


# Diversity and Structure of Fungal Communities in Neotropical Rainforest Soils: The Effect of Host Recurrence

Heidy Schimann<sup>1</sup>  · Cyrille Bach<sup>2</sup> · Juliette Lengelle<sup>2</sup> · Eliane Louisanna<sup>1</sup> · Sandra Barantal<sup>3</sup> · Claude Murat<sup>2</sup> · Marc Buée<sup>2</sup>

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**Abstract** The patterns of the distribution of fungal species and their potential interactions with trees remain understudied in Neotropical rainforests, which harbor more than 16,000 tree species, mostly dominated by endomycorrhizal trees. Our hypothesis was that tree species shape the non-mycorrhizal fungal assemblages in soil and litter and that the diversity of fungal communities in these two compartments is partly dependent on the coverage of trees in the Neotropical rainforest. In French Guiana, a long-term plantation and a natural forest were selected to test this hypothesis. Fungal ITS1 regions were sequenced from soil and litter samples from within the vicinity of tree species. A broad range of fungal taxa was found, with 42 orders and 14 classes. Significant spatial heterogeneity in the fungal communities was found without strong variation in the species richness and evenness among the tree plots. However, tree species shaped the fungal assemblages in the soil and litter, explaining up to 18 % of the variation among the communities in the natural forest. These results demonstrate that vegetation cover has an important

effect on the structure of fungal assemblages inhabiting the soil and litter in Amazonian forests, illustrating the relative impact of deterministic processes on fungal community structures in these highly diverse ecosystems.

**Keywords** Fungal communities · Amazonian forest · Host recurrence · Soil · Litter · Second-generation sequencing

## Introduction

Fungi are critical components of soils in forest ecosystems where they play fundamental roles as decomposers, mutualists, or pathogens, partially driving plant nutrition and carbon cycling in these ecosystems. Fungi comprise some 100,000 described species [1], but the processes that shape the assemblages of fungal communities remain poorly understood. This lack of understanding is especially true in Neotropical rainforests where the diversity and distribution of fungal communities remain poorly described. Recent studies have reported that the distributions of fungi and their sensitivities to biotic and abiotic parameters are various, depending their ecological status [1–3]. If tree species shape endophytic fungal communities in tropical, boreal, and temperate forests [4–6], the major determinants of the composition of the ectomycorrhizal (ECM) fungal community are soil chemistry [7], dispersal ability [8], and host plant composition [9]. In the same vein, the composition of arbuscular mycorrhizal communities may differ depending on habitat type, host species, or soil chemistry [10], even though some authors have recently demonstrated that at global scale, more than 90 % of AM fungi are found on multiple continents [11]. Because mycorrhizal fungi are biotrophic organisms, their host preferences make intuitive sense, and numerous studies have demonstrated that host trees are important factors in shaping the fungal community

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✉ Heidy Schimann  
heidy.schimann@ecofog.gf

<sup>1</sup> INRA, UMR Ecology of Guiana Forests (AgroParisTech, CNRS, CIRAD, Université des Antilles, Université de Guyane), 97310 Kourou, France

<sup>2</sup> INRA, UMR Interactions Arbres-Microorganismes, Université de Lorraine, 54280 Champenoux, France

<sup>3</sup> Equipe Environnement et Microbiologie, Institut Pluridisciplinaire de Recherche en Environnement et Matériaux, Université de Pau et Pays de l'Adour, 64000 Pau, France

assemblage of this ecological guild [12–14]. Although communities of saprophytic soil fungi have received less attention, some studies have highlighted the importance of the soil horizon [15], litter quality [16], or spatial zonation around a tree [17] as environmental parameters shaping the fungal communities in soils. However, very few studies have investigated the effects of tree species on the community structure of free soil fungi and saprophytes, which inherently do not present ecological traits related to biotic interaction, in Neotropical rainforests [18]. In Peay et al. [18], links between trees and soil fungi richness were established in a tropical forest in the western Amazon basin. Nevertheless, the authors showed that these relationships depend on the fungal lineages with a stronger relationship with other lineages that are known for their biotrophic strategies and direct plant–fungal interactions, such as pathogens or mycorrhizae, masking the potential effect on soil saprotrophic fungi.

When studying free soil fungi and their interactions with plants, it seems appropriate to refer to host recurrence as defined by Zhou et al. [19]: “the frequent or predominant occurrence of a symbiotic, parasitic or saprobic fungus on a particular host or range of hosts”. Indeed, fungal host preference is also a non-random structuration of communities of potential host species and suggests that fungi derive their nutrition from a live plant host during some phase of their life cycle [19]. Some studies have already revealed the occurrence of host preference, or recurrence, in endophytic [5], ligninolytic [20], arbuscular mycorrhizal (AM) [21], ECM [14], and pathogenic [22] fungal species in natural forests.

In tropical ecosystems, which harbor great tree diversity, the levels of interactions among plants and fungi are still poorly understood, particularly in comparison to the knowledge that has been accumulated in boreal and temperate forests. In addition, in Neotropical rainforests, the study of these potential interactions is difficult because lowland Amazonia could harbor up to 16,000 tree species [23]. Thus, as potential host diversity increases in tropical ecosystems, the opportunities for specialization may decrease due to the limited successful colonization by fungi because potential host trees become rare. As an example, Cannon [24] suggested that the degree of host preference was low for endophytic fungal communities isolated from the leaves of 12 tree species in Guyana. On the other hand, despite the high levels of diversity and spatial distribution of potential hosts, fungi may display tree host-recurrence, as has already been reported [25], even for some wood-inhabiting fungi [20, 26]. Moreover, the study of the distribution of saprophytic microfungi in leaf litter, which were observed in an Australian tropical rainforest, confirmed the hypothesis that microfungal assemblages are strongly influenced by host phylogeny [27].

