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RESEARCH

Determinants of the Vertical Distribution of the Phyllosphere Differ Between Microbial Groups and the Epi- and Endosphere in a Tropical Forest

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ABSTRACT

The determinants of phyllosphere microbial communities are drawing much attention given their functional importance for their plant host fitness and health. Identifying these determinants remains challenging in neotropical forests, considering the diversity of the tree hosts and the strong vertical heterogeneity of abiotic conditions within the canopy and at the scale of the leaf. Here, we studied fungal and bacterial communities living in the endophytic and epiphytic phyllosphere in tree species across vertical gradients, from the top of the canopy to the ground. We used DNA metabarcoding to characterize microbial communities and measured abiotic variables and foliar traits to characterize environmental heterogeneity. The assembly of fungal communities was more driven by deterministic processes compared with bacteria, with endo- and epiphytic communities being similarly shaped by the host identity and unmeasured parameters. In contrast, in bacterial communities, the relative importance of deterministic processes decreased from endophytic to epiphytic communities. Bacterial epi- and endophytic communities were partly and differently determined by the position within the canopy, the host identity, and leaf traits, suggesting an effect of the vertical gradient and a stronger selection in the inner tissues of the leaf than on its surface. The tree host exerts a selective pressure on microbial communities but the leaf as microhabitat also contributes significantly to the assembly of microbial communities. Discrepancies exist between fungi and bacteria that probably reflect different life-history traits and ecological strategies, emphasizing the need to study these communities jointly if we are to fully understand plant–phyllosphere interactions.

Keywords: assembly rules, bacteria, endophytes, epiphytes, fungi, leaf traits, metabarcoding, neotropical tree, vertical gradient

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Plants have a long history of coevolution with microorganisms, and their interactions have proven to be a key asset to the colonization of land by plants (Strullu-Derrien et al. 2018). Trees, like all plants, interact with microbes. These interactions take place not only in tree roots but also in leaves, an environment called the phyllosphere. A huge diversity of microorganisms colonizes the phyllosphere, either on the surface (epiphytes) or within the internal tissues of leaves (endophytes) (Rosado et al. 2018). These microbial communities encompass most microbial life forms (including filamentous fungi, yeasts, and protists) but are largely dominated by bacteria and fungi (Chaudhry et al. 2021; Rosado et al. 2018; Stone et al. 2018). Phyllosphere microbial communities (PMCs) have received growing attention in recent years due to their influence on plant fitness (Rosado et al. 2018; Vacher et al. 2016; Vorholt 2012).

In particular, PMCs can modify photosynthesis (Rho and Kim 2017; Rosado et al. 2018), be beneficial in plant–insect interactions (Fernandez-Conradi et al. 2018; Omacini et al. 2001; Vacher et al. 2021), and upregulate host defensive pathways (Álvarez-Loayza et al. 2011; Arnold et al. 2003; Christian et al. 2017). Thus, disentangling the mechanisms shaping PMCs should help in understanding how trees would respond to a variety of stresses or disturbances. As for communities of macroorganisms, deterministic and stochastic processes mutually regulate the assembly of PMCs (Dini-Andreote et al. 2015; Nemergut et al. 2013; Vellend 2010, 2016; Zhou and Ning 2017). In this determinism-stochastic framework, microbial communities are dynamically structured by four ecological processes: selection (deterministic niche-based recruitment of a particular set of species; that is, environmental filtering and biotic interactions) (Vellend 2010), dispersal (movement and establishment of species across space either deterministic or stochastic) (Chase and Myers 2011; Hanson et al. 2012), ecological drift (neutral stochastic demographic processes) (Chase and Myers 2011; Hubbell 2011), and evolutionary diversification (stochastic processes generating new genetic variation) (Nemergut et al. 2013). In the case of PMCs, the host may participate in selection and dispersion by controlling the quantity and quality of the nutrient pool available, or by producing antimicrobial molecules, thereby selecting particular microbial taxa (Chaudhry et al. 2021). Moreover, the physiology of the host, mediated or not by environmental constraints, could also exert strong effects on communities (Vacher et al. 2016). However, the effect of the identity of the host plant species remains inconsistent, with some studies reporting a significant effect (Kembel et al. 2014; Laforest-Lapointe et al. 2016) while others report no effect (Griffin et al. 2019). Part of this inconsistency probably lies in the fact that previous studies did not necessarily consider the environmental heterogeneity occurring within the canopy. Indeed, PMCs are exposed to vertical gradients in light and water availability, which influence their composition and diversity (Harrison et al. 2016; Izuno et al. 2016). As a matter of fact, in tropical forests, vertical gradients are particularly steep and the biotic and abiotic conditions in the canopy are very different from those of the understory. The forest top of the canopy is exposed to strong winds (Bittar et al. 2018), sunlight, and high temperatures (Shaw 2004), while the understory receives only 3% of full irradiance and 30% of the rainfall (Calder 2001). Hence, the resultant environmental heterogeneity within the canopy in tropical forests could partly drive the assembly of PMCs, with leaves at the top of the canopy being prone to drier and hotter conditions. In addition, PMCs live in two distinct microenvironments: the internal part (endophytic PMCs) and the surface (epiphytic PMCs) of the leaf, which is more exposed to external environmental conditions (Vacher et al. 2016). Colonization success of the leaf surface may depend on particular microbial functions such as the ability to extract nutrients from the internal tissues while developing on the leaf surface or growing from the few nutrients occurring at the leaf surface, and the ability to withstand dry environments and UV radiation, through motility or biofilm formation (Chaudhry et al. 2021; Vacher et al. 2016). In contrast, leaf internal tissues are richer in nutrients but also contain more plant defense metabolites. In neotropical forests, the wide range of ecological strategies developed by the trees (Allié et al. 2015; Fortunel et al. 2012) is partly translated into the chemistry and the morphology of their leaves (Courtois et al. 2012; Hättenschwiler et al. 2008) with, consequently, a high degree of heterogeneity between tree species. Therefore, leaf endophytic and epiphytic communities should be driven by different factors and processes. Indeed, in mangrove trees, the composition of fungal endophytic communities in leaves are more strongly shaped by the host than epiphytic ones (Yao et al. 2020). In contrast, the plant identity had a stronger effect on bacterial epiphytic communities (Yao et al. 2019). In olive trees, fungal endophytic and epiphytic communities in leaves are also differentially driven by environmental factors and plant organs (Gomes et al. 2018), whereas variations of nutrients and environmental conditions between internal and external plant tissues strongly influence the composition of bacterial communities and tend to recruit bacterial taxa resistant to desiccation and radiation at the leaf surface (Mina et al. 2020). In the light of all of these studies, and concomitantly with the effects of environmental heterogeneities within the vertical gradient between trees species and at the leaf scale, the assembly of bacterial and fungal members of the phyllosphere should differ (Wei et al. 2022).

In this study, we analyzed the assembly processes shaping the microbial communities living in the endophytic and epiphytic phyllosphere in three neotropical tree species in French Guiana. Specifically, we analyzed the epi- and endophytic bacterial and fungal communities across vertical environmental gradients present from the top of the canopy to the ground. We used DNA metabarcoding to characterize microbial communities and measured leaf morphological and chemical traits of host trees in parallel to describe their microhabitat along the vertical gradient. Foliar traits were selected mainly in the leaf economic spectrum (Wright et al. 2004) because, for leaf microbial communities, they can be indicative of water (Hetherington and Woodward 2003) and sugar availability (Evans and Seemann 1989; Field and Mooney 1986) or defense metabolism and space available for microbial colonization (Chaudhry et al. 2021). We specifically tested the following hypotheses: (H1) vertical stratification is stronger in epiphytic than in endophytic communities because epiphytes are more exposed to the within-canopy microclimate gradients; (H2) leaf traits contribute more to the assembly of endophytic than epiphytic communities because endophytes are intimately linked to their host tree; and (H3) the assembly of bacterial and fungal members of the phyllosphere should differ, because fungi and bacteria differ substantially in their growth habits and dispersal capability.

MATERIALS AND METHODS

Study site and sampling design. Leaf *s*ampling was conducted in October 2017 at the Nouragues Experimental Station (4.02°N, 52.414°W) with the help of the Canopy Operating Permanent Access System and tree climbers. Three tree species abundant in the studied area were selected: *Eperua falcata* Aubl. (Caesalpiniaceae), *Macrolobium bifolium* Aubl. (Caesalpiniaceae), and *Tetrasgastris* Gaertn. Sp. (Burseraceae). *E. falcata* is a shade hemitolerant dominant species growing in relatively dense clusters and on a large spectrum of edaphic conditions (Baraloto et al. 2005; Roggy et al. 1999). *Macrolobium* is a widespread genus throughout the Amazonian basin (Murphy et al. 2017). *Tetragastris* sp. belongs to the family Burseraceae, an important neotropical tree lineage known for its chemical defenses, including terpenes and phenolics (Fine and Kembel 2011). The sampling area was approximately 2 ha of homogeneous forest habitat. Five individuals of each tree species were selected, tall enough to have leaves on top of the canopy in full light, and geolocalized (Fig. 1A). On each individual, we sampled leaves at three heights and also in the litter (Fig. 1B): on top of the crown (top canopy [TC]), in the middle of the crown (middle canopy [MC]), on the lower branch of the crown (bottom canopy [BC]), and on the soil beneath each tree individual (litter [L]) Three leaves were collected at each height for DNA analysis. Each leaf was collected in a separate plastic bag to avoid cross contamination, resulting in 180 leaf samples (three tree species × five individuals × four heights × 3 leaves). In addition, we collected two leaves for

each height and each individual that were further used for leaf trait measurements. Leaves were sealed immediately after sampling in preweighted ziplock plastic bags and placed in a cooler until return to the lab.

Microclimate measurements. Irradiance at each height (TC, MC, BC, and L) was recorded with a photosynthetically active radiation linear sensor (Accepter model LP-80 PAR/LAI Ceptometer; Decagon Devices). Instantaneous measurements were realized at the level of sampled leaves for four trees and values were then compared with a reference sensor located at the top of a nearby flux tower (NOURAFLUX; https://www.cnrs.fr/fr/dsi), which provided an estimate of the incident irradiance above the canopy (i.e., the full irradiance). For each height, relative irradiance (percentage) was computed as the ratio of the local and above-the-canopy irradiance. Air temperature and relative humidity were measured with environmental HOBO sensors (model U23-001, HOBO Pro V2 Temp/RH Data logger; Amanvillers, France) placed at each canopy height (TC, MC, and BC) on two trees over 24 h.

