



Beneficial ecological networks dominate the dynamic root endosphere microbiome during long-term symbiosis with host plants

Jilu Che · Yaqiong Wu · Hao Yang · Wenlong Wu · Lianfei Lyu · Xiaomin Wang · Weilin Li

Received: 29 August 2023 / Accepted: 24 January 2024
© The Author(s), under exclusive licence to Springer Nature Switzerland AG 2024

Abstract

Aims Endophytes could assist the host plant in the acquisition of nutrients and tolerance of stresses during their long symbiotic life cycle with the host plant. Blueberry (*Vaccinium* spp.) is adapted to acidic soils and can inhabit harsh soil conditions. However, the dynamic variation of its endosphere microbial community under long-term cultivation remains unclear.

Methods We collected blueberry root samples of four age groups (4a, 8a, 17a, and 33a). The diversity and composition of bacterial and fungal communities in the root endosphere were analyzed, and their co-occurrence networks were constructed to explore interkingdom interactions and keystone taxa.

Results As the major phylum of the endosphere, the relative abundance of Actinobacteria decreased

with the age of the blueberry, whereas Proteobacteria increased. The fungal communities were dominated by Sordariomycetes, Eurotiomycetes, and Agaricomycetes, and these were not regularly altered with the increased blueberry age. The co-occurrence network revealed that positive interactions were observed in the bacterial and fungal communities and were consistently dominant within both intrakingdom and interkingdom networks. Among the interkingdom network, keystone taxa were identified for bacteria mainly belonging to Proteobacteria and Actinobacteria of bacteria, as well as for fungi of Ascomycota, which have potentially beneficial effects on host plants.

Conclusions Our study reveals although the diversity and composition varied with blueberry age in the root endosphere, their positive interactions of intrakingdom or interkingdom consistently dominate the

Responsible Editor: Beatriz Vazquez-de-Aldana.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11104-024-06519-8>.

J. Che · H. Yang · W. Li (✉)
State Key Laboratory of Tree Genetics and Breeding;
Co-Innovation Center for Sustainable Forestry in Southern
China, Nanjing Forestry University, 159 Longpan Road,
Nanjing 210037, China
e-mail: wlli@njfu.edu.cn

J. Che
e-mail: jlche@njfu.edu.cn

H. Yang
e-mail: yanghao_19940720@163.com

J. Che · Y. Wu (✉) · W. Wu · L. Lyu · X. Wang
Institute of Botany, Jiangsu Province and Chinese
Academy of Sciences (Nanjing Botanical Garden Mem.
Sun Yat-Sen), Jiangsu Key Laboratory for the Research
and Utilization of Plant Resources, Qian Hu Hou Cun No.
1, Nanjing 210014, China
e-mail: ya_qiong@126.com

W. Wu
e-mail: 1964wwl@163.com

L. Lyu
e-mail: njbglq@163.com

X. Wang
e-mail: xmwang525@163.com

network, and it may be such positive beneficial interactions that maintain the health of host plant during long-term symbiosis.

Keywords Blueberry · Endosphere · Bacterial and fungal community · Co-occurrence networks · Interkingdom interactions

Introduction

As the second genome of the plant, the plant root-associated microbiome provides a variety of critical beneficial effects on the growth and development of the host plant as well as resistance to the biotic/abiotic stresses (Berendsen et al. 2012; Riva et al. 2022). These microorganisms profoundly affect plant phenotypes, such as shaping root structure, increasing aboveground biomass, and activating the immune system (Bai et al. 2022). Plant phenotypes are the consequence of holobiont genetic expression of the host and its microbiota, where the plant and microorganism profiles are firmly intertwined (Matsumoto et al. 2021; Ravanbakhsh et al. 2021). The plant endosphere microbiota are the microorganisms that inhabit the interior of the plant and spend part or the entire life cycle inside the plant (Hardoim et al. 2015). In most terrestrial plants, fungi, bacteria, and to a lesser extent archaea are known to colonize the endosphere of the root (Vandenkoornhuyse et al. 2015). Distinct from external habitats, the root endosphere harbors highly specific microbial communities that exhibit much lower diversity (Bulgarelli et al. 2012, 2013). The endosphere microbiome is guided by two mutually unexclusive mechanisms, heritability and inheritance, to co-evolve with the host in a long-term symbiotic life cycle (Theis et al. 2016; Delaux and Schornack 2021; Wagner 2021). The composition of the host-associated microbiome may be conserved on evolutionary time scales, implying that it harbors phylogenetic signals of phyllosymbiosis (Mazel et al. 2018). Microbes living internal to the host, such as root endosphere, have a stronger phyllosymbiotic pattern with their host than those living external to the host, such as rhizosphere or epiphytic microorganisms (Fraune and Bosch 2007; Brucker and Bordenstein 2013; Theis et al. 2016; Abdelfattah et al. 2022). Unraveling the diversity and composition of the endosphere microbiome provides an invaluable

fundamental basis for exploring beneficial microorganisms with the potential to promote plant fitness and health.

Interactions or higher-order interactions between numerous microorganisms in a natural community typically contribute to the strong influence of its endosphere microbiota on the host plant (Michalska-Smith et al. 2021). These interactions comprised mutualistic and antagonistic effects, such as the corporation of nitrogen-fixing rhizobia bacteria and arbuscular mycorrhizal fungi supplying limiting nutrients to the host plant (van der Heijden et al. 2016). Mutualistic fungal and bacterial symbionts highlight the beneficial effects of microorganisms on host plants during microbe-plant coevolution, and these microbe-microbe associations are critical for the growth and health of host plants (Martin et al. 2017). Microbial co-occurrence network analysis enables us to visualize the microbial interactions and identify keystone taxa in the root endosphere (Barberán et al. 2012; van der Heijden et al. 2016; de Vries et al. 2018). It was found that bacterial communities play an important role in maintaining the balance of the interkingdom network of bacteria, fungi and oomycetes by offering protection to plants from pathogens (Durán et al. 2018). Fewer modules, edges, connectivity, and keystone taxa were observed in microbial networks in diseased plants, whereas healthy plants were abundant in beneficial microbial taxa with antimicrobial and plant growth-promoting capabilities (Li et al. 2023c). In addition, keystone taxa are critical participants in plant growth and nutrient utilization, and their variation may be relevant to plant growth under different long-term tillage practices (Jia et al. 2022). Of these, some of the keystone taxa identified through the co-occurrence network have been observed to be strongly associated with plant secondary metabolites (Li et al. 2023b). Also, long-term fertilization strategies influence the interactions of the root endosphere multi-kingdom communities, which markedly reduces the complexity of microbial networks and enables the identification of keystone root endophytic taxa positively associated with wheat yield via the co-occurrence network (Ma et al. 2022). Thus, the characterization of microbial cross-border interactions in the root endosphere under long-term cultivation can be explored through symbiotic networks, which provide a basis for the utilization of microbial communities to benefit host plants.

Blueberry (*Vaccinium* spp.), as an agricultural crop adapted to acidic soils, has been widely cultivated worldwide. Its fruit is highly nutritious and rich in phenols and anthocyanins (Silva et al. 2020; Pescie et al. 2021). The adaptation to harsh soil conditions may be related to their distinctive microbiome. It has been found that typical fungi can form a symbiotic association with blueberry root systems known as ericoid mycorrhizal (ERM) (Kariman et al. 2018; Vohník 2020). These fungi secrete a variety of enzymes that decompose complex organic compounds in soil environments with lower soil pH and slow transformation of organic matter, assisting the host plant in the acquisition of nutrients (Yurgel et al. 2017; Wei et al. 2020; Cai et al. 2021). In addition, plants with ERM facilitate mycorrhizal effects and increase the enrichment of particulate organic matter in surface soils, which is significant for enhancing soil carbon and nitrogen stocks in temperate forest ecosystems in the light of global climate change (Ward et al. 2023). Our previous studies have shown that the endosphere has a higher proportion of positive bacterial-fungal interkingdom interactions compared to the rhizosphere, which could be a potentially critical ecological niche for exploring functional microorganisms (Che et al. 2023). Several studies have shown that the microbiome of plants changes with age during long-term cultivation, which may be intimately related to plant growth, health status, and fertilization strategies (Marques et al. 2014; Dai et al. 2018; Huang et al. 2019; He et al. 2022). The abundance of bacteria colonized in apoplasts was found to be controlled by the age and genotype of the host plant, with a higher abundance of the bacterial community in the younger stage than in the mature stage (Jat et al. 2021). Blueberry is a perennial plant whose rhizosphere microbiome changes as the plant ages during long-term cultivation (Che et al. 2022). As an important source of assemblage for the rhizosphere microbiome, such changes have the potential to affect the rhizosphere microbiome, which is more closely related to the host plant. However, there is a lack of insight into the symbiotic relationships developed between root endosphere microbiome and their host plants over long periods of cultivation.

In this perspective, our study explores the characteristics of the blueberry root endosphere microbial community diversity and composition among different plant ages, along with the bacterial-fungal

communities interkingdom interactions. We hypothesized that the positive interkingdom interactions dominant among the co-occurrence network and beneficial taxa abundant in the root endosphere. Hence, our objectives were to (i) determine how blueberry root endosphere microbial community diversity and composition varied within plant ages, (ii) explore the bacterial-fungal communities interkingdom interaction patterns, and (iii) identify the core and keystone taxa in the root endosphere of blueberry.

Materials and methods

Study area and sampling

The sampling area was located in Lishui, Nanjing, Jiangsu Province (31°60' N, 119°20' E). Blueberry (*Vaccinium ashei* Reade) “Brightwell” cultivar of different ages were selected from the four plantations established in 1988 (33a), 2004 (17a), 2013 (8a), and 2017 (4a), respectively.

