Endophytic Bacterial and Fungal Community Compositions in Different Organs of Ginseng (*Panax Ginseng*)

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Abstract

Panax ginseng (*Panax ginseng* C. A. Mey.) is a perennial herb of the genus ginseng, which is used as medicine with dried roots and rhizomes. With the deepening of research on ginseng, the chemical components and pharmacological effects of ginseng have gradually been discovered. Endophytes are beneficial to host plants. However, the composition of endophytes in different organs from ginseng is poorly elucidated. The report of ginsenoside production by endophytic microbes isolated from *Panax* sp., motivated us to explore the endophytic microbial diversity related to the roots, stems, and leaves. In this study, the V5-V7 variable region of endophytic bacteria 16S rRNA gene and V1 variable region of endophytic fungi ITS gene in different organs were analyzed by high-throughput sequencing. The diversity and abundance of endophytic microbes in the three organs are different and are affected by the organs. For example, the most abundant endophytic bacterial genera in roots was *Mycobacterium*; while, the stems and leaves were *Ochrobactrum*. Similarly, the fungal endophytes, *Coniothyrium* and *Cladosporium*, were also found in high abundance in stems, in comparison to roots and leaves. The Shannon index shows that the diversity of endophytic bacteria in roots is the highest ($p<0.05$), and the richness of endophytic bacterial was root>stem ($p<0.05$). Principal coordinate analysis showed that there were obvious microbial differences among the three groups, and the endophytic bacterial composition of the leaves was closer to that of the roots. This study provides an important reference for the study of endophytic microorganisms in ginseng.

Introduction

Panax ginseng is a perennial herb of the genus ginseng that is used as medicine with dried roots and rhizomes (Lee et al. 2019). It is mainly produced in Northeast China and rarely distributed in Japan, North Korea, and other places (Singh et al. 2019). Ginseng contains ginsenosides, polysaccharides, vitamins, sterols, and other effective ingredients, which have pharmacological effects such as anti-tumor, antibacterial, immune regulation, and treatment of liver fibrosis (Yang et al. 2017; Han and Kim 2020; Yuan et al. 2021). Ginsenosides were distributed in different parts of ginseng, and their composition and content were different (Pang et al. 2015). After ginseng was approved as a new resource food and circulated in the market in 2012, its demand soared (Xu et al. 2018). However, ginseng has a long growth cycle and often suffers from diseases, which restricts the crop and quality of ginseng. Although there have been reports on the ginseng genome (Hu et al. 2019; Xu et al. 2017; Liao et al. 2021), the diversity of endophytes and plant-related microorganisms is still in its infancy and needs further research.

Endophytes are microorganisms that live in the internal tissues of living plants, usually bacteria or fungi (Pimente et al. 2011; Gouda et al. 2016). Some endophytes have benefits to host plants, including promotion of plant growth and resistance to plant diseases (Roodi et al. 2021). Endophytes can be used as a promising alternative to obtain bioactive compounds since the report of Taxol production by the endophytic fungus (*Taxomyces andreanae*) separating from *Taxus brevifolia* (Andrea et al. 1993). As a new microbial resource, endophytes have received widespread attention, and their diversity has become a vital factor affecting plant productivity and health, which have a wide range of application potential. It

has been shown that distinct groupings of microbial communities were related to different plant organs (Ottesen et al. 2013). At present, a variety of endophytes such as *Bacillus*, *Pantoea*, *Serratia*, *Enterobacter*, *Yersinia*, and *Pseudomonas*, have been separated from plants (Mashiane et al. 2018). The distribution of endophytes is related to plant genotypes, types, organs, and growth stages. The number and types of endophytes vary due to these factors (Huang 2019; Liu et al. 2019). At present, the research on the diversity of endophytes in ginseng is mainly through traditional isolation methods (Cao et al. 2021), and it is relatively rare to use high-throughput sequencing technology to study the diversity of endophytes in ginseng. To compare the diversity of endophytes in different organs of ginseng, this study used amplicon sequencing technology to analyze the diversity and structure of endophytic microbial communities in the organs. The aim is to obtain more comprehensive and accurate information on the diversity of ginseng endophytes, and lay a theoretical foundation for the future development and utilization of ginseng endophytes, a beneficial biocontrol resource.

In this study, ginseng samples were used as the research object, and high-throughput sequencing technology was used to study the diversity of endophytic microbial communities related to roots, stems, and leaves. The purpose of this research is to analyze the composition, diversity, and potential functions of endophytes in three different organs of ginseng, and provide new ideas for in-depth study of endophytes in ginseng.

Materials And Methods

Plant Materials

We collected fresh five-year-old ginseng from Baishan Lincun Medicine Development Co., Ltd. in Jingyu County of Jilin Province, China (N42°21'18.85", E126°45'44.94"). A total of 21 plant samples were collected for sequencing. The experiment set up 3 groups (roots, stems, and leaves) of sample processing, each group of samples 7 repeated processing. These ginseng plants were cultivated in the planting base which managed according to the WHO guidelines on good agricultural and collection practices for medicinal plants.

Surface sterilization of plant samples and genomic DNA isolation

To obtain endophytes, the sample was washed with tap water and disinfected with 70% ethanol for 1 min and 12% sodium hypochlorite for 3 min, followed by five rinses with sterile water. The successful removal of epiphytes by this disinfection method was confirmed by the absence of colonies in a culture of the final rinse water. These samples were frozen in liquid nitrogen. Use OMEGA Soil DNA Kit (D5625-01) (Omega Bio-Tek, Norcross, GA, USA)to extract total genomic DNA samples, following the manufacturer's instructions, and kept at -20 °C before additional analysis.

PCR Amplification

The forward primer 799F (5'-AACMGGATTAGATACCCKG-3') and the reverse primer 1193R (5'-ACGTCATCCCCACCTTCC-3') were used to obtain amplified fragments representing the V5-V7 variable region of the 16S rRNA gene. The PCR components contained 0.25 μl of Fast pfu DNA Polymerase (5U/μl), 1 μl of each (10 uM) Forward and Reverse primer, 5 μl of buffer (5×), 2 μl (2.5 mM) of dNTPs, 1 μl of Template DNA, and 14.75 μl of ddH_2O. The thermal cycle program is 5 min initial denaturation at 98 °C, followed by 25 cycles consisting of denaturation at 98 °C for 30 s, 30 s annealing at 53 °C, and extension at 72 °C for 45 s, and a final extension at 72 °C for 5 min. The forward primer ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and the reverse primer ITS2R (5'-GCTGCGTTCTTCATCGATGC-3') were used to obtain amplified fragments representing the V1 variable region of the ITS gene. The PCR components contained 0.25 μl of Fast pfu DNA Polymerase (5U/μl), 5 μl of buffer (5×), 2 μl (2.5 mM) of dNTPs, 1 μl of Template DNA, 1 μl of each (10 uM) Forward and Reverse primer and 14.75 μl of ddH_2O. The thermal cycle program is 5 min initial denaturation at 98 °C, followed by 28 cycles consisting of denaturation at 98 °C for 30 s, 30 s annealing at 55 °C, and extension at 72 °C for 45 s, and a final extension at 72 °C for 5 min. PCR amplicons were purified with Vazyme VAHTSTM DNA Clean Beads (Vazyme, Nanjing, China) and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA).

