sp. 1 †	458/111/86	372/231/190	359/230/147/129
Tomentella sp. 2 †	480/165/79	366/219/189	345/127
Ascomycetous fungi			
Cenococcum spp. †	550/440/200	300/230/160	260/180/120/90
Tuber sp. ‡	600	372/250	232/179/158
Wilcoxina mikolae ‡	645	220/95	295/193/167
Wilcoxina mikolae-like ‡	660	329/223	365/108
Wilcoxina sp. ‡	421/200	305/212	278/210/135
Unidentified fungi			
Unknown 9	500/440	250/240/200/160	360/340
Unknown 10	457	232/130	333/172
Unknown 11	620/500	360/300/250/200	220/190/170
Unknown 12	Not determined	350/230	270/220

† These taxa were identified by nucleotide sequencing of partial regions of the mitochondrial DNA and ribosomal DNA.
‡ These taxa were identified by RFLP-matching.

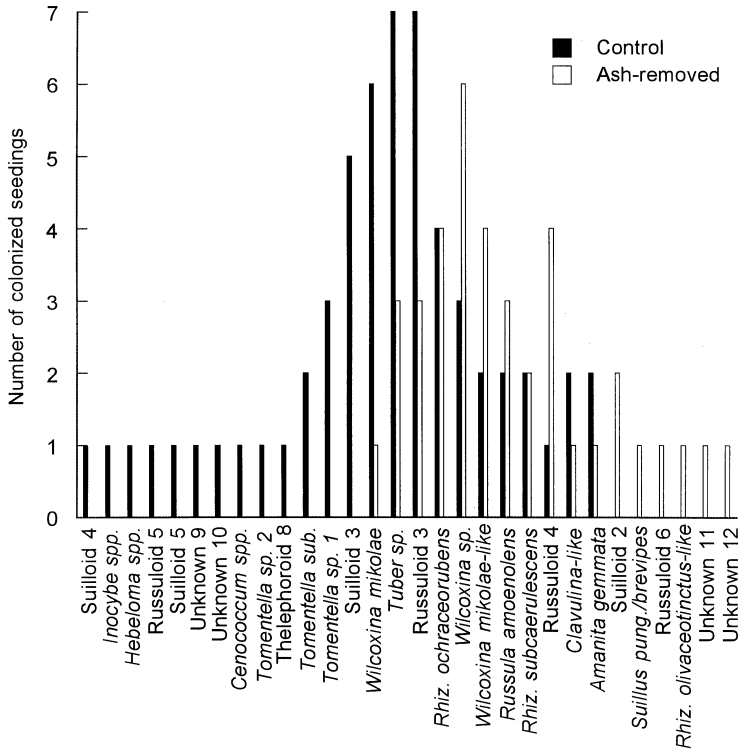

Fig. 1 The composition of mycorrhizal taxa on *P. muricata* seedlings in a recently burned forest stand (control: $n = 21$; ash-removed $n = 18$).

ber of fungal taxa scattered at low frequencies on *P. muricata* seedlings across the site.

Neither soil pH nor soil ammonium levels were significantly affected by the ash-removal treatment at the time of mycorrhizal harvest in the second growing season after fire (data not shown), even though there had been strong ash effects on these parameters in the previous growing season (Grogan *et al.* 2000). Furthermore, there were no significant treatment effects on *P. muricata* shoot or root biomass or on leaf nitrogen or phosphorus concentrations (data not shown). A multivariate principal components analysis of the biomass distribution of each mycorrhizal taxon in relation to soil pH and soil ammonium did not separate out the taxa into distinct clusters (data not shown).

The mycorrhizal root tip biomass distribution of fungi at the site (Fig. 2) also indicated that the EM community consisted of a few dominant taxa and a relatively large number of rare taxa. Furthermore, the eight most abundant taxa in terms of EM root tip biomass were amongst the 11 taxa most frequently found on seedlings (Fig. 1), indicating some correlation between mycorrhizal biomass and seedling colonization frequency.

When treatment and control seedlings were combined, the colonization frequency and spatial distributions of fungal taxa strongly suggested that development of individual mycorrhizal associations was random across the site. First, the frequency distribution of number of taxa per seedling (Fig. 3) resembled a Poisson distribution (Goodness-of-fit: $\chi^2 = 4.61$; $\nu = 3$; $P = 0.20$), indicating that the majority of mycorrhizal taxa were randomly distributed across the seedlings, and that there was no competitive exclusion of additional taxa once initial seedling colonization had occurred. This community-level analysis indicates that, even if there were individual taxa with non-random colonization patterns, they were not frequent enough to alter significantly an overall random distribution of mycorrhizal taxa.

