

# Metagenomic Analysis of Bacterial and Fungal Community Composition Associated with *Paulownia elongata* × *Paulownia fortunei*

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The dynamics and interactions of microbial communities in *Paulownia*'s life cycle are poorly understood. The main goal of this study was to compare the rhizospheric soil and endophytic microbiome and mycobiome of hybrid *Paulownia elongata* and *Paulownia fortunei*. The comparison was based on highly efficient Illumina MiSeq sequencing of bacteria and fungi from the rhizosphere and endosphere of bioenergetic trees *P. elongata* × *P. fortunei*. The general richness of bacteria and rhizospheric fungi (based on Chao 1, Shannon, and Simpson indicators) was higher than in endosphere samples from the same plants. Actinobacteria and Proteobacteria were dominant in the rhizosphere and endosphere of plants in healthy conditions. The rhizosphere fungal communities in both trials were dominated by Ascomycota, Mortierellomycota, and Basidiomycota. Most root endophytes came from Olpidiomycota, Oomycota, and Ascomycota, while most leaf endophytes were from Ascomycota and Basidiomycota. This study was the first report on the composition of bacteria and fungi associated with the endosphere and rhizosphere of *Paulownia* trees. These studies showed that bacterial and fungal communities from the rhizosphere and endosphere were separate communities. It also showed that the health conditions of trees did not affect the composition of endophytic microorganisms in *Paulownia* tissues.

**Keywords:** *Paulownia* spp.; Bioenergy tree; Endophytic and rhizosphere microorganisms; NGS; Structural diversity

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## INTRODUCTION

In the past few years, the atmospheric concentration of crucial greenhouse gases has increased as a result of human activities. Climate change will be one of the main challenges society faces in the coming decades. In this context, some national governments and international organizations have developed various strategies to promote the exchange of fossil fuels for renewable energy (Solomon *et al.* 2009). Plantations of trees with short rotations have been introduced to produce biomass for the energy industry. These plantations are also a promising tool for decreasing the concentration of carbon dioxide in the atmosphere (Lucas-Borja *et al.* 2011). Currently, some of the most popular trees in such plantations are species that belong to the *Populus*, *Salix*, *Betula*, *Alnus*, *Robinia*, *Nothofagus*, and *Paulownia* genera (Lucas-Borja *et al.* 2011; Woźniak *et al.* 2018). *Paulownia* spp. are fast-growing deciduous trees that belong to the Paulowniaceae family. This tree is characterized by high biomass production and a fast growth rate. The bioenergy

tree *Paulownia* is used to produce pellets as well as other forms of biofuel (the calorific value of *Paulownia* is 20.90 kJ/g) (Icka *et al.* 2016). This tree species is well adapted to growing and functioning in a wide range of changing soil and climatic conditions. *Paulownia* spp. are characterized by a well-developed root system and occur in different soil types, such as sands, clays, and even degraded soils (Popović *et al.* 2015; Woźniak *et al.* 2018). The leaves of *Paulownia* spp. are characterized by high contents of proteins (approximately 20%), fats, sugars, nitrogen, phosphorus, and potassium (El-Showk and El-Showk 2003; Yadav *et al.* 2013).

When studying interactions between above- and below-ground components of ecosystems, it is important to understand the direction of community ecology. Plant–microbe interactions may be fundamental to understanding the role of microbes in plant growth and promoting bioremediation and other functions (Turner *et al.* 2013). The plant microbiome is often defined as the host’s second genome because it contains diverse microbial groups, including bacteria, archaea, fungi, oomycetes, and viruses (Turner *et al.* 2013). Plants also live in close relation with microorganisms that inhabit the soil rhizosphere. The microbiome colonizing the rhizosphere soil represents the largest reservoir of biodiversity. An increased amount of data has confirmed that the whole microbiome (bacteria and fungi) associated with rhizosphere soil, its genetic elements, and its interactions, have a key significance in the determination of plant health (Berendsen *et al.* 2012). The structure of the microbial community varies among plant species, and the plant microbial diversity is influenced by a number of biotic and abiotic factors, including soil type, climate, agrotechnical managements, and many others (Köberl *et al.* 2013).

Specific interactions between model organisms, such as the symbiotic system of *Rhizobium* and bean plants (Oldroyd *et al.* 2011), are well known. However, not much is known about most of the microorganisms' interactions with other plant hosts, including *Paulownia*. In addition, the *Paulownia tomentosa* species is among the top ten invasive plants of Asian origin. Therefore, particular attention should be paid to other species as they can act as vectors of new diseases and have a negative impact on global biodiversity (Ding *et al.* 2006; Essl 2007). The occurrence of fungi on *Paulownia* spp. trees is available in literature. The best known are phytoplasmas (mycoplasmatic microorganisms), *e.g.*, *Candidatus Phytoplasma australiense* (Fan *et al.* 2016). However, there is still a lack of information on the bacteria and fungi inhabiting the tissues and rhizospheric soil of *Paulownia*, specifically non-culturable microorganisms. The study of the structure and function of microorganisms in various environments is possible, among other reasons, due to the application of molecular biology techniques, *e.g.*, next generation sequencing (NGS). The continuing development of next generation sequencing, metagenomics techniques, metatranscriptomics, and metabolomic approaches has increased knowledge of the microbiome associated within the plants and their related functions. A comprehensive combination of these analyses makes it possible to study microorganisms associated with plants that live below-ground in the rhizosphere, above-ground in the phyllosphere, and within plant tissues as endophytes.

The main goal of this study was to compare the rhizosphere and endophytic microbiome and mycobiome of hybrid *Paulownia elongata* and *Paulownia fortunei*. A 2-month-old healthy tree (non-symptomatic plant) and a tree with chlorosis, necrosis, and without a fully formed root system (symptomatic plant), grown independently in a common soil medium, were tested. The detailed objectives of the study were to estimate which taxa of bacteria and fungi occur in the leaf, root, and rhizosphere of *Paulownia* spp., and to determine how plant health conditions affect the microbiome and mycobiome.

