

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

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Summary

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- Relatively little is known about diversity or structure of tropical ectomycorrhizal communities or their roles in tropical ecosystem dynamics. In this study, we present one of the largest molecular studies to date of an ectomycorrhizal community in lowland dipterocarp rainforest.
- We sampled roots from two 0.4 ha sites located across an ecotone within a 52 ha forest dynamics plot. Our plots contained > 500 tree species and > 40 species of ectomycorrhizal host plants. Fungi were identified by sequencing ribosomal RNA genes.
- The community was dominated by the Russulales (30 species), Boletales (17), Agaricales (18), Thelephorales (13) and Cantharellales (12). Total species richness appeared comparable to molecular studies of temperate forests. Community structure changed across the ecotone, although it was not possible to separate the role of environmental factors vs host plant preferences. Phylogenetic analyses were consistent with a model of community assembly where habitat associations are influenced by evolutionary conservatism of functional traits within ectomycorrhizal lineages.
- Because changes in the ectomycorrhizal fungal community parallel those of the tree community at this site, this study demonstrates the potential link between the distribution of tropical tree diversity and the distribution of tropical ectomycorrhizal diversity in relation to local-scale edaphic variation.

Introduction

There is increasing evidence that below-ground processes play a major role in determining the structure of plant communities (Wardle, 2002). Mycorrhizal symbioses are ubiquitous in terrestrial ecosystems and have been shown to exert significant influence on recruitment, species composition, richness and productivity of plant communities (van der Heijden *et al.*, 1998; Terwilliger & Pastor, 1999; Weber *et al.*, 2005). While there has been strong debate regarding the factors influencing community dynamics of tropical forests (Hubbell *et al.*, 1999; Condit *et al.*, 2006;

John *et al.*, 2007; Kraft *et al.*, 2008), little empirical work exists on the potential role of mycorrhizal symbionts (Alexander & Lee, 2005). This is partly because mycorrhizal communities in species-rich tropical rainforests remain poorly characterized relative to temperate counterparts as a result of the difficulty of implementing DNA identification techniques in remote locations and a tendency of the field to focus on gymnospermous host plants (Dickie & Moyersoen, 2008).

Most lowland tropical trees are thought to form arbuscular mycorrhizal associations (Malloch *et al.*, 1980; Read, 1991; McGuire *et al.*, 2008). Where ectomycorrhizal asso-

ciations have been noted, they are often associated with tree species that form monodominant stands (Newbery *et al.*, 2000; Torti *et al.*, 2001; McGuire *et al.*, 2008). The most notable exception to this pattern is the paleotropical rainforests of southeast Asia. These forests are among the most diverse plant communities in the world and are characterized by high abundance of trees in the family Dipterocarpaceae (Lee *et al.*, 2002; Slik *et al.*, 2003). The Dipterocarpaceae are known to form associations with ectomycorrhizal fungi, a relationship that likely has ancient Gondwanan origins (Lee, 1990; Moyersoen, 2006). Because the biochemical capabilities of ectomycorrhizal fungi differ substantially from those of arbuscular mycorrhizal fungi (Read, 1991; Smith & Read, 2008), the below-ground dynamics in these forests are likely to be significantly different from those of other lowland tropical rainforests.

Ectomycorrhizal fungal community structure is influenced strongly by both the abiotic and biotic soil environments. Soil environmental factors, such as water availability, base cation concentration, pH and nitrogen content, are often correlated with mycorrhizal community structure (Parrent *et al.*, 2006; Schechter & Bruns, 2008; Peay *et al.*, 2009). Such changes in community structure are likely related to differences between these fungi in optimum conditions for growth and resource acquisition (Smith & Read, 2008). Because ectomycorrhizal fungi are obligate biotrophs, partner preferences or specificity can also create strong biotic links between plant and fungal community structure (Ishida *et al.*, 2007; Tedersoo *et al.*, 2008). Feedback between these plant–soil–fungus interactions has the potential to alter species richness, composition and ecosystem function significantly (van der Heijden *et al.*, 1998; Terwilliger & Pastor, 1999).

In tropical forests, local-scale edaphic variation plays an important role in influencing plant community structure (Fine *et al.*, 2004; Paoli *et al.*, 2006; John *et al.*, 2007). This effect is evident for individual species (Russo *et al.*, 2005) and at higher phylogenetic levels (e.g. genus, family) when closely related species are adapted to similar habitats (Webb, 2000). Because of their important role in mediating plant–soil interactions, there is high potential that tropical mycorrhizal communities affect plant response to edaphic variation and thus contribute to observed patterns in the distribution of tropical tree diversity.

Few studies of natural dipterocarp mycorrhizal fungal communities have been conducted. The majority of these have involved surveys of fruiting bodies or identification through root morphotyping (Smits, 1994; Watling & Lee, 1995; Lee *et al.*, 1997; Sims *et al.*, 1997) and molecular studies have only recently been undertaken in dipterocarp forests (Sirikantaramas *et al.*, 2003; Yuwa-Amornpitak *et al.*, 2006; Tedersoo *et al.*, 2007). Two molecular surveys of dipterocarp forests have been conducted in relatively

species-poor forests in the Seychelles and Thailand (Yuwa-Amornpitak *et al.*, 2006; Tedersoo *et al.*, 2007), but Dipterocarpaceae species diversity reaches a peak on the island of Borneo, where 276 species from 13 genera have been described. One previous study from Borneo has been published (Sirikantaramas *et al.*, 2003), but sample size was fairly limited.

In this study, we examined the ectomycorrhizal fungal community in lowland, mixed-dipterocarp forest in a 52 ha forest dynamics plot at Lambir Hills National Park, Sarawak, on the island of Borneo. We used nucleotide sequence analysis to identify ectomycorrhizal fungi from field collections of host roots. In addition, we located our sampling across an ecotone with distinct differences in soil chemistry and plant community. The differences in soil chemistry and their effects on plant community composition and demographic rates have been thoroughly documented in previous studies (Davies *et al.*, 2005; Russo *et al.*, 2005; Baillie *et al.*, 2006).

Our primary goals were to characterize below-ground ectomycorrhizal diversity and taxonomic structure in this forest, and to test whether ectomycorrhizal fungal community composition changed across the ecotone. Because soil nutrient status and plant community composition covary across this gradient, it is unlikely that observational studies can separate the abiotic vs biotic determinants of ectomycorrhizal fungal community structure in this forest. However, demonstrating a potential link between the distribution of tropical tree diversity and the distribution of tropical ectomycorrhizal diversity with respect to the soil environment is a first step in establishing the role of ectomycorrhizal fungi in influencing tropical forest structure.

