RESEARCH ARTICLE

Ectomycorrhizal fungal traits reflect environmental conditions along a coastal California edaphic gradient

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Abstract

Multispecies mutualisms, such as the association between trees and ectomycorrhizal fungi, are often shaped by environmental context. Here, we explored the functional mechanisms underlying this environmental filtering. Using a single population of *Pinus muricata* (Bishop pine) growing along a strong edaphic gradient, we examined how environmental stress affected ectomycorrhizal fungi. The gradient spans *c.* 400 000 years of soil age, and reduced nutrient availability and increased water stress dwarf trees on older sites. Fungal community composition shifted with nutrient and water availability and with the stature of the *P. muricata* host trees. Not only did pygmy trees host a taxonomically different fungal subset as compared to nonpygmy trees, but associated fungal communities also differed in life history strategies: trees in more stressful conditions hosted fungi with more carbon-intensive foraging strategies. Our results indicate a link between environmental controls of host nutritional status and turnover in the ectomycorrhizal fungal community. The transition to more energy-intensive strategies under nutrient stress may allow for close recycling of recalcitrant nutrient pools within the root zone and facilitate transport of nutrients and water over long distances. These results highlight the value of life history data to understanding the mechanistic underpinnings of species distributions.

Introduction

The maintenance of plant–microorganism mutualisms can be influenced by a number of biotic and abiotic factors (Ehrenfeld *et al.*, 2005). For example, fungal host specificity (Newton & Haigh, 1998; Tedersoo *et al.*, 2009), niche partitioning among fungi (Bruns, 1995), and host tree sanctions and rewards (Cowden & Peterson, 2009) have been suggested as potential biotic factors maintaining the mutualistic interactions between trees and mycorrhizal fungi and the diversity of species involved in the mutualisms. Furthermore, mycorrhizal community composition often varies along environmental gradients (Swaty *et al.*, 1998; Robertson *et al.*, 2006; Toljander *et al.*, 2006; Peay *et al.*, 2010b), suggesting either direct selection by the environment for the most tolerant (or most competitively dominant) fungi, or indirect selection by host trees that preferentially reward the partners that best match their environment. The latter mechanism may be especially important for ectomycorrhizal fungi, whose environmental tolerances seem wider than their observed occurrence in natural systems (Branco, 2010). However, because many factors co-vary in natural systems, disentangling the relative roles of abiotic factors (i.e. edaphic conditions) and biotic factors (i.e. host tree identity) has proven difficult.

Here, we take advantage of the environmental variation along a well-known California chronosequence (Westman & Whittaker, 1975) to examine turnover of the ectomycorrhizal community hosted by a single tree species, *Pinus muricata* (Bishop pine). The soils of the 'ecological staircase' in Mendocino County range from approximately 100 000 to 500 000 years in age. The youngest soils support tall, diverse forests including *P. muricata*. However, on the oldest soils, where nutrient levels are low and a shallow iron hardpan produces seasonal drought and waterlogged conditions, members of the same *P. muricata* population are dwarfed, forming the California pygmy forest ecosystem (Westman, 1975; Westman & Whittaker, 1975).

Variable nutrient and water supply of individual *P. muricata* trees along this environmental gradient can potentially affect the interaction between host trees and their complement of ectomycorrhizal fungi through multiple mechanisms. For example, under stressful conditions, reduced tree carbon supplies could limit payments to fungi on pygmy terraces and, subsequently, limit fungal-mediated nutrient cycling (Kaiser *et al.*, 2010, 2011). Simultaneously, fungal enzymatic activity varies as a function of environmental features and season (Courty *et al.*, 2006, 2010), suggesting that alterations in fungal foraging efficiency could shift the tree's optimal investment landscape. Therefore, by surveying turnover in the ectomycorrhizal community while holding the biotic environment (i.e. host tree) constant, we can obtain direct insight into the interaction between environmental variation and host physiology in shaping this mutualism.

Specifically, we test two hypotheses regarding ectomycorrhizal diversity on the host tree *P. muricata* across the environmental gradient of the ecological staircase: (1) ectomycorrhizal fungal species composition turns over along the gradient, reflecting fungal niche differences in nutrient and water acquisition, and (2) fungi hosted by pygmy trees have life history traits associated with accessing recalcitrant organic compounds. Our results support both hypotheses but also highlight the need for more fungal trait data.

Materials and methods

Study site and field sampling

Samples were collected at the Ecological Staircase of Jug Handle State Reserve, Mendocino, California [39°22′ N, 123°47′ W; mean annual temperature 12.5 °C; mean annual precipitation 983 mm (Northup *et al.*, 1995a, b)] on April 2, 2011. The 'staircase' refers to a series of marine-derived terraces, which step back in age, up in elevation, and away from the coastline. As soils age, they lose nutrients, become more acidic, and form subsurface hardpans impermeable to water (Table 1). Our samples were taken on terraces 1 through 5. Terraces 1 and 2 host mixed conifer forests including *P. muricata*, while terraces 3 through 5 display pygmy forest-type vegetation, in which short (< 5 m in height) *P. muricata* and the occasional *Pinus contorta* (Lodgepole pine) are surrounded by a dense understory of ericaceous shrubs (see Westman & Whittaker, 1975 for a detailed site description).

