Effects of applied microbial cultures on the structure and diversity of bacterial communities associated with Phyllostachys edulis

Fang Liu
Fujian Agriculture and Forestry University
Zong-sheng Yuan
yuanzs369@163.com
Minjiang University
Zhi-hao Zeng
Fujian Agriculture and Forestry University
Hui Pan
Minjiang University

Article

Keywords: Moso bamboo (P. edulis), microbial cultures, high-throughput sequencing, bacterial community composition

Posted Date: June 26th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3060905/v1

Additional Declarations: No competing interests reported.

Abstract

In order to study the feasibility of microbial cultures on the artificial regulation of Moso bamboo (*Phyllostachys edulis*) forest. This study used the microorganisms isolated in the previous research to prepare microbial cultures, and studied the degree of its influence on the bacterial community of Moso bamboo tissue and soil. We collected 36 samples of bamboo whip, whip root, rhizosphere soil, and non-rhizosphere soil of *P. edulis* before and after the application of microbial cultures. Genomic DNA was extracted and Illumina high-throughput sequencing technology was used to analyze the composition and changes of bacterial communities before and after the application of microbial cultures. Twenty-nine phyla, 96 classes, 229 orders, 444 families, and 974 genera of bacteria were identified from all samples. The dominant phyla of the sample bacteria were Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Firmicutes, Myxomycetes, and Bacteroidetes. Treatment with microbial cultures did not alter the bacterial community in the rhizomes, rhizome roots, rhizosphere, and non-rhizosphere soil of *P. edulis*. However, the bacterial diversity indices of the rhizomes and rhizome roots of *P. edulis* increased with time after treatment. The relative abundance of Firmicutes was most affected by the application of microbial cultures, and the rhizosphere soil samples were least affected by the application. Venn diagram and principal coordinate analyses confirmed that the composition of the bacterial community was affected by microbial cultures, but with time, the effect became smaller. Our findings provide a theoretical basis for studies on relationships between the growth of *P. edulis* and the microbiome, and further provide experimental evidence for the transformation of *P. edulis* through microbial regulation.

Introduction

The rhizosphere is composed of many diverse microorganisms [1]. The diversity and abundance of soil bacteria in part determine many important aspects of ecosystem function [2,3,4]. Plants evolved to select microbes that formed plant-specific microbial communities. In normal plant growth and development, many microorganisms form habitats inside the plant, and they are termed plant endophytes, some of which increase the host's nutrient absorption capacity [1]. Relatedly, soil microorganisms fulfill an important function in soil nutrient cycling [5]. Together, both these sets of microbes contribute to plant growth and development, disease resistance, and stress tolerance [5]. However, different microbial communities are shaped by microenvironmental conditions [6].

Recently, in the fields of botany, breeding, and environmental protection, viable cultures of microorganisms with either different functions or mutual or symbiotic relationships were combined in appropriate proportions and applied biotechnologically [7]. Such microbial cultures contain large numbers of beneficial and viable bacteria that when applied to the soil, colonize the rhizosphere of plants and stimulate indigenous microorganisms, which enhances the species richness of the soil microbial community, optimizes the structure of soil aggregates, regulates the nutrient environment of roots, and degrades some of the organic pollutants of the environment to provide nutrients for plants [8]. Moreover, following colonization of the rhizosphere, these bacteria produce various plant growth stimulants [9].

Therefore, in the natural ecosystem, these microbial culture-derived bacteria, as well as rhizosphere and endophytic microorganisms, are plant symbionts that contribute to preventing and controlling plant diseases and promoting plant growth [9]. In recent years, researchers have made outstanding progress in the development of biologics [10,11]. Rhizosphere growth-promoting bacteria are also important biocontrol resources [12,13]. Therefore, it is of great significance to study and explore a way to modify the microbial community to improve the growth environment of plant roots and ultimately improve plant resistance and yield. These microbial communities have the ability to adapt to a variety of environmental conditions established for sustainable crop production [14].

Moso bamboo (*Phyllostachys edulis*) is an important forest resource in southern China, which is of high economic and ecological value. Today, few studies exist on the effects of the application of mixed microbial cultures on soil microbial communities in the *P. edulis* rhizosphere and the soil enzyme activities, and the mechanisms by which mixed microbial cultures promote *P. edulis* growth [15,16]. Therefore, we treated *P. edulis* plants by applying mixed microbial cultures to investigate their effects on the bacterial community composition. We aimed to provide a theoretical framework for studying stress resistance mechanisms of *P. edulis* and assess the feasibility of microbial culture-based artificial regulation. Understanding soil microbes and making targeted modifications may provide new strategies for sustainable agricultural system management [17]. In this study, the metagenomic DNA samples of the bamboo rhizomes, rhizome roots, rhizosphere soil, and non-rhizosphere soil of *P. edulis* were sequenced via an Illumina high-throughput platform [18,19] to determine changes in the structure and composition of the rhizosphere and endophytic bacterial communities of *P. edulis* after treatment with mixed microbial cultures.

Results

Species annotation and assessment

OTU and rarefaction curve analyses

A total of 7,948,668 high-throughput reads, with an average length of 375 bp, were obtained from the 36 samples. These were culled to a final data set that was clustered into OTUs, each of which was composed of *16s rDNA* sequences that shared at least 97% nucleotide identities. A total of 26,336 bacterial OTUs were obtained, which represented 29 phyla, 96 classes, 229 orders, 444 families, and 974 prokaryotic genera. As shown in Fig. 1, the rarefaction curves of the 36 samples flattened out with increasing sequence reads, whereas the number of OTUs gradually stabilized, indicating that the sequencing data were reliable as they sufficiently represented almost the entire microbial community. Hence, the sequencing data was sufficient to fully reflect species diversity in the samples, and their concomitant bacterial microbiome could be reliably described by our results [20].

Alpha diversity analysis

The alpha diversity indices based on the number of OTUs are shown in Table 1. The Sobs index represented the number of OTUs in the sample. The abundance-based coverage estimator (ACE) and the Chao1 indices indicated the number of observed bacterial species. The Shannon index reflected the diversity of bacterial communities. Higher values for these alpha diversity indices indicated higher prokaryotic diversity.

From Fig. 2, the Sobs, ACE, Chao1, and Shannon indices had a similar trend: soil samples had higher values than plant tissue samples. The alpha diversity indices of YA_A and YA_B samples treated with microbial cultures increased with the treatment duration. The diversity indices of YA_E samples did not significantly differ before and after treatment and with treatment duration, indicating that their diversity was barely affected by the application of microbial cultures. Diversity indices of YA_F samples fluctuated with time: after three months of treatment, diversity indices significantly decreased and recovered only in part, after six months of treatment.

