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Research

Investigating niche partitioning of ectomycorrhizal fungi in specialized rooting zones of the monodominant leguminous tree *Dicymbe corymbosa*

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Summary

- Temperate ectomycorrhizal (ECM) fungi show segregation whereby some species dominate in organic layers and others favor mineral soils. Weak layering in tropical soils is hypothesized to decrease niche space and therefore reduce the diversity of ectomycorrhizal fungi.
- The Neotropical ECM tree *Dicymbe corymbosa* forms monodominant stands and has a distinct physiognomy with vertical crown development, adventitious roots and massive root mounds, leading to multi-stemmed trees with spatially segregated rooting environments: aerial litter caches, aerial decayed wood, organic root mounds and mineral soil. We hypothesized that these microhabitats host distinct fungal assemblages and therefore promote diversity.
- To test our hypothesis, we sampled *D. corymbosa* ectomycorrhizal root tips from the four microhabitats and analyzed community composition based on pyrosequencing of fungal internal transcribed spacer (ITS) barcode markers.
- Several dominant fungi were ubiquitous but analyses nonetheless suggested that communities in mineral soil samples were statistically distinct from communities in organic microhabitats. These data indicate that distinctive rooting zones of *D. corymbosa* contribute to spatial segregation of the fungal community and likely enhance fungal diversity.

Introduction

Researchers have long attempted to explain why ectomycorrhizal (ECM) fungi exhibit high diversity in ecosystems where host plant diversity is low (Trappe, 1977; Bruns, 1995; Gardes & Bruns, 1996). Vertical segregation of the ECM fungal community across soil profiles ('vertical niche partitioning') was first hypothesized by Bruns (1995) as a factor that could promote the high ECM fungal species richness observed within stands of a single host tree species. Several authors have subsequently tested this hypothesis in temperate soils using molecular approaches to examine the distribution of ECM fungi across soil profiles. The pioneering study of Dickie *et al.* (2002) used TRFLP analysis of the internal transcribed spacer (ITS1-5.8s-ITS2 ribosomal DNA, ITS) to examine fungal community structure in vertically stratified soil layers in a Pennsylvania, USA pine forest. They found that fungal assemblages differed markedly across the soil profile, with some taxa proliferating in organic litter and others in deeper mineral soil. Additional primary studies in temperate ecosystems have analyzed both roots and soil using sequence-based techniques to elucidate the partitioning of fungal communities within different soil and litter layers (Rosling *et al.*, 2003; Tedersoo *et al.*, 2003; O'Brien *et al.*, 2005; Genney *et al.*, 2006; Courty *et al.*, 2008; McGuire *et al.*, 2013; Taylor *et al.*, 2014; Voříšková *et al.*, 2014). Most of these studies found at least some evidence of spatial differentiation of fungal communities in well-stratified temperate soils.

A recent meta-analysis by Bahram *et al.* (2015) concluded that ECM fungal communities in the temperate zone are more strongly differentiated across vertical soil layers and less influenced by horizontal spatial effects (but see Talbot *et al.*, 2014). Although two tropical datasets were also included in the meta-analysis of Bahram *et al.* (2015), neither exhibited significant differentiation of ECM fungi across different soil layers (Tedersoo *et al.*, 2011). Tedersoo & Nara (2010) and Bahram *et al.* (2015) noted that most tropical soils have relatively weak soil profile development relative to temperate soils. They hypothesized that this lack of soil stratification may partially explain the lower diversity of ECM fungi in some tropical ecosystems. However, forest ecosystems across the tropics are highly variable in terms of their ECM host plant density, soil profile development, and the number of host plant species

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(Tedersoo *et al.*, 2012, 2014; Corrales *et al.*, 2015 and references therein).

At one end of the continuum are tropical forests where one ECM tree species dominates all size classes and thick litter accumulation leads to strong soil profile development (Connell & Lowman, 1989; Newbery *et al.*, 1998; Peh *et al.*, 2011). Theoretically, these 'monodominant' forests should provide robust opportunities for partitioning of fungal communities in specialized rooting zones that are comparable to those in temperate systems. Monodominant forests of the endemic Guiana Shield tree *Dicymbe corymbosa* Spruce ex Benth. (Fabaceae subfam. Caesalpinioideae) are an excellent Neotropical example of a forest type with strong soil profile development and specialized rooting zones. *Dicymbe corymbosa*-dominated forests contain large, mast-fruiting canopy trees, high-density recruitment banks of seedlings and saplings, and diverse communities of ECM fungi (Henkel, 2003; Henkel *et al.*, 2005, 2012; McGuire, 2007). Large *D. corymbosa* trees exhibit reiterative shoot and root growth that result in long-lived, multi-stemmed individuals with litter-trapping root mounds. Extensive layers of organic matter are trapped within stem interstices and root mounds of these trees, are heavily colonized by ECM roots, and are putative sites for nutrient recycling (Mayor & Henkel, 2006; Woolley *et al.*, 2008). A study by McGuire *et al.* (2013) suggested that the organic litter layers of *D. corymbosa* hosted more saprotrophic fungi, whereas ECM fungi were more diverse and dominant in the mineral soil. The organic soils associated with *D. corymbosa* may function as unique fungal microhabitats, as suggested by observations that some ECM fungal species fruit at specific positions on elevated organic deposits in *D. corymbosa* forests (Henkel *et al.*, 2012). For example, locally common species such as *Inocybe epidendron*, *Tylopilus exiguus* and *Russula campinensis* only fruit on organic deposits 1–2 m above the ground on *D. corymbosa* trunks. By contrast, species such as *Clavulina amazonensis*, *Pseudotulostoma volvatum*, and *Amanita craseoderma* only fruit directly from mineral soil of the forest floor. Such fruiting patterns led us to hypothesize that these different soil microhabitats would provide unique rooting environments and host distinct ECM fungal communities, thereby enhancing overall ECM fungal diversity in *D. corymbosa* forests (Henkel *et al.*, 2012).

