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Spore heat resistance plays an important role in disturbance-mediated assemblage shift of ectomycorrhizal fungi colonizing *Pinus muricata* seedlings

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Summary

1. Abiotic disturbance plays an important role in determining assemblage structure and maintaining species richness in many ecosystems. Disturbances events are complex, often affecting multiple environmental parameters simultaneously and causing selective removal of biomass. For this reason, observational studies often fail to elucidate the primary mechanism by which disturbance modifies assembly structure. This is particularly true of microbial systems where reduction in biomass or removal of competitive dominants is not visually obvious.

2. Fire is a major disturbance in pinaceous forests, which are characterized by ectomycorrhizal fungal root associations. To determine the specific mechanisms through which this disturbance affects ectomycorrhizal communities, we experimentally simulated the effects of fire by growing seedlings in field soil exposed to factorial combinations of soil heating and ash addition. Ectomycorrhizal colonists in each of the treatments were then identified from seedling roots using DNA sequences.

3. Both soil heating and ash addition caused changes to the soil chemical environment and altered the fungal assemblages found on seedling roots. The effect on the ectomycorrhizal assembly was most pronounced from soil heating, which caused an overall simplification and increasing dominance by a few taxa with ruderal life-history strategies.

4. We also conducted a follow-up experiment to see whether selective mortality of ectomycorrhizal spores might contribute to these patterns. Heat exposure had strong negative effects on all species tested. However, spore heat tolerance was highest for the species that showed the greatest increase in abundance on seedlings grown in heated soils.

5. *Synthesis*. Fire alters below-ground communities through changes in soil chemistry and mortality of resident organisms. Our results show that soil heating and selective mortality are key mechanisms determining post-fire assemblage structure of ectomycorrhizal fungi. These disturbances favour competitively inferior species and may help maintain diversity of ectomycorrhizal assemblages at the landscape scale.

Key-words: competition, fugitive species, functional traits, intermediate disturbance hypothesis, microbe, mutualism, mycorrhiza, *Pinus muricata*, Point Reyes, succession

Introduction

Abiotic disturbances play a major role in the community structure and function of most ecosystems (reviewed by Sousa 1984). Disturbances affect community structure by creating spatial and temporal heterogeneity in environmental conditions that may favour particular species (Wiens 1976; Caswell & Cohen 1991) as well as removing existing biomass and opening up habitat space for species with life-history strategies geared for effective dispersal and colonization syndromes (Pianka 1970; Grubb 1977; Connell & Slatyer 1977; Walker & Chapin 1987). As a result, events such as fires, hurricanes or floods are frequently associated with major changes in species composition (Sousa 1984; Pickett & White

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1985; Walker & Chapin 1987). Because disturbances often favour species that would otherwise be excluded by competitive dominants, they are considered to play a major role in the maintenance of species diversity (Hutchinson 1951; Connell 1978; Sousa 1979, 1984).

Fire is a particularly common disturbance in arid and Mediterranean ecosystems across the globe. The effects of fire on plant community structure and ecosystem function have been studied extensively for many years (Pyne *et al*. 1996; Sugihara *et al*. 2006). Although fires have clear effects on above-ground vegetation, their effect on below-ground systems are often highly complex, spatially heterogeneous and difficult to observe (reviewed in Neary *et al*. 1999; Certini 2005; Hart *et al*. 2005). Aside from direct mortality of soil dwelling organisms, fires modify the soil physical and chemical environment, increase solar irradiation and generate large inputs of ash from burned plant material. Perhaps because of this complexity, we still know fairly little about how soil microbial communities respond to fire (Neary *et al*. 1999; Hart *et al*. 2005).

Ectomycorrhizal fungi are a key component of the soil community. These fungi form an obligate symbiosis with plant roots and are particularly important in dominant temperate tree families such as the Pinaceae and Fagaceae. The sole energy source for these fungi is photosynthetically derived carbon which the fungi obtain from their hosts in exchange for mineral nutrients or water they scavenge from the soil (Smith & Read 1997). These fungi are critical to the growth of their hosts, and the absence of appropriate mycorrhizal symbionts can prevent trees from establishing at a site (Terwilliger & Pastor 1999; Weber *et al*. 2005). This symbiosis is a major component of terrestrial nutrient cycles, with the fungi receiving approximately 15% of the host's net primary production and providing up to 80% of the host's nitrogen in some cases (Hobbie & Hobbie 2006; Hobbie 2006). Because fine roots are the sole sites of exchange in the symbiosis, all ectomycorrhizal fungi must compete for the same limited resource, and a number of recent studies have demonstrated competitive exclusion among co-occurring fungi (Wu *et al*. 1999; Koide *et al*. 2005; Kennedy *et al*. 2007a). Despite this, ectomycorrhizal assemblages are remarkable for their high diversity (Ishida *et al*. 2007; Parrent and Vilgalys 2007; Peay *et al*. 2007) and it is clear that some mechanism(s) is or are required to explain their co-existence (e.g. Bruns 1995).

Wildfires have been shown to cause significant changes in ectomycorrhizal assemblage structure (Visser 1995; Horton *et al*. 1998; Smith *et al*. 2004). However, field studies often confound many factors important to these fungi, such as soil chemistry, host species composition and successional status (Last *et al*. 1987; Mason *et al*. 1987; Erland & Taylor 2002; Ishida *et al*. 2007). Experimental studies have attempted to individually manipulate two of the major components of fire, heat and ash, (Grogan *et al*. 2000a,b; Izzo *et al*. 2006) but never together. In this study we simulated the effects of fire on ectomycorrhizal assemblages by manipulating both heat and ash to see if they led to predictable shifts. To find out whether selective mortality from soil heating plays a role in determining post-fire assemblage structure, we also tested one potentially important trait, spore heat resistance, for some of the fungi found within our simulated fire treatments.

Because wildfires are infrequent and difficult to replicate, we simulated the effects of fire through experimental heat and ash treatments applied to field collected soils containing ectomycorrhizal propagules. Molecular methods were used to identify the ectomycorrhizal fungi colonizing the roots of pine seedlings that were grown as bioassays in the respective soil treatments, and fluorescent vital stains were used to assay spore heat resistance. We also assessed the direct effects of heat and ash treatments on the soil chemical environment. Based on previous work, we predicted that fungi in the genus *Rhizopogon* would increase in abundance after heat treatments (Horton *et al*. 1998; Smith *et al*. 2004; Izzo *et al*. 2006) and that spores of these fungi would exhibit greater heat tolerance than most other fungi.

Methods

STUDY SYSTEM

Our study is based on the *Pinus muricata* D. Don (Bishop Pine) ectomycorrhizal system found at Point Reyes National Seashore, located in west Marin County, California (38 04′ N, 122 50′ W). The area has a Mediterranean climate with cool, wet winters and warm, dry summers. Near the coast *P. muricata* is the only major ectomycorrhizal host plant and forms monodominant tree stands that intergrade with non-ectomycorrhizal coastal grasslands and scrub. *Pinus muricata* is a closed-cone pine and recruits almost exclusively after large, stand-replacing fires. Because all parent trees are dead, post-fire seedlings must be colonized by ectomycorrhizal propagules that remain in the soil or by aerial dispersal from living stands located in the adjacent landscape. However, previous studies have shown that most of the immediate post-fire colonization comes from resident soil propagules (Baar *et al*. 1999). Thus, while growth chamber experiments are clearly unrealistic in many ways, they likely provide a reasonable approximation of early establishment conditions from the perspective of *P. muricata* and its ectomycorrhizal associates at Point Reyes.