Morphological and chemical leaf traits measurements. We measured 21 leaf traits that may influence the diversity, composition, and function of leaf microbial communities. We measured leaf water content (LWC) and stomatal density (SD) and four morphological traits: leaf dry matter content (LDMC), leaf mass per area (LMA), leaf thickness (LT), and leaf area (LA). All four are informative of the production of biomass by the plant and the density of leaf (Garnier et al. 2001). We also measured chlorophyll (Chl), nitrogen (N), carbon (C), and phosphorus (P) and major cations (calcium [Ca], magnesium [Mg], and potassium [K]), some trace metals (copper [Cu], manganese [Mn], iron [Fe], and zinc [Zn]), and sodium (Na) contents. One sampled leaf was used to measure SD while the other one was used to measure LWC and all other traits. To measure SD, a band of lamina was fixed with 5% formalin, 5% acetic acid, and 50% ethanol buffer. SD of the adaxial surfaces of the lamina (number of stomata per square centimeter) was then determined in the lab with imprints made using transparent nail varnish. The number of stomata was recorded using an optical microscope for each lamina as the average of five randomly selected areas of 1 cm^2 each (Leroy et al. 2009). To measure the other traits, fresh weight (fw) (in grams) was determined by weighing the sealed preweighted bags containing the fresh leaves. LA (in square centimeters) of fresh leaves was measured with a scanner and the ImageJ software (Schneider et al. 2012). LT (in micrometers) was estimated as the mean of three to eight measurements (four per simple leaf and one per leaflet for a compound leaf) with a digital micrometer (Digimatic micrometer; Mitutoyo, Japan). We took care to avoid the main veins for these measurements. Chl contents (in micrograms per square centimeter) were estimated with a portable Chl meter (SPAD-502, Osaka, Japan). Four to eight SPAD measurements per leaf (one per leaflet for a compound leaf) were averaged to obtain the SPAD estimate of the leaf, and Chl content was computed using the calibration equation proposed by Coste et al. (2010). Leaves were then dried at 60°C for 72 h and weighed (dry weight [dw] in grams). LWC (fw − dw/fw; percentage), LMA (dw/LA; in grams per square meter), and LDMC) (dw/fw; in milligrams per gram) were calculated. Leaves were then ground to determine C, N, P, ash, and elemental (Ca, Cu, Fe, Mg, Mn, K, Na, and Zn) contents (percentage) at the USRAVE platform of Bordeaux (France). Leaf specific construction cost (CCM) (grams of glucose per gram dw) was estimated from C, N, and ash contents, according to Poorter (1994). As in Coste et al. (2011), this computation assumed that all N was absorbed as nitrate. Hence, an additional cost for nitrate reduction in nonphotosynthetic tissues was taken into account. Altogether, these measurements resulted in 21 morphological and chemical leaf traits.

Phyllosphere microbial communities sampling. To collect epiphytic PMCs, the whole upper and lower surfaces of each leaf were carefully wiped with a piece of Whatman paper (2 by 2 cm) sterilized by autoclaving (120°C, 20 min) and soaked in sterile cetyltrimethylammonium bromide (CTAB) buffer (2% cetyltrimethylammonium bromide, 1% polyvinyl pyrrolidone, 100 mM Tris-HCl, 1.4 M NaCl, and 20 mM EDTA), within 4 h after leaves were harvested. The Whatman paper was stored in a 2-ml Eppendorf tube filled with sterile CTAB buffer for downstream DNA extraction. The surface sterilization protocol recommended by Compant et al. (2021) was used to collect endophytic PMCs. Each leaf was surface sterilized in 0.525% sodium hypochlorite (2 min) and 70% ethanol (2 min). Two segments of 2 cm^2 in area from the lamina were cut and stored in a 2-ml Eppendorf tube filled with sterile CTAB buffer for downstream DNA extractions and close to a field Bunsen burner to create a sterilized environment. This resulted

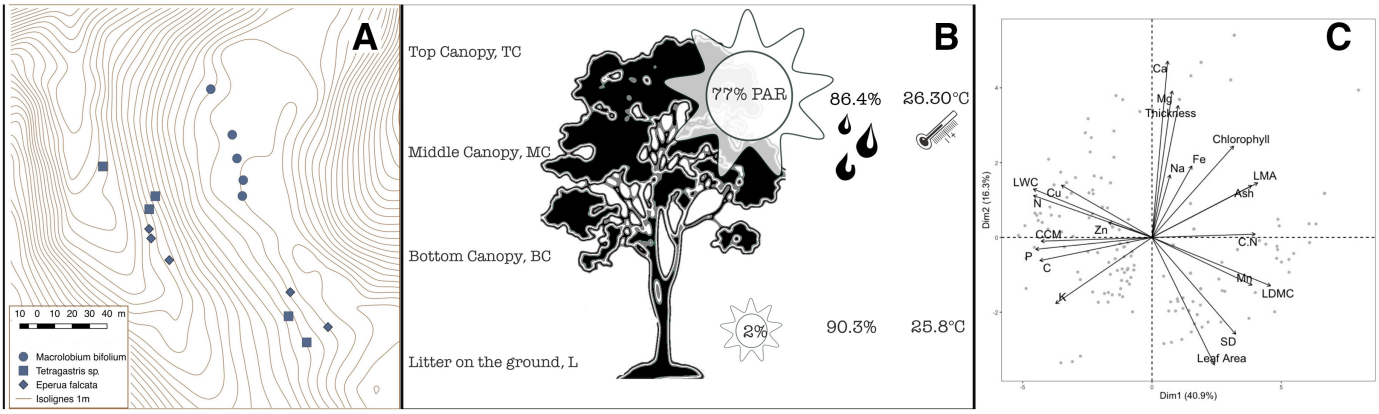

Fig. 1. A, Localization of each tree in the sampling area. **B,** Variations of relative irradiance, temperature, and relative humidity levels along the vertical gradient; the four positions of sampling are shown. **C,** Principal components analysis (PCA) of traits of individual leaves on the two first PCA axes with open circles, filled circles, and triangles corresponding to leaves from *Eperua falcata, Macrolobium bifolium*, and *Tetragastris* sp., respectively. Axes 1 and 2 represent 50 and 17%, respectively, of the overall leaves' inertia. Abbreviations: leaf water content (LWC), stomatal density (SD), leaf dry matter content (LDMC), leaf mass per area (LMA), nitrogen (N), carbon (C), carbon/nitrogen ratio (C:N), phosphorus (P), calcium (Ca), magnesium (Mg), potassium (K), copper (Cu), manganese (Mn), iron (Fe), and zinc (Zn), sodium (Na), and leaf specific construction cost (CCM).

in 360 DNA samples (three tree species × five individuals × four heights × three leaves × two DNA types [epiphytic or endophytic]).

DNA extraction, amplification and sequencing. DNA was extracted using a CTAB extraction method (Carrell and Frank 2014). We added 1,400 µl of CTAB solution to 0.6 g of tissue or Whatman paper, incubated the mixture for 2 h at 60°C, and homogenized it with glass beads for 3 min. Proteins were removed by adding a two-step addition of 600 µl of chloroform, centrifuging for 10 min at 16,000 × *g*, and isolating the top aqueous phase in a sterile tube. Nucleic acids were precipitated by adding a 120-µl volume of cold 3 M sodium acetate and 1 ml of cold isopropanol, then frozen at −20°C for 12 h and centrifuged for 30 min at 16,000 × *g*. The supernatant was discarded, and 700 µl of 70% ethanol was added to the solution and centrifuged for 10 min. The air-dried pellet was resuspended with 30 µl of DNA resuspension fluid (1.0 M Tris-HCL and 0.1 M EDTA) and stored at −20°C.

To characterize bacterial communities, the V5-V6 region of the bacterial 16S ribosomal RNA (rRNA) gene was amplified using the chloroplast-excluding forward primer 799f (Chelius and Triplett 2001) and the reverse primer 1115R (Reysenbach and Pace 1995). For fungi, the internal transcribed spacer 2 (ITS2) region of the rRNA gene was amplified using the ITS86F (Turenne et al. 1999) forward and the ITS4 (White et al. 1990) reverse primers, as recommended by Op de Beek et al. (2014). Forward and reverse primers were tagged in 5′ with a combination of two different 8-nucleotide labels. The PCR amplification was done in 25 µl with 1× buffer, 0.22 mM dNTP each, 0.45 µM each tagged primer, 2.84 mM $MgCl_2$, bovine serum albumin at 0.11 mg/ml. and Taq polymerase (Solis Biodyne) at 0.04 U/µl. The thermocycling conditions were as follows: 5 min at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s at 58°C, and 40 s at 78°C. The PCR reactions were done for each sample separately and amplicons were quantified with a fluorescence-based method (Qubit 3.0; Invitrogen Life Tech) and pooled in equimolar conditions. The library was built using the Fasteris MetaFast protocol (FASTERIS SA, Plan-les-Ouates, Switzerland) to minimize the amounts of tag switches (Esling et al. 2015), and sequenced on one MiSeq Illumina run (FASTERIS SA,) using the paired-end sequencing technology (Metafast protocol, FASTERIS SA). To control for potential contaminants and false-positive sequences caused by tag-switching events (Esling et al. 2015), the molecular experimental design comprised both extractions and PCR negative controls that were systematically sequenced, as well as unused tag combinations.

Bioinformatics. In total, 3,181,610 sequencing reads were obtained and curated using the OBITools3 package (Boyer et al. 2016) and R software (R Core Team 2022) following the procedure described by Zinger et al. (2019). Paired-end reads were assembled and assigned to their respective samples. After dereplication, low-quality sequences (i.e., containing Ns, being shorter than 80 pb or singletons) were excluded. PCR or sequencing errors were removed from the dataset. Briefly, cross-sample contaminations and reagent contaminants were removed on the basis of negative and empty controls, and dysfunctional PCRs were detected with the metabaR R package (Zinger et al. 2020) as indicated by Zinger et al. (2019). They represented <1% in the bacterial dataset and approximately 6% of reads in the fungal dataset (Supplementary Table S1). The remaining sequences were clustered into operational taxonomic units (OTUs) at 97% similarity using the Sumaclust algorithm (Mercier et al. 2013). The most abundant sequence was considered representative of the OTU and assigned a taxon using the SILVAngs r138.1 (Quast et al. 2013) for bacteria and the RDP Classifier (Wang et al. 2007) for fungi. Finally, we checked the taxonomic assignments and kept only sequences assigned to the Bacteria and Fungi kingdoms. Curated data yielded 812,253 reads corresponding to 10,096 OTUs in 274 samples for Bacteria and 1,105,257 reads, 9,582 OTUs, and 313 samples for Fungi. These data were then split into endophytic and epiphytic communities (Supplementary Table S2). Subsequently, each taxonomically defined OTU was assigned with a trophic group using the FungalTraits database (Põlme et al. 2020) for Fungi. This resulted in three trophic groups for Fungi (symbiotroph, saprotroph, and pathogens). For Bacteria, we assigned each OTU several putative functional groups based on OTU taxonomy. Functional information was retrieved from several functional databases and studies. First, we classified them into six main trophic classes: saprotroph, photolithoautotroph, chemolithoautotroph, osmotroph, phytoparasite, and zooparasite (Louca et al. 2016; Madin et al. 2020; Wardeh et al. 2015). We also classified OTUs into two groups according to their efficiency in resource utilization (i.e., Copiotroph versus Oligotroph) based on Ho et al. (2017). We finally classified OTUs into two key functional groups linked to the N cycle (i.e., N-fixing and nitrifying bacteria) (Louca et al. 2016; Nelson et al. 2016). We acknowledge that inferring a function from the taxonomy makes the implicit assumption that the affiliation of functions is well conserved, which is not completely the case. We also acknowledge that this functional analysis will be partial, because the databases and studies used here remain incomplete for many bacterial clades.