To test this hypothesis of spatial partitioning in specialized rooting zones in a tropical forest, we sampled ECM roots from four distinct microhabitats on 20 large *D. corymbosa* trees in four plots and determined their ECM fungal communities via 454 pyrosequencing. We also used our extensive local database of ITS sequences derived from sporocarps of 172 species to refine the identification of the ECM fungi associated with *D. corymbosa*.

Materials and Methods

Study site, root sampling and sporocarp sampling

The study site is located in primary rainforest in the Upper Potaro River Basin of the Pakaraima Mountains of Guyana, in the central Guiana Shield region (Fig. 1). This remote region lies within one of the world's largest intact tropical forests (Potapov *et al.*, 2008). The area receives 3500–4000 mm rainfall per year with peaks in May–July and December–January. Mean daily maximum temperatures range from 25 to 29°C and minima from 19 to 21°C. Fieldwork was conducted in May 2010 in four *Dicymbe corymbosa* plots located within a 5-km radius of a permanent base camp near the Potaro River (5°18′04.8″N; 59°54′40.4″W) with elevations of 700–750 m above sea level. Details on the climate, geology, soils, forest structure and distribution of forest types have been published previously (Henkel *et al.*, 2005 and references therein).

Three of the plots utilized here (plots 1–3 in the present study) correspond to the 1-ha monodominance plots D3, D4 and D5 in Henkel (2003) that were sampled for a long-term census of macrofungi (Henkel *et al.*, 2012). Plot 4 encompasses two adjacent 0.25-ha sites (LP1, LP4) where Henkel *et al.* (2005) studied masting reproduction in *D. corymbosa*. Plots 1 and 2 are located on ridges of highly weathered, ironstone-rich lateritic clay soils east of the Potaro River, whereas plots 3 and 4 are west of the river (Fig. 1). Plot 3 has lateritic clay soils with more silt and sand, whereas plot 4 soils are alluvial sand deposits with minimal silt. All soils have low pH (< 5.3), and have low concentrations of phosphorus (< 12 mg kg^{-1}) and high concentrations of soluble aluminum (> 800 mg kg^{-1}) (Henkel, 2003; Henkel *et al.*, 2005). All plots are strongly monodominant with *D. corymbosa* comprising 63–94% of the basal area. There are no other known ECM hosts within these plots (McGuire *et al.*, 2008).

In each of the four plots we randomly selected five large *D. corymbosa* trees ($n = 20$ trees) with: (1) diameter at breast height (dbh) > 100 cm, (2) extensive shoot and root reiteration, and (3) development of ECM roots in each of the four microhabitats: aerial litter (AL), aerial decayed wood (W), root mounds (RM) and mineral soil (MS) (Fig. 2). The aerial litter samples were composed of leaf litter trapped 1–2 m above the ground on the *D. corymbosa* trunks and lower stems, whereas wood samples were composed of decayed wood enmeshed in the pseudotrunks. The aerial litter and decayed wood microhabitats were never in contact with the forest floor. The root mound soils are structurally similar to the deep litter layers in some temperate forests and these samples were composed of decomposing leaves, small branches and other organic materials. All of the rooting zones have high concentrations of ECM roots (Woolley *et al.*, 2008). The mineral soil samples were taken from the mineral soil horizon directly below the root mound samples at the bases of the trees. The root mound and mineral soil samples are comparable to the spatially segregated soil layers that have been studied by researchers in the temperate zone (Bahram *et al.*, 2015).

In May 2010 we extracted *c.* 1000 cm^3 samples of ECM roots from each of the four microhabitats, yielding four samples from each tree ($n = 80$ root samples). Samples were placed in plastic bags and transported to base camp where they were rinsed with water and inspected under a dissecting microscope within 24 h. From each of these samples 100 healthy ECM roots were randomly sampled following the methods of Smith *et al.* (2007) and rapidly dried with silica gel.

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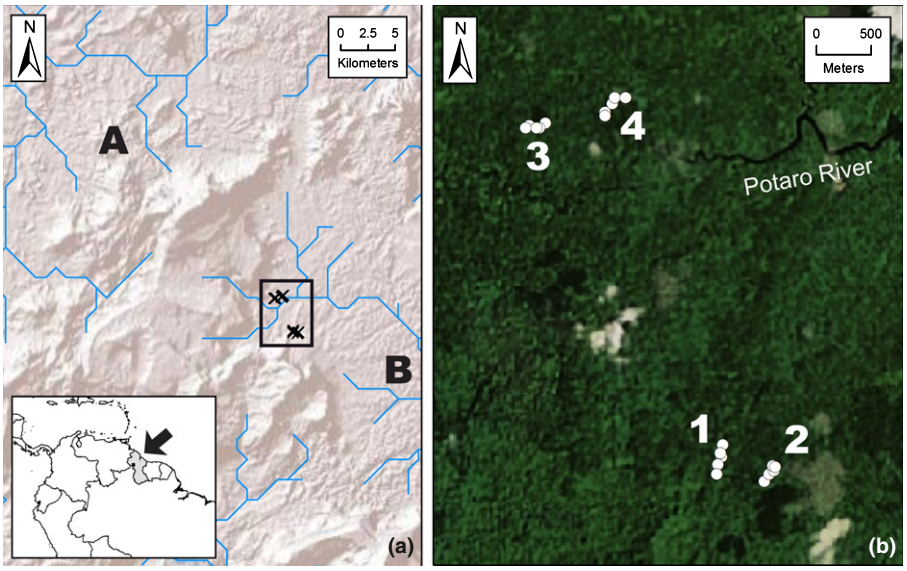

Fig. 1 Map of *Dicymbe corymbosa* study sites in the Upper Potaro River Basin in the Pakaraima Mountains of Guyana. (a) Map shows Guyana (black arrow) in South America (inset) and the locations of the current study sites (black crosses) relative to sites studied by (A) Smith *et al.* (2013) and (B) Smith *et al.* (2011). (b) The Potaro River and relative locations of the four study plots as well as positions of the sampled *Dicymbe corymbosa* trees (white circles).

