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Community structure of soil microorganisms and endophytes of honeysuckle at different ecological niche specificities

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Abstract

Background The plant microbiome is one of the key determinants of healthy plant growth. However, the complexity of microbial diversity in plant microenvironments in different regions, especially the relationship between subsurface and aboveground microorganisms, is not fully understood. The present study investigated the diversity of soil microorganisms in different regions and the diversity of microorganisms within different ecological niches, and compared soil microorganisms and endophytic microorganisms.

Methods 16 S and ITS sequencing was used to sequence the soil and endophytes microbiome of honeysuckle. Alpha diversity analysis and principal component analysis (PCoA) were used to study the soil and endophyte microbial communities, and the function of endophyte bacteria and fungi was predicted based on the PICRUST2 process and FUNGuild.

Results In total, there were 382 common bacterial genera and 139 common fungal genera in the soil of different producing areas of honeysuckle. There were 398 common bacterial genera and 157 common fungal genera in rhizosphere soil. More beneficial bacteria were enriched in rhizosphere soil. Endophytic bacteria were classified into 34 phyla and 770 genera. Endophytic fungi were classified into 11 phyla and 581 genera, among which there were significant differences in the dominant genera of roots, stems, leaves, and flowers, as well as in community diversity and richness. Endophytic fungal functions were mainly dominated by genes related to saprophytes, functional genes that could fight microorganisms were also found in KEGG secondary functional genes.

Conclusion More beneficial bacteria were enriched in rhizosphere soil of honeysuckle, and the microbial network of the rhizosphere is more complex than that of the soil. Among the tissues of honeysuckle, the flowers have the richest diversity of endophytes. The endogenous dominant core bacteria in each part of honeysuckle plant have a high degree of overlap with the dominant bacteria in soil. Functional prediction suggested that some dominant core bacteria have antibacterial effects, providing a reference for further exploring the strains with antibacterial function of honeysuckle. Understanding the interaction between honeysuckle and microorganisms lays a foundation for the

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study of growth promotion, quality improvement, and disease and pests control of honeysuckle from the perspective of microorganisms.

Keywords Honeysuckle, High-throughput sequencing, Rhizosphere soil, Endophytic bacteria, Function prediction

Background

Inter-organismal associations between eukaryotic and prokaryotic organisms are one of the most studied research areas in microbiology in recent years. Similar to the microbiome in the human gut, a large number and variety of microorganisms, including bacteria, archaea, fungi, and viruses, inhabit the exterior and interior of different organs of plants, and these microorganisms, their genetic information and metabolites, etc., collectively constitute the plant microbiome [1]. The plant microbiome plays an important role in plant growth and health, and is regarded as the second or extended genome of plants [2]. In recent years, with increasing research, it has been found that almost all tissues of plants host microbial communities, including the soil-root interface, the interior of plant tissues, and the air-plant interface (the interfollicular environment), which influence plant growth [3], enhance host plant tolerance to abiotic and biotic stresses [4], and promote the accumulation of secondary metabolites in the host plant [5] by promoting host plant growth and health. In plant-microbe research, most attention has been focused on the study of rhizosphere microorganisms and endophytes, e.g., Xu et al. [6] found that the bacterial diversity and community composition of the soil microbial community in the rhizosphere soil of peach tree varied among different regions in a study on the structure and diversity of the soil microorganisms in different regions; Gottel et al. [7] used pyrophosphate sequencing of the 16 S ribosomal RNA gene to compare the bacterial community of mature poplar (*Populus deltoides*) and revealed that the root-endophytic bacterial communities differed significantly compared to the rhizosphere soil. This provides guidance for studying plant-microbe interactions and differences in soil microbes and plant endophytes in different regions.

Lonicera japonica Thunb. is a semi-evergreen vine of the genus Lonicera in the family Loniceraceae with dried flower buds or flowers with first bloom as honeysuckle and dried stem branches as Lonicera japonica caulis [8]. Honeysuckle and Lonicera japonica caulis are two commonly used Chinese medicinal herbs. Honeysuckle has the effect of clearing away heat and detoxifying and evacuating wind-heat [9]. It is a commonly used drug for clearing away heat and detoxifying and anti-virus. It has many clinical effects and is mostly used to treat febrile fever, wind-heat cold, pneumonia, sore throat and so on. And domestic and international studies have confirmed that they both contain a variety of natural active ingredients, such as flavonoids, organic acids, volatile oils, and cycloartenoid terpenes [10], which have antimicrobial and anti-inflammatory [11], antioxidant [12], hepatoprotective and choleretic [13], and hypoglycemic [14] effects, among which, their antibacterial effects have been focused on, and in vitro studies have demonstrated that honeysuckle extracts has some antibacterial effects on a variety of pathogenic bacteria, such as *Escherichia coli*, *Staphylococcus aureus*, Group B *streptococcus*, *Shigella dysenteriae*, *Proteus mirabilis*, *Salmonella enterica*, *Penicillium* spp., *Aspergillus flavus*, *Aspergillus niger*, and so on [15–17]. This broad-spectrum antimicrobial effect possessed by honeysuckle is mainly achieved by inhibiting pro-inflammatory factors as well as inhibiting or killing pathogenic bacteria to achieve anti-inflammatory and antimicrobial effects, in which phenolic acid has a better antimicrobial effect than other phenols [18, 19]. Li et al. [20] and others found that the extracts of different organs of *Lonicera japonica* Thunb. had antibacterial effects, among which the extracts of leaves, flowers and buds had stronger antibacterial ability, and the extracts of vine had weaker antibacterial ability. Therefore, honeysuckle is mainly used in clinical applications nowadays. Honeysuckle is not only used in the field of clinical medicine, but also in food, daily necessities, cosmetics industry and aquaculture industry. Wang et al. [21] used the active ingredients of honeysuckle to have certain antioxidant, anti-aging, antibacterial and anti-inflammatory effects, and added its extract into cosmetics to achieve the effects of clearing heat and eliminating acne, promoting cell metabolism, keeping the skin vitality, delaying skin aging as well as making the skin smooth and white. In addition, Zhi et al. [22] found that honeysuckle extract can improve the gastrointestinal health of piglets by promoting the growth of intestinal villi and regulating the structure of intestinal flora. It can enhance the immune function of piglets by increasing the level of immunoglobulin, regulating the immune balance and enhancing the immune response of the body. Therefore, honeysuckle extract can be used as a feed additive. The wide application of honeysuckle makes it widely planted in China. The traditional genuine producing areas of honeysuckle are Shandong and Henan, and Hebei Julu is the induction area. These three producing areas are the main producing areas of honeysuckle [23]. However, there are still some problems in the cultivation of honeysuckle, such as root rot and powdery mildew, and with the increase of planting years, the diseases are increasing year by year, mainly endangering leaves and roots [24, 25]. These pathogens

usually propagate and spread in the soil, so it is necessary to study the soil microorganisms of honeysuckle.

There are fewer studies on the diversity of microbial communities in plants with bacteriostatic components and the differences between them and those in soil. Li et al. [26] studied the culturable endophytic bacterial species in different parts of dandelion and found that there were differences in the number of endophytic bacteria in dandelion roots, stems, and leaves, and the differences in the number of bacteria were significantly and positively correlated with the content of chlorogenic acid ($P<0.05$). Lai [27] in her study on endophytes of *Lonicera confusa* DC. found that the difference in the presence of its endophytes in different tissues was more pronounced, in which the flowers had the least number of endophytic fungi and endophytic bacteria compared to other tissues, which may be related to the antimicrobial activity of its different parts. Similar to dandelion and Lonicera japonica, honeysuckle, as a herb with good antimicrobial activity, deserves to be studied for its microbial composition and differences in different parts.

Therefore, this study will start from the plant-microbe symbiosis, analyze the structural characteristics of soil and endophyte communities of honeysuckle from different main production areas based on high-throughput sequencing, clarify the relationship between rhizosphere and soil microorganisms and endophytes of honeysuckle, and search for the differences of endophytes in different ecological niches, and study the relationship between the differences of endophytes in different ecological niches of honeysuckle and its bacteriostatic activity to lay the basis for further excavation of honeysuckle with antibacterial microorganisms with bacteriostatic function in honeysuckle, laying the foundation for further excavation.

Methods

Sample handling

For soil (S) samples, the soil near the 7 plants of honeysuckle (depth of 0–20 cm, diameter of 10 cm) was mixed evenly according to the origin, randomly sampled three times, and divided into sterile 10-mL centrifuge tubes. For rhizosphere soil (R) samples, the 7 plants roots were dug out of the soil, and most of the soil was removed by shaking the roots until soil no longer fell off. The roots were placed into a centrifuge tube containing sterile water, and the tube was swirled for 1 min. Plant tissue and impurities were removed with a sterile non-woven cloth, and the samples were centrifuged at 8000 rpm for 10 min at 4 ℃. The supernatant was then removed. Each rhizosphere soil sample was mixed evenly according to the origin, randomly sampled three times, placed into sterile 10-mL centrifuge tubes, and stored at -80 ℃.