EXPERIMENTAL DESIGN

In order to test the effects of fire on ectomycorrhizal fungi colonizing *P. muricata* seedlings, we used factorial combinations of soil heating and ash addition. Fires in coastal California occur primarily during the peak of the dry season in late summer and early fall. Because heat transfer differs significantly between dry and wet soils, we tried to best approximate natural conditions by collecting soils during the peak fire season when soils are also at their driest (Kennedy & Sousa 2006; Kennedy & Peay 2007). On 30 June 2006, approximately 2 L samples of live pine forest soil were collected from eight different locations (16 L total) on Mount Vision (38 05′44″ N, 122 53′13″ W) in Point Reyes National Seashore. Half of the samples were collected from beneath mature stands of *P. muricata* and another half of the samples from young stands regenerating after the 1997 Vision Fire. After collection each sample was sifted through a 5 mm sieve to remove rocks and roots. Samples were then combined, mixed thoroughly and stored at 4 C. On 11 and 12 July the soil was split into two even batches. One batch was mixed 1 : 1 vv with autoclaved sand and put directly into 'cone-tainers' (Stuewe and Sons, Corvallis,

OR). The second batch was subjected to heat treatments using a protocol simplified from Izzo *et al*. (2006) and intended to be used for recreating the rapid warming and slow cooling of soils exposed to fire. Soil was spread into a 1-cm layer in sterile metal pans and placed into a soil drying oven. Soils temperatures between 65 and 70 C are common in low- to moderate-intensity fires and have strong biological effects without total sterilization (Neary *et al*. 1999). For this reason, soil was allowed to reach 70 C (approximately 10–15 min) using a thermocouple and then placed in an incubator set for 45 C for 40 min. After 40 min the soil was removed and allowed to cool at room temperature. Soil temperatures were monitored continuously using a HOBO datalogger (Onset, Pocasset, ME) set to record every 30 s with two thermoprobes. After the soil had cooled to room temperature it was mixed 1:1 vv with autoclaved sand and placed into cone-tainers. These conditions are a good approximation for mineral horizon temperatures achieved in low- to mid-severity fires and the temperature levels at which biological disruptions begin to occur (reviewed in Neary *et al*. 1999).

Three pre-germinated *P. muricata* seeds were placed into each cone-tainer in both treatments and covered with a small layer of sand. Half of the cone-tainers from each heat treatment were then selected randomly to receive the ash treatment. This consisted of 1.5 g (dry weight) ash collected in 2005 from four prescription burn sites at Point Reyes. The ash was collected within 7 days of each prescribed burn and stored in zip-lock bags at 4 C until used in this experiment. This amount approximates post-fire ash levels per unit area observed at Point Reyes after the 1995 Vision Fire (Grogan *et al*. 2000a). We used a fully crossed factorial design with 25 replicates per treatment combination for a total of 100 seedlings (2 treatments × 2 levels × 25 replicates = 100). All seedlings were placed in a growth chamber set to 20 C and 16 h of light and watered twice weekly. On 16 August 2006 all cone-tainers were weeded down to a single seedling. The remaining seedlings were grown in the growth chamber for approximately one year until 28 June 2007.

SEEDLING AND ROOT HARVEST

To characterize patterns of ectomycorrhizal colonization, we sampled individual fine roots from each seedling for molecular identification. Seedlings were first pre-soaked in deionized water while still in their cone-tainers so that soil could be washed away easily while leaving the root system intact. To ensure even sampling, the root system was first divided vertically into thirds and two colonized root tips were randomly selected from each third under a 10× dissecting scope, to get a total of six root tips per seedling. Each root tip was placed into 1 mL of double-distilled water (ddH_2O), flash frozen in liquid nitrogen and lyophilized for 48 h.

The remaining shoot and root system were placed in separate coin envelopes, dried at 64 C for 2 days and weighed. Colonization appeared close to 100% on all seedlings but was not quantified.

MOLECULAR IDENTIFICATION

DNA was extracted using a modified protocol from the REDExtract-N-Amp Tissue PCR Kit (Sigma-Aldrich, Saint Louis, MO). For this protocol, each lyophilized root was placed into 10 L of extraction buffer and incubated in a thermocycler at 65 C for 10 min and subsequently at 95 C for 10 min. Finally, 30 L of neutralization solution B was added and the extracts were stored at 4 C.

PCR was used to amplify the internal transcribed spacer (ITS) region of the nuclear ribosomal RNA genes using the fungal specific primer pair ITS1f and ITS4 (Gardes & Bruns 1993; White *et al*. 1990). PCR products were visualized with gel electrophoresis and 7–10 L of successful amplifications were cleaned by adding 0.5 L of ExoSAP IT (USB Corp, Cleveland, OH) and incubating at 37 C for 15 min followed by 80 C for 15 min. All samples were sequenced in one direction using an ABI3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). For those samples that yielded multiple PCR products or mixed sequences with the initial ITS1f and ITS4 primer pair, we attempted a second round of PCR and sequencing using the ITS4a (ascomycete-specific) and ITS4b (basidiomycete-specific) reverse primers (Gardes & Bruns 1993, Larena *et al*. 1999). PCR was unsuccessful using the published 62–64 C annealing temperature for ITS4a (Larena *et al*. 1999), but worked well at 55 C while retaining specificity for ascomycetes.

FUNGAL IDENTIFICATION

ITS sequences were edited using the program Sequencher 4.2 (Gene Codes Corp., MI). Sequences were initially placed into larger taxonomic groups (e.g. Boletales, Thelephorales) using the National Center for Biotechnology Information's Basic Local Alignment Search Tool (BLAST), which allows query sequences to be matched against a large public data base (GenBank). Within each taxonomic group sequences were grouped into species-level operational taxonomic units (OTUs) using the contig feature of sequencher with parameters set to a minimum of 20% overlap and 97% sequence similarity, a cut-off that has been found to correspond well with morphologically defined species in many fungal groups (Vellinga *et al*. 2003; Garnica *et al*. 2005; Smith *et al*. 2007). Representative sequences from each OTU were then searched against the GenBank data base using BLAST and named to the lowest taxonomic rank possible based on the level of match. Because of the long history of molecular work at Point Reyes many of the fungi colonizing our root tips could be reliably named at the species level. A representative sequence from each OTU has been deposited in GenBank (Table 1).

SPORE HEAT RESISTANCE ASSAY

In order to test for variation in heat resistance between ectomycorrhizal fungi we exposed spores to various heat levels and assayed their viability using fluorescent vital stains. For this experiment we used spores from four fungi: *Rhizopogon olivaceotinctus*, *R. salebrosus*, *Suillus pungens* and *Laccaria proxima*. These fungi were chosen because (i) they were relatively common on seedlings in the study, (ii) their spores were obtainable in sufficient quantity, and (iii) they showed different preferences for heat treatments (Table 1, Fig. 2). Spores were collected from multiple fruiting bodies found during field visits to Point Reyes. For most species, spores were collected by obtaining a spore print onto aluminium foil and scraping the spore residue into ddH_2O. This mixture was stored at 4 C until use. Because *Rhizopogon* species produce gasteroid fruit bodies with no active spore discharge, their fruit bodies were mixed in a stainless steel blender with ddH_2O and the mixture was strained through cheesecloth before it was stored at 4 C. Spores of all species other than *R. olivaceotinctus* were collected during the 2007–2008 mushroom fruiting season (November to April). Although it was common in this study, *R. olivaceotinctus* is rarely observed on roots in mature forests and has only been found fruiting twice at Point Reyes. Because attempts to locate it were unsuccessful during the 2007–2008 season we used a spore slurry made from fruit bodies collected in the 2006–2007 mushroom season.

