



# Influence of Growth Support on the Diversity, Composition, and Functionality of Microbial Communities Associated with *Tillandsia recurvata*

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## Abstract

*Tillandsia recurvata* is an epiphytic plant commonly found in tropical regions and colonizes tree trunks, fences, and power wires. This plant plays an important role in interacting with trees, sharing microorganisms, and performing specific functions in the process of tree colonization. The objective of this study was to evaluate and compare the microbiomes of *T. recurvata* collected from two different locations (trees and fences) and two plant tissues (leaves and roots). The hypothesis of this study was that the microbiome of *T. recurvata* is composed of microorganisms that would provide nutritional support to compensate for the lack of nutrients in a particular growth support. The results showed significant differences in microbial diversity between trees and fences, with trees exhibiting higher richness and more complex microbial networks. *Proteobacteria* was the most prevalent bacterial phylum, with *Actinobacteria* and *Sphingomonas* also playing key roles in nitrogen fixation and plant growth. Fungal communities were similar across locations, with *Ascomycota* and *Basidiomycota* being predominant, but *Paraconiothyrium* and *Nigrospora* showed significant differences in abundance between trees and fences. Functional analysis indicated similar metabolic profiles across leaf and root samples, with key functions for *T. recurvata* including carbohydrate and amino acid metabolism, stress control, and biofertilization.

**Keywords** Microbial ecology · Epiphytic · Plant growth-promoting · Trees · Fence

## Introduction

*Tillandsia recurvata* is an atmospheric epiphyte that occupies canopy trees in many parts of tropical America and plays a crucial role in rain and cloud forests [1]. Epiphytes are a significant element of the forest canopy, and they not only interact with one another, but also with their host plant and the surrounding wildlife [2]. The coexistence of various species that occupy similar ecological roles necessitates precise differentiation of their respective niches, which in turn helps reduce competition between them. This differentiation is often brought about by life-history trade-offs, wherein competitive advantages are gained by superior competitors confined to fewer locations, while colonizers with higher fecundity and broader dispersal ranges are better suited to exploit harsh environments [3].

*Bromeliaceae* represents the only epiphytic lineage within the order *Poales* and comprises a highly diverse group of plants that encompasses both grasses and sedges [4]. Most epiphytic *Bromeliaceae* obtain mineral nutrients

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through their leaves via modified trichomes, a process that is facilitated by atmospheric deposition or rainwater flow over their host. These and several other morphophysiological characteristics are extensively examined in the comprehensive review conducted by Benzing [5], which also addressed the crucial ecological functions of bromeliad in the environment.

Colonization of plants by microorganisms is a widely recognized phenomenon, both aboveground in the phyllosphere and belowground in the rhizosphere [6, 7]. However, studies examining the bacterial communities of epiphyte plants have been conducted under adverse environmental conditions and have primarily focused on specific plant species [6]. These studies revealed differences in microbial community composition between plant compartments, species, temporal changes, and biogeographic patterns [8]. The distribution of *T. recurvata* colonization usually occurs on trees, fences, or power wires. Numerous studies have employed *T. recurvata* to identify regions where pollution is caused by human activities and to distinguish areas with superior soil and air quality [9].

Limited information is available regarding the microbiome of *T. recurvata*, and it remains uncertain whether this plant serves as a reservoir for microorganisms of agronomic interest, particularly in areas with limited available nutrients, such as fences. Joseph et al. [10] evaluated some endophyte fungi isolated from *T. recurvata*, and this research demonstrated that of the seven fungal morphotypes analyzed, five were identified as *Sordariomycetes* through DNA sequencing, revealing a substantial representation of this class within the plant. Phylogenetic analysis using ITS and  $\beta$ -tubulin sequences corroborated the taxonomic classification and uncovered hidden diversity among the isolates, indicating intricate fungal relationships. This study highlights the varied and potentially distinct fungal endophytes present in epiphytic bromeliads such as *T. recurvata* that can be used for agricultural purposes. Previous research has concentrated on the dynamics of bacterial communities in the phyllosphere to assess the influence of deposited bacteria on plant growth and to enhance the comprehension of their significance in biogeochemical processes [11].

The present study aimed to evaluate and compare the microbiome of *T. recurvata* obtained from two distinct locations, trees and fences, and two different plant tissues, specifically the leaves and roots. The hypothesis underlying this study posits that the microbiome of plants located on fences consists of microorganisms with high diversity and specialized functions capable of providing nutrients and conditions for plant growth and development to compensate for the lack of nutrients and adverse conditions in the microenvironment.

## Methods

### Collection of *Tillandsia recurvata* Samples

Ten specimens of *T. recurvata* were collected: five from the fence and five from phorophyte trees. Each plant was carefully collected using gloves and was transported to the laboratory belonging to the São Paulo State University (UNESP), Jaboticabal City, Brazil. The plants of *T. recurvata* were collected from an iron fence (− 21,2,421,684, − 48,2,895,118) and from three different phorophyte trees: *Andira surinamensis* (− 21,2,465,929, − 48,2,940,464), *Stryphnodendron pulcherrimum* (− 21,2,439,687, − 48,2,931,995), and *Cedrela fissilis* (− 21,2,447,914, − 48,2,937,735). The trees were near to each other and subjected to identical climatic conditions. The climate is characterized by hot, rainy summers, and cold, dry winters. According to Köppen-Geiger, the climate classification is Aw.

### Characteristics of Phorophyte Trees

The *T. recurvata* specimens were collected from three distinct phorophyte trees. These trees were as follows: (1) *Andira surinamensis* (Fabaceae): a medium to large-sized tree species typically found in tropical forests, particularly near rivers and swampy areas. This species can reach heights of 20–30 m, making it a significant component of its ecosystem. *A. surinamensis* produces fragrant flowers that attract pollinators, notably bees, which may play a role in the plant's reproductive success and local pollinator dynamics. The tree bears large, fibrous fruits and is valued for its durable wood, which finds applications in construction and furniture-making. Additionally, the species has reported medicinal properties. (2) *Stryphnodendron pulcherrimum* (Fabaceae): a medium-sized tree species adapted to tropical and subtropical regions, typically thriving in well-drained soils. This species reaches heights of 15–20 m, contributing to the mid-canopy structure of its native habitats. *S. pulcherrimum* produces small, whitish-yellow flowers and elongated seed pods, characteristics that may influence its interactions with pollinators and seed dispersers. Notably, the bark of *S. pulcherrimum* has documented medicinal properties, particularly in traditional medicine practices where it is employed for wound healing and as an astringent; and (3) *Cedrela fissilis* (Meliaceae): a large tree species native to tropical and subtropical regions, typically found in fertile, well-drained soils. This species can attain heights of 20–35 m, establishing itself as a dominant canopy component in its habitat. *C. fissilis* produces small, inconspicuous flowers and woody capsules containing winged seeds, adaptations that likely

influence its reproductive strategies and dispersal mechanisms. This tree is particularly notable for its high-quality wood, which is characterized by its workability and pleasant aroma. These properties make *C. fissilis* timber highly valued for furniture, cabinetry, and musical instrument construction [12].

The phorophyte species selected for this study, despite belonging to different families (*A. surinamensis* and *S. pulcherrimum* from Fabaceae, and *C. fissilis* from Meliaceae), share several ecological and economic characteristics. These medium to large trees, typically reaching heights of 15–35 m, are common components of tropical and subtropical forest ecosystems. All three species have recognized economic value, particularly in timber production, construction, and traditional medicine. Notably, the Fabaceae species likely form symbiotic associations with nitrogen-fixing bacteria, potentially influencing soil fertility and nutrient cycling in their habitats. Furthermore, these trees produce flowers and seed pods that play crucial roles in local ecosystems by attracting pollinators and seed dispersers [12].

