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Microbiomes of the Amazon forest: bacterial diversity and community structure in the phyllosphere, litter and soil

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Thesis presented to obtain the degree of Doctor in Science.
Area: Soil and Plant Nutrition

Piracicaba
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1 Interações planta-bactéria 2. Florestas tropicais 3. 16S rRNA 4. Biodiversidade 5. Seca I. Título

This thesis is dedicated to my family
for their love, endless support
and encouragement.

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CONTENT

RESUMO

Microbiomas da floresta Amazônica: diversidade e estrutura da comunidade bacteriana na filosfera, serapilheira e solo

Biomas flroestais cobrem aproximadamente 38 milhões de km² do globo terrestre, dos quais um terço é representado por florestas tropicais e subtropicais. Dentre esses biomas, a floresta Amazônica é uma das mais importantes, uma vez que possui papéis chave na regulação climética e na manutenção da diversidade vegetal, animal e microbiana. A floresta Amazônica representa 60 % do território brasileiro e tem sido constantemente ameaçada pela expanção da agricultura e pecuária. A redução dos níveis de biodiversidade na floresta Amazônica podem resultar em impactos graves e desconhecidos na estabilidade do bioma, uma vez que es papéis desempenhados por microganismos são desconhecidos. Em geral, a diversidade e estrutura da comunidade microbiana na floresta Amazônica, bem como os fatores que moldam essas comunidade são pouco estudados. Tem sido observado na Mata Atlântica que comunidades bacterianas associadas a filosfera, dermosfera e rizosfera de diversas espécies vegetais são únicas e dependentes da espécie vegetal. Com o intuito de revelar quais são os fatores moduladores das comunidades bacterianas associadas a espécies vegetais em micro-ambientes específicos da floresta Amazônica, nós avaliamos a comunidade bacteriana associada a filosfera, serapilheira e solo rizosférico de nove espécies vegetais em três épocas ao longo de um ano em uma parcela natural de floresta Amazônica no Brasil, utilizando plataforma de sequenciamento de alto rendimento do gene 16S rRNA. Nossos resultados destacam que alfa diversidade bateriana na rizosfera é maior que na filosfera. Contudo, a filosfera apresentou alto níveis de heterogeneidade, (altos valores de beta diversidade). Nós também observamos que a extreme seca ocasionada durante o evento climático ENSO 2015-2016 afetou principalmente as comunidade bacterianas na filosfera, induzindo a diminuição da alfa diversidade e o aumento da beta diversidade. Nossos resultados também mostraram que a espécie vegetal e parâmetros funcionais relaciados a espécie vegetal foram importantes moduladores das comunidades bacterianas na floresta Amazônica. Em geral, nossos dados indicam que a embora a espécie vegetal seja um importante determinante das comunidades bacterianas associadas a filosfera, serapilhiera e solo rizosférico, eventos climáticos extremos (tal como secas severas) podem induzir significantes mudanças na diversidade e estrutura das comunidades bacterianas das espécies vegetais da floresta Amazônica, com possíveis mudanças na funcionalidade.

Palavras-chave: Interações planta-bactéria; Florestas tropicais; 16S rRNA; Biodiversidade; Seca

ABSTRACT

Microbiomes of the Amazon forest: bacterial diversity and community structure in the phyllosphere, litter and soil

Forest biomes cover approximately 38 million km² worldwide, from which one third represent tropical and subtropical forests. Among these biomes, the Amazon forest is one of the most important for its roles in global climate regulation and dueling high levels of plant, animal and microbial diversity. The Amazon forest represents 60% of Brazilian territory and has been constantly threatened by the expansion of agricultural and animal husbandry areas. The reduction of the biodiversity levels in the Amazon may result in unforeseen impacts on the stability of the biome. The role of the microorganisms in this process is unknown. In general, the knowledge about the microbial diversity and community structure in the Amazon forest, as well the drivers of these community are poorly understood. It has been observed in the Brazilian Atlantic forest that the bacterial communities associated to the phyllosphere, dermosphere and rhizosphere of several tree species are unique and depend on the plant taxon. In order to unravel the drivers of the bacterial communities associated to plants of the Amazon forest in specific microenvironments, we evaluated the bacterial communities associated with the phyllosphere, litter and rhizospheric soil of nine tree species at three time points in a pristine Amazon forest in Brazil, using high-throughput sequencing of 16S rRNA genes. Our results showed that bacterial alpha diversity in the rhizosphere is higher than in the phyllosphere. However, the phyllosphere showed higher levels of heterogeneity (i.e. higher beta diversity). We also observed that an extreme drought during the ENSO 2015-2016 affected mainly the phyllosphere bacterial communities, inducing decreases in alpha diversity and increases in beta diversity. Our results also showed that plant species and plant functional traits are important drivers of the bacterial communities in the Amazon forest. In general, our data indicate that even though plant species is an important determinant of phyllosphere, litter and rhizospheric soil bacterial community structures, extreme climatic events (such as drought) may induce significant changes in bacterial diversity and community structure of the Amazon forest trees, with possible changes in functionality.

Keywords: Plant-bacteria interaction; Tropical forest; 16S rRNA; Biodiversity; Drought

1. INTRODUCTION

Forest biomes may harbor more than three trillion trees and cover approximately 38 million km^2. One third of these trees are in tropical and subtropical forest biomes (Crowther et al., 2015). In Brazil, the Amazon forest covers approximately five million km^2, representing 60% of country territory (Tilio Neto, 2010). The Amazon forest is a global reservoir of biodiversity, harboring at least 10% of the global biodiversity, which is responsible for key roles in the regulation of the forest functioning, such as biogeochemical cycling, with possible consequences in global climate regulation.

Microbiomes refer to entire habitats, including the microorganisms, their genomes and environmental conditions (Marchesi and Ravel, 2015), even though some authors refer to microbiomes as the collection of all microorganisms of a specific environment. Forests harbor several habitats that can be colonized by microorganisms, such as the leaf surfaces, bark surface, roots and rhizosphere, the interior of the plants, litter, soil, rock surfaces, and the atmosphere, each one with specific features, such as nutrient availability or temporal dynamics and specific drivers that affect microbial abundance, as well as the composition of their communities (Baldrian, 2016).

One of most important forest microbiomes is the phyllosphere, comprising one of the largest habitats for microbial life, with a global surface area of approximately 6.4×10^8 km^2 (Morris and Kinkel, 2002;Ledford, 2015). It has been estimated that the overall phyllosphere may contain more than 10^{26} bacterial cells (Lindow and Brandl, 2003), mostly representing species not yet described (Lambais et al., 2006). A broad range of factors can modulate the microbial communities in the phyllosphere, including plant genotype and physiology (e.g. plant traits) (Lambais et al., 2014;Rosado et al., 2018). Besides, environmental factors, such as UV light, extreme temperatures, water availability, osmotic conditions, high concentration of reactive oxygen species and low availability of nutrients may also affect microbial communities in the phyllosphere (Vorholt, 2012).

Forest litter is composed mainly by leaves, but may also contain brunches, flowers and fruits from surrounding plants. Leaf litter is the major source of organic matter in soils under tropical forests (Baldrian, 2016), and is a key substrate for nutrient cycling (Chave et al., 2010). Microorganisms play important roles in litter decomposition, and their communities are highly affected by litter attributes, such as pH and C:N ratios (Smith et al., 2015;Urbanová et al., 2015).

It has been proposed that shifts in leaf litter composition, as well as plant root inputs can promote changes in the soil microbial communities (Bardgett et al., 2005). However, one of

the most important factors determining the microbial community structure in the rhizosphere is the plant species (Grayston et al., 1998;Berg and Smalla, 2009;Lambais et al., 2014). Plant species, through root exudates, select specific groups of microorganisms, and determine the structure of the microbial communities in the rhizosphere (Chaparro et al., 2013;Sasse et al., 2018). Soil attributes, such as pH, moisture, organic matter and nutrient concentration, also strongly affect the rhizosphere microbial communities (Grayston et al., 1998;Berg and Smalla, 2009;Richter et al., 2018). In contrast, microbial communities in the rhizosphere may play key roles in regulating plant growth and development controlling nutrient availability and uptake, hormone metabolism and defense responses (Matson et al., 2015).

A range of factors, including random deposition following atmospheric dispersal, stochastic events, geographical location, exposure to UV radiation, high temperatures, desiccation and plant species and stage of development (DeForest et al., 2004;Stegen et al., 2012;Lebeis, 2015), may drive the dynamic changes in microbial communities over time (Peñuelas et al., 2012). Even though reversible changes in the microbial communities occur over short periods of time, irreversible changes might occur over long periods of time due to extreme climate events, whose frequency has increased significantly in the past years (Jiménez-Muñoz et al., 2013;Gloor et al., 2015).

One of the most important sources of natural climatic variability in the Amazon forest is the El Niño-Southern Oscillation (ENSO) (Collins et al., 2010). Approximately half the Amazon exhibits clear seasonality in rainfall and is subject to additional high-magnitude water deficits triggered by positive phases of the ENSO (Marengo et al., 2011;Jiménez-Muñoz et al., 2016), as it has been recorded in the last decade (Marengo et al., 2008;Espinoza et al., 2011;Lewis et al., 2011;Marengo et al., 2011). The last extreme drought at Amazon forest was induced by the El Niño 2015–2016 (Jiménez-Muñoz et al., 2016), and occurred during the course of this study.

In this study we determined the diversity and the drivers of the bacterial communities in the phyllosphere, litter and rhizospheric soil of a pristine Amazon forest. The results were reported in two chapters. The first one focusing in determining the drivers of bacterial communities in the phyllosphere and rhizospheric soil at typical climatic conditions. The second one focusing in determining the drivers of the phyllosphere, litter and rhizospheric soil bacterial communities at three time points, including a time point under the impact of a severe drought induced by an ENSO effect.

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2. HOST AND PLANT FUNCTIONAL TRAITS DRIVE PHYLLOSPHERE AND SOIL BACTERIAL COMMUNITY STRUCTURE IN THE AMAZON RAINFOREST

Abstract

It has been observed in the Brazilian Atlantic forest that the bacterial community structures associated with the phyllosphere, dermosphere and rhizosphere of tree species are unique and depend on the plant taxon. However, for the Amazon forest there is no information on the drivers shaping the microbial communities in forest microenvironments. In order to unravel the drivers of the assemblage of bacterial communities associated with the phyllosphere and rhizospheric soil of trees of the Amazon forest, nine abundant tree species were sampled. The results showed that in the rhizosphere the alpha diversity is higher than in the phyllosphere bacterial community. However the phyllosphere bacterial community showed higher levels of heterogeneity (i.e. high beta diversity). Our data showed that the tree taxon is the main driver of the phyllosphere bacterial community. The phyllosphere bacterial communities was dominated by Proteobacteria, while the rhizosphere soil was dominated by Acidobacteria phylum. We identified a phyllosphere core bacteriome composed by five ASVs, represented by *Alphaproteobacteria*, *Gammaproteobacteria*, *Spartobacteria* and *Thermoleophilia* classes. In the rhizosphere soil the core bacteriome was composed by 34 ASVs, with representants of *Acidobacteria*, *Proteobacteria*, *Actinobacteria*, *Verrucomicrobia* and *Firmicutes* phyla. Phylogenetic relationships among tree species were correlated with the phyllosphere bacterial community structure and several plant traits significantly explained differences in the leaf bacterial community structure. In contrast, plant taxon relationships with the rhizospheric soil bacterial communities were weak. Nonetheless, soil attributes related to fertility were able to explain part of the variability of these bacterial communities. Among the predicted functions in the phyllosphere, oxygenic photoautotrophy, photoheterotrophy, dark oxidation of sulfur compounds, biological nitrogen fixation, methanol oxidation, methanotrophy, and ureolysis were identified, while in the rhizosphere soil were identified aerobic ammonia oxidation, denitrification, hydrocarbon degradation, iron respiration, and chemoheterotrophy. In this study, we have shown that the drivers of bacterial communities of the phyllosphere and rhizosphere of trees in the Amazon forest are distinct and that in the phyllosphere the plant-bacteria interaction might be more specific.

Keywords: Bacteria; Diversity; Plant-bacteria interaction; Microbiome; Tropical forest

2.1. Introduction

Globally, forest biomes cover an estimated 38 million square kilometers and harbor more than 3 trillion trees. Approximately one third of these trees are in tropical and subtropical forest biomes (Crowther et al., 2015). Tropical forests are distributed under environments subjected to a broad range of biotic and abiotic conditions, including climate, plant diversity and soil conditions (Hedin et al., 2009). Such conditions dictate specific plant-nutrient-soil dynamics (Pastor and Post, 1986;Hobbie, 1992) and make comparisons between tropical forests or other forest ecosystems challenging.

An intrinsic part of the forest phytobiomes, the phyllosphere comprise one of the largest habitats for microbial life, with a global surface area of approximately 6.4 x 10^8 km^2 (Morris and Kinkel, 2002;Ledford, 2015). It has been estimated that the overall phyllosphere may contain more than 10^{26} bacterial cells (Lindow and Brandl, 2003), mostly representing species not yet described (Lambais et al., 2006). A broad range of factors can modulate the microbial communities in the phyllosphere, including plant genotype and physiology (e.g. plant traits) (Lambais et al., 2014;Rosado et al., 2018). Besides, environmental factors, such as UV light, extreme temperatures, water availability, osmotic conditions, high concentration of reactive oxygen species and low availability of nutrients may also affect microbial communities in the phyllosphere (Vorholt, 2012).

Another intrinsic part of forest phytobiomes is the rhizosphere (Ledford, 2015). One of the most important factors determining the microbial community structure in the rhizosphere is the plant species (Grayston et al., 1998;Berg and Smalla, 2009;Lambais et al., 2014). The plant effects are probably due to differences in the composition of root exudates, selecting specific groups of microorganisms at different developmental stages and changing the ecological interactions between them, that together control the composition and the relative abundance of microorganisms in the rhizosphere (Chaparro et al., 2013;Sasse et al., 2018). Soils attributes also strongly affect the rhizosphere microbial communities. The combination of soil pH, moisture, and physicochemical attributes may cause high levels of variability of microbial communities in the rhizosphere (Grayston et al., 1998;Berg and Smalla, 2009;Richter et al., 2018). On the other hand, microbial communities in the rhizosphere may play key roles in regulating plant growth and development controlling nutrient availability and uptake, hormone metabolism and defense responses (Matson et al., 2015).

It has been shown that the structure of bacterial communities in the phyllosphere and rhizosphere of plant species in the Brazilian Atlantic forest is highly determined by the host taxon (Lambais et al., 2014). The host species effect in the phyllosphere has been showed also in temperate forests (Laforest-Lapointe et al., 2016). However, this effect cannot be generalized, since a weak association between tree species and phyllosphere community structure has been observed in a tropical forest in Malaysia (Kim et al., 2012). In the soil, the tree species can affect microbial community in several ways, including rhizodeposition and production of litter (Prescott and Grayston, 2013). Therefore, the belowground microbial communities can be also be driven by the the taxonomy and phylogeny of the plant species (Barberán et al., 2015).

Plant microbiome play several functional key roles, including plant protection against pathogens, nitrogen fixation, nitrification and methanol degradation (Corpe and Rheem,

1989;Papen et al., 2002;Kürkcüoglu et al., 2007;Reed et al., 2008;Vogel et al., 2012). Lambais et al. (2017) showed that although bacterial composition differs, most of the functional protein groups did not vary between phyllospheres of trees of the Atlantic forest, suggesting that bacterial associated to different plant species shared a group of specific functions important to adaptation to the environmental conditions. In general, the most abundant proteins in the phyllosphere are involved in controlling epiphytic growth, biofilm formation, UV radiation resistance and nitrogen and iron metabolism, suggesting that the ability of bacteria to respond to stress and to assimilate nitrogen are important characteristics of the phyllospheres of plant species of the Atlantic Forest (Lambais et al., 2017). Even though the Amazon forest is functionally different from the Atlantic Forest, it is possible that the bacterial communities in the phyllosphere and rhizosphere have similar structuring patterns.

In this study, we determined the effect of host plant taxon, as well plant functional traits and soil chemical attributes on the bacterial communities in phyllosphere and rhizospheric soil of nine abundant tree species in a preserved lowland forest in the eastern Brazilian Amazon, using high-throughput sequencing of bacterial 16S rRNA genes. Our main goals were to identify bacterial phylotypes present in the phyllosphere and rhizosphere of Amazon forest tress, to determine the patterns of association between plant host taxa and bacterial phylotypes; and to identify and quantify the influence of plant traits and chemical soil attributes on the composition of phyllosphere and rhizosphere bacterial communities.

2.2. Material and methods

2.2.1. Study area

Samples were collected at the Tapajós National Forest (TNF, 54º58'W and 2º51'S, altitude of 350-450 m), Santarém, Pará State, Brazil, on July 14-24, 2015. This pristine site is part of the Large Scale Biosphere-Atmosphere Experiment in the Amazonia – LBA – (Schimel 2004) and chosen as a model to represent the driest quartile of evergreen forest, and hence susceptible to drought events. Mean annual rainfall is 1,909 mm with an average temperature is 25 ºC and relative humidity of approximately 85% (Vieira et al., 2004).

The sampled trees were located within the 20 ha of permanently preserved experimental parcel distributed along a transect of 1,000 meters. Individual trees of *Amphirrhox longifolia* (n = 10, 9; phyllosphere and soil, respectively), *Aparisthmium cordatum* (n = 5, 8), *Chamaecrista xinguensis* (n = 8, 9), *Coussarea albescens* (n = 9, 8), *Erisma uncinatum* (n = 4, 6), *Manilkara huberi* (n = 13, 12),

Miconia lepidota (n = 5, 10), *Protium apiculatum* (n = 9, 9) and *Rinorea pubiflora* (n = 5, 9) were selected based on their relative abundances in the study area and canopy position.

2.2.2. Leaf and soil sampling

Tree branches were cut using an extended pole clipper or through tree climbing and clippers. Only fully developed leaves were selected from sampling and handling of branches through the stems to avoid contamination. Individual leaves were cut with a hand shears directly into 4 L sterile plastic bags and transported to the laboratory where they were immediately processed to dislodge microbial cells from the leaf surface. Soils were sampled from the top 10 cm after removal of the litter layer under the canopy of individual trees at a distance of 30 cm from the trunk. Soil for molecular analyses was stored at -20°C in sterile polypropylene tubes until DNA extraction, whereas soils for chemical analyses were stored at 4°C.

2.2.3. Host plant traits determination

A total of 12 functional plant traits were determined for each tree species: diameter at breast height (DBH), height (H), canopy illumination (CI), leaf area (LA), leaf mass per area (LMA), drip tips (DP), leaf water content (LWC), leaf water repellency (LWR), index of epiphyllous cover (IEC), leaf nitrogen content (LNC), leaf carbon content (LCC) and leaf C:N ratio (LCN).

In order to determine leaf-associated traits, the collected branch leaves were hydrated for at least 3 h and subsequently three leaves per sample were used to measure morphological leaf traits. The leaves were digitized (100 dpi) to estimate LA and DP using ImageJ, version 1.37, (National Institutes of Health, USA, http://www.rsd.info.nih.gov/ij/). The length of drip tips was determined from the deformation of the elliptical shape of the leaf (cm) to the tip, and DP was calculated dividing the length of the drip tips (cm) by the leaf area (cm^2). Leaves were then oven-dried for at least 72 h at 70°C and weighed. From these data, the LMA ($g\ m^{-2}$) was calculated as leaf mass per unit of leaf area (Witkowski and Lamont, 1991). LWC was calculated according to the following equation: $□□□ = 1 - (\frac{□□}{□□})$; where DM corresponds to dry mass (g) and FM corresponds to the fresh mass (g). LWR was determined on the adaxial leaf surface for each tree and estimated as the contact angle (θ) between a water droplet and the leaf surface (Holder, 2007;Rosado et al., 2010). The leaf was pinned onto a Styrofoam platform to flatten the leaf

surface and expose the leaf's horizontal profile. A droplet of distilled water (10 μL) was placed onto the leaf surface using a micropipette to represent a raindrop. A photograph of a profile of the water droplet resting on the leaf surface was taken with a digital camera (Kodak Pixpro Az501) and the angle between the leaf surface and the line tangent to the droplet was measured using the software ImageJ, version 1.37 (National Institutes of Health, USA, http://www.rsd.info.nih.gov/ij/). The θ angle was measured relative to the horizontal leaf surface. Leaves surfaces were classified as super-hydrophilic ($\theta < 40°$), highly wettable ($40° < \theta < 90°$), wettable ($90° < \theta < 110°$), non-wettable ($110° < \theta < 130°$), highly non-wettable ($130° < \theta < 150°$) and super-hydrophobic ($\theta > 150°$) (Aryal and Neuner, 2009). The IEC was estimated based on the method proposed by Dirzo & Dominguez (1995) to estimate the herbivory index. Leaves of each individual tree were classified visually into six categories: (0) absence of epiphyllic coverage, (1) 1 to 6% of covered leaf surface, (2) 7 to 12%, (3) 13 to 25%, (4) 26 to 50% and (5) above 51% of covered leaf surface. The IEC was calculated for each individual according to the equation:

$$\text{IEC} = \frac{\left(\sum_{i=0}^{5} ni * i\right)}{N}$$

Where *i* corresponds to the foliar coverage category, *ni* corresponds to the number of leaves category *i* and N is the total number of leaves per individual.

DBH was measured at 130 cm above ground level. The plant height was measured using measuring tapes in plants less than 2 meters high. The heights of higher plants were estimated visually. The luminous environment of each individual tree was described by visual classification of the crown illumination index (1 to 5) (Clark Deborah and Clark David, 1992). Index values were: 1, no direct light (crown not lit directly either vertically or laterally); 1.5, low lateral light; 2, medium lateral light; 2.5, high lateral light; 3, some overhead light (10-90% of the vertical projection of the crown exposed to vertical light); 3.5, lateral light (< 10% of the vertical projection of the crown exposed to vertical light; crown lit laterally); 4, full overhead light (> 90% of the vertical projection of the crown exposed to vertical light; 4.5, lateral light blocked within some or all of the 90° inverted cone encompassing the crown; 5, crown completely exposed to vertical and lateral light.

2.2.4. Leaf microbial cell dislodgment

Depending of the foliar area of each tree species, between 5 to 10 leaves were placed into a sterile 500 mL polypropylene beaker containing washing solution (0.1 M potassium

phosphate buffer, pH 7.0) and sonicated at 22.5 kHz for 10 min using an ultrasonic cell disrupter (Misonix Inc., Atlantic Beach, NY, USA), according to Lambais et al. (2006). The resulting cell suspension was vacuum-filtered through a 0.25 μm pore size nitrocellulose membrane (Merck Millipore Ldt., Tullagreen, Ireland) and the filter was stored at -20ºC until DNA extraction.

2.2.5. Soil physicochemical analyses

Soil samples were air-dried and sieved through 2-mm mesh before analytical procedures were carried out according to the methodology described by Raij *et al.* (2001). Soil pH was measured in a 1:2.5 (v:v) soil suspension in 0.01 mol L^{-1} $CaCl_2$. Exchangeable P, Ca, Mg and K were extracted using an ion-exchange resin. Exchangeable Al was extracted with KCl (1 mol L^{-1}). P was determined by colorimetry. Ca and Mg were determined by atomic absorption spectrometry, K by atomic emission spectrometry and Al by acid–base titration. Organic matter was determined by dichromate oxidation $Na_2Cr_2O_7$ (0,667 mol L^{-1}) and colorimetry. Sulfur was extracted with 0.01 mol L^{-1} $Ca(H_2PO_4)_2$ and determined by turbidimetry. Boron was extracted with hot water and determined by spectrophotometry with azomethine-H at 420 nm. Fe, Mn, Zn, and Cu were extracted by DTPA pH 7.3 (diethylenetriaminepentaacetic acid) and determined by atomic absorption spectrometry. Potential acidity (H + Al) was estimated based on the pH determined in SMP buffer solution (pH SMP). Exchangeable bases (SB) is the sum of Ca, Mg and K; cation exchange capacity (CEC) is the sum of Ca, Mg, K, Al, and H; base saturation (V) is the percentage of bases (SB) in relation to the CEC. Al saturation (m%) is the percentage of exchangeable Al in relation to the CEC. Soil texture was determined using a Bouyoucos densimeter after vigorous shaking with 1M NaOH as dispersant.

2.2.6. DNA extraction, 16S rRNA gene amplification, and sequencing

Total phyllosphere microbial DNA was extracted using the Fast DNA kit (Qbiogene, Inc., Irvine, CA, USA), according to the manufacturer's instructions. Total soil DNA was extracted from 0.25 g soil using the MoBio PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA), according to the manufacturer's instructions. DNA integrity was determined after electrophoresis in 0.8% agarose gels prepared with 0.5X Tris-Borate-EDTA buffer, and staining with Syber Green (Thermo Fisher Scientific, São Paulo, Brazil). DNA concentration was determined using the Qubit dsDNA HS assay kit (Thermo Fisher Scientific, São Paulo, Brazil), according to the manufacturer's instructions.

The V4 region of the 16S rRNA gene was amplified with the primer set 515f (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806r (5′-GGACTACHVGGGTWTCTAAT-3′) (Caporaso et al., 2011), which included sequencing adapters for the Illumina sequencing platform. PCR amplification was performed in triplicate for each sample, in 25 μL of solution containing: 0.1X PCR buffer, 2 mM $MgCl_2$, 0.2 μM of each dNTPs, 0.2 μM of each primer, and 2.5 U of Taq Platinum (Invitrogen Life Technology, Carlsbad, CA), and 10 ng genomic DNA. The PCR amplification program included initial denaturation at 94°C for 3 min, followed by 30 cycles of 94°C for 45 s, 50°C for 60 s, and 72°C for 30 s, and final extension at 72°C for 10 min.

Next, Illumina Nextera XT overhang adapter nucleotide sequences were included in addition to the 16S rRNA gene-specific sequences. Each PCR amplification was performed using KAPA HiFi PCR in a final volume of 25 μL, according to the manufacturer's instructions. PCR products were purified and samples were pooled in equimolar concentrations before sequencing. The purified library was diluted with PhiX 20% and loaded into the MiSeq Desktop Sequencer (Illumina Inc.) in a paired-end reads run (2 x 250 bp).

2.2.7. Sequence analyses

Processing and quality control of reads were performed using the DADA2 package version 1.4.0 (Callahan et al., 2016) in R v.3.4.2 (R Core Team, 2017). After graphic inspection of quality profiles, raw reads were subjected to trimming and filtering with forward and reverse reads being truncated at 220 and 160 nucleotides, respectively. The first 10 bp of all reads were trimmed from both forward and reverse reads. Next, all reads containing remaining uncalled bases, ambiguous bases or more than two expected errors were removed. Parameters of the DADA2 error model were learned from a random subset of 25% of all reads (~ 4 million reads). Error rates were estimated for each sequencing run to reduce batch effects arising from run-to-run-variability. Amplicon sequence variants (ASVs) were independently inferred from the forward and reverse sequences of each sample using the run-specific error rates, and then read pairs that were merged with any forward/reverse pair that contained a mismatch in their overlapping region were removed. Chimeric sequences were identified in each sample and removed using the function "removeBimeraDenovo".

Taxonomic assignment was performed against the Silva database v.123 using the implementation of the RDP Naive Bayesian classifier available in the DADA2 R package (Wang et al., 2007;Quast et al., 2013). Three bioinformatics products from the above procedures, "ESV

table", "Taxa table", and "Sample Data table", were merged into a phyloseq object (version 1.22.3) (McMurdie and Holmes, 2012).

