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LETTER

Lack of host specificity leads to independent assortment of dipterocarps and ectomycorrhizal fungi across a soil fertility gradient

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Abstract

Plants interact with a diversity of microorganisms, and there is often concordance in their community structures. Because most community-level studies are observational, it is unclear if such concordance arises because of host specificity, in which microorganisms or plants limit each other's occurrence. Using a reciprocal transplant experiment, we tested the hypothesis that host specificity between trees and ectomycorrhizal fungi determines patterns of tree and fungal soil specialisation. Seedlings of 13 dipterocarp species with contrasting soil specialisations were seeded into plots crossing soil type and canopy openness. Ectomycorrhizal colonists were identified by DNA sequencing. After 2.5 years, we found no evidence of host specificity. Rather, soil environment was the primary determinant of ectomycorrhizal diversity and composition on seedlings. Despite their close symbiosis, our results show that ectomycorrhizal fungi and tree communities in this Bornean rain forest assemble independently of host-specific interactions, raising questions about how mutualism shapes the realised niche.

Keywords

Borneo, Lambir Hills, mutualism, mycorrhiza, plant–soil feedback, tropical rainforest.

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INTRODUCTION

The activity of belowground organisms greatly influences the structure of plant communities. Empirical studies show that changing the composition (Bever 1994; Klironomos 2002; Mangan *et al*. 2010), richness (van der Heijden *et al*. 1998) or abundance (Bagchi *et al*. 2014) of soil microorganisms directly alters the growth and survival of individual plants, thereby affecting community structure. In tropical forests, particular attention has been paid to the importance of negative feedbacks with fungal pathogens in maintaining diverse plant communities (Gilbert 2002; Mangan *et al*. 2010). Far fewer studies have examined the importance of beneficial feedbacks with soil microbial mutualists, such as mycorrhizal fungi, but the available evidence suggests that they, too, play critical, but as yet poorly understood roles, in determining tropical forest structure and dynamics (McGuire 2007; Batterman *et al*. 2013).

One way that microbial mutualists could affect tropical tree communities is by mediating edaphic specialisation, which is an important component of tropical tree diversity (Ashton 1964; Harms *et al*. 2001). Indeed, the relatively few molecular surveys that have been conducted to date of soil microbial communities underlying tropical forests show that the soil microbiota often change in parallel with variation in floristic communities across edaphic gradients (Russo *et al*. 2012; Peay *et al*. 2013). Mycorrhizal fungi are particularly important in plant nutrition (Smith & Read 2008) and also covary with soil environment in temperate and tropical ecosystems (Toljander *et al*. 2006; Peay *et al*. 2010). Despite the tight linkages between plants and mycorrhizal fungi, the mechanisms producing covariation in these communities are not known, prompting Janos to ask whether mycorrhizal fungi are "agents or symptoms of tropical communities" (Janos 1985).

Covariation in the community structure of tropical trees and mycorrhizal fungi may arise if there is strong host-specificity, resulting in specialised interactions between particular tree and mycorrhizal taxa. In this case, either the fungus or tree species may be the edaphic specialist, and one simply follows the other (Janos 1985). In other words, mycorrhizal fungi may be agents of tropical tree communities, if the trees follow the fungi, or the symptom of tree communities, if the fungi follow the trees. Indeed, it was long thought that the distribution of trees conditioned the distribution of fungal mutualists (Bisby 1943). Alternatively, this may be an overly simplistic view, and mycorrhizal fungi may be neither agents nor symptoms of tree communities. Covariation between tree and fungal mutualist communities may instead arise in the absence of specialised interactions, if they respond similarly, but independently, to edaphic variation. In other words, both

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tree species and mycorrhizal fungi may display edaphic specialisation, but not because of host-specificity. Without experimental evidence, however, it is impossible to distinguish between these alternative hypotheses.

In the southeast Asian tropics, the ectomycorrhizal Dipterocarpaceae, a species-rich family, dominate most lowland rain forests, often accounting for ~ 20% of individual trees and ~ 40% of stand basal area (Ashton 1964; Lee *et al*. 2002). In a previous study (Peay *et al*. 2010), ectomycorrhizal communities were found to be highly differentiated across a clay-sand soil ecotone varying in soil fertility in old-growth dipterocarp rain forest of northern Borneo (Baillie *et al*. 2006). Tree species composition varies strongly across the edaphic gradient. Indeed, the distributions of 73% of species are significantly aggregated on only one soil type (Davies *et al*. 2005). As a result, the direct effects of specialised interactions between ectomycorrhizal fungi (EMF) and tree species versus soil type in controlling ectomycorrhizal distributions could not be differentiated. Likewise, tree species' edaphic specialisations may arise though associations with particular ectomycorrhizal taxa that are themselves only found in certain soil environments (Janos 1985).

To disentangle the role of soil environment versus host-specificity in driving the distributions of both EMF and trees across this edaphic gradient in northwest Borneo, we conducted a reciprocal transplant experiment across two soil and light environments using seedlings of 13 dipterocarp species with different edaphic specialisations, and identified EMF communities on seedling roots using DNA sequencing. We tested the hypothesis that host-specificity is responsible for covariation between tree and EMF communities along this edaphic gradient and thus contributes to edaphic specialisation of these taxa. We expected that when specialist tree species were sown in their non-preferred soil type, their EMF colonists would exhibit reduced taxonomic richness and divergent composition, compared with co-occurring seedlings growing on their preferred soil type. Alternatively, if host-specificity is not responsible for covariation between tree and EMF communities, then the richness and composition of EMF colonists on a particular soil type should not depend on a seedlings' edaphic specialisation. In addition, because canopy gaps are the main form of disturbance in tropical rainforests, a secondary objective was to quantify their effects on EMF, as little is known about the basic ecology of tropical EMF communities.

METHODS

Study system

Lambir Hills National Park (Lambir) is in northern Borneo, in Sarawak, Malaysia, (4°20′ N, 113°50′ E). Lambir is a hyper-diverse, aseasonal, tropical rain forest, receiving approximately 3000 mm of rainfall per year, with maximum and minimum daily temperatures between 32 and 24 °C (Lee *et al*. 2002). The study was conducted adjacent to a 52-ha forest dynamics plot (FDP) established in 1991. The Dipterocarpaceae are the dominant tree family in the FDP, with 87 species that account for 42% of the basal area and 16% of the total number of individuals (Lee *et al*. 2002). Other potential ectomycorrhizal host lineages occur less frequently in the plot, with the Myrtaceae, Leguminosae and Fagaceae comprising only 3.6, 2.2 and < 1% of individuals respectively.

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, with the two ends represented by high fertility clay (formerly named udult) and a lower fertility sandy loam, (formerly named humult) soil types (Baillie *et al*. 2006). The clay has significantly higher nitrogen, phosphorous, magnesium and calcium concentrations, significantly greater moisture content and significantly lower pH and organic matter accumulation, relative to sandy loam (Davies *et al*. 2005; Baillie *et al*. 2006). Tree community composition and demographic rates also vary significantly across these soil types, with many species showing distinct soil specialisation (Davies *et al*. 2005; Russo *et al*. 2005). The distribution of ectomycorrhizal fungi is also highly structured across this edaphic gradient (Peay *et al*. 2010).