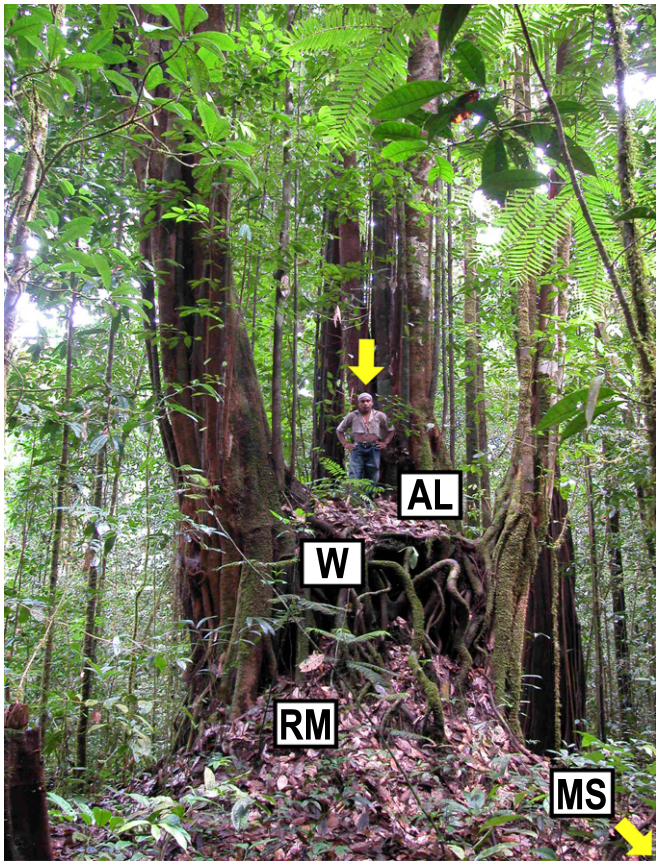

Fig. 2 Mature *Dicymbe corymbosa* tree exhibiting typical reiterative architecture, including multiple stems and roots, a large root mound, and accumulations of elevated organic materials. The four microhabitats sampled for ectomycorrhizal (ECM) roots are: aerial litter (AL), aerial pockets of decayed wood (W), root mound with deep organic deposits (RM) and mineral soil on the forest floor (MS, yellow arrow, bottom right). The top yellow arrow points to field assistant Peter Joseph, included for scale.

Fungal sporocarps were collected in 2000–2015. Specimens were photographed in the field and dried with silica gel. Tissues were ground using a bead beater and DNA extractions were performed using a modified CTAB method (Gardes & Bruns, 1993) or Extract-N-Amp (Sigma-Aldrich). Standard protocols were used for PCR and Sanger sequencing of ITS rDNA from sporocarps using primers ITS1F and ITS4 or ITS4B (White *et al.*, 1990; Gardes & Bruns, 1993).

Pyrosequencing methods and data pipeline

Dried roots were frozen in liquid nitrogen and pulverized with a micropestle. DNA was extracted using the PowerSoil DNA Kit (MoBio, Carlsbad, CA, USA) according to the manufacturer's instructions. All 80 samples were subjected to 454 pyrosequencing of the ITS rDNA using the methods of Bonito *et al.* (2014). Briefly, PCR was set-up on ice using the fungal-specific primer ITS1F and the eukaryote primer ITS4 that had been tagged for pyrosequencing. Primers were modified to contain the 454 A and B primers, with each A-ITS1F primer tagged with an additional 10-bp barcode. PCR was performed with PlatinumTaq (Invitrogen) as follows: 5 min at 95°C, 30 cycles of 95°C for 1 min, 55°C for 45 s and 72°C for 1 min, with a final extension at 72°C for 7 min. Amplicons were visualized on 1.5% agarose gels with SYBR Green I (Molecular Probes, Eugene, OR, USA). To remove small fragments, amplicons were titrated to equimolar concentrations and subjected to two rounds of cleaning with Agencourt AMPure XP beads (Beckman Coulter Inc., Brea, CA, USA) while being monitored with a 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA) on a DNA 1k chip. Amplicons were sequenced in the forward direction using titanium chemistry and the Lib-L kit on a 1/8th plate of the Roche 454 GS-FLX (454 Life Sciences, CT, USA). Raw data are available at NCBI's Sequence Read Archive (Accession SRP073265). DNA sequencing was performed at the Duke Genome Sequencing Center and the University of Florida Interdisciplinary Center for Biotechnology Research.

Pyrosequencing generated 125 202 raw sequence reads and the QIIME software package v.1.6 (Caporaso *et al.*, 2010) was used to clean and assign each sequence to a sample. We used stringent

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quality filters to remove all sequences with primer mismatches, lengths < 300 or > 800 bp, homopolymers > 8 bp, and > 5 ambiguous nucleotides. Sequences were denoised using Denoiser (Reeder & Knight, 2010) followed by extraction of ITS1 using ITS Extractor (Nilsson *et al.*, 2010). Filtering followed by ITS1 extraction yielded 77 081 sequences. We identified 781 reads removed by filtering that corresponded to sequences of *Craterellus atratus* so these were added back into the dataset. The resulting 77 862 ITS1 sequences were then clustered into 383 operational taxonomic units (OTUs) at the 97% similarity level in UCLUST v.1.2.2 (Caporaso *et al.*, 2010). To eliminate potential inflated diversity due to retention of NGS sequence artifacts, we removed all OTU occurrences that accounted for < 1% of sequence reads in a given sample.

Representative sequences from each retained OTU were subjected to BLAST searches against GenBank and UNITE (Kõljalg *et al.*, 2013). Representative OTU sequences were determined to belong to ECM or non-ECM fungal lineages as defined by Tedersoo & Smith (2013). OTUs are named as follows: (1) described taxa have Latin binomials, (2) sequences from sporocarps of undescribed species are labeled with genus name and collector number (T. Henkel (TH), M.C. Aime (MCA), M. Smith (MES), S. Miller (SLM)), (3) ECM sequences from Smith *et al.* (2011, 2013) are labeled as in the original publications, (4) OTUs from an unpublished cloning-based dataset but not found in previous studies are labeled by a representative clone number, (5) taxa found only via pyrosequencing are labeled with the closest determined taxonomic group followed by consecutive numbers (e.g. Thelephoraceae4).