2.2.8. Data analysis and statistics

Owing to the unequal number of sequences among samples, a rarefaction step to 20,000 sequences was performed for 148 samples representing nine tree species and two forest compartments (phyllosphere and rhizospheric soil). Rarefaction and all subsequent statistical analyses were repeated 20 times. Results did not differ qualitatively between iterations, therefore results from a single random rarefaction are provided.

Phylogenetic analyses were performed with the phyloseq (McMurdie and Holmes, 2012), ggplot2 (Wickham, 2009) and vegan (Oksanen et al. 2007) packages in R (Team, 2018). Phyllosphere and rhizospheric soil bacterial alpha diversities were calculated using the Shannon index based on ASV relative abundances for each forest compartment. Phylogenetic beta diversity was defined as the average distance-to-centroid, measured as the average distance (or compositional dissimilarity) from an individual tree to the centroid of the group of all trees within a tree species. Distance-to-centroid values were obtained with the 'betadisper' function in the vegan package in R (Oksanen et al. 2012). Analyses of variance (Kruskal-Wallis) and subsequent post-hoc Tukey's tests were performed to compare differences in diversity across species for each forest compartment.

The effects of host plant species on bacterial phyla and classes relative abundances were determined using a generalized linear model (GLM) constructed and validated using the 'Stats' package (Team, 2018). We modeled the bacterial taxa relative abundance using a gamma distribution model with a log link function.

In order to compare the structure of the bacterial communities among tree species, a non-metric multidimensional scaling (NMDS) ordination of Bray-Curtis dissimilarities between all samples of the phyllosphere or rhizosphere was performed. Relationships between bacterial community structure, host species identity and forest compartment were identified by performing a permutational multivariate analysis of variance (PERMANOVA) on the community matrix (Anderson Marti, 2008). Environmental variables were fitted to the ordination plots using the *envfit* function in the Vegan package. To limit co-linearity effects between variables, only environmental variables from the *envfit* results with $R^2 > 0.25$ and $P < 0.01$ were selected for further analyses. In case of correlations ($r \geq 0.7$) between those variables, the most-commonly measured environmental variable (**Tables 1 and 2**) were selected. The following phyllosphere

traits were selected: CN, H, LMA and IEC. For the soil attributes, the same approach was used and the variables Fe, pH, OM and CEC were selected.

For testing the correlation between tree phylogeny and bacterial community structure (Bray–Curtis dissimilarity) in the phyllosphere and soil we performed a Mantel test (Mantel, 1967). When a significant effect of tree phylogeny on bacterial community structure was detected, we merged bacterial taxa into the genus level and performed an indicator species analysis using *labdsv* package in R (Dufrêne and Legendre, 1997). We applied an IndVal cutoff of 0.3 to select bacterial genera.

To determine changes in specific bacterial taxa distribution associated with a key plant trait or soil attribute, the Threshold Indicator Taxa ANalysis (TITAN) was used (Baker and King, 2010). This analysis uses taxa occurrence, abundance, and directionality of response to estimate confidence limits of taxa response thresholds to key environmental variables.

Predicted bacterial functional groups were identified by comparing the genus table against the Functional Annotation of Prokaryotic Taxa (FAPROTAX) database (Louca et al., 2016). FAPROTAX extrapolates functions of cultured prokaryotes (identified at the genus or species level) to the rest of the prokaryotic genus to estimate putative functions. FAPROTAX have two main limitations: (1) the FAPROTAX database was constructed mainly to analyze biogeochemistry of water bodies and (2) the FAPROTAX database is not comprehensive. Therefore, only a small percentage of our genera may be assigned to at least one functional group. Despite these limitations, FAPROTAX is an appropriate alternative to begin to identify functions that might be associated with particular plant host traits.

2.3. Results

2.3.1. The bacterial communities in the phyllopshere and rhizosphere of Amazonian trees are distinct

Bacterial communities associated with the phyllosphere and rhizosphere of tree species of the Amazon forest were analyzed by sequencing a fragment of the bacterial 16S rRNA gene from the leaf surface and rhizospheric soil. Sequences were clustered into 30,576 amplicon sequence variants (ASV) from 148 phyllosphere and soil samples.

A total of 19,468 ASVs were detected in the phyllosphere of all tree species, with a mean ASV richness per tree of 850 (±52, standard error, s.e.), whereas in the soil a total of 12,220 ASVs were detected, with the mean richness per tree of 917 (±30, s.e.). The non-parametric-

derived Shannon index of bacterial alpha diversity was significantly lower for the phyllosphere than soil (P = 1.8e^{-8}, **Figure 1A**). In contrast, the beta diversity, estimated by the Bray-Curtis dissimilarity index, was significantly higher for phyllosphere than for soil bacterial communities (P = 2.2e^{-16}, **Figure 1B**).

Pairwise comparisons of the bacterial communities inhabiting the same tree compartment showed that there were significantly more differences in the phyllosphere than in the rhizospheric soil bacterial communities, for alpha and beta diversity (**Figure 2 and Figure 3,** respectively). Beta diversity of bacteria did not show any significant differences when soil samples obtained from different trees were compared.

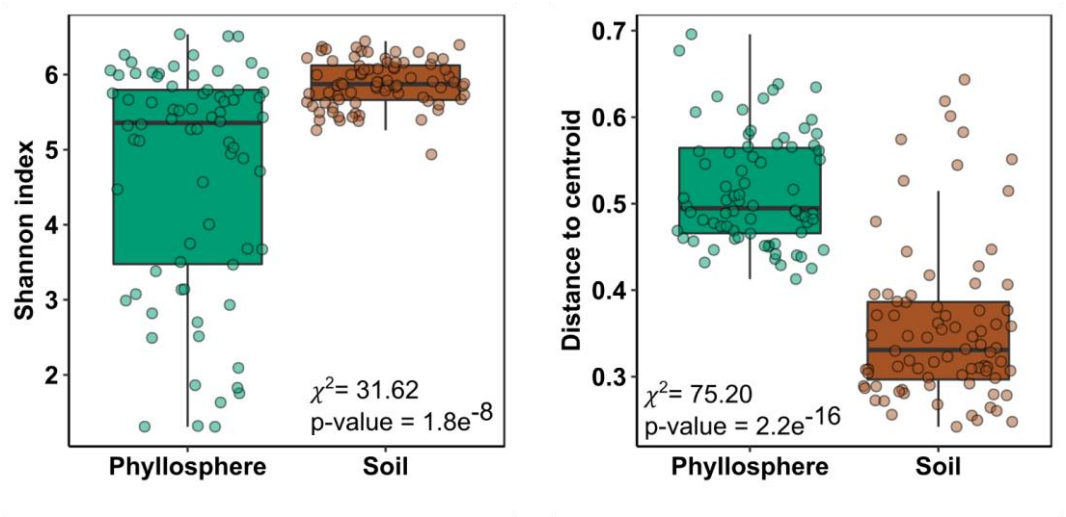

Figure 1. (A) Alpha diversity measured by Shannon index and (B) beta diversity measure by mean distance to centroid for phyllosphere and soil compartments. Error bars represent standard deviation. Means were compared by Kruskal-Wallis test.

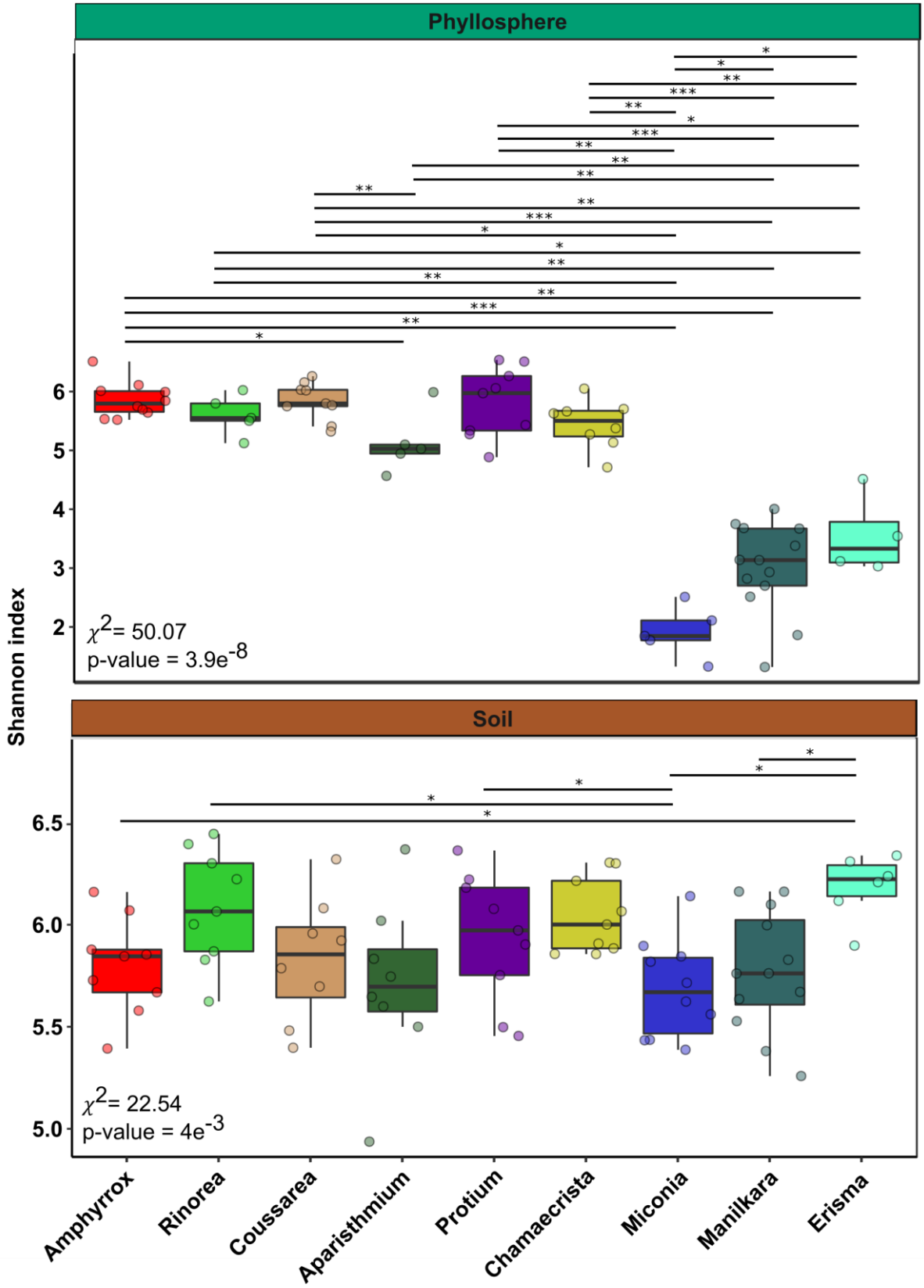

Figure 2. Alpha diversity of bacterial communities in the phyllosphere and in rhizospheric soil of nine Amazonian tree species. Error bars represent standard deviation. Means were compared by the Kruskal-Wallis test. Pairwise comparisons were performed using the Wilcoxon test. Horizontal bars connecting tree species are only shown for significantly different results. Asterisks indicate: * $p<0.05$; ** $p<0.01$; *** $p< 0.001$.

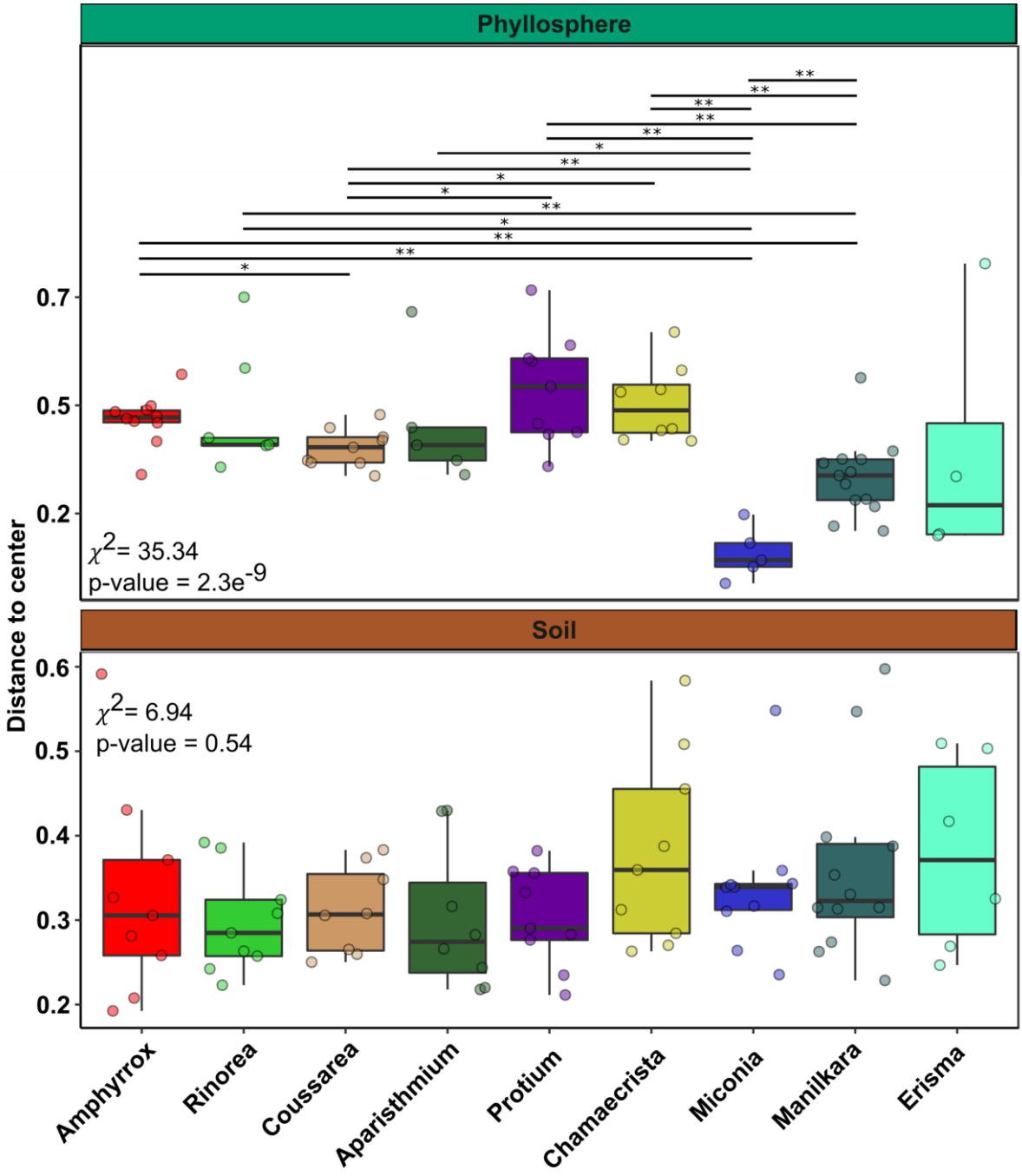

Figure 3. Beta diversity of bacterial communities in the phyllosphere and in rhizospheric soil of nine Amazonian tree species. Error bars represent standard deviation. Means were compared by the Kruskal-Wallis test. Pairwise comparisons were performed using the Wilcoxon test. Horizontal bars connecting tree species are only shown for significantly different results. Asterisks indicate: * p<0.05; ** p<0.01; *** p< 0.001.

A non-metric multidimensional scaling (NMDS) ordination of the phyllosphere and rhizosphere bacterial communities dissimilarities showed that phyllosphere communities were significantly different from those observed in the rhizosphere soil (ANOSIM R = 0.95, *P* = 0.001; **Figure 4A**). Similar results were observed when phyllosphere and rhizosphere were

compared among samples from the plant species. Overall, phyllosphere and rhizosphere shared approximately 7.1% (2,114) of the total ASVs detected (**Figure 4B**).

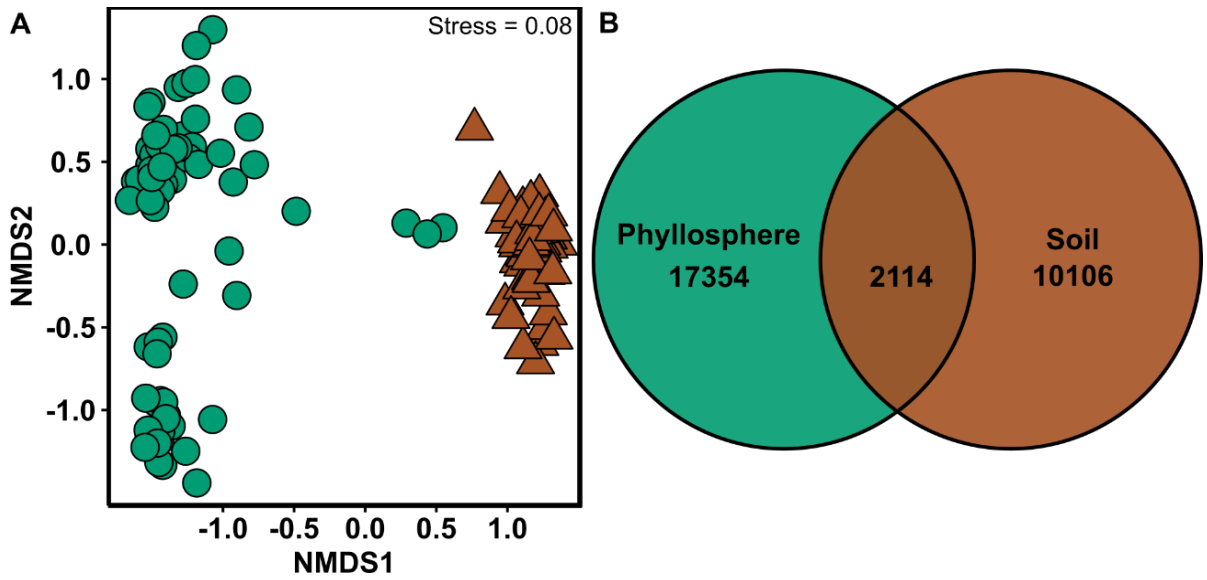

Figure 4. (A) Non-metric multidimensional scaling (NMDS) ordination of Bray-Curtis dissimilarities in bacterial community structures of phyllosphere and soil compartments from nine Amazonian trees. (B) Venn diagram representing unique ASVs from two tree compartments and their overlap for all nine Amazonian trees.

The bacterial communities in the phyllosphere of the trees were dominated by ASVs affiliated to *Proteobacteria* (64% of all sequences), *Actinobacteria* (11%), *Bacteroidetes* (7%), *Acidobacteria* (6%) and *Verrucomicrobia* (5%). AVSs affiliated to *Planctomycetes*, *Firmicutes*, and *Cyanobacteria* represented less than 1% of all sequences (**Figure 5A**). The relative abundances of all phyla, except *Firmicutes*, were influenced by the tree species (Generalized Linear Model, $P < 0.05$). At the class level, phyllosphere bacterial communities were dominated by *Gammaproteobacteria* (29%) and *Alphaproteobacteria* (29%). *Actinobacteria* (8%), *Sphingobacteria* (7%), *Acidobacteria* (6%), *Spartobacteria* (5%), *Betaproteobacteria* (4%), *Thermoleophilia* (3%), *Deltaproteobacteria* (2%) and *Bacilli* (1%) altogether comprised approximately 36% of all sequences (**Figure 6A**). Among the bacterial classes with relative abundance greater than 1%, *Spartobacteria* and *Bacilli* relative abundances were not influenced by tree species ($P > 0.05$).

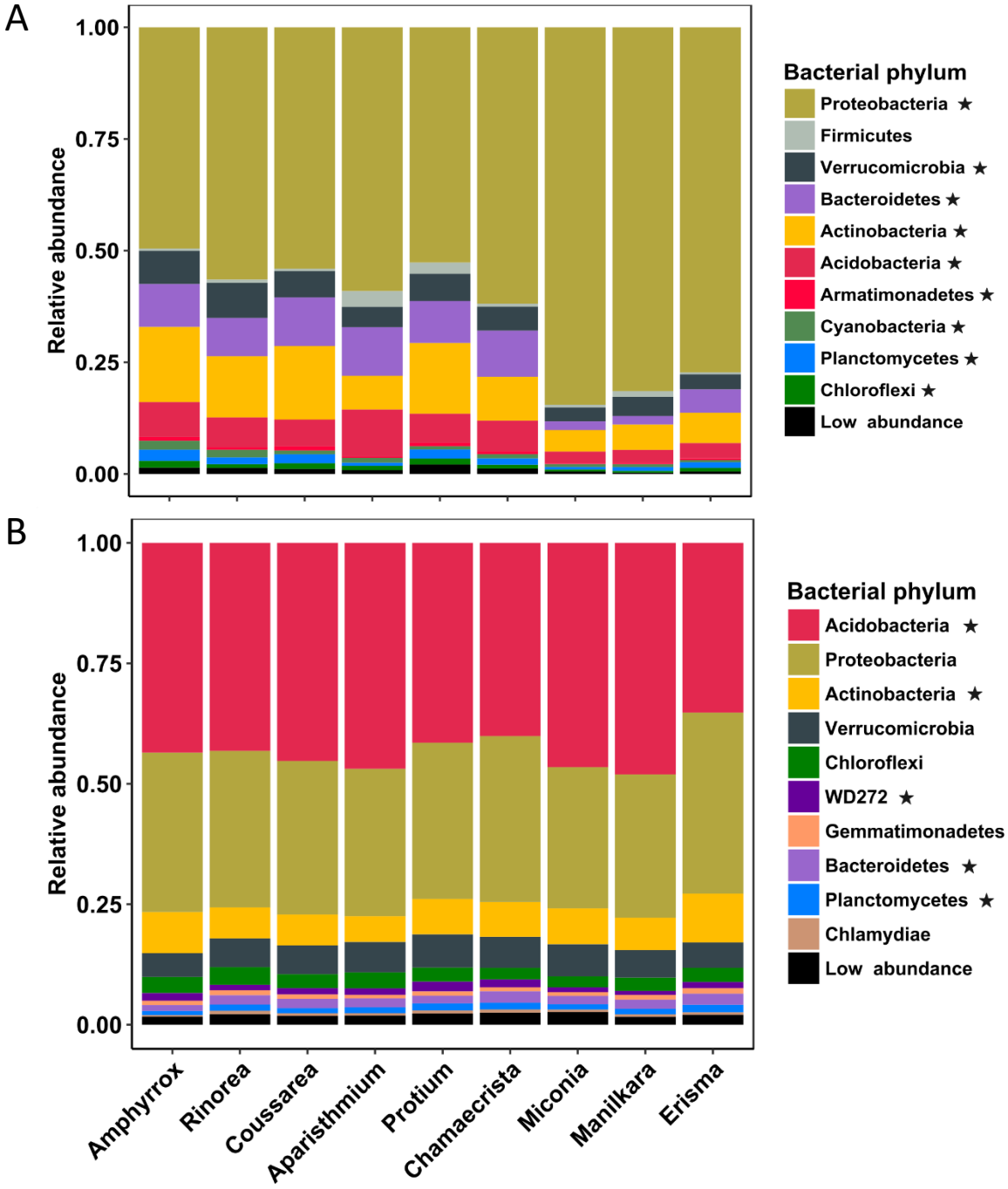

Figure 5. Bacterial taxonomic composition for the top ten most abundant phyla present in the phyllosphere (A) and rhizospheric soil (B). Phyla with relative abundances below 1% were grouped together at the Low abundance class. Black stars indicate phyla significantly affected by host plant species according to the Generalized Linear Model ($P < 0.05$).

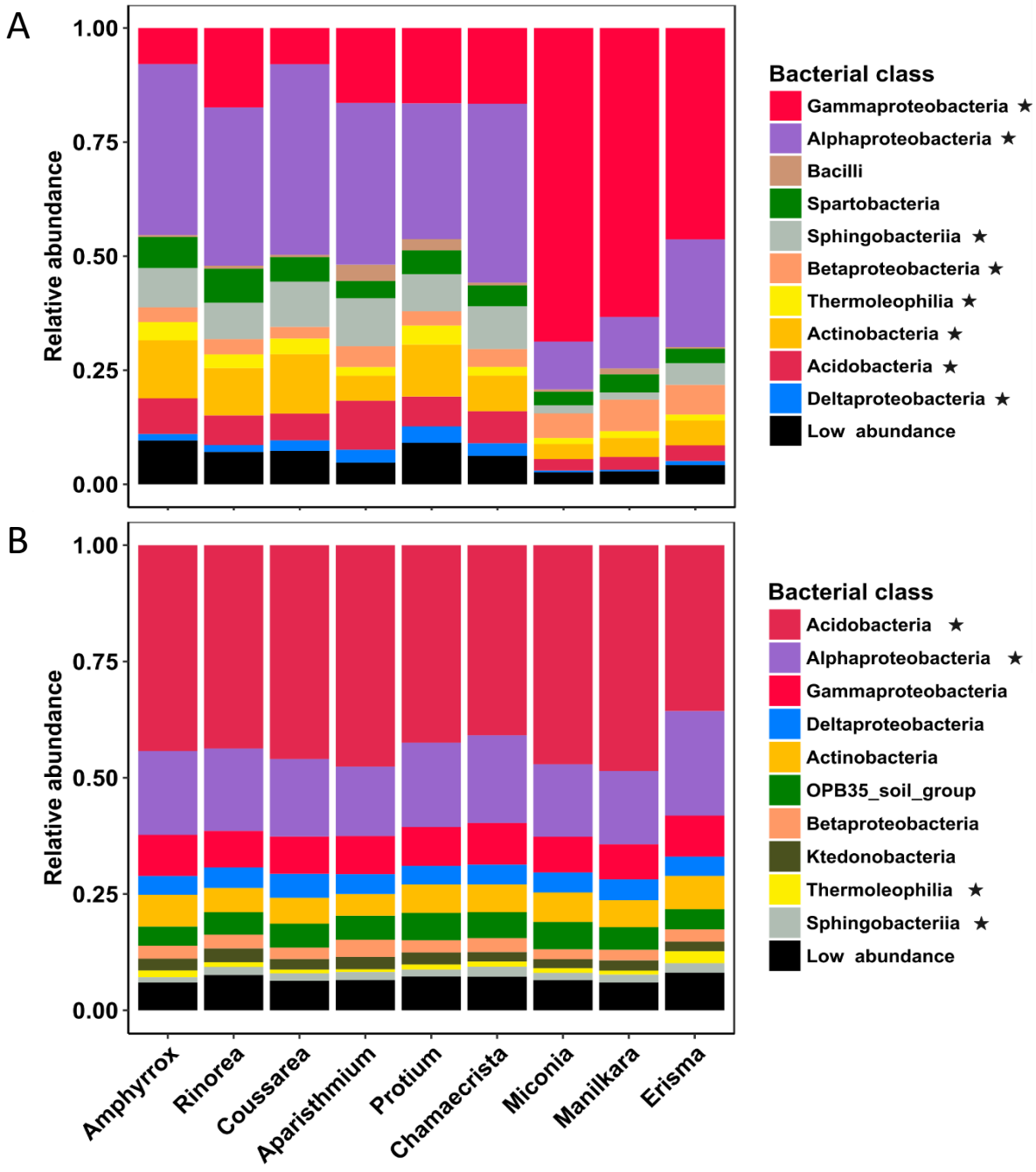

Figure 6. Bacterial taxonomic composition for the top ten most abundant classes present in the phyllosphere (A) and rhizospheric soil (B). Classes with relative abundances below 1% were group together at the Low abundance class. Black stars indicate bacterial classes significantly affected by host plant species according to the Generalized Linear Model ($P < 0.05$).

