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ORIGINAL ARTICLE

Low diversity and high host preference of ectomycorrhizal fungi in Western Amazonia, a neotropical biodiversity hotspot

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Information about the diversity of tropical microbes, including fungi is relatively scarce. This study addresses the diversity, spatial distribution and host preference of ectomycorrhizal fungi (EcMF) in a neotropical rainforest site in North East Ecuador. DNA sequence analysis of both symbionts revealed relatively low richness of EcMF as compared with the richness of temperate regions that contrasts with high plant (including host) diversity. EcMF community was positively autocorrelated up to 8.5 ± 1.0-m distance—roughly corresponding to the canopy and potentially rooting area of host individuals. *Coccoloba* (Polygonaceae), *Guapira* and *Neea* (Nyctaginaceae) differed by their most frequent EcMF. Two-thirds of these EcMF preferred one of the host genera, a feature uncommon in boreal forests. Scattered distribution of hosts probably accounts for the low EcMF richness. This study demonstrates that the diversity of plants and their mycorrhizal fungi is not always related and host preference among EcMF can be substantial outside the temperate zone.

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Introduction

Biodiversity of animals and plants peaks in tropical rain forests due to relatively stable climate and high speciation rates (Jablonski *et al.*, 2006; Lomolino *et al.*, 2006). Similar latitudinal biodiversity gradient is suggested to occur in microbes such as fungi and bacteria (Hawksworth, 2001; Pommier *et al.*, 2007), but remains thus far little explored because of logistic problems and high cost of molecular techniques. By using molecular tools, recent case studies revealed overwhelming richness of tropical foliar endophytes (Arnold and Lutzoni, 2007). The relative biodiversity of arbuscular mycorrhizal fungi is similar in tropical and temperate regions (Husband *et al.*, 2002; Öpik *et al.*, 2009). Local richness and community composition of biotrophic fungi, endophytes, mycorrhizal and hexapod symbionts, is largely driven by their host preference or specificity (Husband *et al.*, 2002; Currie *et al.*, 2003; Arnold, 2008). Here we refer to both phenomena as host preference, because exclusive specificity is difficult to prove. Host taxon may have a substantial role in structuring the ectomycorrhizal (EcM) fungal communities in subtropical *Quercus* (Morris *et al.*, 2009) and temperate mixed (Richard *et al.*, 2005; Ishida *et al.*, 2007; Tedersoo *et al.*, 2008; Smith *et al.*, 2009) forest ecosystems, but its role remains unknown in tropical savanna and rain forest habitats. Fruit-body surveys and inoculation experiments rather suggest host promiscuity in tropical EcM fungi (EcMF) (Lee *et al.*, 2003; Diedhiou *et al.*, 2005).

EcM associations were long considered to be rare in the tropics, but present knowledge suggests that all tropical regions support at least five lineages of host plants (Alexander and Lee, 2005). Among hosts, Dipterocarpaceae and Amhersteae (Caesalpinioideae) form monodominant stands particularly in Tropical Asia and Africa. In neotropical forests, the monodominance of EcM vegetation is limited to a few regions in Northern Amazonia (ter Seege *et al.*, 2006). Instead, many neotropical EcM plant lineages such as Gnetaceae, Pisonieae (Nyctaginaceae), Coccolobeae (Polygonaceae) and Aldineae (Papilionoideae) are usually scattered as shrubs and understorey trees among the dominant arbuscular

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mycorrhizal vegetation (Alexander and Lee, 2005 and references therein). The distribution of EcM hosts *Quercus* and *Alnus* (Fagales) extends into South America along the mountain chain of the Cordilleras. Subtropical *Quercus* forests support a high diversity of EcM fungi in Mexico (Morris *et al.*, 2009).