## EXPERIMENTAL

### Materials

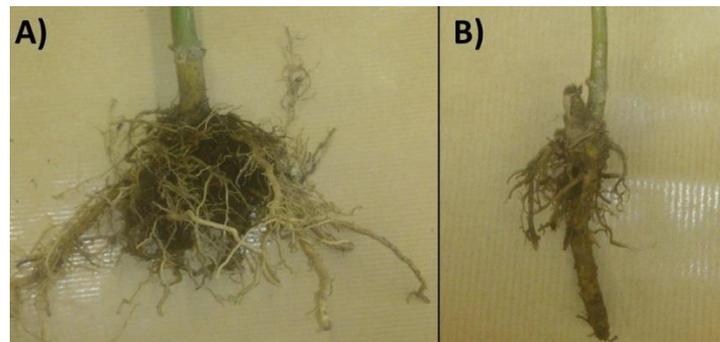
#### *Samples collection*

Plant samples were collected in the summer, specifically in July 2016. Two *Paulownia* plants (*Paulownia elongata* × *Paulownia fortunei*) were selected from Podkarpac, Mazowieckie Voivodeship, Poland (52° 14'N, 20° 27'E):

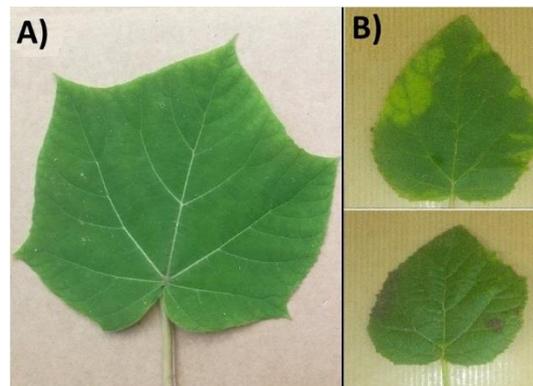
A): a plant with a fully shaped root system (Fig. 1) without physical symptoms of chlorosis and necrosis

B): a plant displaying symptoms of chlorosis and necrosis (Fig. 2) without a fully shaped root system and with a small number of lateral roots (Fig. 1).

The samples were brought to the lab on ice and then stored at -20 °C before processing.



**Fig. 1.** A) *Paulownia* tree with a fully shaped root system; B) *Paulownia* sapling without a fully shaped root system and with a small number of lateral roots (photo: M. Woźniak)



**Fig. 2.** A) Typical *Paulownia* leaf; B) Chlorotic stripes and symptoms of necrosis in *Paulownia* leaves (photo: M. Woźniak)

#### *Sample preparation*

The rhizosphere soil samples were collected from the soil adhering to the roots of the *Paulownia* tree. Next, each sample of plants was washed under running tap water to remove soil and dust particles and allow for drainage. Surface sterilization was completed following modified protocol (Yang *et al.* 2001; Zinniel *et al.* 2002). Each of the plant tissues was surface-sterilized using a series of washes (70% ethanol; 2% sodium hypochlorite NaClO, and 70% ethanol for 30 s) followed by three washes with sterile distilled water. The final rinse was in 12.5 mM potassium phosphate buffer (pH 7.1). The

plant tissues were ground with an aqueous solution (0.9 % NaCl) using a sterile mortar and pestle. The tissue extracts were then diluted in 0.85% NaCl. To confirm the disinfection protocol, aliquots of 0.85% NaCl used in the final rinse were plated in tryptic soy agar (TSA) (Sigma-Aldrich, St. Louis, MO, USA) and potato dextrose agar (PDA) (Difco, Sparks, MD, USA). A summary of the sample names, abbreviations, and number of samples examined by current research is shown in Table 1.

**Table 1.** Samples of Plant Tissue and Rhizosphere Soil for Analysis

Habitat	Samples	
	A (Plant with a fully shaped root system and without physical symptoms of chlorosis and necrosis)	B (Plant without a fully shaped root system and a small number of lateral roots; displayed symptoms of chlorosis and necrosis)
Leaf	LA	LB
Root	RA	RB
Rhizosphere soil	SA	SB

## Methods

### *DNA extraction and sequencing (NGS)*

Total genomic DNA was extracted from the leaves and roots samples using a Fast DNA™ SPIN Kit (MP Biomedicals, Solon, OH, USA) and from the rhizosphere soil using a Fast DNA™ SPIN Kit for soil (MP Biomedicals) in compliance with the manufacturer's instructions. The extracted genomic DNA was quantified and checked for purity at A260/280 nm (1.7 to 2.0) using Nanodrop (Thermo Fisher Scientific, Waltham, MA, USA). Next, polymerase chain reaction (PCR) amplification with universal primers that target V3 to V4 hypervariable regions of the 16S rRNA gene was performed. The primers used to amplify the 16S rRNA genes for bacteria were forward primer 5'-CCTACGGGNGGCWGCAG-3' and reverse 5'-GACTACHVGGGTATCTA-3' (Klindworth *et al.* 2013). The fungal universal primer sets TS1FI2 (5'-GAACCGGCGGARGGATCA-3') and 5.8S (5'-CGCTGCGTTCTTCATCG-3') were used to amplify the internal transcription spacer-1 (ITS-1) as described in previous studies (Schmidt *et al.* 2013; Gałazka and Grządziel 2018). Next generation sequencing was conducted at Genomed S.A. (Warsaw, Poland) on an Illumina MiSeq (San Diego, CA, USA).

### *Bioinformatics analysis*

From fastq files, amplicon sequence variants (ASVs) were selected using the DADA2 version 1.6 package (Callahan *et al.* 2016) in R version 3.4.3 (Vienna, Austria) (R Core Team 2016). Using 'FilterAndTrim' based on quality plots, forward sequences were trimmed to 250 bp, reverse reads to 230 bp, and the first 20 bp were removed (comprising primers and low-quality bases) from both read directions. The filtering of sequences was set to  $maxN = 0$ ,  $maxEE = 3$  and 5 (forward and reverse reads, respectively), and  $truncQ = 2$ , where  $maxN$  was the maximum number of "N" bases,  $maxEE$  corresponded to the maximum expected errors calculated from the quality score ( $EE = \sum (10^{-(Q/10)})$ ), and the  $truncQ$  parameter truncate read at the first instance of a quality score was lower than or equal to 2. Other parameters were set to the default option. The error

rates were evaluated by ‘learnErrors’, where  $n$ -reads were set to  $10^6$ . The sequences were dereplicated using ‘derepFastq’ and default parameters and exact sequence variants were resolved with the use of ‘dada’. Next, ‘removeBimeraDenovo’ was applied to remove chimeric sequences using the consensus method. At this step, 0.062% sequences were recognized as chimeric and removed. Taxonomy was assigned using the latest version of the RDP database (The Ribosomal Database Project) (Callahan *et al.* 2016). The RDP taxonomic training data was formatted for DADA2 (RDP trainset 16/release 11.5) using a naïve Bayesian classifier (Wang *et al.* 2007) and then implemented in assignTaxonomy with the minBoot parameter set to 50. The resulting taxonomy and reads-count tables constructed in DADA2 were appropriately converted and imported into the phyloseq package (McMurdie and Holmes 2013). The comparison of the taxonomic profile of the samples at bacterial and fungal genus level was computed using platform statistical analysis of metagenomic profiles (STAMP) (Parks *et al.* 2014). The reads were then rarefied, setting the seed to 10,000 and the new sample size to 11,247. Different alpha (Chao 1, Shannon, and Simpson indices) and beta diversity measures (non-metric multidimensional scaling (NMDS)) were calculated with the use of the phyloseq package. A permutational multivariate analysis of variance (PERMANOVA) was calculated using the vegan R package (Oksanen *et al.* 2013) using Bray-Curtis distance calculation with permutation set to 999.

