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The effects of heat treatments on ectomycorrhizal resistant propagules and their ability to colonize bioassay seedlings

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ABSTRACT

The effect of disturbance on the resistant propagule community (RPC) of ectomycorrhizal fungi has been given relatively little attention. In this study we investigate the effects of heat, one important factor of fire disturbances, on the ability of ectomycorrhizal RPC fungi to colonize *Pinus jeffreyi* seedlings in greenhouse bioassays. Prior to planting the seed, soils were collected from an old growth mixed-conifer forest in the southern Sierra Nevada, California, USA and then subjected to four heat treatments of none, 45 °C, 60 °C, and 75 °C. After eight months, seedlings were harvested and the ectomycorrhizal fungi colonizing the roots were characterized by molecular methods (PCR-RFLP and DNA sequencing). *Rhizopogon* species increased in dominance on seedlings grown in soils receiving the 75 °C heat treatment. One species significantly increased in frequency, *Rhizopogon olivaceotinctus*, and two species (*Cenococcum geophilum* and *Wilcoxina* sp.) significantly decreased in frequency in the 75 °C treatment. The increase of *R. olivaceotinctus*, coupled with other features of its behavior, suggests that substantial heat disturbances may benefit this species in competing for roots.

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Introduction

As sessile organisms, plants and fungi face similar problems in establishing and competing in biologically complex environments. For this reason fungi might be expected to have developed strategies parallel to those employed by plants. One situation where this may be clear is post-fire recolonization, because fire is a common force in many communities that causes predictable environmental changes, and typically creates a short period where the competitive environment is altered.

Both plants and fungi produce propagule banks in the soil that can respond after disturbance. Seed banks of plants are

widespread and are important in post-disturbance recolonization (Grime 1977). Their ability to germinate and occupy a site is generally contingent upon a disturbance sufficient enough to disrupt the competitively dominant species. Certain ectomycorrhizal fungi, such as *Rhizopogon*, *Tuber*, *Cenococcum*, and *Wilcoxina*, show similar patterns, in that they stockpile an abundance of spores or sclerotia in the soil that are important sources of inoculum in post-disturbance settings (Horton et al. 1998; Baar et al. 1999; Taylor & Bruns 1999). Taylor & Bruns (1999) referred to such species as the resistant propagule community (RPC).

Fire is one important form of disturbance that drives biotic and abiotic patterns of many ecosystems (Barnes et al. 1998)

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and can be important to maintain some seed bank species. Seeds of some plants germinate in response to physical or chemical cues of a fire, such as heat or smoke (Barbour *et al.* 1987; Brown & van Staden 1997) allowing them to take advantage of temporary reductions in interspecific competition to grow to reproduce, and replenish the seed bank. Similarly, ascospores of fungi such as *Neurospora* are also known to germinate in response to heat or fire (e.g. Emerson 1948; Jesenska *et al.* 1993; Pandit & Maheshwari 1996). Some fungi fruit in nature immediately following a fire (Petersen 1970; Carpenter & Trappe 1985; Jacobson *et al.* 2004). *Geopyxis carbonaria* is a good example of an ectomycorrhizal fungus with such a fruiting response. This species fruits in post-fire environments following the death of its host, but is present on roots in the undisturbed forest (Vralstad *et al.* 1998). However, it is not clear if the RPC species are responding to specific cues or simply taking advantage of the new competitive space.

Based on the importance of fire in many ectomycorrhizal forest types, it seems likely that there are spores or propagules of ectomycorrhizal fungi that respond specifically to some aspect of a fire disturbance. There are many soil properties (e.g. nutrient availability, pH, and hydrophobicity) that are altered as a result of the heat and drying stress of fire (Agee 1993). Additionally, these effects vary across space and soil depth. To partition the effects of these different factors, Grogan *et al.* (2000) examined the effect of removing post-fire ash, an important nutrient source, on ECM community composition on field seedlings, but due to high species richness and spatial variability they found no clear effects. Baar *et al.* (1999) found that propagules of some ECM species responded positively to soil drying in greenhouse experiments, and that these were the same species that colonized seedlings in nature following fire. While studies such as these have examined some variables of fire, no study has specifically looked at the possible role that the heat of a fire may play in post-fire colonization of roots by ECM fungi.