### Identification and Characterization of *Tillandsia recurvata* Specimens

Plants of *T. recurvata* were collected and identified for this study using taxonomic keys [13] at the Plant Taxonomy Laboratory of the Department of Biology. Only fully developed plants were collected, and the uniformity of specimen size between groups was considered. This epiphytic bromeliad, commonly known as “ball moss,” adapted to arid environments, forms distinctive spherical or rounded clumps measuring 10–20 cm in diameter. *T. recurvata* specimens exhibit narrow, grayish-green leaves that bend backward (recurve), contributing to the plant’s characteristic ball-like structure. The leaves are thin, pointed, and covered with trichomes and microscopic hair-like structures that impart a silvery appearance and likely play a role in water and nutrient absorption [5, 13, 14].

### DNA Extraction from the Leaves and Roots

The leaves and roots were collected manually using surgical gloves and immediately deposited in sterilized plastic containers. Subsequently, the leaves and roots were placed in a 50-ml conical tube containing 35 ml of phosphate buffer with 0.02% surfactant (Tween 20). The tubes were vortexed for 2 min to separate the root system from the rhizosphere. Then, using sterilized tweezers, the leaves and roots were placed on paper towels and transferred to centrifuge tubes (50 ml). Superficial sterilization of the leaves and the roots was performed according to the method described by [15], with modifications. The tissues were maintained in 100% ethanol for 3 min, followed by 2% sodium hypochlorite for

2 min, and 70% ethanol for 3 min. The disinfected plant tissues were washed thrice with sterile distilled water, and the last wash was inoculated onto nutrient agar plates to validate the effectiveness of the superficial sterilization procedure. Sterilized leaves and roots were macerated in liquid nitrogen using a sterile mortar and pestle. A PowerMax soil DNA extraction kit (Mo Bio Laboratories, Carlsbad, CA, USA) was used to extract genomic DNA from all samples, according to the manufacturer’s instructions. The concentration of the extracted DNA was determined by fluorometry (Qubit™ 3.0, Invitrogen), and the purity was estimated by calculating the A260/A280 ratio via spectrophotometry (NanoDrop™ 1000, Thermo Fisher Scientific). The V3-V4 hypervariable region of the 16S rRNA gene was amplified using primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′) [16]. Also, the internal transcribed spacer (ITS) region was amplified using primers ITS3 (5′-GCATCGATGAAGAACGCAGC-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) to investigate the diversity of fungal communities in the samples [17]. PCR was performed in 30 cycles using the HotStarTaq Plus Master Mix kit (Qiagen) under the following conditions: 94 °C for 3 min, followed by 28 cycles at 94 °C for 30 s, 53 °C for 40 s, and 72 °C for 1 min, and a final elongation step at 72 °C for 5 min. PNA clamp sequences (PNA Bio) were added to block amplification of the 16S rRNA gene from the ribosomes and mitochondria. The amplification products were analyzed on a 2% agarose gel to determine the success of amplification and relative intensity of the bands. Amplicons were sequenced using 2 × 300bp paired-end protocol in the Illumina MiSeq™ platform at the GoGenetic facility (Curitiba, Brazil).

### Data Processing

Initial quality assessment of sequencing data was performed using FastQC (v. 0.11.9) [18]. For further analysis, USEARCH (version 11.0.667) [19] was employed, utilizing the “fastx\_info” and “fastq\_eestats2” functions to examine the quality distribution, sequence length, and expected errors. The “search\_oligodb” function in USEARCH was used to identify the presence and location of the primers 341F and 805R, targeting the V3-V4 region of the 16S rRNA gene, as well as primers ITS3 and ITS4 for ITS sequences. Primers and adjacent barcodes were removed using Atropos (version 1.1.31) [20]. To ensure data quality, Fastp (version 0.23.2) [21] was used to remove sequences with an average Phred quality score below Q25, using the parameter “-average\_qual 25.” Given the paired-end sequencing approach, sequences were merged using PEAR (version 0.9.11) [22] with a minimum overlap criterion of 10 base pairs (-min-overlap 10).

Merged reads were processed using the DADA2 pipeline [23], implemented through the dada2 package (version 1.22.0) in R (version 4.1.2) [24]. The process began with filtering and truncation of reads using the “filterAndTrim” function, with a maximum expected error threshold of 2 (“maxEE = 2”). Error probabilities per base were estimated using the “learnErrors” function. Based on this error model, sequences were corrected with the “dada” function, leading to the identification of amplicon sequence variants (ASVs) specific to each sample. Potential chimeric sequences were removed using the “removeBimeraDenovo” function. Taxonomic classification of 16S rRNA ASVs was performed against the “RefSeq + RDP” database (RefSeq from the National Center for Biotechnology Information [NCBI] supplemented with sequences from the Ribosomal Database Project [RDP]) (version 16.0) [25]. ITS ASVs were compared against the UNITE database (version 2022.11.29) [26]. ASVs that could not be classified to the respective kingdoms or identified as potential contaminants, including chloroplast and mitochondrial sequences, were excluded from the analysis.

ASV counts and taxonomic annotations were exported in “phyloseq” format using the phyloseq package (version 1.38.0) [27]. The phyloseq object was transformed into compositional data using the “phyloseq\_standardize\_otu\_abundance” function from the metagMisc package (version 0.04) [28] for downstream microbiome analyses.

## Descriptive and Statistical Analyses of the Microbiome

Sampling efficiency was assessed using rarefaction curves generated by the “amp\_rarecurve” function in the ampvis2 package (version 2.7.17) [29]. Alpha diversity was quantified by examining species richness and diversity indices (Shannon and Gini-Simpson), using the “alpha” function in the microbiome package (version 1.16.0) [30]. Comparative analysis of means was conducted using Student’s *t*-test, with a 95% confidence interval ( $p$ -value  $\leq 0.05$ ). Additionally, alpha diversity metrics were calculated for rare ASVs, defined as those with an average relative abundance of less than 0.001% within a group or present in only one sample. Beta diversity was analyzed by calculating Bray–Curtis dissimilarity between samples using the “distance” function in the phyloseq package. Significant differences between leaves and roots samples of *T. recurvata* collected from trees and fences were assessed using PERMANOVA and implemented via the “adonis” function in the vegan package (version 2.6.2) [31], with significance set at  $p$ -value  $\leq 0.05$ . Principal coordinate analysis (PCoA) was performed to interpret multidimensional distances, and results were visualized in subsequent plots.

Differentially abundant taxa between leaves samples of *T. recurvata* collected from trees and fences were identified using the DESeq2 methodology (R package version 1.34.0; Love et al., 2014), which applies a negative binomial model to compare means, with the Wald test used for significance (adjusted  $p$ -value  $\leq 0.05$ ). The resulting analyses were visualized using the ggplot2 package (version 3.3.6) [32] in R.

To evaluate the structural characteristics of microbial communities in response to growth support, co-occurrence network analysis was performed at the Genus taxonomic level. Pearson correlation coefficients were calculated using the “corr.test” function in the psych package (version 2.2.5) [33]. Only significant correlations ( $p$ -value  $\leq 0.05$ ) with a Pearson coefficient of  $\pm 0.75$  or greater were considered, focusing on strong positive or negative relationships. Network construction and analysis of topological properties were conducted using the igraph package (version 1.3.4) [34]. Topological properties included the total number of correlated genera (nodes), total number of connections (edges), average and maximum degrees, and centrality measures (average and maximum betweenness centrality). Key hubs were identified by calculating the Kleinberg’s hubbiness score [35], highlighting the most influential genera in the networks. The mean values of degrees and betweenness centrality were compared with Student’s *t*-test, with a 95% confidence interval ( $p$ -value  $\leq 0.05$ ).

To infer the functional potential of bacteria (16S rRNA data), PICRUSt2 (version 2.5.2) [36] was used to predict the functional capacity in terms of KEGG Orthology (KO) counts per sample. Additionally, KOs were mapped to the PLaBAs database (version 1.0) [37] to associate functions with pathways relevant to plant–microbe interactions. Differentially abundant functions between conditions were identified using the DESeq2 methodology (R package version 1.34.0) [38], with significance determined at an FDR adjusted  $p$ -value  $\leq 0.05$ .