All blueberry was planted with consistent and regular practices of management in the field. Three sampling sites were selected randomly in each plantation, being 15 × 15 m² for each site. For the representative of the samples, one endosphere multipoint composite sample from 12 individual root samples was used for subsequent analysis (Che et al. 2022). The sampled plants were in the same growth stage and in good growing condition in October 2021. Root samples were stored in the ice bags and immediately transferred to the laboratory. Root samples were cleaned with sterile water and then sonicated at 60 Hz for 3 min to remove the microorganisms from the rhizosphere and rhizoplane, flash-frozen with liquid nitrogen, and stored in an ultralow temperature refrigerator until analysis (Ren et al. 2020; Che et al. 2023). A total of 12 composite root endosphere samples of four different ages of blueberry were analyzed.

DNA extraction and illumina sequencing

DNA samples were extracted from 0.5 g of root samples using FastDNA SPIN Kits (MP Biomedicals, Santa Ana, CA, USA) following the manufacturer's instructions.

The V5-V7 region and the ITS1 region of the bacterial 16 S rRNA gene and the fungal rRNA gene

were amplified, respectively. The Illumina MiSeq PE300 platform (Illumina, San Diego, USA) was used for sequencing the PCR amplification. Detailed descriptions of the DNA extraction and amplification methods are available in the [Supplementary Information](#). The raw sequences were denoised and classified, the operational taxonomic units (OTUs) were clustered using UPARSE (v7.1) with a 97% similarity, and chimeric sequences were identified and removed using UCHIME. The taxonomy of each sequence was based on our previous methods using the SILVA (v13.8, <http://www.arb-silva.de>) and UNITE (v8.0, <http://unite.ut.ee/index.php>) databases for bacteria and fungi, respectively (Che et al. 2022). In total, 683,513 bacterial and 779,069 fungal sequence reads were acquired after quality filtering, with an average of 56,959 reads and 64,130 reads, respectively. These reads were clustered into 2327 bacterial and 900 fungal OTUs, respectively.

Statistical analysis

Alpha diversity indices (Chao1 and Shannon) were assessed by Mothur software (v1.30.2), and beta diversity was assessed by Principal component analysis (PCA) using QIIME software (v1.9.1) based on the Bray–Curtis dissimilarity matrix with soil physico-chemical properties as covariates (Oksanen et al. 2022). Significant differences in alpha diversity between age groups were evaluated by Student's *t* test (Yuan et al. 2018). Permutational multivariate analysis of variance (PERMANOVA) was conducted to determine differences in beta diversity of microbial communities in the root endosphere among different age groups (Miura et al. 2019). The core microbiome was identified as the microbial species shared by all four age groups without setting cutoffs for relative abundance (Vandenkoornhuyse et al. 2015; Guo et al. 2021), and then UpSet diagrams were performed to visualize the core microbiome using the online tool of the Majorbio Cloud Platform (Ren et al. 2022). Analysis of Composition of Microbiomes (ANCOM) were performed using the ANCOM 2.0 package in R to identify taxa that differed significantly between age groups (Annavajhala et al. 2019; Lin and Peddada 2020), and all *W*-statistic cutoffs from ANCOM output (0.7, 0.8, and 0.9) are provided in the Supplementary files (Tables S4 and S5). The bacterial–fungal interkingdom co-occurrence networks were constructed using OTUs with relative abundances

greater than 0.5%. Valid co-occurrence correlations between OTUs were determined to be statistically significant (SparCC correlation $|r| > 0.7$ and $p < 0.01$) (Friedman and Alm 2012; Kurtz et al. 2015; Mendes et al. 2018). The co-occurrence network was conducted by “igraph” and “Hmisc” in R (Csardi Gabor and Tamas Nepusz 2006; Frank E Harrell Jr 2008) and visualized in Gephi software (v0.9.3). Topological characteristics describe the complex patterns of interkingdom interactions within bacterial and fungal communities (Bastian et al. 2009), in which metrics such as nodes, edges, node degree, modularity, closeness centrality, and betweenness centrality were used in our study. Nodes and edges represent taxa with significant correlation and positive/negative connections, respectively. Node degree indicates the connectivity of nodes, and modularity was used to reveal the ability of nodes to form highly connected communities (Mendes et al. 2018). In addition, closeness centrality and betweenness centrality can reflect the central importance of nodes in information dissemination and their role as bridges between the components of the network (Banerjee et al. 2019).

Results

Diversity patterns of root endosphere microbial communities

Chao1 and Shannon indices of the bacterial communities showed a trend of increasing and then decreasing with the increased age of blueberry trees in the root endosphere (Fig. 1A and B).

Among them, the Shannon index of bacterial communities in group 17a was significantly higher than those in groups 4a and 8a. A different trend was observed in that the Chao1 index of fungal communities decreased with increasing age of blueberry trees, whereas the Shannon index was affected by the ages without a clear pattern of dynamic changes with age (Fig. 1C and D). Interestingly, the Chao1 index for group 33a and the Shannon index for group 17a of the fungal communities were significantly lower than the other groups. Apparently, the alpha diversity of bacterial and fungal communities in the endosphere showed a different trend. It was observed that soil moisture, pH, TP, AK, and NO_3^- -N were significantly correlated with bacterial community beta diversity, while pH, TP, TK, AK, and NO_3^- -N were

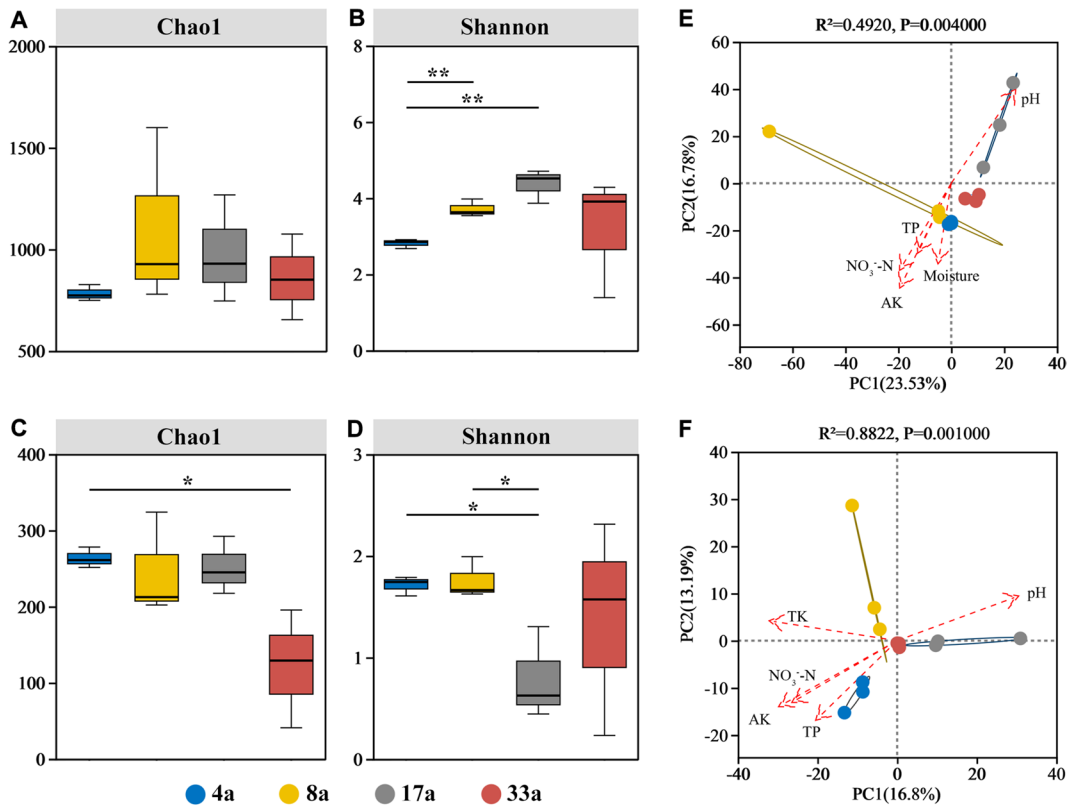


Fig. 1 Alpha diversity (Chao1 and Shannon) of bacteria (A, B) and fungi (C, D) in the endosphere of blueberry at different ages. Significant differences between the different ages are indicated in each figure panel (* $p < 0.05$, ** $p < 0.01$). Principal component analysis (PCA) of bacterial (E) and fungal (F) communities with soil physicochemical properties as covari-

ates in the endosphere of blueberry in different age groups. The 4a, 8a, 17a, and 33a represent the age (years) of the blueberry plants, respectively. TP, total phosphorus content; TK, total potassium content; AK, available potassium content; NO₃⁻-N, Ammonium nitrogen

significantly correlated with fungal community beta diversity (Table S1). In particular, soil pH is strongly related to the beta diversity of bacterial and fungal communities. Principal component analysis (PCA) with soil physicochemical properties as covariates showed a clear difference in the taxonomic composition of bacterial and fungal communities between different blueberry age groups (Fig. 1E and F). These results indicated differences in the diversity and community composition of bacterial and fungal communities within blueberry roots of different ages.

Composition of the endosphere microbial communities

Actinobacteria (77.2%, 58.0%, 45.7%, 34.7%) and Proteobacteria (18.8%, 30.6%, 43.0%, 55.6%) were

the dominant phylum in the endosphere of the bacterial community in four age groups (4a, 8a, 17a, 33a). The relative abundance of Actinobacteria gradually decreased while that of Proteobacteria gradually increased with age (Fig. 2A). *Mycobacterium* was the dominant genus decreased with age in 4a, 8a, and 17a groups, while *Ralstonia* was the dominant genus in 33a (Fig. 2B). For fungal communities, Sordariomycetes (43.0%, 48.7%) and Eurotiomycetes (36.9%, 28.5%) being the dominant class in groups 4a and 8a. And Dothideomycetes (6.7%) and Agaricomycetes (62.6%) were the most abundant class in groups 17a and 33a, respectively (Fig. 2C). In addition, higher relative abundance of ERM fungi Chaetothyriales (36.3%, 27.7%) was observed in both groups 4a and 8a, and the genus of *Oidiodendron* was more abundant in 8a group compared to the other groups (Figs. 2D and S2).

