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Ecological niche imprints the structure and network of microbial communities in Lonicera japonica Thunb

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Abstract

Background and aims

Plant-associated microbiomes play pivotal roles in promoting plant health, productivity, and resistance to diseases. However, the differential adaptability of microorganisms to different microenvironments in *Lonicera japonica* remains unclear, which hinders their application in agriculture.

Methods

Here, the structure and network of bacterial and fungal communities in six compartment niches (bulk soil, rhizosphere soil, root, stem, leaf, and flower) of *L. japonica* were explored using three host ecotypes.

Results

Results demonstrated that the structure and assembly of microbiomes along the soil–plant continuum were primarily governed by compartment niches rather than host ecotypes. Bacterial diversity and network complexity incrementally decreased from the soil to the root and flower; however, fungal diversity and network complexity gradually decreased from the soil to the stem but increased from the stem to the leaf and flower. The bacterial keystone taxa Proteobacteria, *Pseudomonas*, *Pantoea*, *Kluyvera*, and *Enterobacter* were dominated in endosphere samples. Meanwhile, the core fungal keystone taxa *Cryptococcus*, *Alternaria*, and *Erysiphe* were dominated by endosphere samples. However, *Mortierella* and *Fusarium* were dominated by rhizosphere soil.

Conclusion

Microbiota was mainly derived from bulk soil and then filtered in each compartment niches. This finding provides empirical evidence for host selection and soil–plant–microbiome interaction along the soil–plant continuum.

Introduction

Plant-associated microbiota, which consists of diverse but taxonomically structured communities (such as bacteria, fungi, and archaea), is considered as the second genome of host plants, and it varies among different plant species (Brown et al., 2020). Interactions between plant and microbes confer adaptive advantages to plant hosts, including nutrient cycling, growth promotion, stress tolerance, and resistance to pathogens (Trivedi et al., 2020). Most recent studies focusing on the roots and rhizosphere soil have demonstrated that the assembly and structure of microbial communities are shaped by various biotic and abiotic factors, including plant genetics and age, soil types, and soil properties (such as pH and nutrients) (Yu et al., 2018). The assembly and network of microbial communities have been reported to

exhibit niche differentiation between soils (bulk soil and rhizosphere soil) and endosphere niches (root, stem, leaf, and flower) (Lundberg et al., 2012). Compared with endophytic bacterial and fungal communities of *Populus deltoides*, rhizosphere microorganisms have lower inter-sample variation and higher biodiversity (Beckers et al., 2017). However, most of the studies focused on one aspect, and a systemic report of how compartment niche, host species, edaphic factors, and agricultural management perturbations interactively drive the assembly of crop microbiomes is limited. The mechanism of microbiome assembly in plants is incomplete, and some key questions need to be addressed. Therefore, elucidating the effects of compartment niches and host species on the assembly and structure of microbiomes is necessary for the accurate control of the microbiome.

The transmission models of plant microbiota mainly involve horizontal transmission and vertical transmission (Wei and Ashman, 2018). The horizontal transmission model may be achieved as follows: recruitment of rhizosphere microorganisms by the roots, concentration around the rhizoplane of the root tissues, and final colonization of the root tissues (Compant et al., 2016). The vertical model involves the transmission of root microflora to the aerial parts of the host (i.e., stem, leaf, flower, fruit, and seed) (Compant et al., 2010). In addition, the phylloplane is an important interface between plants and microorganisms, and microorganisms from the surrounding environment can colonize the leaf and transform to other parts (Lindow and Brandl, 2003). The microflora along the soil–plant continuum remains unclear, and the role of microorganisms in different ecological niches with different ecotypes has yet to be determined. Thus, comprehensively understanding the origin and dispersal of plant microbiota is necessary to harness the beneficial plant-associated microbiome for sustainable agriculture.

Lonicera japonica Thunb. is an economically important species of honeysuckle belonging to the Caprifoliaceae family (Xu et al., 2023). Historically, it has been regarded as an herbal remedy for treating diseases such as exopathogenic wind-heat and epidemic febrile diseases in China for thousands of years (Chi et al., 2024). It is also used in the production of tea, cosmetics, and healthy beverages (Cai et al., 2020). Given the roles of microorganisms in regulating the growth and health of host plants (Santoyo et al., 2022), elucidating the ecology of microbial communities is important to improve the application performance of *L. japonica*. In addition, *L. japonica* and its endophytes synergistically regulated the toxic effects of Cd accumulation via multiple plant metabolic defensive pathways to increase resistance to metal-induced stress (Xie et al., 2023). In this study, a comprehensive knowledge of the fungal community diversity and composition on the surface of *L. japonicae* Flos was provided and suggested that the fungal community was shaped by the collection area and processing method (Dao et al., 2023). The endophytic fungus *Penicillium ochrochloron* YT2022-65 isolated from *L. japonica* could produce six structurally diversified indole alkaloids (i.e., peniochroloid A, flavonoid B, brocaeloid C, isoroquefortine C, roquefortine C, and dihydrocarneamide A) (Liu et al., 2023). However, the mechanism through which the compartment niches and host ecotypes imprint *L. japonica*-associated microbial communities remains unclear, which prevent us to promote the agricultural practices of *L. japonica* by modulating microbiome. In addition, information about the origin and transmission mode of *L. japonica*-associated microbiome is limited.

Given the abovementioned issues, a comprehensive understanding of the fluctuations of microbial communities along the soil–plant continuum is necessary. In the present study, the bacterial and fungal communities in microenvironments (bulk soil, rhizosphere soil, root, stem, leaf, and flower) of *L. japonica* were analyzed using high-throughput sequencing. This study aims to (i) evaluate the effects of compartment niches and host ecotypes on the assembly and co-occurrence network of microbial communities and (ii) identify the dominant and differential taxa in each compartment and the potential sources of plant microbial communities. We hypothesized that compartment niches strongly imprint the assembly and co-occurrence network of microbiome. Moreover, plant microbial communities migrate from the soil to roots and then to aerial parts.

