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Rethinking ectomycorrhizal succession: are root density and hyphal exploration types drivers of spatial and temporal zonation?

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ABSTRACT

Ectomycorrhizal exploration types have become an increasingly popular functional explanation for observed patterns of fungal community structure. In this study, we examined the relationship between exploration types of ectomycorrhizal fungi and root density. We did so by sampling across a root density gradient formed by the edge-interior transition on 'tree islands', patches of ectomycorrhizal forest in a non-ectomycorrhizal vegetation matrix. We found evidence that long-distance exploration types were more prevalent in areas of low root density while short-distance exploration types were more common in areas of high root density. Gradients in root density are common in ectomycorrhizal forests and change predictably at forest edges, within a soil profile, or over early succession. Based on these results, we propose a general model using the concept of exploration types that could explain some of the spatial or temporal patterns commonly observed in ectomycorrhizal assemblages.

Introduction

Ecological communities are composed of species assemblages that are variable in both space and time (Begon *et al.* 2006). Temporal change in species composition, known as succession, was among the earliest patterns documented by ecologists (Cowles 1899; Gleason 1926; Clements 1936). For ectomycorrhizal (EM) fungi, patterns of succession have been documented in both sporocarp surveys (Mason *et al.* 1983; Last *et al.* 1984; Last *et al.* 1987; Nara *et al.* 2003a; Ashkannejhad & Horton 2006) and root tip analyses (Nara *et al.* 2003b; Ashkannejhad & Horton 2006). Two particular aspects of EM successional patterns have been reproduced in multiple systems. The first is that in primary successional settings (i.e.

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settings not previously colonized by EM fungi), EM assemblages are simple (Nara *et al.* 2003a) and predictably dominated by a set of species that colonize seedlings well by spore (Nara 2008). These fungi have been termed "early-stage", ruderal or pioneer species (Deacon & Fleming 1992). They are joined, but not necessarily replaced, by a much larger set of species whose spores cannot be used to experimentally inoculate seedlings, termed "late-stage" or c- and s-selected species. The second pattern of EM succession is that spatial zonation is often observed, where early-stage fungi occupy the periphery of the root system, and late-stage fungi occupy the area of the roots nearest to the trunk as the host ages (Nara *et al.* 2003a). Significantly, however, seedlings establishing near the trunk in undisturbed soil are colonized by late-stage fungi, showing that tree age alone is not the primary determinant of EM successional patterns (Fleming 1983, 1984).

Many researchers have used "space-for-time" substitutions (Fukami & Wardle 2005) to study how EM assemblages change over time (Visser 1995; Lilleskov *et al.* 2002; Nara *et al.* 2003a; Nara *et al.* 2003b) because documenting succession in one location can take long periods of time. In a previous study (Peay *et al.* 2007), we observed a spatial assemblage patterning similar to successional changes in EM fungi (Gardes & Bruns 1996; Horton *et al.* 1998). Specifically, we found that EM assemblage composition was closely related to the size of the patch (i.e., number of trees) of EM vegetation surveyed, even though tree age remained constant across host patches. Single trees and small tree clusters contained EM species typically thought of as "early-stage" fungi, while larger clumps contained these species plus many additional species, including typical "late-stage" genera such as *Amanita*, *Cortinarius*, *Russula* and others.

We hypothesized that the pattern on these patches, which we term 'tree islands', was driven by tradeoffs in the dispersal and competitive abilities among fungi (Peay *et al.* 2007). Although the primary dispersal mechanism of EM fungi is likely spores, mycelial spread among host roots can be thought of as a more local form of dispersal. Along with differences in patch size, average root density also changed from small to large host patches due to decreasing root density at forest edges and the decreasing edge-interior ratios. Thus, the shifts in EM assemblage composition we observed may have been caused in part by differences in mycelium-based dispersal among roots. The significance of mycelial dispersal and root density in EM succession was first proposed by Newton (1992) who stated, "the phenomenon of [EM] succession can be attributed solely to the relative ability to colonize and spread from different sources of inoculum; no other mechanisms need be invoked" and "… a higher rooting density provides a higher number of sites available for colonization and must favor those fungi that spread by mycelium".