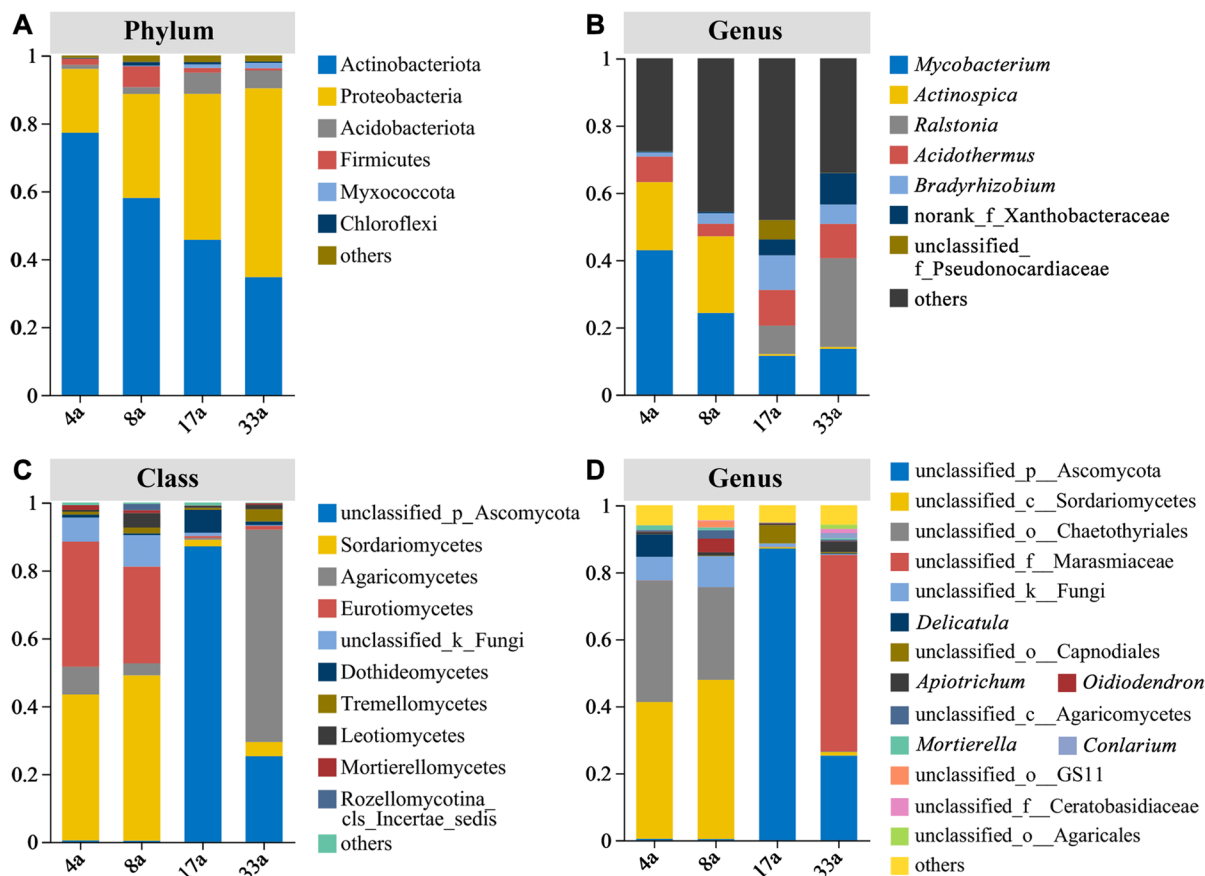


Fig. 2 Taxonomic composition of bacterial (phylum and genus level) (A, B) and fungal (class and genus level) (C, D) communities in the endosphere of blueberry at different ages. “No rank” represents no explicit taxonomic information or

name at the corresponding level, and “unclassified” represents no taxonomic information for the sequence at the corresponding level in the database

Core endosphere bacteria and fungi taxa of blueberry

We identified 427 OTUs and 40 OTUs in bacterial and fungal communities, respectively, as the core endosphere microbiome of blueberry (Fig. 3A, B). *Mycobacterium* (OTU2385, 24.69%; OTU2174, 2.90%), *Actinospica* (OTU2789, 6.55%; OTU1536, 6.45%), *Bradyrhizobium* (OTU2685, 4.26%; OTU2591, 1.86%), and *Acidothermus* (OTU2347, 3.58%; OTU2144, 3.23%; OTU2616, 1.74%) were the most abundant genus taxa in the bacterial community, with *Mycobacterium* (OTU2385, 24.69%) being the highest relative abundance (Fig. 3C; Table S2). Among the fungal community, high relative abundance was observed for the class Sordariomycetes (OTU1878, 34.96%), order Chaetothyriales (OTU1693, 25.58%), and family Marasmiaceae (OTU1720, 23.58%)

(Fig. 3D; Table S3). In particular, the fungi of the orders Chaetothyriales and Helotiales are both the core endosphere microbiome, of which those belonging to such orders may form mutually beneficial symbiotic relationships with the blueberry.

Age-associated endosphere microbiome signature

The distinct taxa among different ages were identified by using ANCOM. A total of 21 and 7 distinct bacterial and fungal taxa were identified (W-statistic > 0.9), respectively (Table 1). Significantly differentially taxa of bacteria primarily belonged to the phylum Proteobacteria and Actinobacteria, while those of fungi belonged to the phylum Ascomycota, Rozellomycota, and Basidiomycota.

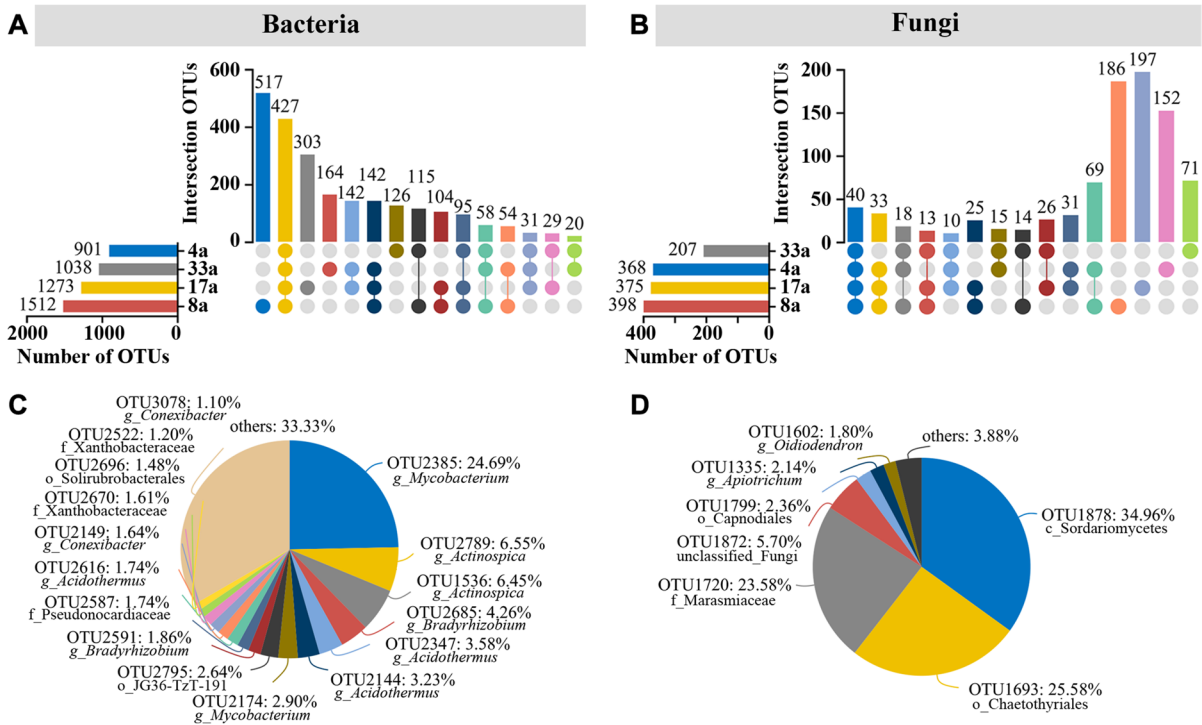


Fig. 3 Core taxa of blueberry endosphere microbiome. UpSet diagram showing specific and shared operational taxonomic units (OTUs) of the endosphere bacterial (A) and fungal (B)

Interkingdom co-occurrence network and keystone taxa of endosphere microbiome

We further characterized the bacterial–fungal interkingdom interactions in the co-occurrence network of the endosphere microbiome of blueberry (Fig. 4A, D; Table S6). The interkingdom microbial co-occurrence network revealed that OTUs of bacteria and fungi were mainly clustered in five modules, with a significantly higher degree in module 1 compared to the other modules (Fig. 4A, B). The bacterial community accounted for 77% of module 1, with the most dominant phylum being Proteobacteria and Actinobacteria. Module 1 had the highest total number of nodes and was more abundant in bacteria than the other modules, and module 5 had a higher number of fungal nodes (Fig. 4C). In the interkingdom network, the average degree of bacterial communities was much higher than that of fungal communities, with

communities of blueberry at different ages. The relative abundance of core taxa of bacterial (C) and fungal (D) taxa in all samples of each group

average degrees of 4.161 and 3.333, respectively, as well as the number of nodes was much higher than that of the fungal community (Fig. 4E, F; Table S6). The positive interactions dominated the bacterial–fungal interkingdom network, with the number of edges of positive and negative interactions being 213 and 70, respectively. More intense interactions were observed in the intrakingdom in the bacterial community, and the positive interactions were consistently dominant the both within intrakingdom and interkingdom (Fig. 4G). Thus, a greater abundance of bacterial nodes with higher degree were observed to interact positively within the endosphere of the blueberry. Interestingly, all the interactions in the intrakingdom were observed to be positive in the fungal community. The keystone taxa were identified in the interkingdom network, which belong to the phyla Proteobacteria, Actinobacteria, and Acidobacteriota of bacteria, and to the phyla Ascomycota of fungi (Fig. 5; Table S7).

