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Assembly processes of bacterial endophytic and epiphytic communities in the canopy of
neotropical trees

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Abstract:

Bacterial phyllosphere communities play a key role in the fitness of plants. Understanding the factors and mechanisms shaping them is necessary to predict how trees would respond to a variety of stresses or disturbances. In tropical forests, the steep vertical gradients in light, rainfall or temperature within the canopy are likely to drive the assembly of bacterial communities in leaves. We explored the assembly processes driving epiphytic and endophytic bacterial communities within the canopy of three Neotropical tree species in French Guiana. We used 16S high-throughput sequencing to characterize leaf bacterial communities and measured foliar traits of host trees to describe variations within the canopy. We found that selection by foliar traits was more important in endophytic communities with a particular effect of traits related to space and available nutrients. Epiphytic communities were also subjected to selection by foliar traits but were more prone to dispersal limitation on top of the canopy. This study provides evidence that the assembly bacterial communities in leaves of tropical trees is jointly driven by determinism and stochasticity. Moreover, the clear differences found between endophytic and epiphytic communities emphasize the major role of the plant host, and provide new perspectives to further investigating the phyllosphere-bacteria interactions.

Key-words: assembly rules, bacterial phyllosphere communities, foliar traits, 16S metabarcoding, endophytes, epiphytes, neotropical tree, within-canopy vertical gradient

Plants have a long history of co-evolution with microorganisms and their interactions have proved a key asset to the colonization of land by plants (Strullu-Derrien *et al.*, 2018). Trees, as all plants, interact with microbes. These interactions do not only take place 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 (Rosado *et al.*, 2018; Stone *et al.*, 2018; Chaudhry *et al.*, 2021). Phyllosphere microbial communities (PMCs) have received growing attention in recent years due to their influence on plant fitness (Vorholt, 2012; Vacher *et al.*, 2016; Rosado *et al.*, 2018). In particular, PMCs can modify photosynthesis by inducing an increase in leaf chlorophyll content, or hydraulics through an increase of the stomatal conductance (Rho & Kim, 2017; Rosado *et al.*, 2018). PMCs produce several beneficial effects on plant/insect interactions by reducing predator insect developmental rate, causing feeding deterrence, or by reducing their survival and oviposition (Omacini *et al.*, 2001; Fernandez-Conradi *et al.*, 2018; Vacher *et al.*, 2021). Last, PMCs upregulate host defensive pathways leading to increased seedling survival and a better plant defense (Arnold *et al.*, 2003; Álvarez-Loayza *et al.*, 2011; Christian *et al.*, 2017). Disentangling the factors and mechanisms shaping PMCs should thus improve our understanding of how trees would respond to a variety of stresses or disturbances.

As for communities of macro-organisms, the assembly of PMCS is driven by four fundamental processes: selection (niche-based mechanisms recruiting a particular set of species, i.e. environmental filtering and biotic interactions, Vellend, 2010), ecological drift (neutral stochastic demographic processes, Chase & Myers, 2011; Hubbell, 2011), dispersal (movement and establishment of species across space preventing local extinction through immigration or mass effect, Chase & Myers, 2011; Hanson *et al.*, 2012) and evolutionary diversification

(processes generating new genetic variation, Nemergut *et al.*, 2013). In the case of PMCs, the host may participate in selection and dispersion, e.g. by controlling the quantity and quality of the nutrient pool available, or by producing antimicrobial molecules, thereby selecting particular microbial taxa. The host traits responsible for these selective pressures can be conserved within host phylogenetic clades and leave an imprint on the structure of the microbial community (Kohl, 2020). In the case of a selection by the host, the composition of PMCs should partly result from interactions with the host plant and the host response to its environment, rather than stochastic processes (Brooks *et al.*, 2016; Kohl, 2020). Hence, the effects of host phylogeny and environmental determinants on the beta-diversity of microbial communities have been disentangle in several studies (for a review see Lim & Bordenstein, 2020). However, host genotype or physiology can also exert strong effects on communities independently of host phylogeny (Amato *et al.*, 2019a,b), or environmental conditions may obscure signals (Brooks *et al.*, 2016). Last, priority effects could also play a role in the assembly of plant microbiomes (Zheng & Lin, 2020).

In tropical trees, PMCs remain understudied (Harrison & Griffin, 2020). So far, available studies showed variations of PMCs among tree species (Redford *et al.*, 2010; Kembel *et al.*, 2014), but few have analyzed the processes driving their assembly (Donald *et al.*, 2020). Because of the huge diversity in tree species (Steege *et al.*, 2013) which develop a wide range of ecological strategies (Fortunel *et al.*, 2012; Allié *et al.*, 2015) , partly translated into the chemistry and the morphology of their leaves (Hättenschwiler *et al.*, 2008; Courtois *et al.*, 2012), neotropical trees could thus recruit either generalist or specialist PMCs. Recent studies report, however, contradicting conclusions on the effect of the identity of the host plant species, with some studies reporting a significant effect (Laforest-Lapointe *et al.*, 2016, 2017; Gomes *et al.*, 2018; Yao *et al.*, 2019, 2020), while others reporting no effect (Griffin *et al.*, 2019). Part of this inconsistency may lie in that previous studies did not necessarily consider environmental

variability. Indeed, PMCs are exposed to strong vertical gradients in light and water availability, which have been previously suggested to influence their composition and diversity (Harrison *et al.*, 2016; Izuno *et al.*, 2016) and could also modify community assembly processes themselves. Such gradients are particularly steep in tropical forests, where the biotic and abiotic conditions in the canopy are very different from those of the understory. The forest top canopy is exposed to strong winds (Bittar *et al.*, 2018), sunlight and high temperatures (Shaw, 2004), while the understory receives only 3% of the light and 30% of the rainfalls (Calder, 2001). These environmental heterogeneities are likely to drive PMCs assembly. In addition, PMCs live in two distinct microenvironments: the internal part (endophytic PMCs) and the surface (epiphytic PMCs) of the leaf more exposed to external environmental conditions (Vacher *et al.*, 2016). Consequently, 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 live on the few nutrients that leak to the leaf surface, and the ability to withstand dry environments and UVs, through motility or biofilm formation (Vacher *et al.*, 2016; Chaudhry *et al.*, 2021). In contrast, internal tissues of the leaf are richer in nutrients, but contain more plant defense metabolites suggesting that endophytic and epiphytic communities could be driven by different factors. Accordingly, in mangrove tree species, epiphytic PMCs are more strongly shaped by the host than endophytic ones (Yao *et al.*, 2020). Additionally, in Olive trees, variations of nutrients and environmental conditions between internal and external plant tissues determined strong differences in the structure of bacterial communities with notably a dominance of phyla resistant to desiccation and radiation at the surface of leaves (Mina *et al.*, 2020).

In this study, we quantified the assembly processes shaping epiphytic and endophytic bacterial communities from the bottom to the top of the canopy of three Neotropical tree species in French Guiana. We used DNA metabarcoding to characterize leaf bacterial communities and

measured morphological and chemical foliar traits of host tree to describe variations in the microhabitat of bacteria along the vertical gradient. We specifically tested the hypothesis: (H1) vertical stratification is stronger in epiphytic than in endophytic communities, because epiphytes respond to the within-canopy microclimate gradient ; (H2) the assembly of epiphytic bacterial communities is more deterministic at the top than at the bottom of the canopy, because of harsher abiotic conditions (higher UV radiations and lower humidity) at the top of the canopy ; (H3) foliar traits contribute more to the assembly of endophytic than epiphytic communities, because endophytes are intimately linked to their host tree.