Materials and Methods

Study system

Lambir Hills National Park (Lambir) is located in northern Borneo, in the Malaysian state of Sarawak (4°20'N, 113°50'E). Lambir is an aseasonal, tropical rainforest, receiving *c.* 3000 mm of rainfall per year, with maximum and minimum daily temperatures between 32 and 24°C (Lee *et al.*, 2002). The study was carried out within a 52 ha forest dynamics plot (FDP) established in 1991 by the Sarawak Forest Department, the Center for Tropical Forest Science, and the Plant Ecology Laboratory of Osaka City University, Japan (Lee *et al.*, 2004). The forest has not been logged and can be considered primary rainforest. Although the trees have not been aged directly, the dominant overstory is likely >> 100 yr old. All free-standing woody plants within the plot ≥ 1 cm diameter at breast height (dbh) have been mapped and identified. At the last census (2003) the 52 ha plot included 1182 tree species, making it among the

most diverse tropical forests in the world. The Dipterocarpaceae are the most abundant tree family in the plot, with 87 species that account for 42% of the basal area and 16% of the total number of individuals (Lee *et al.*, 2002). Five of the ten most abundant species (no. of individuals) and six of the 10 species with greatest basal area are also in the Dipterocarpaceae (Davies *et al.*, 2005). As far as is known, all Dipterocarpaceae are ectomycorrhizal (Wang & Qiu, 2006). Other potential ectomycorrhizal host lineages occur in the plot but are relatively rare, with the Myrtaceae, Leguminosae and Fagaceae making up only 3.6, 2.2 and < 1% of individuals, respectively. Additionally, while no systematic survey of ectomycorrhizal associations has been performed in this forest, many genera within the Myrtaceae and Leguminosae are not thought to be ectomycorrhizal (Wang & Qiu, 2006).

Lambir contains two geological formations that give rise to four distinct soil types within the FDP (Davies *et al.*, 2005). These soil types form a gradient from low to high soil fertility. The two ends of this gradient are represented by high-fertility clay, udult (clay), and low-fertility sandy loam, humult (sand), soil types. The clay soils have significantly higher nitrogen, phosphorus, magnesium and calcium concentrations, and significantly lower pH and organic matter accumulation (Davies *et al.*, 2005; Baillie *et al.*, 2006). Differences between the two soil types are summarized in Supporting Information (Table S1) and photographs of typical samples are shown in Fig. S1. Tree community composition and demographic rates have also been shown to vary significantly across these soil types (Davies *et al.*, 2005; Russo *et al.*, 2005, 2008). Notably for this study, many dipterocarps also show soil preferences (Davies *et al.*, 2005). The most abundant include *Dryobalanops aromatica* and *Dipterocarpus globosus* (sandy soils) and *Dryobalanops lanceolata* and *Hopea dryobalanoides* (clay soils). Tree community data from our specific plots were

extracted from the greater Lambir FDP 2003 census and are presented in Tables 1 and S2.

Sampling design

To sample ectomycorrhizal fungi at the ends of the plant–soil ecotone at Lambir, we chose to locate our plots within two 4 ha sites identified by Lee *et al.* (2002) as typical of the clay and sand soil types, and for which soil chemistry and tree distribution have been shown to differ significantly (Davies *et al.*, 2005). The two sites are separated by *c.* 500 m (Fig. S2). Between 25 May and 1 June 2008, we established four plots in the clay soils and three in the sand soils (seven plots total, Fig. S2). Because clay soils and sand soils are spatially aggregated within the Lambir FDP (Davies *et al.*, 2005; Fig. S2), it is possible that samples of ectomycorrhizal fungi within a soil type could be more similar to each as a result of spatial proximity (i.e. spatial autocorrelation). For this reason, we used a sampling design that would allow us to estimate the scale of spatial autocorrelation of ectomycorrhizal fungal communities at Lambir and determine whether or not our plots were spatially independent. This sampling design consisted of nested squares with sides of 0.5, 1, 2.5, 5 and 20 m, somewhat similar to a design used by Green *et al.* (2004). Soil samples were taken at the shared zero point and the three corners of each square, for a total of 16 samples per plot (16 samples \times seven plots = 112 samples total). In order to ensure that we sampled in an area with potentially ectomycorrhizal roots, we located the zero point for each plot within 5 m of an overstorey dipterocarp. For each sample, unincorporated litter was removed and then a 10 \times 10 \times 10 cm cube of soil was cut through the remaining humus and surface soil horizons using a sharp putty knife. Samples were placed in a plastic bag on site and brought back to the field station where they were

Table 1 Summary of ectomycorrhizal fungal sampling results by plot and soil type

Soil type	No. of samples	No. of tips	S_{obs}	S_{est}	$E_{1/D}$	α	S_{plant}	S_{dipt}	% Dipt
Clay (plot 1)	16	84	18	36.5	0.34	7.0	122	21	27.2
Clay (plot 3)	13	83	4	9.3	0.30	0.9	156	18	26.2
Clay (plot 5)	12	61	7	13.2	0.35	2.0	143	18	13.7
Clay (plot 7)	15	83	15	23.6	0.67	5.3	151	18	16.7
Sand (plot 2)	16	58	24	50.7	0.88	15.3	207	19	15.3
Sand (plot 4)	15	61	25	60.4	0.75	15.8	187	14	16.7
Sand (plot 6)	15	75	26	53.4	0.63	14.1	184	14	16.9
Clay total	56	311	41	75.3	0.26	12.6	326	28	20.1
Sand total	46	194	65	134.1	0.75	34.3	351	25	15.7
Total	102	505	105	204.8	0.25	40.3	578	42	18.4

Species were delineated using a 97% sequence similarity cutoff across the ITS1, 5.8S and ITS2 regions of the nuclear ribosomal RNA genes. Richness metrics were calculated from 500 randomizations drawn without replacement in EstimateS (Colwell, 2005).

S_{obs} , observed species richness; S_{est} , estimated minimum richness based on the nonparametric Jack2 estimator; $E_{1/D}$, Simpson evenness; α , Fisher's α ; S_{plant} , tree species richness; S_{dipt} , Dipterocarpaceae species richness; % Dipt, % of stems in Dipterocarpaceae.

stored in a refrigerator until they could be examined for ectomycorrhizal roots (always within 24 h).

