Effects of microbial cultures on bacterial communities in the roots of Phyllostachys edulis

Zong-sheng Yuan

yuanzs369@163.com

Minjiang University https://orcid.org/0000-0002-5306-3318

Si-fan Wang

Xiao-ling Wang

Fang Liu

Research Article

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Abstract

Aims This study investigated the effects of applying endophytic bacterial cultures isolated from *Phyllostachys edulis* on the bacterial communities in the bamboo root system and rhizosphere, as well as soil chemical properties.

Methods A mixed culture of four bacteria was applied to experimental plots. Seven root samples were collected from growing bamboo shoots: those treated with the bacterial culture at months 0, 3, and 6, untreated ones at the same times.Seven rhizosphere soil samples were collected from growing bamboo shoots: treated ones at months 0, 3, and 6; untreated ones at the same times.These samples were used to assess changes in root endophyte and rhizosphere bacterial communities, as well as in rhizosphere soil available nutrients and chemical properties.

Results Our results showed that the richness of endophytic bacteria in bamboo rhizomes increased with the application time of microbial cultures. The relative abundance of Firmicutes was significantly affected by the application of the microbial cultures. Furthermore, over time, the relative abundance of endophytic bacteria in the culture-treated rhizomes at the genus level gradually converged with that in the high-yield forest. Venn diagram analysis further confirmed the influence of the microbial cultures on bacterial community composition. Principal component analysis showed that culture-treated samples were closer to samples from the high-yield forest than untreated samples.

Conclusions Microbial cultures positively impacted the bacterial communities in *P. edulis*' rhizome roots and rhizosphere soil. Artificially manipulating the bacterial community in *P. edulis* forest soils via microbial cultures could be feasible, provided beneficial strains are carefully selected.

Introduction

The rhizosphere harbors a highly diverse assemblage of microorganisms (Shelake et al. 2019). The diversity and abundance of soil bacteria play a pivotal role in determining various critical aspects of ecosystem function, as extensively documented in previous studies (Wagg et al. 2014; Singh et al. 2018). During the normal growth and development of plants, numerous microorganisms establish habitats within plant tissues, collectively referred to as plant endophytes (Chu et al. 2023). Among them, certain endophytes have been shown to enhance the nutrient - uptake capabilities of their host plants (Shelake et al. 2019).

Soil microorganisms, meanwhile, are integral to soil nutrient cycling processes (Müller et al. 2016). Both rhizosphere and endophytic microorganisms synergistically contribute to multiple aspects of plant biology, including growth promotion, disease resistance, and stress tolerance (Müller et al. 2016). It is important to note, however, that microenvironmental conditions play a crucial role in shaping the composition and structure of these diverse microbial communities (Ma et al. 2016).

Recently, in botany, breeding, and environmental protection, viable microbial cultures with diverse functions or symbiotic relationships have been combined in appropriate proportions and applied biotechnologically (Fasusi et al. 2021). Such microbial cultures, when applied to the soil, colonize the rhizosphere of plants and stimulate indigenous microorganisms, enhancing the species richness of the soil microbial community, optimizing the structure of soil aggregates, regulating the root nutrient environment, and degrading some environmental organic pollutants to provide nutrients for plants (Cong et al. 2017). Moreover, after rhizosphere colonization, these bacteria produce various plant growth stimulants (Ling et al. 2022). Thus, in natural ecosystems, these bacteria derived from microbial cultures, along with rhizosphere and endophytic microorganisms, act as plant symbionts, contributing to plant disease control and growth promotion (Ling et al. 2022). In recent years, researchers have made significant progress in biologic development (Fitzpatrick et al. 2020; Compant et al. 2019). Rhizosphere growth - promoting bacteria are also crucial biocontrol resources (Martins et al. 2013; Santiago et al. 2015). Therefore, studying ways to modify microbial communities is of great significance for improving plant root growth environments, enhancing plant resistance, and increasing yields. These microbial communities can adapt to various environmental conditions for sustainable crop production (Keswani et al. 2019).

In previous research, we isolated and identified endophytic bacteria of *Phyllostachys edulis* (et al. 2021): CT-B09-2, WYS-A01-1, JL-B05, and JL-B06. Preliminary experiments showed that WYS-A01-1 had phosphorus-solubilizing, potassium-solubilizing, and nitrogen-fixing effects; CT-B09-2 had a phosphorus-solubilizing effect; and JL-B05 and JL-B06 exhibited antagonistic effects

against some pathogenic fungi. This study used a mixture of these four bacterial cultures to investigate their effects on the bacterial communities in *P. edulis* roots. Metagenomic DNA samples from *P. edulis* rhizome roots and rhizosphere soil were sequenced using an Illumina high-throughput platform (Liu et al. 2016; Ramakrishnan et al. 2020) to determine the changes in the structure and composition of *P. edulis* rhizosphere and endophytic bacterial communities after treatment with the mixed microbial cultures.

Moso bamboo [*Phyllostachys edulis* (Carr.)] is an important forest resource in China, highly valued for its economic and ecological benefits. Currently, few studies have investigated the effects of mixed microbial cultures on soil microbial communities in the *P. edulis* rhizosphere, soil enzyme activities, and the mechanisms by which they promote *P. edulis* growth (Peng et al. 2013; Li et al.2019). Therefore, we treated *P. edulis* by applying a mixed culture of endophytic bacteria from *P. edulis* to investigate their effects on the bacterial community composition of the rhizome root and rhizosphere soil. Our aim was to provide a theoretical framework for studying the 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 (Wang et al. 2019). In the future, such knowledge can be used to improve soil and maintain environmental safety.

Materials and Methods

Study site

Yong'an City, Sanming City, Fujian Province is the "Hometown of Bamboo Shoots in China", with a total bamboo forest area of 68,000 hm². The average annual temperature and precipitation in the past five years are 19.7℃ and 1600 mm respectively. The sampling site, located in the *P. edulis* forest base of Sanshe Village, Xiyang Town, Yong'an City, Sanming City, Fujian Province, China, has geographic coordinates of 117°46' E and 26°29' N (Fig. 1). The sampling locations feature mountainous and hilly landforms and have a subtropical monsoon climate. Built in the 1980s, the base covers about 20 hm². With concentrated plots, its main soil type is red soil, rich in iron and aluminum oxides, and acidic (pH 4.0–5.5). The organic matter content is medium - low, but the thick surface humus layer suits the growth of bamboo economic forests. Nearly 5 hm² serves as a special forest for teaching, scientific research, and experiments. This plot is natural forest land without artificial fertilization. The rest is high - yield economic forest. We selected an approximately 200 m² experimental area in the plot as the site for applying microbial cultures.

Bacteria strains and microbial cultures application methods

Bacteria strains: The experimental strains were all deposited in the General Microbiology Center of the China National Microbiological Culture Collection Administration (Yuan et al. 2021). CTB09-2, an endophytic bacterium with phosphorus-solubilizing, potassium-solubilizing, and nitrogen-fixing functions, was isolated and purified from bamboo. It was identified as *Enterobacter aerogenes*, and its deposit number is CGMCC No.14377. WYS-A01-1, also an endophytic bacterium with the same phosphorus-solubilizing, potassium-solubilizing, and nitrogen-fixing functions, isolated from bamboo, was identified as *Acinetobacter calcoaceticus*, with a deposit number of CGMCC No.14378. JL-B05 and JLB06, both endophytic *Bacillus amyloliquefaciens* isolated and purified from bamboo, were identified as such. Their deposit numbers are CGMCC No.24215 and CGMCC No.18999, respectively. JL-B05 and JLB06 exhibit slightly different physical and chemical properties (Yuan et al. 2021).

