Scientific and Social Research, 2026, Volume 8, Issue 3
https://ojs.bbwpublisher.com/index.php/SSR
Online ISSN: 2981-9946
Print ISSN: 2661-4332

Metagenomic Analysis of Rhizosphere Microbial Community Structure in Tea-Fruit Intercropping Systems

Mingyan Li[1], Kuan Yan[2], Jianghua Liu[1]*

[1]Southwest Forestry University, Kunming 650000, Yunnan, China
[2]Yibin University, Yibin 644000, Sichuan, China

**Author to whom correspondence should be addressed.*

Abstract: In the tea fruit intercropping system, intercropping between tea trees and fruit trees can enhance plant nutrient utilization advantages, and plant growth is closely related to the rhizosphere microorganisms of plants. In order to explore the community structure characteristics of rhizosphere soil microorganisms under tea fruit intercropping mode, *Method:* This study used the Illumina Novaseq sequencing platform to perform metagenomic sequencing on three types of rhizosphere soil, with tea tree monoculture and loquat tree monoculture as controls, to analyze the differences in rhizosphere microbial community structure between tea fruit intercropping. The NR annotation results showed that under the tea fruit intercropping mode, the abundance of the dominant phylum Actinobacteria showed a decreasing trend, while the abundance of Acidobacteria and Proteobacteria increased. The relative abundance of unclassified Acidobacteria, unclassified Actinobacteria of the dominant phylum Actinobacteria, and unclassified Actinobacteria of the phylum Actinobacteria all increased. The annotation results of COG functional genes showed that in the functional composition of rhizosphere soil microorganisms in tea fruit intercropping, functions related to amino acid transport metabolism occupy a dominant position, and compared with tea monoculture, the relative abundance of functions related to amino acid transport metabolism, energy generation, and conversion is significantly increased. The annotation results of KEGG functional genes showed that pathways related to metabolism occupy a dominant position in the functional composition of the three rhizosphere microorganisms, and compared with tea monoculture, the abundance of functions related to energy metabolism was significantly increased in tea fruit intercropping. After intercropping tea and fruit, their nitrogen fixation and disease resistance abilities were increased, soil fertility was improved, and energy metabolism was enhanced, significantly improving nitrogen metabolism and enhancing the growth status of tea tree roots.

Keywords: Metagenomic sequencing; Rhizosphere microorganisms; Tea fruit intercropping; Soil microorganisms; Differences in community structure

1. Materials and methods

1.1. Experimental site description

Field experiments were conducted in May 2024 in Yezhucao, Jinping Town, Cuiping District, Yibin City, Sichuan Province (104°72’E, 28°93’N). The soil type was brown loam with a moist texture, and the terrain was predominantly hilly. The experimental tea garden covered an area of 510 m². The region features a subtropical monsoon climate combining central and southern subtropical characteristics: an average annual temperature of 18.7°C, an annual rainfall of 1143.5 mm, and distinct synchronous rainfall and heat distribution, providing abundant water and heat resources suitable for tea tree growth. Organic fertilizer was applied to the soil every December [1]. Three sampling sites with the same south-north orientation and an altitude difference of 50 m were selected [2]. Both tea trees and loquat trees in the garden were 8 years old, with the tea tree cultivar being Fuding Dabai. The experiment included three treatments: loquat tree monoculture (PP), tea tree monoculture (CS), and tea-loquat intercropping (PP-CS). The planting densities were: 40 cm × 50 cm for tea tree monoculture, 440 cm × 440 cm for loquat tree monoculture, and 180 cm × 77 cm for tea trees combined with 180 cm × 300 cm for loquat trees in the intercropping system [3–5].

1.2. Soil sample collection

Healthy loquat and tea trees were selected for sampling using the five-point method. Shovels disinfected with 75% ethanol were used to excavate main and lateral roots 10 cm from the trunk at a depth of 10–30 cm. Rhizosphere soil adhering to the root surface (0–4 mm from the root) was collected with sterile brushes, weighing approximately 5–10 g per sample. The samples were mixed, sealed with tinfoil, frozen in liquid nitrogen, and then stored in labeled sterile bags placed in a foam box filled with dry ice for preservation and transportation.