Table 1
Alpha diversity indices of *P. edulis* samples

Sample	sobs	shannon	ace	chao1
YA.A0	666.33 ± 193.58e	2.49 ± 0.99b	809.12 ± 231.56e	810.39 ± 242.32e
YA.A1	1521.00 ± 297.18de	4.15 ± 0.36a	1976.15 ± 370.89de	1943.35 ± 299.48de
YA.A2	3892.67 ± 337.67ab	5.09 ± 0.24a	4916.47 ± 238.28ab	4543.86 ± 204.99ab
YA.B0	4653.33 ± 196.01a	5.21 ± 0.14a	6105.51 ± 614.33a	5544.89 ± 496.11a
YA.B1	616.33 ± 363.11e	2.46 ± 1.19b	896.81 ± 605.28e	780.08 ± 499e
YA.B2	2355.00 ± 371.53cd	4.72 ± 0.35a	3056.87 ± 457.72cd	2899.85 ± 437.24cd
YA.E0	3792.33 ± 114.21ab	5.03 ± 0.08a	5036.51 ± 113.41ab	4653.37 ± 87.21ab
YA.E1	3724.33 ± 325.55ab	4.82 ± 0.21a	4896.20 ± 359.37ab	4504.55 ± 353.50ab
YA.E2	1739.67 ± 1266.10de	4.19 ± 0.84a	2062.40 ± 1515.76de	1974.84 ± 1361.05de
YA.F0	2872.67 ± 739.62bc	4.63 ± 0.95a	3758.75 ± 1081.89bc	3465.06 ± 977.47bc
YA.F1	3718.67 ± 771.52ab	4.91 ± 0.34a	4927.48 ± 830.91ab	4500.09 ± 778.58ab
YA.F2	4158.33 ± 104.31ab	4.95 ± 0.10a	5368.75 ± 70.99ab	4922.21 ± 63.92ab

Species composition analyses

Species Venn diagram analysis

Venn diagrams depict the number of shared and unique OTUs in multiple groups of samples, and thus intuitively show both the similarity and overlap of OTU composition in environmentally different samples [21]. The OTU numbers in YA_A and YA_B samples increased with the treatment duration (Fig. 3), whereas those of YA_E soil samples remained constant. The OTU numbers in YA_F samples increased and then decreased with the treatment duration.

Among the four types of samples, YA_A and YA_F samples had the least (6,765) and most (22,989) OTUs, respectively. After six months of treatment, the unique OTUs of YA_A and YA_B samples were 7.48 and 4.08 times, respectively, of those before application. The unique OTU of YA_E1 and YA_F1 samples were 14.86% and 42.76%, respectively, less than those of YA_E0 and YA_F0. After six months of treatment, YA_E2 and YA_E3 OTUs were 4.42% and 25.02%, respectively, more than those of YA_E1 and YA F1. The OTU numbers did recover, but not to the level before the application of microbial agents. It shows that after treatment, the number of endophytic bacterial OTU in the tissue has been rising. However, the number of bacterial OTUs in rhizosphere and non-rhizosphere soils decreased and did not return to the level before the application of microbial agents after 6 months.

Community composition analysis

To further investigate the changes of specific taxa in the bacterial communities in the four types of samples, we compared the relative abundances of different taxonomic levels of the bacterial communities.

At the phylum level, the bacteria in the rhizome, rhizome root, rhizosphere soil, and non-rhizosphere soil samples mainly included Proteobacteria, Acidobacteriota, Actinobacteriota, Chloroflexi, Firmicutes, Myxococcota, and Bacteroidota.

The relative abundances of a few phyla in some samples increased and then decreased after three and six months of treatment, respectively. For example, the relative abundance of Firmicutes in the rhizome root sample increased after the three months of treatment from 1.9–11.1%, then dropped to 2.7% after the six months of treatment. Likewise, that of Bacteroidota in the rhizome root samples was only 0.1% before treatment, then increased after three months of treatment to 4.7% before dropping to 2.0% after six months of treatment. Chloroflexi and Firmicutes in the bamboo rhizome samples and Acidobacteriota and Actinobacteriota in the rhizosphere soil samples also showed this pattern, but the changes were not obvious. For the non-rhizosphere soil samples, only Acidobacteriota showed this pattern; the abundances of other Phyla either continuously decreased or first decreased and then increased (Fig. 4).

The relative abundances of a few taxa consistently increased at first, such as Acidobacteriota and Actinobacteriota in the rhizome root samples.

The relative abundances of some taxa showed a trend contrary to the above: they decreased in the third month and increased in the sixth month of treatment. These included Actinobacteriota in the bamboo rhizome samples, and Chloroflexi in the rhizome root, rhizosphere soil, and non-rhizosphere samples.

For some taxa, their relative abundances gradually decreased, and these included Proteobacteria in the rhizome, rhizome root and rhizosphere soil samples, Firmicutes in the rhizosphere soil samples, and Actinobacteriota in the non-rhizosphere soil samples.

At the genus level, bacteria in the rhizome, rhizome root, rhizosphere soil, and non-rhizosphere soil samples of *P. edulis* mainly included *Acidothermus*, *Bradyrhizobium*, *Candidatus-Solibacter*, *Burkholderia-Caballeronia-Paraburkholderia*, *Marinobacter*, and *Halomonas* (Fig. 5).

The relative abundance of taxa in some samples increased and decreased in the third month and sixth month, respectively, after the application of agents. The relative abundance of *Bradyrhizobium* in the rhizome root samples increased from 2.0–5.1% after three months of treatment then dropped to 2.3% after six months of treatment. *Brachyrhizobium* and *Burkholderia-Caballeronia- Paraburkholderia* in the rhizome root samples displayed a similar trend. Their relative abundance was only 0.6% before treatment, increased to 8.2% then fell to 6.5% after three and six months, respectively. The relative abundances of *Halomonas* in the rhizome root samples, *Candidatus-Solibacter* in the bamboo rhizome samples, *and Bradyrhizobium* and *Candidatus-Solibacter* in the rhizosphere soil samples displayed a similar pattern, but no taxa with such a pattern was found in the non-rhizosphere soil samples.

The relative abundance of a few taxa gradually decreased, including *Candidatus-Solibacter* in the rhizome root samples and *Acidothermus* in the bamboo rhizome samples.

In contrast, the relative abundances of some taxa communities gradually increased, including *Bradyrhizobium* and *Burkholderia-Caballeronia-Paraburkholderia* in the bamboo rhizome samples, and *Acidothermus* in the rhizosphere soil samples.

The relative abundances of some taxa also decreased and increased after three and six months of treatment, respectively, such as *Acidothermus* in the rhizome root samples and *Bradyrhizobium* in the bamboo rhizome samples.

Ternary phase diagram analysis

Ternary phase diagrams were used to visualize the composition and distribution ratio of dominant species in the three different groups (samples). Circles of the same color represent the same genus, and the size of the circle area represents abundance [22].

The bamboo rhizome samples YA_A0 and YA_B0 had high abundances of *Brachyrhizobium*, and *Unclassified_f_Alcaligenaceae*, respectively (Fig. 6). For other samples, no highly abundant prokaryotic taxon was detected after three months of treatment. Unclassified_f_*Alcaligenaceae* was observed in the bamboo rhizome sample YA_A1, and *Marinobacter* and *Barkholderia-Caballcronia-Paraburkholderia* in the rhizome root sample YA_B1. After six months of the treatment, *Staphylococcus, Acidothermus* and norank_f_*Xanthobacteraceae* were observed in the rhizome root sample YA_B2. The rhizosphere soil sample YA_E1 lacked a highly abundant prokaryotic taxon, whereas *Xanthobacteraceae* was highly abundant in the non-rhizosphere soil sample YA_F1. After six months of treatment, *Acidothermus* was

observed in the rhizosphere soil sample YA_E2 and *Marinobacter* and norank_f_*Xanthobacteraceae* in the non-rhizosphere soil sample YA_F2. These results indicate that the effect of the application of microbial cultures on bacterial communities in soil samples was either minor or delayed.

Comparative analysis of samples

Hierarchical cluster analysis

Here, samples could be divided into three different groups as per their constituent bacteria. The bacterial community composition of the plant tissue and soil samples of *P. edulis* significantly differed but did not cluster together [23]. The compositions of samples of the rhizosphere soil and the control soil were similar and clustered together, whereas those of the rhizome and rhizome root No. zero, one, and two were relatively dispersed (Fig. 7). Two samples of YA_B1 and a sample of YA_A2 formed a small independent cluster.