Soil bacterial communities were structured differently than phyllosphere communities, with a highest proportion of the sequences affiliated to *Acidobacteria* (44%), *Proteobacteria* (32%), *Actinobacteria* (7%), *Verrucomicrobia* (6%) and *Chloroflexi* (2%). Members of the phyla *Bacteroidetes*, *WD272* and *Planctomycetes* had a total relative abundance greater than 1%, whereas sequences affiliated to *Gemmatimonadetes* and *Chlamydiae* represented less than 1% of all sequences each

(**Figure 5A**). In the rhizosphere, the relative abundances of five phyla were affected by plant species ($P < 0.05$), i.e. *Acidobacteria*, *Actinobacteria*, *WD272*, *Bacteroidetes* and *Planctomycetes*. The most abundant classes of bacteria in the rhizosphere were affiliated to *Acidobacteria* (44%), *Alphaproteobacteria* (17%), *Gammaproteobacteria* (8%), *Actinobacteria* (6%), OPB35 (5%), Deltaproteobacteria (4%), *Betaproteobacteria* (3%), *Ktedonobacteria* (2%), *Sphingobacteria* (1%) and *Thermoleophilia* (1%) (**Figure 6B**).

2.3.2. Host species are drivers of bacterial community structure in the phyllosphere of Amazonian trees

Host plant species had a significant effect on the structure of the bacterial communities in the phyllosphere and rhizosphere ($P<0.05$). This effect was more pronounced for the phyllosphere than for the rhizosphere bacterial communities. The NMDS analysis revealed a distinct separation of the bacterial communities in the phyllosphere by tree species, explaining 54% of variation (PERMANOVA, $P = 0.001$). The distribution of phyllosphere bacterial communities in the NMDS plots revealed two groups of trees based on similarities. The first one clustering *Manilkara huberi*, *Erisma uncinatum* and *Miconia lepidota*, and the second one clustering *Amphyrrox longifolia*, *Aparisthmium cordatum*, *Chamaecrista xinguensis*, *Coussarea albescens*, *Protium apiculatum* and *Rinorea pubiflora* (**Figure 7A**).

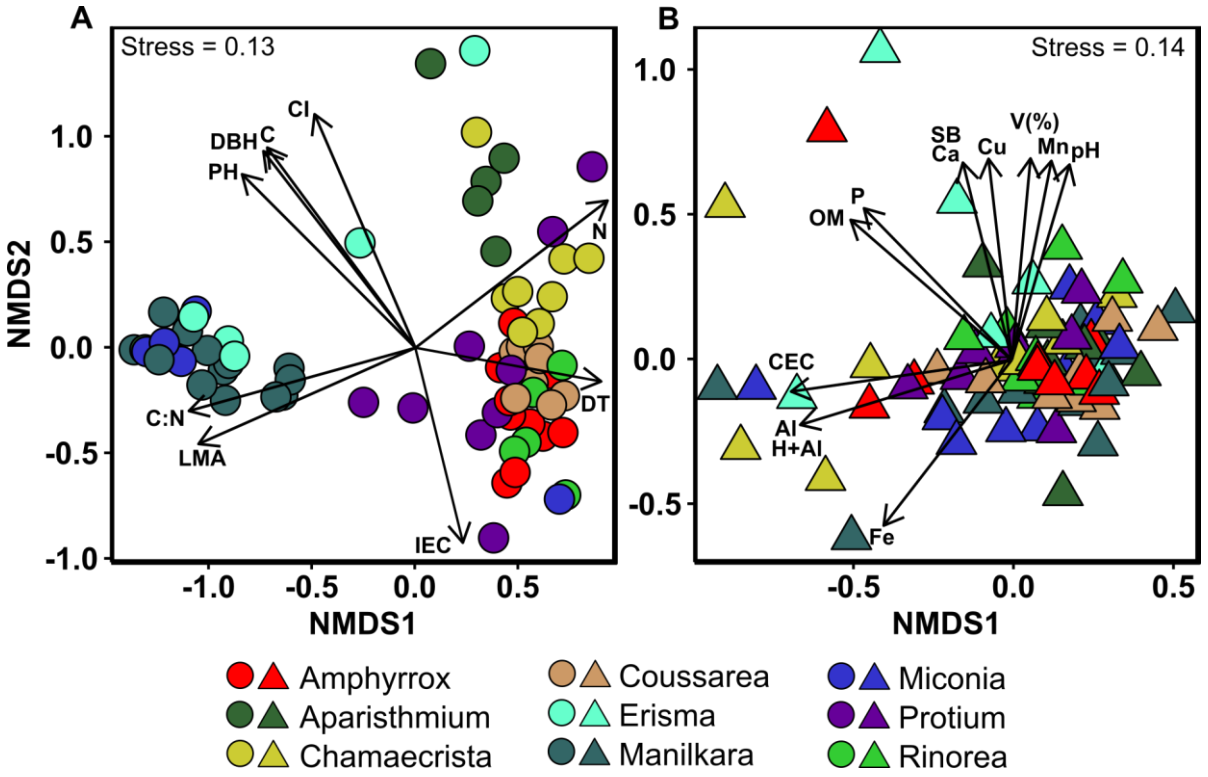

Figure 7. Non-metric multidimensional scaling (NMDS) ordination of variation in bacterial community structure associated with phyllosphere (A, circles) and rhizospheric soil (B, triangle) of Amazonian trees. Ordinations were based on Bray-Curtis dissimilarities among samples. Points represent individual samples, and arrows indicate correlations between host plant traits and bacterial community structure. The arrow shows the direction of the increasing gradient, and the length of the arrow is proportional to the correlation between the variable and the ordination. Only host traits that were significantly correlated with the NMDS ordination axes ($P < 0.01$) are shown. Trait abbreviation are: PPH, plant height; C, carbon content; DBH, diameter at breast height; CI, canopy illumination; N, nitrogen content; DT, drip tips; IEC, index of epiphyllous cover; LMA, leaf mass per area; C:N„carbon nitrogen ratio; Cu, soil copper concentration; Ca, soil calcium concentration; SB, sum of bases; P, soil phosphorus concentration; OM, soil organic matter concentration; CEC, cation exchange capacity; Al, soil exchangeable aluminum; H+Al, soil potential acidity; Fe, soil iron concentration; pH, soil pH; Mn, soil manganese concentration; V%, soil base saturation.

When fitting nine plant functional traits with significant effect on the structuring of bacterial communities in the phyllosphere ($P < 0.01$) onto the NMDS, the bacterial communities of *M. huberi*, *E. uncinatum* and *M. lepidota* were associated with DBH, C, CI, C:N ratio, LMA, and PH, whereas the bacterial communities of the other tree species were associated with DT, N and IEC (**Table 1**).

In contrast to the phyllosphere, the rhizosphere bacterial communities were more similar to each other, with tree species explaining 13% of the variation (PERMANOVA, $P = 0.015$, **Figure 7B**). Soil attributes showed significant correlations with bacterial community structure in the rhizosphere. However, no significant clustering based on tree species was observed (**Figure 7B**). Fitting soil attributes on the NMDS plot resulted in 12 out of 20 attributes linked to the structure of the bacterial communities, i.e. pH, OM, P, Ca, Al, H+Al, CEC, Cu, Fe, Mn, V, and SB ($P < 0.02$) (**Table 2**).

Table 1. Variance of the phyllosphere bacterial community structure explained by different host plant functional traits.

Host trait	R^2	*P* value
Nitrogen content (N)	0.36	0.001
Carbon content (C)	0.19	0.002
C:N ratio (CN)	0.48	0.001
Diameter at breast height (DBH)	0.18	0.006
Plant Height (PH)	0.25	0.001
Index of canopy illumination (ICI)	0.12	0.023
Leaf water content (LWC)	0.01	0.654
Leaf area (LA)	0.00	0.825
Drip tips (DP)	0.15	0.009
Leaf water repellency (LWR)	0.04	0.233
Leaf mass per area (LMA)	0.40	0.001
Index of epiphyllous cover (IEC)	0.26	0.001

Table 2. Variance in soil bacterial community structure explained by different soil attributes.

Attribute	R^2	*P* value
pH (in $CaCl_2$)	0.35	0.001
OM (g dm^{-3})	0.30	0.001
P (mg dm^{-3})	0.12	0.006
K (mmolc dm^{-3})	0.02	0.326
Ca (mmolc dm^{-3})	0.15	0.008
Mg (mmolc dm^{-3})	0.04	0.193
Al (mmolc dm^{-3})	0.15	0.003
H+Al (mmolc dm^{-3})	0.37	0.001
SB (mmolc dm^{-3})	0.14	0.013
CEC (mmolc dm^{-3})	0.39	0.001
V (%)	0.11	0.018
m (%)	0.07	0.059
B (mg dm^{-3})	0.05	0.127
Cu (mg dm^{-3})	0.16	0.003
Fe (mg dm^{-3})	0.29	0.001
Mn (mg dm^{-3})	0.21	0.001
Zn (mg dm^{-3})	0.05	0.098
Sand (g kg^{-1})	0.06	0.081
Silt (g kg^{-1})	0.03	0.268
Clay (g kg^{-1})	0.01	0.520

2.3.3. There are indicator bacterial taxa for most of the Amazonian tree phyllospheres

Host species was a major factor explaining the structuring of the bacterial communities in the phyllosphere of Amazonian trees. To identify which bacterial genera were associated to the phyllosphere of specific tree species, we performed an indicator analysis using the indicator species analysis (**Figure 8**). A total of 68 bacterial genera of the phyllosphere were significantly

associated to host species: two genera associated to *A. longifolia*, ten genera to *A. cordatum*, eight genera to *C. xinguensis*, twenty-two genera to *C. albescens,* eight genera to *E. uncinatum*, five genera to *M. huberi*, four genera to *M. lepidota*, and seven genera to *R. pubiflora*. No bacterial genera were associated to *P. apiculatum* (**Figure 8**).

Overall, genera from Acidobacteria phylum were associated only with *C. albescens*; the Actinobacteria phylum were associated with practically all tree species, except *P. apiculatum* that was not associated with any bacterial taxa; Bacteroidetes phylum was associated with *A. longifolia, C. xinguensis, C. albescens, E. uncinatum, M. huberi* and *R. publiflora*; Deinococcus-Thermus phylum was specifically associated with *C. xinguensis*; Firmicutes phylum was associated with *C. albescens, R. uncinatum* and *M. huberi;* two bacterial genera (*Atopostipes* and *Lactococcus*) are known to be involved in fermentation; Proteobacteria phylum was associated practically all tree species, except *A. longifolia* and *P. apiculatum;* several bacterial genera in the phyllosphere of *M. lepidota* and *E. uncinatum* are putatively involved in fermentation, nitrate reduction and nitrogen fixation, and bacterial genera associated with *A. cordatum* and *C. albescens* are putatively involved in methanotrophy and methylotrophy; Thermotogae and Verrucomicrobia were associated specifically with *C. albescens* and *A. apiculatum*, respectively. Verrucomicrobia is putatively involved in fermentation, nitrate reduction and chemoheterotrophy (**Figure 8**).

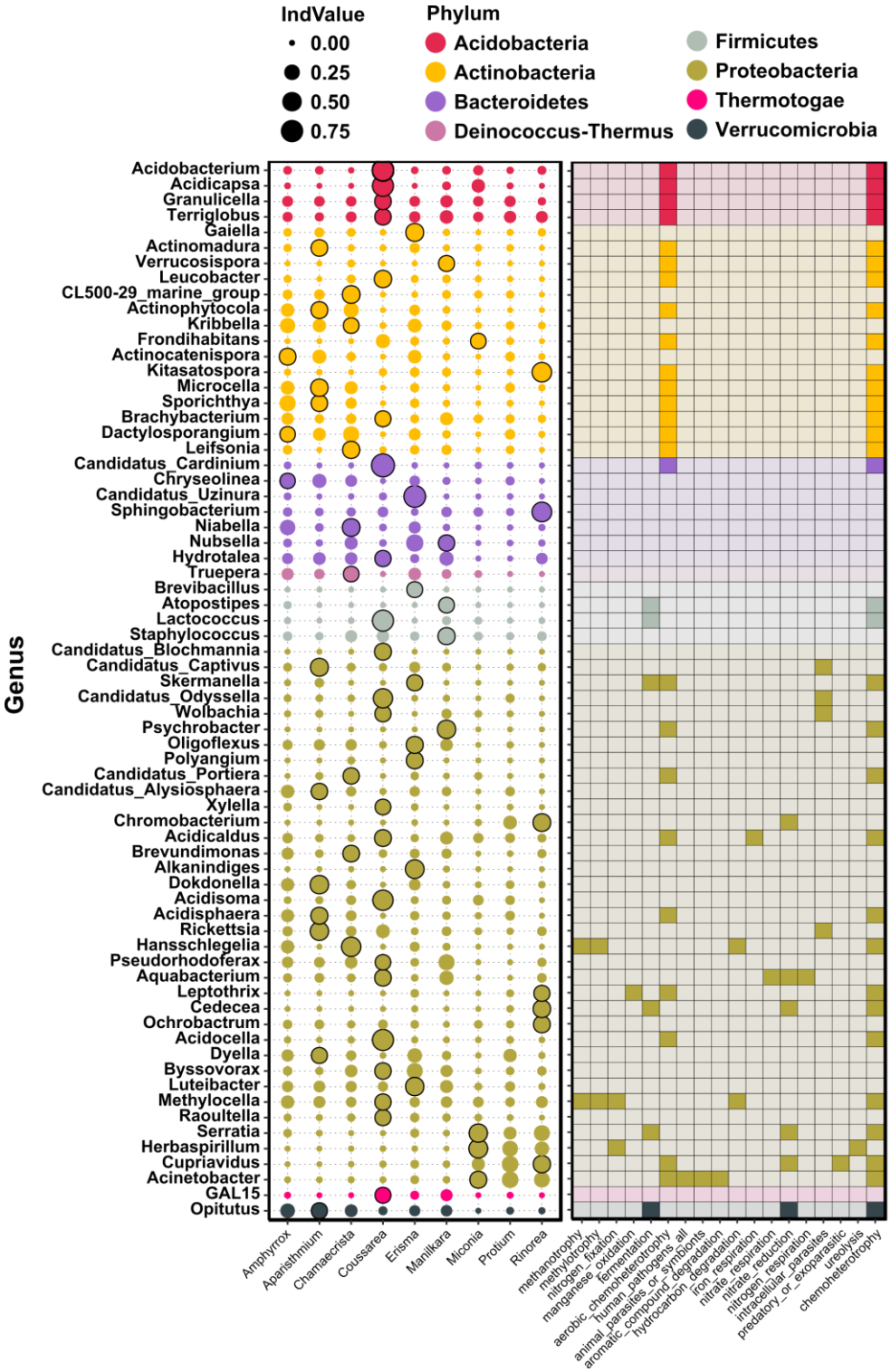

Figure 8. Indicator analysis of bacterial genera from the phyllosphere of tree species and predicted functions based on FAPROTAX.

2.3.4. The core microbiome of the phyllosphere of Amazonian trees

In this study, we defined the core bacteriome as ASVs observed in 99% or more of the samples collected for one tree species (intra-species core microbiome) or among different tree

species (inter-species core bacteriome). The number of phyllosphere ASVs shared between individuals of the same tree species ranged from 0 (in *M. huberi*) to 30 (in *A. cordatum*). Five ASVs (0.025% of all phyllosphere ASVs) were shared between all tree individuals. The shared inter-species ASVs were affiliated to three phyla and four classes of bacteria: *Alphaproteobacteria* (one ASV at 0.7% relative abundance), *Gammaproteobacteria* (one ASV at 1.8%), *Spartobacteria* (two ASVs at 1.3 and 0.5%) and *Thermoleophilia* (one ASV at 0.4%). Overall, they represent 4.8% of all phyllosphere ASVs observed in our study (**Table 3**). When only two or more tree species were considered, the inter-species core microbiome increased to 157 ASVs (1.3%).

Table 3. Taxonomic identity, number of sequences, and relative proportion of the five ASVs observed in the interspecies core bacteriome in the phyllosphere of 68 individuals of nine species of Amazonian trees.

ID	Phylum[a]	Class	Family	Seq[b]	Prop[c] (%)
SV143	*Actinobacteria*	*Thermoleophilia*	480-2	5,882	0.43
SV207	*Verrucomicrobia*	*Spartobacteria*	NA	8,024	0.59
SV4	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriaceae*	25,349	1.86
SV61	*Verrucomicrobia*	*Spartobacteria*	NA	17,922	1.31
SV69	*Proteobacteria*	*Alphaproteobacteria*	*Sphingomonadaceae*	10,126	0.74

[a]Taxonomic assignment was performed against the Silva database v.123 using the implementation of the RDP Naive Bayesian classifier available in the DADA2 R package.
[b]Seq represents the number of reads identified per ASV.
[c]Prop represents the percentage of reads for an ASV relative to the total number.

When we applied the same core bacteriome definition to the rhizosphere, the intra-species core bacteriome varied from 0 (in *M. huberi*) to 29 ASVs (in *R. pubiflora*). A total of 34 ASVs were shared between all tree species. The inter-species core microbiome represented only 0.27% of the ASVs, but 25% of total number of sequences. The shared bacterial ASVs of the rhizosphere core bacteriome were affiliated to three phyla and seven classes, i.e. *Acidibacter* (three ASVs at 1.6, 1 and 0.5% of relative abundances), *Acidothermus* (three ASVs at 1.2, 0.4 and 0.1% relative abundances), *Bacillus* (one ASV at 0.2%), *Bradyrhizobium* (one ASV at 0.5%), *Burkholderia* (one ASV at 0.7%), *Acidobacteriaceae* Subgroup 3 (two ASVs at 0.9 and 0.6%), *Acidobacteriaceae* Subgroup 1 (four ASVs at 1.9, 1.2, 1.1 and 0.8%), *Acidobacteriaceae* Subgroup 2 (three ASVs at 3.7, 1.7 and 1.1%), Subgroup 6 (one ASV at 0.2%), *Acidobacteriaceae* Subgroup 5 (one ASV at 0.2%), *Rhizobiales* (two ASVs at 0.6 and 0.3%), *Rhodospirillales* (two ASVs at 0.2% each), GR-WP33-30 (two ASVs at 0.9 and 0.2%), OPB35 soil group (three ASVs at 0.8, 0.4 and 0.3%), *Rhizomicrobium* (one ASV at 0.2 %), *Telmatobacter* (two ASVs at 0.3 and 0.2 %) and *Variibacter* (two ASV at 1.1 and 0.4 %) (**Table 4**).

Table 4. Taxonomic identity, number of sequences, and relative proportion of the 34 ASVs observed in the soil interspecies core bacteriome of 80 individuals of nine species of Amazonian trees.

ID	Phylum[a]	Class	Family	Seq[b]	Prop[c] (%)
SV8	*Acidobacteria*	*Acidobacteria*	NA	59,626	3.7%
SV10	*Acidobacteria*	*Acidobacteria*	Acidobacteriaceae_(Subgroup_1)	30,387	1.9%
SV15	*Acidobacteria*	*Acidobacteria*	NA	27,556	1.7%
SV23	*Proteobacteria*	*Gammaproteobacteria*	Xanthomonadales_Incertae_Sedis	26,345	1.6%
SV20	*Acidobacteria*	*Acidobacteria*	Acidobacteriaceae_(Subgroup_1)	18,734	1.2%
SV27	*Actinobacteria*	*Actinobacteria*	Acidothermaceae	18,527	1.2%
SV24	*Acidobacteria*	*Acidobacteria*	Acidobacteriaceae_(Subgroup_1)	18,328	1.1%
SV19	*Acidobacteria*	*Acidobacteria*	NA	17,977	1.1%
SV17	*Proteobacteria*	*Alphaproteobacteria*	Xanthobacteraceae	17,692	1.1%
SV14	*Proteobacteria*	*Gammaproteobacteria*	Xanthomonadales_Incertae_Sedis	16,536	1.0%
SV18	*Acidobacteria*	*Acidobacteria*	Unknown family	13,897	0.9%
SV42	*Verrucomicrobia*	OPB35_soil_group	NA	12,771	0.8%
SV35	*Acidobacteria*	*Acidobacteria*	Acidobacteriaceae_(Subgroup_1)	12,566	0.8%
SV25	*Proteobacteria*	*Deltaproteobacteria*	NA	12,014	0.8%
SV48	*Proteobacteria*	*Betaproteobacteria*	Burkholderiaceae	10,714	0.7%
SV46	*Proteobacteria*	*Alphaproteobacteria*	Xanthobacteraceae	9,891	0.6%
SV28	*Acidobacteria*	*Acidobacteria*	Unknown family	9,593	0.6%
SV13	*Proteobacteria*	*Alphaproteobacteria*	Bradyrhizobiaceae	8,784	0.5%
SV52	*Proteobacteria*	*Gammaproteobacteria*	Xanthomonadales_Incertae_Sedis	8,776	0.5%
SV49	*Proteobacteria*	*Alphaproteobacteria*	Xanthobacteraceae	6,761	0.4%
SV93	*Actinobacteria*	*Actinobacteria*	Acidothermaceae	6,435	0.4%
SV104	*Verrucomicrobia*	OPB35_soil_group	NA	5,843	0.4%
SV82	*Proteobacteria*	*Alphaproteobacteria*	alphaI_cluster	5,503	0.3%
SV113	*Acidobacteria*	*Acidobacteria*	Acidobacteriaceae_(Subgroup_1)	4,723	0.3%
SV110	*Verrucomicrobia*	OPB35_soil_group	NA	4,709	0.3%
SV108	*Proteobacteria*	*Alphaproteobacteria*	DA111	3,765	0.2%
SV147	*Proteobacteria*	*Alphaproteobacteria*	Rhizobiales_Incertae_Sedis	3,599	0.2%
SV174	*Acidobacteria*	*Acidobacteria*	NA	3,586	0.2%
SV195	*Acidobacteria*	*Acidobacteria*	Acidobacteriaceae_(Subgroup_1)	3,457	0.2%
SV54	*Firmicutes*	*Bacilli*	Bacillaceae	3,135	0.2%
SV193	*Acidobacteria*	*Acidobacteria*	NA	2,870	0.2%
SV148	*Proteobacteria*	*Deltaproteobacteria*	NA	2,560	0.2%
SV140	*Proteobacteria*	*Alphaproteobacteria*	DA111	2,451	0.2%
SV175	*Actinobacteria*	*Actinobacteria*	Acidothermaceae	2,288	0.1%

[a]Taxonomic assignment was performed against the Silva database v.123 using the implementation of the RDP Naive Bayesian classifier available in the DADA2 R package.
[b]Seq represents the number of reads identified per ASV.
[c]Prop represents the percentage of reads for an ASV relative to the total number.

2.3.1. Phyllosphere and rhizoshere bacteriomes are constrained by plant traits and soil attributes

Considering our findings that particular microbial communities were associated to distinct tree species (**Figure 9**), we attempted to identify plant traits that explained phyllosphere community structure using the Threshold Indicator Taxa ANalysis (TITAN). Three plant traits that were significantly correlated to bacterial communities in the phyllosphere ($R^2 > 0.25$, $P < 0.001$) were selected for further evaluation: C:N ratio, index of epiphyllous cover (IEC), and plant height (PH). Two other plant traits were excluded due to co-linearity ($r \geq 0.7$) (**Table 1**). According to TITAN analysis, *Empedobacter, Herbaspirillum, Cupriavidus, Chromobacterium, Serratia*, *Acinetobacter* and *Extensimonas* were indicator genera that increased in abundance with increasing foliar C:N ratio (**Figure 10A**). Positive indicator z scores for these seven genera varied from 20.63 to 27.44 [95% CI, confidence interval] with a filtered sum(z) change point of $z+ = 22.39$ (**Figure 10B**). There was a decrease in abundance for 69 taxa with increasing C:N ratio, with a range in negative indicator z scores varying from 24.03 to 28.75 [95% CI] and a change point of $z- = 26.26$. The abundance of 56 indicator genera increased in response to IEC, whereas nine taxa showed a decrease in abundance (**Figure 10C**). Positive indicator z scores varied between 1.6 and 4 [95% CI], with a filtered sum(z) change point of $z+ = 3.3$. Taxa abundance decline was observed between 1.15 and 3 [95% CI], with a change point of $z- = 1.6$ (**Figure 10D**). Finally, TITAN analysis identified 11 indicator genera that increased in abundance in response to plant height, whereas 50 genera showed decrease in abundance with increasing PH (**Figure 10E**). Positive indicator taxa were observed to increase their abundances when PH was between 9.97 and 19.25 m [95% CI], with filtered sum(z) change point of $z+ = 13$ m, and negative indicator taxa showed abundance declines between 5.5 and 12.5 m [95% CI], with a sum(z) change point of $z- = 5.65$ m (**Figure 10F**). Bacterial genera showing increasing abundance with increasing PH were: *Empedobacter, Cupriavidus, Ralstonia, Acidiphilium, Kitasatospora, Hymenobacter, Telmatobacter, Amnibacterium, Agromyces, Terriglobus* and *Spirosoma*.

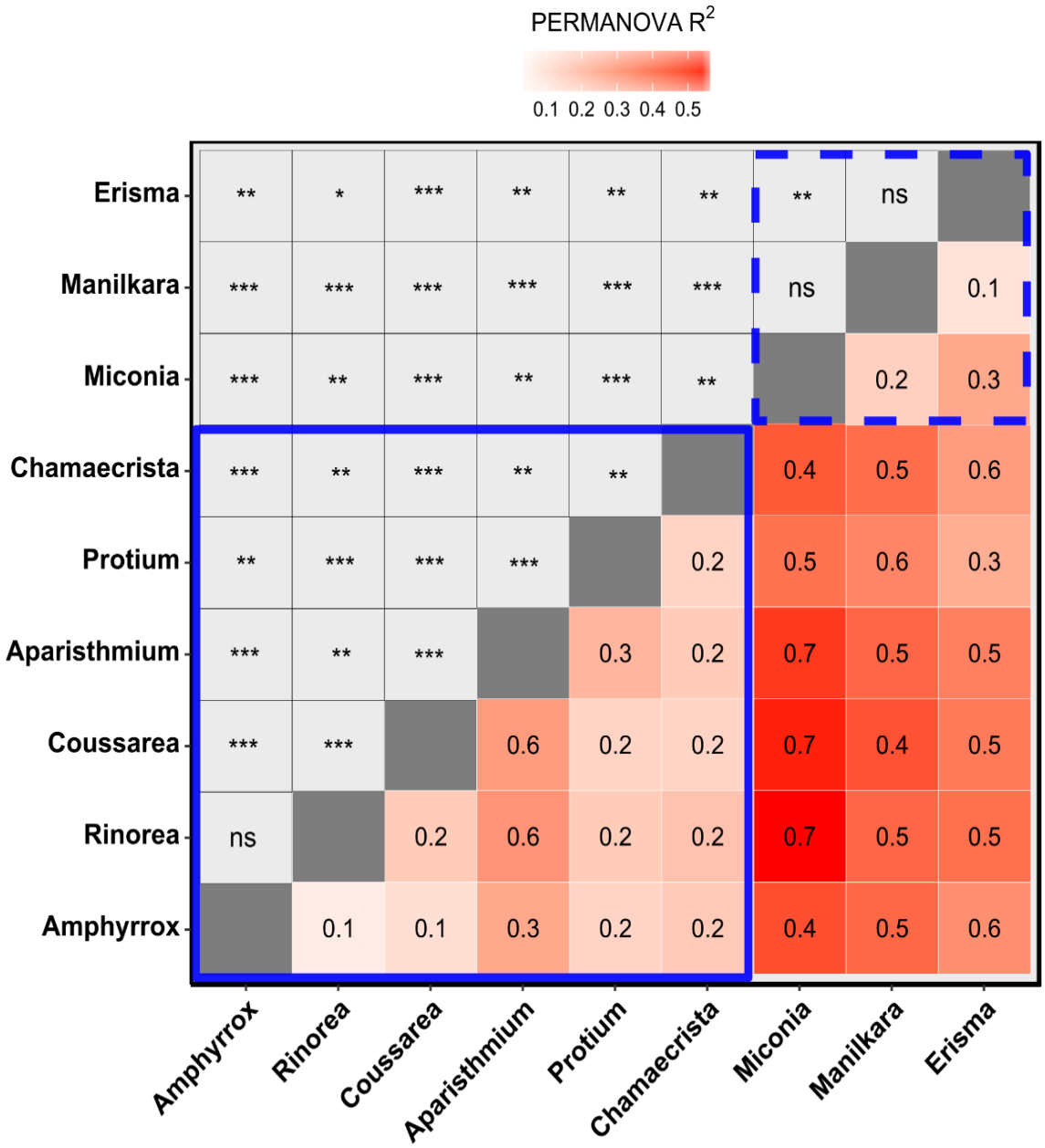

Figure 9. PERMANOVA pairwise comparisons of bacterial community structure between nine Amazonian tree species. Dashed line highlights the first group of tree species and solid line highlights the second group.