## Results

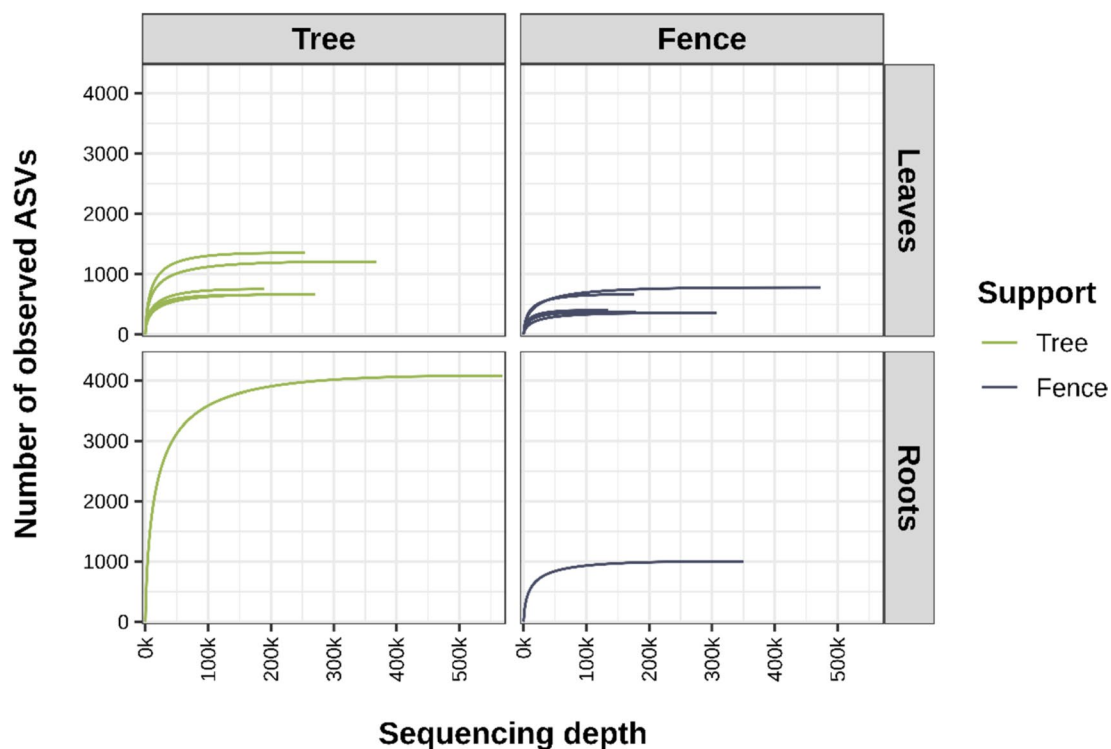
The high-throughput sequencing process generated a total of 3,726,967 (16S rRNA) and 3,161,214 (ITS) reads distributed among the different samples analyzed, including the leaves and roots of epiphytic plants with growth support on trees or fences. The data were organized into two sets, 16S rRNA and ITS, each grouped by plant tissue and growth support conditions. For leaf samples obtained from *T. recurvata* collected from trees, the average raw reads were 295,565.8 for 16S rRNA and 232,207.4 for ITS, while for roots, the total values were 391,499 and 334,619 for roots, respectively. After quality control, the retained 16S rRNA sequences were 272,080.60 for leaves and 347,050 for roots. Additional filtering to remove contaminants resulted in

69,549.80 usable reads for leaves and 336,515 usable reads for roots. For samples collected from fences, the average raw reads were 349,855.80 for 16S rRNA and 265,528.80 for ITS, 108,360, and 337,914, respectively, for roots. The valid reads post-filtering were 20,982.00 for 16S rRNA and 233,110.60 for ITS in leaves, and 68,909 and 281,795 for roots, respectively. The complete read counts for all libraries sequenced are detailed in Table S1 of the Supplementary Material.

The remaining reads, after quality control, processing, and filtering of amplicon sequence variants (ASVs), proved to be adequate for capturing the microbial diversity present under different conditions. This conclusion is supported by the stabilization of rarefaction curves (Fig. 1), indicating that the sequencing depth achieved was sufficient to represent the diversity of microbial communities under the evaluated conditions. Therefore, even considering the inherent losses during quality control and subsequent data filtering, the obtained sequencing coverage ensures representative sampling of the biodiversity present on leaves and root samples of *T. recurvata*. Individualized rarefaction curves for bacterial and fungal domains further confirmed this conclusion, with each data set demonstrating stabilization, as shown in the supplementary material (Fig. S1).

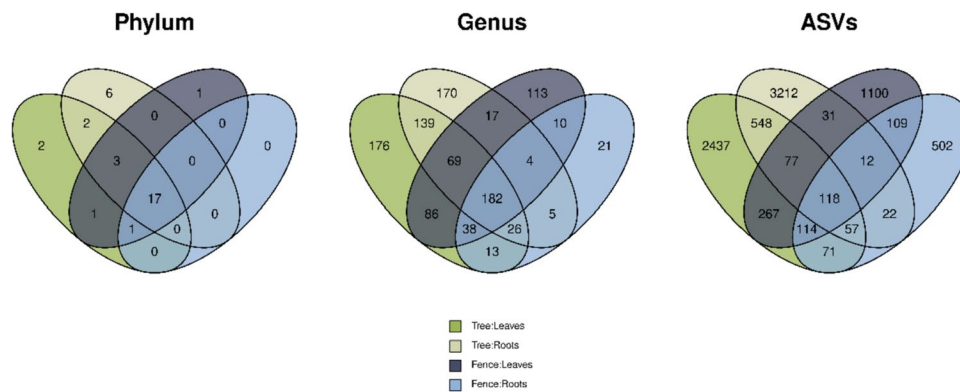
Taxonomic assignments of the obtained ASVs demonstrated a variation in the classification efficiency across different taxonomic levels and marker types. For 16S rRNA sequences, 95.56% of the reads were classified up to the phylum level, 77.40% up to the family level, 68.46% up to the genus level, and 4.91% up to the species level. In contrast, ITS sequences showed even greater efficiency at higher levels, with 99.98% of the reads classified up to the phylum level, 93.27% to the family level, and 90.01% to the genus level. Species-level classification also performed better than 16S rRNA, with 24.39% of the reads correctly assigned. These results suggest high accuracy at higher taxonomic levels but considerable limitations in species-level classification, highlighting the difficulties of using taxonomic markers for finer taxonomic resolution. Additionally, cumulative relative abundance curves presented in the Supplementary Material (Figs. S2 and S3) indicate that a significant portion of the microbial abundances is concentrated within a relatively small number of taxa or ASVs in both the 16S rRNA and ITS datasets. This pattern suggests that while a broad diversity of taxa is present, the community structure is dominated by a few highly abundant groups.

The analysis of Venn diagrams (Fig. 2) revealed significant taxon sharing at higher taxonomic levels, as well



**Fig. 1** Rarefaction curves illustrating the observed amplicon sequence variants (ASVs) of microbial (bacterial and fungal) communities associated with *Tillandsia recurvata* growing on trees and fences, separated by plant tissue (leaves and roots). The y-axis rep-

resents the number of observed ASVs, while the x-axis denotes sequencing depth. The curves show the stabilization into a plateau, indicating sufficient sequencing coverage to capture the microbial diversity within each sample



**Fig. 2** Venn diagrams depicting the shared and unique microbial (bacterial and fungal) taxa across taxonomic ranks (Phyla, Genera, and ASVs) among *Tillandsia recurvata* samples collected from two different growth supports (trees and fences) and two plant tissues

(leaves and roots). Each diagram represents the overlap and exclusivity of microbial diversity across the specified groups, illustrating the extent of taxonomic sharing among the samples

as substantial variations at the ASV level across different conditions. At the phylum level, of a total of 33 phyla, 17 (51.52%) were shared among all conditions, while 10 (30.30%) and 1 (3.03%) were exclusive to trees and fences, respectively. At the genus level, out of 681 genera, 182 (26.73%) were shared among all conditions, while 485 (71.22%) and 144 (21.14%) were exclusive to trees and fences, respectively. Regarding ASVs, out of 8677, 118 (1.36%) were shared among all conditions, while 6197 (71.42%) and 1711 (19.72%) were exclusive to trees and fences, respectively. This pattern was consistent across both microbial domains (bacteria and fungi) studied, as shown in the supplementary material (Fig. S4). These results indicate significant conservation of major taxonomic groups among the conditions, whereas differences at the ASV level reflect substantial variations in population composition.

