Analysis of Microbial Community Structure and Diversity in Crop Rhizosphere Soil Based on Illumina NovaSeq Sequencing Platform

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Abstract [**Objectives**] To make full use of crop rhizosphere microbial resources. [**Methods**] Illumina NovaSeq sequencing platform was used to analyze the richness and diversity of microbial community structure in rhizosphere soil of rice and maize crops in Baitu Town, Gaoyao District, Zhaoqing City. [**Results**] A total of 14 936 OTUs of bacteria and 1 905 OTUs of fungi were obtained from three samples of rice rhizosphere soil, and 13 437 OTUs of bacteria and 1 413 OTUs of fungi were obtained from three samples of maize rhizosphere soil. The diversity and richness of bacterial communities were higher than those of fungi. There are differences in soil bacterial and fungal communities among different crop samples. The analysis of species with bacteria difference at genus level among crop rhizosphere soil samples showed that 18 genera with significant differences were obtained from 6 samples; species analysis of fungi at the genus level showed that 3 genera with significant differences were obtained from 6 samples. [**Conclusions**] The research results of this paper have positive significance for the development and utilization of soil resources in Zhaoqing City and the full exploitation of rice and maize rhizosphere microbial resources.

Key words Rhizosphere soil, Microbial flora, Community diversity, Illumina NovaSeq sequencing

1 Introduction

Microorganisms are the most active components in soil and are important biological indicators to measure soil health[1]. The structure of soil microbial community plays an important role in the improvement of soil microecological environment and the sustainable use of soil, especially the rhizosphere soil microorganisms[2]. The rhizosphere is generally a few millimeters away from the surface of the root axis, which is the micro-region of soil-root-microorganism interaction and the interface between plant roots and soil[3]. Rhizosphere microorganisms exist closely in rhizosphere soil, are an important part of rhizosphere soil, and are closely related to plant disease and pest defense and yield[4-5]. Rhizosphere growth-promoting bacteria are a kind of natural soil bacteria that can significantly improve plant physiological indexes and control plant diseases, and can provide new ideas for the development of ecological agriculture[6]. Pan Liyuan *et al.* found that there is a greater number of rhizosphere microorganisms in high-yield paddy soil, the metabolic capacity is stronger, the microbial community distribution is more uniform, and the degree of diversity is higher[7].

Guangdong Province is a major economic province in China, but there are few studies and analyses based on soil microorganisms. At present, Xiao Qianwen *et al.* used phospholipid fatty acid (PLFA) biomarker method to analyze the characteristics and differences of soil microbial biomass and community structure in green spaces of various functional areas in Guangzhou University Town[8]. Zhao Lanfeng *et al.* used Biolog ecological microplate method to analyze the community structure of soil microbial carbon metabolism in vegetable gardens in four different regions of Guangdong: northern Guangdong, eastern Guangdong, western Guangdong and central Guangdong[9]. Rice and maize are important food crops, and it is of great significance to study the rhizosphere microbial diversity of rice and maize for maintaining and improving the productivity of paddy and maize field ecosystems[10]. In this study, the 16S rDNA of rice and maize rhizosphere bacteria and ITS gene sequence of fungi were amplified by PCR, and the PCR amplification products were sequenced by high-throughput to analyze the diversity of bacteria and fungi in soil, and understand the community structure, species composition and differences of rice and maize rhizosphere soil bacteria and fungi, so as to provide a reference for promoting the growth and development of rice and maize and cultivating higher quality rice and maize varieties.

2 Materials and methods

2.1 Soil sample collection Crop rhizosphere soil was collected on March 1, 2022, using a five-point sampling method (point spacing 1 m), three points of rhizosphere soil were taken at each of rice (SD) and maize (YM) plots, numbered as SD1, SD2, SD3, YM1, YM2, YM3, respectively. During sampling, dead branches and leaves on the surface were properly removed, soil drills were used to dig to the roots of the crops, and the root sys-

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tems were gently shaken to remove large grains of soil and plant residues. The plant root systems and the attached soil samples were sealed in sterile bags to be brought back to the laboratory and stored in liquid nitrogen at −80 ℃ for subsequent tests.