Table 1. List of species observed across experimental treatments and the frequency of their sequences recovered. Dual colonization occurred on < 5% of extracted root tips, thus sequence frequency is approximately equivalent to the number of root tips colonized in each treatment. Total species richness for each treatment is shown in the bottom row

Species	Total	Heat–Ash–	Heat–Ash+	Heat+Ash–	Heat+Ash+	GenBank accession numbers
Ascomycete sp. MF3	1	0	1	0	0	FJ197199
Athelioid sp. MF1	1	1	0	0	0	FJ197201
Cantharellales sp. MF2	4	0	4	0	0	FJ197202
Helotiales sp. MF5	12	7	5	0	0	FJ197203
Helotiales sp. *MF21*	1	0	1	0	0	FJ197204
Humicola sp. MF4	40	0	0	25	15	FJ197200
Laccaria proxima	31	31	0	0	0	FJ197205
Phialocephala fortinii	6	2	4	0	0	FJ197206
Rhizopogon occidentalis	66	10	16	19	21	FJ197207
Rhizopogon olivaceotinctus	43	0	0	15	28	FJ197208
Rhizopogon salebrosus	17	1	0	7	9	FJ197209
Rhizopogon vulgaris	5	0	1	4	0	FJ197210
Sebacinaceae sp. MF12	2	0	2	0	0	FJ197211
Suillus brevipes	4	1	3	0	0	FJ197212
Suillus pungens	9	0	0	9	0	FJ197213
Suillus pseudobrevipes	26	4	11	11	0	FJ197214
Suillus quiescens sp. MF20	5	0	0	0	5	FJ197215
Suillus tomentosus	6	2	4	0	0	FJ197216
Tomentella sublilacina	15	10	5	0	0	FJ197217
Tylospora sp. *MF17*	10	6	4	0	0	FJ197218
Wilcoxina mikolae	170	56	47	25	42	FJ197219
Species richness	21	12	14	8	6	

To assay heat resistance, spores from each species were diluted in ddH_2O to a concentration of 1 million spores per mL as determined using a haemocytometer. For each species, 1 mL of spores suspended in ddH_2O was placed in a water bath set at 65 C for 0, 60, 120, 240, or 360 s. After removal from the water bath spores were allowed to cool to room temperature before they were stained with 3 L 1 mg^{-1} mL^{-1} fluoroscein diacetate (FDA) and 0.5 L 2 mg^{-1} mL^{-1} propidium iodide (PI).

FDA and PI are common vital stains for filamentous fungi (Miller *et al*. 1993, Hickey *et al*. 2004). FDA will only fluoresce when the fluorophore is cleaved through active uptake across a cell membrane and thus is indicative of metabolic activity. PI is a nuclear stain that cannot pass through an intact cell membrane and thus indicates cell death or damage. The two molecules fluoresce at different wavelengths and are easily distinguishable using fluorescent microscopy or flow cytometry (Hickey *et al*. 2004). In preliminary trials we found that a 24-h incubation at 4 C was necessary to effectively stain most spores. To quantify the number of spores stained with FDA and PI in each sample we used flow cytometric analysis on a Beckmann-Coulter FC500 Analyzer (Beckmann Coulter, Fullerton, CA) at the UC Berkeley Cancer Research Laboratory Flow Cytometry Facility. Control runs indicated no autofluorescence of the spores of species used in the experiment (although Thelepheroid spores were excluded for this reason). For each species 20 000 spores were analysed per treatment and the number of spores with no stain, FDA positive, PI positive or dual positive was recorded.

Although assaying heat resistance of spores in soil (as opposed to spores suspended in water) would have allowed more realistic comparison to the results of our community study, soil autofluorescence and the difficulty of recovering spores from soil made this unfeasible for our experiment. Traditional viability assessment methods that involve observing spore germination are also challenging for ectomycorrhizal fungi since many species will not germinate in culture or require the presence of host roots to do so (Ishida *et al*. 2008). While our approach is not perfect, it represents the best alternative available to us at this time.

SOIL CHEMICAL ANALYSES

In order to directly assess the effects of heat and ash treatments on soil chemistry, we replicated the same 2×2 factorial experimental design on remaining soils from the unheated controls. Treatments were conducted identically to those previously described except that soil volumes for each replicate consisted of 250 mL (1 : 1 field soil and autoclaved sand), approximately twice the amount in each cone-tainer, to produce enough for chemical analysis. Similarly, 3 g of ash were added to each ash treatment. There were five replicates per treatment combination for a total of 20 replicates (two treatments $\times$ 2 levels $\times$ 5 replicates). Each replicate was established in a small 300-mL pot, watered to saturation and allowed to air dry for 1 week. Samples were sent to A & L Western Laboratories (Modesto CA) and analysed for pH, percent organic matter (OM), weak-bray phosphorous (P), calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+), cation exchange capacity (CEC), nitrate (NO_3^-) and ammonium (NH_4^+) content.

STATISTICAL ANALYSES

All statistical tests and graphics were produced using the program R v.2.6.1 (R Development Core Team, 2007). To visualize ectomycorrhizal assemblage patterns across treatments we used non-metric multidimensional scaling (NMDS) as implemented by the metaMDS command in the R package Vegan. To test for statistical differences

Table 2. Two-way ANOVA results for the effects of heat and ash treatments on soil chemistry. Results significant at the $P < 0.05$ level are indicated with an asterisk

Variable†	Heat		Ash		Heat × Ash	
	$F_{1,20}$	P	$F_{1,20}$	P	$F_{1,20}$	P
pH	0.00	1.00000	120.33	<0.00001*	0.33	0.57174
Percentage OM (%)	0.57	0.46082	0.43	0.52182	0.00	0.96044
P	2.68	0.12119	19.96	0.00039*	3.62	0.07530
Ca^{2+}	2.45	0.13687	17.48	0.00071*	0.07	0.79927
Log (Mg^{2+})	0.24	0.63109	23.08	0.00019*	0.78	0.38949
Log (K^{+})	0.08	0.77763	102.96	<0.00001*	0.21	0.64939
CEC	0.98	0.33802	7.81	0.01298*	0.89	0.35839
NO_3^-	39.56	<0.00001*	10.96	0.00442*	57.97	<0.00001*
Log (NH_4^+)	2.80	0.11369	11.20	0.00409*	0.77	0.39256

†pH, organic matter (OM), weak-bray phosphorous (P), calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^{+}), cation exchange capacity (CEC), nitrate (NO_3^-) and ammonium (NH_4^+)

in community similarity between treatments we used analysis of variance using distance matrices (ADONIS), which is equivalent to Anderson's (2001) nonparametric multivariate ANOVA. ADONIS is more robust than analysis of similarity (ANOSIM) and allows simultaneous analysis of multiple treatments and their interactions (Oksanen 2008). Our ADONIS model included the main effects from the heat and ash treatments as well as their interaction. Both NMDS and ADONIS were performed using the Raup-Crick similarity index, a probabilistic measure based on presence–absence (Raup & Crick 1979). Other indices available in Vegan (e.g. Bray-Curtis, Morisita-Horn and β-sim) gave very similar results, but poorer NMDS fits (i.e. higher stress) and are not reported. Seedlings where five or more root tips failed to sequence were excluded from these analyses. In addition, one seedling that was colonized entirely by a rare fungus that occurred on no other seedlings (*Cantherellales* sp. MF2) was excluded as there was no basis for comparison with other seedling assemblages.

We used a logistic regression model to assess the effects of heat exposure on spore viability. The model was fit with maximum likelihood techniques using the GLM procedure in R with quasibinomial errors (due to overdispersion). Logistic regression models the log-odds of the probability of an event occurring [ln (p/1 – p)] as a linear function of the predictor variables. In our full model, log-odds of spore survival was predicted by time of heat exposure, species identity and their interaction. Insignificant terms were eliminated from the final model and overall explanatory power was calculated using the maximum likelihood version of R^2 as 1 – (residual deviance/null deviance) (Quinn & Keough 2006). To ensure that our results were not driven by any differences in the starting number of viable spores, we also reran the same analysis but using percent change from the initial number of viable spores for a given species as the response.

We used two-way fixed factor ANOVA to test for differences in total seedling biomass and species richness between treatments with the predictor variables heat, ash and their interaction. Total seedling biomass and species richness were log-transformed to improve normality and the Fligner-Killeen test (Conover *et al*. 1981) showed no heteroscedasticity of variance among treatment groups ($P > 0.05$). We also performed a series of two-way ANOVAs to see how heat and ash affected the soil chemical parameters we measured. One variable, NO_3^-, was heteroscedastic regardless of the transformation employed; however, heteroscedasticity is not as severe a problem for fully balanced ANOVA designs like the one we use (Underwood 1997, pp. 182–183). All statistical tests were considered significant at $P < 0.05$.