The goal of this study was to analyze the effect of heat on the ability of the RPC to colonize seedlings. Based on plant ecology examples, we expected heat to have differential effects on the species within the RPC and to reduce species richness. Differential effects could occur if propagules of some species are better at tolerating heat, are more abundant and therefore less likely to be eliminated, or are stimulated to germinate by heat. While the RPC does not appear to be affected by sustained temperatures of 37 °C (Parke *et al.* 1983), surface soil temperatures during a fire range considerably higher (e.g. Haase & Sackett 1998). Very little is known about the differential effect of disturbance types on different RPC members, and therefore this study should provide new views into ECM autecology of common RPC species.

We chose to study the RPC in an old-growth forest in the Sierra Nevada (California, USA) in which we already had studied several components of the ectomycorrhizal community including: (1) the fungi present on the roots of mature trees (Izzo *et al.* 2005a); (2) the fungi present in the RPC (Izzo *et al.*, unpubl.); and (3) the fungi actively vectored in small mammal scat (Izzo *et al.* 2005b). Fire has historically been a recurring disturbance across these mountains (McKelvey *et al.* 1996) however, due to active fire suppression, a large-scale fire disturbance has not occurred in this forest in over 90 y (North

et al. 2002). Fungi that would benefit from aspects of a fire such as soil heating therefore should have a very limited presence on roots or in the fruiting record making it easier to identify them as being true ruderal species.

Methods

Site description

This study was conducted at the Teakettle Experimental Forest, in the Sierra National Forest (SNF), CA (36°58'N; 119°2'W) on the southwestern slope of the Sierra Nevada. This forest has been described in greater detail by North *et al.* (2002). The elevation at this site is approx. 2100 m. *Abies concolor* (white fir) and *Abies magnifica* (red fir) are the dominant ectomycorrhizal conifers throughout the study range. *Pinus jeffreyi* (Jeffrey pine), and *Pinus lambertiana* (sugar pine) are the other prominent ectomycorrhizal conifers that occur in the overstory. The historical fire regime included recurrent low-intensity surface fires at 15-20 y intervals. Due to active suppression, however, over 90 y have passed since the last large-scale fire (North *et al.* 2002).

Preparation of bioassays

In September 2001, 2 l of mineral soil was collected from eight mature fir stands arbitrarily chosen across approximately a half kilometer. All soil was pooled, sifted through a 2 mm sieve, mixed, and stored at 4 °C for 3 m. The heat treatments of the soil were performed a few days before the seed was planted. We attempted to mimic temperature profiles (Fig 1) reported under *Pinus lambertiana* (Sugar pine) and *Sequoiadendron giganteum* (Giant sequoia) in a Sierra Nevada burn (Haase & Sackett 1998) by creating conditions in which the temperatures rose quickly but cooled slowly. While soil temperature profiles in the Teakettle Experimental Forest could possibly be quite different, we felt that these sorts of heating and cooling rates would better reflect the stress that the RPC undergoes in a fire. To ensure even heating, soil was added to aluminum pans no deeper than 1 cm. A thermocouple was positioned in the middle of the soil and then the pan was covered with aluminum foil. Soils were brought up to final temperatures of 25 °C (no heat treatment control), 45 °C, 60 °C, and 75 °C by placing the pan in a 150 °C drying oven until reaching the target temperature. To lower the soil cooling rate, the pans were placed in successively cooler drying ovens (50 °C and 37 °C). When the soil reached 40 °C the pans were allowed to cool at room temperature. The final target temperatures of the treatments were chosen to span across 65-67 °C. This temperature range is known to germinate *Neurospora* ascospores (e.g. Jacobson *et al.* 2004) and generally kill plant tissue (Agee 1993).