Culture medium: Modified NA culture medium (1L): It contains 10g of peptone, 3g of beef extract, 5g of sodium chloride and 100ml of bamboo - root tissue extracts (To prepare the bamboo - root tissue extracts, first take 100g of bamboo - root tissue. Wash it thoroughly, then chop it into small pieces. Soak the chopped tissue in 1L of distilled water at 65℃ for 3 hours. After soaking, filter the mixture to remove impurities, and the resulting filtered liquid serves as the extract.).

In the designated sampling area, holes were excavated around each *P. edulis* plant. These holes were positioned 10 cm away from the main root and dug to a depth ranging from 20 to 40 cm. A total of 50 holes were randomly dug, and their locations were carefully marked. Subsequently, 50 ml of our mixed microbial cultures were administered to the holes through a root-irrigation system. The mixed microbial cultures consisted of equal volumes of CT-B09-2, WYS-A01-1, JL-B05, and JL-B06 cultures, with each having a concentration of at least 1×10^8 CFU/ml. The microbial cultures were reapplied to the roots every three days, and this

process was repeated three times in total. Sampling commenced on the third day following the final treatment, and the initial sample is designated as the 0th sample.

Sample collection

At the 0th, 3rd, and 6th months post - treatment, *P. edulis* rhizome roots and rhizosphere soil samples, both treated and untreated with microbial cultures, were collected (We started applying the cultures on September 21, 2021, collected the first sample on October 1, the second sample on December 30, and the third sample on March 30, 2022. These six months are the period from the formation of bamboo baby shoots to the emergence of spring bamboo shoots.). In the 6th month, samples from high - yielding *P. edulis* forests were collected to our study.

For bamboo root samples, root tissue was removed from rhizomes. A sterile brush was used to remove the rhizosphere soil of the roots. The roots were then placed in sterile water, ultrasonicated for 1 min to clean the surface, dried with filter paper, and stored in sterile ziplock bags.

For rhizosphere soil samples, the soil within 2 mm of the rhizome roots was brushed off and collected into sterile ziplock bags.

Each sample had three biological replicates, resulting in 42 samples. All samples were promptly stored at -20 ˚C and processed within 24 hours for subsequent experiments.

The labels of each group of samples are shown in Table 1, as follows: "YA" represents the abbreviation of the sampling location. "CK" indicates samples without microbial cultures treatment. "R" denotes rhizome roots, while "S" stands for rhizosphere soil. "H" signifies that the sample is from a high-yielding *P. edulis* forest. Seven root samples were collected from growing bamboo shoots: those treated with the bacterial culture at months 0, 3, and 6 were labeled YA_R0, YA_R1, and YA_R2; untreated ones at the same times were CK_R0, CK_R1, and CK_R2; high-yield forest root samples (YAH_R) were also collected. Seven rhizosphere soil samples were collected from growing bamboo shoots: treated ones at months 0, 3, and 6 were YA_S0, YA_S1, and YA_S2; untreated ones at the same times were CK_S0, CK_S1, and CK_S2; and high-yield forest soil samples (YAH_S) were collected.

Table 1
Labels for samples

	high-yield forest (At the 6th month)	Month 0 (application of the microbial cultures)	3 rd month (application of the microbial cultures)	6th month (application of the microbial cultures)	Month 0 (without treatment)	3rd month (without treatment)	6th month (without treatment)
Rhizome roots	YAH_R	YA_R0	YA_R1	YA_R2	CK_R0	CK_R1	CK_R2
Rhizosphere soil	YAH_S	YA_S0	YA_S1	YA_S2	CK_S0	CK_S1	CK_S2

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

Genomic DNA from *P. edulis* tissue samples was extracted using the Tiangen Plant Genomic DNA Extraction Kit (DP305), and genomic DNA from soil samples was extracted using the Tiangen Soil Genomic DNA Extraction Kit (DP336) (Huang et al. 2024). DNA quality and quantity were detected by agarose gel electrophoresis, and the DNA samples were stored at -20°C until further use.

Primers were designed targeting the conservative and highly variable V5-V7 region to amplify the 16S rRNA gene of bacteria. The primers were 799F (5-AACMGGATTAGATACCCCKG-3) and 1391R (5-GACGGGCGGTGWGTRCA-3) (Liu et al. 2016). A sequencing adaptor was added to the end of the primers before PCR amplification.

The PCR reaction system consisted of: 5 μL 10× Buffer, 5 μL dNTPs (2 mmol·L^{-1}), 1 μL DNA polymerase, 1.5 μL of each primer, 50 ng DNA template, and double-distilled water to make up a total volume of 50 μL. The reaction conditions were: initial denaturation

at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 56°C for 30 s, 72°C for 45 s, and a final extension at 72°C for 10 min (Kumar et al. 2018).

The PCR products were detected and quantified using the QuantiFluor™-ST blue fluorescence quantification system (Promega). Qualified PCR products were purified, quantified, and normalized to construct a sequencing library. The constructed library was first evaluated for quality, followed by the construction of small fragment libraries. Sequencing was performed on the Illumina Hiseq 2500 platform using the paired-end sequencing (Paired-End) method by Majorbio (Beijing).

Data analysis

Data preprocessing and one - way analysis of variance (ANOVA) was conducted using Excel 2019 and SPSS 23 to examine significant differences in indicators among samples (Wahid et al. 2017). Statistical significance was determined at a threshold of $P < 0.05$. For paired - end sequence splicing, Flash (v1.2.11) was employed (Magoč et al. 2011). The RDP Classifier (v2.13) was utilized for OTU cluster annotation, while Usearch (v11) was used to conduct OTU statistics (Robert C Edgar 2010). Qiime (v1.9.1) was applied to generate the abundance table for each taxonomic level.

The sequence reads underwent processing to yield the final high - quality reads. These reads were then clustered into operational taxonomic units (OTUs), where sequences within an OTU shared at least 97% nucleotide identity (Robert C Edgar 2013). Following statistical analyses in R (R 4.2.3, UpsetR), Venn diagrams were constructed (Conway et al. 2017).

The Mothur pipeline (Schloss et al. 2009) was utilized to calculate alpha diversity indices. To analyze the inter - group differences in alpha diversity, R (R 4.2.3, vegan) was used (Lou Jost 2007). PCA statistical analyses and corresponding visualizations were carried out in R (R4.2.3, ade4 & vegan) (Lutsiv et al. 2021). Hierarchical cluster analysis was carried out using R (R 4.2.3, vegan, and ggplot2) (Mitter et al. 2017). Cumulative bar graphs depicting the relative bacterial abundances were constructed, presenting the top 10 taxa at the phylum level and the top 20 at the genus level. The species correlation network was constructed using Networkx v1.11 software, the correlation was calculated using the spearm method, and the top 200 genera in relative abundance were selected for analysis (Han et al. 2024). The FAPROTAX v1.2.1 software was employed to predict the functions of bacterial communities. For visualizing the top 30 functions based on relative expression abundances, the pheatmap package in R v4.2.3 was utilized (Wang et al. 2024). Cluster calculations were conducted using the stats package of R v4.2.3 with the complete linkage method (Murtagh et al. 2014). RDA analysis of soil chemical properties and bacterial communities (genus level) was performed using the R v4.2.3 software vegan package (Si et al. 2017).