Table 1 Significantly differentially taxa of the bacterial and fungal communities in the root endosphere of blueberry among different age groups using ANCOM 2.0 (W-statistic > 0.9)

OTU ID	W_stat.	Phylum	Class	Order	Family	Genus
Bacteria						
OTU1752	1504	Proteobacteria	Gammaproteobacteria	Burkholderiales	Alcaligenaceae	Bordetella
OTU3045	1480	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Pseudolabrys
OTU2798	1460	Actinobacteriota	Actinobacteria	unclassified	unclassified	unclassified
OTU3221	1459	Actinobacteriota	Actinobacteria	Micrococcales	Microbacteriaceae	Humibacter
OTU1738	1457	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	Acidocella
OTU2795	1451	Proteobacteria	Gammaproteobacteria	JG36-TzT-191	norank	norank
OTU2581	1445	Acidobacteriota	Acidobacteriae	Subgroup_2	norank	norank
OTU1577	1443	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Pseudolabrys
OTU2935	1430	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	Chujaibacter
OTU2717	1428	Proteobacteria	Alphaproteobacteria	Micropepsales	Micropepsaceae	norank
OTU2081	1425	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	unclassified
OTU1733	1424	Actinobacteriota	Actinobacteria	Micrococcales	Microbacteriaceae	Galbitalea
OTU2789	1421	Actinobacteriota	Actinobacteria	Catenulisporales	Actinospicaceae	Actinospica
OTU2746	1400	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	Acidocella
OTU1557	1399	Chloroflexi	Ktedonobacteria	Ktedonobacterales	Ktedonobacteraceae	unclassified
OTU4262	1399	Actinobacteriota	Actinobacteria	Kineosporiales	Kineosporiaceae	unclassified
OTU2379	1393	Proteobacteria	Alphaproteobacteria	norank	norank	norank
OTU1536	1389	Actinobacteriota	Actinobacteria	Catenulisporales	Actinospicaceae	Actinospica
OTU2823	1374	Firmicutes	Bacilli	Thermoactinomycetales	Thermoactinomycetaeaceae	Novibacillus
OTU4496	1360	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	norank
OTU2522	1358	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	norank
Fungi						
OTU1872	448	unclassified	unclassified	unclassified	unclassified	unclassified
OTU1720	436	Basidiomycota	Agaricomycetes	Agaricales	Marasmiaceae	unclassified
OTU1693	435	Ascomycota	Eurotiomycetes	Chaetothyriales	unclassified	unclassified
OTU1391	426	Rozellomycota	Rozellomycotina_cls_Incertae_sedis	GS11	unclassified	unclassified
OTU4586	417	unclassified	unclassified	unclassified	unclassified	unclassified
OTU1717	416	Ascomycota	Leotiomycetes	Helotiales	unclassified	unclassified
OTU1385	415	Rozellomycota	Rozellomycotina_cls_Incertae_sedis	GS11	unclassified	unclassified

Discussion

Variation in the endosphere microbial communities of blueberry among different age

Endosphere microbiomes dynamically change with the growth and development of plants (Xiong et al. 2021a). Our results suggested that the Chao1 and Shannon indices of the bacterial community followed an increasing and then decreasing trend with the increasing age of the blueberry, whereas those

of the fungal community showed a decreasing trend. Previous studies on the halophyte plant *Suaeda salsa* indicated that the Shannon index of bacterial community was significantly higher in the mature ages compared to the young ages, which is consistent with our findings, with the exception of the Shannon index of group 33a (Zhou et al. 2023). However, the study of root endosphere bacterial microbiomes of licorice (*Glycyrrhiza uralensis* Fisch.) showed different patterns that the alpha diversity did not clearly differ among different years old seedlings of 1-y, 2-y, and

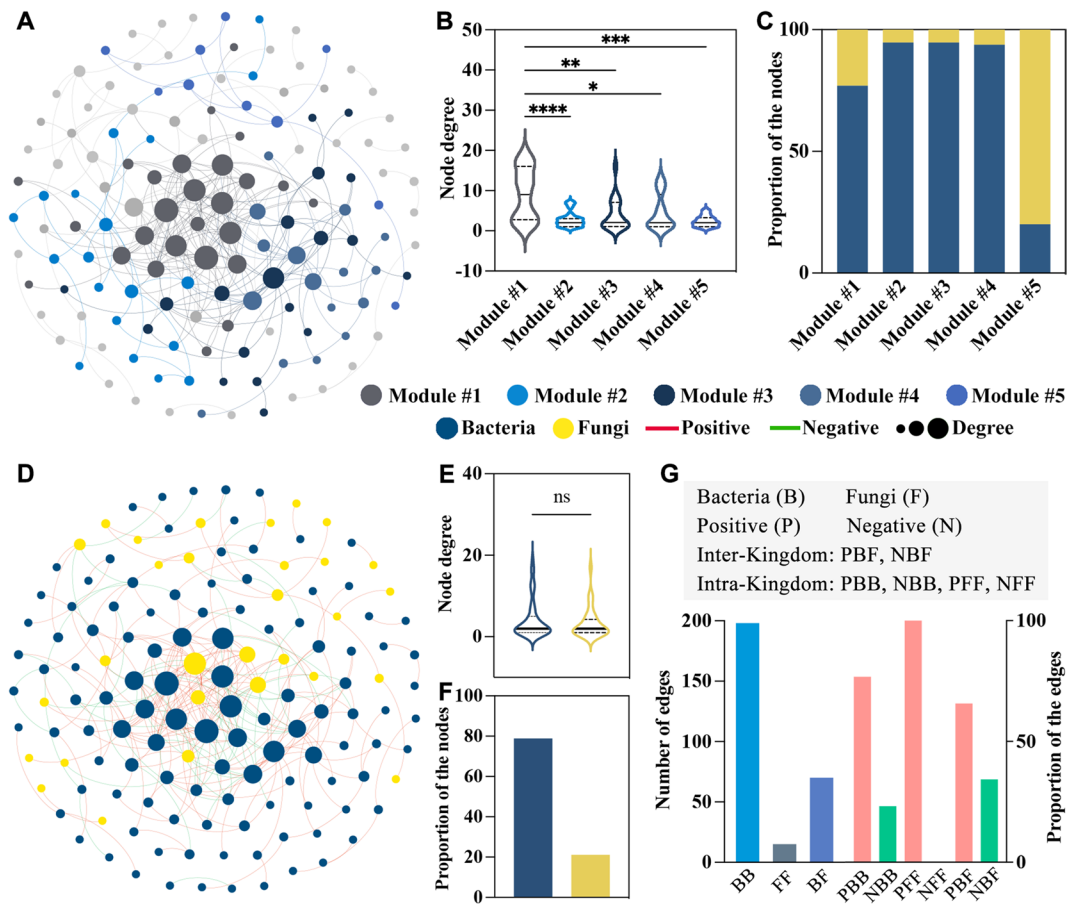


Fig. 4 Co-occurrence network analysis of bacterial–fungal interkingdom network in the endosphere of blueberry. The networks are colored based on the modularity class (A) and bacterial–fungal interkingdom network patterns (D). Node degree in each modularity class (B), and node degree of the bacteria and fungi in the interkingdom network (E). Proportion of the

bacterial and fungal nodes in each modularity class (C) and the interkingdom network (F). The relative abundances of multiple correlations between bacterial and fungal taxa in the interkingdom networks (G). Connections indicated significant ($p < 0.01$) correlations, which were divided into positive (SparCC's $r > 0.7$; red) or negative (SparCC's $r < -0.7$; green) correlations

4-y (Liu et al. 2020). This may be due to the differences in the length of years the plants have been cultivated, thus long-term or short-term cultivation may also have distinct impacts on the diversity of the endosphere bacterial community. Among the fungal community, groups 4a and 8a showed no significant changes in Chao1 and Shannon indices, which is consistent with the finding that vine age influences fungal community composition without affecting richness (Biget et al. 2021). Microbial communities develop under conditions of complex factors over a long period of time; for example, endophytic bacteria thrive in spring and summer with seasonal timing (Fuchs et al. 2017), in relation to iron and potassium

content in leaves (Khorsandy et al. 2016), and are correlated with manganese content and pH in roots (Lee and Hawkes 2021). We also analyzed correlations between each soil physicochemical property and the overall composition of bacterial and fungal communities using Mantel test analyses (Table S8). It was found that endosphere bacterial communities were predominantly correlated with AK ($R^2 = 0.554$, $P < 0.001$), while fungal communities were largely associated with TK ($R^2 = 0.572$, $P < 0.001$). In addition, both endosphere bacterial and fungal communities were correlated with soil pH, TP, AP, and NO_3^- -N. Thus, the lower richness of the 33a group and abundance of the 17a group possibly have been