Tissue samples from three honeysuckle plants were collected and placed on a sterile super-clean workbench for surface sterilization treatment. The samples were washed with sterile water for 30 s, soaked in 75% ethanol for 2 min, soaked in 2.5% NaClO for 5 min, soaked in 75% sterile ethanol for 30 s, and washed 3–5 times with sterile water. The sterilized samples were then placed in a 15 mL sterile centrifuge tube and stored at -80 ℃ for later use.

Bacterial DNA extraction and PCR amplification

Approximately 1 g of soil sample was used for bacterial genomic DNA extraction. DNA was extracted using the Universal Genomic DNA Extraction Kit following the protocol provided by the manufacturer (Solarbio, China). PCR amplification was performed using the following primers: 338 F (5' - ACTCCTACGGGAGGCAGCAG −3 ') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The PCR system (20 μL) contained 10 × buffer (2 μL), 2.5 mM dNTPs (2 μL), 5 μM forward primer (0.8 μL), 5 μM reverse primer (0.8 μL), Taq polymerase (0.2 μL), bovine serum albumin (BSA; 0.2 μL), template DNA (10 ng), and ddH2O (added to a final volume of 20 μL). The PCR thermocycler conditions were as follows: 95 ℃ for 3 min; 35 cycles of 95 ℃ 30 s, 55 ℃ 30 s, and 72 ℃ 45 s; 72 ℃ for 10 min; and hold at 10 ℃ [28]. Each sample was set up with three replicates, and the PCR products of the same sample were mixed (3 μL) and detected by 2% agarose gel electrophoresis. The PCR products were recovered using the AxyPrepDNA gel recovery kit (AXYGEN), and fluorescence quantification was performed according to the preliminary quantitative results of electrophoresis.

Fungal DNA extraction and PCR amplification

Approximately 1 g of soil sample was utilized for fungal genomic DNA extraction. DNA was extracted using the Universal Genomic DNA Extraction Kit following the protocol provided by the manufacturer (Solarbio, China). And the extracted genomic DNA was detected by 1% agarose gel electrophoresis. PCR amplification was performed using the following primers: ITS1F (5'-CTTGGT CATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCG TTCTTCATCGATGC-3'). The PCR system (20 μL) contained 10 × buffer (2 μL), 2.5 mM dNTPs (2 μL), 5 μM forward primer (0.8 μL), 5 μM reverse primer (0.8 μL), Taq polymerase (0.2 μL), BSA (0.2 μL), template DNA (10 ng), and ddH2O (added to a final volume of 20 μL). The PCR thermocycler conditions were as follows: 95 ℃ for 3 min; 35 cycles of 95 ℃ 30 s, 55 ℃ 30 s, and 72 ℃ 45 s; 72 ℃ for 10 min; and hold at 10 ℃ [29]. Each sample was set up with three replicates, and the PCR products of the same sample were mixed (3 μL) and detected by 2% agarose gel electrophoresis. The PCR products were recovered using the AxyPrepDNA gel recovery kit (AXYGEN), and fluorescence quantification was performed according to the preliminary quantitative results of electrophoresis.

Illumina library construction and sequencing

The sequencing library was constructed using the TruSeqTM DNA Sample Prep Kit and sequenced by Illumina. The soil bacterial 16 S rRNA V3-V4 region, and soil fungal ITS1 region were amplified with primers 338 F/806R, and ITS1F/ITS2R. The endogenic bacterial 16 S rRNA V5-V7region, and soil fungal ITS1 region were amplified with primers 799 F/1193R, and ITS1F/ITS2R. The purified amplicons were then run on the Illumina Miseq platform (Shanghai Majorbio Bio-pharm Technology Co., Ltd.). The paired-end (PE) reads obtained by MiSeq sequencing were bidirectionally spliced, and the sequence quality was controlled and filtered. Based on 97% similarity, the obtained high-quality sequences were combined and filtered into different operating units (OTUs). The Bayes algorithm in the Ribosomal Database Project (RDP) classifier was used to conduct bioinformatics statistical analysis of OTUs at 97% similar levels, and the composition of each sample community was analyzed at each taxonomic level [30]. The endophytic bacterial community was predicted based on PICRUST2 process, and the fungal function of endophytic fungal community was predicted by FUNGuild.

Statistical analysis

The software Mothur was used to calculate the alpha diversity metric, including the Shannon diversity index, Simpson index, Ace index and Chao1 richness estimator. The beta diversity was analyzed through the principal-coordinate analysis (PCoA) based on Bray–Curtis distance metrics using the *microeco* R package. R (Version 4.2.2) VennDiagram package draw the Venn diagram, Gephi (Version 0.9.2) map fungi and bacteria network. Calculate topological properties with the R igraph package and perform Zi and Pi calculations and plotting. All statistical analyses were calculated using SPSS v25.0 software with a significance level threshold of $P<0.05$ [31].

Results

Composition of soil core microbiome of honeysuckle

The core microbiome plays a crucial role in the process of crop growth. Because the common microbiome of soil and rhizosphere soil in the three producing areas was the microbiome that existed regardless of region and plant, it was defined as the core microbiome of honeysuckle soil. The Venn diagrams shown in Fig. 1A-C indicated that there were 398 genera of bacteria in rhizosphere soil, 382 genera of bacteria in soil, and 333 genera of core bacteria in rhizosphere soil and soil, which belong to 23 bacterial phyla. The core bacteria mainly included *Proteobacteria*, *Actinobacteriota*, *Firmicutes*, *Bacteroidota*, *Chloroflexi*, *Myxococcota*, and *Acidobacteriota*. In addition, according to the dominant bacteria abundance heatmap, 22 of the 25 dominant bacteria genera were core bacteria genera (abundance > 2%) (Fig. 1D). The relative content of *Bacillus* in soil (2.93%) was higher than that in rhizosphere soil (2.39%), whereas the relative content of *Streptomyces* in rhizosphere soil (2.48%) was higher than that in soil (1.54%). The relative content of *Burkholderia-Caballeronia-Paraburkholderia* was 2.23% in rhizosphere soil and 0.67% in soil. The contents of *Arthrobacter* and *Sphingomonas* in rhizosphere soil were 2.50% and 2.48%, respectively, which were higher than those in soil. The average relative contents of *Bradyrhizobium* and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* in rhizosphere soil were 0.85% and 1.89% higher than those in soil, respectively, which indicated that more beneficial bacteria were enriched in rhizosphere soil.

The Venn diagrams in Fig. 2A-C demonstrated that there were 157 genera of core fungi in the rhizosphere soil of the three producing areas, 139 genera of core fungi in the soil, and 115 genera of core fungi in the rhizosphere soil and soil. The genera were classified into the following seven phyla: *Ascomycota*, *Basidiomycota*, *Chytridiomycota*, unclassified Fungi, *Mortierellomycota*, *Rozellomycota*, and *Glomeromycota*. According to the abundance heatmap of dominant fungi, 31 of the 41 genera of dominant fungi were core fungi (abundance > 2%) (Fig. 2D). The average relative content of *Mortierella* in rhizosphere soil (11.92%) was higher than that in soil (11.72%). Moreover, the average contents of *Metarhizium* and *Beauveria* in rhizosphere soil were 5.63% and 2.29%, respectively, which were higher than those in soil (2.64% and 0.14%, respectively). The average relative content of *Fusarium* in soil (4.75%) was higher than that in rhizosphere soil (2.25%), but the average relative content of pathogens, such as *Alternaria*, *Paraphoma*, *Cladosporium*, and *Rhizoctonia*, in rhizosphere soil was higher than that in soil. These results demonstrated that rhizosphere soil enriches both beneficial fungi and pathogenic fungi to a greater extent than soil.

