ECTOMYCORRHIZAL FUNGAL COMMUNITY ASSEMBLY ON SEEDLINGS OF A NEOTROPICAL MONODOMINANT TREE

By

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ABSTRACT

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Formation of ectomycorrhizae may facilitate early seedling survival of ectomycorrhizal tree species due to enhanced nutrient acquisition. This could be especially important in heavily shaded understories of tropical monodominant forests where host plant photosynthetic capacity is limited. Little information is available on ectomycorrhizal (ECM) fungal colonization or community development of monodominant seedling cohorts, which have high survival rates. Following a 2016 mast seeding event, we sequentially measured percent colonization and species composition of ECM fungi on live and recently dead seedlings of the tropical monodominant tree *Dicymbe corymbosa*. We also compared seedling ECM fungi to those of nearby adult conspecifics. Ectomycorrhizal fungal communities were remarkably different between seedling age classes, as well as between seedlings and adults. While the /russula-lactarius and /tomentella-thelephora lineages were species-rich throughout, there was 80–90% species turnover between 6- and 12-month-old seedlings. There was no difference in age-class fungal communities across sampling plots, indicating little spatial effect. Fungal colonization extent did not correlate with seedling age or differ markedly between live

and dead seedlings. The number of ECM morphotypes increased with seedling age and tended to be greater on live versus dead seedlings. Interspecific competition between ECM fungi or soil nutrient fluxes may influence community assembly of ECM fungi in this tropical monodominant host tree.

ACKNOWLEDGEMENTS

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INTRODUCTION

Most lowland tropical forests have high tree species richness (Richards, 1996), but stands occur in which a single tree species is dominant (Alexander & Högberg, 1986; Corrales, Mangan, Turner, & Dalling, 2016a; Henkel, 2003). In these "monodominant" stands, alpha diversity of trees is extremely low by tropical standards, with the dominant species comprising 60% or more of the canopy trees (Connell & Lowman, 1989). Tropical monodominance is rare relative to temperate and boreal forests (ter Steege et al., 2019), but examples are known from Central and South America (e.g., Corrales et al., 2016a; Halling & Mueller, 2002; Henkel, 2003), Guineo-Congolian Africa (e.g., Alexander & Högberg, 1986; Letouzey, 1968), SE Asia (e.g., Corner, 1972; Lee, 1998; Singh, 1966), and tropical Australia (e.g., Brundrett & Abbott, 1991). Tropical monodominant trees commonly associate with root symbiotic ectomycorrhizal (ECM) fungi, which exchange soil nutrients for photosynthates from their host trees (Francis & Read, 1994; Read, Francis, & Finlay, 1985; Smith & Read, 2008). This type of mutualism is patchily distributed in the tropics (Connell & Lowman, 1989; Corrales et al., 2016b; Henkel, 2003; See, 1990), where arbuscular mycorrhiza (AM) is the dominant mycorrhizal type (Alexander, 1989; Janos, 1983; St. John, 1980). Ectomycorrhizal fungi cycle litter nutrients more efficiently than AM (Smith & Read, 2008). This may facilitate monodominance through competitive exclusion of AM trees (Corrales, Henkel, & Smith, 2018; Finlay & Read, 1986; Henkel, Mayor, & Woolley, 2005; Hobbie & Colpaert, 2003; Mayor, Schuur, & Henkel, 2009; Simard et al., 1997).

In addition to forming ectomycorrhizae, many tropical monodominant trees reproduce by supra-annual mast seeding. Masting establishes large, even-aged seedling cohorts in heavy shade under parental adults (e.g., Henkel & Mayor, 2019; Torti, Coley, & Kursar, 2001). In tree-diverse lowland tropical forests, high-density conspecific seedlings are prone to intensified pathogen pressure and early mortality (Bagchi et al., 2014; Connell, 1971; Janzen, 1970; Mangan et al., 2010). In tropical monodominant stands high seedling survival rates are necessary for multi-generational persistence (Connell & Lowman, 1989; Hart, 1990; Read, Hallam, & Cherrier, 1995). Despite the importance and prevalence of high seedling survival rates across tropical monodominant species, little is known about contributing factors (Henkel & Mayor, 2019).

Ectomycorrhizae could contribute to high survival rates of tropical monodominant seedlings. Seedling survival can be strongly influenced by below-ground competition for soil resources (e.g., Berntson & Wayne, 2000). In a tropical dipterocarp co-dominant forest in SE Asia, ECM colonization of *Hopea helferi* and *H. odorata* seedlings directly improved uptake of phosphorus (P) and calcium (Lee & Alexander, 1994), both of which are limiting in lowland tropical forest soils (Mayor & Henkel, 2006; Vitousek, 1984). Ectomycorrhizal fungi may also link seedlings to adult trees and allow access to their resource pools, thereby improving seedling nutrient acquisition, water uptake, and, ultimately, survival (Booth & Hoeksema, 2010; Finlay & Read, 1986; Nara, 2006; Newman, 1988; Simard et al., 2012; Teste et al., 2009). Proximity to adult conspecific ECM roots may also determine how quickly a seedling is inoculated by ECM fungi (Alexander, Ahmad, & See, 1992).