Material and Methods:

Study site and sampling design

Leaf sampling was conducted in October 2017 at the Nouragues Experimental Station (4°02 N, 52°414 W) with the help of COPAS (Canopy Operating Permanent Access System) and tree climbers (Figure 1a). Three tree species abundant in the studied area were selected: *Eperua falcata* Aubl. (Caesalpiniaceae), *Macrolobium bifolium* Aubl. (Caesalpiniaceae) and *Tetragastris* Gaertn. sp (Burseraceae). *E. falcata* is a shade hemi-tolerant dominant species growing in relatively dense clusters and on a large spectrum of edaphic conditions (Roggy *et al.*, 1999; Baraloto *et al.*, 2005). *Macrolobium* is a widespread genus throughout the Amazonian basin (Murphy *et al.*, 2018). *Tetragastris* sp. belongs to the family Burseraceae, an important Neotropical tree lineage known for their chemical defenses including terpenes and phenolics (Fine & 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 (Figure 1b). On each individual, we sampled leaves at 3 heights (Figure 1c): on top of the crown (Top Canopy, TC), in the middle of the crown (Middle Canopy, MC) and on the lower branch of the crown (Bottom Canopy, BC). Three

leaves were collected at each height for DNA analysis. Each leaf was collected in a separate plastic bag to avoid cross contaminations, resulting in 135 leaf samples (3 tree species x 5 individuals x 3 heights x 3 leaves). In addition, we collected 2 leaves for each height and each individual, that were further used for leaf traits measurements. Leaves were sealed immediately after sampling in pre-weighted zip-lock plastic bags and placed in a cooler until return to the lab.

Microclimate measurements

Irradiance at each height (TC, MC, BC) was recorded with a photosynthetically active radiation linear sensor (Acceptor model LP-80 PAR/LAI Ceptometer, Decagon Devices). Instantaneous measurements were realized at the level of sampled leaves for 4 trees and values were then compared to a reference sensor located at the top of a nearby flux tower (NOURAFLUX, nouragues.cnrs.fr), which provide an estimate of the incident irradiance above the canopy (i.e. the full irradiance). For each height, relative irradiance (%) 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, BC) on two trees over 24 hours.

Morphological and chemical leaf traits measurements

We measured 10 leaf traits that may influence the diversity, composition and function of leaf bacterial communities, because they are indicative of (1) water availability, (2) space available for bacterial colonization, (3) sugars availability, or (4) defense metabolism.

To approximate (1) water availability for leaf bacteria, we measured leaf water content (LWC) and stomatal density (SD). The aperture and density of stomata control the exchange of gases

176 (water and carbon dioxide) between the interior of the leaf and the atmosphere, participating in
 177 the control of water by the whole plant (Hetherington & Woodward, 2003). Stomata are also a
 178 major route of bacterial colonization inside the leaf (Chaudhry *et al.*, 2021). To approximate
 179 (2) space available for bacterial colonization, we measured 4 morphological traits: leaf dry
 180 matter content (LDMC), leaf mass per area (LMA), leaf thickness (LT) and leaf area (LA). All
 181 four are informative of the production of biomass by the plant and the density of leaf (Garnier
 182 *et al.*, 2001). To approximate (3) sugars availability for leaf bacteria, we measured Chlorophyll
 183 and Nitrogen contents, which reflect the photosynthesis and the metabolism activities of the
 184 plant (Field & Mooney, 1986; Evans & Seemann, 1989). Finally, leaf carbon content and leaf
 185 construction cost (CCM) were used as proxies of (4) plant defense, given that the defense
 186 response involves a wide array of mechanisms which demand a massive redistribution of energy
 187 (Chaudhry *et al.*, 2021). One sampled leaf was used to measure SD while the other one was
 188 used to measure LWC and all other traits. To measure SD, a band of lamina was fixed with a
 189 FAA buffer (5% formalin, 5% acetic acid, 50% ethanol). Stomatal density of the adaxial
 190 surfaces of the lamina (SD, number of stomata per cm²) was then determined in the lab with
 191 imprints made using transparent nail varnish. The number of stomata was recorded using an
 192 optical microscope for each lamina as the average of five randomly selected areas of 1cm² each
 193 (Leroy *et al.*, 2009). To measure the other traits, fresh weight (fw, g) was determined by
 194 weighing the sealed pre-weighted bags containing the fresh leaves. Leaf area (LA, cm²) of fresh
 195 leaves was measured with a scanner and the ImageJ software (Schneider *et al.*, 2012). Leaf
 196 thickness (LT, μ m) was estimated as the mean of three to eight measurements (four per simple
 197 leaf and one per leaflet for compound leaf) with a digital micrometer (Digimatic micrometer,
 198 Mitutoyo, Japan). We took care to avoid the main veins for these measurements. Chlorophyll
 199 contents (Chl, μ g cm⁻²) were estimated with a portable chlorophyll meter (SPAD-502 Konica-
 200 Minolta, Osaka, Japan). Four to eight SPAD measurements per leaf (one per leaflet for

compound leaf) were averaged to obtain the SPAD estimate of the leaf. We then used the calibration equation proposed by (Coste *et al.*, 2010). Leaves were then dried at 60°C for 72h and weighted (dry weight, g). Leaf water content ($LWC = \frac{fw - dw}{fw}$, %), leaf mass per area ($LMA = \frac{dw}{LA}$, g m⁻²) and leaf dry matter content ($LDMC = \frac{dw}{fw}$, mg g⁻¹) were calculated. Leaves were then ground to determine C, N and ash contents (%) at the USRAVE platform of Bordeaux (France). Leaf specific construction cost (CCM, g glucose g⁻¹ dw) was estimated from C, N and ash content, according to (Poorter, 1994). As in Coste *et al.* (2011), this computation assumes all nitrogen is absorbed as nitrate hence an additional cost for nitrate reduction in non-photosynthetic tissues was taken into account.

DNA sampling

To collect epiphytic DNA, the whole upper and lower surfaces of each leaf were carefully wiped with a piece (2x2 cm) of Whatman paper sterilized by autoclaving (120°C, 20 min) and soaked in sterile CTAB buffer (2% cetyl trimethylammonium bromide, 1% polyvinyl pyrrolidone, 100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA), within 4 hours after leaves harvesting. The Whatman paper was stored in a 2ml Eppendorf tube filled with CTAB for downstream DNA extraction. Surface-sterilization protocol was performed as recommended by Compant *et al.*, (2021) to collect endophytic communities. Each leaf was surface-sterilized in 0.525% sodium hypochlorite (2 min) and 70% ethanol (2 min) and two segments of 2 cm² area from the lamina were cut and stored in a 2ml Eppendorf tube filled with CTAB for downstream DNA extractions close to a field Bunsen burner to create a sterilized environment. This resulted in 270 DNA samples (3 tree species x 5 individuals 3 heights x 3 leaves x 2 DNA types (epiphytic or endophytic)).