1.3. Experimental methods

Metagenomic sequencing was entrusted to Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China), with the following workflow: Genomic DNA was extracted using the Fast DNA™ Spin Kit for Soil (MP Biomedicals, USA). After DNA extraction, the isolated genomic DNA was verified by 1% agarose gel electrophoresis. DNA was fragmented into approximately 350 bp segments using a Covaris M220 ultrasonic disruptor.PE library construction was performed with the NEXTFLEX Rapid DNA-Seq Kit (Bioo Scientific, Austin, TX, USA), including end repair, adapter ligation, and fragment selection. After library preparation, target DNA fragments were enriched by bridge PCR amplification, followed by sequencing on the Illumina HiSeq 4000 high-throughput sequencing platform to obtain raw metagenomic sequence data.

1.4. Metagenomic sequencing and data analysis

Raw sequencing data were subjected to paired-end sequence splitting and quality filtering using fastp software (v0.20.0) to remove adapters and low-quality sequences. Clean reads were assembled using MEGAHIT (v1.1.2), an assembler based on succinct de Bruijn graphs, and contigs ≥ 300 bp were selected as the final assembly result. Open Reading Frames (ORFs) were predicted using Prodigal, and genes with nucleic acid lengths exceeding 100 bp were translated into corresponding amino acid sequences [6]. To construct a non-redundant database, predicted genes were clustered by CD-HIT, and the longest sequence was selected as the representative sequence. Finally, based on the SOAPaligner tool, high-quality reads from each sample were aligned with the non-redundant gene set to analyze and calculate the gene abundance in the corresponding samples [7].

2. Results and analysis

2.1. Rhizosphere microbial diversity analysis

2.1.1. α-Diversity of rhizosphere microorganisms

α-diversity reflects the level of species diversity within samples, with indices including Ace, Sobs, Chao, and Shannon. Among these, species richness is mainly characterized by the Sobs, Chao, and Ace indices, while species diversity is primarily represented by the Shannon index. The Chao and Ace indices are positively correlated with community species richness—higher values directly correspond to a greater number of species in the sample [8]. The Shannon index is positively correlated with community species diversity—higher values indicate increased community diversity.

The α-diversity analysis results of the three rhizosphere soil microbial communities are shown in **Table 1**. The order of species richness (from lowest to highest) is: PP3, PP1, PP2, PP_CS3, PP_CS2, PP_CS1, CS1, CS2, CS3. The order of species diversity (from lowest to highest) is: PP1, PP_CS3, PP_CS2, PP2, PP3, CS1, CS2, PP_CS1, CS3. These results indicate that after tea-fruit intercropping, the soil microbial richness is higher than that in loquat monoculture but lower than that in tea tree monoculture. Additionally, the soil microbial diversity after intercropping is lower compared to both monoculture systems.

Table 1. Diversity indices of three rhizosphere soil microbial communities

Type	sobs	ace	chao	shannon
PP1	12896	12896	12896	4.884322
PP2	12904	12904	12904	5.054283
PP3	12725	12725	12725	5.264712
PP_CS1	17774	17774	17774	5.631592
PP_CS2	15866	15866	15866	4.892212
PP_CS3	15595	15595	15595	4.905566
CS1	19102	19102	19102	5.545478
CS2	20452	20452	20452	5.474727
CS3	21171	21171	21171	5.747096

2.1.2. Analysis of rhizosphere microbial β-diversity

There are various analytical methods for β-diversity, among which Principal Component Analysis (PCA) is widely used as a typical approach. This method constructs a multivariate data matrix based on the species composition or functional characteristics of community samples. During application, high-dimensional ecological data is transformed into coordinate points in a low-dimensional space through dimensionality reduction, thus clearly showing the ecological distance relationships between different samples. In the coordinate system, the closer the spatial distance between samples, the more similar their compositions are.

The PCA results of microbial community structure in the rhizosphere soils of CS, PP_CS, and PP are shown in **Figure 1**. There are distances between the three groups, indicating that the microbial community structures of the three types of soils are significantly different.