Results

Soil treatments achieved the desired heating profiles, reaching a maximum temperature of approximately 70 C, remaining above 60 C for 15 min and cooling over 3 h to room temperature (data not shown). Of the initial 100 seedlings in the experiment, 95 survived until harvest, thus DNA was extracted from a total of 570 root tips. Fungal colonists were successfully identified from sequence data on 450 (79%) root tips from 94 seedlings (476 sequences in total, including instances of dual colonization). Seedlings grown in heat-treated soils were significantly larger than those grown in unheated soils (treatment means ± 1 SE: heated = 1.20 ± 0.06 g; unheated = 0.77 ± 0.3 g; $F_{1,88} = 45.1$, $P < 0.0001$), while ash addition appeared to have no effect on seedling growth (treatment means ± 1 SE: ash = 0.95 ± 0.05 g; no ash = 1.0 ± 0.06 g; $F_{1,88} = 0.07$, $P = 0.78$).

Ash addition had strong effects on nearly all measured soil parameters (Table 2), increasing pH, phosphorous and the concentration of base cations (Fig. 1). None of these factors were affected by the heat treatment. The effects of heat and ash on nitrogen were more complex. Both heat and ash significantly increased concentrations of nitrate (NO_3^-) nitrogen relative to control soils (no heat, no ash). However, although levels of ammonium (NH_4^+) nitrogen increased slightly (but insignificantly) from the heat treatment, ash addition significantly decreased NH_4^+ content (Fig. 1). Therefore, the seedlings growing in heat-treated soils in our experiment likely experienced a net increase in available nitrogen whereas the ash-treated seedlings primarily experienced a shift in form from NH_4^+ to NO_3^-. None of the treatments affected OM content of the soils.

Using a 97% sequence similarity cut-off we identified a total of 21 species of ectomycorrhizal fungi (Table 1). Most taxa, such as *Wilcoxina*, *Rhizopogon*, *Laccaria*, *Suillus* and *Tomentella*, are common ectomycorrhizal associates at Point Reyes. Some taxa, such as *Phialocephala fortinii* and *Humicola* sp. MF4, are of uncertain mycorrhizal status, but were retained in our analyses as they were identified from apparently healthy roots with ectomycorrhizal mantles. In total, more species were found on seedlings growing in unheated

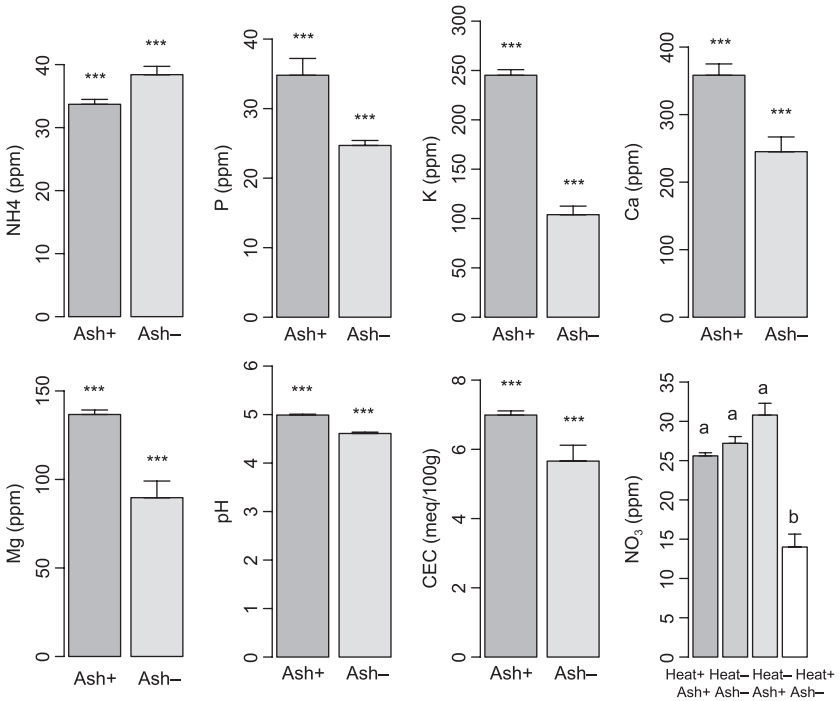

Fig. 1. Significant differences in soil chemistry caused by heat and ash treatments. Asterisks show differences between main effects as indicated by two-way ANOVA, and letters indicate significantly different groups based on Tukey's HSD test.

soils, while species numbers were equivalent on ash-treated soils (Table 1). Mean richness per seedling was 2.29 species (minimum = 1, maximum = 5) and was not affected by any of the treatments (overall model $F_{3,88} = 0.38$, $P = 0.76$).

NMDS plots revealed strong clustering of ectomycorrhizal assemblages on seedlings within respective treatment categories (Stress = 7.89, non-metric fit $R^2 = 0.99$, Fig. 2). The different NMDS axes highlighted different aspects of the assemblage structure. Axes 1 and 2 (Fig. 2a) showed the general simplification of the ectomycorrhizal assemblage on seedlings in the heat-treated soils to a smaller subset of the overall assemblage. Despite sharing many taxa, Axes 1 and 3 (Fig. 2b) showed a tendency towards greater similarity in assemblages on seedlings occurring in the same heat treatments. Axes 2 and 3 (Fig. 2c) showed the greatest separation between all four heat- and ash-treatment combinations and indicated that effects of ash operated within the respective heat treatments. These patterns were supported by the ADONIS analysis, which showed a strong effect of heat, a insignificant effect of ash and a significant heat × ash interaction (Table 3), indicating that all of the treatments had significant effects on assemblage structure. Visual inspection of NMDS Axes 2 and 3 (Fig. 2c) indicated that the interaction was driven by a greater divergence between ash and control assemblages on seedlings in the unheated soils compared with the heated soils, and greater divergence of the ash-treated seedlings across the heat treatments. Partial R^2 values showed that 26% of the variation in species composition was explained by the heat treatment and 3% was explained by the heat × ash interaction (Table 3).

Initial spore viability as measured by FDA was close to 100% in all of the species tested (Fig. 3), indicating no differences in the starting viability of the spores used despite differences in the timing of their collection. PI staining was

Table 3. Effects of heat exposure and ash addition on ectomycorrhizal assemblages. The table shows output from an ADONIS model including the effects of heat, ash and their interaction on similarity of ectomycorrhizal assemblages. ADONIS is an analogue of ANOVA that can be used on distance matrices. Significance tests are based on 10 000 permutations of the Raup-Crick dissimilarity matrix

Parameter	d.f.	SS	MS	F	R^2	Pr (> F)
Heat	1	4.10	4.10	32.31	0.26	<0.001
Ash	1	0.25	0.25	2.00	0.02	0.178
Heat × Ash	1	0.42	0.42	3.33	0.03	0.048
Residuals	87	22.39	0.26	NA	0.69	NA
Total	90	26.71	NA	NA	1.00	NA

broadly consistent with our expected results: all spores of *L. proxima* and *S. pungens* stained positive for PI after 6 min of exposure to 65 C and visual inspection revealed bright staining of the spore nuclei, indicating breakdown of the cell membrane. However, overall we found PI to be an unreliable stain for our experiment. This was because it appeared to also bind and fluoresce on the ornamentation of *L. proxima*, resulting in a number of dual positive spores identified through flow cytometric analysis (Fig. S1). Additionally, even after extended heat exposure and total loss of FDA fluorescence, the cell membranes of both *Rhizopogon* species appeared to remain intact and did not stain with PI. For this reason, all statistical tests of viability are based on the FDA stain results only. Because nearly all spores appeared metabolically active before heat treatments were applied, loss of FDA fluorescence is likely a good indicator of spore death in these experiments.