Illumina MiSeq sequencing

After the individual quantification step, the purified amplifiers were equally pooled, and paired-end 2×250 bp sequencing was performed at Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China) using the Illumina MiSeq platform and MiSeq Reagent Kit v3.

Data Processing and Statistical Analysis

Microbiome bioinformatics were implemented with QIIME2 2019.4 (Bolyen et al. 2019) with slight modification according to the official tutorials (https://docs.qiime2.org/2019.4/tutorials/). Briefly, raw sequence data were demultiplexed using the demux plugin following by primers cutting with the cutadapt plugin. Sequences were then quality filtered, denoised, merged and chimera removed using the DADA2 plugin (Callahan et al. 2016). Taxonomy was assigned to amplicon sequence variants (ASVs) using the classify-sklearn naive Bayes taxonomy classifier in the feature-classifier plugin (Bokulich et al. 2013) against the SILVA Release 132/UNITE Release 8.0 Database. Use QIIME2 and R package (v3.2.0) for sequence data analysis. During the analysis process, non-bacterial and fungal sequences have been removed to avoid the contamination of host plant chloroplasts and mitochondria. ASV-level alpha diversity estimators (Chao1, Shannon, and Good's coverage) were calculated using the ASV table in QIIME2 and visualized as box plots. The ASV files generated in sequencing analysis were used in PICRUSt2 (Douglas and Langille 2019) algorithm to predict the biological function(s) associated with endophytes communities of *P. ginseng*. The ITS and 16S rRNA functional genes were identified from MetaCyc (https://metacyc.org/) databases.

Results

ASV Full Form

After quality control and chimera sequences removal, a total of 1,003,042 effective sequences and 8928 ASVs were obtained of bacterial 16S rRNA genes. The average number of the obtained bacterial sequence was 34,449, and the average length was about 376bp. Similarly, a total of 8,06,544 effective sequences and 303 ASVs were obtained of fungal ITS regions after quality control and chimera sequences removal. The average length of the obtained fungal sequence was 243 bp. Amusingly, for both root and leaf samples, the amount of ASVs in 16S rRNA gene sequencing analysis was > 28 times more than the ASVs in ITS analysis (Supplementary Table 1). When searched in the SILVA database, these bacterial ASVs corresponded to 3458 ASVs in roots, 2142 ASVs in stems, and 2952 ASVs in leaves. These fungal ASVs corresponded to 82 ASVs from roots, 35 ASVs from stems, and 70 ASVs from leaves were identified using the UNITE database (Fig.1).

Alpha diversity of bacterial and fungal communities

Calculate alpha diversity to analyze the richness and diversity of ginseng endophytes. The diversity of endophytes in three different organs were statistically analyzed by the Kruskal-Wallis test and Dunn post hoc tests. The alpha diversity indexes of different ginseng organs are different (Fig. 2). The coverage rate of the sample is greater than 84.5%, indicating that the possibility of not detecting the sequence is small, and there are enough sequences for endophyte diversity analysis. We found that roots had the highest Shannon diversity index, which indicated that the root has the highest endophytic bacterial community diversity. The index of Chao1 indicates that roots have the highest endophytic bacterial community richness. Further analysis revealed that the Shannon index of endophytic bacteria of the root was significantly higher than the stem (p<0.05) (Fig. 2b). The endophytic fungal community diversity in the ginseng roots, stems, and leaves was less affected by the organ and showed no marked change in the diversity.

Beta diversity of bacterial and fungal communities

Use Bray-Curtis distance matrix to perform Principal coordinate (PCoA) analysis and Non-Metric multidimensional scaling (NMDS) analysis on each sample, combined with Anosim analysis, to show the overall relationship of endophytic bacterial and fungal communities structure between the samples. PCoA was achieved to determine the overall resemblance of endophytic microbial community structure among organs; it showed a considerably different community compositions of endophytic bacteria (R = 0.6459, p =0.001) and endophytic fungi (R = 0.6803, p =0.001) in different organs (Fig. 3a, b). PCoA analysis showed that the abscissa was the main coordinate component that caused differences in the composition of the endophytic microbial community in different organ samples. In terms of ASV, PC1 contributed 36.1% and 28.6% to the differences in community composition of endophytic bacteria and fungi, respectively (Fig. 3a, b). The NMDS map showed that the microbial community structure of the three organs was different (bacteria: R = 0.6459, p =0.001, fungus: R = 0.6803, p =0.001) (Fig. 3c, d).

Relative Microbial Abundances

The bacterial phyla detected in different organs of ginseng were *Proteobacteria*, *Bacteroidetes*,, and *Actinobacteria* (Fig. 4a). In the root, the main phyla were *Proteobacteria* (63.74%), *Bacteroidetes* (8.58%), *Actinobacteria* (17.77%). The main phylum in stems and leaves were *Proteobacteria* (81.80% and 66.57%, respectively). Analyze the top 10 genera of the three organs (Fig. 4b). The main genera observed in roots were *Ochrobactrum* (7.07%), *Cupriavidus* (6.14%), and *Mycobacterium* (7.77%). *Ochrobactrum* was the main genus of stems and leaves (15.91% and 13.12%, respectively). Both phylum and genus account for more than 1%.

Fungal ASVs primarily consisted of phyla *Basidiomycota*, *Ascomycota*, and *Mortierellomycota*. *Ascomycota* was the most abundant in ginseng roots, stems, and leaves (Fig. 5a). In the root, the main phyla were *Ascomycota* (86.89%), *Mortierellomycota* (2.44%), *Basidiomycota* (10.18%). The main phyla in the stem were *Ascomycota* (80.42%) and *Basidiomycota* (17.27%). The main phyla in leaves were *Ascomycota* (80.95%), *Basidiomycota* (18.99%), *Mortierellomycota* did not exist in the leaf. Analyze the top 10 genera of the three organs (Fig. 5b). The main genera observed in roots were *Aspergillus* (15.48%), *Cadophora* (13.40%), *Tetracladium* (7.2%). The main genera of stems were *Umbilicaria* (10.56%), *Simplicillium* (7.46%), *Monilinia* (6.80%). *Monilinia* (33.52%) was the main genus of leaves.

LEfSe analysis of the endogenous microbiome

To further clarify the possible interactions between the identified endophytic microbes dependencies in ginseng organ samples, linear discriminant effect size (LEfSe) was used to quantitatively analyze the biomarkers of different organs. We detected significant differences in biomarker abundance of endophytic bacteria from different groups, and identified a total of 9 biomarkers from all organ samples, as shown in the branch diagram (Fig. 6b). In the root, the significantly abundant taxa were the family *Mycobacteriaceae*, genus *Mycobacterium* and *Devosia*. In the stem, the significant taxa belonged to the class *Gammaproteobacteria*, orders *Betaproteobacteriales*, *Sphingomonadales*, family *Burkholderiaceae*, *Sphingomonadaceae*, and genus *Sphingomonas*, which were all abundant.