Statistical analyses. All analyses were conducted using the R software (R Core Team 2022) and with packages vegan 2.5-7 (Oksanen et al. 2020), adespatial 1.7-17 (Dray et al. 2021), ade4 1.7-19 (Dray et al. 2022), lme4 1.1-27.1 (Bates et al. 2015), FactoMineR 2.4 (Lê et al. 2008), metabaR (Zinger et al. 2021), hillR (Li 2018), and ggplot2 3.3.5 (Wickham 2016).

First, we tested the effects of vertical positions in the crown, the host species, and their interactions on each leaf trait with analysis of variance. Normality of each variable was assessed with a Shapiro test and variables were box-cox transformed when necessary. The processes involved in the assembly of endophytic and epiphytic PMCs was assessed by estimating their variation in α and β diversity across host species, vertical positions (TC, MC, BC, and L), and geographic locations of the host trees. The diversity of PMCs (fungal and bacterial endophytes and epiphytes, respectively) was estimated using the exponential of the Shannon index (Jost et al. 2010). These indices correspond to the Hill numbers at $q = 1$, which have been shown to provide more robust estimates of diversity (Calderón-Sanou et al. 2020; Haegeman et al. 2013). To stay in the framework of Hill numbers, the composition of PMCs was assessed using Morisita-Horn distances with $q = 1$ (Chao et al. 2014; Jost et al. 2010). This index is more resistant to undersampling because the relatively rare species have little effect. Because spatial autocorrelation may arise from both spatially structured environmental factors and dispersal, spatial variation was accounted for by including principal components of neighbors matrices (PCNMs) spatial eigenvectors based on the geographical coordinates of tree individuals in the model. We only considered downstream the PCNMs having a positive and significant Moran's I, as assessed with 1,000 permutations. To study α-diversity responses, we built linear models to quantify the effects of geographical position of trees (significant PCNMS), host species identity (*E. falcata*, *M. bifolium*, and *Tetragastris* sp.), vertical position in the canopy (TC, MC, BC, and L), and all leaf traits as variables on the Shannon index. We checked the potential collinearity among variables with a variance inflation factor (VIF) and removed variables with VIF values >5. As a result, LWC, LDMC, CCM, and C content had the highest values and were removed from further analysis. To minimize the model overfitting, we then conducted a stepwise selection based on the Akaike information criterion (Ripley 2021).

The relationships between the composition of PMCs and their environments were estimated by performing distance-based redundancy analyses based on Morisita-Horn distances (and $q = 1$), as mentioned above. The models included the same factors as above (i.e., significant PCNMs, host species identity, vertical position in the canopy, and all leaf traits) (21 variables) as explanatory variables. We first checked the potential collinearity among variables with a VIF and found no variables with VIF values >10 (our threshold). We then checked the significance of the test, including all constraints, and selected the most parsimonious model with a stepwise removal of the least contributing variable based on their adjusted R^2. We illustrated the patterns of the structure of bacterial and fungal communities by performing a nonmetric multidimensional scaling ordination on Morisita-Horn distance matrices.

In parallel, we examined the variability of bacterial and fungal trophic groups across the vertical gradient by performing a Kruskal-Wallis rank test ($P < 0.05$) followed by a Dunn's test for multiple comparisons. We illustrated the differences across the vertical gradient with boxplots for each group.

To supplement our statistical approach and better disentangle the relative contribution of deterministic and stochastic processes in the assembly of epiphytic and endophytic communities in leaves, we also implemented an abundance-based β-null modeling approach (Dini-Andreote et al. 2015; Tucker et al. 2016). The model calculated the deviation between the observed β diversity and the null-expected β diversity of randomly assembled pair of communities. We used the R code proposed by Luan et al. (2020) to generate a null scenario by random resampling OTUs and reads in the community matrix. The total occurrences and abundances of OTU were used as probabilities of selecting an OTU and its number of reads. Null expectations of community dissimilarities for each sample pair were obtained based on average Bray-Curtis dissimilarities of simulated communities. The null deviation value (NDV) was then defined as the difference between the observed and the averaged null dissimilarities. Close to 0, NDV indicated higher influence of stochasticity whereas, close to -1 or $+1$, it indicated a higher influence of deterministic processes. We computed NDVs for fungal and bacterial communities globally and separately for endophytic and epiphytic communities. Averaged NDVs were then compared with a Wilcoxon test.

Last, we further characterized how the identity of the host, the position within the vertical gradient, and leaf traits select for particular epiphytic and endophytic communities by estimating each OTU's niche breadth. To this end, we used the outlying mean index (OMI). More specifically, for each OTU, the OMI measured the distance between the mean environmental conditions where it occurs and the mean environmental conditions of the study area (Dolédec et al. 2000; Thuiller et al. 2005). A lower OMI value indicates that the species has a larger niche breadth, thus suggesting that the species is less subjected to environmental selection. We computed OMI values for fungal and bacterial OTUs globally and separately for endophytic and epiphytic communities. Because the datasets had many OTUs with very low abundances that could have blurred the signal, we decided to computed OMI only with the most abundant OTUs (i.e., with an abundance higher than the first quartile of sequence number across samples). Averaged OMIs were then compared with a Wilcoxon test.

RESULTS

Variations in leaf microclimate, physiology, and chemical composition within the canopy and between tropical tree species. Temperature and relative humidity showed no significant differences between the top and the bottom of the canopy (86 ± 12% relative humidity and 26 ± 3°C) but the relative irradiance varied from 77 ± 34% of the photosynthetic active radiation at the top of the canopy to 1.55 ± 1.5% on the soil (Fig. 1B). The majority of leaf traits exhibited strong and significant variations between host species and across positions within the canopy (Supplementary Table S3; Supplementary Figs. S1 and S2). Interactions between both factors were only significant for CCM, C:N, Ca, Cu, Fe, Mn, Mg, Na, P, K, and Zn contents. Half of the traits (10 of 20) decreased across positions in the canopy. The other tenors remained constant or increased across the positions in the canopy (Supplementary Table S3; Supplementary Figs. S1 and S2).

Composition of bacterial and fungal communities in the phyllosphere. Phyllosphere bacterial communities were dominated by six classes, including *Alphaproteobacteria* (49.06%), *Actinobacteria* (23.64%), *Sphingobacteria* (5.79%), *Gammaproteobacteria* (4.50%), *Chloroflexia* (5.43%), and *Cytophagia* (2.44%) (Supplementary Fig. S3B). Five orders represented 76% of the OTUs: *Rhizobiales, Actinomycetales, Sphingomonadales*, *Sphingobacteriales*, and *Rhodospirillales*. We were able to identify 200 families and 630 genera of kingdom Bacteria, the latter being mainly represented by genera *Methylobacterium* (12.60%) and *Sphingomonas* (10.58%). Leaf fungal communities were dominated by Dothideomycetes (36.17%), Sordariomycetes (25.74%), Lecanoromycetes (10.74%), and Eurotiomycetes (8.36%) (Supplementary Fig. S3A). Approximately 60% of the OTUs corresponded to five orders: Capnodiales, Xylariales, Chaetothyriales, Hypocreales, and Pleosporales. In total, fungal communities included 271 families and 621 genera. In all, 86% of fungal OTUs and 23% of bacterial OTUs could be assigned a functional group. Among fungi, 25.57% were pathogens, 35.73% were saprotrophs, and 4.45% were symbiotrophs (Supplementary Fig. S4A to C). Among bacteria, 5.41% of OTUs were categorized as photolithoautotroph, 38.94% as copiotroph (and 6.71% as oligotroph), 5.33% as N-fixing, and 1.67% as nitrifying (Supplementary Fig. S4D to H).

Drivers of phyllosphere bacterial and fungal communities. Overall, the diversity of fungal communities was poorly explained by our models (12 and 4% of the variation for endophytes and epiphytes respectively) (Supplementary Table S4). Endophytic fungal communities were mainly influenced by the geographical position of trees and SD, and epiphytic ones by one PCNM vector and position within the canopy (Supplementary Table S4). The variance explained for bacterial α diversity was overall much higher (49 and 31% for endophytic and epiphytic communities, respectively). The diversity of bacterial endophytic communities was mainly influenced by the position in the canopy (MC and L); the thickness of the leaf; Chl, Mg, and Na contents; and SD, whereas the position within the canopy (L); geographical position of the trees (three PCNMs); LMA; and Chl, Ca, Cu, and K contents were the main drivers of the diversity of epiphytic communities (Supplementary Table S4). Likewise, the structures of communities were differently driven by host species, geographical position of the trees, position within the canopy, and leaf traits (Fig. 2; Supplementary Table S5). More specifically, for fungi, host species and geographical position (the later only for endophytic communities) were the only factors retained in our models but the variance explained was only 2%. In bacterial communities, the variation of endophyte communities was significantly driven by the position within the canopy, the host species, some morphological traits (LT, SD, and LDMC) and chemical traits (K, Na, Mg, Cu, Chl contents, and C:N), and geographical position of the trees (five PCNMs). The full model explained up to 20% of the variation. The composition of epiphytic bacterial communities was significantly driven by their position within the canopy, the host species, some morphological traits (LT, LMA, CCM, LWC, and SD) and chemical traits (C, N, C:N, K, Ca, Cu,

Mn, Mg, and Chl contents), and geographical position (all PCNMs), the full model explaining up to 23% of the variation. We found a clear differentiation of L samples from the rest of samples for both endophytic and epiphytic bacterial communities (Fig. 3A and B) but no clear patterns of fungal community structure across the position in the vertical gradient or among host tree species (Fig. 3C and D). In parallel, the position within the vertical gradient had no significant effect on any of the three fungal trophic groups and only on bacterial photolithoautroph (Supplementary Fig. S4A to D). We detected no significant effects of position within the vertical gradient for any fungal trophic groups (Supplementary Fig. S4A to C). There were significantly more copiotrophic (and less oligotrophic)