While there are few morphological traits that are easily measurable for EM fungi, the concept of hyphal exploration type has become increasingly popular for EM fungal ecologists. Agerer (2001) proposed that EM fungi can be classified into exploration types based on patterns of hyphal development and rhizomorph structure. Specifically, he proposed that such morphological features are closely related to their ability to explore different distances from the colonized root tip. Since its publication, that paper has been widely cited as an anecdotal functional explanation for observed patterns in community structure, but EM exploration type research to date has focused on different modes of mineral nutrient acquisition. For example, Hobbie & Agerer (2010) recently demonstrated a consistent correlation between EM exploration type and $\delta^{15}N$ sporocarp values and suggested differences among exploration types were related to utilization of labile (shorter distance types) *versus* non-labile (longer distance types) nitrogen sources.

It is also likely that there is a second form of resource exploration by EM fungi related to exploration type: to find uncolonized host roots. Given that host roots are the exclusive carbon source for EM fungi, and that a single colonized root is a temporary structure that is likely to lose functionality within weeks or months (Tierney & Fahey 2001; Hogberg *et al.* 2002; Smith & Read 2008), exploration for new roots is likely to be a crucial function of the mycelium. Therefore, exploration type will likely correlate with different strategies for mycelial dispersal and root colonization. Specifically, we hypothesize that investment in long-distance exploration will be best suited to settings with low root density, and conversely shorter-range exploration will be best suited to settings with high root density.

In this study, we tested this hypothesis by sampling EM roots across a root density gradient represented by the edge and interior of EM tree islands. We predicted that: (a) root density would decrease at the forest edge; (b) assemblage composition would differ between forest edge and interior; and (c) long-distance exploration types would be favored in zones of low root density and short-distance exploration types in zones of high root density.

Methods

Site description

The study was conducted at Point Reyes National Seashore (PRNS), located in West Marin County, California (38°04′ N by 122°50′ W). The area has a Mediterranean climate with cold, wet winters and hot, dry summers. Mean annual temperature is ~11 °C and mean annual precipitation is ~430 mm. The precipitation falls almost exclusively in the winter months, although there can be substantial inputs from coastal fog during the summer. *Pinus muricata* (Bishop Pine), an EM host plant, is a closed cone pine that normally requires high intensity fires for seed release. As a result of this autecology, it tends to form even-aged, monodominant stands where it occurs at PRNS. Near the coast, stands of *P. muricata* intergrade with grasslands and scrub characterized by *Baccharis pilularis*, *Toxicodendron diversiloba* and *Rubus ursinus*, all of which are non-EM host plants. In 1995, a large fire burned significant areas of PRNS and caused significant regeneration of monodominant *P. muricata* in the coastal areas, and facilitated widespread invasion of *P. muricata* into previously non-ectomycorrhizal scrub and grassland communities.

Sampling methodology

In Jan. 2009, we selected three tree islands (Island 1—38° 5.33′ N, 122° 52.55′ W; Island 2—38° 3.56′ N, 122° 52.48′ W; Island

3–38° 5.30′ N, 122° 52.98′ W) of regenerating *P. muricata* forest where we could delineate a sharp boundary between pine forest and non-EM coastal scrub (Fig S1). Along 100 m of edge of each *P. muricata* island, we established two 30 m transects for EM root tip sampling. We sampled two 664 cm^3 soil cores at three randomly selected locations along each transect. At each location, one core was taken 10 m under the forest canopy perpendicular to the forest boundary (interior) while the second was taken at the drip-line (edge). These distances were chosen based on previous work in this system showing that community spatial autocorrelation is absent for samples taken >3 m apart (Lilleskov *et al.* 2004). After removal, soil cores were stored in plastic bags and transported back to the field station where they were processed. In total, we sampled 36 soil cores (3 islands × 2 locations × 6 replicates).

Cores were washed gently over a 2 mm soil sieve to remove soil and the remaining root pool was examined under a stereomicroscope for the presence of EM roots. From each core, we removed the first eight colonized EM roots encountered while searching under the microscope through the root pool for DNA extraction and molecular analysis. To determine root density the remaining roots were placed in a coin envelope, dried at 65 °C for 48 hr and weighed on a microbalance.