In this study, we characterized the fungal communities occupying the soils and litters in the vicinity of selected tree species in a Neotropical rainforest. Using monospecific plots

at an experimental tree plantation, we specifically investigated the effect of tree cover on fungal communities in comparison to a stochastic distribution. Focusing on non-biotrophic fungi, the main hypothesis was that modifications to environmental and soil properties induced by tree species (e.g., the chemical composition of the litter and top soil) primarily explained the non-biotrophic fungal community structure. We first described the fungal communities in six different tropical tree plantations located in French Guiana; then, we coupled this approach to sampling from a nearby natural rainforest where sufficient representatives of four of these six tree species have been found. We then used 454 pyrosequencing to (i) characterize local-scale diversity and variation in the species composition of these fungal communities and (ii) investigate whether this variation depends on the tree species.

## Materials and Methods

### Ethics Statement

Field sampling was permitted by the CIRAD ([www.cirad.fr](http://www.cirad.fr)), and no protected species was sampled.

### Study Sites

To test the hypotheses related to the effects of tree species and host recurrence on the structure and composition of fungal communities, we selected two different sites: a monospecific tree species plantation and a nearby natural forest plot characterized by the same climatic and soil conditions.

Both sites were located at the Paracou experimental station in French Guiana (5° 18' N, 52° 53' W). The climate, which is mainly driven by the north/south movement of the intertropical convergence zone, is characterized by a long-term average annual temperature of 25.7 °C (1971–2001) and an average annual precipitation of 3041 mm (1971–2001). The temperature varies little over the course of the year (<2 °C), but variations in precipitation are substantial with less rainfall (<100 mm per month) during two drier periods that occur in March and from mid-August to mid-November [28, 29]. The soils are nutrient-poor acrisols developed on a Precambrian metamorphic formation called the Bonidoro series and composed of schist sandstone. The first sampling site is an experimental plantation established by the CIRAD in 1983, which consists of 24 monospecific forest tree plots (21 × 21 m) planted with 3-year-old seedlings of tree species selected for their timber value and their natural abundance (Supporting Material (SM), Figure S1). In comparison to natural forest, the monospecific plots of the plantation offered the advantage of having leaf litter with homogeneous chemical characteristics within each plot (SM, Table S1). The soils in the tree plots were relatively similar (SM, Table S2, [30]). Monospecific

plots of six tree species were selected to conduct this study: *Carapa surinamensis* FAW Miquel (Sapindales: Meliaceae), *Goupia glabra* JBCF Aublet (Malpighiales: Goupiaceae), *Hymenaea courbaril* C Linnaeus (Fabales: Fabaceae), *Platonia insignis* KFP von Martius (Malpighiales: Clusiaceae), *Simarouba amara* JBCF Aublet (Sapindales: Simaroubaceae), *Vochysia tomentosa* AP De Candolle (Myrtales: Vochysiaceae). All tree species are heliophilic or hemi-tolerant species of the upper story and are relatively abundant in the tropical rainforest of French Guiana [30]. These six species were selected because they are relatively taxonomically distant (different families) and their leaf litters have contrasting chemical characteristics [31]. Moreover, these plants form AM associations and are representatives of Guianan tropical forests [32]. The second sampling site consisted of a permanent plot of natural forest that has been followed over time to assess the dynamics of its tree communities and is located close to the plantation (plot P16 at the Paracou experimental station, SM Figure S2). This lowland tropical forest is typical of Guiana Shield forests, with Leguminosae, Chrysobalanaceae, Lecythidaceae, and Burseraceae as the dominant tree families [23]. At this site, annual inventories of mortality, circumference, and taxonomic identification have been conducted for all trees  $\geq 10$  cm DBH in 6.25-ha plots since 1984 [29].

### Sampling Strategy

All sampling was conducted in April 2009 at the beginning of the rainy season. At the plantation, the monospecific tree plots were  $21 \times 21$  m in size and were separated by 20 to 120 m (SM, Figure S1). At six monospecific tree plots, three litter samples together with the underlying soil were randomly collected at locations that were at least 5 m apart, resulting in a total of 36 samples (6 plots  $\times$  3 replicates per plot  $\times$  2 matrices = 36 samples). The samples were collected manually using a stainless steel cylinder (diameter 0.15 m) to a depth of 0.10 m. We carefully removed the fine roots from the soil samples. We also carefully separated the litter from the soil in the field and stored the samples in separate plastic bags, which were then rapidly frozen at  $-20$  °C before DNA extraction. At the P16 plot of the nearby natural forest (SM, Figure S2), we selected five trees of only four species (*C. surinamensis*, *G. glabra*, *P. insignis*, and *V. tomentosa*). These individuals were randomly selected across the 25 ha of the P16 plot, avoiding areas of swamp forest. We did not manage to find enough individuals of the two remaining species in this plot. The individuals with the greatest trunk girths were selected to provide the widest crown coverage. The means of the girths were 74, 122, 104, and 42 cm for *C. surinamensis*, *G. glabra*, *P. insignis*, and *V. tomentosa*, respectively. Beneath each tree, one composite sample (corresponding to three subsamples taken within 1 m from the trunk

of each individual tree) of both litter and soil (two matrices) was collected, resulting in a total of 40 samples (4 tree species  $\times$  5 spatial replicates  $\times$  2 matrices = 40 composite samples).

### DNA Extraction and PCR Amplicon Pyrosequencing

The procedures for DNA extraction and fungal ITS pyrosequencing were adapted from Coince et al. [33], and the detailed protocol for the extraction, amplification, and sequencing are provided in the Supporting Material. The extraction of genomic DNA was carried out using 500 mg of homogeneous soil or 500 mg of ground litter per sample using the Fast DNA Spin Kit for Soil (MP Biomedicals) following the manufacturer's instructions.