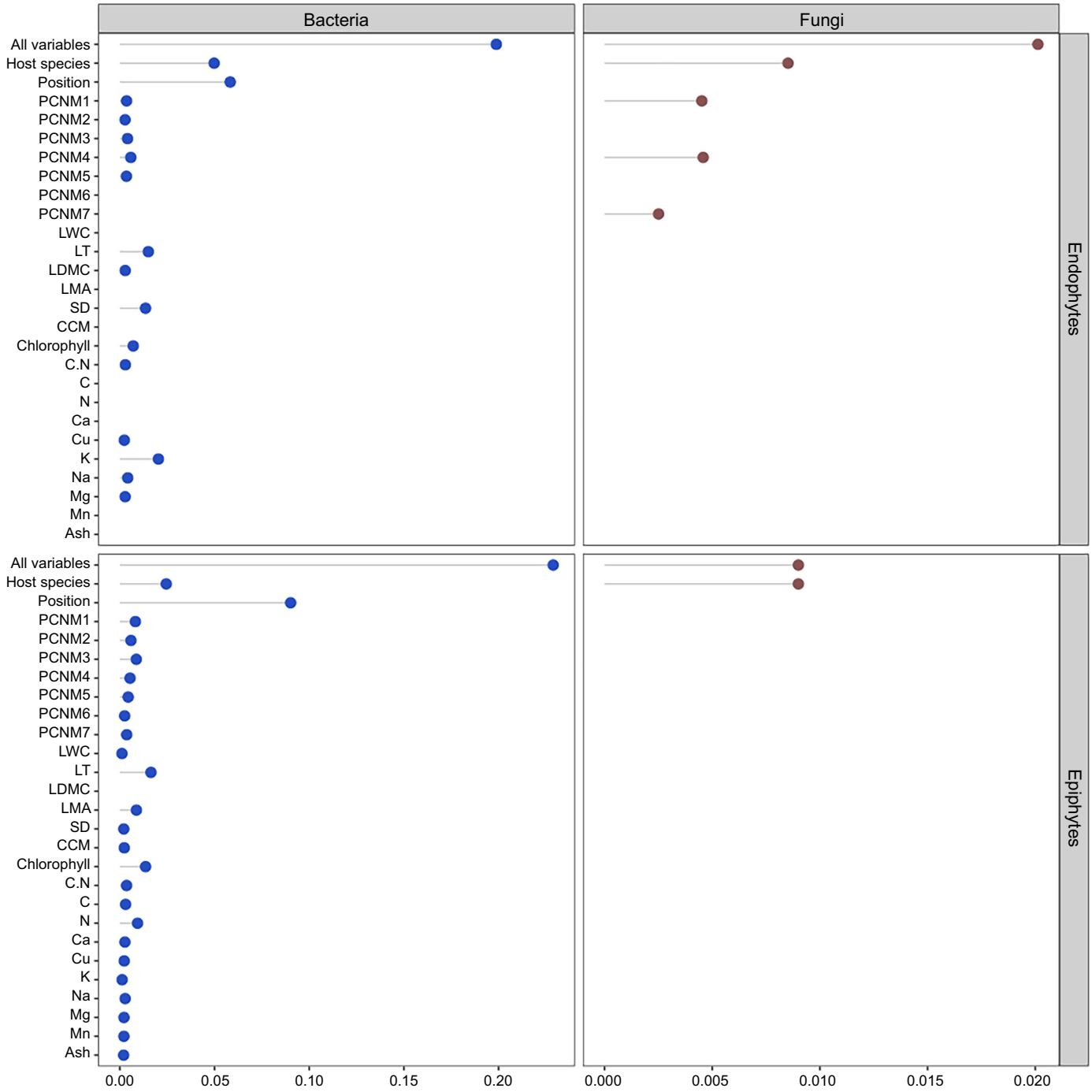

Fig. 2. Structure of fungal (left) and bacterial (right) communities explained by host species, vertical position in the canopy, geographical position (significant principal components of neighbors matrices), and leaf traits. Results are shown of a stepwise selection of variables using dbRDA for each endophytic and epiphytic fungal or bacterial community. For clarity, cumulative adjusted R^2 for all variables and adjusted R^2 for each individual variable are presented in the *x*-axis and the list of traits in the *y*-axis. Abbreviations: leaf water content (LWC), leaf thickness (LT), stomatal density (SD), leaf dry matter content (LDMC), leaf mass per area (LMA), nitrogen (N), carbon (C), carbon/nitrogen ratio (C:N), phosphorus (P), calcium (Ca), magnesium (Mg), potassium (K), copper (Cu), manganese (Mn), iron (Fe), and zinc (Zn), sodium (Na), and leaf specific construction cost (CCM). Overall results are available in Supplementary Table S5.

and more N-fixing and nitrifying bacteria in L samples than in other positions in the canopy (Supplementary Fig. S4E to H).

Stochasticity and niche breadth analyses of endophytic and epiphytic communities. The extent of stochasticity in the assembly of bacterial and fungal communities was evaluated with an abundance-based β-null model approach. We found that both fungal and bacterial communities deviated from the null expectations, with fungal communities exhibiting significantly higher deviations than bacterial ones (Fig. 4A). More specifically, NDVs for fungal endophytes were significantly lower than those for epiphytic communities (Fig. 4B). Bacterial endophytic communities' NDVs were, on average, significantly higher than those of bacterial epiphytic ones (Fig. 4C). The impact of selection by leaf traits, host species, and position within the vertical gradient on community assembly was evaluated by computing niche breadth for each OTU. The average OMI of fungal communities was significantly higher than that of bacterial ones (Fig. 5A) ($P < 0.001$). For fungal communities, there was no significant differences between average OMI values

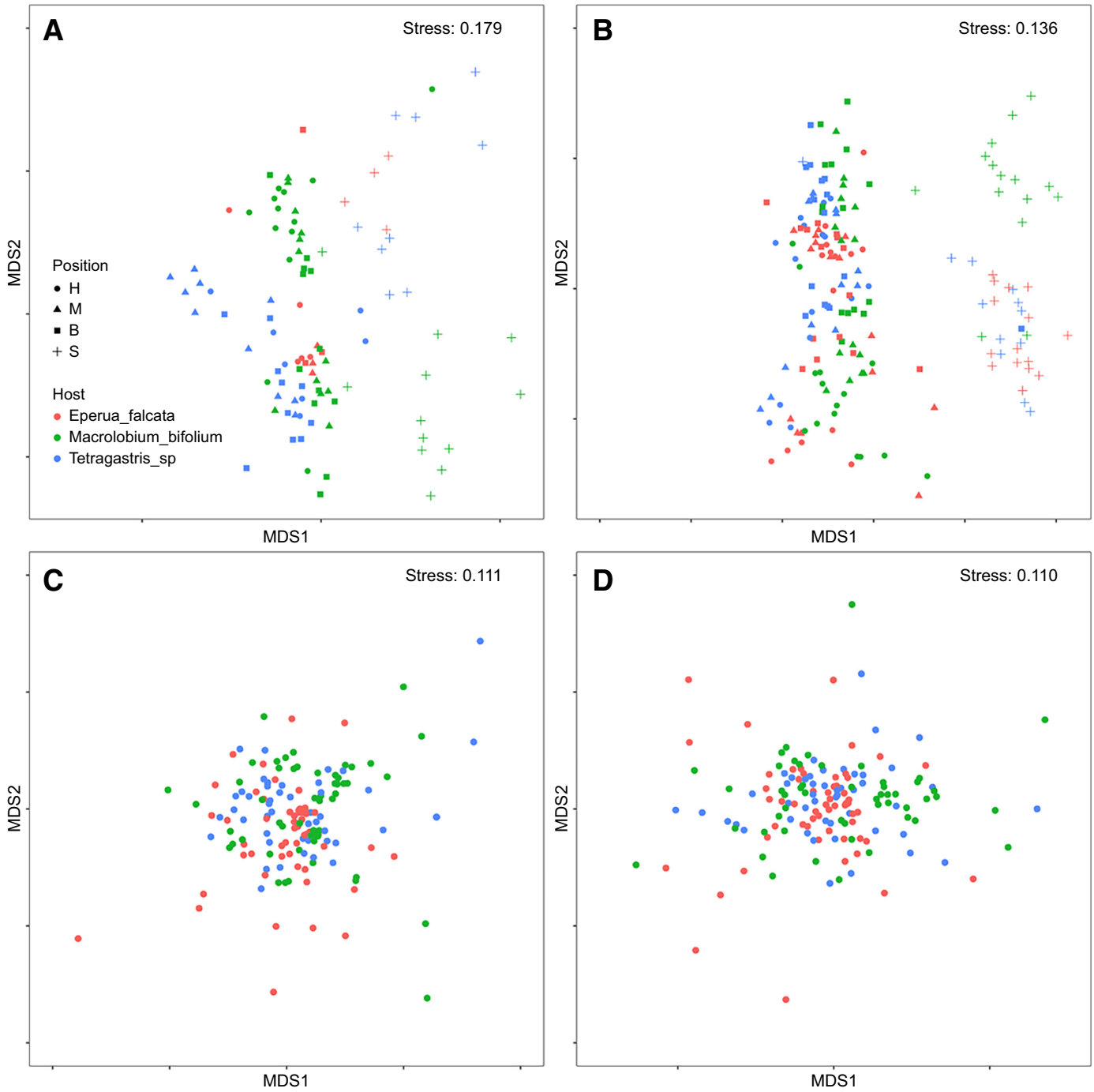

Fig. 3. Nonmetric multidimensional scaling (NMDS) plot of **A,** bacterial endophytic and **B,** epiphytic communities and **C,** fungal endophytic and **D,** epiphytic communities. Different colors stand for host tree species: red = *Eperua falcata*, green = *Macrolobium bifolium*, and blue = *Tetragastris* sp. Additionally, different shapes stand for different position within the canopy (A and B): circle = top canopy, triangle = middle canopy, square = bottom canopy, and cross = litter on the ground.

of endophytic and epiphytic communities (Fig. 5B) ($P = 0.352$). For bacterial ones, OMI values of endophytic communities were, on average, significantly higher than that of epiphytic communities (Fig. 5C) ($P < 0.001$).

