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ORIGINAL PAPER

Variation in ectomycorrhizal fungal communities associated with *Oreomunnea mexicana* (Juglandaceae) in a Neotropical montane forest

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Abstract Neotropical montane forests are often dominated by ectomycorrhizal (EM) tree species, yet the diversity of their EM fungal communities remains poorly explored. In lower montane forests in western Panama, the EM tree species *Oreomunnea mexicana* (Juglandaceae) forms locally dense populations in forest otherwise characterized by trees that form arbuscular mycorrhizal (AM) associations. The objective of this study was to compare the composition of EM fungal communities associated with *Oreomunnea* adults, saplings, and seedlings across sites differing in soil fertility and the amount and seasonality of rainfall. Analysis of fungal nrITS DNA (nuclear ribosomal internal transcribed spacers) revealed 115 EM fungi taxa from 234 EM root tips collected from adults, saplings, and seedlings in four sites. EM fungal communities were equally species-rich and diverse across *Oreomunnea* developmental stages and sites, regardless of soil conditions or rainfall patterns. However, ordination analysis revealed high compositional turnover between low and high fertility/rainfall sites located ca. 6 km apart. The EM fungal community was dominated by *Russula* (ca. 36 taxa). *Cortinarius*, represented by 14 species and previously reported to extract nitrogen from organic sources under low nitrogen availability, was found only in low fertility/high rainfall sites. Phylogenetic diversity analyses of *Russula* revealed greater evolutionary distance among taxa found on sites with contrasting fertility and rainfall than was expected by chance, suggesting that environmental differences among sites may be important in structuring EM fungal communities. More research is needed to evaluate whether EM fungal taxa associated with *Oreomunnea* form mycorrhizal networks that might account for local dominance of this tree species in otherwise diverse forest communities.

Keywords Beta diversity · Community structure · Fortuna Forest Reserve · Mycorrhizal networks · *Russula* (Russulaceae)

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Introduction

Nutrient uptake and transfer via mycorrhizal associations strongly influences the growth and survival of most plant species in nearly all of earth's most species-rich and threatened terrestrial biomes (Smith and Read 2008; Bonfante and Genre 2010). In tropical forests, trees predominantly form associations with arbuscular mycorrhizal (AM) fungi (Glomeromycota) (Janos 1983; St John and Uhl 1983; Béreau and Garbaye 1994; Onguene and Kuyper 2001; St John 1980; McGuire 2008). However, forests dominated by tree species that associate with ectomycorrhizal (EM) fungi, especially Basidiomycota, have been recognized in all major tropical regions (Becker 1983; Connell and Lowman 1989; Hart et al. 1989; Henkel 2003). Ectomycorrhizal plants in lowland tropical forests belong mostly to the Dipterocarpaceae and Fabaceae (primarily a narrow group of Caesalpinioideae), whereas Fagales (including members of

the Juglandaceae, Betulaceae, and Fagaceae) frequently occur in montane sites (Itoh 1995; Conway and Alexander 1992; Hart et al. 1989; Henkel 2003; Morris et al. 2008). In some cases, these EM species grow in "monodominant" forests, wherein a single tree species accounts for more than 50 % of canopy trees in a stand (Connell and Lowman 1989). Why these monodominant forests persist in otherwise diverse plant communities is not fully understood (Peh et al. 2011).

Mast fruiting, low rates of disturbance, high tolerance of shade by seedlings, slow litter decomposition, and escape from herbivory have been proposed as mechanisms to explain tropical monodominance (reviewed by Peh et al. 2011). Strikingly, a common feature of many monodominant tree species in tropical forests is the formation of EM associations (Malloch et al. 1980; Connell and Lowman 1989; Henkel 2003). In temperate forests, natural isotope abundance and radio-isotopic labeling experiments have shown that some EM tree species can develop EM networks, where hyphal connections transfer water, carbon, and nutrients from adult to juvenile plants (Simard et al. 1997; Plamboeck et al. 2007; Booth and Hoeksema 2010; see Simard et al. 2012 for review). In tropical forests, direct evidence of resource transfer among individuals is currently lacking, but decreased survival and growth of seedlings when isolated from neighboring plants is consistent with EM network effects (Onguene and Kuyper 2002; McGuire 2007).

Ectomycorrhizal networks may increase survival of conspecific seedlings in a spatially structured fashion, disproportionately increasing their abundance near adult trees (Onguene and Kuyper 2002; Henkel 2003; McGuire 2007; Teste et al. 2009; Booth and Hoeksema 2010) in a manner consistent with positive plant–soil feedbacks (reviewed by Bever et al. 2012). In turn, the presence and strength of plant–soil feedback depends on the functional traits and taxonomic composition of the EM fungal community (e.g., Dickie et al. 2002; O'Brien et al. 2010; Kennedy et al. 2012). Determinants of EM fungal community composition remain poorly understood in tropical forests. For example, there is conflicting evidence regarding host specificity in tropical EM fungal communities (e.g., for evidence of host preference, see Tedersoo et al. 2008, 2010a and Morris et al. 2009; for evidence of low host specificity, see Diédhiou et al. 2010; Tedersoo et al. 2011; and Smith et al. 2011, 2013). Similarly, the influence of soil type on EM fungal community composition remains unresolved, in part because EM fungal communities associated with the same host species have rarely been studied across a range of soil conditions.

Here, we examine EM fungal communities associated with *Oreomunnea mexicana* (Standl.) J.-F. Leroy, a widely distributed neotropical tree in the walnut family (Juglandaceae), and one of the few examples of a monodominant EM species in the Neotropics. In montane forests in western Panama, *Oreomunnea* forms locally monodominant stands within otherwise highly species-rich forest comprised mostly of taxa that form AM associations (Andersen et al. 2010). In this region, *Oreomunnea* forms dominant stands on several distinct soil types that are distributed over a scale of only a few kilometers. These soils are derived from contrasting parent materials and occur in areas that differ in the seasonality and quantity of annual rainfall (Andersen et al. 2010), making this system unique for the study of EM fungal ecology. Preliminary field surveys of fungal fruiting bodies indicated that diverse communities of EM fungi associate with *Oreomunnea* in these stands (A. Corrales et al. unpublished data).

In this first characterization of the EM fungal community associated with *Oreomunnea*, we used data generated from root tips of seedlings, saplings, and adult trees across this landscape to test four predictions. First, we predicted that infection frequency of EM fungi would be lower in more fertile soils, consistent with the general view that benefits of EM fungi depend on soil conditions (Treseder 2004). Second, we predicted that the diversity, composition, and phylogenetic diversity of EM fungi would vary with soil fertility. Third, we expected to see (a) commonalities in EM fungal communities shared across seedling, sapling, and adult life stages of *Oreomunnea*, and that (b) community similarity among developmental stages would be particularly strong in the lowest fertility soils, where selection for EM networks or particularly beneficial symbionts would likely be strongest. Fourth, we expected lower phylogenetic diversity of EM fungi in high-fertility sites, reflecting lower colonization rates and consequently lower community diversity.

Methods

The study focused on stands of *Oreomunnea mexicana* (Juglandaceae; hereafter, *Oreomunnea*) in three watersheds in a primary lower montane forest (1000–1400 m.a.s.l.) in the Fortuna Forest Reserve in western Panama (Fig. 1; hereafter, Fortuna; 8°45′N, 82°15′W). *Oreomunnea* is a mid-elevational canopy tree distributed from southern Mexico to western Panama at 900–2600 m.a.s.l. (Stone 1972). It produces ca. 100 mg, wind-dispersed fruits, which can generate high-density seedling patches in the understory (Table 1). *Oreomunnea* is locally dominant at some of our study sites, accounting for up to 70 % of individuals and stand basal area at the Honda watershed (A. Corrales unpublished data). Dominance by *Oreomunnea* is not directly related to particular functional traits such as leaf chemistry (i.e., nitrogen (N) and phosphorus (P)) and wood density, which are close to community averages for the area (K. Heineman unpublished data). However, in contrast to almost all co-occurring tree species at Fortuna, *Oreomunnea* forms EM associations. EM status was reported from Mexican populations of *Oreomunnea* (Quist et al. 1999) and confirmed for populations at Fortuna based on clearing of roots with 10 % KOH and staining with trypan blue. Mantle and Hartig net structures were observed with a light microscope. Other EM tree species