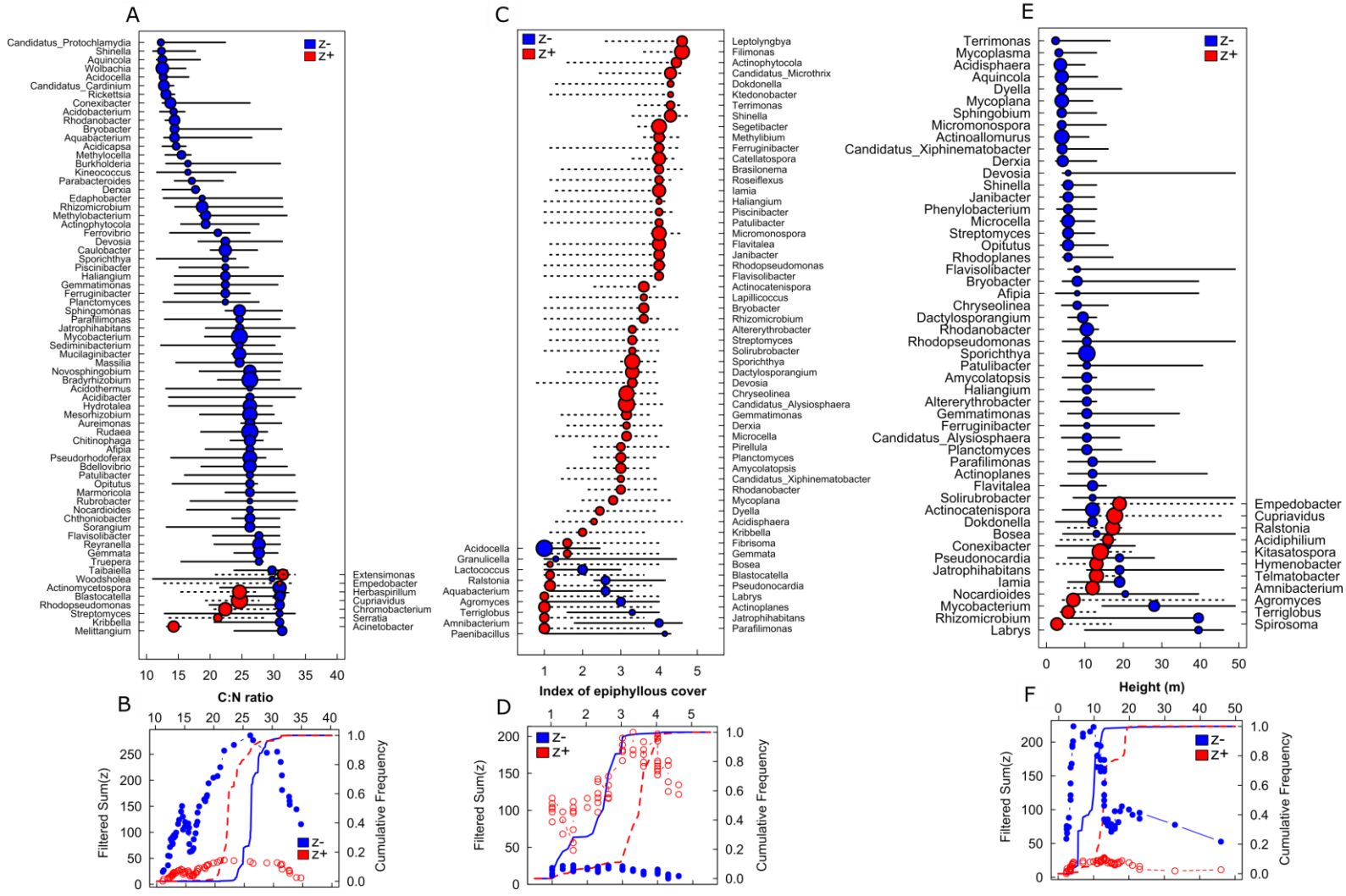

Figure 10. Threshold Indicator Taxa Analysis (TITAN) of the phyllosphere bacterial community response to (A) carbon:nitrogen (C:N) ratio, (C) index of epiphyllous cover, and (E) plant height. Pure indicator taxa (≥0.95) are plotted in increasing order with respect to their observed environmental change point. Blue symbols correspond to negative (z-) indicator taxa, whereas red correspond to positive (z+) indicator taxa. Symbols are sized in proportion to z scores. Horizontal lines overlapping each symbol represent 5th and 95th percentiles among 500 bootstrap replicates. Panels B, D, and F correspond to filtered sum(z-) and sum(z+) values of each taxa along the environmental gradient.

We performed identical analysis for the rhizosphere bacterial communities sampled in the top 10 cm soil layer adjacent to the tree trunk. Four soil attributes provided significant correlations ($R^2 > 0.25$, $P < 0.001$) with the observed community structure: pH, OM, CEC and Fe. A fifth attribute, H+Al, was found to provide variance inflation and was removed from the analyses. TITAN identified 34 genera that increased in abundance with increasing soil pH values (**Figure 11A**). Abundance increases occurred between pH 3.6 and 3.8 for the majority of these positive indicator taxa [95% CI], with an observed sum(z) change point of $z+ = 3.7$. Only seven taxa were observed to decrease in abundance with increasing pH: *Acidothermus, Acidiphilium, Telmatobacter, Actinospica, Mycobacterium, Acidobacterium* and *Isosphaera.* The change point ($z-$) of decline occurred at pH 3.7 (**Figure 11B**). Similarly, a total of 24 indicator genera were observed to increase in abundance with increasing OM concentrations in the soil, whereas the abundances of two genera decreased under the same conditions (**Figure 11C**). Taxa abundance increased between OM concentrations of 47.85 and 56.95 g dm^{-3} [95% CI], with a filtered sum(z) change point of $z+ = 51.85$ g dm^{-3}. Conversely, the other two genera showed abundance declines between OM concentrations of 49.84 and 79.75 g dm^{-3} [95% CI], with a change point of $z- = 62.1$ g dm^{-3} (**Figure 11D**). These to genera were identified as *Candidatus* Koribacter and *Methylovirgula.* In response to increasing CEC values, a total of 16 indicator genera were observed to have increased abundances, whereas 10 genera had decreased abundances (**Figure 11E**). Positive indicator taxa responded to CEC increases between 132.55 and 141.1 $mmol_c$ dm^{-3} [95% CI], with filtered sum(z) change point of $z+ = 140.3$ $mmol_c$ dm^{-3}. Increases in Fe concentration from 65 and 363.13 mg Fe dm^{-3} were associated with increased abundances of nine indicator genera: *Acidobacterium, Aciditerrimonas, Acidiphilium, Telmatobacter, Actinospica, Mucilaginibacter, Acidothermus, Rhizomicrobium* and *Burkholderia* (**Figure 11G**). The filtered sum(z) change point of $z+$ was an Fe concentration of 298.3 mg dm^{-3}. A total of 23 genera having decreasing abundances with increasing Fe concentration were identified, and the main changes were observed between 100.17 and 194 mg Fe dm^{-3} (**Figure 11H**).

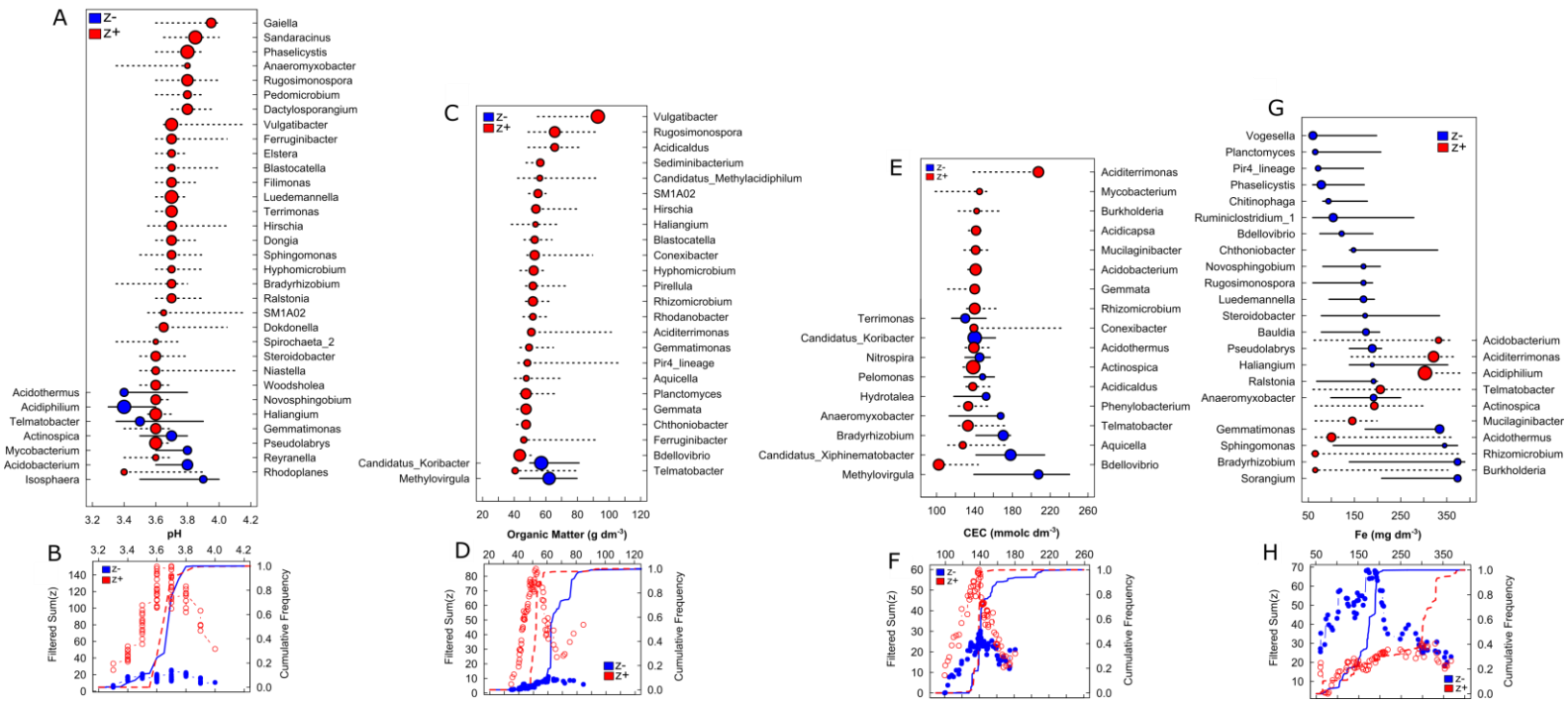

Figure 11. Threshold Indicator Taxa Analysis (TITAN) of the soil bacterial community response to (A) pH, (C) organic matter, (E) cation exchange capacity, and (E) iron. Pure indicator taxa (≥0.95) are plotted in increasing order with respect to their observed environmental change point. Blue symbols correspond to negative (z-) indicator taxa, whereas red correspond to positive (z+) indicator taxa. Symbols are sized in proportion to z scores. Horizontal lines overlapping each symbol represent 5th and 95th percentiles among 500 bootstrap replicates. Panels B, D, F and H correspond to filtered sum(z-) and sum(z+) values of each taxa along the environmental gradient.

2.3.2. Habitat filtering favor unique functional microbial traits

Owing to the different bacterial communities observed in the two forest compartments (ANOSIM R = 0.95, *P* = 0.001), phyllosphere and rhizospheric soil, a functional interpretation for these two distinct datasets was performed. Main functions associated with phyllosphere microbial communities were oxygenic photoautotrophy, photoheterotrophy, dark oxidation of sulfur compounds, biological nitrogen fixation, methanol oxidation, methanotrophy, and ureolysis. Soil microbial communities were enriched for different functional traits, mainly aerobic ammonia oxidation, denitrification, hydrocarbon degradation, iron respiration, and chemoheterotrophy (**Tables 5 and 6**).

Table 5. Predicted functions for bacterial genera observed on the phyllosphere of nine tree species in the Amazon forest. Functional predictions were based on the FAPROTAX curated database.

Functional groups assignments	TITAN indication					
	CN ratio		ICE		H	
	z+	z-	z+	z-	z+	z-
Aerobic chemoheterotrophy	2	27	21	5	5	24
Anoxygenic photoautotrophy						1
Anoxygenic photoautotrophy S oxidizing						1
Aromatic compound degradation	1					1
Cellulolysis		2				
Chemoheterotrophy	3	32	21	6	5	25
Cyanobacteria			2			
Dark oxidation of sulfur compounds			1			1
Denitrification						1
Fermentation	1	2		1		1
Hydrocarbon degradation		1				
Methanol oxidation		1				
Methanotrophy		1				
Methylotrophy		2				
Nitrate denitrification						1
Nitrate reduction	3	3		1	1	2
Nitrate respiration		1		1		1
Nitrite denitrification						1
Nitrite respiration						1
Nitrogen fixation	1	3	1			1
Nitrogen respiration		1				1
Nitrous oxide denitrification						1
Oxygenic photoautotrophy			2			
Photoautotrophy			2			
Photoheterotrophy		1	1			2
Phototrophy		1	3			2
Ureolysis	1	4				1
Genus assigned to at least one group	5	41	26	7	5	29
Genus could not be assigned	2	28	30	2	6	21
Total number of genera	7	69	56	9	11	50

Table 6. Predicted functions for bacterial genera observed in the rhizospheric soil of nine tree species in the Amazon forest. Functional predictions were based on the FAPROTAX curated database.

Functional groups assignments	TITAN indication							
	pH		Fe		OM		CEC	
	z+	z-	z+	z-	z+	z-	z+	z-
Aerobic ammonia oxidation	2			2				1
Aerobic chemoheterotrophy	11	5	5	5	6		6	1
Aerobic nitrite oxidation								1
Anoxygenic photoautotrophy	1	5						
Anoxygenic photoautotrophy S oxidizing	1							
Cellulolysis		1	1	1			1	
Chemoheterotrophy	12		5	6	7		6	1
Denitrification	1							
Fermentation	1							
Hydrocarbon degradation					1			
Iron respiration					1		1	
Methanotrophy					1			
Methylotrophy					1			
Nitrate denitrification	1							
Nitrate reduction	1							
Nitrate respiration	1							
Nitrification	2			2				2
Nitrite denitrification	1							
Nitrite respiration	1							
Nitrogen fixation	1			1				1
Nitrogen respiration	1							
Nitrous oxide denitrification	1							
Photoautotrophy	1							
Photoheterotrophy	1							
Phototrophy	1							
Genus assigned to at least one group	16	5	5	11	10	0	8	4
Genus could not be assigned	18	2	4	12	14	2	8	6
Total number of genera	34	7	9	23	24	2	16	10

2.4. Discussion

In this study we detected a high level of bacterial species richness in the phyllosphere of trees in the Amazon forest. However, the bacterial alpha diversity in the phyllosphere was lower than in the rhizosphere. In addition, bacterial communities in the phyllosphere were more dissimilar among tree species than in the rhizosphere, indicating that bacterial beta diversity was higher in the phyllosphere than in the rhizosphere. Due the higher selective pressure on the leaf surfaces and the higher levels of environmental variability, such as high incidence of UV radiation, water and nutrient restrictions, diurnal temperature variation, besides biotic factors, such as, leaf age and other host tree species intrinsic traits (Vorholt, 2012;Laforest-Lapointe et al., 2016;Rosado et al., 2018) as compared to the soil, phyllosphere microbiomes tend to have a high

rate of community change and be less diverse than belowground microbiomes (Bodenhausen et al., 2013;Lebeis, 2015). Hawkins et al. (2015) have reported that increases of environmental disturbances result in higher beta diversity of stream invertebrates. In their study, beta diversity increase was associated with increased occupancies of rare but tolerant taxa, and decreased occupancies of some relatively abundant but sensitive species (Hawkins et al., 2015). Similar results were reported by Mykrä *et al.* (2017), where anthropogenic disturbances negatively influenced alpha diversity and increased beta diversity in fungal communities during decomposition of leaf litter.

Our analyses of the dissimilarities among the bacterial communities showed that the phyllosphere communities were significantly different from those detected in the rhizosphere. Similar results were observed when phyllosphere and rhizosphere bacterial communities of each tree species individually were compared. Overall, only 7.1% of the ASVs (2,114) were shared between phyllosphere and rhizosphere samples. In terms of taxonomic composition, the bacterial communities in the phyllosphere of trees from the Amazon forest differ from past reports for bacterial communities in the phyllosphere of tropical and temperate forests. In our study, tree phyllosphere communities were dominated by Gammaproteobacteria (29%), contrasting to the observations of Kim et al (2012 in a tropical forest of Malaysia (13%) (Kim et al., 2012) and Laforest-Lapointe, Messier et al. (2016) in a temperate forest of Quebec (Laforest-Lapointe et al., 2016). In contrast, and agreeing with our results, Lambais et al. (2014) observed that approximately 51% of the bacterial OTUs in the phyllosphere of trees from the Brazilian Atlantic forest were assigned to Gammaproteobacteria.

Based on the occurrence of ASVs in the phyllosphere of the studied plant species, we determined the overall core phyllosphere bacteriome. The overall core phyllosphere bacteriome was composed of 5 ASVs, representing 5% of all sequences detected in more than 99% of samples. We also applied an alternative approach to determine the intra- and inter-species core bacteriomes, and found that 45% of the overall core bacteriome ASVs were detected only in the phyllosphere of a single tree species (i.e. all individuals of a single tree species located in different positions). This low intraspecific variation has been shown in tree species of the Atlantic Forest, in which the structure of the bacterial communities in the phyllosphere, dermosphere and rhizosphere are unique and depend on the plant taxon (Lambais et al., 2014). (Redford et al., 2010) also have shown that the bacterial community structure in the phyllosphere of *Pinus ponderosa* is not affect by the geographical position of the trees. The higher interspecies differences observed in the bacterial communities in the phyllosphere may be attributed to the

leaf characteristics such as cuticle structure and composition, leaf age, chemical composition, and/or volatile organic compounds emissions (Redford et al., 2010).

Using the same workflow we found that 29% of all core bacteriome ASVs in the rhizosphere were present only in one tree species. This percentage is lower when compared to the phyllosphere, suggesting that tree taxon effects on the bacterial communities are greater in the phyllosphere than in the phyllosphere. This results contrast to the findings of (Lambais et al., 2014), who observed that the tree species effect on the assemblage of microbial communities were greater in the rhizosphere than in the phyllosphere of trees of the Atlantic forest. The authors collected samples of soil associated with roots under individual tree canopies from the 0–20 cm soil layer, whereas we collected samples from the 0-10 cm layer. A global analysis of root distributions for terrestrial biomes reported that in tropical forests in general, 26% of roots are concentrated in the top 10 cm, 60% in the top 30 cm, and 78% in the top 50 cm (Jackson et al., 1996). Consequently, samples collected from 0-20 cm had more root biomass, and probably a higher influence of the root exudates.

Our results showed that the overall soil core bacteriome harbors a higher number of ASVs than the phyllosphere core bacteriome. On the other hand, we detected a higher number of ASVs in the intra-species core bacteriome of the phyllosphere than rhizosphere. These results suggest a higher selective pressure of the host on the bacterial community of the phyllosphere than on the rhizosphere, and that host tree species have a higher effect on the structuring of phyllosphere than rhizosphere bacterial communities. In addition, the indicator species analyses at the tree species level, strongly suggest that the host tree species play a major role modulating the bacterial community in the phyllosphere. Most of bacterial phyla were associated to the tree species studied here, but Acidobacteria was specifically associated to *C. albescens*. This tree species was specifically associated to *Acidobacterium*, *Acidicapsa*, *Granulicella* and *Terriglobus*. Acidobacteria are broadly distributed across several ecosystems, especially soil, and have a high abundance of genes encoding transporters involved in nutrient acquisition from the environment, suggesting adaptation to oligotrophic conditions, such as nutrient-limited soils and the phyllosphere (Kielak et al., 2016). In our study, the relative abundance of Acidobacteria in the phyllosphere was approximately 6%, contrasting with other reports for different phyllospheres. A study with tree species from a tropical forest in Malaysia showed that Acidobacteria accounted for 17% of phyllosphere bacteria (Kim et al., 2012), whereas studies in the Brazilian Atlantic forest have reported a low frequency or absence of Acidobacteria in the phyllospheres of *O. dispersa*, *M. schottiana*, *M. uleana, E. cuprea*, *E. melanogyna* and *T. serratifolia.* (Lambais et al., 2014).

ASVs affiliated to Actinobacteria, Bacteroidetes and Proteobacteria were associated to most of tree species in our study. These three phyla have also been detected in the phyllosphere of rice (Knief et al., 2012) and Arabidopsis (Bodenhausen et al., 2013). In contrast, ASVs affiliated to Firmicutes, Thermotogae and Verrucomicrobia were associated to few tree species. Verrucomicrobia is a prevalent member of the rhizosphere (Nunes da Rocha et al., 2009) and Thermotogae members are normally found in Amazon soil samples (Mendes et al., 2015), supporting the idea that part of the phyllosphere bacterial community may be originated from the soil through soil particles dispersion. The presence, even in low abundance on the leaf surface, may reflect the ecological importance of these poorly studied phyla. Firmicutes has been described in the rice (Knief et al., 2012), bean, canola and soybean (Copeland et al., 2015).

Host plant species was a significant driver of the structuring of the phyllosphere and rhizosphere bacterial communities in this study. This effect was more pronounced for the phyllosphere than rhizosphere bacterial communities. These results are in agreement with other studies in tropical (Kim et al., 2012;Lambais et al., 2014) and temperate forests (Laforest-Lapointe et al., 2016). It has been shown in tree species of the Atlantic Forest that the structure of the bacterial communities in the phyllosphere, dermosphere and rhizosphere are unique and depend on the plant taxon (Lambais et al., 2014). We found evidence for evolutionary associations between plants and phyllosphere communities (Mantel test; $R = 0.30$, $p = 0.001$), suggesting that evolutionary traits of the trees control the organization of the bacterial communities in the phyllosphere. In contrast, we do not found relationship between plant evolutionary traits and microbial communities in the rhizosphere (Mantel test; $R = 0.02$, $p = 0.26$), reinforcing the low effect of plant species on rhizosphere bacterial communities.

In order to identify the host functional traits most likely associated to the structuring of the bacterial community, nine functional traits related to host leaf chemistry, leaf morphology and plant attributes were evaluated. Our results indicated a significant correlation ($p < 0.01$) between the bacterial community structure in the phyllosphere and two sets of traits (**Figure 7a**). The first set of traits included LMA, C:N ratio, PH, LCC, DBH and CI. The second set of traits included LNC, DP and IEC. In accordance with the findings of Kembel et al. [18] in tropical forests, in our study the phyllosphere bacterial community structure was correlated with traits linked to plant-resource uptake strategies such as leaf nitrogen content, leaf carbon content and leaf mass per area (Wright et al., 2004). PH, DBH and CI are related to plant height, and higher values are related to higher exposure to sun light. Light availability is a key environmental variable in closed-canopy forests because light limits photosynthetic carbon gain, which in turn influences growth

and mortality (Wright et al., 2010). Taken together, these results confirm that phyllosphere bacterial communities are shaped by the ecological strategies of their plant hosts.

To analyze individually how the bacterial community changes with a plant trait gradient we performed TITAN (Threshold Indicator Taxa Analysis). In general, we identified that most bacterial genera in the phyllosphere are associated to tree species with low LCN, suggesting that the content of carbon in leaves is determinant to epiphytic colonization (Lindow and Brandl, 2003). However, these results also suggested that low LNC affects negatively, at least for most groups, the bacterial taxa abundance in the phyllosphere. It has been described that molecules containing carbon and nitrogen, mainly simple sugars such as glucose, fructose and sucrose are the dominant carbon sources on leaf surfaces (Mercier and Lindow, 2000) and can support a high phylogenetic and functional microbial diversity in the phyllosphere. Besides that, plant species with high ICE and low PH were also associated to a higher number of bacterial genera. Those are the predominant conditions of understory tree species, which live under low light availability, have high particle deposition rates and consequently higher epiphyll cover, which might favor microbial colonization and growth.

The increase of IEC values affected positively the bacterial genera, i.e. leaves with a high epiphytic coverage showed higher microbial abundance (**Figure 10b**). The opposite was showed for PH, since the abundance of most bacterial genera decreased with increasing PH values. Light is one of the major determinants of plant growth, development and survival in tropical forests (Osunkjoya et al., 1992;Nicotra et al., 1999). Additionally, variations in the temperature and radiation intensity contribute to turn the phyllosphere into a stressful environment for microbial growth (Sundin and Jacobs, 1999;Lindow and Brandl, 2003). Those conditions are intensified in canopy plant species, usually more exposed to sunlight and environmental variability. It has been shown that microbial communities in the phyllosphere change the basic physicochemical proprieties of the leaf surface through of release of biosurfactants, which increase the leaf surface wettability and water availability, and consequently the leaching of inorganic and organic substances from the leaf interior to the leaf surface (Schreiber et al., 2005), contributing to the increase in microbial fitness.