DISCUSSION

Microbial communities in the phyllosphere were dominated by fungal and bacterial clades that are in line with previous observations in tropical trees (Bulgarelli et al. 2013; Donald et al. 2020; Griffin and Carson 2018; Kembel and Mueller 2014; Kembel et al. 2014) and the particularities of the phyllosphere environment (Stone et al. 2018). Fungal communities mainly consisted of saprotrophic and pathogen clades, with few symbiotroph, and were quite homogeneously spread across the vertical gradient in the trees. In contrast, we found differences in bacterial trophic and functional groups between communities in fresh leaves within the canopy and litter leaves on the ground. We also report strong discrepancies in the drivers of endophytic and epiphytic bacterial and fungal communities in the phyllosphere. Deterministic and stochastic processes are two nonexclusive determinants of the assembly of leaf PMCs (Stegen et al. 2013; Vacher et al. 2016; Vass et al. 2020; Zhou and Ning 2017). Overall, the assembly of fungal communities had a greater deterministic component, and fungal OTUs had a relatively narrower niche breadth than bacterial ones. However, a very small part of the variation among communities was explained, suggesting that niche-based factors play only a minor role in controlling the assembly of fungi. This could result from an incomplete selection of biotic and abiotic factors in the models. For example, leaf fungal communities are also driven by seasonality in temperature and rainfall (Gomes et al. 2018; Oita et al. 2021). In tropical areas with a strong seasonality, as is the case in French Guiana, dry seasons lead to a robust physiological filter at the leaf scale, where hot temperatures, lower humidity, and UV irradiance could limit survival of propagules and the colonization of leaves, with consequently lower richness of leaf fungal communities (Oita et al. 2021).

The balance between deterministic and stochastic processes were similar for fungal endo- and epiphytic communities. In line with the few studies that have specifically compared fungal endophytic and epiphytic communities (Gomes et al. 2018; Santamaría and Bayman 2005; Yao et al. 2019), the host, through leaf traits such as SD, LA, and some chemical components, had a small and similar effect. We noted also a small and significant effect of the vertical position within the canopy. In addition, no factors seemed to clearly drive the composition of either endophytic or epiphytic communities or the distribution of the three fungal trophic groups (saprotroph, symbiotroph, and pathotroph). This overlap between communities might be the consequence of the intrinsic particularities of some foliar fungi that could be both epiphytes and endophytes (Gomes et al. 2018), or their being able to modify their trophic mode between green and dead leaves (Vacher et al. 2016).

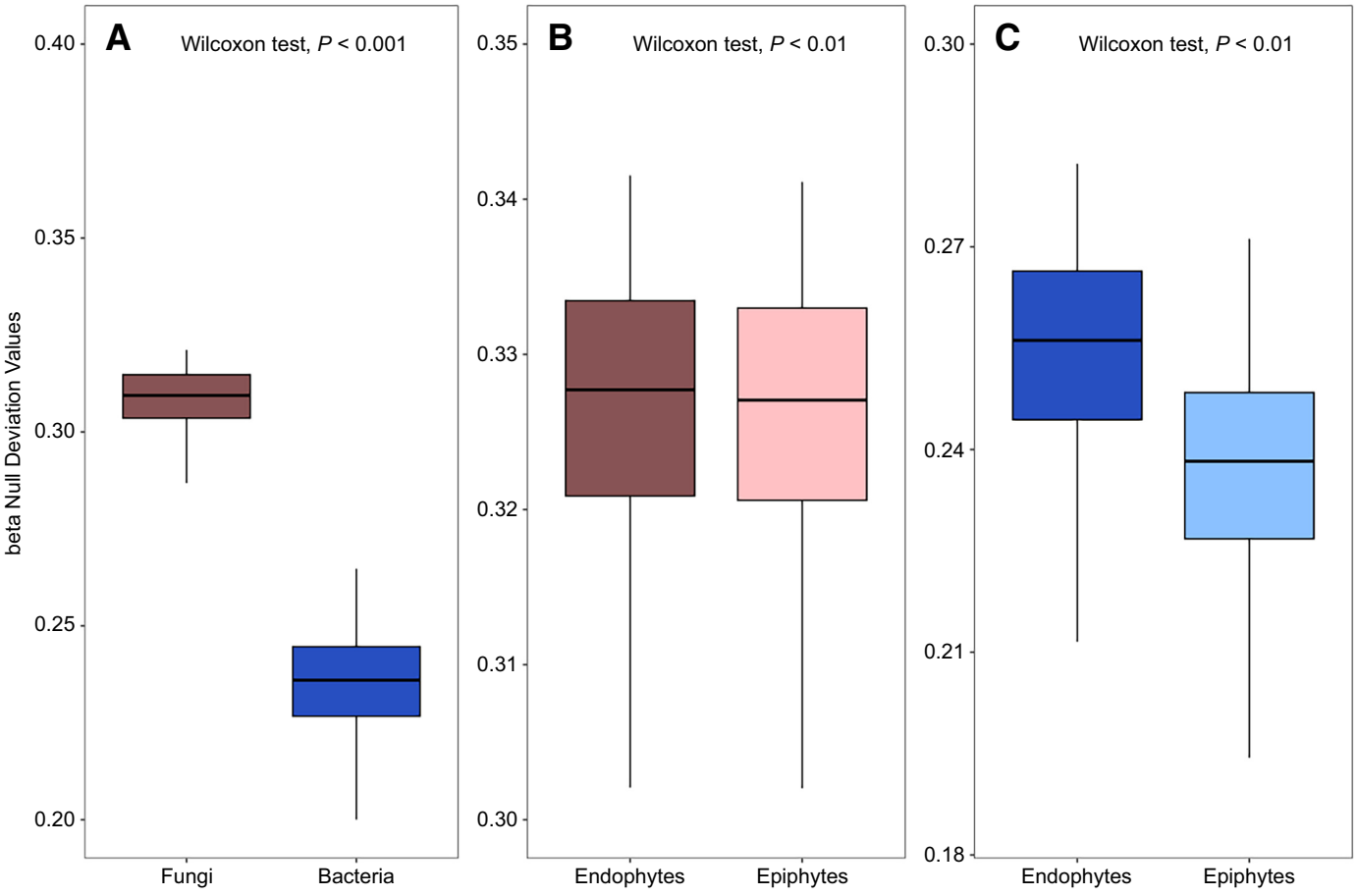

Fig. 4. Boxplots of β-null deviation values: **A,** Fungi (brown) compared with Bacteria (blue); **B,** fungal endophytic (brown) compared with fungal epiphytic (light brown) communities; and **C,** bacterial endophytic (blue) compared with bacterial epiphytic (light blue) communities. Results of Wilcoxon test for each pair are shown.

A different pattern emerged when considering bacterial communities, where the relative importance of deterministic processes decreased from endophytic to epiphytic communities. In the same way, niche breadth was wider in bacterial epiphytic communities, suggesting that the chemical and physical characteristic of the inner tissues of leaf are more selective than the conditions of the leaf surface. This is indicative of a higher control by the host over colonization of internal than external tissues (Mina et al. 2020; Trivedi et al. 2020). Host species identity and the position within the canopy were also important drivers of bacterial communities (up to 15% of the variation), in line with previous studies (Harrison and Griffin 2020; Herrmann et al. 2021; Kembel and Mueller 2014; Kembel et al. 2014; Laforest-Lapointe et al. 2016). Bacterial responses to the identity of host species manifested themselves either directly, suggesting an effect of host evolutionary history or of unmeasured traits, or through interspecific leaf traits variations. Most of the values of leaf traits investigated decreased or increased significantly with the vertical light gradient within the canopy (Markesteijn et al. 2007; Poorter et al. 2019), which may indicate an indirect effect of the irradiance on the composition of bacterial communities in leaves. However, the vertical environmental gradient affected bacterial endophytic and epiphytic communities at different levels and through different factors. Specifically, bacterial endophytic communities were more affected through their diversity and epiphytic ones were more affected through their composition. The physiology of the leaf and its variations within the canopy could explain this discrepancy: leaf resource uptake strategies would affect local leaf nutrients and water availability to bacterial communities (Kembel et al. 2014; Lajoie and Kembel 2021). LT and LMA, related to space for colonization, were involved in the variations along the gradient of both bacterial communities. Stomatal chambers or apoplastic spaces inside leaves (large intercellular spaces known to host microbial communities) (Vacher et al. 2016) can explain the effect of the LT we found. The environment at the surface of a leaf and its chemistry (cuticle layer, stomata, and hydathodes) is known to create stressful and nutrient-poor conditions where bacteria must adjust to multiple fluctuations (season and day/night cycles, for example) (Bringel and Couée 2015). To cope with them, bacteria develop growth strategies such as forming large aggregates to successfully colonize the leaf (Chaudhry et al. 2021; Vacher et al. 2016; Vorholt 2012). Interestingly, leaf traits related to water content (LWC and SD) had a significant impact on both epi- and endophytic bacterial communities. Leaf surface microtopographic features such as SD or leaf vein density have been reported to regulate the assembly of the bacterial communities because they improve the supply of available water to the