Materials and methods

Sample collection

Three *L. japonica* cultivars were collected in accordance with their biological characteristics and types: Sijihua (SJ), Beihua No. 1 (BH), and Huajin No. 3 (HJ). All *L. japonica* plants were grown in the same site without fertilization and watered two times a week. The soils away from *L. japonica* roots (20 cm) at a depth of 10 cm were obtained as bulk soil. The soils attached to the roots (< 3 mm) were sampled as rhizosphere soil. The bulk and rhizosphere soil samples were ground, sieved, and stored at − 80°C for DNA extraction. The root, stem, leaf, and flower of *L. japonica* were collected, treated with 70% ethanol for 3 min, immersed in 2.5% NaClO for 5 min, and washed with sterile water five times (Barra et al., 2016). Then, the last washing water was plated on Luria–Bertani and potato dextrose agar for incubation, and we checked whether the surface of plants was disinfected (Carrión et al., 2019). The sterile samples were shredded, ground to powder, and then stored at − 80°C for DNA extraction.

DNA extraction, PCR, and sequencing

Total DNA was extracted in accordance with the instructions of the FastDNA Spin Kit for soil. The 16S rRNA gene fragments were amplified using the bacterial primers 341F/805R (5'-CCTACGGGNGGCWGCAG-3')/(5'-GACTACHVGGGTATCTAATCC-3'). The obtained ITS rRNA gene fragments were amplified using the fungal primer fITS7/ITS4(5'-GTGARTCATCGAATCTTTG-3')/(5'-TCCTCCGCTTATTGATATGC-3'). The PCR reaction mixture consisted of Phusion Hot Start Flex 2× Master Mix (12.5 μL), a primer (2.5 μL, 200 mM), template DNA (50 ng), and PCR-grade water with a final volume of 25 μL (Lin et al., 2022). The amplification program was set as follows: pre-denaturation for 30 s at 98°C; 32 cycles of 98°C for 10 s, 50°C for 30 s, and 72°C for 45 s; and final extension for 10 min at 72°C. The PCR products were purified as previously described (Wei et al., 2022). The purified PCR products were sequenced using the NovaSeq PE250 platform, and paired-end reads with a length of 250 bp were acquired. Then, the fast files were available in the Sequence Read Archive database of the National Center for Biotechnology Information (accession number: PRJNA1132304 [bacteria] and PRJNA1132307 [fungi]).

Data processing and statistical analysis

To obtain effective tags, the raw fastq files were demultiplexed and filtered by UPARSE (v8) and QIIME (v1.9.1) (Edgar et al., 2013; Bolyen et al., 2019). The paired-end reads were truncated, and quality filtering (average Phred quality score of ≥ Q20) was carried out (Marasco et al., 2018). The resulting amplified sequence variants (ASVs) were assigned with taxonomic identification by Ribosomal Database Project classifier (v.2.2) against the 16S rRNA (Silva v.138) and ITS (Unite v.8.0) gene database (Izawa et al., 2020). Overall, 3,274,177 bp bacterial clean reads and 4,148,079 bp fungal clean reads were obtained (Table S1). The representative sequences of ASVs were classified into taxonomy (at the phylum, class, order, family, genus, and species levels) (Nilsson et al., 2019).

Non-metric multidimensional scaling (NMDS) analysis based on three distances (Bray–Curtis distance, weighted UniFrac distance, and unweighted UniFrac distance) were performed to visualize the variation patterns of β-diversity (Caporaso et al., 2010). The significance of β-diversity was tested by permutational multivariate ANOVA (Oksanen et al., 2007). Alpha diversity indices (including Chao 1 and Shannon) were calculated using mothur (v.1.30.2) (Schloss et al., 2009). The significance of alpha diversity indices was tested by using the Kruskal–Wallis test. Circos plots were carried out using a Circos table viewer to display the distribution proportion of dominant microbiota (Krzywinski et al., 2009). The linear discriminant analysis effect size (LEfSe) was applied to identify the biomarkers (Wilcoxon *P*-value < 0.05 and logarithmic linear discriminant analysis score > 5) (Guerrero-Preston et al., 2016). Co-occurrence analysis among microbial taxa was performed on the basis of Spearman's correlation scores (Spearman's $r > 0.8$ or $r < -0.8$, $P < 0.05$). Gephi was used for network visualization and property measurements (Bastian et al., 2009). The source values of endophytic bacteria and fungi were calculated by source tracking analysis (Liu et al., 2018).

Results

Compartment niches imprint the microbial community to a greater extent than plant ecotypes

The variations in the bacterial community of *L. japonica* were shaped by compartment niches (Bray–Curtis distance, $R^2 = 55.97\%$, $P < 0.001$; weighted UniFrac distance, $R^2 = 53.88\%$, $P < 0.001$; unweighted UniFrac distance, $R^2 = 54.93\%$, $P < 0.001$) to a greater extent than host cultivars (Bray–Curtis distance, $R^2 = 6.16\%$, $P < 0.05$; weighted UniFrac distance, $R^2 = 0.76\%$, $P > 0.05$; unweighted UniFrac distance, $R^2 = 0.58\%$, $P > 0.05$; Fig. 1A and Figure S1A). In addition, NMDS revealed clear and separate clustering of bacterial community between the soil and plant samples.

Variations in fungal community were also largely imprinted by compartment niches (Bray–Curtis distance, $R^2 = 64.50\%$, $P < 0.001$; weighted UniFrac distance, $R^2 = 71.09\%$, $P < 0.001$; unweighted UniFrac distance, $R^2 = 63.93\%$, $P < 0.001$) compared with host cultivars (Bray–Curtis distance, $R^2 = 2.06\%$, $P >$

0.05; weighted UniFrac distance, R^2 = 3.31%, P > 0.05; unweighted UniFrac distance, R^2 = 4.88%, P > 0.05; Fig. 1B and Figure S1B). Clear and separate clustering of fungal community between the soil and pant samples was also displayed. These results indicate that the variations in bacterial and fungal communities can be strongly influenced by host compartments. Therefore, considerable attention should be given to the effect of compartment niches on the microbial community of *L. japonica* in future studies.

Microbial alpha diversity decreased from soil to plant samples

Significant differences in bacterial alpha diversity were observed among different compartments (Kruskal–Wallis, P < 0.001; Fig. 1C, S2A and Table S2). Bacterial Chao 1 was greatest in soil samples (bulk soil, 1165.47; rhizosphere soil, 1185.77), and it incrementally decreased from the root (88.62) and stem (43.08) to the leaf (39.93) and finally to the flower (35.99). The bacterial Shannon value was significantly higher in bulk soil (9.06) and rhizosphere soil (8.73) than in plant samples (root, 3.26; stem, 3.99; leaf, 4.95; flower, 3.77).