## RESULTS AND DISCUSSION

### Bacterial and Fungal Richness and Diversity

To the best of the authors’ knowledge, this was the first reported use of PCR-based Illumina Miseq technology to determine the fungal and bacterial diversity in the *Paulownia* endosphere and rhizosphere in Poland. Metagenomics studies provides access to the genetic diversity of samples received directly from natural environment. It is emphasized, that metagenomics analysis does not require prior isolation and cultivation or the knowledge of microbiological communities. Summarizing, metagenomics allows studying non-cultured microorganisms, their composition and physiological profile. Illumina-based analysis was chosen among others techniques (*e.g.* 454 sequencing) because of fewer reported errors for this approach. Moreover, Illumina Miseq technology made it possible to obtain 10 times or more sequences per sample (Smith *et al.* 2008; Eida *et al.* 2018). The latest next-generation sequencing (NGS) methods allow the detecting of a high number of phylogenetic taxa, including with low-abundance taxa. Metagenomic analysis based on the Illumina has been used to characterize the community of different plant species: *Olea europaea* L. (Müller *et al.* 2015), *Aloe vera* (Akinsanya *et al.* 2015), *Oryza sativa* L. (Wang *et al.* 2016), and *Phoenix dactylifera* L. (Al-Bulushi *et al.* 2017). In these studies, different levels of endophyte variability were reported as a result of mostly environmental conditions. The current study depicted, for the first time, the microbiome and mycobiome changes in the leaves, roots, and rhizosphere of *Paulownia* depending on plant health conditions by using the Illumina sequencing protocol. Very little is known about *Paulownia* interactions with the bacterial and fungal communities in natural ecosystems, specifically with respect to trees in the early stages of growth. Moreover, it should be emphasized that the *Paulownia* microbiome and mycobiome analysis should be given priority because species of this genus can act as vectors of new diseases and have a negative impact on global biodiversity (Ding *et al.* 2006; Essl 2007).

Bacterial communities in all samples were comprised of 17 to 610 individual ASVs per sample with an average of 220 ASVs. The average length of the retained sequences was  $381 \pm 51$  base pairs (mean  $\pm$  standard deviation [SD]). The numbers of different fungal ASVs ranged from 63 to 1,891 per sample with an average of 557. The average length of the retained sequences was  $337 \pm 62$  base pairs (mean  $\pm$  SD) (Table 2). The complete data sets were submitted to the NCBI Sequence Read Archive (SRA) under accession numbers, which are given in Table 3 (SRA Database Accession No. SRP192943, BioProject No. PRJNA532872).

**Table 2.** Number of ASVs and a Comparison of Alpha Diversity between Samples of *Paulownia* Rhizosphere, Root and Leaf Organs Based on the Shannon Index, Chao 1 Estimator, and Simpson Diversity Index

Sam- ples	Bacteria				Fungi			
	AVSs	Chao1	Shannon	Simpson	AVSs	Chao1	Shannon	Simpson
LA	17	7	1.583	0.750	63	74	3.623	0.963
RA	114	90	4.127	0.978	87	77	3.067	0.915
SA	389	247	5.166	0.992	986	625.474	5.249	0.989
LB	17	6	1.654	0.793	108	124	3.566	0.951
RB	172	133	3.778	0.949	210	136.75	2.306	0.755
SB	610	343	5.315	0.992	1891	341.813	4.040	0.965

Note: Amplicon sequence variants (ASVs) - recently this unit replaces OTU (Operational taxonomic unit) in metagenomics analysis. ASV method shows more sensitivity and specificity. Moreover, it allows differentiating of sequence variants that differing by only one nucleotide. Chao 1 estimator - This index includes the observed number of species, more specifically the number species singletons (species observed once) and doubletons (species observed twice). Shannon's index ( $H'$ ) measures for both abundance and evenness of the species present in sample. The Simpson index allows estimation the odds that two individual microorganisms sampled at random will belong to the same AVS.

**Table 3.** Metagenome Sequences Data Available on NCBI, SRA Database Accession No. SRP192943, and BioProject No. PRJNA532872

Samples	Bacteria	Fungi
LA	SRX5707406	SRX5707416
RA	SRX5707412	SRX5707415
SA	SRX5707410	SRX5707408
LB	SRX5707405	SRX5707409
RB	SRX5707411	SRX5707414
SB	SRX5707413	SRX5707407