The fungal ITS1 region [34] was amplified from 76 DNA samples with the ITS1F and ITS2 primers [32] because the applied primer pair shows good specificity for the detection of Dikarya [28]. In total, we obtained two independent sets of libraries: 36 and 40 libraries corresponding to the samples from the plantation and the P16 natural forest plot, respectively. Beckman Coulter Genomics (Danvers, MA, US) performed the 454 pyrosequencing. The raw data were deposited on the Sequence Read Archive website (<http://www.ncbi.nlm.nih.gov/sra>) under the BioProject identifier PRJNA270839.

### Bioinformatics Analysis

The steps of the bioinformatics analysis are detailed in the Supporting Material. Briefly, the trimming and denoising of reads were done using a pipeline of analyses with Mothur (version 1.22.2; [29] and Usearch version 7.0.1001; [35]). The clusterization of operational taxonomic units (OTUs) was done using abundance-sorted sequences with the `cluster_otus` command of Usearch with a 97 % similarity threshold. The taxonomic assignment of a consensus sequence generated by UPARSE was performed against the curated UNITE database release 5.0 [36] using the Basic Local Alignment Search Tool algorithm, Blastn version 2.2.23 [32]. Unassigned OTUs were considered unknown ("no hits found" in Table S3, SM). Using the literature [2], we were able to classify 16.5 % of the OTUs into different trophic status and lifestyle categories (Table S2).

### Statistical Analyses

We removed unassigned OTUs from the main dataset and also conducted all statistical analyses with the complete dataset. The results are presented in the Supplementary Material (SM, Table S4 and Figure S3). Contingency tables containing the abundance of OTUs in each sample rarefied with the minimum sample size ( $n = 3189$  for P16 natural forest,  $n = 877$  for the plantation) were used, and all statistical analyses were

performed with R v 3.2.3 (R project for Statistical Computing, R Core Team, 2004–2013) and the package *vegan* [37].

Because ITS sequences seem to be particularly prone to 454 sequencing errors [38] and because diversity indices based on counts can be overestimated [39], the analyses were performed only using OTUs with the abundance greater than two; OTUs representing one pyrosequencing sequence were removed from the datasets. The suitability of the sequencing depth and sampling effort was tested by computing species accumulation and rarefaction curves using the function *specaccum* of the package *vegan*.

For the community analyses, we computed randomly rarefied communities with the minimum sample size of the datasets for each sample ( $n = 877$ ) using the subsample command in *Mothur*. The richness of OTUs was compared by calculating rarefaction curves for each site. We included all OTUs that were assigned (an accession number in GENBANK) and at least with two reads in the analyses. The differences in the richness of rarefied communities at the plantation and the P16 plot in the natural forest were analyzed through a Kruskal–Wallis rank sum test. Similarly, Simpson's index was computed to evaluate the average evenness of the rarefied fungal communities under each tree species in the soil and litter from the plantation and the P16 plot of the natural forest, and these values were compared using a Kruskal–Wallis rank sum test.

The variation in the fungal community composition was analyzed as follows: pairwise dissimilarity/distance matrices were computed using the Morisita–Horn distance, an abundance-based index that is not strongly sensitive to species richness and sample sizes [40], after the transformation of the data with the Hellinger distance to down-weight the effect of rare species [41]. The dissimilarity in the fungal communities was visualized using non-metric multidimensional scaling (NMDS) graphs using the *metaMDS* function (package *vegan*). The spatial autocorrelation among the fungal communities in the samples was analyzed using the Mantel test with the function *mantel* (package *vegan*). The effects of habitat (soil or litter), tree species, geographical distances among the samples, and their interactions on the composition of the fungal communities were tested with permutational multivariate analysis of variance (PERMANOVA).  $F$  statistics, partial  $R$ , and  $p$  values were based on 999 permutations. Spatial factors were included as follows: we generated a principal coordinates of neighborhood matrix (PCNM, function *pcnm*, package *vegan*) to transform the spatial distances to rectangular data and kept the first vector as a variable for the PERMANOVA.

We tested for correlations between soil or litter characteristics and the corresponding fungal community composition as follows and only for the plantation data: we took advantage of existing soil and litter data for the plantation (SM, Table S1 and Table S2). We used soil data (C, N, and P contents, pH, CEC, and clay content) from Bréchet et al. [30] or litter data (C/N,

C/P, N/P, lignin, cellulose, hemicellulose, and holocellulose contents and resorption time) from Hättenschwiler et al. [31]. We first performed a principal component analysis on the soil or litter data and extracted the coordinates of the first axis. Second, we performed a Mantel test between the dissimilarity matrix of the fungal communities and the first axis coordinates for the soil/litter data. We conducted this analysis both for litter fungal communities and litter chemical data and for soil fungal communities and soil physico-chemical data.