Second, most of the recurring taxa (e.g. *Tuber* sp., Russuloid 3, *Wilcoxina* sp.) occurred randomly on seedlings that were separated from their nearest colonized neighbour by distances ranging from 0.02 m to 35 m (Fig. 4). A chi-square test of all recurring fungal taxa indicated that colonization frequencies on seedlings located within the same plot (separated by distances < 0.5 m) did not differ significantly from those on seedlings located in different plots (separated by distances of > 2 m) ($\chi^2 = 11.75$; $\nu = 14$; $P = 0.63$). These analyses strongly suggest that seedling colonization by the majority of recurring taxa was independent of proximity to neighbouring seedlings colonized by the same taxa, and that the differences in seedling density due to initial sowing of

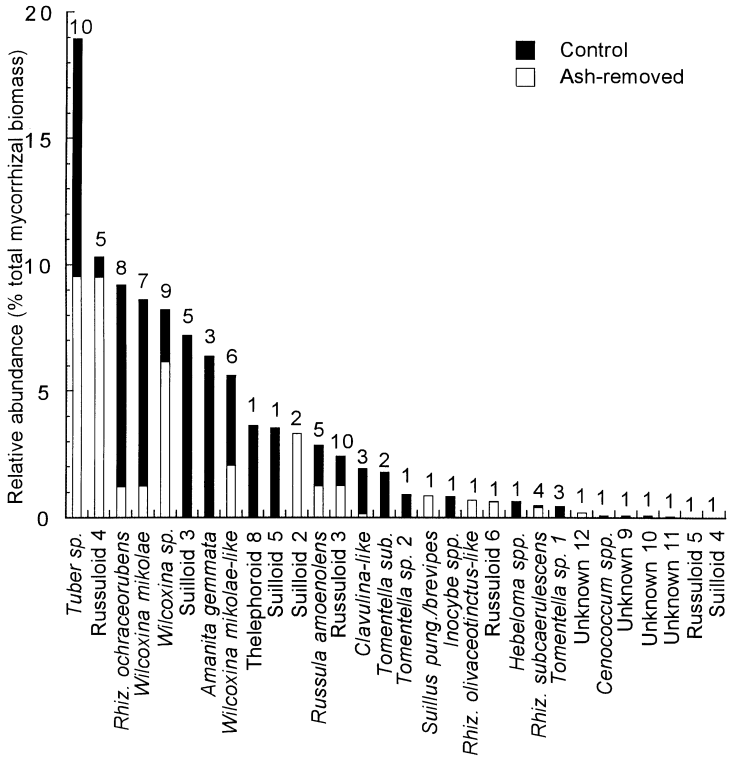

Fig. 2 The relative abundances of mycorrhizal taxa on *P. muricata* in relation to total mycorrhizal root tip biomass. The numbers above each column indicate total number of seedlings colonized. In certain cases, relative mycorrhizal biomass for a particular taxon was so small that it is beyond the resolution of the figure.

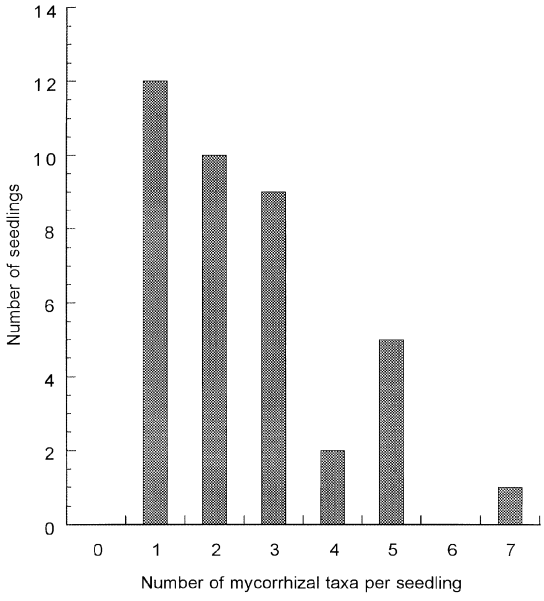

Fig. 3 Frequency distribution of mycorrhizal taxa per seedling. All seedlings were colonized by at least one mycorrhizal taxon. Treatment and control seedlings were combined for this analysis.

both treatment and control plots did not result in confounding effects on mycorrhizal colonization. Since half of all observed mycorrhizal taxa at the site were non-recurring (Fig. 1), these results collectively indicate that the post-fire mycorrhizal community structure on *P. muricata* arose primarily from successful colonization by randomly distributed point-source fungal inocula.