Data based on frequency (the number of samples where an OTU was detected) and abundance (total number of pyrosequencing reads) were qualitatively similar. Because pyrosequencing abundance data is considered to be 'semi-quantitative' (Amend *et al.*, 2010), we relied on relative abundance data for all analyses except when otherwise noted.

Statistical analyses

All statistical analyses were conducted using the software R (R Core team, 2016). For comparisons of diversity the Shannon–Wiener diversity index (H'), Simpson's diversity index ($1 - D$), and evenness (E) were calculated for all ECM fungi and for each of the microhabitats separately based on a dataset randomly subsampled to 500 ECM sequences per sample. Samples with < 500 ECM sequences were excluded from this reduced dataset so that ECM diversity estimates were based on only 53 of the 80 samples.

We used Nonmetric Multidimensional Scaling (NMS) ordination based on Sørensen (Bray–Curtis) distances to visualize data and interpret patterns in fungal community structure. The NMS analysis was conducted using a full matrix of all ECM fungal OTUs vs all root samples.

In order to examine the effect of microhabitat type on fungal community composition and structure we performed Permutational Multivariate Analysis of Variance (PERMANOVA) analyses. The PERMANOVA analyses were performed with microhabitat (aerial litter, decayed wood, root mound, mineral soil) as the grouping variable blocked by plot ($n = 4$). We used 999 randomized runs and Sørensen distances. The PERMANOVA is a nonparametric, multivariate analysis used to test for differences in mean within-group distances with a null hypothesis of no differences between groups. The PERMANOVA analyses were conducted first using only ECM fungi and then using all fungi. For both methods, global and pairwise comparisons were performed between microhabitats for all groups. Comparisons were considered significant at $P < 0.05$ for global comparisons whereas pairwise comparisons between microhabitats were considered significant at $P < 0.0083$ based on Bonferroni corrections.

In order to determine which ECM fungi were most associated with each of the microhabitats, we used Indicator Species Analysis (ISA). The ISA calculates indicator values that reveal the relative abundance and constancy of each OTU for each microhabitat. A small P-value rejects the null hypothesis that the observed maximum indicator value is no larger than expected by chance and therefore that a particular OTU is more abundant and occurs more consistently in that microhabitat (McCune & Mefford, 2011). All P-values < 0.05 were considered significant.

Results

Fungal community structure

A total of 77 477 sequences were retained for the final analysis ranging from 106 to 3753 sequences per sample (mean = 968). The mean number of sequences per sample (with standard deviations) was as follows: 1340 (± 971) in aerial litter, 975 (± 601) in root mounds, 896 (± 681) in mineral soil and 663 (± 393) in decayed wood. After removal of low-quality and low-abundance reads, chimeras and nonfungal sequences, a total of 191 fungal OTUs were detected. We then applied a conservative filter by removing all OTUs from any sample where they comprised < 1% of all sequence reads, thereby reducing the dataset to 131 OTUs. Of these, 97 OTUs were resolved in ECM lineages whereas the remaining 34 OTUs represented parasites, saprotrophs or other trophic modes.

The overall diversity of ECM fungi detected in this study (as estimated by diversity indices calculated using rarefied data) was slightly lower than in a nearby forest with three ECM host trees (*D. corymbosa*, *D. altsonii* and *A. insignis*; Smith *et al.*, 2011) but similar to a nearby *Pakaraimaea–Dicymbe* forest (Smith *et al.*, 2013; Supporting Information Table S1). Twelve ECM fungal lineages were represented, with the highest richness in /russula–lactarius (23 OTUs), /clavulina (18 OTUs), /tomentella–thelephora (13 OTUs) and /boletus (13 OTUs). Basidiomycota ECM fungi were dominant (10 lineages, 95 OTUs) as compared to Ascomycota (2 lineages, 2 OTUs). Two additional ECM lineages (/coltricia, /ramaria–gautieria) were represented by three OTUs found only at very low abundance and frequency (< 25 reads in only one sample).

Of the 97 ECM fungi included in the final analyses, 47 OTUs (49%) had an ITS1 sequence that matched a species from our

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regional database of 172 sporocarp-forming species (Tables S2, S3). When low-frequency and low-abundance OTUs were also included in the comparison, the number increased to 61 OTUs (Table S2). The most frequent and abundant fungi were overwhelmingly ECM; of 73 OTUs with > 100 sequence reads, 62 were ECM and 11 non-ECM. Many of the ECM OTUs were found in previous studies in Guyana, including 52 OTUs found by Smith *et al.* (2011), 16 OTUs found by Smith *et al.* (2013), and 12 OTUs found in both of these studies. Although non-ECM fungi were generally uncommon in our dataset, the richness of non-ECM fungi was considerably higher in the aerial litter samples (25 OTUs) than in the other microhabitats (4–12 OTUs) (data not shown).

Influence of microhabitat on fungal communities

Species accumulation curves for ECM fungi indicated that each microhabitat was equitably sampled and that most of the ECM diversity was detected (data not shown). Diversity of ECM fungi was similar among the different microhabitats with richness ranging from 50 (aerial litter) to 64 OTUs (mineral soil). Although many of the common OTUs were detected across multiple different microhabitats (Figs 3, 4), there are nonetheless several lines of evidence suggesting that microhabitat has a strong influence on fungal communities.