PCoA analysis

The colored ellipses represented the confidence intervals of each group of samples. The application of the growth-promoting microbial cultures had a greater impact on the compositions of bacterial communities in the bamboo rhizome and rhizosphere soil samples of *P. edulis* than that in the rhizome root samples (Fig. 8). The confidence interval [24] of the No. zero bamboo rhizome and rhizosphere soil samples did not overlap with the confidence interval of the No. one samples, but the confidence intervals of the No. zero, and one samples slightly overlapped with that of the No. two samples. However, the confidence interval of the No. zero non-rhizosphere soil samples did not overlap with the corresponding No. one and two samples, and the confidence interval of the No. one samples also overlapped with the No. two samples to a certain extent. These results suggest that the bacterial community compositions were greatly affected by microbial agents. However, with time, the compositions of the plant samples, and the soil samples from around the plants recovered, which corroborates the Venn diagram findings.

Analysis of differences in taxon abundances

Using relative abundance data of the samples, statistical analyses were conducted to detect taxa whose abundances differed between *P. edulis* samples (bamboo rhizomes, rhizome roots, and rhizosphere soil). Hypothesis testing was then performed to evaluate the significance of these differences (Fig. 9).

The abundances of *Nocardioides*, *Cellulomonas*, *Microbacterium*, *Rhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Mesorhizobium* in rhizome No. two samples were significantly higher than those of corresponding No. zero and one samples. The abundance of unidentified *Alcaligenes* in the rhizosphere soil No. zero samples was significantly higher than that of the corresponding No. one and two samples. The abundance of *Conexibacter* in the rhizosphere soil samples increased then decreased, whereas the abundance of unidentified *Myxococcus* decreased then increased. The abundance of

Chujaibacter in the rhizosphere No. two samples was significantly high than those of the corresponding No. zero and one samples.

Discussion

During growth and reproduction, microorganisms exhibit both mutualistic symbiosis and mutual competition. When a certain taxon in the rhizosphere microdomain increases, it inevitably affects the growth and reproduction of other taxa. For example, when the number of bacteria in the rhizosphere soil significantly increased, the number of certain pathogenic microorganisms reduced [25,26]. Therefore, this study applied microbial agent to a *P. edulis* forest in Yong'an, China, and determined whether they had an impact on the soil microorganisms through a series of tests.

Current similarly applied microbial cultures mainly contain beneficial bacteria —they produce activating substances and a variety of natural fermentation substances—, which proliferate in the root soil zone to form a dominant microbial community that promotes plant growth [27]. The rhizosphere microdomain environment is optimized by these microbes via their growth and metabolic processes [28], and this thus promotes the absorption of nutrients by plant roots and their growth [29]. For example, *Bacillus* secreted organic acids, which reduced soil pH, activated the soil, and fixed phosphorus and potassium nutrients [30]. *Acinetobacillus* bacteria had a remediation effect on polluted soil [31]. *Klebsiella aerogenes* exhibited phosphorolysis and had a growth-promoting effect on *P. edulis* plants [32]. Therefore, we identified previously reported endophytic bacteria of *P. edulis* [33,34,35] and created a mixed microbial culture of *Klebsiella aerogenes* strain CT-B09-2, the *Acinetobacter calcoaceticus* strain WYS-A01-1, and the *Bacillus amyloliquefaciens* strains JL-B05 and JL-B06 for application.

The diversity indices of the bamboo rhizome, rhizome root, rhizosphere soil, and non-rhizosphere soil samples had variation trends after the application of the mixed microbial cultures. The diversity indices of the bamboo rhizome and rhizome root samples increased with treatment duration. Specifically, the relative abundance of Firmicutes in the rhizome root samples increased from 1.9–11.1% after three months of treatment and dropped to 2.7% after six months of treatment. In the applied cultures were *B. amyloliquefaciens* strains JL-B05 and JL-B06, which are members of Firmicutes. This indicates that our microbial cultures may have influenced the endophytic bacterial community of *P. edulis* rhizome roots. In the non-rhizosphere soil samples, only the relative abundance of Acidobacteriota increased after three months of treatment, while that of other taxa either continuously decreased or first decreased then increased. However, the microbial cultures had little effect on the diversity of the rhizosphere soil samples, and the alpha diversity indices of the rhizosphere soil samples did not significantly change with treatment or treatment duration. Particularly, from the ternary phase diagram, the rhizosphere soil sample YA_E1 (after the three-month treatment duration) lacked a single highly abundant taxon. Moreover, the number of OTUs in the *P. edulis* bamboo rhizome and rhizome root samples increased with treatment duration, and that of the rhizosphere soil samples was barely affected by treatment duration. For the bamboo rhizome and rhizome root samples, the similarity in OTU composition between the No. one and

two samples was higher than that between the No. zero and one samples. This shows that the application of microbial cultures affected *P. edulis*, and the responses of bamboo rhizome and rhizome root tissues to these effects were stronger than those of the soil samples. Notably, when the abundance of a certain taxon in the bamboo rhizome and rhizome root tissues significantly changed or became the dominant taxon, it inhibited the growth of the original dominant taxon, resulting in a decrease in its abundance and ultimately leading to an altered diversity.

The applied microbial cultures had little effect on the rhizosphere soil, which may be due to the continuous influence of rhizosphere exudates. The rhizosphere exudates of *P. edulis* plants first attract organisms to the rhizosphere environment, and thus concomitant bacteria must be highly competitive to successfully colonize this niche. Therefore, the microbial community in rhizosphere soil is mainly influenced by the rhizosphere exudates. The rhizosphere exudates of healthy *P. edulis* plants are stable [35], which in turn results in a stable microbial diversity. Moreover, we found that in different *P. edulis* tissue samples, the order of the diversity indices was : soil samples > rhizome root samples > bamboo rhizome samples. Thus, soil samples inherently had a high diversity, and the application of microbial cultures had a greater impact on the alpha diversity indices of the microbiome of the bamboo rhizome and rhizome root tissues than the microbiome of the rhizosphere and non-rhizosphere soils.

At the same time, the PCoA analysis indicated that the effects of the microbial culture application on the bacterial community compositions in the bamboo rhizome and rhizosphere soil of *P. edulis* were greater than those of the rhizome root samples; the confidence intervals of the No. zero and one samples both slightly overlapped with that of the No. two samples, whereas the confidence intervals of the No. zero and one samples did not overlap. Relatedly, the bacterial community compositions of the rhizosphere soil and non-rhizosphere soil samples barely changed with increasing treatment duration. This suggests that the effects of the application of microbial cultures on the microbiome of *P. edulis* are significant in the short term. However, with increasing treatment duration, the composition of the bacterial community recovered to an extent, and the effect of the microbial cultures weakened or even disappeared.

By assessing the significance of differences between groups, we showed that the abundances of *Nocardioides*, *Cellulomonas*, *Microbacterium*, *Bradyrhizobium* of the bamboo rhizome No. two samples were significantly higher than those of the corresponding No. zero and one samples. Moreover, the abundance of *Chujaibacter* in the rhizosphere soil No. two samples was significantly greater than those of the corresponding No. zero and one samples. *Nocardioides* contributes to the degradation of crude oil and sewage treatment [36]. Some strains of *Cellulomonas* can degrade cellulose, and thus play a positive role in environmental carbon cycle promotion [37]. *Microbacterium* is a common hydrocarbon-degrading bacteria [38]. *Bradyrhizobium* performs nitrogen fixation and participates in the restoration of nitrogen in the ecosystem [39]. The known functions of these bacteria indicate that after the application of the microbial cultures, its constituent, *A. calcoaceticus* WYS-A01-1, probably improved the microenvironment, promoted the degradation of complex organic matters, and stimulated growth of indigenous microorganisms to a certain extent, thereby increasing the abundance of some functional taxa.