DNA extraction, amplification and sequencing

DNA was extracted using a CTAB extraction method (Carrell & Frank, 2014). We added 800 µl of CTAB solution to 0.6 g of tissue, incubated for 2 h at 60°C, and homogenized with glass beads for 3 min. Proteins were removed with the addition of an equal volume of chloroform, centrifuged for 10 min at 16 000 g, and placed the top aqueous phase in a sterile tube. Nucleic acids were precipitated with the addition of 1/10 volume of cold 3 M sodium acetate and 1/2 volume cold isopropanol, froze them at -20°C for 12 h, and centrifuged for 30 min at 16 000 g. The supernatant was discarded, 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 it at -20°C. The V5-V6 region of the bacterial 16S rRNA gene was amplified using the chloroplast-excluding forward primer 799f (Chelius & Triplett, 2001) and the reverse primer 1115R (Reysenbach & Pace, 1995). 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 1x buffer, 0.22 mM dNTP each, 0.45 µM each tagged primer, 2.84 mM MgCl₂, 0.11 mg/ml bovine serum albumin and 0.04 U/µl Taq polymerase (Solis Biodyne). The thermocycling conditions were as follows: 5 min at 94°C, 30 cycles of 30 sec at 94°C, 30 sec at 58°C and 40 sec 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) and sequenced on one run of a MiSeq Illumina platform (FASTERIS SA,) using the paired-end sequencing technology. To control for potential contaminants (Salter *et al.*, 2014) and false positive sequences caused by tag-switching events (Esling *et al.*, 2015) the sequenced multiplexes comprised extractions/PCR negative controls and unused tag combinations.

Bioinformatics

A total of 3,181,610 sequencing reads were obtained and curated using the OBITools3 package (Boyer *et al.*, 2016) and the R software version 3.5.3 (R Core Team 2019) following the procedure described in (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/sequencing errors were removed from the dataset and remaining reads were clustered into operational taxonomic units (OTUs) at 97% similarity using the Sumacust algorithm (Kopylova *et al.* 2016). The most abundant read was considered representative of the OTU and taxonomically assigned using the SILVAngs r138.1 (Quast *et al.*, 2013). Finally, we checked the taxonomic assignment and kept only sequences assigned to the Bacteria kingdom. Curation procedure yielded to 649,212 reads corresponding to 10152 bacterial OTUs in 203 samples. Finally, because the sequencing depths were uneven across samples (Figure S1), the sequencing depth of each sample was standardized by randomly resampling a number of reads equal to the first quartile of read number across samples (n=1864) which removed 51 samples and kept 152 samples for subsequent analyses.

Statistical analyses

All analyzes were conducted using the R software (R Development Core Team) and with packages vegan 2.5-7 (Oksanen *et al.*, 2020), adespatial 1.7-17 (Dray *et al.*, 2021), ape 5.5 (Paradis & Schliep, 2019), phyloseq 1.36.0 (McMurdie & Holmes, 2013), picante 1.8.2 (Kembel *et al.*, 2010), lme4 1.1-27.1 (Bates *et al.*, 2015), FactoMineR 2.4 (Lê *et al.*, 2008) and ggplot2 3.3.5 (Wickham, 2016, p. 2).

The importance of deterministic processes in the assembly of PMCs was assessed by estimating their variation in composition (i.e. beta-diversity) and diversity (i.e. species richness) among host species, within the canopy (TC, MC, BC), within the leaf (epiphytes or endophytes), and across spatial location of the host trees. The diversity and composition of bacterial communities

were assessed using exponential of the Shannon index (see Supplementary) and Bray-Curtis distance (Lou Jost & Chazdon, 2010). Because spatial autocorrelation may arise from both spatially structured environmental factors and dispersal, spatial variation was account for by including Principal Components of Neighbours Matrices (PCNM) spatial eigenvectors based on the geographical coordinates of tree individuals in the model. PCNMs significance were tested by computed Moran's I with 1000 permutations and retained only three PCNMs that showed a significant and positive value of Moran's I. Variations in PMCs composition were estimated by performing a Permutational Multivariate Analyses of Variance (PERMANOVA) with 999 permutations (Anderson, 2001) and dissimilarities among samples were visualized using a Principal Coordinate Analyses (PCA). The model included geographical position of trees (3 significant PCNMs), host species (*E. falcata*, *M. bifolium*, *Tetragastris sp.*), vertical position in the canopy (low, middle and top of the canopy), position on the leaf (epiphytes and endophytes) and their interactions as fixed effects. The homogeneity of variance among groups (host species, position in the canopy and on the leaf) groups were tested as PERMANOVA analysis are sensitive to heterogeneity in within-group variance (Oksanen *et al.*, 2020).

The relationships between foliar traits and the alpha- (Shannon index) and beta-diversity (Bray-Curtis distance) of endophytic and epiphytic communities were assessed using a linear mixed model and a permutational multivariate analysis of variance, respectively. Both models included leaf traits related to water resources (LWC, SD), available space (LDMC, LMA, LT, LA), available sugars (Chlorophyll and N contents) and defense metabolism (CCM and C content) implemented as fixed effects, and host species implemented as random effect. Homogeneity of variance among host species was also tested after performing the PERMANOVA model.

Significant variations of the leaf traits mentioned above were beforehand tested individually by performing an analysis of variance (ANOVA, $p < 0.01$). The model included the vertical

positions in the crown, the host species and their interactions. Normality of each variable was tested with a Shapiro test and variables were transformed with a box-cox when necessary.

To disentangle the relative contribution of niche-based and neutral processes in the assembly of epiphytic and endophytic communities in leaves separately, we implemented a null modeling approach as proposed by Stegen *et al.* (2013) and Vass *et al.* (2020). Pairwise phylogenetic turnover between communities were calculated with the mean-nearest-taxon-distance (β MNTD) metric (Fine & Kembel, 2011) to infer the strength of selection. MUSCLE (Edgar 2004) with default parameters was used to align sequences found in communities of each vertical position (TC, MC, BC). Pairwise distances were computed with a Juke-Cantor model (JC69) and built using a phylogenetic tree with the UPGMA method for each community. The pairwise phylogenetic turnover between communities was calculated as the mean nearest taxon distance metric (β MNTD) while accounting for taxa relative abundance. According to Stegen *et al.* (2013), the degree to which β MNTD deviates from a null model expectation should reflect the degree to which community composition is driven by selection, which is expected to recruit phylogenetically related OTUs due to niche conservatism. To quantify the deviation, we built a null model by shuffling randomly taxa names and abundances across tips of the phylogeny, and then calculated β MNTD. This allowed us to obtain the null distribution of β MNTD, after 999 randomizations. Then, β -nearest taxon index (β NTI) was computed as the difference between observed β MNTD and the mean of the null distribution in units of s.d. of the null distribution. For this procedure we used the R codes provided by (Tripathi *et al.*, 2018).

According to Stegen (2013), when $|\beta$ NTI|>2, the observed difference in phylogenetic turnover between communities is the result of selection. When $|\beta$ NTI|<2, the observed difference in phylogenetic community composition should result from dispersal limitation, ecological drift or homogenizing dispersal. We quantified these processes by computing Bray-Curtis based Raup-Crick metric (RC_{bray}) on pairwise comparisons when $|\beta$ NTI|<2. Following the method

proposed by Dini-Andreote *et al.* (2015), the relative contribution of each process to variations in community composition were estimated as follow: dispersal limitation was estimated as the percentage of pairwise comparisons with $|\beta\text{NTI}| < 2$ and $\text{RC}_{\text{bray}} > +0.95$; homogenizing dispersal was estimated as the percentage of pairwise comparisons with $|\beta\text{NTI}| < 2$ and $\text{RC}_{\text{bray}} < -0.95$; and ecological drift was estimated as the percentage of pairwise comparisons with $|\beta\text{NTI}| < 2$ and $|\text{RC}_{\text{bray}}| < 0.95$. We then computed the contribution of each process (selection, dispersal limitation, homogenizing dispersal or ecological drift) for each position in the canopy in epiphytic and endophytic communities.