2.2 Determination of soil microbial community The total DNA of soil bacteria and fungi was extracted by DNA kit, and the operation procedure was carried out according to the instructions. The extracted soil microbial DNA was detected by 1% agarose gel electrophoresis, and then the DNA was sent to Beijing Nuohe Zhiyuan Technology Co., Ltd. for high-throughput sequencing through Illumina NovaSeq sequencing platform.

2.3 Statistical analysis of bioinformatics Based on the original data obtained for sequencing on the Illumina NovaSeq analysis platform, the original data was spliced and filtered to obtain effective data. Then based on a valid data similarity level of 97%, OTUs (Operational Taxonomic Units) clustering and species classification analysis were carries out. According to the clustering results of OTUs, species annotation was made on the representative sequence of each OTU, and the corresponding species information and abundance distribution based on species were obtained. Alpha diversity was calculated to obtain the information of species richness and uniformity within the sample. In order to further explore the differences of community structure among grouped samples, *T*-test statistical analysis method was used to test the significance of species composition and community structure of grouped samples.

3 Results and analysis

3.1 Analysis of microbial OTU number in crop rhizosphere soil Based on the sequencing data obtained by IIlumina NovaSeq analysis platform, the sequence information on three samples of rice (SD) and maize (YM) was read and clustered. In order to study the bacterial and fungal species composition of each sample, OTUs (Operational Taxonomic Units) clustering was performed with identity of 97%. The number of bacterial and fungal species OTUs in each sample is shown in Fig. 1. The results showed that the OTU numbers of rhizosphere soil bacteria in samples SD1, SD2, SD3, YM1, YM2, and YM3 were 5 115, 4 948, 4 873, 4 206, 4 958, and 4 273, and the OTU numbers of rhizosphere soil fungi were 568, 623, 714, 366, 551, and 496, indicating that the OTU numbers of bacteria and fungi among samples were not much different, but rice and maize in the same area changed slightly depending on their planting locations and environments.

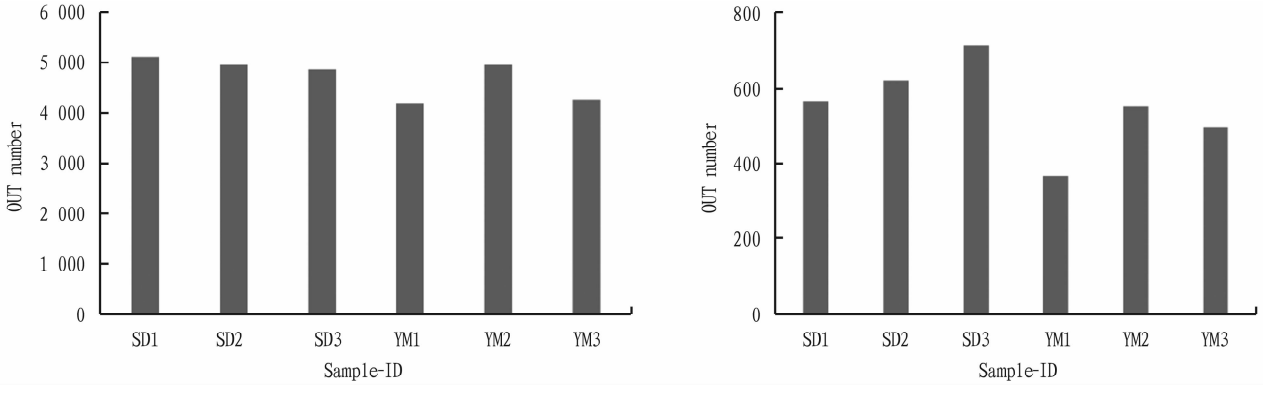

Fig. 1 Statistics of OTU clustering of soil bacteria and fungi under the condition of similarity at 97%

3.2 Analysis of microbial community structure and composition of crop rhizosphere soil

3.2.1 Analysis of bacterial community structure and composition. At the phylum level, there were 9 with clear classification, and most genera were unclassified (Fig. 2). They were Proteobacteria, Firmicutes, Bacteroidota, Acidobacteriota, Chloroflexi, Myxococcota, Actinobacteriota, Actinobacteria, Crenarchaeota, among which Proteobacteria was very obvious, and the relative abundance in rice and maize samples was above 17.3%; the second dominant phylum, Bacteroidetes, showed certain superiority, especially the relative abundance in maize samples reached more than 13.0%.