Similarly, a total of 6 biomarkers have been identified for endophytic fungi in different organs, as shown in the branch diagram (Fig. 6d). The significant taxa in the leaves were affiliated with different phylogenetic groups, including the order *Capnodiales*, family *Sclerotiniaceae*, and genus *Monilinia*. In the stem, the significant taxa belonged to the class *Sordariomycetes*, class *Saccharomycetes*, and order *Saccharomycetales*, which were all abundant.

Functional Characteristics of the endophytic microbiome

According to the function prediction results based on 16S rRNA analysis, we found that the microbiota of three organ-associated endophytic bacteria had relatively similar functions in 60 MetaCyc functions, and was mainly enriched in the amino acid biosynthesis; carbohydrate biosynthesis; Cofactor, Prosthetic Group, Electron Carrier, and Vitamin Biosynthesis, and so on. According to the function prediction results based on ITS analysis, we found that the microbiota of three organ-associated endophytic fungal also

had relatively similar functions in 29 MetaCyc functions, and was mainly enriched in the nucleoside and nucleotide biosynthesis; fatty acid and lipid biosynthesis; etc.

Discussion

Endophytes are important microbial resources that can interact beneficially with plants (Ahmad et al. 2021; Wu et al. 2021). In this study, the composition of the microbial communities in the roots, stems, and leaves of ginseng was collected to determine the invisible majority of these endophytic microbial communities and to gain an in-depth understanding of these endophytic microbial communities. The Venn diagram preliminarily revealed the differences in ASVs of endophytes among the three organs (Fig. 1). Bacterial communities appear to be much more abundant than fungal communities in the plant endosphere. Consistent with the number of sequencing reads obtained by 16S and ITS sequencing analysis.

In this study, the Chao1 and Shannon diversity indexes of endophytic bacterial communities in roots were significantly higher than stems (Fig. 2a), indicating that the richness and diversity of endophytic bacterial communities were higher than stems. PCoA and NMDS analysis showed that there were differences in the microbial communities between the three organs. The bacterial composition of roots and leaves was similar (Fig. 3). This is similar to the research results of wild ginseng: the bacterial composition of the leaves closer to the root rather than the stem (Khan et al. 2017). The diversity of endophytic microbial communities in plants will be affected by plants, age, tissue type, etc [24, 27]. Compared with stems, root endophytic bacteria have a higher diversity (Fig. 2), probably because most endophytic bacteria originate from rhizosphere soil (Wang et al. 2016).

The main bacterial phyla in the three different organs of ginseng were *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria*. The relative abundance of these species varied between the different organs of ginseng, which is consistent with the results of most studies (Singha et al. 2021; Hong et al. 2018; Gouda et al. 2016). Previous studies have shown that *Actinobacteria*, and *Proteobacteria* were common phyla in plant tissues, they play a key role in participating in host metabolism and maintaining the stability of the endophytic microflora (Bashir et al. 2020; Müller et al. 2015). *Actinobacteria* are widely found in plants and have antibacterial activity (Conn and Franco 2004). The relative abundance of *Actinobacteria* in roots (17.77%) is about 5 times that in stems (3.17%) and about 2 times that in leaves (9.45%); it may be the result of selective enrichment of roots that need to inhibit certain pathogenic bacteria.

The results show that *Ochrobactrum* was dominant in all three organs at the endophytic bacteria community abundance on genus level. *Mycobacterium* and *Lactobacillus* were more common in the root. Previous studies have shown that Mycobacterium and Sphiugomouas bacteria isolated from the roots of *Dendrobium moschatum* could significantly increase the germination rate of *D. moschatum* seed (Tsavkelova et al. 2007). *Pseudomonas* was found in ginseng roots, stems, and leaves. Studies have found that siderophore-producing *Pseudomonas* has the potential to promote plant growth (Sharma and Johri 2003). In comparison to stem samples, the root samples of P. *ginseng* contain a more than five-fold

higher abundance of *Actinobacteria*. *Actinobacteria* have been reported to produce a variety of microbe-derived secondary metabolites, which have a wide range of applications in agriculture, industry, and medicine (Mishra et al. 2021). Our results indicate that *Nocardioides* are present in three ginseng organ samples. Literature search shows that *Nocardioides* have been isolated as endophytes from numerous medicinal plants (Liu et al. 2019; Qin et al. 2012) and testified to produce economically vital metabolites like Artemisinin, Actinomycin, etc (Mishra et al. 2021; El-Refai et al. 2011). In this study, the composition and diversity of endophytic microbial communities related to the three organs of ginseng were compared. However, it is necessary to link the physiological characteristics and physiological indicators of the three organs with the conclusion of endophytes diversity.

The ITS analysis performed in this experiment showed that *Periconia* is only present in the leaves. Kim et al. proved that *Periconia* sp. has the ability to produce Periconicins, a diterpene compound with antibacterial activity (Kim et al. 2004). *Cladosporium*, another endophytic fungus, was about 10 times more abundant in stems in this study. Earlier, it was reported to produce Brefeldin A, cardiac glycosides, and Taxol (Khan et al. 2016). It was reported in literature that the endophytic fungi isolated from ginseng are *Aspergillus*, *Penicillium*, etc (Wu et al. 2013). In this study, they were also found by high-throughput sequencing analysis. In addition, *Monilinia*, *Simplicillium*, *Cadophora* were also abundant. The results of this study enriched the types and resources of ginseng endophytic fungi.

Endophytes present in medicinal plants are considered to be potential sources for producing various secondary metabolites, which can reduce disease symptoms caused by plant pathogens (Passari et al. 2017). The function prediction indicated that the metabolic functions of the microbial groups in the roots, stems, and leaves of ginseng were similar. The functional classification "Amino Acid Biosynthesis and Carbohydrate Biosynthesis", which were significantly abundant in all three organs. Carbohydrate catabolism can provide energy for the growth of mycelium and provide a carbon skeleton for another metabolism (Deveau et al. 2008). According to the definition proposed by Hardoim et al (Hardoim et al. 2015), endophytes should be defined in terms of habitat rather than function. The differences in the composition of endophytes in roots, stems, and leaves can be explained by differences in physiological structure and nutritional components. It is speculated that the selection preference of endophytes may be related to the secondary metabolites secreted by the host. In addition, external environmental factors also play a significant role in regulating plant growth. For example, humidity, environmental temperature, soil structure, etc., are also related to the function of root endophytes (You et al. 2016).

There are two main methods for studying the plant microflora :(I) culture-dependent methods, which are developed by developing appropriate nutrient media and/or under appropriate culture conditions; (□) culture-free methods (e.g., next-generation sequencing), which are culture-independent methods that involve sequencing microbial DNA directly from the target microorganism (Oita et al. 2021). Culture-dependent analysis of microbial community diversity reveals only a small fraction of the actual microbial population. Studies have shown that ~99% of microorganisms are unculturable (Schloss and Handelsman 2005). Recently, metagenomics has gained a lot of attention in the research of plant endophytes, which can help identify different microorganisms in the environment. The diversity and

abundance of microorganisms can be estimated through amplification and sequence analysis of specific marker genes like the 16S ribosomal RNA (rRNA) gene for bacteria and ITS regions for fungi (Fadiji et al. 2020). This method can also help to identify different microorganisms (nonculture and culturableable) present in the environment (Fadiji et al. 2020). It has been reported that high-throughput sequencing data can supplement the prediction of fungal diversity based on traditional mycology methods, and improve our understanding of fungal diversity (Baldrian et al. 2021).