In addition, the applied microbial cultures lacked toxic and inhibitory effects on the growth of *P. edulis*, which demonstrated their feasible field application. Moreover, all our findings support the feasibility of using microbial cultures to regulate the growth of *P. edulis* plants and alter their microbiome. Furthermore, our findings are a possible foundation for studies on the relationship between the growth of *P. edulis* and its microbiome. However, apart from the influence of host plants, microorganisms are affected by various environmental factors, such as local temperatures, light, moisture, and geographical location. These factors should be considered when using microbial cultures to improve plant growth.

Materials and Methods

Sample collection

The geographic coordinates of the sampling site were 117°46' E and 25°89' N, which is in the *P. edulis* Forest Base in Sanshe Village, Xiyang Town, Yongan City, Sanming City, Fujian Province, China. This region has a subtropical monsoon climate. In the selected sampling area, holes, which radiated 10 cm from the main root of each *P. edulis* plant, were dug to a depth of 10–20 cm. Then, 50 ml of our custom-made growth-promoting mixed microbial culture was applied to the holes via a root-irrigation system. The mixed microbial cultures were an even mixture of *Klebsiella aerogenes* strain CT-B09-2, *Acinetobacter calcoaceticus* strain WYS-A01-1, and *Bacillus amyloliquefaciens* strains JL-B05 and JL-B06 cultures, each of concentration $\geqq 1 \times 10^{8}$ CFU/ ml. Samples of bamboo rhizomes, rhizome roots, rhizosphere soil, and non-rhizosphere soil of *P. edulis* were collected before treatment, then at three and six months of treatment. For the bamboo rhizome, samples were collected from rhizome buds within 50 cm of the root. For the rhizome root, the root tissue was sampled. For the rhizosphere soil, soil that had adhered (within 2 mm) to the root was sampled. The soil that was 30 cm from the root system was sampled as non-rhizosphere soil. Each of the 12 types of samples was in triplicates, resulting in a total of 36 samples. Immediately after collection, samples were placed in sterile sample bags, stored at -20˚C, and processed within 24 h for subsequent experiments.

Labeling of each group of samples (Table 2) was as follows: bamboo rhizome samples were represented by A; rhizome root samples were represented by B; rhizosphere soil samples were represented by E; non-rhizosphere soil samples were represented by F; labels of untreated samples were suffixed with “0”, and collectively referred to as No. zero samples; labels of samples treated with growth-promoting microbial agents for three months were suffixed with “1”, and collectively referred to as No. one samples; labels of samples treated with growth-promoting microbial agents for six months were suffixed with “2”, and collectively referred to as No. two samples.

Table 2
Labels for samples

Sample	No application of growth-promoting microbial cultures	Treated with growth-promoting microbial cultures for three months	Treated with growth-promoting microbial cultures for six months
Bamboo rhizomes	YA_A0	YA_A1	YA_A2
Rhizome roots	YA_B0	YA_B1	YA_B2
Rhizosphere soil	YA_E0	YA_E1	YA_E2
Non-rhizosphere soil	YA_F0	YA_F1	YA_F2

Genomic DNA extraction and high-throughput sequencing on 16S rRNA

The genomic DNA of the samples was extracted using a Tiangen-based kit following the manufacturer's instructions. DNA quality and quantity were detected through agarose gel electrophoresis, and the DNA samples were stored at -20°C until use. Primers were designed based on the conservative and highly variable V5-V7 region to amplify the 16s rRNA gene of bacteria. The primers were 799F (5 $-\forall CMGGA\top AGATA\mathbb{CC}KG-3$) and 1391R (5$-GACGGGCGGTGWGTRCA-3$). A sequencing adaptor was added at the end of the primers prior to PCR amplification. PCR products were purified, quantified, and normalized to construct a sequencing library. Quality evaluation for the constructed library was first conducted, after which a small fragment library was built and sequenced on an Illumina NovaSeq sequencing platform using the paired-end sequencing method (Majorbio, Beijing).

Data analysis

The sequence reads were processed to obtain the final high-quality reads, which were clustered in operational taxonomic units (OTU) containing sequences that had at least 97% nucleotide identities with each other [40]. The R Programming Language (R) was used to plot sample rarefaction curves [41] and Venn diagrams were drawn after statistical analyses in R. The Mothur pipeline [42] was used to calculate two alpha diversity indices —Chao 1 and Shannon indices— and R was used to analyze inter-group differences in alpha diversity. PCoA statistical analyses and related plotting were done in R. Hierarchical clustering analyses and drawing of the ternary plot, and bar charts of bacterial relative abundance were done in R, to assess the statistical significance of inter-group differences.

Declarations

Author Contributions: F Liu Z. S. Yuan and H. Pan wrote the main manuscript text and Z.H. Zeng prepared figures 1-9. All authors reviewed the manuscript.

Funding: This research was funded by the Forestry Science and Technology Project of Fujian Province, grant number 2021FKJ07.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original sequences obtained by sequencing have been uploaded to the NCBI SRA database. The relevant accession numbers were PRJNA900781.

Acknowledgments: We thank the anonymous reviewers and the editor for their valuable comments.

Conflicts of Interest: The authors declare no conflict of interest.

Corresponding author: Correspondence to Zongsheng Yuan.

Ethical declaration statements: We have obtained the permission to collect the moso bamboo (*Phyllostachys edulis*) in Sanshe village, Xiyang town, Yong'an city, and Wu village, Huangtan town, Jiangle county in Sanming city of Fujian province, China.The voucher specimens used in this study were deposited in the Mycological Research Center of Fujian Agriculture and Forestry University, Fujian Province, China. The specimens in the center are publicly available, and specimens of the samples are readily available to researchers, the specimen deposit number for this study is 2022-113-8 and identified by Fang Liu. The use of moso bamboo in this study complied with all local, national and international guidelines and regulations.We comply with the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