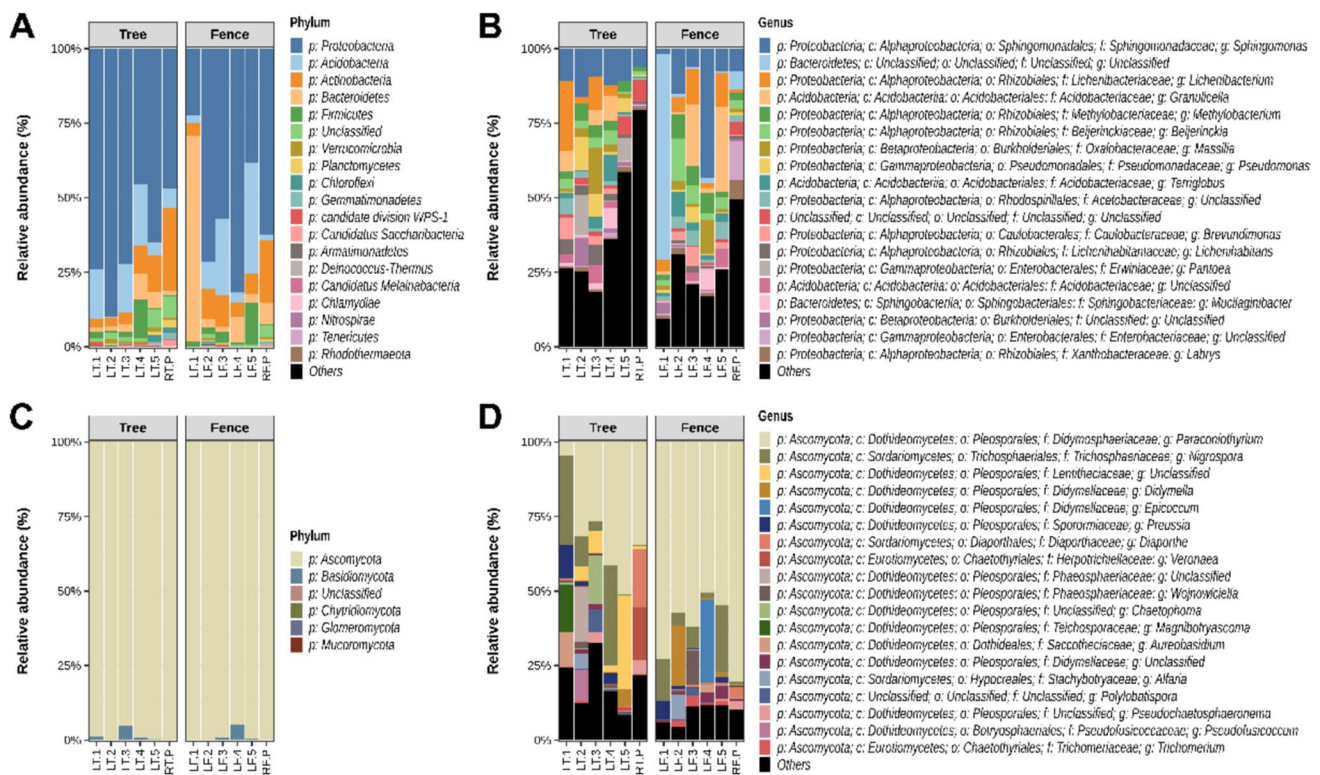
Detailed taxonomic analysis revealed the most prevalent taxa on the studied samples, ranging from the phylum to the species level. As an illustrative example of the observed taxonomic diversity, the levels of “Phylum” and “Genus,” grouped by condition, are highlighted in Fig. 3.

The composition of the bacterial community varied significantly among the different sampling environments (Fig. 3A). In the leaf samples, *Proteobacteria* was the dominant phylum, representing 69.37% of the relative abundance in tree epiphytes and 54.20% in fence epiphytes. This dominance is primarily due to the *Sphingomonadaceae* family, which constitutes a large part of the community. *Actinobacteria* and *Acidobacteria* were also notable, with 6.35% and 11.53% in tree plant leaves and 7.37% and 15.53% in fence plant leaves, respectively. In the root samples, *Proteobacteria* still dominated, but to a lesser extent, with 46.79% in trees and 62.28% in fences. *Actinobacteria* made significant contributions, with 27.71% in tree epiphyte roots and 21.14% in fence epiphyte roots, while *Acidobacteria* made

contributions of 6.58% and 1.88% in tree and fence plants, respectively. At the genus level (Fig. 3B), *Sphingomonas* was the dominant genus in the leaves, with 11.89% in the trees and 14.80% in the fences. *Lichenibacterium* was also prevalent, at 8.08% in tree plant leaves and 6.87% in fence plant leaves. *Granulicella* accounted for 4.09% of tree leaves and 10.37% of fence leaves. In roots, *Sphingomonas* represented 6.02% of trees and 7.66% of fences, whereas *Lichenibacterium* and *Granulicella* had lower proportions.

The fungal community composition varied among sampling conditions (Fig. 3C). In the leaf samples, *Ascomycota* was the predominant phylum, comprising 98.56% of trees and 98.64% of fences. *Basidiomycota* were present in lower proportions, with 1.37% in trees and 1.35% in fences. In the roots, *Ascomycota* dominated with 99.85% of trees and 99.94% of fences, while *Basidiomycota* had 0.13% and 0.05%, respectively. At the genus level (Fig. 3D), *Paraconiothyrium* was the dominant genus in the leaves, with 31.01% in the trees and 59.46% in the fences. *Nigrospora* was prevalent in 15.45% of the tree leaves and 9.93% of the fence leaves. *Diaporthe* was notably present in roots, with 19.42% in trees and 3.62% in fences.

The results of the alpha diversity analysis showed significant differences in richness and Shannon and Gini-Simpson indices between leaf samples of epiphytic plants grown on trees and fences (Table 1; Fig. 4). The leaves of plants growing on trees had higher average values for richness (928.8), Shannon (3.62), and Gini-Simpson (0.89) compared to the leaves of plants grown on fences, which had averages of 518.2, 2.18, and 0.67, respectively (Table 1). These differences were statistically significant, with *p*-values of 0.05 (Richness), 0.003 (Shannon), and 0.014 (Gini-Simpson), indicating greater diversity and balance in the microbial community associated with the leaves of plants on trees (Fig. 4).



**Fig. 3** Taxonomic profile representing the distribution of relative abundances of major microbial taxa identified in *Tillandsia recurvata* samples. The plots are separated into Bacteria (A, B) and Fungi (C, D), displaying the relative abundance at the Phylum (A, C) and

Genus (B, D) levels for samples collected from trees and fences. Up to 19 of the most abundant taxa are shown in each plot, with taxa of lower abundance grouped under the category “Others” to enhance the visualization of dominant taxa

**Table 1** Summary of alpha diversity metrics—Richness, Shannon, and Gini-Simpson Index—of microbial (bacterial and fungal) communities across leaves and roots samples of *Tillandsia recurvata* collected from trees and fences. Averages are accompanied by ± standard deviation (only for leaves)