In the rhizospheric soil, our results showed that the an increase of pH, OM and CEC impact positively the abundance of the majority of the bacterial taxa, whereas a decrease in Fe concentrations favors the majority of the bacterial taxa. In general, the pattern observed showed that the abundance of these bacterial groups was directly related to soil fertility, mainly due to pH increase. Ours results are in accordance with the study by Fierer and Jackson (2006) in different locations of North and South America and Shen et al. (2013) in the northeast China, who

described similar patterns of variation for some specific phyla across pH gradients. For example, the abundance of Acidobacteria has been shown, in general, to increase with lower pH (Jones et al., 2009;Shen et al., 2013). However, it has been shown that the pH might affect differently the Acidobacteria subgroups, i.e. subgroups 1-3 decrease in relative abundance as soil pH increases, and subgroups 4 and 6 exhibit an opposite pattern (Shen et al., 2013) (Jones et al., 2009). In our study, we found that *Blastocatella* (Subgroup_4) increased and *Telmatobacter* and *Acidobacterium* (both Subgroup_1) decreased in abundance with increasing soil pH values (**Figure 11a**). The effect of pH on bacterial abundance is not specific to Acidobacteria members, we also identified changes of relative abundances of Actinobacteria and Proteobacteria phyla (Alpha- and Betaproteobacteria) in response of pH variations. Although less frequently, we identified also genera from Planctomycetes, Bacteroidetes, Gemmatimonadetes affected by pH, indicating that the pH affects also rare members of the bacterial community (**Figure 11a**).

It has been shown that increases in soil pH are mainly associated with organic matter content and CEC increases (Helling et al., 1964), which together enhance soil fertility. In our study line, the majority of the bacterial genera had significant increases of relative abundance with increasing pH, OM and CEC values, as well as decreasing of Fe concentrations. However, two genera were found to increase in abundance with decreasing of OM and CEC, *Methylovirgula* and *Candidatus_Koribacter*. Both genera have been described in environmental soil samples, from acidic soils and decaying wood (Vorob et al., 2009) and from pasture soil (Sait et al., 2002), respectively.

In this study, we found that tree species is the main driver of phyllosphere bacterial community in the Amazon forest. Our results showed that phylogenetic relationship among tree species was correlated with phyllosphere bacterial community structure and that several plant functional traits explained the differences in the phyllosphere community structures, which might have affect differentially several bacterial taxa across plant trait gradient. It is also possible that additional leaf functional traits, not measured in this study (i.e., increased leaf cuticle thickness, wax composition and volatile organic compounds) could also play a key role in the phyllosphere by limiting carbon availability on the leaf surface (Vorholt, 2012). In contrast, we identified a weak influence of plant species on soil bacterial community structure, probably because the high complexity of roots in the forest topsoil. Multiple plant individuals can release compounds in this soil layer, and our results could reflect a combined effect of several plant species. Nonetheless, groups of soil parameters were associated to shifts the bacterial communities. Our results suggest that decreases of soil acidity (pH increase), associated with high OM contend and CEC values and decreases of soil Fe concentrations, are favorable to most bacterial taxa in the rhizosphere of

trees in the Amazon forest. Taken together, our results give important insights into an unknown microbial world of the Amazon forest.

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3. EXTREME CLIMATIC EVENTS HAVE DIFFERENTIAL IMPACTS ON THE BACTERIAL COMMUNITIES OF THE PHYLLOSPHERE, LITTER AND RHIZOSPHERIC SOIL IN THE AMAZON RAINFOREST

Abstract

Tropical forests are important for global environmental sustainably, contributing to the regulation of global climatic patterns and harboring a huge, mostly unknown, microbial diversity involved in key ecosystem functions, as nutrient cycling. Is has been show that extreme climatic events, such as prolonged drought and increases in temperature, may affect the ecosystem functionally in forests like the Amazon. However, the effects of extreme climatic events on the microbial communities in the Amazon are not known. To further examine the drivers of bacterial community structure in specific plant microenvironments and the effect of a prolonged drought period in the Amazon forest, we evaluated the bacterial community structures associated with the phyllosphere, litter and rhizospheric soil of nine tree species at three time points in a pristine forest in Brazil, using high-throughput sequencing of the 16S rRNA genes. Results indicated that seasonality is an important factor modulating the bacterial community in the phyllosphere, litter and rhizospheric soil. The extreme climatic event, prolonged drought due to the influence of the ENSO 2015-2016, decreased the bacterial richness and alpha diversity, and increase the dissimilarities of the bacterial communities (i.e. beta diversity). Tree species was shown to be an important driver of the bacterial communities in the phyllosphere and plant functional traits explained most of the variability of microbial communities. In addition, chemical composition of the leaves and litter, and soil attributes changing along the seasons also explained partially the variability of the bacterial communities in the phyllosphere, litter and rhizospheric soil. Indicator species analyses revealed that few bacterial genera indicators of phyllosphere, litter or rhizospheric soil were not affect by seasonality. In contrast, at the tree species level, no phyllosphere bacterial genus was indicator of a specific tree species across seasons. Our results indicate that climatic factors are the main force determining the diversity and structure of the microbial communities in the bacteriomes of the trees in the Amazon forest. However, the magnitude of this effect is dependent on the bacteriome.

Keywords: Phyllosphere; Litter; Rhizosphere soil; Bacteria; Diversity; Plant-bacteria interaction; Microbiome; Tropical forest

3.1. Introduction

Tropical forest may harbor a great diversity of microorganisms, which are involved in key functions in the ecosystems and may colonize a distinct habitat available , such as the phyllosphere, litter and soil (Baldrian, 2016). Within the forest, microhabitats suitable for microbial growth can be found in close proximity, but exhibit distinct environmental properties, processes and dynamics, which altogether affect microbial abundance and community composition (Baldrian, 2016).

Microbial community composition is the result of a combination of dispersal history, host selection (Grayston et al., 1998;Chaparro et al., 2013;Prescott and Grayston, 2013;Lambais

et al., 2014;Laforest-Lapointe et al., 2016a), growth, and survival in the face of environmental conditions and competition (Vorholt, 2012;Baldrian, 2016). Several studies suggest that plant species select and attract specific microbes, shaping the diversity and structure of microbial communities in the phyllosphere and rhizosphere in a plant-specific manner (Broeckling et al., 2008;Lambais et al., 2014;Laforest-Lapointe et al., 2016a). Plant species select their microbial communities mainly by secreting organic compounds, usually carbohydrates and amino acids, into the rhizosphere (Huang et al., 2014) and phyllosphere (Vacher et al., 2016).

The phyllosphere, i.e. the leaves surfaces, comprise one of the largest habitats for microbial life, with a global surface area of approximately 6.4 x 10^8 km^2 (Morris and Kinkel, 2002;Ledford, 2015). Previous studies have estimated that the overall phyllosphere may contain more than 10^{26} bacterial cells (Lindow and Brandl, 2003), mostly representing species not yet described (Lambais et al., 2006). Phyllosphere represents an environment exposed to multiple stressors, with dynamic changes in solar irradiation, temperature and moisture (Vorholt, 2012). For surviving in such environment, microorganisms depend on a wide range of metabolic process, allowing them to cope with stressful conditions and oligotrophy (Mercier and Lindow, 2000). Although previous studies have reported that the phyllosphere harbors a relatively low abundance and diversity of microorganisms, as compared to soil and litter (Vorholt, 2012;Baldrian, 2016), the combination of an extensive foliar area and biotic and abiotic stressors differentially modulating the microbial communities can result in extremely diverse communities in certain biomes, yet poorly understood.

Features of tropical rainforests, such as physiognomy and plant species composition, associated with elevated temperatures and rainfall, contribute to the high levels of biomass productivity observed, as well and litter deposition and decomposition (Brando et al., 2008;Rowland et al., 2018). Leaf litter is the major source of organic matter in soils under tropical forests (Baldrian, 2016), and is a key substrate for nutrient cycling (Chave et al., 2010). Microorganisms play important roles in litter decomposition, and their communities are highly affected by litter chemical characteristics, such as pH and C:N ratios (Smith et al., 2015;Urbanová et al., 2015).

Shifts in leaf litter and plant root inputs can promote changes in the soil microbial communities (Bardgett et al., 2005). However, one of the most important factors determining the microbial community structure in the rhizosphere is the plant taxon (Grayston et al., 1998;Berg and Smalla, 2009;Lambais et al., 2014). Plant taxa, mostly through root exudates, select specific groups of microorganisms, modulating the microbial communities in the rhizosphere (Chaparro et al., 2013;Sasse et al., 2018). Soil attributes, such as pH, moisture, and organic matter and

nutrient concentration, also strongly affect the rhizosphere microbial communities (Grayston et al., 1998; Berg and Smalla, 2009; Richter et al., 2018). Conversely, microbial communities in the rhizosphere may play key roles in regulating plant growth and development controlling nutrient availability and uptake, hormone metabolism and defense responses (Matson et al., 2015). Changes in microbial community structure, physiology and function may alter ecosystem processes, for example plant litter decomposition, soil organic matter transformations and nutrient cycling (Schimel et al., 2007;McGuire and Treseder, 2010;Smith et al., 2015). Since microbial communities are also affected by seasonal variations of environmental attributes, it is also important to evaluate the effects of climatic variation and extreme climatic events, on the microbiomes and their functional roles in the ecosystems.

The Intergovernmental Panel on Climate Change (IPCC, 2013) has predicted an increase of the mean global air temperature by 2-3ºC until 2050. The main impacts associated with warming is related to shifts in pre-existing natural variability, or magnitude of the climatic events, such as precipitation distribution. One of the most important sources of natural climatic variability is the El Niño-Southern Oscillation (ENSO) (Collins et al., 2010). Approximately half of the Amazon basin exhibits clear seasonality in rainfall and is subject to additional high-magnitude water deficits triggered by positive phases of the ENSO (Marengo et al., 2011;Jiménez-Muñoz et al., 2016). During the last 40 years, the mean temperature at the Amazon basin increased by 0.5 ºC, with peak warming during the dry season (Jiménez-Muñoz et al., 2013;Gloor et al., 2015). In addition to warming, measurements from the last decade have shown extreme drought periods associated with the ENSO (Marengo et al., 2008;Espinoza et al., 2011;Lewis et al., 2011;Marengo et al., 2011). The last extreme drought in the Amazon forest was during the course of the ENSO 2015–2016 (Jiménez-Muñoz et al., 2016), within the period of our study. The frequent occurrence of extreme climatic events raises concerns about the resilience of the Amazon forest towards extreme droughts and warming (Jiménez-Muñoz et al., 2016). It has been shown that extreme droughts and warming may affect the communities of plants and animals in the Amazon forest (Brando et al., 2008;Adams et al., 2009;Sheik et al., 2011;Peñuelas et al., 2012;Doughty et al., 2015) However, whether extreme climatic events affect the diversity, abundance and structure of the microbial communities in the soil or associated to plants in the Amazon forest is not known.

3.2. Material and methods

3.2.1. Study area

Samples were collected at the Tapajós National Forest (TNF, 54º58'W and 2º51'S, altitude of 350-450 m), Santarém, Pará State, Brazil, on July 14-24, 2015, February 9-18, 2016 and July 13-23, 2016 (**Figure 12**). This pristine site is part of the Large Scale Biosphere-Atmosphere Experiment in the Amazonia – LBA – (Schimel 2004). The mean total annual precipitation (1998–2013) at this location is 2,037 mm (Brum et al., 2018). During the prolonged dry season of 2015 (August–December), monthly precipitation averaged 64 mm (Restrepo-Coupe et al., 2017). Mean annual temperature and humidity for the site are 25°C and 85%, respectively (Rice et al., 2004).

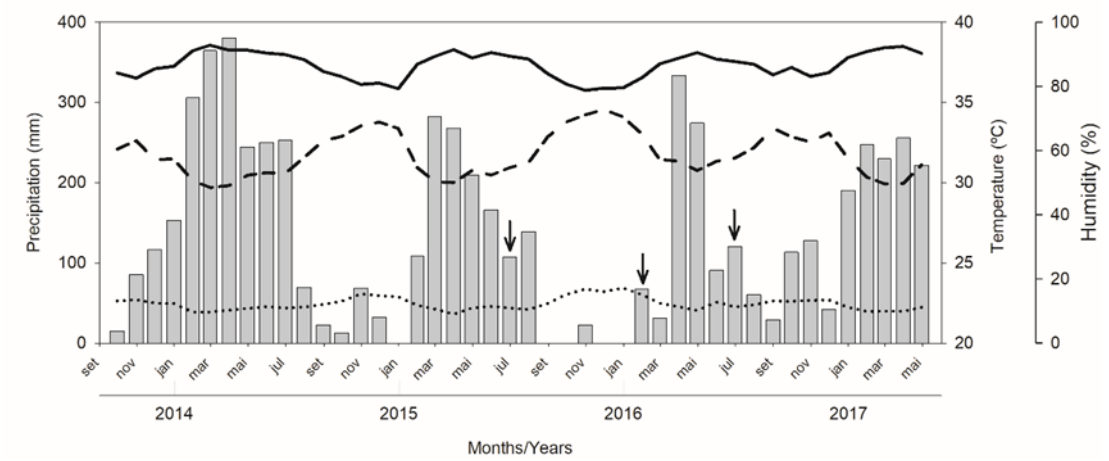

Figure 12. Climate data obtained from the INMET weather station in Belterra, Para State, Brazil, from November 2014 to May 2017. Bars represent precipitation, dashed line represents maximum temperature, dotted line represents minimum temperature and solid line represents the air humidity. Arrows indicate sampling times.

The sampled trees were located within 20 ha of a permanently preserved experimental parcel distributed along a transect of 1,000 meters. At three time points (July 2015, February 2016 and July 2016), samples from the phyllosphere, litter and rhizospheric soil of individual trees of *Amphirrhox longifolia* (n = 29, 18, 29; phyllosphere, litter and soil, respectively), *Aparisthmium cordatum* (n = 24, 20, 26), *Chamaecrista xinguensis* (n = 27, 19, 27), *Coussarea albescens* (n = 28, 19, 25), *Erisma uncinatum* (n = 16, 12, 17), *Manilkara huberi* (n = 39, 25, 37), *Miconia lepidota* (n = 22, 18, 29), *Protium apiculatum* (n = 27, 16, 28) and *Rinorea pubiflora* (n = 25, 20, 28) were collected. Tree species were selected based on their relative abundances in the studied area and canopy position.

3.2.2. Leaf, litter and soil sampling

Tree branches were cut using an extended pole clipper or through tree climbing and clippers. Only fully developed leaves were selected from branches handled through the stems to avoid contamination. Individual leaves were cut with a hand shears directly into 4 L sterile plastic bags and transported to the laboratory where they were immediately processed to dislodge microbial cells from the leaf surface. Leaf litter was collected using a 20x20 cm frame (0.04 m^2) randomly placed under the canopy of individual tree species, allowing for mass-to-area conversions. Soils were sampled from the top 10 cm after removal of the litter layer under the canopy of individual tree species at a distance of 30 cm from the tree trunk. Soil for molecular analyses was stored at -20 °C in sterile polypropylene tubes until DNA extraction, whereas soils for chemical analyses were stored at 4 °C until processing.

3.2.3. Host plant traits determination

A total of 12 functional plant traits were determined for each tree species: diameter at breast height (DBH), height (H), canopy illumination (CI), leaf area (LA), leaf mass per area (LMA), drip tips (DP), leaf water content (LWC), leaf water repellency (LWR), index of epiphyllous cover (IEC), leaf nitrogen content (LNC), leaf carbon content (LCC) and leaf C:N ratio (LCN).

In order to determine leaf-associated traits, the collected branch leaves were hydrated for at least 3 h and subsequently three leaves per sample were used to measure morphological leaf traits. The leaves were digitized (100 dpi) to estimate LA and DP using ImageJ, version 1.37, (National Institutes of Health, USA, http://www.rsd.info.nih.gov/ij/). The length of drip tips was determined from the deformation of the elliptical shape of the leaf (cm) to the tip, and DP was calculated dividing the length of the drip tips (cm) by the leaf area (cm^2). Leaves were then oven-dried for at least 72 h at 70°C and weighed. From these data the LMA (g m^{-2}) was calculated as leaf mass per unit of leaf area (Witkowski and Lamont, 1991). LWC was calculated according to the following equation: $LWC = 1 - (\frac{DM}{FM})$; where DM corresponds to dry mass (g) and FM corresponds to the fresh mass (g). LWR was detremined on the adaxial leaf surface for each species and estimated as the contact angle (θ) between a water droplet and the leaf surface (Holder, 2007;Rosado et al., 2010). The leaf was pinned onto a Styrofoam platform to flatten the leaf surface and expose the leaf's horizontal profile. A droplet of distilled water (10 µL) was placed onto the leaf surface using a micropipette to represent a raindrop. A photograph of a

profile of the water droplet resting on the leaf surface was taken with a digital camera (Kodak Pixpro Az501) and the angle between the leaf surface and the line tangent to the droplet was measured using software ImageJ, version 1.37 (National Institutes of Health, USA, http://www.rsd.info.nih.gov/ij/). The θ was measured relative to the horizontal leaf surface. Leaves surfaces were classified as super-hydrophilic (θ < 40°), highly wettable (40° < θ < 90°), wettable (90° < θ < 110°), non-wettable (110° < θ < 130°), highly non-wettable (130° < θ <150°) and super-hydrophobic (θ >150°) (Aryal and Neuner, 2009). The IEC was estimated based on the method proposed by Dirzo & Dominguez (1995) to estimate the herbivory index. Leaves of each individual were classified visually into six categories: (0) absence of coverage, (1) 1 to 6% of covered leaf surface, (2) 7 to 12%, (3) 13 to 25%, (4) 26 to 50% and (5) above 51% of covered leaf surface. The IEC was calculated for each individual according to the equation:

$$IEC = \frac{\left(\sum_{i=0}^{5} ni * i\right)}{N}$$

Where *i* corresponds to the foliar coverage category, *ni* corresponds to the number of leaves category *i* and N is the total number of leaves per individual.

DBH was measured at 130 cm above ground level. The plant height was measured using measuring tapes in plants less than 2 meters high. The heights of higher plants were estimated visually. The luminous environment of each individual was described by visual classification of the crown illumination index (1 to 5) (Clark Deborah and Clark David, 1992). Index values were: 1, no direct light (crown not lit directly either vertically or laterally); 1.5, low lateral light; 2, medium lateral light; 2.5, high lateral light; 3, some overhead light (10-90% of the vertical projection of the crown exposed to vertical light); 3.5, lateral light (< 10% of the vertical projection of the crown exposed to vertical light; crown lit laterally); 4, full overhead light (> 90% of the vertical projection of the crown exposed to vertical light; 4.5, lateral light blocked within some or all of the 90° inverted cone encompassing the crown; 5, crown completely exposed to vertical and lateral light.

3.2.4. Leaf and litter microbial cell dislodgment

Depending of the foliar area of each tree species, between 5 to 10 leaves or litter subsamples of 10 g were placed into a sterile 500 mL polypropylene beaker containing 0.1 M potassium phosphate buffer (pH 7.0) and sonicated at 22.5 kHz for 10 min using an ultrasonic cell disrupter (Misonix Inc., Atlantic Beach, NY, USA), according to (Kinkel et al., 1996). The resulting phyllosphere cell suspension was vacuum-filtered through a 0.25 μm pore size

nitrocellulose ester membrane (Merck Millipore Ldt., Tullagreen, Ireland) and the membranes were stored at -20 ºC until DNA extraction.

3.2.5. Soil physicochemical analysis

Soil samples were air-dried and sieved through 2-mm mesh before analytical procedures were carried out according to the methodology described by Raij *et al.* (2001). Soil pH was measured in a 1:2.5 (v:v) soil suspension in 0.01 mol L^{-1} $CaCl_2$. Exchangeable P, Ca, Mg and K were extracted using an ion-exchange resin. Exchangeable Al was extracted with KCl (1 mol L^{-1}). P was determined by colorimetry. Ca and Mg were determined by atomic absorption spectrometry, K by atomic emission spectrometry and Al by acid–base titration. Organic matter was determined by dichromate oxidation $Na_2Cr_2O_7$ (0,667 mol L^{-1}) and colorimetry. Sulfur was extracted with 0.01 mol L^{-1} $Ca(H_2PO_4)_2$ and determined by turbidimetry. Boron was extracted with hot water and determined by spectrophotometry with azomethine-H at 420 nm. Fe, Mn, Zn, and Cu were extracted by DTPA pH 7.3 (diethylenetriaminepentaacetic acid) and determined by atomic absorption spectrometry. Potential acidity (H + Al) was estimated based on the pH determined in SMP buffer solution (pH SMP). Exchangeable bases (SB) is the sum of Ca, Mg and K; cation exchange capacity (CEC) is the sum of Ca, Mg, K, Al, and H; base saturation (V) is the percentage of bases (SB) in relation to the CEC. Al saturation (m%) is the percentage of exchangeable Al in relation to the CEC. Soil texture was determined using a Bouyoucos densimeter after vigorous shaking with 1M NaOH as dispersant.

3.2.6. DNA extraction, 16S rRNA gene amplification, and sequencing

Total phyllosphere and litter microbial DNA was extracted using the Fast DNA kit (Qbiogene, Inc., Irvine, CA, USA), according to the manufacturer's instructions. Total soil DNA was extracted from 0.25 g of soil using the MoBio PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA), according to the manufacturer's instructions. DNA integrity was determined by electrophoresis in 0.8% agarose gels prepared with 0.5X Tris-Borate-EDTA buffer, and stained with Syber Green (Thermo Fisher Scientific, São Paulo, Brazil). DNA concentration was determined using the Qubit dsDNA HS assay kit (Thermo Fisher Scientific, São Paulo, Brazil), according to the manufacturer's instructions.

The hypervariable region V4 of the 16S rRNA gene was amplified with the primers 515f (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806r (5′-GGACTACHVGGGTWTCTAAT-3′)

(Caporaso et al., 2011), which included sequencing adapters for the Illumina sequencing platform. PCR amplification was performed in triplicate for each sample in a solution (25 μL) containing: 0.1X PCR buffer, 2 mM $MgCl_2$, 0.2 μM of each dNTPs, 0.2 μM of each primer, 2.5 U of Taq Platinum (Invitrogen Life Technology, Carlsbad, CA), and 10 ng of genomic DNA. PCR conditions were: initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C for 45 s, 50 °C for 60 s, and 72 °C for 30 s, and final extension at 72 °C for 10 min.

Next, Illumina Nextera XT overhang adapter nucleotide sequences were included in addition to the 16S rRNA gene-specific sequences. PCR amplifications were performed using KAPA HiFi PCR in a final volume of 25 μL per reaction according to the manufacturer's instructions. PCR products were purified and samples were pooled in equimolar concentrations before sequencing. The purified library was diluted with PhiX 20% and loaded into MiSeq Desktop Sequencer (Illumina Inc.). The sequencing consisted of paired-end reads (2 x 250 bp).

3.2.7. Sequence analyses and statistics

Processing and quality control of reads were performed using the DADA2 package version 1.4.0 (Callahan et al., 2016) in R v.3.4.2 (Team, 2018). After graphic inspection of quality profiles, raw reads were subjected to trimming and filtering with forward and reverse reads being truncated at 220 and 160 nucleotides, respectively. The first 10 bp were trimmed from all forward and reverse reads. Next, all reads containing remaining uncalled bases, ambiguous bases or more than two expected errors were removed. Parameters of the DADA2 error model were learned from a random subset of 25% of all reads (~4 million reads). Error rates were estimated for each sequencing run to reduce batch effects arising from run-to-run-variability. Amplicon sequence variants (ASVs) were independently inferred from the forward and reverse reads of each sample using run-specific error rates. Read pairs were merged with any forward/reverse pair that contained a mismatch in their overlapping region and were removed. Chimeric sequences were identified in each sample and removed using the function "removeBimeraDenovo".

Taxonomic assignment was performed against the Silva database v.123 using the implementation of the RDP Naive Bayesian classifier available in the DADA2 R package (Wang et al., 2007;Quast et al., 2013). Three bioinformatics products from the above procedures, i.e. "ESV table", "taxa table" and "sample data table", were merged into a phyloseq object (version 1.22.3) (McMurdie and Holmes, 2012).

Owing to the unequal number of sequences among samples, a rarefaction step to 20,000 sequences was performed for 650 samples representing nine tree species and three forest

compartments (phyllosphere, litter and soil) at three time points (July 2015, February 2016 and July 2016). Rarefaction and all subsequent statistical analyses were repeated 20 times. Results did not differ qualitatively across iterations, therefore results from a single random rarefaction are provided.

Phylogenetic analyses were performed with the phyloseq (McMurdie and Holmes, 2012), ggplot2 (Wickham, 2009) and vegan (Oksanen et al., 2013) packages in R (Team, 2018). Phyllosphere and soil microbial alpha diversities were calculated using the Shannon index based on ASV relative abundances for each forest compartment. Phylogenetic beta diversity was defined as the average distance-to-centroid, measured as the average distance (or compositional dissimilarity) from an individual tree to the centroid of the group of all trees within a tree species. Distance-to-centroid values were obtained with the 'betadisper' function in the vegan package in R (Oksanen et al., 2013). Analysis of variance (Anova type III) and subsequent post-hoc pairwise Tukey's tests were performed to test for differences in diversity across species for each forest compartment.

In order to compare the structure of the bacterial communities among tree species, a non-metric multidimensional scaling (NMDS) ordination of Bray-Curtis dissimilarities among all samples per compartment was performed. Relationships between bacterial community structure, host species identity and forest compartment were identified performing a permutational multivariate analysis of variance (PERMANOVA) on the community matrix (Anderson Marti, 2008).

To identify significant indicator genera from the bacterial communities, we merged taxa into the genus level and performed an indicator species analysis using the *labdsv* package in R (Dufrêne and Legendre, 1997).

3.3. Results

3.3.1. Richness and diversity of bacterial communities in the phyllosphere, litter and rhizospheric soil

Bacterial communities in the phyllosphere, litter and rhizospheric soil of tree species of the Amazon forest were analyzed by removing the attached bacteria from the leaf surface and litter for DNA extraction, or by extracting DNA directly from the soil, and characterizing their 16S rRNA gene profiles by sequencing. High throughput sequencing were performed and

sequences clustered into 72,468 amplicon sequence variants (ASVs) in 650 phyllosphere, litter and rhizosphere soil samples

A total of 26,795 ASVs were detected in the phyllosphere across all tree species and sampling seasons, with a mean ASV richness per tree of 603 (±23 standard error, s.e.). In the leaf litter a total of 34,832 ASVs were detected, with a mean AVS richness per tree of 1,442 (±37 s.e.), whereas in the rhizospheric soil a total of 23,833 ASVs were detected, with a mean AVS richness per tree of 1078 (±18 s.e.). In general, the alpha diversity, based on the non-parametric-derived Shannon index, was significantly lower for the phyllosphere than for litter and rhizospheric soil bacterial communities (**Figure 13A**). The opposite was observed for the beta diversity, estimated based on the Bray-Curtis dissimilarity index. Beta diversities in the phyllosphere and litter bacterial communities were significantly higher than in the rhizospheric soil bacterial communities (**Figure 13B**).