Information is scarce on whether the species composition and diversity of ECM fungi impact seedling growth and survival. This is especially true for tropical monodominant trees. The ECM trait has independently arisen at least 66 times in Kingdom Fungi (Tedersoo, May, & Smith, 2010). Given such phylogenetically diverse origins, ECM fungi may be expected to have diverse functional traits operating along a mutualism-parasitism continuum (Johnson, Graham, & Smith, 1997). In *Salix reinii* seedlings in Japan, single species pairings of ECM fungi showed considerable variation in their impacts on seedling growth and nutrient acquisition (Nara, 2006). A *Pseudotsuga menziesii* seedling's ability to withstand drought was also influenced by associated ECM fungal species (Park, Linderman, & Black, 1983). Species richness of associated ECM fungi can also affect seedling growth and survival. *Pinus patula* seedlings inoculated with two ECM fungal species grew more than those with single-species inoculations (Reddy & Natarajan, 1997). Additionally, percent ECM colonization of dipterocarp seedlings was positively correlated with host foliar P in a selectively logged tropical forest (Lee & Lim, 1989). Brearley et al. (2017) found a positive correlation between ECM colonization and organic nitrogen (N) uptake in dipterocarp seedlings.

Multiple factors of the ECM mutualism may therefore contribute to seedling growth and survival. However, the aforementioned studies have tested a limited number of ECM fungal species on mainly temperate seedlings. Tropical monodominant forests can have very high ECM fungal species diversity (e.g., Ebenye et al., 2017; Henkel et al., 2012). All told, tropical ECM monodominant trees provide a compelling case in which to study the importance of ECM fungi to seedling survival.

Here we examined natural community assembly of ECM fungi on an even-aged seedling cohort of the Guyanese monodominant tree *Dicymbe corymbosa* Spruce ex Benth. (Fabaceae subfam. Detarioideae). Following a July 2016 mast seeding, newly established seedlings were sequentially sampled over a 12-month period (Henkel & Mayor, 2019). Following each sampling event, we: (a) measured the extent of ECM colonization on individual root systems and compared this between seedling age classes; (b) selected ECM fungal morphotypes for individual morphotype and age-aggregate DNA sequencing; and (c) compared ECM fungal communities of seedlings with those of nearby conspecific adults known from a previous study (Smith et al., 2017). This was the first study to temporally track ECM fungal community assembly on seedlings of a tropical monodominant tree. Throughout the study we aimed to determine aspects of the ECM symbiosis that may facilitate seedling establishment and survival, key components in persistent tropical monodominance (Henkel & Mayor, 2019).

METHODS

Study Site

The study was carried out in monodominant *D. corymbosa* forest of the Upper Potaro River Basin in Guyana's Pakaraima Mountains. Mean daily temperatures range from 19 to 29°C. Annual rainfall averages 3500–4000 mm, with peak rainy seasons in May–July and December–January. The site was situated in an undulating valley 20 km east of Mt Ayanganna, and was densely forested with primarily *Dicymbe*-dominated and, to a lesser extent, mixed forests
of the *Eschweilera–Licania* association (Degagne et al., 2009; Fanshawe, 1952). Study plots were established in *D. corymbosa* monodominant stands within a 5-km radius of a permanent base camp near the Potaro River (5°18′05″ N; 59°54′40″ W) at elevations 700–750 m a.s.l. In these plots *D. corymbosa* comprises upwards of 85% of the basal area of canopy trees, heavily dominates the seedling, sapling, and pole classes, and no other ECM tree species occur (Henkel, 2003; McGuire et al., 2008). Further details of climate, geology, soils, forest structure, and mast seeding cycles can be found in Henkel (2003), Henkel et al. (2005), Henkel and Mayor (2019), and Henkel, Terborgh, and Vilgalys (2002).

In 2003, five 0.25-ha plots were established to study forest dynamics following a *D. corymbosa* mast seeding event (Henkel et al., 2005). In 2005 two 0.25-ha plots were added within the same 5-km range of the Potaro base camp (Woolley, Henkel, & Sillett,

2008). All seedlings collected for this study resulted from the 2016 mast seeding event from these seven plots (reported as plots “P1–P7” in Henkel et al., 2005 and Henkel & Mayor, 2019).

Root Sampling

To measure changes in the extent of colonization and community assembly of ECM fungi during seedling development, we harvested 30 newly established seedlings per plot at 6-month intervals between July 2016 and July 2017, following the 2016 masting event. Seedling age classes were defined as: <1-month-old (July 2016; 2–3 weeks after germination), 6-month-old (January 2017), and 12-month-old (July 2017). At each time ten recently dead 2016 cohort seedlings were also collected from each plot. Whole seedlings were carefully extracted from the soil, transported back to base camp, and their roots rinsed with water. Root system width and depth and stem height were measured on 6- and 12-month live seedlings. Due to time constraints, percent ECM colonization estimation and morphotype selection for <1-month seedlings were completed in the field (see details below). For 6- and 12-month seedlings, all lateral roots of each seedling were cut from the main root axis, placed in a collection envelope, and field-dried with silica gel. Roots were transported to the USA to measure percent ECM colonization, sort morphotypes, and sequence DNA.

Seedling Mycorrhization

For <1-month seedlings, entire root systems were viewed under a dissecting microscope in the field. We estimated ECM percent colonization (PC) of root systems using the following categories: 0 (0 PC), 1 (1–25 PC), 2 (26–50 PC), 3 (51–75 PC), and 4 (76–100 PC). For each 6- and 12-month seedling, the dried lateral roots were cut into 0.5-cm segments and placed in a 10 cm Petri dish with a 1 × 1 cm grid on its underside. Roots were rehydrated with distilled water and viewed under a dissecting microscope at 40× magnification. Each vertical and horizontal gridline on the Petri dish was visually scanned, and each time a root intersected a gridline it was scored as "ECM" or "non-ECM" based on presence or absence of a fungal mantle (Giovannetti & Mosse, 1980). Seedling PC was calculated by dividing the number of ECM roots by the total number of root tips scored, multiplied by 100. Given the different techniques used, we converted the categorical scores of <1-month seedlings to percentage scores, allowing comparison with 6- and 12-month seedlings. For <1-month seedlings within each categorical score (0, 1, 2, 3, 4), we randomly generated a numerical percentage that fell within the category's percentage range.