The depth distribution for each mycorrhizal taxon indicated that most of the recurring fungi successfully colonized seedlings throughout the upper 20 cm of soil (Fig. 5). By contrast, fine (and thick) root biomass decreased at deeper soil depths over this interval (data not shown). In order to test whether colonization by a particular taxon influenced subsequent colonization on the same seedling, we calculated the frequency distribution of number of successive 5-cm depth increments on each seedling colonized by the same taxon. A test of 'goodness of fit' to the Poisson distribution indicated a random distribution of colonization events with depth along each seedling root system ($\chi^2 = 0.33$; $\nu = 1$; $P = 0.57$), implying that, even if competitive exclusion by initial colonizing taxa on subsequent colonization of roots at other depths occurred, it was not frequent enough to make the community-wide pattern differ significantly from random. In combination,

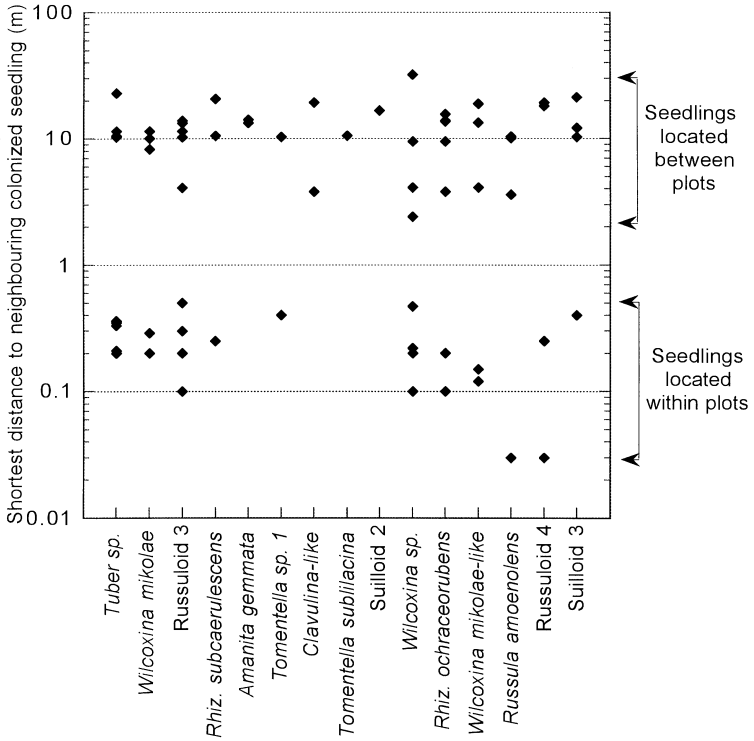

Fig. 4 The distribution of shortest distances between colonized *P. muricata* seedlings for each recurring mycorrhizal taxon (log scale). Treatment and control seedlings were combined for this analysis.