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Figures

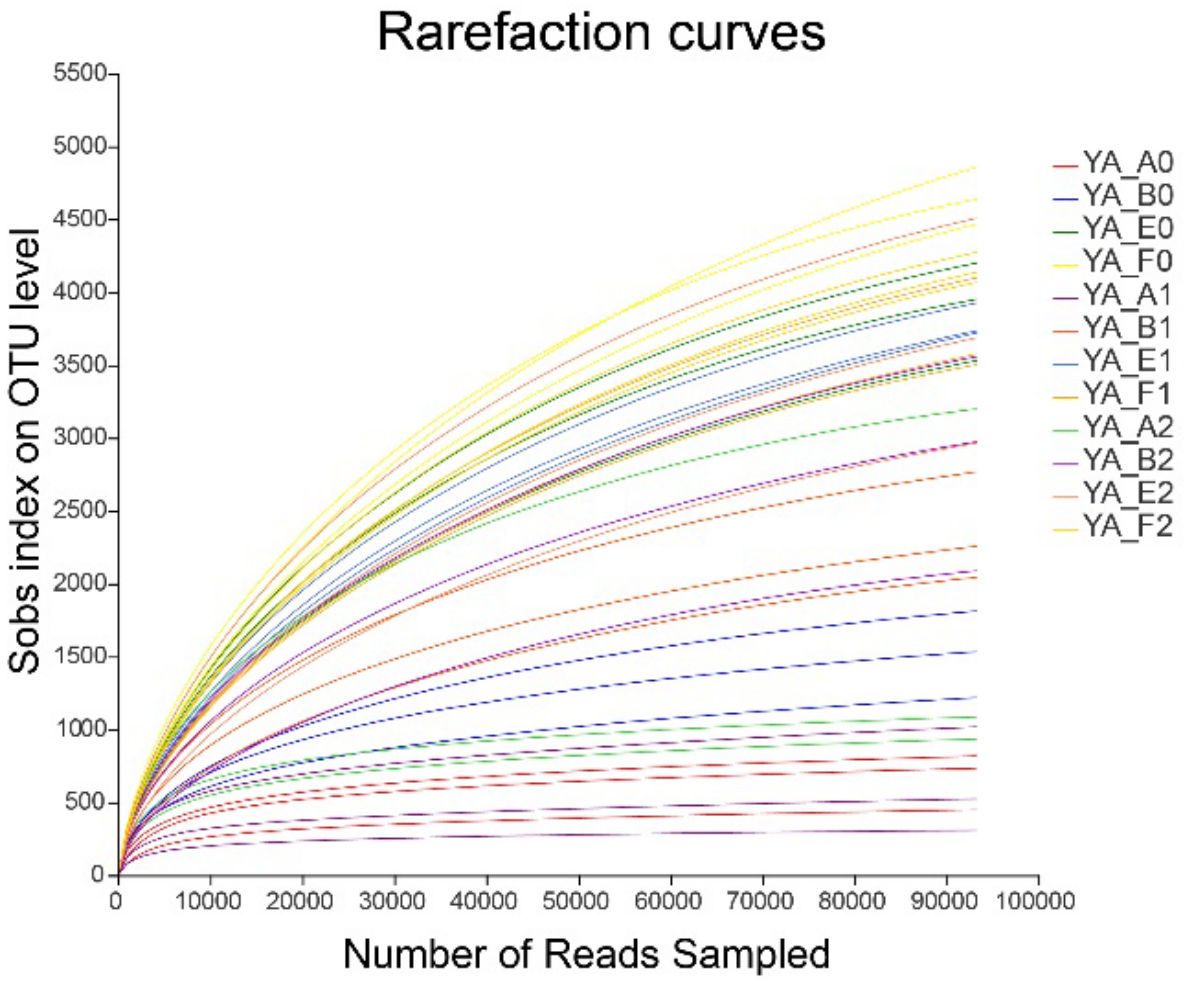

Figure 1

Rarefaction curves of *P. edulis* samples. Nucleotide sequence identities of >97% were used to define operational taxonomic units

