Bacterial assembly as part of immune system in Paulownia responses to witches' broom caused by phytoplasmas

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Research Article

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Abstract

Purpose

Paulownia witches' broom (WB) usually affects part of the infected tree, rarely spreading throughout the tree. WB was infected by phytoplasmas, but the associated microbiome assembly remains unknown. This study aims to clarify the possible mediating mechanism of WB outbreaks in *Paulownia*.

Methods

We collected leaves, branches, roots and rhizosphere soil of *Paulownia* trees with or without WB, and analyzed the bacterial community on the basis of 16S rRNA high-throughput sequencing.

Results

All leaf and branch samples contained phytoplasmas, but high phytoplasma levels were required for WB. Low bacterial community diversity and richness, a lack of beneficial bacteria, and an unstable bacterial network might be conducive to a WB outbreak. Phytoplasmas were more abundant in the roots of diseased trees than in the roots of healthy trees. Moreover, phytoplasma infections altered the bacterial community composition, but did not induce morphological changes. Phytoplasma did not present in the rhizosphere soil of diseased trees, but their bacterial community composition changed comparing with the corresponding samples of healthy trees. Asymptomatic leaves and branches of diseased trees recruited beneficial bacteria and increased the bacterial network stability, which might contribute to the restriction of phytoplasma infections and WB development further. The abundance of several beneficial bacteria in the endosphere was significantly negative correlation with that of phytoplasmas, implying their inhibitory effects on phytoplasmas.

Conclusion

Our findings have clarified the possible mechanism mediating WB outbreaks in *Paulownia* trees. Furthermore, we demonstrated that *Paulownia* trees inhibit phytoplasma infections by modulating the endosphere and rhizosphere bacterial communities.

Introduction

Many microbes inhabit various plant tissues, including the leaves, branches, and roots, as part of a microbiome (Hassani et al. 2018; Xiong et al. 2021; Podolich et al. 2015). These endophytes coexist in special communities comprising mutualistic, commensal, or parasitic microbes that are influenced by host–microbe and microbe–microbe interactions (Hassani et al. 2018; Shalev et al. 2022). The functions of plant microbiomes have typically been neglected, but there is increasing evidence that the plant

bacterial community can affect host health by modulating nutrient acquisition, plant hormone production, and defense responses to pathogen infections (Hassani et al. 2018). The host plant also provides the habitat and nutrients for the microbiome, while also influencing the microbial composition (Hassani et al. 2018). Pathogen infections can disturb the endophytic microbial composition, while the host plant can actively reshape the microbiome to minimize the negative effects of a pathogen infection (Carrion et al. 2019). These phenomena have been observed in various plant species, including chili pepper (Gao et al. 2021), wheat (Liu et al. 2021), sugar beet (Carrion et al. 2019), and *Arabidopsis thaliana* (Berendsen et al. 2018). Some important beneficial bacteria, such as *Bacillus* (Santoyo et al. 2012; Lopes et al. 2018) and *Pseudomonas* (D'Amelio et al. 2011; Santoyo et al. 2012; Biessy and Filion, 2018) species, are usually actively selected by the host plant to defend against a pathogen infection. Induced systemic resistance involves the activation of plant defense systems by beneficial bacteria (e.g., *Bacillus* and *Pseudomonas* species) in response to a pathogen infection (Kloepper et al. 2004; Han et al. 2006; Biessy and Filion, 2018). For example, *Pseudomonas* sp. CMR12a produces two types of cyclic lipopeptides that can induce the systemic resistance of rice and bean to *Magnaporthe oryzae* and the web blight pathogen *Rhizoctonia solani* AG2-2, respectively (Ma et al. 2016). Beneficial bacteria have important effects on the plant immune system. Besides, *Bacillus* and *Pseudomonas*, the genus *Methylobacterium* (Zhang et al. 2021), *Sphingomonas* (Innerebner et al. 2011; Asaf et al. 2020) and *Novosphingobium* (Hahm et al. 2012; Duan et al. 2013) can produce plant growth-stimulating factors or inhibit pathogen growth. In addition to endophytes, many microbes in the rhizosphere are closely related to plant health because of their effects on nutrient acquisition or phytohormone production (Berendsen et al. 2012). The rhizosphere microbiome is also crucial for suppressing pathogen infections, especially soil-borne infections (Berendsen et al. 2012). Some studies suggested that beneficial bacteria in the rhizosphere can also control aboveground pathogen infections (Berendsen et al. 2018; Liu et al. 2021). Additionally, Rhizosphere bacteria are an important source of endophytes (Berendsen et al. 2012) and plants can select potential endophytes through their root exudates (Papik et al. 2020). Therefore, exploring the microbial communities in the endosphere and rhizosphere is critical for preventing plant diseases.

Paulownia is an important timber species native to China and Southeast Asia (Rodriguez-Seoane et al. 2020), wherein it can grow in low-quality soil. It is economically valuable because it can be included in multiple products (e.g., furniture and traditional medicines). Moreover, it contributes to ecosystem services because of its effects on sand fixation as well as water and soil conservation, while also being useful for farmland intercropping (Pérez 2005; Brundu and Richardson, 2016; He et al. 2016). Additionally, *Paulownia* trees are a rich source of biologically active secondary metabolites, such as benzoic acids and flavonoids (Rodriguez-Seoane et al. 2020). During the growth period, *Paulownia* trees are highly susceptible to witches' broom (WB), which can cause economic losses of billions of dollars per year in China alone (Yue et al. 2008). Typical WB symptoms include stunting, yellowing, and proliferating secondary shoots (Yue et al. 2008) and it is believed to be caused by phytoplasmas (Yue et al. 2008; Cao et al. 2021). Phytoplasmas are prokaryotes that lack a cell wall and can infect more than 700 plant species (Bertaccini et al. 2014; Maejima et al. 2014; Huang et al. 2021). Interestingly, phytoplasmas have

a dual host cycle that alternates between plants and sap-feeding insects (Hogenhout et al. 2008). In plants, phytoplasmas colonize the cytoplasm of vascular phloem sieve cells (Musetti et al. 2013). The mechanism underlying the induction of WB by phytoplasmas has been revealed in *A. thaliana* plants. More specifically, SAP05 protein effectors from phytoplasmas are key factors for the development of WB because they mediate the concurrent degradation of SPL and GATA developmental regulators (Huang et al. 2021). Their obligatory parasitic lifestyle makes it impossible to culture phytoplasmas under *in vitro* conditions (Contaldo et al. 2016), which is an obstacle for developing an effective treatment for *Paulownia* trees affected by WB. There are three methods for detecting phytoplasmas in plant tissues, namely high-throughput sequencing of 16S rRNA, real-time PCR, and nested PCR, of which high-throughput sequencing is reportedly the most sensitive (Eichmeier et al. 2019).

Our long-term analysis indicated that *Paulownia* WB usually affects part of the infected tree, rarely spreading throughout the tree, indicative of some resistance to WB. Although the plant microbiome is closely associated with host health, the microbiome of *Paulownia* trees with or without WB symptoms remains relatively uncharacterized. In this study, we collected different parts of *Paulownia* trees to compare the endosphere and rhizosphere bacterial communities between trees with and without WB on the basis of the high-throughput sequencing of 16S rRNA. We clarified the pathogen mechanism underlying the development of *Paulownia* WB as well as the microbe-associated mechanism mediating tree resistance to WB.

Materials And Methods

Field trial

A field trial was conducted on a 1-ha plot of land in Yuzhou city, Henan province, China. *Paulownia* variety ‘Baihua’ was replanted in 2017, with 4 m separating trees and 5 m separating rows. The average tree height was approximately 5–6 m and 10% of the trees had WB symptoms.

Sample collection and processing

Samples were collected from six randomly selected *Paulownia* trees with or without WB (Fig. 1). For the healthy trees, one complete branch was removed from each tree. For the diseased trees, one symptomatic branch and one asymptomatic branch were collected from each tree. The maximum diameter of each branch was 1 cm. Eight segments (1 cm long) were prepared for each branch (i.e., branch compartments). Ten randomly selected leaves were collected from each branch (i.e., leaf compartments). Randomly selected root segments (1 cm diameter) were collected at a soil depth of 5–10 cm (i.e., root compartments). The soil adhering to the roots were collected (i.e., rhizosphere soil compartments).

The collected leaf, branch, and root compartments were washed sequentially with sterile Millipore water (30 s), 70% (v/v) ethanol (1 min), 2.0% sodium hypochlorite solution (3 min), and sterile Millipore water (30 s) (Beckers et al. 2017). The surface-sterilized tree samples and the soil samples were stored at −80°C prior to the DNA extraction.

DNA extraction

Bacterial community genomic DNA was extracted from the tree and soil samples using the E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek, USA). The extracted DNA was analyzed by 1% agarose gel electrophoresis and the DNA concentration and purity were determined using the NanoDrop 2000 UV-vis spectrophotometer (Thermo Fisher Scientific, USA).