Characteristics of soil microbial community structure and interaction network analysis in honeysuckle soil

The Shannon index and Simpson index describe the diversity and uniformity of the community. The larger the Shannon index and the smaller the Simpson index, the higher the species diversity of the community. Chao1 and Ace indices describe the number of species in a community, and the larger the value, the greater the number of communities. Alpha diversity analysis (Table 1) showed that there was no significant difference in the Shannon, Simpson, Chao1, and Ace indices of soil and rhizosphere soil in the core bacteria, but the mean values of the Shannon, Chao1, and Ace indices of rhizosphere soil were greater than those of soil. These results indicated that the community diversity and species quantity of bacteria in the rhizosphere soil core were higher. To further

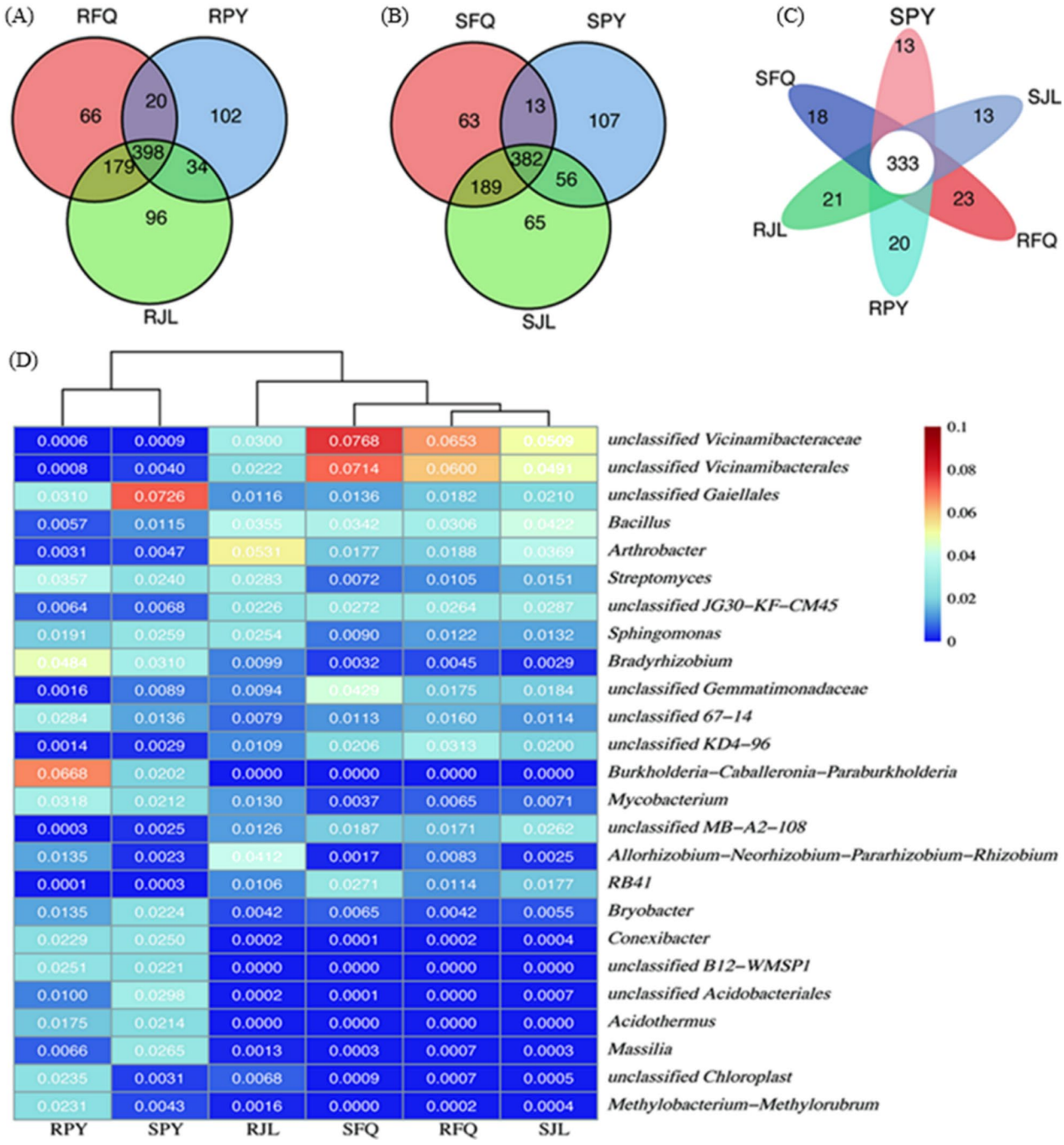

Fig. 1 Abundance map of dominant bacteria and core bacteria genera. **A:** Venn diagram of Fengqiu, Pingyi, and Julu rhizosphere soils. **B:** Venn diagram of Fengqiu, Pingyi, and Julu soils. **C:** Venn diagram of *Coreobacteria* from Fengqiu, Pingyi, and Julu soils and rhizosphere soils. **D:** Heatmaps of rhizosphere soil and soil dominant bacteria (abundance > 2%) in Fengqiu, Pingyi and Julu. (RPY: Pingyi rhizosphere soils; RJL: Julu rhizosphere soils; RFQ: Fengqiu rhizosphere soils; SPY: Pingyi soils; SJL: Julu soils; SFQ: Fengqiu soils)

clarify the difference in bacterial community structure between rhizosphere soil and soil, the core bacteria of rhizosphere soil and soil at the genus level were evaluated by linear discriminant analysis effect size (LEfSe) analysis. Figure 3A shows that there were 25 different bacteria between the two groups. Among these, 18 rhizosphere soils had significantly higher bacterial abundance than soils, and 7 rhizosphere soils had lower bacterial abundance than soils, which indicated that the differences in core bacteria were attributed to the rhizosphere soils. The differential bacteria belonging to the dominant core bacteria (marked in red) were mainly beneficial bacteria,

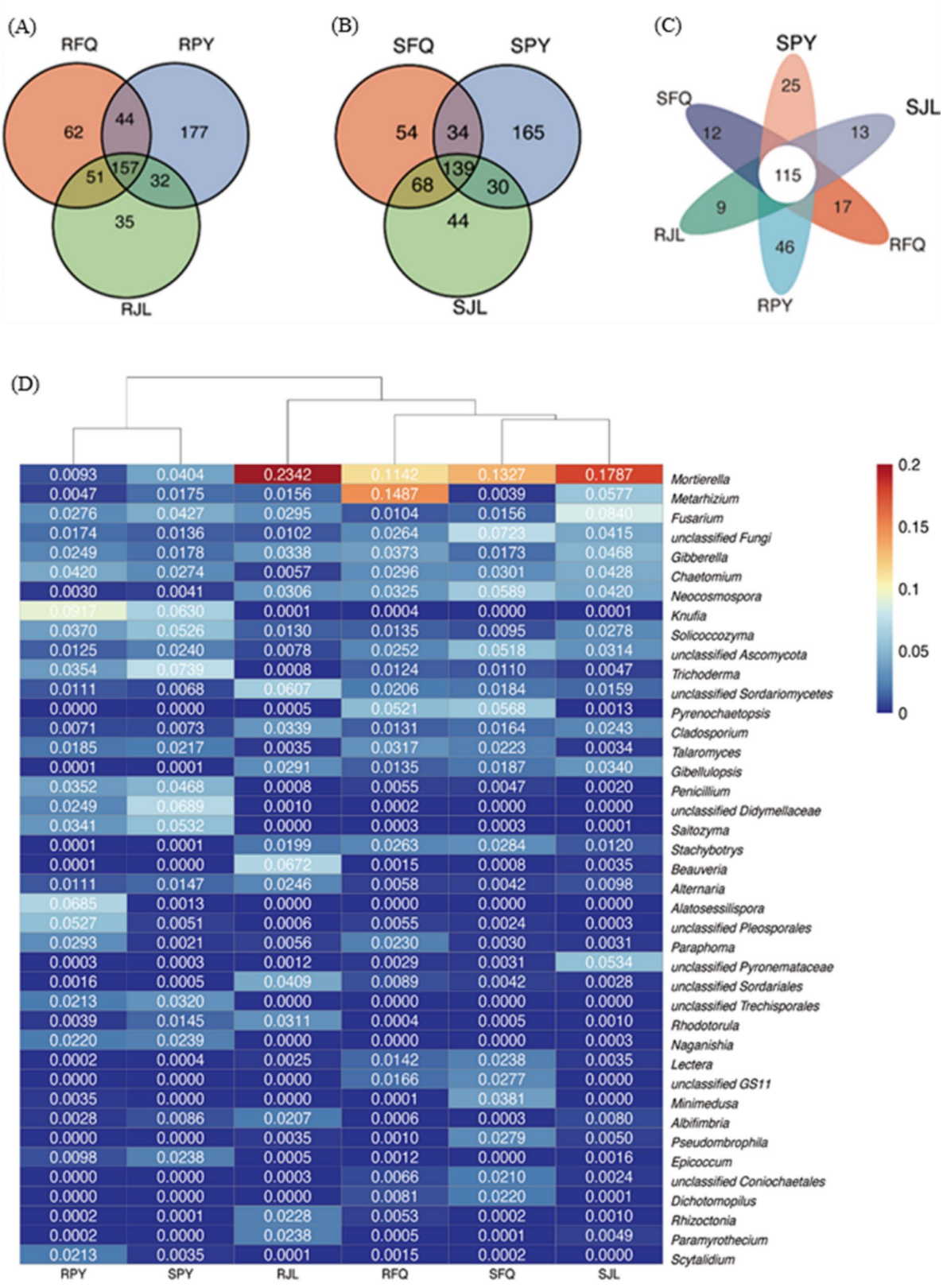

Fig. 2 Abundance map of dominant fungi and core fungal genera. **A:** Venn diagram of Fengqiu, Pingyi, and Julu rhizosphere soils. **B:** Venn diagram of Fengqiu, Pingyi, and Julu soils. **C:** Venn diagram of core fungi from Fengqiu, Pingyi, and Julu. **D:** Heatmap of rhizosphere soil and soil dominant bacteria (abundance > 2%) in Fengqiu, Pingyi, and Julu. (RPY: Pingyi rhizosphere soils; RJL: Julu rhizosphere soils; RFQ: Fengqiu rhizosphere soils; SPY: Pingyi soils; SJL: Julu soils; SFQ: Fengqiu soils)