Results

Variations in leaf microclimate, physiology and phenology within the canopy and across tropical tree species

Temperature and relative humidity showed no significant differences between the top and the bottom of the canopy (86% \pm 12 % of relative humidity and 26 \pm 3°C), but the relative irradiance varied from 77 % \pm 34% of the PAR at the top of the canopy to 1.55% \pm 1.5% on the soil (Figure 1c). All leaf traits exhibited strong and significant variations among host species and positions within the canopy (Figure 2). Interactions between both factors were only significant for C, N contents and CCM (Figure 2). Most leaf trait variations followed the same trends across the different tree species. Leaf SD, LDMC, LMA, LT and chlorophyll content decreased from the top to the bottom of the canopy while LWC and LA increased.

Leaf bacterial community composition associated to tropical tree species

Leaf bacterial communities were dominated by 6 classes including Alphaproteobacteria (49.06%), Actinobacteria (23.64%), Sphingobacteria (5.79%), Gammaproteobacteria (4.50%), Chloroflexia (5.43%) and Cytophagia (2.44%) (Figure S2). 5 orders represented 76% of the

OTUs: Rhizobiales, Actinomycetales, Sphingomonadales, Sphingobacteriales and Rhodospirillales (Figure S3). We were able to assign 200 families and 630 genera with mainly *Methylobacterium* (12.60%), and *Sphingomonas* (10.58%).

Factors driving leaf bacterial communities at the leaf, the canopy and tree scales

Bacterial community composition varied significantly across host species, geographical position and within the leaf (Table 1). Host species was the main driver of the bacterial assemblage in leaves, explaining 10.7% (Table 1) of bacterial composition and influencing significantly its diversity (Table S1). However, tree species showed no deep differences regarding their bacterial communities at the Class or Family taxonomic rank (Figure S2) indicating that variations of bacterial communities among their host species occur at lower taxonomic ranks. Surprisingly, the variation in community composition within the canopy was less important than differences between leaf inside tissues and its surface suggesting that community assemblage of Bacteria in tropical tree species occur at the leaf spatial scale.

Assembly processes in endophytic and epiphytic communities

In endophytic communities, deterministic assembly processes ($\beta\text{NTI} < |2|$) represented 50.3%, 52.1% and 43.7% at the top, middle and bottom of the canopy respectively (Figure 3a). Overall, no significant difference of pairwise comparisons of βNTI was observed suggesting that deterministic and stochastic factors are equally important in endophytic communities within the canopy. However, when estimating the effect of each process within the canopy we found that endophytic communities were mainly driven by selection (52.10% at TC, 43.7% at MC and 50.3% at BC) and ecological drift (41% at TC, 39.5% at MC and 47 % at BC) whereas dispersal limitation had limited effects at the top (6.8%) and bottom of the canopy (2.6%) (Figure 4a). Interestingly, the latest had more effect in the middle of the canopy (16.8%).

In epiphytic communities, deterministic assembly processes ($\beta\text{NTI} < |2|$) represented 34.1%, 49.6% and 43.1% at the top, middle and bottom of the canopy respectively (Figure 3b). We found significant differences of pairwise comparisons of βNTI values overall among the three vertical positions (Figure 3b). As for endophytic communities, epiphytic communities were also mainly driven by selection (34% at TC, 49.6% at MC and 43.1% at BC) and ecological drift (46.5% at TC, 44% at MC and 50.6% at BC). Dispersal limitation had increasing effect on epiphytic communities from the top to the bottom of the canopy (19.4% at TC, 6.55% at MC and 6.25% at BC, Figure 4b).

Effects of leaf traits in bacterial epiphytic and endophytic communities:

Leaf traits accounted for 21.6 % and 18.4% of the variation in endophytic and epiphytic community composition, respectively (Table 2). In endophytic communities, 9.17% of the variation were explained by LDMC (3.6%), Chlorophyll (2.97%) and C contents (2.60%). In epiphytic communities, 9.17% of the variation were significantly explained by LT (2.57%), Chlorophyll (1.88%), N (2.07%) and C (2.48%) contents. Leaf traits had no significant effects on endophytic and epiphytic alpha diversity (Table S2).

Discussion

Host species is a strong driver of bacterial leaf communities

Bacterial communities in the phyllosphere were largely dominated by Alphaproteobacteria, Actinobacteria and Bacteroidetes which is in line with previous observations in tropical trees (Kembel *et al.*, 2014; Griffin & Carson, 2018; Donald *et al.*, 2020). Proteobacteria are known to carry methylotrophy, nitrification or nitrogen fixation, Bacteroidetes phyllosphere bacteria are often aerobic and pigmented suggesting a good adaptation to this environment and Actinobacteria are plant pathogens, nitrogen-fixers, fungal antagonists or decomposers (Stone

et al., 2018). *Sphingomonas* (Sphingomonales) and *Methylobacterium* (Rhizobiales), the two most abundant genera, are common in phyllosphere communities (Bulgarelli *et al.*, 2013). *Methylobacterium* uses the methanol produced by the leaf metabolism as source of carbon and energy and *Sphingomonas* produces multiple transport proteins suggesting an ability to scavenge diverse plant metabolites (Delmotte *et al.*, 2009). In line with previous studies on tropical tree species (Kembel *et al.* 2014, Laforest-Lapointe *et al.* 2016, Harrison and Griffin 2020), host species were the most important drivers of PMC, together with the geographical factors. Simultaneously, the position on the leaf was also significant to a lesser extent, contrary to what was observed in Laforest-Lapointe *et al.* (2016) and Herrmann *et al.* (2021). Contrary to our first hypothesis, no prominent impact of the vertical stratification on diversity or composition was observed, neither in endophytic or in epiphytic communities.

Deterministic and stochastic processes equally govern the assembly of endophytic and epiphytic communities across the vertical gradient

According to the β -nearest taxon index (β NTI), both deterministic and stochastic processes are jointly responsible for the assembly of leaf PMCs (Stegen *et al.* 2013, Vass *et al.* 2020, Zhou & Ning 2017, Vacher 2016). Niche-based factors included ecological filters imposed by the environment, here we showed a wide variation in microclimate (mainly irradiance) from the top of the canopy to the base of the crown, but also by the host, namely the physiological and morphological variations of leaves across tree species and induced by the irradiance vertical gradient. Overall, and contrary to our expectations, separate analyses of endophytic and epiphytic bacterial communities showed that endophytic communities were more prone to determinism (46%) than epiphytic ones (37%), but with variations according to the position in the canopy. The assembly of the endophytic and epiphytic was also significantly driven by stochastic processes such as dispersal limitation, ecological drift or diversification. The

importance of these effects differed between epiphytes and endophytes communities and across the vertical gradient. Dispersal limitations had more effects on endophytic PMCs assembly in the middle of the crown of the tree whereas it had higher effects at the top of the canopy for epiphytic PMCs. Beside the seed and germination environment, bioaerosols are one of the major sources of the colonization of the leaf by bacteria (Bulgarelli *et al.*, 2013); together with rainfall and subsequent splashing of raindrops (Morris, 2002) or transmission by herbivorous insects or animals (Vacher *et al.*, 2016, 2021). While it is easy to imagine that the top of the canopy is more subject to wind and rainfalls (Bittar *et al.*, 2018), their effects inside the crown are more difficult to understand. Moreover, the contribution of ecological drift (or evolutionary diversification) on the turnovers was similar on endophytic or epiphytic communities and whatever the positions. Here, we cannot distinguish between ecological drift and evolutionary diversification. It is assumed that ecological drift should occur in communities where the strength of deterministic processes is low (Chase & Myers, 2011), which is rather not the case in this study, and interactions between ecological drift and other processes may be important in host-associated microbial communities (Kohl, 2020). On the other hand, host-associated communities should evolve in time in a manner that is congruent with host phylogeny (Brooks *et al.*, 2016; Kohl, 2020; Lim & Bordenstein, 2020).