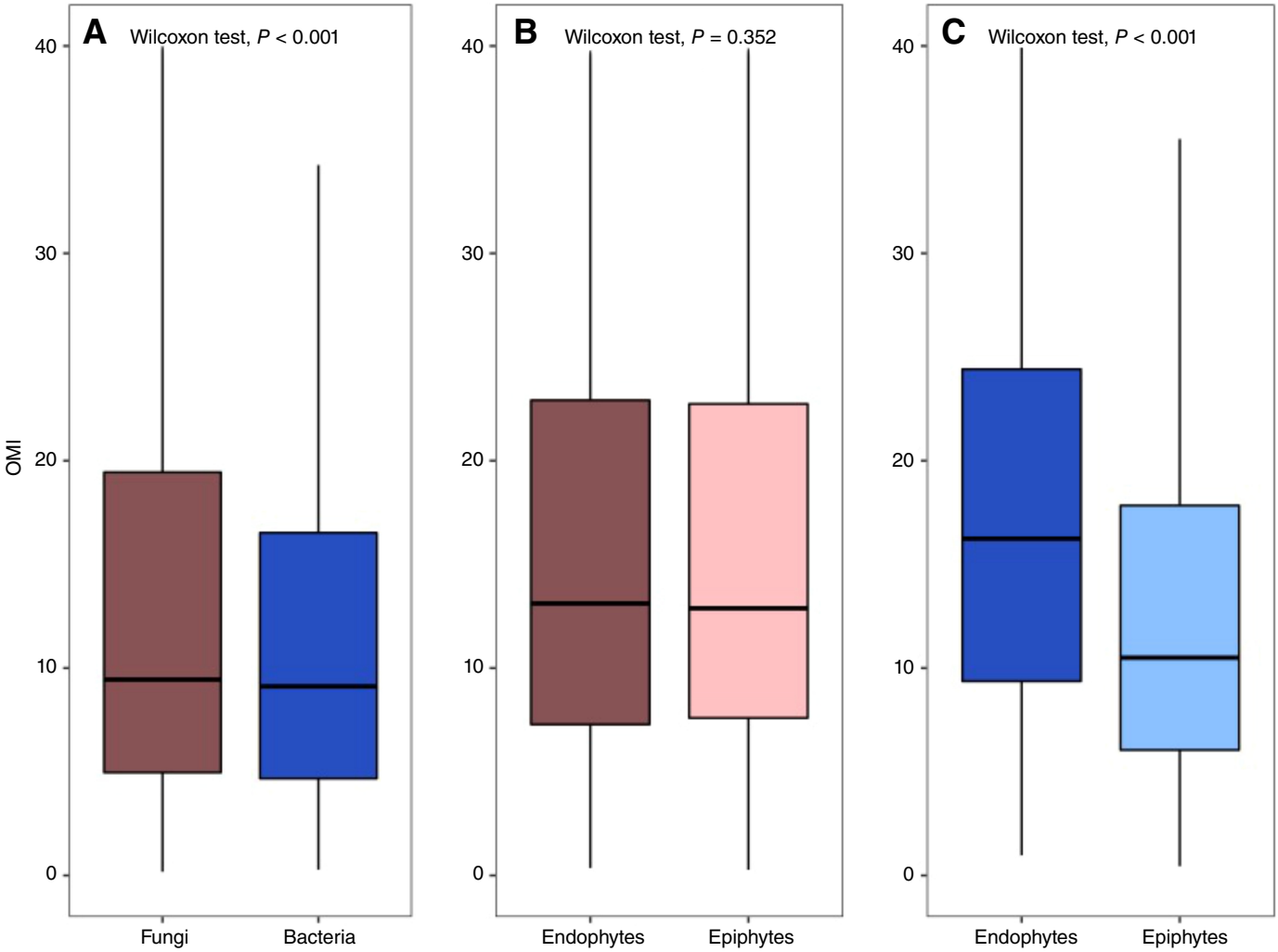

Fig. 5. Boxplots of outlying mean index of **A,** Fungi compared with Bacteria; **B,** fungal endophytic compared with fungal epiphytic communities; and **C,** bacterial endophytic compared with bacterial epiphytic communities. Results of Wilcoxon test for each pair are shown.

surrounding microorganisms (Vorholt 2012, Yan et al. 2022); and stomata are entry points in the inner leaf tissues and one of the major ways to regulate dispersal (Vacher et al. 2016). Chl, C, and N contents and several chemical elements also significantly and differently shaped bacterial endo- and epiphytic communities, suggesting a selection by the nutrients resulting from photosynthesis. Indeed, leaf N content is linked to the maximum photosynthetic rate (Cornelissen et al. 2001) and influences the composition of phyllosphere bacterial communities (Kembel et al. 2014; Laforest-Lapointe et al. 2016; Lajoie et al. 2020). Unmeasured leaf traits such as volatile organic compounds exported to the surface of the leaf (i.e., methanol) could also explain the abundance of *Methylobacterium* we found in bacterial communities (Lemanceau et al. 2017; Vacher et al. 2016). There are different ways to explain that epiphytic communities were less driven by deterministic processes. At the leaf scale, dynamic interactions between microbes or between the microbes and the plant, highly heterogeneous plant responses to colonization (Schlechter et al. 2019), but also the microtopography at the surface of the leaf (Yan et al. 2022) drive bacterial composition and probably increase the contribution of stochasticity to bacterial community assembly. Moreover, the bacterial colonization of the leaf from bioaerosols (Bulgarelli et al. 2013), rainfall, and subsequent splashing of raindrops (Morris 2002) or transmission by herbivorous insects or animals (Vacher et al. 2016, 2021) may also add stochasticity in the assembly.

To conclude, our study provides evidence that the assembly of endophytic and epiphytic microbial communities in tropical leaves was governed by a balance of deterministic and stochastic effects. This resulted in different effects of the host and the position within the vertical environmental gradient on microbial community diversity and composition. Moreover, strong discrepancies exist between the assembly of fungal and bacterial communities, probably due to their different life-history traits and ecological strategies. This emphasizes the selective pressure (i.e., the phyllosphere effect) of the plant on microbial communities but underlines that, at the leaf scale, the microhabitat (inside or outside the leaf) substantially drives the microbial communities. Few studies have specifically compared endophytic and epiphytic communities (Bodenhausen et al. 2013; Hunter et al. 2010; Mina et al. 2020; Yao et al. 2019, 2020). They showed an intimate link between endophytic communities and the plant and suggest that epiphytic communities are more resistant to environmental disturbances. Extreme climate events are expected to be more frequent in neotropical forests. It is then of great importance to understand how the functional and molecular interactions between microbial communities (endophytic and epiphytic) and their host plant will be a key asset to cope with these changes.

Data availability. Leaf traits measurements, all metadata on tree individuals, raw and processed sequence data used for this article, additional files needed, and R scripts are available from the Zenodo repository (https://doi.org/10.5281/zenodo.7802975).

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LITERATURE CITED

Allié, E., Pélissier, R., Engel, J., Petronelli, P., Freycon, V., Deblauwe, V., Soucémarianadin, L., Weigel, J., and Baraloto, C. 2015. Pervasive local-scale tree-soil habitat association in a tropical forest community. PLoS One 10:e0141488.

Álvarez-Loayza, P., White, J. F., Jr., Torres, M. S., Balslev, H., Kristiansen, T., Svenning, J.-C., and Gil, N. 2011. Light converts endosymbiotic fungus to pathogen, influencing seedling survival and niche-space filling of a common tropical tree, *Iriartea deltoidea*. PLoS One 6:e16386.

Arnold, A. E., Mejia, L. C., Kyllo, D., Rojas, E. I., Maynard, Z., Robbins, N., and Herre, E. A. 2003. Fungal endophytes limit pathogen damage in a tropical tree. Proc. Natl. Acad. Sci. U.S.A. 100:15649-15654.

Baraloto, C., Forget, P.-M., and Goldberg, D. E. 2005. Seed mass, seedling size and neotropical tree seedling establishment. J. Ecol. 93:1156-1166.

Bates, D., Mächler, M., Bolker, B., and Walker, S. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67:1-48.

Bittar, T. B., Pound, P., Whitetree, A., Moore, L. D., and Van Stan, J. T. 2018. Estimation of throughfall and stemflow bacterial flux in a subtropical oak-cedar forest. Geophys. Res. Lett. 45:1410-1418.

Bodenhausen, N., Horton, M. W., and Bergelson, J. 2013. Bacterial communities associated with the leaves and the roots of *Arabidopsis thaliana*. PLoS One 8:e56329.

Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P., and Coissac, E. 2016. obitools: A unix-inspired software package for DNA metabarcoding. Mol. Ecol. Resour. 16:176-182.

Bringel, F., and Couée, I. 2015. Pivotal roles of phyllosphere microorganisms at the interface between plant functioning and atmospheric trace gas dynamics. Front. Microbiol. 6:486.

Bulgarelli, D., Schlaeppi, K., Spaepen, S., van Themaat, E. V. L., and Schulze-Lefert, P. 2013. Structure and functions of the bacterial microbiota of plants. Annu. Rev. Plant Biol. 64:807-838.

Calder, I. R. 2001. Canopy processes: Implications for transpiration, interception and splash induced erosion, ultimately for forest management and water resources. Pages 203-214 in: Tropical Forest Canopies: Ecology and Management, Vol. 69. K. E. Linsenmair, A. J. Davis, B. Fiala, and M. R. Speight, eds. Springer, Dordrecht, The Netherlands.

Calderón-Sanou, I., Münkemüller, T., Boyer, F., Zinger, L., and Thuiller, W. 2020. From environmental DNA sequences to ecological conclusions: How strong is the influence of methodological choices? J. Biogeogr. 47:193-206.

Carrell, A. A., and Frank, A. C. 2014. *Pinus flexilis* and *Picea engelmannii* share a simple and consistent needle endophyte microbiota with a potential role in nitrogen fixation. Front. Microbiol. 5:333.

Chao, A., Chiu, C.-H., and Jost, L. 2014. Unifying species diversity, phylogenetic diversity, functional diversity, and related similarity and differentiation measures through hill numbers. Annu. Rev. Ecol. Evol. Syst. 45:297-324.

Chase, J. M., and Myers, J. A. 2011. Disentangling the importance of ecological niches from stochastic processes across scales. Philos. Trans. R. Soc. B Biol. Sci. 366:2351-2363.

Chaudhry, V., Runge, P., Sengupta, P., Doehlemann, G., Parker, J. E., and Kemen, E. 2021. Shaping the leaf microbiota: Plant–microbe–microbe interactions. J. Exp. Bot. 72:36-56.

Chelius, M. K., and Triplett, E. W. 2001. The Diversity of archaea and bacteria in association with the roots of *Zea mays* L. Microb. Ecol. 41:252-263.

Christian, N., Herre, E. A., Mejia, L. C., and Clay, K. 2017. Exposure to the leaf litter microbiome of healthy adults protects seedlings from pathogen damage. Proc. Biol. Sci. 284:20170641.

Compant, S., Cambon, M. C., Vacher, C., Mitter, B., Samad, A., and Sessitsch, A. 2021. The plant endosphere world—Bacterial life within plants. Environ. Microbiol. 23:1812-1829.

Cornelissen, J., Aerts, R., Cerabolini, B., Werger, M., and van der Heijden, M. 2001. Carbon cycling traits of plant species are linked with mycorrhizal strategy. Oecologia 129:611-619.

Coste, S., Baraloto, C., Leroy, C., Marcon, É., Renaud, A., Richardson, A. D., Roggy, J.-C., Schimann, H., Uddling, J., and Hérault, B. 2010. Assessing foliar chlorophyll contents with the SPAD-502 chlorophyll meter: A calibration test with thirteen tree species of tropical rainforest in French Guiana. Ann. For. Sci. 67:607.

Coste, S., Roggy, J.-C., Schimann, H., Epron, D., and Dreyer, E. 2011. A cost–benefit analysis of acclimation to low irradiance in tropical rainforest tree seedlings: Leaf life span and payback time for leaf deployment. J. Exp. Bot. 62:3941-3955.

Courtois, E. A., Baraloto, C., Timothy Paine, C. E., Petronelli, P., Blandinieres, P.-A., Stien, D., Höuel, E., Bessière, J.-M., and Chave, J. 2012. Differences in volatile terpene composition between the bark and leaves of tropical tree species. Phytochemistry 82:81-88.