Determination of soil chemical properties

The pH of the soil was measured using a pH meter (PHS – 3E, INESA, China) in a 1:5 soil - solution ratio with 0.01 mol L^{-1} $CaCl_2$. The electrode method was adopted to determine soil conductivity. For soil total phosphorus (TP) and available phosphorus (AP) (Gu et al. 2019), the $HClO_4$ - H_2SO_4 digestion method and extraction with 0.5 mol L^{-1} $NaHCO_3$ solution were applied, respectively. Subsequently, a continuous flow analyzer (SAN++, SKalar, the Netherlands) was used for their measurement. Soil total potassium (TK) and available potassium (AK) were extracted with 1.0 mol L^{-1} CH_3COONH_4 solution and then analyzed using a flame atomic absorption spectrophotometer (PinAAcle 900H, Platinum Elmer, USA) (Liu et al. 2024). After pretreating soil samples with 0.5 mol L^{-1} HCl to eliminate inorganic carbonates, soil organic matter (OM), total nitrogen (TN), and hydrolyzable nitrogen were quantified by an elemental analyzer (EA3000, EuroVector, Italy) (Ji et al. 2017).

Results

Alpha diversity analysis

Alpha diversity is primarily determined by two factors: one is species richness, which refers to the number of species present; the other is species evenness, which describes how evenly individuals are distributed among different species within the community. Community richness indices, such as ACE, Chao1, and the Richness index, quantify the observed number of bacterial species. Meanwhile, the Shannon index is a key metric for measuring community diversity, taking into account both the richness and evenness of species in the community.

Therefore, from Fig. 2, we can roughly observe the changes in the endophytic bacterial community of rhizome roots. Firstly, for the samples treated with the cultures, the community richness indices, including ACE, Chao1, and the Richness index, of root endophytic bacteria (YA_R) continuously increase. These indices gradually approach those of the high-yielding forest (YAH_R). In contrast, for the samples without microbial cultures treatment, these indices increase initially and then decline, with a relatively small overall variation range. Secondly, based on the Shannon index of community diversity, the high-yielding forest exhibits the highest value. Both the culture-treated and untreated samples show low diversity, but the Shannon index of the culture-treated samples shows a downward trend. As the treatment time extends, the diversity decreases to a level similar to that of the untreated samples.

The Fig. 3 provides an overview of the changes in the rhizosphere soil bacterial community. Firstly, regarding community richness, the richness indices of the culture-treated samples were initially close to those of high - yielding forests. As time progressed, by the sixth month, these indices exceeded those of high - yielding forests. For the untreated samples, the CK_S0 community richness index was the lowest among all samples. It increased initially and then declined over time, resulting in an overall minimal net change. Secondly, based on the Shannon index of community diversity, high yielding forests exhibited relatively high values, whereas the culture-treated samples showed the lowest diversity. The diversity of culture-treated samples increased over time. In contrast, the untreated samples initially had lower diversity than high - yielding forests but also showed an upward trend. By the sixth month, their diversity index slightly surpassed that of high - yielding forests.

Species Venn diagram analysis

Venn diagrams illustrate the number of shared and unique operational taxonomic units (OTUs) across multiple sample groups, thereby intuitively revealing both the similarity and overlap of OTU composition in samples from different environments (Ji et al., 2017). As shown in the Venn diagram of root endophytic bacteria (Fig. 4), the number of shared genera among all rhizome root endophytic bacteria samples is 61, while the number of shared genera in rhizosphere soil samples is 47. The number of genera in the samples treated with microbial cultures increased gradually, eventually surpassing that of the high-yield forest samples. In contrast, the number of genera in untreated samples fluctuated but remained lower than that in high-yield forest samples.

Community composition analysis

The relative abundances of rhizome roots samples at the phylum and genus levels (Fig. 5) showed that the endophytic bacterial communities in the rhizome roots of the bamboo forest were mainly composed of Proteobacteria, Acidobacteriota, and Actinobacteriota (Fig. 5a). Secondly, the endophytic bacterial communities in the roots treated with cultures (YA_R series) (YA_R series) differed significantly from those in the untreated roots. In the roots samples treated with cultures, the relative abundance of Proteobacteria decreased over time, whereas it remained stable in the untreated samples. In the treated samples, the relative abundance of Firmicutes increased from 1.9% initially to a peak of 11.1% at 3 months. In contrast, Firmicutes showed a downward trend in the untreated samples. The relative abundance of Chloroflexi in the untreated samples (CK_R series) was significantly lower than that in the treated samples (YA_R series) and the high-yield forest (YAH_R) (Fig. 5c).

Furthermore, changes in the relative abundances of rhizome roots bacterial communities at the genus level (Fig. 5b) also indicated that, over time, the relative abundances of endophytic bacterial communities in the rhizome roots of the cultured treatments gradually converged with those of the high-yield forest. In the untreated samples, the total proportion of relative abundance of five genera — *Acidothermus, Bradyrhizobium*, unknown_bacteria, *Acidibacter*, and *Candidatus_solibacter* — reached as high as 50%. while in the treated samples, the total relative abundances of these five genera did not exceed 25%, and steadily decreased over time, trending towards the levels in the high-yield forest (Fig. 5d).

Additionally, compared with other samples, the relative abundances of Acidobacteria and Unidentified Bacteria in the high-yield forest sample (YAH_R) were significantly higher (Fig. 5c). The relative abundances of other bacterial genera in the high-yield forest (YAH_R) also exceeded 50%. This suggests that the high-yield forest harbors abundant, untapped bacterial resources (Fig. 5d).

Rhizosphere soil samples revealed that the bacterial colonies in the bamboo forest's rhizosphere soil were predominantly composed of Acidobacteriota, Proteobacteria, and Actinobacteriota (Fig. 6a). In the treated samples (YA_S), the relative abundances of Acidobacteria and Proteobacteria exceeded 30%, while those of Actinobacteria and Chloroflexi surpassed 10%. Notably, the relative abundances of Proteobacteria, Actinobacteria, and Chloroflexi were significantly higher than in the untreated

samples (Fig. 6c). The untreated samples (CK_S) exhibited the following characteristics: the relative abundance of Acidobacteria was higher than in other samples, exceeding 60%, and even surpassing 75% during the 3-month period. However, by the 6th month, the relative abundances of all phyla remained relatively unchanged compared to the initial (0 month) time point.

The changes in the relative abundances of rhizosphere soil bacterial communities at the genus level (Fig. 6b) indicated that the relative abundances of the rhizosphere soil bacterial communities in the treated samples (YA_S) were comparable to those in the high-yield forest (YAH_S). The dominant bacterial genera in each sample were Candidatus_solibacter and Acidothermus. In the untreated samples, the combined relative abundance of the five genera of Candidatus_solibacter, Acidothermus, Acidobacteriia, unknown_bacteria, and Acidibacter reached 50%. Finally, a distinctive feature of the high-yield forest samples was that the relative abundance of Acidobacteriota was significantly higher than that in the YA_R series samples, and the relative abundance of Chloroflexi was significantly lower than in the treated samples. Among all samples, the relative abundance of Unidentified Bacteria at the phylum level and other bacteria at the genus level (Fig. 6d) in the high-yield forest (YAH_S) was also the highest.

