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BACTERIAL COMMUNITY COMPOSITION AND FUNCTIONS IN RHIZOSPHERE SOIL OF FOUR DECIDUOUS FRUIT TREES

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Abstract

The purpose of this study was to explore the composition of bacterial communities in the rhizosphere soil of four deciduous fruit trees (pomegranate, peach, cherry, and ginkgo) and to study the bacterial community structure and metabolic function of them. 16S rRNA sequencing technology was adopted to analyze the bacterial community structure, and Tax4Fun was employed to predict the metabolic profiles of the bacterial community. The results showed that the Shannon index, Ace index, and Chao1 index values of the ginkgo rhizosphere soil samples were the largest,indicating that the bacterial diversity of ginkgo rhizosphere soil was the highest. 4764 OTUs were identified for bacteria and were classified as 46 phylums, 113 classes, 175 orders, 247 families, 339 genera, and 504 species. Whether at the phylum level, at the order level, or at the genus level, no significant differences in the composition of bacterial communities were detected among the rhizosphere soils of the four deciduous fruit trees. Proteobacteria, Actinobacteria, Bacteroidota, and Acidobacteriota were the most abundant phyla, and Gammaproteobacteria, Bacteroidota, Alphaproteobacteria, and Alphaproteobacteria were the most abundant orders across all samples. The KOs (KEGG orthology) were mainly involved in 6 KEGG level 1 pathways and 35 KEGG level 2 pathways. The relative abundance of these six pathways was not significantly different in rhizosphere soils among the four deciduous fruit trees at the first KEGG level. The main enrichment pathways of microorganism metabolism at the second KEGG level are consistent, mainly in carbohydrate metabolism, amino acid metabolism, energy metabolism, lipid metabolism, nucleotide metabolism, and the metabolism of cofactors and vitamins.

Introduction

Increasing evidence indicates that rhizosphere microorganisms have a significant influence on plant growth, root structure, and nutrient uptake (Vacheron *et al.* 2013), and the diversity and composition of rhizosphere microbial communities are essential for maintaining soil quality and plant health. Plant roots can promote or prevent the recruitment of rhizosphere microorganisms by secreting root exudates and volatile compounds (Berendsen *et al.* 2012; Schmidt *et al.* 2019). Some root exudates, such as various organic acids and amino acids, as well as other compounds, are released into the rhizosphere and thus become nutrients for rhizosphere microbes (Babalola 2010), affecting the composition and diversity of rhizosphere microbial communities (Aira *et al.* 2010). Moreover, the release of root exudates is affected by plant species and environmental factors, which create a unique environment for microorganisms (Bais *et al.* 2006). Different plant species may develop unique microbial communities through the interactions between plant roots and microorganisms, which may also lead to changes in the functions of microorganisms in the rhizosphere soil (Turner *et al.* 2013). In particular, understanding the effect of plant species on soil microbial communities is very helpful for the cultivation of fruit trees.

A deciduous fruit tree is a kind of fruit tree that defoliates at the end of autumn and sprouts in the spring of the second year (Cheng *et al.* 2022). Bengbu, situated in the northern part of Anhui Province, is a transitional zone between temperate and tropical monsoon climate zones. It is suitable for planting a variety of fruit trees. The main deciduous fruit trees planted in Bengbu are

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pomegranate, peach, cherry, and ginkgo. High-throughput sequencing technology has been widely used in microbial diversity analysis because of its advantages of high speed, high efficiency and precision (Gao *et al.* 2021). At present, there are many studies on the bacterial diversity in the rhizosphere soil of grain crops, but there are few studies on the bacterial diversity in the rhizosphere soil of deciduous fruit trees (Shao *et al.* 2020).

In this study, high-throughput sequencing was used to analyze the bacterial diversity of soil in the rhizosphere of deciduous fruit trees, such as pomegranate, peach, cherry and ginkgo, in order to provide theoretical and technical support for the cultivation and management of deciduous fruit trees.