PCR amplification and pyrosequencing

The rhizosphere bacterial community was analyzed by amplifying the V3–V4 hypervariable region of the bacterial 16S rRNA gene by PCR using the primer pair 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), whereas the endosphere bacterial community was analyzed by amplifying the V5–V6 hypervariable region of the bacterial 16S rRNA gene by PCR using the primer pair 799F (5'-AACMGGATTAGATACCCKG-3') and 1193R (5'-ACGTCATCCCCACCTTCC-3'). The PCR amplifications were performed using the ABI GeneAmp® 9700 PCR thermocycler (ABI, USA), with the following program: 95°C for 3 min; 27 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s; 72°C for 10 min and then 4°C. The PCR mixtures contained 4 μL 5× *TransStart* FastPfu buffer, 2 μL 2.5 mM dNTPs, 0.8 μL forward primer (5 μM), 0.8 μL reverse primer (5 μM), 0.4 μL *TransStart* FastPfu DNA Polymerase, 0.2 μL BSA, 10 ng template DNA, and ddH_2O for a final volume of 20 μL. The PCR amplifications were performed in triplicate. The amplified products were analyzed in 2% agarose gels, purified using the AxyPrep DNA Gel Extraction Kit (Axygen, USA), and quantified using the Quantus™ Fluorometer (Promega, USA). Purified amplicons were pooled (equimolar concentrations) for the paired-end sequencing analysis, which was performed using the Illumina MiSeq PE300 platform (Illumina, USA) according to the standard protocols provided by Majorbio Bio-Pharm Technology Co. Ltd. The raw reads were deposited into the NCBI Sequence Read Archive database (Accession Number: PRJNA821669).

Sequence processing

The raw 16S rRNA gene sequencing reads were demultiplexed, filtered for quality using fastp (version 0.20.0), and merged using FLASH (version 1.2.7), with the following criteria: (i) 300-bp reads were truncated at any site with an average quality score < 20 over a 50-bp sliding window, and the truncated reads shorter than 50 bp were discarded, as were reads containing ambiguous bases; (ii) only overlapping sequences longer than 10 bp were assembled according to the overlapping sequence. The maximum mismatch ratio for the overlapping region was 0.2. Reads that could not be assembled were discarded; (iii) samples were distinguished according to barcodes (i.e., barcode matching) and primers (up to two mismatched nucleotides were acceptable) and the sequence direction was adjusted. Abundance data of sequences matching “Chloroplast” and “Mitochondria” were were removed from the data sets.

Operational taxonomic units (OTUs) with a 97% similarity cutoff were clustered using UPARSE (version 7.1). Chimeric sequences were identified and removed. The taxonomy of each representative OTU sequence was analyzed using RDP Classifier (version 2.2) and the 16S rRNA database, with a confidence threshold of 0.7.

Analysis of sequencing data

To compare the α diversity of the microbial community between samples, a principal co-ordinate analysis (PCoA) was performed on the basis of the Bray–Curtis distance. The R package “vegan” was used for the analysis and the results were visualized using “ggplot2”. A heatmap of the bacterial community at the genus level in the examined compartments was constructed using the R package “pheatmap”. A volcano plot illustrating the enrichment and depletion patterns of the tree-associated bacterial microbiomes between compartments was prepared and the enriched and depleted genera were analyzed using the R package “limma”. A bacterial co-occurrence network was constructed by determining the pair-wise Spearman correlations using the R package “Hmisc”. The cutoff for Spearman’s coefficient was 0.65 and the adjusted *P* value was 0.01. The unconnected nodes (degree = 0) were discarded.

Statistical analysis

Statistical analyses were performed using SPSS (version 20.0). The significance of the differences between means was assessed by ANOVA with Duncan’s *post hoc* tests.

Results

Comparison of the asymptomatic and symptomatic *Paulownia* branches

We collected and analyzed three types of *Paulownia* branch samples, namely the branches from healthy trees (i.e., HB) and the asymptomatic and symptomatic branches (i.e., ASB and SB, respectively) from trees with WB (Fig. S1). The HB samples exhibited very limited virgation and the twig was thick and strong, with no detectable abnormalities. Compared with the ASB and HB samples, which were similar in appearance, the SB samples had more twigs, which were thick (i.e., typical WB symptom). Notably, the diseased *Paulownia* trees were infected with WB for about 3 years, but the symptoms were restricted to individual branches rather than the whole tree, indicative of some immunity of plant. Additionally, there were no detectable abnormalities in the roots of diseased tree compared to the healthy.

Effects of WB on the α diversity of bacterial communities

We calculated the α diversity of the bacterial community in different tree compartments according to the 16S rRNA gene sequencing data (Fig. 2A). Among the leaf samples, the Shannon index was significantly lower for the symptomatic leaf (SL) than for the healthy leaf (HL) and asymptomatic leaf (ASL); there were no significant differences between the HL and ASL samples. Similar results were obtained for the branch samples. In terms of the root and rhizosphere soil samples (Fig. 2B), the Shannon index of the diseased root (DR) was significantly lower than that of the healthy root (HR), whereas there were no significant differences in the Shannon index between the healthy rhizosphere soil (HRS) and diseased rhizosphere soil (DRS) samples. These results suggested WB decreased the endophyte diversity in the SL and SB samples, but had little effect on the endophyte diversity of the asymptomatic samples. Additionally, WB decreased the endophyte diversity in the roots of diseased trees.

The analysis of the leaf samples revealed the Chao1 index was lowest for SL, followed by ASL and HL (Fig. 2C). Of the branch samples, the Chao1 index of SB was significantly lower than that of HB, but it was not significantly different from that of ASB (Fig. 2C). Among the root and rhizosphere soil samples, there was no significant difference in the Chao1 index between DR and HR or between HRS and DRS (Fig. 2C and D). These results suggested WB decreased bacterial community richness in SL and SB samples as well as in ASL samples, but it had little effect on the roots and rhizosphere soil.

Effects of WB on the β diversity of bacterial communities

Bray–Curtis distances were calculated on the basis of the bacterial community composition and visualized following the PCoA. For the endophyte community (Fig. 3A), PCoA1 and PCoA2 explained 31.9% and 16.3% of the total variation, respectively. Healthy and asymptomatic samples clustered in one group, which was separated from the corresponding symptomatic samples across PCoA1, implying that the variation in the bacterial communities in the leaf and branch compartments was primarily due to WB. The leaf and branch samples were separated from the root samples across PCoA2, suggesting that the tree compartments were also an important factor for the variation. Regarding the rhizosphere bacterial community (Fig. 3B), PCoA1 and PCoA2 explained 28.4% and 13.8% of the total variation, respectively. The rhizosphere soil samples of the diseased and healthy trees were separated across PCoA1 or PCoA2. These findings indicated that WB affected the β diversity of the bacterial communities in the endosphere and in the rhizosphere. Hence, WB and tree compartments were the two main factors associated with the differences in the β diversity in the endosphere.

Effects of WB on the bacterial community composition

An analysis of the bacterial community composition at the phylum level (Fig. S2) indicated that Proteobacteria was the most common phylum (44.7–78.4%) among the different compartments of healthy trees, followed by Firmicutes, Actinobacteriota, and Bacteroidota. In the SL and SB samples, Firmicutes was the most common phylum (87.6% and 99.1%, respectively). In the ASL, ASB, and DR samples, the phylum distribution was similar to that of the corresponding healthy compartments. More specifically, Proteobacteria was the most common phylum, followed by Firmicutes, Actinobacteriota, and Bacteroidota. The HRS and DRS samples had similar bacterial community structures, with Actinobacteriota, Proteobacteria, and Acidobacteriota species detected as the most common bacteria. These findings indicated that WB altered the bacterial community composition in SL and SB samples at the phylum level, whereas it had little effect on the bacterial community composition in asymptomatic samples as well as in the roots and rhizosphere soil.

An examination of the bacterial genera revealed differences among the analyzed compartments (Fig. S3). In the leaf samples, *Escherichia*, *Streptococcus*, and *Lactobacillus* were the three most common genera in HL samples, whereas *Sphingomonas*, *Pseudomonas*, and *Escherichia* were the top three genera in ASL samples. However, *Candidatus*_Phytoplasma was the most common genus in SL samples. In the branch samples (Fig. S3), *Pseudomonas*, *Curtobacterium*, and *Methylobacterium* were the three most common genera in HB samples, whereas *Curtobacterium*, *Sphingomonas*, and *Methylobacterium* were the

dominant genera in ASB samples. However, similar to the leaf samples, *Candidatus*_Phytoplasma was the primary genus in SB samples. In the root samples (Fig. S3), *Allorhizobium*, *Rhizobacter*, and *Steroidobacter* were the top three genera in HR samples, whereas *Candidatus*_Phytoplasma, *Allorhizobium*, and *Variovorax* were the main genera in DR samples. The predominant genera were consistent in the two types rhizosphere samples (Fig. S3). Thus, WB modified the bacterial community composition in the symptomatic and asymptomatic tree samples (i.e., leaves and branches) as well as in the roots, but it had little effect on the rhizosphere bacterial community.

Notably, *Candidatus*_Phytoplasma was detected in not only diseased trees but also healthy trees, with abundances of 98.8%, 86.4%, and 17.7% in SL, SB, and DR samples, respectively (Fig. 4). It was detected at significantly lower levels in HL (0.1%), HB (0.2%), HR (2.4%), ASL (1.7%), and ASB (1.0%) samples. Phytoplasmas were less abundant in HL and HB samples than in ASL and ASB samples, respectively, but the differences were not significant ($P > 0.05$). Phytoplasmas were undetectable in DRS and HRS samples. Accordingly, WB symptoms were consistent with the phytoplasma abundances in the branches and leaves (Fig. 4 and Fig. S1). Moreover, WB was induced by only high phytoplasma levels.