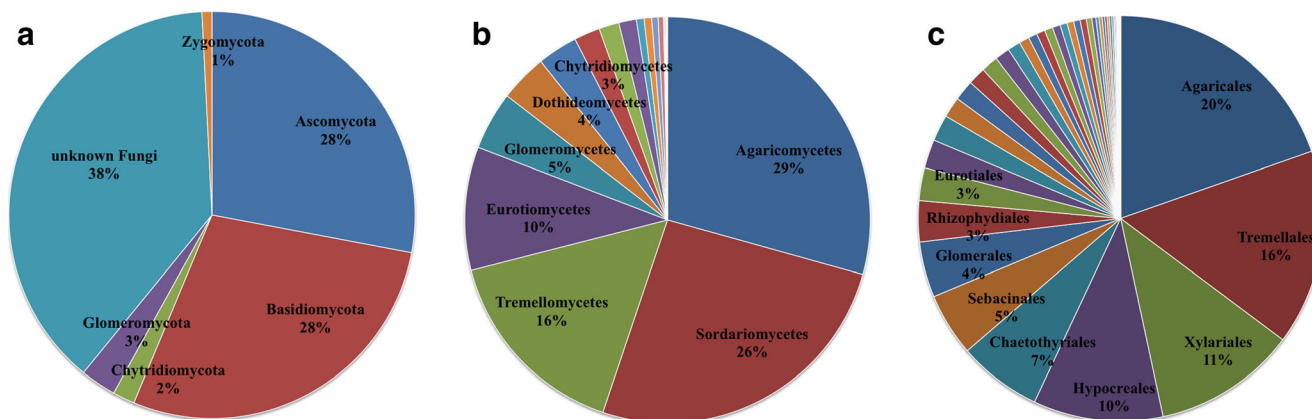
## Results

### Fungal Taxonomic Diversity

The majority of sequences (67 %) were assigned to fungi, and we found no correspondence in the UNITE database for 33 % of the reads (no hits found, SM, Table S3). We also confirmed that these reads were not assigned in GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>). Among the 67 % assigned reads, 38 % were not assigned to the phylum level and were considered to be unknown fungi (Fig. 1a). For the samples from the plantation, we were able to identify 42 orders and 14 classes, with 28 % of the sequences corresponding to Ascomycota, 28 % to Basidiomycota, 3 % to Glomeromycota, 2 % to Chytridiomycota, and 1 % to Zygomycota. At the class level, 26 % of the sequences corresponded to Sordariomycetes, 29 % of Agaricomycetes, and 16 % of Tremellomycetes for the most abundant (Fig. 1b). The most abundant orders were the Agaricales (20 %), the Tremellales (16 %), the Xylariales (11 %), the Hypocreales (10 %), the Chaetothyriales (7 %), the Sebaciales (5 %), the Glomerales (4 %), and the Eurotiales (3 %; Fig. 1c). Similar proportions of the major phyla and classes appeared in the P16 plot of the natural forest site (SM Figure S4 a, b, c) with slight differences in the proportions of the most abundant orders: 26 % Agaricales, 17 % Hypocreales, 9 % Tremellales, and 8 % Polyporales and Xylariales.

At the plantation site, we found 352 unknown fungal OTUs (approximately 20 % of all OTUs), and 16 of these were supported by more than 1000 reads (SM, Table S3). The next most abundant OTUs in the soil and litter at the plantation were three OTUs that were only assigned to the phylum level and were classified as Fungi in the UNITE database, followed by *Cryptococcus* (Tremellaceae: Agaricomycotina), a common filamentous fungal genus in soil [42]. In the P16 plot in the natural forest, the two first OTUs were unknown, followed by another *Cryptococcus*, and then an unknown fungal OTU (SM, Table S2). Based on the literature [2], some of the assigned OTUs have been classified to different ecological guilds (Table S3). At both sites, approximately 16.5 % of the OTUs were assigned to the genus level with a corresponding ecological status. Among them, only 2 % could be ECM





**Fig. 1** A broad range of fungal taxa was found at the plantation. The proportion of reads for which we found a hit for the plantation (of a total of 125,372 reads) at the kingdom (a), class (b), and order (c) levels, calculated from the 67 % of reads for which we could assign a hit in the databases

fungi, suggesting that this category of fungi is most likely not associated with the six selected host trees. Because the ITS primer pair targeted the Dikarya subkingdom, we obtained a very limited description of the AM fungal assemblage. Nevertheless, we identified five AM fungal species specifically associated with different tree species (SM, Table S3).

The total number of OTUs in the P16 plot in the natural forest was higher than that found at the plantation, with 1921 and 1148 OTUs, respectively, indicating a higher species richness in the natural forest than the plantation, but neither curve reached an asymptote (SM, Figure S5). However, the OTU richness per tree species varied between 48 and 74 OTUs (for *Hymenaea* and *Goupia*, respectively) at the plantation and between 74 and 92 OTUs (for *Platonia* and *Vochysia*, respectively) at the P16 plot in the natural forest, but these differences were not significant (Kruskal–Wallis rank sum test,  $p$  value = 0.42). At both sites, the evenness of the fungal communities was relatively similar in the litter and soil (Fig. 2a, b), and there was no significant difference (Kruskal–Wallis rank sum test,  $p$  value = 0.72). In addition, no significant differences in evenness were found among the six tree species at the plantation or among the four selected species within the P16 plot in the natural forest, with Simpson's index values ranging from 0.70 for *C. surinamensis* and 0.93 for *P. insignis* (Fig. 2c, d).

