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Ectomycorrhizal fungi are shared between seedlings and adults in a monodominant *Gilbertiodendron dewevrei* rain forest in Cameroon

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ABSTRACT

Ectomycorrhizal networks may facilitate the establishment and survival of seedlings regenerating under the canopies of tropical forests and are often invoked as a potential contributor to monodominance. We identified ectomycorrhizal fungi in a monodominant *Gilbertiodendron dewevrei* (Fabaceae) rain forest in Cameroon, using sporocarps and ectomycorrhizae of three age categories (seedlings, intermediate trees, and large trees) and tentatively revealed nutrient transfer through ectomycorrhizal networks by measuring spontaneous isotopic (^{13}C and ^{15}N) abundances in seedlings. Sporocarp surveys revealed fewer ectomycorrhizal fungal taxa (59 species from 1030 sporocarps) than molecular barcoding of ectomycorrhizal roots (75 operational taxonomic units from 828 ectomycorrhizae). Our observations suggest that ectomycorrhizal fungal diversity is similar to that in other mixed tropical forests and provide the first report of the *Tuber-Helvella* lineage in a tropical forest. Despite some differences, all age categories of *G. dewevrei* had overlapping ectomycorrhizal fungal communities, with families belonging to Thelephoraceae, Russulaceae, Sebacinaceae, Boletaceae, and Clavulinaceae. Of the 49 operational taxonomic units shared by the three age categories (65.3% of the ectomycorrhizal fungal community), 19 were the most abundant on root tips of all categories (38.7% of the shared taxa), supporting the likelihood of ectomycorrhizal networks. However, we obtained no evidence for nutrient transfer from trees to seedlings. We discuss the composition of the ectomycorrhizal fungal community among the *G. dewevrei* age categories and the possible role of common ectomycorrhizal networks in this rain forest.

Abstract in French is available with online material.

Key words: ^{13}C; ^{15}N; Caesalpinioideae; common ectomycorrhizal network; ectomycorrhiza; Fabaceae subfamily; internal transcribed spacer; sporocarps.

MANY TREES FORM ECTOMYCORRHIZAL SYMBIOSES WITH DIVERSE BASIDIOMYCOTA AND ASCOMYCOTA, which play a key role in many tropical forests (Bâ *et al.* 2012), affecting tree growth and nutrient absorption as well as protection against pathogens. These associations also produce sporocarps of ectomycorrhizal (EM) fungi, many of which are of economic interest (Yun & Hall 2004). Most known EM tree species are found in temperate and boreal regions (Smith & Read 2008, Van Der Heijden *et al.* 2015). Despite recently increasing interest (Bâ *et al.* 2012), tropical EM associations are poorly understood, particularly in monodominant forests.

Monodominant forests are large stands in which a single tree species comprises more than 60 percent of canopy-level trees (Torti & Coley 1999, McGuire 2007). Factors that create and maintain monodominant forests within a matrix of otherwise high-diversity tropical rain forests remain unexplained (Peh *et al.*

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2011). The observation that monodominant forests typically contain ectomycorrhizal trees has led to the hypothesis that EM fungi, supported by adult trees, could facilitate the establishment of seedlings growing under limited light, thereby competitively excluding non-EM seedlings (Torti & Coley 1999, McGuire 2007). Enhanced establishment and survival of EM seedlings near mature EM trees have been reported in monodominant or mixed stands (Newbery *et al.* 2000, Onguene & Kuyper 2002, McGuire 2007). In some cases, seedlings are integrated into an EM fungal network already supported by mature trees (Selosse *et al.* 2006, McGuire 2007, Corrales *et al.* 2016a). Recently, a Panamanian monodominant species (*Oreomunnea mexicana,* Juglandaceae) was shown to have potential for such common mycorrhizal networks (CMNs) (Corrales *et al.* 2016a). Although some aspects remain controversial, CMNs are known to drive nutrient transfers (including carbon) between plants in some conditions (Selosse *et al.* 2006, Teste *et al.* 2010, Klein *et al.* 2016). Plants receiving carbon from CMNs are found in temperate and some tropical ecosystems, and the compounds received from EM fungal partners usually increase their natural ^{13}C abundance (Selosse & Roy 2009). These plants also show high N content and ^{15}N abundance, indicating modifications of their nitrogen nutrition, although the mechanism for this remains poorly understood (Selosse & Roy 2009, Selosse *et al.* 2016). Candidates for nutrient transfer can thus be detected by isotopic analyses. However, isotopic analyses of seedlings in a mixed Guinean EM forest where adults and seedlings shared most EM fungi failed to detect resource transfer to seedlings (Diédhiou *et al.* 2010). To the best of our knowledge, resource transfer has not yet been tested in monodominant tropical rain forests.

The Fabaceae timber tree *Gilbertiodendron dewevrei* (De Wild.) J. Léonard of the subfamily Caesalpinioideae (hereafter caesalpinioids) provides a case of almost monospecific forest stands (>90% of the canopy trees) on >1000–5000 ha patches in the Congo basin (Letouzey 1985, Hart 1995, Peh *et al.* 2011), where this species dominates in all subcanopy age categories (Hart 1990, 1995). These stands harbor diverse EM sporocarps (*e.g.* Buyck 1993, 1994, 1997, Verbeken & Walleyn 2010). Fassi (1960) described ectomycorrhizae (ECMs) on *G. dewevrei*, but the distribution of EM fungal communities on roots of the different age categories of *G. dewevrei* remains unknown.

Although EM fungi have been identified on *G. ogoouense* and *Gilbertiodendron* sp. roots from mixed Cameroon forests (Tedersoo *et al.* 2011), our study is the first to investigate aboveground and belowground EM fungal communities of *G. dewevrei* and their potential role on seedlings. *Gilbertiodendron dewevrei* maintains high densities of seedlings, suggesting that established trees may inoculate seedlings, and perhaps even provide carbon resources through CMNs (Onguene & Kuyper 2002, McGuire 2007). To identify the potential for CMNs between adult trees and seedlings, we determined the EM fungal communities of different age categories by performing ECM barcoding and sampling sporocarps to increase the data set and to obtain specific references for barcodes. We investigated the following: (1) if trees and seedlings share EM fungal taxa; (2) if the composition of the EM fungal communities varies among different age categories; and (3) if EM fungi provide carbohydrates and nitrogen to *G. dewevrei* seedlings.

METHODS

Study Area.—We conducted this project in Southeast Cameroon during the rainy seasons of 2009–2012 (Fig. S1). We located our study area within an 8 ha forest near Nkondon I. Annual rainfall averaged 1512 mm and fell mainly from August to November near our study area (Meyibot I: Djuikouo *et al.* 2010). The area is densely forested with *G. dewevrei,* which comprises 81.1 percent of the basal area and densely regenerates (Djuikouo *et al.* 2010).