Ectomycorrhizal root tips were sorted into morphotypes based on morphological variations. The organization of an ectomycorrhiza is a layer of often interwoven fungal hyphae ("mantle") surrounding the root, hyphae penetrating between the outer root cells to form the interface for nutrient exchange ("Hartig net"), and extramatrical hyphae extending out from the mantle (Smith & Read, 2008). Observable differences in color

and hyphal density of the mantle and extramatrical hyphae generally reflect the species of fungus forming the ectomycorrhiza (Agerer, 2006). As the host plant did not vary in this study, such differences provided the basis for sorting ectomycorrhizas into putative species-level morphotypes.

Sanger Sequencing of ECM Morphotypes

When a new morphotype was observed in a seedling age class, it was isolated and stored in nuclei lysis buffer (NLB) (Promega, Madison, WI, USA) prior to DNA extraction using the Wizard® Purification Kit (Promega, Madison, WI, USA). For each morphotype, we amplified the internal transcribed spacer (ITS) region, the primary fungal barcode (Schoch et al., 2012), using the primer pair ITS1F and ITS4 (Gardes & Bruns, 1993). All reactions included 12.5 μL of MyTaq Mix (Bioline, Memphis, TN, USA), 1.00 μL of each primer (at 10 μM), and approximately 5–10 ng of DNA. The final reaction volume was 25 μL. For unsuccessful PCRs, the PCR product was cleaned with ExoSap-IT™ PCR Product Cleanup Reagent (ThermoFisher Scientific, Waltham, MA, USA) and that was used as the template for a nested PCR using the primer pair ITS1 and ITS4 (Gardes, White, Fortin, Bruns, & Taylor, 1991; White, Bruns, Lee, & Taylor, 1990). The PowerClean© DNA Clean-Up Kit (MoBio Laboratories, Carlsbad, CA, USA) was used on DNA samples that contained PCR inhibiting substances (e.g., humics). If multiple bands were imaged following gel electrophoresis, each band was excised and the DNA was gel-extracted using Promega's Wizard® SV Gel Clean-Up System, followed by a template-concentrated PCR reaction. PCR products were sent to GeneWiz® (South

Plainfield, NJ, USA) for Sanger sequencing, and the resulting sequences were manually edited using Sequencher 5.2.3 (Gene Codes Corporation, Ann Arbor, MI, USA).

Illumina Sequencing of ECM Bulked Samples

Each distinctive morphotype encountered on an individual seedling was placed in cetyltrimethyl ammonium bromide (CTAB). These were grouped by sampling plot and by age class, yielding seven bulk extractions per age class and 21 total. Extraction of bulked root tips allows capture of molecular taxa that may have been missed through morphotype Sanger sequencing. The roots from each bulk extraction were ground in liquid nitrogen and the DNA was extracted using the CTAB method outlined in Vilgalys & Hester (1990). The DNA pellets were resuspended with 50 μL TE (10 mM tris hydrochloride, 1 mM disodium EDTA) and stored at −20°C until use. Extracts were then amplified using the primer pair ITS1F and ITS2 (Gardes & Bruns, 1993; White et al., 1990). Those primers were amended with a TruSeq back end 33 bp flanking region (Illumina, San Diego, CA, USA), so that unique barcodes could be ligated during the next round of PCRs. These PCR products were cleaned with ExoSAP-IT (ThermoFisher Scientific, Waltham, MA, USA) and a second PCR was performed using TruSeq DNA amplicon combinatorial dual index adaptors (Illumina, San Diego, CA, USA). The libraries were pooled in equal concentrations, along with a non-biological synthetic control, SynMock (Palmer, Jusino, Banik, & Lindner, 2018), and 250 bp paired-end reads were sequenced on one half of a lane of the Illumina MiSeq at the Purdue Genomics Core.

The resulting MiSeq amplicon data were analyzed using the AMPtk pipeline, which processes next-generation sequencing amplicon data from Illumina platforms (Palmer, Jusino, Banik, & Lindner, 2018). Forward and reverse primer sequences were trimmed, and paired end reads were merged using USEARCH v. 9.2.64 (Edgar, 2010). Reads were then clustered into operational taxonomic units (OTUs) with UPARSE (Edgar, 2013) using our custom sequence database, which has data resulting from 15 years of ECM sporocarp and root sampling in the study area (e.g., Henkel et al., 2012; Smith et al., 2011, 2017). When sequences did not match any in the custom database, they were BLAST searched on GenBank to estimate the closest taxonomic group. All OTU assignments were based on ≥ 97% global alignment (Lindahl et al., 2013; Selosse, Vincenot, & Öpik, 2016). Singletons were removed following clustering. Names were applied to OTUs as follows: (a) binomials for described taxa; (b) genus names with collection numbers from undescribed Guyanese sporocarp collections of T. Henkel (TH), M. C. Aime (MCA), M. E. Smith (MES), and S. L. Miller (SLM), or from ECM root tip collections of K. McGuire (GUYSOILD11, ##GadID) or M. Bidartondo & T. Bruns (Dinkey2230CA); (c) genus names with a clone number (e.g., 62MS_1F) from unpublished cloning-based datasets; (d) closest taxonomic group followed by a number (e.g., Helotiales4) for taxa found only from previous Guyana pyrosequencing (Smith et al., 2017); and (e) closest taxonomic group followed by an underscore and number (e.g., Clavulina_1) for taxa found only from Illumina sequencing in the current study.