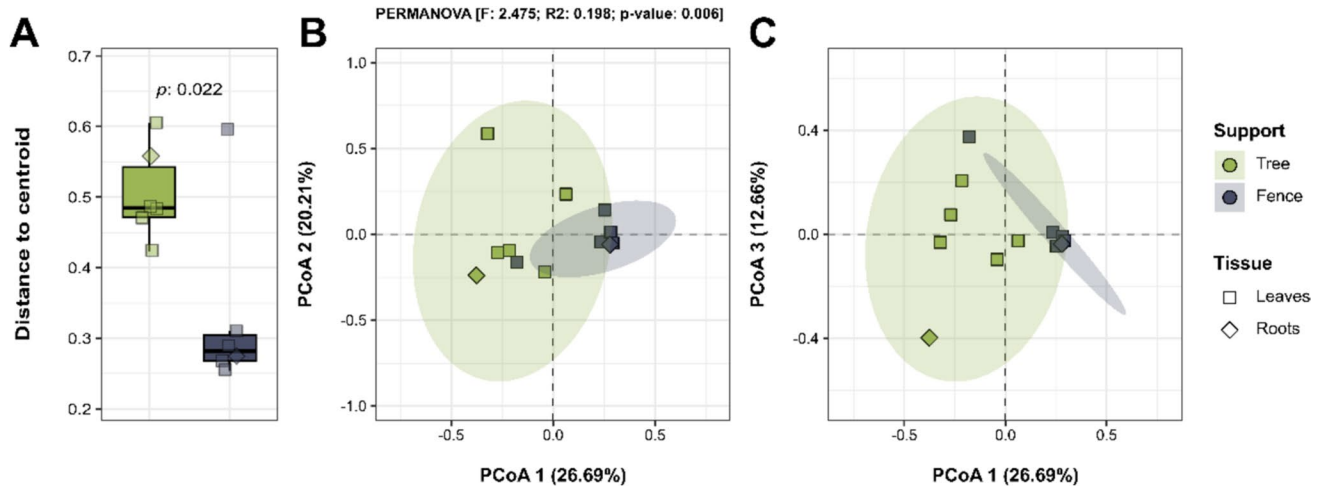
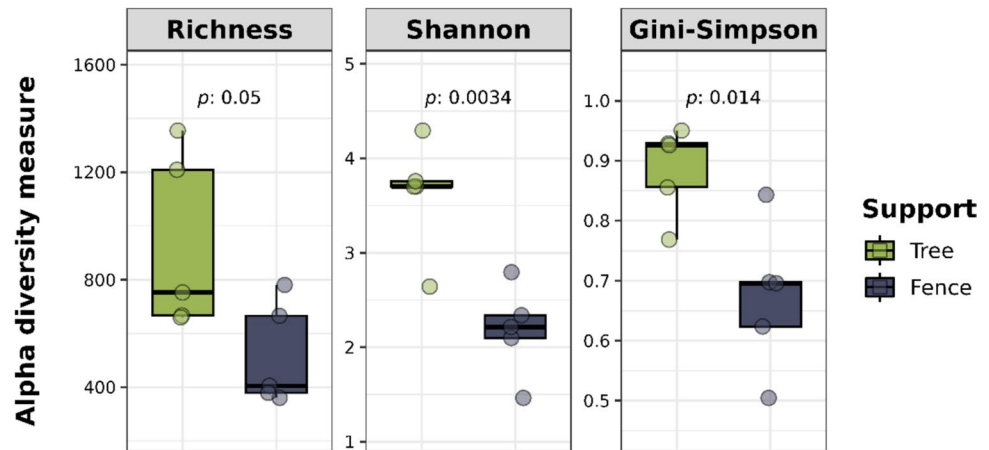
Group	Richness	Shannon	Gini-Simpson
Tree (leaves)	928.8 ± 328.58	3.62 ± 0.60	0.89 ± 0.07
Tree (roots)	4077	5.73	0.97
Fence (leaves)	518.20 ± 192.44	2.18 ± 0.48	0.67 ± 0.12
Fence (roots)	1005	2.54	0.59

For roots, richness was considerably higher in plants grown on trees (4077) compared to those grown on fences (1,005). The Shannon and Gini-Simpson indices were also higher in the roots of plants on trees, with values of 5.73 and 0.97, respectively, compared to values of 2.54 and 0.59 in plants on fences (Table 1). These results suggest that epiphytic plants growing on trees support a more diverse and balanced microbial community in both leaves and roots, reflecting the distinct influences of different growth supports on the associated microbial communities. Individualized values for each sample and microbial domain are provided in the Supplementary Material (Table S2). These data

show that bacterial richness is more significantly affected by the growth support, while fungal diversity metrics exhibit more pronounced differences (Fig. S5). Additionally, the analysis of rare species (ASVs with a mean relative abundance < 0.001% or present in only one sample) revealed that the diversity (Shannon index) of rare bacterial ASVs is higher in samples from plants grown on trees, whereas fungal diversity appears unaffected by the different growth supports (Table S3 and Fig. S6).

Principal coordinates analysis (PCoA) based on Bray–Curtis distances was used to determine the similarity of microbial compositions between epiphytic plant samples grown on trees and fences (Fig. 5). The distances from the centroid indicated a greater dispersion of samples from trees compared to those from fences, suggesting more variability within the microbial communities associated with tree-supported plants (Fig. 5A). The PCoA results showed significant separation of samples according to growth support, explaining 26.69%, 20.21%, and 12.66% of the total variability observed in the first three principal axes, respectively, totaling 59.56% of the explained variability (Fig. 5B and C). PERMANOVA analysis confirmed the statistical difference between the microbial compositions of epiphytic plants from trees and fences, with a *p*-value of 0.006, indicating

**Fig. 4** Box plots displaying alpha diversity metrics—Richness, Shannon, and Gini-Simpson Index—of microbial (bacterial and fungal) communities across leaves samples of *Tillandsia recurvata* collected from trees and fences. The box plots illustrate the variation in diversity within these samples. Statistical comparisons were performed using Student's *t*-test to identify significant differences ( $p \leq 0.05$ ) between the two groups



**Fig. 5** Beta diversity analysis of microbial communities (Bacteria and Fungi) associated with *Tillandsia recurvata* growing on trees versus fences. The panel includes **A** box plots showing the distribution of centroid distances based on Bray–Curtis dissimilarities, with statistical comparisons performed using Student's *t*-test ( $p \leq 0.05$ ), and

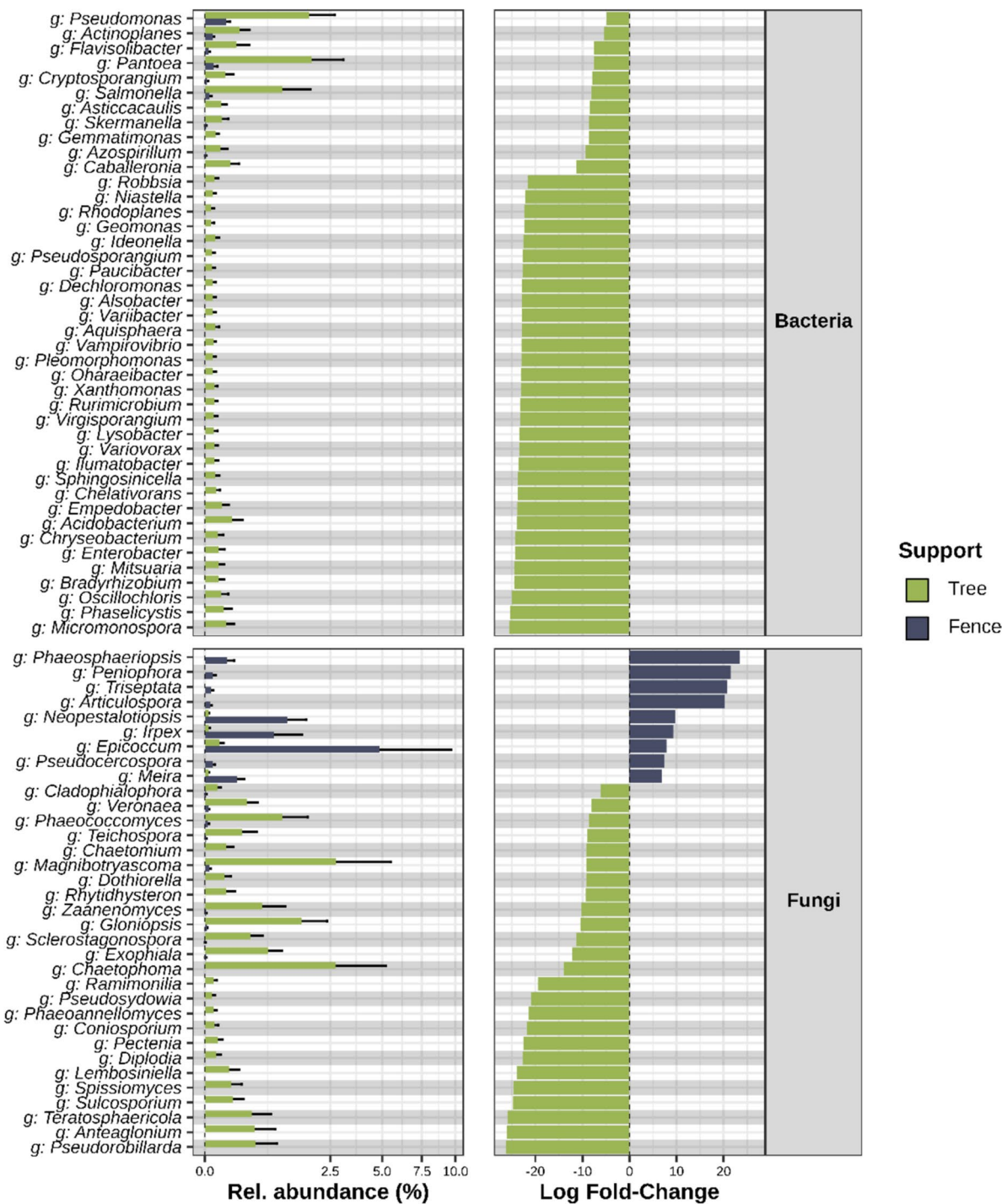
principal coordinate analysis (PCoA) plots displaying the dispersion of leaves and roots samples across combinations of axes 1 and 2 (**B**), and axes 1 and 3 (**C**). The influence of growth support on community composition was assessed using permutational multivariate analysis of variance (PERMANOVA) ( $p \leq 0.05$ )