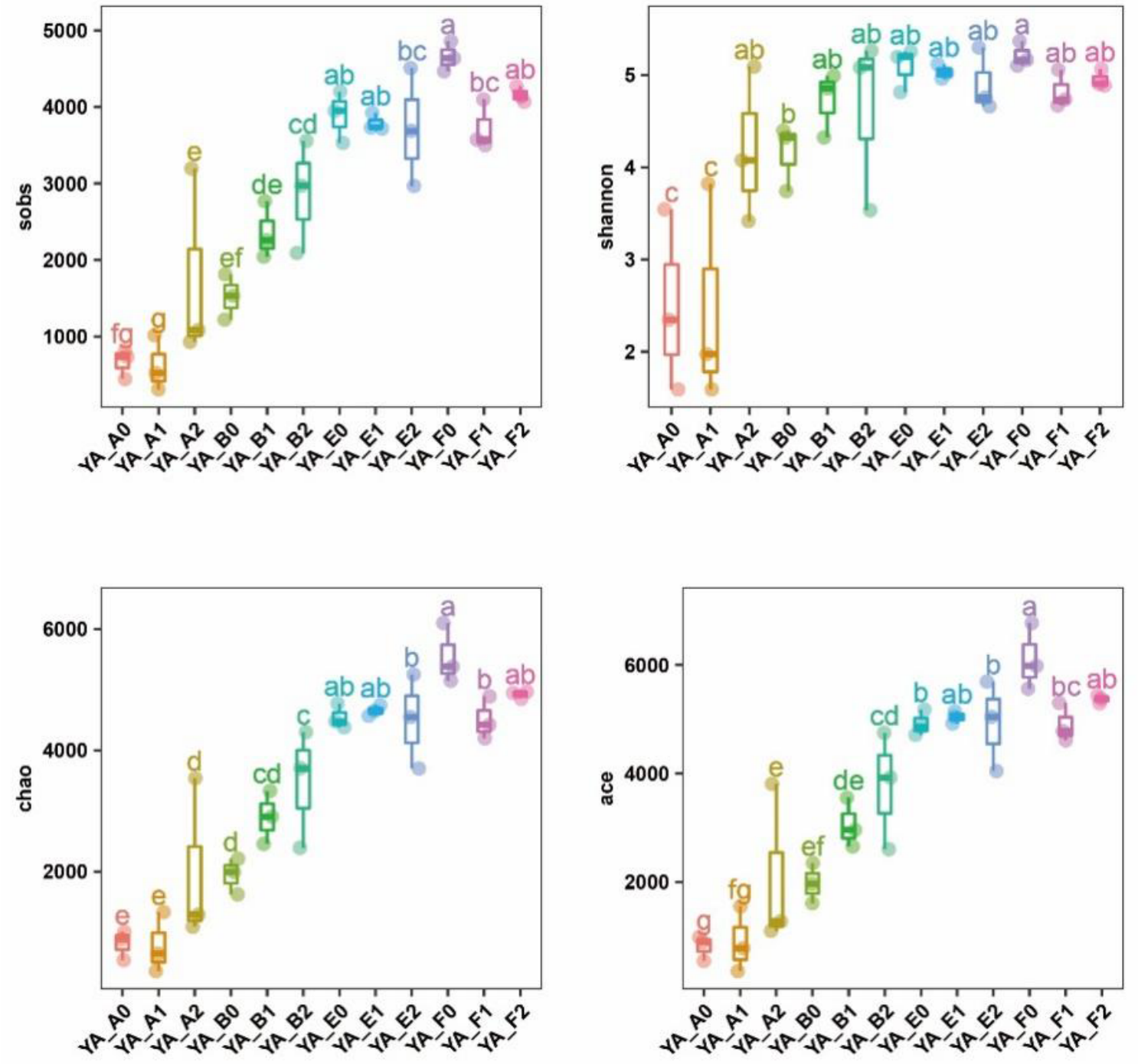

Figure 2

Boxplots of the alpha diversity indices of *P. edulis* samples

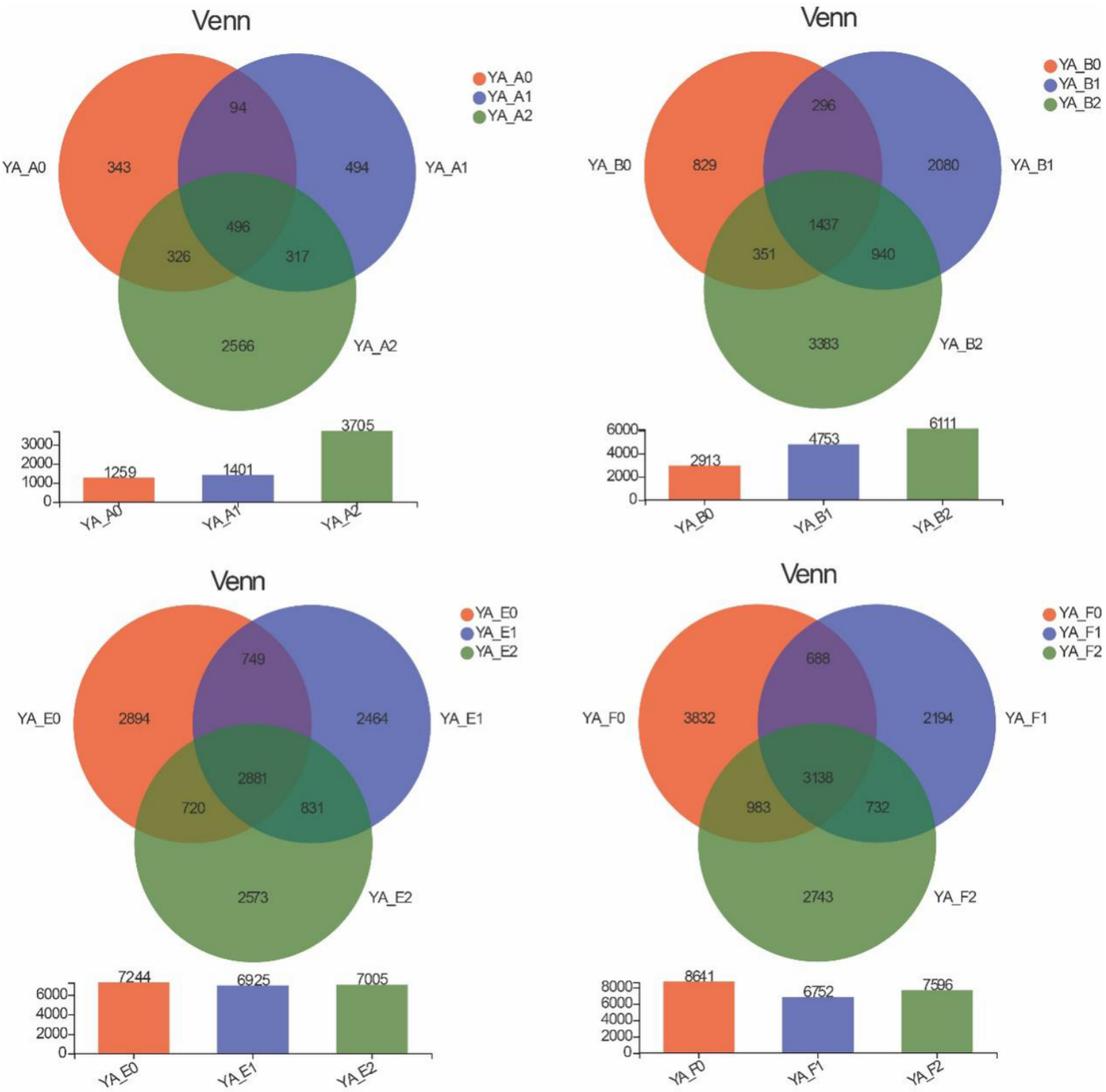

Figure 3

Venn diagrams of *P. edulis*samples

(A: Bamboo rhizome samples; B: Rhizome root samples; C: Rhizosphere soil samples; D: Non-rhizosphere soil samples)

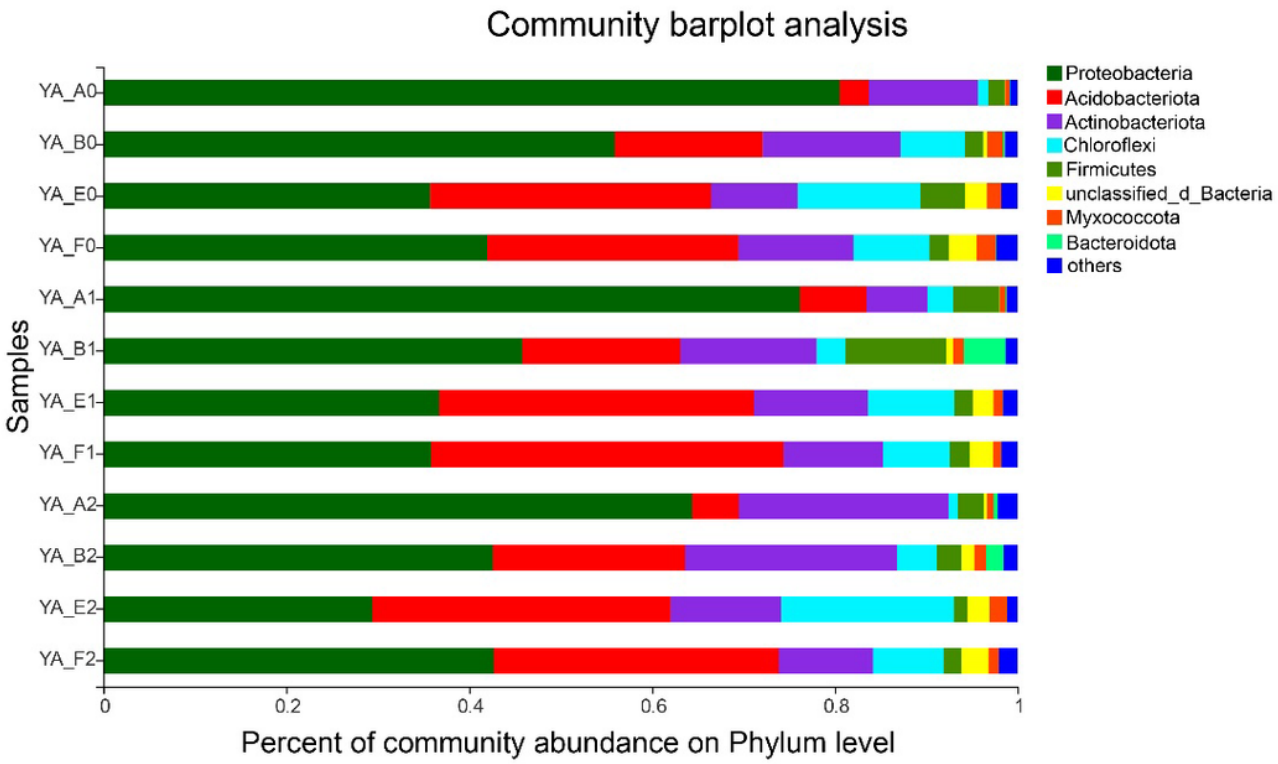

Figure 4

Relative abundances of bacterial communities in the *P. edulis* samples at the phylum level