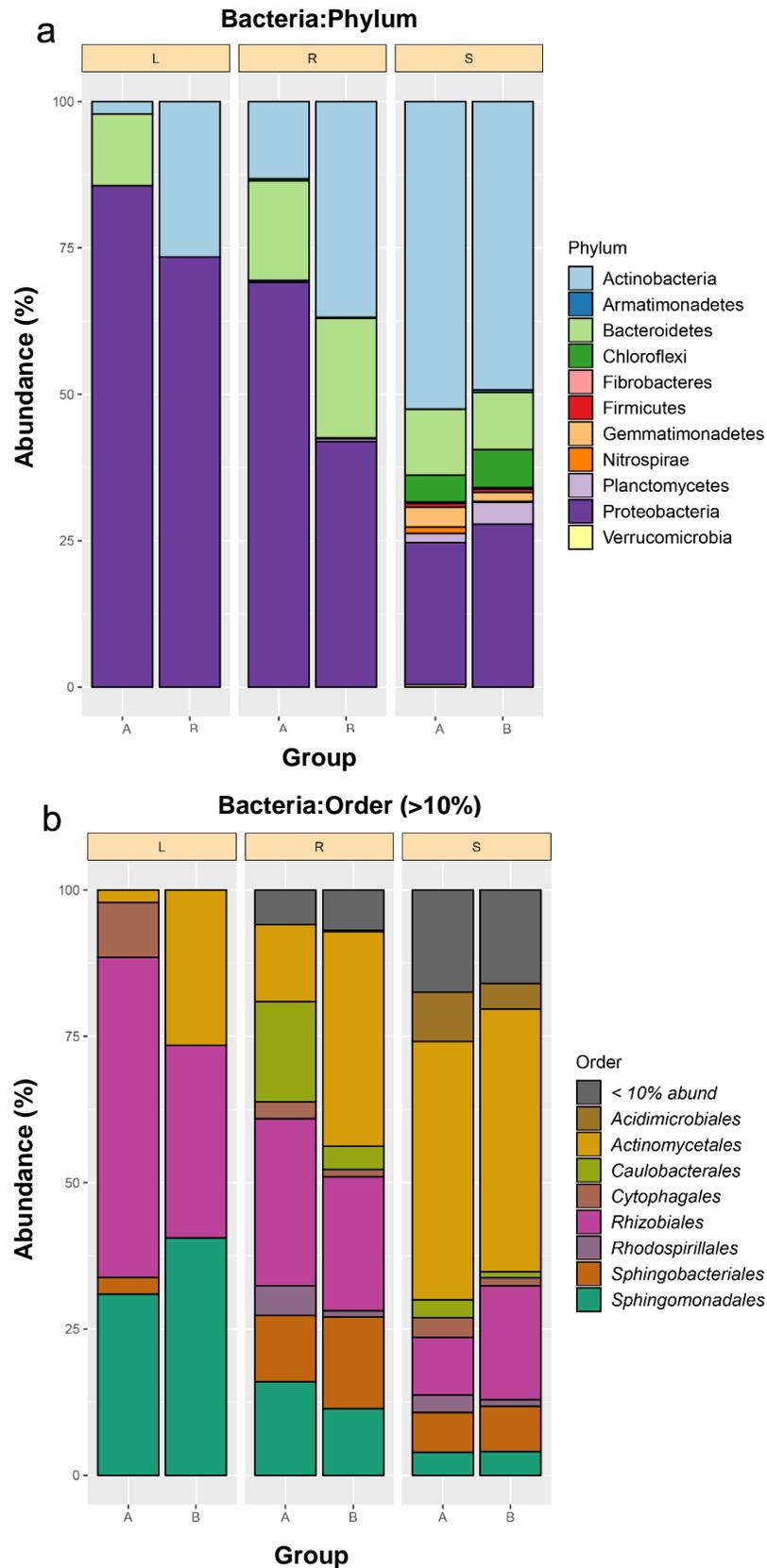
Note: The number of deposited sequences given in the table are a way of organizing the ever-increasing number of metagenomics data. Moreover, this notations of the data allows users more easily locate, understand and analyse metagenomics data.

The first definition of "alpha diversity" was proposed by Robert Whittaker in 1960 and is referred to as "The richness in species of a particular stand or community, or a given stratum or group of organisms in a stand" (Whittaker 1960). Overall, alpha-diversity is determined as the diversity within a single sample or set of repetition. Alpha diversity in the rhizosphere and endosphere was calculated using the Chao 1 estimator, Shannon index, and Simpson's diversity index, all of which demonstrated a higher bacterial and fungal richness and diversity in the rhizosphere than in the endosphere, both in healthy and infected *Paulownia* samples. The alpha diversity indices (Chao 1, Shannon, and Simpson)

presented differences among the plant parts of *P. elongata* × *P. fortunei* (Table 2). The study indicated that rhizosphere samples had the highest number of ASVs relative to that found in the organs of the plants, followed by roots and leaves, regardless of the plant's health condition. Chao 1 indicated a high number of bacterial phylotypes in the SB sample and a low number of species in the leaf samples (LB). The Shannon index (H') showed that the rhizosphere soil sample from a plant that displayed symptoms had the highest diversity, whereas the leaf sample from a healthy plant had the lowest diversity. In contrast, the highest number of fungal phylotypes based on the Chao 1 index was found in the SA sample, while the lowest was found in the LA sample. The H' revealed that the SA sample had the highest diversity, whereas the root sample (RB) had the lowest diversity. The results suggested that the diversity and composition of the associated microorganisms were mostly maintained regardless of the plant's health condition. The number of ASVs in the bacterial and fungal endophytic samples was much lower than in the rhizosphere and less diverse (Table 2). Similar results have been described for poplar trees (Gottel *et al.* 2011). Additionally, more diverse fungi were found in leaves than in roots, while more diverse bacteria were found in roots than in leaves. This may have resulted from air fungal spores being the main source of the endophytic mycobiome and rhizosphere soil being the source of most endophytic bacteria (Oberhofer and Leuchtmann 2014; Wang *et al.* 2016; Li *et al.* 2017; Liu *et al.* 2017; Perez-Rosales *et al.* 2017; Chen *et al.* 2018). Differences in the variability and diversity of microorganisms in the rhizosphere as well as the endosphere of *Paulownia* were probably caused by the availability of nutrients. The compounds secreted by the plant roots function as chemical attractants for a large number of diverse and metabolically active soil microbial communities (Ahemad and Kibret 2014). The number and species diversity of endophytic bacteria are variable along the axis of the plant (Gaiero *et al.* 2013). The most quantitatively and qualitatively diverse plant microbiome is observed in the roots, which results from their direct contact with soil and is the main reservoir of endophytes (Thijs *et al.* 2014). As the distance from the colonization site increases and the amount of minerals supplied by xylem gets smaller, the number and variety of endophytic bacteria decreases (Gaiero *et al.* 2013).