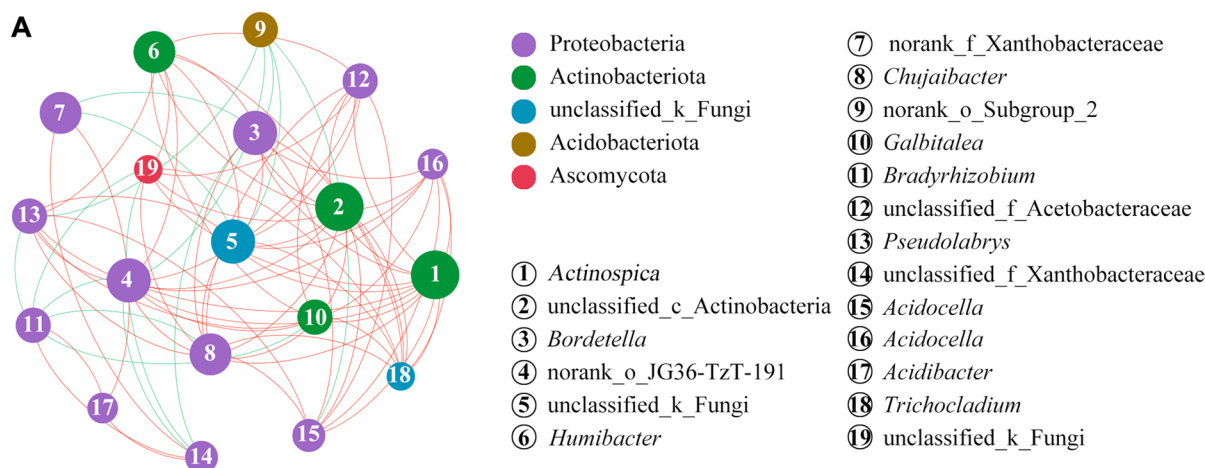


Fig. 5 Keystone taxa in bacterial–fungal interkingdom co-occurrence network of the blueberry in the endosphere. The co-occurrence network was colored at the phylum level. The 19 nodes with the highest node degree were numbered to

show taxonomic information. Connections indicated significant ($p < 0.01$) correlations, which were divided into positive (SparCC's $r > 0.7$; red) or negative (SparCC's $r < -0.7$; green) correlations

affected by complex factors during long-term cultivation. The rhizosphere microbial community serves as a source for the assembly process to the endosphere microbial, and its community composition has an impact on the endosphere to some extent. Different blueberry cultivars showed similar bacterial community composition and diverse fungal community composition in the rhizosphere (Kawash et al. 2023). In conjunction with our previous findings, the trends in the alpha diversity of the endosphere bacterial community dynamics with age were similar to those of the rhizosphere bacterial community, whereas the fungal community was quite different (Che et al. 2022). This suggests that fungi are more susceptible to differentiation under varied habitats and therefore may lead to variations in the endosphere. The different patterns of bacterial and fungal communities may be due to different driving forces in overcoming barriers to entry into the root system in the assembly process, with endophytic fungi being largely influenced by biogeography (Hacquard 2016; Zheng and Gong 2019). In addition, the fungi evolutionary conservation mechanisms may lead to a different extent of community alteration associated with the same impact factor (Zhang et al. 2022a).

The results of bacterial community composition were consistent with previous findings that Actinobacteria and Proteobacteria were the major phyla in the endosphere (Zhang et al. 2021; Che et al. 2023),

and our result showed a marked decreasing trend in the abundance of Actinobacteria and a marked increasing trend in the abundance of Proteobacteria with increasing blueberry ages. Host-associated bacterial microbial communities in the endosphere of roots, stems, and leaves have been reported to be abundant in members belonging to the phylum Proteobacteria, which are distributed among a variety of unrelated plant host species (Edwards et al. 2015; Hacquard et al. 2015). These Proteobacteria contain a variety of taxa, some of which have the potential to fix nitrogen in low-nutrient conditions, while some promote nutrient uptake and plant growth, such as *Anaeromyxobacter* and *Bacillus* (Fan et al. 2019; Zhang et al. 2022b). Their enrichment and depletion are determined by active selection of the host plant and/or opportunistic colonization of available ecological niches rather than being a passive process (Bulgarelli et al. 2013; Beckers et al. 2017). In comparison with our previous study, it was found that the relative abundance of dominant taxa in the root endosphere differed from the rhizosphere of the same plants (Che et al. 2022), which may be due to the host selection effects in diverse microhabitats (Xiong et al. 2021b; Li et al. 2022a). Therefore, after the endophytes have experienced a long lifetime with their hosts, these beneficial taxa may have established a more stable and mutually beneficial symbiotic relationship with the host plants, leading to an increasing

abundance of these taxa. In addition, it was reported that *Actinospica* belonging to Actinobacteria have the potential to suppress plant pathogens (Shi et al. 2017). Therefore, decreased abundance of *Actinospica* may be responsible for stabilizing the beneficial relationships, which may result in plants selectively sacrificing a portion of functional microorganisms capable of coping with biotic stresses in order to compensate for microbial taxa associated with nutrient availability (Ren et al. 2020). In our study, the fungal communities showed high proportions of Sordariomycetes in the endosphere (Fig. 2B). The class Sordariomycetes is worldwide distributed in predominantly terrestrial taxa, which are pathogens and endophytes of plants, arthropod and mammals, as well as mycoparasites and saprobes involved in decomposition and nutrient cycling (Zhang et al. 2006; Hongsanan et al. 2017). Some species of Sordariomycetes are biocontrol agents with significant economic value or produce chemical metabolites of importance to agricultural, pharmaceutical, or other biotechnology industries (Helaly et al. 2018; Hyde et al. 2019). Members of Sordariomycetes are not only found in our studies, but are also frequently isolated from a wide variety of plants (Chen et al. 2023). Meanwhile, Agaricomycetes and Eurotiomycetes had higher proportions in the endosphere (Fig. 2B). Many of the identified ERM fungi primarily belonging to the Eurotiomycetes, Agaricomycetes, and Leotiomycetes, which form a symbiotic relationship with blueberry root systems to promote nutrient absorption and adaptation to harsh habitats (Bizabani et al. 2016). Here, we found the known ERM fungi of the genus *Oidiendron* abundant in the 8a group, which may provide a basis for the construction of synthetic communities.

Interkingdom interactions in bacterial–fungal communities

Beneficial interactions between microorganisms can have significant effects on the growth and development of host plants (Santos and Olivares 2021). Our results indicated that positive interactions dominated the interkingdom as well as the intrakingdom network of bacterial and fungal communities in the root endosphere of blueberry. In particular, there were significant positive interactions at 66% of the edges within bacterial–fungal interactions (Table S6). Previous studies have shown that the positive interkingdom

interactions increased along the soil-rhizosphere-root continuum, and that these interactions were much higher in the root endosphere than in the other root compartment niches (Che et al. 2023). Endophytic root microbial communities and host plants are an evolutionary homeostatic unit, evolving adaptive strategies over time (Bai et al. 2022). The positive interactions between microorganisms at the same trophic level can generate facilitation, which is a mutually beneficial process rather than resource competition or direct antagonism (Li et al. 2022a). It was found that this cooperation between plant-growth-promoting endophytes could be reinforced to provide long-lasting beneficial effects on host plants (Yurgel et al. 2022). Furthermore, such positive interactions can have a synergistic effect on the abiotic/biotic stresses of host plants. Previous studies demonstrated that the arbuscular mycorrhizal fungi can exert synergistic effects by offering varied nutrients to help plants in response to low nutrient stress (van der Heijden et al. 2016). Both bioaugmentation with antagonistic microorganisms and inhibition of naturally-occurring helper microbes can directly or indirectly suppress pathogens (Li et al. 2019, 2022b), and thus there is growing evidence confirming that pathogen suppression and subsequent disease development can be achieved by manipulating these interactions (Poppiers et al. 2023). Collectively, the positive bacterial–fungal interkingdom interactions within the root endosphere may act as an essential driver to meet the functional requirements of the host plant in maintaining ecosystem homeostasis during their growth and development.

Potential ecological functions of dominant microbes in the endosphere of blueberry

Both core microbiota and keystone microbial species may have potential critical functions associated with host plant fitness (Kariman et al. 2018). Our result showed that OTU2385, belonging to the *Mycobacterium* genus, was the most abundant genus taxa in the core bacterial community of endosphere, with relative abundance being 24.69% (Table S2). Several members of the genus *Mycobacterium* have been found to host plants with significantly lower relative abundance in diseased plants than in healthy plants, which potentially promotes plant growth (Koskimäki et al. 2010; Hardoim et al. 2015; Li et al. 2023c;

Taniguchi et al. 2023). *Actinospica*, which have previously been identified as biocontrol agents that are effective in protecting plants from soil-borne pathogens (Shi et al. 2017), had the highest node degree in the co-occurrence network and were identified as a keystone taxon (Fig. 5; Table S7). In addition, our results revealed that OTU2591 of *Bradyrhizobium* genus was abundant in the endosphere with a relative abundance of 1.86% (Table S2), while another OTU2685 of *Bradyrhizobium* genus was identified as a keystone taxon (Fig. 5; Table S7). It was reported that *Bradyrhizobium* genus is able to establish symbiotic relationships with a wide range of legume hosts via the Nod factor mechanisms contributing to nitrogen cycling and utilization, whereas a number of them have the capacity for plant growth promotion with non-legume plants in endophytic associations without the Nod factor mechanism (Zhang et al. 2014; Klepa et al. 2021, 2022). We identified the keystone taxa belonging to the genera *Pseudolabrys*, *Acidibacter*, and *Trichocladium*. Strains of these genera have been reported to have the ability to influence the soil carbon, nitrogen, and phosphorus cycling (Peng et al. 2022; Wu et al. 2022), to be associated with soil nutrient and iron cycling (Li et al. 2023a), and to contribute to lignocellulose degradation (Uke et al. 2021). Despite the fact that the keystone taxa belonging to the genera *Chujaibacter*, *Acidocella*, and *Galbitalea* were identified and some studies have reported their occurrence and isolated cultures, functional studies related to plants have not been reported (Sanguin et al. 2009; Kim et al. 2014; Shaffer et al. 2022; Ding et al. 2023).