To characterize community turnover across the terraces, we sampled the ectomycorrhizal community of six *P. muricata* trees (five on Terrace 2; 29 trees in total) on each terrace (Supporting information, Fig. S1). Individual trees were selected haphazardly with the condition that

Table 1. Terrace soil characteristics reflect substrate age along the Mendocino chronosequence

Terrace	Estimated soil age* (years)	Elevation† (m)	Litter layer depth‡ (cm)	Litter layer pH† (H_2O)	Litter layer N† (g kg^{-1})	Litter layer available P† (mg kg^{-1})	Oa horizon depth‡ (cm)	Oa horizon NH_4^+‡ (mg kg^{-1})	Oa horizon available P‡ (mg kg^{-1})	Hard pan depth† (cm)	*P. muricata* ecotype
1	100 000	22	3.2 ± 1.2	5.01	10	314	7.8 ± 4.4	4.6 ± 2.1	7.2 ± 4.7	n/a	Nonpygmy
2	200 000	61	3.0 ± 1.3	4.11	8.7	284	8.7 ± 3.6	3.2 ± 1.4	5.1 ± 4.5	n/a	Nonpygmy
3	> 240 000	90	0.5 ± 0.2	3.89	5.8	128	2.5 ± 1.1	1.4 ± 1.1	3.5 ± 2.9	43	Pygmy
4	330 000	130	1.8 ± .2	3.93	6.1	123	4.3 ± 3.4	1.8 ± 0.7	3.5 ± 4.9	60	Pygmy
5	> 400 000	160	1.7 ± 0.9	4.05	6.5	118	6.1 ± 4.5	2.4 ± 1.4	4.7 ± 6.2	53	Pygmy

*Data from Merritts *et al.* (1991).
†Data from Northup *et al.* (1995a).
‡Means ± 1 SD.

no ectomycorrhizal host tree other than *P. muricata* occurred within 10 m. Sampled trees were at least 15 m from one another. The maximum distance between trees was 3.5 km. On terraces 1 and 2, sampling was constrained to monotypic *P. muricata* stands. The GPS location of each tree was recorded to within 5 m.

Two soil cores (cylinders 10 cm deep and 6 cm in diameter) were taken from the surface root zone of each tree using a bulb planter (OXO Product #1068280; New York, NY). The first core was placed 15 cm from the base of the trunk at a predetermined and randomly generated compass heading. The second core was placed six inches from the base of the trunk 180° from the first core (i.e. on the opposite side of the tree). Cores were immediately sealed in plastic and were stored at 4 °C until processing.

Fungal identification and trait matching

Within 7 days, all cores were processed by homogenization, followed by sequential washing of material in tap water through three soil sieves of 5.6-, 2.0-, and 0.5-mm mesh sizes (USDA Standard Testing Sieves #3½, #10, and #35; Fisher Scientific Co., Hampton, NH). All materials passed through the largest mesh size, and proportional amounts from the other two sieves were homogenized and examined under a dissecting microscope. The first 16 ectomycorrhizal root tips to be encountered were washed in deionized water prior to DNA extraction. The homogenization steps randomized the distribution of the sample's root tips, so that the 16 selected tips can be considered a randomly drawn sample from the pool of tips in each core. When multitip clusters were encountered, only one tip from the cluster was selected as a sample. To extract DNA, tips were heated in 10 μL Extraction Solution (SKU E7526; Sigma-Aldrich Co. LLC, St. Louis, MO) for 10 min at 65 °C, then 10 min at 95 °C, before addition of 10 μL of Neutralization Solution B (SKU N3910; Sigma-Aldrich Co. LLC).

The internal transcribed spacer (ITS) region of the nuclear ribosomal RNA genes of each root tip was amplified using the ITS-1F (Gardes & Bruns, 1993) and ITS-4 primers (White *et al.*, 1990) and sequenced by Beckman Coulter Genomics (Danvers, MA). When the initial PCR amplification yielded multiple bands, or when sequencing results were poor, PCR and sequencing attempts were repeated using ITS-1F in combination with ITS-4a (Larena *et al.*, 1999) or ITS-4b (Gardes & Bruns, 1993). Sequencing efforts were halted when usable sequence was obtained for > 75% of sampled root tips.