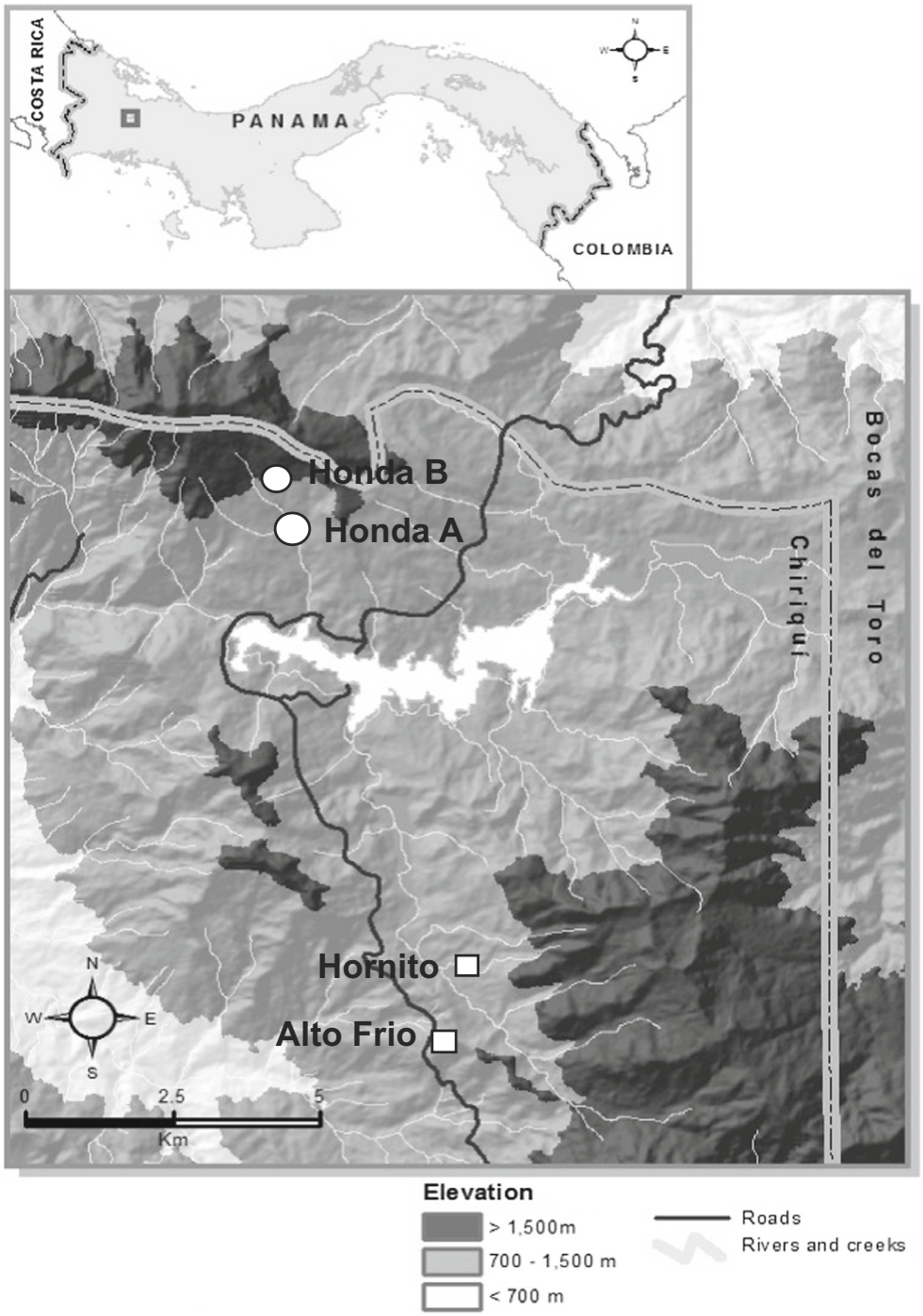

Fig. 1 *Upper panel*, location of Fortuna Forest Reserve, Panama. *Lower panel*, sampling sites at Fortuna: *circles* represent low fertility/high rainfall sites (Honda A and B) and *squares* represent high fertility/low rainfall sites (Hornito and Alto Frio). Reproduced with modifications from Andersen et al. (2010)

that are present in the study area (i.e., *Quercus insignis*, *Q.* cf *lancifolia*, and *Coccoloba* spp.) occur at low densities (typically <10 individuals >10 cm DBH per hectare) both within and outside of *Oreomunnea*-dominated stands.

Climate records indicate that the mean annual temperature for Fortuna ranges from 19 to 22 °C (Cavelier 1996). Annual rainfall averages ca. 5800 mm at our sites in Hornito and Alto Frio, and 9000 mm at our sites in Honda A and Honda B, although all were drier during our study (Table 1; Fig. 1). Hornito and Alto Frio typically have 1–2 months per year with <100 mm of precipitation; in contrast, no months with <100 mm of rainfall have been recorded over the 7-year period for which records are available at Honda A and Honda B (Andersen et al. 2012; J. Dalling unpublished data).

Table 1 Characteristics of the study sites at Fortuna Forest Reserve, Panama

Site	Honda A	Honda B	Hornito	Alto Frio
Elevation (m)	1175	1266	1404	1176
Annual rainfall 2013 (mm)	6055	6440	1990	1895
Soil variables				
Geology	Rhyolite	Rhyolite	Dacite	Andesite
NaOH–EDTA inorg. P (μg cm^{-3})	17.9	15.1	24.3	27.7
NaOH–EDTA org. P (μg cm^{-3})	73.4	60.8	122.7	248.8
NH_4 (μg cm^{-3})	2.2	1.8	1.8	3.8
NO_3 (μg cm^{-3})	1.2	0.4	1.2	2.6
K_2SO_4 extract. org. C (μg cm^{-3})	152.2	92.0	95.3	92.8
pH in water	4.63	3.63	5.76	5.62
Total N (mg cm^{-3})	2.92	2.39	2.87	4.72
Total C (mg cm^{-3})	43.9	40.9	35.0	51.1
Total P (μg cm^{-3})	180.6	127.7	280.2	503.0
Resin P (μg cm^{-3})	0.2	1.9	2.2	1.4
Bulk density (g cm^{-3})	0.11	0.13	0.39	1.00
Al (cmol (+) L^{-1})	1.1	1.3	0.5	0.0
Ca (cmol (+) L^{-1})	0.05	0.15	4.94	8.47
K (cmol (+) L^{-1})	0.02	0.02	0.18	0.12
Light variables				
Canopy openness (%)	6.66	7.90	8.70	9.32
Vegetation variables				
Community basal area >10 cm dbh (m^2ha^{-1})	45.6	46.5	52.9	40.7
Number of *Oreomunnea* seedlings m^{-2}	9.9	7.8	0.7	0.2
Annual seedling mortality rate (%)	0.31	0.17	0.19	0.5
Oreomunnea adults per 0.1 ha	31	79	71	42
Oreomunnea basal area >10 cm (m^2 0.1 ha^{-1})	1.59	2.48	2.58	1.80

Honda A and Honda B are low fertility/high rainfall sites, and Hornito and Alto Frio are high fertility/low rainfall sites. Soil data are expressed in volume basis due to large variation in bulk density among plots

In addition to differences in rainfall, these sites differ markedly in soil characteristics, with contrasting pH, N, P, and base cation availability (Table 1). These distinctive soil traits are related to underlying geology: low-fertility Ultisols at Honda A and Honda B are derived from rhyolite, whereas high fertility soils at Hornito (Ultisol) and Alto Frio (Inceptisol) are derived from dacite and andesite (Andersen et al. 2012; B. Turner unpublished data). Sites differing in soil fertility are ca. 6 km apart, with 200 m separating the two low fertility sites (Honda A and B) and 1 km separating the two high fertility sites (Hornito and Alto Frio). A third soil type of intermediate fertility derived from andesite separates the high and low fertility sites in this study, and does not support populations of *Oreomunnea* (Andersen et al. 2010). Characteristics of our study sites are shown in Table 1, and methods for distinguishing potential effects of spatial proximity, fertility, and rainfall on community structure of EM fungi are described below.

Sampling of ectomycorrhizas

Root tips of *Oreomunnea* were collected at all sites between January and July 2012 (Table 2). At Honda A, Honda B, and Hornito, samples were collected from a total of 44 individuals per site: four adults per site (mean DBH=50 cm) located >50 m apart, five seedlings (5–20 cm height) within 20 m of each adult, and five saplings (40–100 cm height) within 20 m of each adult. The area where the trees were sampled was approximately 4500±3500 m^2 per site. Adults and juveniles of *Oreomunnea* were less common at Alto Frio, such that 17 individuals were sampled there (four adults, nine seedlings, and four saplings).

Five lateral roots were excavated 2–3 m from the trunk of each adult tree until fine roots that were clearly connected to the tree were found. From each adult we collected up to 50 cm of total root length representing multiple root branches. All roots obtained from adult trees were included in field

Table 2 Number of ectomycorrhizal root tips sequenced, root tips collected, individuals sampled, and OTUs observed for each site in Fortuna, and each developmental stage of *Oreomunnea*

Site	Honda A	Honda B	Hornito	Alto Frio	Total
Root tips sequenced/total root tips collected (individuals sampled)					
Adults	22/48 (4)	17/33 (4)	30/57 (4)	25/49 (4)	94/187 (16)
Saplings	30/52 (20)	35/67 (20)	19/54 (19)	4/10 (5)	88/183 (64)
Seedlings	12/25 (20)	15/34 (20)	14/30 (20)	11/14 (9)	52/103 (69)
Number of OTUs					
Adults	17	13	23	15	55
Saplings	26	25	18	4	59
Seedlings	11	13	12	7	39

collections even if EM fungal infection was not visible macroscopically. The entire root system of each focal seedling and sapling was collected.

Roots were stored in plastic bags and refrigerated within 2 h of collection. Each sample was carefully cleaned with tap water, cut into 1-cm pieces, and observed with a dissecting stereoscope. Three 1-cm pieces with EM structures were collected haphazardly from each sample and preserved in 95 % alcohol at 4 °C for DNA extraction. Infection frequency was calculated for each sample as the number of root tips among ten haphazardly chosen 1-cm fragments with presence of an EM mantle observed under a dissecting microscope.

Molecular analysis

Molecular analyses followed Peay et al. (2011) with slight modifications. Genomic DNA was extracted from EM root tips and the internal transcribed spacers and 5.8S rDNA of fungal associates was amplified directly using the REDExtract-N-Amp plant PCR kit (following the manufacturer's instructions; Sigma-Aldrich) with primers ITS1F and ITS4 or ITS4B. PCR conditions consisted of 95 °C for 1 min, and then 35 cycles of 95 °C for 30 s, 52 °C for 30 s, and 72 °C for 45 s, with a final extension time at 72 °C for 10 min. PCR amplicons were visualized on 1.5 % agarose gel stained with ethidium bromide or SYBR Green. Positive products were cleaned using ExoSap-IT (Affymetrix, Santa Clara, CA, USA; 1.5 μL Exosap, 7.5 μL PCR product) and sequenced bidirectionally using the Applied Biosystems BigDye Terminator v3.1 cycle sequencing kit and the original PCR primers on an Applied Biosystems 3730*xl* DNA Analyzer (Foster City, CA, USA) at the University of Arizona Genetics Core (UAGC). Sequences were assembled and quality scores were assigned using *phred* and *phrap* (Ewing and Green 1998; Ewing et al. 1998) with orchestration by Mesquite v. 1.06 (http://mesquiteproject.org), and then manually verified and edited in Sequencher 5.1 (Gene Codes Corporation, MI, USA) following U'Ren et al. (2012). Sequences were assigned to operational taxonomic units (OTUs) using a 97 % sequence similarity cutoff (see Smith et al. 2007a; Hughes et al. 2009) with Sequencher 5.1 (see Arnold et al. 2007; U'Ren et al. 2009).

Statistical analyses

Two-way ANOVA was used to assess the effect of developmental stage (seedling, sapling, and adult) and site (Honda A, Honda B, Hornito, and Alto Frio) on infection frequency. An alternative model was also assessed where sites were grouped into high fertility/low rainfall sites (Hornito and Alto Frio) and low fertility/high rainfall sites (Honda A and B). Species accumulation curves were used to compare OTU richness among developmental stages and sites. Total species richness was estimated using the bootstrap estimator (Smith and van Belle 1984; U'Ren et al. 2012).

To explore broader patterns of EM fungal diversity, we compiled records from EM fungal inventories of temperate and tropical forests, including studies reviewed by Tedersoo et al. (2012) and three more recent studies by Diédhiou et al. (2014), Smith et al. (2013), and Kennedy et al. (2012). We calculated diversity for data presented in each study using Fisher's alpha, which is robust to differences in sample size, and compared temperate versus tropical forests using a one-way ANOVA.