In all ectomycorrhizal fungi tested, spore viability was negatively affected by progressive exposure to heat (Fig. 3). The interaction between heat exposure and species identity

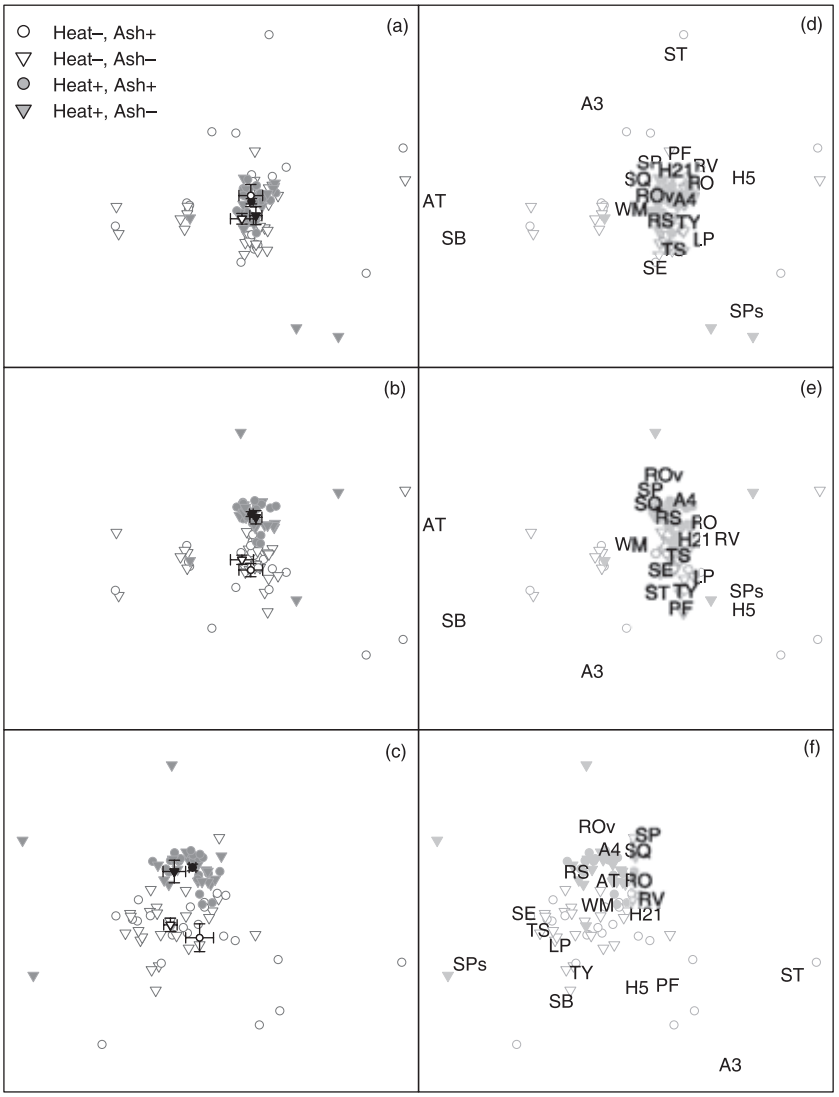

Fig. 2. NMDS plots of seedling ectomycorrhizal assemblages. (a–c) Ordination of *Pinus muricata* seedlings for Axes (*x*,*y*) 1 and 2, 1 and 3 and 2 and 3, respectively. Each point represents a single *P. muricata* seedling. Seedlings closer together in the ordination have more similar ectomycorrhizal assemblages. A small amount of jitter was introduced to the plot to separate overlapping points. Treatment groups are indicated in the legend. Black symbols are centroids ± 1 SE for each treatment group calculated from the NMDS ordination. Hollow and filled centroids indicate unheated and heated treatments, respectively. Centroid symbols for ash treatments follow the legend. Centroids and SEs are for visualization purposes only – all statistical tests of assemblage structure were done using ADONIS (Table 3). (d–f) Ordination of ectomycorrhizal species for NMDS axes 1 and 2, 1 and 3 and 2 and 3, respectively. Species plotted closer together occurred on more similar sets of seedlings. Seedlings and treatments are shown in background for reference. Species abbreviations are *Ascomycete* sp. MF3 (A3), *Ascomycete* sp. MF4 (A4), *Athelioid* sp. MF1 (AT), *Helotiales* sp. MF5 (H5), *Helotiales* sp. MF21 (H21), *Laccaria proxima* (LP), *Phialocephala fortinii* (PI), *Rhizopogon occidentalis* (RO), *R. olivaceotinctus* (ROv), *R. salebrosus* (RS), *R. vulgaris* (RV), *Sebacinaceae* sp. MF12 (SE), *S. brevipes* (SB), *Suillus pseudobrevipes* (SPs), *S. pungens* (SP), *S. quiescens* (SQ), *S. tomentosus* (ST), *Tomentella sublilacina* (TS), *Tylospora* sp. MF17 (TY), *Wilcoxina mikolae* (WM).

was insignificant and removed from the final statistical model (both main effects were significant in the full model). Both heat exposure time and species identity were highly significant in the final model (time effect: $F_{1,18} = 94.42$, $P < 0.0001$; species effect: $F_{3,15} = 9.98$, $P = 0.0007$), which had good explanatory power ($R^2 = 0.91$). The significant species effect indicated overall differences in spore heat tolerance (Table S1 in Supporting Information, Fig. 3). For a given period of heat exposure, spore viability tended to be greatest in *R. olivaceotinctus* > *L. proxima* > *R. salebrosus* > *S. pungens*, with our logistic model predicting 50% spore mortality after 261, 162, 96, and 79 s of heat exposure, respectively. Results were nearly identical when the analysis was based on rescaled spore viability (i.e. all species rescaled to start at 100% spore viability) and so are not presented here.

Discussion

Ectomycorrhizal assemblages colonizing *P. muricata* seedlings were affected by both heat and ash treatments. These effects were strongest for the heat treatments, with a number of both common and rare species eliminated entirely in the heated soils. Most notable were *L. proxima* and *T. sublilacina*, which were abundant on seedlings grown in unheated soils but totally absent from seedlings grown in the heated soils. Conversely, *R. olivaceotinctus*, which was observed on 43 root tips in the heat-treated soils, was never observed on seedlings in the unheated soils. Other *Rhizopogon* species, although present in the unheated treatments, also tended to increase in frequency in the heat-treated soils. The most common species observed in the study, *Wilcoxina mikolae*, appeared relatively unaffected by the heat treatments. Because there were only a few new species in the heat treatment, the overall effect was both a simplification and shift in assemblage structure. These results suggest that the main effect of heat was to reduce the number and diversity of viable ectomycorrhizal propagules available to colonize bioassay seedlings.

The effects of ash were subtler and appeared to operate within the respective heat treatments, as evidenced by the significant interaction term in the ADONIS model and treatment centroids in the NMDS plots. For example, *L. proxima* was observed on root tips 31 times in the unheated

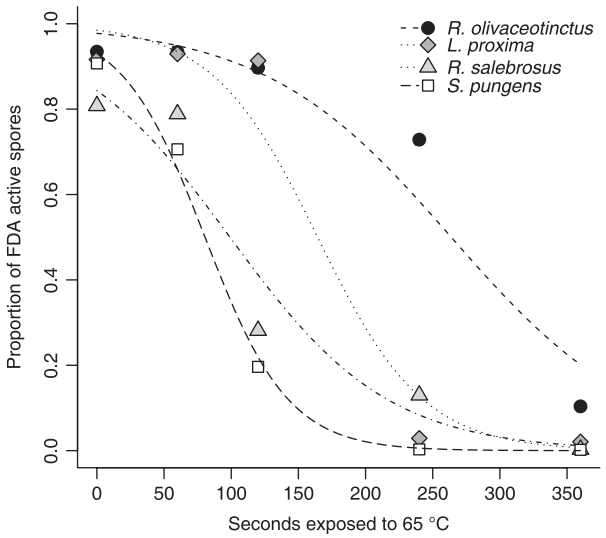

Fig. 3. Percentage spore viability with progressive heat exposure for four species of ectomycorrhizal fungi. Points are actual data values and dashed lines are predicted values. Logistic regression equations for each species are *Rhizopogon olivaceotinctus* = 5.06 – 0.02 s^{-1} 65 C, *Laccaria proxima* = 3.35 – 0.02 s^{-1} 65 C, *Rhizopogon salebrosus* = 1.94 – 0.02 s^{-1} 65 C and *Suillus pungens* = 1.63 – 0.02 s^{-1} 65 C (significance tests for model coefficients are given in Table S1). Predicted lines are given by solving the logistic equation, $p = 1/1 + e^{-(ax+b)}$, using the logistic regression coefficients. All species tested were negatively affected by heat exposure, but there were clear differences between species in their ability to resist heat stress. *R. olivaceotinctus* displayed the greatest heat tolerance and was also the species that increased colonization most on seedlings grown in heat treated field soils.

soils, but not once in conjunction with ash addition. *L. proxima* was also totally absent from heated soils so a similar pattern was not observed in those treatments. Failure to colonize ash treatments was also observed in the heat-treated soils for *S. pungens* and *S. pseudobrevipes*. However, *S. pseudobrevipes* was observed to colonize ash-treated seedlings in unheated soils. This suggests that in post-fire settings heat is the primary filter determining membership of the initial post-fire assemblage and that chemical changes in the soil environment caused by variability in ash concentrations play a secondary role in determining patterns of ectomycorrhizal abundance.