Statistical Analyses

Statistical analyses were carried out using R software v. 1.1.456 (R Core Team, 2016). Percent colonization and the number of morphotypes per seedling were compared among seedling age classes using nonparametric Kruskal-Wallis rank sum tests, as the data were not normally distributed (Kruskal & Wallis, 1952). Post hoc Mann-Whitney U-tests were performed when rank sum tests showed significant differences between groups. Linear regression modeling was used to examine relationships between percent colonization or the number of morphotypes per seedling and seedling morphological traits, including root system width, root system depth, seedling height, and the number of root tips per seedling as explanatory variables. Models included data from 6-month seedlings, 12-month seedlings, and combined 6- and 12-month seedlings.

Ectomycorrhizal fungal species richness was estimated for each plot and seedling age class based on OTUs assigned to Illumina sequences. Temporal and spatial turnover in ECM fungal species were quantified between 6- and 12-month seedling age classes and the seven sampling plots.

We compared the 26 most frequently sequenced ECM fungal OTUs from each of the 6-month-old, 12-month-old, and adult *D. corymbosa* seedling age classes. This number was chosen because it was the maximum amount of OTUs recovered from 6-month seedlings through Illumina sequencing, and therefore the greatest number of OTUs we could compare among the three groups. Seedling OTU frequency was calculated from the number of plots from which the OTU was recovered. Adult tree OTU frequency was

calculated from the number of soil cores (of 80 total) from ten trees that it occurred in, as reported in Smith et al. (2017). Frequencies were scaled between 0 and 1 and visualized with a heat map generated in ggplot2 in R (Wickham, 2016).

Nonmetric Multidimensional Scaling (NMDS) ordination of OTUs based on Bray-Curtis dissimilarity distances was performed to examine influences of seedling age and sampling plot on ECM fungal community structure, utilizing the vegan package in R (Kruskal, 1964; Oksanen et al., 2018). The ordination used a matrix of OTUs assigned to Sanger-sequenced morphotypes versus the number of root tips collected for a given morphotype per plot. Low read number (< 5) OTUs were excluded and data were rarefied to the lowest abundance sample to account for unequal sample sizes. Significance of NMDS groupings was subsequently tested using the Adonis statistical method (Anderson, 2001).

RESULTS

Seedling Colonization

Percent ECM colonization (PC) of live *D. corymbosa* seedlings was significantly higher in 6-month seedlings than <1-month and 12-month seedlings ($P < 0.001$) but did not differ between <1-month and 12-month seedlings ($P = 0.70$; Figure 1). Total live seedlings had insignificantly higher PC than total dead seedlings ($\bar{x}_{live} = 40.32 \pm 23.16$; $\bar{x}_{dead} = 36.74 \pm 20.70$; $P = 0.091$).

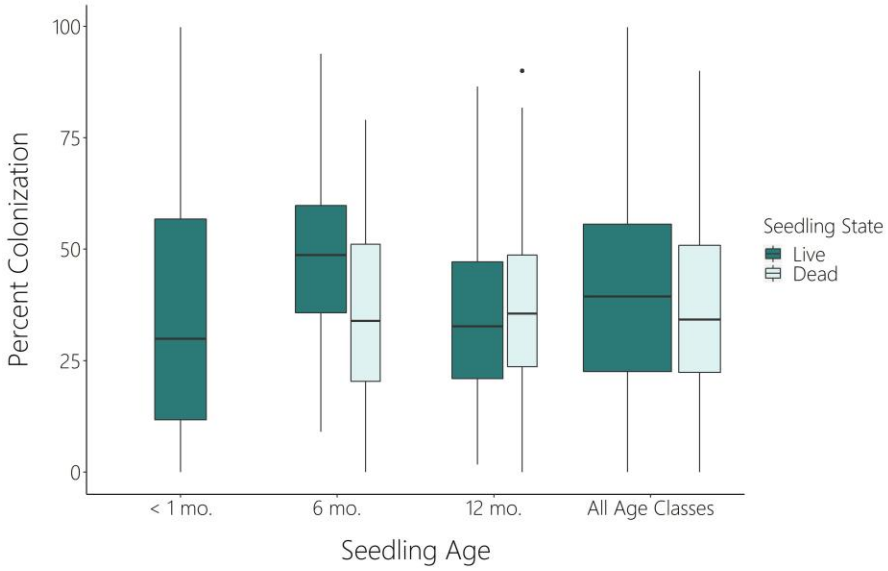

Figure 1. Percent ECM colonization of living and dead seedling fine lateral roots at three age classes and for all age classes combined. Box width corresponds to the number of seedlings sampled.

Relationships between seedling morphological characters and PC differed between 6-month and 12-month age classes. At 6 months, shorter seedlings had higher colonization (R^2 = 0.036, P = 0.017). At 12-mo, PC was best predicted by a model that used all measured characters, including root system width, root system depth, seedling height, and the total number of root tips (R^2 = 0.043, P = 0.012). When combining data from 6- and 12-month age classes, seedling root system width and seedling height were the best predictors of PC (R^2 = 0.018, P = 0.009; Table 1). Shorter seedlings with wider root systems tended to have higher PC. The strength of these correlations between seedling morphological characteristics and PC was, however, weak (all *R*-squared values less than 0.05).

Table 1. Linear regression modeling of seedling morphological features and percent colonization by ECM fungi from combined 6- and 12-month seedling age classes (*Adj.* R^2 = 0.016, P = 0.032). Only root system width was significantly positively correlated with percent colonization, but the effect was weak.