Differences in EM fungal community composition among sites and developmental stages were visualized by Nonmetric Multidimensional Scaling (NMDS). Only nonsingleton OTUs were used in these analyses, allowing us to evaluate the distributions of the more common species while reducing the potential for rare species, whose occurrence in the dataset may be influenced by undersampling, to influence inferences about composition. Nonetheless, results with and without singletons were very similar (results not shown). NMDS analyses were based on Bray-Curtis dissimilarity matrices using abundance and presence-absence data. Significance of visualized

differences was determined using permutational analyses of dissimilarity (ADONIS) using 200 permutations and a Euclidean distance matrix (Oksanen et al. 2008).

Because location, soil fertility, and rainfall patterns were correlated in this study, we used a statistical approach to explore the interplay of these factors with regard to observed community structure. A Mantel test based on 999 permutations first was used to examine the relationship of community composition to geographic distance among sites. However, geographic proximity also reflected environmental similarity (Fig. 1, Table 1). Therefore, a principal component analysis (PCA) was used to reduce 14 environmental variables (see Table 1) to two axes (describing 62.56 and 15.30 %, respectively, of the total variance). Environmental variables included in the PCA were site-specific annual rainfall from May 2012 to April 2013 and soil characteristics: bulk density (g cm^{-3}), total C, inorganic N, and P (mg cm^{-3}), NaOH–EDTA inorganic P (μg cm^{-3}), NaOH–EDTA organic P (μg cm^{-3}), resin P (μg cm^{-3}), NH_4 (μg cm^{-3}), NO_3 (μg cm^{-3}), K_2SO_4 extractable organic C (μg cm^{-3}), and base cations Al, Ca, and K (cmol (+) L^{-1}). The first two of the resulting PCA axes were used in a NMDS analysis, with correlation coefficients between the PCA axes and the NMDS axes identifying differences between sites with contrasting fertility and rainfall patterns (Ter Braak 1995). All statistical analyses were carried out using the package *vegan* 2.0-6 in R 2.15.1 (R Development Core Team 2011).

Taxonomic placement

Taxonomic placement of OTUs was estimated by comparisons via BLAST with GenBank (blastn; Altschul et al. 1990) and the UNITE database (Kõljalg et al. 2013). The databases gave matching results with high confidence at the genus level for 92 % of sequences (Table S1). The remaining 8 % of sequences showed <50 % query length and were left as undetermined (19 sequences representing 12 OTUs). Sequences that matched named sequences at 91–97 % identity in GenBank and UNITE were identified only to genus. Genus names were only assigned to OTUs when all sequences within the OTU returned species in the same genus after BLAST/UNITE searches. For *Russula*, species-level taxonomic placement of OTUs also was informed by phylogenetic inference including GenBank sequence data from vouchered and identified specimens (see below).

Phylogenetic analysis of *Russula* species

Preliminary collections of fruiting bodies in the study area indicated that *Russula* was common in the EM fungal community associated with *Oreomunnea*. Given that this genus is an important component of many EM fungal communities in the tropics (e.g., Peay et al. 2010; Smith et al. 2011; Tedersoo et al. 2011) and appears to shift in community composition with changes in soil N availability (Lilleskov et al. 2002; Avis et al. 2003; Avis et al. 2008), it was chosen to test hypotheses concerning phylogenetic diversity. To augment data on *Russula* distribution obtained from root tips, *Russula* fruiting bodies were collected every 2 weeks throughout the study period from January to July 2012 along four 50×4 m transects in each site. Transects were established in *Oreomunnea* stands at the same sites from which root tips were sampled, averaging 150 m (±110 m) linear distance from root tip sampling points. Macromorphology of fresh *Russula* fruiting bodies was recorded in the field, and a sample of tissue was preserved for DNA extraction. Sequences from fruiting bodies were obtained as described above. Vouchers of fruiting bodies are deposited at the University of Arizona Robert L. Gilbertson Mycological Herbarium (MYCO-ARIZ).

To evaluate the structure of *Russula* communities as a function of *Oreomunnea* developmental stage and soil fertility/rainfall level, we inferred phylogenetic relationships of species within the genus using 109 sequences representing root tips and fruiting bodies collected in this study (Table S2), and 32 sequences downloaded from GenBank. Sequences were selected from GenBank only if they were obtained from vouchered and identified specimens, and if there was a >90 % BLAST match with one or more sequences from the study site. This approach permitted us to select high-quality, fully identified sequences from GenBank to estimate taxonomic placement of *Oreomunnea*-associated species. Four sequences from voucher specimens of *Stereum hirsutum* (Willd.) Pers. (AY854063), *Amylostereum laevigatum* (Fr.) Boidin (AY781246), *Gloeocystidiellum porosum* (Berk. & M.A. Curtis) Donk (AY048881), and *Bondarzewia montana* (Quél.) Singer (DQ200923) were used for the outgroup following Miller and Buyck (2002) and Buyck et al. (2008).

Sequences were aligned using MUSCLE (Edgar 2004). The resulting alignment was edited using Gblocks 0.91b (Castresana 2002) to exclude positions that were poorly or ambiguously aligned. The final data set consisted of 639 characters and 144 terminal taxa. The tree was inferred using maximum likelihood analysis using the GTR+I+Gamma model of evolution in GARLI 2.0 (Zwickl 2006). Support was assessed using 1000 bootstrap replicates.

The resulting phylogenetic tree was used as input for two subsequent analyses. First, we calculated Faith's phylogenetic diversity index (PD) using the package Picante 1.5-2 in R (Kembel et al. 2010) to compare the phylogenetic diversity of *Russula* within each fertility/rainfall environment (i.e., alpha diversity). Faith's PD, defined as the sum of the branch lengths connecting all taxa within a local community (Faith 1992), was calculated based on random subsets of 19 OTUs (the smallest number of *Russula* OTUs recovered per site).

Second, UniFrac permutation analysis (Lozupone et al. 2006, 2010) was used to determine whether the phylogenetic structure of communities differed in a manner consistent with environmental filtering. For comparisons among communities associated with different sites, soil fertility/rainfall conditions, or developmental stages of *Oreomunnea*, the lengths of branches in the phylogeny that were unique to each community were calculated and compared to 50 permutations in which assignment of taxa to communities was randomized. If the environment or developmental stage selects for fungi that share phylogenetically conserved traits, then we would expect communities from similar environments or stages to share more of their branch length (i.e., be more closely related) than the random expectation (Lozupone et al. 2006).

Results

Ectomycorrhizal fungi were common in roots of *Oreomunnea* at all developmental stages and sites at Fortuna, Panama. Seedlings, saplings, and adults of *Oreomunnea* did not differ significantly in infection frequency (i.e., the percentage of root tips with visible EM infection) (F=2.69, df=2, 160, P=0.07), although a trend suggested somewhat lower incidence in seedlings (Fig. 2).

Infection frequency by EM fungi differed significantly among sites (F=2.88, df=3, 159, P=0.04), reflecting a significantly higher infection frequency at Alto Frio than Honda B (Tukey HSD P=0.043). Consistent with the differences among sites, we observed significant differences in infection frequency when the sites were grouped by soil fertility/rainfall (P=0.0195; Fig. 2). There was no evidence that variation in infection frequency reflected a meaningful interaction of site and developmental stage (F=0.16, df=6, 151, P=0.98).

Richness and diversity of EM fungi

In total, 473 mycorrhizal root tips were collected from adult trees, saplings, and seedlings. Sequences obtained from 234 root tips (49.3 %; Table 2) yielded 115 OTUs (Fig. 3). Overall EM fungal diversity was high (Shannon-Wiener Index, H'= 4.56; Simpson's Index, 1-D=0.99, Fisher's alpha=89.5; Table 3; see also Fig. 3).

Diversity of EM fungi was similar among developmental stages of *Oreomunnea* (Table 4, Fig. 4). In contrast to our prediction, EM fungal diversity was also similar among sites, with no apparent relationship to soil fertility/rainfall (Table 4, Fig. 4). Although the community of EM fungi was highly diverse, accumulation curves were asymptotic once singletons were removed (Fig. 4).

Community composition of EM fungi

Consistent with our prediction, we found that communities of EM fungi did not differ as a function of *Oreomunnea* developmental stage (ADONIS; F=1.09, df=2, 30, P=0.34). However, ADONIS revealed significant differences in EM fungal community composition among sites (F=1.81, df=3, 29, R^2=0.17, P=0.005). Only one species (*Laccaria* sp. 4) was found in all sites. Six OTUs occurred in at least three sites (Table S1). Overall community composition was most similar between Honda A and Honda B, which shared 16 OTUs.

NMDS suggested differences in the EM fungal community as a function of soil fertility/rainfall: the low fertility/high rainfall sites (Honda A and B) grouped separately from the high fertility/low rainfall sites (Hornito and Alto Frio) (Fig. 5). However, the Mantel test also revealed a significant, positive correlation between geographic proximity and community similarity (R=0.61, P=0.001). Because geographic proximity is positively associated with environmental similarity in our

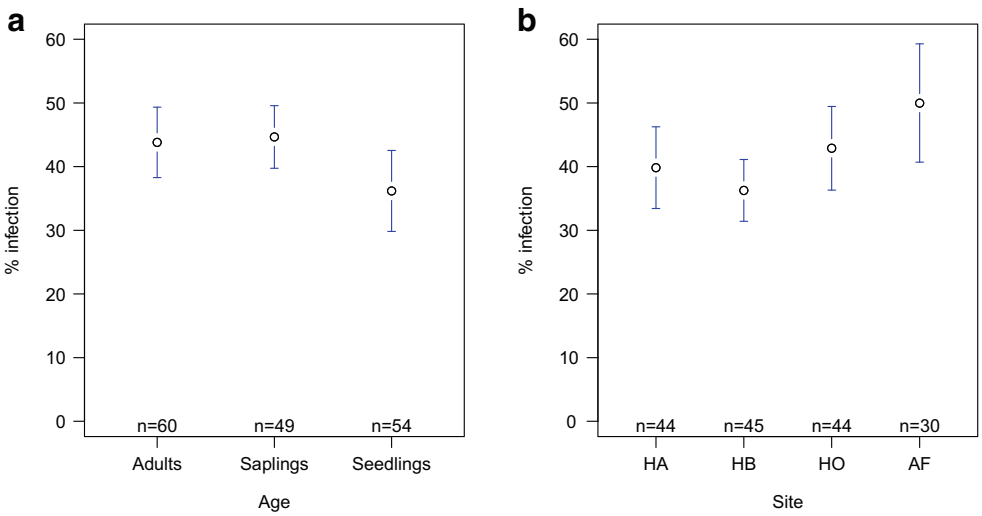

Fig. 2 Infection frequency (i.e., percent of root tips visibly infected by ectomycorrhizal fungi) in roots of *Oreomunnea mexicana* adults, saplings, and seedlings (**a**) in four study sites (**b**) in Fortuna, Panama. *Error bars* indicate the 95 % confidence interval around the mean. Honda A (*HA*) and Honda B (*HB*) are low fertility/high rainfall sites; Alto Frio (*AF*) and Hornito (*HO*) are high fertility/low rainfall sites. Sample sizes (*n*) indicate the number of root tips sequenced