A large number of field studies have shown a similar pattern of community shift and simplification after fire (Visser 1995; Horton *et al*. 1998; Stendell *et al*. 1999; Smith *et al*. 2004), frequently with the increase in abundance of *Rhizopogon* species. Using a seedling bioassay approach, Schoenberger & Perry (1982) and Pilz & Perry (1984) found no or slightly negative effects of slash burning on ectomycorrhizal colonization in clear cuts. However, most of the aforementioned studies do not control for the separate effects of heat, ash or successional status. In one of the few comparable studies to manipulate soil heating, Izzo *et al*. (2006) also found a substantial increase in *R. olivaceotinctus* in heat-treated soils from a Sierran mixed-conifer forest. *Wilcoxina mikolae*, the most common species in this study, has also been frequently observed in other fire-effects studies. This is perhaps because *Wilcoxina* spp. produce sclerotia (a small, hardened aggregation of hyphae) that may confer some heat tolerance. Izzo *et al*. (2006) found that heat treatments decreased *Wilcoxina* abundance, but only in their 75 C treatment, a heat level not tested in this study.

Studies explicitly examining the effects of ash on ectomycorrhizal assemblages are less common. Ash addition or removal experiments have shown that ash can cause significant changes in soil chemistry and plant nutrition (Tryon 1948; Grogan *et al*. 2000a; Mahmood *et al*. 2003). Despite this, no studies have been able to make strong conclusions regarding the effects of ash on ectomycorrhizal assemblages (Grogan *et al*. 2000b; Mahmood *et al*. 2002). This may be because ash has relatively small biological effects (Tryon 1948, this study), but also may be due to the high species richness and low abundance of individual species of ectomycorrhizal fungi in the field (Erland & Söderström 1991; Grogan *et al*. 2000b; Mahmood *et al*. 2002). By using a highly replicated factorial design we are able to show that ash does have a significant effect on ectomycorrhizal assemblage structure. However, our results concur with field studies of Erland & Söderström (1991), Grogan *et al*. (2000b) and Mahmood *et al*. (2002) that the magnitude of this effect is probably fairly small at the assemblage level.

There are a number of mechanisms that could explain the patterns of community shift we observed. First, it is well-documented that heat and ash additions change soil chemistry and increase nutrient availability (St. John & Rundel 1976; Grogan *et al*. 2000a; Mahmood *et al*. 2003; Certini 2005). Both heat and ash treatments in this study significantly affected the soil chemical environment, however the effect was much stronger for ash compared with heat treatments. This is likely because temperatures >180 C are usually required to induce major physical and chemical changes in soil, while the temperatures in this study (60–70 C) were aimed at causing biotic disruption without total sterilization (reviewed in Neary *et al*. 1999; Hart *et al*. 2005). This is also indicated by the failure of our heat treatments to cause combustion and loss of OM (Table 2). The effects of natural wildfires on soil nutrients are also highly variable in space and time and vary greatly depending on fire intensity. For instance, intense fires can often lead to long-term losses in total nitrogen (through volatilization), but short-term increases in plant available nitrogen (NO_3^- or NH_4^+), as observed in this study (St. Paul & Rundel 1976; Neary *et al*. 1999; Grogan *et al*. 2000a; Hart *et al*. 2005). This effect is probably at least partly responsible for the increased growth in seedlings we observed in the heat-treated soils. It is unclear, however, whether increased nitrogen mineralization at this level would contribute significantly to shifting ectomycorrhizal community structure, as the increases we observed (for both NH_4^+ and NO_3^-) are much smaller than those usually investigated in studies of anthropogenic N deposition (e.g. Lilleskov *et al*. 2002). The primary effects of ash or charcoal addition – increased pH, calcium, magnesium, potassium and phosphate – are fairly

predictable and may be very long-lasting (Tryon 1948; Zackrisson *et al*. 1996; Wardle *et al*. 1998). Although our ash had been stored for a year prior to use, the effects were still quite consistent with those reported from other studies using fresh ash or charcoal. Ectomycorrhizal fungi have been shown to differ in their preference for and ability to utilize ash-amended substrates (Mahmood *et al*. 2001; Wallander *et al*. 2003). Responses of ectomycorrhizal species to ash addition thus likely indicate that changes in soil chemistry affected the competitive balance between certain species. Mahmood (2003) found this to be the result in a two-species competition experiment involving addition of hardened wood ash. More generally, nutrient addition was found to favour colonization of a *Rhizopogon* species in a two-species competition experiment (Lilleskov & Bruns 2003). Competition experiments using ash addition in our system could shed more light on this result in the future.

The second mechanism that could explain these results is heat-induced mortality of ectomycorrhizal propagules. Our heat resistance experiment agrees well with other studies that have also shown significant mortality for fungi at temperatures in the range of 60–80 C (reviewed in Neary *et al*. 1999). No studies we know of have directly examined heat resistance of ectomycorrhizal propagules due to the difficulty in assessing viability with standard germination tests (Ishida *et al*. 2008). Unfortunately, we were not able to obtain and assay spores for most species that appeared in the study. Fungal fruit body production is highly unpredictable and some species, such as *W. mikolae*, do not produce macroscopic fruiting bodies from which large number of spores can be harvested. Additionally, while our spore heating assays assume that most colonization came from spores, it is possible that some colonization we observed in the study came from hyphal material remaining in the soil (e.g. the sclerotia of *Wilcoxina*). Future studies could address these problems by using ectomycorrhiza free soils with spores of target species added directly.

Because we could not test spore heat resistance for all species, there are two ways that the heat treatments could have resulted in the observed assembly shift and simplification, (i) given equal spore banks between species, through differences in spore heat tolerance, or (ii) given equal spore heat tolerance among species, through differences in the size of the spore bank. One novel conclusion from this experiment is that rates of heat-induced propagule mortality can differ significantly between ectomycorrhizal fungi. While we did not test heat resistance for all species in the assemblage, it appears ecologically relevant that the species with the greatest increase in abundance in heat-treated soils (*R. olivaceotinctus*) also had the most heat resistant spores tested. This strongly suggests that heat resistance is a key trait in determining post-fire colonization patterns. To our knowledge this connection has not been directly demonstrated before in ectomycorrhizal assemblages. In contrast, both *R. salebrosus* and *S. pungens* appeared to do well in the heat-treated soils and yet had relatively low heat tolerance, a pattern which suggests that for some species the presence of relatively large spore banks is crucial for post-fire success. Together, these results show that post-fire success can likely be achieved through a number of life-history strategies that allow a species to be active soon after disturbance.

We believe our results support the assertion that disturbance plays a role in maintaining the high diversity of ectomycorrhizal assemblages (Bruns 1995). The relative rarity of species of the genus *Rhizopogon* on seedlings grown in unheated soils, despite presence of their spores, strongly suggests competitive inferiority (e.g. Kennedy *et al*. 2007a,b), particularly, as all of these species have been observed to colonize seedlings well in the absence of competitors (Bruns *et al*. 2009). Because soil heating causes high mortality of below-ground organisms at even relatively moderate temperatures, fires likely open up a large amount of competitor-free space. When a colonization-competition trade-off occurs (Skellam 1951; Tilman 1994), fires should lead to increase in abundance of competitive inferiors. Species in the genus *Rhizopogon* have traits that match 'fugitive' or r-selected life histories (Hutchinson 1951; Pianka 1970), including competitive inferiority, fast growth and high production of reactive, long-lived spores (Bruns *et al*. 2009). Thus, in these systems fires may maintain ectomycorrhizal species richness by creating a landscape mosaic of environmental conditions and competitor-free space that is necessary for the long-term persistence of 'fugitive' components of the assembly (Hutchinson 1951; Wiens 1976; Grubb 1977; Sousa 1984; Caswell & Cohen 1991).