### Bacterial and Fungal Composition and Community Structure

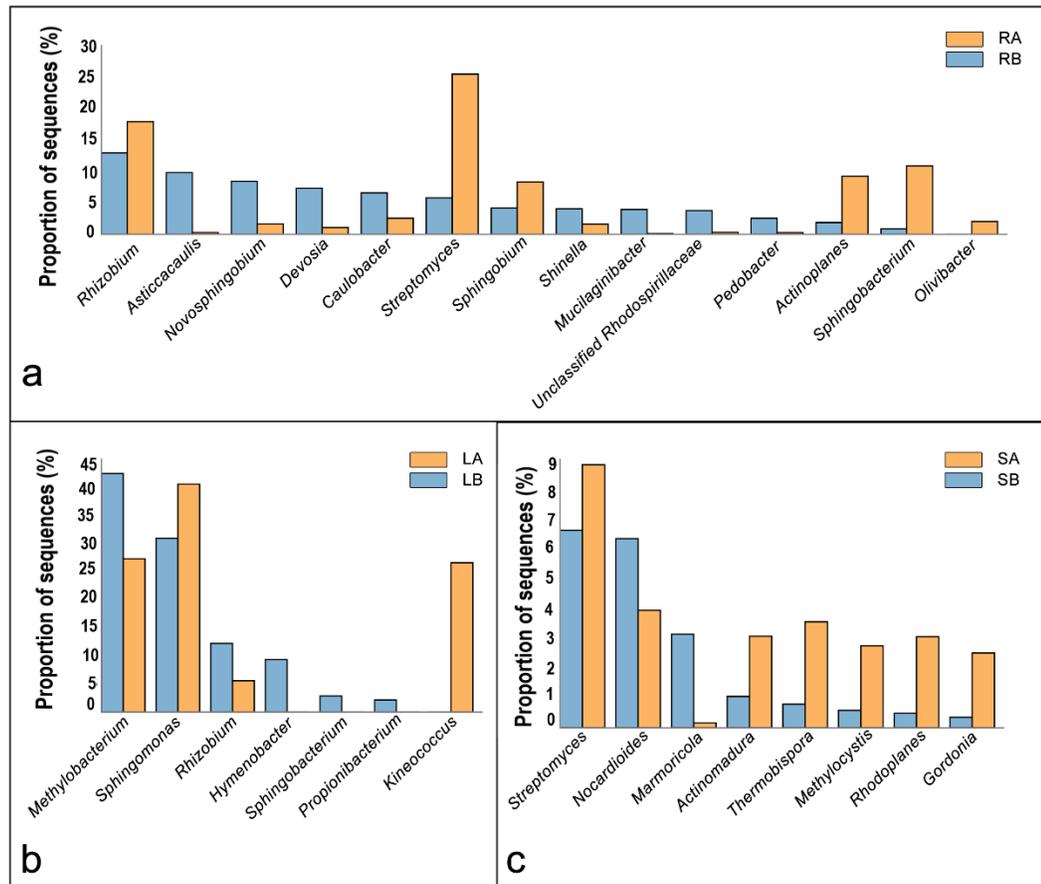
Representative sequences in all ASVs were compared with the RDP database to assign a taxonomy classification to determine the community composition. The relative abundance of bacterial communities, as obtained in the three habitats evaluated, is shown in Fig. 3. The results demonstrated that the root and leaf endophytes and rhizobacteria had noticeably different community structures and abundance. Proteobacteria was the predominant bacterial group in the *Paulownia* root and leaf endophytes. Contrarily, Actinobacteria was the dominant bacterial among the rhizobacteria. *Rhizobiales* and *Actionomycetales* were the most frequently detected bacterial order in the rhizosphere soil. *Rhizobiales* and *Actionomycetales* were the dominant bacterial order in the roots while *Actionomycetales* was the most frequent bacterial order in the leaves (Fig. 3). The relative abundance of bacteria from the Actinobacteria phylum shows a decreasing tendency from the soil, through the roots, and up to the leaves. In contrast, bacteria classified in the phylum Proteobacteria showed increasing tendencies in the same range of habitats. This ranking of relative abundance was in agreement with general knowledge. Most of the Actinobacteria are soil-dwelling organisms, so they constitute an important part of the rhizosphere microbial community.



**Fig. 3.** The composition and relative abundance of bacterial phyla (a) and order (b) Abbreviation: root samples (R), leaf sample (L); rhizosphere soil (S); non-symptomatic plant tissue samples (A); symptomatic plant tissue samples (B)