Molecular identification

EM roots were stored in 1 ml of 2× cetyltrimethylammonium bromide (CTAB) at 4 °C until DNA extraction could be performed. DNA was extracted using a modified protocol from the REDExtract-N-Amp Tissue PCR Kit (Sigma–Aldrich, Saint Louis, MO USA). For this protocol, each root was removed from CTAB, blotted dry with a Kimwipe, placed into 10 μl of extraction buffer and incubated in a thermocycler set for 65 °C for 10 min and 95 °C for 10 min. Finally, 30 μl of neutralization solution B was added and the extracts stored at 4 °C.

PCR cycling was done under standard conditions with the fungal specific primer pair ITS1f and ITS4 (White *et al.* 1990; Gardes & Bruns 1993). PCR products were visualized with gel electrophoresis and 7.5 μl of successful amplifications were cleaned by adding 1.5 μl of ExoSAP IT (USB Corp, Cleveland, OH USA) and incubating at 37 °C for 15 min followed by 80 °C for 15 min. All samples were sequenced in one direction using an ABI3170 Genetic Analyzer (Applied Biosystems, Foster City, CA USA). 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 basidiomycete specific reverse primer ITS4b (Gardes & Bruns 1993) to eliminate root inhabiting endophytes (EM ascomycetes are relatively rare in this system) (Gardes & Bruns 1996; Peay *et al.* 2007). Species were defined using a 97 % sequence similarity cutoff and named according to the nearest BLAST match.

Determination of EM exploration types

Exploration types were determined for each species using the online database Determination of Ectomycorrhizae (DEEMY; www.deemy.de), a review of the literature (e.g. Agerer 2001, 2006) and our field and laboratory experience working with particular species. If exploration type could not be determined at the species level, we based our determination on the most closely related species for which this information was available. This is likely a reasonable assumption given that exploration type appears to be fairly well conserved for most genera. For some species there was insufficient information or taxonomic resolution to assign exploration type and these were left out of subsequent analyses. We then ranked species in terms of exploration distance (Table 1) based on the morphological classification scheme of Agerer (2001).

Soil Sampling

To characterize the soil abiotic environment we took additional samples using the same soil corer from each patch. Samples were spaced 10 m apart at three locations at one of the transects on each island. At each location, we sampled both edge and interior sites as described above. The soils were sent to Western Agricultural Laboratories (Modesto, CA) for measurement of pH, P (weak Bray), NO_3, K, Ca, Mg, cation exchange capacity (CEC) and % organic matter (OM).

Statistical analyses

We used analysis of variance (ANOVA) to determine significant differences in root density and soil chemistry between

Table 1 – Exploration type and abundance of ectomycorrhizal fungi observed in this study. Classifications of exploration type are based on Agerer (2001) and the online "Determination of Ectomycorrhizae" database. Exploration distances are ranked as Short < Medium-smooth < Medium-fringe < Long

Taxa	No. root tips	Exploration type	Accession no.
Amanita muscaria	2	Medium-smooth	HM021158
Atheliaceae sp. MF1	11	Unknown	HM021159
Cantharellaceae sp. RT541	7	Unknown	HM021160
Cantharellaceae sp. RD4	2	Unknown	HM021161
Cortinarius sp. KGP42	2	Medium-fringe	HM021162
Hebeloma sp. KGP23	3	Short	HM021163
Helvella sp. RD7	27	Short	HM021164
Inocybe sp. KGP109	3	Short	HM021165
Inocybe sp. TAM205	11	Short	HM021166
Inocybe sp. TAM354	6	Short	HM021167
Lactarius luculentus	5	Medium-smooth	HM021168
Rhizopogon occidentalis	7	Long	HM021169
Rhizopogon salebrosus	16	Long	HM021170
Russula amoenolens	3	Short	HM021171
Gymnomyces fallax	2	Unknown	HM021172
Suillus pungens	27	Long	HM021173
Tomentella sp. RD18	1	Medium-smooth	HM021175
Tomentella sp. RD19	6	Medium-smooth	HM021176
Tomentella sp. RD20	9	Medium-smooth	HM021177
Tomentella sp. RD21	3	Medium-smooth	HM021178
Tomentella sp. RD22	2	Medium-smooth	HM021179
Tomentella sublilacina	12	Medium-smooth	HM021180
Tomentellopsis sp. RD27	1	Medium-smooth	HM031461
Tricholoma flavovirens	1	Medium-fringe	HM021181
Tricholoma imbricatum	4	Medium-fringe	HM021182
Tuber sp. RT420	16	Short	HM021183

samples taken at the edge or interior of *P. muricata* stands. Our ANOVA models included sample location (edge *versus* interior) and island identity as fixed factors in a two-way ANOVA. Where there was a significant interaction between sample location and island identity, we tested for differences among groups using Tukey's Honestly Significant Difference test.