The final temperature profile of the soil resembled that of the prescribed burn of Haase & Sackett (1998) with its sharp rise and slow decline, although our treatment temperatures did not reach nearly as high nor last as long. With each successively hotter temperature treatment the soils remained at hotter temperatures longer than the previous treatment and therefore the intensity of each successive treatment was not linear. For example, the soil in the 75 °C treatment stayed above 60 °C for over 20 min. The increasing heat had

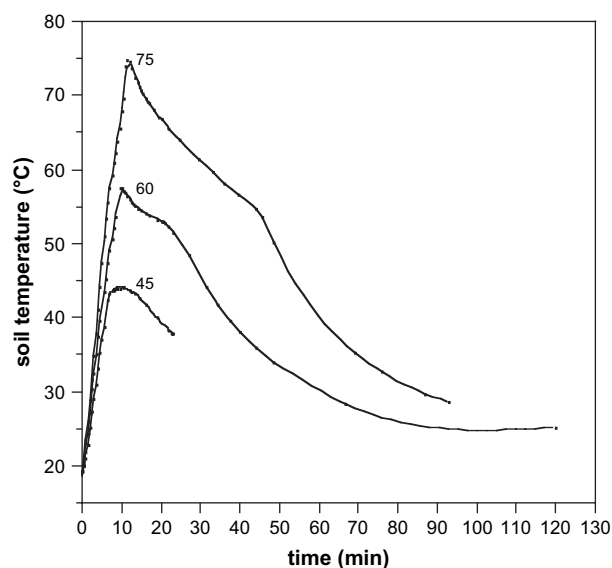


Fig 1 – Temperature profiles of soils during the heat treatments. Numbers on the chart indicate the approximate peak temperature reached in each treatment.

a noticeable effect of increasing hydrophobicity of the soil, which is a common effect of fire in nature (Agee 1993).

Following the heat treatments soil was mixed at roughly 50:50 (mass) with sterilized coarse sand. The coarse sand was added to help drainage and had been autoclaved with a 250 °C wet cycle for 30 min followed by a 250 °C dry cycle for 30 min. To assay for airborne contamination, we set up negative controls using plot soil that was autoclaved in the same manner as the sand. Polyester fiber fill (JoAnn Fabrics, Emeryville, CA, USA) was added to the bottom of the cones to help contain the soil. To avoid contamination, all work with the soils was performed in an airflow hood that was scrubbed with soapy water before working with a new soil.

Bioassays of the soils were performed with Jeffrey pine (*Pinus jeffreyi*) seedlings. This species was used rather than *Abies* species because it is known to be an early colonizer following fire in this forest type (Mooney & Conrad 1977) and therefore should be the most relevant host to any fire-requiring fungi. Additionally, in bioassays of soils from this forest using both *P. jeffreyi* and *Abies concolor*, *P. jeffreyi* seedlings retrieved a greater diversity of fungal species (Izzo et al. unpubl.). Seeds were surface-sterilized in hydrogen peroxide for 10 min, soaked in running water for 48 h, and then stratified at 4 °C for 2 m. Pots (RLC-4 Super ‘Stubby’ Cell Cone-tainers, Steuwe & Sons, Corvallis, OR) were sterilized and prepared by soaking them in 10 % bleach for 30 min then in distilled water for 30 min. Following the stratification period, one seed was placed on the surface of each of ten bioassay tubes per treatment and control for a total of 50 seedlings. Each cone was covered with its own saran wrap while germinating to maintain humidity.

Within two weeks, all the seed had germinated and seedlings had fully emerged. At this point, the top of the soil was covered with a layer of sterile coarse sand. Seedlings within a treatment were grouped together and had at least 5 cm of

space from other treatments to further reduce concerns about soil splashing between treatments. To avoid edge effects of light that might result from this, the entire rack was rotated and shifted randomly every two weeks. Additionally, the collecting trays that were underneath the seedlings were washed after every watering to remove any soil or propagules that may have washed through. Seedlings were watered twice a week and were grown for 14 h of light and 10 h of dark under a laboratory bench fitted with fluorescent lighting ($75 \mu\text{mol m}^{-2} \text{s}^{-1}$). Greenhouse conditions were avoided to lessen the chance of *Thelephora* contamination.