Processing of ectomycorrhizal root samples

To extract ectomycorrhizal roots, soil samples were washed gently over a stack of three soil sieves with 4, 1 and 0.5 mm mesh sizes. Each fraction was then examined under a dissecting microscope for the presence of colonized ectomycorrhizal roots. We found that the majority of colonized ectomycorrhizal roots remained attached to coarse roots or in mid-sized soil clusters, and thus most roots were removed from the 4 mm fraction in this study. Roots were identified as ectomycorrhizal based on physical characteristics such as the presence of a fungal mantle, turgidity and the absence of root hairs. Individual roots were placed directly into 300 μ l of 2 \times CTAB buffer (100 mM Tris-HCl (pH 8.1), 1.4 M NaCl, 20 mM EDTA, 2% cetyl trimethyl ammonium bromide). No attempt to morphotype roots was made. Roots were kept refrigerated (except during transportation), shipped back to UC Berkeley, and then stored at -20°C until DNA extraction.

Molecular identification of ectomycorrhizal roots

We extracted DNA from up to eight ectomycorrhizal root tips per soil sample (a few samples had fewer than eight colonized root tips) using the protocol of Peay *et al.* (2007), with the one modification that root tips were amalgamated directly in the CTAB storage buffer.

To identify fungi on root tips we used PCR to amplify the internal transcribed spacer (ITS) region of the ribosomal RNA genes using the fungal-specific primer set ITS1f and ITS4 (White *et al.*, 1990; Gardes & Bruns, 1993). Positive samples with a single amplicon were sequenced according to Peay *et al.* (2007) and samples with multiple amplicons or mixed sequences were reamplified from a new aliquot using the basidiomycete-specific primer set ITS1f and ITS4b to eliminate possible endophytes or soil contaminants (Gardes & Bruns, 1993). While this may have biased our dataset somewhat towards basidiomycetes, the majority of ascomycetes recovered from direct sequencing belonged to saprophytic or endophytic lineages (see the Results section).

Taxonomic designations

We used a 97% sequence similarity cutoff as our fungal operational taxonomic unit (OTU). To assign names to OTUs, we used a combination of the Basic Local Alignment Search Tool (BLAST) and phylogenetic analysis. BLAST matches query sequences against a reference database (GenBank) maintained by the National Center for Biotechnology Information. The usefulness of this approach

depends on the sequences available in GenBank. Because few molecular studies have been performed in southeast Asia, we found that few of our ITS sequences had very close matches (Table S3). To help improve taxonomic placement, we conducted a second round of sequencing using a more conserved gene region, the 25S nuclear ribosomal large subunit (LSU) gene, for each OTU we identified using the ITS locus. Representative samples were amplified using the primers ITS1f and TW13 (White *et al.*, 1990) and sequenced with the internal 25S primer LROR (<http://www.biology.duke.edu/fungi/mycolab/primers.htm>) or TW13. All LSU sequences were also queried using BLAST and this information was factored into our taxonomic designations. In addition, we took into consideration the position of OTUs within a phylogenetic tree based on 25S and 5.8S rDNA (see later discussion). Representative ITS and LSU sequences for each OTU have been deposited in GenBank (Table S3).

Statistical analyses

Minimum estimates of ectomycorrhizal fungal species richness were generated for each plot, each soil type, and the entire study using the program EstimateS (Colwell, 2005). Each estimate was based on 500 randomizations of sample order without replacement. From the EstimateS output, we calculated evenness ($E_{1/D}$) as the reciprocal of Simpson's D divided by species richness (Magurran, 2004). We compared within-plot species richness (α -diversity) for the clay and sand soils using Welch's t -test for heterogeneous variances, because of higher variance in the clay plots.

To compare estimates of species richness at Lambir with other studies, we plotted species accumulation curves from published studies of high diversity temperate ectomycorrhizal fungal communities in Japan (Ishida *et al.*, 2007), Tasmania (Tedersoo *et al.*, 2008), North Carolina, USA (Parrent *et al.*, 2006), and from an unpublished study conducted in California, USA (Peay, 2008). The unpublished study consisted of 151 soil cores sampled from around 16 mature individuals of *Pinus muricata* D. Don and two plots laid out in a similar nested fashion in contiguous, mature *P. muricata* forest. Eight roots tips were randomly selected and sequenced from each soil core. Sampling methodology was not identical between these two studies, but use of the ITS sequence for identification, the number of root tips sampled per core, and the use of spatially clustered samples within sites make the two studies useful for comparative purposes. Data from published studies were obtained directly from the authors or extracted from figures and tables in the paper. All studies were rarefied by sample and plotted both by sample and by the number of root tips processed (if available). To account for the potential of our spatially clustered sampling design to bias richness estimates at Lambir, we also plotted a species accumulation curve where cores

taken within the determined zone of spatial autocorrelation (see Results section) were treated as a single sample.

Species turnover between plots (β -diversity) was calculated using the β_{sim} similarity coefficient using the Vegan package in the statistical program R version 2.7.2 (R Development Core Team, 2008). β_{sim} is a presence-absence-based measure of β -diversity that controls for differences in species richness. β_{sim} is calculated as $\min(b, c) / (\min(b, c) + a)$, where a is the number of shared species between plots, b is the number of species unique to the comparison plot, and c is the number of species unique to the focal plot (Koleff *et al.*, 2003). β_{sim} is 0 when there is total species overlap between two plots, and 1 when two plots share no species common. Patterns of β -diversity were visualized using nonmetric multidimensional scaling (NMDS). We tested whether species sharing was greater within soil types with analysis of variance using distance matrices (ADONIS, Oksanen, 2008), which is equivalent to Anderson's (2001) nonparametric multivariate ANOVA. ADONIS is similar to analysis of similarity (ANOSIM) but is thought to be more statistically robust (Oksanen, 2008). Statistical significance was tested against 1000 null permutations.

To quantify the degree of spatial autocorrelation in our samples, we calculated a mantel correlogram for community similarity of individual soil cores. A mantel correlogram tests the similarity of samples within a distance class against the similarity of all other samples. Samples are not spatially independent within distances where significant, positive values of the Mantel r occur. Distance classes for our analysis were based on the sampling distances within our nested sampling design and average distances between plots. Significance values across distance classes were adjusted using the progressive Bonferroni method recommended by Legendre & Legendre (1998). In order to remove any confounding factors, we calculated the mantel correlogram separately for each soil type as well as for all samples combined. These analyses were done with the *mgram* function in the R package *Ecodist*.