Coefficient	Estimate	Std. Error	*t* value	*P*
Root System Width	0.5712	0.2637	2.166	0.0309
Root System Depth	-0.1925	0.1813	-1.062	0.2890
Stem Height	-0.3893	0.2208	-1.764	0.0786
Total No. Root Tips	0.0043	0.0078	0.552	0.5810

Morphotypes

The number of ECM morphotypes on live seedlings increased over time. Morphotypes per seedling averaged 1.20 ± 0.69 at < 1 month, 3.17 ± 1.27 at 6 months, and 3.55 ± 1.47 at 12 months (Figure 2). For dead seedlings age did not affect the number of morphotypes per seedling ($P = 0.824$). Across age classes, a live seedling had significantly more morphotypes than a dead seedling ($\bar{x}_{live} = 2.64 \pm 1.57$; $\bar{x}_{dead} = 1.88 \pm 1.05$; $P < 0.001$).

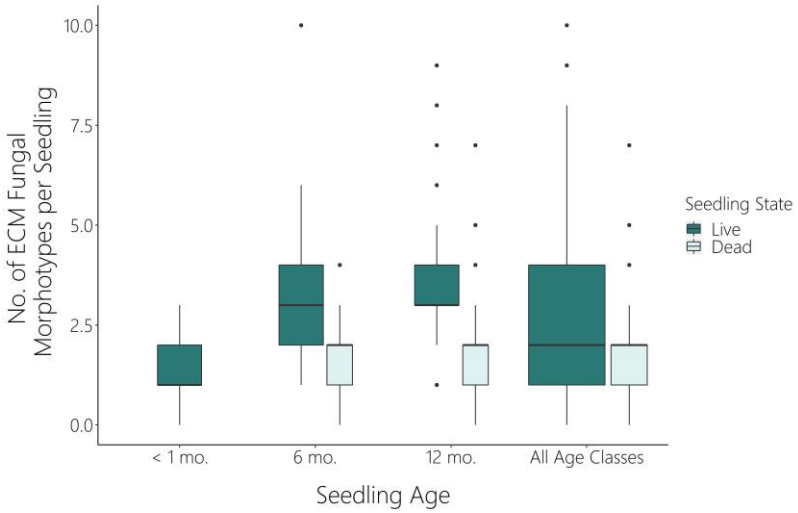

Figure 2. Mean number of ECM morphotypes per seedling at three age classes and for all age classes combined. Box width corresponds to the number of seedlings sampled.

Visual recognition of different ECM fungal morphotypes within an age class often overestimated species richness relative to ITS Sanger sequencing. In <1-month seedlings, of seven recognized morphotypes, five belonged to ITS-distinct OTUs; at 6 months, 22 OTUs were recovered from 27 morphotypes, and at 12 months, 34 recognized

morphotypes yielded 17 OTUs, seven of which were putatively non-ECM fungi. The five morphotypes shared between <1-month and 6-month seedlings were assigned to four ITS-distinct OTUs. Between 6- and 12-month seedlings, six of 11 shared morphotypes belonged to distinct OTUs. One- and 12-month-old seedlings shared only two morphotypes, both of which were ITS-distinct.

Communities

Illumina community sequencing showed an increase in the number of ECM fungal OTUs over time. Additionally, numerous OTUs assigned after Illumina sequencing were missing from Sanger sequencing, and vice versa. Overall, community sequencing recovered 158 distinct ECM fungal OTUs from 20 ECM fungal lineages (Tedersoo et al., 2010) (Figure 3a). Species richness increased over time, with 6 OTUs at <1 month, 26 OTUs at 6 months, and 114 OTUs at 12 months. The most species-rich ECM fungal lineages included /russula-lactarius (35 OTUs) and /tomentella-thelephora (28 OTUs), followed by /boletus and /sebacina with 22 OTUs each. Prominent lineages from Sanger sequencing of morphotypes were /clavulina (11 OTUs), /tomentella-thelephora (11 OTUs), /russula-lactarius (6 OTUs), and /boletus and /inocybe (4 OTUs each) (Figure 3b).

Species turnover was high between 6- and 12-month seedlings as indicated by morphotype Sanger sequences ($\beta_A = 0.80$) and Illumina community sequences ($\beta_A = 0.91$). Beta-diversity of ECM morphotypes was low among sampling plots at 6 months ($\beta_w = 0.21 \pm 0.06$) and 12 months ($\beta_w = 0.20 \pm 0.06$). Illumina sequencing detected nearly

twice as much β-diversity among plots relative to morphotype sequencing in 6-month (β_w = 0.36 ± 0.11) and 12-month (β_w = 0.44 ± 0.08) seedlings.

The NMDS ordination indicated that ECM fungal communities differed by seedling age (R^2 = 0.20, P < 0.001) (Figure 4a) but not by plot (R^2 = 0.30, P = 0.091) (Figure 4b).