3.2.2 Analysis of fungal community structure and composition. Through comparison with UNITE database, species annotation was carried out, and statistics of different taxonomic levels showed that there were 1 322 OTUs in total. And the number of OTUs that can be annotated to the database was 1 288 (97.43%), the proportion of OTUs annotated to the phylum level was 72.09%, and the proportion of OTUs annotated to the genus level was 70.73%. According to the results of species annotation, the top 10 species with maximum abundance at each taxonomic level (phylum, class, order, family, genus, species) of each sample were selected to generate a column cumulative chart of species relative abundance. Column chart of relative species abundance at phylum level (Fig. 3). At the taxonomic level of phyla, there were many fungal species that can be annotated in rice samples and maize samples. The top ten phyla in abundance were Mucoromycota, Kickxellomycota, Zoopagomycota, Rozellomycota, Glomeromycota, Mortierellomycota, Basidiomycota, Chytridiomycota, Ascomycota and Olpidiomycota. Among them, the dominant phylum was Ascomycota, especially in maize samples, the relative abundance of Ascomycota was as high as 59%. The second dominant phylum was Olpidiomycota, which showed certain superiority in rice, but it was not obvious in rice samples.

3.3 Alpha diversity analysis of soil microbial community

3.3.1 Alpha diversity analysis of soil bacterial microbial communities. According to the sequencing results of bacterial 16S rDNA, various indicators of bacterial community abundance and diversity in 6 soil samples were analyzed (Table 1). According to the data analysis, for Observed_species, SD1 had the largest number of species, indicating the largest species diversity. For Coverage, it reached more than 97% for all six kinds of soils, and the average coverage rate was 98.35%, far exceeding 90%, indicating that the sequencing results can represent the authenticity and effectiveness of the experimental results. For Simpson, among the six soils, SD1 and SD3 had the highest Simpson index, indicating the highest diversity and uniformity of species distribution within the community. For Shannon, the Shannon index of maize 1 soil (YM1) and maize 3 soil (YM3) among the six soils was low (9.693 and 9.928, respectively), but it was not much different from the other four soils, while the average Shannon index of the other four soils reached 10.379 – 10.785, indicating that the bacterial community diversity of these four soils was higher than that of maize 1 soil (YM1) and maize 3 soil (YM3). In the four soils SD1, SD2 and YM1 and SD3, the Shannon index gradually increased from left to right, indicating that their community diversity was higher and higher, and the species distribution was more and more uniform. For Chao1 index, the ACE index of paddy soil was generally higher than that of maize soil, indicating that the number of OTU was the highest in paddy soil community. All in all, the OTU number, Shannon index, ACE index and Chao1 index of bacteria in paddy soil were mostly higher than those in maize soil, indicating that the bacterial diversity in paddy soil was relatively richer.

Table 1 Alpha diversity index of soil samples

Sample ID	Observed species	Coverage %	Shannon index	Simpson	Chaol index	ACE index
SD1	5 115	98.40	10.781	0.999	5 542.681	5 643.789
SD2	4 948	98.50	10.538	0.998	5 334.529	5 422.832
S03	4 873	98.60	10.785	0.999	5 292.640	5 329.667
YM1	4 206	98.50	9.693	0.994	4 705.722	4 753.314
YM2	4 958	97.40	10.379	0.998	6 812.382	6 237.266
YM3	4 273	98.70	9.928	0.995	4 657.350	4 700.074