Biodiversity and community ecology of EcM fungi is relatively poorly documented in tropical ecosystems (Sirikantaramas *et al.*, 2003; Tedersoo *et al.*, 2007). In South America, this information relies on fruit-body observations (Pegler, 1983; Singer *et al.*, 1983; Henkel *et al.*, 2002; Læssøe and Petersen, 2008) and limited root sampling of Nyctaginaceae (Haug *et al.*, 2005) and Dipterocarpaceae (Moyersoen, 2006). Here we tested the hypothesis that particularly Nyctaginaceae hosts associate with a few EcM fungi that belong exclusively to the Thelephoraceae and Russulaceae families (Chambers *et al.*, 2005; Haug *et al.*, 2005). Based on the general latitudinal biodiversity gradient (Hillebrand, 2004) and the anticipated correlation between EcM fungal and host plant diversity (Dickie, 2007), we hypothesized that EcM fungi are highly diverse in a primary neotropical forest site. By using rDNA sequence analysis for *in situ* identification of both fungal and plant symbionts, we addressed spatial autocorrelation, host and habitat preference of EcM fungi.

Materials and methods

Sampling of EcM root tips and fruit-bodies was performed at an approximately 30-ha site in the 50-ha Forest Dynamics Plot in Yasuni National Park, North East Ecuador (0°41′S; 76°24′W). On a fully censused 25-ha half of the plot, richness of vegetation is among the highest in the world and comprises >1100 tree species (diameter >1 cm), including the EcM hosts *Coccoloba* (9 spp.), *Guapira* (2 spp.) and *Neea* (15 spp.) (Valencia, 2004; Valencia *et al.*, 2004). The site lies 216–248 m above sea level and comprises small hills and valleys that form a local soil and moisture gradient. Topography contributes to niche differentiation of the vegetation (Valencia *et al.*, 2004), but gradients of micro- and macroelements are hypothesized to explain this phenomenon (John *et al.*, 2007; Kraft *et al.*, 2008). The Yasuni region receives an annual average of 3081 mm rainfall that peaks in October–November (Valencia, 2004). The soil is yellow clay in upland parts and clay loam in valley beds, and is characterized by a shallow organic horizon (A; 0–2 cm deep). Raw humus is found only around decaying boles and large-leaved palms.

Around individuals of each three host genera, a total of 120 root samples (15 × 15 cm to 10 cm depth) were taken and precisely located based on 200–400 replicate measurements by using a GPS Garmin CS60 (Garmin International Inc., Olathe, KS, USA). Due to infrequence of certain host species, one to four root samples were taken at least 4 m apart from each other and up to 6-m distance from stems or tree trunks of *Coccoloba*, *Guapira* and *Neea* individuals. EcM roots were virtually absent further distant. Around trees, where EcM roots could not be found, large roots were traced to ensure inclusion of host roots, whether or not EcM. Generally, the host taxa were well segregated at the study site. Only *Neea* '*comun*' (sensu Valencia, 2004) tends to aggregate in sparse patches of up to 10 individuals. With a few exceptions, individuals of different EcM host species were located at least 15–20 m apart, rendering overlap of the EcM root systems unlikely. EcM roots were separated from bulk soil and obvious non-EcM roots and transported to the Yasuni Field Station.

All root samples were processed within 10 h of collection. Based on the occurrence of a fungal mantle, the proportion of root tips bearing EcM was scored on roots of host trees by using a portable stereomicroscope. To facilitate molecular typing, EcM root tips were sorted into morphotypes based on the color and texture of fungal mantle, hyphae and rhizomorphs. The relative abundance of each morphotype was recorded and several root tips from each morphotype were mounted into CTAB buffer (1% cetyltrimethylammonium bromide, 100 mM Tris–HCl (pH 8.0), 1.4 M NaCl, 20 mM EDTA) for shipping. Due to low EcM colonization, scarcity of targeted host roots and the moribund state of several root systems, the number of informative root samples was reduced to 60, including 34 host individuals and nine host species. The root systems of *Coccoloba* and *Guapira* overlapped in a single soil core. *Coccoloba*, *Guapira* and *Neea* were present in 26, 21 and 14 samples, respectively.