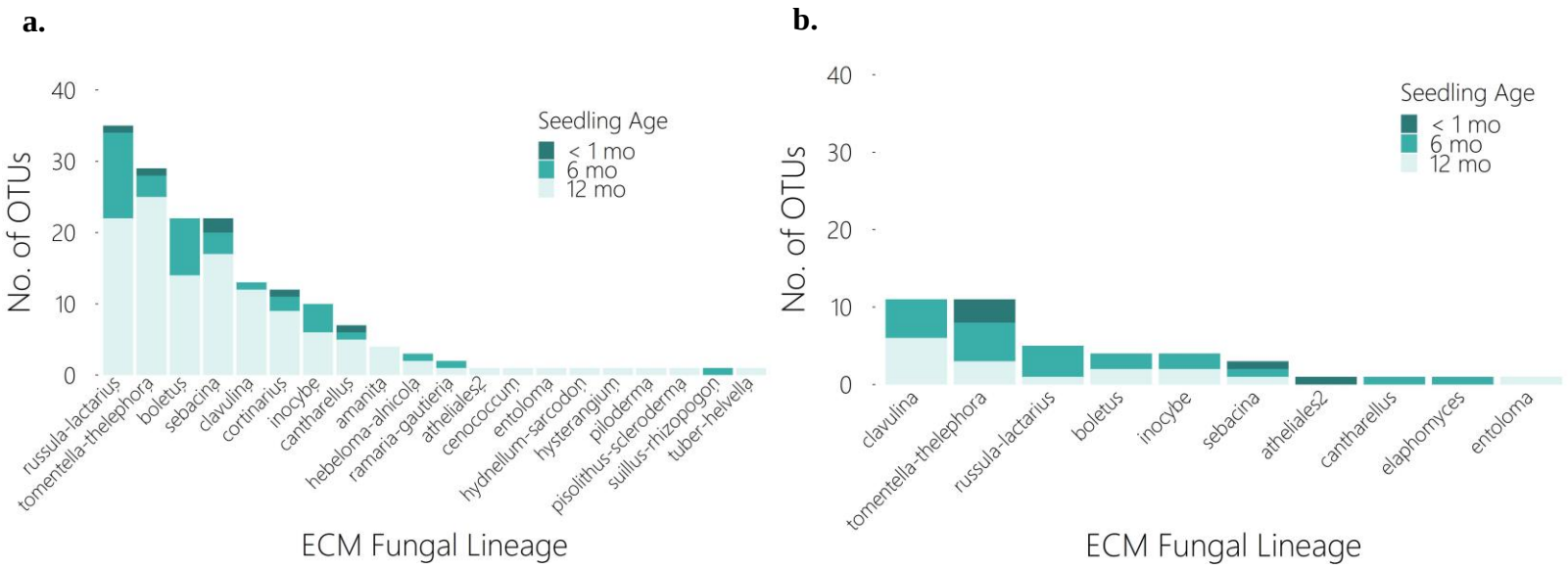

Figure 3. Species richness of ECM fungal lineages from (a) Illumina sequencing of bulked seedling root tips and (b) Sanger sequencing of selected ECM morphotypes.

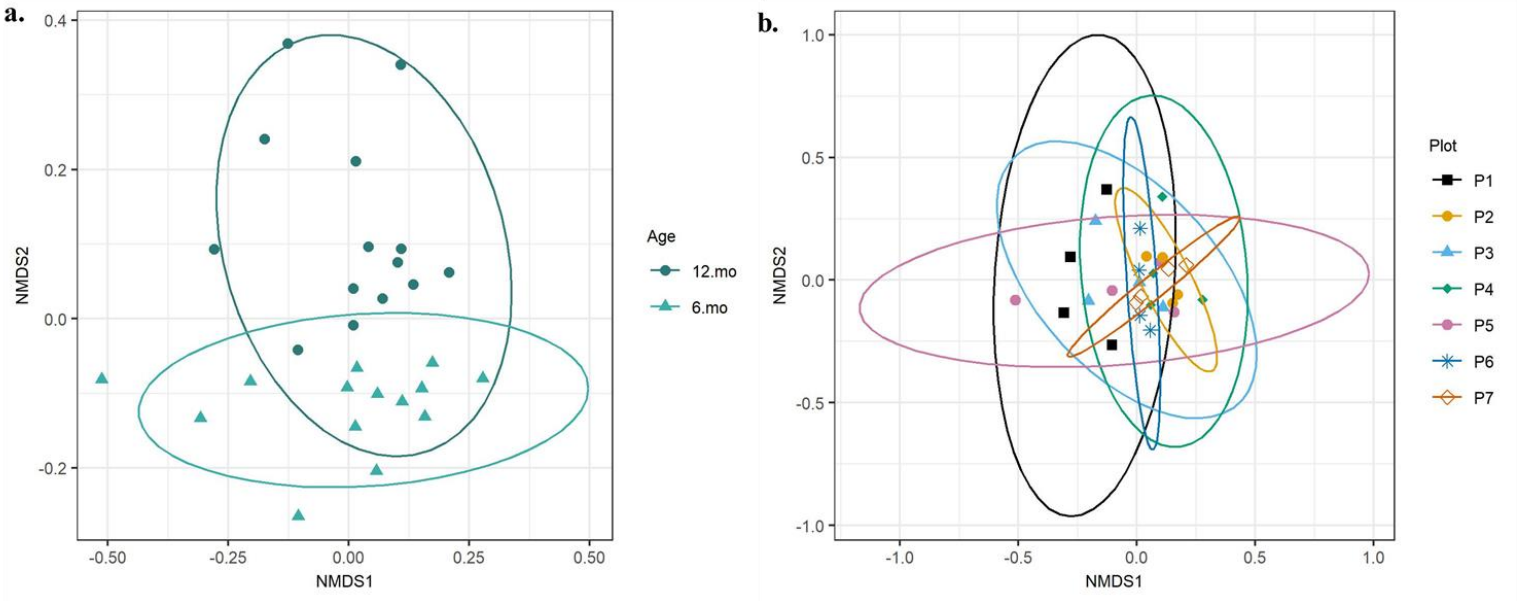

Figure 4. NMDS ordination of Sanger-sequenced ECM fungal communities and (a) seedling age class or (b) seedling sampling plot.

Comparisons with Adult *D. corymbosa*

Species richness of ECM fungi was lower on <1-month (6 OTUs) and 6-month (26 OTUs) seedlings than that of nearby adult *D. corymbosa* trees (97 OTUs) (Smith et al., 2017). Conversely, 12-month seedlings had more OTUs (114) than adults.