The resultant sequences were clustered into operational taxonomic units (OTUs) using Geneious (version 5.3.6; Biomatters, Auckland, New Zealand) and a 97% sequence homology cutoff. Analyses were repeated using a less stringent 95% sequence homology cutoff, but no statistically significant differences in results were observed (data not shown), and so results from the 97% threshold are presented here. Taxonomy was assigned to OTUs using the Basic Local Alignment Search Tool (BLAST, http://blast.ncbi.nlm.nih.gov). Because of the limitations of database coverage, in many cases, assignment was only possible to the genus level. Using the BLAST-based taxonomy, we screened our dataset to remove species that were obviously nonmycorrhizal. Because the 97% sequence similarity threshold has been shown to be a reasonable approximation for fungal species (Smith *et al.*, 2007), we refer to these OTUs as species.

For sequences that were identifiable to species (i.e. > 97% match to a described species), we used the DEEMY (An information system for characterization and DEtermination of EctoMYcorrhizae.) database (http://www.deemy.de/) to gather information on foraging distance, rhizomorph formation, and hydrophobicity. These traits relate to fungal foraging strategy (Hobbie & Agerer, 2010). Because information in the database comes primarily from European studies, many taxa in our study were not represented in DEEMY. However, foraging-related functional traits for fungal hyphae are typically conserved at the genus level (Agerer, 2006). Therefore, when no species-level match was present in DEEMY for a particular taxon, entries for *Pinus*-hosted congeners were surveyed and, if 90% of the entries agreed, consensus trait values were assigned to that taxon. This also allowed incorporation of data for some sequences that could only be identified to genus.

Soil environment

To directly test the response of ectomycorrhizal fungi to local soil chemistry, on September 18, 2013, additional soil samples were obtained using GPS measurements to relocate trees. (In 8 of 29 cases, the identity of the originally sampled tree was confirmed by remnants of flagging tape placed on April 2, 2011. In other cases, the nearest *P. muricata* tree to the previously recorded GPS coordinates was sampled.) At each tree, litter depth and organic layer depth were recorded. Two soil fractions (one organic and one mineral) were collected at each tree by combining material from two cores placed opposite to one another, six inches from the base of the tree. Because of the organic layer depth and high amount of woody debris at the most westward tree (on Terrace 1), only an organic soil sample was obtained.

Within 48 h of completion of field sampling, soils were sieved and homogenized for moisture, pH, and nutrient analysis. A fraction of soil was weighed, and then dried at

65 °C for 72 h to determine moisture content. A subsample of this fraction was ground with liquid nitrogen and analyzed for carbon and nitrogen content using a Carlo Erba NA1500 elemental analyzer (CE Elantech Inc., Lakewood, NJ). Nitrate and ammonium were extracted with potassium chloride, and plant-available phosphorus was extracted using resin-P bags (Soil Survey Laboratory Staff, 1992). Concentrations of these nutrients were measured using a WestCo SmartChem 200 discrete analyzer (Unity Scientific Inc., Brookfield, CT).

Data analysis

We looked for environmental drivers of ectomycorrhizal community composition by comparing species and trait assemblages in pygmy forests (terraces 3, 4, and 5) with assemblages in nonpygmy forests (terraces 1 and 2), which is between-ecotype comparisons. We also performed parallel within-ecotype comparisons to assess the relative importance of environmental drivers.

We used linear models to regress species richness against core, tree, and terrace number, as our samples were sequentially ordered along the chronosequence. Using the 'VEGAN' package (Oksanen *et al.*, 2012) in R (R Core Team, 2012), we also calculated a Jaccard dissimilarity matrix measuring the difference in trees' ectomycorrhizal fungal species assemblage. We then ordinated trees using nonmetric multidimensional scaling (NMDS) according to their ectomycorrhizal fungal species assemblage and used a permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001) calculation with ecotype (pygmy vs. nonpygmy) as a factor to determine the statistical significance of community divergence. We repeated this analysis using two additional dissimilarity indices to test the robustness of our results. In particular, we used a Chao index to correct for unsampled species and an original Raup–Crick index to test the relative importance of unique species by converting data to presence/absence and ignoring abundance. An identical analysis was performed using DEEMY fungal trait data assigned to each identified root tip in the dataset (instead of fungal species identity). We also used a Pearson's chi-squared test to compare the trait distribution of ectomycorrhizal tips at the ecotype and terrace level.

To test the role of the soil environment in shaping fungal communities, we first looked for patterns in measured soil characteristics across terraces (ANOVA; Tukey's honest significant difference test) and between pygmy and nonpygmy ecotypes (*t*-test). We then condensed soil variation into its principal components and tested for the explanatory power of these axes, as well as individual soil parameters, using permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001).

To test for the effect of spatial distance and, implicitly, the role of distance-dependent processes such as dispersal in driving community composition, we used a Mantel test to compare our tree ectomycorrhizal community dissimilarity matrices with the physical distance matrix. Because the terraces and ecotypes are spatially autocorrelated (i.e. the terraces fall in a rough line moving away from the ocean), we expected a significant spatial autocorrelation over the entire dataset. Therefore, we also performed within-ecotype Mantel tests (i.e. looked for spatial autocorrelation within terraces 1 and 2, and within terraces 3 through 5) and used these results to supplement our within-ecotype PERMANOVA results.