3.3.2 Alpha diversity analysis of soil fungal microbial communities. According to the sequencing results of fungal ITS, various indicators of bacterial community abundance and diversity in 6 soil samples were analyzed (Table 2). According to the data analysis, for Observed_species, SD3 had the largest number of species, indicating the largest species diversity. For Coverage, it reached more than 99.8% for all six kinds of soils, and the average coverage rate was 99.83%, which was close to 100%, indicating that the sequencing results can represent the authenticity and effectiveness of the experimental results. For Simpson, among the six soils, SD3 had the highest Simpson index, indicating the highest diversity and uniformity of species distribution within the community. For Shannon, the Shannon index of rice soil 1 (SD1) and maize soil 1 (YM1) was low among the six soils (2.114 and 3.739, respectively), but not much different from the other four soils, while the average Shannon index of the other four soils reached 4.731 – 6.406, indicating that the diversity of fungal communities in these four soils was higher in rice soil 1 (SD1) and maize soil 1 (YM1). In the four soils YM2, SD2, YM3 and SD3, the Shannon index gradually increased from left to right, indicating that their community diversity was higher and higher, and the species distribution was more and more uniform. For Chao1 index, the ACE index of paddy soil was higher than that of maize soil, indicating that the number of OTU was the highest in paddy soil community. All in all, the OTU number, ACE index and Chao1 index of fungi in paddy soil were mostly higher than those in maize soil, indicating that the fungal diversity in paddy soil was relatively richer.

Table 2 Alpha diversity index of soil samples

Sample ID	Observed species	Coverage %	Shannon index	Simpson index	Chaol index	ACE index
SD1	568	99.80	2.114	0.418	610.933	625.430
SD2	623	99.90	5.514	0.937	651.714	655.528
SD3	714	99.80	6.406	0.971	950.478	828.521
YM1	366	99.90	3.739	0.847	404.647	417.080
YM2	551	99.80	4.731	0.880	610.348	625.407
YM3	496	99.80	5.148	0.939	553.722	561.641

3.4 Analysis of species with difference between crop rhizosphere soil sample groups The analysis of species with bacterial difference at the genus level between groups of crop rhizosphere soil samples is shown in Fig. 4. Eighteen genera with significant differences were obtained from 6 samples: RB41, Rhodanobacter, Anaeromyxobacter, Qdoribacter; Acidipila – Silvibacterium, Blastomonas spp., UTCFX1, Rothia, Mle1-7, pathogenic bacteria-nitrosobacteria, granule, Subgroup-10, Citrinfermentans, Terrimonas, GOUTA6, unidentified bacteria, SWB02, *Ranunculus* L. The abundance of Rhodanobacter, Qdoribacter, Acidipila – Silvibacterium and Blastomonas in maize samples was higher than that in rice group, and the difference was significant ($P<0.05$). The abundance of granules in maize group was higher than that in rice group, and the difference was extremely significant ($P<0.01$). The abundance of GOUTA6 in rice group was higher than that in maize group, and the difference was extremely significant ($P<0.01$). The abundance of 10 genera including RB41, *Anaeromyxobacter*, UTCFX1, *Rossbacteria*, Mle1-7, pathogenic bacteria-nitrite bacteria, Subgroup-10, *Citrinfermentans*, *Terrimonas* and *Ranunculus* in rice samples was significantly higher than that in maize group, with significant difference ($P<0.05$). Compared with the rice group, the dominant bacterial communities in the maize group were *Rhodanobacter*, *Qdoribacter*, *Acidipila – Silvibacterium*, *Blastomonas* spp., and granule. Compared with the maize group, the dominant bacterial communities in the rice group were RB41, *Anaeromyxobacter*, UTCFX1, *Rothia*, Mle1-7, pathogenic bacteri-

a-nitrite bacteria, Subgroup-10, *Citrinfermentans*, *Terrimonas*, GOUTA6, and *Ranunculus* L.