Table 1 Alpha index of core bacteria community

different position	Shannon	Simpson	Chao1	Ace
rhizosphere soil	4.5266 ± 0.1751	0.9786 ± 0.0043	330.3668 ± 23.2139	326.4352 ± 15.5529
soil	4.4136 ± 0.1831	0.9742 ± 0.0074	322.9235 ± 12.4469	319.4915 ± 10.2108

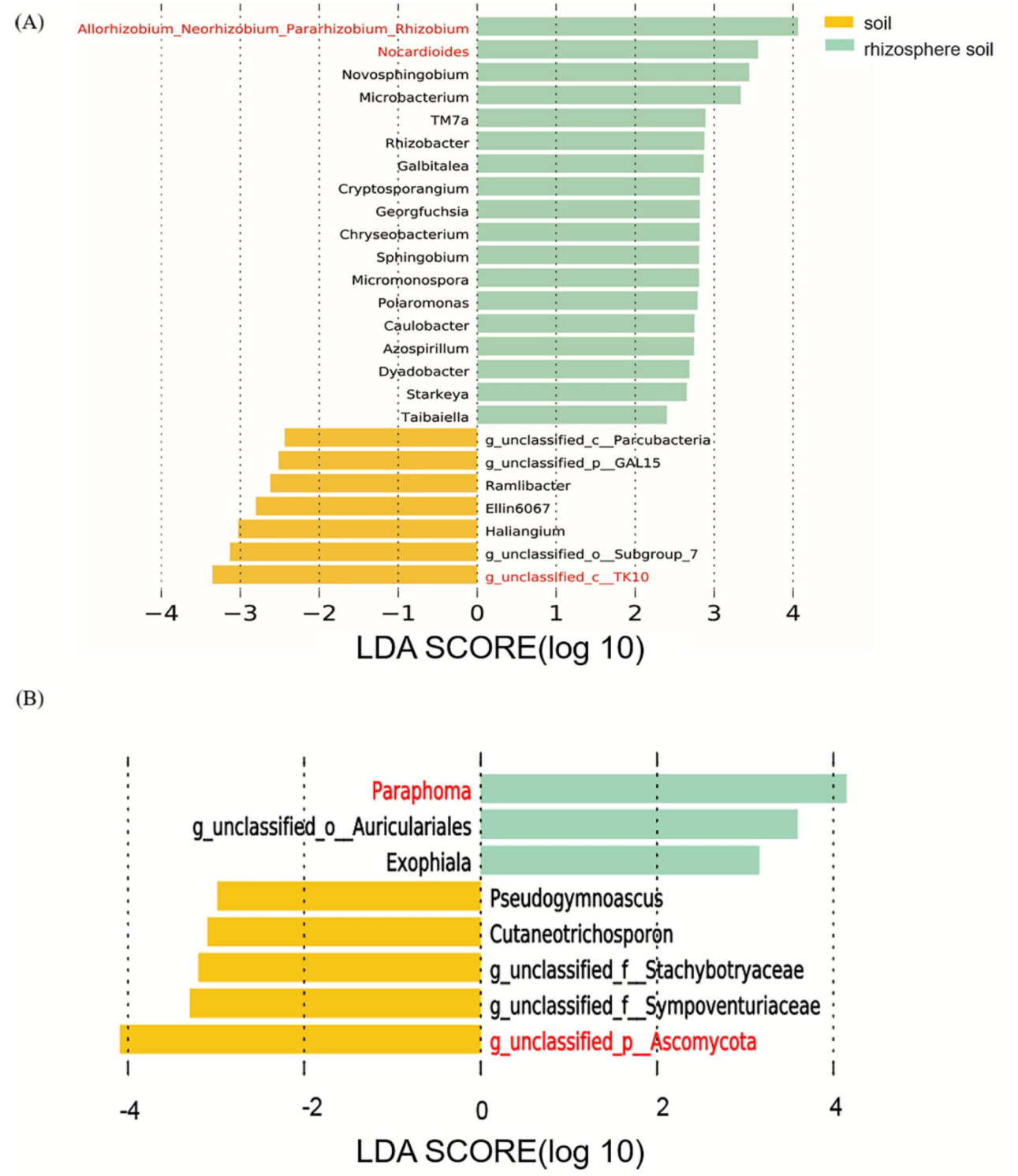

Fig. 3 **A:** LEfSe analysis of core bacteria in soil and rhizosphere soil. **B:** LEfSe analysis of core fungi in soil and rhizosphere soil

Table 2 Alpha index of core fungi community

different position	Shannon	Simpson	Chao1	Ace
rhizosphere soil	3.2536 ± 0.3012	0.9116 ± 0.0584	108.8788 ± 4.4488	108.8556 ± 5.1277
soil	3.2387 ± 0.1515	0.9289 ± 0.0151	103.4418 ± 6.4327	102.9103 ± 5.2077

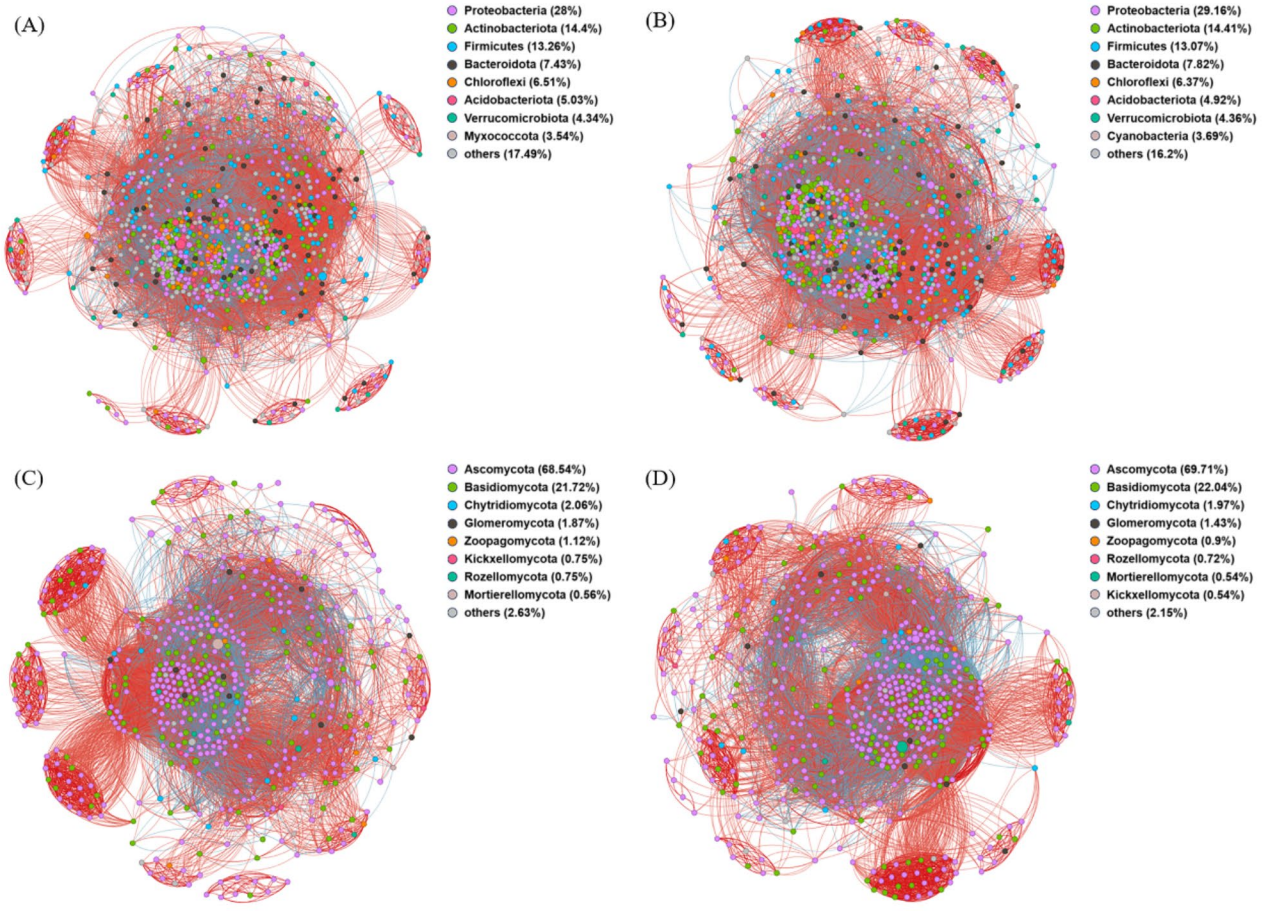

Fig. 4 Graph of correlation network analysis of microbial community at genus level. **A:** soil bacterial network; **B:** rhizosphere soil bacterial network; **C:** soil fungal network; **D:** rhizosphere soil fungal network;

such as *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, which have a nitrogen fixation effect.