Depending on the amount of material, 1–5 single root tips of each morphotype per root sample were subjected to replicate DNA extraction (Tedersoo *et al.*, 2007), amplification of the rDNA Internal Transcribed Spacer (ITS) region and 28S gene. Tomentelloid and other morphotypes were amplified by using primer pairs ITSOF-T (5′-acttggtcatttag aggaagt-3′), LR5-Tom (5′-ctaccgtagaaccgtctcc-3′), and ITSOF-T, LB-W (5′-cttttcatctttccctcacgg-3′), respectively. DNA yielding no PCR product was re-amplified with primers ITSOF-T, ITS4 (5′-tcctccgcttattgat atgc-3′); 28S rDNA gene was amplified with primers LR0R (5′-acccgctgaacttaagc-3′) and LB-Z (5′-aaaaatgg cccactagaaact-3′). Thermal cycling parameters are described in reference Tedersoo *et al.* (2006). PCR products were purified and sequenced as described by Tedersoo *et al.* (2008). Sequences were edited using Sequencher 4.7 (GeneCodes Corp., Ann Arbor, MI, USA) and assigned to molecular species based on 97% sequence similarity (bar-coding threshold) of the ITS region (Tedersoo *et al.*, 2003, 2008). Due to high level of nucleotide substitution in South American indigenous fungi relative to other continents, and ambiguous affinities to existing genera, fungal species were assigned to phylogenetic lineages (cf. Tedersoo *et al.*, 2010) based on BLASTN searches against the International Sequence Database

(INSD). All unique ITS-28S sequences were submitted to INSD and the UNITE database (Kõljalg *et al.*, 2005) under accession numbers UDB004231–81. To confirm identification of host plants, the plastid trnL region was amplified and sequenced in one to four EcM root tips of each sample as described by Tedersoo *et al.* (2008). Plant taxonomy and provisional names of EcM trees follow Valencia (2004), due to lack of their taxonomic treatment in Western Amazonia.

Spatial patterns of EcM species density (that is, richness per root sample) and colonization were assessed based on Euclidean distance and Moran's *I* as implemented in the Vegan package of R (R Core Development Team, 2007). The distances among root samples were continuous and therefore we arbitrarily established 18 distance classes (3.0–5.0, 5.0–7.0, 6.5–7.5, 6.0–8.0, 7.0–9.0, 7.5–9.5, 8.0–10.0, 8.5–10.5, 9.0–11.0, 11.0–23.0, 18.0–28.0, 23.0–33.0, 28.0–38.0, 32.0–48.0, 48.0–96.0, 96.0–160.0, 160.0–404.0, 404.0–760.0 m). The lowest distance classes overlapped to improve the detection of critical distances. Moran's *I* was not significant ($P>0.05$) in any of the spatial scales and therefore, root samples were used as replicates in a non-nested design to address differences in fungal species density (square-root-transformed) and EcM colonization (arcsin-square-root-transformed) among host genera and topographic positions. Two-way analyses of variance followed by Tukey–Kramer tests for unbalanced design were applied in these analyses. To account for spatial effects on EcM fungal community, we performed a partial Mantel test by using the above distance classes, Jaccard distance and species occurrence data as implemented in the Ecodist package of R. The test revealed significant spatial autocorrelation in the EcM fungal community at 8.5 ± 1.0-m distance that roughly matched the collection of multiple root samples from large tree individuals (Figure 1). When root samples were pooled by tree individuals, no spatial trends were evident. Therefore, we conservatively used tree individuals as sampling units in subsequent analyses of fungal data. Host preference of fungal species was assessed at the host genus level, because of too few replicate individuals for each tree species. Biases in the distribution of six dominant fungal species in relation to host and topography (lowland, ridge and hill top) were studied by using Fisher's Exact Tests followed by Benjamini–Hochberg correction to reduce false discovery rate (alternative to Bonferroni correction; Verhoeven *et al.*, 2005). To compare species diversity among host genera, species accumulation curves and their 95% confidence intervals were calculated by using EstimateS 8 (Colwell, 2006). The effects of host genus and topographic position on the fungal community were addressed using Sørensen distance measure as implemented in Adonis routine of the Vegan package of R. Significance level of $\alpha < 0.05$ was used throughout the study.