Epiphytic communities are selected by similar traits as endophytic communities in leaves

The identity of host species and leaf traits related to the plant economics spectrum strongly shaped the composition (beta-diversity) of both endophytic and epiphytic communities. We expect this to be mainly due to the physiology of the leaf, as suggested by our results, as already shown by (Kembel *et al.*, 2014). The impact of leaf resource uptake strategies on bacterial communities can be explained through their impact on local leaf nutrients and water availability for bacteria (Kembel *et al.*, 2014; Lajoie & Kembel, 2021). As expected, foliar traits explained

up to 21.6% and 18.4 % of the variation of beta-diversity between samples in endophytic and epiphytic bacterial communities respectively, which suggests a more important role of leaf attributes on endophytic than on epiphytic communities. Leaf dry matter content for endophytic communities and leaf thickness for epiphytic ones, i.e. traits related to space for colonization, explained similar proportions of variation. The environment at the surface of a leaf and its chemistry (cuticle layer, stomata, hydathodes) is known to create more stressful and nutrient-poor conditions for bacteria. To cope with them, bacteria develop growth strategies such as forming large aggregates, key to successful colonization (Vorholt, 2012; Vacher *et al.*, 2016; Chaudhry *et al.*, 2021). On another hand, stomatal chambers or apoplastic spaces inside leaves are large intercellular spaces known to host microbial communities (Vacher *et al.*, 2016) which can explain the effect of leaf thickness we showed. These host-adapted colonizers are also more tolerant to harmful UV radiation oxidative stress and desiccation, particularly important for top-canopy leaves (Delmotte *et al.*, 2009; Vorholt, 2012). Epiphytic communities were more strongly shaped by traits related to available sugars (3.95% of the variation), namely chlorophyll and nitrogen contents than epiphytic communities (2.97 %), and similarly by traits linked to defense such as carbon content (2.48% and 2.6% of the variation in epiphytic and endophytic communities respectively). These results suggest a selection by nutrients resulting from photosynthesis. Indeed, leaf nitrogen content is linked to the maximum photosynthetic rate (Cornelissen 2003) and correlates with composition of bacterial communities (Kembel *et al.*, 2014; Laforest-Lapointe *et al.*, 2016; Lajoie *et al.*, 2020). Leaf nutrients include also volatile organic compounds such as methanol exported to the surface of leaf (Vacher *et al.*, 2016; Lemanceau *et al.*, 2017), which may explain the slightly higher abundance of *Methylobacterium* we found in epiphytic communities. Interestingly, leaf traits related to water content (leaf water content and stomatal density) showed no impact on communities contrary to what is expected in the literature. Leaf surface microtopographic features is known to regulate the assembly of

the bacterial communities (Vorholt, 2012); in particular stomatal density of leaf vein density because they improve the supply of available water to the surrounding microorganisms (Yan *et al.*, 2022). Last, most of the leaf traits investigated decreased or increased significantly with the vertical light gradient within the canopy, which was expected (Markesteijn *et al.*, 2007; Poorter *et al.*, 2019), and suggest an indirect effect of the irradiance on the composition of endophytic and epiphytic communities in leaves.

To conclude, our study provides evidence that the assembly of bacterial communities in tropical leaves was governed by selection imposed on endophytic communities; and with epiphytic communities more exposed to dispersal limitation on top of the canopy. We found that internal features of leaf were selective factors for epiphytic communities in the same way as for endophytic ones. This emphasizes the global selective pressure (i.e. the phyllosphere effect) of the plant on microbial communities, but with marked differences. However, stochastic process is also important and ecological drift (or evolutionary diversification) was similar in both communities within the canopy, which raises questions about how strongly the phylogeny of the host might also drives the composition of bacterial communities.

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502 Data availability: Leaf traits measurements, all metadata on tree individuals, processed
503 sequence data used for this manuscript, R scripts is available from the Zenodo repository, under
504 DOI: 10.5281/zenodo.5794608.

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506 Literature cited:

507 Allié E, Péliissier R, Engel J, Petronelli P, Freycon V, Deblauwe V, Soucémarianadin L, Weigel
508 J, Baraloto C. 2015. Pervasive Local-Scale Tree-Soil Habitat Association in a Tropical Forest
509 Community. *PLOS ONE* 10: e0141488.

510 Álvarez-Loayza P, White JF, Torres MS, Balslev H, Kristiansen T, Svenning J-C, Gil N. 2011.
511 Light Converts Endosymbiotic Fungus to Pathogen, Influencing Seedling Survival and Niche-
512 Space Filling of a Common Tropical Tree, *Iriartea deltoidea* (J-H Yu, Ed.). *PLoS ONE* 6:
513 e16386.

514 Amato KR, G. Sanders J, Song SJ, Nute M, Metcalf JL, Thompson LR, Morton JT, Amir A, J.
515 McKenzie V, Humphrey G, *et al.* 2019a. Evolutionary trends in host physiology outweigh
516 dietary niche in structuring primate gut microbiomes. *The ISME Journal* 13: 576–587.

517 Amato KR, Mallott EK, McDonald D, Dominy NJ, Goldberg T, Lambert JE, Swedell L,
518 Metcalf JL, Gomez A, Britton GAO, *et al.* 2019b. Convergence of human and Old World
519 monkey gut microbiomes demonstrates the importance of human ecology over phylogeny.
520 *Genome Biology* 20: 201.

521 Anderson MJ. 2001. Permutation tests for univariate or multivariate analysis of variance and
522 regression. *Canadian Journal of Fisheries and Aquatic Sciences* 58: 626–639.

523 Arnold AE, Mejia LC, Kyllö D, Rojas EI, Maynard Z, Robbins N, Herre EA. 2003. Fungal
524 endophytes limit pathogen damage in a tropical tree. *Proceedings of the National Academy of*
525 *Sciences* 100: 15649–15654.

526 Baraloto C, Forget P-M, Goldberg DE. 2005. Seed mass, seedling size and neotropical tree
527 seedling establishment. *Journal of Ecology* 93: 1156–1166.

528 Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting Linear Mixed-Effects Models Using
529 lme4. *Journal of Statistical Software* 67: 1–48.

530 Bittar TB, Pound P, Whitetree A, Moore LD, Stan JTV. 2018. Estimation of Throughfall and
531 Stemflow Bacterial Flux in a Subtropical Oak-Cedar Forest. *Geophysical Research Letters* 45:
532 1410–1418.

533 Boyer F, Mercier C, Bonin A, Le Bras Y, Taberlet P, Coissac E. 2016. obitools: a unix-inspired
534 software package for DNA metabarcoding. *Molecular Ecology Resources* 16: 176–182.

535 Brooks AW, Kohl KD, Brucker RM, van Opstal EJ, Bordenstein SR. 2016. Phyllosymbiosis:
536 Relationships and Functional Effects of Microbial Communities across Host Evolutionary
537 History (D Relman, Ed.). *PLOS Biology* 14: e2000225.

538 Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P. 2013. Structure
539 and Functions of the Bacterial Microbiota of Plants. *Annual Review of Plant Biology* 64: 807–
540 838.