We tested for differences in plant community structure (species richness, richness of Dipterocarpaceae, and percentage of stems in the Dipterocarpaceae) within our plots using Welch's t -test for heterogeneous variances. We tested for relationships between plant community structure and ectomycorrhizal fungal richness using Pearson correlation. Plot-level plant species composition and Dipterocarpaceae species composition were ordinated using the methods described earlier and differences between soil types were tested using ADONIS.

Phylogenetic community analyses

Phylogenetic methods have the potential to yield significant insight into the factors that influence ecological community

structure (Webb *et al.*, 2002). To test if co-occurring ectomycorrhizal communities exhibited nonrandom community phylogenetic structure, we built a phylogenetic tree of all ectomycorrhizal taxa found in the study. All available 5.8S and 25S LSU sequences were aligned using the online program MAFFT (Katoh *et al.*, 2002) and hand-aligned using the program MacClade v4.06 (Sinauer Associates, Sunderland, MA, USA). The phylogeny was estimated using maximum likelihood with the program GARLI (Zwickl, 2006) with a general time-reversible model of nucleotide substitution rates. The run was terminated after 10 000 generations without an increase in log-likelihood > 0.01 and the single most likely tree was saved for phylogenetic analyses. While the unconstrained analysis accurately grouped all taxa at the familial and ordinal levels, the branching order of some deeper lineages (e.g. Sebaciales, Cantharellales) was not consistent with the most recent multigene phylogenies of the kingdom Fungi (Hibbett, 2006; James *et al.*, 2006). For this reason we used a backbone constraint based on a phylogeny of the Agaricomycotina presented by Hibbett (2006). This site-wide phylogeny was used as the pool against which we calculated measures of phylogenetic α and β -diversity for sites or soil types, with the program *Phylocom* (Webb *et al.*, 2008a), using either the command line version or the R package *Picante* (Kembel *et al.*, 2009). For phylogenetic α -diversity we calculated two common metrics, net relatedness index (NRI) and nearest taxon index (NTI) (Webb *et al.*, 2002). NRI considers the mean pairwise phylogenetic distance (along our Lambir-wide ectomycorrhizal phylogeny) between all co-occurring taxa within a sample, while NTI incorporates the mean distance of each taxon in the sample to the phylogenetically closest relative with which it co-occurs. Departures from a random model of community assembly (where phylogenetic relatedness and its correlates do not influence membership in the community) were assessed by comparing the observed relatedness measures to a null expectation generated by creating random communities of equal richness from the entire Lambir-wide ectomycorrhizal phylogeny. Both NRI and NTI are standard effect sizes of the null model analysis; significant positive values of NRI and NTI indicate that co-occurring taxa tend to be more closely related than expected under a random model of community assembly (phylogenetic clustering), while significant negative values indicate co-occurring taxa tend to be drawn from more distantly related clades (phylogenetic even dispersion) than expected. Phylogenetic clustering or even dispersion may result when ecological processes, such as habitat filtering or limiting similarity, act on functional traits which exhibit some degree of correlation with phylogenetic distance (i.e. traits are conserved or convergent; see Kraft *et al.*, 2007 for a review). In addition, we tested for significant soil associations by particular ectomycorrhizal clades using the *nodesig* function in *PhyloCom*. *Nodesig* tests whether the

abundance of terminal taxa beneath a given node is significantly greater or less than expected under a random model of community assembly. To test the robustness of our results to changes in phylogenetic reconstruction, we also calculated NRI and NTI on 10 alternative topologies selected randomly from 100 bootstrap trees generated by GARLI. To detect phylogenetic patterns that might occur at finer spatial scales, we calculated NRI and NTI for each individual soil core. To avoid problems with multiple comparisons, we then used the Wilcoxon signed-rank test to see whether NRI or NTI for soil cores as a whole was significantly different from zero.

Phylogenetic β -diversity measures the degree of phylogenetic similarity between two sites (Graham & Fine, 2008; Webb *et al.*, 2008b). We calculated phylogenetic β -diversity in PhyloCom using mean pairwise phylogenetic distance and minimum nearest-neighbor distance. Compared with traditional species-based measures of β -diversity, phylogenetic β -diversity has the advantage that it can allow the comparison of multiple sites that may not share any common species. Patterns of phylogenetic β -diversity were visualized using NMDS. We tested for greater phylogenetic similarity within soil types using ADONIS on the phylogenetic distances.

Results

In total, 102 out of 112 (91%) soil samples were found to contain ectomycorrhizal roots. From these samples, DNA was extracted from 830 root tips. PCR success was high and fungi were successfully sequenced from 589 root tips (71%). Using a 97% sequence similarity cutoff, we identified 146 fungal OTUs (hereafter species) (Table 1). Seventy-two percent (105) of species and 86% (505) of root tips were colonized by fungi derived from lineages generally considered to be ectomycorrhizal. The remaining species and tips were either associated with fungal lineages traditionally considered saprophytic (e.g. Polyporales, Mycenaceae), endophytic (e.g. Chaetothyriales) or of unknown trophic status (e.g. Trechisporales). A larger number of non-ectomycorrhizal fungal species and root tips were found in the sand soils (total no. of species: sand = 26, clay = 16; total no. of tips: sand = 64, clay = 20), but differences in plot-level means were not significant between soil types ($P > 0.05$). The large number of saprotrophic tips in sand plots was primarily the result of one taxon, Polyporales LH76, isolated from 25 root tips that formed hyphal root coverings that appeared superficially similar to ectomycorrhizal mantles (Fig. S3). There were no close BLAST matches for the ITS regions of this taxon, but the LSU sequences showed good homology with wood-rotting genera such as *Ganoderma* and *Fometopsis*. While it is likely that some of these species may constitute novel ectomycorrhizal associations, verification of trophic status awaits more

detailed anatomical study and controlled mycorrhizal synthesis. For the purposes of this study, these putatively nonmycorrhizal taxa were excluded from further statistical analyses of community structure. LSU sequences were generated for 78% (82) of the ectomycorrhizal fungal species, representing 92% (465) of all ectomycorrhizal root tips.

The most diverse and abundant ectomycorrhizal groups were the Russulales and Boletales (Table 2). Ectomycorrhizal ascomycetes were relatively rare, and the most common were closely affiliated with a new group of *Sordariales* ectomycorrhizas described by Tederloo *et al.* (2007) from a dipterocarp in the Seychelles. Species accumulation curves and Jackknife 2 (Burnham & Overton, 1979; Colwell, 2005) estimates of minimum species richness (Fig. S4) indicated that a significant number of additional species could be identified through a greater sampling effort. Estimated species richness appeared similar to high-diversity temperate studies, however the result varied depending on whether richness was plotted by number of samples or number of root tips, or whether we combined spatially autocorrelated soil cores (Fig. S4).