Characterization of the seedling ectomycorrhizal community

Seedlings were harvested after 8 months. Each seedling was removed from its pot and rinsed under tap water to remove soil. Roots were considered to be ectomycorrhizal if: (1) they had no root hairs showed swelling, and visible hyphae; or (2) a hyphal sheath was present. Roots of each seedling were analyzed under a dissecting microscope for 15 min to look for different morphotypes. Two representatives of each morphotype from each seedling were placed into a tube together for molecular analysis. Freeze-dried tips were crushed with a 3.5 mm glass bead in a bead-beater for 25 s and suspended in 1000 μl CTAB/PVPP buffer (2 % CTAB, 1 % PVPP, 0.1 M Tris pH 8.0, 1.4 M NaCl, 0.02 M EDTA). Following a 60 min incubation at 65 °C, samples were vortexed with 600 μl chloroform:isoamyl alcohol (24:1) and centrifuged (13000 g) for five minutes. Samples were further cleaned with Qiagen DNeasy genomic isolation kits (Qiagen, Valencia, CA) and resuspended in 50 μl AE buffer (Qiagen). PCR was performed in a PTC-100 thermal cycler (MJ Research, Inc., Waltham, MA, USA) in conditions previously described, (Gardes & Bruns 1993) with 5 μl of the isolated nucleic acids in a 50 μl total volume for the reaction. The primer ITS region was amplified with the fungal-specific ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) primer pair that targets both ascomycetes and basidiomycetes. PCR product was digested enzymatically with *Hinf*I and *Alu*I (New England Biolabs, Ipswich, MA, USA), analyzed by agarose gel electrophoresis and the resulting fragments sized using the program GelReader (NCBI). We sequenced the ITS1/5.8S/ITS2 region to analyze for identification of the fungi. Multiple representatives of each unique RFLP type were sequenced to confirm the validity of the RFLP type groupings. Each sequence was compared to both GenBank and our own internal database of local accessions using BLAST searches (Altschul et al. 1997). RFLP taxa were named based on the closest taxonomic level that we could identify them based on the GenBank database and in accordance with the sequence types named in other studies in this and nearby forests (Izzo et al. 2005a, 2005b). A more detailed analysis was carried out in PAUP (Swofford 2002) on subsets of taxa identified as closely related. RFLP taxa that were less than 2 % different were combined into a single ITS sequence group. In the genus *Rhizopogon*, where the databases are more complete, sequence groups were identified based on the nomenclature of Grubisha et al. (2002) and on a previously published alignment of spore bank *Rhizopogon* species (Kjøller & Bruns 2003).

Measuring heat effects

To test the effects of heat on ECM colonization and species richness, we used one-way analyses of variance (ANOVA). Prior to each ANOVA, the variances were determined to be homogeneous (Bartlett's test, $P > 0.05$) and the data were normally distributed (D'Agostino-Pearson K^2 test, $P > 0.05$). Tukey HSD tests were used for *a posteriori* comparisons of means. Calculations were performed in JMP v3.2.6 (SAS Institute, Cary, NC). Fisher exact tests (computed online at <http://faculty.vassar.edu/lowry/tab2x2.html>, Zar 1996) were used to test for corresponding increases or decreases in the frequency of each species in response to heat. For each of these tests we used pairwise comparisons of each of the heat treatments to the original control because it was not clear at what temperature an effect would take place and there was no reason to assume the response would be linear nor the same with all fungi.

Results

Of the 50 seedlings, two died leaving only nine seedlings in both the 45 °C and the 60 °C treatments. Overall root colonization was 57 ± 3 % (mean \pm s.e.) across all treatments with a significantly higher percentage on seedlings in all of the heat-treated soils (Fig 2). Low levels of contamination (two seedlings with a theleporoid contaminant) were detected in the autoclaved controls; however, this taxon was not detected in any of the untreated or treated soil bioassays, and therefore did not affect our results.

In all, 253 root tip samples were analyzed by molecular means. Of these, 66 % (191) were successfully named as a sequence type, 9 % (23) had mixed patterns with no clear total band matches, and 25 % (62) did not amplify. Of the samples that appeared mixed, roughly two-thirds had morphotypes that fit *Rhizopogon* species, many of which were noted to be of questionable health and therefore it was assumed that mixes of endophytic or saprophytic fungi were primarily being detected. Twenty-three RFLP types were initially identified and sequenced. To minimize the likelihood of