Conclusions

In this study we confirm results from studies manipulating heat and ash separately (Grogan *et al*. 2000a,b; Mahmood *et al*. 2002; Izzo *et al*. 2006), and show that both have definite effects on ectomycorrhizal assemblage structure. Our data suggest that heat and ash treatments both modify the soil environment in ways that may favour particular species. However, we also demonstrate that a functional trait, spore heat resistance, can lead to differential survival and post-fire success as well. Thus, the primary disturbance agent in this system alters species composition and maintains richness of the ectomycorrhizal assemblage by creating a mosaic of environmental conditions and causing mortality of competitive dominants.

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References

Anderson, M.J. (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, **26**, 32–46.

Baar, J., Horton, T.R., Kretzer, A. & Bruns, T.D. (1999) Mycorrhizal recolonization of *Pinus muricata* from resistant propagules after a stand-replacing wildfire. *New Phytologist*, **143**, 409–418.

Bruns, T.D. (1995) Thoughts on the processes that maintain local species-diversity of ectomycorrhizal fungi. *Plant and Soil*, **170**, 63–73.

Bruns, T.D., Peay, K.G., Boynton, P.J., Grubisha, L.C., Hynson, N.A., Nguyen, N.H. & Rosenstock, N.P. (2009) Inoculum potential of *Rhizopogon* spores increases with time over the first four years of a 99-year spore burial experiment. *New Phytologist*, **181**, 463–470.

Caswell, H. & Cohen, J.E. (1991) Disturbance, Interspecific Interaction and Diversity in Metapopulations. *Biological Journal of the Linnean Society*, **42**, 193–218.

Certini, G. (2005) Effects of fire on properties of forest soils: A review. *Oecologia*, **143**, 1–10.

Connell, J.H. (1978) Diversity in tropical rain forests and coral reefs – high diversity of trees and corals is maintained only in a non-equilibrium state. *Science*, **199**, 1302–1310.

Connell, J.H. & Slatyer, R.O. (1977) Mechanisms of succession in natural communities and their role in community stability and organization. *American Naturalist*, **111**, 1119–1144.

Conover, W.J., Johnson, M.E. & Johnson, M.M. (1981) A comparative study of tests for homogeneity of variances, with applications to the outer continental shelf bidding data. *Technometrics*, **23**, 351–361.

Erland, S. & Söderström, B. (1991) Effects of lime and ash treatments on ectomycorrhizal infection of *Pinus sylvestris* L. seedlings planted in a pine forest. *Scandinavian Journal of Forest Research*, **14**, 209–217.

Erland, S. & Taylor, A.F.S. (2002) Diversity of ecto-mycorrhizal fungal communities in relation to the abiotic environment. *Mycorrhizal Ecology* (eds M.G.A. van der Heijden & I.R. Sanders), pp. 163–200. Springer, Berlin.

Gardes, M. & Bruns, T. (1993) ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology*, **2**, 113–118.

Garnica, S., Weiss, M., Oertel, B. & Oberwinkler, F. (2005) A framework for phylogenetic classification in the genus *Cortinarius* (Basidiomycota, Agaricales) derived from morphological and molecular data. *Canadian Journal of Botany*, **83**, 1457–1477.

Grogan, P., Baar, J. & Bruns, T.D. (2000a) Below-ground ectomycorrhizal community structure in a recently burned bishop pine forest. *Journal of Ecology*, **88**, 1051–1062.

Grogan, P., Bruns, T.D. & Chapin, F.S. (2000b) Fire effects on ecosystem nitrogen cycling in a Californian bishop pine forest. *Oecologia*, **122**, 537–544.

Grubb, P.J. (1977) Maintenance of species-richness in plant communities – importance of regeneration niche. *Biological Reviews of the Cambridge Philosophical Society*, **52**, 107–145.

Hart, S.C., DeLuca, T.H., Newman, G.S., MacKenzie, M.D. & Boyle, S.I. (2005) Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. *Forest Ecology and Management*, **220**, 166–184.

Hickey, P.C., Swift, S.R., Roca, M.G. & Read, N.D. (2004) Live-cell imaging of filamentous fungi using vital fluorsescent dyes and confocal microscopy. *Methods in Microbiology*, **34**, 63–87.

Hobbie, E.A. (2006) Carbon allocation to ectomycorrhizal fungi correlates with belowground allocation in culture studies. *Ecology*, **87**, 563–569.

Hobbie, J.E. & Hobbie, E.A. (2006) 15N in symbiotic fungi and plants estimates nitrogen and carbon flux rates in Arctic tundra. *Ecology*, **87**, 823–828.

Horton, T.R., Cázares, E. & Bruns, T.D. (1998) Ectomycorrhizal, vesicular-arbuscular and dark septat fungal colonization of bishop pine (*Pinus muricata*) seedlings in the first 5 months of growth after wildfire. *Mycorrhiza*, **8**, 11–18.

Hutchinson, G.E. (1951) Copepodology for the Onithologist. *Ecology*, **32**, 571–577.

Ishida, T.A., Nara, K. & Hogetsu, T. (2007) Host effects on ectomycorrhizal fungal communities: Insight from eight host species in mixed conifer-broadleaf forests. *New Phytologist*, **174**, 430–440.

Ishida, T.A., Nara, K., Tanaka, M., Akihiko, K. & Hogetsu, T. (2008) Germination and infectivity of ectomycorrhizal fungal spores in relation to their ecological traits during primary succession. *New Phytologist*, **180**, 491–500.

Izzo, A., Canright, M. & Bruns, T.D. (2006) The effects of heat treatments on ectomycorrhizal resistant propagules and their ability to colonize bioassay seedlings. *Mycological Research*, **110**, 196–202.

Kennedy, P.G. & Peay, K.G. (2007) Different soil moisture conditions change the outcome of the ectomycorrhizal symbiosis between *Rhizopogon* species and *Pinus muricata*. *Plant and Soil*, **291**, 155–165.

Kennedy, P.G. & Sousa, W.P. (2006) Forest encroachment into a Californian grassland: examining the simultaneous effects of facilitation and competition on tree seedling recruitment. *Oecologia*, **148**, 464–474.

Kennedy, P.G., Bergemann, S.E., Hortal, S. & Bruns, T.D. (2007a) Determining the outcome of field-based competition between two *Rhizopogon* species using real-time PCR. *Molecular Ecology*, **16**(4), 881–890.

Kennedy, P.G., Hortal, S., Bergemann, S.E. & Bruns, T.D. (2007b) Competitive interactions among three ectomycorrhizal fungi and their relation to host plant performance. *Journal of Ecology*, **95**, 1338–1345.

Koide, R.T., Xu, B., Sharda, J., Lekberg, Y. & Ostiguy, N. (2005) Evidence of species interactions within an ectomycorrhizal fungal community. *New Phytologist*, **165**, 305–316.

Larena, I., Salazar, O., Gonzalez, V., Julian, M.C. & Rubio, V. (1999) Design of a primer for ribosomal DNA internal transcribed spacer with enhanced specificity for ascomycetes. *Journal of Biotechnology*, 2–3, 187–194.

Last, F.T., Dighton, J. & Mason, P.A. (1987) Successsions of sheathing mycorrhizal fungi. *Trends in Ecology and Evolution*, **2**, 157–161.

Lilleskov, E.A. & Bruns, T.D. (2003) Root colonization dynamics of two ectomycorrhizal fungi of contrasting life history strategies are mediated by addition of organic nutrient patches. *New Phytologist*, **159**, 141–151.

Lilleskov, E.A., Fahey, T.J., Horton, T.R. & Lovett, G.M. (2002) Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. *Ecology*, **83**, 104–115.

