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ORIGINAL ARTICLE

Study on the soil microbial community structure of the Rhizosphere of *Camellia sinensis* L. in Anping Village, Kaiyang County, Guizhou Province

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Abstract

Background To determine the differences in the microbial communities in the *Camellia sinensis* L. hairy root, lateral root, and main root rhizospheres in Anping Village, Kaiyang County, Guizhou Province, the community structure, diversity, and main dominant species of bacteria and fungi in different parts of the soil were analyzed by ITS and 16S sequencing.

Results In the rhizosphere soil of the main root, lateral root, and hairy root of *Camellia sinensis* L., there were significant differences in the diversity and richness of the bacterial and fungal communities. The bacterial diversity was the highest and the fungal richness was the lowest in the rhizosphere soil of the main root. In the bacterial community, Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, and Gemmatimonadetes were the common dominant bacteria. Rhodospirillaceae, Bradyrhizobiaceae, Hyphomicrobiaceae, Solibacteraceae, and Koribacteraceae were the common dominant bacteria in the rhizosphere soil of different parts of the root system, but the relative abundance of bacteria in different parts of the rhizosphere soil varied greatly. The dominant groups of fungal communities in different parts of the rhizosphere soil were Basidiomycota, Ascomycota, Mortierellomycota, and Sebacinaceae. The structure of the fungal community is similar in different parts.

Conclusions Compared with the different parts of the hairy root, lateral root, and main root of rhizosphere soil of *Camellia sinensis L*. in Anping village, it was found that the abundance of fungal community decreased with the increase of bacterial community abundance, and there were significant differences in bacterial community diversity and structure. However, the fungal community maintained stability among different parts.

Keywords *Camellia sinensis* L., Rhizospheric soil, High-throughput sequencing, Microbial community structure

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Introduction

Microorganisms are an important part of the soil ecosystem, and the community structure and diversity of soil microorganisms are closely related to changes in soil texture (Meng et al. 2017). They play a decisive role in maintaining the stability of the ecosystem and restoring system function after disturbance (Acosta-Martínez et al. 2010). The quantity, composition, and distribution of root exudates mainly depend on root morphology, plant development, and plant species (Pausch et al.

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2016). Dynamic changes in rhizospheric microorganisms affect plant growth, metabolism, and stress resistance (Schirmer et al. 2015). At the same time, plant root exudates are also key factors affecting the microbial diversity in the rhizosphere of different plants (Costa et al. 2007; George et al. 2002). The roots of *Camellia sinensis* L. are composed of main roots, lateral roots, absorptive roots, and root hairs. Absorptive roots are fine roots that originate from the main roots and lateral roots (Ofek et al. 2014).

Camellia sinensis (L.). O. Ktze. is an economically important crop in China. Guizhou is rich in germplasm resources of *Camellia sinensis L.*; these resources are widely distributed (Jin et al. 2018) and exhibit good development potential for germplasm innovation of *Camellia sinensis L.*. Endophytes and rhizospheric microorganisms of *Camellia sinensis L.* are among the main growth-promoting bacteria. The varieties, growth years, seasons, pH, soil organic carbon, and organic nitrogen of *Camellia sinensis L.* can affect the community structure of soil microorganisms of *Camellia sinensis L.*(Pausch et al. 2016). The diversity of microorganisms in the rhizosphere soil of *Camellia sinensis L.* not only as species diversity but also functional diversity. Some studies have noted that nitrogen-fixing bacteria, phosphorus-solubilizing bacteria, ammonifying bacteria, and cellulose-decomposing bacteria in Rhizosphere soil of *Camellia sinensis L.* can promote growth, improve soil fertility, and play an important role in increasing production (Knight et al. 2018). To date, studies on the rhizospheric microorganisms of *Camellia sinensis L.* plants have focused mainly on germplasm resource investigation, physiological growth, and metabolism, genetic diversity etc. (Lee et al. 2008; Bokulich et al. 2013). However, considerable uncertainties exist about how root morphological traits influence soil microbial community composition. There is no report on the distribution specificity of rhizospheric soil microbial communities among different root tissues of *Camellia sinensis L.* in Kaiyang County.