Materials and Methods

*Sample collection and experimental design:*The sample location is the campus of Bengbu College. On May 8, 2022, three deciduous fruit trees (pomegranate, peach, cherry, and ginkgo) were randomly selected to remove the topsoil and collect the rhizosphere soil at a depth of 10-20 cm. There were 4 groups of samples with 3 replicates: pomegranate samples (SL); peach tree samples (TS); cherry samples (XS) and ginkgo (YX) samples.

*Sequencing:*Total genome DNA from samples was extracted using the CTAB/SDS method. DNA concentration and purity were monitored on 1% agarose gels. According to the concentration, DNA was diluted to lug/μL using sterile water.

16S rRNA genes of V3-V4 regions were amplified used specific primer 515F (5'-GCGGTAA TTCCAGCTCCAA-3') and 806R (5'-AATCCRAGAATTTCACCTCT-3') with the barcode. All PCR reactions were carried out with 15 μl of Phusion® High-Fidelity PCR Master Mix (New England Biolabs); 0.2 μM of forward and reverse primers, and about 10 ng template DNA. Thermal cycling consisted of initial denaturation at 98°C for 1 min, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 30 s, and elongation at 72°C for 30 s. Finally, 72°C for 5 min.

The same volume of IX loading buffer (containing SYB green) was mixed with PCR products, and electrophoresis was performed on a 2% agarose gel for detection. PCR products were mixed in equidensity ratios. Then, the mixture of PCR products was purified with the Qiagen Gel Extraction Kit (Qiagen, Germany).

Sequencing libraries were generated using the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) following the manufacturer's recommendations, and index codes were added. The library quality was assessed on the Qubit@2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. At last, the library was sequenced on an Illumina NovaSeqplatform, and 250 bp paired-end reads were generated.

Data analysis: Paired-end reads were assigned to samples based on their unique barcode and truncated by cutting off the barcode and primer sequence.

Paired-end reads were merged using FLASH (VI.2.7, http://ccb.jhu.edu/software/FLASH/) (Magoč and Salzberg 2011), a very fast and accurate analysis tool that was designed to merge paired-end reads when at least some of the reads overlap the read generated from the opposite end of the same DNA fragment, and the splicing sequences were called raw tags.

Quality filtering on the raw tags wasperformed under specific filtering conditions to obtain ahigh-quality clean tag (Bokulich*et al.* 2013) according to the QIIME(V1.9.1, http://qiime.org/ scripts/split libraries fastq. html (Caporaso*et al.* 2010) quality controlled process.

The tags were compared with the reference database (Silva database, https://www.arb-silva.de/) using the UCHIME algorithm (UCHIME Algorithm, http://www.drive5.com/

usearch/manual/uchim e_algo.html) (Edgar *et al.* 2011) to detect chimera sequences, and then the chimera sequences were removed (Haas *et al.* 2011). Then the effective tags were finally obtained.

Results and Discussion

*Diversity of the rhizosphere bacterial community:*Alpha diversity reflects the richness and diversity of individual sample species. OTU coverage, the higher the value, the higher the probability that the species in the sample will be measured. The coverage of each sample was greater than 98.6%, indicating that the information about microbial species was fully reflected (Table 1). The larger the Shannon index value, Ace index value, and Chao1 index value, the higher the species diversity of the sample (Grice *et al.* 2009). The Shannon index, Ace index, and Chao1 index values of the ginkgo rhizosphere soil samples were the largest. The results showed that the bacterial diversity of ginkgo rhizosphere soil was the highest.

Table 1. Alpha diversity index of bacteria in four deciduous fruit trees.

Sample	Observed species	Shannon	Ace	Chao1	Coverage
SL	2190	9.214	2545.914	2504.448	0.987
TS	2137	9.151	2523.826	2491.076	0.987
YT	2107	9.106	2446.619	2681.171	0.987
YX	2196	9.221	2554.251	2567.736	0.986

A total of 4,764 OTUs were identified for bacteria with 97% sequence similarity, which were classified as 46 phyla, 113 classes, 175 orders, 247 families, 339 genera, and 504 species. The number of OTUs of bacterial communities detected for SL, TS, YT, and YX was 3416, 3109, 3021, and 3154, respectively (Table 2).

Table 2.Statistical table of species abundance of bacteria in four deciduous fruit trees.