Alpha diversity analysis (Table 2) indicated that the mean values of the Shannon, Chao1, and Ace indices of core fungi in rhizosphere soil were greater than those of soil, while the mean value of the Simpson index of rhizosphere soil was less than that of soil, which indicated that the community diversity and species number of core fungi in rhizosphere soil were higher. To further clarify the differences between rhizosphere soil and soil fungal community structure, LEfSe was used to analyze the different core fungi in rhizosphere soil and soil at the genus level. Figure 3B shows that there were eight genera with different fungi between the two groups. Among these, three genera had significantly higher fungal abundance in rhizosphere soil than in soil, and five genera had lower fungal abundance in rhizosphere soil than in soil, indicating that the differences in core fungi were mainly attributed to soil, which contrasted the differences in core bacteria. Among these fungi, the dominant core differentiator, *Paraphoma*, was the pathogenic fungus.

To investigate the interaction of microbial communities in soil and rhizosphere soil, bacteria at the classification level were analyzed. Bacteria with correlations greater than 0.6 and $P<0.05$ were selected to draw the interaction network among microbial communities (Fig. 4A-B), and the network attribute parameters were calculated (Table S1). As shown in Table S1, the average degree of rhizosphere soil was higher than that of soil, which indicated that one node in the rhizosphere soil bacterial network had more connections with other nodes, suggesting a more complex network. In addition, the average clustering coefficient of rhizosphere soil was higher than that of soil, indicating that the adjacent nodes of rhizosphere

soil were more connected, and the nodes were more easily clustered together. These findings suggested that rhizosphere soil bacteria are more densely connected than soil and that their structures are more compact and complex.

The key species were further determined according to the topological characteristics of the nodes in the network. For this experiment, all the network nodes of soil and rhizosphere soil bacteria were peripheral nodes when the average relative abundance of the bacteria at the taxonomy level was greater than 1%, which allowed calculation of the Zi and Pi network attributes. In total, 12 nodes of soil bacteria and 25 nodes of rhizosphere soil bacteria fell in the connector, and neither node fell in the module hub or network hub (Fig. S1A-B). According to the bacteria corresponding to each key node, the distribution and abundance of the critical bacteria in soil and rhizosphere soil at the gate level were analyzed (Table S2). The key bacteria group in the soil belonged to 12 genera and 5 phyla, including *Firmicutes* (5 genera), *Proteobacteria* (4 genera), *Desulfobacterota* (1 genus), *Verrucomicrobiota* (1 genus), and *Myxococcota* (1 genus). The key bacteria groups in the rhizosphere soil belonged to 25 genera and 9 phyla, including *Proteobacteria* (8 genera), *Firmicutes* (6 genera), *Desulfobacterota* (3 genera), *Actinobacteriota* (2 genera), and *Bacteroidota* (2 genera). All other phyla belonged to 1 genus. In addition, according to the calculation, the abundance of key bacteria groups in soil and rhizosphere soil were almost all low-abundance species without dominant bacteria.

Fungi at the genus classification level were selected, and those with correlations greater than 0.6 and $P<0.05$ were used to plot the interaction network among microbial communities (Fig. 4C-D), and the network attribute parameters were calculated (Table S3). As shown in Table S3, the average path length and network diameter were lower in the rhizosphere soil network than in the soil network. The average degree of rhizosphere soil was higher than that of soil, which indicating that one node in the rhizosphere soil fungal network had more connections with other nodes, suggesting a more complex network. In addition, the average clustering coefficient of rhizosphere soil was higher than that of soil, which indicated that the adjacent nodes of rhizosphere soil were more connected, allowing the nodes to be more easily clustered together. Thus, these findings demonstrated that rhizosphere soil fungi have more dense network connections than soil and that their structures are more compact and complex.

According to the topological characteristics of the nodes in the network, the key species were determined. Zi and Pi calculations indicated that 10 nodes of soil fungi fell in the connector, and the other nodes all fell in the peripheral nodes. Five nodes of rhizosphere soil fungi fell within the connector, and one node was located within the module hub. The remaining nodes were located within the peripheral nodes (Fig. S1C-D). According to the fungi corresponding to each key node, the distribution and abundance of the critical fungi in soil and rhizosphere soil at the gate level were analyzed (Table S4). The key fungi groups in soil belonged to 3 phyla in 10 genera, including *Ascomycota* (7 genera), *Basidiomycota* (2 genera), and *Zoopagomycota* (1 genus). The key fungi groups in rhizosphere soil belonged to 6 genera and 3 phyla, including *Ascomycota* (4 genera), *Basidiomycota* (1 genus), and *Glomeromycota* (1 genus). In addition, the abundance of key bacteria groups in soil and rhizosphere soil was comprised of low abundance species without dominant bacteria, and the key genera of soil and rhizosphere soil had no intersection.

Comparison of microbial composition and analysis of microbial diversity in roots, stems, leaves, and flowers of honeysuckle

Based on the OTU level, a Venn diagram was used to show the OTU number of unique endophytes and common endophytes in each tissue of honeysuckle. Endophytic bacteria Venn diagram as in Fig. 5A and endophytic fungi Venn diagram as in Fig. 5B.

For further analysis of species composition, the top 11 species were classified based on the phylum level and genus level (Fig. S2A-D). Endophytic bacteria were identified in 34 phyla and 770 genera. Endophytic fungi were noted in 11 phyla and 581 genera. Among them, the top four endophytic bacteria were *Proteobacteria* (83.99%), *Actinobacteriota* (6.83%), *Firmicutes* (6.16%), and *Bacteroidota* (2.35%). The abundance of other phyla was less than 1% in the root, stem, leaf, and flower tissues. The top two phyla of endophytic fungi were *Ascomycota* (54.42%) and *Basidiomycota* (44.32%), and the abundance of the other phyla was less than 2% in the roots, stems, leaves, and flowers.

At the genera level, the top four genera with the highest abundance of root endophytic bacteria were *Pseudomonas* (50.13%), *g_unclassified_f__Enterobacteriaceae* (10.96%), *Allorhizobium-Neorhizobium-Pararhizobium -Rhizobium* (5.92%), and *g_unclassified_f__Rhodocyclaceae* (5.27%). The top four genera with high abundance of stem endophytic bacteria were *Ensifer* (31.09%), *g_unclassified_f__Rhodocyclaceae* (9.45%), *Pseudomonas* (7.99%), and *Bradyrhizobium* (6.21%). The top four genera with high abundance of leaf endophytic bacteria were *Ensife* (25.25%), *g_unclassified_f__Rhodocyclaceae* (17.59%), *Pseudomonas* (13.41%), and *Bradyrhizobium* (7.04%). The top four genera with the highest abundance of floral endophytes were *g_unclassified_f__Rhodocyclaceae* (19.23%), *Pseudomonas* (18.70%), *Pantoea* (10.20%), and *Aquabacterium* (4.46%). At the endophytic fungal level, *Gibberella* and *Fusarium* were the highest in roots,