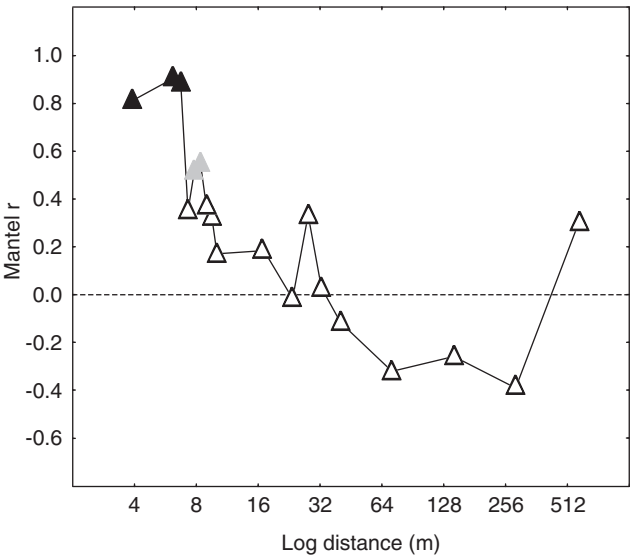

Figure 1 Mantel variogram demonstrating changes in similarity of root samples based on EcM fungal species composition with increasing distance. Closed triangles, $P<0.001$; shaded triangles, $P<0.05$; open triangles, $P>0.05$. Note the logarithmic scale. EcM, ectomycorrhizal.

Results

DNA sequence analysis of EcM fungi was successful for 105 of 111 (94.6%) morphotype and root sample combinations. With three exceptions, molecular tools confirmed the identity of a host plant at the genus level. Species within each genus displayed no sequence variation in the plastid trnL intron. Thus, plant species-level identification relies solely on leaf and bark morphology.

Bar-coding of the rDNA ITS region revealed 38 species (belonging to seven monophyletic lineages) of EcM fungi on root systems of nine host species (three genera) in Yasuni (Table 1). Species density (richness per root sample) averaged 1.42 across all hosts, with no significant differences among host genera or topographic positions (Supplementary Figure S1a). Between 0 and 92% of host root tips were EcM in all root samples, but there were no significant differences among genera or topographic positions (Supplementary Figure S1b). Moran's *I* for EcM species density and root colonization was non-significant in all distance classes, indicating lack of spatial autocorrelation above 4-m distance.

Of host trees, genera and the most common species displayed no specificity to certain fungal lineages or species (Supplementary Tables S1, S2). *Coccoloba*, *Guapira* and *Neea* were associated with 25, 9 and 11 species of EcM fungi that belong to five, five and four lineages, respectively. Based on 95% confidence interval, *Coccoloba* was found to be associated with significantly more EcM fungal species as compared with *Guapira* when root samples were treated as sampling units, but not when individuals were considered (Supplementary