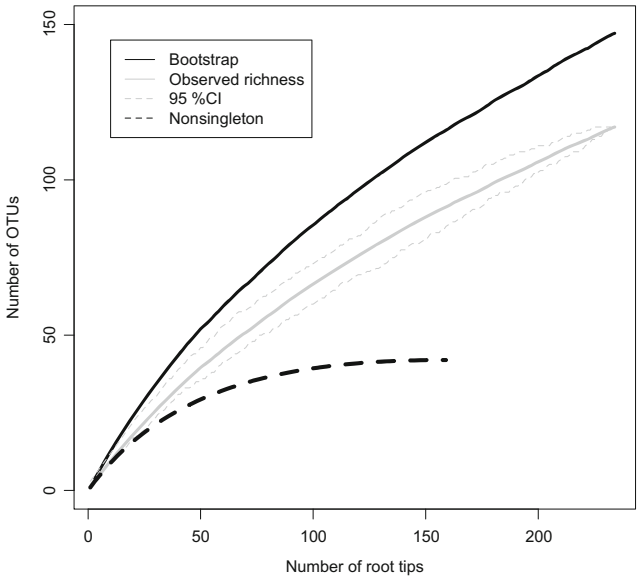

Fig. 3 Species (OTU) accumulation curves of EM fungi colonizing root tips of *Oreomunnea mexicana* at Fortuna. OTUs were based on 97 % sequence similarity. The analysis includes all sequences obtained in the present study. The *gray solid line* indicates observed OTU richness; *dotted gray lines* represent the 95 % confidence interval around observed richness; the *black solid line* indicates the bootstrap estimate of total species richness; the *dashed black line* indicates the accumulation curve for nonsingleton OTUs

study, the relative importance of spatial and environmental factors is difficult to interpret. We therefore examined the importance of environmental factors alone by evaluating correlations of PCA axes with NMDS. Only the first PCA axis (PC1) was significantly correlated with the first two axes of the NMDS (R^2=0.69, P=0.001; Fig. 5). PC1 accounted for 62.5 % of the variation in environmental variables and was negatively associated with soil fertility and positively associated with annual rainfall. Correlation of the PCA axis with the NMDS suggests environmental filtering driven by rainfall and/or soil fertility influences EM fungal community composition in addition to geographic proximity.

Taxonomic placement of EM fungi

Overall, 99 % of sequenced root tips were colonized by EM fungi belonging to 13 lineages sensu Tedersoo et al. (2010b): /amanita, /byssocorticium, /boletus, /clavulina, /laccaria, /cortinarius, /elaphomyces, /cantharellus, /coltricia, /inocybe, /russula-lactarius, /sebacina, /tomentella-thelephora, and /tricholoma. The most OTU-rich lineages were /russula-lactarius (36 OTUs from 97 root tips), /tomentella-thelephora (25 OTUs from 39 root tips), /cortinarius (14 OTUs from 27 root tips), /boletus (10 OTUs from 12 root tips), and /laccaria (5 OTUs from 17 root tips). Three root tips were colonized by members of the Strophariaceae, Marasmiaceae, and a genus of Atheliaceae considered to be saprotrophic; these were excluded from further analysis.

Russula, *Tomentella*, and *Laccaria* were present in all sites. *Laccaria* was especially abundant in the high fertility/low rainfall sites (Table S1). *Cortinarius* was not observed in Alto Frio and was rarely observed in Hornito (high fertility/low rainfall sites), but was abundant in Honda A and B, making up 16 and 26 % respectively, of the total number of sequenced root tips. *Boletus* and *Clavulina* were found only in low fertility/high rainfall sites (Honda A, Honda B), but were not common.

The four most abundant OTUs represented *Russula* (16 % of sequences). These included one unidentified species (*Russula* sp. 2), *Russula cyanoxantha* (*Russula* sp. 3), *Russula puellaris*, and *Russula* cf. *pectinata* (see Table S1 for information about OTUs and taxonomic distributions across sites). *Russula* spp. were found in association with roots of adults, saplings, and seedlings (Fig. 6).

Phylogenetic diversity of *Russula*

The most commonly found clades within Russula included representatives from roots as well as fruiting bodies (Fig. S1). In some clades, BLAST matches were consistent with estimated taxonomy based on phylogenetic analysis, but in other cases taxonomic placement at the species level differed between the two approaches. For example, one of the more common morphotypes is related to *R. cyanoxantha* (sequences from this study form a clade with identified vouchers from Hibbett (GQ452059) and Smith et al. (2007b) (DQ974758)).

Table 3 Diversity of the EM fungal community for each site and for all developmental stages of *Oreomunnea*

Index	Honda A	Honda B	Hornito	Alto Frio	Seedlings	Saplings	Adults
Simpson	0.94	0.94	0.95	0.94	0.97	0.97	0.97
Shannon	3.07	3.07	3.09	2.57	3.67	3.64	3.61
Fisher's alpha	73.3	33.9	64.7	28.5	79.4	78.3	55.5
PD	0.87	0.99	1.33	0.98			

Simpson and Shannon indexes are based on 99 randomizations and restricted to 24 samples per site and 42 samples per developmental stage (consistent with the minimum number of samples recovered per site or stage; see Table 2). Faith's phylogenetic diversity index (PD) was calculated based on sampling 19 *Russula* OTUs per site

Table 4 Comparison of the results of this study with other studies of ectomycorrhizal species in tropical and temperate biomes (adapted from Tedersoo et al. 2012)

Reference	Country	Lat	Lon	Elev	Host	MAP	Fisher's alpha	*N*	Number of species
Corrales et al. (this study)	Panama	9°02′91″ N	82°32′46″ W	1000	Juglandaceae	4000	89.5	234	115
Diédhiou et al. (2014)	Southern Guinea	8°51′ and 7°60′ N	9°31′ and 8°49′ W	500–1752	Fabaceae	2500–3000	182.1	332	189
Diédhiou et al. (2010)	Guinea	8°50′60″ N	9°31′01″ W	600	Fabaceae	3000	16.9	370	53
Morris et al. (2009)	Mexico	18°36′ N	99°36′ W	2450–2550	Fagaceae	1200–1500	27.1	8000	154
Phosri et al. (2012)	Thailand	16°51′14″ N	100°31′4″ E	160	Dipterocarpaceae	1250	38.3	194	69
Peay et al. (2010)	Malaysia	4°19′59″ N	113°49′59″ E	140	Dipterocarpaceae	3000	37.7	589	106
Smith et al. (2011)	Guyana	5°16′1.2″ N	59°50′24″ W	710	Fabaceae	3866	34.5	1020	118
Tedersoo et al. (2007)	The Seychelles	4°41′17″ S	55°29′6″ E	480	Dipterocarpaceae	3500	4.3	135	15
Tedersoo et al. (2010a)	Ecuador	0°41′ S	76°24′0″ W	232	Caryophyllales	3081	20.4	105	37
Smith et al. (2013)	Guyana	5°26′21″ N	60°04′43″	800	Dipterocarpaceae and Fabaceae	2000–>2400 mm	19.8	255	52
Temperate studies									
Avis et al. (2003)	USA	45°25′ N	93°10′ W	450	*Quercus*/*Corylus*	790	20.7	648	72
Avis et al. (2008)	USA	41° 37′51″ N	87°05′13″ W	206	*Quercus*	945	129.5	1333	314
Bahram et al. (2011)	Estonia	58°17′ N	27°19′ E	35	*Populus*	620	312.55	122	103
Bahram et al. (2012)	Iran	36°27′ N	51°06′ E	100–2700	Several spp	237–552	141.34	1755	367
Bergemann and Garbelotto (2006)	USA	40°00′30″ N	123°57′00″ W	580	*Lithocarpus*	1125	59.28	382	119
Courty et al. (2008)	France	48°75′ N	6°35′ E	250	*Quercus*	744	48.3	180	75
Dickie et al. (2010)	New Zealand	43°9′12″ S	171°43′48″ E	1000	*Pinus* and *Nothofagus*	1447	61.98	354	118
Douglas et al. (2005) (Lodgepole pine site)	USA	44°27′21″ N	110°29′34″ W	2430	*Pinus contorta*	510	13.43	5570	81
Douglas et al. (2005) (mixed conifer site)	USA	44°27′21″ N	110°29′34″ W	2430	Several spp	510	4.93	5933	35
Gao and Yang (2010)	China	27°50′ N	99°24′ E	4300	*Kobresia* spp	2000	48.11	150	70
Ishida et al. (2007)	Japan	35°56′–35°57′ N	138°48′–138°49′ E	1350–1500	Several spp	1596	66.25	1396	205
Izzo et al. (2005)	USA	36°58′ N	119°2′ W	2100	Several spp	1250	27.05	1105	101
Jones et al. (2008)	Canada	50°18′43 N	125°28′29 W	37–160	Several spp	2240	31.48	138	53
Kennedy et al. (2003)	USA	37°54′ N	122°37′ W	600	Several spp	1250	16.5	442	56
Kennedy et al. (2012)	USA	42°10′52 N	122°43′85 W	1082	*Arbutus*/Pinaceae	611	51.85	537	126
Kjøller and Clemmensen (2009)	Sweden	57°56′	13°47′	132–300	*Pinaceae*	582–818	30.72	969	107
Krpata et al. (2008)	Austria	46°33′ N	13°42′ E	578	*Populus tremula*	1297	7.28	12,020	54
Lang et al. 2011	Germany	51°05′20″ N	10°31′24″ E	350	Several spp	670	14.83	94,893	130
Lian et al. (2006)	Japan	39°56′ N	141°14′ E	360–380	*Pinus densiflora*	1145	5.67	5499	39
Mühlmann and Peintner (2008a, b); Mühlmann et al. (2008)	Austria	46°50′ N	11°01′ E	2280–2450	Several spp		6.86	10,000	50
Nara (2006)	Japan	35°19′ N	138°11′ E	1450–1600	Several spp	4854	4.99	6698	36

Table 4 (continued)