In total, 41 and 65 species of ectomycorrhizal fungi, respectively, were found on clay and sand plots, despite greater sampling on clay soils (Table 1). Plot-level richness was also significantly higher on sand compared with clay (Table 1, $t = 4.33$, $P = 0.02$). This pattern was also true for comparisons using Fisher's α ($t = 7.48$, $P = 0.002$) or the Jack2 estimate of species richness ($t = 5.07$, $P = 0.006$). Species abundance distribution also appeared different between the two soil types, with high dominance by a few taxa (primarily *Russula* spp.) on clay and greater evenness as measured by $E_{1/D}$ on sand ($t = 3.02$, $P = 0.03$, Table 1, Fig. S5). Mean plant species richness was significantly higher in sand plots ($t = 4.70$, $P = 0.006$, Table 1), but species richness of Dipterocarpaceae and number and percentage of stems in the Dipterocarpaceae did not differ

Table 2 The distribution of ectomycorrhizal fungal species within orders of ascomycetes and basidiomycetes detected on roots at Lambir Hills National Park, Sarawak, Malaysia

Order	No. of species	No. of root tips	% root tips
Agaricales	18	53	10.5
Atheliales	3	9	1.8
Boletales	17	61	12.1
Cantharellales	12	48	9.5
Elaphomycetales	1	2	0.4
Helotiales	1	3	0.6
Hymenochaetales	2	2	0.4
Hysterangiales	1	5	1.0
Russulales	30	264	52.3
Sebacinales	2	3	0.6
Sordariales	5	13	2.6
Thelephorales	13	42	8.3

significantly by soil type ($P > 0.05$, Table 1). There were only seven species of Fagaceae within the plots, which comprised $< 1\%$ of the total stem number. There were no significant correlations between any of the plant community variables presented in Table 1 and ectomycorrhizal fungal species richness (data not shown, $P > 0.05$).

Species turnover between plots was high, with an average β -diversity value between plots of 0.95 (min = 0.76, max = 1), and 14 out of 21 plot pairs sharing no species in common. A mantel correlogram indicated that composition of ectomycorrhizal core samples was positively autocorrelated up to distances of 5 m (Fig. 1). Distance between plots within a soil type was greater than the scale of sample autocorrelation, as the average distance between plot centers within a soil type was 83 m (min = 35 m, max = 150 m). Significant negative correlations corresponded with mean distances between plots, appearing to be the result of the positive autocorrelation at small scales within plots. The threshold for positive spatial autocorrelation was identical when the correlogram was calculated for all core samples irrespective of soil type (data not shown).

Nonmetric multidimensional scaling showed clustering of ectomycorrhizal communities in plots sampled on similar soil types (Fig. 2). ADONIS indicated that this clustering was significant, although soil type explained just over a quarter of the variation in species composition ($F = 1.78$, $R^2 = 0.26$, $P < 0.001$). NMDS (not shown) and ADONIS

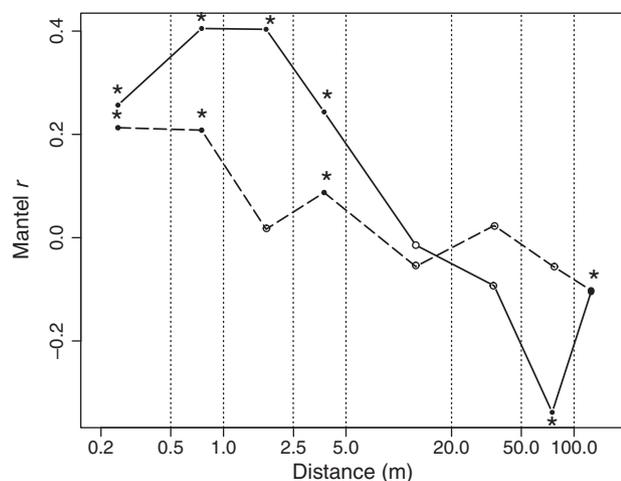


Fig. 1 Mantel correlogram showing the strength of spatial autocorrelation of ectomycorrhizal fungal core samples within different distance classes. Results are plotted separately for sand (dashed line) and clay (solid line) soils. Vertical lines demarcate distance classes, with the corresponding Mantel r plotted at the midpoint. Distance classes are based on the nested sampling design used in the study. The x-axis is plotted on a log scale for clarity. Filled circles with an asterisk indicate a significant Mantel r -value after progressive Bonferroni correction. Positive values indicate greater than expected community similarity of cores sampled within a given distance class, and negative values indicate greater than expected dissimilarity.