Fungal communities were significantly structured by microhabitat, whether analyzing only ECM fungi or all fungi ($P = 0.001$, Table 1). Significant differences were detected in all pairwise comparisons of microhabitats for ECM fungi except for aerial litter vs root mounds. Results were similar when all fungi were included in the analysis except that both aerial litter vs root mounds and aerial litter vs wood were not significant (Table 1). Consistent with these results, the NMS ordination showed that ECM fungal communities in mineral soil were the most distinctive. Despite some overlap with aerial litter and root mounds in the NMS ordination (Fig. 3), ECM fungal communities in wood were also statistically distinct (Table 1). Nonetheless, the NMS ordination and relative abundances of ECM fungal OTUs indicated that the aboveground microhabitats rich in organic matter (e.g. aerial litter, root mounds, decayed wood) hosted fungal communities more similar to one another than to those in mineral soil (Figs 3, 4). In contrast to the strong effect of microhabitat, the sampling plot exerted only a weak influence on fungal community structure (Fig. 3).

Indicator species analysis indicated that some taxa were preferentially associated with a particular microhabitat ($P \leq 0.05$; Table 2). Of the 19 OTUs that were significantly associated with one or more microhabitat, nine were associated with mineral soil whereas fewer OTUs were strongly associated with the organic microhabitats, particularly root mounds and wood.

Discussion

Niche partitioning in a tropical forest

Niche partitioning among ectomycorrhizal (ECM) fungal communities in organic vs mineral soils has been implicated as a driver of high alpha diversity because increased niche space should facilitate the packing of more species into a smaller area (Bruns, 1995; Dickie & Koide, 2014). Although vertical

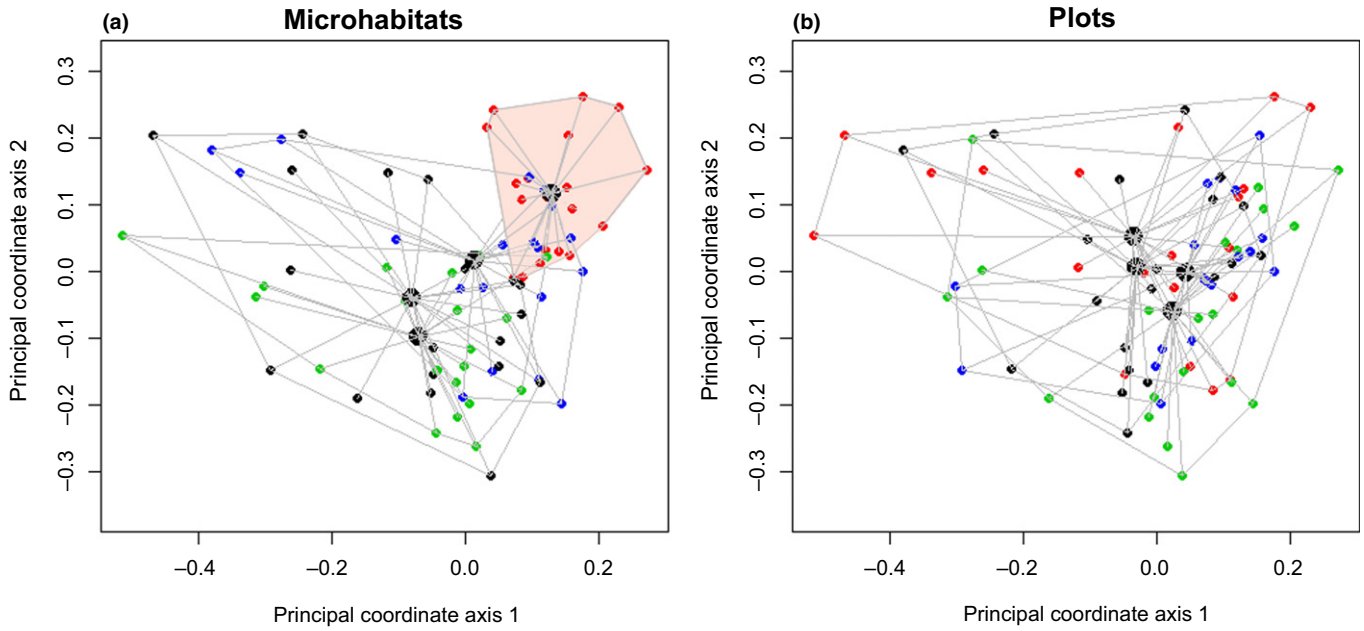

Fig. 3 Nonmetric multidimensional scaling (NMS) ordination of ectomycorrhizal (ECM) fungi communities on roots of *Dicymbe corymbosa* depicting the influence of microhabitat (a) vs plot (b). Plots have overlapping distributions and close centroids, indicating that ECM fungal communities are similar between plots. Ectomycorrhizal fungal communities from the root mounds (green) and aerial litter (black) have close centroids and mostly overlapping distributions, whereas those from decayed wood have less overlap and are more distinct. The fungal communities in mineral soil (red, highlighted with pink fill) are clearly the most unique. Analysis based on all fungal operational taxonomic units (OTUs) reveals similar results (data not shown).