Experimental design

The reciprocal transplant experiment used 13 species from five genera of Dipterocarpaceae, 11 of which specialise on sandy loam or clay (10 congeneric species pairs plus an additional sandy loam specialist), plus two generalist species (Table 1). Species' edaphic specialisation patterns were based on a Poisson cluster model analysis of tree distribution data from the FDP (Davies *et al*. 2005). Seeds from each species were collected from local mother trees (1-5 trees per species, depending on seed availability) in January 2010 during a general flowering and mast fruiting event. Vouchers from all mother trees are stored in the FDP herbarium. Seeds were randomly sown directly into 24 experimental plots (5 × 5 m) in clay or sandy loam under closed or open forest canopy (six plots per *Soil Type* × *Canopy Status* combination). Closed canopy plots had completely closed canopies with no quantifiable gaps, whereas open canopy plots had noticeable canopy gaps resulting in greater insolation for most of the day. Measurements using photosynthetically active radiation sensors (LI-190; Li-Cor, Lincoln, Nebraska, USA) confirmed the far greater photon flux density in gap versus closed canopy plots (data not shown). Therefore, our plots encompassed a range of canopy closure environments found in the forest, as differences in proximity to overstory trees and photosynthetic carbon assimilation due to variation in light could influence EMF colonisation.

Seeds were randomly sown into 33 × 33 cm squares of each plot (one seedling/square, but not all squares were used). Seeds experienced heavy predation from termites and vertebrates, and so multiple seeds of a species were sown in each square to ensure that one seedling established. Not all tree species were represented in each plot in the final molecular data set, consisting of 251 seedlings (8–25 seedlings/species; Table 1). Due to multiple sowing, seedling mortality was not quantified; we focus on the EMF communities of surviving seedlings (i.e. our results are conditioned on seedlings surviving to be sampled). Soil nutrient supply rates were characterised for a companion set of plots in the same areas from another study (S.E. Russo, unpublished data). Thus, the experiment is a factorial recipro-

Table 1 Edaphic specialisation and estimated richness of ectomycorrhizal fungi colonising dipterocarp seedlings in a reciprocal transplant experiment in Bornean forest

			Clay-Closed			Clay-Gap			Sand-Closed			Sand-Gap		
Species	Code	Edaphic Specialisation	S	SD	N	S	SD	N	S	SD	N	S	SD	N
Anisoptera grossivenia	ANI2GR	Generalist	7.32	2.07	3	4.65	3.94	6	8.22	4.29	3	6.60	3.23	6
Dipterocarpus acutangulus	DIPTAC	Generalist	3.99	NA	1	4.57	2.82	7	9.37	1.96	2	7.93	4.00	5
Dipterocarpus globosus	DIPTGL	Sandy loam	7.38	5.20	6	5.23	0.68	5	6.47	0.98	4	5.69	3.95	5
Dipterocarpus palembanicus	DIPTPA	Clay	8.00	4.24	2	6.48	5.26	5	4.96	NA	1	NA	NA	0
Dryobalanops aromatica	DRYOAR	Sandy loam	4.62	5.54	3	5.37	2.56	4	4.00	2.83	2	7.66	2.17	6
Dryobalanops lanceolata	DRYOLA	Clay	7.19	1.62	4	3.57	2.43	7	8.87	4.85	4	7.25	3.06	6
Hopea beccariana	HOPEBE	Sandy loam	4.50	2.37	4	4.00	1.73	6	NA	NA	0	5.12	2.80	4
Hopea dryobalanoides	HOPEDR	Clay	3.50	2.64	4	4.10	2.50	9	NA	NA	0	6.00	NA	1
Shorea beccariana	SHORBE	Sandy loam	5.00	NA	1	3.91	2.34	6	6.08	2.53	7	7.65	2.09	3
Shora laxa	SHORLA	Sandy loam	4.00	1.41	2	4.10	3.27	5	7.95	4.44	6	6.00	2.31	10
Shorea macrophylla	SHORML	Clay	3.40	1.52	5	5.48	2.36	4	7.46	1.44	5	4.20	1.32	7
Shorea xanthophylla	SHORXA	Clay	5.48	1.33	6	3.91	2.17	11	6.99	NA	1	4.99	2.95	7
Vatica nitens	VATINT	Sandy loam	4.99	3.89	7	3.34	1.87	9	7.50	4.95	2	6.06	2.55	7
Summary			5.34	2.89	48	4.52	2.61	84	7.08	3.14	37	6.26	2.76	67

The number of seedlings (N) and average richness (S) and standard deviation (SD) based on rarefaction to 5000 sequences are shown for each species across factorial combinations of soil type (Clay, Sandy Loam) and canopy status (Open, Closed). Species names are followed by six letter species codes and each species edaphic specialisation: soil generalist, clay specialist or sandy loam specialist.

cal transplant design crossing *Tree Species Edaphic Specialisation* × *Soil Type* × *Canopy Status* (Supplemental Methods).

Molecular characterisation ECM fungi

In order to identify ectomycorrhizal fungi, we excavated seedling root systems after 2.5 years of *in situ* growth and extracted DNA from 15 EMF root-tips (Supplemental Methods) using a modified chloroform-CTAB (cetyl trimethyl ammonium bromide) buffer extraction coupled with a commercial DNA extraction kit (Qiagen Tissue Kit; Qiagen, Valencia, CA, USA), as in Peay *et al.* (2010). The fungal internal transcribed spacer 1 (ITS1) region of the nuclear ribosomal RNA genes were amplified using modified ITS1F and ITS2 primer constructs and sequenced on an Illumina MiSeq using the protocol of Smith & Peay (2014). Low-quality reads and non-ectomycorrhizal taxa were removed bioinformatically (Supplemental Methods) to generate the final species table. Sequences and sample data are deposited in the NCBI sequence read archive (SRP057798).

Statistical analysis

Based on rarefaction curves, the EMF species table was rarefied to 5000 sequences per sample as this produced asymptotic richness estimates for all nearly all samples (Fig. S1c). We analysed variation in EMF richness per seedling using a linear mixed effects model with *Soil Type*, *Tree Species Edaphic Specialisation, Canopy Status* and their interactions as fixed effects and *Plot Number* and *Tree Species Identity* as random effects using the package lme4 1.1-6 as implemented in lmerTest v2.0-6 in R statistical software. Based on inspection of residuals, EMF richness was square root transformed to improve normality, and homogeneity of variances was confirmed using Levene's test. We calculated pseudo-R^2 (pR^2) values, which estimate the goodness of fit for the fixed factors alone and the random and fixed factors together in multilevel models, based on the method of Nakagawa & Schielzeth (2013) as implemented in R (Jon Lefcheck, pers. comm.). We also fit a linear model using average richness for each species by treatment combination, excluding species with no seedlings (HOPEBE, HOPEDR, DIPTPA; Table 1, Fig. S2) in any single treatment combination, with *Soil Type*, *Tree Species Edaphic Specialisation, Canopy Status* and their interactions as fixed effects and *Tree Species Identity* as a random effect. Richness was log-transformed to improve normality of model residuals, and Levene's test confirmed homogeneity of variances. As an additional test of whether host identity affected EMF richness, we used the full data set to estimate a linear mixed effects model with random effects for *Plot* and *Tree Species Identity* and a single fixed factor (*Match*) with two levels indicating whether or not the seedling was matched with its preferred soil type. We used correlation tests to evaluate whether seedling growth parameters (growth rates in seedling mass, root mass and leaf area, relative growth rates in seedling diameter and height, leaf area ratio and root mass ratio) affected EMF richness (Supplemental Methods).