We randomly located three representative sites (0.5-ha per site) within the 8 ha forest: S1 (2°49′02.4″N, 13°56′54.8″E), S2 (2°49′30.6″N, 13°56′40.2″E), and S3 (2°49′53.2″N, 13°56′18.2″E). Sites S1 and S2 were 970 m apart, while sites S2 and S3 were 977 m apart. Soil properties are analyzed in supporting information (Table S3). A single individual of EM *Uapaca* sp. (at S1) and several individuals of *Carapa procera* (a non-EM Meliaceae, Béreau *et al.* 1997, Wang & Qiu 2006), *Irvingia gabonensis* (a non-EM Irvingiaceae, Onguene & Kuyper 2001), and *Pentaclethra macrophylla* (a non-EM Fabaceae, Henkel *et al.* 2002) co-occurred with *G. dewevrei*. We divided each site into five rectangles of 100 m × 10 m in which we identified *G. dewevrei* individuals from three main age categories: seedlings, intermediate trees, and large trees (Table S1).

Sampling Strategy and Morphotyping of ECMs.—At each site, we sampled ECMs from six large trees, six intermediate trees, and 48 seedlings during the rainy season of 2010. Sampled trees were at least 8 m apart. For each large or intermediate tree, eight soil cores were taken at 1–2 m from the base of the tree trunk by inserting a 15 cm diameter soil corer to a depth of 30 cm (approximately 300 g of fresh soil remained attached to the roots) at sites locally devoid of seedlings. We randomly chose seedlings (6–15 leaves, and height <1 m) and fully harvested them by digging with a spade (20 cm width and 28 cm depth) to recover approximately 300 g of fresh soil. All cores were stored in a cooler for <5 days before being processed. Thus, we evaluated 144 cores containing the roots of large trees, 144 cores containing these of intermediate trees, and 144 root systems of seedlings.

We gently washed all root systems over a 250-μm sieve under running tap water, dispersed them in a dish of water, and examined them at 10× magnification under a dissecting microscope for EM root tips. EM root tips were classified into morphotypes according to Thoen and Bâ (1989). We counted the numbers of ECMs and noncolonized root tips to determine the percentage of EM colonization. In total, we morphotyped 49,888 ECMs.

We maximized the detection of EM fungal diversity at the barcoding step by choosing representatives of different morphotypes: we subsampled for molecular analyses 15 EM root tips from large and intermediate trees and two EM root tips per seedling (*i.e.*, 96 EM root tips per site) by maximizing the number of

morphotypes represented. These 828 samples were stored in CTAB buffer according to Séne *et al.* (2015).

DNA Extraction and Sequencing.—We extracted sporocarp (Table S2) and ECM DNA using an Extract-N-Amp™ Plant PCR Kit (Sigma-Aldrich, St. Louis, U.S.A.). We performed PCR amplification and sequencing of the internal transcribed spacers and 5.8S region (ITS) of the ribosomal DNA with primers ITS1-F and ITS4, or otherwise ITS1-F and ITS4B as in Séne *et al.* (2015). We edited sequences using CodonCode Aligner v.4.1.1 (LI-COR, Inc., MA). We partitioned sequences (accession numbers KR819005 to KR819138) into operational taxonomic units (OTUs) by grouping sequences with more than 97 percent similarity level. In the following section, 'species' describe fungal taxa identified from sporocarps, and 'OTUs' describe taxa identified by barcoding. OTUs were identified at the species level when a sequence presented more than 97 percent full-length similarity to (i) sequences derived from sporocarps in this study or (ii) well-identified sequences from the GenBank database using the BLAST-N algorithm. We identified OTUs at the genus or family level based on BLAST-N results. Taxa identified from sporocarps and root tips were assigned to the phylogenetic lineages of EM fungi defined by Tedersoo *et al.* (2010a). We considered Helotiales to be EM fungi, although some debate exists in this respect, and some species may not be EM; Sordariales and Eurotiales are likely not EM taxa but were taken into account since our knowledge of EM taxa remains limited in African tropical ecosystems.

^{13}C and ^{15}N Analyses.—We investigated the natural ^{13}C and ^{15}N abundance in seedlings to assess, by comparison with older autotrophic plants or EM fungi, whether they showed any isotopic enrichment that may reflect the gain of compounds from CMNs. At each site in 2010, we harvested five leaves from large trees, intermediate trees, and seedlings of *G. dewevrei*, as well as large trees of *Pentaclethra macrophylla*, an arbuscular mycorrhizal (AM) tree, as a control for autotrophic biomass. We also collected *G. dewevrei* seeds to determine the isotopic abundance of reserves that contribute to early seedling growth, as well as $n = 3$ replicates taken from the pileus of sporocarps of five fungal species (*Clavulina* sp.2, *Lactifluus longipes*, *Russula brunneoderma*, *Russula* sp.1, and *Scleroderma* sp., Table S4) to estimate the isotopic abundance of the fungal biomass. All leaves were from similar light conditions and distance to soil and therefore had comparable photosynthetic conditions and CO_2 sources. Isotopic abundances were measured as in Tedersoo *et al.* (2007) and expressed in $\delta^{13}C$ and $\delta^{15}N$ values in parts per thousand relative to international standards (respectively, V-PDB and atmospheric N_2).

Statistical Analyses.—We performed one-way ANOVA with XLSTAT2010 software (Addinsoft SARL, Paris) to compare (i) soil properties at the three sites and (ii) stable isotope abundances between ectomycorrhizal trees, arbuscular mycorrhizal trees, seeds, and EM sporocarps. Data were normally distributed. Significant differences between pairs were revealed by a Newman-Keuls test, which is more sensitive to differences than a *post-hoc* Tukey test (Abdi & Williams 2010). Species-accumulation curves, estimates of species richness (first-order jackknife), and diversity indices (Fisher's alpha, Shannon index) were inferred using EstimateS software version 9.1.0 (Colwell 2013). We assessed the effects of site, age category, and their interaction on the EM fungal community composition *via* perMANOVA (permutational multivariate analysis of variance, Anderson 2001) analysis, using the function Adonis from the R package Vegan 2.2-1 (Oksanen *et al.* 2015). Due to high inter-individual variance, we merged individual fungal communities by site and age. We, therefore, performed NMDS on the community matrix with OTUs as rows and nine communities (three sites * three ages). For each significant factor detected by perMANOVA (site and tree category), we computed pairwise perMANOVA (Anderson 2001) on balanced subsamples (*e.g.*, to test for the difference in EM fungal composition between sites S1 and S2, we sampled the maximum number of individuals at each site while keeping the number of samples per site equal). The relative abundance of EM fungal taxa was calculated as the ratio of the number of ECMs or sporocarps of a given taxon over the total number of ECMs or sporocarps per plot and site. Relative frequency of EM fungal lineages was calculated as the ratio of the number of occurrences of a given lineage over the total occurrence of lineages per plot and per site. The α type I error was set at 5 percent.