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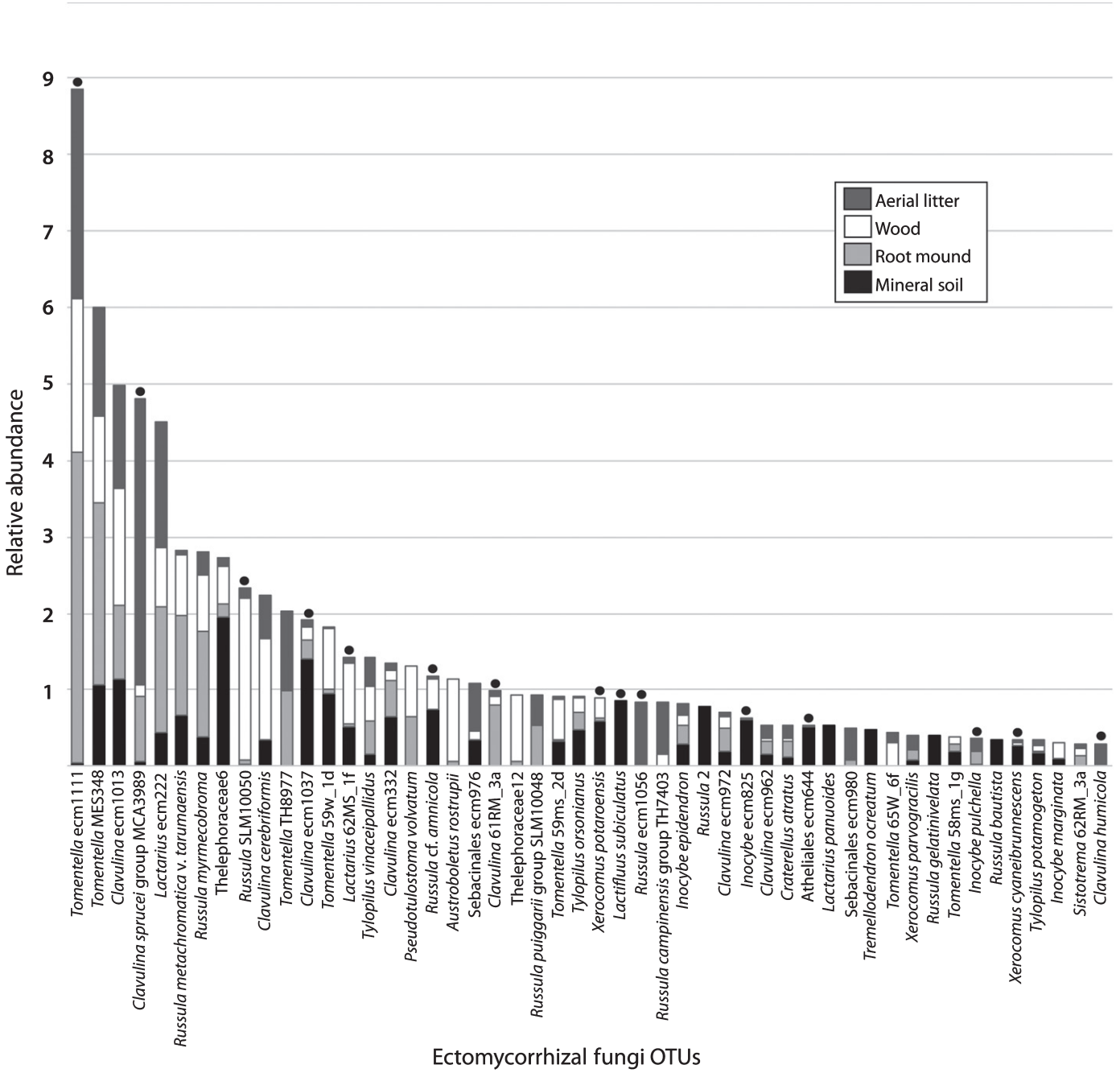

Fig. 4 Relative abundance of the 50 most common operational taxonomic units (OTUs) of ectomycorrhizal (ECM) fungi on roots of *Dicymbe corymbosa* as detected by pyrosequencing. Each abundance bar is shaded to designate the proportion of occurrences for each microhabitat: aerial litter, decayed wood, root mound and mineral soil. OTUs most closely associated with one or more microhabitats based on indicator species analysis ($P < 0.05$) are indicated by a black dot. Binomials designate named fungal species whereas species with TH, MCA or MES numbers matched voucher specimens of undescribed species. The ECM numbers correspond to fungal OTUs known only from ECM root sequences.

stratification across soil layers has been repeatedly documented in temperate forests, no evidence of an analogous pattern has thus far been found in the few studies conducted in the tropics (Bahram *et al.*, 2015). Based on temperate studies and our observations of specialized fruiting sites for key fungal species, we expected that ECM fungi would be strongly segregated among the microhabitats found on *Dicymbe corymbosa*. We inferred that this pattern should be particularly strong because elevated soils often have distinct properties and nutrient cycling, and may therefore host unique fungi (Cardelus, 2010; Hertel, 2011; Orlovich *et al.*, 2013). Furthermore, a previous study by McGuire *et al.* (2013) suggested that the mineral soil fungal communities in *D. corymbosa* forests are likely distinct from those in leaf litter.

Our data indicate that fungal communities are differentiated among the spatially segregated rooting zones of *D. corymbosa*

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Table 1 Results of permutational MANOVA (PERMANOVA) examining effects of *Dicymbe corymbosa* rooting microhabitat on community composition of ectomycorrhizal (ECM) fungi only (a) and all fungal OTUs (b)

	Degrees of freedom	Sums of squares	Mean of squares	F^1	R^2	*P*
(a) Ectomycorrhizal (ECM) fungi only						
Microhabitat	3	2.482	0.82722	1.837	0.06931	**0.001**
Residuals	74	33.322	0.4503	0.93069		
Total	77	35.804	1			

Pairwise comparisons	*F*	R^2	*P*-value	Adjusted *P*-value
Mineral soil vs Aerial litter	2.374886241	0.060314745	0.001	**0.006**
Mineral soil vs Root mound	2.495653258	0.061627682	0.001	**0.006**
Mineral soil vs Wood	1.654398753	0.042799754	0.001	**0.006**
Aerial litter vs Root mound	1.376444088	0.0358669	0.022	0.132
Aerial litter vs Wood	1.487722462	0.039685592	0.007	**0.042**
Root mound vs Wood	1.605090599	0.041577175	0.002	**0.012**

	Degrees of freedom	Sums of squares	Mean of squares	F^1	R^2	*P*
(b) All fungi						
Microhabitat	3	2.435	0.81159	1.796	0.06787	**0.001**
Residuals	74	33.439	0.45188	0.93213		
Total	77	35.874	1			

Pairwise comparisons	*F*	R^2	*P*-value	Adjusted *P*-value
Mineral soil vs Aerial litter	2.162062981	0.055208097	0.001	**0.006**
Mineral soil vs Root mound	2.559413007	0.063102812	0.001	**0.006**
Mineral soil vs Wood	1.637090982	0.042370969	0.001	**0.006**
Aerial litter vs Root mound	1.336480451	0.034861845	0.036	0.216
Aerial litter vs Wood	1.390950772	0.037200198	0.009	0.054
Root mound vs Wood	1.663406836	0.043022769	0.001	**0.006**

Samples were partitioned into four rooting microhabitats; aerial litter, decayed wood, root mound and mineral soil. All tests utilized the Sørensen distance measure. Significant *P*-values are highlighted in bold. Global comparisons are considered significant at $P < 0.05$, whereas pairwise comparisons between microhabitats are considered significant at $P < 0.0083$ based on Bonferroni corrections (0.05×6 comparisons $= 0.0083$). Both original *P*-values and Bonferroni adjusted *P*-values are shown for the pairwise comparisons.