In summary, this study used high-throughput sequencing technology to analyze the composition, diversity, and differences of microbial communities in the three organs of ginseng. Similar to many studies, *Proteobacteria* was the most abundant endophytic bacterial phyla and *Ascomycota* was the most abundant endophytic fungal phyla of all samples. In addition, this study also revealed the compositions of the endophytic microbial communities. The diversity of endophytic bacterial communities in ginseng roots was significantly higher than that in stems ($p<0.05$). Ginseng organs are one of the important factors affecting the diversity of endophytic microbial communities, and the soil characteristics can provide an essential contribution to the understanding of their effects on the endophytic microbial communities associated with P. *ginseng* roots. This research helps fill in the lack of scientific understanding of ginseng endophytic microbial communities. However, in-depth research on plant-endophyte symbiosis is needed in the future.

Declarations

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Availability of data and materials

Raw amplicon sequence data related to this study were deposited in the NCBI Sequence Read Archive (NCBI SRA) under Bioprojects PRJNA766834.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Andrea S, Gary S, Donald S (1993) Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. Science 260:214-216. https://doi.org/doi:10.1126/science.8097061
2. Ahmad T, Farooq S, Mirza DN, Kumar A, Mir RA, Riyaz-Ul-Hassan S (2021) Insights into the endophytic bacterial microbiome of Crocus sativus: functional characterization leads to potential agents that enhance the plant growth, productivity, and key metabolite content. Microb Ecol. https://doi.org/10.1007/s00248-021-01810-y
3. Bokulich NA, Subramanian S, Faith JJ, Gevers D, Gordon JI, Knight R, Mills DA, Caporaso JG (2013) Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nat Methods 10:57-59. https://doi.org/10.1038/nmeth.2276
4. Bolyen E, Rideout JR, Dillon MR et al (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:852-857. https://doi.org/10.1038/s41587-019-0209-9
5. Bashir S, Iqbal A, Hasnain S (2020) Comparative analysis of endophytic bacterial diversity between two varieties of sunflower *Helianthus annuus* with their PGP evaluation. Saudi J Biol Sci 27:720-726. https://doi.org/10.1016/j.sjbs.2019.12.010
6. Baldrian P, Větrovský T, Lepinay C, Kohout P (2021) High-throughput sequencing view on the magnitude of global fungal diversity. Fungal Divers 1-9. https://doi.org/10.1007/s13225-021-00472-y
7. Conn Vanessa M, Franco Christopher MM (2004) Effect of microbial inoculants on the indigenous actinobacterial endophyte population in the roots of wheat as determined by terminal restriction fragment length polymorphism. Appl Environ Microb 70:6407-6413. https://doi.org/10.1128/AEM.70.11.6407-6413.2004
8. Callahan BJ, Mcmurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 13:581-583. https://doi.org/10.1038/nmeth.3869
9. Cao K, Gao FQ, Dong JW, Xu HW (2021) Isolation and identification of endophytic fungi in ginseng and preliminary verification of its biocontrol function. Journal of Jilin Normal University(Natural Science Edition) 42(02):122-126. https://doi.org/10.16862/j.cnki.issn1674-3873.2021.02.020
10. Deveau A, Kohler A, Frey-Klett P, Martin F (2008) The major pathways of carbohydrate metabolism in the ectomycorrhizal basidiomycete *Laccaria bicolor* S238N. New Phytol 180:379-390. https://doi.org/10.1111/j.1469-8137.2008.02581.x
11. Douglas GM, Langille MGI (2019) Current and promising approaches to identify horizontal gene transfer events in metagenomes. Genome Biol Evol 11:2750-2766. https://doi.org/10.1093/gbe/evz184
12. El-Refai H, Abdelrahman H, Abdulla H, Hanna AG, Hashem A, El-Refai A, Ahmed E (2011) Studies on the production of actinomycin by *Nocardioides luteus*, a novel source. Curr Trends Biotechnol Pharm 5:1282-1297. https://doi.org/10.5958/2250-0499.2021.00042.2

13. Fadiji AE, Babalola OO (2020) Exploring the potentialities of beneficial endophytes for improved plant growth. Saudi J Biol Sci 27:3622-3633. https://doi.org/https://doi.org/10.1016/j.sjbs.2020.08.002
14. Fadiji AE, Babalola OO (2020) Metagenomics methods for the study of plant-associated microbial communities: a review. J Microbiol Meth 170:105860. https://doi.org/https://doi.org/10.1016/j.mimet.2020.105860
15. Gouda S, Das G, Sen SK, Shin H-S, Patra JK (2016) Endophytes: a treasure house of bioactive compounds of medicinal importance. Front Microbiol 7. https://doi.org/10.3389/fmicb.2016.01538
16. Hardoim Pablo R, Van Overbeek Leonard S, Berg G, Pirttilä Anna M, Compant S, Campisano A, Döring M, Sessitsch A (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiology and Molecular Biology Reviews 79:293-320. https://doi.org/10.1128/MMBR.00050-14
17. Hong CE, Kim JU, Lee JW, Lee SW, Jo IH (2018) Diversity of bacterial endophytes in *Panax ginseng* and their protective effects against pathogens. 3 Biotech 8:397. https://doi.org/10.1007/s13205-018-1417-6
18. Huang Y (2019) Illumina-based analysis of endophytic bacterial diversity of four *Allium* species. Sci Rep-UK 9:15271. https://doi.org/10.1038/s41598-019-51707-7
19. Hu H, Shen X, Liao B, Luo L, Xu J, Chen S (2019) Herbgenomics: A stepping stone for research into herbal medicine. Sci China Life Sci 62:913-920. https://doi.org/10.1007/s11427-018-9472-y
20. Han MJ, Kim D (2020) Effects of red and fermented ginseng and ginsenosides on allergic disorders. Biomolecules 10. https://doi.org/10.3390/biom10040634
21. Kim S, Shin D, Lee T, Oh K-B (2004) Periconicins, two new fusicoccane diterpenes produced by an endophytic fungus *Periconia* sp. with antibacterial activity. J Nat Prod 67:448-450. https://doi.org/10.1021/np030384h
22. Khan MIH, Sohrab MH, Rony SR, Tareq FS, Hasan CM, Mazid MA (2016) Cytotoxic and antibacterial naphthoquinones from an endophytic fungus, *Cladosporium* sp. Toxicol Rep 3:861-865. https://doi.org/10.1016/j.toxrep.2016.10.005
23. Khan Chowdhury MDE, Jeon J, Ok Rim S, Park Y-H, Kyu Lee S, Bae H (2017) Composition, diversity and bioactivity of culturable bacterial endophytes in mountain-cultivated ginseng in Korea. Sci Rep-UK 7:10098. https://doi.org/10.1038/s41598-017-10280-7
24. Liu Y, Fang BZ, Mohamad OAA, Zhang YG, Jiao JY, Dong ZY, Xiao M, Li L, Li WJ (2019) *Nocardioides ferulae* sp. nov., isolated from root of an endangered medicinal plant *Ferula songorica* Pall. ex Spreng. Int J Syst Evol Micr 69:1253-1258. https://doi.org/10.1099/ijsem.0.003261
25. Liu F, Hewezi T, Lebeis SL, Pantalone V, Grewal PS, Staton ME (2019) Soil indigenous microbiome and plant genotypes cooperatively modify soybean rhizosphere microbiome assembly. BMC Microbiol 19:201. https://doi.org/10.1186/s12866-019-1572-x
26. Liao B, Hu H, Xiao S, Zhou G, Sun W, Chu Y, Meng X, Wei J, Zhang H, Xu J, Chen S (2021) Global pharmacopoeia genome database is an integrated and mineable genomic database for traditional

medicines derived from eight international pharmacopoeias. Sci China Life Sci. https://doi.org/10.1007/s11427-021-1968-7