The top 26 most frequently encountered ECM fungal OTUs from each of the 6-month and 12-month seedling groups and adult *D. corymbosa* comprised 68 distinct ECM fungal OTUs and their composition varied considerably between the groups (Figure 5). Only *Russula myrmecobroma* and *Tomentella* ecm1111 occurred in each group. Six- and 12-month-old seedlings shared more OTUs with adult *D. corymbosa* than they did with each other. Six-month seedlings shared two additional OTUs with adult trees (*Inocybe pulchella* and *Tylopilus potamogeton*) and 12-month seedlings shared three additional OTUs with adult trees (*Clavulina* ecm1037, *Inocybe epidendron, Tylopilus vinaceipallidus*).

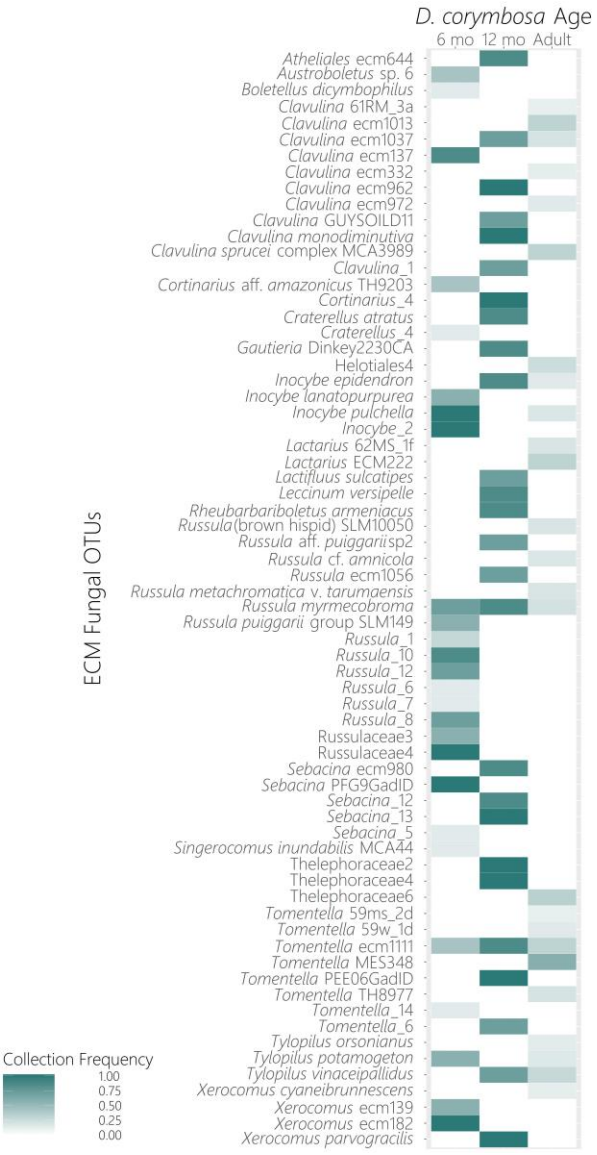

Figure 5. Collection frequency of the 26 most frequently encountered ECM fungal OTUs on each of the 6- and 12-month seedling age classes and on adult *D. corymbosa* (Smith et al., 2017). Frequencies were calculated using the number of sequence reads from Illumina sequencing (seedlings) or pyrosequencing (adults), and correspond to the number of plots an OTU was sampled out of seven (seedlings) or the number of soil cores an OTU was recovered from, out of 80 (adults).

DISCUSSION

We found little overlap in ECM fungal OTUs occurring either across seedling age classes or between seedlings and adults of *D. corymbosa*. This contrasts with the Central African leguminous monodominant *Gilbertiodendron dewevrei* in which 65% of ECM fungal symbionts were shared between seedlings and larger trees (Ebenye et al., 2017). However, they collected seedling and adult roots at the same time and sampled far fewer seedlings than this study (n = 48 versus n = 770). Through repeated sampling of an even-aged cohort of new seedlings over 12 months following a masting event, we detected high temporal turnover of ECM fungal species, but no spatial effects on community composition. The strong temporal effect suggests that undetected biotic or abiotic factors operating throughout the study site may have driven changes in the ECM fungal community.

One environmental factor that could have driven ECM fungal community change is soil nutrient availability. In temperate forests, increase in soil N can decrease ECM fungal diversity and alter species composition (Avis, McLaughlin, Dentinger, & Reich, 2003; Lilleskov, Fahey, Horton, & Lovett, 2002; Peter, Ayer, & Egli, 2001). Following masting, a large amount of reproductive biomass is shed, from which ECM fungi may directly access organic N and transport it to seedlings (Leake & Read, 1997; Perez-Moreno & Read, 2000). However, in our system, ECM fungi had no consistent effect on N concentrations in *D. corymbosa* leaf litter over a 12-month decomposition period (Mayor & Henkel, 2006). Slowed N mineralization in addition to the lack of N limitation

in tropical soils (Vitousek & Sanford, 1986) weakens the possibility that N fluxes influence ECM community composition in *D. corymbosa* monodominant stands.

Phosphorus fluxes could potentially have more impact on ECM fungi than those of N (Vitousek 1984). In tropical monodominant forests masting can affect the availability of soil P (Newbery, Alexander, & Rother, 1997). In Guyana, where soils are extremely low in available P, *D. corymbosa* masting releases an average of ~1.5 kg ha^{-1} of P in reproductive litter to the soil environment, which is high compared to other tropical masting trees (Green & Newbery, 2002; Henkel et al., 2005; Henkel & Mayor 2019). In the year after a mast year, however, trees are P-depleted and soil P concentrations drop, as their leaf litter fall is substantially lacking in P (Henkel & Mayor, 2019; Newbery et al., 1997). Large P pulses directly after mast seeding may decrease fungal community diversity, as observed in temperate old-growth beech-maple forests following seasonal P increases (Burke, López-Gutiérrez, Smemo, & Chan, 2009). In *Eucalyptus diversicolor* seedlings, percent colonization by four ECM fungal species was reduced with increasing soil P concentrations (Bougher, Grove, & Malajczuk, 1990). Soil P fluxes could potentially affect mycorrhization in *D. corymbosa* seedlings, but their link with ECM fungal diversity remains to be tested.