PCA (Principal Co-ordinates Analysis) analys

The PCA diagram reveals that for the endophytic bacterial community, the treated root samples are clearly separated from the untreated root samples. The untreated samples appear closer to the high-yield forest samples, but the treated root samples are getting closer to the high-yield forest samples over time (Fig. 7a). In terms of rhizosphere soil bacterial communities, both culture-treated and untreated samples were at a certain distance from the high-yield forest samples, but the distance between the untreated samples and the high-yield forest samples was greater than that between the treated samples and the high-yield forest samples (Fig. 7b).

Through the inter-generic correlation network, we can find that each sample in the sixth month is divided into six related clusters of bacterial genera, but there are still differences in details. The cluster of untreated root sample CK_R2 indicates more positive correlation red lines and closer interactions (Fig. 8a); the red lines of the high-yield forest root YAH_R sample (Fig. 8c) are less than CK_R2, while the treated sample YA_R2 (Fig. 8b) is in the middle. The untreated rhizosphere soil sample CK_S2 (Fig. 8d) also has more red lines in the cluster than the treated sample YA_S2 (Fig. 8e). It can be seen that there is a difference between treated and untreated samples.

Functional expression abundance

We selected the 30 functions with the highest functional expression abundances in rhizomes root samples for comparison (Fig. 9). The expression abundances of all functions in the culture treated samples were higher than those in the untreated samples. The functional expression abundances of high - yield forest samples were at a moderate level. The functions related to cell communication and the sensory system exhibited a pattern of increasing first, reaching a peak, and then decreasing. Notably, these two functions showed the lowest expression levels in the CK_R0 group of untreated samples, after which they gradually increased.

We selected the 30 functions with the highest abundances in rhizosphere soil samples for comparison (Fig. 10). The abundances of all functions in the culture - treated samples were higher than those in other samples. The functional abundances in high - yield forest samples were at a moderate level. Specifically, the abundances of the functions related to the sensory system, cell communication, and immune system were the highest immediately after the initial cultures application, and then declined over time. In contrast, these three functions showed the lowest expression levels in the untreated samples.

Chemical characteristics of rhizosphere soil and rhizome root appearance characteristics

As presented in Table 2, in the sixth month, the culture - treated samples showed greater similarity to the high - yield forest samples than the untreated samples did, specifically regarding available phosphorus, available potassium, pH, total potassium, total phosphorus, total nitrogen, and available nitrogen (Fig. 11). The conductivity and pH values of both the untreated samples and the culture - treated sample YA_S2 exhibited substantial fluctuations and were significantly distinct from those of the high - yield forest samples (Table 2). During the six - month period, the available phosphorus, total potassium, and conductivity levels in the untreated samples increased, whereas those in the culture - treated samples declined. Among the untreated samples, only the untreated sample CK_S2 in the sixth month had an organic matter content approaching that of the high - yield forest sample

YAH_S. The Redundancy Analysis (RDA) ordination plot indicates that treated samples and untreated samples cluster in distinct regions (Fig. 12). Treated samples are positioned closer to high - yield forest samples. Moreover, soil chemical indicators such as organic matter (OM), pH, total nitrogen (TN), etc., show a stronger association with treated samples and high - yield forest samples.

Table 2
Chemical indicators of soil

Sample	Available nitrogen (mg/kg)	Available phosphorus (μmol/g)	Available potassium (mg/kg)	Total nitrogen (g/kg)	Total phosphorus (mg/kg)	Total potassium (g/kg)	Conductivity (mS/cm)	pH	Organic matter (g/kg)
CK_S0	32.12 ± 0.03c	2.17 ± 0.03b	26.19 ± 0.08c	0.46 ± 0.02d	145.63 ± 0.03d	6.13 ± 0.04c	41.52 ± 0.03d	3.42 ± 0.03e	14.12 ± 0.14e
CK_S1	86.37 ± 0.03a	4.47 ± 0.05a	32.15 ± 0.06b	1.00 ± 0.03b	507.04 ± 0.05b	14.78 ± 0.07b	62.02 ± 0.03b	5.69 ± 0.04b	20.81 ± 0.01a
CK_S2	68.93 ± 0.04b	4.53 ± 0.03a	34.57 ± 0.05a	1.02 ± 0.03b	531.04 ± 0.04a	15.87 ± 0.07a	65.02 ± 0.03a	6.14 ± 0.05a	17.85 ± 0.03c
YAH_S	28.32 ± 0.03f	1.64 ± 0.06d	22.90 ± 0.06d	0.78 ± 0.03c	148.36 ± 0.04c	5.65 ± 0.05d	48.82 ± 0.03c	4.22 ± 0.03c	19.07 ± 0.06b
YA_S0	32.12 ± 0.03c	2.20 ± 0.05b	26.21 ± 0.05c	0.46 ± 0.02d	145.69 ± 0.1d	6.13 ± 0.04c	41.52 ± 0.03d	3.41 ± 0.03e	14.24 ± 0.05e
YA_S1	29.82 ± 0.03d	1.84 ± 0.06c	17.51 ± 0.06f	1.08 ± 0.03a	118.72 ± 0.03f	5.14 ± 0.05e	35.82 ± 0.03e	3.87 ± 0.03d	19.05 ± 0.05b
YA_S2	28.62 ± 0.03e	1.62 ± 0.06d	19.85 ± 0.1e	0.76 ± 0.03c	138.62 ± 0.03e	5.72 ± 0.03d	30.08 ± 0.08f	4.26 ± 0.03c	15.35 ± 0.05d

Different lowercase letters indicate significant differences between groups ($P < 0.05$)

As depicted in Fig. 13, in the sixth month, when rhizomes treated with microbial cultures were excavated from the soil, it was observed that they had more nodes compared to the untreated samples. The untreated samples had 7.35 nodes per meter, whereas the treated samples had 8.12 nodes per meter, which was close to the 8.58 nodes per meter found in the high - yield forest. When dug out, the fibrous roots of the treated samples were less prone to breakage, and the length of the retrievable fibrous roots was greater than that of the untreated samples. Nevertheless, the fibrous root tissue of the high - yield forest rhizome samples was more abundant.

Discussion

Selection of beneficial endophytic bacteria from P. edulis as microbial cultures and apply them at the appropriate time

Currently, similarly applied microbial cultures mainly contain beneficial bacteria. These bacteria produce activating substances and various natural fermentation products. They proliferate in the root - soil zone, forming a dominant microbial community that promotes plant growth (Chaparro et al. 2012). Through their growth and metabolic activities, these microbes optimize the rhizosphere microdomain environment (Li et al. 2024). This optimization, in turn, enhances the absorption of nutrients by plant roots and promotes plant growth (Shahane et al. 2019).

The sampling times for this study were: the first sample in October, the second in December, and the third in March of the following year. These six months are the period from the formation of bamboo shoots to the emergence of spring bamboo shoots. During this time, precipitation at the sampling site was moderate. Particularly in March of the following year, the weather

was favorable, with an average temperature of around 15℃, reaching 30℃ on sunny days, rainfall was approximately 50–100 mm. This period is thus highly conducive to the proliferation of soil bacteria, making it suitable for applying cultures and collecting samples. In contrast, from April to May, there are often over ten days of rain, June sees frequent heavy rainfall, July has a high - temperature dry season with temperatures ranging from 30–40℃, and August - September is the typhoon season with recurrent heavy downpours. The erosion caused by abundant rain and high temperatures are unsuitable for the experiments. Therefore, we selected October to March of the following year as the experimental cycle.