Among the core microbiome of endosphere fungi, OTU1878 (Sordariomycetes class), OTU1693 (Chaetothyriales order), and OTU1720 (Marasmiaceae family) had relatively high abundance, but all were taxonomically unclassified at the genus level (Table S3). And the genus *Oidiodendron* was identified as the core microbiome, which has also been reported in previous studies to dominate the endosphere among various blueberry cultivars (Che et al. 2023). Previous studies showed that the fungi of the order Chaetothyriales and genus *Oidiodendron* comprise identified ERM fungi (Morvan et al. 2020), and thus these core microbes potentially have crucial functional characteristics for facilitating blueberry fitness in nutrient-deficient habitats (Wei et al. 2020; Pescie et al. 2021). Previous studies have shown that ERM fungi

have the ability to decompose complex organic compounds, which can facilitate plant nutrient acquisition (Kowal et al. 2018; Martino et al. 2018). In addition, the identified high relative abundant taxa OTU1878 (34.96% in the core fungal community) and keystone taxa OTU4581 both belonged to the Sordariomycetes class (Tables S3 and S7), which is consistent with previous studies indicating that the Sordariomycetes are the more abundant fungal taxa adapted to plant-associated lifestyles in the endosphere (Yurgel et al. 2018). Overall, our findings indicate that both the core and keystone taxa may have potential benefits in promoting plant nutrition or defending against pathogens. The plant-related beneficial functions of these functional microorganisms can be further explored to deepen our understanding of the complex co-evolutionary unit of plants and endophytes.

Conclusions

Our study revealed that the alpha diversity of Chao1 and Shannon indices were influenced by the age of the blueberry trees, as well as the beta diversity of the blueberry root endosphere microbiome. These community compositions were affected by plant age. Within the bacterial community, Actinobacteria and Proteobacteria were the major phylum in the endosphere of the bacterial community, with the relative abundance of Actinobacteria decreasing with the age of blueberry and that of Proteobacteria increasing with the age of blueberry. Among fungal communities, Sordariomycetes, Eurotiomycetes, and Agaricomycetes were the most abundant class, with abundant ERM fungi of *Oidiodendron* in group 8a. 427 OTUs and 40 OTUs were identified as core endosphere microbiome of blueberry in bacterial and fungal communities, respectively. 21 and 7 taxa of bacteria and fungi were identified with significant differences among different age groups, respectively. Positive interactions predominate in the bacterial–fungal interkingdom network and consistently dominate in both intrakingdom and interkingdom networks. Several keystone taxa were identified in the interkingdom network, mainly belonging to the phyla Proteobacteria and Actinobacteria of bacteria, and to the phyla Ascomycota of fungi. These identified core and keystone taxa may have potentially beneficial functions for the host plant. In conclusion, our results show that

plant age evidently influences the diversity and composition of the microbiome in the root endosphere, nevertheless, positive interactions within intrakingdom or interkingdom consistently dominate the network, and it is likely that such positive and beneficial interactions maintain homeostasis as a long-term co-evolutionary unit.

Acknowledgements This research was supported by the “JBGS” Project of Seed Industry Revitalization in Jiangsu Province (JBGS [2021]021), the Key Project of Modern Agriculture of Science and Technology Department of Jiangsu Province (BE2022372), the earmarked fund for Jiangsu Agricultural Industry Technology System (JATS [2023]466), the Chinese Central Financial Project for Cooperative Extension of Major Agricultural Technology (2022-ZYXT-06), and the Postgraduate Research & Practice Innovation Program of Jiangsu Province (KYCX23_1248).

Author contributions Jilu Che: Conceptualization, Investigation, Visualization, Data curation, Writing -original draft. Yaqiong Wu: Supervision, Conceptualization, Writing - review & editing. Hao Yang, Lianfei Lyu, Xiaomin Wang and Wenlong Wu: Investigation, Data curation. Weilin Li: Supervision, Conceptualization, Writing - review & editing.

Data availability The data that support the findings of this study are available from the National Center for Biotechnology Information (NCBI) Sequence Read Archive (<http://trace.ncbi.nlm.nih.gov/Traces/sra/>) under accession number PRJNA1005460. Detailed metadata on soil physicochemical characteristics are provided in Supplementary Table S9.

Declarations

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.

References

- Abdelfattah A, Tack AJM, Wasserman B et al (2022) Evidence for host–microbiome co-evolution in apple. *New Phytol* 234:2088–2100. <https://doi.org/10.1111/nph.17820>
- Annavajhala MK, Gomez-Simmonds A, Macesic N et al (2019) Colonizing multidrug-resistant bacteria and the longitudinal evolution of the intestinal microbiome after liver transplantation. *Nat Commun* 10:4715. <https://doi.org/10.1038/s41467-019-12633-4>
- Bai B, Liu W, Qiu X et al (2022) The root microbiome: community assembly and its contributions to plant fitness. *J Integr Plant Biol* 64:230–243. <https://doi.org/10.1111/jipb.13226>
- Banerjee S, Walder F, Büchi L et al (2019) Agricultural intensification reduces microbial network complexity and the abundance of keystone taxa in roots. *ISME J* 13:1722–1736. <https://doi.org/10.1038/s41396-019-0383-2>
- Barberán A, Bates ST, Casamayor EO, Fierer N (2012) Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME J* 6:343–351. <https://doi.org/10.1038/ismej.2011.119>
- Bastian M, Heymann S, Jacomy M (2009) Gephi: an open source software for exploring and manipulating networks. *Proc Int AAAI Conf Web Soc Med* 3(1):361–362. <https://doi.org/10.1609/icwsm.v3i1.13937>
- Beckers B, De Beeck MO, Weyens N et al (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 CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. *Trends Plant Sci* 17:478–486. <https://doi.org/10.1016/j.tplants.2012.04.001>
- Biget M, Mony C, Aubry M et al (2021) The drivers of vine-plum root microbiota endosphere composition include both abiotic and plant-specific factors. *OENO One* 51:299–315. <https://doi.org/10.20870/oeno-one.2021.55.3.4616>
- Bizabani C, Fontenla S, Dames JF (2016) Ericoid fungal inoculation of blueberry under commercial production in South Africa. *Sci Hortic* 209:173–177. <https://doi.org/10.1016/j.scienta.2016.06.029>
- Brucker RM, Bordenstein SR (2013) The hologenomic basis of speciation: Gut bacteria cause hybrid lethality in the genus *Nasonia*. *Science* (1979) 341:667–669. <https://doi.org/10.1126/science.1240659>
- Bulgarelli D, Rott M, Schlaeppi K et al (2012) Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. *Nature* 488:91–95. <https://doi.org/10.1038/nature11336>
- Bulgarelli D, Schlaeppi K, Spaepen S et al (2013) Structure and functions of the bacterial microbiota of plants. *Annu Rev Plant Biol* 64:807–838. <https://doi.org/10.1146/annurev-arplant-050312-120106>
- Cai B, Vancov T, Si H et al (2021) Isolation and characterization of endomycorrhizal fungi associated with growth promotion of blueberry plants. *J Fungi* 7. <https://doi.org/10.3390/jof7080584>
- Che J, Yang H, Wang S (2022) Long-term cultivation drives dynamic changes in the rhizosphere microbial community of blueberry. *Front Plant Sci* 13:962759. <https://doi.org/10.3389/fpls.2022.962759>
- Che J, Wu Y, Yang H et al (2023) Root niches of Blueberry Imprint increasing bacterial-fungal interkingdom interactions along the Soil-Rhizosphere-Root Continuum. *Microbiol Spectr* 11:1–15
- Chen YP, Su PW, Hyde KD, Maharachchikumbura SSN (2023) Phylogenomics and diversification of Sordariomycetes. *Mycosphere* 14:414–451. <https://doi.org/10.5943/mycosphere/14/1/5>
- Csardi G, Nepusz T (2006) The igraph software package for complex network research. *InterJ Complex Syst* 1695. <https://igraph.org/>
- Dai Z, Su W, Chen H et al (2018) Long-term nitrogen fertilization decreases bacterial diversity and favors