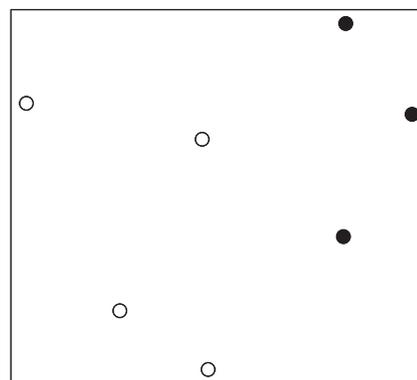
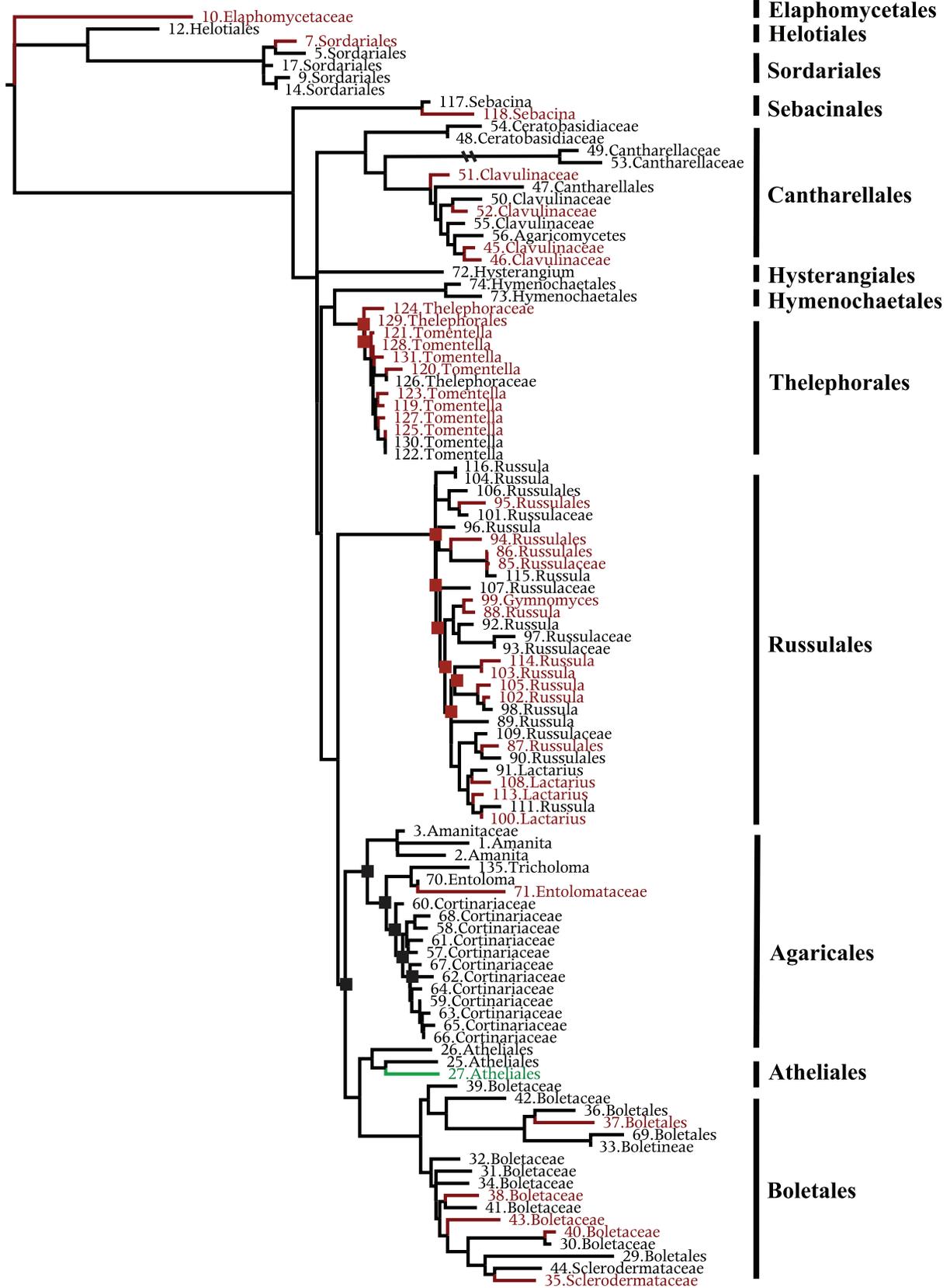


Fig. 2 Patterns of β -diversity for ectomycorrhizal fungi in lowland dipterocarp forest at Lambir Hills National Park, Sarawak, Malaysia. Black and white points represent plots from sand and clay soils, respectively. Plots closer together in the ordination are more similar to each other (i.e. have lower β -diversity). This clustering was significant when tested by ADONIS.

showed that species composition of all plants ($F = 4.29$, $R^2 = 0.46$, $P < 0.01$) and members of the Dipterocarpaceae ($F = 5.90$, $R^2 = 0.54$, $P < 0.01$) were also more similar within the same soil type. It should be pointed out that this analysis does not construe a test of habitat associations of plant species, but is rather a demonstration that the biotic environment experienced by the ectomycorrhizal fungi is more similar within soil types. For rigorous demonstration that plant community composition and demographic rates are affected by soil type at Lambir, refer to Davies *et al.* (2005) or Russo *et al.* (2005).

The ectomycorrhizal community found on the clay site exhibited significant phylogenetic clustering based on mean pairwise phylogenetic distance between communities (NRI = 2.13, $P = 0.036$), but not mean nearest neighbor distance (NTI = 0.38, $P = 0.72$). The sand site appeared to be assembled randomly with respect to phylogenetic distance (NRI = -1.68, $P = 0.079$, NTI = -0.23, $P = 0.85$). These results did not change significantly for the 10 alternative tree topologies that we examined. There was no evidence of phylogenetic community structure at the scale of soil cores (mean NRI = 0.12, Wilcoxon signed-rank test, $P = 0.64$, mean NTI = 0.13, Wilcoxon signed-rank test = 0.79). NMDS phylo-ordination based on nearest neighbor distance clustered sites by soil type, but this tendency was marginally insignificant (ADONIS $F = 5.65$, $R^2 = 0.53$, $P = 0.055$, Fig. S6a). NMDS phylo-ordination based on mean pairwise phylogenetic distance showed clustering of clay sites but a wide dispersion between sand sites ($F = 1.32$, $R^2 = 0.21$, $P = 0.006$, Fig. S6b). Some fungal clades also showed significant soil associations (Fig. 3). Overall, clay sites had higher representation of the Russulales and Thelephorales and significantly lower representation of the Agaricales, particularly Cortinariaceae (Fig. 3).



0.2

Fig. 3 Phylogenetic tree of ectomycorrhizal fungi found on roots in Lambir Hills National Park, Sarawak, Malaysia. The phylogeny was generated using maximum-likelihood methods with a combined 5.8S and 25S ribosomal RNA gene dataset and a backbone constraint based on recent multilocus phylogenies of the kingdom Fungi. Red labels and branch tips represent taxa sampled in clay soils, and black represent those found in sand soils. Only one taxon, *Atheliales* sp. LH27 (green), was found in both soil types. Red and black nodes indicate clades where significantly more terminal taxa were associated with clay or sand soils, respectively. Major orders found in the study are indicated by vertical bars.