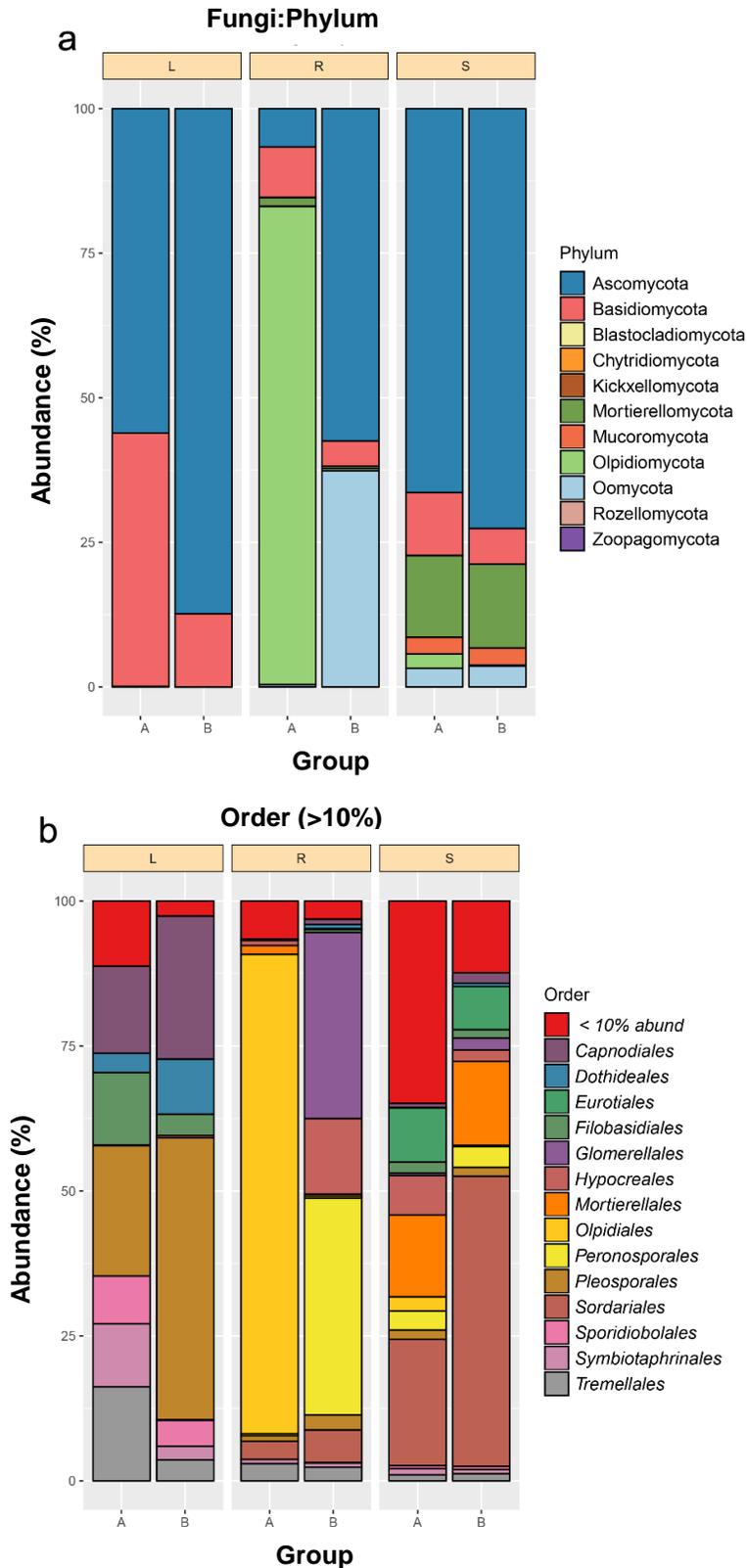
Proteobacteria are commonly found in various environments, especially the endosphere. Beckers *et al.* (2017) as well as Ding and Melcher (2016) demonstrated that Proteobacteria are dominant groups in the endosphere of different plants. In the authors' own studies, it was found that Bacteroidetes and Proteobacteria were dominant in the *Paulownia* leaf endosphere, which has been confirmed in current studies on healthy leaves (Woźniak *et al.* 2018). Actinobacteria, Proteobacteria, Bacteroidetes, and Chloroflexi were the most abundant phyla in the rhizosphere associated communities. This was only partly in line with the study of Tu and co-authors (2018) that showed that Acidobacteria was the most abundant phylum in the soil of *Paulownia*, followed by Proteobacteria, Actinobacteria, Gemmatimonadetes, and Bacteroidetes. Domination of Actinobacteria and Proteobacteria in the rhizosphere of *Paulownia* is probably connected to the nutrient-rich conditions of the rhizosphere (Castro *et al.* 2010; Gottel *et al.* 2011). Similar results have been observed in other plant species, such as *Zea mays* L. (Sanguin *et al.* 2006) and *Glycine max* L. (Xu *et al.* 2009). Then again, the analysis of the microbiome of other woody species, such as *Castanea dentata* (Lee *et al.* 2008) and *Populus deltoides* (Gottel *et al.* 2011), showed that members of Acidobacteria are dominant in the rhizosphere systems. In the authors' studies, bacteria belonging to the phylum Acidobacteria were not detected in the rhizosphere soil samples. Several of the bacterial genera observed, including *Asticcacaulis*, *Streptomyces*, and *Sphingobacterium*, differed in their abundance in samples of the rhizosphere. There were also differences in the abundance of *Hymenobacter*, *Sphingobacterium*, and *Kineococcus* in samples of the endosphere of leaves as well as of *Thermobispora*, *Rhodoplanes*, and *Gordonia* in samples of the endosphere of roots. Members of the genera *Rhizobium*, *Streptomyces*, and *Sphingobacterium* are well-known for their capacity in plant growth promotion. They also exhibit an ability to biodegrade potentially hazardous compounds (Vurukonda *et al.* 2018; Yadav and Saxena 2018).

Based on the analysis of the internal transcription spacer-1 (ITS1) data set, members of the phylum Ascomycota were dominant in all samples, excluding the sample RA (Fig. 4). The abundance of Ascomycota varied in different samples. Remarkably, phylum Olpidiomycota absolutely dominated the fungal content of endophytes in the fully shaped root system of plant A, accounting for approximately 82.66% of the total root associated microbiome. The endophyte microbiome associated with plant A demonstrated a differing structure composition and a relative abundance of microbial communities in which Ascomycota and Oomycota were dominant. The overall fungal composition of the leaves A *versus* leaves B sample was similar, while the abundance of each phylum varied in the samples. It was similar in the case of the rhizosphere soil samples, whereby the SA sample additionally contained the Olpidiomycota phylum. The most abundantly detected orders were *Pleosporales*, *Capnodiales*, *Dothideales*, and *Sporidiobolales* in leaves of *P. elongata* × *P. fortunei*. The dominant fungal orders *Sordariales*, *Mortierellales*, and *Eurotiales* were coexistent in the SA and SB samples (Fig. 4). The differences between fungal communities were primarily due to large numbers of individual ASVs, such as Ascomycota that dominated most endophyte samples of leaves B, comprising on average 78.31%, as well as to large members of Olpidiomycota that dominated the root endosphere samples of plant A. The dominant presence of *Olpidium* (82.66%) in the roots of a healthy plant in this study was baffling. The results of Lay *et al.* (2018) and Tkacz *et al.* (2015) showed that *Olpidium* species constitute a large proportion of the fungal community in plant roots and the rhizosphere but not at that level. *Olpidium* spp. are common biotrophic, symptomless fungal parasites of many plants, and are also considered to be vectors of plant viruses (Lay *et al.* 2018).