Effects of WB on specific bacterial taxa

The volcano plot indicated that WB affected the abundance of a number of bacterial taxa. In the leaf compartments (Fig. 5A, B), the abundances of 119 and 76 bacterial genera differed significantly between HL and SL samples and between ASL and SL samples, respectively. With the exception of *Candidatus*_Phytoplasma, all of the taxa were significantly more abundant in HL or ASL than in SL samples ($P < 0.05$). The differentially abundant bacteria mainly belong to Proteobacteria, Firmicutes, Bacteroidota, and Actinobacteriota. Additionally, some were beneficial bacteria in plants, including *Bacillus* (Santoyo et al. 2012; Lopes et al. 2018), *Pseudomonas* (D'Amelio et al. 2011; Santoyo et al. 2012; Ma et al. 2016; Biessy and Filion, 2018), *Novosphingobium* (Duan et al. 2013), *Methylobacterium* (Zhang et al. 2021; Alibrandi et al. 2018), and *Sphingomonas* species (Innerebner et al. 2011; Asaf et al. 2020). There were also significant differences in the abundances of genera between HL and ASL samples (Fig. 5C). The *Sphingomonas* and *Methylobacterium* beneficial bacterial species were more abundant in ASL than in HL ($P < 0.05$).

In the branch compartments, the abundances of 41 and 39 bacterial genera differed significantly between HB and SB samples and between ASB and SB samples, respectively (Fig. 5D, E). Most of these genera belong to Proteobacteria, Firmicutes, Bacteroidota, and Actinobacteriota. The taxa included *Pseudomonas*, *Novosphingobium*, *Sphingomonas*, *Bacillus*, and *Methylobacterium* species, which are beneficial for plants. All of the beneficial bacteria were more abundant in HB or ASB than in SB ($P < 0.05$).

In the root compartments, eight endosphere bacterial species were significantly differentially abundant between HR and DR samples ($P < 0.05$) (Fig. 5G). All but one of these species were significantly more abundant in HR than in DR ($P < 0.05$), including a beneficial bacterial species (*Streptomyces*). There were significant differences in the abundances of 15 taxa between DRS and HRS ($P < 0.05$) (Fig. 5H), including one beneficial bacterial species (*Brevibacillus*), which was more abundant in DRS than in HRS.

Bacterial co-occurrence networks in different tree compartments

To further clarify the bacterial communities in different tree compartments, we constructed bacterial co-occurrence networks (Fig. 6). In the leaf and branch compartments, the average degree and proportion of negative edges were lower for SL and SB than for the corresponding healthy samples. On the basis of a comparison with the corresponding healthy samples, the average degree of ASL and ASB was relatively unchanged, but the proportion of negative edges increased. In the root compartments, the average degree and proportion of negative edges were respectively higher and slightly lower for DR than for HR. In the rhizosphere soil compartments, the average degree and the proportion of negative edges were lower for DRS than for HRS. Therefore, WB altered the endosphere and rhizosphere bacterial network properties. The changes in the bacterial network average degree and proportion of negative edges were most notable because they reflected the complexity of the bacterial community and the competitive interactions.

Among the leaf and branch samples, 15 and 6 bacterial genera were significantly negatively correlated with the phytoplasma abundance, respectively (Fig. 7). Some of the genera, such as *Novosphingobium*, *Pseudomonas*, and *Sphingomonas*, included beneficial bacterial species. In the root samples, six genera were significantly correlated with the phytoplasma abundance; all but one of the correlations were negative. These results suggested that beneficial bacteria, such as *Novosphingobium*, *Pseudomonas*, and *Sphingomonas* species, might be the key taxa involved in *Paulownia* tree defense responses to phytoplasma infections.

Discussion

In this study, an analysis of 16S rRNA gene sequencing data revealed the substantial abundance of phytoplasma (86.4%-98.8%) presented in symptomatic samples (SL and SB) (Fig. 4A, B). These abundances might be the highest recorded for phytoplasma-associated plant diseases (Ren et al. 2021). Astonishingly, phytoplasmas were also detected in healthy samples (HL and HB) without any WB symptom, albeit at very low levels, suggesting that WB symptom development is closely related to the abundance of phytoplasmas (Fig. 4 and Fig. S1). In fact, phytoplasma was the primary taxon in asymptomatic samples (SL and SB) (Fig. S3). Additionally, WB resulted in major changes to the endophyte community compositions in *Paulownia* trees. For example, Proteobacteria, which is a common phylum in plants (Beckers et al. 2017), was the dominant phylum in healthy samples, but it was replaced by Firmicutes as the main phylum in symptomatic samples (Fig. S2). *Escherichia* and *Pseudomonas* were the main genera in healthy samples, respectively, whereas phytoplasma was the predominant taxon in the corresponding symptomatic samples (Fig. S3). The SL *vs* HL and SB *vs* HB comparisons detected a number of differentially abundant genera (Fig. 5A, D). Expect for phytoplasma, all the bacterial abundances decreased in asymptomatic samples, implying that phytoplasmas can inhibit the growth of other endophytes, which may be related to resource competition and the secretion of antimicrobial compounds (Hassani et al. 2018). Unfortunately, characterizing the underlying mechanism is difficult because phytoplasma cannot be cultured under *in vitro* conditions (Eichmeier et al. 2019). Notably, some important beneficial bacteria (e.g., *Bacillus*, *Pseudomonas*, *Methylobacterium* and *Sphingomonas*

species) decreased in abundance in the SL and SB samples (Fig. 5A, D). Particularly, previous research has confirmed that *Pseudomonas* spp. strains, combined with *Glomus mosseae*, can inhibit phytoplasma infections and delay the expression of associated symptom in *Chrysanthemum carinatum* (D'Amelio et al. 2011), implying enriched *Pseudomonas* in plant might also played an important role in WB suppression. Our findings confirmed the point of Podolich (2015), who suggest that the endophyte-mediated disease resistance can develop, in case the plant contained a sufficient diversity of 'protective' endophytes; Alternatively, the plant can become susceptible to pathogens upon if it lack of strategic members from endophytic microbial cohorts. Considered together, these findings indicate that a threshold abundance of beneficial bacteria might be a critical factor for preventing WB in heathy plants that may contain phytoplasmas in their tissues (Fig. 4). In contrast, low endophyte diversity and a lack of beneficial bacteria might exacerbate WB severity in symptomatic tissues.

The WB symptoms were detected in only parts of the *Paulownia* trees, not the whole, even though the tested trees had been affected by WB for about 3 years (Fig. S1). In this study, phytoplasma was also detected in asymptomatic samples (ASB and ASL) from diseased trees, which was similar to the levels in the corresponding healthy samples (HB and HL), but much lower than the levels in the corresponding symptomatic samples (SB and SL) (Fig. 4A, B). These observations indicated WB symptoms would outbreak only when phytoplasma abundance reach certain level. Additionally, the α diversity and endophyte community structure of asymptomatic samples were more similar to those of healthy samples than to those of symptomatic samples (Fig. 2 and Fig. 3). We determined that a number of taxa were more abundant in asymptomatic samples than in the corresponding symptomatic samples, respectively (Fig. 5B, E), including some beneficial bacteria (e.g., *Bacillus*, *Pseudomonas*, *Methylobacterium*, and *Sphingomonas* species). These findings may help to explain the lack of WB symptoms in the asymptomatic parts of diseased trees. Though the endophyte community in the asymptomatic samples was very similar to that in the healthy samples, a number of genera were differentially in abundance. Among these taxa, only two were for known beneficial bacteria *Sphingomonas* and *Methylobacterium*, both of which were more abundant in asymptomatic samples than in healthy samples, indicative of the active recruitment of beneficial bacteria by *Paulownia* trees to restrict the infection by phytoplasmas. There is increasing evidence that plants can actively select endophytes under biotic and abiotic stress conditions, including pathogen infections (Berendsen et al. 2012; Carrion et al. 2019; Jones et al. 2019). For example, in response to a fungal pathogen, Chitinophagaceae and Flavobacteriaceae species are enriched within the sugar beet endosphere to suppress fungal root disease development (Carrion et al. 2019). Diseased pepper plants can also recruit beneficial bacteria, including *Pseudomonas*, *Streptomyces*, and *Bacillus* species, to defend against Fusarium wilt disease (Berendsen et al. 2018). Considering that the susceptibility of asymptomatic plant parts to phytoplasma infections may increase because they are close to the symptomatic tissue, the enrichment of beneficial taxa might be important for preventing WB in asymptomatic tissues.