The fungal alpha diversity was also largely shaped by compartment niches (Kruskal–Wallis, P < 0.001; Fig. 1D, S2B and Table S3). The Fungal Chao 1 index was significantly greater in soil samples (bulk soil, 207.10; rhizosphere soil, 172.54) than in plant samples (root, 26.72; stem, 24.74; leaf, 38.65; and flower, 24.00). The fungal Shannon index was significantly greater in soils (bulk soil, 5.32; rhizosphere soil, 5.22) than in plant samples (root, 2.18; stem, 1.93; leaf, 2.51; and flower, 2.22). These results demonstrated that the alpha diversity (Shannon and Chao 1) of microbial communities associated with *L. japonica* along the soil–plant continuum was strongly shaped by compartment niches.

Microbial network complexity and connectivity incrementally decreased from soil to plants

The co-occurrence profiles were used to explore the increasing effect of host selection on bacterial and fungal communities. For bacterial co-occurrence networks, the average degree was the greatest in soil samples (bulk soil, 23.82; rhizosphere soil, 24.77), followed by that in the root (10.91) and stem (7.54), and was the lowest in the leaf (6.06) and flower (5.72; Table 1 and Fig. 2A). Higher values of topological properties (i.e., nodes, edges, positive edges, negative edges, modularity, network diameter, average path length, average weighted degree, and modularity with resolution) were detected in soil samples (bulk soil, 712, 8479, 7460, 1019, 0.89, 10, 3.76, 26.59, and 0.89, respectively; rhizosphere soil, 721, 8929, 8199, 730, 0.88, 9, 3.61, 30.00, and 0.88, respectively) than in plant samples (root, 152, 829, 803, 26, 0.82, 8, 2.91, 16.47, 0.82, respectively; stem, 79, 298, 293, 5, 0.75, 4, 1.13, 12.48, and 0.75, respectively; leaf, 72, 218, 217, 1, 0.8, 5, 1.26, 9.69, and 0.80, respectively; flower, 71, 203, 195, 8, 0.82, 6, 1.69, 8.62, and 0.82, respectively).

Table 1
Topological properties of co-occurring networks of microbial community associated with *L. japonica* plants.

Types	Network properties	Bulk soil	Rhizosphere soil	Root	Stem	Leaf	Flower
Bacteria	Number of nodes	712	721	152	79	72	71
	Number of edges	8479	8929	829	298	218	203
	Positive edges	7460	8199	803	293	217	195
	Negative edges	1019	730	26	5	1	8
	Modularity	0.89	0.88	0.82	0.75	0.8	0.82
	Number of communities	33	30	34	27	25	28
	Network diameter	10	9	8	4	5	6
	Average path length	3.76	3.61	2.91	1.13	1.26	1.69
	Average degree	23.82	24.77	10.91	7.54	6.06	5.72
	Average clustering coefficient	0.70	0.71	0.88	0.95	0.94	0.93
	Average weighted degree	26.59	30.00	16.47	12.48	9.69	8.62
	Density	0.03	0.03	0.07	0.10	0.09	0.08
	Modularity with resolution	0.89	0.88	0.82	0.75	0.80	0.82
	Total triangles	57514	66604	2877	695	339	270
Fungi	Number of nodes	227	213	48	33	60	41
	Number of edges	900	765	86	48	113	92
	Positive edges	773	722	85	48	113	92
	Negative edges	127	43	1	0	0	0
	Modularity	1.04	0.93	0.74	0.46	0.75	0.45
	Number of communities	50	39	30	24	35	23
	Network diameter	12	15	2	1	2	1
	Average path length	4.48	4.98	1.03	1	1.02	1
	Average degree	7.93	7.18	3.58	2.91	3.77	4.49

Types	Network properties	Bulk soil	Rhizosphere soil	Root	Stem	Leaf	Flower
	Average clustering coefficient	0.79	0.79	0.91	0.87	0.94	0.93
	Average weighted degree	8.37	8.92	4.99	3.68	5.34	6.84
	Density	0.04	0.03	0.08	0.09	0.06	0.11
	Modularity with resolution	1.04	0.93	0.74	0.46	0.75	0.45
	Total triangles	1563	1191	48	13	75	102

The fungal network complexity gradually decreased from the soil to the stem but increased from the stem to leaf and flower (Table 1 and Fig. 2B). Subsequently, the average degree decreased from bulk soil (7.93) and rhizosphere soil (7.18) to the root (3.58) and stem (2.91) but increased to the leaf (3.77) and flower (4.49). Similarly, the values of other topological properties (i.e., nodes, edges, positive edges, modularity, average clustering coefficient, average weighted degree, and total triangles) decreased from bulk soil (227, 900, 773, 1.04, 0.79, 8.37, and 1563, respectively) and rhizosphere soil (213, 765, 722, 0.93, 0.79, 8.92, and 1191, respectively) to the root (48, 86, 85, 0.74, 0.91, 4.99, and 48, respectively) and stem (33, 48, 48, 0.46, 0.87, 3.68, and 13, respectively) but increased to the leaf (60, 113, 113, 0.75, 0.94, 5.34, and 75, respectively) and flower (41, 92, 92, 0.45, 0.93, 6.84, and 102, respectively).