The temporal shift in ECM fungal community structure on *D. corymbosa* seedlings may have been influenced by root competition among ECM fungal species (Landeweert et al., 2003; Lilleskov & Bruns, 2003; Jonsson, Nilsson, Wardle, & Zackrisson, 2001; Wu, Nara, & Hogetsu, 1999). *Pinus densiflora* seedling roots initially colonized by *Pisolithus tinctorius* were overtaken by an introduced, unidentified ECM

fungus (Wu et al., 1999). Lilleskov and Bruns (2003) found that *Tomentella sublilacina* eventually replaced *Rhizopogon occidentalis* on seedlings of *Pinus muricata*. *Tomentella* species may be particularly adept competitors for root colonization, as in the current study the OTU *Tomentella* ecm1111 was a top colonizer of *D. corymbosa* seedlings across age classes and of previously sampled adults (Smith et al., 2017). Simple priority effects may also drive interspecific competition as found by Kennedy and Bruns (2005), with "first colonizing" species of *Rhizopogon* tending to remain dominant on seedlings of *P. muricata*. As *Tomentella* ecm1111 was prominent in our <1-month seedlings, it is possible that this OTU may have remained dominant via a priority effect.

Interspecific competition among ECM fungi may be intensified due to limited niche availability on seedling root systems. For species to coexist, partitioning among multiple niches should occur (Chase & Leibold, 2003). This appears to be the case with highly reiterated adult *D. corymbosa*, where vertically arranged heterogenous soil rooting zones promote ECM fungal niche partitioning and specialization and increase their stand-level alpha-diversity (Smith et al., 2017). *Dicymbe corymbosa* seedlings have comparatively limited root heterogeneity, with no significant change in root morphological parameters between 6- and 12-month seedlings. Additionally, a majority of the seedling root systems were left uncolonized, indicating that colonizable space was not a limiting factor. Without significant vertical growth of seedling root systems, fungal species dependent on vertical niche partitioning would be unable to coexist, which may explain why we found different ECM fungal communities on seedlings and adults. Aside from spatial partitioning, temporal partitioning may arise due to species-level differences

in physiological tolerances, environmental variability over time, and the lifespan of individual fungal genets (Koide, Shumway, Xu, & Sharda, 2007).

In our study ECM colonization was not correlated with seedling age, in contrast to previous results found with *G. dewevrei* (Torti & Coley, 1999). In our case colonization extent may have been influenced by the composition of fungal species present as inoculum for the seedlings, and interactions between them. In a previous study *P. menziesii* seedlings were inoculated with species of *Rhizopogon, Hebeloma,* or *Laccaria,* and these exhibited different levels of colonization—36, 93, and 73%, respectively (Dosskey, Linderman, & Boersma, 1990). When Nara (2006) individually inoculated *Salix reinii* seedlings with one of 11 ECM fungal species from six genera, the resulting colonization levels varied from 5 to 78%, and were significantly different between both hetero- and congeneric species. Multiple independent origins of the ECM trait across Kingdom Fungi may have led to variation in functional traits related to the ECM symbiosis, including colonization potential. We found that ECM fungal communities are very diverse on *D. corymbosa* seedlings. Each ECM fungal species may vary in colonization potential, and this may have been reflected by the high overall variation in percent colonization we observed (std. dev. = ± 23.16%).

The number of ECM morphotypes per seedling was comparable to other tropical monodominant seedlings. Up to 11 ECM morphotypes per 7-month-old seedling were recovered from the monodominant dipterocarp *Shorea leprosula* in Malaysia (Alexander et al., 1992), similar to the maximum of 10 for a single 6-month-old *D. corymbosa* seedling. In a Congolian *Gilbertiodendron* monodominant forest, 19 ECM morphotypes

were recovered from a seedling sample of indeterminate age, compared to 27 morphotypes from our 6-month *D. corymbosa* (Torti & Coley, 1999). This same study also found fewer ECM morphotypes per seedling than we did in 6-month seedlings (1.3 ± 0.9 vs. 3.17 ± 1.27). Morphotyping is, however, a subjective method that can under- or overestimate ECM fungal diversity. More accurate comparisons of ECM fungal communities may be facilitated by root tip selection and a combination of sequencing techniques (Menkis, Visiliauska, Taylor, Stenlid, & Finlay, 2005). Regardless, we found through both Sanger and Illumina sequencing that seedlings of *D. corymbosa* are species rich as individuals, and as a cohort.

Ectomycorrhizal colonization of seedlings in this tropical monodominant forest is a dynamic process, characterized by high overall ECM fungal species richness and high species turnover over time. Seedlings do not appear dependent on extensive levels of colonization, but the number of colonizing fungal species may play an important role in their survival. Tropical monodominant forests harbor a significant portion of global ECM fungal diversity (Corrales et al., 2018). *Dicymbe corymbosa* seedlings may be important local reservoirs of ECM fungal diversity, as 12-month seedlings had more OTUs than adults (Smith et al., 2017). If ECM fungi contribute to the near-term, and potentially long-term, survival of seedlings, this could ultimately promote persistence of tropical monodominant forests.

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