Reference	Country	Lat	Lon	Elev	Host	MAP	Fisher's alpha	*N*	Number of species
Palmer et al. (2008)	USA	43°57′ N	91°2′ W	275	Several spp	820	17.13	233	46
Parrent and Vilgalys (2007)	USA	35°57′ N	79°7′ W	130	*Pinus taeda*	1140	33.55	1787	134
Pena et al. (2010)	Germany	47°59′ N	8°45′ E	800	*Fagus sylvatica*	776	31.03	At least 515	89
Richard et al. (2005)	France	42°20′ N	8°49′ E		Several spp	750	77.73	393	140
Richard et al. (2011)	France	43°44′29″ N	3°35′45″ E	270	*Quercus ilex*	908	38.11	1147	131
Roy et al. (2013)	France	41°59′–45°46′ N	0°38′–9°17′ E		*Alnus* spp	284	21.34	1178	86
Ryberg et al. (2009)	Sweden	68°21′ N	18°30′ E	1010–1040	*Dryas octopetala*, *Salix reticulata*	850	27.08	389	74
Ryberg et al. (2010)	Sweden	68°20′ N	18°30′ E	980	Several spp	847	13.50	154	34
Smith et al. (2004)	USA	43.5° N	118.5° W	1600–1700	*Pinus ponderosa*, *Cercocarpus ledifolius*	173	66.44	480	140
Smith et al. (2005)	USA	45.4° N	117.3° W	1300–1600	Several spp	642	92.25	543	178
Smith et al. (2007b, 2009); Morris et al. (2008)	USA	39°17′ N	121°17′ W	400–600	Several spp	710	30.65	11,590	182
Taniguchi et al. (2007)	Japan	35°32′ N	134°13′ E		Several spp	1083	11.79	284	38
Tedersoo et al. (2003)	Estonia	58°17′ N	27°19′ E	36	Several spp	620	43.22	85	47
Tedersoo et al. (2006)	Estonia	58°27′ N	22°00′ N	8	Several spp	550	98.15	468	172
Toljander et al. (2006)	Sweden	64°39′ N	18°30′ E	235	Several spp	570	12.50	2442	66
Twieg et al. (2007)	Canada	50°22′–50°58′	118°32′–119°23′	500–1200	*Pseudotsuga menziesii*, *Betula papyrifera*	663	30.31	938	105
Walker et al. (2005)	USA	35°02′29″ N	83°27′16″ W		*Quercus rubra*, *Q. prinus*	1800	32.72	291	75

Columns indicate latitude in degrees (Lat), longitude in degrees (Lon), elevation in meters (Elev), dominant host lineage (Host), Mean Annual Precipitation in millimeters (MAP), diversity (Fisher's alpha), number of samples analyzed (*N*), and number of ectomycorrhizal species found in study site (OTUs based on 97% sequence similarity)

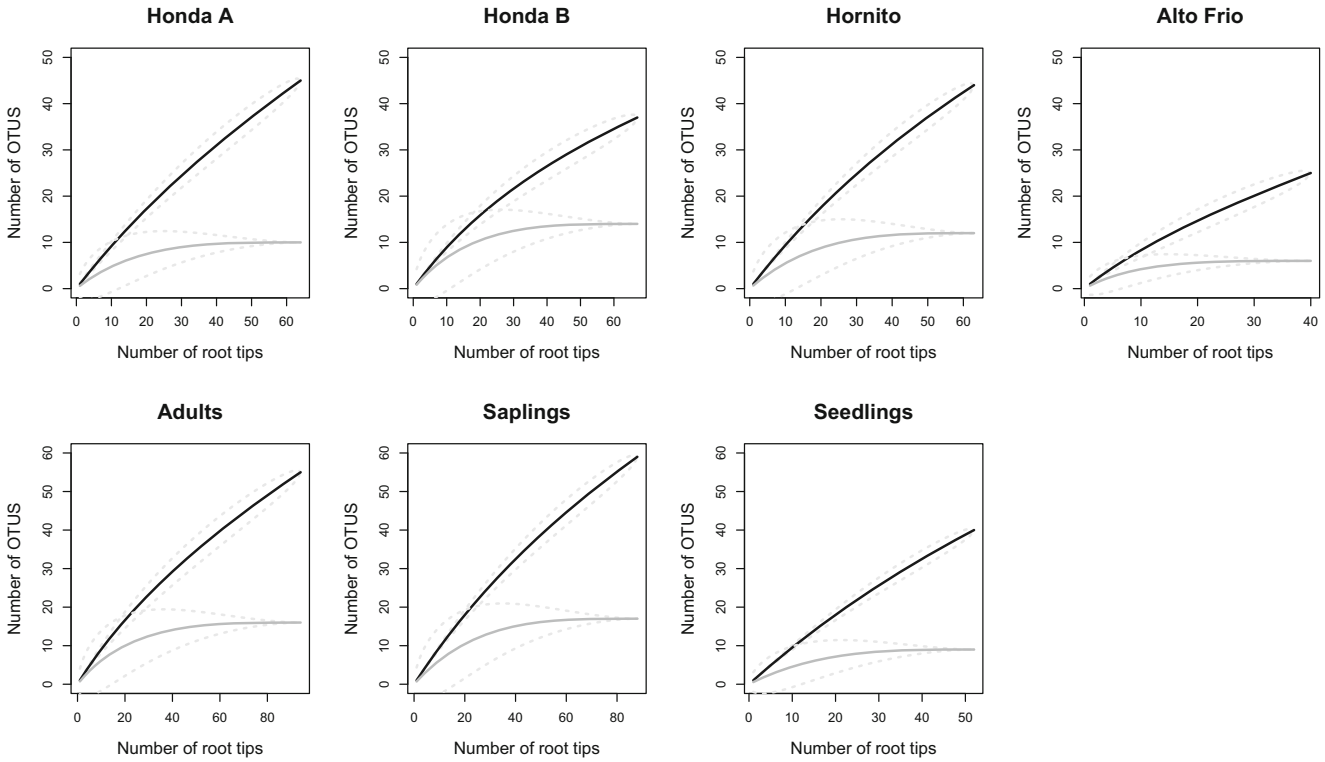

Fig. 4 Ectomycorrhizal species accumulation curves and 95 % confidence intervals based on 1) All OTUs (*black*) and 2) excluding singletons (*gray*) OTUs derived from 97 % sequence similarity. *Upper panel*: accumulation curves per site for Honda A, Honda B, Hornito, and Alto Frio. *Lower panel*: accumulation curves for three developmental stages: seedlings 5–20 cm in height, saplings 40–100 cm in height, and adults

Phylogenetic diversity of *Russula* did not differ as a function of fertility/rainfall ($PD_{high\ fertility/low\ rainfall}$=2.15, $PD_{low\ fertility/high\ rainfall}$=3.53, t=−0.9521, df=2, P=0.506) (Table 4). We found no significant structure in the *Russula* phylogeny as a function of *Oreomunnea* developmental stage (Fig. S1), either when comparing adults and seedlings (UniFrac analysis, P=0.29) or seedlings and saplings (P=0.73). However, consistent with our prediction, the phylogenetic composition of *Russula* communities differed significantly among sites (P<0.001) and when sites were grouped according to fertility/rainfall (P<0.001). Significant differences were observed between Alto Frio and Honda A (P<0.001), Alto Frio and Honda B (P<0.001), and Honda B and Hornito (P=0.01–0.05), but not between sites with similar fertility/rainfall.

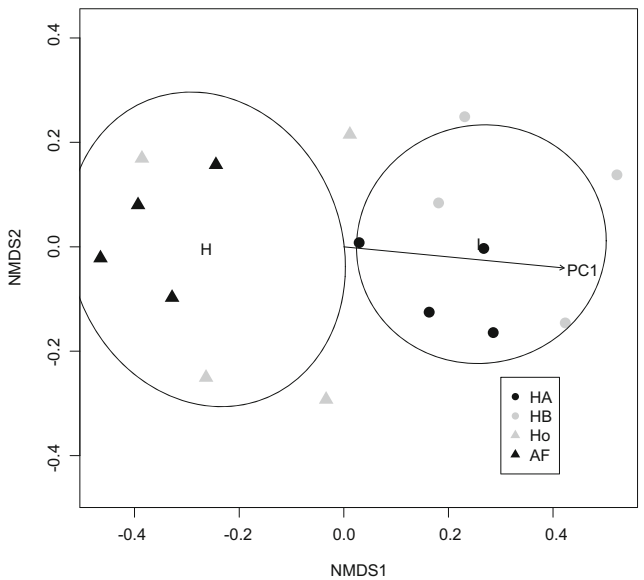

Fig. 5 Nonmetric multidimensional scaling (NMDS) plot showing differences in the EM fungal community composition among sites that differ in fertility and rainfall (high fertility/low rainfall—*triangles*; low fertility/high rainfall—*circles*). The first PCA axis of environmental variables (PC1) was significantly correlated with compositional variation (R^2=0.69, P<0.001; see text for further explanation). Stress=0.116

Discussion

This is the first detailed inventory of EM fungi associated with the widely distributed neotropical tree *O. mexicana* and one of the few analyses of EM fungal community diversity in a tropical montane forest. Ectomycorrhizal tree species that have been studied in detail in the neotropics include *Dicymbe corymbosa* (Fabaceae), *Pakaraimaea dipterocarpacea* (Dipterocarpaceae), *Quercus crassifolia* and *Q. laurina* (Fagaceae), and *Coccoloba* spp. (Henkel 2003; Miller et al. 2000; Moyersoen 2006; Morris et al. 2009; Tedersoo et al.

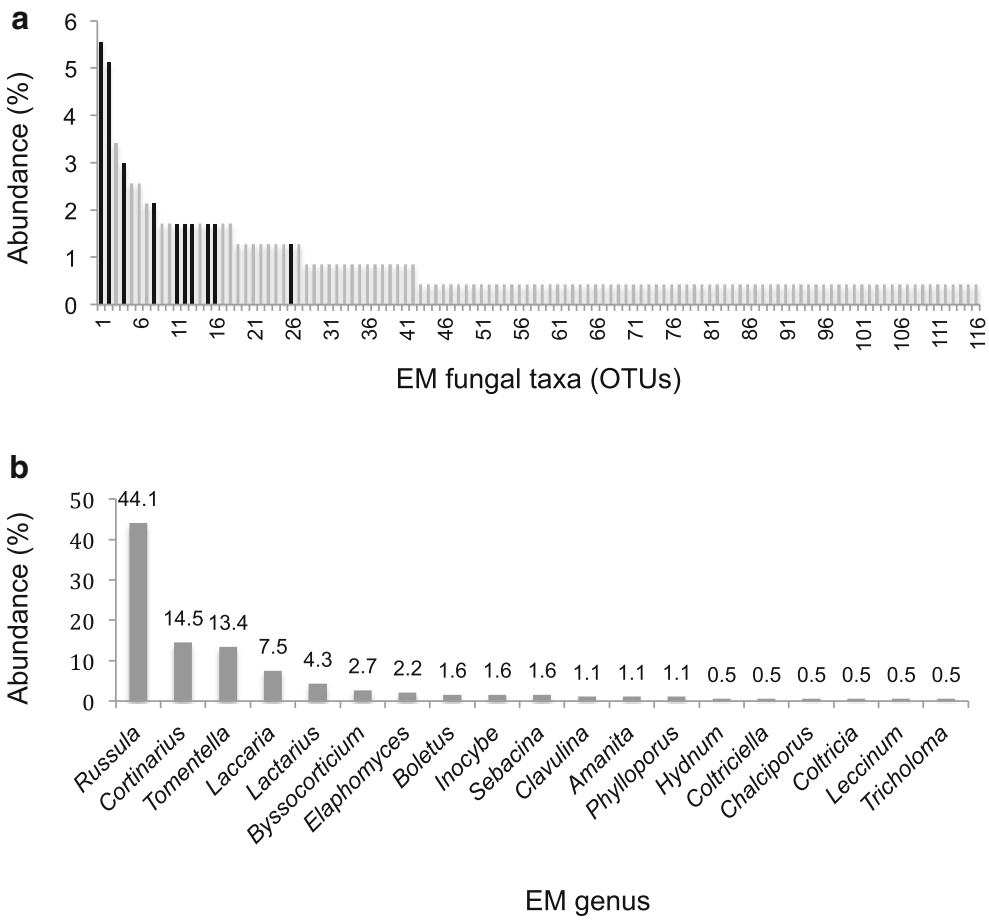

Fig. 6 **a** Relative abundance of OTUs based on 97 % sequence similarity. Columns in *black* show OTUs infecting adults, saplings, and seedlings. **b** Relative abundance of fungal genera

2010a; Smith et al. 2011, 2013). Our study adds new data for a representative of the Juglandaceae in an area of high plant species richness, and dramatic local-scale differences in soil types and rainfall patterns.