RESULTS

Sporocarp Identification.—The 1030 sampled sporocarps (Tables 1 and S2) fell into 11 EM fungal lineages proposed by Tedersoo *et al.* (2010a), including 10 from Basidiomycota and 1 from Ascomycota (Fig. 1): /russula-lactarius-lactifluus contributed the most species (21), followed by /boletus (5), /amanita (5), /cantharellus (5), /clavulina (4), /ramaria-gautieria (2), /cortinarius (1), /pisolithus-scleroderma (1), /thelephora-tomentella (1), /tricholoma (1), and /tuber-helvella (1). Depending on the site, 5–9 lineages were fruiting during our surveys. The most fruiting species were found in /russula-lactarius-lactifluus, followed by /boletus (Fig. S2). Morphological and molecular identification (GenBank accessions in Table S4) showed 59 species dominated by *Russula concolora* Buyck, *R. diffusa* Buyck, *Russula* sp.16, *Russula* sp.15, Boletaceae 4, *Russula* sp.5, *Lactifluus sesemotani* (Beeli) Buyck, and *Russula* sp.2, each contributing more than 3 percent of the total abundance of sporocarps (Fig. 2). At the species level, the communities revealed nonuniform composition for the three sites, with 14–34 EM fungal species recorded per site (Fig. 2). The number of EM fungal species that occurred in two sites ranged from 6 (S1 and S2) to 12 (S1 and S3). The 55 EM fungal species collected in 2010 (when above- and belowground sampling can be compared) were similar in number to the other sampling years (52, 51, and 50 species in 2009, 2011, and 2012, respectively; Table S2). Of the 55 species collected in 2010, /russula-lactarius-lactifluus (25 species) was most prevalent, followed by /boletus (12), /amanita (4), /cantharellus (4), /clavulina (4), /ramaria-gautieria (2), /cortinarius (1), /pisolithus-scleroderma (1),

TABLE 1. *Number of EM root tips, sporocarps and OTUs, percentage of mycorrhizal colonization, species richness, and diversity indices from* G. dewevrei *seedlings (S), intermediate trees (T), and large trees (LT) in a monodominant forest in Cameroon.*

	Site 1			Site 2			Site 3			All sites		
	S	T	LT	S	T	LT	S	T	LT	S	T	LT
Total No. of EM root tips observed	8604	6607	9420	4681	3919	3789	5803	3455	3610	19,088	13,981	16,819
Percentage EM colonization[a]	92.9% a	83.4% b	87.6% c	91.3% a	85.3% b	94.2% ab	94.5% a	88.1% b	85.5% b	92.9% a	85.0% b	88.5% b
No. of EM root tips extracted	101	93	101	91	92	86	81	98	85	273	283	272
No. of sequences	78	81	72	72	67	61	74	59	65	224	207	198
No. of OTUs	33	45	38	24	42	33	29	41	35	65	68	63
Rarefied number of OTUs[b]	29.2 ± 2.4	38.5 ± 2.6	33.5 ± 4.4	21.7 ± 2.9	38.7 ± 4.2	32.5 ± 2.5	25.5 ± 3.1	41.0 ± 5.0	33.8 ± 1.7	62.0 ± 3.8	67.3 ± 2.5	63.0 ± 2.2
Jackknife1 richness estimator	43.2 ± 3.2	60.0 ± 3.7	54.8 ± 3.6	32.8 ± 3.0	63.9 ± 3.8	46.7 ± 3.3	40.0 ± 3.3	69.5 ± 3.8	46.9 ± 3.2	85.9 ± 4.6	84.1 ± 4.0	76.9 ± 3.6
Shannon's diversity index	3.1 ± 0.1	3.6 ± 0.0	3.3 ± 0.1	2.6 ± 0.1	3.5 ± 0.0	3.4 ± 0.0	2.9 ± 0.1	3.6 ± 0.0	3.4 ± 0.0	3.7 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
Fisher's alpha per age	23.0 ± 5.1	48.6 ± 12.3	32.7 ± 7.7	12.5 ± 2.6	48.9 ± 12.4	29.7 ± 6.8	17.1 ± 3.7	59.6 ± 15.9	33.2 ± 7.8	31.9 ± 3.6	36.0 ± 4.1	30.9 ± 3.5
Fisher's alpha per site	35.01 ± 5.49			33.45 ± 5.55			31.90 ± 5.35			22.20 ± 3.03		
No. of sporocarps[c]	148			74			172			394		
No. of fruiting species[c]	29			13			33			55		
Common OTUs[d]	12.7%			9.2%			19.0%			65.3%		
Below-/ aboveground similarity[e]	44.8%			46.2%			33.3%			27.3%		

±Standard deviations.

[a]At each site, percentages followed by different alphabets differ significantly ($P < 0.05$).

[b]Number of OTUs rarefied at $n = 59$ sequences for each site and $n = 198$ sequences for all sites, respectively.

[c]Collected in 2010 (year where above- and belowground samplings can be compared).

[d]Percentage of OTUs occurring on seedlings, intermediate trees, and large trees at each site.

[e]Percentage of OTUs found belowground corresponding to species forming sporocarps at each site.

FIGURE 1. Some EM sporocarps harvested under the three age categories of *Gilbertiodendron dewevrei*: (A) *Cantharellus congolensis* Beeli, (B) *Cantharellus rufopunctatus* var. *rufopunctatus* (Beeli) Heinem., (C) *Clavulina* sp.1, (D) Gomphaceae sp.1, (E) *Lactifluus pelliculatus* (Beeli) Buyck, (F) *Lactarius melanogalus* Heim., (G) *Lactifluus longipes* (Verbeken) Verbeken, (H) *Lactifluus sesemotani* (Beeli) Buyck, (I) *Pulveroboletus aberrans* Heinem. & Goss.-Font., (J) *Russula concolora* Buyck, (K) *Russula diffusa* Buyck, (L) *Russula* sp.4, (M) *Russula subfistulosa* var. *apsila* Buyck, (N) Thelephoraceae sp., (O) *Tylopilus* sp.3, (P) *Xerocomus virescens*.

/thelephora-tomentella (1), and tricholoma (1), and (Table S2). Species-accumulation curves reached an asymptote (Figs. S3 and S4A), indicating that the sporulating EM fungal diversity was well recovered at all sites.