541 Calder IR. 2001. Canopy processes: implications for transpiration, interception and splash
542 induced erosion, ultimately for forest management and water resources. In: Linsenmair KE,
543 Davis AJ, Fiala B, Speight MR, eds. Forestry Sciences. Tropical Forest Canopies: Ecology and
544 Management. Dordrecht: Springer Netherlands, 203–214.

545 Carrell AA, Frank AC. 2014. Pinus flexilis and Picea engelmannii share a simple and consistent
546 needle endophyte microbiota with a potential role in nitrogen fixation. *Frontiers in*
547 *Microbiology* 5.

548 Chase JM, Myers JA. 2011. Disentangling the importance of ecological niches from stochastic
549 processes across scales. *Philosophical Transactions of the Royal Society B: Biological Sciences*
550 366: 2351–2363.

551 Chaudhry V, Runge P, Sengupta P, Doehlemann G, Parker JE, Kemen E. 2021. Shaping the
552 leaf microbiota: plant–microbe–microbe interactions (S Kopriva, Ed.). *Journal of Experimental*
553 *Botany* 72: 36–56.

554 Chelius MK, Triplett EW. 2001. The Diversity of Archaea and Bacteria in Association with the
555 Roots of *Zea mays* L. *Microbial Ecology* 41: 252–263.

556 Christian N, Herre EA, Mejia LC, Clay K. 2017. Exposure to the leaf litter microbiome of
557 healthy adults protects seedlings from pathogen damage. *Proceedings of the Royal Society B:*
558 *Biological Sciences* 284: 20170641.

559 Compant S, Cambon MC, Vacher C, Mitter B, Samad A, Sessitsch A. 2021. The plant
560 endosphere world – bacterial life within plants. *Environmental Microbiology* 23: 1812–1829.

561 Coste S, Baraloto C, Leroy C, Marcon É, Renaud A, Richardson AD, Roggy J-C, Schimann H,
562 Uddling J, Hérault B. 2010. Assessing foliar chlorophyll contents with the SPAD-502
563 chlorophyll meter: a calibration test with thirteen tree species of tropical rainforest in French
564 Guiana. *Annals of Forest Science* 67: 607–607.

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

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

571 Delmotte N, Knief C, Chaffron S, Innerebner G, Roschitzki B, Schlapbach R, von Mering C,
572 Vorholt JA. 2009. Community proteogenomics reveals insights into the physiology of
573 phyllosphere bacteria. *Proceedings of the National Academy of Sciences* 106: 16428–16433.

574 Dini-Andreote F, Stegen JC, van Elsas JD, Salles JF. 2015. Disentangling mechanisms that
575 mediate the balance between stochastic and deterministic processes in microbial succession.

576 *Proceedings of the National Academy of Sciences* 112: E1326–E1332.

577 Donald J, Roy M, Suescun U, Iribar A, Manzi S, Péllissier L, Gaucher P, Chave J. 2020. A test
 578 of community assembly rules using foliar endophytes from a tropical forest canopy. *Journal of*
 579 *Ecology* 108: 1605–1616.

580 Dray S, Bauman D, Blanchet G, Borcard D, Clappe S, Guenard G, Jombart T, Larocque G,
 581 Legendre P, Madi N, *et al.* 2021. *adespatial: Multivariate Multiscale Spatial Analysis*.

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

584 Evans J, Seemann JR. 1989. The allocation of protein nitrogen in the photosynthetic apparatus:
 585 Costs, consequences, and control. *Photosyn Thesis*: 183–205.

586 Fernandez-Conradi P, Jactel H, Robin C, Tack AJM, Castagneyrol B. 2018. Fungi reduce
 587 preference and performance of insect herbivores on challenged plants. *Ecology* 99: 300–311.

588 Field C, Mooney HA. 1986. photosynthesis--nitrogen relationship in wild plants. *On the*
 589 *economy of plant form and function : proceedings of the Sixth Maria Moors Cabot Symposium,*
 590 *Evolutionary Constraints on Primary Productivity, Adaptive Patterns of Energy Capture in*
 591 *Plants, Harvard Forest, August 1983*.

592 Fine PVA, Kembel SW. 2011. Phylogenetic community structure and phylogenetic turnover
 593 across space and edaphic gradients in western Amazonian tree communities. *Ecography* 34:
 594 552–565.

595 Fortunel C, Fine PVA, Baraloto C. 2012. Leaf, stem and root tissue strategies across 758
 596 Neotropical tree species. *Functional Ecology* 26: 1153–1161.

597 Garnier E, Laurent G, Bellmann A, Debain S, Berthelie P, Ducout B, Roumet C, Navas M-L.
 598 2001. Consistency of species ranking based on functional leaf traits. *New Phytologist* 152: 69–
 599 83.

600 Gomes T, Pereira JA, Benhadi J, Lino-Neto T, Baptista P. 2018. Endophytic and Epiphytic

601 Phyllosphere Fungal Communities Are Shaped by Different Environmental Factors in a
602 Mediterranean Ecosystem. *Microbial Ecology* 76: 668–679.

603 Griffin EA, Carson WP. 2018. Tree Endophytes: Cryptic Drivers of Tropical Forest Diversity.
604 In: Pirttilä AM, Frank AC, eds. Forestry Sciences. Endophytes of Forest Trees: Biology and
605 Applications. Cham: Springer International Publishing, 63–103.

606 Griffin EA, Harrison JG, Kembel SW, Carrell AA, Wright SJ, Carson WP. 2019. Plant host
607 identity and soil macronutrients explain little variation in sapling endophyte community
608 composition: Is disturbance an alternative explanation? *Journal of Ecology* 107: 1876–1889.

609 Hanson CA, Fuhrman JA, Horner-Devine MC, Martiny JBH, Hanson CA. 2012. Beyond
610 biogeographic patterns: processes shaping the microbial landscape. *Nat. Rev. Microbiol.*: 497–
611 506.

612 Harrison JG, Forister ML, Parchman TL, Koch GW. 2016. Vertical stratification of the foliar
613 fungal community in the world’s tallest trees. *American Journal of Botany* 103: 2087–2095.

614 Harrison JG, Griffin EA. 2020. The diversity and distribution of endophytes across biomes,
615 plant phylogeny and host tissues: how far have we come and where do we go from here?
616 *Environmental Microbiology* 22: 2107–2123.

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

620 Hetherington AM, Woodward FI. 2003. The role of stomata in sensing and driving
621 environmental change. *Nature* 424: 901–908.

622 Hubbell SP. 2011. *The Unified Neutral Theory of Biodiversity and Biogeography (MPB-32)*.
623 Princeton University Press.

624 Izuno A, Kanzaki M, Artchawakom T, Wachrinrat C, Isagi Y. 2016. Vertical Structure of
625 Phyllosphere Fungal Communities in a Tropical Forest in Thailand Uncovered by High-

626 Throughput Sequencing (AM Yurkov, Ed.). *PLOS ONE* 11: e0166669.

627 Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP,
628 Webb CO. 2010. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26:
629 1463–1464.

630 Kembel SW, O’Connor TK, Arnold HK, Hubbell SP, Wright SJ, Green JL. 2014. Relationships
631 between phyllosphere bacterial communities and plant functional traits in a neotropical forest.
632 *Proceedings of the National Academy of Sciences* 111: 13715–13720.

633 Kohl KD. 2020. Ecological and evolutionary mechanisms underlying patterns of
634 phyllosymbiosis in host-associated microbial communities. *Philosophical Transactions of the*
635 *Royal Society B: Biological Sciences* 375: 20190251.