This study aims to utilize bamboo root secretions to enable microbial cultures to promote the development of *P. edulis* roots and colonize in the soil, thereby achieving mutualistic symbiosis. In this study, we selected the following endophytic bacteria from *P. edulis* (Yuan et al. 2021): CT-B09-2 (*K. aerogenes*), WYS-A01-1 (*A. calcoaceticus*), JL-B05, and JL-B06 (*B. amyloliquefaciens*). These bacteria were chosen as the mixed microbial cultures for the experiment for the following reasons. Firstly, they are endophytic bacteria isolated from healthy *P. edulis* tissues in high - yield forests in previous experiments. Seed germination experiments using these bacteria showed that they do not inhibit the germination of *P. edulis* seeds, indicating their harmlessness to *P. edulis* roots. Secondly, *Bacillus* species belonging to Firmicutes can secrete organic acids, lower soil pH, activate the soil, and fix phosphorus and potassium nutrients (Liu et al. 2014). *Acinetobacter* species within Proteobacteria have the ability to remediate contaminated soil (Soares et al. 2020), while *Klebsiella* from Proteobacteria exhibits phosphorus - solubilizing and growth - promoting effects (Bruto et al. 2014). Thirdly, preliminary experiments demonstrated that WYS-A01-1 has phosphorus - solubilizing, potassium - solubilizing, and nitrogen - fixing properties. CT-B09-2 shows phosphorus - solubilizing activity, and JL-B05 and JL-B06 have antagonistic effects against certain pathogenic fungi.

Based on the above - mentioned reasons, this study selected the four strains described above for experimentation. The objective is that they can not only enrich the bacterial community in poor - quality soil but also enhance soil fertility.

The positive effects of microbial cultures on endophytes bacteria and rhizosphere soil bacteria

Other studies have demonstrated that an increase in a specific taxon within the rhizosphere microdomain inevitably impacts the growth and reproduction of other taxa. For instance, when the abundance of bacteria in rhizosphere soil significantly rises, the population of certain pathogenic microorganisms decreases (Adesina et al. 2022; Deng et al. 2022). Therefore, another objective of this study was to investigate whether the microbial cultures modify the relative abundance of rhizosphere and endophytic bacterial communities.

In our research, following the application of microbial cultures to bamboo rhizomes, the alpha diversity index of endophytic bacteria in bamboo roots and rhizosphere soil bacteria, along with the relative abundance of bacterial phyla and genera, converged towards the characteristics of high-yield forest samples.

Firstly, for the samples treated with the microbial cultures, community richness indices, including ACE, Chao1, and the richness index, exhibited an upward trend. In contrast, the untreated CK_R series samples showed no significant alterations, while the CK_S series samples consistently maintained the lowest values for these indices. Secondly, strains JL-B05 and JL-B06 within the applied cultures both belong to the phylum Firmicutes. After the treatment, the relative abundance of Firmicutes in the rhizome root samples of the treated group increased. In the untreated group, however, the change in this phylum was decline. Thirdly, regarding the relative abundance at the genus level, the whip root and rhizosphere soil samples treated with microbial cultures differed significantly from the untreated samples at the beginning. Six months later, their relative abundances grew more similar to those of the high-yield forest samples. Lastly, PCA indicated that the treated rhizosphere soil samples were more similar to those from high - yield forest rhizosphere soils. The inter - genus correlation network analysis revealed that the treated root samples resembled the root samples from high - yield forests more closely.

These results demonstrate that, in this study, the bacterial communities in roots and rhizosphere soils indeed underwent changes following the application of microbial cultures. We hypothesize that our microbial cultures may actively modify the diversity and abundance of rhizosphere and endophytic bacterial communities.

Microbial cultures bring benefits to P. edulis

To verify the benefits of the microbial cultures for *P. edulis*, we collected rhizome root and rhizosphere soil samples from the high-yield forest during the sixth month of the experiment and conducted comparative analyses. Firstly, the Venn diagram demonstrated that the number of genera in both endophytic and rhizosphere bacteria of the microbial culture-treated samples increased gradually, eventually surpassing that of the high-yield forest samples. Secondly, the 30 functions with the highest functional expression abundances in the culture-treated samples was higher than that in the high-yield forest samples and the untreated samples. In particular, it includes functions related to growth and metabolism, such as replication and repair, signal transduction, carbohydrate metabolism, membrane transport, cell communication, etc. Finally, an analysis of the chemical characteristics of the rhizosphere soil samples revealed that, for most indicators in the sixth month, the culture-treated samples were more similar to the high-yield forest samples than the untreated ones. When excavating the bamboo rhizome samples, we observed that the rhizomes treated with the culture medium had more nodes and their fibrous roots were less prone to breakage. More rhizome nodes provide more possibilities for bamboo shoot growth. Collectively, these findings suggest that the bacterial community in the experimental forest may have undergone positive changes, shifting towards the characteristics of the high-yield forest. At the end of 2024, the workers at the experimental field commended our experimental results. They noted that bamboos grew robustly and yielded more shoots in the plots where the cultures were applied, and thus asked that we re - apply the cultures.

Conclusion

In summary, the relative abundance of endophytic bacteria and rhizosphere bacteria in bamboo rhizomes increased over time with the application of a bacterial mixed culture. Bacterial mixed cultures affected both bacterial community composition and soil chemistry. Culture-treated samples resembled those from high-yield forests more closely than untreated samples. Our results support the feasibility of using microbial cultures to regulate bamboo growth and alter its microbiome. They also provide a potential basis for studying the relationship between bamboo growth and its microbiome.

Declarations

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Author contributions Si-fan Wang: Writing – original draft, methodology, formal analysis, data curation. Xiao-ling Wang: Writing – review & editing, methodology. Zongsheng Yuan: Writing – review & editing, supervision, project administration, Funding acquisition. Fang Liu: Writing – review & editing, supervision, project administration, conceptualization.

Data availability The datasets used or analyzed during the current study are available from the corresponding authors on reasonable request.

Competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

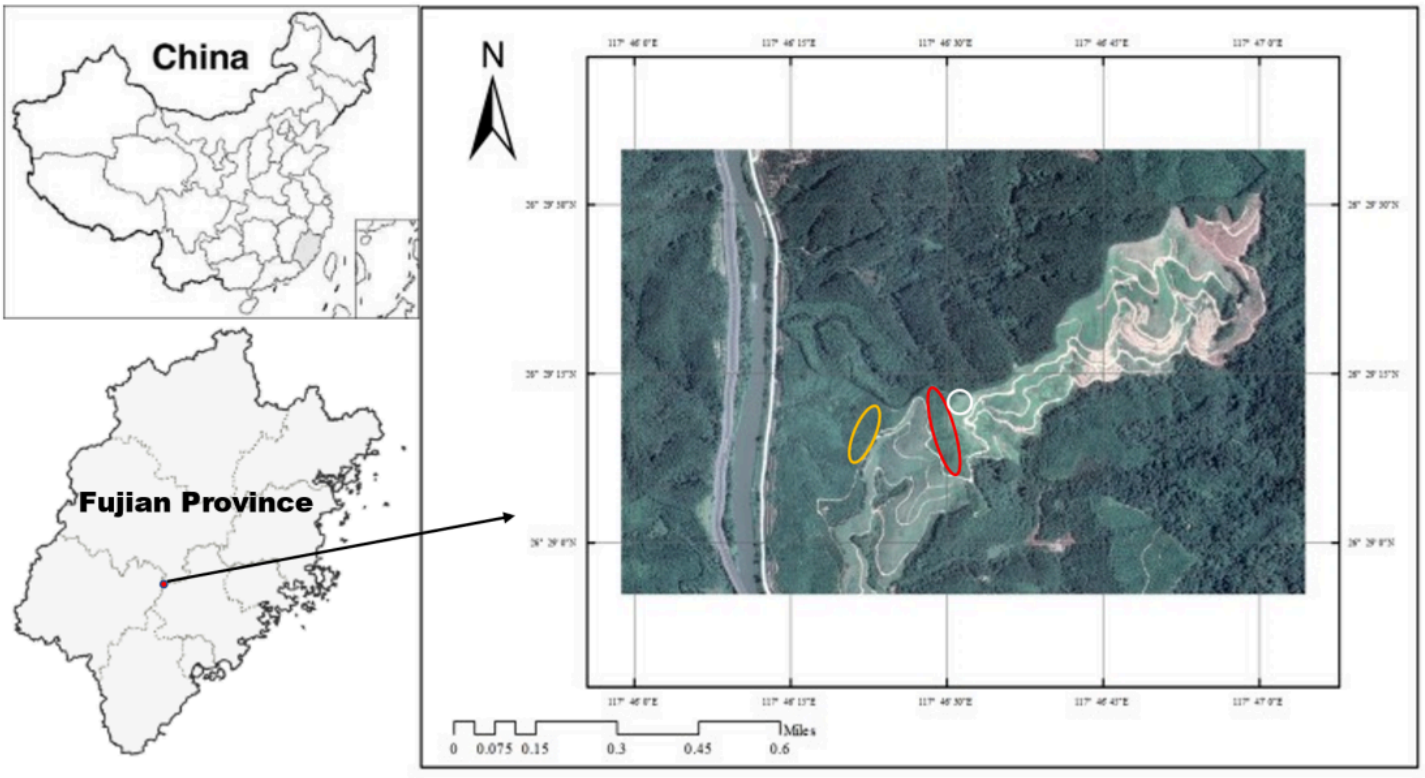

Figure 1

Geographic location and topography of the forest base in Sanshe Village, Xiyang Town, Yong'an City, Sanming City, Fujian Province, China. The yellow circle represents the high-yield forest plot, the red circle denotes the plot with microbial culture application, and the white circle indicates the untreated plot (CK).

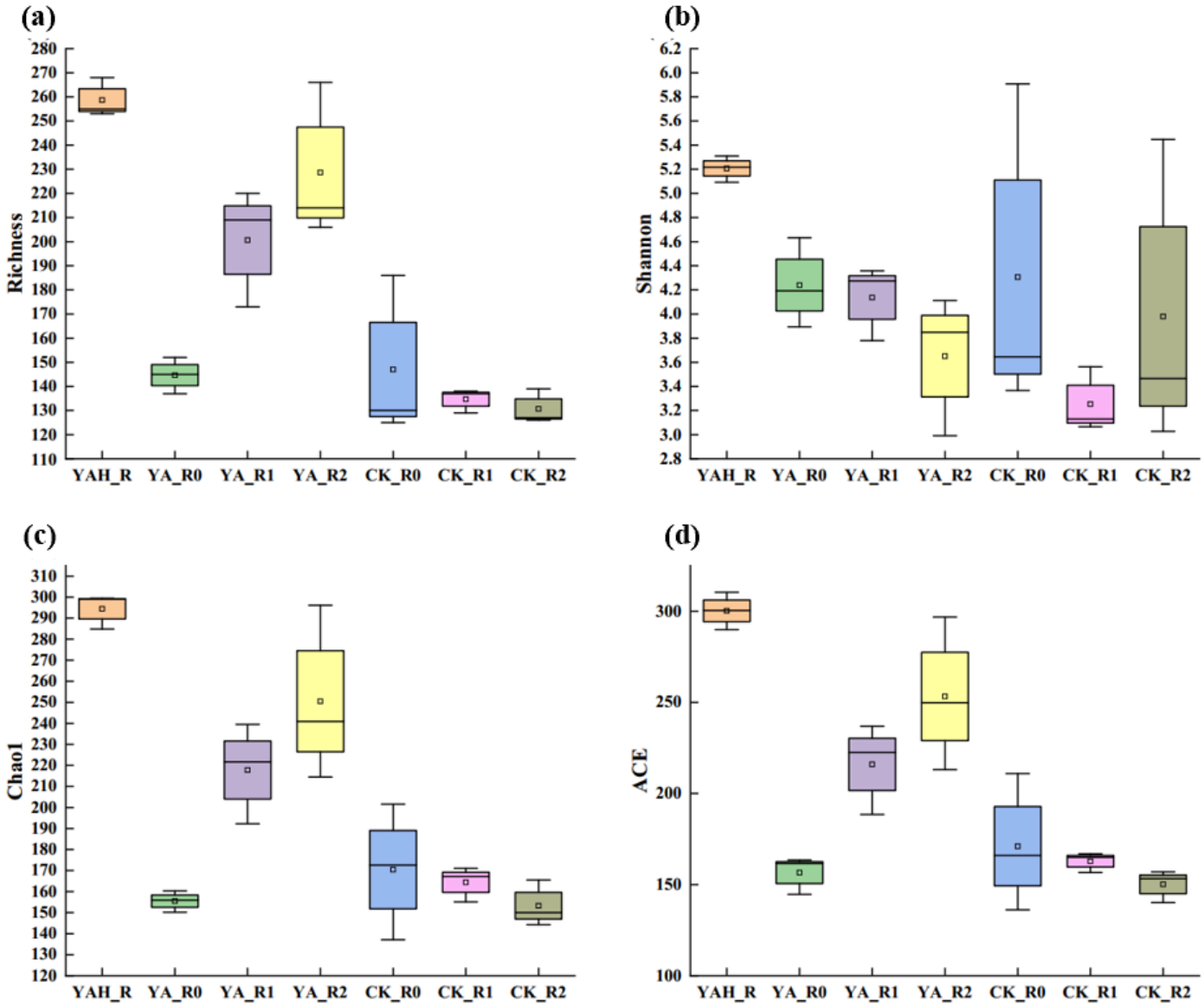

Figure 2

Boxplots of the alpha diversity indices of *P. edulis* rhizome roots samples. **(a)**Richness index. **(b)**Shannon index. **(c)** Chao index. **(d)** Ace index.