Species composition across samples was visualized using non-metric multidimensional scaling (NMDS) based on the Bray–Curtis index of community dissimilarity (Oksanen *et al.* 2008). We tested for significant differences in species composition between islands and sampling locations (edge *versus* interior) with an ANOVA using distance matrices (ADONIS; Anderson 2001; Oksanen *et al.* 2008) on the Bray–Curtis dissimilarity matrix. Because of significant differences among islands (see below) the effects of location were nested within island.

We also looked for differences between edge and interior samples based on hyphal exploration type. We did this by tallying the number of root tips that could be assigned to each exploration type and using a χ^2 test to see if the distribution of exploration types was random with respect to sampling location. To avoid violating assumptions of the χ^2 test, medium-smooth and medium-fringe exploration types were combined for this analysis due to low frequencies of the medium-fringe type. We also used the same NMDS visualization and ADONIS approach to examine functional composition of ECM communities using exploration types rather than species identity, and looked for correlations between the functional NMDS axis scores and root density. Finally, we used logistic and Poisson regression to examine the quantitative relationship between exploration type and root density. All statistics were calculated using the program R v. 2.10 (R Development Core Team 2009) and considered significant at $P \leq 0.05$.

Results

Mean root density was significantly higher for cores sampled on the interior *versus* edge locations of the tree islands (Location $F_{1,29} = 10.6$, $P = 0.003$). However, there was a significant location by island interaction, driven by the low interior root density on Island 3 (Fig 1; Interaction $F_{1,29} = 4.5$, $P = 0.02$). There were no significant differences ($P > 0.05$) in edge *versus* interior samples for any of the soil variables measured (Table S1).

In total, we identified 27 species of EM fungi from 191 (66 %) of 288 root tips (Table 1). NMDS ordination (Stress = 15.2, Fig S2) and ADONIS ($r^2 = 0.08$, $P = 0.001$) showed weak clustering of samples by island, but no effect of edge or interior sample location ($r^2 = 0.02$, $P = 0.43$) on species composition.

We were able to classify exploration types for 24 species, which accounted for 89 % of the identified root tips. The distribution of exploration types was not equal between edge and interior habitats, ($\chi^2_3 = 23.6$, $P \leq 0.001$; Table 2), with more root tips belonging to long-distance exploration types present in edge cores and more root tips belonging to short-distance exploration types present in interior cores. The functional ordination (Stress = 13.3, Fig 2) showed the opposite pattern as the species based ordination, with a significant effect of sample location (ADONIS $r^2 = 0.10$, $P = 0.018$) but no effect of island (ADONIS $r^2 = 0.037$, $P = 0.226$). There was not a significant ($P > 0.05$) correlation between functional NMDS axis scores and root density. Generalized linear models of exploration type and root density based on logistic or Poisson regression showed the same patterns of increasing abundance of long-distance exploration types with decreased root density. However, they were highly overdispersed (data not shown), indicating a failure of the data to fit model assumptions.

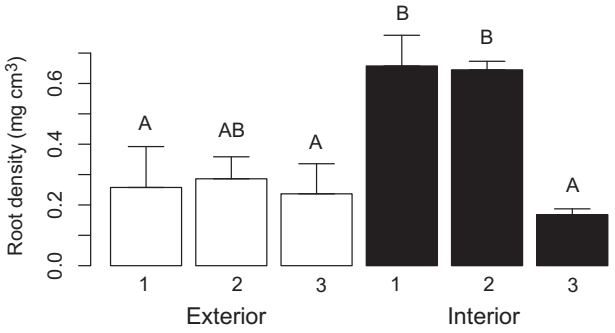

Fig 1 – Root density for samples taken at the edge and interior locations of three *Pinus muricata* tree islands. Root density was generally higher for samples taken at interior locations, with the exception of Island 3. Different letters indicate significant differences ($P \leq 0.05$) based on Tukey's HSD test.