The soil in Kaiyang County, Guizhou Province, China is a special acidic selenium-rich soil, which is rare in the world, but *Camellia sinensis L.* is widely distributed here. At present, the distribution characteristics of soil microbial communities in the rhizosphere of *Camellia sinensis L.* trees in Kaiyang County have not been reported. In this study, a high-throughput amplification sequencing technique was used to classify and screen bacteria and fungi in the rhizosphere soil of *Camellia sinensis L.* in Anping Village, Fengsan Town, Kaiyang County, Guizhou Province. the community structure and composition were determined, and the α diversity was analyzed. The purpose of this paper is to clarify the effects of different parts of the root system on the composition and function of rhizosphere soil microflora through the comparison of microbial diversity and relative abundance and to determine whether bacteria and fungal microorganisms have different ecological functions in rhizosphere soil different parts of the *Camellia sinensis L.* to provide a research basis for the development and utilization of rhizosphere microbial resources.

Materials and methods
Site description
The sampling site is in the natural woodland of Anping Village, Fengsan Town, Kaiyang County, central Guizhou (latitude 106°59′ 48.77′′, longitude 27°12′ 10.04′′) at an elevation of 1086 m. The forest coverage in this area is approximately 54%, and the annual average temperature is approximately 10.6–15.2 ℃, which is conducive to the growth of *Camellia sinensis L.*

The soil is red-brown soil, and the soil pH is 4.32–4.68. Under the influence of the soil parent material, the background is higher than the average value of 0.29 mg/kg in China (Shuang-Mei et al. 2013).

Sample collection
Ten *Camellia sinensis L.* seedlings were randomly selected from the sampling sites. After the whole seedlings were dug up, the rhizospheric soil attached to hairy roots (S1), lateral roots (S2) and main roots (S3) was collected by shaking off, the rhizospheric soils from each part were fully mixed, and the 16S and ITS genes were amplified and sequenced.

DNA extraction and high-throughput sequencing
Total DNA was extracted according to the instructions of the E.Z.N.A.® Soil Kit, the concentration and its purity was determined by a NanoDrop2000 and quality determined by 1% agarose gel electrophoresis, and the V3-V4 variable region was amplified by PCR with the 338F and 806R primers.

The PCR products were recovered by 2% agarose gel electrophoresis, purified by an AxyPrep DNA Gel Extraction Kit, eluted with Tris–HCl, and detected by 2% agarose electrophoresis. Quantitative ST (Promega, USA) was used for detection and quantification. According to the standard operating procedures for the Illumina MiSeq platform, the purified amplified fragments were used to construct the PE 2*300 library.

The Illumina MiSeq PE300 platform was used for sequencing.

Data analysis and processing
A high-throughput MiSeq sequencer was used for sequencing, and the Data2 plug-in in QIIME2 software was used to control the quality of all the original

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sequences of all samples to form an amplified feature sequence (OTU). In species classification, bacteria were based on the sklearn algorithm, and fungi were based on the vsearch algorithm.

For alpha diversity, Chao1 and Ace indexes were used to estimate the richness of the microbial community; in both cases, larger values indicated higher community richness. Shannon's and Simpson's indexes were used to estimate the diversity of the microbial community. For Shannon's index, larger values indicated higher diversity; in contrast, for Simpson's index, lower values indicated higher diversity.

Results

Diversity indexes of bacteria and fungi in different parts of the rhizosphere soil

Based on a similarity level greater than or equal to 99%, the number of bacterial OTUs in the hairy root, lateral root, and main root rhizospheric soils was 29,116, 29,747, and 33,156, respectively, and the number of fungal OTUs was 49,817, 58,219, and 41,056, respectively. In the soil samples from different parts of the rhizosphere, the number of sequences that could be used for the classification of fungi was higher than that of bacteria.