Changes in the core microbiota of microbial communities associated with L. japonica

For bacterial community, the Venn diagram revealed that 6,358, 6,678, 453, 217, 194, and 185 ASVs were observed in bulk soil, rhizosphere soil, root, stem, leaf, and flower, respectively (Figure S3A). At the phylum level, Proteobacteria, Acidobacteriota, Actinobacteriota, Cyanobacteria, and Planctomycetota were the highest phyla, and they demonstrated significant compartment-specific effects ($P < 0.05$; Fig. 3A, and Table S4). The soil was dominated by Acidobacteriota (bulk soil, 20.77%; rhizosphere soil, 20.28%), Actinobacteriota (bulk soil, 14.29%; rhizosphere soil, 12.12%), and Planctomycetota (bulk soil, 3.97%; rhizosphere soil, 5.06%), whereas the leaf was dominated by Cyanobacteria (11.79%). In addition, Proteobacteria were mostly enriched in the plant samples (root, 99.20%; stem, 98.52%; leaf, 88.07%; and flower, 96.61%). At the genus level, the top five genera, namely, *Pseudomonas*, *Pantoea*, *Kluyvera*, *Enterobacter*, and *Chloroplast unclassified*, presented significant compartment-specific effects ($P < 0.05$; Fig. 3B, and Table S4). These genera were dominated in plant samples, with *Pseudomonas* (54.09%) and *Chloroplast unclassified* (11.79%) in the leaf, *Pantoea* in the stem (40.54%) and flower (41.15%), *Kluyvera* in the root (12.82%) and stem (11.01), and *Enterobacter* in the root (7.83%) and leaf (7.09%). At the species level, *Pantoea_sp. Pot1 f*, *Pantoea_sp._MG 2011 90 GD*, *Pseudomonas_sp._TM16*, *Pseudomonas* sp. *TA_AY2*, and *Kluyvera_sp.* were the top five species (Fig. 3C, and Table S4). *Pantoea*

sp. *MG 2011 90 GD* and *Pseudomonas* sp. *TM16* were dominant in the flower (23.15%) and leaf (11.53%), respectively. By contrast, *Kluyvera* sp. was dominant in the root (12.63%) and stem (11.00%).

Regarding fungal community, several unique ASVs were detected in the bulk soil (937) and rhizosphere soil (823) compared with the root (135), stem (117), leaf (163), and flower (101) (Figure S3B). Ascomycota, Basidiomycota, and Zygomycota were the most abundant fungal phyla, and they displayed significant compartment-specific effects ($P < 0.05$; Fig. 3D, and Table S5). Ascomycota was dominant in the flower (81.27%), bulk soil (76.17%), and rhizosphere soil (77.53%). Basidiomycota was dominant in the stem (93.41%) and root (87.97%), whereas Zygomycota was dominant in the rhizosphere soil (9.63%). Significant compartment-specific effects were also found in the top five fungal genera, namely, *Cryptococcus*, *Alternaria, Erysiphe*, *Phellinus*, and *Eurotiomycetes unclassified* ($P < 0.05$; Fig. 3E, and Table S5). The stem was dominated by *Cryptococcus* (91.61%); the flower was dominated by *Alternaria* (46.93%) and *Erysiphe* (31.52%); the root was dominated by *Phellinus* (31.05%), and the rhizosphere soil was dominated by *Mortierella* (9.46%) and *Fusarium* (5.45%). In addition, the top five species *Cryptococcus magnus*, *Alternaria unclassified*, *Erysiphe symphoricarpi*, *Phellinus ribis f ulicis*, and *Eurotiomycetes unclassified* showed significant compartment-specific effects ($P < 0.05$; Fig. 3F, and Table S5). *Cryptococcus magnus* was dominant in the stem, whereas *Erysiphe symphoricarpi* was dominant in the flower and root. Moreover, *Phellinus ribis f ulicis* was dominant in the bulk soil, rhizosphere soil, and root, compared with those of the aerial parts. These results indicated that the soil and plant samples shared a common structural composition, but they differed in composition proportions.

Different microbial biomarkers in different ecological parts of L. japonica

LEfSe identified the microbial biomarkers of *L. japonica* along the soil–plant continuum (Fig. 4 and S4). A total of 11 bacterial biomarkers (LDA > 5) were identified, and one biomarker was identified as the most significant in the bulk soil, one in rhizosphere soil, two in the root, one in the stem, three in the leaf, and three in the flower (Fig. 4A, 4B and S4A). The class Alphaproteobacteria was identified as the most significant biomarker in the bulk soil. The order Rhizobiales was identified as the most significant biomarker in the rhizosphere soil. The phylum Proteobacteria and the family Enterobacteriaceae were identified as the most significant biomarkers in the root. The class Gammaproteobacteria was identified as the most significant biomarker in the stem. The order Pseudomonadales, the family Pseudomonadaceae, and the genus *Pseudomonadales* were identified as the most significant biomarkers in the leaf. The order Enterobacterales, the family Erwiniaceae, and the genus *Pantoea* were identified as the most significant biomarkers in the flower.

Among the 20 fungal biomarkers (LDA > 5.0), two, four, five, and nine taxa were dominant in the bulk soil, root, stem, and flower, respectively (Fig. 4C, 4D and S4B). The class Sordariomycetes and the order Hypocreales were identified as the most significant biomarkers in the bulk soil. The class Agaricomycetes, the order Hymenochaetales, the family Hymenochaetaceae, and the genus *Phellinus*

were identified as the most significant biomarkers in the root. The phylum Basidiomycota, the classes Tremellomycetes and Agaricomycetes, the order Tremellales, the family Tremellaceae, and the genus *Cryptococcus* were identified as the most significant biomarkers in the stem. The phylum Ascomycota, the class Dothideomycetes, the order Pleosporales, the family Pleosporaceae, and the genus *Alternaria* were identified as the most significant biomarkers in the flower.

Origin and transmission mode of L. japonica-associated microbiomes

Source tracking analysis was performed to determine the potential sources of bacterial and fungal communities in each compartment niche of *L. japonica* (Fig. 5). For bacterial community, 59.58% of rhizosphere community was from the bulk soil, and 43.41% of endophytes in the root were from the rhizosphere soil (Fig. 5A). In particular, the stem and flower potentially selected the majority of taxa from the nearby niches, with known source values > 60%. A similar source phenomenon was also obtained in fungal communities (Fig. 5B). The rhizosphere soil, root, stem leaf, and flower potentially selected the majority of taxa from the nearby niches, with known source values > 50%. Surprisingly, 95.61% of the fungal community in the flower was from the leaf. These results indicated that the bacterial and fungal endophytes of *L. japonica* were primarily obtained from soil and then gradually filtered through different compartment niches of *L. japonica*.

Discussion

To date, few studies have focused on microbial community variations in the niches along the soil–plant continuum, much less simultaneously assessed microbiomes in different plant cultivars. In this study, the microbial communities along the soil–plant continuum of *L. japonica* with three cultivars were investigated to evaluate the influences of compartment niches and host cultivars on the assembly of microbiomes. The results of this study also demonstrated strong niche-dependent variations in the bacterial and fungal communities. Therefore, the *L. japonica* microbiome assemblage was mainly imprinted by the compartment niches.