### Effects of Tree Species on Fungal Community Structure at the Plantation and the P16 Plot in the Natural Forest

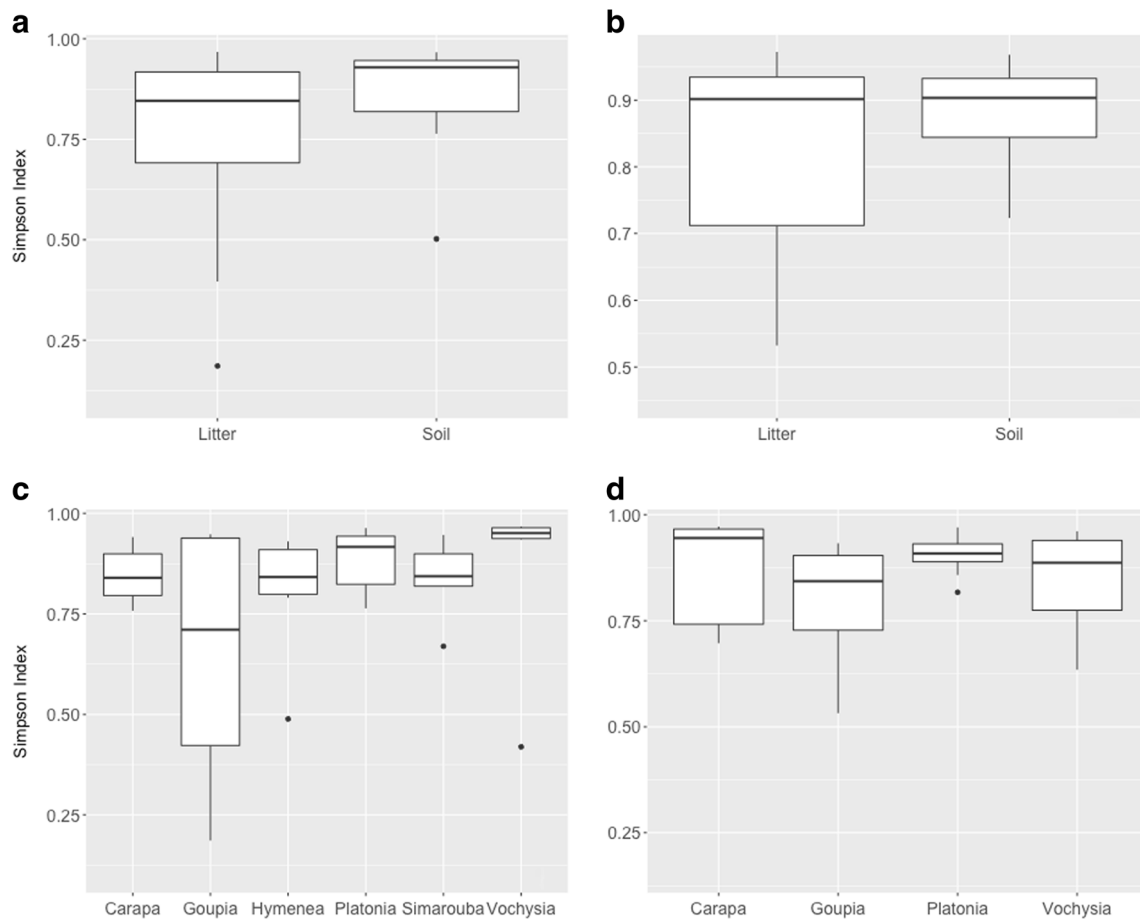
At the plantation, the Morisita–Horn indices were high, with an average value of 0.93 (min = 0.48, max = 1), suggesting large variation in the OTUs among samples. Indeed, among the most abundant OTUs (SM, Table S3), a majority were hyperdominant in only one tree monoculture, suggesting a strong association with the corresponding host tree; for example, 87 % of the reads for OTU-4, which was assigned as *Cryptococcus podzolicus* (AF444321), came from the *G. glabra* and *C. surinamensis* monocultures; *Crinipellis setipes*

was only present in the *H. courbaril* monoculture; and 90 % of the reads of OTU-10, which was assigned as *Pestalotiopsis virgulata* (AY687879), came from the *P. insignis* and *G. glabra* monocultures (Fig. 3a, c). In the P16 natural forest plot, the pattern was similar for these two OTUs: 70 % of the reads assigned as *C. podzolicus* (AF444321) were found under *C. surinamensis* and *G. glabra* and 93 % of the reads assigned as *P. virgulata* (AY687879) were found under *C. surinamensis* and *P. insignis* (Fig. 3b, d).

For the plantation, the NMDS ordination showed clustering of samples by tree species for both the litter and soil (Fig. 1b, c) and to a lesser extent by type of sample—soil or litter (Fig. 1a).

The PERMANOVA showed a low but significant clustering of samples by type of sample—soil or litter (Table 1), but with only 6.4 % of the variance explained: tree species explained 32 % of the variance and the geographical position of the trees explained only 4.1 % of the variance (Table 1). The interactions between factors explained 35 % of the variation, but these were not significant except for the interaction between habitat type and tree species, which resulted in 78 % of the total variation being explained by these three factors. The samples were positively autocorrelated up to 20 m as shown by the Mantel correlogram (Mantel's  $r = 0.34$ ,  $p = 0.01$ , SM Figure S6a). Because there is no true spatial replication at the experimental plantation, we tested the effects of these different variables using the natural forest data, particularly to confirm the impact of tree species.

The dissimilarity in the fungal communities in the soil and litter beneath the trees in the P16 plot in the natural forest (Morisita–Horn indices) showed high values, which ranged from 0.10 to 1, with an average of 0.89. At the plantation, the NMDS ordination showed a low level of clustering of samples by sample type (Fig. 4a) and a stronger clustering by tree species both in the litter samples (Fig. 4e) and in the soil samples (Fig. 4f). In this natural forest, tree species, type of sample, and their interaction had significant effects on the



**Fig. 2** Similar fungal evenness is found in soil and litter regardless of tree species. Simpson's index values for the soil and litter at the plantation (a) and under each tree species (c); in the litter and soil from the P16 plot in the natural forest (b) and under each tree species (d). *C* *Carapa surinamensis*, *G* *Goupia glabra*, *H* *Hymenaea courbaril*, *P* *Platonia*

*insignis*, *S* *Simarouba amara*, *V* *Vochysia tomentosa*. Box plots represent means with standard errors and 95 % confidence intervals. The values were not significantly different (Kruskal–Wallis rank sum test,  $p=0.01$ )

composition of the fungal communities and explained 18, 4, and 8 % of the variation, respectively, adding up to 30 % of the variation in communities explained (PERMANOVA,  $p < 0.05$ , Table 1). The geographical position and the interactions among the other factors had no significant effects. The samples were not autocorrelated according to the Mantel correlogram (SM, Figure S6b).

For the plantation, the results of the Mantel test showed significant correlations between litter characteristics and the litter fungal communities ( $p = 0.001$ , SM Table S5) and less significant correlations between soil characteristics and the soil fungal communities ( $p = 0.08$ , SM Table S5), even though some soil parameters were highly different among the six tree monocultures (Table S2).