To examine variation in composition of EMF with respect to host species and treatments, we used permutational analysis of variance (permanova; Anderson 2001) as implemented in the program Primer v. 6.1.13 with permanova+ v. 1.0.3 (PRIMER-E Ltd, Lutton, Ivybridge, United Kingdom). Community composition across treatments was visualised by nonmetric multidimensional scaling (NMDS) using the R software package vegan. For analysis of the full data set with permanova, we included *Plot* identity as a random factor nested within *Soil Type* and *Canopy Status*. Analysis of community structure with permanova was done using both Bray–Curtis (relative abundance) and β_{sim} (presence-absence, but controls for richness) dissimilarities. We tested for differences in multivariate dispersion between treatments groups using the *betadisper* function in the vegan package. Because of strong effects of

Soil Type and uneven representation of tree species across soil types, we examined host effects with permanova separately on clay and sandy loam sites to avoid the possibility of confounding *Soil Type* and *Host Species Identity*. For clay we used only the gap plots because NMDS appeared to show some separation between gap and closed canopy plots and because all species were well represented on clay-gap plots (Table 1). As with richness, we tried an additional analysis to look for host effects in permanova by using the full data set with a random plot effect and a single fixed factor with two levels specifying whether or not a seedling was matched on its preferred soil (*Match*). We visualised dipterocarp species – EMF interactions using the R package, Bipartite (Dormann *et al*. 2008).

We also used a multivariate generalised linear model (GLM) to test whether EMF species abundance distributions were affected by *Soil Type*, *Canopy Status* and *Tree Species Identity* (Warton *et al*. 2012). These models avoid conflation of location and dispersion that can be associated with distance-based models such as permanova and allow direct tests of whether individual species respond to the treatment factors. Here, we present results using a negative binomial model using sequence count data, but a binomial GLM (presence-absence) gave similar results. We tested the effects of *Soil Type*, *Canopy Status* and their interactions using the full data set and tested for the effects of *Tree Species Identity* separately using the data sub-setted for Clay-Gap and Sandy Loam treatments. Analyses were conducted using the R package mvabund using the 50 most abundant EMF species (Wang *et al*. 2014).

RESULTS

The mean number of EMF species colonising dipterocarp seedlings was 5.53 (± 3.05 SD), but varied significantly with both *Soil Type* and *Canopy Status* (Table 2). Based on pR^2, the full linear mixed effects model explained 18% of variation in EMF species richness with significant main effects of *Soil Type* (pR^2 = 0.095, $P < 0.001$) and *Canopy Status* (pR^2 = 0.033, $P = 0.016$). On average, seedlings growing in sandy loam had 1.81 (37%) more EMF species than in clay, while seedlings growing under closed canopy had 1.06 (20%) more EMF species than under open canopy (Fig. 1). There was no significant effect of tree species edaphic specialisation, nor of any interaction ($P > 0.05$; Table 2), on EMF species richness. In addition, pR^2 showed that the proportion of variation explained by random effects was small for *Plot Identity* (pseudo $R^2 = 0.035$) and essentially zero for *Species Identity* (p$R^2 < 0.001$). Tests using average species richness values of EMF for each tree species × treatment combination yielded similar results for fixed effects and explained a large fraction of variation in EMF richness (Model pR^2 = 0.45; Table S1). As with the model based on non-averaged data, there was virtually zero variance attributable to the random effect included for dipterocarp host *Species Identity* (p$R^2 < 0.001$). A linear mixed effects model with the same random effects but the fixed factor *Match* showed that whether a seedling was matched on its preferred soil type did not affect EMF species richness ($P = 0.164$). While seedling growth varied in predictable ways across treatments (e.g. seedlings generally grew better on high fertility clay soils and in gaps; data not shown), the only seedling growth measure significantly correlated with EMF richness was *Root Mass Ratio* (root dry mass/total seedling dry mass; $r = 0.24$, $P = 0.047$), which was positively associated with the number of EMF species (Fig. S3). Incorporation of seedling growth into more complex statistical models did not reveal any meaningful relationships between EMF richness, seedling growth and whether the seedling was growing on its preferred soil type (data not shown).

The EMF observed on the roots of dipterocarp seedlings represented a broad range of phylogenetic lineages. The 306 species were assigned to 42 genera (Table S2) and 27 families (Table S3, Fig. S4). The most common genera were *Russula* (Russulaceae), *Tomentella* (Thelephoraceae), *Phylloporus* (Boletaceae), *Lactarius* (Russulaceae), *Sebacina* (Sebacinaceae) and *Cortinarius* (Cortinariaceae) (Fig 2, Table S2). The abundance distribution was lognormal with a few common species, but 22% of species were found on only one seedling (Fig. S5). Most EMF taxa had relatively restricted distributions with respect to soil and canopy status. Of the 238 taxa that occurred on more than one seedling, 167 (70%) were detected on a single soil type, and 112 (47%) were found in a single light environment. Only 28 of 143 (19%) taxa that occurred on four or more seedlings were detected in all treatment combinations. Taking into account relative abundance (% of sequences per seedling) of species on seedlings only strength-

Table 2 Factors affecting richness of ectomycorrhizal fungi colonising dipterocarp seedlings based on a linear mixed effects model including fixed effects for *Soil* Type (Clay, Sandy Loam), *Canopy Status* (Open, Closed) and seedling *Edaphic Specialisation* (Clay, Sandy Loam, Generalist) and random effects for *Plot Identity* and *Species Identity*

	Sum Sq	Mean Sq	NumDF	DenDF	*F*	Pr(> *F*)
Soil type	6.31	6.31	1	38.44	12.97	0.001
Canopy status	1.78	1.78	1	38.44	6.40	0.016
Edaphic specialisation	1.00	0.50	2	221.73	1.73	0.179
Soil : Canopy	< 0.01	< 0.01	1	38.44	< 0.01	0.962
Soil : Edaphic specialisation	0.18	0.09	2	221.73	0.03	0.967
Canopy : Edaphic specialisation	0.42	0.21	2	221.73	0.75	0.472
Soil : Canopy : Edaphic specialisation	0.55	0.28	2	221.73	0.77	0.463

Pseudo R^2 for the entire model was 17.8%, with 3.5% and < 0.1% of total model variation attributed to the random effects of *Plot* and *Species Identity* respectively.