Furthermore, the results indicated that the bacterial alpha diversity (i.e., Chao 1 and Shannon) and network complexity (as indicated by the average degree) of *L. japonica* was the greatest in soil samples (bulk soil and rhizosphere soil), but they incrementally decreased from the root to the flower. Similar results were reported in a previous study (Xiong et al., 2021; 33Wei et al., 2021). The fungal diversity and network complexity of *L. japonica* gradually decreased from the soil to the stem but increased from the stem to the leaf and flower. Similar patterns in the fungal communities of the aforementioned compartment niches have been reported in previous literature (Lin et al., 2022). The variations in microbial communities include enrichment and depletion from the surrounding soil. The bulk soil harbored higher diversity and network complexity of bacterial and fungal communities than rhizosphere soil. This observation was consistent with a previous report (Fan et al., 2018). The bulk soil is an important inoculum reservoir, which contributes to the construction of specific microbes in each plant

compartment niche (Che et al., 2023). The diversity and network complexity in the rhizosphere soil were higher than those in the root. The spread of root exudates into the rhizosphere soil enhances the chemical attraction and colonization of microorganisms, which leads to the formation of highly abundant and diverse microbial communities in the rhizosphere. The root serves as a selective barrier responsible for such huge loss in species diversity from soil to plant compartments (Bulgarelli et al., 2012). These host immune system and host selection effects lead to the differentiation of microbial communities in various compartment niches.

Niche differentiation, especially between soils and plants, leads to structural variations of microbial communities in *L. japonica* niches. Keystone taxa are important drivers in microbiome assembly and functioning, and they are positively associated with plant productivity (Banerjee et al., 2018). Our results indicate that Proteobacteria, Acidobacteriota, and Actinobacteriota are predominant bacterial phyla in *L. japonica*. A similar trend was reported in *Cycas panzhihuaensis* in a previous study (Zheng et al., 2019). The members of Actinobacteriota possess potential inhibitory effect on plant pathogens, and they may be involved in the selection of functions corresponding to plant health requirements (Sanguin et al., 2009). The bacterial genera *Pseudomonas*, *Pantoea*, *Kluyvera*, and *Enterobacter* are the core keystone, and they are dominant in plant samples. Similarly, *Pseudomonas* is found to be more abundant in the roots than in the soils of *Casuarina equisetifolia* (Lin et al., 2022). *Pseudomonas* is also dominant in many other plants, and it is known as plant growth–promoting bacteria (Chen et al., 2019). *Pseudomonas* can play a key role in regulating host fitness, pathogen inhibition, and plant tolerance. Ascomycota and Basidiomycota were the most abundant phyla in *L. japonica* microbiota. The abundance of Basidiomycota was lower than that of Ascomycota in bulk soil and rhizosphere soil. This phenomenon may be explained by the fact that Basidiomycota is considered as K-strategists, and it has difficulty surviving in rhizosphere soil that is rich in root exudates (Bastian et al., 2009). The core fungal genera *Cryptococcus*, *Alternaria*, and *Erysiphe* were dominant in plant samples; however, *Mortierella* and *Fusarium* were dominant in the rhizosphere soil. The members of *Fusarium* are identified as core fungal microbiota, and they can cause root rot (Punja et al., 2021). As a core flora in *L. japonica*, *Fusarium* provides a possibility for studying the interactions of host-dependent pathogens to comprehensively understand the soil health of *L. japonica*. Collectively, different compartment niches can recruit microbial groups with specific functions and adaptations (Cordovez et al., 2019). The identification of these dominant taxa provides necessary information for formulating strategies to control the microbial community in *L. japonica*.

Ascertaining potential sources of bacterial and fungal communities in *L. japonica* is important to understand the interactions among soils, microbes, and plants. In a previous study, a large proportion of microbial taxa were shared in soil and plant compartments, and their origin and transmission mode were reported (Wei et al., 2021). Nevertheless, very little is known about the enrichment and assembly of the microbial community in *L. japonica*. Here, the origin and proportion of endophytes in *L. japonica* were analyzed using source tracking analysis. The results indicated that the microbial communities in the rhizosphere soil (bacteria, 59.58%; fungi, 68.37%) were derived from bulk soil, and endophytes (bacteria, 43.41%; fungi, 66.80%) in the root were derived from the rhizosphere soil. These results are not

surprising because the bulk soil is an important microbial reservoir and the main source of microbial communities in rhizosphere soil, and the roots serve as an important transition boundary and play a pivotal role in the selection of microbial communities from rhizosphere soil to the root (Hu et al., 2020). Thus, the diversity of bacteria and fungi in plants is lower than that in soil. These findings demonstrate the potential sources and driving forces of endophytes in *L. japonica*, which will provide an important basis for the interactions among plant, soil, and microbes.

Conclusion

In this study, we provide relatively comprehensive evidence for the contribution of compartment niches to the structure and assembly of bacterial and fungal communities in *L. japonica*. Our results indicate that the microbiome assembly of *L. japonica* along the soil–plant continuum is profoundly affected by the compartment niches rather than host ecotypes. Bacterial diversity and network complexity decreased from the soil to the root and flower. Meanwhile, fungal diversity and network complexity gradually decreased from the soil to the root and stem but increased from the stem to the leaf and flower. The bacterial and fungal keystone taxa were found in different compartment niches, and they showed significant differences. Furthermore, the bacterial and fungal communities of *L. japonica* were predominantly from bulk soil. These findings provide comprehensive understanding of the assembly and origin of the bacterial and fungal communities in *L. japonica* with different cultivars and emphasize the importance of the host selection effect.

Declarations

Author contributions

G.F.W., L.L.D. and Z.Y.Z.: Conceptualization. G.Z.Z., X.C and Y.D.D.: Writing-original draft preparation and Data curation. Y.X.Z. and Z.Y.H..: Methodology. G.Z.Z., L.P.S. and Z.Y.Z.: Formal analysis, Software. L.L.G. and X.C.: Resources. L.L.D.: Writing-review and editing. L.L.D. and S.L.C.: Supervision. L.L.D. and S.L.C.: Funding acquisition.