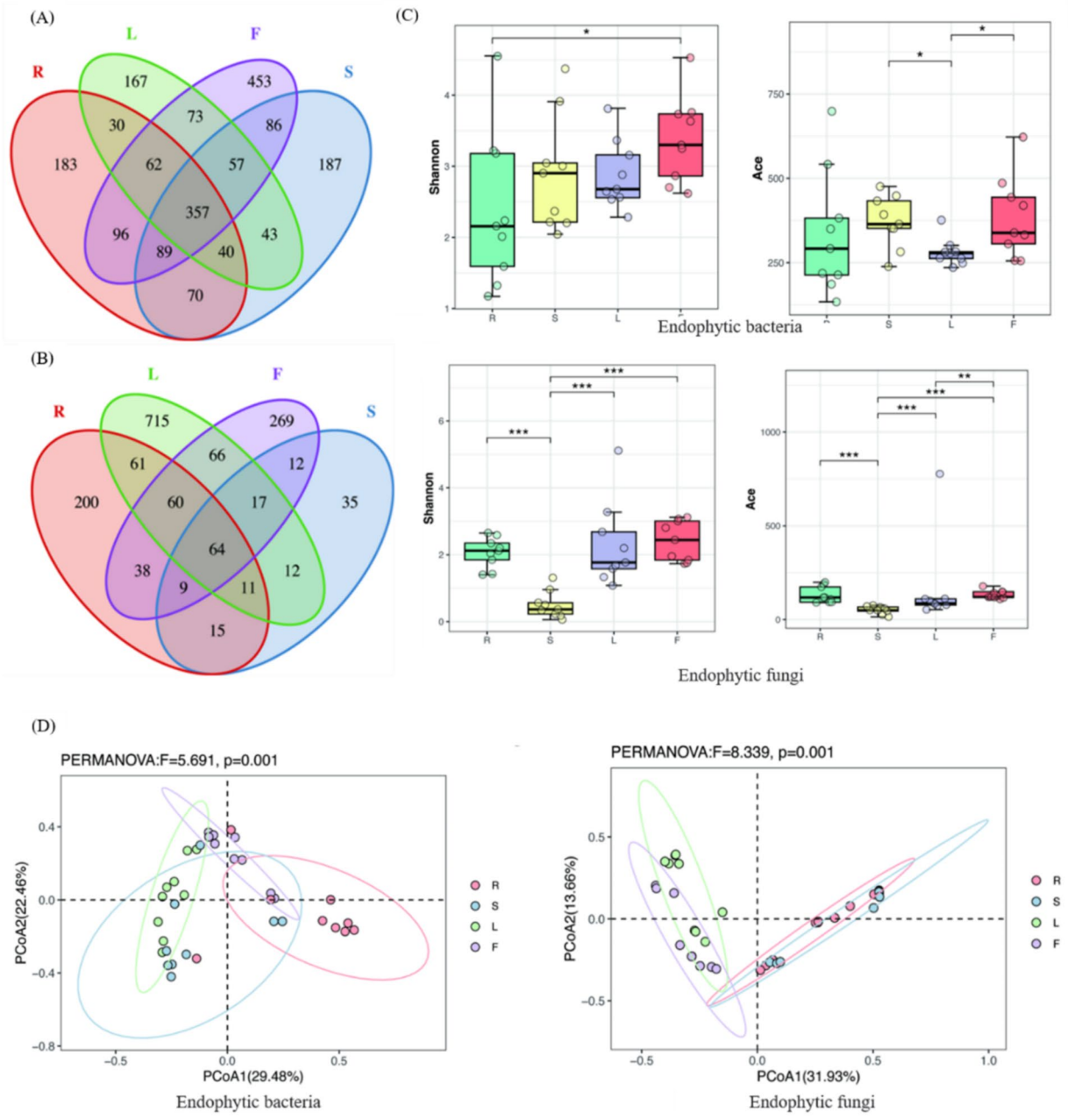

Fig. 5 **A:** The endophytic bacteria in the roots, stems, leaves, and flowers of honeysuckle unique and common OTUs. **B:** The endophytic fungi in the roots, stems, leaves, and flowers of honeysuckle unique and common OTUs. **C:** Alpha diversity index of endophytes of roots, stems, leaves and flowers of honeysuckle. **D:** PCoA analysis of endophytes from roots, stems, leaves and flowers of honeysuckle. (R, root; S, stem; L, leaf; F, flower; $P \le 0.05$:*; $P \le 0.01$:**; $P \le 0.001$:***)

with abundances of 17.55% and 7.94%, respectively. *Phyllosporia* had the highest abundance in stems (59.13%), followed by roots (27.30%). *Ceratobasidium* was the highest in stems (33.37%) but less than 1% in roots, leaves, and flowers. *Alternaria* and *Erysiphe* were enriched in leaves with an abundance of 20.93% and 29.41%, respectively, followed by flowers with an abundance of 17.41% and 16.25%, respectively. In addition, *Filobasidium* and *Cladosporium* had the highest abundance in flowers, with an abundance of 21.13% and 13.77%, respectively.

As shown in Fig. 5C, according to the Shannon index analysis of the roots, stems, leaves, and flowers of honeysuckle, there were significant differences in the Shannon index of the bacterial communities of the roots and flowers, indicating that the endophytic bacteria of the flowers had higher diversity than that of the roots. In

addition, the Ace index of stems and flowers was significantly higher than that of leaves, indicating that endophytic bacteria were more abundant in stems and flowers than in leaves. In addition, the Shannon and Ace indices of the fungal communities in roots, leaves, and flowers were significantly higher than those in stems, indicating that the diversity and richness of stems were lower than those in roots, leaves, and flowers. In addition, the Ace indices of endophytic fungi in leaves were significantly lower than those in flowers, indicating that the richness of flowers was higher than that in leaves.

PCoA analysis of endophyte communities based on Bray-curtis distance and testing of PCoA results were shown in Fig. 5D, which indicated that the overall structure of endophytic bacterial and endophytic fungal communities in roots, stems, leaves, and flowers of Honeysuckle was significantly different from that of the other endophytes. Further pairwise comparison showed that there were significant differences in endophytic bacterial community structure between the two pairs of each site in both endophytic bacteria and fungi (Fig. S3, Fig. S4).

Relationship between endophytes and soil microorganisms of honeysuckle and prediction of endophyte functions

By screening the common microorganisms of soil core bacteria of honeysuckle and endophytic core bacteria of honeysuckle, 60 genera of common microorganisms were obtained, which were defined as common core bacteria, and their content distribution in rhizosphere soil and soil is shown in Figure S5A. In addition, the common core dominant bacteria in honeysuckle plants were further screened (Fig. S5B), which indicated that the common core dominant bacteria in honeysuckle belonged to the dominant bacteria in soil (marked in red), indicating that the endogenous common core dominant bacteria may come from the soil. *Pseudomonas*, which was in the top five endogenetic dominant bacteria, is a conditioned pathogen, but it also has an anti-inflammatory effect, indicating that other bacteria may be beneficial bacteria. The results also suggested that honeysuckle flower selected some functional bacteria from the soil to colonize its body.

Through screening the common microorganisms of soil core fungi of honeysuckle and endophytic core fungi of honeysuckle, 30 genera of common core fungi were obtained, and their content distribution in rhizosphere soil and soil is shown in Fig. S6A. In addition, the common core dominant fungi in honeysuckle plants were further screened (Fig. S6B). The common core dominant fungi in honeysuckle belonged to the dominant fungi in soil (marked in red), indicating that the endogenous common core dominant fungi may also come from the soil. Most of the endophytic dominant fungi were common pathogens. The results suggested that honeysuckle selects for certain functional bacteria from the soil, as well as pathogenic fungi, speculating on their potential for certain functions.

Endophytic bacterial communities were predicted based on the PICRUST2 process. According to the abundance distribution of primary functional genes of endophytic bacteria in roots, stems, leaves, and flowers, genes related to metabolism had the highest abundance among the six known biological metabolic pathways (Fig. 6A), indicating that metabolism was the main function. Regarding the abundance of secondary functional genes of endophytic bacteria in roots, stems, leaves, and flowers, endophytic bacteria also had microbial resistance and other functions in addition to numerous metabolic functions (Fig. 6B), indicating that endophytic bacteria in various tissues of honeysuckle may function to resist some pathogenic microorganisms. FUNGuild was used to predict the fungal function of endophytic fungal communities in the roots, stems, leaves, and flowers of honeysuckle, which demonstrated that wood saprophytes, plant pathogens, and other saprophytic functions were more abundant (Fig. 6C).