Discussion

Taxonomic representation at Lambir

Ectomycorrhizal communities of angiosperm forests in southeast Asia and the southern hemisphere have received less study than temperate forests of the northern hemisphere (Alexander & Lee, 2005; Dickie & Moyersoen, 2008). This situation has begun to be rectified with the increasing number of molecular studies from tropical and southern hemisphere forests (Sirikantaramas *et al.*, 2003; Haug *et al.*, 2005; Yuwa-Amornpitak *et al.*, 2006; Riviere *et al.*, 2007; Tedersoo *et al.*, 2007, 2008). The need for more studies from these areas is evident by their poor representation in GenBank. While our BLAST results did not indicate close matches to species in temperate molecular studies, we found that major ectomycorrhizal families that are most abundant and diverse on roots in temperate forests – Russulaceae, Boletaceae, Cortinariaceae and Thelephoraceae – were also the most common in this study (Gardes & Bruns, 1996; Horton & Bruns, 2001). Thelephoraceae, Russulaceae and Boletaceae were also found to be common on roots in other studies of dipterocarp forest (Lee *et al.*, 1997; Sirikantaramas *et al.*, 2003; Manassila *et al.*, 2005; Yuwa-Amornpitak *et al.*, 2006) as well as other tropical and southern hemisphere angiosperm forests (Riviere *et al.*, 2007; Tedersoo *et al.*, 2008), suggesting that this may be a global pattern.

The below-ground structure of this community shows both differences from and similarities with that revealed by fruit-body surveys of Bornean dipterocarp forests (Smits, 1994). As noted by Sirikantaramas *et al.* (2003), Thelephoraceae are rarely reported from fruit-body surveys of dipterocarp forests, yet are abundant and diverse on dipterocarp root tips. The failure of some fruit-body surveys to detect cryptic taxa such as the Thelephoraceae has also been noted from temperate systems (Horton & Bruns, 2001). Otherwise, our results are fairly consistent with what might have been predicted from previous fruit-body studies (Lee *et al.*, 2003). Interestingly, while the Boletales are commonly a significant part of the fruit-body communities in many temperate forests, they often have correspondingly low abundance on root tips (Gardes & Bruns, 1996). In this system, Boletes appear to be common and diverse both above and below ground (Corner, 1972; Smits, 1994). There are certainly differences in the community structure between this forest and temperate forests surveyed thus far,

but the broad similarity at the family level between temperate and tropical ectomycorrhizal fungal communities is much greater than the minimal overlap in dominant plant families between temperate and tropical forests.

Ectomycorrhizas in tropical forests

Our study is one of the few large-scale molecular characterizations of a natural ectomycorrhizal fungal community in mixed-dipterocarp forest. We found that ectomycorrhizal fungi were common and diverse, in terms of both species and known ectomycorrhizal lineages. While our sampling was somewhat biased based on the orientation of plots close to an established Dipterocarpaceae, this family is a ubiquitous, dominant component in these and other forests in Borneo. While paleotropical mycologists and forest ecologists have long known about the association between dipterocarps and ectomycorrhizal fungi (Corner, 1972; Alexander *et al.*, 1992), it has been postulated that ectomycorrhizal symbiosis should be relatively rare or consist of communities with few species in mixed, lowland tropical rainforests (Malloch *et al.*, 1980; Janos, 1983; Read, 1991). One reason cited for this claim is that ectomycorrhizal symbiosis is based primarily on nitrogen acquisition, and that nitrogen limitation in tropical forests is low relative to their temperate counterparts (Malloch *et al.*, 1980; Janos, 1983; Read, 1991). The second reason cited is that ectomycorrhizal fungi are relatively host-specific compared with arbuscular mycorrhizal fungi. In high-diversity plant communities, the rarity of most plant species could put specialized mycorrhizal fungi (and their hosts) at a disadvantage because of the difficulty of locating suitable symbiotic partners (Malloch *et al.*, 1980; Janos, 1985; May, 1991). The fact that many tree species capable of forming monodominant stands in tropical forests form ectomycorrhizal associations has sometimes been viewed as the exception that proves this rule. This is because host plants are very abundant and available nitrogen is often tied up in recalcitrant organic matter (Connell & Lowman, 1989; Torti *et al.*, 2001; McGuire, 2007). We found that ectomycorrhizal fungi were common in both low- and high-fertility soil types, although species richness was higher in the low-fertility sand site. Moyersoen *et al.* (2001) found no difference when comparing ectomycorrhizal root colonization in a low-nutrient heath forest and a lowland rainforest in Borneo. Given that the tropical rainforests of Borneo are among the

largest and most diverse in the world, the common occurrence of ectomycorrhizal fungi here suggests that there is nothing inherent in the nutrient dynamics of tropical rainforests that should restrict occurrence of ectomycorrhizal symbiosis (Alexander & Lee, 2005).

Host diversity, ectomycorrhizal fungal species richness and the latitudinal gradient

Two slightly contradictory views have been put forward regarding the relationship between plant diversity and ectomycorrhizal fungal species richness. As mentioned previously, one hypothesis is that ectomycorrhizal fungi are relatively host-specific compared with arbuscular mycorrhizal fungi, and that specialized fungi (and their hosts) should be at a disadvantage in high-diversity plant communities (Malloch *et al.*, 1980; Janos, 1985; May, 1991; Ferrer & Gilbert, 2003). On the other hand, some recent studies have suggested that preference for particular host plants increases the number of available fungal niches, and thus greater diversity of host plants increases ectomycorrhizal fungal species richness (Dickie, 2007; Ishida *et al.*, 2007). Given the widely recognized latitudinal gradient in plant species richness, a strong relationship between plant species richness and ectomycorrhizal fungal species richness (positive or negative) might determine the type of latitudinal gradient that exists for ectomycorrhizal fungi.

While comparing species richness between studies is difficult because of differences in sampling methodologies and the failure of most studies to saturate species accumulation curves (Fig. S4), ectomycorrhizal fungal species richness at Lambir appears to fall within the range of variability exhibited by temperate studies from lower-diversity plant communities. The observed (105) and minimum estimate (205) of species richness at Lambir fall at the high end of richness from recent molecular studies reviewed by Dickie (2007), which ranged from 16 to 205 observed and 35 to 387 estimated species. However, more specific inferences about relative richness at Lambir compared with high-diversity temperate forests are difficult to make given the differences in sampling methods, as demonstrated by Fig. S4. Additionally, while our plots at Lambir contained at least 49 species of ectomycorrhizal host plants (counting just Dipterocarpaceae and Fagaceae), we did not directly identify host plants from root tips, making it difficult to quantify the actual number of host plants sampled.

Ectomycorrhizal richness is likely affected by a combination of α -, β - and phylogenetic diversity of host plants. Unraveling this complex relationship and its effects on global patterns of fungal richness will require studies that employ similar sampling methodology across a range of plant communities. In addition, phylogenetically explicit approaches will likely be needed to take into account the relationship between phylogenetic relatedness and host

specificity in symbiotic interactions (Gilbert & Webb, 2007). Given the small number of ectomycorrhizal studies from tropical forests currently available and the lack of a strong pattern in our results, it is still too early to make definitive conclusions about the strength of the latitudinal diversity gradient for ectomycorrhizal fungi. However, this is certainly an area where greater research would be extremely useful.