- the growth of Actinobacteria and Proteobacteria in agro-ecosystems across the globe. *Glob Chang Biol* 24:3452–3461. <https://doi.org/10.1111/gcb.14163>
- de Vries FT, Griffiths RI, Bailey M et al (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>
- Delaux PM, Schornack S (2021) Plant evolution driven by interactions with symbiotic and pathogenic microbes. *Science* (1979) 371. <https://doi.org/10.1126/science.aba6605>
- Ding JYM, Ho LS, Ibrahim J et al (2023) Impact of sterilization and chemical fertilizer on the microbiota of oil palm seedlings. *Front Microbiol* 14:1–17. <https://doi.org/10.3389/fmicb.2023.1091755>
- Durán P, Thiergart T, Garrido-Oter R et al (2018) Microbial interkingdom interactions in roots promote Arabidopsis Survival. *Cell* 175:973–983e14. <https://doi.org/10.1016/j.cell.2018.10.020>
- Edwards J, Johnson C, Santos-Medellín C et al (2015) Structure, variation, and assembly of the root-associated microbiomes of rice. *Proc Natl Acad Sci U S A* 112:E911–E920. <https://doi.org/10.1073/pnas.1414592112>
- Fan K, Delgado-Baquerizo M, Guo X et al (2019) Suppressed N fixation and diazotrophs after four decades of fertilization. *Microbiome* 7:1–10. <https://doi.org/10.1186/s40168-019-0757-8>
- Fraune S, Bosch TCG (2007) Long-term maintenance of species-specific bacterial microbiota in the basal metazoan Hydra. *Proc Natl Acad Sci U S A* 104:13146–13151. <https://doi.org/10.1073/pnas.0703375104>
- Friedman J, Alm EJ (2012) Inferring correlation networks from genomic Survey Data. *PLoS Comput Biol* 8:1–11. <https://doi.org/10.1371/journal.pcbi.1002687>
- Fuchs B, Krischke M, Mueller MJ, Krauss J (2017) Plant age and seasonal timing determine endophyte growth and alkaloid biosynthesis. *Fungal Ecol* 29:52–58. <https://doi.org/10.1016/j.funeco.2017.06.003>
- Guo J, Ling N, Li Y et al (2021) Seed-borne, endospheric and rhizospheric core microbiota as predictors of plant functional traits across rice cultivars are dominated by deterministic processes. *New Phytol* 230:2047–2060. <https://doi.org/10.1111/nph.17297>
- Hacquard S (2016) Disentangling the factors shaping microbiota composition across the plant holobiont. *New Phytol* 209:454–457. <https://doi.org/10.1111/nph.13760>
- Hacquard S, Garrido-Oter R, González A et al (2015) Microbiota and host nutrition across plant and animal kingdoms. *Cell Host Microbe* 17:603–616. <https://doi.org/10.1016/j.chom.2015.04.009>
- Hardoim PR, van Overbeek LS, Berg G et al (2015) The Hidden World within plants: ecological and evolutionary considerations for defining functioning of Microbial endophytes. *Microbiol Mol Biol Rev* 79:293–320. <https://doi.org/10.1128/mmb.00050-14>
- Harrell FE (2014) Hmisc: a package of miscellaneous R functions. <https://hbiostat.org/R/Hmisc/>
- He C, Wang R, Ding W, Li Y (2022) Effects of cultivation soils and ages on microbiome in the rhizosphere soil of Panax ginseng. *Appl Soil Ecol* 174:104397. <https://doi.org/10.1016/j.apsoil.2022.104397>
- Helaly SE, Thongbai B, Stadler M (2018) Diversity of biologically active secondary metabolites from endophytic and saprotrophic fungi of the ascomycete order Xylariales. *Nat Prod Rep* 35:992–1014. <https://doi.org/10.1039/c8np00010g>
- Hongsanan S, Maharachchikumbura SSN, Hyde KD et al (2017) An updated phylogeny of Sordariomycetes based on phylogenetic and molecular clock evidence. *Fungal Divers* 84:25–41. <https://doi.org/10.1007/s13225-017-0384-2>
- Huang F, Liu Z, Mou H et al (2019) Effects of different long-term farmland mulching practices on the loessial soil fungal community in a semiarid region of China. *Appl Soil Ecol* 137:111–119. <https://doi.org/10.1016/j.apsoil.2019.01.014>
- Hyde KD, Xu J, Rapior S et al (2019) The amazing potential of fungi: 50 ways we can exploit fungi industrially. *Fungal Divers* 97:1–136. <https://doi.org/10.1007/s13225-019-00430-9>
- Jat SL, Suby SB, Parihar CM et al (2021) Microbiome for sustainable agriculture: a review with special reference to the corn production system. *Arch Microbiol* 203:2771–2793. <https://doi.org/10.1007/s00203-021-02320-8>
- Jia L, Wang Z, Ji L et al (2022) Keystone microbiome in the rhizosphere soil reveals the effect of long-term conservation tillage on crop growth in the Chinese Loess Plateau. *Plant Soil* 473:457–472. <https://doi.org/10.1007/s11104-022-05297-5>
- Kariman K, Barker SJ, Tibbett M (2018) Structural plasticity in root-fungal symbioses: diverse interactions lead to improved plant fitness. *PeerJ* 2018:1–38. <https://doi.org/10.7717/peerj.6030>
- Kawash J, Oudemans PV, Erndwein L, Polashock JJ (2023) Assessment and comparison of rhizosphere communities in cultivated *Vaccinium* spp. provide a baseline for study of causative agents in decline. *Front Plant Sci* 14:1–11. <https://doi.org/10.3389/fpls.2023.1173023>
- Khorsandy S, Nikbakht A, Sabzalian MR, Pesarakli M (2016) Effect of fungal endophytes on morphological characteristics, nutrients content and longevity of plane trees (*Platanus orientalis* L.). *J Plant Nutr* 39:1156–1166. <https://doi.org/10.1080/01904167.2015.1109113>
- Kim SJ, Lim JM, Ahn JH et al (2014) Description of *Galbitalea Soli* gen. nov., sp. nov., and *Fron dih abitans sucicola* sp. nov. *Int J Syst Evol Microbiol* 64:572–578. <https://doi.org/10.1099/ijms.0.058339-0>
- Klepa MS, Helene LCF, O'hara G, Hungria M (2021) *Bradyrhizobium agreste* sp. Nov., *bradyrhizobium glycinis* sp. nov. and *bradyrhizobium diversitatis* sp. nov., isolated from a biodiversity hotspot of the genus *glycine* in Western Australia. *Int J Syst Evol Microbiol* 71. <https://doi.org/10.1099/ijsem.0.004742>
- Klepa MS, Helene LCF, O'hara G, Hungria M (2022) *Bradyrhizobium cenepequi* sp. nov., *Bradyrhizobium semiaridum* sp. nov., *Bradyrhizobium hereditatis* sp. nov. and *Bradyrhizobium australafricanum* sp. nov., symbionts of different leguminous plants of Western Australia and South Africa and definition of three.

- Int J Syst Evol Microbiol 72. <https://doi.org/10.1099/ijsem.0.005446>
- Koskimäki JJ, Hankala E, Suorsa M et al (2010) Mycobacteria are hidden endophytes in the shoots of rock plant [*Pogonatherum Paniceum* (Lam.) Hack.] (Poaceae). *Environ Microbiol Rep* 2:619–624. <https://doi.org/10.1111/j.1758-2229.2010.00197.x>
- Kowal J, Pressel S, Duckett JG et al (2018) From rhizoids to roots? Experimental evidence of mutualism between liverworts and ascomycete fungi. *Ann Bot* 121:221–227. <https://doi.org/10.1093/aob/mcx126>
- Kurtz ZD, Müller CL, Miraldi ER et al (2015) Sparse and compositionally robust inference of Microbial Ecological Networks. *PLoS Comput Biol* 11:1–25. <https://doi.org/10.1371/journal.pcbi.1004226>
- Lee MR, Hawkes CV (2021) Plant and soil drivers of whole-plant microbiomes: variation in switchgrass fungi from coastal to mountain sites. *Phytobiomes J* 5:69–79. <https://doi.org/10.1094/PBIOMES-07-20-0056-FI>
- Li M, Wei Z, Wang J et al (2019) Facilitation promotes invasions in plant-associated microbial communities. *Ecol Lett* 22:149–158. <https://doi.org/10.1111/ele.13177>
- Li F, Jin Z, Wang Z et al (2022a) Host plant selection imprints structure and assembly of fungal community along the soil-root continuum. *mSystems* 7. <https://doi.org/10.1128/msystems.00361-22>
- Li M, Pommier T, Yin Y et al (2022b) Indirect reduction of *Ralstonia solanacearum* via pathogen helper inhibition. *ISME J* 16:868–875. <https://doi.org/10.1038/s41396-021-01126-2>
- Li L, Yang X, Tong B et al (2023a) Rhizobacterial compositions and their relationships with soil properties and medicinal bioactive ingredients in *Cinnamomum migao*. *Front Microbiol* 14:1–14. <https://doi.org/10.3389/fmicb.2023.1078886>
- Li Y, Jin J, Li P et al (2023b) Regional variations and plant compartments shape the community structures of the endophytic microbiome and secondary metabolites of *Astragalus mongholicus*. *Ind Crops Prod* 192:116037. <https://doi.org/10.1016/j.indcrop.2022.116037>
- Li Y, Qi G, Xie Z et al (2023c) The endophytic root microbiome is different in healthy and *ralstonia solanacearum*-infected plants and is regulated by a consortium containing beneficial endophytic bacteria. *Microbiol Spectr* 11. <https://doi.org/10.1128/spectrum.02031-22>
- Lin H, Peddada SD (2020) Analysis of compositions of microbiomes with bias correction. *Nat Commun* 11:1–11. <https://doi.org/10.1038/s41467-020-17041-7>
- Liu Y, Li Y, Luo W et al (2020) Soil potassium is correlated with root secondary metabolites and root-associated core bacteria in licorice of different ages. *Plant Soil* 456:61–79. <https://doi.org/10.1007/s11104-020-04692-0>
- Ma Y, Wang D, Guo X et al (2022) Root stoichiometry explains wheat endophytes and their link with crop production after four decades of fertilization. *Sci Total Environ* 846:157407. <https://doi.org/10.1016/j.scitotenv.2022.157407>
- Marques JM, da Silva TF, Vollu RE et al (2014) Plant age and genotype affect the bacterial community composition in the tuber rhizosphere of field-grown sweet potato plants. *FEMS Microbiol Ecol* 88:424–435. <https://doi.org/10.1111/1574-6941.12313>
- Martin FM, Uroz S, Barker DG (2017) Ancestral alliances: Plant mutualistic symbioses with fungi and bacteria. *Science* (1979) 356. <https://doi.org/10.1126/science.aad4501>
- Martino E, Morin E, Grelet GA et al (2018) Comparative genomics and transcriptomics depict ericoid mycorrhizal fungi as versatile saprotrophs and plant mutualists. *New Phytol* 217:1213–1229. <https://doi.org/10.1111/nph.14974>
- Matsumoto H, Fan X, Wang Y et al (2021) Bacterial seed endophyte shapes disease resistance in rice. *Nat Plants* 7:60–72. <https://doi.org/10.1038/s41477-020-00826-5>
- Mazel F, Davis KM, Loudon A et al (2018) Is host filtering the main driver of phyllosymbiosis across the Tree of Life? *mSystems* 3. <https://doi.org/10.1128/msystems.00097-18>
- Mendes LW, Raaijmakers JM, De Hollander M et al (2018) Influence of resistance breeding in common bean on rhizosphere microbiome composition and function. *ISME J* 12:212–224. <https://doi.org/10.1038/ismej.2017.158>
- Michalska-Smith M, Song Z, Spawn-Lee SA et al (2021) Network structure of resource use and niche overlap within the endophytic microbiome. *ISME J* 1–12. <https://doi.org/10.1038/s41396-021-01080-z>
- Miura T, Sánchez R, Castañeda LE et al (2019) Shared and unique features of bacterial communities in native forest and vineyard phyllosphere. *Ecol Evol* 9:3295–3305. <https://doi.org/10.1002/ece3.4949>
- Morvan S, Megloulou H, Lounès-Hadj Sahraoui A, Hijri M (2020) Into the wild blueberry (*Vaccinium angustifolium*) rhizosphere microbiota. *Environ Microbiol* 22:3803–3822. <https://doi.org/10.1111/1462-2920.15151>
- Oksanen J, Simpson G, Blanchet F et al (2024) Vegan: community ecology package. R package version 2.6-5. <https://github.com/vegandevs/vegan>
- Peng Z, Guo X, Xiang ZX et al (2022) Maize intercropping enriches plant growth-promoting rhizobacteria and promotes both the growth and volatile oil concentration of *Atractylodes lancea*. *Front Plant Sci* 13:1–14. <https://doi.org/10.3389/fpls.2022.1029722>
- Pescio MA, Fradkin M, Lavado RS, Chiochio VM (2021) Endophytic fungi in blueberry cultivars, in three production areas of Argentina. *Physiol Mol Plant Pathol* 115:101662. <https://doi.org/10.1016/j.pmp.2021.101662>
- Poppeliers SW, Sánchez-Gil JJ, de Jonge R (2023) Microbes to support plant health: understanding bioinoculant success in complex conditions. *Curr Opin Microbiol* 73:102286. <https://doi.org/10.1016/j.mib.2023.102286>
- Ravanbakhsh M, Kowalchuk GA, Jousset A (2021) Targeted plant hologenome editing for plant trait enhancement. *New Phytol* 229:1067–1077. <https://doi.org/10.1111/nph.16867>
- Ren Y, Xun W, Yan H et al (2020) Functional compensation dominates the assembly of plant rhizospheric bacterial community. *Soil Biol Biochem* 150:107968. <https://doi.org/10.1016/j.soilbio.2020.107968>
- Ren Y, Yu G, Shi C et al (2022) Majorbio Cloud: a one-stop, comprehensive bioinformatic platform for multiomics analyses. *iMeta* 1:1–7. <https://doi.org/10.1002/imt2.12>