Molecular Identification of EM Fungi on Root TIPS.—Over all sites, 49,888 EM root tips were observed from seedlings, intermediate trees, and large trees. The percentage of EM roots ranged from 83.4 to 94 percent, depending on age category (Table 1). Of the 828 EM root tips subsampled for DNA analysis, 629 (76%) were successfully sequenced and revealed 75 distinct OTUs (Table 1) from 11 different EM fungal lineages (Fig. S5). The 75 OTUs included 68 Basidiomycota (90.7% of the total abundance of OTUs) and 7 Ascomycota (9.3%, Fig. 3). Among basidiomycetes, /russula-lactarius-lactifluus contributed the most (40% of all OTUs), followed by /thelephora-tomentella (23%), /sebacina (12%), /clavulina (8%), /amanita (2.5%), /boletus (2.5%), and /cantharellus (2.5%). Among ascomycetes, the most highly represented lineages were /marcelleina-peziza gerardii (4%), /sordariales (2.5%), /elaphomyces (1.5%), and /helotiales (1.5%). The abundance of OTUs on root tips varied among sites (Fig. S6): among OTUs representing >5 percent of the total number of tips, Thelephoraceae 2 dominated S1 (7.3%), *Russula* sp.5, Thelephoraceae 1 and Thelephoraceae 2 dominated S2 (10.5%, 10.5%, and 6.5%, respectively), and *Russula* sp.16, Russulaceae 11, *Russula* sp.13, and Sebacinaceae 2 dominated S3 (8%, 6.5%, 5%, and 5%, respectively) (Figs. S6 and 3). Overall, 15 OTUs (20%) matched species found as sporocarps: nine species from /russula-lactarius-lactifluus, three from /clavulina, one from /amanita, /boletus, and /thelephora-tomentella. The similarity between belowground and aboveground EM fungal taxa at each site ranged from 33.3 to 46.2 percent (Table 1). When considering OTUs from all sites and all samples, the species-accumulation curves reached an asymptote, suggesting that the EM fungal community was exhaustively sampled at this scale (Fig. S4A). We detected a total of 119 EM fungal taxa from EM root tips and sporocarps (Table S4).

Ectomycorrhizal Distribution Among Studied Sites.—The number of OTUs detected on root tips was 71, 65, and 63 at S1, S2, and S3, respectively (Table 1; Fig. S6). Species-accumulation curves indicate that the EM fungal community was not exhaustively sampled at each site and that there were no significant differences in the EM fungal species richness among sites (Fig. S7A). This contrasts with the accumulation curves for sporocarps, which suggest that although S1 and S3 had similar diversity, S2 had less than half diversity (Fig. S7B). Although the three sites shared 69.3 percent (52/75) of OTUs on roots, composition differed markedly among sites (df = 628, $F = 2.99$, $P = 0.001$) based on perMANOVA (Table 2). The NMDS

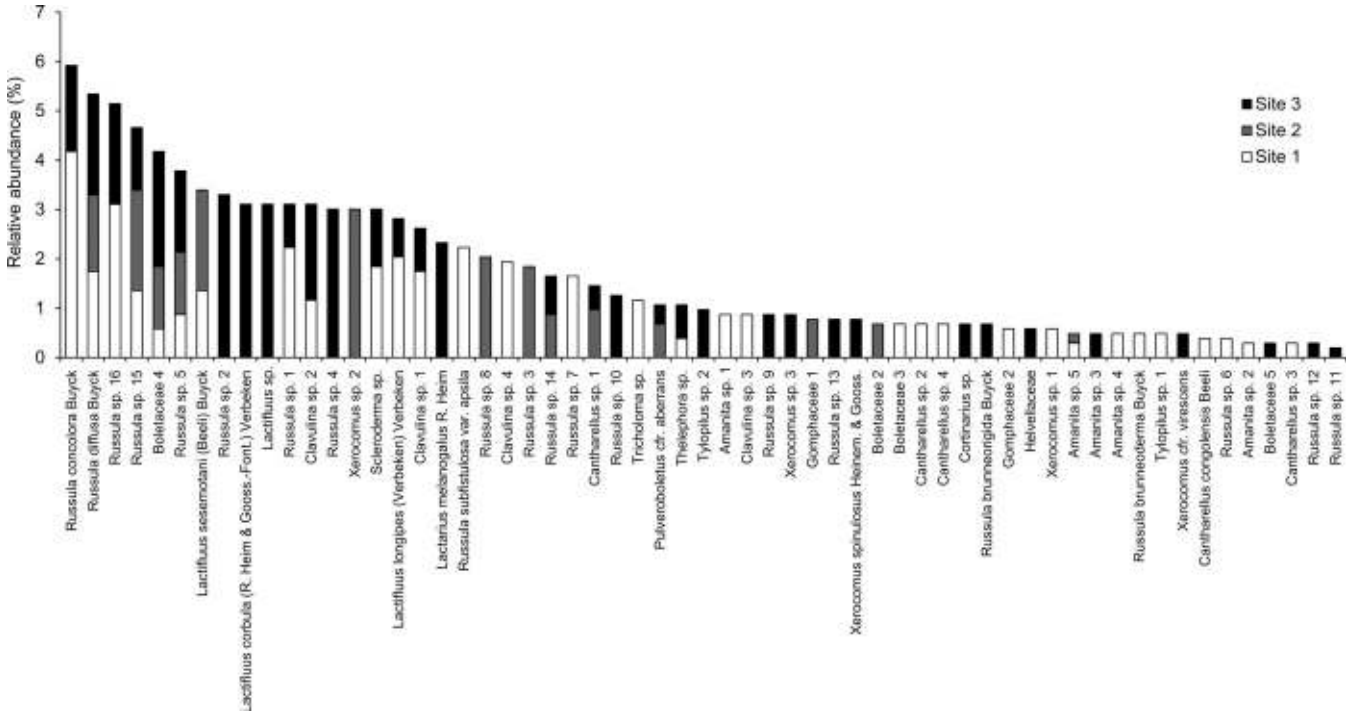

FIGURE 2. Abundance distribution of EM species found among sporocarps collected over 4 years at each site.