All statistical analyses were performed using R (R Core Team, 2012), and test results were considered significant at the $P < 0.05$ level.

Results

Community composition

Across the study, we harvested 928 root tips (29 trees × 2 cores × 16 randomly encountered tips per core). From these tips, we successfully recovered 716 sequences (77% sequencing coverage) that were clustered into 133 OTUs. We removed 33 obviously nonectomycorrhizal species from our dataset (including the most abundant species, an undescribed, saptrotrophic *Leptosporomyces* which was evenly distributed across all 5 terraces, and sequences from three lichen species) and culled 18 unique sequences of low quality and short length.

At a 97% sequence homology cutoff, 82 species remained, representing 518 tips across all 29 trees. Thirty-four of these taxa were encountered only once (Fig. S2). Many, but not all, of the most common taxa displayed clear patterns in their distribution. For example, *Russula bruneola* was found only on nonpygmy host trees, whereas *Suillus tomentosus* was found only on pygmy host trees (Fig. 1). However, these species-specific patterns did not produce any significant trends in OTU richness across terraces when species numbers were pooled by core, by tree, or within and across the entire terrace (Fig. S3).

Soil characteristics

Overall, mean soil properties mirrored previous results at this site (Westman, 1975, 1978; Westman & Whittaker, 1975; Merritts *et al.*, 1991; Northup *et al.*, 1995a, b). Soils on terraces with pygmy ecotype trees had shallower litter and organic layers ($P < 0.001$, $P < 0.05$, respectively) than soils on terraces with nonpygmy ecotypes. Pygmy soils

were also drier (organic layer $P < 0.001$; mineral layer $P < 0.01$). In the organic layer, pygmy soils had less total carbon, total nitrogen, and ammonium than nonpygmy soils ($P < 0.01$ for all three measurements). In both organic and mineral soils, there was a nonsignificant trend toward decreasing pH with increasing terrace number (Fig. S4). Nitrate and plant-available P were low in all samples, and no trend was detected (Figs S4 and S5). At the local scale, soil properties showed a complex relationship with terrace age, highlighting local heterogeneity within terraces (Figs S4 and S5).

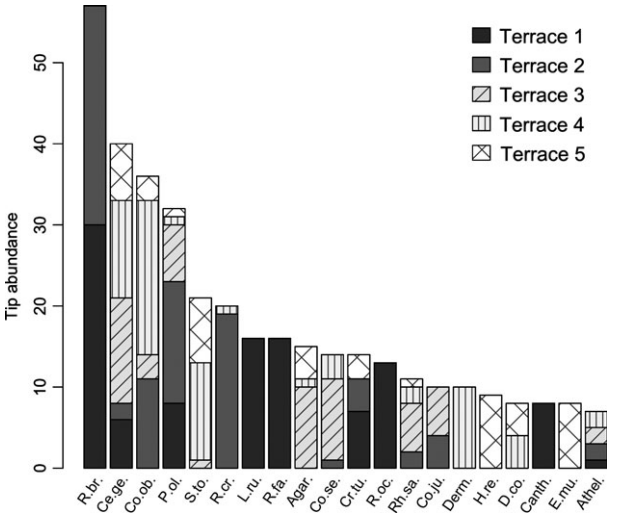

Fig. 1. Tip encounters by taxon and terrace for the 20 most abundant ectomycorrhizal taxa. Fungi were identified from individual root tips by sequencing of the ITS region of the nuclear ribosomal RNA genes. Operational taxonomic units were defined at approximately the species level using a 97% homology cutoff, and taxonomic identity assigned by searching for matching sequences using BLAST. Taxonomic names are abbreviated (see Table S1 for full names).

Environmental drivers of species turnover

We found significant differences in fungal community composition between pygmy and nonpygmy trees regardless of dissimilarity index (Fig. 2, compare a and b, PERMANOVA d.f. = 28, F-statistic = $2.152_{\text{ecotype d.f. = 1, residual d.f. = 27}}$, $R^2 = 0.07382$, $P < 0.001$). In part, these differences were driven by the presence of several species that were restricted to terrace 1 (Fig. 2). Terrace 1 also had a relatively high total richness (Fig. S3c). The addition of the first two principal component axes of soil characteristics (PCA1 and PCA2, which explained 81% and 14% of variance, respectively; Table S2) doubled the amount of variance explained by the model, although inclusion of PCA1 and PCA2 was not statistically supported [(tree-level fungal community ~ ecotype + PCA1 + PCA2); cumulative $R^2 = 0.15101$, $P > 0.05$ for all factors except ecotype regardless of order]. However, two individual soil characteristics explained small amounts of fungal community variance (Oa horizon percent moisture, F-statistic = 2.17, $R^2 = 0.07$, $P < 0.05$ after Bonferroni correction for multiple hypothesis testing; Oa horizon total N, F-statistic = 1.76, $R^2 = 0.06$, $P < 0.05$ after Bonferroni