636 Kopylova E, Navas-Molina JA, Mercier C, Xu ZZ, Mahé F, He Y, Zhou H-W, Rognes T,
637 Caporaso JG, Knight R. 2016. Open-Source Sequence Clustering Methods Improve the State
638 Of the Art. *mSystems* 1: e00003-15.

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

641 Laforest-Lapointe I, Paquette A, Messier C, Kembel SW. 2017. Leaf bacterial diversity
642 mediates plant diversity and ecosystem function relationships. *Nature* 546: 145–147.

643 Lajoie G, Kembel SW. 2021. Plant-bacteria associations are phylogenetically structured in the
644 phyllosphere. *Molecular Ecology* 00: 1–16.

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

647 Lê S, Josse J, Husson F. 2008. FactoMineR: A Package for Multivariate Analysis. *Journal of*
648 *Statistical Software* 25: 1–18.

649 Lemanceau P, Barret M, Mazurier S, Mondy S, Pivato B, Fort T, Vacher C. 2017. *Plant*
650 *communication with associated microbiota in the spermosphere, rhizosphere and phyllosphere.*

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

653 Lim SJ, Bordenstein SR. 2020. An introduction to phylosymbiosis. *Proceedings of the Royal
654 Society B: Biological Sciences* 287: 20192900.

655 Lou Jost AC, Chazdon RL. 2010. Compositional similarity and β (beta) diversity.

656 Markesteijn L, Poorter L, Bongers F. 2007. Light-dependent leaf trait variation in 43 tropical
657 dry forest tree species. *American Journal of Botany* 94: 515–525.

658 McMurdie PJ, Holmes S. 2013. phyloseq: An R Package for Reproducible Interactive Analysis
659 and Graphics of Microbiome Census Data (M Watson, Ed.). *PLoS ONE* 8: e61217.

660 Mina D, Pereira JA, Lino-Neto T, Baptista P. 2020. Epiphytic and Endophytic Bacteria on Olive
661 Tree Phyllosphere: Exploring Tissue and Cultivar Effect. *Microbial Ecology* 80: 145–157.

662 Morris CE. 2002. Phyllosphere. In: eLS. American Cancer Society.

663 Murphy B, de la Estrella M, Schley R, Forest F, Klitgård B. 2018. On the Monophyly of
664 *Macrolobium* Schreb., an Ecologically Diverse Neotropical Tree Genus (Fabaceae-
665 Detarioideae). *International Journal of Plant Sciences* 179: 75–86.

666 Nemergut DR, Schmidt SK, Fukami T, O'Neill SP, Bilinski TM, Stanish LF, Knelman JE,
667 Darcy JL, Lynch RC, Wickey P, *et al.* 2013. Patterns and Processes of Microbial Community
668 Assembly. *Microbiology and Molecular Biology Reviews* 77: 342–356.

669 Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara
670 RB, Simpson GL, Solymos P, *et al.* 2020. *vegan: Community Ecology Package*.

671 Omacini M, Chaneton EJ, Ghersa CM, Müller CB. 2001. Symbiotic fungal endophytes control
672 insect host–parasite interaction webs. *Nature* 409: 78–81.

673 Paradis E, Schliep K. 2019. ape 5.0: an environment for modern phylogenetics and evolutionary
674 analyses in R. *Bioinformatics* 35: 526–528.

675 Poorter H. 1994. Construction costs and payback time of biomass: a whole plant perspective.

676 Poorter H, Niinemets Ü, Ntagkas N, Siebenkäs A, Mäenpää M, Matsubara S, Pons ThijsL.
 677 2019. A meta-analysis of plant responses to light intensity for 70 traits ranging from molecules
 678 to whole plant performance. *New Phytologist* 223: 1073–1105.
 679 Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013.
 680 The SILVA ribosomal RNA gene database project: improved data processing and web-based
 681 tools. *Nucleic Acids Research* 41: D590–D596.
 682 Redford AJ, Bowers RM, Knight R, Linhart Y, Fierer N. 2010. The ecology of the phyllosphere:
 683 geographic and phylogenetic variability in the distribution of bacteria on tree leaves.
 684 *Environmental Microbiology* 12: 2885–2893.
 685 Reysenbach A-L, Pace NR. 1995. Reliable amplification of hyperthermophilic archaeal 16S
 686 rRNA genes by the polymerase chain reaction. *Archaea: a laboratory manual*. Cold Spring
 687 Harbor Laboratory Press, Cold Spring Harbor, NY: 101–107.
 688 Rho H, Kim S-H. 2017. Endophyte Effects on Photosynthesis and Water Use of Plant Hosts: A
 689 Meta-Analysis. In: Doty SL, ed. Functional Importance of the Plant Microbiome: Implications
 690 for Agriculture, Forestry and Bioenergy. Cham: Springer International Publishing, 43–69.
 691 Roggy JC, Prévost MF, Gourbiere F, Casabianca H, Garbaye J, Domenach AM. 1999. Leaf
 692 natural ^{15}N abundance and total N concentration as potential indicators of plant N nutrition in
 693 legumes and pioneer species in a rain forest of French Guiana. *Oecologia* 120: 171–182.
 694 Rosado BHP, Almeida LC, Alves LF, Lambais MR, Oliveira RS. 2018. The importance of
 695 phyllosphere on plant functional ecology: a phyllo trait manifesto. *New Phytologist* 219: 1145–
 696 1149.
 697 Salter SJ, Cox MJ, Turek EM, Calus ST, Cookson WO, Moffatt MF, Turner P, Parkhill J,
 698 Loman NJ, Walker AW. 2014. Reagent and laboratory contamination can critically impact
 699 sequence-based microbiome analyses. *BMC Biology* 12: 87.
 700 Schneider C, Rasband W, Eliceiri K. 2012. NIH Image to ImageJ: 25 years of image analysis.

701 *Nature Methods* 9.

702 Shaw DC. 2004. Vertical organization of the canopy biota. In: Forest Canopies. Academic
703 Press, 73–101.

704 Steege H ter, Pitman NCA, Sabatier D, Baraloto C, Salomão RP, Guevara JE, Phillips OL,
705 Castilho CV, Magnusson WE, Molino J-F, *et al.* 2013. Hyperdominance in the Amazonian Tree
706 Flora. *Science* 342.

707 Stegen JC, Lin X, Fredrickson JK, Chen X, Kennedy DW, Murray CJ, Rockhold ML, Konopka
708 A. 2013. Quantifying community assembly processes and identifying features that impose
709 them. *The ISME Journal* 7: 2069–2079.

710 Stone BWG, Weingarten EA, Jackson CR. 2018. The Role of the Phyllosphere Microbiome in
711 Plant Health and Function. In: Annual Plant Reviews online. American Cancer Society, 533–
712 556.

713 Strullu-Derrien C, Selosse M-A, Kenrick P, Martin FM. 2018. The origin and evolution of
714 mycorrhizal symbioses: from palaeomycology to phylogenomics. *New Phytologist* 220: 1012–
715 1030.

716 Tripathi BM, Stegen JC, Kim M, Dong K, Adams JM, Lee YK. 2018. Soil pH mediates the
717 balance between stochastic and deterministic assembly of bacteria. *The ISME Journal* 12:
718 1072–1083.

719 Vacher C, Castagneyrol B, Jouselin E, Schimann H. 2021. Trees and Insects Have
720 Microbiomes: Consequences for Forest Health and Management. *Current Forestry Reports* 7:
721 81–96.