Sample	Phylum	Class	Order	Family	Genus	Species	OTU
SL	42	103	133	214	297	439	3416
TS	39	101	141	222	293	424	3109
YT	40	101	134	215	296	405	3021
YX	43	101	137	222	301	417	3154
Total	46	113	175	247	339	504	4764

*Composition of the bacterial community:*Species composition analysis reflects the community structure of the samples at different taxonomic levels. I assessed the taxonomic distributions of bacterial OTUs at different levels. Whether at the phylum level, at the orderlevel, or at thegenus level, no significant differences in the composition of bacterial communities were detected among the rhizosphere soils of the four deciduous fruit trees. At the phylum level, 10 dominant bacterial phyla were assigned, namely, Proteobacteria, Actinobacteria, unidentified bacteria,Bacteroidota, Acidobacteriota, Firmicutes, Verrucomicrobiota, Actinobacteriota, Myxococcota, and Chloroflexi, which made up 90% of the entire bacterial community. Proteobacteria, Actinobacteria, Bacteroidota, and Acidobacteriota were the most abundant phyla across all samples, accounting for 22.79% ~ 27.30%, 9.74% ~ 13.21%, 7.71% ~ 13.02%, and 9.17% ~ 12.14% of the total valid reads in all samples, respectively (Fig. 1, Table 3).

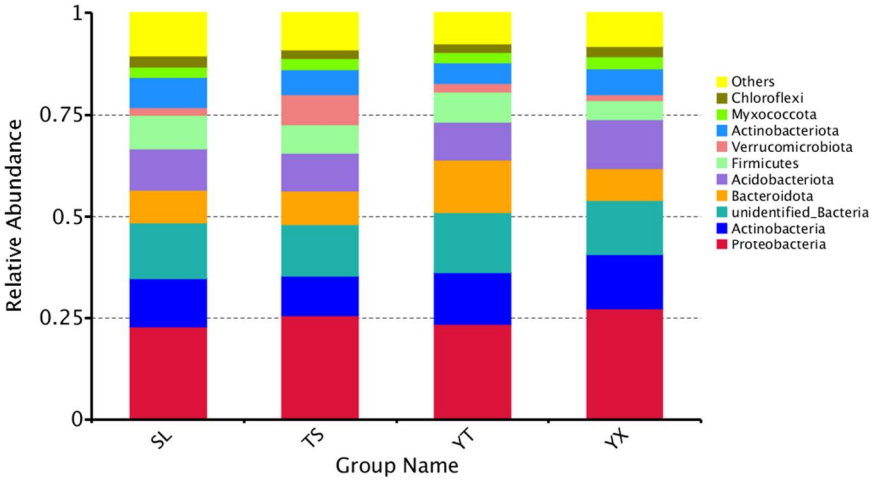

Fig.1. The composition and structure of bacterial community from four deciduous fruit trees at the phylum level

Table 3.Percent of dominant bacterial community at the phylum level.

Phylum level	SL	TS	YT	YX
Proteobacteria	0.2279	0.2557	0.2348	0.2730
Actinobacteria	0.1184	0.0974	0.1259	0.1321
unidentified_Bacteria	0.1388	0.1276	0.1496	0.1356
Bacteroidota	0.0815	0.0821	0.1302	0.0771
Acidobacteriota	0.0999	0.0930	0.0917	0.1214
Firmicutes	0.0839	0.0710	0.0739	0.0461
Verrucomicrobiota	0.0190	0.0737	0.0212	0.0147
Actinobacteriota	0.0736	0.0615	0.0504	0.0631
Myxococcota	0.0250	0.0279	0.0270	0.0295
Chloroflexi	0.0280	0.0202	0.0203	0.0249
Others	0.1040	0.0899	0.0750	0.0825

At the order level, 10 dominant bacterial orders were assigned, namely, Gammaproteobacteria, unidentified Actinobacteria, Bacteroidia, Alphaproteobacteria, Verrucomicrobiae, Vicinamibacteria, Bacilli, Clostridia, Desulfovibrionia and Blastocatellia, which made up 60% of the entire bacterial community. Gammaproteobacteria, Alphaproteo-bacteria,Alphaproteobacteria andBacteroidota were the most abundant orders across all samples (Fig. 2, Table 4).