In the current study, there were no morphological changes in the roots of *Paulownia* trees exhibiting WB symptoms; however, a significant phytoplasma abundance was detected in the roots, which was higher than the abundance in the roots of healthy trees (Fig. 4C), suggesting phytoplasmas may infect both the

aboveground and belowground parts of *Paulownia* trees, especially in the diseased plant. Furthermore, WB changed the endophyte composition at the genus level, but not at the phylum level (Fig. S2 and Fig. S3). The most abundant genus, *Allorhizobium*, was replaced by phytoplasma and the diversity of the endophyte community decreased in the roots of diseased trees (Fig. 2A and Fig. S3). Compared with the healthy samples, the abundances of several genera (except phytoplasma) decreased in the roots of diseased trees (Fig. 5G), including the beneficial genus *Streptomyces* (Viaene et al. 2016).

The infection of the plant endosphere by pathogens can alter the rhizosphere microbial community composition. For example, *Candidatus* Liberibacter asiaticus, which is related to phytoplasmas, can modulate the composition of rhizosphere microbial communities after it infects the aboveground parts of citrus trees (Trivedi et al. 2012). A similar phenomenon was observed in this study, in which the rhizobacterial community composition of diseased trees differed from that of healthy trees. There were significant differences in the abundances of a number of genera between the rhizosphere soil of diseased and healthy trees (Fig. 5H and Fig. S4). Moreover, *Brevibacillus*, which is a beneficial taxon with antagonistic effects on many pathogens (Joo et al. 2015), was more abundant in the rhizosphere soil of healthy trees than in the rhizosphere soil of diseased trees (Fig. 5H). Beneficial bacteria in rhizosphere soil may have important functions that control pathogens in aboveground plant parts. For example, wheat plants can recruit the beneficial microbe *Stenotrophomonas rhizophila* to suppress the infection of aboveground tissues by the soil-borne pathogen *Fusarium pseudograminearum* (Liu et al. 2021). These results suggested that *Paulownia* trees might select beneficial bacteria in the rhizosphere soil to defend against phytoplasma infections.

Microbial network properties, which reflect the interactions among co-existing organisms, can influence the response of communities to environmental stimuli, including pathogens (Jakuschkin et al. 2016; Wei et al. 2017; De et al. 2018). The positive edges between two species are suggestive of a mutualistic interaction, whereas negative edges are indicative of a competitive interaction (Jakuschkin et al. 2016). In this study, the results indicated that WB affected the endosphere and rhizosphere bacterial co-occurrence network properties (Fig. 6 and Table 1). The proportion of negative edges seemed to be related to the abundance of beneficial bacteria in tree compartments. Specifically, a high abundance of beneficial bacteria was associated with a high proportion of negative edges (Table 1, Fig. 6 and Fig. 5), likely because of the competition between the beneficial bacteria and the pathogenic phytoplasmas. Microbial networks with low positive correlations and high negative correlations among members are believed to be relatively stable (Coyte et al. 2015; Gao et al. 2021; Hernandez et al. 2021), with beneficial effects on the host (Coyte et al. 2015). This is consistent with the results of the analyses of *Paulownia* WB and the related bacterial community in this study. For example, the bacterial networks of asymptomatic samples (ASL or ASB) had a high proportion of negative edges, making them relatively stable and beneficial for *Paulownia* trees, even more than the bacterial networks of the corresponding healthy samples probably because of the selection of specific microbes by the trees (Table 1). The bacterial networks of the asymptomatic samples (SL or SB) had the lowest proportion of negative edges (Table 1), making them relatively unstable and ineffective for preventing WB.

Table 1
Bacterial co-occurrence networks along the different of compartments of Paulownia plant

Samples	Node	Total edge	Positive edge / %	Negative edge / %	Average degree	Average clustering coefficient	Average path distance
HL	158	252	62.7	37.3	3.190	0.518	9.016
ASL	94	138	53.6	46.4	2.936	0.434	3.848
SL	14	16	75.0	25.0	2.286	1.000	1.000
HB	66	90	75.6	24.4	2.727	0.483	3.676
ASB	68	97	62.9	37.1	2.853	0.504	4.728
SB	19	21	90.5	9.5	2.211	0.583	1.471
HR	126	282	77.7	22.3	4.476	0.607	7.980
DR	119	342	78.4	21.6	5.748	0.626	5.936
HRS	421	2118	59.4	40.6	10.062	0.509	5.211
DRS	411	1780	56.4	43.6	8.662	0.512	5.603

A correlation analysis revealed that the abundances of 27 bacterial genera were significantly correlated with phytoplasma abundances in the leaf, branch, and root samples (Fig. 7). All but one of these genera were negatively correlated with phytoplasmas, suggestive of a competitive interaction. Specifically, the *Novosphingobium*, *Pseudomonas* and *Sphingomonas* beneficial bacterial species might be important for limiting phytoplasma infections. *Enterococcus* was the only taxon that was positively correlated with phytoplasmas, implying its mutualistic relationship with phytoplasma. These findings suggest that beneficial bacteria (e.g., *Novosphingobium*, *Pseudomonas*, and *Sphingomonas* species) may be crucial for maintaining the health of *Paulownia* trees. Furthermore, they may be useful for developing effective treatments for *Paulownia* WB.

Conclusions

We demonstrated that *Paulownia* WB symptoms are closely related to the severity of the phytoplasma infection in leaves and branches. In SL and SB samples, the phytoplasma abundance was very high, which resulted in decreased endophytic diversity and richness and altered microbial community compositions. Additionally, beneficial bacteria (e.g., *Bacillus*, *Pseudomonas*, *Methylobacterium*, and *Sphingomonas* species) decreased in abundance, thereby enabling WB symptoms to worsen. Phytoplasmas were detected in healthy tissues, as well as asymptomatic leaves and branches from diseased trees at a very low level. Moreover, the endophyte community compositions of asymptomatic samples and the corresponding healthy samples were similar. However, beneficial bacteria, including *Sphingomonas* and *Methylobacterium* species, were more abundant in the asymptomatic samples than

in the healthy samples, probably because the trees infected with phytoplasmas were actively recruiting beneficial bacteria as part of their defense response. The phytoplasma abundance in the roots of diseased trees reached considerable level, which was higher than the phytoplasma abundance in the corresponding healthy samples, but there were no morphological differences. Furthermore, WB changed the compositions of the root endosphere and rhizosphere bacterial communities. The observed increase in the abundance of beneficial bacteria in the rhizosphere might be associated with the active recruitment by trees to minimize the detrimental effects of the phytoplasma infection in the endosphere. The results of the network analysis were in accordance with the observed changes in the endophytes. The bacterial networks of asymptomatic (ASL and ASB) samples were relatively stable and had the most beneficial effects on *Paulownia* trees, possibly because of the enrichment of beneficial bacteria. The abundances of several beneficial bacteria in the endosphere, including *Novosphingobium*, *Pseudomonas*, and *Sphingomonas* species, were negatively correlated with the phytoplasma abundance, implying these species may have important inhibitory effects on phytopathogens. Our findings have elucidated the phytoplasma-related mechanism underlying the development of *Paulownia* WB as well as the *Paulownia* resistance-related response to WB involving the active selection of beneficial bacteria in the endosphere and rhizosphere.

Declarations

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Competing interests The authors have no relevant financial or non-financial interests to disclose.

Author Contributions Li Xuanzhen and Fan Guoqiang planned and designed the research. Huang Jing and Pan Yanshuo performed experiments, conducted fieldwork and analysed data. Li Xuanzhen, Zhai Xiaoqiao and Zhao Zhenli wrote the manuscript. All authors read and approved the final manuscript.

Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonablerequest.

References

Alibrandi P, Cardinale M, Rahman MM, Strati F, Cina P, De Viana ML, Giamminola EM, Gallo G, Schnell S, De Filippo C, Ciaccio M, Puglia AM (2018) The seed endosphere of *Anadenanthera colubrina* is inhabited by a complex microbiota, including *Methylobacterium* spp. and *Staphylococcus* spp. with potential plant-growth promoting activities. Plant Soil 422:81-99 https://doi.org/10.1007/s11104-017-3182-4

Asaf S, Numan M, Khan AL, Al-Harrasi A (2020) *Sphingomonas*: From diversity and genomics to functional role in environmental remediation and plant growth. Crit Rev Biotechnol 40:138-152. https://doi.org/10.1080/07388551.2019.1709793

Beckers B, Op DBM, Weyens N, Boerjan W, Vangronsveld J (2017) Structural variability and niche differentiation in the rhizosphere and endosphere bacterial microbiome of field-grown poplar trees. Microbiome 5:1-17. https://doi.org/10.1186/s40168-017-0241-2

Berendsen RL, Pieterse CM, Bakker PA (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478-486. https://doi.org/10.1016/j.tplants.2012.04.001

Berendsen RL, Vismans G, Yu K, Song Y, de Jonge R, Burgman WP, Burmolle M, Herschend J, Bakker P, Pieterse C (2018) Disease-induced assemblage of a plant-beneficial bacterial consortium. ISME J 12:1496-1507. https://doi.org/10.1038/s41396-018-0093-1

Bertaccini A, Duduk B, Paltrinieri S, Contaldo N (2014) Phytoplasmas and phytoplasma diseases: A severe threat to agriculture. Am J Plant Sci 5:1763-1788. https://doi.org/10.4236/ajps.2014.512191

Biessy A, Filion M (2018) Phenazines in plant-beneficial *Pseudomonas* spp.: Biosynthesis, regulation, function and genomics. Environ Microbiol 20:3905-3917. https://doi.org/10.1111/1462-2920.14395