27. Lee H, Jeong J, Alves AC, Han S, In G, Kim E, Jeong W, Hong Y (2019) Metabolomic understanding of intrinsic physiology in *Panax ginseng* during whole growing seasons. J Ginseng Res 43:654-665. https://doi.org/https://doi.org/10.1016/j.jgr.2019.04.004
28. Müller H, Berg C, Landa BB, Auerbach A, Moissl-Eichinger C, Berg G (2015) Plant genotype-specific archaeal and bacterial endophytes but similar *Bacillus* antagonists colonize mediterranean olive trees.Front Microbiol 6. https://doi.org/10.3389/fmicb.2015.00138
29. Mashiane AR, Adeleke RA, Bezuidenhout CC, Chirima G (2018) Community composition and functions of endophytic bacteria of Bt maize. S Afr J Sci 114:88-97. https://doi.org/10.17159/sajs.2018/20170018
30. Mishra S, Goyal D, Phurailatpam L (2021) Targeted 16S rRNA gene and ITS2 amplicon sequencing of leaf and spike tissues of *Piper longum* identifies new candidates for bioprospecting of bioactive compounds. Arch Microbiol 203:3851-3867. https://doi.org/10.1007/s00203-021-02356-w
31. Ottesen AR, González Peña A, White JR et al (2013) Baseline survey of the anatomical microbial ecology of an important food plant: *Solanum lycopersicum* (tomato). BMC Microbiol 13:114. https://doi.org/10.1186/1471-2180-13-114
32. Oita S, Carey J, Kline I, Ibáñez A, Yang N, Hom EFY, Carbone I, U'ren JM, Arnold AE (2021) Methodological approaches frame insights into endophyte richness and community composition. Microb Ecol 82:21-34. https://doi.org/10.1007/s00248-020-01654-y
33. Pimentel MR, Molina G, Dionísio AP, Maróstica Junior MR, Pastore GM (2011) The use of endophytes to obtain bioactive compounds and their application in biotransformation process. Biotechnol Res Int 2011:576286. https://doi.org/10.4061/2011/576286
34. Pang SF, Li YL, Xu SQ, Sun CH, Zhao JH, Wang YP, Zhang ZD (2015) Investigation of ginsenosides in different parts of *Panax ginseng*. Ginseng Research 27:5-8. https://doi.org/10.19403/j.cnki.1671-1521.2015.01.002
35. Passari AK, Mishra VK, Singh G, Singh P, Kumar B, Gupta VK, Sarma RK, Saikia R, Donovan AO, Singh BP (2017) Insights into the functionality of endophytic actinobacteria with a focus on their biosynthetic potential and secondary metabolites production. Sci Rep-UK 7:11809. https://doi.org/10.1038/s41598-017-12235-4
36. Qin S, Yuan B, Zhang Y, Bian G, Tamura T, Sun B, Li W, Jiang J (2012) *Nocardioides panzhihuaensis* sp. nov., a novel endophytic actinomycete isolated from medicinal plant *Jatropha curcas* L. Anton Leeuw Int J G 102:353-360. https://doi.org/10.1007/s10482-012-9745-8
37. Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN (2008) Bacterial endophytes: recent developments and applications. FEMS Microbiol Lett 278:1-9. https://doi.org/10.1111/j.1574-6968.2007.00918.x
38. Roodi D, Millner JP, Mcgill CR, Johnson RD, Hea S-Y, Brookes JJ, Glare TR, Card SD (2021) Development of plant–fungal endophyte associations to suppress phoma stem canker in Brassica.

Microorganisms 9:2387. https://doi.org/10.3390/microorganisms9112387

39. Sharma A, Johri BN (2003) Growth promoting influence of siderophore-producing *Pseudomonas strains* GRP3A and PRS9 in maize (*Zea mays* L.) under iron limiting conditions. Microbiol Res 158:243-248. https://doi.org/10.1078/0944-5013-00197
40. Schloss PD, Handelsman J (2005) Metagenomics for studying unculturable microorganisms: cutting the Gordian knot. Genome Biol 6:229. https://doi.org/10.1186/gb-2005-6-8-229
41. Singh P, Kim YJ, Wang C, Mathiyalagan R, Yang DC (2016) The development of a green approach for the biosynthesis of silver and gold nanoparticles by using *Panax ginseng* root extract, and their biological applications. Artif Cell Nanomed B 44:1150-1157. https://doi.org/10.3109/21691401.2015.1011809
42. Singha KM, Singh B, Pandey P (2021) Host specific endophytic microbiome diversity and associated functions in three varieties of scented black rice are dependent on growth stage. Sci Rep-UK 11:12259. https://doi.org/10.1038/s41598-021-91452-4
43. Tsavkelova EA, Cherdyntseva TA, Klimova SY, Shestakov AI, Botina SG, Netrusov AI (2007) Orchid-associated bacteria produce indole-3-acetic acid, promote seed germination, and increase their microbial yield in response to exogenous auxin. Arch Microbiol 188:655-664. https://doi.org/10.1007/s00203-007-0286-x
44. Wu H, Yang H, You X, Li Y (2013) Diversity of endophytic fungi from roots of *Panax ginseng* and their saponin yield capacities. SpringerPlus 2:107. https://doi.org/10.1186/2193-1801-2-107
45. Wang W, Zhai Y, Cao L, Tan H, Zhang R (2016) Endophytic bacterial and fungal microbiota in sprouts, roots and stems of rice (*Oryza sativa* L.). Microbiol Res 188-189:1-8. https://doi.org/10.1016/j.micres.2016.04.009
46. Wu T, Li X, Xu J, Liu L, Ren L, Dong B, Li W, Xie W, Yao Z, Chen Q, Xia J (2021) Diversity and functional characteristics of endophytic bacteria from two grass species growing on an oil-contaminated site in the Yellow River Delta, China. Sci Total Environ 767:144340. https://doi.org/10.1016/j.scitotenv.2020.144340
47. Xu J, Chu Y, Liao B et al (2017) *Panax ginseng* genome examination for ginsenoside biosynthesis. GigaScience 6. https://doi.org/10.1093/gigascience/gix093
48. Xu J, Shen L, Chen SL, Li G, Li XW, Dong Linlin, Kon Do KJ (2018) Pollution-free criterion and technical regulations of radix ginseng cultivation in the farmland. World Science and Technology/Modernization of Traditional Chinese Medicine and Materia Medica 20:1138-1147. https://doi.org/CNKI:SUN:SJKX.0.2018-07-013
49. You Y, Park JM, Park J, Kim J (2016) Endophyte distribution and comparative analysis of diversity in wetlands showing contrasting geomorphic conditions. Symbiosis 69:21-36. https://doi.org/10.1007/s13199-015-0363-x
50. Yang Y, Ren C, Zhang Y, Wu X (2017) Ginseng: an nonnegligible natural remedy for healthy aging. Aging Dis 8:708-720. https://doi.org/10.14336/AD.2017.0707