As shown in Table 1, taking the Chao1 index as an example, the values were in the order S3 > S1 > S2, indicating that the soil bacteria in the main root rhizosphere had higher species richness, followed by those in the hairy root rhizosphere and lateral root rhizosphere. This result is exactly the same as that for the Faith-pd index and observed index. The Shannon index showed that the bacterial diversity in the main root rhizospheric soil was the highest.

As shown in Table 2, the observed features index and Chao1 index showed that the fungal richness in the main root rhizosphere was the lowest, and the species richness in the lateral root rhizosphere was relatively high. The Shannon index and Simpson index showed that the fungal diversity in the main root rhizosphere was the lowest.

Composition of the soil microbial community in different parts

The bacteria in the three samples of *Camellia sinensis L.* rhizospheric soil were annotated to 27 phyla and 83 families, and fungi were annotated to 7 phyla and 47 families. There were 22 bacterial phyla in the hairy root rhizosphere soil, 24 bacterial phyla in the lateral rhizosphere soil, and 23 bacterial phyla in the main rhizosphere soil. The groups with relative abundances greater than 1% in the different parts are shown in Table 3. Based on the level of phylum classification, it was found that the bacteria in different parts of rhizosphere soil were Proteobacteria, Actinobacteria, Acidobacteria, AD3, and Chloroflexi, but the relative abundance varied greatly, among which Acidobacteria accounted for the largest proportion in the main root rhizospheric soil.

As shown in Table 4, at the family classification level, only Rhodospirillaceae, Bradyrhizobiaceae, Hyphomicrobiaceae, Solibacteraceae, and Koribacteraceae were the dominant bacterial families. Thermogemmatisporaceae was not a dominant bacterial family in the hairy root rhizospheric soil, but it was the dominant bacterial family in the main root and lateral root rhizospheric soils. The proportion of Thermomonosporaceae in the rhizospheric soil of the main roots and hairy roots was very low, but the relative abundance in the lateral root rhizospheric soil was relatively high. The main root rhizospheric soil

Table 1 Alpha diversity analysis of soil bacteria in different parts of the rhizosphere soil

Samples	Chao1 index	Faith-pd index	Observed features index	Shannon index	Simpson index	OTUs
S1	933.900	43.597	915.000	8.695	0.994	29,116
S2	914.556	42.910	907.000	8.730	0.994	29,747
S3	976.167	46.691	975.000	8.947	0.996	33,156

Table 2 Alpha diversity analysis of soil fungi from different parts of the rhizosphere soil

Samples	Chao1 index	Faith-pd index	Observed features index	Shannon index	Simpson	OTUs
S1	139.000	23.152	139.000	4.163	0.881	49,817
S2	161.000	26.123	161.000	3.471	0.826	58,219
S3	133.000	15.207	133.000	5.237	0.918	41,056

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Table 3 The relative abundance of bacteria accounts for more than 1% at the phylum level

Bacteria species	% Bacterial composition of the lateral (S1), hairy (S2) and main root (S3) respectively		
	S1	S2	S3
Acidobacteria	15.490%	19.892%	28.278%
Actinobacteria	23.458%	18.473%	18.696%
AD3	13.065%	16.240%	5.761%
Bacteroidetes	1.020%	-	1.00%
Chloroflexi	6.828%	11.987%	14.739%
Gemmatimonadetes	1.522%	1.184%	1.028%
Planctomycetes	-	-	2.009%
Proteobacteria	36.482%	27.716%	23.742%
Verrucomicrobia	-	-	1.059%
WPS_2	-	1.315%	1.873%

Table 4 The relative abundance of bacteria in the sample accounts for the top 10 at the family level