Table 1 Identification of EcMF in Yasuni National Park, Ecuador

Recovered species		*Identification*	
Name	*Accession no.*	*Best blastN match*	*Identity (%)*
/amanita Y01	UDB004231	AY436470 *Amanita pseudovaginata*	83.2
/cantharellus Y01	UDB004232	AB445116 *Pterygellus polymorphus*	Partial
/clavulina Y01	UDB004233	EF559274 *Clavulina cristata*	83.2
/clavulina Y02	UDB004234	EU118616 *Clavulina cinerea*	92.3
/clavulina Y03	UDB004235	EU819415 *Clavulina cristata*	79.1
/clavulina Y04	UDB004236	EU118616 *Clavulina cinerea*	90.7
/clavulina Y05	UDB004237	EU819415 *Clavulina cristata*	78.1
/inocybe Y01	UDB004238	AM882757 *Inocybe sambucina*	85.6
/inocybe Y02	UDB004239	AM882780 *Inocybe squamata*	82.8
/russula-lactarius Y01	UDB004240	AY606973 *Lactarius edulis*	80.5
/russula-lactarius Y02	UDB004241	DQ422032 *Russula pallidospora*	83.7
/russula-lactarius Y03	UDB004242	DQ422032 *Russula pallidospora*	81.9
/russula-lactarius Y04	UDB004243	AY667425 *Russula puiggarii*	97.6
/russula-lactarius Y05	UDB004244	EU819422 *Russula brevipes*	89.0
/russula-lactarius Y06	UDB004245	AY606973 *Lactarius edulis*	79.6
/russula-lactarius Y07	UDB004246	AY606974 *Lactarius phlebophyllus*	77.9
/russula-lactarius Y08	UDB004247	FJ845430 *Russula densifolia*	82.2
/russula-lactarius Y09	UDB004248	GQ166898 *Lactarius glaucescens*	90.5
/sebacina Y01	UDB004249	AJ966753 *Sebacina incrustans*	86.2
/sebacina Y02	UDB004250	EU819445 *Tremellodendron pallidum*	84.0
/sebacina Y03	UDB004251	EU326155 *Sebacina incrustans*	82.4
/sebacina Y04	UDB004252	AJ966753 *Sebacina incrustans*	89.5
/tomentella-thelephora Y01	UDB004253	UDB000777 *Tomentella sublilacina*	87.2
/tomentella-thelephora Y02	UDB004256	UDB000030 *Tomentella sublilacina*	87.6
/tomentella-thelephora Y03	UDB004258	TPU83484 *Thelephora penicillata*	89.3
/tomentella-thelephora Y04	UDB004259	DQ068971 *Tomentella ellisii*	92.7
/tomentella-thelephora Y05	UDB004260	AY230244 *Thelephora terrestris*	89.7
/tomentella-thelephora Y06	UDB004261	DQ068971 *Tomentella ellisii*	91.7
/tomentella-thelephora Y07	UDB004262	AJ889980 *Thelephora caryophyllea*	88.2
/tomentella-thelephora Y08	UDB004264	EU819523 *Tomentella stuposa*	89.2
/tomentella-thelephora Y10	UDB004265	AJ889980 *Thelephora caryophyllea*	88.3
/tomentella-thelephora Y11	UDB004267	FN393106 *Tomentella botryoides*	88.6
/tomentella-thelephora Y12	UDB004268	DQ068971 *Tomentella ellisii*	91.8
/tomentella-thelephora Y13	UDB004269	AJ889980 *Thelephora caryophyllea*	88.2
/tomentella-thelephora Y14	UDB004271	DQ068971 *Tomentella ellisii*	87.8
/tomentella-thelephora Y15	UDB004274	DQ482002 *Tomentella sublilacina*	88.0
/tomentella-thelephora Y16	UDB004275	EU819523 *Tomentella stuposa*	89.6
/tomentella-thelephora Y17	UDB004276	FN393106 *Tomentella botryoides*	86.2

Abbreviation: EcM, ectomycorrhizal fungi.

Figure S2). *Neea* had accumulating species richness level similar to that of other host genera.

The /tomentella-thelephora, /russula-lactarius and /clavulina were the most species-rich EcM fungal lineages in Yasuni (Table 1). Different fungal species, /inocybe Y01, /cantharellus Y01 and /tomentella–thelephora Y05, were the most frequent mycobionts on species *Coccoloba*, *Guapira* and *Neea*, respectively. All these fungal species displayed significantly biased distribution for their hosts. Based on host individuals as sampling units, statistically significant differences in host preference were recovered in four out of six most frequent fungal species (Figure 2). These taxa were distributed throughout the study site and showed no evidence of spatial aggregation (Supplementary Figure S3). Biased host associations among fungal species occurred at the host genus, but not host species level (Supplementary Table S1). There was no evidence for host specificity at the fungal lineage level, except in the /cantharellus lineage, the only species of which exclusively associated with two *Guapira* species.

The fungal community was significantly spatially autocorrelated among root samples up to 8.5 ± 1.0-m distance (Figure 1). The multivariate analysis based on root samples pooled by host individual suggested that host species has the strongest effect on the fungal community composition by explaining 19.5% of the variation in fungal species distribution data ($F_{2,29} = 3.70$; $P < 0.001$). By contrast, topographic position and an interaction term between topography and host remained non-significant ($P > 0.1$).