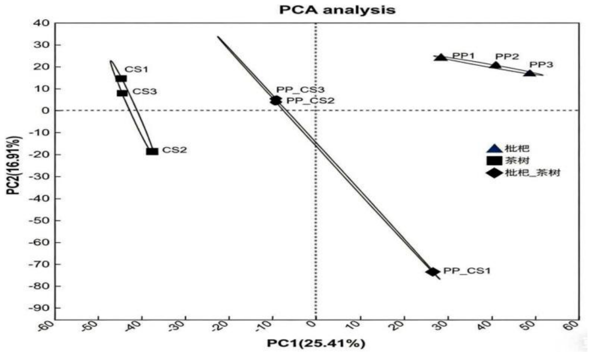

Figure 1. PCA analysis of microbial community structure in three types of rhizosphere soils

2.2. Analysis of rhizosphere microbial community composition

2.2.1. Analysis of rhizosphere microbial community composition at the phylum level

The results of species annotation based on the NR database indicated that the microorganisms in the samples were dominated by bacteria, with a relative abundance of approximately 89.86%. Fungal taxa followed, accounting for about 5.60%, while archaea and other microorganisms only made up 4.54%. As shown in **Figure 2**, the dominant microbial phyla in the three soil samples mainly included Actinobacteria, Proteobacteria, Acidobacteria, Chloroflexi, Gemmatimonadetes, Thaumarchaeota, Planctomycetota, Bacteroidota, and Verrucomicrobia. The microbial communities in the three rhizosphere soil samples were classified into a total of 161 phyla. As shown in **Figure 3**, further analysis revealed that the composition of dominant phyla showed similar characteristics among different samples, but their relative abundance distributions exhibited certain differences. Actinobacteria, Proteobacteria, and Acidobacteria were the dominant phyla in all three rhizosphere soil samples. In the PP_CS samples, the relative abundance of Actinobacteria decreased by 1.46% compared with that in the CS samples, but increased by 3.27% compared with that in the PP samples. The relative abundance of Proteobacteria increased by 3.23% and 6.55% compared with that in the CS and PP samples, respectively. The relative abundance of Acidobacteria increased by 4.09% and 3.53% compared with that in the CS and PP samples, respectively. These results indicated that different cropping patterns had significant effects on the structure of the tea tree rhizosphere microbial community.

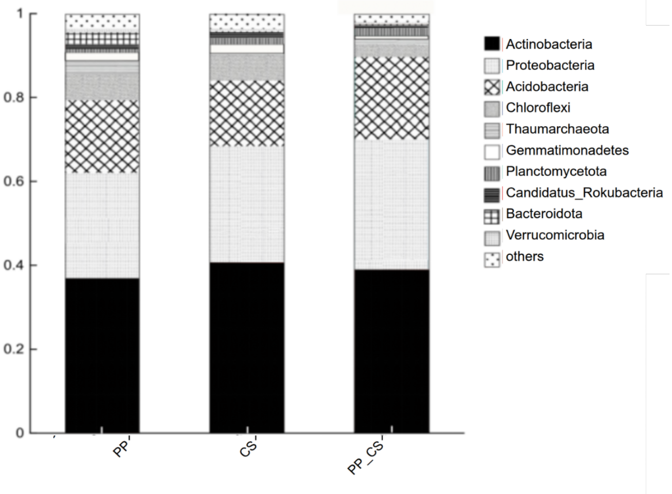

Figure 2. Community composition of rhizosphere microorganisms at the phylum level in three types of rhizosphere soils

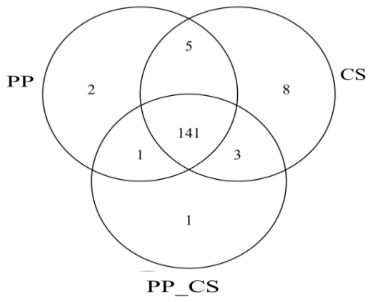

Figure 3. Venn diagram of rhizosphere microbial species at the phylum level in three types of rhizosphere soils

2.2.2. Analysis of rhizosphere microbial community composition at the genus level

The information on the dominant taxa of the three rhizosphere microbial communities at the genus level is presented in **Figure 4**. The main taxa included unclassified Acidobacteria (phylum Acidobacteria, with an average relative abundance of 8.96%), unclassified Actinomycetia (class Actinomycetes, 8.20%), unclassified Actinobacteria (phylum Actinobacteria, 4.95%), unclassified Solirubrobacterales (order Solirubrobacterales, 3.42%), and unclassified Chloroflexi (phylum Chloroflexi, 3.35%).