Bacteria species	% Bacterial composition of the lateral (S1), hairy (S2) and main root (S3) respectively		
	S1	S2	S3
Acetobacteraceae	2.057%	1.785%	1.873%
Bradyrhizobiaceae	6.443%	4.741%	5.996%
Burkholderiaceae	2.311%	–	–
Conexibacteraceae	1.971%	–	1.951%
EB1017	1.662%	1.557%	–
Hyphomicrobiaceae	5.440%	4.183%	3.532%
Koribacteraceae	2.899%	5.824%	7.917%
Ktedonobacteraceae	–	–	2.156%
RB40	–	–	1.686%
Rhodospirillaceae	6.615%	5.282%	4.723%
Sinobacteraceae	2.126%	1.765%	–
Solibacteraceae	3.356%	2.676%	2.8045%
Thermogemmatisporaceae	–	5.484%	10.948%
Thermomonosporaceae	–	1.409%	–

Table 5 The main groups of fungi in the samples at the phylum level

fungi species	% Bacterial composition of the lateral (S1), hairy (S2) and main root (S3) respectively		
	S1	S2	S3
Ascomycota	9.615%	12.167%	28.953%
Basidiomycota	78.718%	78.121%	66.556%
Cercozoa	–	0.009%	–
Chytridiomycota	–	0.007%	–
Mortierellomycota	11.040%	8.842%	4.344%
Mucoromycota	0.111%	0.071%	0.125%
Rozellomycota	0.496%	0.533%	0.034%

Table 6 The relative abundance of fungi accounted for more than 1% of the family level in the sample

Fungi species	% Bacterial composition of the lateral (S1), hairy (S2) and main root (S3) respectively		
	S1	S2	S3
Archaeorhizomycetaceae	1.159%	4.296%	2.835%
Aspergillaceae	2.069%	1.358%	5.432%
Geminibasidiaceae	7.633%	4.318%	1.972%
Hypocreaceae	2.831%	3.957%	11.594%
Inocybaceae	–	1.133%	–
Lyophyllaceae	4.459%	11.182%	3.519%
Mortierellaceae	11.014%	8.807%	4.343%
Myxotrichaceae	–	–	1.847%
Ophiocordycipitaceae	–	–	1.746%
Pyronemataceae	1.019%	–	1.663%
Russulaceae	1.030%	–	–
Sebacinaceae	29.892%	35.277%	25.374%

contained the highest number of annotated families and genera, and there were unannotated nondominant bacteria in the hairy root and lateral root rhizospheric soils.

As shown in Table 5, there were 6 fungal phyla in the hairy root rhizospheric soil. A comparison with the database indicated that the phyla were Basidiomycota (78.718%), Mortierellomycota (11.040%), Ascomycota (9.615%), Rozellomycota (0.496%), Mucoromycota (0.111%), and unspecified fungi (0.018%). There were 8 fungal phyla in the lateral root rhizospheric soil, including Cercozoa (0.009%), and Chytridiomycota (0.007%).

There were 5 fungal phyla in the main root rhizospheric soil.

As shown in Table 6, at the family level, the most abundant fungi in the hairy root, lateral root, and main root rhizospheric soils were Sebacinaceae. Among the groups accounting for more than 1%, only Russulaceae had different proportions among the rhizospheric soils; it was the dominant bacterial family in the hairy root rhizospheric soil but had a small proportion in the main root rhizospheric soil. In the main root rhizospheric soil, Myxotrichaceae (1.847%), and Ophiocordycipitaceae (1.746%) were the dominant bacteria with high abundance. These two families and their genera accounted for a very low proportion in the hairy root and lateral root rhizospheric soils, while the other families and genera were common dominant bacteria.

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Discussion

The balance of the soil ecosystem is closely related to the structure and diversity of the soil microbial community (Mousavi et al. 2018). The rhizospheric soil is a unique soil ecosystem (Shi et al. 2015) that is significantly different from non-rhizospheric soil ecosystems (Surette et al. 2003). The rhizospheric soil microbial community not only plays an important role in soil ecosystems but is also an important index of soil health.

The soil community diversity in the rhizosphere of the main root was the highest, with the most abundant species.