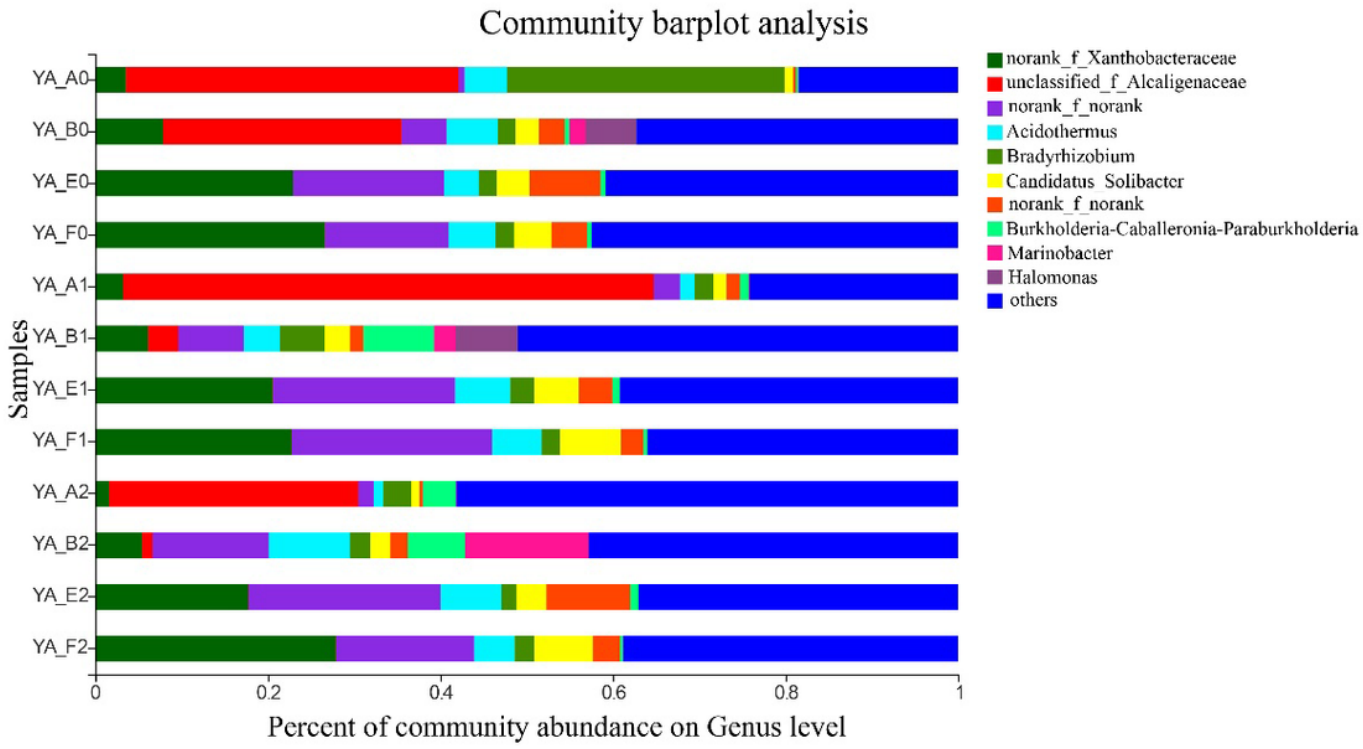

Figure 5

Genus relative abundances of bacterial communities in *P. edulis* samples

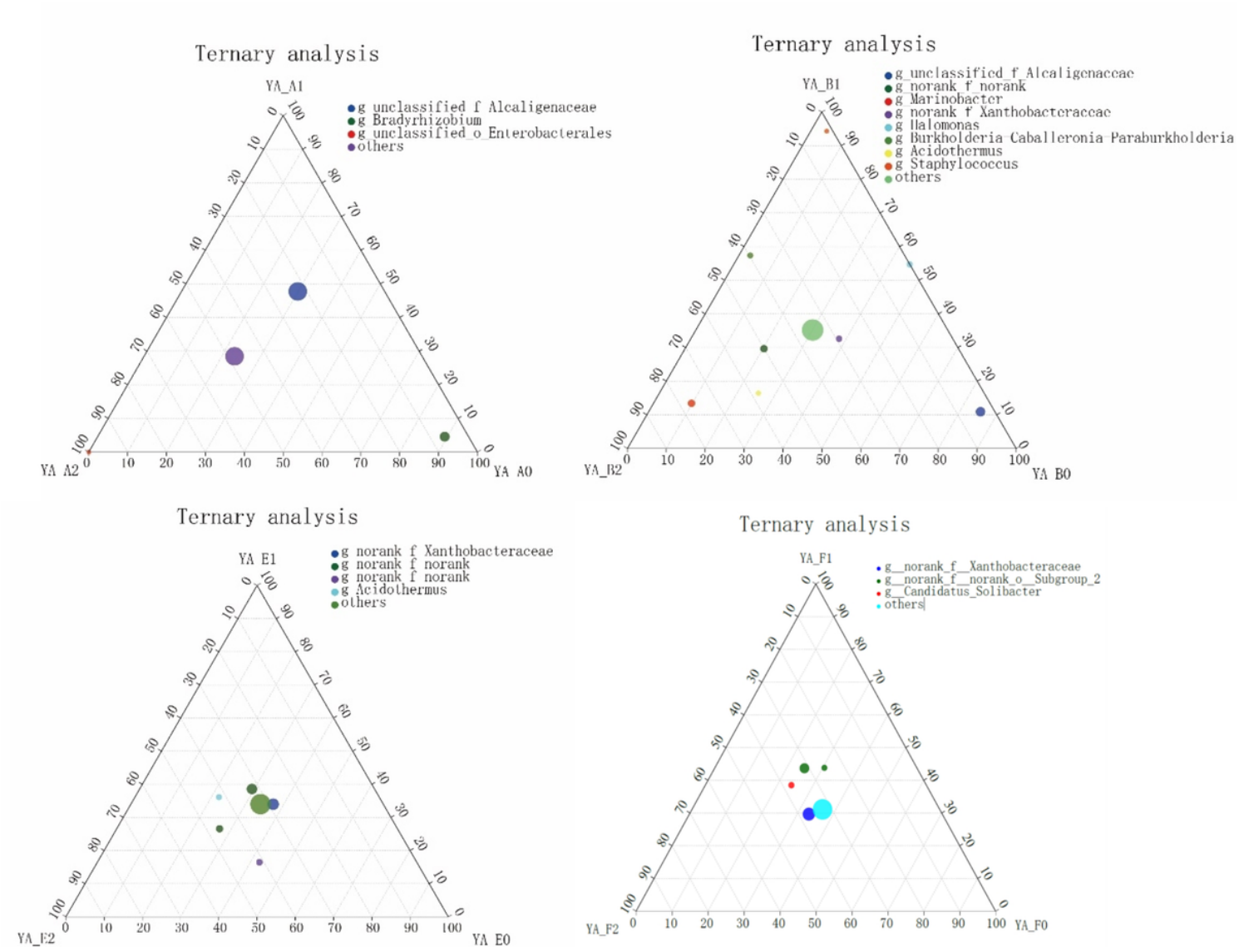

Figure 6

Ternary phase diagram of the *P. edulis* samples

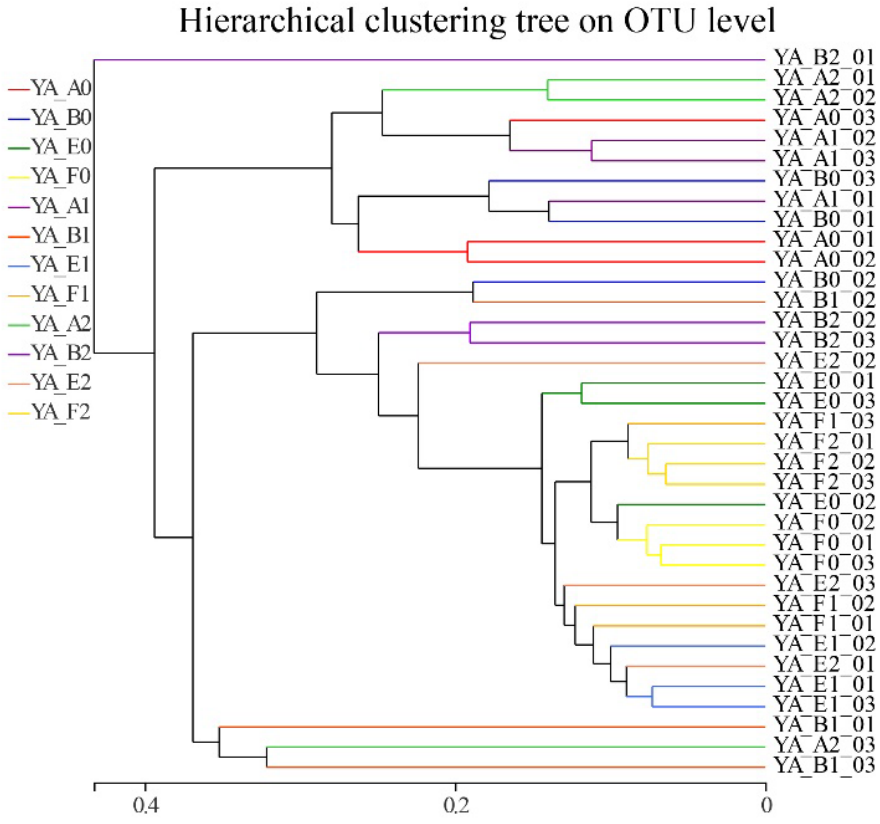

Figure 7