[1]*F*, a pseudo *F*-statistic calculated as a ratio of between-group sum of squares vs within-group sum of squares.

trees. The Permutational Multivariate Analysis of Variance (PERMANOVA) analyses indicated that microhabitat significantly influences the fungal community, regardless of whether the analyses included all fungi or only ECM taxa ($P = 0.001$, Table 1). Pairwise comparisons between the communities in different microhabitats also found statistical differences between most communities. Mineral soil hosted the most distinctive ECM communities with more taxa preferentially associated with mineral soil samples and fewer taxa preferentially associated with the organic microhabitats (aerial litter, root mound, wood) (Figs 3, 4; Tables 1, 2). Likewise, the Indicator Species Analysis (ISA) found more species to be statistically associated with mineral soil samples than with any other microhabitat (Table 2). Several operational taxonomic units (OTUs) were also rare or absent in mineral soil samples, but were more evenly distributed across aerial litter, root mound and wood samples (Fig. 4). These results suggest that the organic microhabitats share more ECM fungi in common and that mineral soil fungal communities are more unique (Fig. 2). Among different organic microhabitats, the distinctive ECM fungal assemblage associated with decayed wood may be related to the unique physicochemical properties of this substratum as compared to litter (Woolley *et al.*, 2008). We specifically sampled ECM roots so non-ECM fungi were generally uncommon in our study. However, it was notable that aerial litter samples hosted a much higher species richness of non-ECM fungi when compared to other microhabitats. These data are consistent with the observations of McGuire *et al.* (2013) that non-ECM fungi are more prevalent in the litter of *D. corymbosa* forests. The aerial litter caches are completely removed from soil on the forest floor where ECM inoculum is likely highest. This may explain why non-ECM fungi might be more diverse on epicormic root surfaces or within ECM mantles in the highest positions on *D. corymbosa* trees.

Although a few taxa formed sporocarps and ectomycorrhizal root tips in the same microhabitats (e.g. *Tremellodendron ocreatum* in mineral soil, *Russula myrmecobroma* in root mounds, *Clavulina humicola* in aerial litter), many other fungi colonized roots in multiple microhabitats and showed no correlation between ectomycorrhiza formation and sporocarp production (Fig. 4). These include some species that fruit on organic substrata (e.g. *Inocybe epidendron*, *Clavulina cerebriformis*) but formed mycorrhizas in all substrata, and other species that always

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Table 2 Indicator species analysis showing microhabitat species preferences of ectomycorrhizal (ECM) fungi associated with *Dicymbe corymbosa* among the four rooting microhabitats: aerial litter, decayed wood, root mound and mineral soil

Species	Substrate	A	B	Statistic	*P*-value
Clavulina sprucei group MCA3989	Aerial litter	0.8248	0.5263	0.659	0.001***
Russula ecm1056	Aerial litter	0.9712	0.2632	0.506	0.004**
Clavulina humicola	Aerial litter	0.95	0.2632	0.5	0.002**
Russula campinensis group TH7403	Aerial litter	0.93	0.2105	0.442	0.019*
Lactifluus subiculatus	Mineral soil	1	0.3	0.548	0.003**
Xerocomus cyaneibrunnescens	Mineral soil	0.748	0.35	0.512	0.004**
Atheliales ecm644	Mineral soil	0.9895	0.25	0.497	0.003**
Inocybe ecm825	Mineral soil	0.9685	0.25	0.492	0.008**
Xerocomus potaroensis	Mineral soil	0.8646	0.25	0.465	0.013*
Inocybe 65ms_3g	Mineral soil	1	0.15	0.387	0.049*
Clavulina 61RM_3a	Root mound	0.8953	0.25	0.473	0.026*
Russula SLM10050	Wood	0.8325	0.3684	0.554	0.013*
Clavulina cinereoglebosa	Wood	1	0.1579	0.397	0.021*
Tomentella TH8977	Aerial litter + Root mound	0.9902	0.359	0.596	0.001***
Inocybe pulchella	Aerial litter + Root mound	0.9098	0.3077	0.529	0.026*
Lactarius 62MS_1f	Mineral soil + Wood	0.8805	0.2821	0.498	0.04*
Russula cf. *amnicola*	Mineral soil + Wood	0.979	0.2308	0.475	0.029*
Xerocomus ecm1082	Mineral soil + Wood	1	0.1795	0.424	0.047*
Tomentella ecm1111	Aerial litter + Root mound + Wood	0.9903	0.3793	0.613	0.009**

Fungal species are considered significantly associated with one or more microhabitat at $P < 0.05$. Species significance at: *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.001$.
A is the probability that if the species is present in the sample that the site belongs to the group whereas B is the probability of finding the species in sites belonging to the group.

fruit in mineral soil (e.g. *Pseudotulostoma volvatum*) but formed mycorrhizas in elevated sites.

Preferential fruiting in elevated positions is commonplace in *D. corymbosa* forests but this phenomenon appears not to be linked to ectomycorrhiza formation for many species. This disconnect between sporocarps and ectomycorrhizal root tips suggests that many taxa may form large, long-lived genets (fungal individuals) with medium- or long-distance exploration type ectomycorrhizas (e.g. /boletus lineage; Agerer, 2001). Many taxa also belong to lineages that spread vegetatively across wide areas or persist perennially (e.g. /russula–lactarius, /boletus; Douhan *et al.*, 2011). Furthermore, some species that fruit from elevated sites on *D. corymbosa* (e.g. *Amanita calochroa*, *Boletellus ananas*) show hyphal cord extension up trees to sporocarps, providing circumstantial evidence for genets that extend across rooting zones (Mayor *et al.*, 2008).