Our results reveal that species diversity of EM fungi associated with *Oreomunnea* is high despite the small spatial scale at which this study was carried out. In conjunction with other inventories, our data suggest that tropical forest EM fungal communities can be as species-rich as those found in temperate forests (i.e., Smith et al. 2011; Henkel et al. 2012; Diédhiou et al. 2014). A comparison of EM fungal diversity in root tips using Fisher's alpha showed no significant difference between temperate forests (mean Fisher's alpha=46.87, SD=55.23) and tropical forests studied thus far (mean Fisher's alpha=47.06, SD=52.65; $F<0.001$, $df=1, 46$, $P=0.992$). When comparisons were restricted only to angiosperms, the results still did not differ significantly (temperate forests, mean Fisher's alpha=62.02, SD=80.97; $P=0.618$).

All of the major EM fungal clades *sensu* Tedersoo et al. (2010b) encountered in this study have been reported previously in both temperate and tropical EM fungi inventories (Peay et al. 2010; Smith et al. 2011; Tedersoo et al. 2011; Phosri et al. 2012; Diédhiou et al. 2014). Members of the /russula-lactarius, /cortinarius, and /tomentella-thelephora lineages were particularly abundant, accounting for 72 % of OTUs. An overall dominance by *Russula* has been found in other above- and belowground EM fungal inventories in tropical forests (Peay et al. 2010; Smith et al. 2011; Tedersoo et al. 2011; Henkel et al. 2012; Phosri et al. 2012; Diédhiou et al. 2014), suggesting that this is an important taxonomic group to focus on in future biogeographic and systematics-based studies.

Consistent with the few previous studies of EM fungal communities in tropical forests, we observed strong community dissimilarity of EM fungi across sites. For example, in *Dicymbe*-dominated forest in Guyana, Smith et al. (2011) found significant differences in EM fungal species composition among 19 sites with an average inter-site distance of 689 m. Peay et al. (2010) found significant clustering in community composition in sites with similar soil types in dipterocarp-dominated forest in Lambir Hills National Park, Sarawak (Malaysia). Our results suggest that variation in EM fungal communities at small spatial scales also may be a feature in montane tropical forests, expanding the scope for local turnover to greatly enhance alpha and beta diversity at small spatial scales.

Geographic distance and environmental similarity are confounded in our study area, as the distance between sites within the same soil/rainfall characteristics was small (0.2–1 km)

compared to the distance between sites with different characteristics (6 km). However, our analyses suggest an important role of environmental factors, here defined as soil fertility and rainfall patterns, in potentially filtering community structure.

At present, our sampling is not sufficient to define the relative importance of soil fertility versus rainfall patterns in shaping EM fungal communities in *Oreomunnea*. In general, high rainfall sites tend to have low nutrient availability due to increased leaching (Austin and Vitousek 1998); however, variation in fertility in our sites is determined by differences in underlying geology. Variation in fungal community composition may also in part be driven by dispersal limitation, which also could underlie a significant Mantel correlation between geographic distance and community dissimilarity. In spite of our inability to identify the exact environmental variables that drive EM fungal beta diversity, our study suggests that abiotic factors can drive high levels of species turnover in EM fungal communities that associate with the same host species.

Turnover in the EM fungal community as a whole was echoed by turnover in OTU assemblages among sites in key fungal genera. For example, even when singletons were removed, 14 of 21 *Russula* OTUs found at the high fertility/low rainfall sites were not found in either of the low fertility/high rainfall sites. UniFrac results also showed significant differences in the phylogenetic relatedness of *Russula* communities across sites consistent with environmental filtering: *Russula* communities associated with the two high fertility/low rainfall sites were less closely related with the ones found in low fertility/high rainfall sites than expected by chance.

Temperate *Russula*, particularly from the subsection Foetentineae, tend to inhabit more fertile habitats with relatively higher N availability (Avis et al. 2003; Avis et al. 2012) and have been reported to increase in abundance in response to N fertilization in permanent plots (N addition of 5.4 or 17 g N m^{-2} $year^{-1}$ in oak savanna; Avis et al. 2003). Several authors have also noted that EM fungi associated with N-rich habitats may form less beneficial or parasitic relationships with their hosts (Johnson et al. 1997; Egger and Hibbett 2004; Avis 2012). It is possible that less beneficial EM fungi reduce the competitive advantage of *Oreomunnea* relative to co-occurring tree species in the more fertile sites studied here (Table 1).

In contrast, all of the 14 species of *Cortinarius* observed here were found infecting root tips at the low fertility/high rainfall sites. Some *Cortinarius* species have been shown extract N from organic sources under conditions of low N availability (Taylor et al. 2000; Lilleskov et al. 2002; Avis et al. 2003). More research on functional traits may help us understand the influence of fertility and rainfall on fungal community composition, and its effect on associated plant communities.

We found that diversity and composition of EM fungal communities did not differ among developmental stages of *Oreomunnea*. Strikingly, some of the most abundant *Russula* OTUs were found in all stages, and UniFrac analyses revealed the similarity of *Russula* communities in both adults and seedlings, and seedlings and saplings. A similar result was found in seedlings and adults of EM tree species in Guinean tropical rain forest, where the OTUs infecting several developmental stages were the most abundant in the EM fungal community (Diédhiou et al. 2010).

Russula as a candidate for ectomycorrhizal network effects in *Oreomunnea*

Indirect evidence consistent with the existence of EM networks in tropical forest comes from experiments showing that hyphal exclusion increases the mortality and decreases the growth rate of EM seedlings (Onguene and Kuyper 2002; McGuire 2007). However, no study has yielded direct evidence of resource transfer (i.e., nutrients or water) to seedlings, nor identified the EM fungi potentially involved in such transfer, in tropical forest. Analyses of EM fungal composition is important, as EM networks effects are unlikely to strongly impact recruitment of host trees unless common EM fungal taxa infect both seedlings and adults. At Fortuna, network effects on *Oreomunnea* might be more likely to occur at the low fertility/high rainfall sites of Honda A and B, where seedling densities are exceptionally high, and seedling mortality rates are low below crowns (Table 1). In our surveys, OTUs representing *Russula cyanoxantha*, *Russula* sp3, *Russula puellaris*, and *Russula* cf. *pectinata* accounted for 24 % of sequences in the low fertility/high rainfall sites. Further studies might profitably target whether resource transfer to seedlings occurs via members of this group.

Although inventories of EM fungal fruiting bodies and root tips have been made in oak-dominated forests in Central America (Halling and Mueller 2005; Mueller et al. 2006; Morris et al. 2009), this is one of the first belowground surveys of EM fungi in a tropical montane forest and provides insight into the diversity of EM fungal species associated with *Oreomunnea* across sites varying in fertility and in the amount and seasonality of rainfall. The rationale for this project was to provide information for future experiments that will explicitly test for the existence of mycorrhizal networks and their effects on *Oreomunnea* seedling performance. This information will be useful to uncover the factors driving plant–soil feedback and EM host tree dominance in tropical montane forests.

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References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Andersen KM, Turner BL, Dalling JW (2010) Soil-based habitat partitioning in palms in lower montane tropical forests. J Biogeogr 37:278–292

Andersen KM, Endara MJ, Turner BL, Dalling JW (2012) Trait-based community assembly of understory palms along a soil nutrient gradient in a lower montane tropical forest. Oecologia 168:519–531

Arnold AE, Henk DA, Eells RL, Lutzoni F, Vilgalys R (2007) Diversity and phylogenetic affinities of foliar fungal endophytes in loblolly pine inferred by culturing and environmental PCR. Mycologia 99: 185–206

Austin AT, Vitousek PM (1998) Nutrient dynamics on precipitation gradient in Hawai'i. Oecologia 113:519–529

Avis PG, McLaughlin DJ, Dentinger BC, Reich PB (2003) Long-term increase in nitrogen supply alters above- and below-ground ectomycorrhizal communities and increases the dominance of *Russula* spp. in a temperate oak savanna. New Phytol 160:239–253

Avis PG, Mueller GM, Lussenhop J (2008) Ectomycorrhizal fungal communities in two North American oak forests respond to nitrogen addition. New Phytol 179:472–483

Avis PG (2012) Ectomycorrhizal iconoclasts: the ITS rDNA diversity and nitrophilic tendencies of fetid *Russula*. Mycologia 104:998–1007

Bahram M, Põlme S, Kõljalg U, Tedersoo L (2011) A single European aspen (*Populus tremula*) tree individual may potentially harbor dozens of *Cenococcum geophilum* ITS genotypes and hundreds of species of ectomycorrhizal fungi. FEMS Microbiol Ecol 75:313–320

Bahram M, Põlme S, Kõljalg U, Zarre S, Tedersoo L (2012) Regional and local patterns of ectomycorrhizal fungal diversity and community structure along an altitudinal gradient in the Hyrcanian forests of northern Iran. New Phytol 193:465–473

Becker (1983) Ectomycorrhizae on *Shorea* (Dipterocarpaceae) seedlings in a lowland Malaysian rainforest. Malay For 46:146–170

Béreau M, Garbaye J (1994) First observations on the root morphology and symbioses of 21 major tree species in the primary tropical rain forest of French Guyana. Ann Sci For 51:407–416

Bergemann SE, Garbelotto M (2006) High diversity of fungi recovered from the roots of mature tanoak (*Lithocarpus densiflorus*) in northern California. Can J Bot 84:1380–1394

Bever JD, Platt TG, Morton ER (2012) Microbial population and community dynamics on plant roots and their feedbacks on plant communities. Annu Rev Microbiol 66:265–83

Bonfante P, Genre A (2010) Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. Nat comm 1:48

Booth MG, Hoeksema JD (2010) Mycorrhizal networks counteract competitive effects of canopy trees on seedling survival. Ecology 91: 2294–2302

Buyck B, Hofstetter V, Eberhardt U, Verbeken A, Kauff F (2008) Walking the thin line between Russula and Lactarius: the dilemma of Russula subsect. Ochricompactae. Fungal Divers 28:15–40

Castresana J (2002) Gblocks server v. 0.91b, Institut de Biologia Evolutiva (CSIC-UPF). http://molevol.cmima.csic.es/castresana/Gblocks_server.html

Cavelier J (1996) Fog interception in montane forests across the central cordillera of Panama. J Trop Ecol 12:357–369

Connell JH, Lowman MD (1989) Low-diversity tropical rain forests: some possible mechanisms for their existence. Am Nat 134:88–119

Conway D, Alexander IJ (1992) Soil conditions under monodominant *Gilbertiodendron dewevrei* and mixed forest Ituri Forest Reserve, Zaire. Tropical Biology Newsletter 62:[unpaginated].