Discussion

Our results show that there were significant differences in the occurrence of EM exploration types between the interior and edge of the tree islands, which we propose are related to the observed lower root density at island edges. These effects were present despite the fact that changes in community structure at the species level were not detectable across the same edge-interior gradient of root density. A lack of any significant differences in the soil variables with sampling location also indicated that the observed differences in EM

Table 2 – Distribution of ectomycorrhizal exploration types by sampling location. Cores were sampled either under closed canopy forest (Interior) or at the forest edge beneath the canopy drip-line (Exterior) on three *Pinus muricata* tree islands. Values indicate the number of root tips belonging to each exploration type. χ^2 test showed that exploration types were not distributed randomly with respect to sampling location ($\chi^2_2 = 19.6$, $P < 0.001$)

Location	Exploration type		
	Short	Medium	Long
Exterior	28	11	34
Interior	41	37	17

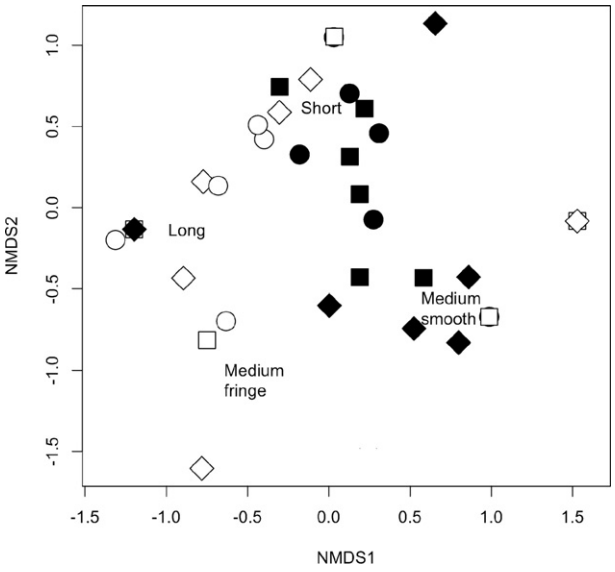

Fig 2 – Functional NMDS ordination based on hyphal exploration type for soil cores sampled across the edge-interior of three *Pinus muricata* tree islands. Each point represents a single soil core. Points closer together have more similar composition of hyphal exploration types. Symbols denote different tree islands (Circle = 1, Square = 2, Diamond = 3) and black and white colors represent interior and edge samples, respectively. Text indicates the portion of the ordination with which each hyphal exploration type was most associated. Despite the fact that there were no significant differences in species composition between edge and interior samples, functional composition varied significantly with sampling location.

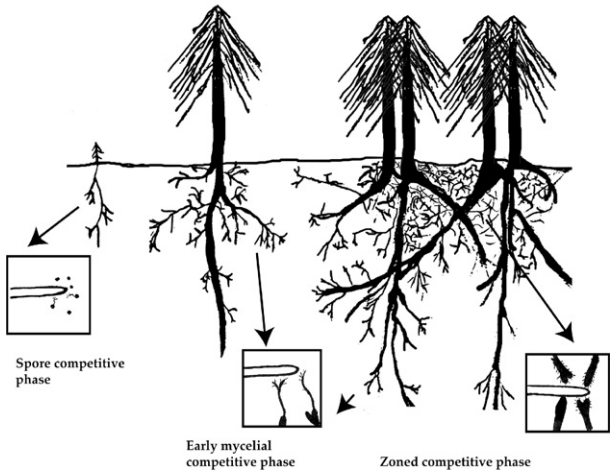

Fig 3 – A model for EM fungal succession based on spatial and temporal changes in root density. Boxes depict the most common modes by which non-mycorrhizal roots (white) are colonized by spores or existing EM roots (shaded). During the spore competitive phase the EM species with the most reactive spores establish first. During the early mycelial competitive phase less abundant less reactive spores establish at low frequency and vegetative competition among long- and moderate-range exploration types ensues. During the zoned competitive phase, high density root zones are dominated by short-range exploration types and lower density areas (such as deeper roots and edges) are dominated by moderate to long-range types.

exploration type were more likely influenced by root density than nutrient availability.