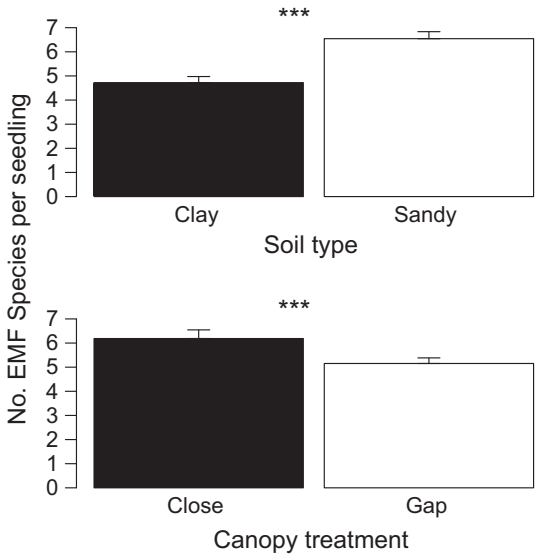

Figure 1 Richness of ectomycorrhizal fungal species colonising dipterocarp seedlings in a reciprocal transplant experiment in Bornean forest. Richness estimates are based on a subsample of 15 root tips and rarefaction to 5000 sequences per seedling. Linear mixed effects models showed that richness per seedling was higher on sandy loam compared with clay soils and in closed canopy forest compared with gaps. ***indicates $P < 0.05$. Only main effects are shown as there was no interaction between *Soil Type* and *Canopy Status*.

ened these patterns. For example, 84% of species had > 90% of their relative abundance on a single soil type. Similar patterns were not seen with respect to host species identity and plot (Fig. S5). The more common EMF species colonised the majority of dipterocarp hosts: only 8 (3%) of the EMF species that were found on more than one seedling were found on a single host species, and only 39 (16%) were found in a single plot. Abundant EMF species (i.e. those found on many seedlings) were also found to colonise most dipterocarp species used in the experiment (Fig. S5). The same was not true with respect to plots, and even abundant EMF were not present in a large number of plots (Fig. S5).

Analysis by permanova and NMDS ordination provided evidence for distinctive communities of EMF on seedlings based on the *Soil Type* they were grown in (Fig. 3, Table S4). The effect of *Canopy Status* on EMF community composition was marginally insignificant, as was the interaction between *Soil Type* and *Canopy Status*. Similar results were obtained when using a presence-absence metric controlling for richness differences (β_{sim}). The ordination represented the data structure well (stress = 0.1079), and there was a strong correlation between ordination axes and our treatments (Axis 1 R^2 = 0.51, Axis 2 $R^2 = 0.12$). A Tukey HSD test on group multivariate dispersions showed significant differences across some treatment groups ($F_{3,285} = 3.39$, $P = 0.019$): there was greater dispersion in EMF community dissimilarities between seedlings in open versus closed canopy plots on sandy loam ($P = 0.027$) and marginally significant differences between open canopy plots on sandy loam and closed canopy plots on clay ($P = 0.068$) and between open canopy plots on sandy loam versus clay ($P = 0.097$). A permanova test based on plot EMF composition also showed a significant effect of *Soil Type* ($r^2 = 0.10$, $P < 0.001$), but not *Canopy Status* ($r^2 = 0.05$, $P = 0.11$), nor any interaction ($r^2 = 0.05$, $P = 0.153$). Examination of EMF host associations in sandy loam and clay gap plots showed no evidence for differential colonisation by EMF of the different dipterocarp species ($P > 0.05$, Fig. S6). Similarly, a permanova model including the factor indicating whether a seedling was matched on its preferred soil type (*Match*) showed no effect on community composition ($P = 0.163$), and models including seedling growth parameters were uninformative (data not shown).

The use of multivariate species abundance GLMs gave a similar picture of ectomycorrhizal community structure: both *Soil Type* (Dev = 401.1, $P = 0.001$) and *Canopy Status* (Dev = 165.5, $P = 0.001$) had significant effects, with a significant interaction (Dev = 49.3, $P = 0.002$). Univariate tests for the fifty most abundant taxa showed that 68% had distributions significantly ($P < 0.05$) biased towards one soil type (32% after correction for multiple comparisons), 32% to one canopy type (6% after correction) and 8% with significant interactions (none after correction). EMF distributions generally showed high fidelity to a particular habitat, but rampant partner-sharing within soil types (Fig. 4). Multivariate abundance GLMs testing for host specificity separately on clay-gap and sandy loam were both non-significant ($P > 0.05$).

DISCUSSION

Host identity and soil environment have been the primary focus of ectomycorrhizal ecology since the symbiosis was first described over a century ago (Frank 1885, translatation by Trappe 2005). Using a reciprocal transplant experiment to disentangle these two factors, we demonstrate extreme edaphic specialisation of EMF across a natural soil ecotone, but no detectable specificity in the relationships between EMF and their dipterocarp seedling hosts. Neither tree species identity, nor edaphic specialisation, showed any influence on the composition and richness of the EMF colonists of seedlings. Our study involved young trees of 13 dipterocarp species, and further investigation is required to evaluate whether our results hold for more mature trees, other dipterocarps, or non-dipterocarp ectomycorrhizal species. However, available evidence suggests that our findings for seedlings would likely apply to more mature trees in this forest. To date no studies have found differential ectomycorrhizal specificity as plants age. Moreover, seedlings growing near mature trees have EMF communities similar to adults, generally consisting of the later-successional taxa that we found to be abundant (Last *et al*. 1987; Tedersoo *et al*. 2008). Our study therefore provides strong experimental evidence that host-specificity does not contribute to EMF and tree species' edaphic distributions in this forest. Instead, our results support the alternative hypothesis that tree and mycorrhizal communities assemble independently of each other in this forest, but in response to similar environmental factors, implying that the mechanisms generating the soil habitat specialisation observed in this forest are not directed by symbiotic partners, even in an obligate mutualism. While having a suite of locally adapted mycorrhizal fungi is beneficial for plant performance (Johnson *et al*.