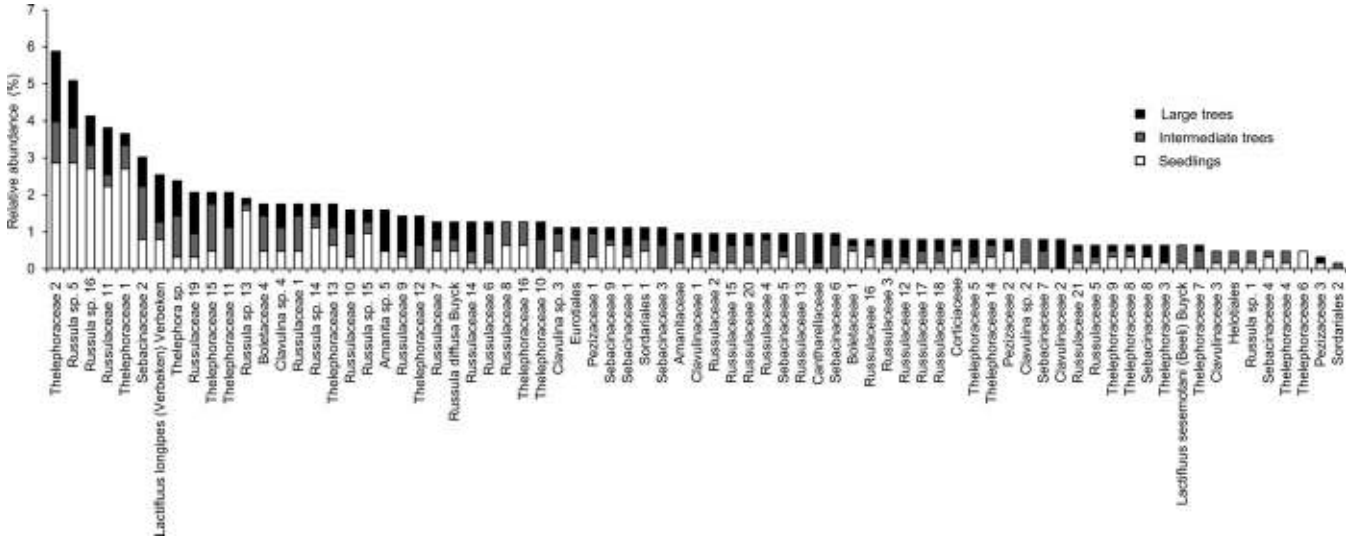

FIGURE 3. Abundance distribution of OTUs on EM root tips of *G. dewevrei* seedlings, intermediate trees, and large trees at all sites.

ordination (stress = 0.09) showed an incomplete separation of communities based on site (Fig. 4B).

Ectomycorrhizal Composition Between Large Trees, Intermediate Trees, and Seedlings.—The percentage of EM root tips was significantly higher for seedlings than for other age categories at all sites (except for large trees at S2, Table 1). Ectomycorrhizal fungal species-accumulation curves per age category did not reach an asymptote, suggesting that the EM fungal community was not exhaustively sampled in the three age categories of trees at each site (Fig. S7A), or even when pooling all sites (Fig. S4B). In all, we detected 65, 68, and 63 OTUs on seedlings, intermediate trees, and large trees, respectively (Table 1). The three age categories shared 49 OTUs, representing

65.3 percent of the diversity found on roots (Table 1; Fig. S8), and all fungal lineages detected were shared except/helotiales, which occurred only on seedlings and intermediate trees at all sites (Fig. S5). S3 had the highest proportion of OTUs shared between the three tree age categories (19%), followed by S1 (12.7%) and S2 (9.2%, Table 1). The most abundant OTUs, Thelephoraceae sp.2, *Russula* sp.5, *Russula* sp.16, and Russulaceae sp.11, were associated with the three age categories at all sites (Fig. 3). Our analyses of diversity (Table 1) revealed homogeneous patterns, with no difference among tree categories for Fisher's alpha (ANOVA: df = 55, $F = 0.97$, $P = 0.385$; Table 1) in accordance with the species-accumulation curves (Fig. S4B). However, there was a significant difference for Shannon diversity index (ANOVA: df = 55, $F = 4.002$, $P = 0.024$) among tree categories (intermediate tree: 1.83 ± 0.33; large tree: 1.69 ± 0.38; seedlings: 1.40 ± 0.67). Using pairwise *t*-test with *P*-value adjustment, the only significant difference in diversity was found for seedlings against intermediate tree (*t*-test: $t = 2.529$, $P = 0.022$). There is a weak but significant effect of the tree age categories (perMANOVA: df = 628, $F = 2.16$, $P < 0.001$) in the EM fungal community composition (Table 2) reflected in NMDS ordinations (Fig. 4A). Pairwise perMANOVA detected significant differences between seedlings and large trees (df = 407, $F = 2.28$, $P < 0.001$), as well as between seedlings and intermediate trees (df = 407, $F = 2.41$, $P < 0.001$), but not between large trees and intermediate trees (df = 407, $F = 1.40$, $P = 0.06$, Table 2).

TABLE 2. *Nonparametric perMANOVA on Bray-Curtis distance to test the effects of site and age category of* G. dewevrei *on the distribution of OTUs.*

	SS	MS	*F*	R^2	*P*
(A) Effect of site and tree category					
Site	2.883	1.44152	2.9904	0.00938	0.001***
Age category	2.089	1.04468	2.1672	0.00680	0.001***
Site × age category	3.968	0.99206	2.0580	0.01291	0.001***
(B) Pairwise comparison					
S1 vs. S2	2.080	1.03988	2.1315	0.00993	0.001***
S1 vs. S3	1.437	1.43740	2.9514	0.00688	0.001***
S2 vs. S3	1.022	1.02154	2.0895	0.00488	0.001***
(C) Pairwise comparison					
Seedlings vs. large trees	1.111	1.11090	2.2852	0.0056	0.001***
Large trees vs. intermediate trees	0.688	0.68799	1.4008	0.00344	0.063
Seedlings vs. intermediate trees	1.320	1.32043	2.4109	0.00663	0.001***

SS, sums of squares; MS, mean squares.
***$P < 0.001$.

^{13}C AND ^{15}N Natural Abundances.—At all three sites, the foliage of *G. dewevrei* and the AM species *P. macrophylla* was strongly depleted in ^{13}C (Figs. 5A–C) compared with EM sporocarps: the foliage and seeds of *G. dewevrei* were, on average, depleted by 9.3 per mille and 7.9 per mille, respectively, for δ^{13}C and by 8.5 per mille and 9.6 per mille, respectively, for δ^{15}N. The ^{13}C abundance increased with age for the *G. dewevrei* category at two sites (S1 and S2, Figs. 5A–C), whereas the ^{13}C abundance of foliage of the AM *P. macrophylla* was not different from that of large *G. dewevrei* trees (Figs. 5A–C). Foliage of *G. dewevrei* did not differ significantly as a function of age category in ^{15}N abundance and C/N (which did not differ between sites, data not shown) (Figs. 5D–E and S10) but had significantly higher values than those of *P. macrophylla*. Seeds had higher C/N and similar ^{15}N abundance compared with foliage for *G. dewevrei*. EM sporocarps