Mahmood, S. (2003) Colonisation of spruce roots by two interacting ectomycorrhizal fungi in wood ash amended substrates. *FEMS Microbiology Letters*, **221**, 81–87.

Mahmood, S., Finlay, R.D., Erland, S. & Wallander, H. (2001) Solubilisation and colonisation of wood ash by ectomycorrhizal fungi isolated from a wood ash fertilised spruce forest. *FEMS Microbiology Ecology*, **35**, 151–161.

Mahmood, S., Finlay, R., Wallander, H. & Erland, S. (2002) Ectomycorrhizal colonisation of roots and ash granules in a spruce forest treated with granulated wood ash. *Forest Ecology and Management*, **160**, 65–74.

Mahmood, S., Finlay, R.D., Fransson, A.M. & Wallander, H. (2003) Effects of hardened wood ash on microbial activity, plant growth and nutrient uptake by ectomycorrhizal spruce seedlings. *FEMS Microbiology Ecology*, **43**, 121–131.

Mason, P.A., Last, F.T., Wilson, J., Deacon, J.W., Fleming, L.V. & Fox, F.M. (1987) Fruiting and successions of ectomycorrhizal fungi. *Fungal Infection in Plants* (eds G.F. Pegg & P.G. Ayres), pp. 253–268.

Miller, S.L., Torres, P. & McClean, T.M. (1993) Basidiospore viability and germination in ectomycorrhizal and saprotrophic basidiomycetes. *Mycological Research*, **97**, 141–149.

Neary, D.G., Klopatek, C.C., DeBano, L.F. & Ffolliott, P.F. (1999) Fire effects on belowground sustainability: a review and synthesis. *Forest Ecology and Management*, **122**, 51–71.

Oksanen, J. (2008) Multivariate analysis of ecological communities in R: Vegan tutor. Available at http://cc.oulu.fi/~jarioksa/opetus/metodi/vegantutor.pdf.

Parrent, J.L. & Vilgalys, R. (2007) Biomass and compositional responses of ectomycorrhizal fungal hyphae to elevated CO_2 and nitrogen fertilization. *New Phytologist*, **176**, 164–174.

Peay, K.G., Bruns, T.D., Kennedy, P.G., Bergemann, S.E. & Garbelotto, M. (2007) A strong species-area relationship for eukaryotic soil microbes: island size matters for ectomycorrhizal fungi. *Ecology Letters*, **10**, 470–480.

Pianka, E.R. (1970) R-Selection and K-Selection. *American Naturalist*, **104**, 592–597.

Pickett, S.T.A. & White, P.S. (1985) *The Ecology of Natural Disturbance and Patch Dynamics*. Academic Press, Orlando.

Pilz, D. & Perry, D.A. (1984) Impact of clearcutting and slash burning on ectomycorrhizal associations of Douglas-fir seedlings. *Canadian Journal of Forest Research*, **14**, 94–100.

Pyne, S.J., Andrews, P.L. & Laven, R.D. (1996) *Introduction to Wildland Fire*. John Wiley & Sons, New York.

Quinn, G.P. & Keough, M.J. (2006) *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, New York.

R Core Development Team. (2007) *R: A Language and Environment For Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Raup, D.M. & Crick, R.E. (1979) Measurment of faunal similarity in paleontology. *Journal of Paleontology*, **53**, 1213–1227.

Schoenberger, M.M. & Perry, D.A. (1982) The effect of soil disturbance on growth and ectomycorrhizae of Douglas-fir and western hemlock seedlings: A greenhouse bioassay. *Canadian Journal of Forest Research*, **12**, 343–353.

Skellam, J.G. (1951) Random dispersal in theoretical populations. *Biometrika*, **38**, 196–218.

Smith, J.E., McKay, D., Niwa, C.G., Thies, W.G., Brenner, G. & Spatafora, J.W. (2004) Short-term effects of seasonal prescribed burning on the ectomycorrhizal fungal community and fine root biomass in ponderosa pine stands in the Blue Mountains of Oregon. *Canadian Journal of Forest Research*, **34**, 2477–2491.

Smith, S.E. & Read, D.J. (1997) *Mycorrhizal Symbiosis*. Academic Press, New York.

Smith, M.L., Douhan, G.W. & Rizzo, D.M. (2007) Intra-specific and intra-sporocarp ITS variation of ectomycorrhizal fungi as assessed by rDNA sequencing of sporocarps and pooled ectomycorrhizal roots from a *Quercus* woodland. *Mycorrhiza*, **18**, 15–22.

Sousa, W.P. (1979) Experimental investigations of disturbance and ecological succession in a rocky inter-tidal algal community. *Ecological Monographs*, **49**, 227–254.

Sousa, W.P. (1984) The role of disturbance in natural communities. *Annual Review of Ecology and Systematics*, **15**, 353–391.

St. John, T.V. & Rundel, P.W. (1976) The role of fire as a mineralizing agent in a Sierran coniferous forest. *Oecologia*, **25**, 35–45.

Stendell, E.R., Horton, T.R. & Bruns, T.D. (1999) Early effects of prescribed fire on the structure of the ectomycorrhizal fungus community in a Sierra Nevada ponderosa pine forest. *Mycological Research*, **103**, 1353–1359.

Sugihara, N.G., van Wagtendonk, J.W., Shaffer, K.E., Fites-Kauffman, J. & Thode, A.E. (2006) *Fire in California's Ecosystems*. University of California Press, Berkeley.

Terwilliger, J. & Pastor, J. (1999) Small mammals, ectomycorrhizae, and conifer succession in beaver meadows. *Oikos*, **85**, 83.

Tilman, D. (1994) Competition and biodiversity in spatially structured habitats. *Ecology*, **75**, 2–16.

Tryon, E.H. (1948) Effect of charcoal on certain physical, chemical and biological properties of forest soils. *Ecological Monographs*, **18**, 81–115.

Underwood, A.J. (1997) *Experiments in Ecology*. Cambridge University Press, New York.

Vellinga, E., de Kok, R.P.J. & Bruns, T.D. (2003) Phylogeny and taxonomy of *Macrolepiota* (Agaricaceae). *Mycologia*, **95**, 442–456.

Visser, S. (1995) Ectomycorrhizal fungal succession in jack pine stands following wildfire. *New Phytologist*, **129**, 389–401.

Walker, L.R. & Chapin, F.S. (1987) Interactions among Processes Controlling Successional Change. *Oikos*, **50**, 131–135.

Wallander, H., Mahmood, S., Hagerberg, D., Johannesson, H. & Pallon, J. (2003) Elemental composition of ectomycorrhizal mycelia identified by PCR-RFLP analysis and grown in contact with apatite or wood ash in forest soil. *FEMS Microbiology Ecology*, **44**, 57–65.

Wardle, D.A., Zackrisson, O. & Nilsson, M.-C. (1998) The charcoal effect in Boreal forests: Mechanisms and ecological consequences. *Oecologia*, **115**, 419–426.

Weber, A., Karst, J., Gilbert, B. & Kimmins, J.P. (2005) *Thuja plicata* exclusion in ectomycorrhiza-dominated forests: testing the role of inoculum potential of arbuscular mycorrhizal fungi. *Oecologia*, **143**, 148–156.

White, T.J., Bruns, T.D., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A Guide to Method and Applications* (eds M. Innis, D.H. Gefland, J.J. Sninsky & T.J. White), pp. 315–322. Academic Press, San Diego.

Wiens, J.A. (1976) Population responses to patchy environments. *Annual Review of Ecology and Systematics*, **7**, 81–120.

Wu, B., Nara, K. & Hogetsu, T. (1999) Competition between ectomycorrhizal fungi colonizing *Pinus densiflora*. *Mycorrhiza*, **9**, 151–159.

Zackrissson, O., Nilsson, M.-C., Wardle, D. (1996) Key ecological function of charcoal from wildfire in the Boreal forest. *Oikos*, **77**, 10–19.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Effects of heat exposure and species identity on spore viability of four ectomycorrhizal fungi.

Fig. S1. Fluorescent microscopy pictures taken of spores of (a) *Laccaria proxima*, and (b) *Rhizopogon olivaceotinctus* after a 5 min immersion in a 65 C water bath.

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