Dini-Andreote, F., Stegen, J. C., van Elsas, J. D., and Salles, J. F. 2015. Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession. Proc. Natl. Acad. Sci. U.S.A. 112:E1326-E1332.

Dolédec, S., Chessel, D., and Gimaret-Carpentier, C. 2000. Niche separation in community analysis: A new method. Ecology 81:2914-2927.

Donald, J., Roy, M., Suescun, U., Iribar, A., Manzi, S., Péllissier, L., Gaucher, P., and Chave, J. 2020. A test of community assembly rules using foliar endophytes from a tropical forest canopy. J. Ecol. 108:1605-1616.

Dray, S., Bauman, D., Blanchet, G., Borcard, D., Clappe, S., Guenard, G., Jombart, T., Larocque, G., Legendre, P., Madi, N., and Wagner, H. H. 2021. adespatial: Multivariate multiscale spatial analysis. https://CRAN.R-project.org/package=adespatial

Dray, S., Dufour, A.-B., and Thioulouse, J. 2022. ADE4: Analysis of ecological data: Exploratory and Euclidean methods in environmental sciences. http://pbil.univ-lyon1.fr/ADE-4/

Esling, P., Lejzerowicz, F., and Pawlowski, J. 2015. Accurate multiplexing and filtering for high-throughput amplicon-sequencing. Nucleic Acids Res. 43:2513-2524.

Evans, J., and Seemann, J. R. 1989. The allocation of protein nitrogen in the photosynthetic apparatus: Costs, consequences, and control. Pages 183-205 in: Photosynthesis, 1st ed. W. R. Briggs, ed. Wiley-Liss, New York.

Fernandez-Conradi, P., Jactel, H., Robin, C., Tack, A. J. M., and Castagneyrol, B. 2018. Fungi reduce preference and performance of insect herbivores on challenged plants. Ecology 99:300-311.

Field, C., and Mooney, H. A. 1986. The photosynthesis–nitrogen relationship in wild plants. Pages 25-56 in: On the Economy of Plant Form and Function: Proceedings of the Sixth Maria Moors Cabot Symposium, Evolutionary Constraints on Primary Productivity, Adaptive Patterns of Energy Capture in Plants, Harvard Forest, August 1983. T. J. Givnish, ed. Cambridge University Press, London, U.K.

Fine, P. V. A., and Kembel, S. W. 2011. Phylogenetic community structure and phylogenetic turnover across space and edaphic gradients in western Amazonian tree communities. Ecography 34:552-565.

Fortunel, C., Fine, P. V. A., and Baraloto, C. 2012. Leaf, stem and root tissue strategies across 758 neotropical tree species. Funct. Ecol. 26:1153-1161.

Garnier, E., Laurent, G., Bellmann, A., Debain, S., Berthelier, P., Ducout, B., Roumet, C., and Navas, M.-L. 2001. Consistency of species ranking based on functional leaf traits. New Phytol. 152:69-83.

Gomes, T., Pereira, J. A., Benhadi, J., Lino-Neto, T., and Baptista, P. 2018. Endophytic and epiphytic phyllosphere fungal communities are shaped by different environmental factors in a Mediterranean ecosystem. Microb. Ecol. 76:668-679.

Griffin, E. A., and Carson, W. P. 2018. Tree endophytes: Cryptic drivers of tropical forest diversity. Pages 63-103 in: Endophytes of Forest Trees: Biology and Applications. A. M. Pirttilä and A. C. Frank, eds. Springer International Publishing, New York, NY, U.S.A.

Griffin, E. A., Harrison, J. G., Kembel, S. W., Carrell, A. A., Wright, S. J., and Carson, W. P. 2019. Plant host identity and soil macronutrients explain little variation in sapling endophyte community composition: Is disturbance an alternative explanation? J. Ecol. 107:1876-1889.

Haegeman, B., Hamelin, J., Moriarty, J., Neal, P., Dushoff, J., and Weitz, J. S. 2013. Robust estimation of microbial diversity in theory and in practice. ISME J. 7:1092-1101.

Hanson, C. A., Fuhrman, J. A., Horner-Devine, M. C., and Martiny, J. B. H. 2012. Beyond biogeographic patterns: Processes shaping the microbial landscape. Nat. Rev. Microbiol. 10:497-506.

Harrison, J. G., Forister, M. L., Parchman, T. L., and Koch, G. W. 2016. Vertical stratification of the foliar fungal community in the world's tallest trees. Am. J. Bot. 103:2087-2095.

Harrison, J. G., and Griffin, E. A. 2020. The diversity and distribution of endophytes across biomes, plant phylogeny and host tissues: How far have we come and where do we go from here? Environ. Microbiol. 22: 2107-2123.

Hättenschwiler, S., Aeschlimann, B., Coûteaux, M.-M., Roy, J., and Bonal, D. 2008. High variation in foliage and leaf litter chemistry among 45 tree species of a neotropical rainforest community. New Phytol. 179:165-175.

Herrmann, M., Geesink, P., Richter, R., and Küsel, K. 2021. Canopy position has a stronger effect than tree species identity on phyllosphere bacterial diversity in a floodplain hardwood forest. Microb. Ecol. 81:157-168.

Hetherington, A. M., and Woodward, F. I. 2003. The role of stomata in sensing and driving environmental change. Nature 424:901-908.

Ho, A., Lonardo, D. P. D., and Bodelier, P. L. E. 2017. Revisiting life strategy concepts in environmental microbial ecology. FEMS Microbiol. Ecol. 93:fix006.

Hubbell, S. P. 2011. The Unified Neutral Theory of Biodiversity and Biogeography (MPB-32). Monographs in Population Biology. Princeton University Press, Princeton, NJ.

Hunter, P. J., Hand, P., Pink, D., Whipps, J. M., and Bending, G. D. 2010. Both leaf properties and microbe-microbe interactions influence within-species variation in bacterial population diversity and structure in the lettuce (*Lactuca* species) phyllosphere. Appl. Environ. Microbiol. 76:8117-8125.

Izuno, A., Kanzaki, M., Artchawakom, T., Wachrinrat, C., and Isagi, Y. 2016. Vertical structure of phyllosphere fungal communities in a tropical forest in Thailand uncovered by high-throughput sequencing. PLoS One 11:e0166669.

Jost, L., Chao, A., and Chazdon, R. L. 2010. Compositional similarity and β (beta) diversity. Pages 66-84 in: Biological Diversity: Frontiers in Measurement and Assessment. A. E. Magurran and B. J. McGill, eds. Oxford University Press, Oxford, U.K.

Kembel, S. W., and Mueller, R. C. 2014. Plant traits and taxonomy drive host associations in tropical phyllosphere fungal communities. Botany 92: 303-311.

Kembel, S. W., O'Connor, T. K., Arnold, H. K., Hubbell, S. P., Wright, S. J., and Green, J. L. 2014. Relationships between phyllosphere bacterial communities and plant functional traits in a neotropical forest. Proc. Natl. Acad. Sci. U.S.A. 111:13715-13720.

Laforest-Lapointe, I., Messier, C., and Kembel, S. W. 2016. Host species identity, site and time drive temperate tree phyllosphere bacterial community structure. Microbiome 4:27.

Lajoie, G., and Kembel, S. W. 2021. Plant-bacteria associations are phylogenetically structured in the phyllosphere. Mol. Ecol. 30:5572-5587.

Lajoie, G., Maglione, R., and Kembel, S. W. 2020. Adaptive matching between phyllosphere bacteria and their tree hosts in a neotropical forest. Microbiome 8:70.

Lê, S., Josse, J., and Husson, F. 2008. FactoMineR: A package for multivariate analysis. J. Stat. Softw. 25:1-18.

Lemanceau, P., Barret, M., Mazurier, S., Mondy, S., Pivato, B., Fort, T., and Vacher, C. 2017. Plant communication with associated microbiota in the spermosphere, rhizosphere and phyllosphere. Adv. Bot. Res. 82: 101-133.

Leroy, C., Corbara, B., Dejean, A., and Céréghino, R. 2009. Ants mediate foliar structure and nitrogen acquisition in a tank-bromeliad. New Phytol. 183:1124-1133.

Li, D. 2018. hillR: Taxonomic, functional, and phylogenetic diversity and similarity through Hill numbers. J. Open Source Softw. 3:1041.

Louca, S., Parfrey, L. W., and Doebeli, M. 2016. Decoupling function and taxonomy in the global ocean microbiome. Science 353:1272-1277.

Luan, L., Jiang, Y., Cheng, M., Dini-Andreote, F., Sui, Y., Xu, Q., Geisen, S., and Sun, B. 2020. Organism body size structures the soil microbial and nematode community assembly at a continental and global scale. Nat. Commun. 11:6406.

Madin, J. S., Nielsen, D. A., Brbic, M., Corkrey, R., Danko, D., Edwards, K., Engqvist, M. K. M., Fierer, N., Geoghegan, J. L., Gillings, M., Kyrpides, N. C., Litchman, E., Mason, C. E., Moore, L., Nielsen, S. L., Paulsen, I. T., Price, N. D., Reddy, T. B. K., Richards, M. A., Rocha, E. P. C., Schmidt, T. M., Shaaban, H., Shukla, M., Supek, F., Tetu, S. G., Vieira-Silva, S., Wattam, A. R., Westfall, D. A., and Westoby, M. 2020. A synthesis of bacterial and archaeal phenotypic trait data. Sci. Data 7:170.

Markesteijn, L., Poorter, L., and Bongers, F. 2007. Light-dependent leaf trait variation in 43 tropical dry forest tree species. Am. J. Bot. 94:515-525.

Mercier, C., Boyer, F., Bonin, A., and Coissac, E. 2013. SUMATRA and SUMACLUST: Fast and exact comparison and clustering of sequences. Page 27-29 in: Programs and Abstracts of the SeqBio 2013 workshop (Abstract). GdRBIM and gdrIM, Montpellier, France.

Mina, D., Pereira, J. A., Lino-Neto, T., and Baptista, P. 2020. Epiphytic and endophytic bacteria on olive tree phyllosphere: Exploring tissue and cultivar effect. Microb. Ecol. 80:145-157.

Morris, C. E. 2002. Phyllosphere. In: Encyclopedia of Life Sciences. John Wiley & Sons, Ltd., New York, NY, U.S.A.

Murphy, B., Estrella, M. de la, Schley, R., Forest, F., and Klitgård, B. 2017. On the monophyly of *Macrolobium* Schreb., an ecologically diverse neotropical tree genus (Fabaceae-Detarioideae). Int. J. Plant Sci. 179:75-86,

Nelson, M. B., Martiny, A. C., and Martiny, J. B. H. 2016. Global biogeography of microbial nitrogen-cycling traits in soil. Proc. Natl. Acad. Sci. U.S.A. 113:8033-8040.