Discussion

The present study examined honeysuckle plants from three producing areas and identified 398 genera in rhizosphere soil core bacteria and 382 genera in soil core bacteria. The rhizosphere soil and soil common core bacteria belonged to 333 genera, distributed in 23 bacterial phyla, including *Proteobacteria*, *Actinobacteriota*, *Firmicutes*, *Bacteroidota*, and other bacteria. There are great differences in the geographical environment and climate in Fengqiu, Pingyi, and Julu, resulting in some unique bacterial genera, but the core bacterial genera of these three areas are not easily disturbed by environmental factors, suggesting that these core bacterial genera are important for the biological function of the host [32]. Using high-throughput sequencing technology, the endophytic bacteria of Ginkgo biloba from different regions have been demonstrated to have the same endophytic dominant bacteria in different regions, which may be related to the stable generation of secondary metabolic compounds [33], which agreed with the findings of the present study. In addition, most of the dominant bacteria selected in the present study were core bacteria, and most of them were beneficial bacteria, which were enriched in the rhizosphere soil. Among them, Streptomyces was a higher actinomycete, which was abundant in the soil and rich in secondary metabolites, and can produce antibiotics. Currently, most antibiotic products produced from *Streptomyces* are from this genus. In addition, active substances, such as plant hormones, are produced, which induces systemic resistance of plants and resists plant

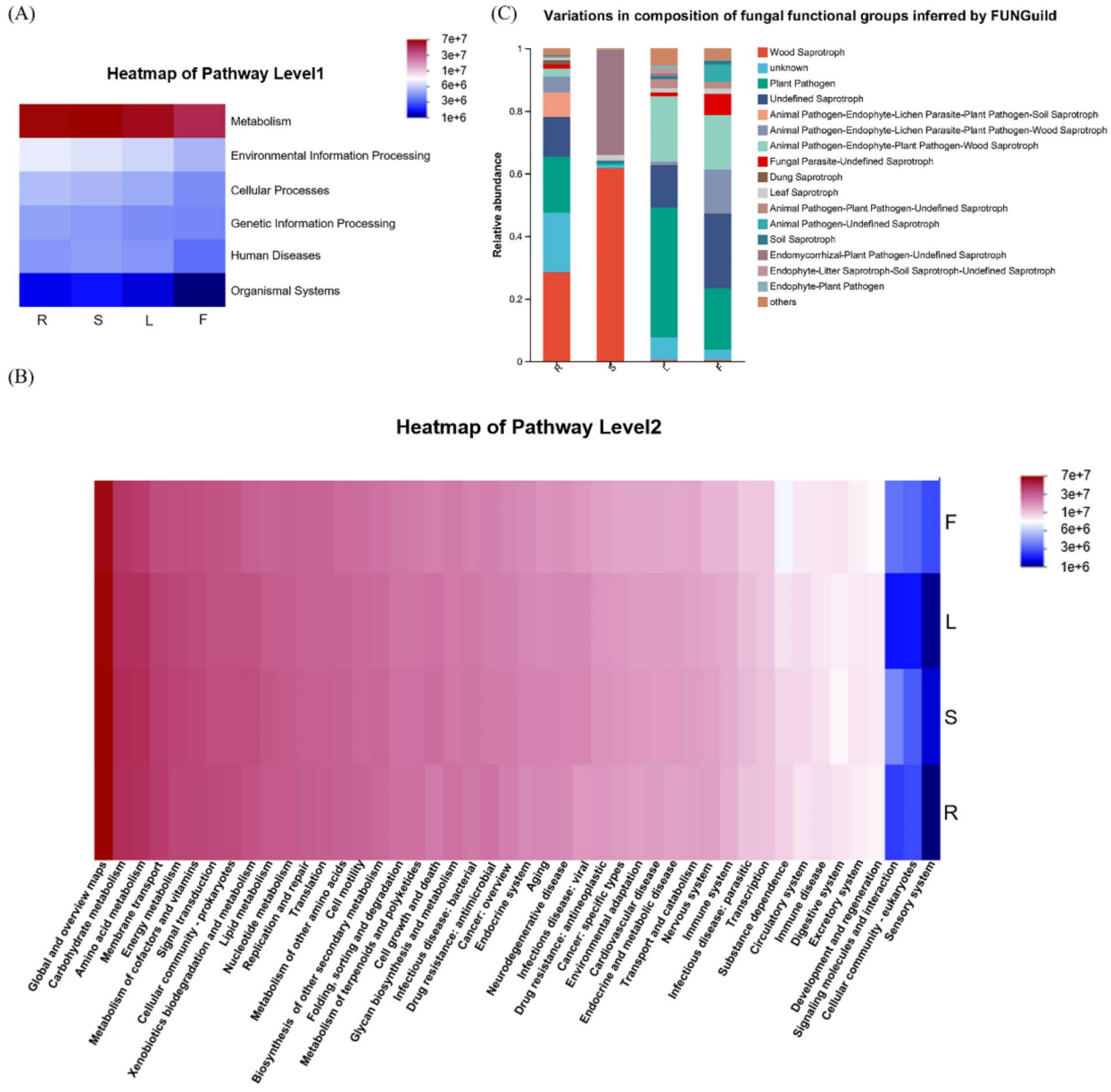

Fig. 6 **A:** Abundance of KEGG first-level functional genes of endophytic bacteria in roots, stems, leaves and flowers of honeysuckle. **B:** Abundance of KEGG second-level functional genes of endophytic bacteria in roots, stems, leaves and flowers of honeysuckle. **C:** FUNGuild analysis of endophytic fungi in roots, stems, leaves, and flowers of honeysuckle (R, root; S, stem; L, leaf; F, flower)

diseases, indicating the important role in improving the disease resistance and stress resistance of plants [34, 35]. *Burkholderia-Caballeronia-Paraburkholderia* are important biocontrol and growth-promoting bacteria that produce secondary metabolites to inhibit different pathogenic fungi [36]. *Arthrobacter* and *Sphingomonas* play an important role in soil environmental restoration, and both have the ability to purify soil [37–39]. *Bradyrhizobium* and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* are nitrogen-fixing bacteria, which have functions of fixing nitrogen in the atmosphere, improving plant nutrient absorption, and promoting plant growth, indicating their importance for plant growth [40].

In the three honeysuckle-producing areas in the present study, there were 157 genera of common core fungi in rhizosphere soil, 139 genera of common core fungi in soil, and 115 genera of common core fungi in rhizosphere soil and soil, belonging to 7 fungal phyla, mainly *Ascomycota* and *Basidiomycota*. In addition, screening of dominant genera identified that *Metarhizium* and *Beauveria*

were enriched in rhizosphere soil, and their distribution in rhizosphere soil and soil mass was further identified. *Metarhizium* and *Beauveria* are a class of parasitic pathogenic fungi with abundant species and wide distribution, and they infect and kill various arthropods and insects [41]. In addition, most *Metarhizium* is also saprophytic bacteria, rhizosphere fungi, and beneficial root endophytes, and the combination of the two bacteria is more virulent to gypsy moths than each bacterium alone [42]. In addition to being a green, effective, and environmentally friendly insecticide, *Metarhizium* is also effective in the prevention and control of some pathogenic fungi, such as gray mold of tomato [43]. Honeysuckle is susceptible to various insect pests, such as aphids, red spiders, and scarab beetles. The enrichment of *Metarhizium* and *Beauveria* in the rhizosphere indicates that honeysuckle plants recruit some pest-resistant bacteria in the root to resist the impact of insect pests on honeysuckle plants. In addition, many pathogenic fungi, such as *Alternaria*, *Paraphoma*, and *Rhizoctonia*, were also enriched in honeysuckle plants. These conditions may indicate the characteristics and microecological balance of the healthy soil of honeysuckle. Liu [44] reported that some common harmful fungi, such as *Aspergillus*, *Penicillium*, *Fusarium*, and other pathogenic fungi, are increased in diseased oil peony, while antagonistic bacteria, such as *Trichochium*, *Metarhizium*, *Bacillus*, and *Delfordia*, are decreased. The existence of some antagonists plays an Important role in maintaining the health of medicinal plants and the homeostasis of microorganisms. Therefore, our study of soil microorganisms in the three main production areas of honeysuckle can well compare the differences of soil microorganisms in each main production area, and select the appropriate main production area for planting honeysuckle according to the types and abundance of beneficial microorganisms. Usually in this process, medicinal plants recruit rhizosphere microbes in the soil through root exudates. The genome, growth stage and environment of medicinal plants can lead to differences in root exudates, thus changing the composition of rhizosphere microbes, recruiting beneficial microorganisms or inhibiting the growth of pathogenic microorganisms, so as to enrich the beneficial microbial community [45]. For example, root exudates in ginseng affect the species and abundance of rhizosphere microbial communities, which in turn affect root growth and ginsenoside accumulation [46]. Honeysuckle may enrich the beneficial microorganisms in the soil in this way, which can affect honeysuckle through direct and indirect mechanisms. Direct mechanisms include the use of microbial characteristics to directly promote plant growth, such as IAA production, nitrogen fixation, and phosphorus solubilization and so on. Indirect mechanisms include inhibiting or hindering the proliferation of plant pathogenic bacteria and fungi, such as producing antibiotics, inducing systemic resistance and so on [47]. At the same time, we compared the differences between the microorganisms of the rhizosphere soil and the soil in the main production areas, and based on the differences between the two, we can learn the beneficial microorganisms enriched by honeysuckle, which lays the foundation for the subsequent research on the honeysuckle growth-promoting microorganisms as well as the biocontrol of pests and diseases.