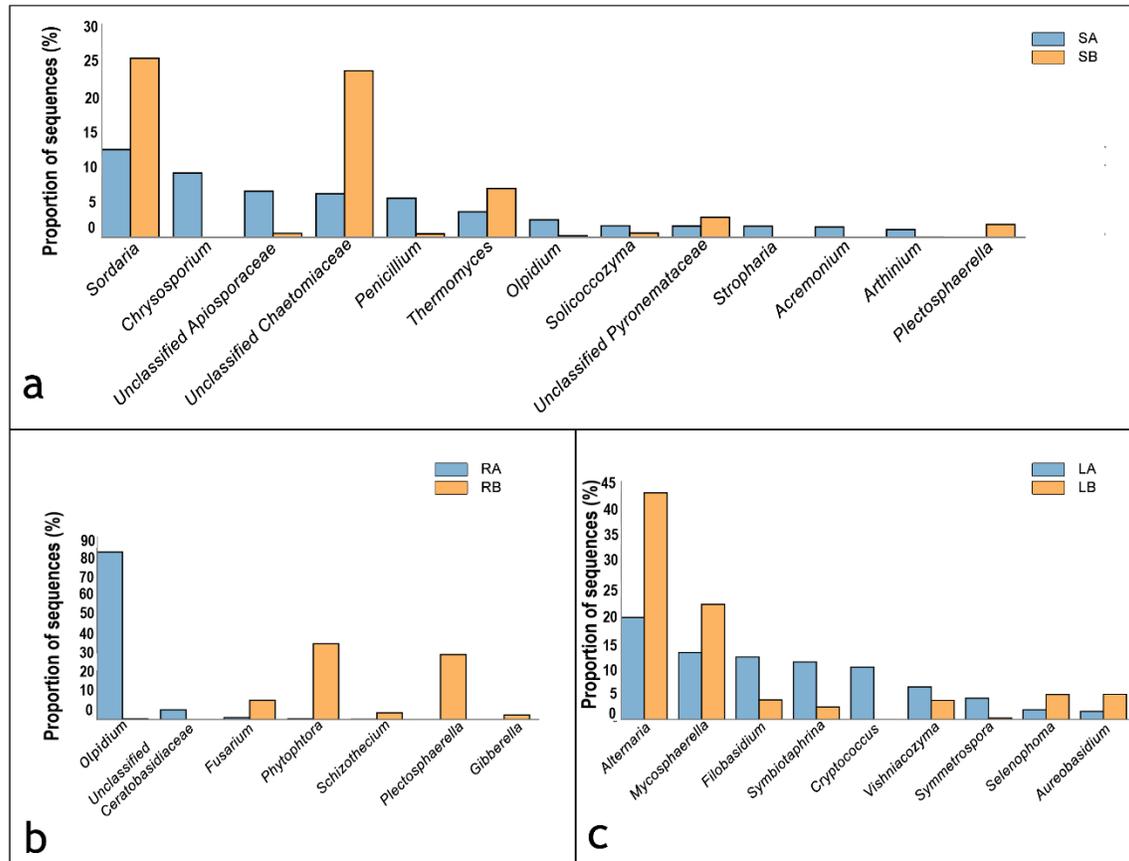


**Fig. 4.** Comparative taxonomic profile of the samples at bacterial genus level, computed by STAMP. The most abundant genera found in each sample are shown; a) in root samples (R); b) in a leaf sample (L); and c) in rhizosphere soil (S)

In the tissues of leaves, fungi of the genus *Mortierella* were identified, which have the ability to solubilize phosphate (Zhang *et al.* 2011). Additionally, genera, including well-known plant pathogens, were detected, but the obtained sequences were dominantly affiliated with plants with unique morphological changes. At the genus level, *Phytophthora* (37.40%), *Plectosphaerella* (32%), and *Fusarium* (9.46%) were the most dominant pathogens in roots B with morphological changes. The fungi of the genus *Phytophthora* and *Fusarium* are known pathogens of *Paulownia* (Ray *et al.* 2005). The results indicated that investigations should be undertaken on the fungi of *Plectosphaerella* due to such a high relative abundance in a root system that was not fully shaped with a small number of lateral roots. The fungi from the genus *Plectosphaerella* are well known as pathogens of several plants such as potato, tomato, melon, tobacco, soybean, pepper, and other plants. Moreover, these fungi are active root rot (Li *et al.* 2017). Previous research based on culture-dependent methods reported that common diseases of *Paulownia* include witches'-broom (caused by a phytoplasma) and anthracnose. Typical pathogens for *Paulownia* are *Sphaceloma paulowniae*, *Phyllosticta paulowniae*, *Rhizoctona solani*, *Cercospora* sp., *Corynespora cassiicola*, *Fusarium* spp., *Ascochyta paulowniae*, and *Phytophthora* spp. (Ray *et al.* 2005). The high prevalence of two genera, *Alternaria* and *Mycosphaerella*, in sample LB over all other detected fungi could be related to the occurrence of morphological changes on leaves.

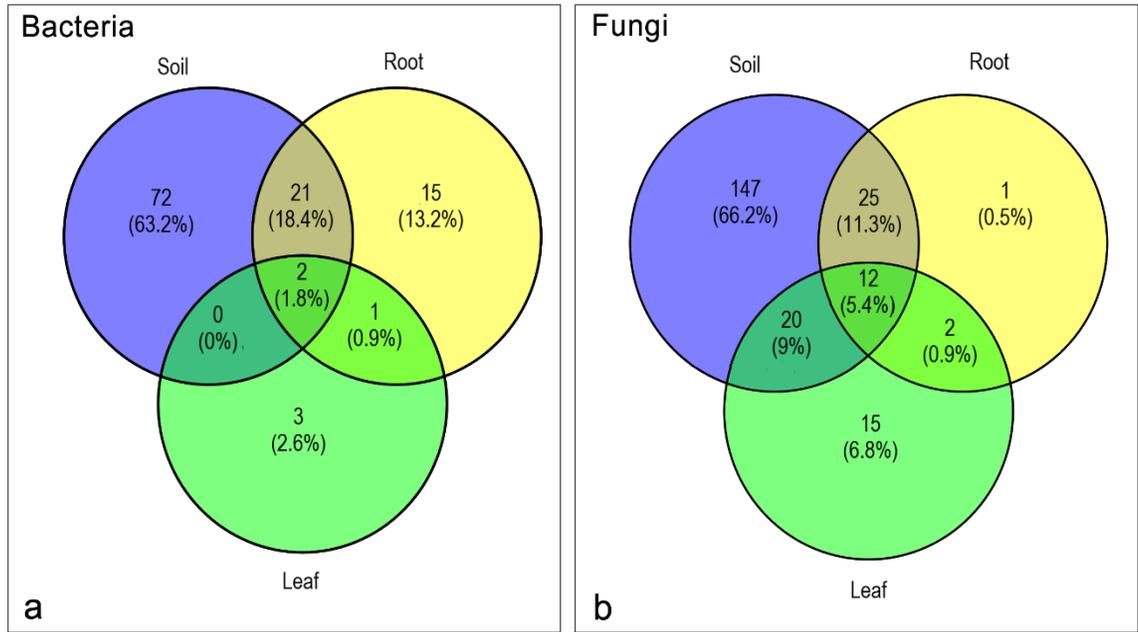


**Fig. 5.** The composition and relative abundance of fungal phyla (a) and order (b) Abbreviation: root samples (R), leaf sample (L); rhizosphere soil (S); non-symptomatic plant tissue samples (A); symptomatic plant tissue samples (B)