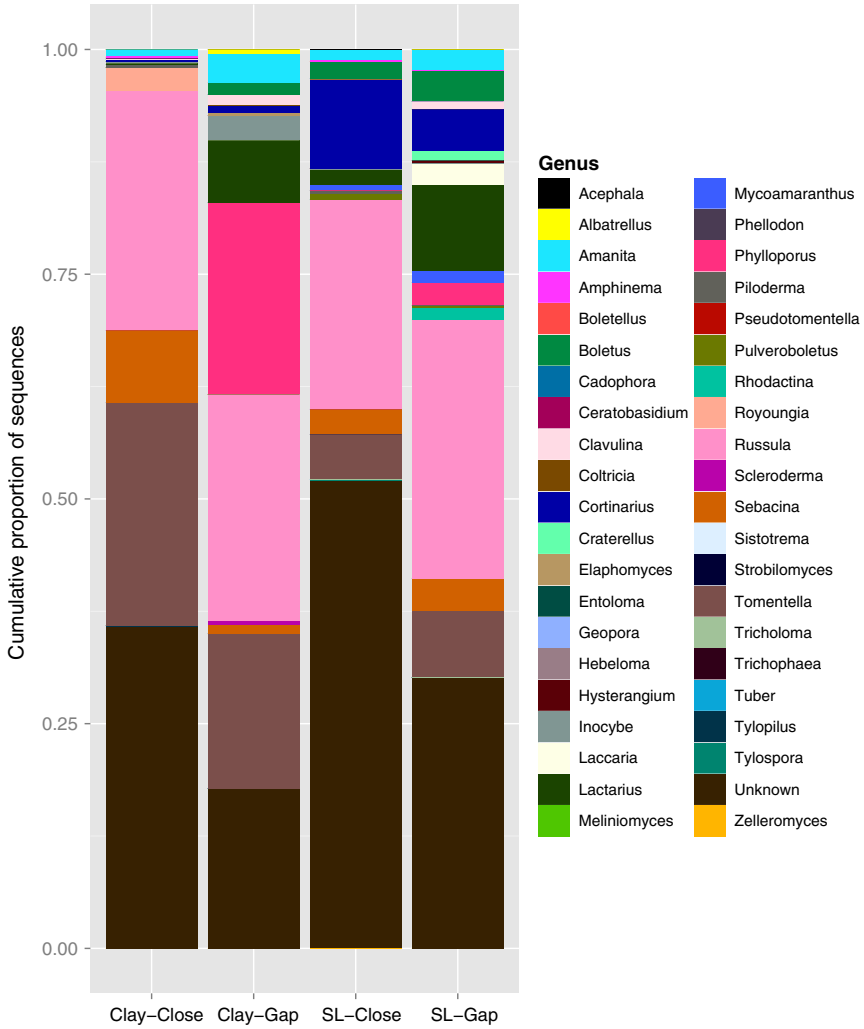

Figure 2 Relative abundance of ectomycorrhizal genera colonising dipterocarp seedlings in a reciprocal transplant experiment in Bornean forest with respect to combinations of *Soil Type* (Clay, Sandy Loam) and *Canopy Status* (Open, Closed). Relative abundance is calculated as the total proportion of DNA sequences in each treatment combination assigned to each genus.

2010), the ability of clay specialist dipterocarps to associate with sandy loam EMF (and vice versa) appears to be insufficient to allow these taxa to maintain large population densities in non-preferred soil types in this forest. Instead, we propose that tree and EMF diversity and distribution patterns may be determined by the extent to which independent adaptations of EMF-tree species associations jointly enable these taxa to overcome limitations imposed by the biotic and abiotic environment on their respective soil habitats.

Lack of host-specificity

Results from our reciprocal transplant suggest that partner specialisation contributes little to the structure of ectomycorrhizal fungal communities on dipterocarp seedlings in this rain forest. This is in contrast with early work suggesting specificity could play an important role in dipterocarp ecology (reviewed in Brearley 2012). Previous work on ectomycorrhizal symbiosis in other systems has been mixed with respect to the importance of host specificity. When strong examples of specificity exist, they tend to be restricted to particular broad taxonomic groups. For example, there is a strong host specificity between members of the Pinaceae and Suillaceae and between mycoheterotrophic plants such as *Monotropa* and the fungi they parasitise (Bruns *et al.* 2002). Introduced trees from different biogeographic regions often fail to associate with native fungi in the introduced range (Dickie *et al.* 2010). However, in New Zealand the specificity of plant-EMF symbioses was not found to be a major barrier to the spread of invasive plants into novel habitats (Bogar *et al.* 2015). In principle, tight host-specificity could be true of the Dipterocarpaceae and their EMF mutualists, but we found no evidence that this was the case in the seedlings of our study species. Instead, our results are more consistent with studies showing the predominance of multi-host fungi. For example, Kennedy *et al.* (2003) found that the majority of EM associations were shared between Pinaceae and Fagaceae in a temperate forest, with no evidence for host specialisation. Similarly, there is no evidence for host specialisation by ectomycorrhizal fungi amongst co-occurring caesalpinoid legumes and the neotropi-

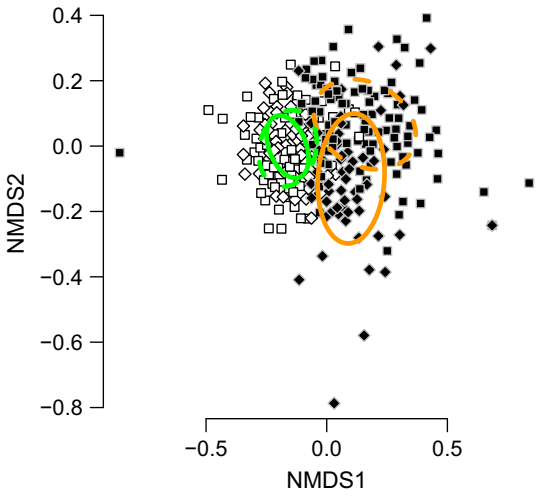

Figure 3 Non-metric multidimensional scaling plot illustrating differences in ectomycorrhizal fungal composition on dipterocarp seedlings grown in Clay (black fill) and Sandy Loam (unfilled) soils and in Gaps (squares) or under Closed canopy (diamonds) in a reciprocal transplant experiment in Bornean forest. Data points represent individual seedlings and lines show 95% confidence ellipses for each treatment combination, with green for sandy loam, orange for clay, dashed for gaps and solid for closed canopy.

cal dipterocarp, *Pakaraimaea dipterocarpacea* (Smith *et al.* 2013) or amongst African Dipterocarpaceae and Sarcolaenaceae (Tedersoo *et al.* 2011). By contrast, other studies have found evidence that host specialisation contributes significantly to diversity and composition of ectomycorrhizal fungi (Ishida *et al.* 2007). For example, Tedersoo *et al.* (2013) estimated that relatedness of tree species explained 20% of variation in EMF community composition amongst European Salicaceae. Sthultz *et al.* (2009) found that genotypes of *Pinus edulis* differing in herbivore susceptibility were colonised by distinct EMF communities, raising the possibility that EMF may also respond to genotypic variation of closely related species if their phenotypes are ecologically divergent. One difficulty in field studies of host-specialisation is the confounding role of environment, since, as with the EMF taxa, most co-occurring tree species also differ in some niche-dimension, as at Lambir (Russo *et al.* 2007). As host environmental specialisation or host effects on local soil environment may give rise to community patterns identical to host specialisation, experimental approaches are essential for parsing these alternatives, and we expect they will reveal even greater degrees of host generalism amongst ectomycorrhizal fungi than previously suspected.