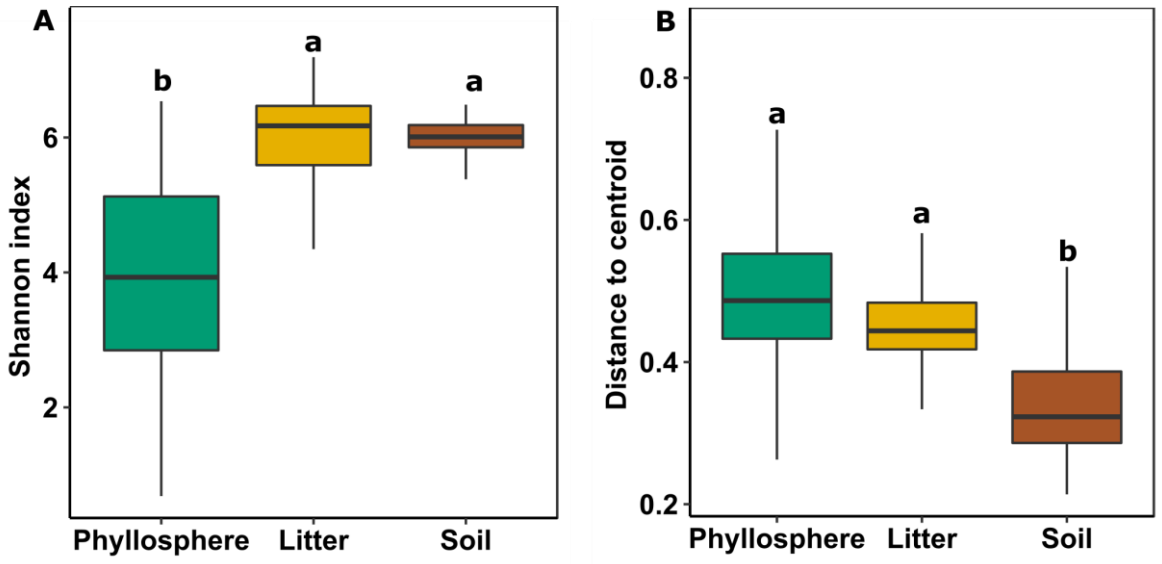

Figure 13. (A) Alpha diversity determined by the Shannon index and (B) beta diversity determined by the mean distance to centroid for phyllosphere, litter and soil compartments. Error bars represent the standard deviation.

The statistical analysis comparing the Shannon indexes between seasons, independently of tree species, showed a decrease in alpha diversity in the phyllosphere in February 2016 and July 2016, as compared to July 2015 (**Figure 14a**). The opposite was observed for the rhizospheric soil bacterial communities (**Figure 14e**). In the litter, a significant lower diversity index was observed in February 2016, as compared to July 2016 (**Figure 14c**). The beta diversity in the phyllosphere was significantly higher in February 2016 and July 2016, as compared to July 2015 (**Figure 14b**). In the litter, significantly higher beta diversity was observed in July 2016,

whereas in the soil beta diversity did not show significant differences between the three time points (**Figure 14d and f**).

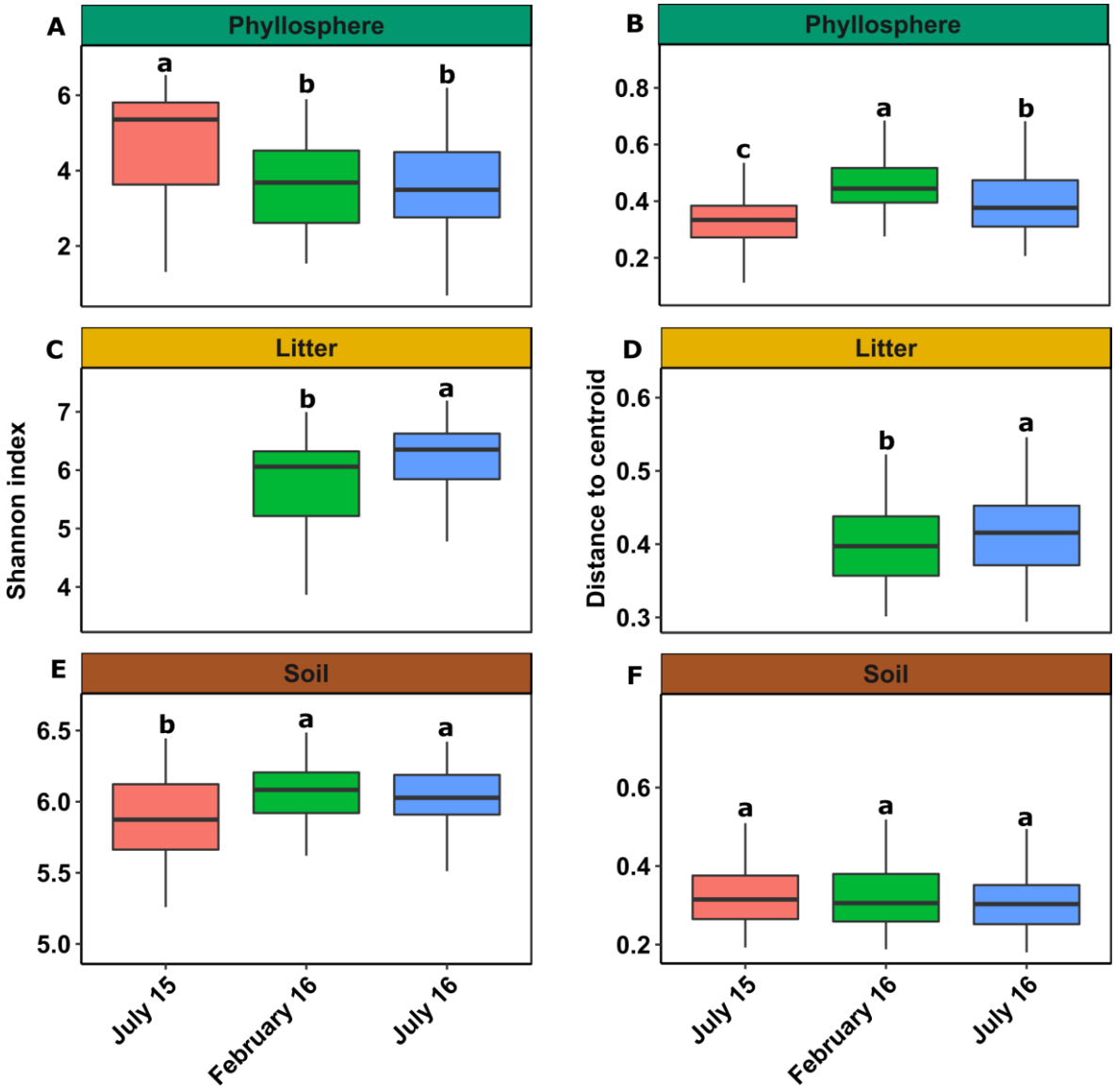

Figure 14. Alpha (A, C and E) and beta (B, D and F) diversities of bacterial communities in the phyllosphere, litter and rhizospheric soil of nine tree species in the Amazon forest at three time points. Error bars represent the standard deviation of the mean.

Alpha diversity of the bacterial communities was affected by tree species and seasonality in all forest compartments ($p < 0.01$, **Figure 15 and Table S1**). In the phyllosphere, the highest values for the Shannon index were observed in July 2015, associated with *A. longifolia*, *C. albescens*, *P. apiculatum*, *C. xinguensis* and *R. pubiflora* (Tukey's test, $p < 0.05$). The effect of seasonality on the Shannon index was dependent on the tree species. The alpha diversity in the phyllosphere of *A. longifolia*, *C. albescens*, *A. cordatum*, *P. apiculatum* and *C. xinguensis* decreased along the time course ($p < 0.05$). The opposite was observed in the phyllosphere of *M. lepidota*, where the Shannon

diversity index increased significantly along the time course ($p < 0.05$) (**Figure 15a and Table S1a**).

The Shannon index in the litter under the canopy of *C. albescens* and *A. pubiflora* was higher in July 2016, as compared to the other sampling times (Tukey's test, $p < 0.05$, **Figure 15b and Table S1b**). Statistically significant differences were not observed for the other tree species studied (Tukey's test, $p > 0.05$, **Figure 15b and Table S1b**).

In the rhizospheric soil, in general, the Shannon index did not vary for the bacterial communities of the different tree species, but was affected by the season in some tree species. Bacterial diversity in the rhizosphere of *M. lepidota* decreased along the time course, whereas in the rhizosphere of *A. cordatum* and *M. huberi* it increased. In the rhizosphere of *A. longifolia* the highest Shannon index values were observed in February 2016 (Tukey's test, $p < 0.05$, **Figure 15c and Table S1c**).

Beta diversity of the bacterial communities was also affected by tree species and seasonality in all forest compartments ($p < 0.01$, **Figure 16 and Table S1**). Beta diversity in the phyllosphere of *C. albescens*, *M. lepidota*, *C. xinguensis*, *M. huberi* and *E. uncinatum* was lower in July 2015 than in February 2016 and July 2016 (Tukey's test, $p < 0.05$, **Figure16a and Table S1a**). In the other plant species, bacterial beta diversity in the phyllosphere did not vary between time points (Tukey's test, $p > 0.05$).

In the litter, the highest values of beta diversity were observed in February 2016 associated to *A. longifolia* and July 2016 associated to *E. uncinatum* (Tukey's test, $p < 0.05$, **Figure 16b and Table S1b**). In the rhizospheric soil, there were no significant effects of tree species or season on the bacterial beta diversity (**$p > 0.05$, Figure 16c and Table S1c**).

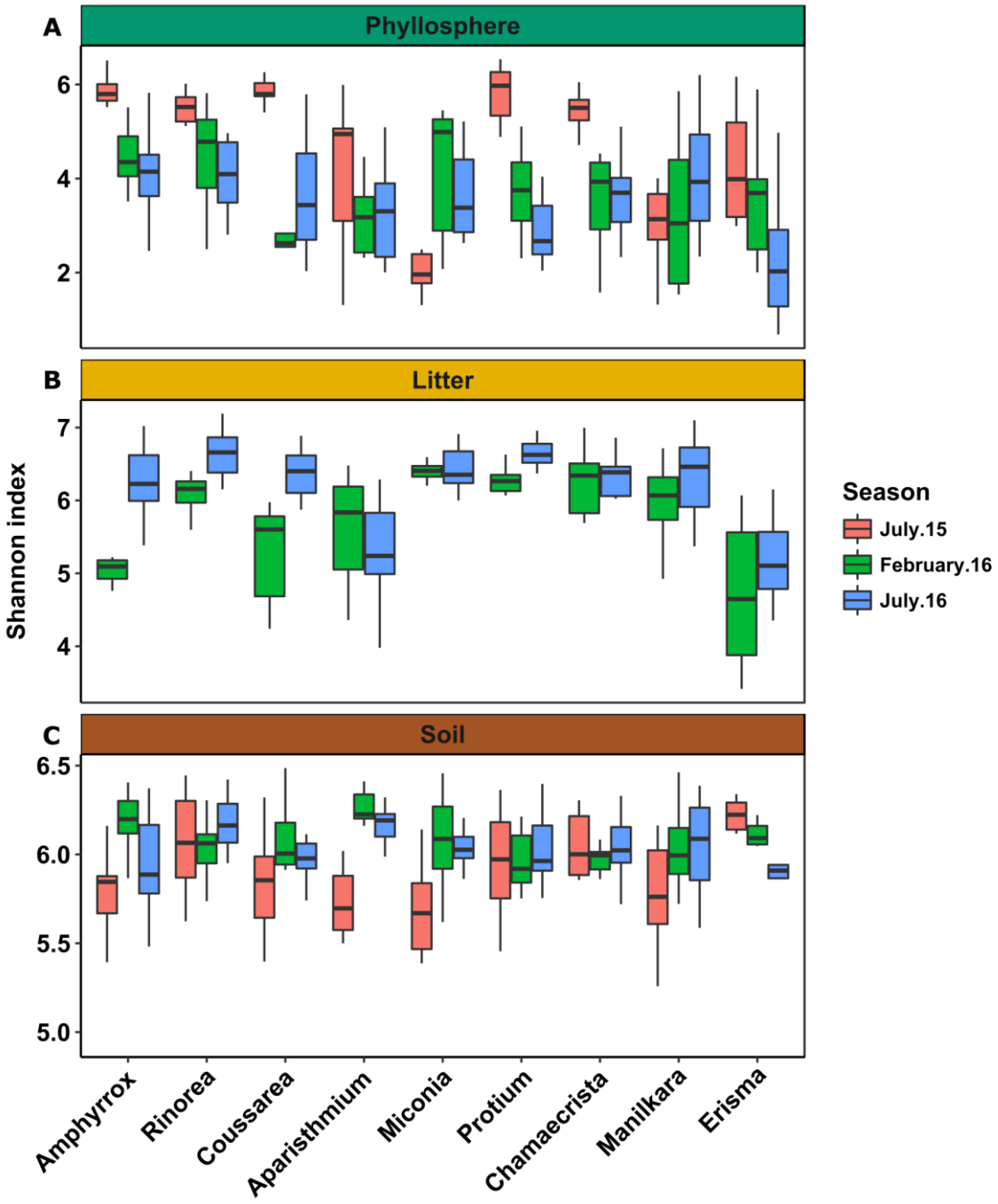

Figure 15. Alpha diversity of bacterial communities in the phyllosphere (A), litter (B) and rhizospheric soil (C) of nine tree species in the Amazon forest at three time points. Error bars represent the standard deviation of the mean.

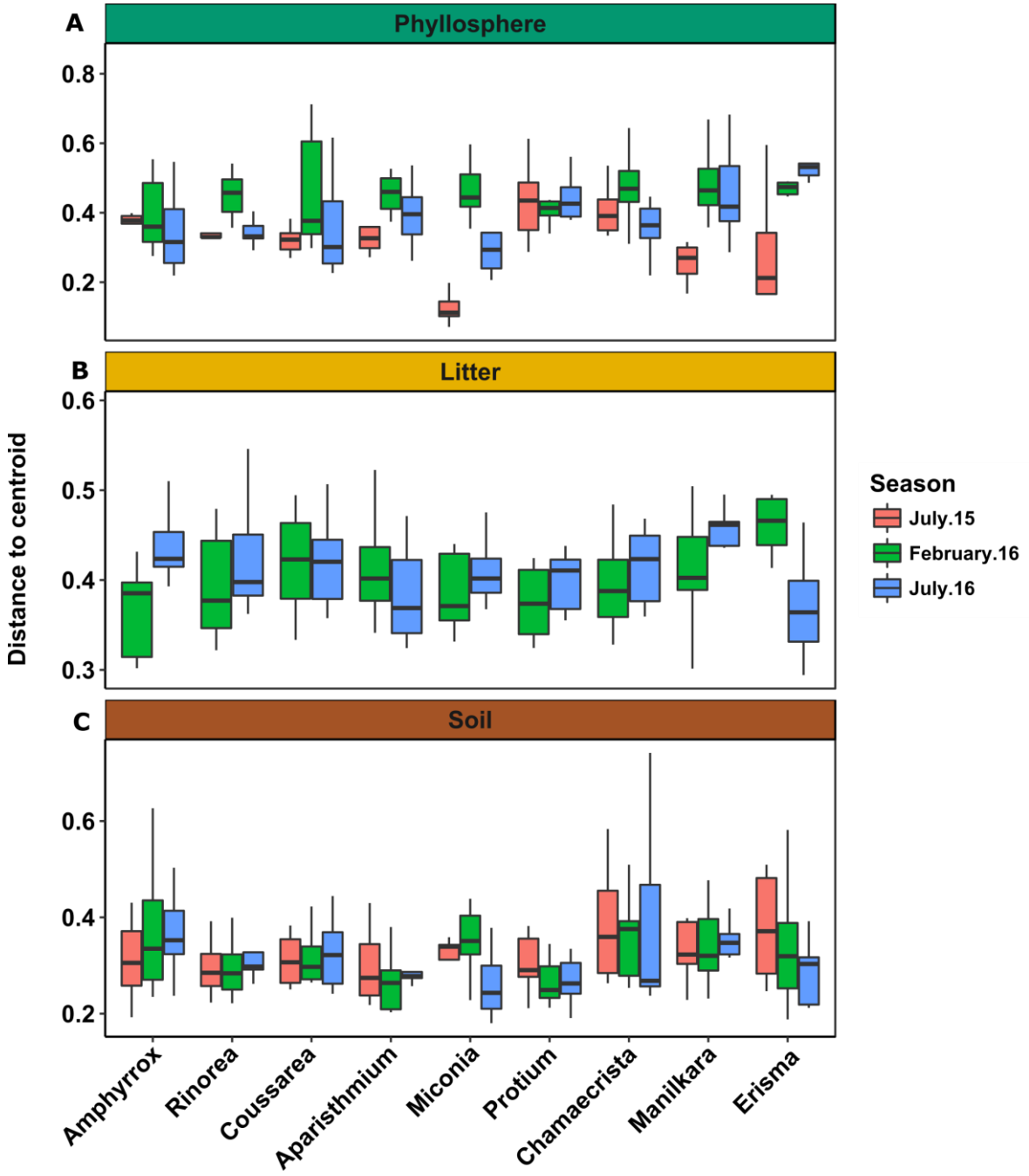

Figure 16. Beta diversity of bacterial communities in the phyllosphere (A), litter (B) and rhizospheric soil (C) of nine tree species in the Amazon forest at three time points. Error bars represent the standard deviation of the mean.

3.3.2. Bacterial community structure in the phyllopshere, litter and rhizospheric soil

A NMDS ordination, based on the relative abundance of ASVs, reveled distinct bacterial community structures in different forest compartments (**Figure 17**). Although variations in community structure were observed for all forest compartments, in the phyllosphere the seasonal effect was higher than in the litter and rhizosphere, explaining 27% of the variation in the bacterial community structure (PERMANOVA on Bray-Curtis distance, P = 0.001), whereas

for the litter and rhizosphere, seasonality explained 11 and 9% of the variation, respectively (PERMANOVA, P = 0.001).

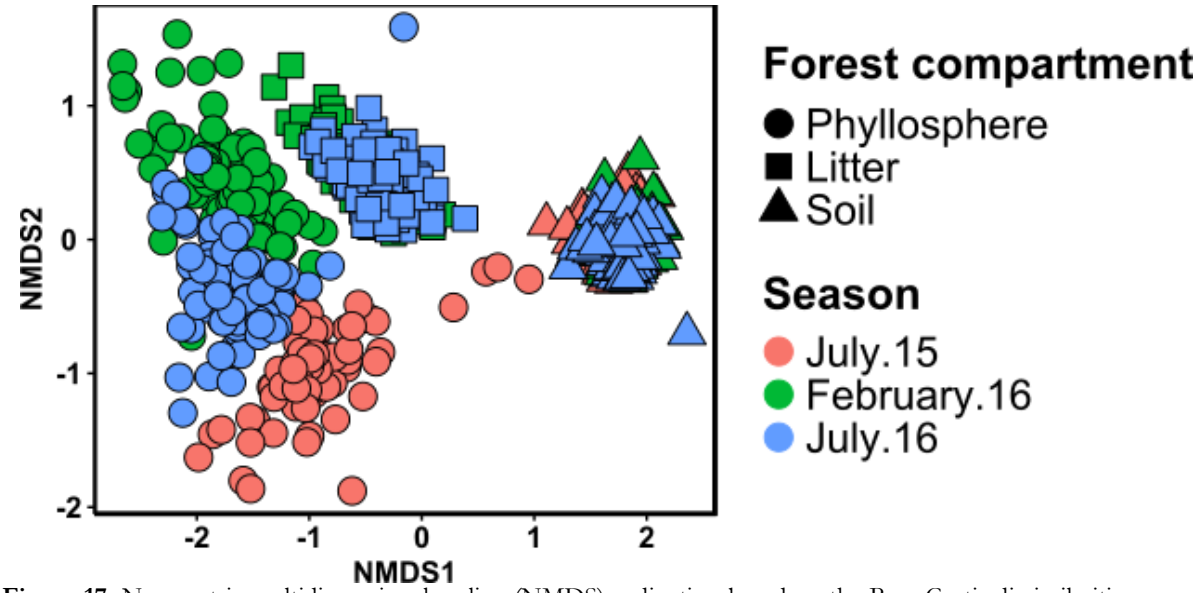

Figure 17. Non-metric multidimensional scaling (NMDS) ordination based on the Bray-Curtis dissimilarities among bacterial communities in the phyllosphere, litter and rhizospheric soil of trees in the Amazon forest sampled in July 2015, February 2016 and July 2016. Points represent individual samples.

The analysis of the factors explaining the variation of the bacterial community structures (PERMANOVA) showed that forest compartment explained 37.9% of the variance (p=0.001), whereas season and tree species explained 4.9% and 2% of the variance (p=0.001), respectively. The interaction between tree species and forest compartment, tree species and season, and forest compartment and season explained 3.5, 2.4 and 6.8% of the variance (p=0.001), respectively. Finally, the interaction between tree species, forest compartment and season explained 3.8% of variance of the bacterial community structure (p=0.001). In total, the model explained 61.1% of variance of the bacterial community structure (**Table 7**).

Table 7. Bacterial community structure variation explained by the studied factors (PERMANOVA on Bray-Curtis dissimilarities)

Factors	Df	R2	P
Tree Specie (Sp)	8	0.020	0.001
Forest compartment (FC)	2	0.379	0.001
Season (S)	2	0.049	0.001
Sp x FC	16	0.035	0.001
Sp x S	16	0.024	0.001
FC x S	3	0.068	0.001
Sp x FC x S	24	0.032	0.001
Residuals	578	0.389	0.001
Total	649	1	0.001

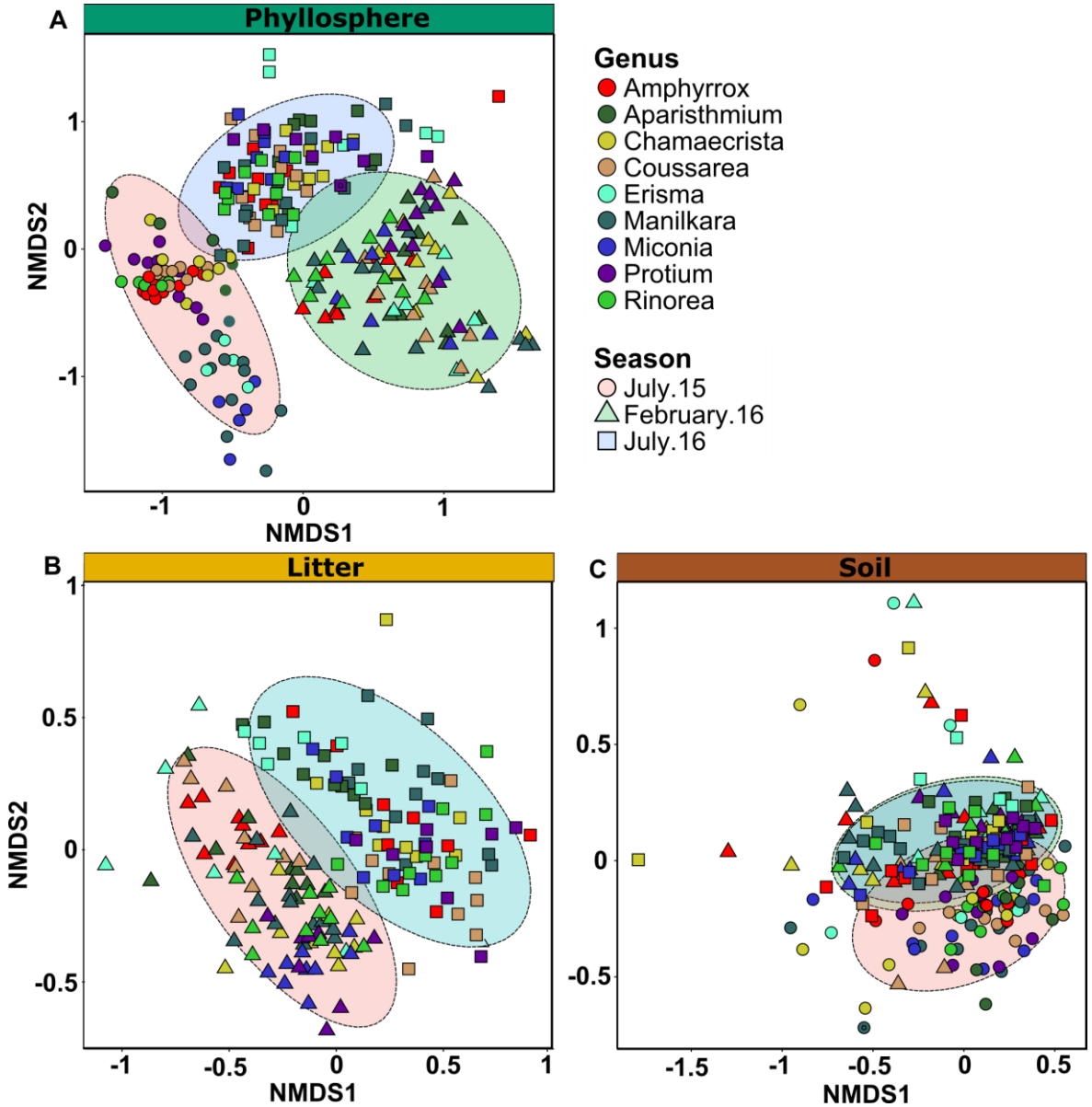

Figure 18. Non-metric multidimensional scaling (NMDS) ordination based on Bray-Curtis dissimilarities among bacterial communities in the phyllosphere (A), litter (B) and rhizospheric soil (C) of trees in the Amazon forest sampled in July 2015, February 2016 and July 2016. Points represent individual samples. Ellipses represent 95% confidence.

Our results showed that forest compartment was the strongest modulator of the bacterial communities in trees of the Amazon forest (**Table 7**). However, besides the forest compartment, seasonality and tree species were also important determinants of the structure of the bacterial communities. To better understand the drivers of the bacterial community structures in the Amazon forest, we evaluated the effect of the factors "tree species" and "season" within each forest compartment separately (**Figure 18a, b and c**). The seasonal effect was more significant on the modulation of the bacterial communities in the phyllosphere, explaining 27% of the total variance (P = 0.001, **Figure 18a**). In the litter and rhizospheric soil, 11 and 9% of the variance, respectively, was explained by a seasonal effect (P = 0.001, **Figure 18b and c**). "Tree

species" was also a significant factor modulating the bacterial communities, explaining 9.6, 9.5 and 6.8 % of total variance in the phyllosphere, litter and rhizospheric soil, respectively (P = 0.001, **Figure 18a, b and c**).

The higher effects of forest compartment on the modulation of the bacterial communities, as compared to tree species and seasonality, can also be observed at the tree species level (**Figure 19 and Table 8**). In addition, in all tree species, significant seasonal effects on the structure of the bacterial communities were observed for all forest compartments. Notwithstanding, in the phyllosphere these effects were more expressive (**Table 8**). In the phyllosphere of all tree species studied, the greatest differences in the bacterial community structures were detected in to February 2016, as compared to July 2015 (**Figure 19**).

Table 8. Bacterial community structure variation explained by studied factors (PERMANOVA on Bray-Curtis dissimilarities) in each tree species.