There were significant differences in the number of genera among the three rhizosphere microbial communities. As shown in **Figure 5**, the microbial communities across the three rhizosphere soil samples were classified into a total of 3,838 genera. The variation trend in the number of detected genera among different treatment groups was as follows: tea tree monocropping (CS, 3,550 genera) > tea-loquat intercropping (PP_CS, 3,288 genera) > loquat monocropping (PP, 2,972 genera). Specifically, 321 unique genera were identified in the CS samples, 148 in the PP_CS samples, and 79 in the PP samples.

The composition of dominant genera showed certain similarities among all groups, but their relative abundances exhibited distinct differences. In the three rhizosphere soil microbial communities, the dominant genera were unclassified Acidobacteria (phylum Acidobacteria), unclassified Actinomycetia (class Actinomycetes), and unclassified Actinobacteria (phylum Actinobacteria). Among them, in the PP_CS samples, the relative abundances of unclassified Acidobacteria, unclassified Actinomycetia, and unclassified Actinobacteria increased by 4.76%, 2.39%, and 0.55%, respectively, compared with those in the CS samples.

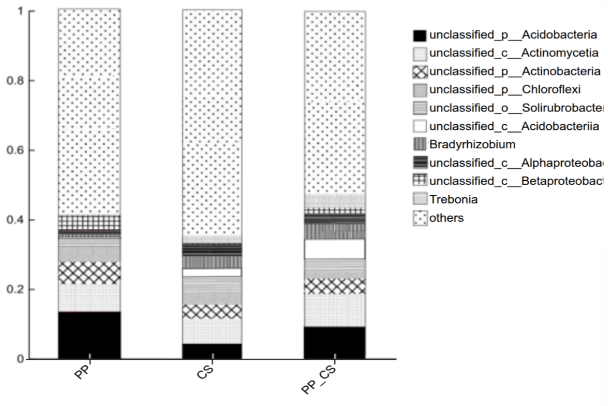

Figure 4. Community composition of rhizosphere microorganisms at the genus level in three types of rhizosphere soils

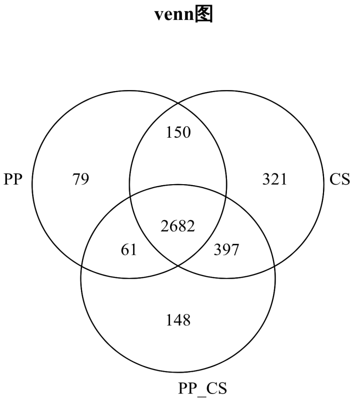

Figure 5. Venn diagram of rhizosphere microbial species at the genus level in three types of rhizosphere soils

3. Discussion

Rhizosphere microorganisms in tea tree soil play a crucial regulatory role in the physiological metabolism and ecological adaptability of tea trees [9]. Based on the metagenomic sequencing method, this study performed high-throughput sequencing of the total genetic material of microbial communities in environmental samples surrounding plant roots, aiming to systematically investigate the population distribution patterns, metabolic functional characteristics, and regulatory roles of tea tree rhizosphere microorganisms in the ecosystem.

In the α-diversity analysis of rhizosphere microorganisms from the three soil types, it was found that both the diversity and abundance of soil microorganisms under tea-fruit intercropping were lower than those under tea tree monocropping, which was inconsistent with the results of previous studies [10–11]. This discrepancy might be attributed to the alterations in soil pH and organic matter content caused by tea-fruit intercropping [12]. Relevant studies have demonstrated that compared with monoculture tea gardens, the soil pH values under three tea-fruit intercropping patterns (loquat-tea, bayberry-tea, and citrus-tea) are lower. The reduced soil pH inhibits the growth of microbial taxa adapted to neutral and alkaline environments, which is consistent with the decreased abundance of Actinobacteria in tea-fruit intercropping soils as revealed by NR annotation results.