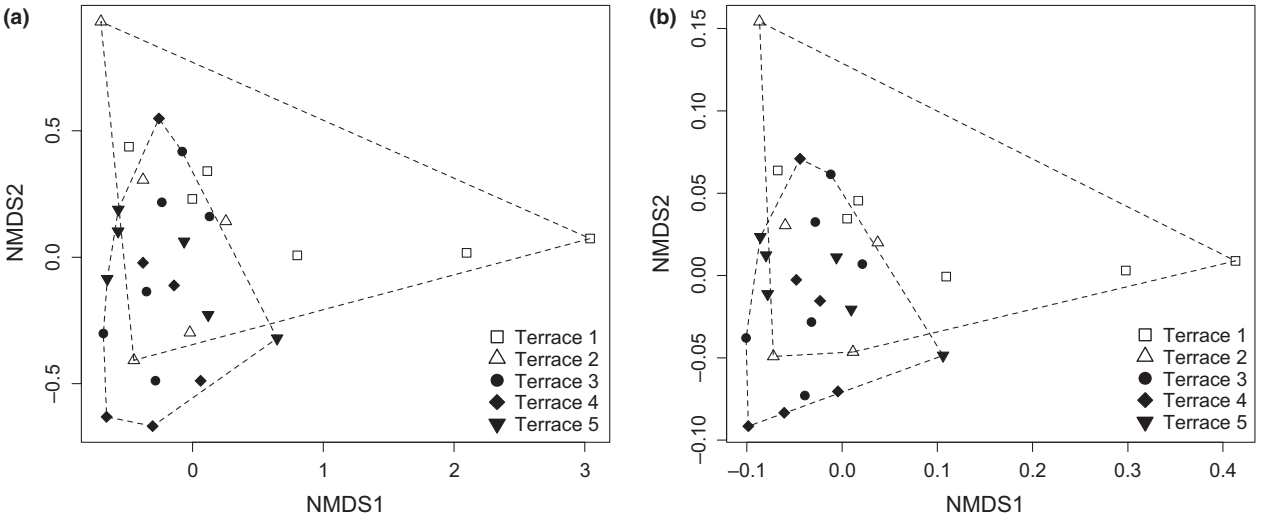

Fig. 2. Two-dimensional NMDS ordination of individual *Pinus muricata* trees by fungal community. Axes explain variance in the data, and physical distance between trees on plots is proportional to distance between the trees' fungal communities, as measured by (a) Jaccard (stress = 0.1005) and (b) a Chao (stress = 0.1002) similarity index. Pygmy (filled symbols) and nonpygmy (open symbols) trees clustered separately from one another. The higher similarity between trees of the same ecotype compared to trees of different ecotypes was statistically significant at the $P < 0.001$ level.

correction). Including Oa horizon percent moisture improved model fit regardless of term order (PERMANOVA d.f. = 28, ecotype F-statistic = 2.2216, $R^2 = 0.07382$, $P < 0.005$; Oa horizon percent moisture F-statistic = 1.8733, $R^2 = 0.06224$, $P < 0.01$). Inclusion of Oa horizon total N content as a third term in the model was order-dependent ($P < 0.05$ for the N content term only when N content was listed first in the model).

As expected, a study-wide Mantel test showed significant correlation between fungal species assemblages and tree physical distance (Pearson's Mantel statistic $r = 0.1898$, $P = 0.002$). However, within-ecotype Mantel tests showed that spatial distance was not a significant driver of community composition at these scales (pooled terraces 1 and 2, $P > 0.05$; pooled terraces 3 through 5, $P > 0.05$). Similarly, our within-ecotype analyses of variance showed no significant difference between terraces (pooled terraces 1 and 2, PERMANOVA d.f. = 10, F-statistic = 1.0593, $R^2 = 0.10531$, $P = 0.424$; pooled terraces 3 through 5, PERMANOVA d.f. = 17, F-statistic = 0.99675, $R^2 = 0.11731$, $P = 0.443$).

Trait analysis

Using the DEEMY database, we were able to collect trait data for 55 of our 82 species (Table S1), accounting for 442 tips (49% of sequenced tips on Terrace 1, 42% on Terrace 2, 74% on Terrace 3, 82% on Terrace 4, and 74% on Terrace 5). Traits were nonrandomly distributed at the terrace level and at the ecotype level. In particular, fungal traits associated with high biomass and high carbon requirements (e.g. long-distance foraging, hydrophobicity, and rhizomorph formation) were more prevalent on terraces 3–5 (Fig. S6; foraging distance $\chi^2 = 162.4572$, d.f. = 12, $P < 0.001$; rhizomorph formation $\chi^2 = 59.6836$, d.f. = 4, $P < 0.001$; hydrophobicity $\chi^2 = 90.2845$, d.f. = 4, $P < 0.001$) and on pygmy trees (Fig. 3; foraging distance $\chi^2 = 153.9368$, d.f. = 3, $P < 0.001$; rhizomorph formation $\chi^2 = 55.7124$, d.f. = 1, $P < 0.001$; hydrophobicity $\chi^2 = 78.5283$, d.f. = 1, $P < 0.001$).