Brundu G, Richardson DM (2016) Planted forests and invasive alien trees in Europe: A Code for managing existing and future plantings to mitigate the risk of negative impacts from invasions. NeoBiota 30:5-47. https://doi.org/10.3897/neobiota.30.7015

Cao Y, Sun G, Zhai X, Xu P, Ma L, Deng M, Zhao Z, Yang H, Dong Y, Shang Z, Lv Y, Yan L, Liu H, Cao X, Li B, Wang Z, Zhao X, Yu H, Wang F, Ma W, Huang J, Fan G (2021) Genomic insights into the fast growth of *Paulownias* and the formation of *Paulownia* witches' broom. Mol Plant 14:1668-1682. https://doi.org/10.1016/j.molp.2021.06.021

Carrion VJ, Perez-Jaramillo J, Cordovez V, Tracanna V, de Hollander M, Ruiz-Buck D, Mendes LW, van Ijcken W, Gomez-Exposito R, Elsayed SS, Mohanraju P, Arifah A, van der Oost J, Paulson JN, Mendes R, van Wezel GP, Medema MH, Raaijmakers JM (2019) Pathogen-induced activation of disease-suppressive functions in the endophytic root microbiome. Science 366:606-612. https://doi.org/10.1126/science.aaw9285

Contaldo N, Satta E, Zambon Y, Paltrinieri S, Bertaccini A (2016) Development and evaluation of different complex media for phytoplasma isolation and growth. J Microbiol Methods 127:105-110. https://doi.org/10.1016/j.mimet.2016.05.031

Coyte KZ, Schluter J, Foster KR (2015) The ecology of the microbiome: Networks, competition, and stability. Science 350:663-666. https://doi.org/10.1126/science.aad2602

D'Amelio R, Berta G, Gamalero E, Massa N, Avidano L, Cantamessa S, D'Agostino G, Bosco D, Marzachi C (2011) Increased plant tolerance against chrysanthemum yellows phytoplasma (*'Candidatus* Phytoplasma asteris') following double inoculation with *Glomus mosseae* BEG12 and *Pseudomonas putida* S1Pf1Rif. Plant Pathol 60:1014-1022. https://doi.org/10.1111/j.1365-3059.2011.02479.x

De V, Griffiths RI, Bailey M, Craig H, Girlanda M, Gweon HS, Hallin S, Kaisermann A, Keith AM, Kretzschmar M, Lemanceau P, Lumini E, Mason KE, Oliver A, Ostle N, Prosser JI, Thion C, Thomson B, Bardgett RD (2018) Soil bacterial networks are less stable under drought than fungal networks. Nat Commun 9:3033. https://doi.org/10.1038/s41467-018-05516-7

Duan J, Li X, Gao J, Wang D, Yan Y, Xue Q (2013) Isolation and identification of endophytic bacteria from root tissues of *Salvia miltiorrhiza* Bge. and determination of their bioactivities. Ann Microbiol 3:1501-1512. https://doi.org/10.1007/s13213-013-0614-0

Eichmeier A, Kiss T, Necas T, Penazova E, Tekielska D, Bohunicka M, Valentova L, Cmejla R, Morais D, Baldrian P (2019) High-Throughput sequencing analysis of the bacterial community in stone fruit phloem tissues infected by "*Candidatus* phytoplasma prunorum". Microb Ecol 77:664-675. https://doi.org/10.1007/s00248-018-1250-9

Gao M, Xiong C, Gao C, Tsui C, Wang MM, Zhou X, Zhang AM, Cai L (2021) Disease-induced changes in plant microbiome assembly and functional adaptation. Microbiome 9:187. https://doi.org/10.1186/s40168-021-01138-2

Hahm MS, Sumayo M, Hwang YJ, Jeon SA, Park SJ, Lee JY, Ahn JH, Kim BS, Ryu CM, Ghim SY (2012) Biological control and plant growth promoting capacity of rhizobacteria on pepper under greenhouse and field conditions. J Microbiol 50:380-385. https://doi.org/10.1007/s12275-012-1477-y

Han SH, Lee SJ, Moon JH, Park KH, Yang KY, Cho BH, Kim KY, Kim YW, Lee MC, Anderson AJ, Kim YC (2006) GacS-dependent production of 2R, 3R-butanediol by *Pseudomonas chlororaphis* O6 is a major determinant for eliciting systemic resistance against *Erwinia carotovora* but not against *Pseudomonas syringae* pv. Tabaci in tobacco. Mol Plant Microbe Interact 19:924-930. https://doi.org/10.1094/MPMI-19-0924

Hassani MA, Duran P, Hacquard S (2018) Microbial interactions within the plant holobiont. Microbiome 6:58. https://doi.org/10.1186/s40168-018-0445-0

He T, Vaidya BN, Perry ZD, Parajuli P, Joshee N (2016) *Paulownia* as a medicinal tree: Traditional uses and current advances. European J Med Plants 14:1-15. https://doi.org/10.9734/EJMP/2016/25170

Hernandez DJ, David AS, Menges ES, Searcy CA, Afkhami ME (2021) Environmental stress destabilizes microbial networks. ISME J 15:1722-1734. https://doi.org/10.1038/s41396-020-00882-x

Hogenhout SA, Oshima K, Ammar E, Kakizawa S, Kingdom HN, Namba S (2008) Phytoplasmas: Bacteria that manipulate plants and insects. Mol Plant Pathol 9:403-423. https://doi.org/10.1111/J.1364-3703.2008.00472.X

Huang W, MacLean AM, Sugio A, Maqbool A, Busscher M, Cho ST, Kamoun S, Kuo CH, Immink R, Hogenhout SA (2021) Parasitic modulation of host development by ubiquitin-independent protein

degradation. CELL 184:5201-5214. https://doi.org/10.1016/j.cell.2021.08.029

Innerebner G, Knief C, Vorholt JA (2011) Protection of *Arabidopsis thaliana* against leaf-pathogenic *Pseudomonas syringae* by *Sphingomonas* Strains in a controlled model system. Appl Environ Microbiol 77:3202-3210. https://doi.org/10.1128/AEM.00133-11

Jakuschkin B, Fievet V, Schwaller L, Fort T, Robin C, Vacher C (2016) Deciphering the pathobiome: Intra- and interkingdom interactions involving the pathogen Erysiphe alphitoides. Microb Ecol 72:870-880. https://doi.org/10.1007/s00248-016-0777-x

Jones P, Garcia BJ, Furches A, Tuskan GA, Jacobson D (2019) Plant host-associated mechanisms for microbial selection. Front Plant Sci 10:862. https://doi.org/10.3389/fpls.2019.00862

Joo HJ, Kim H, Kim L, Lee S, Ryu J, Lee T (2015) A *Brevibacillus* sp antagonistic to mycotoxigenic *Fusarium* spp. Biol Control 87:64-70. https://doi.org/10.1016/j.biocontrol.2015.04.010

Kloepper JW, Ryu CM, Zhang S (2004) Induced systemic resistance and promotion of plant growth by *bacillus* spp. Phytopathology 94(11):1259-1266. https://doi.org/10.1094/PHYTO.2004.94.11.1259

Liu H, Li J, Carvalhais LC, Percy CD, Prakash VJ, Schenk PM, Singh BK (2021) Evidence for the plant recruitment of beneficial microbes to suppress soil-borne pathogens. New Phytol 229:2873-2885. https://doi.org/10.1111/nph.17057

Lopes R, Tsui S, Goncalves P, de Queiroz MV (2018) A look into a multifunctional toolbox: Endophytic *Bacillus* species provide broad and underexploited benefits for plants. World J Microbiol Biotechnol 34:94. https://doi.org/10.1007/s11274-018-2479-7

Ma Z, Hua G, Ongena M, Hofte M (2016) Role of phenazines and cyclic lipopeptides produced by *Pseudomonas* sp. CMR12a in induced systemic resistance on rice and bean. Environ Microbiol Rep 8:896-904. https://doi.org/10.1111/1758-2229.12454

Maejima K, Oshima K, Namba S (2014) Exploring the phytoplasmas, plant pathogenic bacteria. J Gen Plant Pathol 80:210-221. https://doi.org/10.1007/s10327-014-0512-8

Musetti R, Buxa SV, De Marco F, Loschi A, Polizzotto R, Kogel KH, van Bel AJ (2013) Phytoplasma-triggered Ca^{2+} influx is involved in sieve-tube blockage. Mol Plant Microbe Interact 26:379-386. https://doi.org/10.1094/MPMI-08-12-0207-R

Papik J, Folkmanova M, Polivkova-Majorova M, Suman J, Uhlik O (2020) The invisible life inside plants: Deciphering the riddles of endophytic bacterial diversity. Biotechnol Adv 44:1-21. https://doi.org/10.1016/j.biotechadv.2020.107614

Pérez A (2005) *Paulownia*, a fast-growing plant, as a raw material for paper manufacturing. Afinidad 65:100-105.