722 Vacher C, Hampe A, Porté AJ, Sauer U, Compant S, Morris CE. 2016. The Phyllosphere:
723 Microbial Jungle at the Plant–Climate Interface. *Annual Review of Ecology, Evolution, and*
724 *Systematics* 47: 1–24.

725 Vass M, Székely AJ, Lindström ES, Langenheder S. 2020. Using null models to compare

bacterial and microeukaryotic metacommunity assembly under shifting environmental conditions. *Scientific Reports* 10: 2455.

Vellend M. 2010. Conceptual Synthesis in Community Ecology. *The Quarterly review of biology* 85: 183–206.

Vorholt JA. 2012. Microbial life in the phyllosphere. *Nature Reviews Microbiology* 10: 828–840.

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

Yan K, Han W, Zhu Q, Li C, Dong Z, Wang Y. 2022. Leaf surface microtopography shaping the bacterial community in the phyllosphere: evidence from 11 tree species. *Microbiological Research* 254: 126897.

Yao H, Sun X, He C, Li X-C, Guo L-D. 2020. Host identity is more important in structuring bacterial epiphytes than endophytes in a tropical mangrove forest. *FEMS Microbiology Ecology* 96.

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

Zheng Y, Lin X. 2020. Niche Specialization and Functional Overlap of Bamboo Leaf and Root Microbiota. *Frontiers in Microbiology* 11.

Zinger L, Taberlet P, Schimann H, Bonin A, Boyer F, Barba MD, Gaucher P, Gielly L, Giguet-Covex C, Iribar A, *et al.* 2019. Body size determines soil community assembly in a tropical forest. *Molecular Ecology* 28: 528–543.

Tables:

Table 1: Bacterial community structure explained by host species, vertical position in the canopy, position on the leaf (epiphyte vs endophyte), their interactions and geographical position. PERMANOVA analysis of Bray-Curtis dissimilarities. Statistical significance was assessed from 999 permutations

	Df	SumSq	R ²	F	Pr(>F)
Host species (H)	2	5.167	0.107	9.676	0.001
Position in the canopy (C)	2	0.484	0.010	0.907	0.605

Position in the leaf (L)	1	0.857	0.018	3.208	0.001
MEM1	1	1.13	0.023	4.233	0.001
MEM2	1	0.35	0.007	1.311	0.123
MEM3	1	0.425	0.009	1.593	0.043
(H)x(C)	4	0.92	0.019	0.861	0.797
(H)x(L)	2	2.503	0.052	4.688	0.001
(C)x(L)	2	0.589	0.012	1.103	0.287
(H)x(C)x(L)	4	0.819	0.017	0.767	0.961
Residual	131	34.979	0.725		
Total	151	48.223	1.000		

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788 Table 2: Results of a PERMANOVA testing the effects of 10 leaf traits categorized in 4 groups on dissimilarity matrices of endophytic and
 789 epiphytic communities, host species was implemented as random factor and 1000 permutations were done. LWC: Leaf water content, SD: Stomatal
 790 density, LDMC: Leaf Dry Matter Content, LMA: Leaf Mass per Area, LA: Leaf Area, LT: Leaf Thickness, CCM: Leaf construction cost.
 791 Significant p-values (<0.05) and associated Rsquare are indicated in bold. The direction of variation of values of foliar traits from the top (TC) to
 792 the bottom (BC) of the canopy is also indicated (but see also Figure 2)

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		Variations	ENDOPHYTES					EPIPHYTES				
		from TC to	Df	SS	F	R ²	Pr(>F)	Df	SS	F	R ²	Pr(>F)
		BC										
Leaf water resources	LWC	increase	1	0.316	0.943	0.0164	0.511	1	0.234	0.846	0.0081	0.606
	SD	decrease	1	0.326	0.972	0.0169	0.526	1	0.422	1.526	0.0146	0.139
Space for colonization	LDMC	decrease	1	0.693	2.069	0.036	0.059	1	0.612	2.215	0.0212	0.641
	LMA	decrease	1	0.345	1.031	0.0179	0.817	1	0.518	1.875	0.018	0.441
	LA	increase	1	0.333	0.993	0.0173	0.527	1	0.434	1.571	0.015	0.139
	LT	decrease	1	0.403	1.203	0.0209	0.155	1	0.741	2.682	0.0257	0.006

Available sugars	Chlorophyll content	decrease	1	0.571	1.704	0.0297	0.015	1	0.541	1.958	0.0188	0.028
	N content	stable	1	0.326	0.972	0.0169	0.458	1	0.598	2.164	0.0207	0.018
Defense metabolism	C content	stable	1	0.5	1.493	0.026	0.037	1	0.714	2.584	0.0248	0.004
	CCM	decrease	1	0.333	0.994	0.0173	0.326	1	0.475	1.72	0.0165	0.055
Residual			45	15.08		0.784		85	23.494		0.816	
Total			55	19.226		1		95	28.785		1	

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Figures captions

Figure 1: a) Localization of the sampling site in French Guiana, b) localization of each tree in the sampling area, c) variation of light, temperature and humidity levels along the vertical gradient, d) Correlation circle of variables on the two first axis from a PCA performed on the 10 leaf traits. e) PCA of individual leaves on the two first PCA axes with open circles, filled circles and triangles corresponding to leaves from *E.falcata*, *M.bifolium* and *T. sp.* respectively. Axes 1 and 2 represent, respectively, 50 and 17% of the overall leaves' inertia. e and f) Contributions of each variable to the two first dimensions of the PCA.

Figure 2: Variations of each individual foliar leaf trait in the canopy and between host species. Differences between host species, position in the canopy and their interactions were tested with an analysis of variance. Normality of each variable was tested with a Shapiro test and the variable was transformed with a box-cox when necessary. Significant p-values are indicated ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; NS, non-significant. LWC: Leaf water content in percent, SD: Stomatal density in number.cm², LDMC: Leaf Dry Matter Content in mg.g⁻¹, LMA: Leaf Mass per Area in g.m⁻², LA: Leaf Area in cm², LT: Leaf Thickness in μm , Chlorophyll content in $\mu\text{g.cm}^{-2}$, CCM: Leaf construction cost in g of Glucose.g⁻¹, C and N: Carbon and Nitrogen contents in g.g⁻¹. E.f = *Eperua falcata*; M.b = *Macrolobium bifolium*; T.sp = *Tetragastris sp*; white = top canopy, light grey = middle canopy, dark grey = bottom canopy.

Figure 3: Patterns of between-community nearest taxon intext (βNTI) in bacterial endophytic (a) and epiphytic (b) communities within the top (TC), the middle (MC) and the bottom (BC) of the canopy. Box plots of βNTI distributions show the median (thick line), the first quartile (lower box bound), the third quartile (upper box bound), the range of data that deviate from the box no more than 1.5 times the height of the box (vertical lines). p-values resulting from a

821 multiple comparisons Kruskal-Wallis test are shown. Horizontal dashed red lines indicate
822 upper and lower significance threshold at $bNTI = +2$ and -2 respectively.

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824 Figure 4: Relative contribution of deterministic (selection by environment, biotic interactions
825 or nonrandom dispersal) and stochastic processes (dispersal limitation, mass effect, ecological
826 drift or diversification) for each vertical position in the assembly of bacterial endophytic (a)
827 and epiphytic (b) communities. White color corresponds to dispersal limitation or mass effect,
828 grey to ecological drift or diversification and dark grey to selection.