that growth support significantly influenced the structure of microbial communities. Although there was some overlap between the groups, the separation trend observed in the PCoA plots suggests distinct patterns in microbial composition associated with each type of support. The results, when individualized by microbial kingdom (Fig. S7), revealed that the effect of growth support was more pronounced in fungal communities (Fig. S7D–F) compared to bacterial communities (Fig. S7A–C).

Differentially abundant (DA) analysis revealed 180 DA taxa between epiphytic plants grown on trees and fences, comprising 65 bacteria and 115 fungi. These taxa were categorized into one phylum, seven classes, nine orders, 28 families, 76 genera, and 59 species. Among the identified genera, 67 (42 bacterial and 25 fungal) were more abundant in the tree epiphytic plant samples, while nine fungal genera

were more abundant in the fence samples (Fig. 6). Most DA taxa from tree epiphytes were exclusive to this condition and were not detected in fence samples.

Co-occurrence network analysis, based on Pearson correlation coefficients ( $r \geq 0.75$  or  $r \leq -0.75$ ) and a 95% confidence level ( $p \leq 0.05$ ), revealed considerable differences in the structures of microbial networks associated with epiphytic plants grown on trees and fences at the genus level (Fig. 7, Table 2). The network associated with trees showed a higher number of nodes (548) and twice the number of edges (22,986) compared to the fence network, which had 396 nodes and 11,369 edges (Table 2). Both networks had a low number of negative edges, although the ratio of positive to negative edges was higher in the tree-plant sample network. Additionally, the tree network exhibited higher values in terms of average degrees, with an average degree of 83.89,

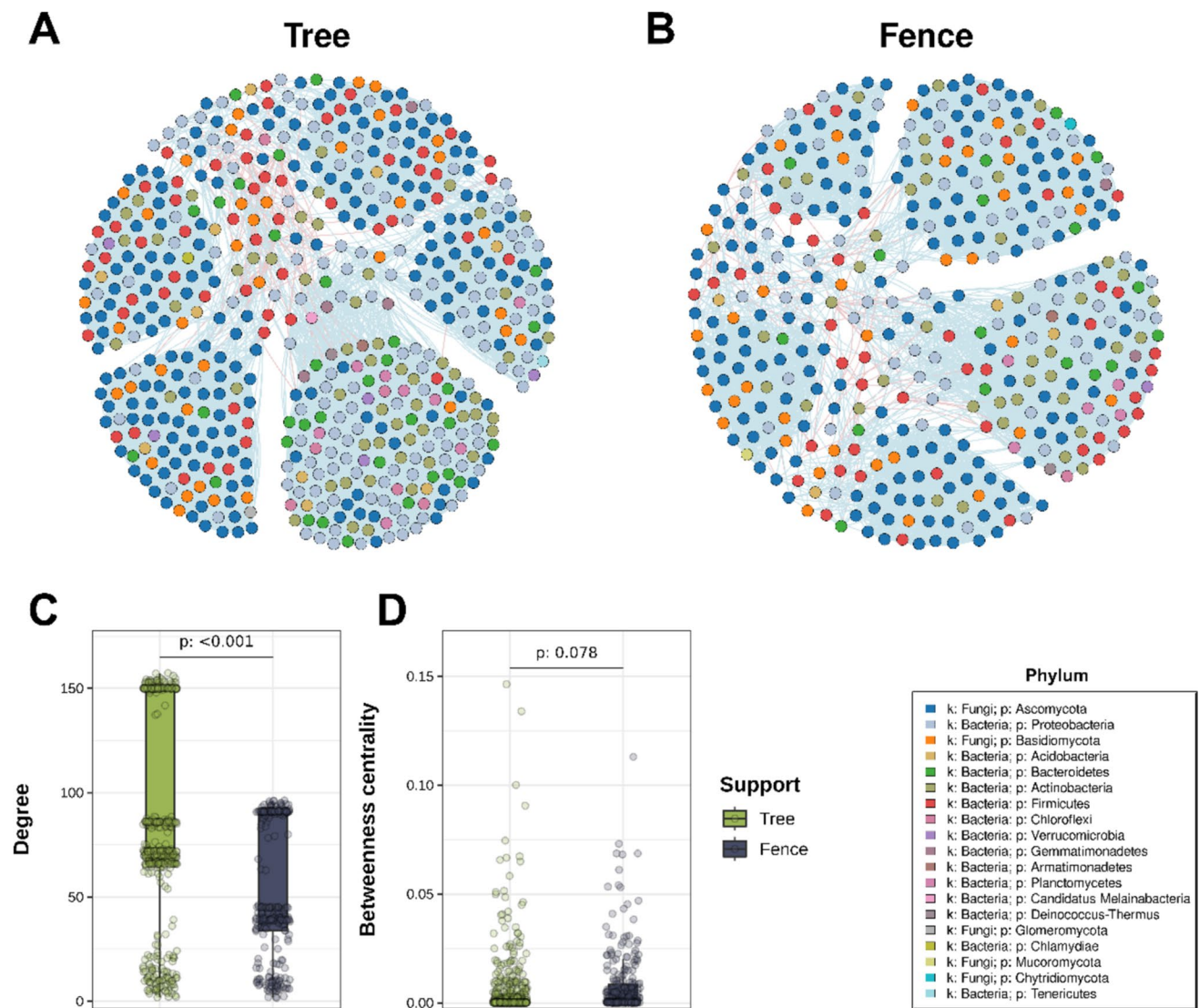


**Fig. 6** Differential abundance analysis of Bacterial and Fungal genera from leaf samples of *Tillandsia recurvata* collected from trees and fences. The left panel shows the relative abundance of differentially abundant (DA) genera, with the x-axis transformed using a square root scale to enhance the visualization of less abundant gen-

era. The right panel presents the mean intensity of the difference in abundance, expressed as Log<sub>2</sub> Fold-Change, for genera that exhibited statistically significant differences between the two groups (FDR corrected  $p \leq 0.05$ )

compared to the fence network, which had an average degree of 57.42. Statistical comparison indicated that these differences were significant ( $p < 0.001$ ) (Fig. 7C). Betweenness

centrality measures, which indicate the extent to which a node lies on the shortest path between other nodes, were relatively similar between the two networks, with an average



**Fig. 7** Co-occurrence networks of microbial genera (Bacteria and Fungi) in leaves and root samples of *Tillandsia recurvata* collected from trees (**A**) and fences (**B**). The networks are constructed based on Pearson correlation coefficients ( $r \geq 0.75$  or  $r < -0.75$ ) with a 95% confidence level ( $p \leq 0.05$ ). Positive correlations are represented

by blue edges, and negative correlations by red edges, with node fill color indicating the phylum to which each genus belongs. The average number of connections (Degree) (**C**) and betweenness centrality (**D**) of the groups were statistically compared using Student's *t*-test ( $p \leq 0.05$ )

betweenness of 0.005 in the tree network and 0.007 in the fence network (Fig. 7D, Table 2).