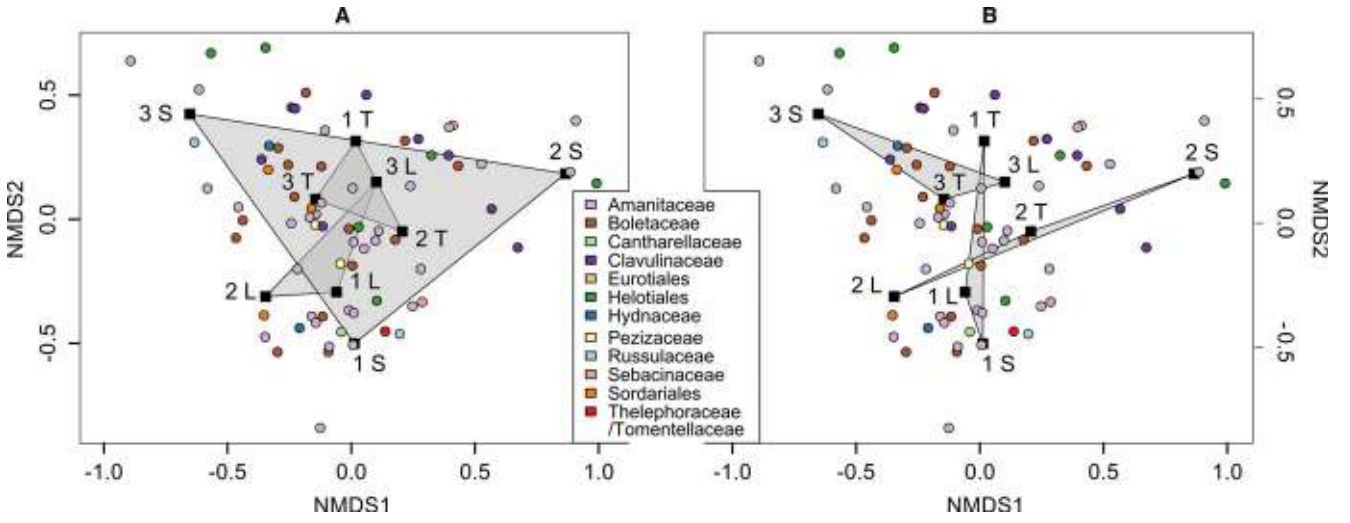

FIGURE 4. Nonmetric multidimensional scaling ordination (NMDS) of EM fungal community (stress = 0.09). The two figures represent the effect of age category of *G. dewevrei* (A) and site (B) on EM fungal community composition in seedlings (S), intermediate trees (T), and large trees (LT) at sites 1, 2, and 3. Colored points represent EM fungal taxa by family. Black squares represent communities.

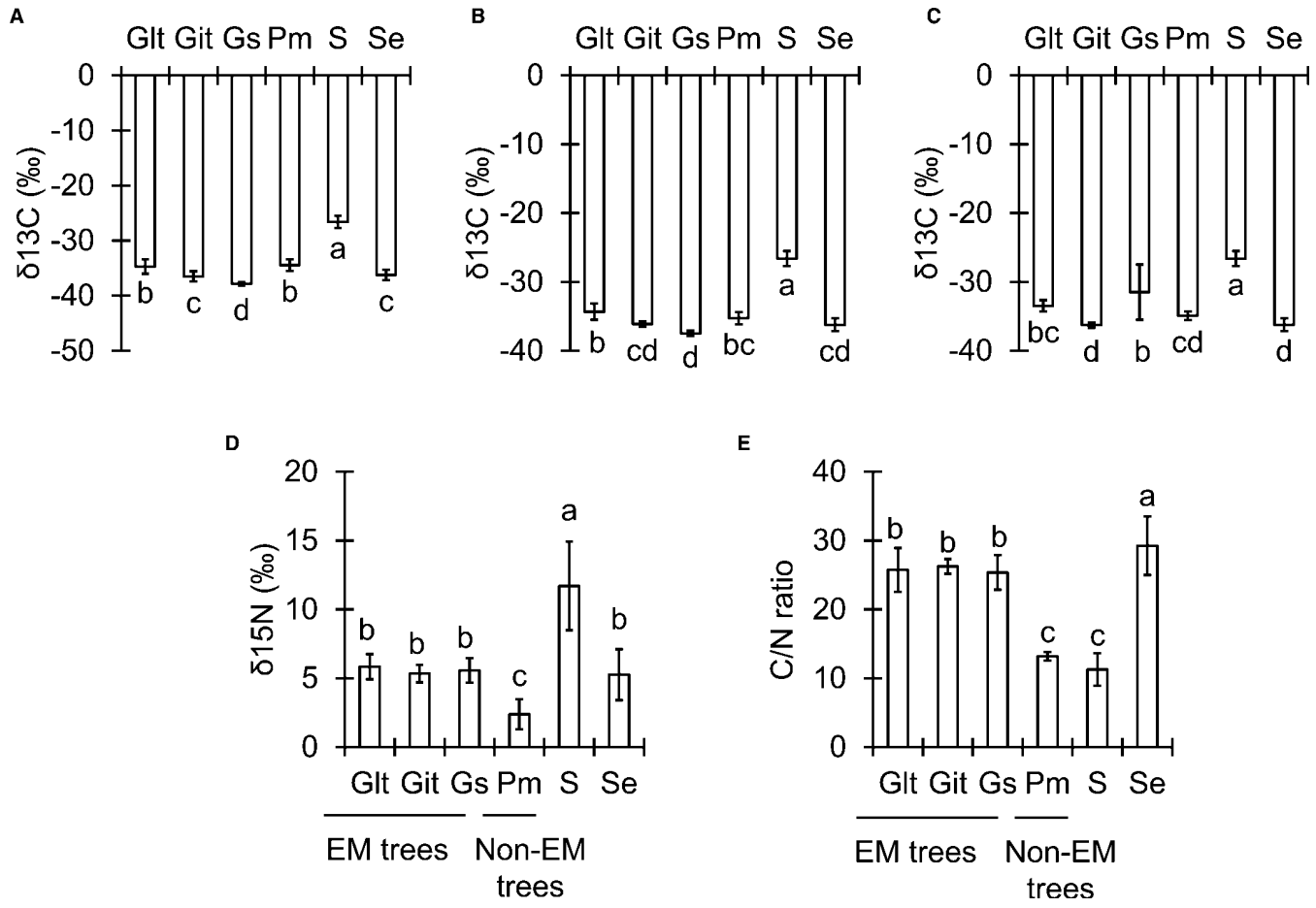

FIGURE 5. Mean values of $\delta^{13}C$ (‰) at site 1 (A), site 2 (B), and site 3 (C), and of $\delta^{15}N$ (D, ‰) and C/N (E) for the three sites pooled, since they did not differ significantly for any age category. Glt, Git, and Gs: *G. dewevrei* large trees, intermediate trees, and seedlings, respectively; Pm: AM *P. macrophylla* large trees; S: EM sporocarps. Se: seeds of *G. dewevrei*. Significant differences revealed by Newman-Keuls tests are indicated by different letters ($P < 0.05$).

had higher ^{15}N abundance and lower C/N than all plants (except *P. macrophylla* for C/N, Figs. 5D–E and S10).

DISCUSSION

At all three sites of the studied monodominant *G. dewevrei* forest, we detected fewer EM fungal taxa from sporocarps (59 species from 1030 sporocarps) than from EM root tips (75 OTUs from 828 barcoded EM root tips). EM fungal diversity on root tips differed significantly depending on tree age category and site. However, a core community was shared among the three investigated tree age categories (colonizing 65.3% of the barcoded ECMs) and among the three sites (69.3%). Shared fungi can form CMNs that link together *G. dewevrei* plants of different age categories growing in close vicinity, but we found no evidence for nutrient transfer from trees to seedlings based on the seedlings' natural isotopic abundances.