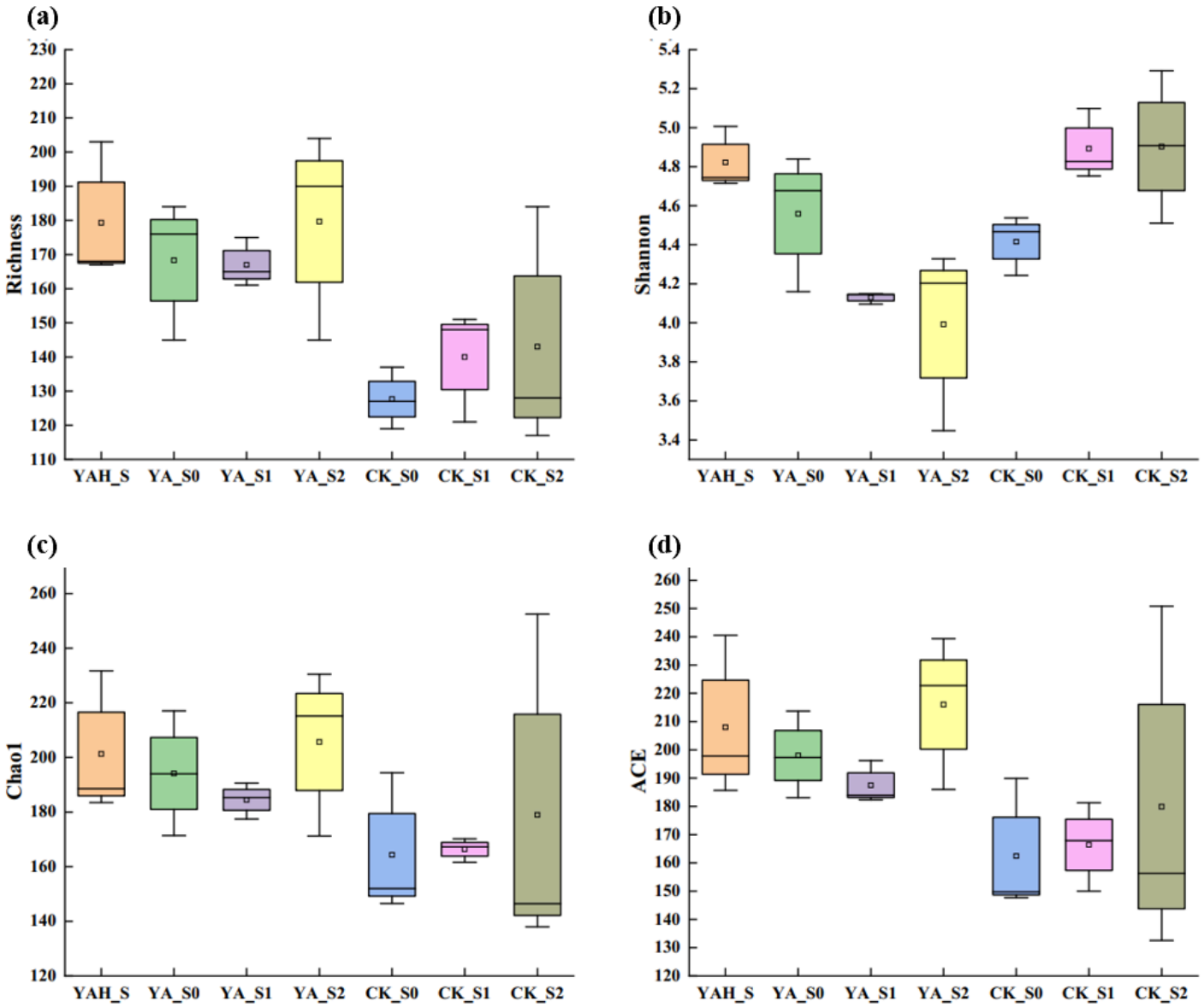

Figure 3

Boxplots of the alpha diversity indices of *P. edulis* rhizosphere soil samples.**(a)**Richness index. **(b)** Shannon index. **(c)** Chao index. **(d)** Ace index.

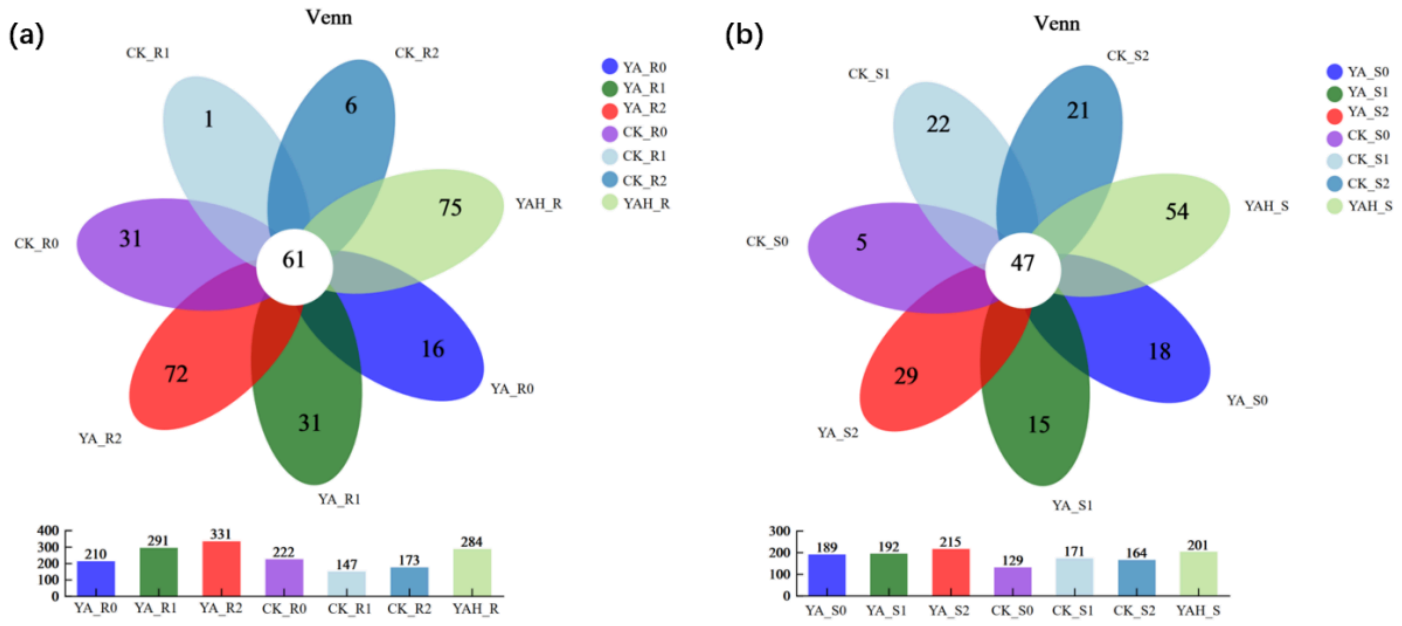

Figure 4

Venn diagrams of *P. edulis* samples. **(a)** Rhizome root samples. **(b)** Rhizosphere soil samples.