Species annotation results based on the NR database indicated that bacteria were the dominant microbial group in the samples, accounting for approximately 89.86% of the relative abundance [13]. Fungal taxa followed with a relative abundance of about 5.60%, while archaea and other microorganisms only made up 4.54%. At the phylum level, the dominant phyla of rhizosphere microorganisms in the three soil types were Actinobacteria, Proteobacteria, and Acidobacteria, which were consistent with the findings of previous studies [14]. However, there were certain differences in both species number and species composition among the three planting patterns. In terms of species number, the number of microbial phyla in tea-fruit intercropping soils was lower than that in tea tree monocropping soils. In terms of species composition, the relative abundances of Proteobacteria and Acidobacteria in tea-fruit intercropping soils were higher than those in both tea tree monocropping and loquat monocropping soils, whereas the relative abundance of Actinobacteria, a dominant phylum, was lower in tea-fruit intercropping soils than in tea tree monocropping soils. As a typical eutrophic anaerobic microbial group, Proteobacteria not only possess nitrogen-fixing functions and participate in organic matter transformation and soil structure formation, but also exert multiple roles in soil ecosystem construction due to their unique physiological characteristics. As key participants in nitrification, Acidobacteria can catalyze the reduction of nitrate and nitrite, and thus have irreplaceable ecological value for nitrogen cycling in the ecological environment. The results of this study suggested that tea-fruit intercropping could enhance nitrogen fixation and disease resistance, and improve nitrogen metabolism capacity, which was basically consistent with the results of previous studies. The community abundance of Actinobacteria was significantly positively correlated with soil available nutrient content, organic matter, and soil-related enzymes, indicating that the organic matter content in soils decreased after tea-fruit intercropping, which was inconsistent with the results of some previous studies. The speculated reason for this phenomenon is that tea trees prefer acidic soils, and their root exudates can further acidify the soil environment. In contrast, Actinobacteria favor slightly alkaline conditions [15]. Therefore, soil acidification in tea gardens would lead to a decrease in the abundance of Actinobacteria and an increase in the proportion of acid-tolerant microbial groups such as Acidobacteria. In addition, Verrucomicrobia was detected in all three rhizosphere soil samples, but its distribution characteristics varied among different planting patterns, with the abundance of Verrucomicrobia in tea-fruit intercropping soils being lower than that in tea tree monocropping soils. Previous studies have shown that the changes in the abundance of Verrucomicrobia are closely related to soil element metabolism: its abundance is positively correlated with carbon metabolism function and negatively correlated with nitrogen metabolism function, and the population size of Verrucomicrobia decreases with the increase of soil nutrient content. These findings indicated that tea-fruit intercropping could improve soil nitrogen metabolism while affecting soil fertility.

At the genus level, the dominant genera of rhizosphere microorganisms in the three soil types were unclassified Acidobacteria (phylum Acidobacteria), unclassified Actinomycetia (class Actinomycetes), and unclassified Actinobacteria (phylum Actinobacteria). In tea-fruit intercropping soils, the relative abundances of these three dominant genera were all higher than those in tea tree monocropping soils.

4. Conclusion

Roots serve as the link between plants and soil. A healthy soil environment can promote root development, thereby facilitating plant growth. Taking the rhizosphere microorganisms under three planting patterns (tea-fruit intercropping, tea tree monocropping, and loquat monocropping) as the research objects, this study analyzed the structure and function of microbial communities by means of metagenomic sequencing technology. The main conclusions are drawn as follows:

(1) After tea-fruit intercropping, both the diversity and abundance of rhizosphere microorganisms decreased.

(2) Actinobacteria, Proteobacteria, and Acidobacteria were the dominant phyla among the rhizosphere microorganisms under the three planting patterns. After tea-fruit intercropping, the relative abundance of Actinobacteria was lower than that under tea tree monocropping, while the relative abundances of Proteobacteria and Acidobacteria were higher than those under tea tree monocropping.