These data support the hypothesis that root density selects for EM species with colonization strategies that are best matched to root spacing. When roots are widely spaced, species with shorter distance exploration types are unlikely to colonize many new roots *via* mycelial-based dispersal, while longer distance types should be able to find and colonize new roots effectively. As roots become denser, short-range exploration would become an effective strategy and may be more efficient in terms of carbon expenditure than longer distance types. Note that we are not saying that other factors such as litter quality, soil pH, and nutrient availability are unimportant in explaining the distribution of EM exploration types (Dighton *et al.* 2000, 2001). Rather, we suggest that root density alone can be predictive of a portion of EM community structure. This is particularly likely to be true in primary successional settings, where root density changes significantly from the time between seedling colonization and canopy closure.

From the data in this study and our previous work in this system, we believe patterns of EM succession in young *P. muricata* forests may be best explained by differences in dispersal ability (of both spores and mycelium) and competition. This hypothesis is modelled in the diagram presented in Fig 3. Our model has two main components: (1) early colonization of seedlings and young saplings (in the absence of adult trees) is driven by quantitative differences in spore abundance and reactivity, while later colonization is dominated by mycelial dispersal among roots and competition; and (2) the optimal strategy for mycelial competition changes with root density. Because of this, natural gradients in root density contribute to predictable patterns of temporal or spatial zonation often observed in EM assemblages. The idea that spore inoculum drives early primary EM succession is almost a tautology, because in a system with no preexisting forest all EM species must arrive by spore. Furthermore, there are many studies showing that the earliest colonizers are EM species that produce abundant fruit bodies and have highly reactive spores (i.e. those that can be observed to inoculate seedlings in controlled experiments) (Fox 1983; Mason *et al.* 1983; Jumpponen *et al.* 1998; Terwilliger & Pastor 1999; Jumpponen *et al.* 2002; Nara *et al.* 2003a; Nara *et al.* 2003b; Ashkannejhad & Horton 2006). We predict that what initially orders these species in succession is early abundance of spores and speed of germination. It has been well documented that timing of colonization matters during the initial phase of spore colonization (Kennedy *et al.* 2009). For example, Kennedy & Bruns (2005) found that *Rhizopogon occidentalis* and *R. salebrosus* were equally capable of colonizing seedlings at equivalent spore concentrations when alone, but when they were co-inoculated onto seedlings, *R. occidentalis* won because its spores

germinated more quickly and preempted the receptive roots. A similar pattern was inadvertently observed with *Suillus pungens*; this species colonized 25 % of the control seedlings in the field experiment of Kennedy *et al.* (2007), but did not colonize any of 180 seedlings that were inoculated in the field with *Rhizopogon* spores at the time seeds were planted.

In contrast, EM mycelium connected to other living roots is generally more efficient than spores in colonizing new roots. This is because the living mycelium has a much larger carbon budget than a spore, and can use it to effectively explore soil and encounter uncolonized roots. In addition, there is likely to be less lag time between encountering a root and colonizing it because the mycelium is already active, whereas a spore must germinate. Early experiments by Fleming (1983, 1984) demonstrated this mycelial effect with trenching experiments that cut mycelial connections to living roots or surrounding trees and allowed seedlings to be colonized by spore. Mycelial-free zones may also occur in natural settings following small-scale disturbance and could allow spore colonizers to have a niche in mature forests. For example, seasonal drying of the organic layer in western U.S. conifer ecosystems is likely to provide such a niche (Izzo *et al.* 2006), and the fringe of root systems of single trees or tree islands is another likely zone for spore colonization (Peay *et al.* 2007).