Data availability statement

16S rRNA gene, ITS rRNA gene and transcriptome sequence data of all samples were submitted to the NCBI SAR database (http://www. ncbi.nlm.nih.gov/) under the accession PRJNA1132304 [bacteria] and PRJNA1132307 [fungi]).

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

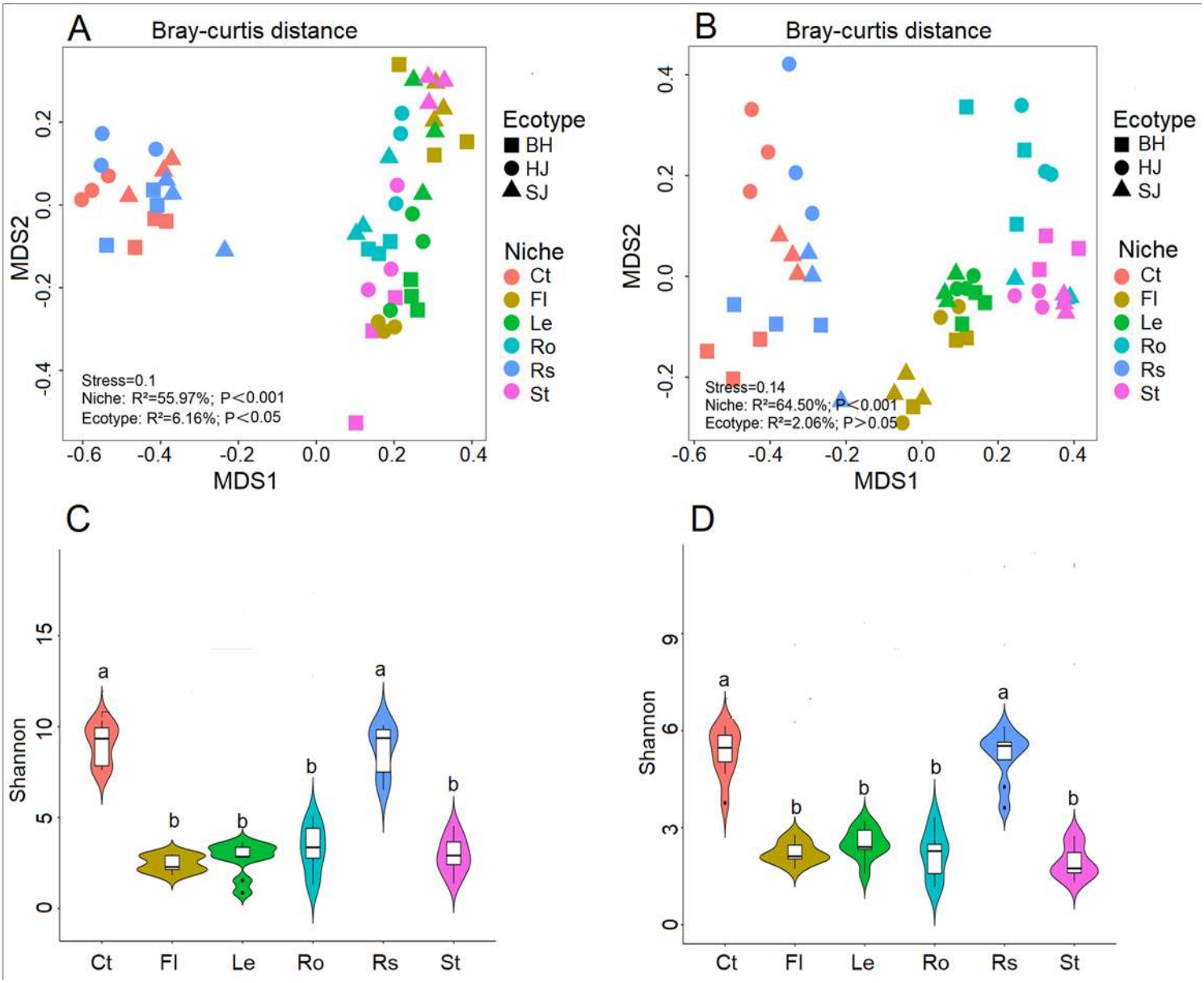


Figure 1

Non-metric multidimensional scaling (NMDS) ordinations based on Bray-curtis distance and Shannon diversity of microbial communities associated with *L. japonica*. **(A)** NMDS of bacteria. **(B)** NMDS of fungi. **(C)** Bacterial Shannon. **(D)** Fungal Shannon. SJ, BH, and HJ represent Sijihua, Beihua No. 1, and Huajin No. 3,respectively. Ct, Rs, Ro, St, Le, and Fl represent bulk soil, rhizosphere soil, root, stem, leaf, and flower, respectively.