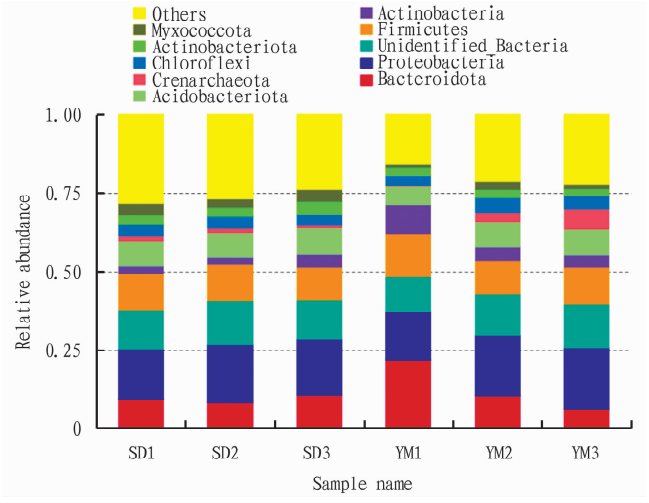

Fig. 2 Bacterial community structure of soil samples at phylum level

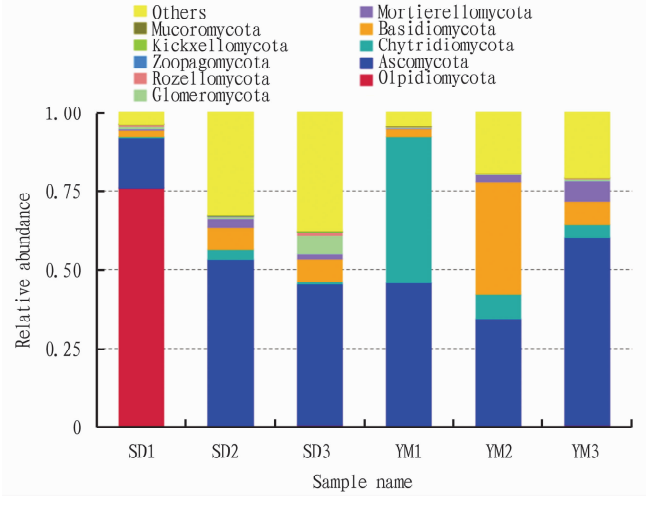

Fig. 3 Fungal community structure of soil samples at phylum level

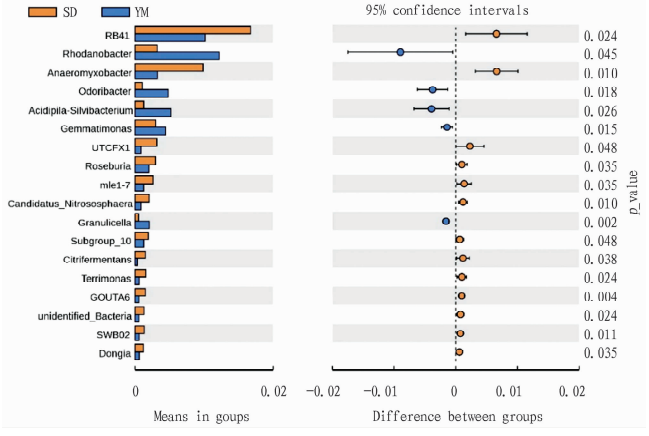

Fig. 4 Analysis of species with bacterial differences at genus level between groups

The species with difference of fungi at the genus level between groups of crop rhizosphere soil samples are shown in Fig. 5. Three genera with significant differences were obtained from 6 samples, namely *Penicillium*, *Apiosordaria* and *Phaeosphaeria*. The average value of *Penicillium* in rice group was 0.004 0, which was significantly lower than that in maize group, and the difference was extremely significant ($P<0.01$). The average value of *Apiosordaria* in maize group was 0.006 0, which was significantly lower than that in rice group, and the difference was significant ($P<0.05$). The average value of *Phaeosphaeria* in the rice group was 0.000 2, which was significantly lower than that in the maize group, and the difference was extremely significant ($P<0.01$) (Table 4). Compared with the bacterial communities of rice group, the dominant bacterial communities in maize group were *Penicillium* and *Phaeosphaeria*, and the dominant bacterial community in rice group was Apiosordaria.