51. Yuan S, Dong M, Zhang H, Jiang X, Yan C, Ye R, Zhou H, Chen L, Lian H, Jin W (2021) Ginsenoside PPD inhibit the activation of HSCs by directly targeting TGFβR1. Int J Biol Macromol S0141-8130:02504-6. https://doi.org/10.1016/j.ijbiomac.2021.11.098

Figures

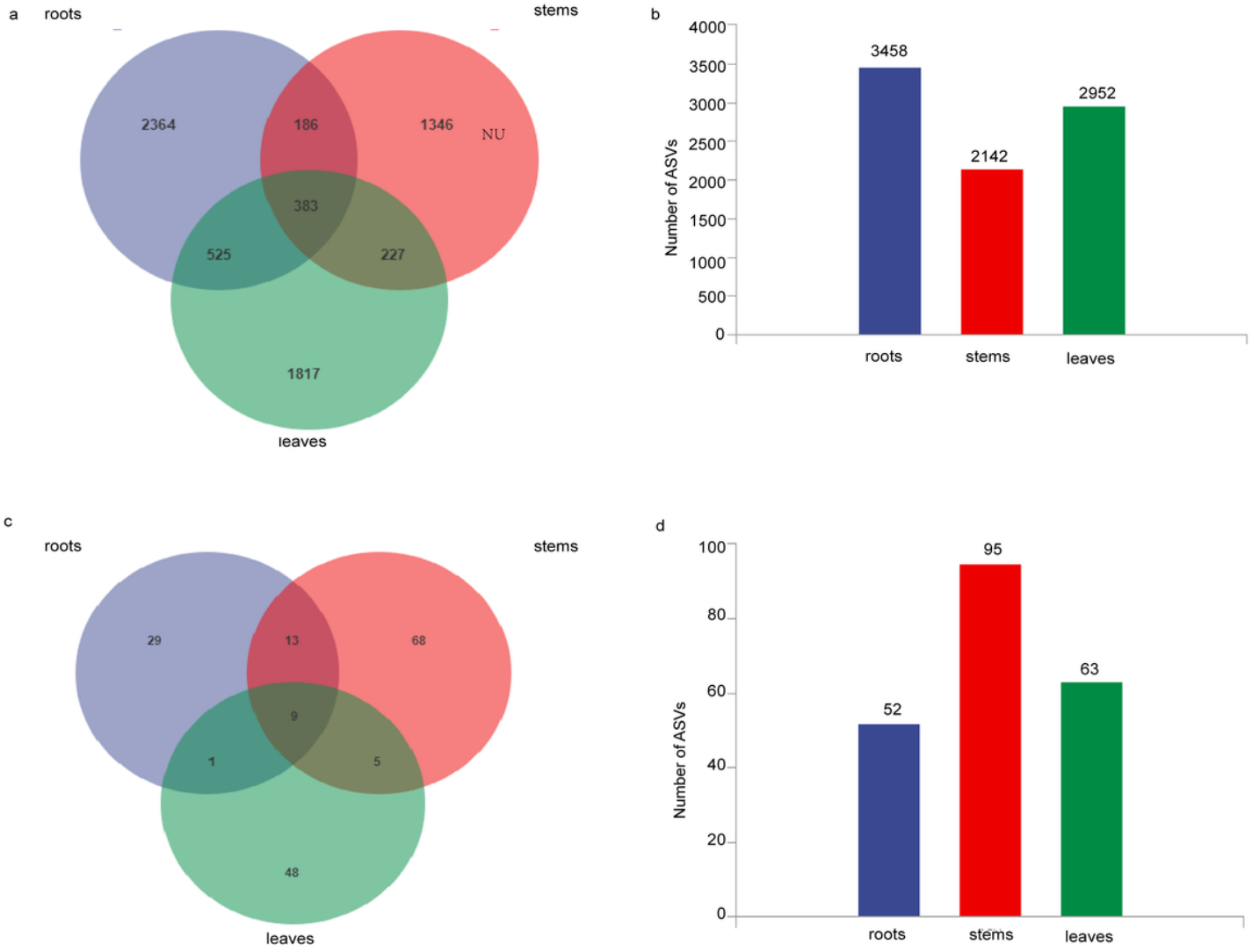

Figure 1

Venn diagram showing the shared ASVs endophytes in different organs (a. bacteria; c. fungi, c, d. fungi). Histogram shows the ASV numbers of endophytes in different organs (b. bacteria; d. fungi)