Podolich O, Ardanov P, Zaets I. Pirttilä AM, Kozyrovska N (2015) Reviving of the endophytic bacterial community as a putative mechanism of plant resistance. Plant Soil 388:367–377 DOI https://doi.org/10.1007/s11104-014-2235-1

Ren F, Dong W, Shi S, Dou G, Yan DH (2021) Chinese chestnut yellow crinkle disease influence microbiota composition of chestnut trees. Microb Pathog 152:1-8. https://doi.org/10.1016/j.micpath.2020.104606

Rodriguez-Seoane P, Diaz-Reinoso B, Moure A, Dominguez H (2020) Potential of *Paulownia* sp. for biorefinery. Ind Crops Prod 155:1-15. https://doi.org/10.1016/j.indcrop.2020.112739

Santoyo G, Del Carmen Orozco-Mosqueda M, Govindappa M (2012) Mechanisms of biocontrol and plant growth-promoting activity in soil bacterial species of *Bacillus* and *Pseudomonas*: A review. Biocontrol Sci Techn 22:855-872. https://doi.org/10.1080/09583157.2012.694413

Shalev O, Karasov TL, Lundberg DS, Ashkenazy H, Pramoj NAP, Weigel D (2022) Commensal *Pseudomonas* strains facilitate protective response against pathogens in the host plant. Nat Ecol Evol 6(4):383-396. https://doi.org/10.1038/s41559-022-01673-7

Trivedi P, He Z, Van Nostrand JD, Albrigo G, Zhou J, Wang N (2012) Huanglongbing alters the structure and functional diversity of microbial communities associated with citrus rhizosphere. ISME J 6:363-383. https://doi.org/10.1038/ismej.2011.100

Viaene T, Langendries S, Beirinckx S, Maes M, Goormachtig S (2016) *Streptomyces* as a plant's best friend? FEMS Microbiol Ecol 92:1-10. https://doi.org/10.1093/femsec/fiw119

Wei Z, Hu J, Gu Y, Yin S, Xu Y, Jousset A, Shen Q, Friman VP (2017) *Ralstonia solanacearum* pathogen disrupts bacterial rhizosphere microbiome during an invasion. Soil Biol Biochem 118:8-17. https://doi.org/10.1016/j.soilbio.2017.11.012

Xiong C, Zhu YG, Wang JT, Singh B, Han LL, Shen JP, Li PP, Wang GB, Wu CF, Ge AH, Zhang LM, He JZ (2021) Host selection shapes crop microbiome assembly and network complexity. New Phytol 229:1091-1104. https://doi.org/10.1111/nph.16890

Yue HN, Wu YF, Shi YZ, Wu KK, Li YR (2008) First report of *Paulownia* Witches'Broom phytoplasma in china. Plant Dis 92:1134. https://doi.org/10.1094/PDIS-92-7-1134A

Zhang C, Wang MY, Khan N, Tan LL, Yang S (2021) Potentials, utilization, and bioengineering of plant growth-promoting *Methylobacterium* for sustainable agriculture. Sustainability 13:1-12. https://doi.org/10.3390/su13073941