We repeated tree-level ordinations using distance matrices of fungal traits, rather than species identity, and again found that pygmy ecotypes clustered differently

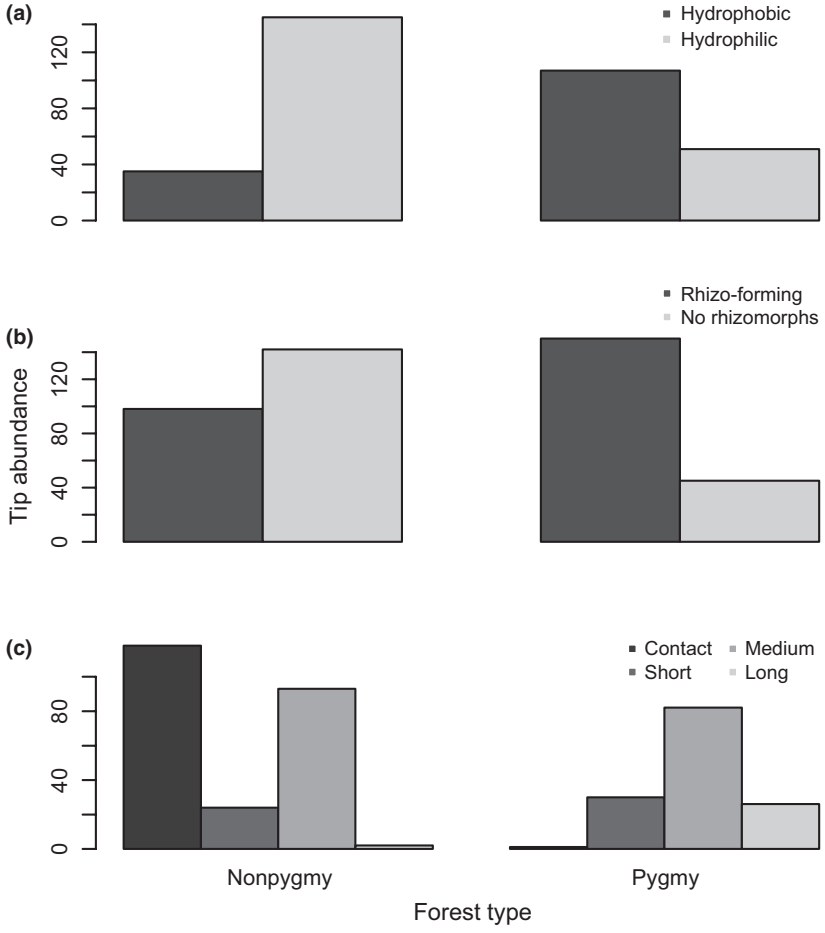

Fig. 3. Distribution of fungal traits across nonpygmy (left column) and pygmy (right column) forest types. The distributions of three foraging-related traits are shown: (a) hydrophobicity, (b) rhizomorph formation, and (c) foraging distance. As soil conditions become harsher, hydrophobic and rhizomorph-forming species become more abundant, and the prevalence of contact foraging types is reduced while the abundance of long-distance foragers rises. Trait distributions are nonrandomly distributed at a significance level of 0.001.

than nonpygmy ecotypes (Fig. 4; PERMANOVA d.f. = 28, F-statistic = 5.0172, $R^2 = 0.1567$, $P = 0.007$). Although our ability to assign traits to root tips differed across terraces (with relatively higher assignment rates on terraces 3–5), the high statistical significance of our tests suggests that the patterns observed are robust. In addition, we repeated our analysis with data unweighted by abundance (i.e. we counted each species' traits only once per tree, instead of weighting traits by root tip abundance), with qualitatively similar and statistically significant results (results not shown).

Discussion

Our work seeks to link taxonomic and life history data to a mechanistic explanation for ectomycorrhizal fungal community turnover along environmental gradients. Because we confined our sampling to a single host tree species, *P. muricata*, along an edaphic gradient, our results suggest that changes in environmental quality, rather than changes in host plant species, shaped the ectomycorrhizal community. Environmental quality may have affected the ectomycorrhizal community either directly or indirectly through changes in tree physiology. Indirect effects could be driven by plastic or genetic variation in tree hosts across the gradient, although we currently lack data to evaluate these possibilities. Regardless, our study revealed two statistically distinguishable fungal communities: (1) a nonpygmy-associated community, found on terraces 1 and 2, whose trait distribution tended toward short-distance, hydrophilic, low biomass exploration types, and (2) a pygmy-associated community, found on terraces 3, 4, and 5, whose trait distribution tended toward long-distance, hydrophobic, high-biomass exploration types. Thus, the traits of the fungal community appear to shift with the environmentally mediated nutritional needs of the host tree.