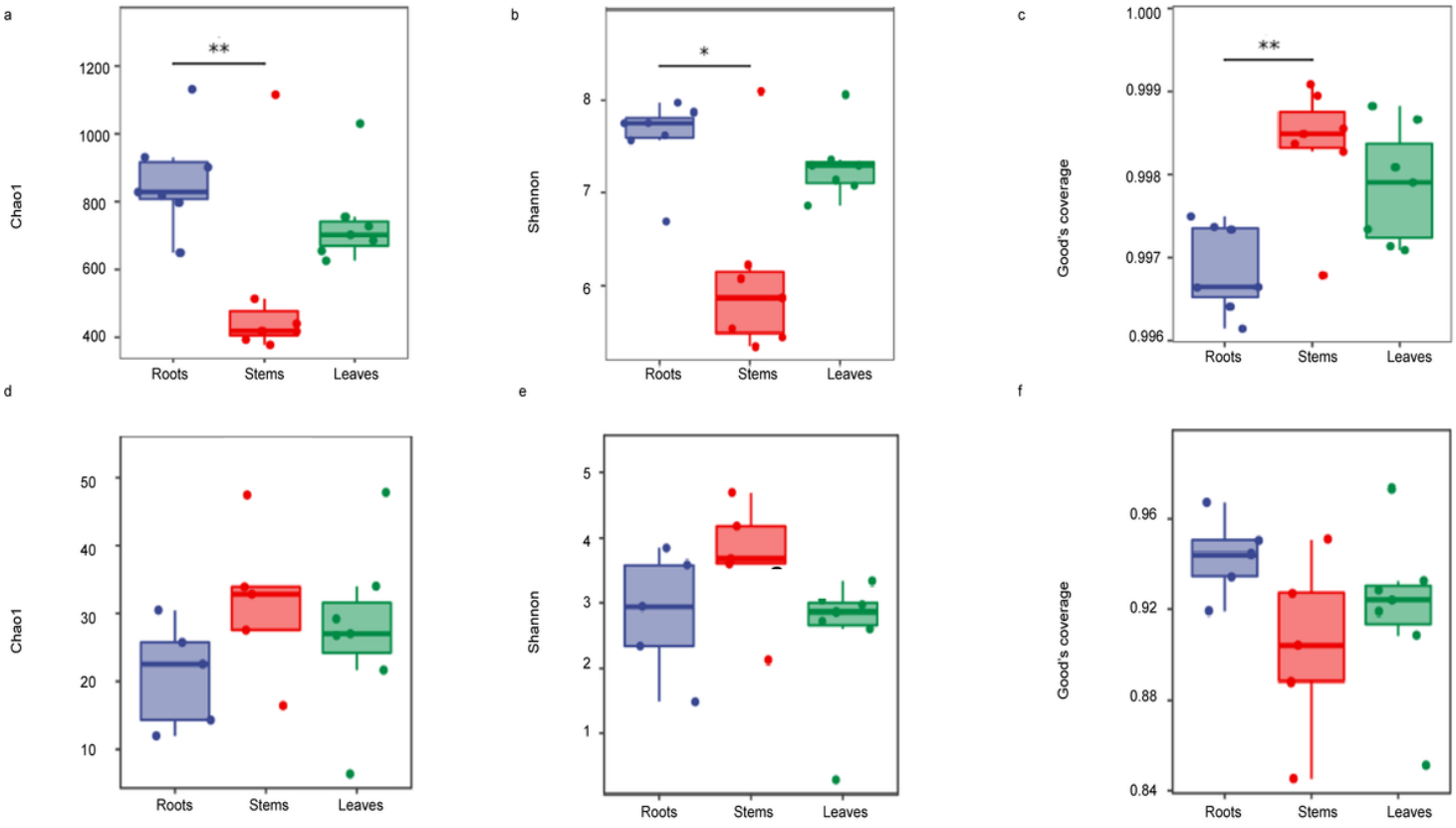

Figure 2

Alpha diversity of bacterial communities in different organs: a. Chao1; b. Shannon; c. Goods coverage; alpha diversity of fungal communities in different organs: d. Chao1; e. Shannon; f. Goods coverage. (*p < 0.05, **p < 0.01, ***p < 0.001)

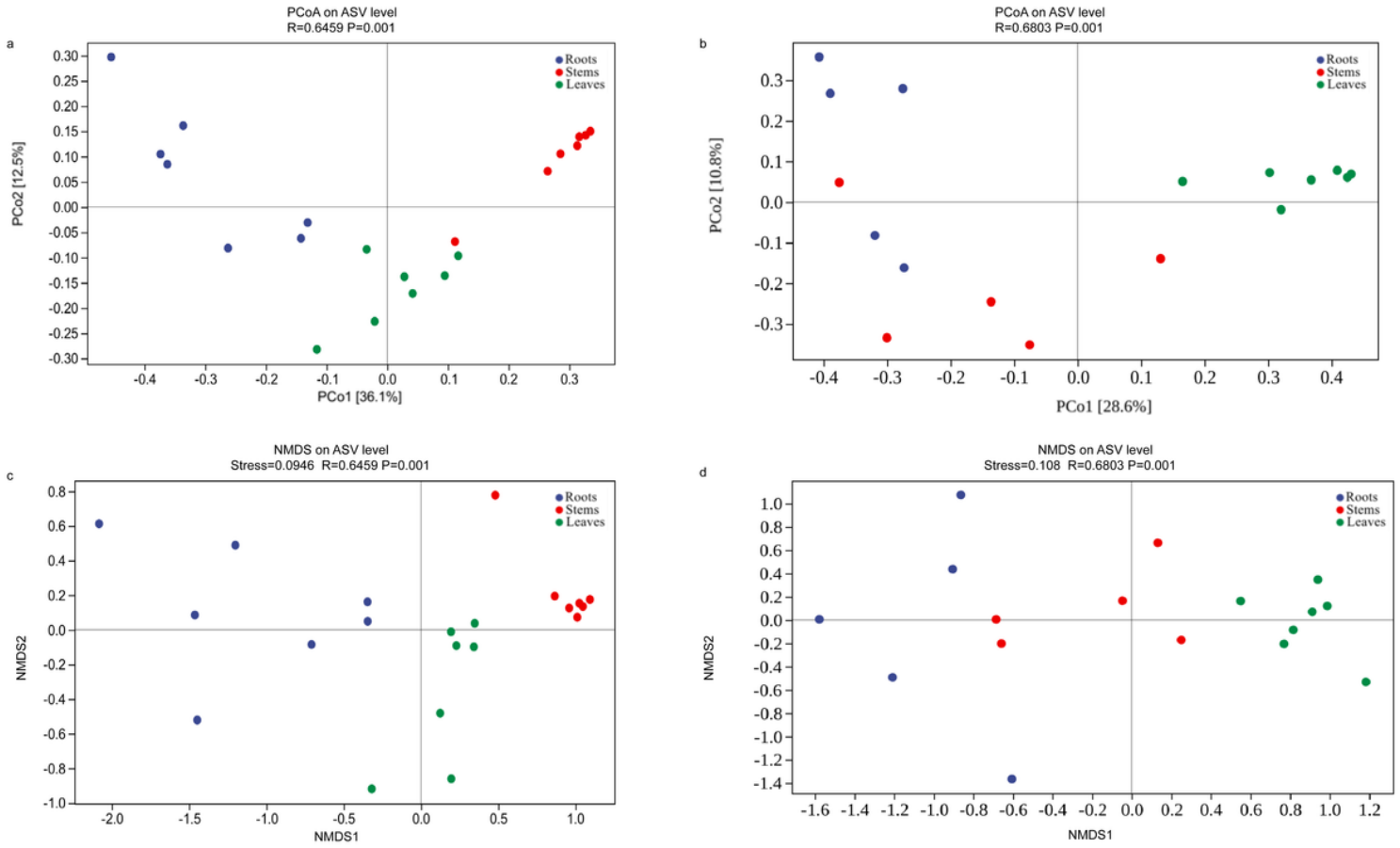

Figure 3

PCoA analysis plot of three organs (a, bacteria; b, fungi); NMDS plot of three organs (c, bacteria; d, fungi)

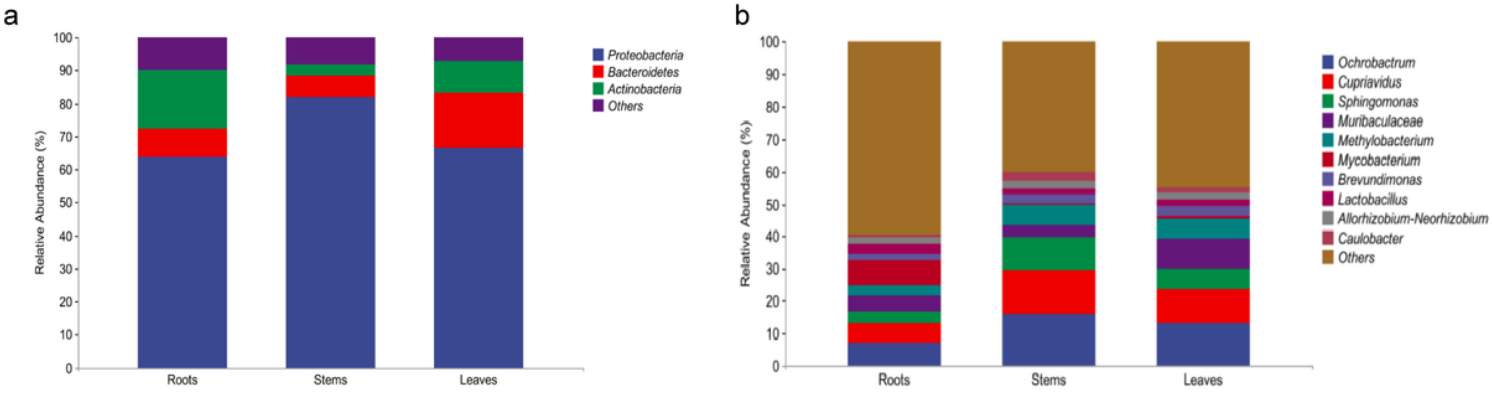

Figure 4

Relative abundance of endophytic bacterial communities classified at phylum (a) and genus (b) level in different organs