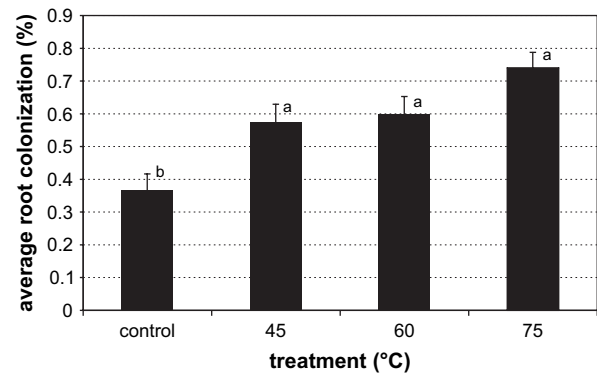


Fig 2 – Effect of the heat treatment on the relative portion of roots on a seedling that were colonized by ectomycorrhizal fungi. Error bars indicate standard error calculated on pooled variance (ANOVA). Letters above the bars indicate averages different at $p < 0.05$ (Tukey HSD).

incorrect grouping due to similar RFLP patterns, more representatives were sequenced from the more common RFLP types. Analysis of DNA sequence data and discarding types resulting from multiple fungi resulted in seven sequence types (Table 1), most of which had been detected in a larger scale study on the RPC in this forest (Izzo et al. unpublished). On average, individual seedlings were colonized by 2.8 ± 0.2 species. The heat treatments did not affect species richness on seedlings relative to the non-treated soils (Fig 3). Three species showed significant responses to the 75 °C treatment. *Cenococcum geophilum* (Fisher exact 2-tailed $p = 0.032$) and *Wilcoxina1* ($p = 0.001$) decreased in frequency, while *Rhizopogon olivaceotinctus* ($p = 0.005$) increased (Table 2). No significant species responses were seen in the 45 °C or 60 °C treatments. *Rhizopogon* species collectively dominated the total occurrences on seedlings at the 75 °C treatment (Fig 4). One fungus that potentially has affinities with the pezizalean lineages was seen only on one seedling in the 75 °C treatment.

Table 1 – Identification of species-groups by BLAST analysis. Study names are based upon general taxonomic affinities of GenBank sequences showing the best BLAST matches. Results of BLAST analysis of the ITS1 region are shown along with the length of the sequence (basepairs) that was used for the search. For simplicity, only the top match is shown. Theleporoid sp. 6 was a contaminant that only appeared in the negative controls. The spacer regions for Ascomycota sp. 12 did not give strong matches to GenBank sequences and therefore the result based on the entire sequence including the 5.8S region is reported

Study name	bp	Best match	% similarity/bp	GenBank no. accession
Ascomycota sp.12 ^a	552	many weak matches	95 %/196	AY587279
<i>Cenococcum geophilum</i>	141	<i>Cenococcum geophilum</i> (AY112935)	98 %/141	AY587280
Helotiales sp. 1	169	Ericoid mycorrhizal sp. (AF072296)	90 %/154	AY587281
<i>Rhizopogon roseolus</i> gr	223	<i>Rhizopogon rubescens</i> (AF158018)	100 %/223	AY587282
<i>Rhizopogon arctostaphyli</i> gr	216	<i>Rhizopogon arctostaphyli</i> (AF377167)	100 %/216	AY587283
<i>Rhizopogon olivaceotinctus</i> gr	273	<i>Rhizopogon olivaceotinctus</i> (AJ515509)	98 %/275	AY587284
Theleporoid sp. 6	198	<i>Thelephora terrestris</i> (AY230244)	98 %/198	AY587285
<i>Wilcoxina</i> sp. 1	189	<i>Wilcoxina rehmsii</i> (AF266708)	96 %/190	AY587286

a Entire ITS1/5.8S/ITS2 sequence used in BLAST analysis.

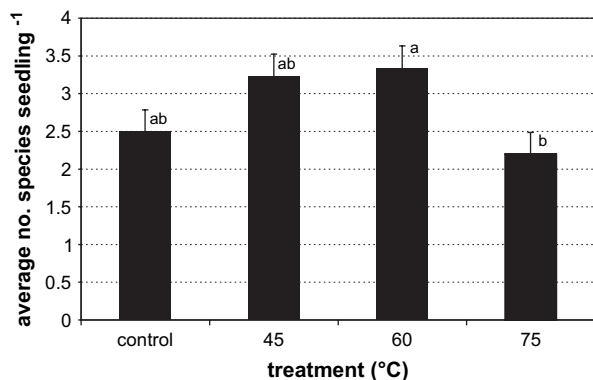


Fig 3 – Effect of treatment on fungal species richness on seedlings. Error bars indicate standard error calculated on pooled variance (ANOVA). Letters above the bars indicate averages different at $p < 0.05$ (Tukey HSD).