Figure 3 and Table 5 show the community structure and classification comparison results at the genus level. The top ten bacteria were the species of Romboutsia, Chthoniobacter, Terrimicrobium, RB41, Ileibacterium, Bacteroides, Sphingomonas, Arthrobacter, MND1, Dongia.

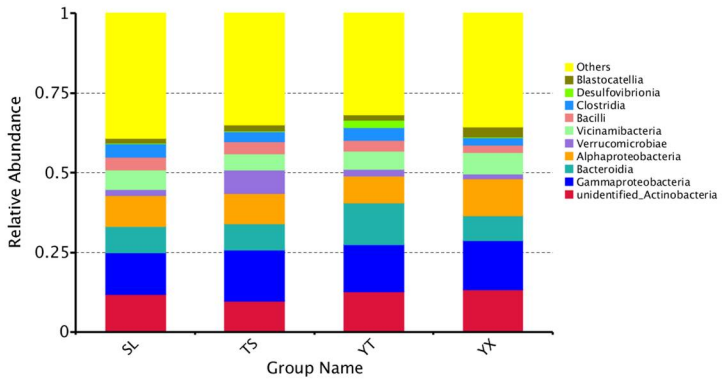

Fig.2.The composition and structure of bacterial community from four deciduous fruit trees at the order level.

Table 4 Percent of dominant bacterial community at the order level.

Order level	SL	TS	YT	YX
Gammaproteobacteria	0.1313	0.1611	0.1493	0.1552
unidentified_Actinobacteria	0.1184	0.0974	0.1259	0.1321
Bacteroidia	0.0815	0.0821	0.1302	0.0771
Alphaproteobacteria	0.0966	0.0946	0.0854	0.1178
Verrucomicrobiae	0.0190	0.0737	0.0212	0.0147
Vicinamibacteria	0.0616	0.0499	0.0561	0.0670
Bacilli	0.0401	0.0385	0.0334	0.0235
Clostridia	0.0434	0.0323	0.0401	0.0224
Desulfovibrionia	0.0007	0.0017	0.0237	0.0017
Blastocatellia	0.0156	0.0198	0.0159	0.0328
Others	0.3918	0.3489	0.3188	0.3557

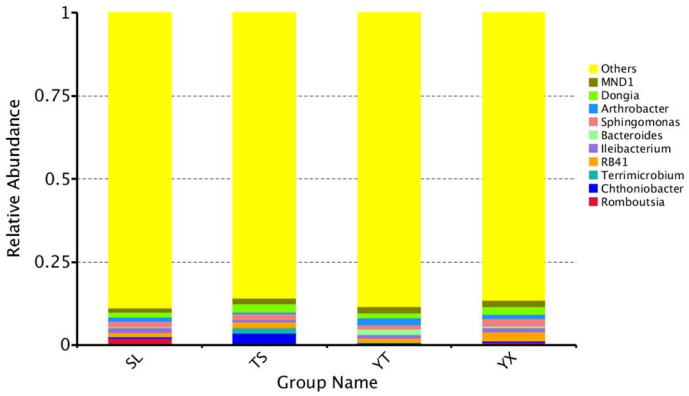

Fig.3.The composition and structure of bacterial community from the four deciduous fruit trees at the genus level.

Table 5.Percent of dominant bacterial community at the genus level.

Genus level	SL	TS	YT	YX
Romboutsia	0.0218	0.0034	0.0009	0.0084
Chthoniobacter	0.0031	0.0326	0.0047	0.0033
Terrimicrobium	0.0016	0.0176	0.0024	0.0014
RB41	0.0116	0.0157	0.0127	0.0275
Ileibacterium	0.0153	0.0088	0.0119	0.0132
Bacteroides	0.0026	0.0006	0.0162	0.0029
Sphingomonas	0.0159	0.0165	0.0134	0.0237
Arthrobacter	0.0126	0.0051	0.0196	0.0118
MND1	0.0140	0.0173	0.0184	0.0201
Dongia	0.0143	0.0240	0.0154	0.0240
Others	0.8872	0.8584	0.8844	0.8637

The functional traits of the rhizosphere microbiome: Tax4Fun software was used to predict the function of community samples by comparing community composition data with the categories of microbial metabolism functions in the KEGG database (Ashauer*et al.*2015). The KOs (KEGG orthology) were mainly involved in 6 KEGG level 1 pathways and 35 KEGG level 2 pathways (Figs 4, 5).