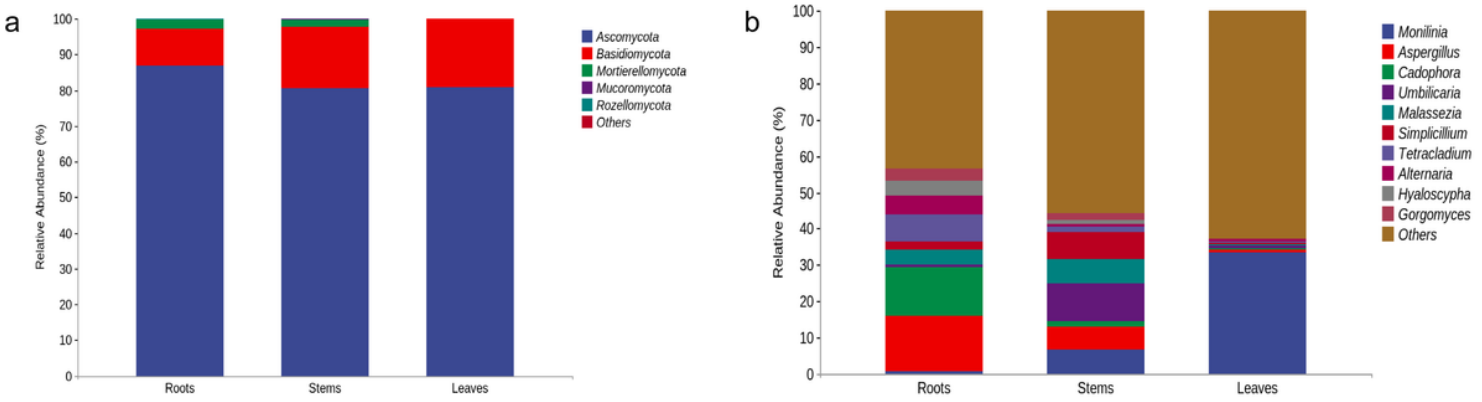

Figure 5

Relative abundance of endophytic fungal communities classified at phylum (a) and genus (b) level in different organs

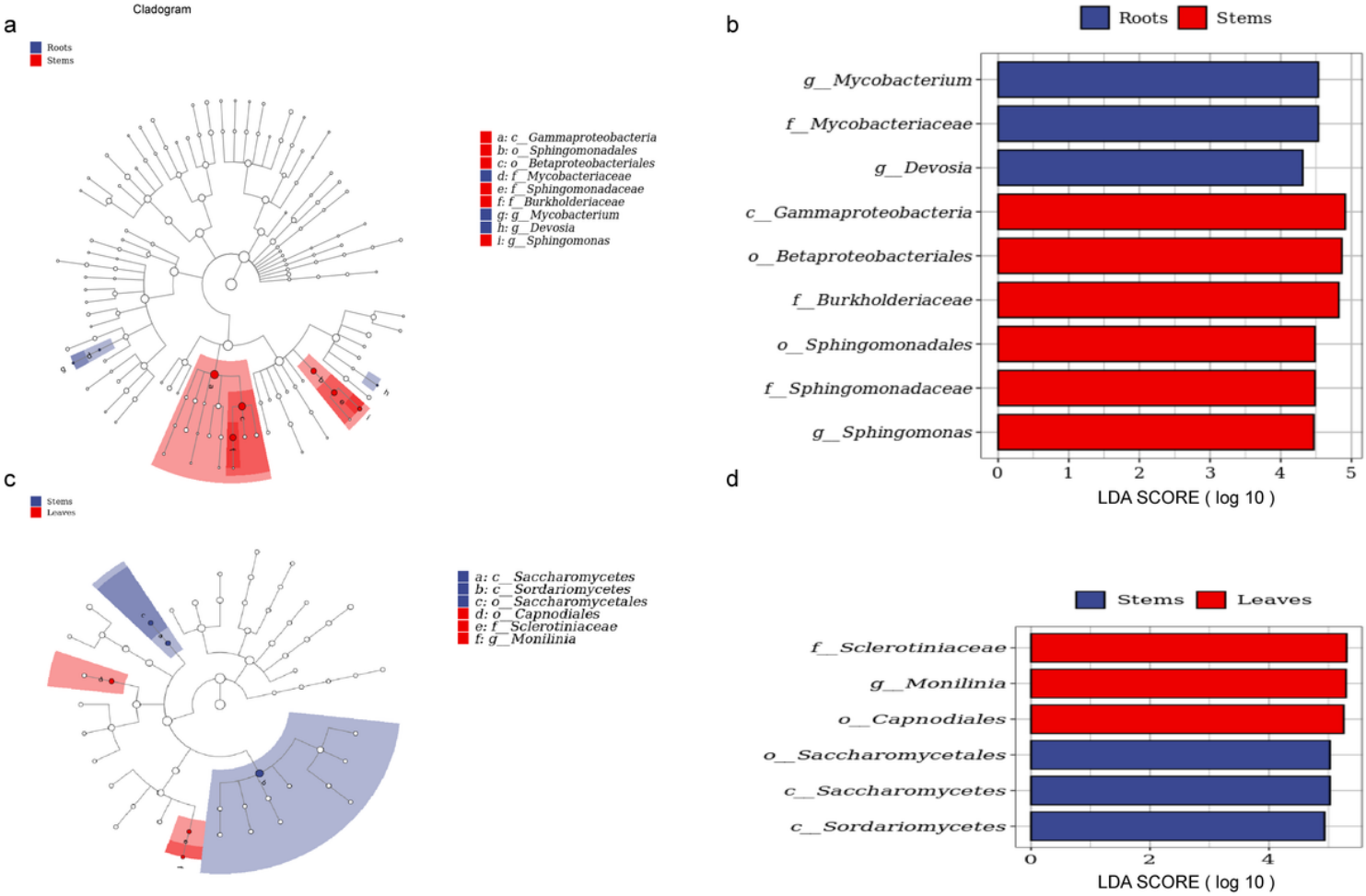

Figure 6

LEfSe analysis of microbial abundance in different organs. The clustering diagram shows the phylogenetic distribution of endophytes in three organs (a, bacteria; c, fungi). The histogram of the LDA

score calculates the differently abundant microbe among different organs, with a threshold of 4.0 (b, bacteria; d, fungi)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1.docx