Courty PE, Franc A, Pierrat JC, Garbaye J (2008) Temporal changes in the ectomycorrhizal community in two soil horizons of a temperate oak forest. Appl Environ Microbiol 74:5792–5801

Dickie IA, Koide RT, Steiner KC (2002) Influences of established trees on mycorrhizas, nutrition, and growth of *Quercus rubra* seedlings. Ecol Monogr 72(4):505–521

Dickie IA, Bolstridge N, Cooper JA, Peltzer DA (2010) Co-invasion by *Pinus* and its mycorrhizal fungi. New Phytol 187:475–484

Diédhiou AG, Selosse MA, Galiana A, Diabate M, Dreyfus B, Ba AM, Miana de Faria S, Bena G (2010) Multi-host ectomycorrhizal fungi are predominant in a Guinean tropical rainforest and shared between canopy trees and seedlings. Environ Microbiol 12:2219–2232

Diédhiou AG, Christelle H, Ebenye M, Selosse MA, Onguene N, Ba AM (2014) Diversity and community structure of ectomycorrhizal fungi in mixed and monodominant African tropical rainforest. In: Bâ AM, McGuire KL, Diédhiou AG (eds) Ectomycorrhizal symbioses in tropical and neotropical forests. CRC Press, pp 1–18

Douglas RB, Parker VT, Cullings KW (2005) Belowground ectomycorrhizal community structure of mature lodgepole pine and mixed conifer stands in Yellowstone National Park. For Ecol Manag 208:303–317

Edgar R (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797

Egger KN, Hibbett DS (2004) The evolutionary implications of exploitation in mycorrhizas. Can J Bot 82:1110–1121

Ewing B, Green P (1998) Base-calling of automated sequencer traces using *Phred.* II. Error probabilities. Genome Res 8:186–194

Ewing B, Hillier L, Wendl M, Green P (1998) Base-calling of automated sequencer traces using *Phred*. I. Accuracy assessment. Genome Res 8:175–185

Faith DP (1992) Conservation evaluation and phylogenetic diversity. Biol Conserv 61:1–10

Gao Q, Yang ZL (2010) Ectomycorrhizal fungi associated with two species of *Kobresia* in an alpine meadow in the Western Himalaya. Mycorrhiza 20:281–287

Halling RE, Mueller GM (2005) Common mushrooms of the Talamanca Mountains. New York Botanical Garden Press, Bronx, NY, Costa Rica

Hart TB, Hart JA, Murphy PG (1989) Monodominant and species-rich forests in the humid tropics: causes for their co-occurrence. Am Nat 133:613–633

Henkel TW (2003) Monodominance in the ectomycorrhizal *Dicymbe corymbosa* (Caesalpiniaceae) from Guyana. J Trop Ecol 19:417–437

Henkel TW, Aime MC, Chin MML, Miller SL, Vilgalys R, Smith ME (2012) Ectomycorrhizal fungal sporocarp diversity and discovery of new taxa in *Dicymbe* monodominant forests of the Guiana Shield. Biodivers Conserv 21:2195–2220

Hughes KW, Petersen RH, Lickey EB (2009) Using heterozygosity to estimate a percentage DNA sequence similarity for environmental species' delimitation across basidiomycete fungi. New Phytol 182: 795–798

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

Itoh A (1995) Regeneration processes and coexistence mechanisms of two Bornean emergent dipterocarp species. Doctorate thesis, Kyoto University, Kyoto

Izzo A, Agbowo J, Bruns TD (2005) Detection of plot level changes in ectomycorrhizal communities across years in an old-growth mixed-conifer forest. New Phytol 166:619–629

Janos DP (1983) Tropical mycorrhizas, nutrient cycles and plant growth. In: Sutton SL, Whitmore TC, Chadwick AC (eds) Tropical rain forest: ecology and management. Blackwell Scientific Publications, Oxford, pp 327–345

Johnson NC, Graham JH, Smith FA (1997) Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phytol 135:575–585

Jones MD, Twieg BD, Durall DM, Berch SM (2008) Location relative to a retention patch affects the ECM fungal community more than patch size in the first season after timber harvesting on Vancouver Island, British Columbia. For Ecol Manag 255:1342–1352

Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP, Webb CO (2010) Picante: R tools for integrating phylogenies and ecology. Bioinforma Appl Note 26:1463–1464

Kennedy PG, Izzo AD, Bruns TD (2003) There is high potential for the formation of common mycorrhizal networks between understory and canopy trees in a mixed evergreen forest. J Ecol 91:1071–1080

Kennedy PG, Smith DP, Horton TR, Molina R (2012) *Arbutus menziesii* (Ericaceae) facilitates regeneration dynamics in mixed evergreen forest by promoting mycorrhizal fungal diversity and host connectivity. Am J Bot 99:1691–1701

Kjøller R, Clemmensen KE (2009) Belowground ectomycorrhizal fungal communities respond to liming in three southern Swedish coniferous forest stands. For Ecol Manag 257:2217–2225

Kõljalg U et al (2013) Towards a unified paradigm for sequence-based identification of fungi. Mol Ecol 22:5271–5277

Krpata D, Peintner U, Langer I, Fitz WJ, Schweiger P (2008) Ectomycorrhizal communities associated with *Populus tremula* growing on a heavy metal contaminated site. Mycol Res 112: 1069–1079

Lian C, Narimatsu M, Nara K, Hogetsu T (2006) *Tricholoma matsutake* in a natural *Pinus densiflora* forest: correspondence between above- and below-ground genets, association with multiple host trees and alteration of existing ectomycorrhizal communities. New Phytol 171:825–836

Lilleskov EA, Fahey TJ, Horton TR, Lovett GM (2002) Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. Ecology 83:104–115

Lozupone C, Hamady M, Knight R (2006) UniFrac—an online tool for comparing microbial community diversity in a phylogenetic context. BMC Bioinformatics 7:371

Lozupone C, Lladser ME, Knights D, Stombaugh J, Knight R (2010) UniFrac: an effective distance metric for microbial community comparison. ISME J 5:169–72

Malloch DW, Pirozynski KA, Raven PH (1980) Ecological and evolutionary significance of mycorrhizal symbioses in vascular plants (A Review). Proc Natl Acad Sci 77:2113–2119

McGuire KL (2007) Common ectomycorrhizal networks may maintain monodominance in a tropical rain forest. Ecology 88:567–574

McGuire KL (2008) Ectomycorrhizal associations function to maintain tropical monodominance. In: Siddiqui ZA et al. (eds) Mycorrhizae: sustainable agriculture and forestry, Springer Science, pp 287–302

Miller SL, Buyck B (2002) Molecular phylogeny of the genus Russula in Europe with a comparison of modern infrageneric classifications. Mycol Res 106:259–276

Miller OK, Lodge DJ, Baroni TJ (2000) New and interesting ectomycorrhizal fungi from Puerto Rico, Mona, and Guana Islands. Mycologia 92:558–570

Morris MH, Smith ME, Rizzo DM, Rejmanek M, Bledsoe CS (2008) Contrasting ectomycorrhizal fungal communities on the roots of co-occurring oaks (*Quercus* spp.) in a California woodland. New Phytol 178:167–176

Morris MH, Pérez-Pérez MA, Smith ME, Bledsoe CS (2009) Influence of host species on ectomycorrhizal communities associated with two co-occurring oaks (*Quercus* spp.) in a tropical cloud forest. FEMS Microbiol Ecol 69:274–287

Moyersoen B (2006) *Pakaraimaea dipterocarpacea* is ectomycorrhizal, indicating an ancient Gondwanaland origin for the ectomycorrhizal habit in Dipterocarpaceae. New Phytol 172:753–762

Mueller GM, Halling RE, Carranza J, Mata M, Schmit JP (2006) Saprotrophic and ectomycorrhizal macrofungi of Costa Rican oak forests. In: Kappelle M (ed) Ecology and conservation of neotropical montane oak forests. Ecological Series 185. Springer, Heidelberg, pp 55–68

Mühlmann O, Peintner U (2008a) Ectomycorrhiza of *Kobresia myosuroides* at a primary successional glacier forefront. Mycorrhiza 18:355–362

Mühlmann O, Peintner U (2008b) Mycobionts of *Salix herbacea* on a glacier forefront in the Austrian Alps. Mycorrhiza 18:171–180

Mühlmann O, Bacher M, Peintner U (2008) *Polygonum viviparum* mycobionts on an alpine primary successional glacier forefront. Mycorrhiza 18:87–95

Nara K (2006) Pioneer dwarf willow may facilitate tree succession by providing late colonizers with compatible ectomycorrhizal fungi in a primary successional volcanic desert. New Phytol 171:187–198

O'Brien MJ, Gomola CE, Horton TR (2010) The effect of forest soil and community composition on ectomycorrhizal colonization and seedling growth. Plant Soil 341:321–331

Oksanen L, Kindt R, Legendre P, O'Hara B, Simpson GL, Solymos P, Henry M, Stevens H, Wagner H (2008) VEGAN: Community ecology package. R package version 1.15-1. http://cran.r-project.org/, http://vegan.r-forge.r-project.org/

Onguenea NA, Kuyper TW (2001) Mycorrhizal associations in the rain forest of South Cameroon. For Ecol Manag 140:277–287

Onguene NA, Kuyper TW (2002) Importance of the ectomycorrhizal network for seedling survival and ectomycorrhiza formation in rain forests of south Cameroon. Mycorrhiza 12:13–17