The extent to which root density and exploration type help explain EM successional dynamics or spatial zonation in other systems is an open question. Evidence from non-pine systems appears to be equivocal. For example, *Hebeloma crustuliniforme* and *Lactarius pubescens*, the early successional edge species of Last *et al.* (1984), are both classified as rhizomorphic but neither are 'long-distance' explorers. Similarly, species of *Laccaria* (medium-smooth) and *Inocybe* (short) were the early colonists of willow islands on Mount Fuji (Nara *et al.* 2003a; Nara *et al.* 2003b). However, in the same system *Scleroderma bovista* (long) was noted for its preference of edge habitats. As noted in our model, spore colonization is also likely the primary factor in generating these patterns and may explain some of the aforementioned discrepancies between exploration type and root density. Additionally, it is unclear how much exploration ability might vary within some of these categories, and in some systems medium-smooth or medium-fringe exploration types may be the successful mycelial dispersers. The presence of late-successional fungi with long-distance exploration types may also represent the vertical gradient in roots within a soil profile. For example, the relative infrequence of boletoid fungi (long-distance) in belowground studies of EM root tips may be related to their prevalence deep in the soil where roots are lower in abundance and are less likely to be sampled by mycorrhizal researchers (Gardes & Bruns 1996; Rosling *et al.* 2003).

There are some important methodological considerations to keep in mind when evaluating these results. The first pertains to the difficulty we had in establishing a direct quantitative relationship between exploration type and root density. This is at least partly due to the presence of additional processes affecting exploration type (see previous paragraphs), which are consistent with the failure of our data to fit strict binomial or Poisson models. However, it is also likely in part due to the fact that exploration type categories are an over-simplification of fungal exploration capabilities. While they are useful in light of the difficulty of *in-situ* measurements, quantitative measurements of maximum exploration distance, hyphal extension rates, hyphal biomass or enzymatic capabilities would be more robust functional trait measures. Second, different species of EM fungi may manipulate fine root architecture (Sohn 1981; van der Heijden & Kuyper 2003) in ways that affect local density of fine roots (e.g. racemose growth *versus* short branching). For this reason selecting an appropriate measure of root density (e.g. fine root density, root mass density, root length, etc.) is not trivial. In addition, root density within a single soil core might not reflect the spatial scale at which exploration type is selected. Third, there is a strong phylogenetic signal to exploration type categories (Agerer 2006). For instance, all of the long-distance exploration types found in this study belonged to the suilloid lineage of the Boletales. As such, it is possible that the patterns observed in this and other studies (e.g. Hobbie & Agerer 2010) are driven by phylogenetically correlated traits that have not been measured. As the ability to develop fungal supertree phylogenies based on ITS sequence data grows, the use of methods to account for phylogenetic signal, such as phylogenetically independent contrasts (Felsenstein 1985), will be important to incorporate in future studies testing our hypothesis. Fourth, our work does not address the specific mechanisms of root take-over and how these might interact with root density in determining competitive outcomes. Given that EM colonization rates are close to 100 % in mature forests, the availability of uncolonized fine roots will depend on rates of fine root turnover and the rate or seasonality of root extension relative to mycelial growth. In cases where rates of turnover and extension are low, uncolonized roots may be rare and direct root take-over may be common (Wu *et al.* 1999). How such situations might interact with exploration types is an interesting area for future study. However, we expect that in most systems seasonal root flushes likely provide a large pool of uncolonized roots within particular time windows.

Another aspect of the model that needs further validation is determining how spore inoculum levels vary across a range of spatial scales. Previous work in other western U.S. forests suggest that individual species distributions of animal-dispersed spores are patchy (Kjöller & Bruns 2003; Izzo *et al.* 2006; Rusca *et al.* 2006) and we are currently testing this question with aerially dispersed spores across a cm–km scale at PRNS. As mentioned above, experimental studies where root density is directly manipulated and its effect on competitive outcomes assessed would also be helpful in determining the costs and benefits of different EM exploration strategies in different environments.

In conclusion, this work demonstrates the utility of a functional trait approach in examining highly diverse communities (McGill *et al.* 2006) and proposes a new hypothesis for EM community assembly. As the ability to identify and measure key fungal functional traits improves, this type of approach will allow for statistical comparisons across diverse systems even if they do not share species. Additionally, we believe our model provides a new perspective on the role of exploration types in mycelial dispersal and generates testable predictions regarding patterns of spatial and temporal zonation in EM fungal communities.

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Supplementary data

Supplementary data associated with this article can be found in online version at doi:10.1016/j.funeco.2010.09.010.

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