Variables	Amphyrrox			Coussarea			Miconia		
	Df	**R2**	**P**	**Df**	**R2**	**P**	**Df**	**R2**	**P**
Forest compartment (FC)	2	0.433	0.001	2	0.415	0.001	2	0.450	0.001
Season (S)	2	0.069	0.001	2	0.089	0.001	2	0.074	0.001
FC x S	3	0.095	0.001	3	0.118	0.001	3	0.123	0.001
Residuals	68	0.402		64	0.377		61	0.353	
Total	75	1		71	1		68	1	
Variables	**Aparisthium**			**Erisma**			**Protium**		
	Df	**R2**	**P**	**Df**	**R2**	**P**	**Df**	**R2**	**P**
Forest compartment (FC)	2	0.454	0.001	2	0.386	0.001	2	0.431	0.001
Season (S)	2	0.067	0.001	2	0.070	0.001	2	0.083	0.001
FC x S	3	0.097	0.001	3	0.098	0.001	3	0.104	0.001
Residuals	62	0.380		37	0.444		63	0.382	
Total	69	1		44	1		70	1	
Variables	**Chamaecrista**			**Manilkara**			**Rinorea**		
	Df	**R2**	**P**	**Df**	**R2**	**P**	**Df**	**R2**	**P**
Forest compartment (FC)	2	0.389	0.001	2	0.398	0.001	2	0.464	0.001
Season (S)	2	0.071	0.001	2	0.080	0.001	2	0.063	0.001
FC x S	3	0.095	0.001	3	0.101	0.001	3	0.093	0.001
Residuals	65	0.443		93	0.419		65	0.378	
Total	72	1		100	1		72	1	

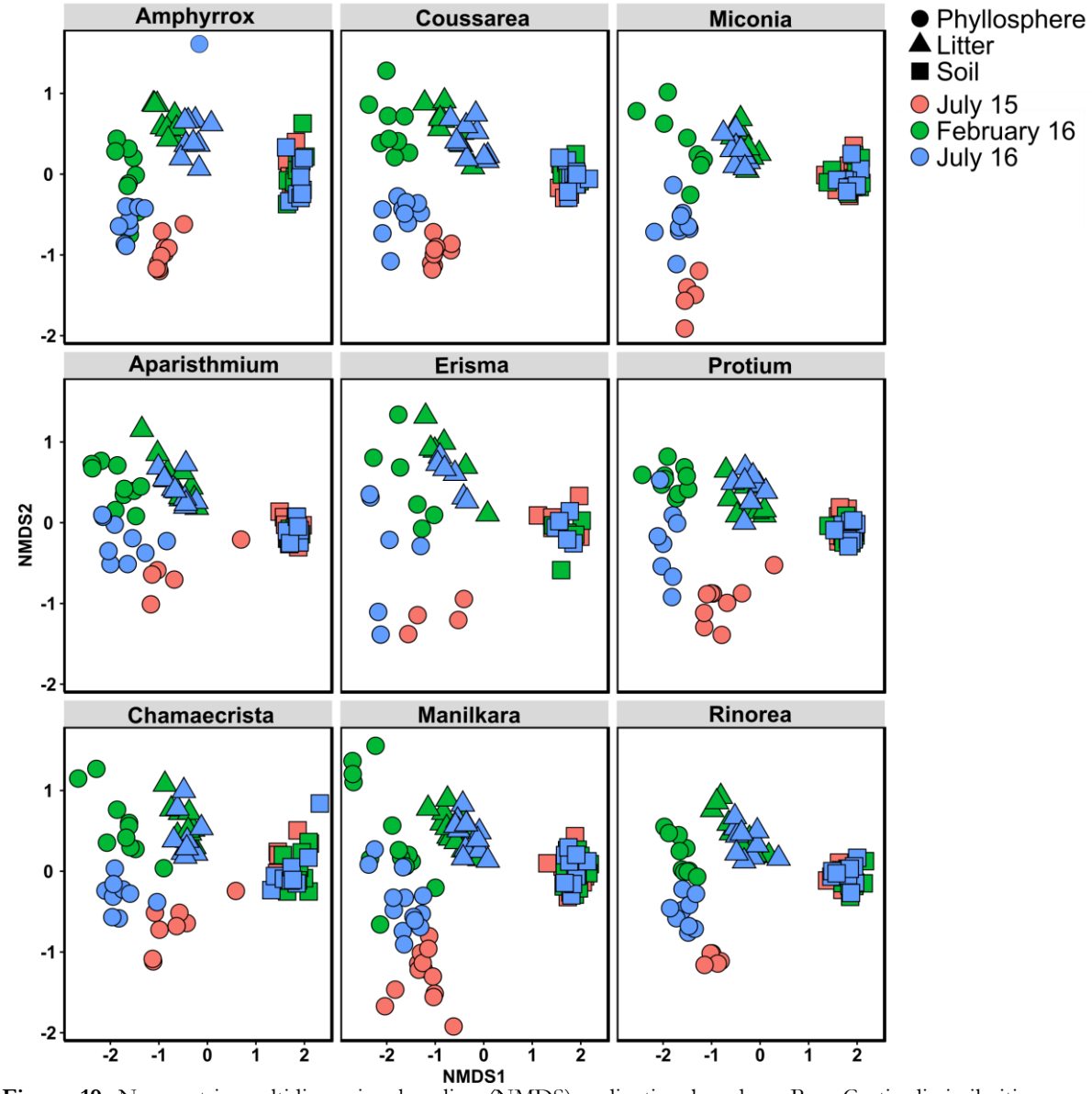

Figure 19. Non-metric multidimensional scaling (NMDS) ordination based on Bray-Curtis dissimilarities among bacterial communities in the phyllosphere, litter and rhizospheric soil of trees in the Amazon forest sampled in July 2015, February 2016 and July 2016. Points represent individual samples.

We have also observed that the similarities between the bacterial communities in the phyllosphere and litter were higher than between phyllosphere and soil or litter and soil, regardless of the tree species (**Figure 19 and 20**). In February 2016, the similarities between the bacterial community structures in the phyllosphere and litter were higher than in July 2016. This pattern was observed for most tree species, except for *M. huberi*, *A. cordatum*, *E. uncinatum* and *A. longifolia*, for which similarities remained unchanged or decreased along time (**Figure 20**).

In the rhizospheric soil, the effects of seasonality and tree species on the variation of the bacterial community structure were smaller than those observed in the other forest compartments (**Figure 20 and Table 8**). Significant variations in the bacterial communities in the rhizosphere of *C. albescens*, *M. lepidota*, *A. cordatum*, *P. apiculatum*, *M. huberi* and *R. pubiflora* were detected (**Figure 20**). The major changes in the bacterial community structures were observed when comparing samples from July 2015 and February 2016. Comparing samples from February 2016 and July 2016, no significant variations in the structure of the bacterial communities were observed, independent of the tree species (**Figure 20**).

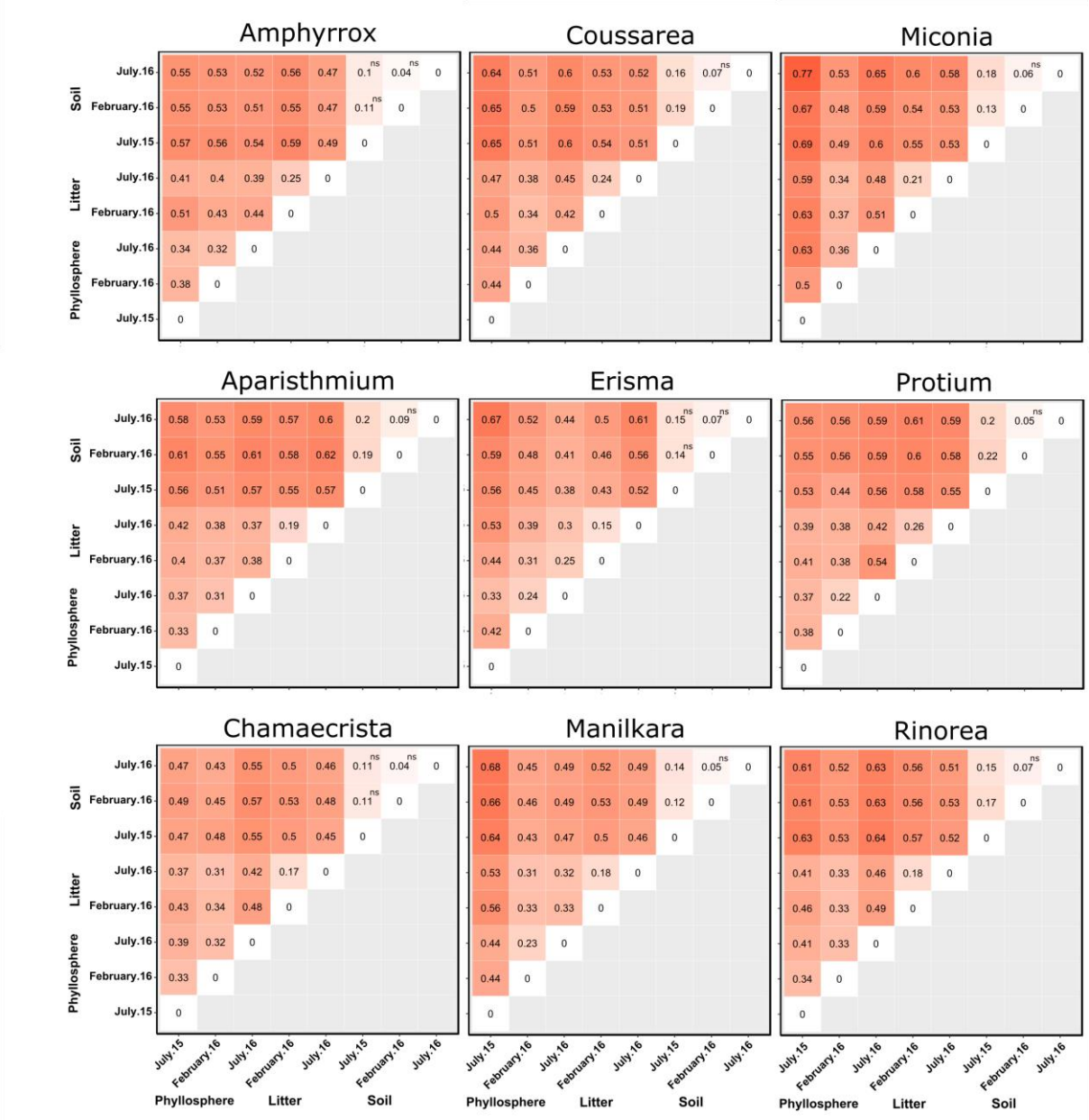

Figure 20. PERMANOVA pairwise comparisons between the bacterial community structures in the phyllosphere, litter and rhizospheric soil of different tree species of the Amazon at three time points. ^{ns} no-significant at $P < 0.01$ (PERMANOVA). The color scale indicates the R^2 values.

3.3.3. Plant traits and environmental factors as modulator of the bacterial community structure in the phyllosphere, litter and rhizospheric soil

In this study, we identified a strong influence of seasonality on the bacterial community structure in trees of the Amazon forest, which may be related to variations in specific plant traits or environmental factors. In order to determine the main plant traits and environmental factors modulating the bacterial community structure in the phyllosphere, litter and rhizospheric soil, a fitting of plant traits (including bacterial richness, alpha and beta diversity) and environmental factors to microbial community structure was performed.

In the phyllosphere, among the parameters measured, IEC, precipitation, and air humidity were the main factors affecting the bacterial communities in July 2015. All of these parameters were also directly related to richness and alpha diversity at this time point. On the other hand, in February 2016, under the affect of severe drought, air temperature, LWR, PH, LMA, C, N and C:N ratio were the main drivers of the bacterial communities in the phyllosphere and were directly related to beta diversity. In contrast, in July 2016, only two plant traits, LWC and C content, were significantly associated to the bacterial community structures in the phyllosphere (**Figure 21a**).

In the litter, the main drivers of the bacterial communities were air humidity in February 2016, and air temperature and C:N ratio in July 2016. Air temperature and N concentration were directly associated to beta diversity. However, no clear trend was observed for alpha diversity and richness, even though these traits significantly fitted the bacterial community data (**Figure 21b**).

In the rhizospheric soil, we observed no significant seasonal effect on the bacterial communities (**Figure 21c**). However, analyzing the factors driving the community structure, we can infer two major tendencies, the first is that Fe, Cu, and B concentrations, CEC and H+Al, and precipitation are mainly associated to the community structure in February 2016. The second is that P, Mg and Ca concentrations, SB and V are directly associated to bacterial ASV richness. Among the physical soil attributes, only silt content was significantly associated to the bacterial community structure, but the vector was perpendicular to the microbial community ordination (**Figure 21c**).

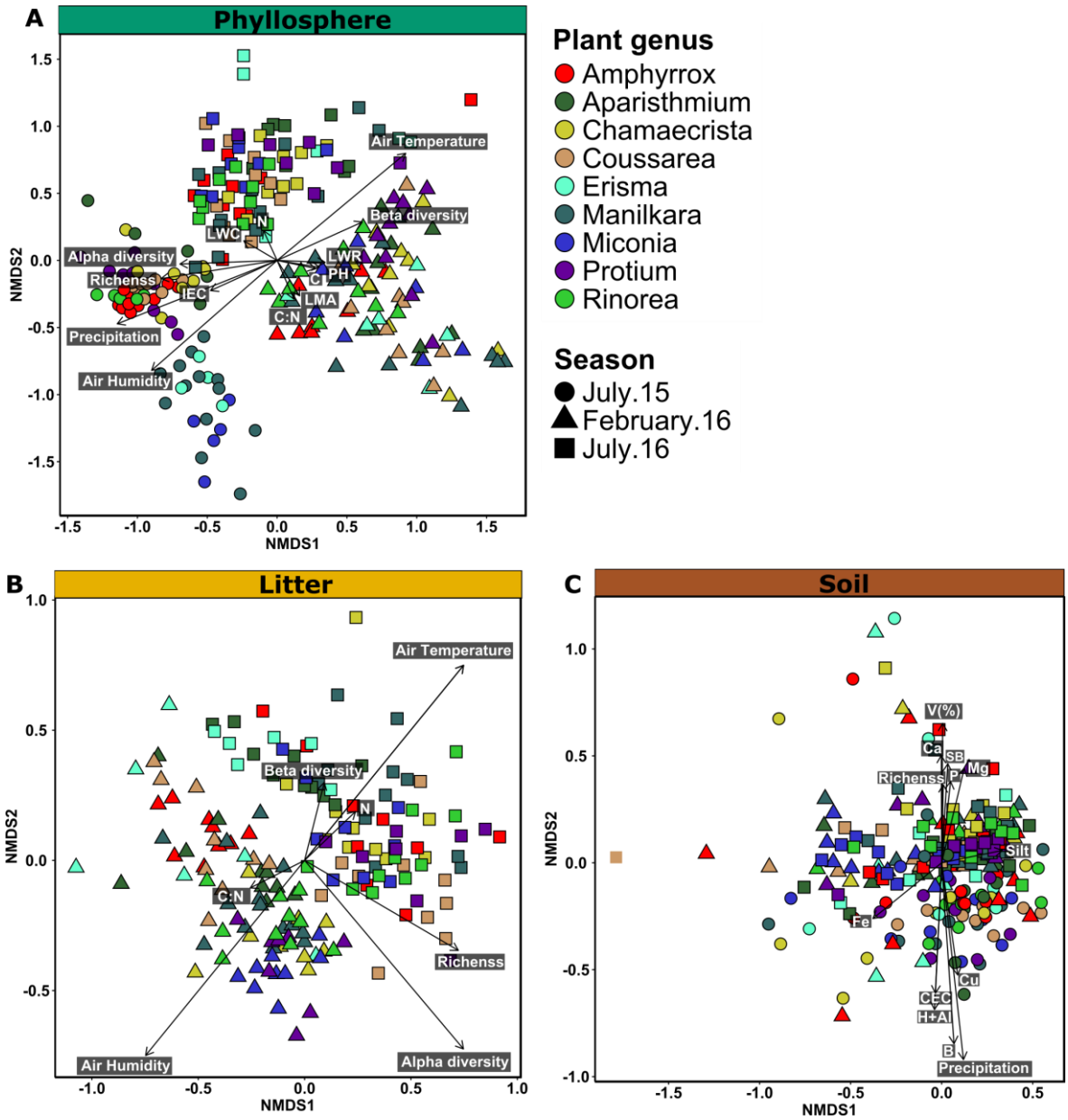

Figure 21. Non-metric multidimensional scaling (NMDS) ordination of the variation in bacterial community structure associated to the phyllosphere (A), litter (B) and rhizospheric soil (C) of trees of the Amazon forest sampled in July 2015, February 2016 and July 2016 . Ordination were based on Bray-Curtis dissimilarities among samples. Points represent individual samples, and arrows indicate correlations between traits and bacterial community structure. The arrows show the direction of the increasing gradient, and the length of the arrow is proportional to the correlation between the variable and the ordination. Only host traits that were significantly correlated with the NMDS ordination axes ($P < 0.01$) are shown. Plant traits are: PH, plant height; C, leaf carbon content; N, leaf nitrogen content; IEC, index of epiphyllous cover; LWR, leaf water repellency; LWC, leaf water contend; LMA, leaf mass per area; C:N, leaf carbon to nitrogen ratio. Soil attributes are: Cu, copper concentration; Ca, calcium **concnetration**; SB, sum of bases; P, phosphorus concentration; OM, organic matter concentration; CEC, cation exchange capacity; Al, exchangeable aluminum concentration; H+Al, potential acidity; Fe, iron concentration; pH, soil pH; Mn**,** manganese concentration; V%**,** base saturation; Environmental parameters are: Mean air temperature, Mean air humidity and Precipitation. Microbial community attributes are: ASV Richness, Alpha diversity (Shannon's index) and Beta diversity (Distance to centroid).

3.3.4. Effects of seasonality on forest compartment indicator bacterial genera

Our results showed that the extreme drought conditions observed in February 2016 caused changes in the abundance of distinct bacterial taxa in each of the compartments evaluated (**Figure 22a, b and c**). In the phyllosphere, in general, we have identified a reduction in the relative abundance of bacteria assigned to the phylum Actinobacteria in February and July 2016, as compared to July 2015. In addition, bacterial genera, such as *Chthonomonas* (Armaimonadetes), *Roseiflexus* (Chloroflexi) and *Deinonoccus* (Deinococcus-Thermus), detected in the phyllosphere in July 2015 were no longer detected in February 2016. We have also observed that the bacterial genera *Methylocella*, *Metlittangium*, *Acinitobacter* and *Rasltonia* (Proteobacteria), *Terriglobus* (Acidobacteria), *Fibrisoma* and *Spirosoma* (Bacteroidetes), which were detected in the phyllosphere in July 2015 but not in February 2016, were considered indicators of the phyllosphere in July 2016. In contrast, *Pantoea*, *Methylobacterium* and *Klebsiella* (Proteobacteria), *Actinomycetospora* (Actinobacteria), *Hymenobacter* (Bacteroidetes) were considered phyllosphere indicator genera at all sampling times (**Figure 22a, b and c**).

For the litter, we have no data on the bacterial community in July 2015, and cannot determine clearly the changes in community structure along the time course. The indicator species methodology is dependent on the members that make up the factor (e.g. forest compartment), thus phyllosphere bacterial indicator genera in July 2015 that were detected as litter indicators in February 2016 and July 2016 were indeed litter indicators. Thus, *Chitinophaga* (Bacteroidetes), *Labris*, *Mesorhizobium* and *Devosia* (Proteobacteria) were most likely litter indicators. Another important issue is that, due to the drought stress in February 2016, there was an increase in the index of leaf senescence, increasing leaf fallout and bulk litter mass. In addition, the low rainfall volume during the sampling period may have contributed to low litter degradation rates, resulting in the observed accumulation of litter on the soil surface. Our results also suggest that *Afifia*, *Bosea*, *Rhodopseudomonas*, *Aureimonas* and *Pseudomonas* (Proteobacteria), identified as litter indicator genera in February 2016 came from the phyllosphere, since those indicators were identified in the phyllosphere in July 2015 (**Figure 22a, b and c**).

In the soil, we identified a greater number of bacterial genera that remained indicators at the three time points studied, as compared to the other forest compartments. Among them were *Pedosphaera* (Verrucomicobria), *GAL15* (Thermotogae), *Acidibacter*, *Variibacter* and *Sorangium* (Proteobacteria), *Acidothermus* and *Actinospica* (Actinobacteria) e *Telmatobacter* (Acidobacteria). We have also identified a few bacterial genera that were affected by seasonality, i.e. *Acidicaldus* and *Methylovirgula* (Proteobacteria) and *Isosphaera* (Plantctomycetes) and were soil indicators in July 2015 and July 2016, but were not detected in February 2016. As described previously for litter,

due to the absence of litter data for July 2015, bacterial genera belonging to litter may have been attributed to soil, due to the similarity of the environments. Thus, we can include the genus Aquicella (Proteobacteria) as litter indicator in July 2015, since it was observed as litter indicator in the other time points (**Figure 22a, b and c**).

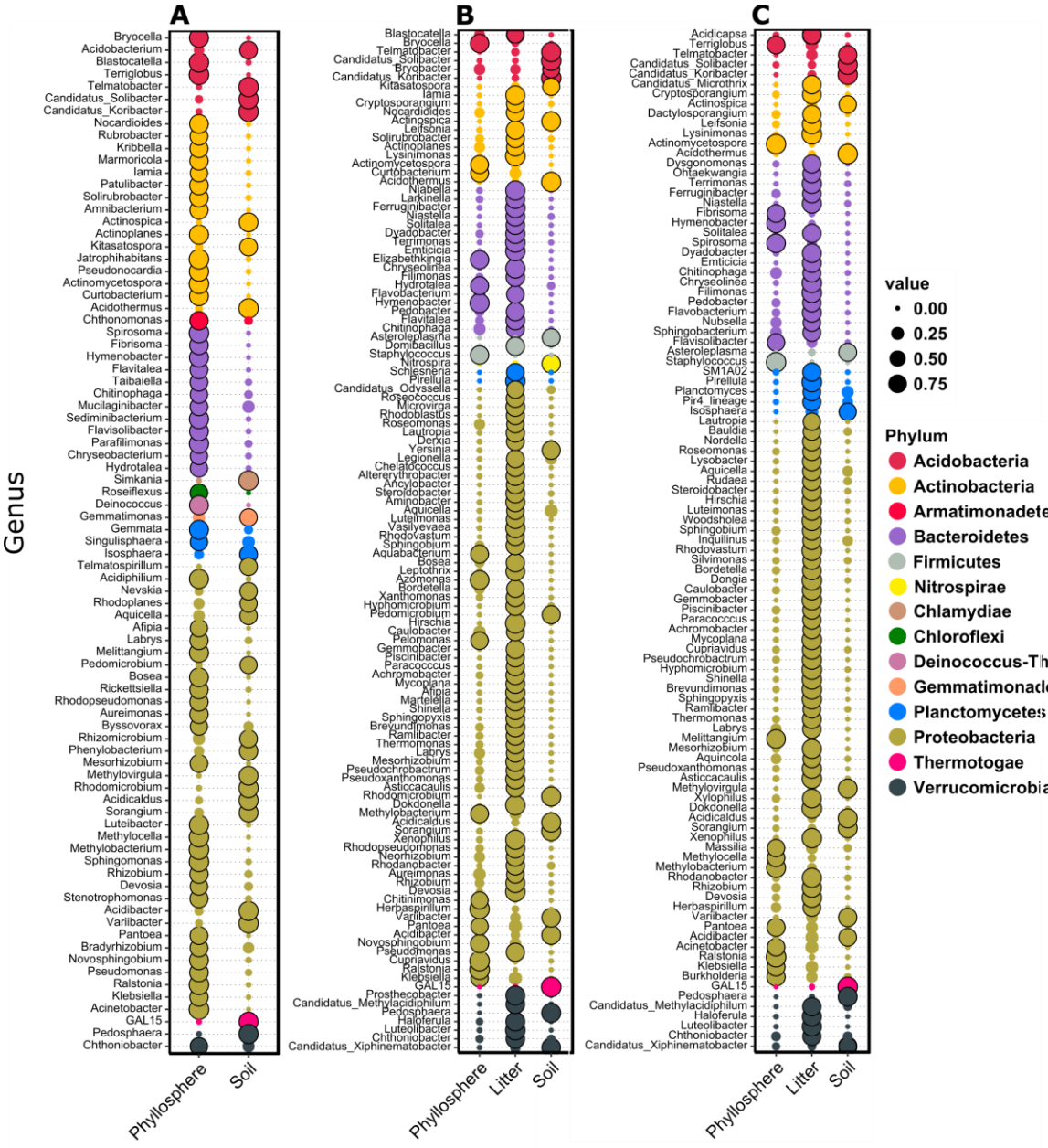

Figure 22. Phyllosphere, litter and rhizospheric soil indicator genera analyses based on 16S rRNA ASVs at the genus level. The circle size represents the indicator Value index (IndVal). Only genera with $p \leq 0.01$ are show. Colors indicate bacterial phylum. Circles delimited by black line indicate that the bacterial genera are indicators. Samples collected in July 2015 (A), February 2016 (B) and July 2016 (C).

3.3.5. Effects of seasonality on plant species indicator bacterial genera

In the phyllosphere**,** the bacterial genera indicators of tree species were highly variable (**Figure 23a, b and c**), and no indicator genera that remained indicator of the same tree species throughout the time course were detected. In contrast, we identified several bacterial genera that switched as indicators between certain tree species at the three time points studied. The major changes in the tree species indicator bacterial community were concentrated in two groups of plants, the first formed by *C. albescens, A. longifolia* and *R. pubiflora*, and the second formed by *A. cordatum* and *M. lepidota*. Within the first group of plants the tree species indicator genera *Actinomycetospora*, *Patulibacter* (Actinobacteria) and *Bosea* (Proteobacteria) could be not considered indicators of *C. albescens*, *A. longifolia* and *R. pubiflora*. at all times points (July 2015, February 2016 and July 2016). The bacterial indicator genera *Opitutus* (Verrucomicrobia), *Bryobacter* (Acidobacteria), *Angustibacter*, *Candidatus_Alysiosphaera*, *Acidisphaera* (Proteobacteria), *Actinophytocola*, *Microcella* and *Pseudonocardia* (Actinobacteria) switched as tree species indicators in in the phyllosphere of *A. longifolia* and *R. pubiflora* across seasons. In the second group of plants, the bacterial genera in the phyllosphere of *Acidisoma*, *Methylocella* (Proteobacteria) and *Granulicella* (Acidobacteria) were not considered indicators of tree species at all time points in *A. cordatum* and *M. lepidota*. We have also identified bacterial genera that were affected by seasonality. *Janibacter* and *Sporichthya* (Actinobacteria) were rhizospheric soil indicators in July 2015 and July 2016, but were not detected in February 2016 (**Figure 23a, b and c**).

In the litter, we identified only two bacterial genera that remained indicators of the same tree species along the seasons, i.e. *Acinetobacter* (Proteobacteria) as indicator of *E. uncinatum* and *Legionella* (Proteobacteria) as indicator of *P. apiculatum*. However, in February 2016, many tree species indicator bacterial genera were identified, most of them assigned to the Actinobacteria phylum, contrasting with July 2016, when most of the indicator genera were assigned to the Proteobacteria phylum (**Figure 24a, b and c**).