(3) At the genus level, the dominant genera of rhizosphere microorganisms were unclassified Acidobacteria (phylum Acidobacteria), unclassified Actinomycetia (class Actinomycetes), and unclassified Actinobacteria (phylum Actinobacteria). In the tea-fruit intercropping system, the relative abundances of these three dominant genera were all higher than those under tea tree monocropping.

(4) Tea-fruit intercropping led to the enrichment of more differential bacterial taxa, but reduced the enrichment of differential fungal taxa.

Disclosure statement

The authors declare no conflict of interest.

References

[1] Parolin P, Bresch C, Desneux N, et al., 2012, Secondary Plants Used in Biological Control: A Review. International Journal of Pest Management, 58(2): 91–100.

[2] Cao QM, Wang YP, Qin JW, et al., 2020, Effects of Compound Planting on Soil Properties and Tea Chemical Quality in Hainan Tea Gardens. Southwest China Journal of Agricultural Sciences, 33(7): 1504–1509.

[3] Handelsman J, Rondon MR, Brady SF, et al., 1998, Molecular Biological Access to the Chemistry of Unknown Soil Microbes: A New Frontier for Natural Products. Chemical Biology, 5(10): R245–R249.

[4] Liu LY, Cui HF, Tian G, 2013, Application of High-throughput Sequencing Technology in Metagenomics. Chinese Medicinal Biotechnology, 8(3): 196–200.

[5] Lin XY, Li AS, Luo T, et al., 2024, Study on Fruit-tea Intercropping Cultivation Patterns. Anhui Agricultural Science Bulletin, 30(5): 21–25.

[6] Wen B, Zhang XL, Ren S, et al., 2019, Characteristics of Soil Nutrients, Heavy Metals and Tea Quality in Different Intercropping Patterns. Agroforestry Systems, 94(3): 963–974.

[7] Wang H, 2015, Responses of Soil Microbial Community Structure to Tea Planting Age, Fertilization and High Temperature Conditions in Tea Gardens, thesis, Zhejiang University.

[8] Hu MY, Li YY, Ge CR, et al., 2021, Research Progress and Application Prospects of Associative Nitrogen Fixation in Gramineous Plants. Chinese Journal of Eco-Agriculture, 29(11): 1815–1826.

[9] Du X, 2021, Microbial Community Structure and Functional Potential of Rhizosphere Soil of Plants in the Coastal Zone of Daihai Lake, thesis, School of Architecture and Civil Engineering, Inner Mongolia University of Science and Technology.

[10] Dong MH, Gu JR, Liu TF, et al., 2015, Comparative Analysis of Soil Nutrients in Different Tea-fruit Intercropping Tea Gardens in Dongting Mountain, Suzhou. China Tea, 37(5): 19–20.

[11] Li QS, Lei WX, Liu JX, et al., 2021, Effects of Ophiopogon japonicus Intercropping on Soil Physical and Chemical Properties and Microbial Community Structure in Tea Gardens. Journal of Southern Agriculture, 52(12): 3366–3374.

[12] Tian YH, Liang YF, Wang GH, et al., 2001, Study on Ecological Effects of Artificial Ecological Tea Gardens. Journal of Tea Science, 21(2): 170–174.

[13] Wang WX, Li XW, Huang WG, et al., 2020, Correlation between Bacterial Community Composition and Diversity of Rhizosphere Soil of Ammopiptanthus mongolicus and Ecological Factors. Acta Ecologica Sinica, 40(23): 8660–8671.

[14] Fierer N, Ladau J, Clemente JC, et al., 2013, Reconstructing the Microbial Diversity and Function of Pre-agricultural Tallgrass Prairie Soils in the United States. Science, 342(6158): 621–624.

[15] Navarrete AA, Soares T, Rossetto R, et al., 2015, Verrucomicrobial Community Structure and Abundance as Indicators for Changes in Chemical Factors Linked to Soil Fertility. Antonie Van Leeuwenhoek, 108(3): 741–752.