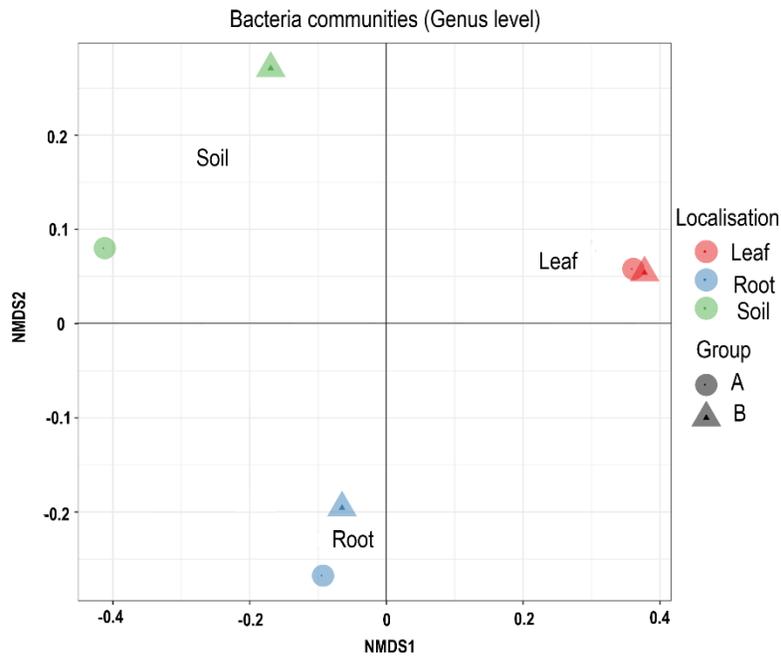


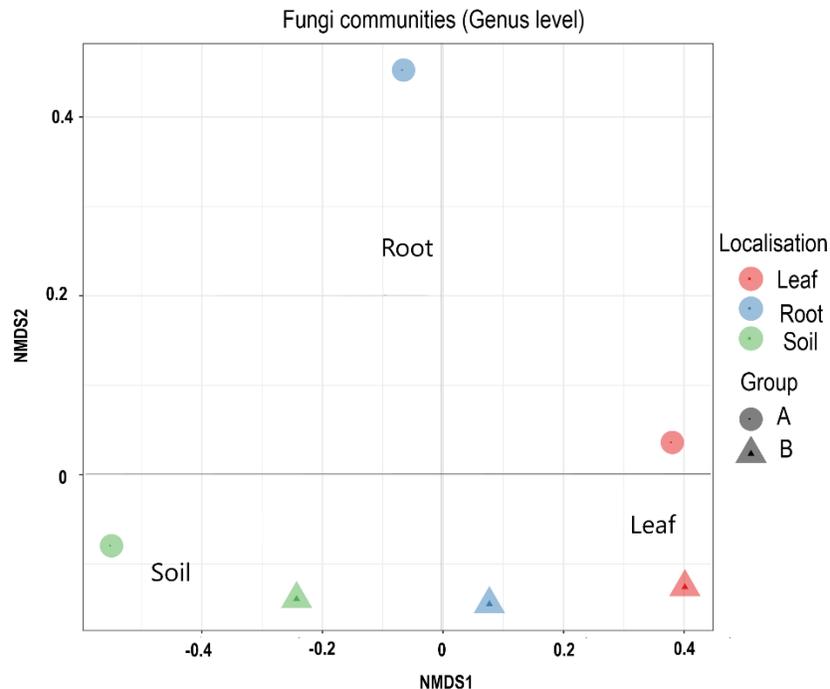
**Fig. 6.** Comparative taxonomic profile of the samples at fungal genus level, computed by STAMP; the most abundant genera found in each samples are shown: a) in rhizosphere soil (S); b) in root samples (R); c) in a leaf sample (L)

According to Venn diagrams (Fig. 7), consistent overlap patterns of genus clusters among different habitats in healthy *Paulownia* were obtained.



**Fig. 7.** Venn diagram of bacterial genera (a) and fungal genera (b) in samples of soil, root and leaf of a healthy plant (non-symptomatic plant) showing the number of shared and unique genera in those samples. The numbers in the overlapping circles show genera shared by two or three samples of the fungal community.





**Fig. 8.** NMDS biplots of the most abundant genera of bacteria (a) and fungi (b). The samples are indicated by symbols and colours.

In the bacterial community, only two genera were detected as core microbiomes in all three samples: *Shingomonas* and *Rhizobium*. No genera were shared between the community of the leaves and the community of the rhizosphere. There were 22 different genera shared only by the microbiome of the rhizosphere and the microbiome of the roots; one genus, *Sphingobacterium*, was shared by the microbial community of the roots and leaves. A core microbiome of 12 genera was found in the fungal community in all habitats: the rhizosphere and endosphere of the leaves and roots. There were 25 genera detected in the soil and roots simultaneously. In the roots' and leaves' core microbiome, two shared genera could be identified, whereas the bacterial community of the leaves and rhizosphere shared 20 genera. The bacteria in the soil, root, and leaf samples harboured 72, 15, and 3 unique genera, respectively. Moreover, the fungi community in the soil, root, and leaf samples harboured 147, 1, and 15 unique genera, respectively (Fig. 7).

The core microbiome and mycobiome may be important in maintaining fundamental functions. Endophytic root bacterial and fungal communities include a subgroup of members originating from the surrounding rhizosphere soil. The rhizosphere and endosphere will have similar overall structures of phylogenetic groups if facultative endophytes occur in the rhizosphere. This dependency was observed in the current study.

The core genera composition results indicated the presence of diverse endophytic bacteria and fungi in the leaves and roots of the *Paulownia* tree and in the rhizosphere soil. For a more detailed explanation, the microbial population correlated with habitat and plant health condition, and non-metric multidimensional scaling (NMDS) was used to visualize the degree of variation of the bacteria and fungi populations in the different samples. The NMDS analysis revealed that habitat was a lead interpretive factor for variability in community composition of the microbiome and mycobiome of *Paulownia* trees. The superimposed circles show that the samples typically clustered based on habitat. The study

indicated that plant health condition was not a factor that shaped the community composition in samples, excluding the mycobiome of the roots (Fig. 8).

## CONCLUSIONS

1. Using Illumina sequencing, conspicuous differences were observed in the bacterial and fungal community structure (leaves, root, and rhizosphere soil) of the *Paulownia* tree in different habitats.
2. The differences in the relative abundance of ASVs and in the structure of the microbial community between a healthy plant and a plant with morphological changes were slight.
3. The health conditions of *Paulownia* in this study did not affect the composition of the bacterial and fungal communities in *Paulownia*.

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