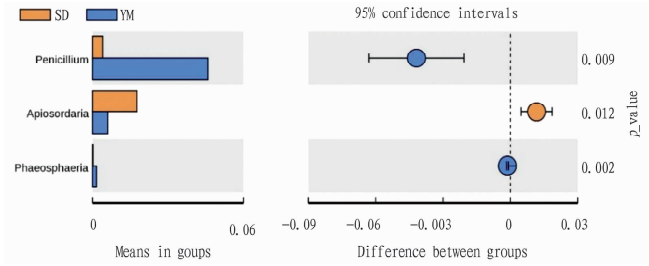

Fig. 5 Analysis of species with fungal differences at genus level between groups

4 Discussion and conclusion

Soil is the material basis for the survival of various plants, and the community structure of microorganisms in different plant varieties and different soil properties is very different[11]. Microorganisms are the most active components in rhizosphere soil, mainly composed of bacteria, fungi and actinomycetes. They carry out oxidation, nitrification, nitrogen fixation and other processes in the soil, promote the decomposition of soil organic matter and the transformation of nutrients, and participate in the material acquisition of plants in the soil, so rhizosphere microorganisms are the key factor to ensure soil ecological stability[1-3]. At present, there are few studies on the microbial community structure of rice and maize soil in China. Li Hongyi analyzed and characterized the bacterial community composition of paddy soil and its surrounding soil through high-throughput sequencing technology, revealing the significant differences in microbial communities and functions between paddy soil and surrounding ecosystems[23]. Liu Quancheng used culture method and plate confrontation method to isolate and screen a strain from maize rhizosphere soil with great biocontrol potential, which can be used to control maize diseases[12].

In this study, based on the sequencing data obtained by Illumina NovaSeq analysis platform, the sequence information of three samples of rice (SD) and maize (YM) was read and clustered. The results showed that the number of bacterial and fungal OTUs among the samples was not very different, but the rice and maize in the same area varied slightly with their planting locations and environments. Bacteria were compared with the database Silva138, species annotation was performed, and different

taxonomic levels were statistically analyzed. At the phylum level, Proteobacteria showed very obvious performance, and the relative abundance in rice and maize samples reached more than 17.3%. The fungi were compared with the database UNITE, species annotation was carried out, and different taxonomic levels were statistically analyzed. At the phylum level, the dominant phylum is Ascomycota. Alpha diversity refers to the number of species in local uniform habitat, which can reflect the coexistence results of microbial communities by competing for resources or utilizing the same habitat[13]. In the analysis of Alpha diversity of soil microbial community, the diversity of bacteria and fungi in rice soil is relatively abundant. In addition, it can enrich China's strain resource bank and promote the application of microbial fertilizers in agriculture.

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generation by installing photovoltaic panels on agricultural land. The results of the study show that agro-photovoltaic systems have significant advantages in increasing land use efficiency, providing renewable energy, and improving agricultural output. However, their economic benefits are affected by a number of factors, including crop type, seasonal variations, and land use structure.

The economics of agro-photovoltaic systems are highly dependent on crop type and cropping structure. Therefore, when designing and implementing agro-photovoltaic systems, priority should be given to selecting suitable crop types to maximize the economic benefits. Policy support and subsidies play a key role in the promotion of agro-photovoltaic systems. The government should formulate favorable legal frameworks and plans, and provide financial subsidies and tax incentives to encourage farmers and enterprises to invest in agro-photovoltaic systems. In addition, improving the efficiency and durability of PV modules through technological innovation and R&D, and lowering the initial investment and operation and maintenance costs of the system will also significantly improve its economics.

In the future, the development of agro-photovoltaic systems should further incorporate region- and crop-specific optimization designs. By comprehensively considering factors such as land use, crop types, and climatic conditions, advanced simulation and optimization tools should be used to develop a scientific and reasonable system configuration plan. At the same time, international cooperation and experience exchange should be strengthened, successful cases and best practices should be promoted, and the widespread application of agricultural and solar complementary systems should be promoted worldwide.

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