The main hubs differed between the networks, with the genus *Marmoricola* being the primary hub in the tree network and *Mucilaginibacter* in the fence network (Table 2). Taken together, these differences reflect the influence of growth support on the structure and interactions of microbial communities associated with epiphytic plants. Thus, the analysis suggests a more complex and interconnected network in trees.

Functional prediction analysis of the microbial communities was performed using the PICRUSt2 program, which predicts functions from 16S rRNA sequences. Principal

component analysis (PCA) of the annotated functions (KOs) revealed considerable overlap in functional composition across different conditions (Fig. 8A). The first three principal components explained 24.07%, 16.71%, and 14.05% of the total variance, respectively, accounting for 54.83% of the explained variance.

The functional profile of the KOs classified in the metabolism class indicated similar profiles between leaf and root samples (Fig. 8B), as well as between plants grown on trees and fences. The most abundant classes were carbohydrate metabolism (23.45%), amino acid metabolism (19.49%), cofactor and vitamin metabolism (11.88%), and energy metabolism (11.32%). Regarding the microbial

**Table 2** Summary of the main topological characteristics and centrality measures of co-occurrence networks for microbial (bacterial and fungal) genera identified in leaf and root samples of *Tillandsia recurvata* grown on trees and fences

Attribute	Tree	Fence
N. of nodes	548	396
N. of edges	22,986	11,369
Positive edges	22,885	11,287
Negative edges	101	82
Clustering coefficient	0.971	0.948
Mean degree	83.89	57.42
Max. degree	157	96
Mean betweenness	0.005	0.007
Max. betweenness	0.146	0.113
Main hubs	<i>Marmoricola</i>	<i>Mucilaginibacter</i>

traits annotated with the PLABase database (Fig. 8C), the functional profile was also similar and conserved among the evaluated conditions. The main functional categories were plant system colonization (27.4%), stress control or biocontrol (19.12%), competitive exclusion (17.98%), and bio-fertilization (13.9%).

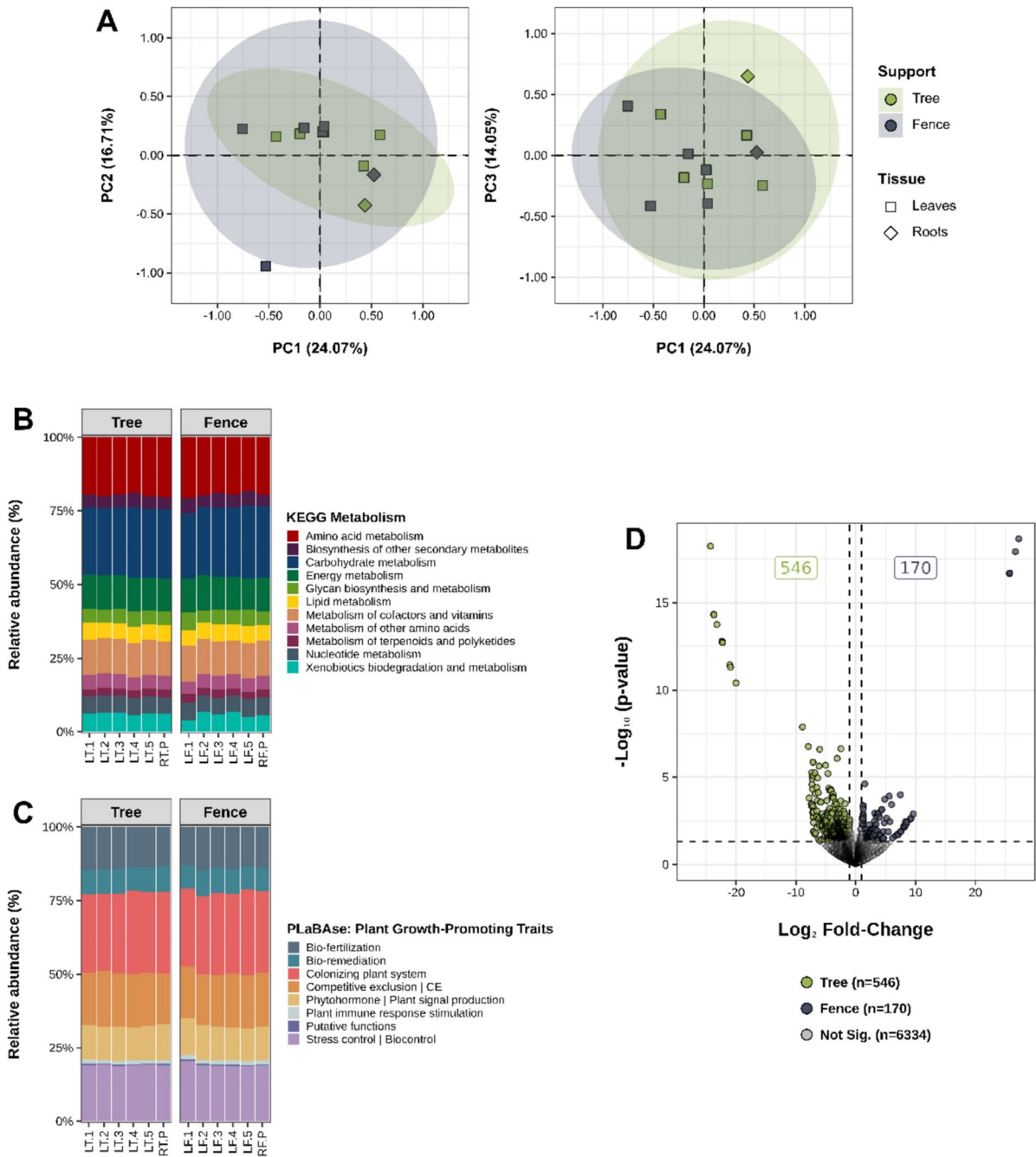
The volcano plot (Fig. 8D) highlights the differentially abundant functions between the leaf samples of plants grown on trees and fences. Although the overall profile is conserved, tree-grown plants exhibited 546 differentially expressed functions (representing 7.74% of the 7050 detected KOs), in contrast to only 170 functions (2.41% of the 7050 detected KOs) that were more expressed in fence plants, indicating a greater functional diversity in *T. recurvata* plants grown on trees.

## Discussion

Taxonomic classification determines the degree of relatedness among organisms recorded on the leaves and roots of the epiphyte *T. recurvata* growing on trees and fences. At the phylum level, 33 major taxonomic groups were identified. In all conditions, 17 phyla (51.52%) were shared. Of these, 10 phyla (30.30%) were exclusive to trees and one phylum (3.03%) was exclusive to fences. At the genus level, 681 specific phyla were identified. Under all conditions, 182 genera (26.73%) were shared. A total of 485 genera (71.22%) were exclusive to trees and 144 genera (21.14%) were exclusive to fences. These findings indicate that fences provide less microbial diversity and sharing than trees. Therefore, it has been suggested that colonizing trees is more advantageous than fencing for epiphytic plants. Many studies have evaluated the distribution of colonization by *Tillandsia* sp. The study examined the intricate dynamics governing the habitat occupancy of epiphytes, such as *T. recurvata*, highlighting

the significance of host traits, tree size, and spatial configuration in shaping the distribution and abundance of these species [1]. Another study showed that *T. flexuosa* growing on electrical cables in Panama showed slow growth and less successful colonization of plants on cables compared to trees, indicating suboptimal conditions for cable-inhabiting populations [39]. The present study reinforces that the microbial diversity was lower than that of the tree beyond the suboptimal conditions of cable-inhabiting. It is fascinating to consider that understanding the complex relationships among various bromeliads can yield valuable insights into the patterns and dynamics of natural communities, particularly in environments with high and low tree densities. The results of this study indicate that positive interactions and high levels of dispersal may have a significant impact on the assembly of atmospheric bromeliads than local competitive interactions [2].