Discussion

Combining molecular identification of plants and fungi revealed a substantial level of partner preference

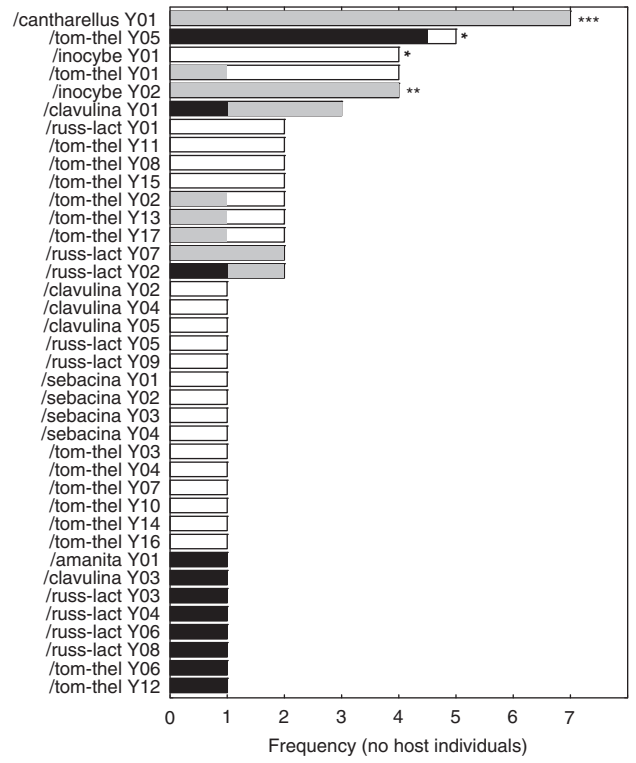

Figure 2 Frequency of EcM fungal species arranged by host genera. Open bars, *Coccoloba*; shaded bars, *Guapira*; closed bars, *Neea*. ***$P<0.001$; **$P<0.01$; *$P<0.05$. /tom-thel, the /tomentella-thelephora lineage; /russ-lact, the /russula-lactarius lineage. EcM, ectomycorrhizal.

among the most frequent fungal species, but not among the species or genera of host plants. Thus, this study lends no support to the hypothesis of general mycobiont specificity in the plant family Nyctaginaceae (Chambers *et al*., 2005; Haug *et al*., 2005; Suvi *et al*., 2010). Re-analysis of the EcM fungal community of shrub 'C' of *Neea* sp1 (sensu Haug *et al*., 2005) in an Andean cloud forest revealed a species of *Clavulina* in addition to members of the Russulaceae and Thelephoraceae reported previously.

In Yasuni, four out of six most frequent EcM fungal species displayed host preference. It is unlikely that this is related to host-mediated soil effect, as these EcM plants are usually non-dominant, understorey trees and contribute little to the litter and root biomass in such diverse ecosystems (John *et al*., 2007). The observed host preference pattern strongly argues against implications from fruit-body observations in tropical forests of South America (Pegler, 1983; Singer *et al*., 1983) and Southeast Asia (Lee *et al*., 2003), where the dominant fungal morphospecies associate with several host genera and families. The discrepancy probably results from inclusion of fungi producing resupinate fruit bodies, more precise mycobiont identification at the cryptic species level and confirmation of the plant host in EcM root tips by use of molecular tools. Sampling effect may also account for these differences, as host preference differs by host taxa in temperate ecosystems (Molina *et al*., 1992; Tedersoo *et al*., 2008). Nevertheless, the fungal community dominants are non-selective of their hosts in subalpine to temperate ecosystems of the Northern Hemisphere (Horton and Bruns, 1998; Kennedy *et al*., 2003; Richard *et al*., 2005; Ishida *et al*., 2007; Ryberg *et al*., 2009). In most ecosystems studied to date, host genus or even species substantially affect the community composition of EcM fungi by slight non-significant shifts in the frequency of individual species (Ishida *et al*., 2007; Morris *et al*., 2008). Historical factors (Singer, 1953), specialized habitats (Molina *et al*. 1992; Chambers *et al*., 2005), partial autotrophy (Bruns *et al*., 2002), phylogenetic (Bruns *et al*., 2002; Ishida *et al*., 2007) and physiological differences (Morris *et al*., 2008) among host trees may directly contribute to the differential development of host preference. We are unaware of the potential ecological differences among the three host genera due to scant data on these plants. Although topography best explains the distribution of vegetation (Valencia *et al*., 2004), it had no effect on EcM fungi, indicating that fungal species are non-selective for differences in soil at the study site. This contrasts with temperate forests, where the EcM fungal community shifts along with both microtopography and host species (Toljander *et al*., 2006; Tedersoo *et al*., 2008).