Limited EM Fungal Lineage Diversity but Large Specific Diversity.—The 119 EM fungal taxa identified here belong to only 16 EM phylogenetic lineages *sensu* Tedersoo *et al.* (2010a). This high species diversity from a limited number of EM fungal lineages is consistent with other tropical rain forests, and it complies with the view that tropical forests have a lower diversity of EM phylogenetic lineages than temperate forests (which usually display >20 lineages: Tedersoo & Nara 2010). For monodominant tropical EM forests, Smith *et al.* (2013) documented 11 EM fungal lineages in a Guyana forest dominated by the dipterocarp *Pakaraimaea dipterocarpaceae*, and Corrales *et al.* (2016a) found 13 EM lineages on *Oreomunnea mexicana* in Panama. For mixed tropical EM forests, Smith *et al.* (2011) found 17 lineages on three caesalpinioid species from Guyana; Tedersoo *et al.* (2011) found 18 lineages on 11 caesalpinioids and one Phyllanthaceae in Cameroon; Peay *et al.* (2010) identified 12 EM fungal lineages under dipterocarps in a Malaysian rain forest; and Diédhiou *et al.* (2010) identified seven EM fungal lineages on four caesalpinioids and one Phyllanthaceae in a Guinean rain forest.

While comparison between studies is difficult because of differences in sampling methodologies and failure to saturate species-accumulation curves, values of Fisher's alpha, a diversity

index that corrects for sampling intensity (Table 1), fall in the range of values reported for tropical forests (Corrales *et al.* 2016a): based on this index, the diversity is lower than in monodominant *Oreomunnea mexicana* (Fisher's alpha = 89.5) but higher than in *Coccoloba uvifera* forests (Fisher's alpha = 3.67 for seedlings and 3.32 for adult trees; Séne *et al.* 2015) or under *Pakaraimaea dipterocarpaceae* (Fisher's alpha = 19.8; Smith *et al.* 2013); yet they are in the range of mixed EM tropical forests, which is equally large (Fisher's alpha = 4–183; Corrales *et al.* 2016a). Thus, the lower host tree diversity does not necessarily translate into lower fungal diversity, and ranges of diversity are large in both mixed and monodominant forests. This finding contrasts with the view that EM diversity correlates with that of tree species (*e.g.*, Dickie 2007, Ishida *et al.* 2007, Tedersoo *et al.* 2008) and suggests that additional factors drive fungal diversity Richard *et al.* For example, host density (Table S1) influences EM fungal richness (Tedersoo *et al.* 2014), perhaps because a greater availability of roots provides more resources. Similarly, a recent origin of the EM symbiosis and/or stress conditions may reduce the fungal diversity (Séne *et al.* 2015). Finally, the lack of difference in richness of EM fungal taxa between the mixed and monodominant forests may, in part, be due to the dominance of generalist EM fungi (Onguene & Kuyper 2002, Richard *et al.* Diédhiou *et al.* 2010, Henry *et al.* 2015).

Although our sampling is globally saturated, many OTUs detected belowground were not found aboveground and vice versa. Sixty OTUs in all were found on roots only, while 44 (58.9% of sporocarps) were obtained from sporocarps exclusively. Only 15 OTUs (28.7% of roots tips and 41.1% of sporocarps) were detected from both approaches. Surveys of sporocarps may have overlooked inconspicuous sporocarps of the /thelephora-tomentella and /sebacina lineages (Horton & Bruns 2001), as well as hypogeous species if any were present. More unexpectedly, species well represented by sporocarps, such as those in the taxa /ramaria-gautieria, /tuber-helvella, /tricholoma, and /pisolithus-scleroderma, were not found on root tips, although they are known to form ECMs (Bâ *et al.* 2012): they may have a lower investment in EM formation than the other lineages, although we cannot exclude that their ECMs were deeper than the sampled soil. Similarly, belowground EM fungal species were sometimes absent aboveground, *e.g.* the lineages /sebacina, /marcelleina-peziza gerardii, /elaphomyces, and /sordariales. Some /russula-lactarius-lactifluus and /thelephora-tomentella species that were dominant on root systems were absent from the sporocarp survey, while the /boletus, /cantharellus, and /clavulina species dominant on sporocarps were less abundant on root systems. Similarly, the few species of ascomycetes fungi found from lineages /elaphomyces (1), /sordariales (2), and /helotiales (1) were present on root only. Interestingly, the lineages /elaphomyces currently emerge as an African clade requiring further research (Buyck *et al.* 2016). These discrepancies further confirm that although the /russula-lactarius-lactifluus and /thelephora-tomentella lineages clearly dominated this *G. dewevrei* monodominant forest, epigeous sporocarps are not perfect indicators of belowground richness (see also Gardes & Bruns 1996, Baptista *et al.* 2015). Moreover, sporocarps revealed >2× lower species diversity in S2 compared with S1 or S3, while OTU diversities on roots did not differ, the discrepancies have also been reported in other studies (*e.g.*, Baptista *et al.* 2015). A better view of the EM fungal diversity is achieved by combining sporocarp and ECM surveys, as concluded for other temperate and tropical ecosystems (Richard *et al.* 2005, Diédhiou *et al.* 2010, Séne *et al.* 2015).

The few species of ascomycetes detected may be due to their specific ecological requirements more than a methodological issue (see Tedersoo & Smith 2013). Previous studies in tropical ecosystems also found low diversity of EM ascomycetes (Peay *et al.* 2010, Smith *et al.* 2011, Tedersoo *et al.* 2011, Henry *et al.* 2015, 2016). Among the 16 discovered EM fungal lineages, /tuber-helvella (one OTU, GenBank accession number KR819045) had not previously been reported from an African tropical forest, to the best of our knowledge (see Tedersoo *et al.* 2007, 2010a, 2011, Jairus *et al.* 2011, Bâ *et al.* 2012). In addition to the Holarctic genus *Tuber* (Bonito *et al.* 2010), the /tuber-helvella lineage includes taxa distributed in the austral regions (Tedersoo & Nara 2010). Members of this lineage are probably poorly represented in the Paleotropic forests. There was also a noticeable absence of common Holarctic and Austral EM fungal lineages such as /cenococcum and /laccaria, as well as some panglobal EM fungal lineages (*e.g.*, /entoloma, /hebeloma-alnicola, and /hysterangium).

Similar Structure but Different Compositions of EM Fungal Communities among the Age Categories.—The composition of EM fungal communities differed as a function of the *G. dewevrei* age category, with communities on seedlings differing from those on older trees (Table 2). Soil properties were unlikely to drive between-sites differences (Supporting information, Table S3).