## Discussion

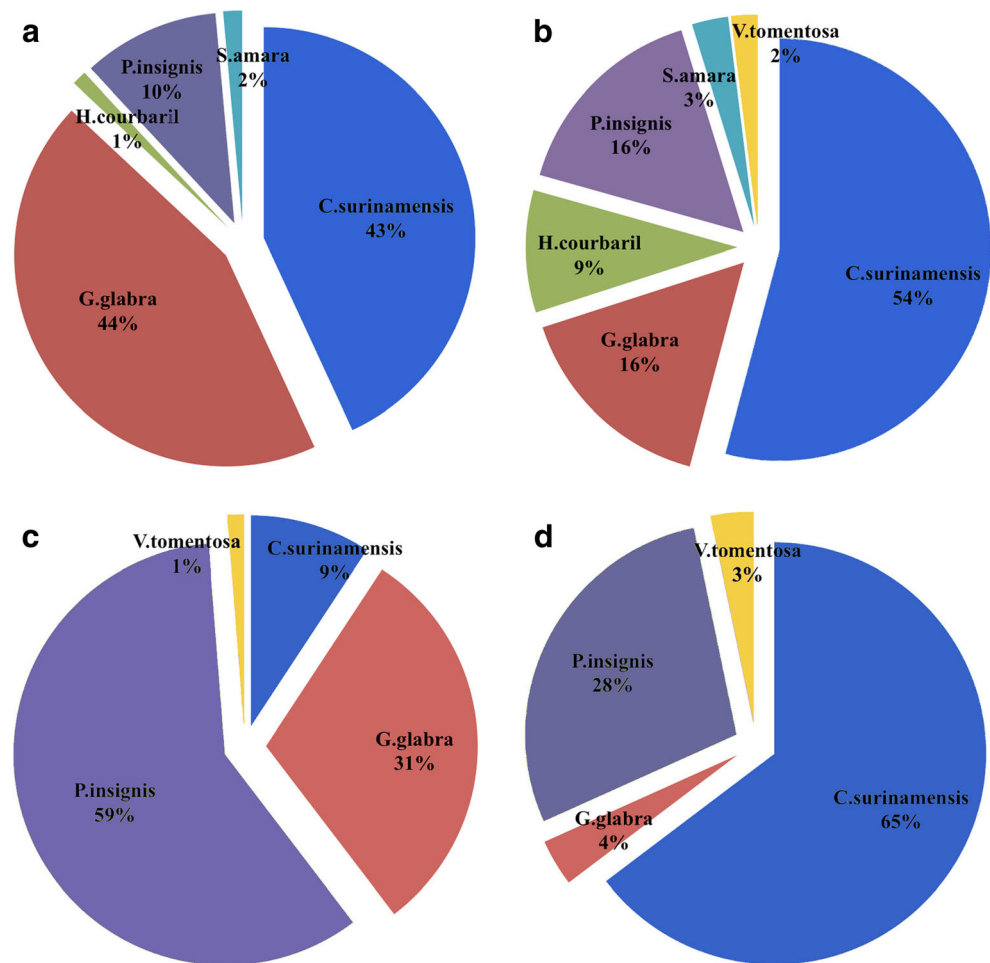
In boreal and temperate forests, pedo-climatic factors and the taxonomic range of potential hosts influence the distribution

of fungi [2, 43, 44], but little is known about soil fungal assemblages in Neotropical rainforests. The number of tree species has been estimated to be approximately 16,000 in the Amazonian basin [23], and recent studies have suggested high levels of diversity in the fungal communities of these ecosystems [18, 37], which are still poorly described [36]. Here, we found that tree species and environmental niche (soil or litter) are among the main local factors explaining the variation in soil fungal species composition, in soil fungal communities in Neotropical rainforest.

After bioinformatics filtering, we identified 5,586-species-level OTUs in the soil and litter from both sites, with 33 % being unassigned OTUs. This proportion was clearly higher than in recent studies that have been conducted on the soils of subtropical or temperate forests using same technologies and fungal primers [45]. The incompleteness of molecular databases, in particular, those concerning Neotropical fungi, which remain poorly described [36], can explain this high proportion.

In the 30-year-old plantation of upper canopy species, each monospecific tree stand harbored a locally homogeneous

**Fig. 3** Proportions of *C. podzolicus* and *P. virgulata* in the soil and litter associated with different tree species at the plantation and the P16 plot in the natural forest. Proportions of *C. podzolicus* (Agaricales, Marasmiaceae) at the plantation (a) and the P16 plot in the natural forest (c) and of *Pestalotiopsis virgulata* (Xylariales, Amphispheariaceae) at the plantation (b) and the P16 plot in the natural forest (d)



litter, which differed in their chemical characteristics. We made the assumption that fungal assemblages reflect substrate specialization in saprobes and host-recurrence in putative fungal endophytes and pathogens. The underlying reasons for this affinity remain unclear, but Paulus et al. [27] suggested that different factors may be involved in such preferential interactions, including fungal adaptation to plant secondary metabolites or the presence of a biotrophic phase in the life cycle of a particular fungus. We found a large range of fungal orders with, interestingly, dominant taxa found mainly in one or two stands in the plantation, which may reflect an impact of the tree species in the overstory and its associated litter and soil. However, all of the studied tree species are known to host endomycorrhizal fungi [39], and the taxonomic repartitions established in this study should be interpreted with caution as Glomeromycota and Chytridiomycota may have been underestimated because we amplified the ITS1 region with a primer pair designed for the Dikarya subkingdom (Ascomycota and Basidiomycota).

Because quantifying microbial communities with 454 pyrosequencing appears to be biased [46], we mainly focused on the variation in fungal community composition.