Nemergut, D. R., Schmidt, S. K., Fukami, T., O'Neill, S. P., Bilinski, T. M., Stanish, L. F., Knelman, J. E., Darcy, J. L., Lynch, R. C., Wickey, P., and Ferrenberg, S. 2013. Patterns and processes of microbial community assembly. Microbiol. Mol. Biol. Rev. 77:342-356.

Oita, S., Ibáñez, A., Lutzoni, F., Miadlikowska, J., Geml, J., Lewis, L. A., Hom, E. F. Y., Carbone, I., U'Ren, J. M., and Arnold, A. E. 2021. Climate and

seasonality drive the richness and composition of tropical fungal endophytes at a landscape scale. Commun. Biol. 4:313.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., and Wagner, H. 2020. vegan: Community Ecology Package. https://CRAN.R-project.org/package=vegan

Omacini, M., Chaneton, E. J., Ghersa, C. M., and Müller, C. B. 2001. Symbiotic fungal endophytes control insect host–parasite interaction webs. Nature 409:78-81.

Op de Beek, M., Lievens, B., Busschaert, P., Declerck, S., Vangronveld, J., and Colpaert, J. V. 2014. Comparison and validation of some ITS primer pairs useful for fungal metabarcoding studies. PLoS One 9:e97629.

Põlme, S., Abarenkov, K., Henrik Nilsson, R., Lindahl, B. D., Clemmensen, K. E., Kauserud, H., Nguyen, N., Kjøller, R., Bates, S. T., Baldrian, P., Frøslev, T. G., et al. 2020. FungalTraits: A user-friendly traits database of fungi and fungus-like stramenopiles. Fungal Diversity 105:1-16.

Poorter, H. 1994. Construction costs and payback time of biomass: A whole plant perspective. Pages 111-127 in: A Whole Plant Perspective on Carbon-Nitrogen Interactions. J. Roy and E. Garnier, eds. SPB Academic Publishing, Amsterdam, The Netherlands.

Poorter, H., Niinemets, Ü., Ntagkas, N., Siebenkäs, A., Mäenpää, M., Matsubara, S., and Pons, T. L. 2019. A meta-analysis of plant responses to light intensity for 70 traits ranging from molecules to whole plant performance. New Phytol. 223:1073-1105.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glöckner, F. O. 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res. 41:D590-D596.

R Core Team. 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Reysenbach, A.-L., and Pace, N. R. 1995. Reliable amplification of hyperthermophilic archaeal 16S rRNA genes by the polymerase chain reaction. Pages 101-107 in: Archaea: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Rho, H., and Kim, S.-H. 2017. Endophyte effects on photosynthesis and water use of plant hosts: A meta-analysis. Pages 43-69 in: Functional Importance of the Plant Microbiome: Implications for Agriculture, Forestry and Bioenergy. S. L. Doty, ed. Springer International Publishing, New York.

Ripley, B. 2021. Support functions and datasets for venables and Ripley's MASS. R package MASS version 7.3-45. https://cran.r-project.org/web/packages/MASS/index.html

Roggy, J. C., Prévost, M. F., Gourbiere, F., Casabianca, H., Garbaye, J., and Domenach, A. M. 1999. Leaf natural 15 N abundance and total N concentration as potential indicators of plant N nutrition in legumes and pioneer species in a rain forest of French Guiana. Oecologia 120:171-182.

Rosado, B. H. P., Almeida, L. C., Alves, L. F., Lambais, M. R., and Oliveira, R. S. 2018. The importance of phyllosphere on plant functional ecology: A phyllo trait manifesto. New Phytol. 219:1145-1149.

Santamaría, J., and Bayman, P. 2005. Fungal epiphytes and endophytes of coffee leaves (*Coffea arabica*). Microb. Ecol. 50:1-8.

Schlechter, R. O., Miebach, M., and Remus-Emsermann, M. N. P. 2019. Driving factors of epiphytic bacterial communities: A review. J. Adv. Res. 19:57-65.

Schneider, C., Rasband, W. S., and Eliceiri, K. W. 2012. NIH Image to ImageJ: 25 Years of image analysis. Nat. Methods 9:671-675.

Shaw, D. C. 2004. Vertical organization of the canopy biota. Pages 73-101 in: Forest Canopies. M. D. Lowman and H. B. Rinker, eds. Academic Press, New York.

Stegen, J. C., Lin, X., Fredrickson, J. K., Chen, X., Kennedy, D. W., Murray, C. J., Rockhold, M. L., and Konopka, A. 2013. Quantifying community assembly processes and identifying features that impose them. ISME J. 7: 2069-2079.

Stone, B. W. G., Weingarten, E. A., and Jackson, C. R. 2018. The role of the phyllosphere microbiome in plant health and function. Annu. Plant Rev. Online. 1:533-556.

Strullu-Derrien, C., Selosse, M.-A., Kenrick, P., and Martin, F. M. 2018. The origin and evolution of mycorrhizal symbioses: From palaeomycology to phylogenomics. New Phytol. 220:1012-1030.

Thuiller, W., Lavorel, S., and Araújo, M. B. 2005. Niche properties and geographical extent as predictors of species sensitivity to climate change. Global Ecol. Biogeogr. 14:347-357.

Trivedi, P., Leach, J. E., Tringe, S. G., Sa, T., and Singh, B. K. 2020. Plant–microbiome interactions: From community assembly to plant health. Nat. Rev. Microbiol. 18:607-621.

Tucker, C. M., Shoemaker, L. G., Davies, K. F., Nemergut, D. R., and Melbourne, B. A. 2016. Differentiating between niche and neutral assembly in metacommunities using null models of β-diversity. Oikos 125:778-789.

Turenne, C. Y., Sanche, S. E., Hoban, D. J., Karlowsky, J. A., and Kabani, A. M. 1999. Rapid identification of fungi by using the ITS2 genetic region and an automated fluorescent capillary electrophoresis system. J. Clin. Microbiol. 37:1846.

Vacher, C., Castagneyrol, B., Jousselin, E., and Schimann, H. 2021. Trees and insects have microbiomes: Consequences for forest health and management. Curr. For. Rep. 7:81-96.

Vacher, C., Hampe, A., Porté, A. J., Sauer, U., Compant, S., and Morris, C. E. 2016. The phyllosphere: Microbial jungle at the plant–climate interface. Annu. Rev. Ecol. Evol. Syst. 47:1-24.

Vass, M., Székely, A. J., Lindström, E. S., and Langenheder, S. 2020. Using null models to compare bacterial and microeukaryotic metacommunity assembly under shifting environmental conditions. Sci. Rep. 10:2455.

Vellend, M. 2010. Conceptual synthesis in community ecology. Q. Rev. Biol. 85:183-206.

Vellend, M. 2016. The Theory of Ecological Communities (MPB-57). Monographs in Population Biology, vol. 57. Princeton University Press, Princeton, NJ.

Vorholt, J. A. 2012. Microbial life in the phyllosphere. Nat. Rev. Microbiol. 10:828-840.

Wang, Q., Garrity, G. M., Tiedje, J. M., and Cole, J. R. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73:5261-5267.

Wei, Y., Lan, G., Wu, Z., Chen, B., Quan, F., Li, M., Sun, S., and Du, H. 2022. Phyllosphere fungal communities of rubber trees exhibited biogeographical patterns, but not bacteria. Environ. Microbiol. 24:3777-3790.

White, T., J., Bruns, T. D., Lee, S. B., and Taylor, J. W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 in: PCR Protocols: A Guide to Methods and Application. M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds. Academic Press, San Diego, CA.

Wickham, H. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.

Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J. H. C., Diemer, M., Flexas, J., Garnier, E., Groom, P. K., Gulias, J., Hikosaka, K., Lamont, B. B., Lee, T., Lee, W., Lusk, C., Midgley, J. J., Navas, M.-L., Niinemets, Ü., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V. I., Roumet, C., Thomas, S. C., Tjoelker, M. G., Veneklaas, E. J., and Villar, R. 2004. The worldwide leaf economics spectrum. Nature 428:821-827.

Yan, K., Han, W., Zhu, Q., Li, C., Dong, Z., and Wang, Y. 2022. Leaf surface microtopography shaping the bacterial community in the phyllosphere: Evidence from 11 tree species. Microbiol. Res. 254:126897.

Yao, H., Sun, X., He, C., Li, X.-C., and Guo, L.-D. 2020. Host identity is more important in structuring bacterial epiphytes than endophytes in a tropical mangrove forest. FEMS Microbiol. Ecol. 96:fiaa038.

Yao, H., Sun, X., He, C., Maitra, P., Li, X.-C., and Guo, L.-D. 2019. Phyllosphere epiphytic and endophytic fungal community and network structures differ in a tropical mangrove ecosystem. Microbiome 7:57.

Zhou, J., and Ning, D. 2017. Stochastic community assembly: Does it matter in microbial ecology? Microbiol. Mol. Biol. Rev. 81:e00002-17.

Zinger, L., Bonin, A., Alsos, I. G., Bálint, M., Bik, H., Boyer, F., Chariton, A. A., Creer, S., Coissac, E., Deagle, B. E., De Barba, M., Dickie, I. A., Dumbrell, A. J., Ficetola, G. F., Fierer, N., Fumagalli, L., Gilbert, M. T. P., Jarman, S., Jumpponen, A., Kauserud, H., Orlando, L., Pansu, J., Pawlowski, J., Tedersoo, L., Thomsen, P. F., Willerslev, E., and Taberlet, P. 2019. DNA metabarcoding—Need for robust experimental designs to draw sound ecological conclusions. Mol. Ecol. 28:1857-1862.

Zinger, L., Donald, J., Brosse, S., Gonzalez, M. A., Iribar, A., Leroy, C., Murienne, J., Orivel, J., Schimann, H., Taberlet, P., and Lopes, C. M. 2020. Advances and prospects of environmental DNA in neotropical rainforests. Pages 331-373 in: Tropical Ecosystems in the 21st Century. Advances in Ecological Research, vol. 62. A. J. Dumbrell, E. C. Turner, and T. M. Fayle, eds. Elsevier, London, U.K.

Zinger, L., Lionnet, C., Benoiston, A.-S., Donald, J., Mercier, C., and Boyer, F. 2021. metabaR: An r package for the evaluation and improvement of DNA metabarcoding data quality. Methods Ecol. Evol. 12:586-592.

Wardeh, M., Risley, C., McIntyre, M. K., Setzkorn, C., and Baylis, M. 2015. Database of host-pathogen and related species interactions, and their global distribution. Sci. Data 2:150049.