These results partly differ from those obtained by Corrales *et al.* (2016a) for *Oreomunnea mexicana*, the only other example of monodominant forests investigated for age effect in which the EM fungal communities of the three age categories did not differ. Moreover, Corrales *et al.* (2016b) did not find evidence for CMN in *O. mexicana* based on mesh exclosure experiments and isotopes analyses. Variation in community composition among sites is consistent with high spatial turnover of tropical EM fungal communities (even at the kilometer scale: Smith *et al.* 2011), a patchiness also known from temperate ecosystems (Richard *et al.* 2005).

Even so, 19 of the 49 OTUs that occurred in more than one age category were most abundant on root tips for all three age categories (38.7% of all sequences). The 30 remaining OTUs were rare (28.1%). This suggests that the most abundant partners of nearby conspecific adult trees may be a source of inoculum for seedlings and potentially for CMN, although physical links are not directly proven here. Similar observations were made in multi-aged stands of tropical rain forests. For instance, adults and seedlings of monodominant *Coccoloba uvifera* forests share three EM fungal taxa representing 80 percent of the EM colonization (Séne *et al.* 2015). In mixed forests of Madagascar, 88 percent of ECMs from adults are formed by EM fungal taxa also found on seedlings, but they encompass less than a half of the taxonomic diversity (Henry *et al.* 2015). In a mixed tropical rain forest in

Guinea, EM fungi shared by adults and seedlings represent 79 percent of the EM colonization (Diédhiou *et al.* 2010). It is difficult to determine whether this simply represents a sampling bias (with common species more likely to reveal a link to different age categories) or whether a multi-age strategy is linked to commonness in EM fungal species. The EM fungal taxa richness (based on jackknife 1 and Fisher's alpha) was not significantly different for the three tree categories (Table 1), consistently with observations on *Oreomunnea mexicana* (Corrales *et al.* 2016a).

No Evidence for Nutrient Transfer from Trees to Seedlings.—Plants from temperate regions that rely on CMNs are enriched in ^{13}C, ^{15}N, and total N (Selosse & Roy 2009, Selosse *et al.* 2016), reflecting values from the biomass of EM fungi. The values observed here for EM fungi were concordant with this pattern, but we detected no ^{15}N and total N deviation in understory seedlings versus adult plants (Fig. 5D). For ^{13}C, seedlings were even slightly depleted compared with adult plants, which is the opposite of what could be predicted based on carbon transfer. Since the main factors likely to influence ^{13}C abundance were controlled (distance to soil to avoid different levels of input of CO_2 from soil respiration; light level to avoid different rates of photosynthesis), this trend was rather unexpected. We consider two possible explanations. First, the result may be affected by carbon from the seed reserves, which are depleted in ^{13}C (Cernusak *et al.* 2009): this carbon source may have been used to build cell wall polymers earlier in plant development. A more sensitive analysis of recently synthesized soluble circulating sugars may reveal some isotopic differences (Hynson *et al.* 2012). Second, the carbon issuing from photosynthesis is likely depleted in ^{13}C for two reasons. Seedlings are generally more shaded than adult plants, so that systemically circulating carbon may be more ^{13}C depleted (a lower photosynthetic rate entails a better fractionation against ^{13}C; Farquhar *et al.* 1989). Moreover, smaller trees are closer to the soil and experience a less elevated $^{13}CO_2$ abundance than higher trees due to the surrounding soil and plant respiration, combined with poor ventilation in the understory (Lüttge 2008). The same reasons could apply for the lower ^{13}C abundance in intermediate trees compared with large trees, because they also occupy a lower position in the canopy.

These results and values are consistent with those reported by Diédhiou *et al.* (2010), who found no isotopic evidence for carbon transfer between seedlings and adults of several EM trees species, including EM caesalpinioid legumes, in mixed rain forest from a South Guinea forest. We do not fully reject the possibility of carbon transfer that does not follow the same physiological pathway as that reported in other CMNs formed by EM fungi, and thus has different isotopic particularities and fractionations. Moreover, the tendency of seedlings to have a lower ^{13}C abundance, as discussed above, may even offset any marginal contribution of fungal C to their biomass. On one hand, we failed to find evidence that a nursery effect of CMNs by way of C transfer could contribute to monodominance; on the other hand, the fact that seedlings could connect to CMNs that are likely pre-established at the expense of older trees may be relevant in terms of carbon budget. This may indeed explain the observed high EM rate of the seedlings, which is higher than for trees (Table S1).

Our study highlights the high specific diversity, but poor lineage diversity, of EM fungal communities associated with roots of *G. dewevrei* in monodominant stands. Although EM fungal communities varied between growth stages of *G. dewevrei*, the three age categories had partially overlapping EM fungal communities, and potentially formed CMNs between adults and seedlings. Nevertheless, there was no evidence of carbon transfer from adults to seedlings. CMNs and their impact on the nutrition, growth, and fitness of regenerating seedlings should be further investigated experimentally in *G. dewevrei* monodominant forests.

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SUPPORTING INFORMATION

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

TABLE S1. Mean number of stems of *G. dewevrei* seedlings, intermediate, and large trees (as defined in main text) in each site.

TABLE S2. Number of EM fungal species per EM fungal lineage from sporocarps in each year and all sites.

TABLE S3. Soil physical and chemical properties of the three studied sites (depth 0–30 cm, $n = 3$, measurement per site).

TABLE S4. Ectomycorrhizal fungal taxa recovered from sporocarps and root tips of the three age categories of *G. dewevrei* in the three sites.

FIGURE S1. Location of studied sites in southeast Cameroon.

FIGURE S2. Distribution of EM fungal lineages from sporocarps at each site over 4 years.

FIGURE S3. Species-accumulation curves of sporocarps collected at each year and at all sites.

FIGURE S4. Species-accumulation curves with ECMs and sporocarp sampling effort at all sites (A), and on EM root tips of the three age categories of *G. dewevrei* at all sites (B) in 2010.

FIGURE S5. Distribution of EM fungal lineages on root tips at each site (A) and on roots tips of seedlings, intermediate trees, and large trees at all sites (B).

FIGURE S6. Distribution of OTUs on EM root tips of seedlings, intermediate trees, and large trees of *G. dewevrei* at site 1 (A), site 2 (B), and site 3 (C).

FIGURE S7. Species-accumulation curves of OTUs on EM root tips of seedlings, intermediate trees, and large trees (A) and sporocarps (B) at each site in 2010.

FIGURE S8. Venn diagram showing the number of OTUs shared by seedlings, intermediate trees, and large trees of *G. dewevrei*.

FIGURE S9. Rarefaction curves of the percentage of OTUs shared by the three age categories (seedlings, intermediate, and large trees).

FIGURE S10. Mean values of $\delta^{15}N$ (‰) and C/N at site S1 (A, D), S2 (B, E), and S3 (C, F).

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