The results showed that in different parts of the rhizosphere, the bacterial richness in the main root rhizospheric soil was the highest, followed by that in the hairy root and lateral root rhizospheric soils. According to the comparison of fungal groups, the richness of fungi in the main root rhizospheric soil was the lowest, followed by that in the hairy root and the lateral root rhizospheric soils. As we all know the main root plays an important role in the growth and development of plants, followed by lateral root. Root hair had the weakest effect on growth and development. The main root has the highest bacterial richness in the rhizosphere soil of *Camellia sinensis L.*, indicating that the bacterial richness in the rhizosphere soil is closely related to the growth and development of *Camellia sinensis L.*

Many scholars have carried out in-depth studies of rhizospheric microflora in model plants and crops such as *Arabidopsis thaliana* (Bressan et al. 2009), corn (Jin et al. 2013), and rice (Fang et al. 2021) and found that substances secreted by plants can drive and shape different rhizospheric microbial processes. In other words, rhizosphere microorganisms mainly attract specific microbial populations through rhizosphere exudates. The quantity, composition, and distribution of rhizosphere exudates mainly depend on root morphology, plant development, and plant species (Liu et al. 2022). It was found that the exudates were unevenly distributed in different root morphologies and structures, such as young lateral roots, root hairs, and old main roots (Acharya et al. 2023). It is reasonable to think that the difference in microbial community diversity in different parts of the root system of *Camellia sinensis L.* is closely related to root exudates.

Pervaiz (Pervaiz et al. 2020) the soil microorganisms in the rhizosphere of peach trees were studied. it was found that the diversity of bacterial community absorbed by fine roots was higher than that of large roots because of its high content of essential minerals. However, the results of this study are different. As Pervaiz pointed out, it is not clear whether there is a general relationship between the chemical or morphological characteristics of roots and the composition of soil microorganisms, especially under field conditions. Although more research is needed to establish these relationships, we have demonstrated that different root types show unique microbial community composition.

The alpha diversity index of bacteria in different parts of the rhizospheric soil of *Camellia sinensis L.* was higher than that of fungi

In the rhizosphere soil of *Camellia sinensis L.*, the alpha diversity index of bacteria was higher than that of fungi. Wu et al. (2021) collected rhizosphere soil samples from poplar plantations of different ages and found that the diversity of fungi was lower than that of bacteria. Jia, X (Jia et al. 2018) found that the bacterial diversity in the rhizosphere soil of *Robinia pseudoacacia* seedlings was higher based on high-throughput sequencing. These results are consistent with our research results.

It should be pointed out that there are differences in the richness of bacteria and fungi in different parts of the rhizosphere soil of *Camellia sinensis L.* We found that the part with the highest bacterial alpha diversity had the lowest fungal alpha diversity, indicating that there was competition between bacteria and fungi in the rhizosphere soil niche, and bacteria seemed to be better at such competition. Previous studies have shown that bacteria can promote the formation and decomposition of organic matter, improve soil fertility, and play an important role in soil ecosystems (FeZ et al. 2008). Although the number and species of fungi in the soil are lower than those of bacteria, fungi in the soil are also a member of a stable ecosystem and are very important for maintaining the normal operation of the ecosystem (Chang et al. 2017). In the rhizosphere soil of *Camellia sinensis L.*, it follows the principle that the diversity of bacteria is higher than that of fungi.

The relative abundances of dominant bacteria vary greatly in different parts of the rhizosphere soil

The compositions of dominant microflora in different parts of the Rhizosphere soil of *Camellia sinensis L.* were similar, including mainly Proteobacteria, Actinobacteria, and Acidobacteria, but the relative abundances were different. According to the analyses of bacterial communities in 237 soil samples from 6 continents by researchers from many countries, Proteobacteria, Acidomycetes, and Actinomycetes are the three phyla with the highest relative abundances. Peralta et al. (Peralta et al. 2012) found that there were three dominant bacterial groups, Proteobacteria, Acidobacteria, and Actinobacteria, in rhizosphere soil of different vegetation types. This is consistent with the analysis of dominant microorganisms in the rhizosphere soil of *Camellia sinensis L.* in this study. Shih also pointed out that the relative abundance of each

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phylum changes under different environmental conditions (Shi et al. 2015).