Figures

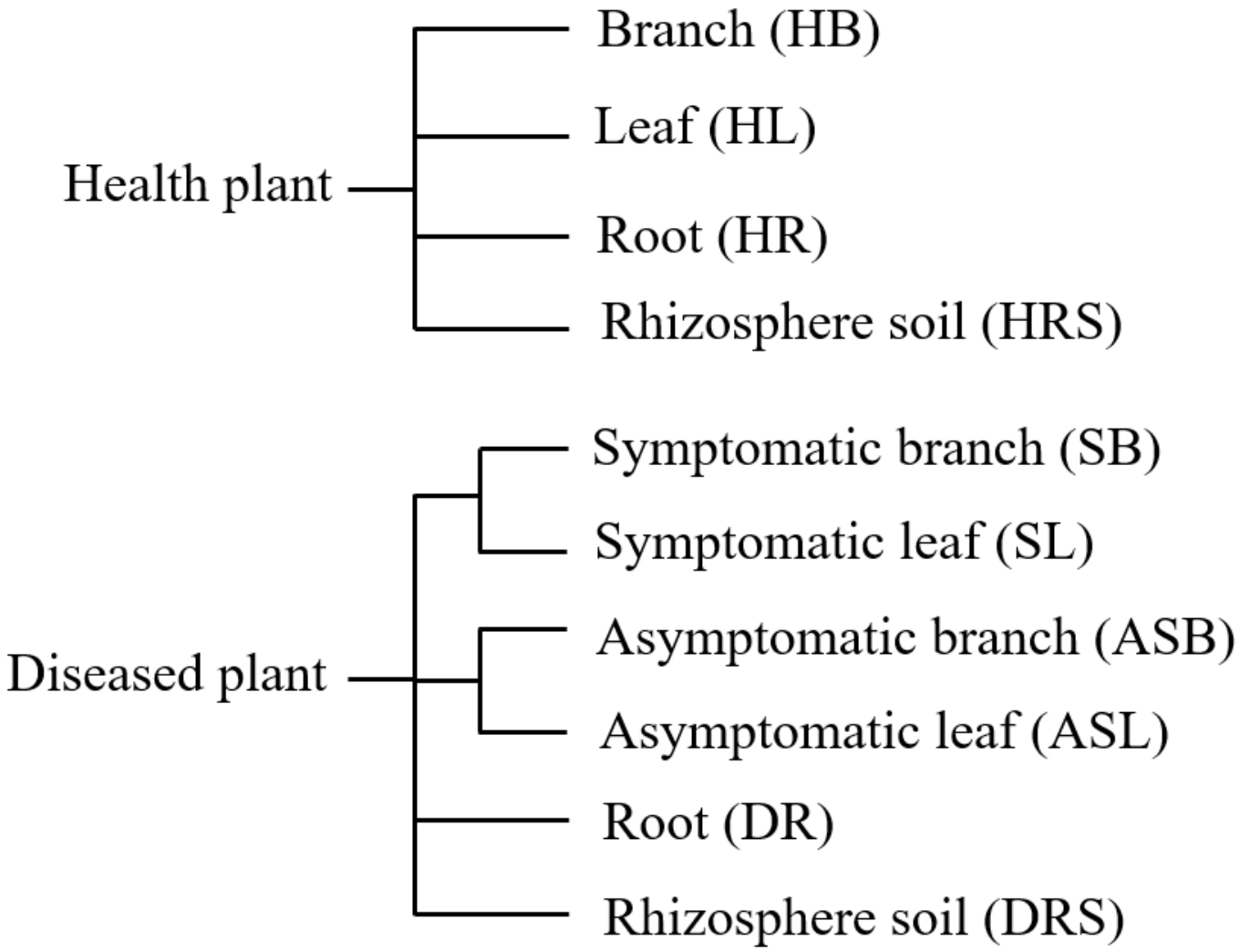

Figure 1

The source of tested samples and their abbreviations

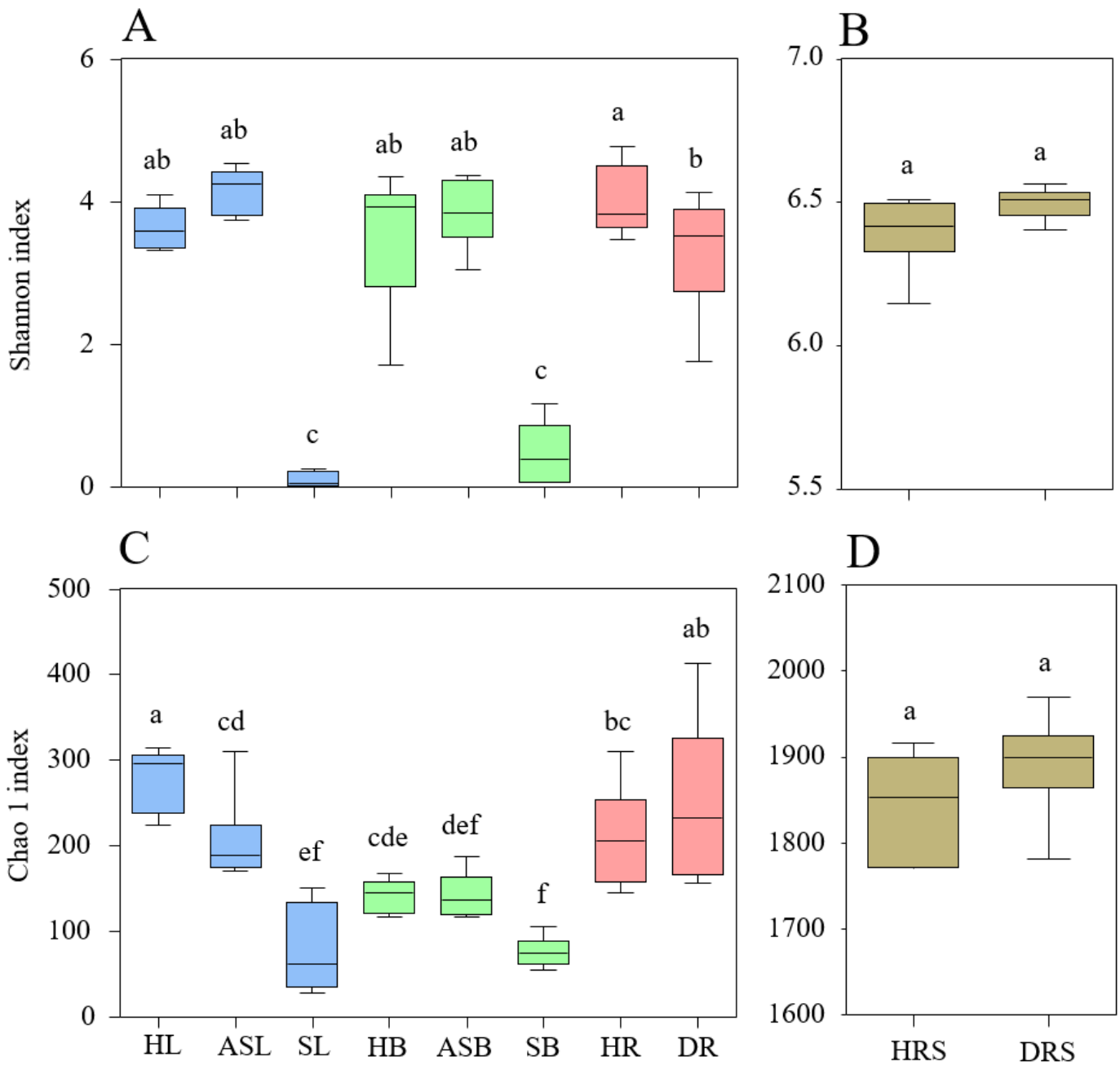

Figure 2

Effects of phytoplasma infections on the α diversity of endosphere and rhizosphere bacterial communities. Different lowercase letters indicate statistically significant differences between treatments as determined by one-way ANOVA with post hoc Tukey HSD test ($P < 0.05$). (A and B presented the Shannon index in endosphere and rhizosphere bacterial community, respectively; C and D presented the Chao 1 index in endosphere and rhizosphere bacterial community, respectively)

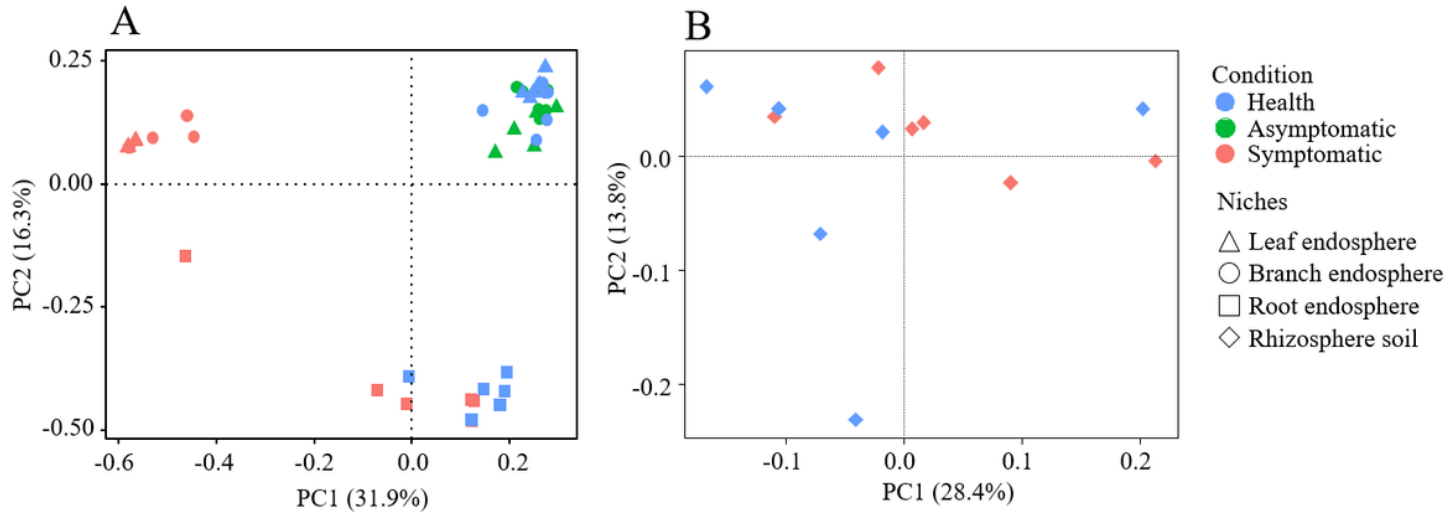

Figure 3

PCoA analysis of endosphere and rhizosphere bacterial communities (A and B presented PCoA analysis of endosphere and rhizosphere bacterial community, respectively)

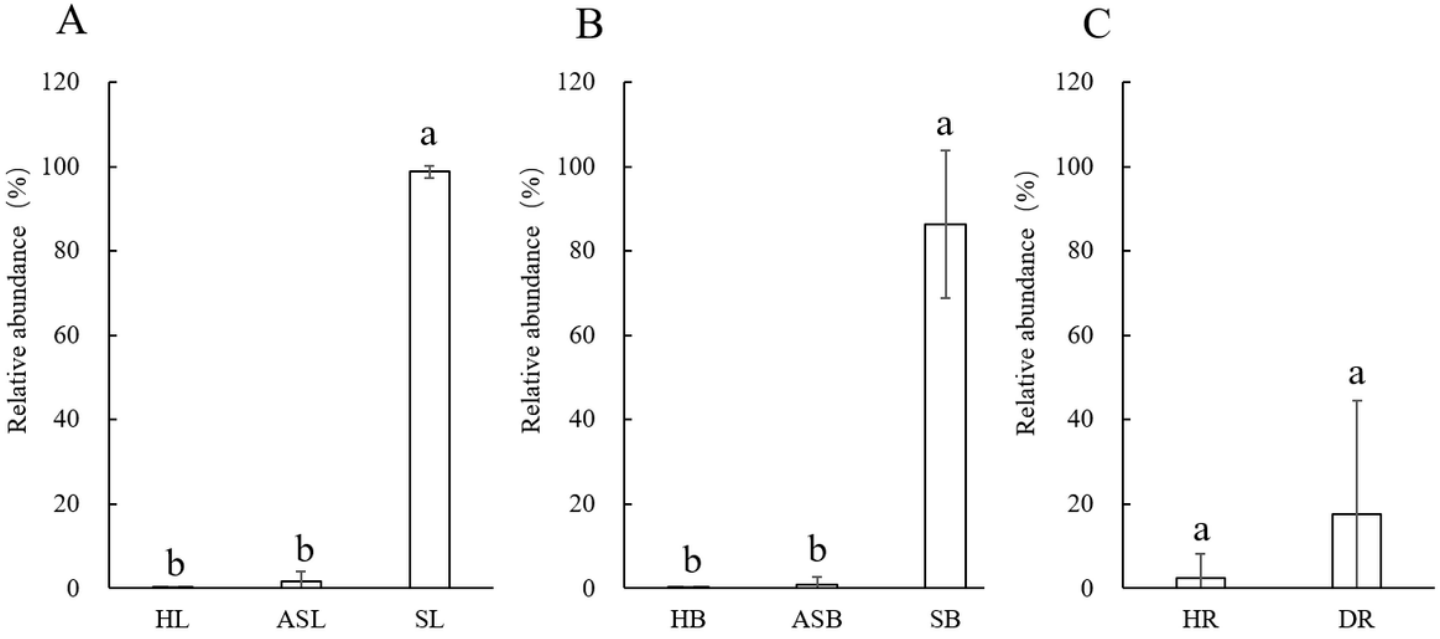

Figure 4

Abundunce of Phytoplasma in the different of compartments of *Paulownia* plant (A: Leaf compartments; B: Branch compartments; C: Root compartments)