Hierarchical cluster analysis of the *P. edulis* samples

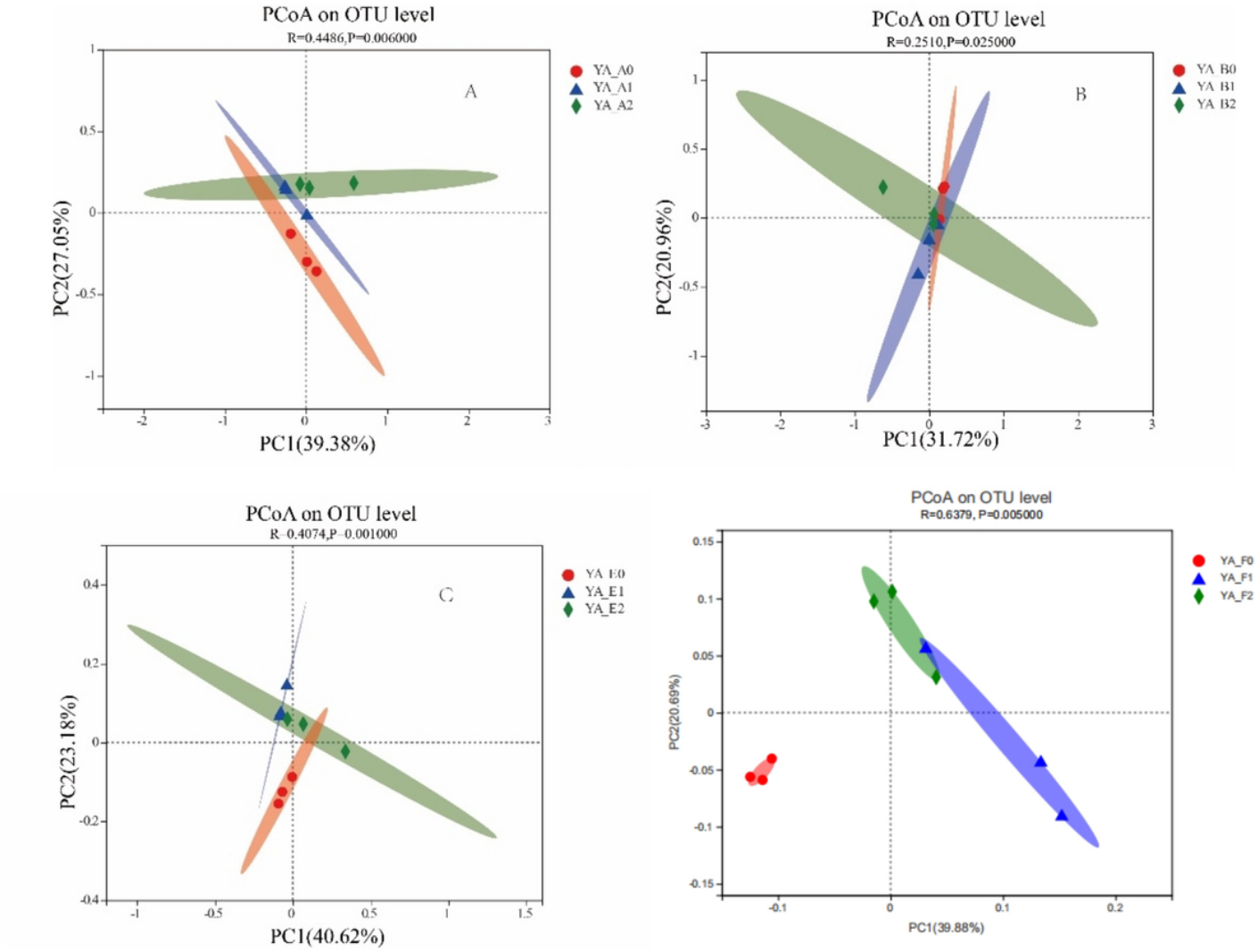

Figure 8

PCoA analysis of the *P. edulis* samples

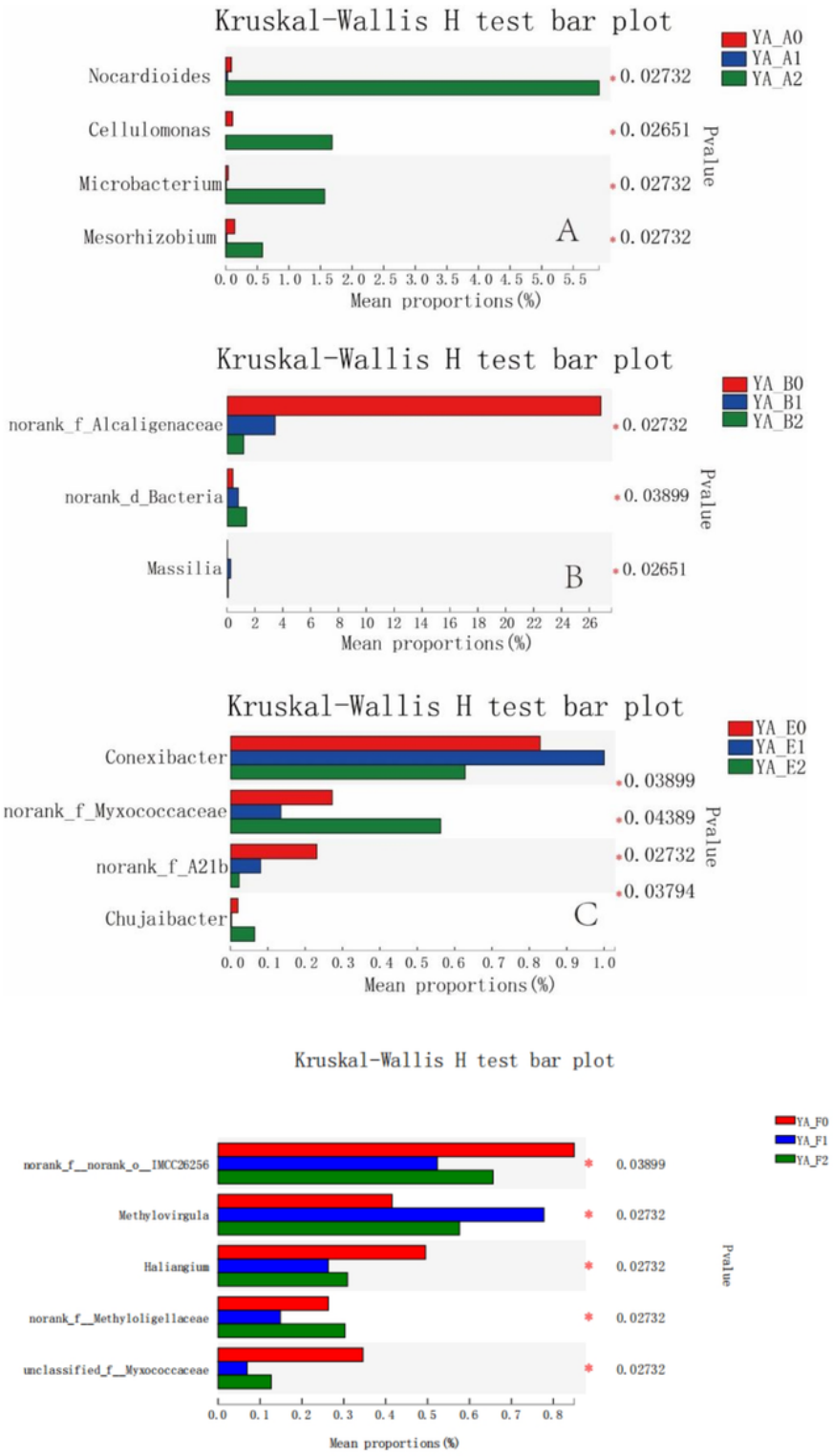

Figure 9

The significance of the differences between *P. edulis* sample groups