At the phylum level, compared with the hairy roots and lateral roots, the group with a significantly high relative abundance in the main roots was Acidobacteria. Acidobacteria can survive in barren environments with low organic carbon content (Sul et al. 2013). The proportion of Acidobacteria in the main root soil was obviously better than that in the lateral root and hairy root soils. Acidobacterium can effectively secrete all kinds of extracellular acids (Kanokratana et al. 2011), thus reducing soil salinity and alkalinity and playing an important role in the control of soil salinization (Mori et al. 2016). Actinomycetes degrade cellulose and chitin, which are the main sources of soil nutrients (Turner et al. 2013). Most Proteobacteria are mainly facultative or specifically anaerobic and heterotrophic, and some Proteobacteria species (Amy et al. 2017) play an important role in removing refractory pollutants. The dominance of Proteobacteria in the hairy root and lateral root soils of Camellia sinensis L. may be related to the environmental factors in the rhizospheric soil of Camellia sinensis L., and the growth rate of these bacteria is faster in the rhizospheric soil (Turner et al. 2013). At the family level, Rhodospirillaceae, Bradyrhizobiaceae, Hyphomicrobiaceae, Solibacteraceae, and Koribacteraceae were the common dominant bacteria, and the composition of dominant bacteria varied greatly among different parts of the rhizospheric soil.

In the rhizosphere soil of different parts, the structure of the fungal community was similar, and the abundance ratio changed, but the difference was not significant.

Together, the relative abundance analysis results indicated that the dominant groups in the three soil samples were basidiomycetes, ascomycetes, and angiosporium, of which basidiomycetes accounted for the largest proportion, i.e., more than 65% of the dominant fungi. There was little difference in the species and relative abundance of dominant fungi among different parts of rhizosphere soil. Cortinarius, Inocybe, and Hebeloma of basidiomycetes have been proven many times to form symbiotic systems with plants under certain conditions, playing an important role in enhancing plant resistance (Liimatainen et al. 2014; Gates et al. 2011; Ammirati et al. 2007).

At the family level, Sebacinaceae accounted for the largest proportion in the rhizospheric soils of hairy roots, lateral roots, and main roots. Liu et al. (Liu et al. 2021) found that the family with the highest relative abundance in the plantation of *Picea mandshurica* was Umbelliferae, while in the natural forest, it was the wax shell family. In the four molecular ecological networks of soil fungal communities, Chrysomelaceae harbored the key species. Sebacinaceae is a common dominant bacterial taxon in the mycorrhizal fungal community (Bell et al. 2020;

Brundrett and Tedersoo 2018). The above results are consistent with the results of this study. The change in the fungal community in the rhizosphere soil of hairy roots, lateral roots, and main roots at the family level was not obvious, but the proportion of Mortieraceae and Mortierella in the three different parts showed the ranking of hairy roots > lateral roots > main roots.

Conclusions

In Anping Village, Kaiyang County, Guizhou Province, the content of bacteria in the rhizosphere soil of *Camellia sinensis* was higher than that of fungi, and the richness, diversity, and species diversity of the bacterial community were higher than those of fungi.

The composition of dominant bacteria in different parts of rhizosphere soil was similar, but the relative abundance of dominant bacteria varied greatly in different parts of the rhizosphere, and the main group with significantly higher relative abundance in the main root was Acidobacteria. Proteus was dominant in the hairy root and lateral root soil. We indicated that different root types showed unique bacterial community compositions.

The results of the relative abundance analysis of fungi in rhizosphere soil showed that the dominant groups in the three soils were basidiomycetes, ascomycetes, and angiosporidium, among which basidiomycetes accounted for the largest proportion. At the family level, the community structure of the fungal community was similar in different parts, and the proportion of abundance changed, but only a small change in abundance.

There are some limitations in our research. On this basis, future studies can further explore the relationship between the formation of dominant microflora in different parts of the rhizosphere and other factors.

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Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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