Potential linkage between ectomycorrhizal fungi and tree distributions

As in the plant communities in Borneo, ectomycorrhizal assembly structure appears to change with soil type (Russo *et al.*, 2005; Paoli *et al.*, 2006). Species richness and evenness were higher on sand plots. Community structure was also more similar within a soil type, despite high β -diversity and nearly total turnover of species between plots. While it is possible that the closer proximity of plots located within the two sites could give rise to dispersal-driven spatial autocorrelation in community structure, our data indicate that positive autocorrelation was gone for samples taken > 5 m apart (Legendre, 1993). This number is in agreement with the degree of spatial autocorrelation found in temperate studies (Lilleskov *et al.*, 2004), and suggests that our plots were spatially independent samples from the ectomycorrhizal fungal community in each soil type. While we believe that the community differentiation across soil types that we observed is biologically meaningful (as has been demonstrated for trees at Lambir), it is still possible that some degree of spatial confounding exists in this study (*sensu* Hurlbert, 1984, but see Oksanen, 2001). For this reason, our results should be interpreted cautiously until they can be confirmed experimentally or replicated at other study sites.

While the differences in ectomycorrhizal fungal community across soil types suggest the operation of some type of habitat filter, it is not clear what biological mechanism drives this pattern. Because plant communities are also more similar within a soil type, ectomycorrhizal fungi could be responding to the soil physicochemical environment or plant community, or both. There is good evidence from field studies that soil environment affects ectomycorrhizal fungal community structure (Swaty *et al.*, 1998; Toljander *et al.*, 2006). Pot studies have also shown some differentiation of dipterocarp mycorrhizas based on particular soil types or nutrient sources (Brearley, 2006; Brearley *et al.*, 2007). In relation to the plant community, while many ectomycorrhizal fungi are host generalists (Horton & Bruns, 1998; Kennedy *et al.*, 2003), there is some evidence that host preferences or specificity can affect ectomycorrhizal fungal community structure (Ishida *et al.*, 2007; Tedersoo *et al.*, 2008). Thus, the habitat filter operating across this ecotone could be biotic or abiotic, or some combination of the two.

Assuming the existence of habitat filtering (biotic or abiotic), the phylogenetic clustering that we observe within the clay site is consistent with greater functional trait similarity among more closely related ectomycorrhizal fungi. Phylogenetic conservatism of soil niches has also been hypothesized by other workers based on morphological similarities or habitat preferences within particular ectomycorrhizal lineages (Tedersoo *et al.*, 2003; Agerer, 2006). It is important to note that nonrandom patterns of phylogenetic community structure may result from numerous combinations of ecological process and patterns of trait conservation (Kraft *et al.*, 2007), and thus it is not possible to infer both ecological process and trait pattern from phylogenetic community structure alone. In this case, the evidence for habitat filtering allows us to make reasonable inferences about trait evolution.

There are a number of functional traits, such as the ability to use organic forms of nitrogen (Abuzinadah & Read, 1986; Lilleskov *et al.*, 2002), foraging morphology (Agerer, 2001), drought tolerance (Mexal & Reid, 1973) and host specificity (Tedersoo *et al.*, 2008), that could be important in habitat filtering for ectomycorrhizal fungi. Of the groups that showed significant phylogenetic habitat associations in this study, there is evidence from temperate systems that some *Russula* species may not use organic N and that *Cortinarius* species are specialized in the acquisition of organically bound nitrogen in low nitrogen settings (Lilleskov *et al.*, 2002; Avis *et al.*, 2003). This pattern matches the lower nutrient concentrations, slower decomposition and increased organic matter found on the sandy soils (Lee *et al.*, 2002; Baillie *et al.*, 2006). Along these lines, the abundance and richness of saprotrophic taxa were higher (although not significantly so) on sand plots. It is important to point out that phylogenetic clustering in this system was fairly weak (most clades occurred at least once in both soil types), and that our knowledge of how functional traits are conserved in ectomycorrhizal lineages is still highly speculative. However, investigations along this line have the potential to provide mechanistic explanations and allow generalizations about the factors that control ectomycorrhizal fungal community assembly across different ecosystems (Peay *et al.*, 2008).

Our study shows that there is a potential link between the distribution of tropical tree diversity with respect to local soil environment (Janzen, 1974; Russo *et al.*, 2005; Paoli *et al.*, 2006; John *et al.*, 2007) and the distribution of tropical ectomycorrhizal diversity. There are several possible (nonexclusive) processes that could generate the pattern we observe: trees and mycorrhizas could be responding independently to the same abiotic gradient; host specificity by mycorrhizal fungi could cause fungi to track host distributions; or host specificity by trees may cause them to track mycorrhizal distributions. Tree demography studies at Lambir have shown strong soil-related performance differ-

ences among closely related species (Russo *et al.*, 2008). Whether those performance differences are related to the advantages of specific mycorrhizal associations is currently unknown. Measurement of ectomycorrhizal functional traits in combination with reciprocal transplants of host seedlings across the soil ecotone would be a powerful way to unravel the relationships between host specificity, environmental habitat filtering and the distribution of tropical diversity.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Photograph of the two soil types sampled at Lambir Hills National Park.

Fig. S2 Location of ectomycorrhizal sample plots from this study in relation to the 52 ha forest dynamics plot at Lambir Hills.

Fig. S3 Typical ectomycorrhizal roots encountered in the soils of dipterocarp forest at Lambir Hills National Park.

Fig. S4 Species accumulation curve for Jack2 estimates of ectomycorrhizal fungi richness from Lambir Hills National Park, Sarawak, Malaysia, plotted against number of samples, and number of root tips.

Fig. S5 Species abundance distributions for ectomycorrhizal fungi sampled from clay and sand soil types in lowland dipterocarp forest at Lambir Hills National Park, Sarawak.

Fig. S6 Patterns of phylogenetic β -diversity for ectomycorrhizal fungi in lowland dipterocarp forest at Lambir Hills National Park, Sarawak.

Table S1 Soil chemistry data presented in Table 1 of Russo *et al.* (2005)

Table S2 Summary of plant communities by family for the study and across soil types

Table S3 Taxa recovered from fungal-specific sequencing of nuclear ribosomal RNA genes from root tips collected in mixed-dipterocarp forest at Lambir Hills National Park, Sarawak, Malaysia

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