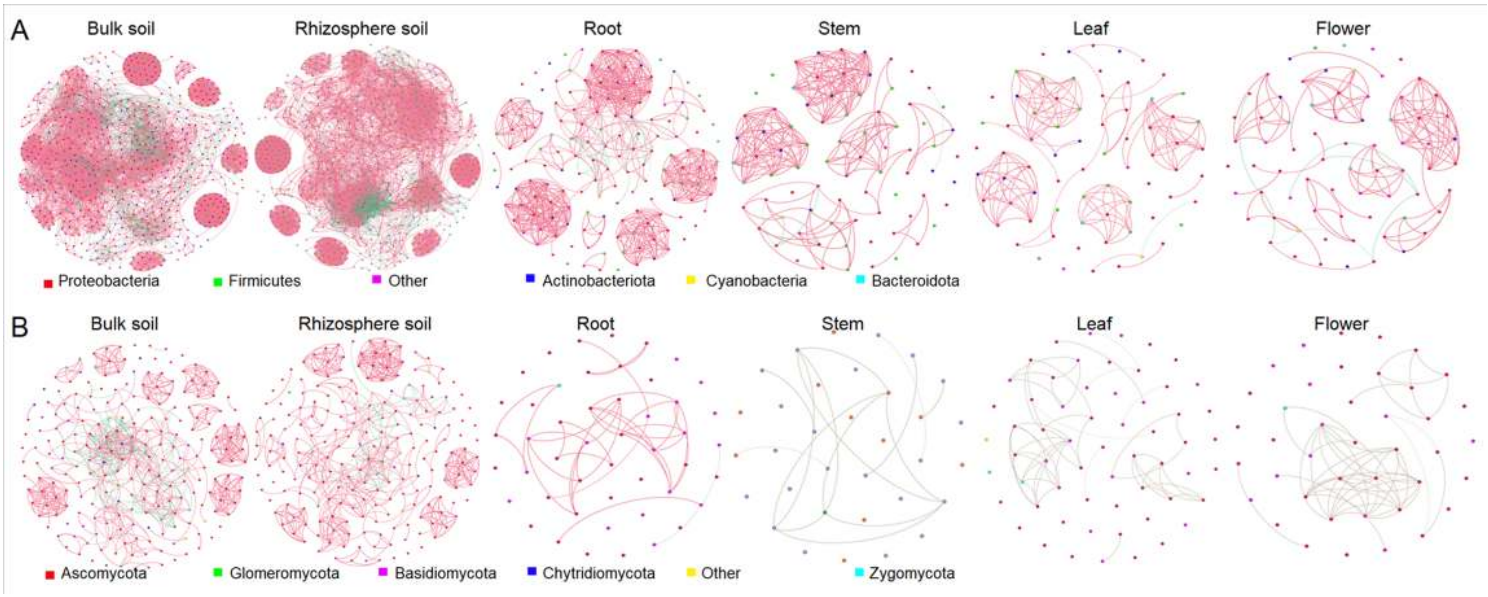


Figure 2

Co-occurrence network analysis of microbial communities associated with *L. japonica* (Spearman's |r| > 0.8 and $P < 0.05$). **(A)** Bacteria. **(B)** Fungi. Red and green represent positive and negative correlations, respectively.

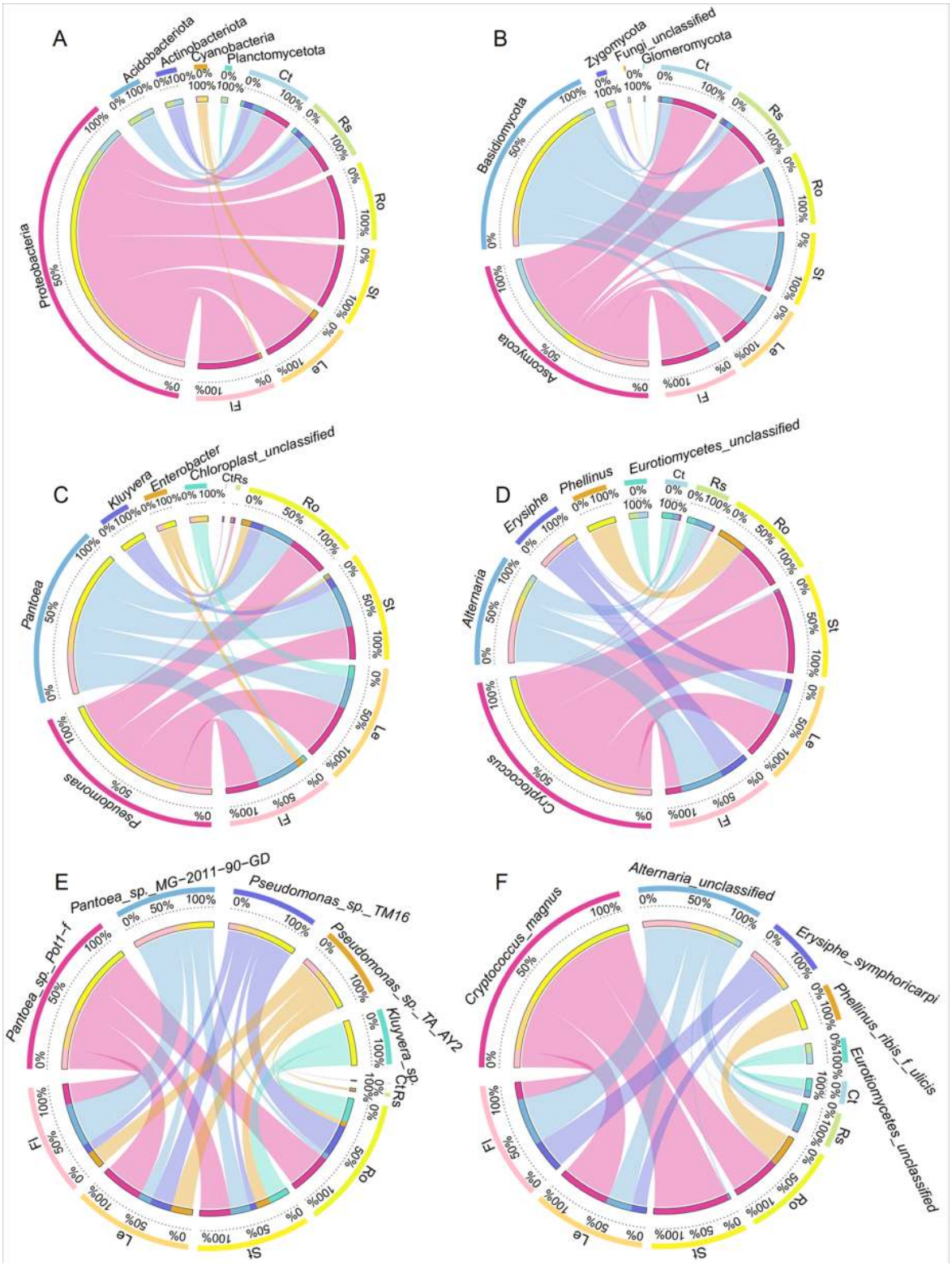


Figure 3

Circos plot displaying the distribution proportion of core microbiota of *L. japonica*. **(A)** Core bacterial phyla. **(B)** Core fungal phyla. **(C)** Core bacterial genera. **(D)** Core fungal genera. **(E)** Core bacterial species. **(F)** Core fungal species. Ct, Rs, Ro, St, Le, and Fl represent bulk soil, rhizosphere soil, root, stem, leaf, and flower, respectively.