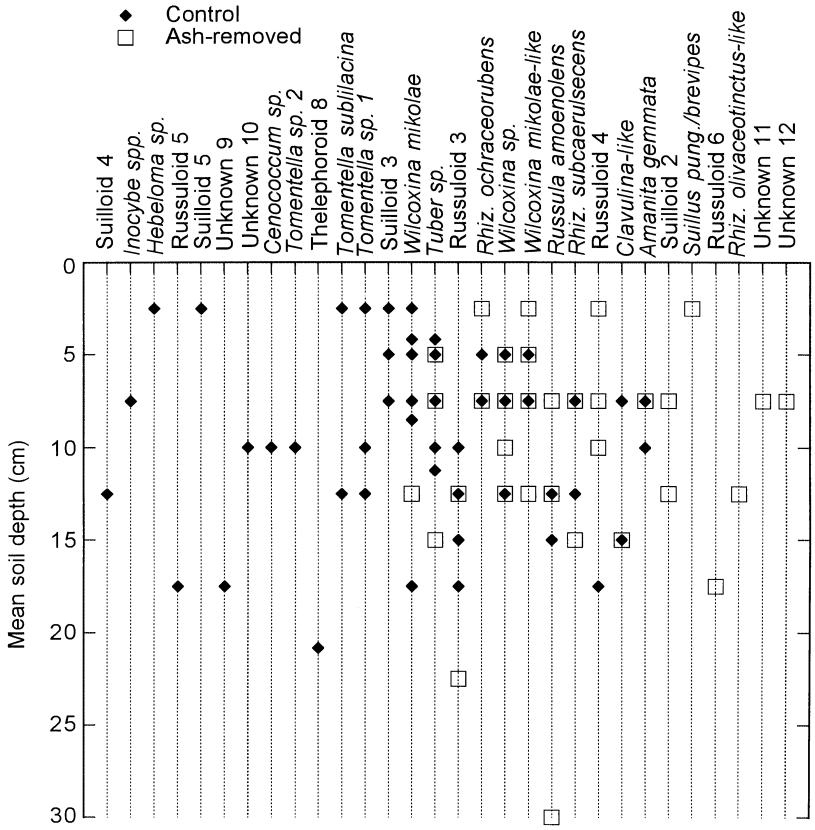

Fig. 5 Distribution of mycorrhizal taxa on *P. muricata* seedlings in relation to soil depth. Each point is the mean soil depth at which a particular mycorrhizal taxon occurs on an individual seedling. ◆, control; □, ash-removed.

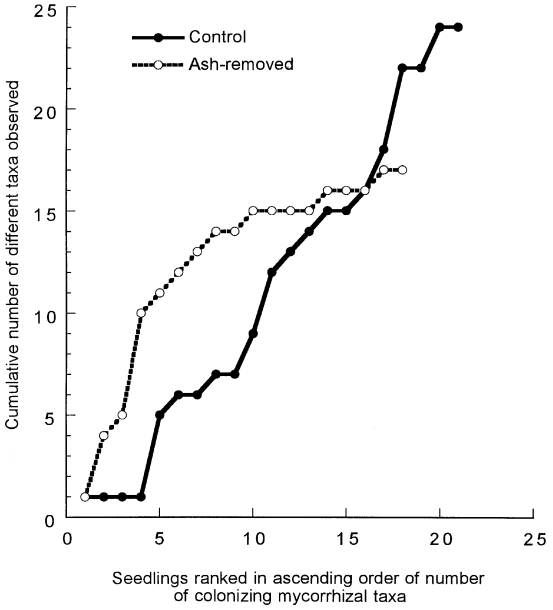

Fig. 6 Richness of mycorrhizal taxa observed at the site as a function of the number of seedlings sampled (control: $n = 21$; ash-removed: $n = 18$).

these colonization and spatial frequency analyses indicate that *P. muricata* associations with most fungal taxa were not influenced by proximity to neighbouring colonized roots on either the same seedling, or on adjacent seedlings.

The relationship between number of mycorrhizal taxa observed and number of seedlings sampled (Fig. 6) indicates that our study underestimated the total richness of mycorrhizal taxa present on either treatment or control seedlings at the site. Therefore, sampling of additional seedlings might have resulted in a significant ash treatment effect on mycorrhizal richness/diversity if the increases in total number of observed taxa on treatment seedlings and control seedlings were not equivalent. In any event, incomplete sampling of total EM richness should not affect the basic patterns of spatial distributions and seedling colonization frequencies that underly the mycorrhizal community structure reported in this study.

Discussion

Our test of the influence of ash deposits on *P. muricata* mycorrhizal community composition 2 years after wildfire did not indicate a significant treatment effect. Although the composition of mycorrhizal taxa differed between seedlings from ash-removed and control plots across the site (Fig. 1), individual taxa that were distinctive to either group were too infrequent to analyse statistically for a direct treatment effect. There were no significant treatment effects on either mycorrhizal community richness or diversity at the replicate plot-level, and no distinct treatment-related clusters in the principal components analysis. An earlier study demonstrated that ash removal significantly lowered soil pH and nitrogen availability relative to control plots during the first growing season after fire (Grogan *et al.* 2000), suggesting that spatial heterogeneity in ash deposition and accumulation could influence spatial patterning in the distribution of mycorrhizal taxa after wildfire. However, there were no treatment effects on any of the measured soil or plant parameters by the time of the current study. Together, our results suggest that although ash may have influenced particular fungal associations (Fig. 1), it was the composition and distribution of EM inocula that were the primary determinants of mycorrhizal community structure in this regenerating *P. muricata* forest stand.

The potential for effects of individual fungal taxa on nutrient acquisition and growth by plant populations under natural conditions depends on the composition and distribution of EM associations. Since *P. muricata* was the only major EM host, and we were able to sample almost all mycorrhizas on each seedling, our study is representative of the overall EM community structure at the site. Several of the fungal genera observed in the study (*Amanita* spp., *Suillus* spp., *Rhizopogon* spp., *Cenoccocum* spp. and *Hebeloma* spp.) have particular ecophysiological capacities to utilize organic nitrogen sources, at least under laboratory and pot experimental conditions (Abuzinadah & Read 1986a, b, 1989a, b). Differences in such ecophysiological capacities within the EM fungal community at our site could influence the performance of individual *P. muricata* seedlings. However, the very low proportions of total seedlings colonized by most taxa (Fig. 1) suggest that these differences are unlikely to have a substantial influence on stand-level nutrient cycling and primary production in the years immediately following fire.