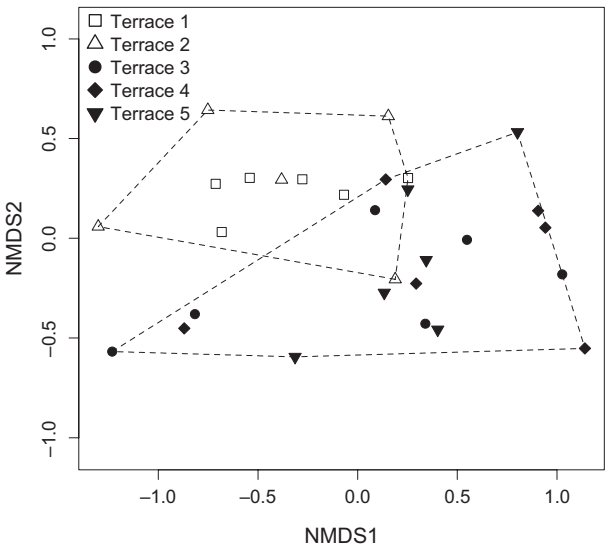

Fig. 4. Two-dimensional ordination (stress = 0.0957) of trees by the foraging traits of their fungal communities. Pygmy (filled symbols) and nonpygmy (open symbols) trees cluster separately ($P < 0.01$).

A number of studies have observed changes in fungal community composition along environmental gradients (Wurzburger *et al.*, 2004; Schechter & Bruns, 2008; Branco & Ree, 2010; Peay *et al.*, 2010a, b). However, the mechanism underlying these patterns is not clear because fungal community response could be related either to direct environmental effects on fungal physiology and competitiveness or indirectly to changes in host plant nutrient demands. One of the few manipulative experiments on this topic (Branco, 2010) showed that even extreme environments are unlikely to pose a severe physiological challenge for ectomycorrhizal fungi. However, no alternative mechanism was demonstrated. Our result, that functional traits related to nutrient acquisition mirror patterns of community turnover, thus provides positive evidence for the importance of an indirect, host-mediated explanation for fungal community turnover.

The observed tree ecotype clustering coincides with previous evaluations of soil chemistry, which show that younger, nonpygmy terraces are more N and P rich and less acidic than terraces with pygmy trees (Westman, 1975, 1978; Westman & Whittaker, 1975; Merritts *et al.*, 1991; Northup *et al.*, 1995a, b). Terrace- and ecotype-level trends in soil characteristics confirmed previous reports of reduced nutrient availability and water stress (e.g. drought during summer months; note that our soil samples were collected in September, at the end of the dry summer season) on older soils. Intriguingly, soil measurements were quite heterogeneous across trees at each terrace. This local-scale soil heterogeneity is not uncommon in natural systems (Jackson & Caldwell, 1993; Parker & Van Lear, 1996).

As our measurements of soil variables both generally supported previously measured edaphic trends along the chronosequence and showed soil heterogeneity at the tree scale, we examined the relative explanatory power of both regional (terrace- and ecotype-scale) and local (tree-scale) processes in structuring the ectomycorrhizal community. We emphasize that it is not possible to draw a direct link between edaphic and fungal data because of the temporal (more than 2 years separated the two sampling dates) and spatial (fungal and edaphic properties were measured in physically separate cores) gaps between the two sets of measurements. In particular, properties such as soil moisture and plant-available nutrients are likely to change as a function of rainfall events, seasonality, and other temporal factors. Nevertheless, we note that in our study,

although inclusion of local-scale soil properties improved our models of fungal community composition, fungal communities were best explained by tree ecotype, suggesting that the community hosted by an individual tree was likely shaped by regional soil and community characteristics.

There are many reasons that regional properties (e.g. tree ecotype) may be better predictors of fungal community composition than local properties. In our study, tree ecotype correlates with a variety of regional factors, including soil chemistry, hydrology, and vegetation, that may all be expected to shape the local ectomycorrhizal species pool. Additionally, individual pine trees typically have lateral root zones that extend for tens of meters (Fogel, 1983; Sudmeyer *et al.*, 2004), and fungal individuals may cover spatial extents ranging from a few to tens of meters belowground (Dahlberg & Stenlid, 1990; Bergemann & Miller, 2002). Thus, the fungal community likely represents an integrated response to soil chemistry at a larger scale, as well as to the immediate chemical properties at the spatial location sampled.