Importance of the soil environment

The results from this study are highly consistent with our previous work in the Lambir FDP (Peay *et al.* 2010), in which minimal overlap in ectomycorrhizal fungal community composition across soil types and a marked decrease in ectomycorrhizal species richness on clay soils were observed. In addition, the 29 species of EMF common to the two studies showed consistent patterns of habitat fidelity. In both studies, we observed similar patterns of variation in taxonomic composition, with high abundance of Russulaceae, Thelephoraceae and Boletaceae, and some indication of lineage-level habitat specificity, for example amongst the Thelephoraceae (clay) and *Cortinarius* (sandy loam). Sandy soils at Lambir are relatively oligotrophic, with decreased litter nutrients (P, N, Ca, Mg, K and Mn) and slower decomposition rates resulting in greater accumulation of humus and plant necromass relative to clay soils (Baillie *et al.* 2006). Previous work in boreal and temperate ecosystems has generally found a strong role for increasing soil fertility, primarily in the form of nitrogen, in driving EMF composition and richness (Lilleskov *et al.* 2002; Toljander *et al.* 2006). The ability to utilise N in organic forms is thought to be an important functional trait for many ectomycorrhizal fungi (Smith & Read 2008) and likely confers a competitive advantage to plant associates, particularly in soils rich in organic matter. Consistent with this, increases in mineral N are often associated with declines in EMF richness and the loss of protein-utilising EMF, such as the Cortinariaceae (Lilleskov *et al.* 2002). While the fertility gradient at Lambir is complex, and it is not yet clear which nutrients limit tree growth (Kochsiek *et al.* 2013), we observed a decline in richness and abundance of *Cortinarius* on the more fertile clay soils. Feedbacks between soil fertility, plant litter quality and decomposition rate appear to be general drivers of EMF composition and richness from Boreal to tropical biomes.

Total P is generally higher in clay-rich soils, and PO_4^{3-}, the form available to plants, is considered the primary limiting nutrient to tree growth in many tropical forests (Vitousek 1984). Differential use of organic and inorganic P forms may be linked to the type of mycorrhizal association of tree species in tropical forests (Steidinger *et al.* 2014), but these dynamics are still poorly understood. Even less is known about how P availability affects ectomycorrhizal communities, although it may be a key structuring factor in N-saturated systems (Walker *et al.* 2014). Interestingly, nutrient addition studies with Dipterocarpaceae do not show P to be the primary nutrient limiting growth (Palmiotto *et al.* 2004), and availability of cations may also have important effects on tree growth at Lambir (Kochsiek *et al.* 2013) and in other tropical forests (Vitousek 1984). It is possible that the ability to overcome P limitation in SE Asian rain forests may partially explain the success of the ectomycorrhizal strategy of dipterocarps. However, the role of P availability in determining the structure and function of tropical EMF communities deserves further investigation.

Role of canopy gaps in EMF colonisation

Tree-fall gaps are one of the most important disturbances in lowland rain forests (Whitmore 1978), increasing insolation available to seedlings and thereby their photosynthetic carbon fixation. Improved plant C balance is generally positively correlated with EMF colonisation (e.g. Ingleby *et al.* 1998). In temperate forests, canopy disturbance has been shown to diminish the availability of EMF hyphae in the soil and lead to reductions in EMF richness and increases in the proportion of spore-colonising ruderal species among

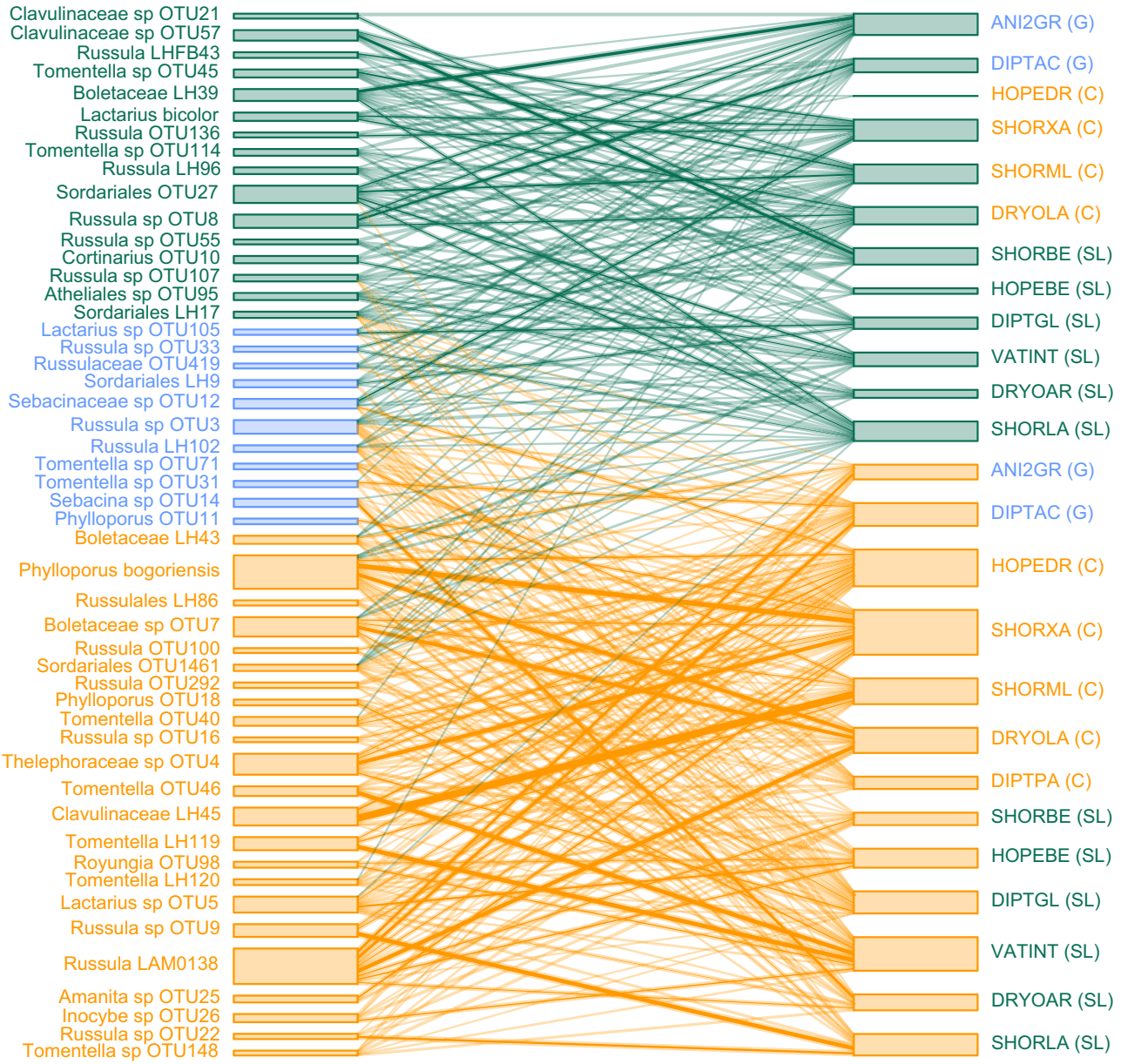

Figure 4 Extreme host generalism and habitat specialisation between dipterocarps and ectomycorrhizal fungi (EMF). Lines represent observed colonisation events, with line thickness proportional to the number of DNA sequences for each EMF. Seedling labels are coloured depending on whether they are edaphic specialists on Clay (Orange), Sandy Loam (Green) or are Generalists (Blue). The colour of seedling boxes and lines show whether the interaction occurred on Clay (Orange) or Sandy Loam (Green) soil. The width of seedling boxes is proportional to the number of seedlings for each dipterocarp species. Labels and boxes for EMF are coloured based on statistically significant association for Clay (Orange), Sandy Loam (Green) or no significant edaphic association (Blue) based on multivariate abundance analysis (see text). For EMF lack of significance may represent habitat generalism or lack of statistical power due to limited data. Only the 50 most abundant EMF are plotted.