The influence of spatial dynamics has rarely been assessed in determining appropriate sampling procedures to characterize EM community composition (Egger 1995; Dahlberg *et al.* 1997; Pritsch *et al.* 1997). Spatial sampling strategy (i.e. distance between sampled seedlings) was relatively unimportant in this study because EM associations were independent of proximity to neighbouring colonized seedlings (Fig. 4). However, an analysis of cumulative mycorrhizal richness on successive seedlings demonstrated that the number of sampled seedlings in our study was insufficient to identify the entire EM community on treatment or control seedlings (Fig. 6). These analyses indicate that characterization of mycorrhizal community composition at our site would have been best achieved by maximizing

the total number of seedlings examined, irrespective of their spatial location.

Wildfire had strong effects on below-ground mycorrhizal community composition in *P. muricata* forest stands at Point Reyes. Previous studies at neighbouring unburned mature *P. muricata* sites indicated dominance by basidiomycetes such as russuloids, thelephoroids and amanitoids (Gardes & Bruns 1996; Horton & Bruns 1998; Taylor & Bruns 1999). After the fire, our study and several others from different sites at Point Reyes indicated that these taxa became relatively uncommon and were replaced in the basidiomycete component by previously rare fungi such as *Rhizopogon ochraceorubens and* other suilloid taxa that remain unidentified (Figs 1 and 2; Horton *et al.* 1998; Baar *et al.* 1999). At a higher taxonomic level, fire frequently results in a shift in dominance from basidiomycetous to ascomycetous fungi such as *Tuber* sp. *and Wilcoxina* spp. (Torres & Honrubia 1997). These latter taxa were very rare in the unburned mature *P. muricata* mycorrhizal community at Point Reyes (Gardes & Bruns 1996; Horton & Bruns 1998; Taylor & Bruns 1999), but were common in post-fire studies (Figs 1 and 2; Horton *et al.* 1998; Baar *et al.* 1999), together constituting 42% of the total mycorrhizal root tip biomass at our site (Fig. 2). Studies in other fire-prone pine ecosystems suggest that the initial strong representation of ascomycetes within post-fire EM communities begins to dissipate within six years of wildfire (Visser 1995). Further research is required to evaluate the functional importance of these higher level taxonomic changes to the dynamics of nutrient cycling in fire-prone ecosystems.

Our analysis of below-ground structure in the EM community on *P. muricata* indicates that point-source inocula dominated early post-fire seedling colonization at this site. Half of all observed taxa at our site occurred only once (Fig. 1), and the recurring fungi had random spatial distributions on seedlings across the site (Fig. 4). Since colonization from mycelial networks would lead to clusters of EM taxa on adjacent seedlings, these results indicate that spores, sclerotia or very small patches of mycelia such as individual surviving EM roots (Ferrier & Alexander 1985) were the principal inoculum-types.

Baar *et al.* (1999) previously concluded that spores and sclerotia were the dominant post-fire inoculum-types at a different *P. muricata* site within Point Reyes. This conclusion was based on the presence of desiccation-resistant inoculum of *Rhizopogon* spp., *Tube*r spp., *Wilcoxina* spp. and *Tomentella sublilicina* in both pre-fire (Taylor & Bruns 1999) and post-fire soil (Baar *et al.* 1999) from the same site, and on the relative abundances of most of these taxa on field-collected seedlings after the fire (Baar *et al.* 1999). The first three of these genera were also relatively frequent at our site (Figs 1 and 2), further supporting the potential importance of spore and sclerotial inocula during the development of EM communities after fire.

Colonization from small patches of surviving mycelia on persistent roots in the mineral soil layer (Ferrier & Alexander 1985) may have contributed to the presence of taxa such as the thelephoroid *Tomentella sublilacina* and certain russuloids (e.g. *Russula amoenolens*) that were relatively abundant on root tips before the fire (Gardes & Bruns 1996; Horton & Bruns 1998; Taylor & Bruns 1999). The fact that russuloids comprise the majority of EM associations at deeper soil depths (i.e. below 15 cm) in our study (Fig. 5) is consistent with possible colonization from surviving mycelia. Nevertheless, the EM community after fire was dominated by previously rare or unobserved taxa, and our study indicates that most fungal taxa were randomly distributed on seedlings across the site (Fig. 4). Thus, our results support the conclusion that spores and sclerotia were the dominant forms of inocula during development of early post-fire EM community structure at Point Reyes.

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