We found very large differences in the species assemblages among the plots, indicating strong variation in the fungal community structures, which is in line with the results of Peay et al. [18] from western Amazonian rainforests. The environmental niche (litter or soil beneath the trees) explained only a minor, but significant, part of the variation in communities, and this result was identical for the plantation and the P16 plot in the natural forest. Thus, this may suggest a shift in the composition between litter and soil samples, which is also confirmed by the proportion of Ascomycota being slightly higher in the litter than in the soil. This finding may reveal a vertical segregation of the composition of fungal communities across these horizons, as has been previously reported [47]. The dominance of Ascomycota fungi has been recently shown in tropical leaf litters [16] and in temperate and tropical tree phyllospheres [33, 42], but this pattern seems more complex in soils. Some studies have reported the dominance of Basidiomycota in temperate soils [48, 49] or associated with temperate tree roots [33, 50], but conversely, Ascomycota seem more abundant in the Mediterranean [51, 52], in tropical soils [18] or associated with tropical mangrove roots [53].

**Table 1** Permutational multivariate analysis of variance (PERMANOVA) testing the effects of sample type, tree species, the geographical positions of the samples, and the interactions among these factors on the dissimilarity matrices calculated for the fungal communities at the plantation and the P16 plot in the natural forest

	<i>df</i>	SS	MS	<i>F</i> value	<i>R</i> <sup>2</sup>	<i>p</i> value
Plantation	1	0.974	0.974	3.580	0.064	<b>0.001</b>
<i>sl</i>	5	4.965	0.993	3.648	0.328	<b>0.020</b>
<i>tree_sp</i>	1	0.630	0.630	2.315	0.041	<b>0.005</b>
<i>geo</i>	5	2.961	0.592	2.176	0.195	<b>0.001</b>
<i>sl:tree_sp</i>	1	0.222	0.221	0.815	0.014	0.723
<i>sl:geo</i>	5	1.156	0.231	0.849	0.076	0.800
<i>tree_sp:geo</i>	5	0.943	0.188	0.693	0.062	0.945
<i>sl:tree_sp:geo</i>	12	3.266	0.272		0.216	
Residuals	35	15.118			1.000	
Total P16 natural forest						
<i>sl</i>	1	0.648	0.648	1.820	0.039	<b>0.001</b>
<i>tree_sp</i>	3	2.972	0.990	2.780	0.179	<b>0.022</b>
<i>geo</i>	1	0.40	0.408	1.145	0.024	0.207
<i>sl:tree_sp</i>	3	1.473	0.491	1.377	0.088	<b>0.007</b>
<i>sl:geo</i>	1	0.421	0.421	1.183	0.025	0.153
<i>tree_sp:geo</i>	3	1.061	0.353	0.992	0.064	0.483
<i>sl:tree_sp:geo</i>	3	1.031	0.343	0.964	0.062	0.598
Residuals	24	8.553	0.356		0.516	
Total	39	16.571			1.000	

*sl* sample type (soil versus litter), *tree\_sp* tree species, *geo* geographical position of samples, *sl:tree\_sp* interaction between sample type and tree species, *sl:geo* interaction between sample type and geographical position, *tree\_sp:geo* interaction between tree species and geographical position, *sl:tree\_sp:geo* interaction between type of sample, tree species and geographical position

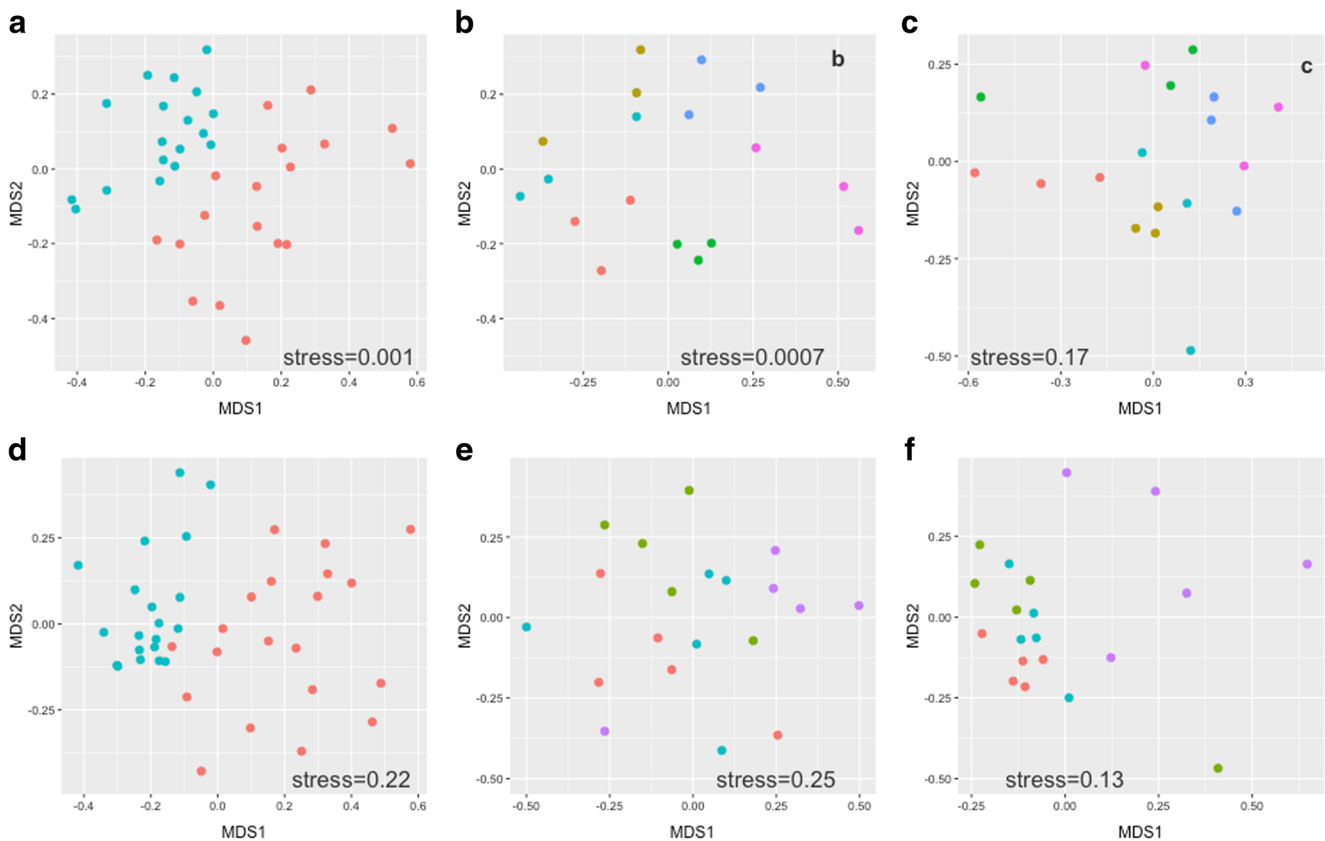
The most interesting result was observed at the tree plantation, where the “tree species” factor significantly structured the fungal assemblages and explained up to 32 % of the variation among the communities. This result is strengthened by the low amount of variation in fungal communities that was explained by the geographical position of the samples (Table 1) and the significant spatial autocorrelation of samples found only at short distances (within a plot, SM Figure S6a), which excludes a possible effect of geographical distance. Overall, these factors and their interactions explained the majority of the variation in the composition of communities. In addition, significant correlations were found between the composition of litter fungal communities and litter chemical characteristics (SM Table S5). This suggests that the 30-year-old monospecific tree stands may have shaped these fungal communities through their contrasting litter chemical characteristics, with a smaller effect of soil characteristics. Altogether, these results suggest that non-random structuration of fungal communities at the plantation has occurred due to the potential effect of tree species combined with