Richness of EcM fungi was relatively low (38 species from seven lineages) in the Ecuadorian Amazon despite the high host plant diversity, relatively large sampling area (30 ha) and moderate sample size ($n=60$). Comparable studies of temperate and tropical sites in the Northern and Southern Hemisphere reveal several-fold more species and lineages than Yasuni (Tedersoo and Nara, 2010). The level of richness compares better with a monodominant *Coccoloba uvifera* coastal forest in Cuba (U Kõljalg and L Tedersoo, unpublished data) and other island ecosystems (Supplementary Table S2; Peay *et al*., 2007; Tedersoo *et al*., 2007; L Tedersoo and A Sadam, unpublished data from São Tomé and Mount Cameroon). The relatively low EcM fungal diversity strongly contrasts with the peaking plant diversity, including high host species richness in the Yasuni 50-ha plot (26 species; Valencia, 2004; Valencia *et al*., 2004), and challenges the hypothesis of a positive fungal diversity, host diversity relationship (Dickie, 2007), on a global scale. Additional sites in South America and other tropical and subtropical ecosystems comprising different hosts need to be studied to understand the relative influence of latitude, historical and sampling effects on diversity and host preference of EcM fungi.

We hypothesize that the low EcM fungal richness in Yasuni results from scattered distribution of host trees. Each tree or a group of trees effectively forms a small, more or less isolated island for EcM fungi, as crowns and probably root systems of individual trees seldom overlap. In such host islands, fungi can cross the non-EcM rain forest matrix only by spore

dispersal, but not by vegetative mycelium growth. This situation may select for EcM fungi with small genetic individuals and efficient dispersal mechanisms, that is, species with a pioneer strategy. In boreal and temperate forests, EcM trees dominate and naturally form a continuous vegetation belt with high root density in humus-rich soils.

The level of spatial autocorrelation within EcM fungal community was higher in Yasuni (8.5 ± 1.0 m) as compared with that of temperate forests (<3 m; Lilleskov *et al*., 2004; M Bahram, unpublished data). This pattern suggests low spatial turnover of the fungal community around the tree individuals and/or higher space requirement of fungal genets in Yasuni. In temperate ecosystems, fungal species density is greater (approximately 5–10 species per core versus <2 in this study) and EcM fungal individuals often cover an area that is larger compared with these host islands (Dahlberg, 2001). Relatively high spatial autocorrelation and low species density support the hypothesis of dispersal limitation of EcM fungi in Yasuni. The presence of a single or a few host individuals on an island indicates no or very low fine-scale genetic diversity of suitable roots. Host genets may affect the community of EcM fungi by differential carbon availability (Korkama *et al.*, 2007). Thus we speculate that genetic uniformity of hosts in vegetation islands may be related to the low species richness. Host preference probably further reduces the chances of establishment and gene flow of EcM fungi in such island ecosystems.

In conclusion, this study demonstrates that the diversity of plant and fungal partners in EcM symbiosis is not necessarily related at the global scale. Host preference among the dominant fungal taxa, relatively high-level spatial autocorrelation and scattered growth habit of host individuals may account for the low richness of EcM fungi in a neotropical rain forest of blooming plant biodiversity.

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