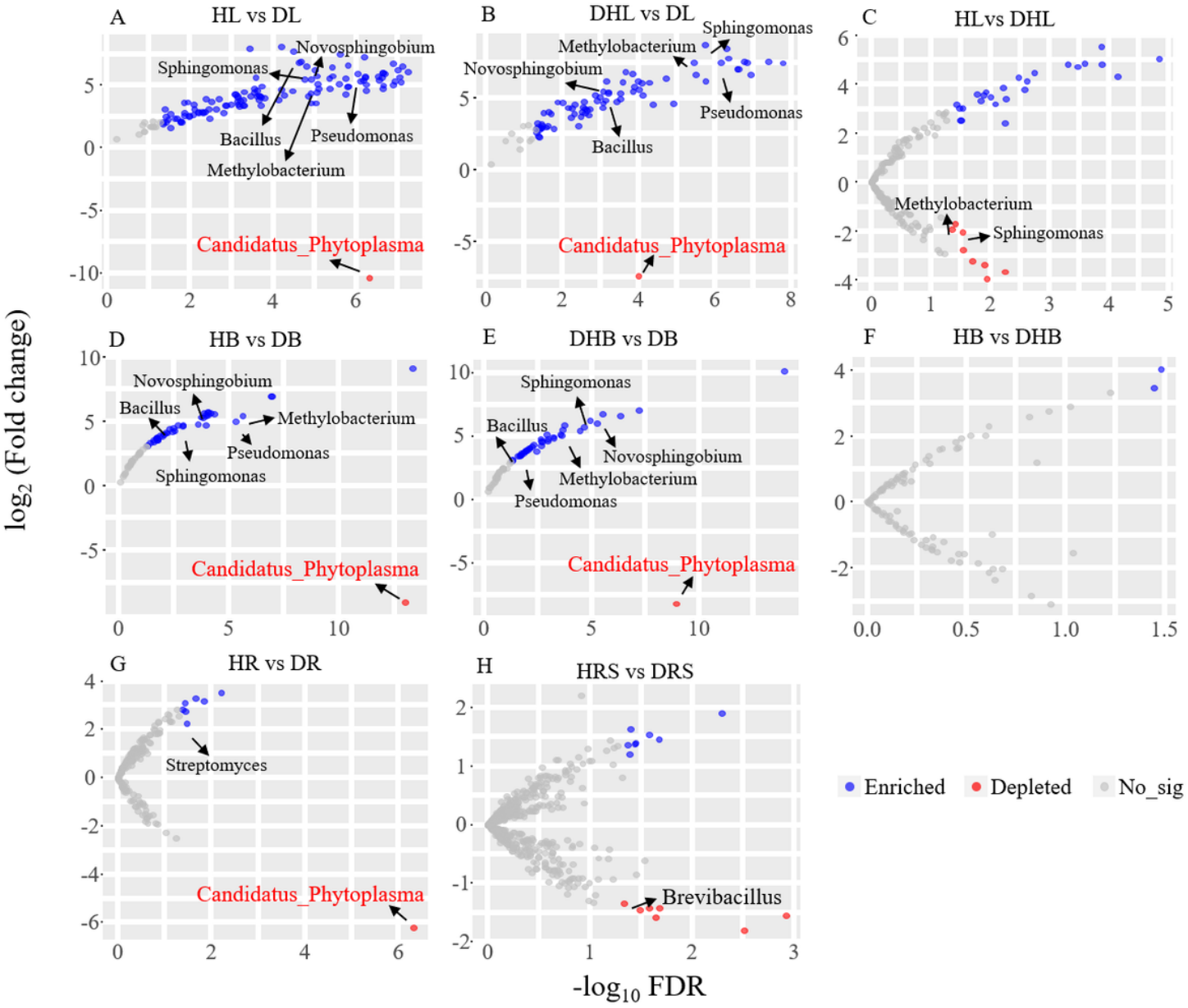

Figure 5

The volcano plot illustrating the enrichment and depletion patterns of the crop-associated bacterial microbiomes beteween different compartment

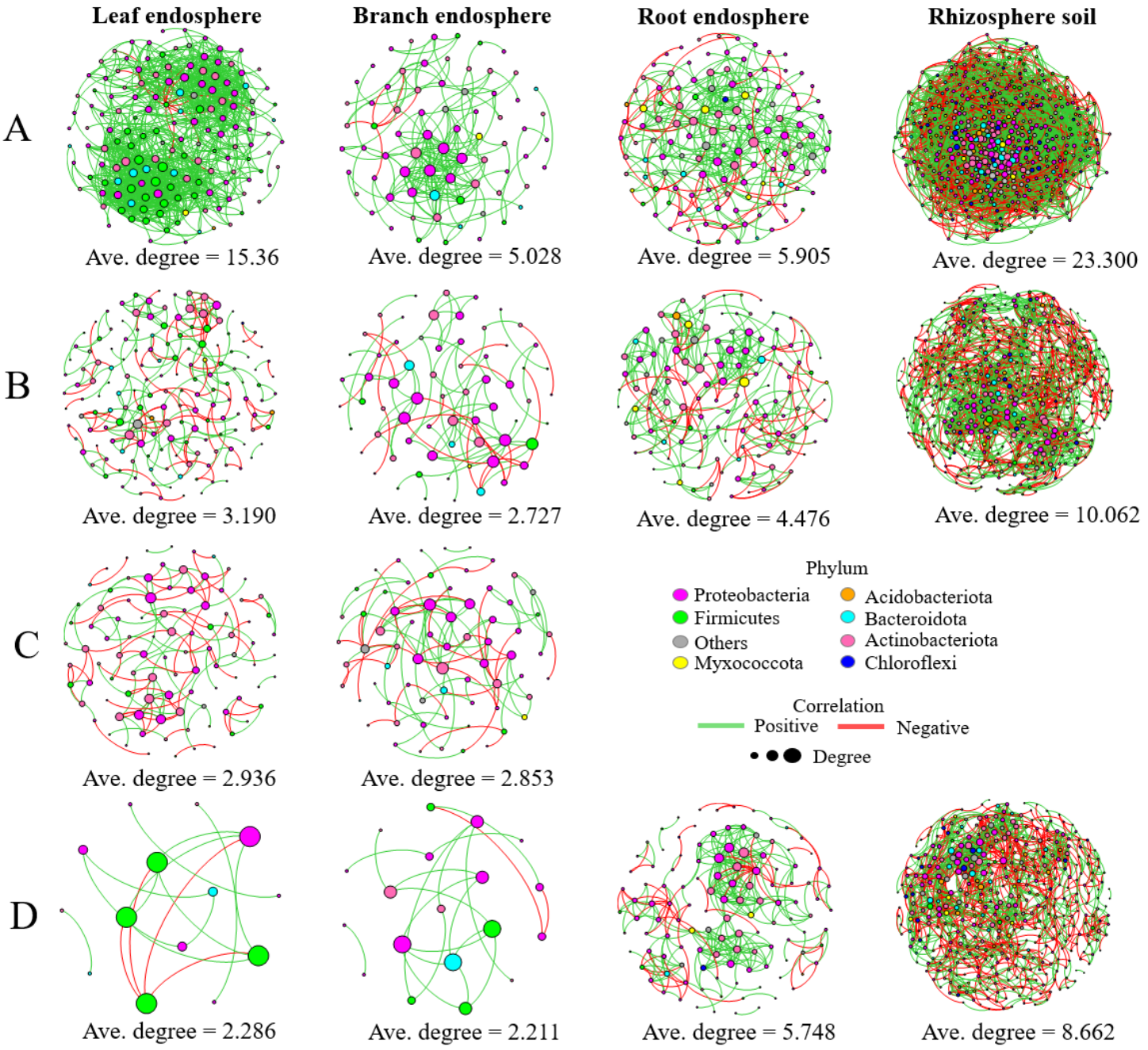

Figure 6

Bacterial co-occurrence networks along the different of compartments of Paulownia plant (A: Total bacterial network; B: Bacterial network in leaf, branch, root and rhizosphere soil compartments in healthy plant; C: Bacterial network in asymptomatic leaf and branch compartments in diseased plant; D: Bacterial network in symptomatic leaf and branch, as well as root and rhizosphere soil compartments in diseased plant)

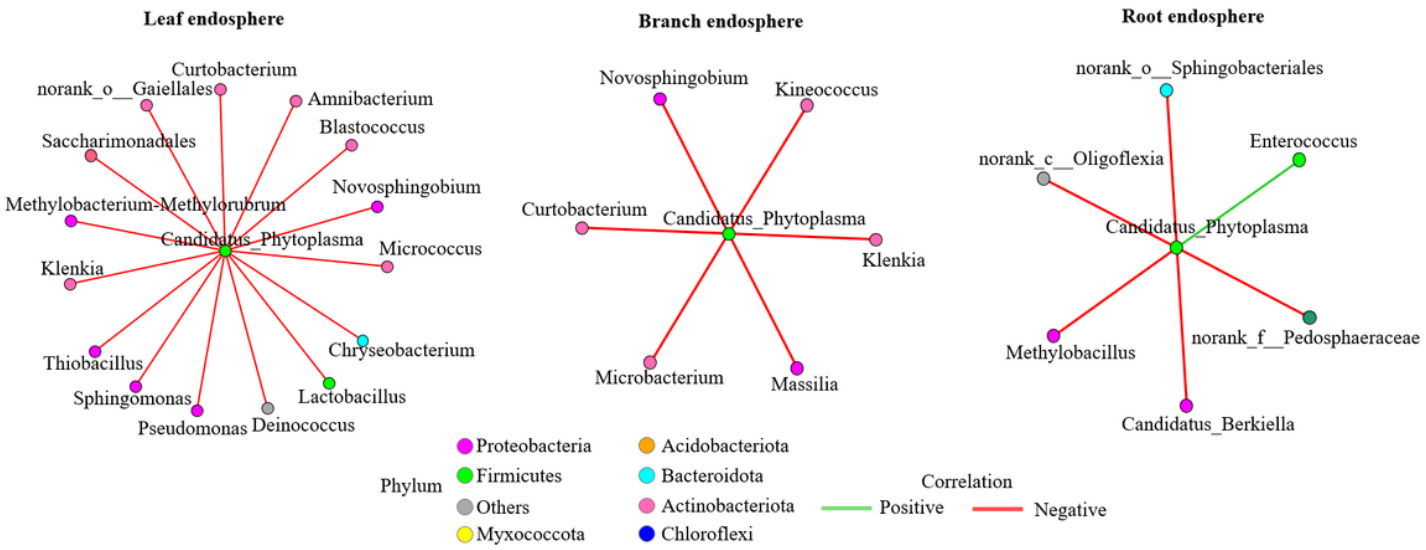

Figure 7

Endophytes co-occurrence networks along the different of compartments of *Paulownia* plant

Supplementary Files

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