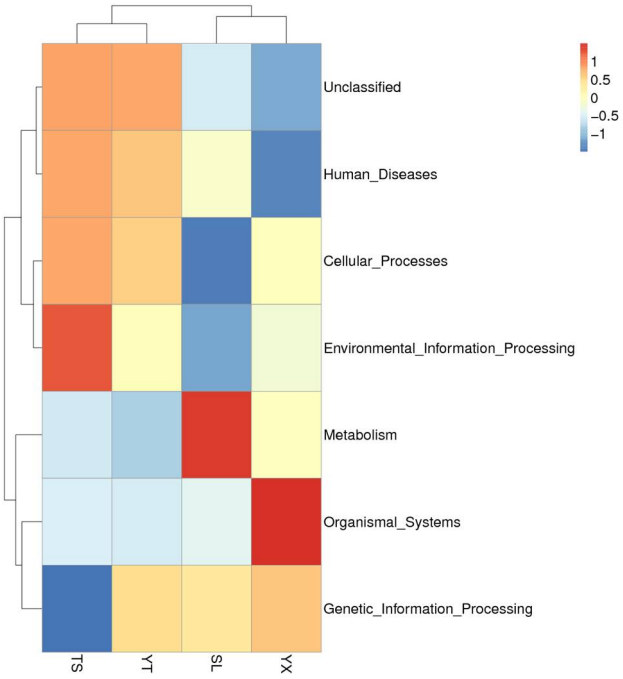

Fig.4.Characterisation of the functional traits in KEGG level 1 pathway of four deciduous fruit trees rhizosphere microbiome.