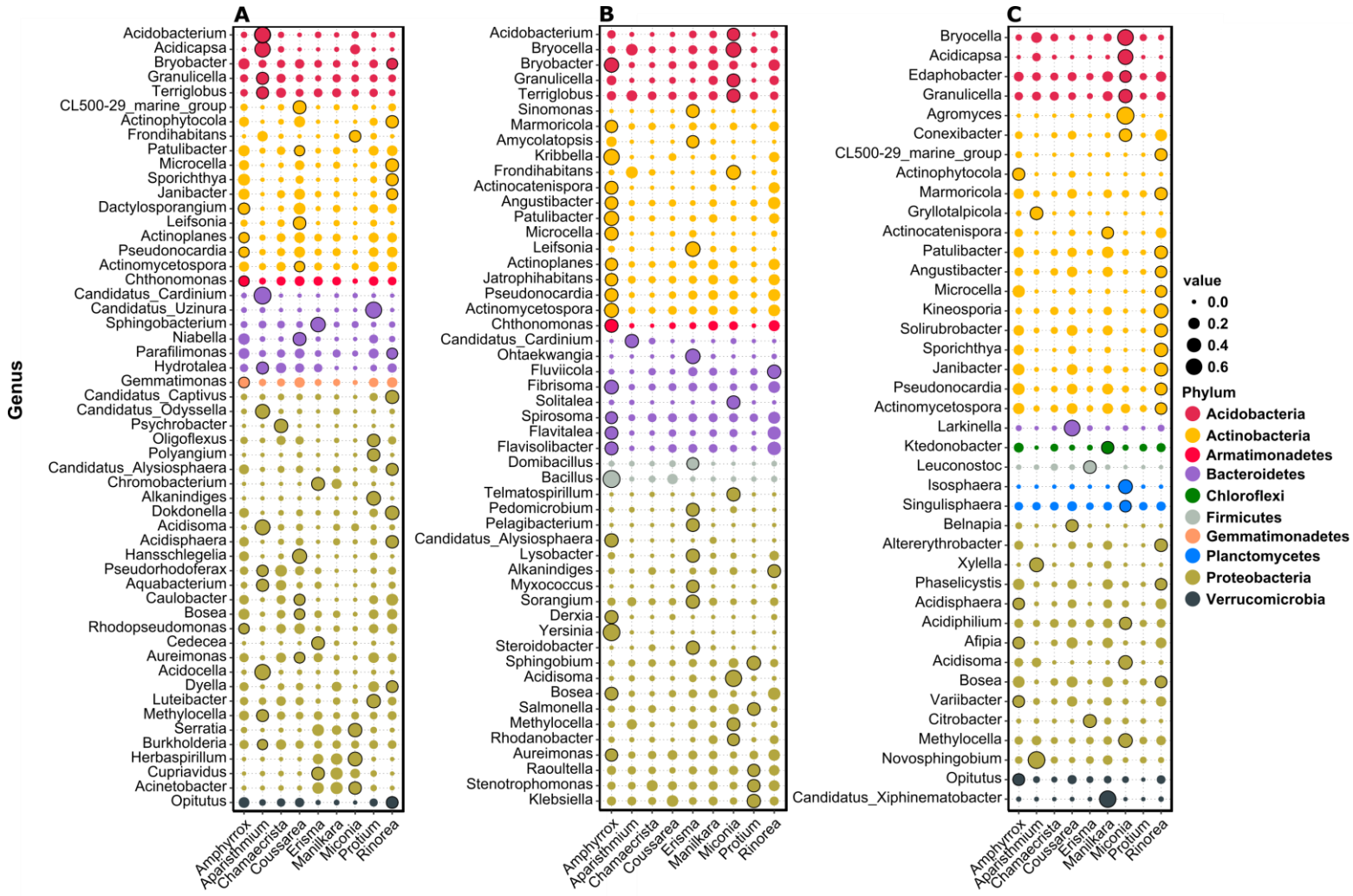

Figure 23. Tree species indicator genera analyses based on 16S rRNA ASVs at genus level in the phyllosphere. The circle size represents the indicator Value index (IndVal). Only genera with $p \leq 0.01$ are show. Colors indicate bacterial phylum. Circles delimited by black line indicate that the bacterial genera are indicators. Samples collected in July 2015 (A), February 2016 (B) and July 2016 (C).

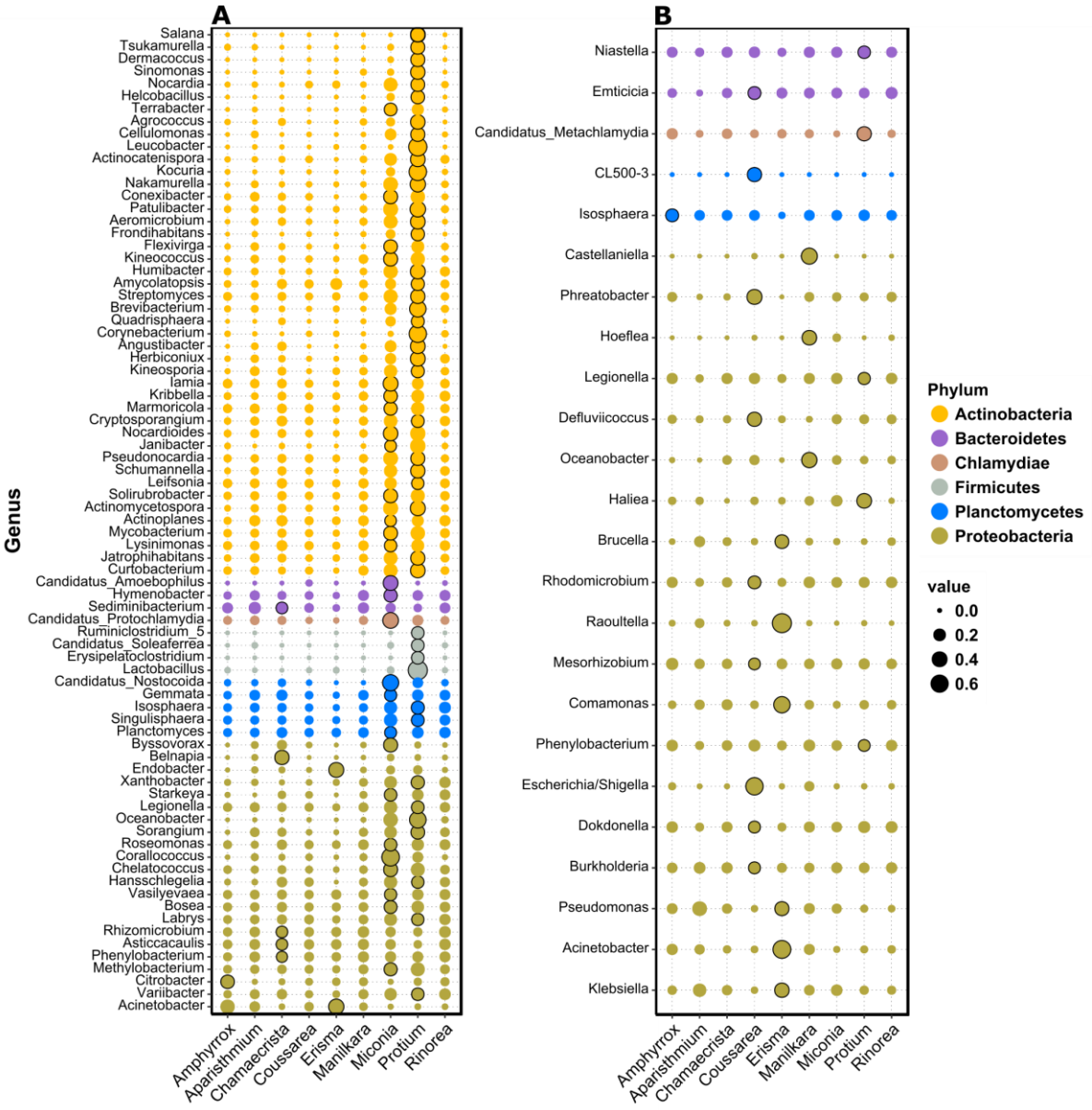

Figure 24. Tree species indicator genera analyses based on 16S rRNA ASVs at genus level in the litter. The circle size represents the indicator Value index (IndVal). Only genera with $p \leq 0.01$ are show. Colors indicate bacterial phylum. Circles delimited by black line indicate that the bacterial genera are indicators. Samples collected in July 2015 (A), February 2016 (B) and July 2016 (C).

In the rhizospheric soil, no indicator genera that remained indicator of the same tree species throughout the sampled time points were detected. *Gemmatimonetes* (Gemmatimonadetes) was indicator of *E. uncinatum* in July 2015 and July 2016, but not in February 2016. *Bdellovibrio* (Proteobacteria) was indicator of *C. xinguenxis* only in July 2015, and indicator of *E. uncinatum* only in February 2016. All other bacterial indicator genera were specifically associated to one tree species at a single season (**Figure 25a, b and c**). *E. uncinatum* rhizosphere selected a larger number of indicator bacterial genera as compared to the other tree species, mostly in July 2015

and July 2016. The indicator genera analyses also showed that the drought stress in February 2016 caused a reduction in the abundance of tree indicator bacterial genera in the rhizosphere.

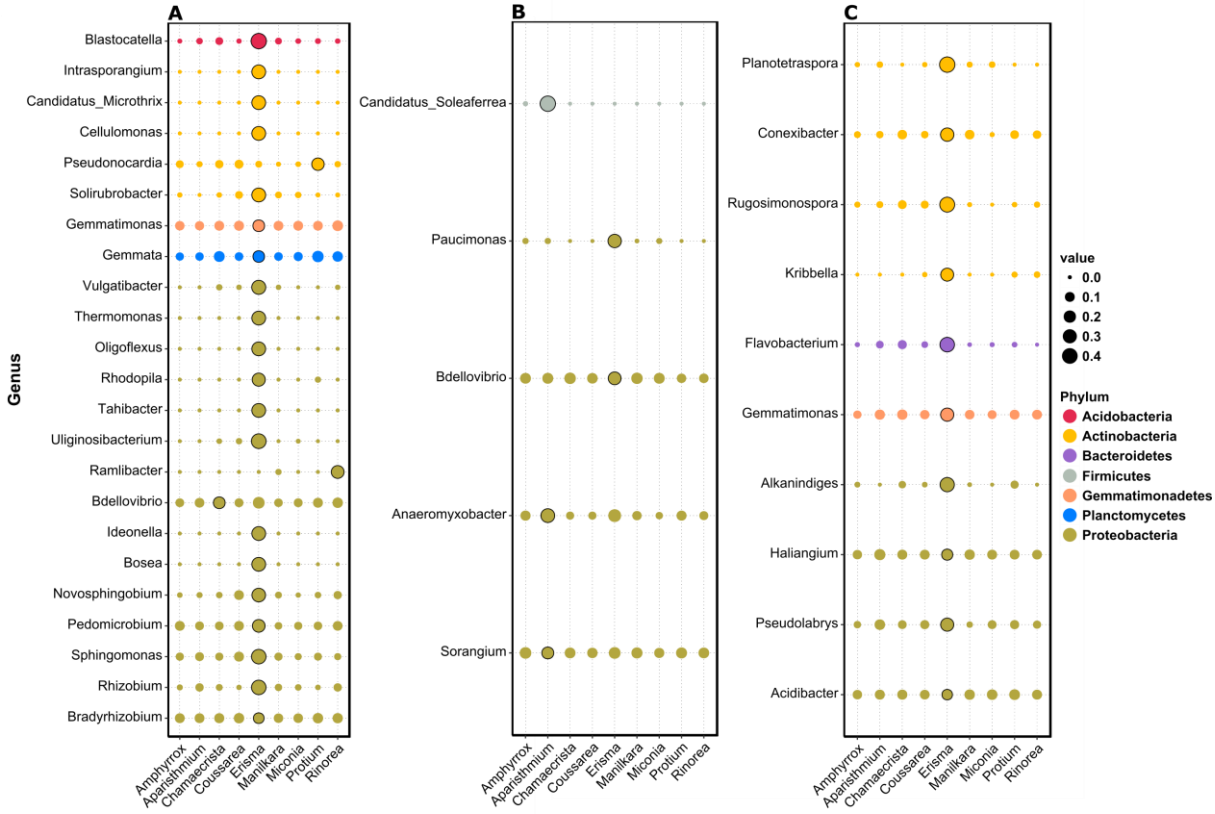

Figure 25. Tree species indicator genera analyses based on 16S rRNA ASVs at genus level in the rhizosphere. The circle size represents the indicator Value index (IndVal). Only genera with $p \leq 0.01$ are show. Colors indicate bacterial phylum. Circles delimited by black line indicate that the bacterial genera are indicators. Samples collected in July 2015 (A), February 2016 (B) and July 2016 (C).

3.4. Discussion

In this study, we evaluated the bacterial diversity and community structure in several tree species of the Amazon forest across three time points, including a time point when the forest was under a severe drought due to the ENSO 2015-2016 (Jiménez-Muñoz et al., 2016), and found that this extreme climatic event promoted significant changes in bacterial diversity and community structure in the phyllosphere, litter and rhizosphere. The highest bacterial ASV richness was detected in the litter, followed by phyllosphere and soil ($P < 0.01$). In addition, the highest values of alpha diversity, measured by the Shannon's index, were observed in the litter and rhizospheric soil ($P < 0.01$). For most of the tree species, we observed that the bacterial alpha diversity in the phyllosphere, litter and rhizospheric soil decreased at the driest sampling season, whereas the beta diversity increased. Moreover, forest compartment and tree species

bacterial indicator genera in the phyllosphere, litter, and soil significantly changed upon the extreme drought.

Bacterial communities in the phyllosphere were more dissimilar among individuals of the same tree species and beta diversity was higher than in the litter and soil. In addition, phyllosphere and litter beta diversity increased in the driest season, as compared to the rainy season, in most of plant species. Phyllosphere and litter are subject to intense biotic and abiotic stresses, including radiation, temperature and humidity oscillations, low nitrogen and phosphorus availability, as well as antibiotic and other defense compounds (Vorholt, 2012;Laforest-Lapointe et al., 2016a;Rosado et al., 2018). Consistent with our findings, Mykrä *et al.* (2017) reported that anthropogenic disturbances can cause decreases in alpha diversity and increases in beta diversity of fungal communities during decomposition of leaf litter. Even though, microbial communities in the litter and soil are subjected to several environmental stresses, in the phyllosphere the stresses may be higher because the leaf surfaces are more exposed to the atmosphere, mainly in the canopy tree species, contributing to high levels of microbial community heterogeneity (beta diversity).

Seasonality was the main driver of bacterial community structure in all bacteriomes (R^2 = 27, 11 and 9% in the phyllosphere, litter and soil, respectively), despite the effect of tree taxon. The tree taxon effect is in agreement with other studies showing that each tree species harbors a distinct bacterial community in the phyllosphere (Redford and Fierer, 2009;Kim et al., 2012;Lambais et al., 2014;Laforest-Lapointe et al., 2016a). However the effect of an extreme climatic condition on the bacterial communities in the Amazon forest was shown for the first time in this study. Our results contrast with the findings of (Laforest-Lapointe et al., 2016b), who observed in a temperate forest that a greater part of the variation in phyllosphere bacterial communities was explained by host species identity rather than by site or time, suggesting that the factors driving the plant-bacteria associations in the phyllosphere might be distinct in temperate and tropical forests. Alternatively, the atypical dry period observed in our sampling site in the Amazon may have inflated the effect of seasonality. However, the differences of the weights of the factors ("seasonality" and "plant species") explaining data variance was large enough to infer that the season is an important environmental factor modulating the bacterial communities in the Amazon forest. Other studies indicate that the phyllosphere bacterial communities are the most affected by drought. For instance, rain exclusion altered the composition of phyllosphere bacterial communities in Holm oak (*Quercus ilex*) with an expressive increase of the richness of epiphytic bacterial populations in the summer (Peñuelas et al., 2012;Rico et al., 2014).

According to Vellend (2010), ecological communities are shaped by four processes: dispersal, speciation, selection and drift. Is plausible to argue that in dense tropical forests, as the Amazon forest, the dispersion of microorganisms occurs constantly, either by wind or rainfall (Morris and Kinkel, 2002), and a "core microbiome" shared among all plant species may exist (Kembel et al. (2014), even though a consistent number of studies have shown that the phyllosphere microbial communities are composed mainly by rare taxa (Lambais et al., 2006;Kembel et al., 2014;Lambais et al., 2014;Laforest-Lapointe et al., 2016a). However, when microorganisms arrive on the leaf surface they need to adapt to a variety of abiotic and biotic stressors that exert an intense selection pressure. These microbial populations are constantly exposed to evolutionary diversification events, which might be higher in the phyllosphere (Vacher et al., 2016). In agreement to the idea that environmental selective pressure on microbial populations, such as temperature and precipitation, are key drivers of microbial community structure, our results indicate that seasonal factors associated with an extreme climatic event is the major driver of the structuring of the bacterial communities in the phyllosphere of tress in the Amazon forest. Besides the environmental drivers, we have also shown that the tree taxon is an important factor driving the assemblage of the microbial communities in the phyllosphere. Tree species explained a considerable part of the variation in the community structure in the phyllosphere, although in a minor proportion than seasonality. Since phyllosphere microbial communities are comprised of few very abundant taxa and a huge number of rare taxa (Lambais et al., 2006;Kembel et al., 2014;Lambais et al., 2014;Laforest-Lapointe et al., 2016a) and the drift process primarily affects rare taxa, small changes in their abundance due to environmental pressures can result in their extinction on a local scale (Nemergut et al., 2013).

Phyllosphere microbial communities are also shaped by plant functional traits, that create a unique microenvironment on the leaf surfaces. In order to identify which and how plant functional traits might explain the variation in microbial community structure, we determined the relationships between leaf chemistry and morphology traits with the bacterial community structure in phyllosphere, in addition to environmental parameters such as air temperature, humidity and monthly precipitation. Our results reinforce the idea that the seasonal variability of the bacterial communities might be explained in terms of plant functional traits and environmental parameters. For example, the bacterial community in July 2015 was positively correlated with IEC leaf trait and precipitation, and air humidity environmental parameters, whereas in February 2016 the microbial community structures were correlated with LWR, PH, C, LMA and C:N ratio, and in July 2016 community structures were correlated only with LWC and N leaf content. It is important to note that air temperature was correlated with community

structure mainly in February and July 2016. Basically, our results indicate that the microbial richness and alpha diversity are strongly influenced by climatic parameters, and are related to the establishment of epiphytic organisms on the leaves. Even though we have not observed a relationship between IEC and bacterial diversity, it is plausible to speculate that microclimate on the leaf surface colonized by epiphytes might favor the microbial diversity, by creating several niches of colonization. In February 2016, the driest period, the microbial community seemed to be influenced mainly by leaf structure and composition. During the prolonged dry season of 2015 (August–December), monthly precipitation averaged only 64 mm (Restrepo-Coupe et al., 2017), and the water stress increased leaf senescence and fallout with posterior growth of new leaves. In February 2016, we observed an increase of LWR, which might be associated to the presence of new leaves. LWR is influenced by the chemistry of waxes on leaf surfaces and microstructures such as trichomes (Jeffree, 2006). These microstructures increase repellency due to an increase in leaf surface roughness (Rosado and Holder, 2013), which might be intrinsic to the plant species. Additionally, high LWR in new leaves, as compared to old leaves (Xiong et al., 2018), may be due to the erosive effect of environmental factors, such as precipitation and atmospheric deposition, on the waxes of the leaf surface (Baker and Hunt, 1986). Our results are consistent with a study measuring leaf wettability that showed that LWR values are higher during dry periods when compared to rainy ones, due to the amount of particles deposited on the leaf surfaces during the dry season (Xiong et al., 2018). We have observed that new leaves have higher carbon concentration, probably due to the higher concentrations of organic compounds in waxes, partially explaining the increase of beta diversity in February 2016, as compared to July 2015. Drought stress has shown to increase coverage in leaves of *A. thaliana*, besides cuticular wax components, such as alkanes, aldehydes and ketones (Kosma et al., 2009). Although there is no clear evidence of the effects of leaf waxes on microbial growth (Vorholt, 2012), it is likely that they affect microbial growth along leaf development.

All indicator bacterial genera identified in the phyllosphere are frequently detected in this habitat (Vorholt, 2012;Kembel et al., 2014;Lambais et al., 2014;Laforest-Lapointe et al., 2016a;Ottesen et al., 2016), and may have high levels of tolerance to the environmental pressures across seasons. Such results suggest that these indicator bacterial genera play key functional roles in the phyllosphere. Ottesen et al. (2016) reported several network connections among *Ralstonia*, *Pantoea*, *Hymenobacter* and *Methylobacterium* in the phyllosphere of tomato. These authors also used artificial plants to identify microbial controls for phyllospheres studies and detected a complex network of relationships between *Ralstonia* and other bacteria in phyllosphere, whereas no relationships between *Ralstonia* and other genera were evident in control samples. In addition,

Methylobacterium is an important bacterial group in the phyllosphere of several plant species (Delmotte et al., 2009;Knief et al., 2012;Laforest-Lapointe et al., 2016a;Ottesen et al., 2016;Lambais et al., 2017), and have been shown to positively influence plant health and development (Abanda-Nkpwatt et al., 2006;Innerebner et al., 2011), contributing to promote plant growth through the production of secondary metabolites (Vorholt, 2012).

At tree species level, only in the litter we identified bacterial indicator genera of plant species across all seasons. In the litter, *Acinetobacter* and *Legionella* (Proteobacteria phylum) were associated with *E. uncinatum* and *P. apiculatum*, respectively. These bacterial genera have been constantly detected in the phyllosphere and litter of several plants (Tláskal et al., 2016;Haas et al., 2018), even though we did not observe these bacterial genera as phyllosphere indicators of tree species. Our data suggest that these bacterial genera might be members of both bacteriomes, without necessarily being associated to a specific plant species. Litter has a high diversity of organic compounds that can be metabolized by microorganisms, explaining the high bacterial diversity observed. In tropical forest, as the Amazon forest, the deposition of a thick litter layer (Rowland et al., 2018) results in an environment with low variability of temperature and humidity, which may be associated to reduced pressure on the processes shaping the microbial communities. Dispersal and succession process in the litter occur constantly, either by wind, rain and animals, or by changes in the chemical composition of the litter during the decomposition process (Šnajdr et al., 2011). Although decomposition rates are regulated by climate, litter quality and decomposer organisms, a rapid deposition of fresh litter on the forest floor under low rain conditions may decrease decomposition rates. In addition, the fresh litter may carry microorganisms from the phyllosphere, and may have bacterial communities more similar to the phyllosphere. It has been observed that the litter microbial community changes during the course of decomposition (Šnajdr et al., 2011). Our data suggest that during the first stages of litter decomposition, the bacterial communities in the litter share a large number of bacterial species with the phyllosphere. In contrast, at late stages decomposition, litter shares a large number of bacterial species with the soil. Soil bacterial communities showed low levels of variability across seasons, as well as among tree species, suggesting that the variation in the environmental conditions in the forest had a low impact on the rhizosphere bacterial communities.

A large number of rhizosphere bacterial indicator genera were associated with *E. uncinatum*, as compared to other trees species. These data suggest that this tree species have a stronger rhizosphere effect on the microbial community. It is interesting that, after the long drought season in the Amazon forest, the rhizosphere bacterial indicators of *E. uncinatum* changed completely, except *Gemmatimonadetes* that was detected as indicator in July 2016 as well.

One possible explanation for this strict selectivity might be the composition of root exudates released by *E. uncinatum*, and the capacity of this tree species to still release root exudates into the rhizosphere even during extreme drought. Consistent with our results, previous studies in rice showed that the composition of root exudates varies with the plant growth stage, such that the exudation of organic acids is replaced by exudation of sugars as the plant develops (Aulakh et al., 2001). In addition, to the biotic pressure shaping the rhizosphere bacterial community, we have also identified a weak environmental influence on the rhizospheric bacterial community. Two groups of soil attributes explained the bacterial community variability, CEC and H+Al, which were correlated with bacterial community structure in July 2015, and SB, V, Ca, Mg and P, which were correlated with bacterial community structure in February and July 2016. In general, our results support the evidence that rhizospheric soil bacterial communities were more resistant to environmental pressures in relation to the other bacteriomes studied.

Together, our results suggest that environmental pressures are the main drivers of bacterial diversity and community structure in the phyllosphere, litter and rhizosphere of trees in the Amazon forest. The sensibility of the bacteriome to environmental pressures was higher in the phyllosphere, median in the litter and lower in the rhizospheric soil. High dynamic changes in the microbial communities in the phyllosphere have already been demonstrated (Jumpponen and Jones, 2009;Redford and Fierer, 2009), but in these studies, plant species was the main factor explaining the microbial community variation. In contrast, in this study we report for the first time that the an extreme climatic event caused a shift in the bacterial community structure in the phyllosphere, litter and rhizospheric soil with possible consequence on the functionality of these bacteriomes.

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Suplementar materials

Table S1. Alpha and beta diversity of bacterial communities on the phyllosphere, litter and in soil for nine Amazonian tree species across three seasons.

A) Phyllosphere

Plant genus	Shannon`s index			Distance of centroid		
	July 15	Feb. 16	July 16	July 15	Feb. 16	July 16
Amphyrrox	5.86 aA	4.49 aB	4.04 aB	0.37 abA	0.39 aA	0.38 abA
Coussarea	5.83 aA	2.85 bB	3.69 abB	0.32 abB	0.44 aA	0.35 abAB
Miconia	1.89 cB	4.17 abA	3.64 abA	0.12 cC	0.46 aA	0.33 bB
Aparisthium	5.12 abA	3.15 abB	3.34 abB	0.36 abA	0.45 aA	0.40 abA
Erisma	3.51 bcAB	3.57 abA	2.32 bB	0.29 abB	0.45 aA	0.53 aA
Protium	5.80 aA	3.71 abB	2.86 abB	0.43 aA	0.44 aA	0.42 abA
Chamaecrista	5.44 aA	3.44 abB	3.63 abB	0.40 aAB	0.47 aA	0.35 abB
Manilkara	2.99 cB	3.32 abAB	4.02 aA	0.26 bB	0.49 aA	0.45 abA
Rinorea	5.59 aA	4.50 aA	4.07 aA	0.34 abA	0.45 aA	0.34 bA

B) Litter

Plant genus	Shannon`s index		Distance of centroid		
	Feb. 16	July 16		Feb. 16	July 16
Amphyrrox	5.03 cdB	6.22 aA		0.36 bB	0.43 aA
Coussarea	5.26 bcdB	6.38 aA		0.42 baA	0.42 aA
Miconia	6.41 aA	6.35 aA		0.38 abA	0.40 aA
Aparisthium	5.62 abcA	5.24 cA		0.41 abA	0.38 aA
Erisma	4.71 dA	5.18 bcA		0.46 aA	0.46 aB
Protium	6.28 aA	6.57 aA		0.37 abA	0.40 aA
Chamaecrista	6.26 aA	6.04 abA		0.39 abA	0.43 aA
Manilkara	5.86 abA	6.35 aA		0.41 abA	0.44 aA
Rinorea	6.09 aA	6.63 aA		0.39 abA	0.42 aA

C) Soil

Plant genus	Shannon`s index			Distance of centroid		
	July 15	Feb. 16	July 16	July 15	Feb. 16	July 16
Amphyrrox	5.79 abcdB	6.18 aA	5.93 aAB	0.32^{ns}	0.36	0.37
Coussarea	5.83 abcdA	5.97 aA	5.96 aA	0.31	0.31	0.32
Miconia	5.68 dB	6.02 aA	6.03 aA	0.33	0.35	0.27
Aparisthium	5.70 cdB	6.23 aA	6.16 aA	0.30	0.26	0.28
Erisma	6.18 aA	6.07 aA	5.88 aA	0.37	0.34	0.28
Protium	5.93 abcdA	5.96 aA	5.98 aA	0.30	0.27	0.26
Chamaecrista	6.04 abcA	5.99 aA	6.05 aA	0.38	0.36	0.36
Manilkara	5.77 bcdB	6.03 aA	6.04 aA	0.36	0.34	0.33
Rinorea	6.08 abA	6.03 aA	6.06 aA	0.29	0.30	0.31

Values with different lowercase letters represent means of different tree species with statistically significant differences within the same compartment (Tukey's test, $P<0.05$). Values with different uppercase letters represent means of different compartments with statistically significant differences within the same sampling time (Tukey's test, $P<0.05$).