- Riva V, Mapelli F, Bagnasco A et al (2022) A meta-analysis approach to defining the culturable core of plant endophytic bacterial communities. *Appl Environ Microbiol* 88:e02537–e02521
- Sanguin H, Sarniguet A, Gazengel K et al (2009) Rhizosphere bacterial communities associated with disease suppressiveness stages of take-all decline in wheat monoculture. *New Phytol* 184:694–707. <https://doi.org/10.1111/j.1469-8137.2009.03010.x>
- Santos LF, Olivares FL (2021) Plant microbiome structure and benefits for sustainable agriculture. *Curr Plant Biol* 26:100198. <https://doi.org/10.1016/j.cpb.2021.100198>
- Shaffer JP, Nothias LF, Thompson LR et al (2022) Standardized multi-omics of Earth's microbiomes reveals microbial and metabolite diversity. *Nat Microbiol* 7:2128–2150. <https://doi.org/10.1038/s41564-022-01266-x>
- Shi L, Du N, Shu S et al (2017) *Paenibacillus polymyxa* NSY50 suppresses *Fusarium* wilt in cucumbers by regulating the rhizospheric microbial community. *Sci Rep* 7:1–13. <https://doi.org/10.1038/srep41234>
- Silva S, Costa EM, Veiga M et al (2020) Health promoting properties of blueberries: a review. *Crit Rev Food Sci Nutr* 60:181–200. <https://doi.org/10.1080/10408398.2018.1518895>
- Taniguchi T, Isobe K, Imada S et al (2023) Root endophytic bacterial and fungal communities in a natural hot desert are differentially regulated in dry and wet seasons by stochastic processes and functional traits. *Sci Total Environ* 899:165524. <https://doi.org/10.1016/j.scitotenv.2023.165524>
- Theis KR, Dheilly NM, Klassen JL et al (2016) Getting the Hologenome Concept Right: an Eco-Evolutionary Framework for Hosts and Their Microbiomes. *mSystems* 1. <https://doi.org/10.1128/msystems.00028-16>
- Uke A, Nakazono-Nagaoka E, Chuah JA et al (2021) Effect of decomposing oil palm trunk fibers on plant growth and soil microbial community composition. *J Environ Manage* 295:113050. <https://doi.org/10.1016/j.jenvman.2021.113050>
- van der Heijden MGA, De Bruin S, Luckerhoff L et al (2016) A widespread plant-fungal-bacterial symbiosis promotes plant biodiversity, plant nutrition and seedling recruitment. *ISME J* 10:389–399. <https://doi.org/10.1038/ismej.2015.120>
- Vandenkoornhuyse P, Quaiser A, Duhamel M et al (2015) The importance of the microbiome of the plant holobiont. *New Phytol* 206:1196–1206. <https://doi.org/10.1111/nph.13312>
- Vohník M (2020) Ericoid mycorrhizal symbiosis: theoretical background and methods for its comprehensive investigation. *Mycorrhiza* 30:671–695. <https://doi.org/10.1007/s00572-020-00989-1>
- Wagner MR (2021) Prioritizing host phenotype to understand microbiome heritability in plants. *New Phytol* 232:502–509. <https://doi.org/10.1111/nph.17622>
- Ward EB, Polussa A, Bradford MA (2023) Dependent effects of ericoid mycorrhizal shrubs on soil carbon and nitrogen pools are accentuated under arbuscular mycorrhizal trees. 1–17. <https://doi.org/10.1111/gcb.16887>
- Wei X, Chen J, Zhang C et al (2020) Ericoid mycorrhizal fungus enhances microcutting rooting of *Rhododendron Fortunei* and subsequent growth. *Hortic Res* 7:140. <https://doi.org/10.1038/s41438-020-00361-6>
- Wu Q, Chen D, Zhou W et al (2022) Long-term fertilization has different impacts on bacterial communities and phosphorus forms in sugarcane rhizosphere and bulk soils under low-P stress. *Front Plant Sci* 13:1–13. <https://doi.org/10.3389/fpls.2022.1019042>
- Xiong C, Singh BK, He JZ et al (2021a) Plant developmental stage drives the differentiation in ecological role of the maize microbiome. *Microbiome* 9:1–15. <https://doi.org/10.1186/s40168-021-01118-6>
- Xiong C, Zhu YG, Wang JT et al (2021b) Host selection shapes crop microbiome assembly and network complexity. *New Phytol* 229:1091–1104. <https://doi.org/10.1111/nph.16890>
- Yuan J, Zhao J, Wen T et al (2018) Root exudates drive the soil-borne legacy of aboveground pathogen infection. *Microbiome* 6:121–127. <https://doi.org/10.1590/S0085-56262009000100026>
- Yurgel SN, Douglas GM, Comeau AM et al (2017) Variation in bacterial and eukaryotic communities associated with natural and managed wild blueberry habitats. *Phytobiomes J* 1:102–113. <https://doi.org/10.1094/PBIOMES-03-17-0012-R>
- Yurgel SN, Douglas GM, Dusault A et al (2018) Dissecting community structure in wild blueberry root and soil microbiome. *Front Microbiol* 9:1–14. <https://doi.org/10.3389/fmicb.2018.01187>
- Yurgel SN, Ajeethan N, Smertenko A (2022) Response of Plant-Associated Microbiome to Plant Root colonization by exogenous bacterial endophyte in perennial crops. *Front Microbiol* 13:1–12. <https://doi.org/10.3389/fmicb.2022.863946>
- Zhang N, Castlebury LA, Miller AN et al (2006) An overview of the systematics of the Sordariomycetes based on a four-gene phylogeny. *Mycologia* 98:1076–1087. <https://doi.org/10.1080/15572536.2006.11832635>
- Zhang XX, Guo HJ, Wang R et al (2014) Genetic divergence of *Bradyrhizobium* strains nodulating soybeans as revealed by multilocus sequence analysis of genes inside and outside the symbiosis island. *Appl Environ Microbiol* 80:3181–3190. <https://doi.org/10.1128/AEM.00044-14>
- Zhang J, Liu YX, Guo X et al (2021) High-throughput cultivation and identification of bacteria from the plant root microbiota. *Nat Protoc* 16:988–1012. <https://doi.org/10.1038/s41596-020-00444-7>
- Zhang M, Wang W, Bai SH et al (2022a) Linking *Phyllostachys edulis* (moso bamboo) growth with soil nitrogen cycling and microbial community of plant-soil system: effects of plant age and niche differentiation. *Ind Crops Prod* 177:114520. <https://doi.org/10.1016/j.indcrop.2022.114520>
- Zhang Y, Hua Q, Xu W et al (2022b) Response of root endosphere bacterial communities of typical rice cultivars to nitrogen fertilizer reduction at the jointing stage. *Arch Microbiol* 204:1–11. <https://doi.org/10.1007/s00203-022-03334-6>
- Zheng Y, Gong X (2019) Niche differentiation rather than biogeography shapes the diversity and composition of microbiome of *Cycas panzhihuaensis*. *Microbiome* 7:1–19. <https://doi.org/10.1186/s40168-019-0770-y>

Zhou Y, Wei Y, Ryder M et al (2023) Soil salinity determines the assembly of endophytic bacterial communities in the roots but not leaves of halophytes in a river delta ecosystem. *Geoderma* 433:116447. <https://doi.org/10.1016/j.geoderma.2023.116447>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.