Although there was a statistically significant difference in the prevalence of bacterial groups between different locations (trees and fences) and plant tissues (leaves and roots), the bacterial communities were fairly conserved at broader taxonomic levels, such as the Phylum (Fig. 3). These similarities between the microbiomes of plants from different locations were found in another study. Aguiar-Cruz [40] evaluated the microbiome of bromeliad plants from five different forests in Mexico and found that despite the environmental differences, there was a high redundancy in the putative metabolic functions of the prokaryotic communities. This suggests that certain metabolic functions, particularly those related to organic carbon and nitrogen cycling, remain relatively constant in these microecosystems. However, differences in microbial communities may be influenced by spatial variability [41]. The prevalent phylum is *Proteobacteria*, which is important in soil ecosystems [42]. *Actinobacteria* contribute to the ecosystem by being involved in atmospheric nitrogen fixation and plant growth [43]. *Sphingomonas* is also present, and some members of this genus may have the ability to fix atmospheric nitrogen and promote plant growth [44]. The presence of nitrogen-fixing microflora on the leaves of *Tillandsia* sp. is potentially significant for the nutrition of these plants, especially in relation to the absorbing role of foliar trichomes. These microorganisms also contribute to the nutrition of other phyllospheric microorganisms, such as yeasts and fungi [45]. *Lichenibacterium* is another genus that might play a role in plant growth. It is worth noting that the phylum *Granulicella* has a lower prevalence in tree leaves (4.09%) and a higher prevalence in fence leaves (10.37%) and plays an important role in the health and ecology of lichens [46]. The presence of these phyla could explain the plant growth-promoting traits including colonization of plant system and stress control as can be seen in Fig. 8C. Likewise, this phylum is the most important when plants live in fences.



**Fig. 8** Functional composition and differentially abundant predicted genes in bacterial communities associated with *Tillandsia recurvata* growing on trees versus fences. **A** Principal component analysis (PCA) of the functional composition (KEGG Orthologs [KOs]) predicted using PICRUST2 on 16S rRNA data. **B** Functional profile of KOs associated with metabolic classes from the KEGG database.

**C** Functional profile of microbial traits annotated using the plant-associated bacteria web resources database (PLaBase). **D** Volcano plots showing differentially abundant KOs between leaf samples from plants grown on trees and fences, with statistical significance set at FDR corrected  $p \leq 0.05$  and a minimum fold-change difference of 2 ( $\log_2 \text{FC} \pm 1$ )

Regarding the prevalence of fungi, it was observed that the similarity between the locations and plant tissues was higher for fungi than for bacteria. At the phylum level, no significant differences were observed in the prevalence of *Ascomycota* and *Basidiomycota*. *Ascomycota* includes

species that are either plant pathogens or edible fungi [47], whereas *Basidiomycota* comprises fungi that play important ecosystem functions and can be both plant pathogens and beneficial fungi [48]. However, there was a statistically significant difference in the phylum *Paraconiothyrium*, with a

31.01% prevalence in tree leaves and 59.46% in fence leaves. This genus may play a role in biological control, bioremediation, and antibiotic production [49]. Additionally, *Nigrospora* was present in tree leaves at 15.45%, and in fence leaves at 9.93%. This genus may also have biocontrol potential or produce secondary metabolites [50]. The genus *Diaporthe* was notably present in the roots, with 19.42% prevalence in tree roots and 3.62% in fence roots. This genus includes endophytic, saprobic, and plant pathogenic fungi, with some species transforming infection-inhibiting factors into their derivatives. This genus includes temperate and tropical species [51]. The fungal species identified in the present study were found in the endosphere (leaves and roots). However, the phyllosphere also shows high fungal diversity. Felix et al. [52] found that more than 180 species of yeasts and yeast-like fungi were recorded from the bromeliad phyllosphere. At least 50 yeast species with biotechnological potential have been isolated from bromeliads, and over 90% of these species are capable of producing extracellular enzymes, indicating significant biotechnological applications.

It is noteworthy that the plants situated in the tree exhibited a greater significance in terms of microbial diversity than those placed in the fences, as evidenced by the higher values observed in all the indices assessed, namely Richness, Shannon, and Gini-Simpson. These findings raise the question of why plants choose to grow in trees rather than fences? This outcome suggests that several factors are involved in this process, and it underscores the importance of trees in maintaining ecological balance, as has been discussed in some studies [2, 17]. Including genera of agronomic interest, *Bradyrhizobium* (Log<sub>2</sub>FC: 24.45;  $p < 0.001$ ), *Azospirillum* (Log<sub>2</sub>FC: 9.29;  $p = 0.011$ ), and *Pseudomonas* (Log<sub>2</sub>FC: 4.82;  $p = 0.029$ ), the results indicated that these taxa were significantly more abundant in the plant from the trees. These findings suggest that the growth conditions of trees promote a greater diversity of microbial genera, including taxa with potential agronomic benefits [53]. This observation highlights the influence of the support environment on the microbial ecology profiles of epiphytic plants.

Co-occurrence analysis revealed that trees displayed a more intricate microbial network with greater connectivity than fences. This suggests that the way plants develop, whether on trees or fences, influences the structure and interactions of associated microorganisms. The microbial communities associated with trees form a more complex network. A study shows that mixed-species plantations exhibit more robust co-occurrence networks than monocultures, indicating stronger microbial interactions. Furthermore, the study revealed that afforestation with functional traits of different tree species significantly enhanced the microbial structures associated with soil carbon and nitrogen cycling [54]. The results of the present study suggest that the microbial community on plants located on the tree trunk may play

an important role in tree health, whereas the microbial community on plants located on the fence is only necessary to support plant growth. On the other hand, the analysis of metabolism class (KOs) results revealed that there were similar profiles between leaf and root samples, as well as between plants on trees and fences. The conservation of these skills is more prevalent in microorganisms, resulting in no discernible variation between the locations where the plants were collected.

## Conclusion

This study suggests that *T. recurvata* individuals growing on trees have a higher microbial diversity and distribution than those growing on fences. Plants on trees are carriers of bacteria, such as *Bradyrhizobium*, *Azospirillum*, and *Pseudomonas*, which are of agricultural interest. In addition, the growth conditions of trees appeared to encourage a greater variety, and co-occurrence analysis revealed that trees formed a more complex microbial network with greater connectivity than that of fences. This suggests that the growth support, whether on trees or fences, affects the structure and interactions of associated microorganisms and that the plants on trees could be a reservoir of microbes of agricultural interest.

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**Author Contribution** JSS: conceptualization, methodology, investigation, writing—original draft, writing—review and editing. LALC: data curation, formal analysis, methodology, investigation, writing—original draft, writing—review and editing. CHB: data curation, methodology, investigation, writing—review and editing. ETF: data curation, methodology, investigation, writing—review and editing. DGP: data curation, investigation, writing—review and editing. DN: data curation, investigation, writing—review and editing. ND: data curation, investigation, writing—review and editing. ECR: conceptualization, formal analysis, funding acquisition, investigation, methodology, resources, supervision, validation, writing—original draft, writing—review and editing.

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**Data Availability** The raw data and analyzed data used during the current study are available from the corresponding author on reasonable request. All the isolated microorganisms were identified using 16 s rRNA gene analysis and deposited in the GenBank as follows: NCBI Sequence Read Archive (SRA) database under BioProject PRJNA1086858 PRJNA1134710 (<https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA1134710>).

## Declarations

**Ethical Approval** Not applicable.

**Competing Interests** The authors declare no competing interests.

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