Community comparisons across sites and biomes

The fungal community in this study was remarkably similar in composition to nearby sites in Guyana. Many ECM fungi from our dataset matched root tips and/or sporocarps from previous studies (Table S2), including 47 of 97 ECM fungi that matched sporocarps reported by Henkel *et al.* (2012) and 58 of 97 ECM fungi detected locally by either Smith *et al.* (2011) or Smith *et al.* (2013). Additional matches to previously sampled ECM taxa also were found among low-abundance OTUs that were removed from the final analysis (Table S2).

With 172 local ECM species known from sporocarps (Table S3) and additional OTUs known only from roots, a conservative estimate of 250+ ECM species in the Upper Potaro Basin reaffirms this region as a biodiversity hotspot for tropical ECM fungi. The high overlap between ECM roots and sporocarps in the present study is partly attributable to our extensive sporocarp database. However, the strong fungal community similarity and resulting low beta diversity among local sites with different host plants, variable soils and separation by distances of 1–100 km is nonetheless striking compared to other studies (Tedersoo *et al.*, 2010; Peay *et al.*, 2015).

The lack of significant horizontal spatial structure of ECM communities across Guyana suggests that this region may be unique. In a recent meta-analysis of spatial structure across ecosystems, Bahram *et al.* (2013) found that the distance decay of similarity (e.g. diminishing similarity with increasing geographical distance) for ECM fungal communities was greater in tropical than temperate sites, implying rapid turnover of species between sites and therefore strong landscape-scale spatial structuring in the tropics. Strong distance decay was found across tropical habitats ranging from Southeast Asian sites with high ECM host density and strong soil gradients (Peay *et al.*, 2010) to Amazonian forest with a low host density of understory ECM trees (Tedersoo *et al.*, 2010). However, the Guiana Shield ECM communities do not follow this pattern.

The low beta diversity of ECM fungi in Guyana's Pakaraima Mountains may reflect a pool of regionally endemic ECM fungi that are widely distributed across sites, regardless of host plants and soil types (Smith *et al.*, 2011, 2013; Henkel *et al.*, 2012). Few Guiana Shield ECM fungi are known to extend into Central America, North America or temperate South America, but several taxa found in Guyana have been reported more widely across the South American lowland tropics (e.g. *Amanita xerocybe*, *Cantharellus guyanensis*, *Clavulina amazonensis*, *Singerocomus*

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inundabilis and others; Bas, 1978; Singer & Aguiar, 1986; Simmons *et al.*, 2002; Henkel *et al.*, 2011, 2014, 2016; Roy *et al.*, 2016; A. Vasco-Palacios, pers. comm.). Other evidence that Guiana Shield ECM fungi are biogeographically distinct includes the presence of novel ECM lineages (Tedersoo & Smith, 2013; Henkel *et al.*, 2016; Sánchez-García *et al.*, 2016), endemic hyperdiversity and phylogenetic uniqueness in the /clavulina lineage (Kennedy *et al.*, 2012), and the universally low BLAST homology between Guiana Shield ECM fungi and other GenBank sequences (Table S2).

The relatively high alpha but low beta diversity of ECM fungi in Guyana rainforests may be related to the weak disturbance regime. The *Dicymbe*-dominated forests differ from many other tropical forests in that landscape-scale disturbances such as fire and hurricanes are essentially absent (Hammond & Steege, 1998). *Dicymbe* forests are distributed over a moderate altitudinal gradient, are always warm, and have two rainy seasons per year with moderate dry periods, thereby yielding stable temperature and moisture regimes (Fanshawe, 1952). The reiterative growth of *D. corymbosa* also means that individual trees are often large, have indeterminate life spans and may be hundreds or thousands of years old. Although tree-fall gaps create important recruitment opportunities in typical tropical forests (Wright, 2002), *D. corymbosa* trees sprout new stems to fill their own light gaps whereas root systems remain intact, thereby helping to maintain monodominance (Woolley *et al.*, 2008). Periodic mast fruiting leads to large pools of seedlings and saplings that also contribute to belowground dominance (Henkel *et al.*, 2005). The result is that *D. corymbosa* monodominant forests provide a stable environment where one host species fixes the majority of carbon, generates massive accumulations of litter and wood, and provides all of the ECM roots. Although significant carbon resources are available to ECM fungi, the minimal disturbance that favors *D. corymbosa* monodominance may also moderate drivers of ECM fungal diversity by decreasing 'edge effects' and reducing opportunities for competition–colonization tradeoffs (Dickie & Reich, 2005; Kennedy, 2010). Theory and available literature suggest that this host and habitat homogeneity may lead to highly competitive ECM fungal species excluding weaker species that depend on disturbance (Bever *et al.*, 2010; Mujic *et al.*, 2015). Spatially segregated rooting zones in *D. corymbosa* stands may provide a buffer against these effects and therefore enhance the fungal diversity by increasing the niche space available to ECM fungi in these monodominant forests.

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Author contributions

M.E.S., T.W.H., and R.V. planned and designed the research; M.E.S., T.W.H., G.C.W., A.K.F. and M.C.A. performed experiments; M.E.S., T.W.H., G.C.W., A.K.F. and M.C.A. conducted fieldwork and analyzed and interpreted data; and M.E.S., T.W.H., A.K.F. and R.V. wrote the manuscript.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Table S1 Diversity statistics for root-associated fungi of *Dicymbe corymbosa*

Table S2 List and identities of all fungal OTUs detected on the roots of *Dicymbe corymbosa* based on pyrosequencing

Table S3 Representative voucher specimens and ITS rDNA GenBank accession numbers for 170 species or morphospecies of ectomycorrhizal fungi from the Upper Potaro Basin, Guyana

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