soil EMF (Baar *et al.* 1999). Although the effect of canopy status on EMF species composition was only marginally significant, and smaller than that of soil type, we found that seedlings establishing in high-light gaps had overall 20% fewer species of ectomycorrhizal fungi on their roots compared to seedlings in closed-canopy plots. This result is more consistent with the availability of EMF to seedlings in gaps being reduced by disturbance-caused increases in colonisation distance, rather than by endogenous plant carbon dynamics, but more work is needed on the responses of EMF to gap formation and variable carbon allocation in tropical forests.

CONCLUSIONS

Two decades ago, David Janos asked whether tropical mycorrhizal fungi were 'agents or symptoms' of tropical tree community structure (Janos 1985). Our results suggest that perhaps they are neither. Although community structure of both ectomycorrhizal fungi and their dipterocarp hosts

responds strongly to the edaphic gradient at Lambir, our seedling experiment supports the hypothesis that this is not due to mutually dependent assembly. Clearly, there are benefits to plant hosts of associating with mycorrhizal fungi matched for a particular environment (Johnson *et al.* 2010), presumably enhancing the growth, survival and reproduction of both parties in complex ways. However, because they were not found to be species-specific, these symbiotic associations are not sufficient to explain the edaphic distributions of either EMF or dipterocarp tree species at Lambir. Rather, we propose that this mutualism is just one component of the respective tree and fungal ecological strategies that govern how interactions with the biotic and abiotic environment are translated into fitness differences across this soil, and potentially other types of environmental gradients. Even if obligate tree-EMF mutualism does not explain differential edaphic specialisation of species within the Dipterocarpaceae, its evolution has likely been a hugely important innovation leading to dominance of this tree family in southeast Asian tropical forests and other predominantly ectomycorrhizal taxa in other tropical forests (McGuire 2007). If our results hold for other systems, then it may be necessary to re-evaluate our understanding of how EMF root mutualisms shape the realised niches of both plants and fungi in the absence of strong host specificity.

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AUTHORSHIP

KGP designed the study, developed molecular methods, analysed data and prepared the manuscript; SER designed the study, established field experiment, analysed data and prepared the manuscript; KM contributed to the manuscript preparation; ZYL harvested seedlings and prepared samples for sequencing; JPC harvested seedlings; ST contributed to initial establishment of field experiment; SJD contributed to study design and manuscript preparation.

REFERENCES

Anderson, M.J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecol.*, 26, 32–46.

Ashton, P.S. (1964). Ecological studies in the mixed dipterocarp forests of Brunei state. *Oxford Forestry Memoirs*, 25, 1–75.

Baar, J., Horton, T.R., Kretzer, A. & Bruns, T.D. (1999). Mycorrhizal recolonization of *Pinus muricata* from resistant propagules after a stand-replacing wildfire. *New Phytol.*, 143, 409–418.

Bagchi, R., Gallery, R.E., Gripenberg, S., Gurr, S.J., Narayan, L., Addis, C.E. *et al.* (2014). Pathogens and insect herbivores drive rainforest plant diversity and composition. *Nature*, 506, 85–88.

Baillie, I.C., Ashton, P.S., Chin, S.P., Davies, S.J., Palmiotto, P.A., Russo, S.E. *et al.* (2006). Spatial association of humus, nutrients and soils in mixed dipterocarp forest at Lambir, Sarawak, Malaysian Borneo. *J. Trop. Ecol.*, 22, 543–553.

Batterman, S.A., Hedin, L.O., van Breugel, M., Ransijn, J., Craven, D.J. & Hall, J.S. (2013). Key role of symbiotic dinitrogen fixation in tropical forest secondary succession. *Nature*, 502, 224–227.

Bever, J.D. (1994). Feedback between plants and their soil communities in an old field community. *Ecology*, 75, 1965–1977.

Bisby, G.R. (1943). Geographical distribution of fungi. *Bot. Rev.*, 9, 466–482.

Bogar, L.M., Dickie, I.A., Kennedy, P.G. & Richardson, D.M. (2015). Testing the co-invasion hypothesis: ectomycorrhizal fungal communities on *Alnus glutinosa* and *Salix fragilis* in New Zealand. *Divers. Distrib.*, 21, 268–278.

Brearley, F.Q. (2012). Ectomycorrhizal associations of the Dipterocarpaceae. *Biotropica*, 44, 637–648.

Bruns, T.D., Bidartondo, M.I. & Taylor, D.L. (2002). Host specificity in ectomycorrhizal communities: what do the exceptions tell us? *Integr. Comp. Biol.*, 42, 352–359.

Davies, S., Tan, S., LaFrankie, J. & Potts, M. (2005). Soil-related floristic variation in the hyperdiverse dipterocarp forest in Lambir Hills, Sarawak. In *Pollination Ecology and Rain Forest Diversity, Sarawak Studies*. (eds Roubik, D., Sakai, S., Hamid, A.). Springer-Verlag, New York, pp. 22–34.

Dickie, I.A., Bolstridge, N., Cooper, J.A. & Peltzer, D.A. (2010). Co-invasion by *Pinus* and its mycorrhizal fungi. *New Phytol.*, 187, 475–484.

Dormann, C.F., Gruber, B. & Fruend, J. (2008). Introducing the bipartite Package: Analysing Ecological Networks. *R news*, 8, 8–11.

Gilbert, G.S. (2002). Evolutionary ecology of plant diseases in natural ecosystems. *Annu. Rev. Phytopathol.*, 40, 13–43.

Harms, K.E., Condit, R., Hubbell, S.P. & Foster, R.B. (2001). Habitat associations of trees and shrubs in a 50-ha neotropical forest plot. *J. Ecol.*, 89, 947–959.

van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T. *et al.* (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, 396, 69–72.

Ingleby, K., Munro, R.C., Noor, M., Mason, P.A. & Clearwater, M.J. (1998). Ectomycorrhizal populations and growth of *Shorea parvifolia* (Dipterocarpaceae) seedlings regenerating under three different forest canopies following logging. *For. Ecol. Manage.*, 111, 171–179.