The key nodes identified in the ecological network module often represent key species that may play an important role in maintaining the stability of microbial community structure. Annotation of the key nodes of the bacterial network in rhizosphere soil and soil of honeysuckle identified 12 genera of bacteria in soil and 25 genera in rhizosphere soil as key bacteria. Of note, almost all of these key species were low-abundance species, while some high-abundance species were found in peripheral nodes. It has been suggested that low-abundance species may be important for the stability of rhizosphere soil and soil structure of *L. japonica*. Li et al. [48] studied the role of low-abundance bacteria in inducing systemic resistance against root rot of *Astragalus* in a simplified artificial community; they found that high-abundance bacterial species promote plant growth and inhibit the growth of pathogens to protect the host, while low-abundance bacterial species increase plant induced systemic resistance (ISR) to control disease. The combination of the two strains induces greater systemic resistance than any single strategy, indicating the importance of low-abundance species in plant growth.

In the present study, the fungal microbial networks in soil and rhizosphere were constructed, and they were similar to the bacterial networks. The network properties of rhizosphere soil fungi showed that the rhizosphere soil fungi network was more complex than that of soil. Secondly, the key species were almost all low-abundance species, which indicated that they have a role in maintaining the entire microbial network. Moreover, the key species of the two soils had no intersection, which indicated that their microbial networks were different.

The analysis of community composition and diversity of medicinal plants and the prediction of bacterial flora function can further guide the screening of functional strains to better realize the sustainable utilization of medicinal plant resources and provide more reliable strain information for the development of medicinal microbial resources. In the present study, the structure and diversity of endophytic bacteria and fungi in honeysuckle plants were analyzed by Illumina MiSeq high-throughput sequencing technology. The results of species composition showed that *Proteobacteria* (83.99%), *Actinobacteriota* (6.83%), *Firmicutes* (6.16%), and *Bacteroidota* (2.35%) were the dominant bacterial phyla in the

roots, stems, leaves, and flowers of honeysuckle. At the genus level, Pseudomonas has high abundance in roots, stems, leaves, and flowers. Pseudomonas aeruginosa is widely present in the plant body and is a dominant species in terms of both number and function. Numerous studies have shown that Pseudomonas aeruginosa has biological functions such as symbiotic nitrogen fixation [49], phosphorus solubilization [50], IAA production [51], and degradation of pesticides [52], which suggests the presence of beneficial bacteria in all tissues of Honeysuckle, thus promoting the growth of Honeysuckle. Moreover, Feng et al. [53] analyzed endophytic bacteria in different tissues of cassava, and they reported that the dominant bacterial phyla of cassava are *Proteobacteria* (41.12%), *Cyanobacteria* (12.96%), *Actinobacteriota* (9.33%), *Bacteroidota* (8.06%), *Firmicutes* (5.95%), and *Acidobacteria* (5.36%). These results suggested that the dominant phylum is different in different plant tissues. In addition, the dominant genera of each honeysuckle part were different, and their diversity was different. A previous study on endophytic bacteria in different tissues of *Glycyrrhiza uralensis* has demonstrated that the diversity of endophytic bacteria is highest in the taproot and lowest in leaves, and endophytic fungi also have the highest diversity in taproots and the lowest diversity in fibrous roots [54]. Several studies have shown that endophytic bacteria have unique tissue specificity, and the diversity structure of endophytic bacteria in different tissues varies with different plants, which may be related to the habitats and the formation of unique community characteristics in the process of long-term adaptation to the environment [55]. Meanwhile, among the various parts we found that *Cladosporium* spp. had the highest abundance in flowers. Cladosporium spp. fungi are widely parasitized in plants, and in addition to synthesizing compounds that are identical or similar to those of their hosts, they also synthesize many types of compounds, such as alkaloids, polyketides, quinones, steroids, and terpenoids, most of which have different degrees of antipathogenic, antitumor, and antiviral activities [56, 57]. Therefore, this difference may be one of the reasons for the remarkable antibacterial effect of Honeysuckle. And the antibacterial function of honeysuckle is one of its main functions, and the strength of the antibacterial effect is closely related to the quality of honeysuckle. Thus, the study of the differences in endophytic microorganisms of honeysuckle, especially the differences in functional microorganisms, has a certain guiding significance in evaluating the quality of honeysuckle.

Prediction analysis of gene function showed that the relative abundance of functional genes involved in metabolism in endophytic bacteria was the highest, which was similar to the abundance of functional genes in endophytic bacteria of different tissues of pyrrhoea, water shield [58], and soil bacteria of wolfberry [59]. In terms of endophytic fungi, the abundance of genes related to saprophytes was mainly higher. In addition, Kyoto Encyclopedia of Genes and Genomes (KEGG) secondary functional genes in different tissues of honeysuckle were also found to be antimicrobial functional genes, thus providing guidance for future studies on antibacterial endophytic bacteria of honeysuckle. In addition, the dominant common core bacteria in the honeysuckle were the dominant bacteria in the soil, which were mostly beneficial bacteria, and the dominant fungi were mostly common pathogens. Similar to *Sichuan peony* [60] and *Dendrobium chinensis* [61], there were common dominant bacteria and fungi in the rhizosphere soil and plants, which have beneficial effects on plant growth. A previous study of *Astragalus* seeds [62] has identified the presence of the common dominant bacterium *Erwinia* in the seed and within germinated seeds, which promotes the germination of seeds, as well as the presence of the dominant fungus *Fusarium* both in the seed and ungerminated seeds, which acts as a plant pathogen.

At present, there is a large demand for honeysuckle in the market, indicating the importance of improving the yield and quality of honeysuckle. The present study investigated the endophytic bacteria of honeysuckle in different regions and predicted gene function, thus laying the foundation for future studies to increase the quality and production of honeysuckle.

Conclusion

The study indicated that there were 333 genera of honeysuckle soil core bacteria distributed in 23 bacterial phyla, including *Proteobacteria*, *Actinomycetes*, *Firmicutes*, and *Bacteroidetes*. There were 115 genera of core fungi distributed in 7 phyla, including *Ascomycetes* and *Basidiomycetes*. More beneficial bacteria, such as *Streptomyces*, *Rhizobium*, *Beauveria bassiana*, *Metarhizium anisopliae*, and *Mortispora* were enriched in rhizosphere soil than in soil, and common pathogenic fungi, such as *Streptomyces* and *Rhizoctonia*, were also enriched in rhizosphere soil. The differences between core bacteria were mainly from the rhizosphere soil, and the differences between core fungi were mainly from the soil. In addition, the microbial network of the rhizosphere soil was more closely connected than that of the soil, and the structure was more complex in rhizosphere soil than in soil. *Proteobacteria* and *Firmicutes* were the key groups of soil bacteria in honeysuckle, especially in rhizosphere soil. Most of the key groups of soil fungi were *Ascomycetes* and *Basidiomycetes*, especially in soil.

In the present study, the species number and differences of endophytes in the roots, stems, leaves, and flowers of honeysuckle in the main producing areas were analyzed. The abundance of endophytes in different parts

was analyzed, and the differences in community structure were further explored. There were significant differences in the diversity and community structure of endophytes in different parts of the honeysuckle, and the diversity of endophytes in flowers was the most abundant. There were 60 genera of core bacteria and 30 genera of common core fungi in honeysuckle. The dominant common core bacteria of plants were the dominant bacteria in soil; most of the dominant bacteria were beneficial bacteria, and most of the dominant fungi were common pathogens. The function prediction of endophytic bacteria of honeysuckle showed that the endophytic bacteria had potential antimicrobial function, thus providing guidance for future research on the antibacterial properties of honeysuckle endophytic bacteria. Meanwhile, understanding the interaction between honeysuckle and microorganisms lays a foundation for the study of growth promotion, quality improvement, and disease and pests control of honeysuckle from the perspective of microorganisms.

Supplementary Information

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Supplementary material 1

Supplementary material 2

Supplementary material 3

Supplementary material 4

Supplementary material 5

Supplementary material 6

Supplementary material 7

Supplementary material 8

Supplementary material 9

Supplementary material 10

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Author contributions
LCS and RGX conceived and designed the study; XQY, JD, ZN, KYQ performed the research; XQY, LM, YCC, LXY analyzed and interpreted the data; MJ, HGD gave advice during the experiments; XQY, RGX wrote the main manuscript text, LCS and RGX gave scientific guidance throughout the research, and aided in editing of the manuscript and critical analysis. All authors reviewed the manuscript.

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Data availability
The datasets generated during the current study are available in the NCBI repository, National Center for Biotechnology Information (nih.gov). Accession number: PRJNA1138513, PRJNA1138551, PRJNA1138291, PRJNA1138277, PRJNA1138180, PRJNA1138297.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Consent.

Competing interests
The authors declare no competing interests.

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