related abiotic effects, such as the chemical characteristics of the different litters and the possible nutrients leaching into the soils beneath and their different environmental niches. Interestingly, the effect of tree species was demonstrated on non-biotrophic Dikarya communities, while the trees species that are present are known to establish endomycorrhizal interactions with the Glomeromycota fungal phylum [63]. The influence of hosts on arbuscular fungi and the endemism characteristic of these fungi has been controversial until now [11]. We did not specifically investigate endomycorrhizal fungi in this study, but our results suggest potential host recurrence and coadaptations between non-mycorrhizal fungi and tropical forest trees. Furthermore, the variation in phyllospheric fungal communities is closely associated with host plant taxonomic identity and functional traits [42], partially explaining the observed structure of the fungal community after senescent leaves have fallen to the soil. This host-recurrence effect on fungal communities was confirmed in the natural forest, with only a minor part of the variation explained by the same factors. Neotropical natural rainforests display high tree  $\alpha$ -diversity [23] and hence litters with heterogeneous chemical characteristics at a very small scale [31], which might lower the effects of a particular tree species on the structure of the fungal communities beneath it.

Taxonomic relatedness among host plants as an important factor governing the effects of hosts have recently been demonstrated in ECM fungal communities [14], fungal plant pathogens [22], and phyllospheric fungal communities [42]. In addition, the high heterogeneity in chemical characteristics that has been measured in the living tropical tree leaves remains in litter after senescence despite the phenomenon of resorption [31]. Consequently, high overlap between the phyllosphere and decomposer communities has been observed [16, 42, 54], which could influence leaf litter and soil fungal assemblages. As shown by the still significant effect of tree species in the natural forest, local-scale ecological processes (host selectivity and habitat specificity) resulting from the high  $\alpha$ -diversity and spatial variability of trees in Amazonian forests may be strong enough to shape fungal communities in the soil and litter and hence plant–fungal interactions in non-ECM forests.

In the present study, we showed that tree species is one of the major factors explaining the differences in the composition of soil fungal communities in a natural Neotropical rainforest. The experimental tree plantation, which provided us the opportunity to study fungi that were influenced by only one tree species, also confirmed this result obtained in the nearby natural forest, even if this effect was partially blurred by differences in plant diversity and the crossed effects of the different host trees and heterogeneous microhabitats. Thus, the interpretations are weakened because we did not have the opportunity to appropriately replicate the study. We cannot fully exclude random effects that could hamper the results and thus





**Fig. 4** Effects of sample type (litter or soil) on fungal community composition. NMDS ordination of fungal communities including only assigned OTUs in all samples (**a**, **d**), litter (**b**, **e**) and soil samples (**c**, **f**) at the plantation (*above*) and the P16 plot in the natural forest (*below*) based on Morisita–Horn dissimilarities performed after Hellinger transformation of the data. At the plantation, the significant effects of tree species and sample type (soil or litter) explained 28 and 11 % of the variation in the composition of fungal communities, respectively (PERMANOVA,

$p < 0.05$ ). In P16 plot in the natural forest, the significant effects of tree species and sample type (soil or litter) explained 14 and 12 % of the variation in the composition of fungal communities, respectively (PERMANOVA,  $p < 0.05$ ). **a**, **d** Green dots = litter samples, red dots = soil samples; **b–f** pink dots = *Vochysia tomentosa*, blue dots = *Simarouba amara*, green dots = *Hymenaea courbaril*, red dots = *Carapa surinamensis*, brown dots = *Goupia glabra*, light blue dots = *Platonia insignis*

affect the full generalization of the analysis. Nevertheless, we are confident that tree species, through leaf and litter chemical characteristics and root exudates, shape fungal communities in the soil and litter of Neotropical forests. The extent of these impacts has yet to be precisely delimited through an appropriate experimental design allowing for true spatial replication. In particular, the extent of host recurrence among fungal lineages and the impacts of different soils are topics of interest to better understand the interactions between tree and fungal communities in these hyperdiverse Neotropical forests.

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