Palmer JM, Lindner DL, Volk TJ (2008) Ectomycorrhizal characterization of an American chestnut (*Castanea dentata*)-dominated community in Western Wisconsin. Mycorrhiza 19:27–36

Parrent JL, Vilgalys R (2007) Biomass and compositional responses of ectomycorrhizal fungal hyphae to elevated CO_2 and nitrogen fertilization. New Phytol 176:164–174

Peay KG, Kennedy PG, Davies SJ, Tan S, Bruns TD (2010) Potential link between plant and fungal distributions in a dipterocarp rainforest: community and phylogenetic structure of tropical ectomycorrhizal fungi across a plant and soil ecotone. New Phytol 185:529–542

Peay KG, Kennedy PG, Bruns TD (2011) Rethinking ectomycorrhizal succession: are root density and hyphal exploration types drivers of spatial and temporal zonation? Fungal Ecol 4:233–240

Pena R, Offermann C, Simon J, Naumann PS, Gessler A, Holst J, Dannenmann M, Mayer H, Kögel-Knabner I, Rennenberg H, Polle A (2010) Girdling affects ectomycorrhizal fungal (EMF) diversity and reveals functional differences in EMF community composition in a beech forest. Appl Environ Microbiol 76:1831–1841

Peh KSH, Lewis SL, Lloyd J (2011) Mechanisms of monodominance in diverse tropical tree-dominated systems. J Ecol 99:891–898

Phosri C, Põlme S, Taylor AFS, Kõljalg U, Suwannasai N, Tedersoo L (2012) Diversity and community composition of ectomycorrhizal fungi in a dry deciduous dipterocarp forest in Thailand. Biodivers Conserv 21:2287–2298

Plamboeck AH, Dawson TE, Egerton-Warburton LM, North M, Bruns TD, Querejeta JI (2007) Water transfer via ectomycorrhizal fungal hyphae to conifer seedlings. Mycorrhiza 17:439–447

Quist D, Garbelotto M, Chapela IH (1999) Mycorrhizal ecology of *Oreomunnea*: implications of fungal community structure on plant distribution and diversity. Preliminary investigations in the Sierra

Juarez, Oaxaca, Mexico. Abstract In: Libro de resúmenes del III Congreso Latinoamericano de Micología, pp 99–100

R Development Core Team (2011) R: a language and environment for statistical computing. In: R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

Richard F, Millot S, Gardes M, Selosse M-A (2005) Diversity and specificity of ectomycorrhizal fungi retrieved from an old-growth Mediterranean forest dominated by *Quercus ilex*. New Phytol 166: 1011–1023

Richard F, Roy M, Shahin O, Sthultz C, Duchemin M, Joffre R, Selosse M-A (2011) Ectomycorrhizal communities in a Mediterranean forest ecosystem dominated by *Quercus ilex*: seasonal dynamics and response to drought in the surface organic horizon. Ann For Sci 68: 57–68

Simard SW, Perry DA, Jones MD, Myrold DD, Durall DM, Molina R (1997) Net transfer of carbon between tree species with shared ectomycorrhizal fungi. Nature 388:579–582

Simard SW, Beiler KJ, Bingham MA, Deslippe JR, Philip LJ, Teste FP (2012) Mycorrhizal networks: mechanisms, ecology and modeling. Fungal Biol Rev 26:39–60

Roy M, Rochet J, Manzi S, Jargeat P, Gryta H, Moreau P-A, Gardes M (2013) What determines *Alnus*-associated ectomycorrhizal community diversity and specificity? A comparison of host and habitat effects at a regional scale. New Phytol 198:1228–1238

Ryberg M, Larsson E, Molau U (2009) Ectomycorrhizal diversity in *Dryas octopetala* and *Salix reticulata* in an Alpine cliff ecosystem. Arct Alp Res 41:506–514

Ryberg M, Andreasen M, Björk RG (2010) Weak habitat specificity in ectomycorrhizal communities associated with *Salix herbacea* and *Salix polaris* in alpine tundra. Mycorrhiza 21:289–296

Smith EP, van Belle G (1984) Nonparametric estimation of species richness. Biometrics 40:119–129

Smith JE, McKay D, Niwa CG, Thies WG, Brenner G, Spatafora JW (2004) Short-term effects of seasonal prescribed burning on the ectomycorrhizal fungal community and fine root biomass in ponderosa pine stands in the Blue Mountains of Oregon. Can J For Res 34:2477–2491

Smith JE, McKay D, Brenner G, McIver J, Spatafora JW (2005) Early impacts of forest restoration treatments on the ectomycorrhizal fungal community and fine root biomass in a mixed conifer forest. J Appl Ecol 42:526–535

Smith ME, Douhan GW, Rizzo DM (2007a) Intra-specific and intra-sporocarp ITS variation of ectomycorrhizal fungi as assessed by rDNA sequencing of sporocarps and pooled ectomycorrhizal roots from a *Quercus* woodland. Mycorrhiza 18:15–22

Smith ME, Douhan GW, Rizzo DM (2007b) Ectomycorrhizal community structure in a xeric *Quercus* woodland based on rDNA sequence analysis of sporocarps and pooled roots. New Phytol 174:847–863

Smith ME, Douhan GW, Fremier AK, Rizzo DM (2009) Are true multihost fungi the exception or the rule? Dominant ectomycorrhizal fungi on *Pinus sabiniana* differ from those on co-occurring *Quercus* species. New Phytol 182:295–299

Smith ME, Henkel TW, Aime MC, Fremier AK, Vilgalys R (2011) Ectomycorrhizal fungal diversity and community structure on three co-occurring leguminous canopy tree species in a Neotropical rainforest. New Phytol 192:699–712

Smith ME, Henkel TW, Uehling JK, Fremier AK, Clarke HD, Vilgalys R (2013) The ectomycorrhizal fungal community in a neotropical forest dominated by the endemic dipterocarp *Pakaraimea dipterocarpacea*. PLoS ONE 8, e55160

Smith SE, Read DJ (2008) Introduction. In: Smith SE, Read DJ (eds) Mycorrhizal symbiosis 3rd edn. Academic Press

St John TV (1980) A survey of mycorrhizal infection in an Amazonian rain forest. Acta Amazon 10:527–533

St John TV, Uhl C (1983) Mycorrhizae in the rainforest at San Carlos de Rio Negro, Venezuela. Acta Cient Venez 34:233–237

Stone DE (1972) New world juglandaceae, III. A new perspective of the tropical members with winged fruits. Ann. Mo. Bot. Gard. 59:297–322

Taniguchi T, Kanzaki N, Tamai S, Yamanaka N, Futai K (2007) Does ectomycorrhizal fungal community structure vary along a Japanese black pine (*Pinus thunbergii*) to black locust (*Robinia pseudoacacia*) gradient? New Phytol 173:322–334

Taylor AFS, Martin F, Read DJ (2000) Fungal diversity in ectomycorrhizal communities of Norway spruce [*Picea abies* (L.) Karst.] and beech (*Fagus sylvatica* L.) along north–south transects in Europe. In: Detlef Schulze ED (ed) Carbon and nitrogen cycling in European forest ecosystems. Ecological Studies v. 142. Springer, Heidelberg, pp 343–365

Tedersoo L, Kõljalg U, Hallenberg N, Larsson K-H (2003) Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. New Phytol 159:153–165

Tedersoo L, Suvi T, Larsson E, Kõljalg U (2006) Diversity and community structure of ectomycorrhizal fungi in a wooded meadow. Mycol Res 110:734–748

Tedersoo L, Suvi T, Beaver K, Kõljalg U (2007) Ectomycorrhizal fungi of the Seychelles: diversity patterns and host shifts from the native *Vateriopsis seychellarum* (Dipterocarpaceae) and *Intsia bijuga* (Caesalpiniaceae) to the introduced *Eucalyptus robusta* (Myrtaceae), but not *Pinus caribea* (Pinaceae). New Phytol 175: 321–333

Tedersoo L, Jairus T, Horton B, Abarenkov K, Suvi T, Saar I, Kõljalg U (2008) Strong host preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon-specific primers. New Phytol 180:479–490

Tedersoo L, Sadam A, Zambrano M, Valencia R (2010a) Low diversity and high host preference of ectomycorrhizal fungi in Western Amazonia, a Neotropical biodiversity hotspot. ISME J 4:465–471

Tedersoo L, Way TW, Smith ME (2010b) Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 20:217–263

Tedersoo L, Bahram M, Jairus T, Bechem E, Chinoya S, Mpumba R, Leal M, Randrianjohany E, Razafimandimbison S, Sadam A, Naadel T, Koljalg U (2011) Spatial structure and the effects of host and soil environments on communities of ectomycorrhizal fungi in wooded savannas and rain forests of Continental Africa and Madagascar. Mol Ecol 20:3071–3080

Tedersoo L, Bahram M, Toots M, Diédhiou AG, Henkel TL, Kjoller R, Morris MH, Nara K, Nouhara E, Peay K, Polme S, Ryberg M, Smith ME, Koljalg U (2012) Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. Mol Ecol 21:4160–4170

Ter Braak CJF (1995) Ordination. In: Jongman RHG, Ter Braak CJF, Van Tongeren OFR (eds) Data analysis in community and landscape ecology. Cambridge University Press, New York, pp 91–173

Teste FP, Simard SW, Durall DM, Guy RD, Jones MD, Schoonmaker AL (2009) Access to mycorrhizal networks and roots of trees: importance for seedling survival and resource transfer. Ecology 90:2808–2822

Toljander JF, Eberhardt U, Toljander YK, Paul LR, Taylor AFS (2006) Species composition of an ectomycorrhizal fungal community along a local nutrient gradient in a boreal forest. New Phytol 170:873–883

Twieg B, Durall DM, Simard SW (2007) Ectomycorrhizal fungal succession in mixed temperate forests. New Phytol 176:437–447

Treseder KK (2004) A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO2 in field studies. New Phytol 164:347–355

U'Ren JM, Dalling JW, Gallery RE, Maddison DR, Davis EC, Gibson CM, Arnold AE (2009) Diversity and evolutionary origins of fungi associated with seeds of a Neotropical pioneer tree: a case study for analysing fungal environmental samples. Mycol Res 113:432–449

U'Ren JM, Lutzoni F, Miadlikowska J, Laetsch AD, Arnold AE (2012) Host and geographic structure of endophytic and endolichenic fungi at a continental scale. Am J Bot 99:898–914

Walker JF, Miller OK, Horton JL (2005) Hyperdiversity of ectomycorrhizal fungus assemblages on oak seedlings in mixed forests in the Southern Appalachian Mountains. Mol Ecol 14:829–838

Zwickl DJ (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation, The University of Texas at Austin