The relative dominance of high-investment exploration strategies in pygmy forests, and low-investment in nonpygmy forests, is consistent with the differential nutrient availability in the two forest types. The longer the distance over which a fungus forages, the greater its biomass investment in exploratory hyphae and long-distance transport structures (i.e. rhizomorphs, hydrophobic mantle). Such an energy-intensive strategy is believed to be competitively dominant in low-nutrient environments where resources are rare and patchy (Hobbie, 2006), and indeed, such strategies often correlate with the ability to break down particularly recalcitrant organic matter (Hobbie & Agerer, 2010). In contrast, in nutrient-rich environments, short-distance and contact foragers, whose exploration strategy is less energy intensive, are thought to be favored.

In this system, elevated tannin concentrations in the needles of dwarfed trees and the subsequent effects on nitrogen release have led researchers to propose the 'short-circuit hypothesis', in which trees respond to N limitation by tying up litter N in recalcitrant organic compounds primarily accessible to their complement of ectomycorrhizal fungi (Northup *et al.*, 1995a, b). Such tight recycling of nutrients within a tree's own root zone would require that pygmy trees partner with mycorrhizal fungi that tend to invest in large amounts of exploratory biomass to best able to break down these recalcitrant compounds. Indeed, these are the dominant traits of fungi we observed in the pygmy forests.

Although we did not measure enzymatic activity in this study, the seasonality of our system, which intensifies the stress experienced by pygmy host trees on terraces 3, 4, and 5, may affect community function over the course of the year. Indeed, many fungal metabolic enzymes show temporally variable activity patterns (Courty *et al.*, 2010), although the temporal pattern may depend on enzyme type. For example, Courty *et al.*, (2006) found that enzymes associated with nitrogen release displayed greater seasonal variation in activity levels than enzymes associated with phosphorus release. In our system, available P is at least 55% lower on pygmy terraces than nonpygmy terraces, suggesting that phosphorus limitation may also play a role in shaping mycorrhizal community structure.

In addition, hydrophobic rhizomorphs are less likely to leak water over long transport distances (Duddridge *et al.*, 1980; Agerer, 2001, 2006), a valuable trait in the drought-prone pygmy forests. Soil moisture has been shown to affect colonization success of ectomycorrhizal fungi (Swaty *et al.*, 1998; Kennedy & Peay, 2007). Although we did not measure colonization levels in this study, we did observe shifts in relative OTU abundance which may have been driven, in part, by taxon-specific responses to water availability (Mexal & Reid, 1973).

Another important distinction between pygmy and nonpygmy forests was the presence of ericaceous understory shrubs in the pygmy forest. These mycorrhizal host plants may affect colonization of pines by certain fungal taxa (Kohout *et al.*, 2011), adding an additional level of environmental filtering to the system. Thus, although we restricted our sampling to sites with only one species of ectomycorrhizal host, this by no means eliminated the impact of other members of the plant community on the belowground community. Additionally, the morphological differences between pygmy and nonpygmy host trees may reflect genotypic differences within the species across the gradient. For example, previous work on lodgepole pine (*P. contorta*) at the ecological staircase has described genetic variation along the chronosequence (Aitken & Libby, 1994; Eckert *et al.*, 2012). However, given the relative generality of many pine-associated ectomycorrhizae, we do not expect genotype differences among conspecifics to play a major role in shaping the ectomycorrhizal community.

While in some cases, dispersal limitation may shape the ectomycorrhizal community (Peay *et al.*, 2010a), this is not likely to be a factor in our study for two reasons. First, the *P. muricata* population in our system is both spatially and temporally continuous, providing ample opportunity for admixture of fungi across terraces. Second, we found significant differences in community composition only when our subsample included both pygmy and nonpygmy trees, regardless of the physical distance between the samples.

Conclusions

Our results suggest that environmental factors drive mycorrhizal fungal community indirectly via their effects

on host nutrient demands. In particular, we observed two fungal communities, one associated with nonpygmy trees on nutrient-rich, well-drained terraces, and one associated with pygmy trees on low-nutrient, water-impermeable terraces. The results of foraging trait analysis supported the idea that, in nutrient- and water-stressed systems, trees rely upon fungi with high-biomass exploration strategies, despite the higher carbon cost of supporting these fungi.

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Supporting Information

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

Fig. S1. Geographic location of trees used in this study.
Fig. S2. Ectomycorrhizal rank-abundance curve, showing 82 unique OTUs at the 97% sequence homology level.
Fig. S3. Number of OTUs (in most cases, equivalent to species in this molecular analysis) in each core (a), pooled for each tree (b), and pooled for each terrace (c).
Fig. S4. Soil characteristics pooled by terrace.
Fig. S5. Soil characteristics pooled by ecotype (non-pygmy = terraces 1 and 2; pygmy = terraces 3–5).
Fig. S6. Distribution of fungal traits across terraces.
Table S1. Fungal taxa, their abbreviations (for 20 most abundant), and their foraging traits.
Table S2. First five principal component axes of soil data and their relative loadings of soil parameters.