Ishida, T.A., 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.

Janos, D.P. (1985). Mycorrhizal fungi: agents or symptoms of tropical community composition. In *Proceedings of the 6th North American Conference on Mycorrhizae*. (ed Molina, R.). Oregon State University, Corvallis, pp. 98–103.

Johnson, N.C., Wilson, G.W., Bowker, M.A., Wilson, J.A. & Miller, R.M. (2010). Resource limitation is a driver of local adaptation in mycorrhizal symbioses. *PNAS*, 107, 2093–2098.

Kennedy, P.G., Izzo, A.D. & Bruns, T.D. (2003). There is high potential for the formation of common mycorrhizal networks between understorey and canopy trees in a mixed evergreen forest. *J. Ecol.*, 91, 1071–1080.

Klironomos, J.N. (2002). Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature*, 417, 67–70.

Kochsiek, A., Tan, S. & Russo, S.E. (2013). Fine root dynamics in relation to nutrients in oligotrophic Bornean rain forest soils. *Plant Ecol.*, 214, 869–882.

Last, F.T., Dighton, J. & Mason, P.A. (1987). Successsions of sheathing mycorrhizal fungi. *Trends Ecol. Evol.*, 2, 157–161.

Lee, H.S., Davies, S.J., LaFrankie, J.V., Tan, S., Yamakura, T., Itoh, A. *et al.* (2002). Floristic and structural diversity of mixed dipterocarp forest in Lambir Hills National Park, Sarawak, Malaysia. *J. Trop. For. Sci.*, 14, 379–400.

Lilleskov, E.A., Hobbie, E.A. & Fahey, T.J. (2002). Ectomycorrhizal fungal taxa differing in response to nitrogen deposition also differ in pure culture organic nitrogen use and natural abundance of nitrogen isotopes. *New Phytol.*, 154, 219–231.

Mangan, S.A., Schnitzer, S.A., Herre, E.A., Mack, K.M.L., Valencia, M.C., Sanchez, E.I. *et al.* (2010). Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. *Nature*, 466, 752–755.

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

Nakagawa, S. & Schielzeth, H. (2013). A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods Ecol. Evol.*, 4, 133–142.

Palmiotto, P.A., Davies, S.J., Vogt, K.A., Ashton, M.S., Vogt, D.J. & Ashton, P.S. (2004). Soil-related habitat specialization in dipterocarp rain forest tree species in Borneo. *J. Ecol.*, 92, 609–623.

Peay, K., Kennedy, P., Davies, S., Tan, S. & Bruns, T. (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, K., Baraloto, C. & Fine, P. (2013). Strong coupling of plant and fungal community structure across western Amazonian rainforests. *ISME J.*, 7, 1852–1861.

R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Russo, S.E., Davies, S.J., King, D.A. & Tan, S. (2005). Soil-related performance variation and distributions of tree species in a Bornean rain forest. *J. Ecol.*, 93, 879–889.

Russo, S.E., Potts, M.D., Davies, S.J. & Tan, S. (2007). Determinants of tree species distributions: comparing the roles of dispersal, seed size, and soil specialization in a Bornean rain forest. In: *Seed Dispersal: Theory and its Application in a Changing World* (eds Dennis, A, Green, R, Schupp, EW & Wescott, D). CAB International, Wallingford, UK, pp. 499–518.

Russo, S.E., Legge, R., Weber, K.A., Brodie, E.L., Goldfarb, K.C., Benson, A.K. *et al.* (2012). Bacterial community structure of contrasting soils underlying Bornean rain forests: inferences from microarray and next-generation sequencing methods. *Soil Biol. Biochem.*, 55, 48–59.

Smith, D. & Peay, K. (2014). Sequence depth, not PCR replication, improves ecological inference from Next Generation DNA Sequencing. *PLoS ONE*, 92, e90234.

Smith, S.E. & Read, D.J. (2008). *Mycorrhizal Symbiosis*, 3rd edn.. Elsevier, San Francisco.

Smith, M.E., Henkel, T.W., Uehling, J.K., Fremier, A.K., Clarke, H.D. & Vilgalys, R. (2013). The ectomycorrhizal fungal community in a Neotropical forest dominated by the endemic dipterocarp *Pakaraimaea dipterocarpacea*. *PLoS ONE*, 8, e55160.

Steidinger, B.S., Turner, B.L., Corrales, A., Dalling, J.W. & Briones, M.J. (2014). Variability in potential to exploit different soil organic phosphorus compounds among tropical montane tree species. *Funct. Ecol.*, doi:10.1111/1365-2435.12325.

Sthultz, C.M., Whitham, T.G., Kennedy, K., Deckert, R. & Gehring, C.A. (2009). Genetically based susceptibility to herbivory influences the ectomycorrhizal fungal communities of a foundation tree species. *New Phytol.*, 184, 657–667.

Tedersoo, L., Suvi, T., Jairus, T. & Koljalg, U. (2008). Forest microsite effects on community composition of ectomycorrhizal fungi on seedlings of *Picea abies* and *Betula pendula*. *Environ. Microbiol.*, 10, 1189–1201.

Tedersoo, L., Bahram, M., Jairus, T., Bechem, E., Chinoya, S., Mpumba, R. *et al.* (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., Mett, M., Ishida, T.A. & Bahram, M. (2013). Phylogenetic relationships among host plants explain differences in fungal species richness and community composition in ectomycorrhizal symbiosis. *New Phytol.*, 199, 822–831.

Toljander, J.F., Eberhardt, U., Toljander, Y.K., Paul, L.R. & Taylor, A.F. (2006). Species composition of an ectomycorrhizal fungal community along a local nutrient gradient in a boreal forest. *New Phytol.*, 170, 873–883.

Trappe, J.M. (2005). On the nutritional dependence of certain trees on root symbiosis with belowground fungi (an English translation of A.B. Frank's classic paper of 1885). *Mycorrhiza*, 15, 267–275.

Vitousek, P.M. (1984). Litterfall, nutrient cycling and nutrient limitation in tropical forests. *Ecology*, 65, 285–298.

Walker, J.K., Cohen, H., Higgins, L.M. & Kennedy, P.G. (2014). Testing the link between community structure and function for ectomycorrhizal fungi involved in a global tripartite symbiosis. *New Phytol.*, 202, 287–296.

Wang, Y., Naumann, U., Wright, S. & Warton, D.I. (2014). Statistical methods for analysing multivariate abundance data. *R package version*, 3.9.3. http://CRAN.R-project.org/package=mvabund.

Warton, D.I., Wright, S.T. & Wang, Y. (2012). Distance-based multivariate analyses confound location and dispersion effects. *Methods Ecol. Evol.*, 3, 89–101.

Whitmore, T.C. (1978). Gaps in the forest canopy. In *Tropical trees and living systems*. (eds Tomlinson, P.B., Zimmerman, M.M.). Cambridge University Press, New York, pp. 639–655.

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