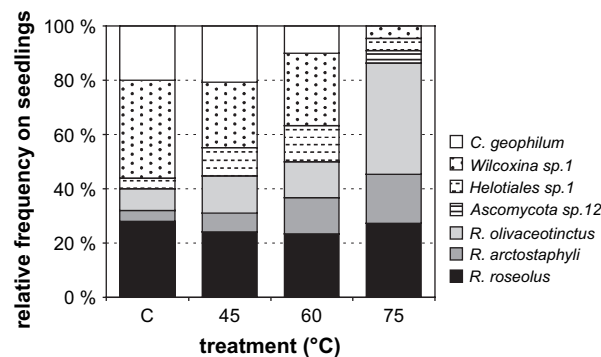


Fig 4 – Relative frequency of fungal types on seedlings grown in heat-treated soils. Numbers indicate the relative proportion of the species across all seedling occurrences within a treatment. *Rhizopogon* species are highlighted in darker shades.

Discussion

The heat treatments in our study altered the composition of the fungi that colonized the bioassay seedlings, and generally favored *Rhizopogon* species at the highest temperature (Fig 4). There are four possible non-exclusive explanations for the overall shift in community composition. First, the propagules may have differed in their ability to withstand the highest temperatures used in our study. Basidiospores of *Rhizopogon* in particular seem able to withstand considerable stress. They are known to maintain viability across multiple years (Miller et al. 1994), are viable after traveling through a mammal gut (Colgan & Claridge 2002), and were the most prominent at the hottest treatment in our study. Additionally, the stress of soil drying, which would be another effect of heat, has also been shown to increase the frequency of *R. olivaceotinctus* in another bioassay study (Baar et al. 1999). Second, the density of spores of *Rhizopogon* spp. appears to be much higher than other species (Baar et al. 1999; Taylor & Bruns 1999; Kjølner &

Bruns 2003), and given an equal effect of heat across species, this may simply allow *Rhizopogon* spp. a greater chance of spore survival. Third, the heat treatment may have changed the competitive environment. As the treatments became hotter, our soils became noticeably more hydrophobic, and the percentage of roots that were colonized increased which can be an indicator of altered nutrient status. Fourth, the heat treatments may have acted as a signal for spore germination. We know that even without heat *R. olivaceotinctus* spores readily colonize seedlings from soils (this study; Izzo et al. unpubl.; Kjølner & Bruns 2003). However, spores of *Neurospora* clearly exhibit heat activation and yet can also be stimulated to germinate in a variety of other conditions that involve carbohydrate sources (Emerson 1948; Pandit & Maheshwari 1996).

The increasing frequency of *R. olivaceotinctus* at the higher temperatures is especially interesting given what we know about this species. As opposed to many other RPC species that seem to maintain themselves in mature forests, *R. olivaceotinctus* is a good candidate to be a true disturbance-requiring fungus. *R. olivaceotinctus* fruit bodies are rare enough that the species has received conservation protection status in the Pacific Northwest (Castellano et al. 1999, 2003). The rarity of this species extends into California as well. It has not been collected in the Sierra National Forest, in spite of multiple years of collecting of hypogeous fungi, nor was it found in the rodent fecal pellet survey of Teakettle Forest (Izzo et al. 2005b). At the same time, based on its detection in bioassay studies its spores are plentiful and viable in Sierra Nevada soils (Izzo et al. unpubl.; Kjølner & Bruns 2003) and was one of the most prominent colonizers of field seedlings following a stand-replacing fire in coastal California (Baar et al. 1999). The viability of *R. olivaceotinctus* spores through our heat treatments supports the premise that they can withstand considerable stress, including heat levels that would only be attained through a fire disturbance. That *R. olivaceotinctus* frequency increased on seedlings in the highest temperature treatment, further suggests that specific levels or types of disturbance might aid it in the competition against other RPC species to colonize roots. Fire would be one type of