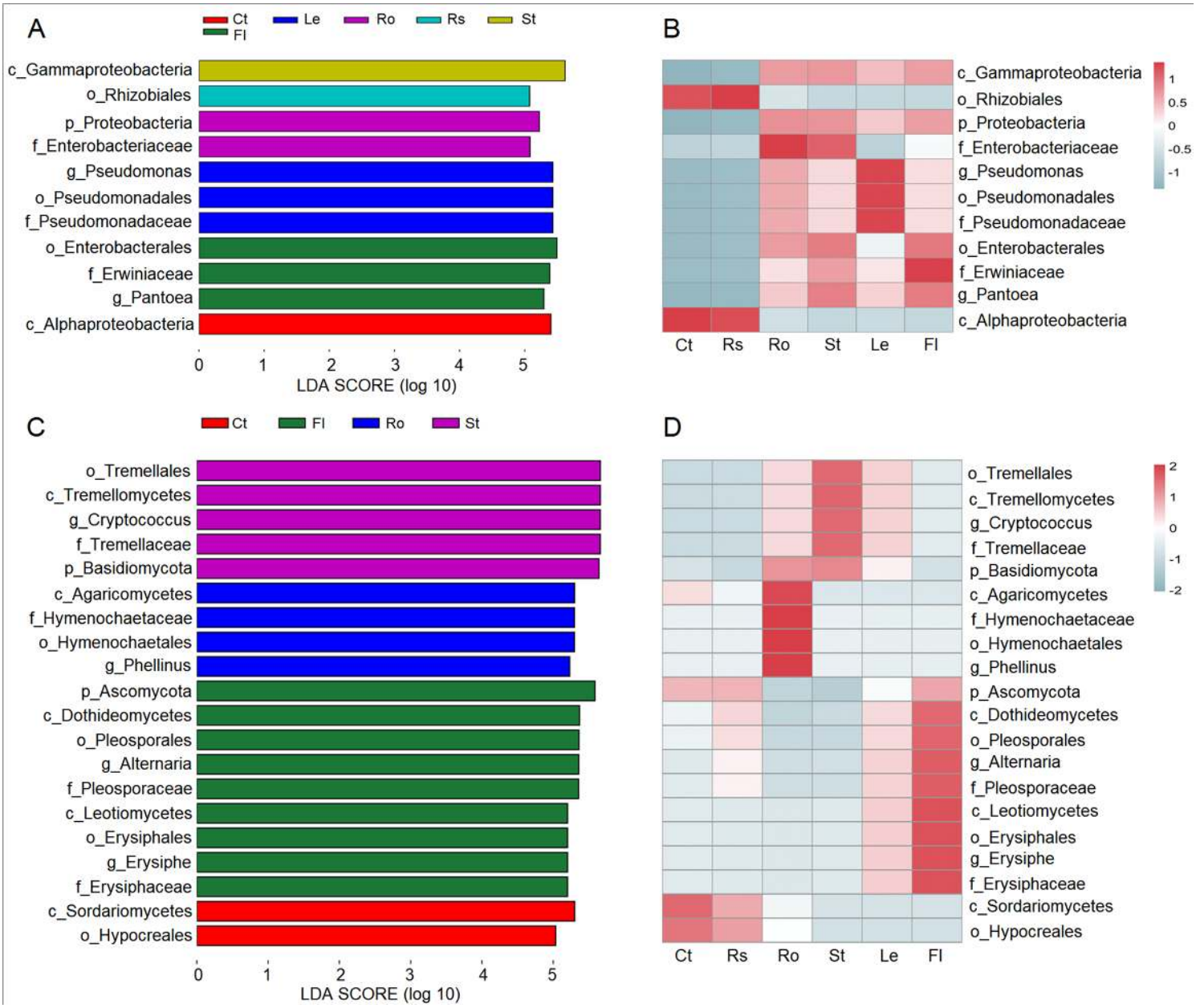


Figure 4

Linear discriminant analysis effect size identifying the potential biomarkers in different compartment niches. **(A)**. Bacterial LDA score. **(B)**. Relative abundance of bacterial biomarkers. **(C)**. Fungal LDA score. **(D)**. Relative abundance of fungal biomarkers.

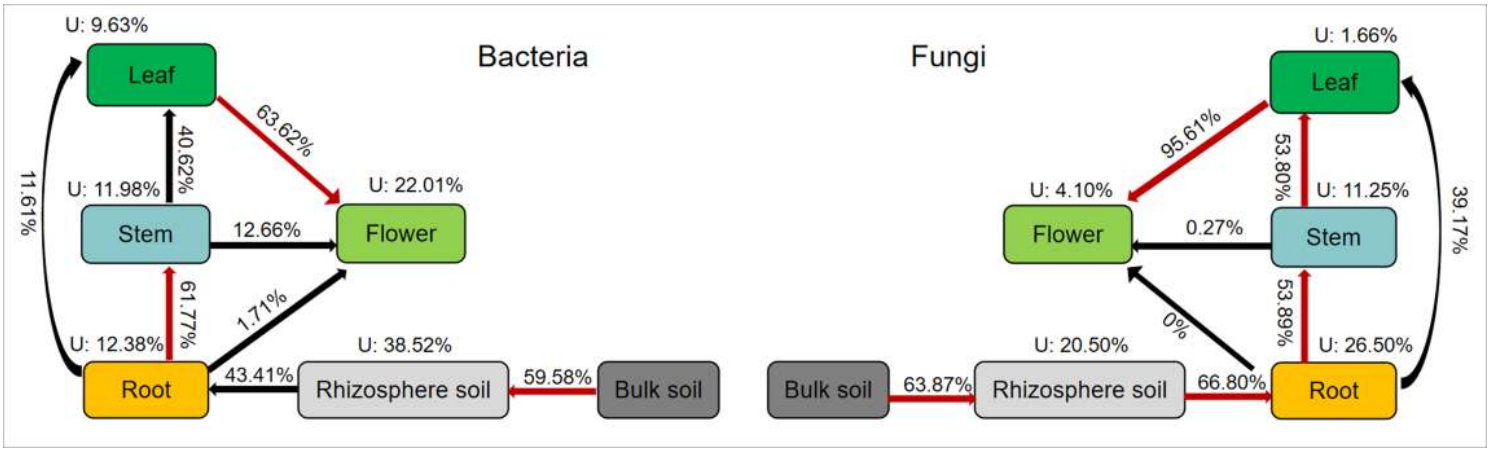


Figure 5

Source model showing the potential sources of *L. japonica* associated microbial communities. U represents the unknown source. The red arrow represents the value greater than 50%.

Supplementary Files

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