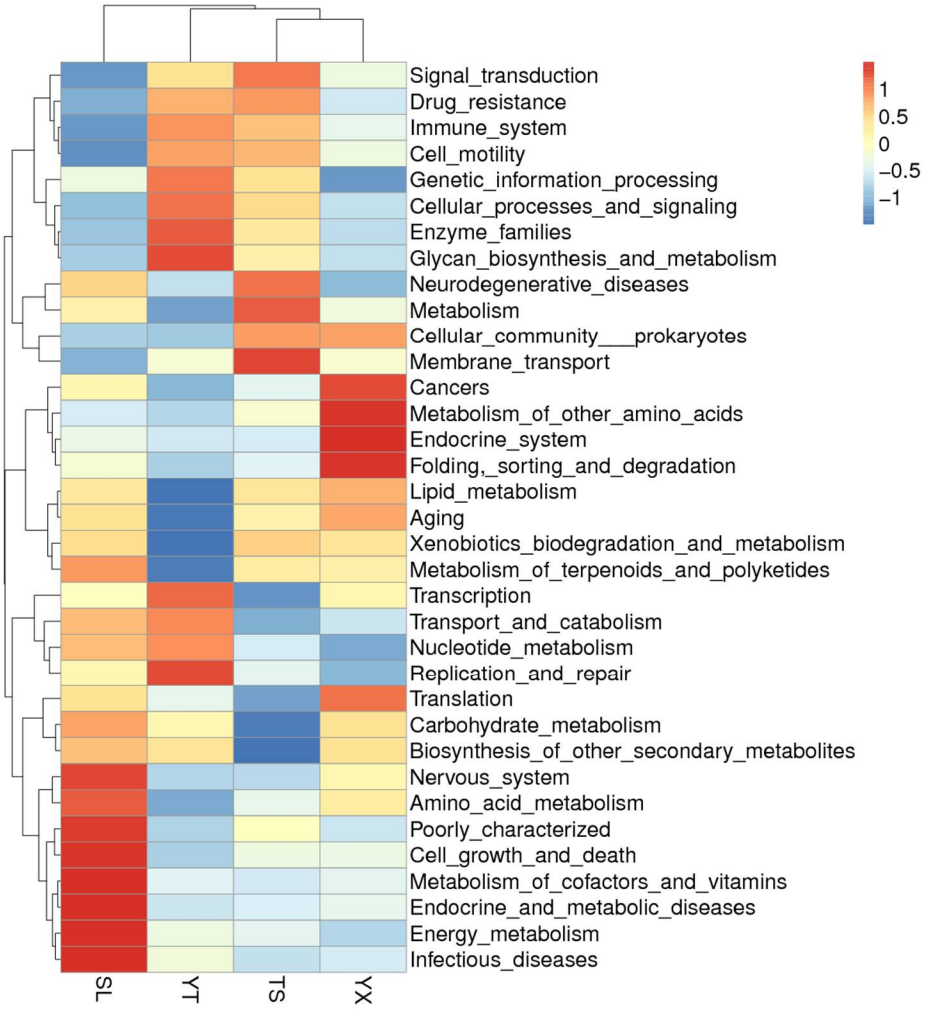

Fig.5.Characterisation of the functional traits in KEGG level 2 pathway of four deciduous fruit trees rhizosphere microbiome.

The relative abundance of cellular processes, environmental information processing, genetic information processing, human diseases, metabolism, and organismal systems pathways was 7.44-7.59, 12.74-13.20, 21.32-21.6472, 2.69-2.71, 47.97-48.44 and 1.83-1.86%, respectively, in therhizosphere soils of four samples at the first KEGG level. The relative abundance of themetabolism pathway was the highest, and genetic information processing was the second highest. The relative abundance of these six pathways was not significantlydifferent in rhizosphere soils among the four deciduous fruit trees (Table 6).

The results of KEGG metabolic pathway analysis show that the main enrichment pathways of microorganisms metabolism at the second KEGG level are consistent, mainly in carbohydrate metabolism, amino acid metabolism, energy metabolism, lipid metabolism, nucleotide metabolism and metabolism of cofactors and vitamins (Table 7).

Table 6. The proportion of predicted functional profiles in four deciduous fruit trees samples (Pathway level 1).

Pathway level 1	SL	TS	YT	YX
Metabolism	0.4844	0.4802	0.4797	0.4814
Genetic_Information_Processing	0.2160	0.2132	0.2162	0.2164
Environmental_Information_Processing	0.1274	0.1320	0.1297	0.1292
Cellular_Processes	0.0744	0.0759	0.0757	0.0753
Human_Diseases	0.0270	0.0271	0.0271	0.0269
Organismal_Systems	0.0184	0.0183	0.0183	0.0186
Unclassified	0.0524	0.0533	0.0533	0.0522

Table 7.The proportion of some predicted functional profiles in four deciduous fruit trees samples (Pathway level 2).

Pathway level 2	SL	TS	YT	YX
Carbohydrate_metabolism	0.1079	0.1065	0.1075	0.1076
Amino_acid_metabolism	0.0996	0.0982	0.0976	0.0988
Energy_metabolism	0.0468	0.0461	0.0462	0.0460
Lipid_metabolism	0.0381	0.0382	0.0370	0.0384
Metabolism_of_cofactors_and_vitamins	0.0341	0.0335	0.0336	0.0336
Nucleotide_metabolism	0.0333	0.0326	0.0334	0.0323
Glycan_biosynthesis_and_metabolism	0.0273	0.0278	0.0283	0.0274
Xenobiotics_biodegradation_and_metabolism	0.0202	0.0203	0.0192	0.0202
Metabolism_of_other_amino_acids	0.0183	0.0183	0.0182	0.0184
Metabolism_of_terpenoids_and_polyketides	0.0172	0.0171	0.0167	0.0171
Biosynthesis_of_other_secondary_metabolites	0.0148	0.0145	0.0147	0.0147

In summary, the taxonomic and functional properties exhibited similarity in **the** rhizosphere microbiome of four deciduous fruit trees in the same environment and at the same time. This work broadens the understanding of the relationship between rhizosphere microbial composition and function and lays a foundation for the exploitation of microbes to improve the microecological environment of the soil.

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