Table 2 – Frequency of species groups across treatments. Asterisks indicate changes significantly different from the treatment where soils were not heated

Species group	Sterile	No heat	45	60	75
Ascomycota sp. 12	0	0	0	0	1
Cenococcum geophilum	0	5	6	3	0*
Helotiales sp. 1	0	1	3	4	1
<i>Rhizopogon olivaceotinctus</i> group	0	2	4	4	9*
<i>R. roseolus</i> group	0	7	7	7	6
<i>R. arctostaphylli</i> group	0	1	2	4	4
Theleporoid sp. 6	2	0	0	0	0
Wilcoxina sp. 1	0	9	7	8	1**
Total seedlings in treatment	10	10	9	9	10

*P < 0.05
**P < 0.005.

disturbance that could mimic some of our treatment conditions and may explain why *R. olivaceotinctus* was frequent on post-fire field roots and more prominent in bioassays of post-fire soils as compared to pre-fire soils in a coastal California forest (Baar *et al.* 1999; Taylor & Bruns 1999), however other forms of disturbance such as long-term soil drying may be sufficient as well.

Two species, *C. geophilum* and *Wilcoxina* sp. 1, showed significant decreases in frequency in the highest heat treatment. Given that sclerotia formed by these genera are generally thought to be tolerant to stress or disturbance conditions and can persist through fire (Visser 1995; Torres & Honrubia 1997; Smith *et al.* 2005) this result may be more reflective of differences in how fast they can germinate and subsequently spread to colonize more roots relative to *Rhizopogon*. Constrained to the space within the pots used in our experiment, *Rhizopogon*'s ability to rapidly spread through the soil by mycelial growth may have given it a distinct advantage compared to *Cenococcum* and *Wilcoxina* species whose hyphal growth appears to be concentrated locally (Agerer 2001). While this did not affect *Cenococcum* and *Wilcoxina* from being easily detected and common in the non-treated soils, such an effect could have been more pronounced in the 75 °C treatment where new soil conditions (e.g. increased hydrophobicity, altered nutrient status, etc.) potentially altered the competitive environment for hyphal growth as has been seen in paired competitions of *Rhizopogon occidentalis* with *Tomentella sublilacina* (Lilleskov & Bruns 2003). At the same time, *Rhizopogon* species were detected 1–2 m earlier than *C. geophilum* on field seedlings following a stand-replacing fire (Horton *et al.* 1998), so these advantages may be comparable to what occurs in some field situations.

Bioassay studies present certain limitations that must be considered when interpreting the results. First, while our treatments have some obvious parallels to fire conditions, the effects of fire on soil are much more complex than those mimicked by our bioassay conditions. As one example, direct heating effects are likely to be localized and limited to the top few centimetres of soil, or to pockets of high fuel loads in most forest fires (Agee 1993). Therefore, the patchiness of fire effects coupled with the apparent widespread distribution of *C. geophilum* and *Wilcoxina* sp. 1 in this forest and others (Izzo *et al.* 2005a) may buffer these species from being negatively affected at the large scale. Similarly, any advantages that may be conferred to species such as *R. olivaceotinctus* may operate on much smaller scales. Second, our light intensities of 75 $\mu\text{m m}^{-2} \text{s}^{-1}$ were considerably low compared to 1600 $\mu\text{m m}^{-2} \text{s}^{-1}$ measured in the understory in this forest (North *et al.* 2004). Bioassays run in similar conditions have produced reasonable facsimiles of post-disturbance field root communities (Baar *et al.* 1999) however a reduction in carbon availability can affect root community composition in defoliation studies (Saikkonen *et al.* 1999; Cullings *et al.* 2001) and may be similarly biasing our results towards RPC species that present less demand for carbon.

Despite the limitations that bioassay studies present, they remain one of our most useful tools for studying ectomycorrhizal spore ecology. From our data we are not able to isolate the specific cause(s) of species differences in colonization from the RPC, that could include spore germination response,

spore resistance to stress, or changes in mycelial colonization capabilities. However, the potential for disturbances to have differential effects on RPC species adds one more dimension to understanding ectomycorrhizal community dynamics.

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