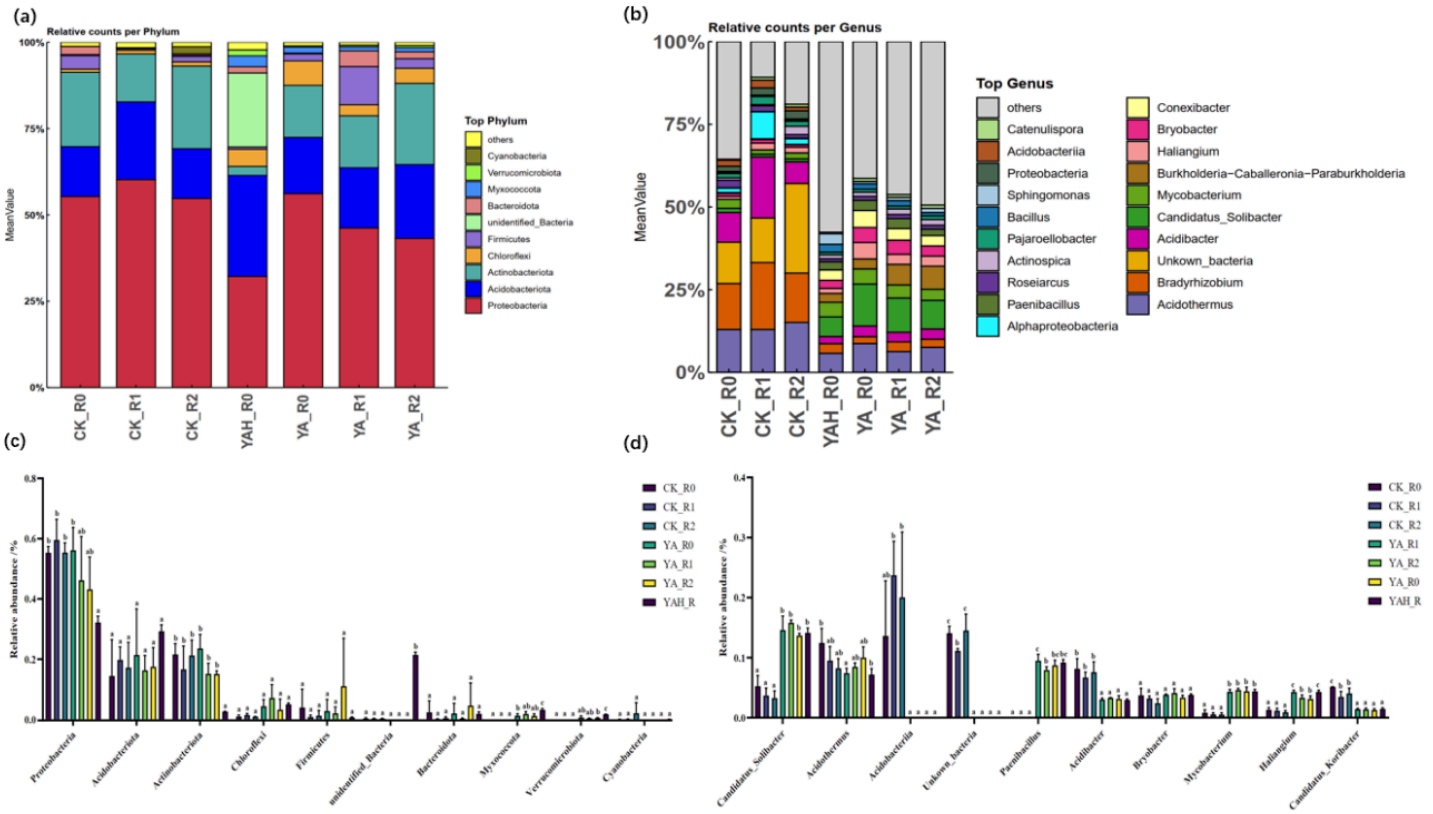

Figure 5

The relative abundances of *P. edulis* rhizome roots samples at the phylum and genus levels. (a) Relative abundances at the top 10 phylum for roots samples. (b) Relative abundances at the top 20 genus for roots samples. (c) Bar graph of top 10 phylum relative abundance changes in roots samples. (d) Bar graph of top 10 genus relative abundance changes in roots samples. Statistical

significance was assessed using the Kruskal-Wallis test. Groups with the same letters are not significantly different at an adjusted p value < 0.05.

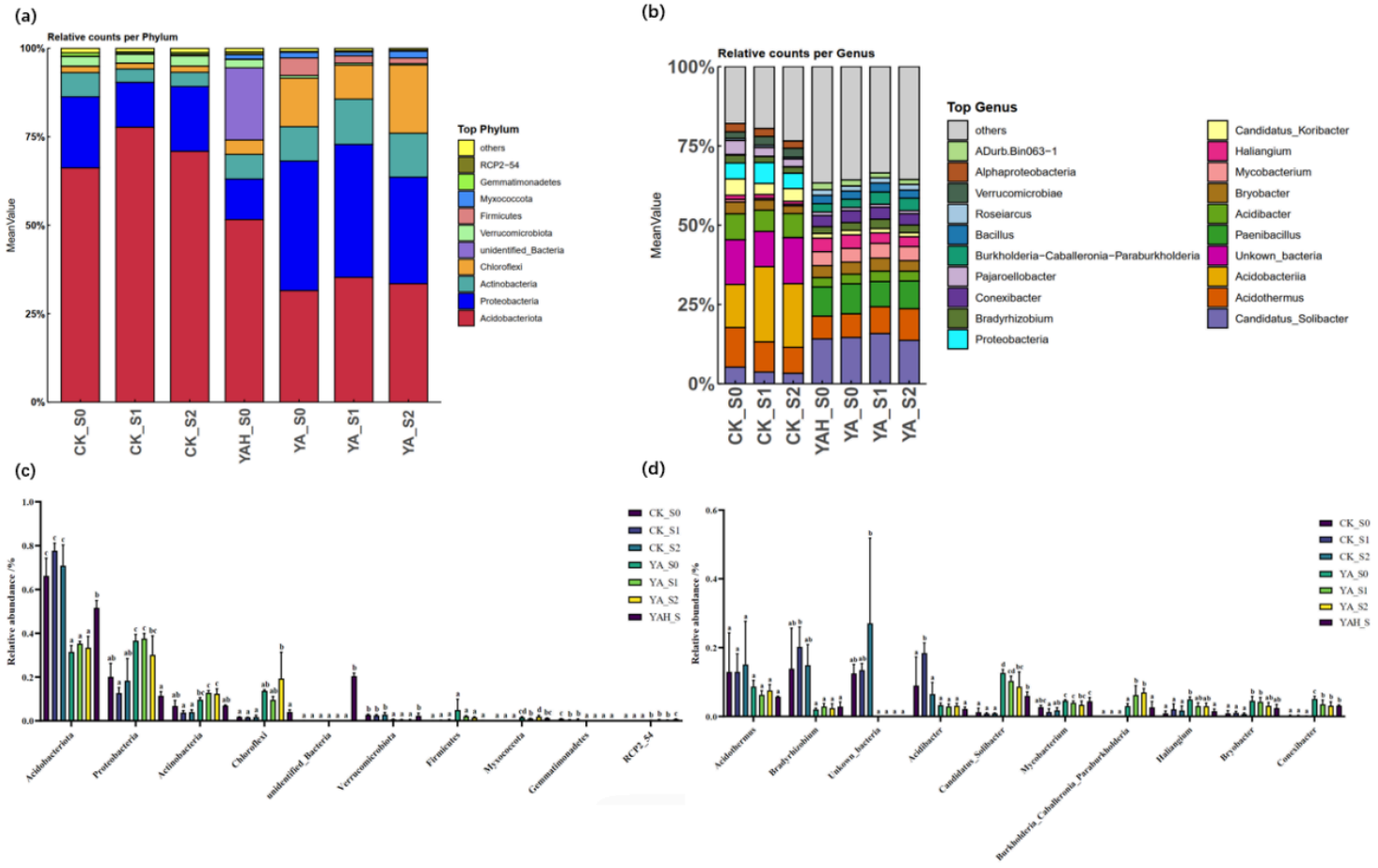

Figure 6

The relative abundances of *P. edulis* rhizosphere soil samples at the phylum and genus levels. (a) Relative abundances at the top 10 phylum for rhizosphere soil samples. (b) Relative abundances at the top 20 genus for rhizosphere soil samples. (c) Bar graph of top 10 phylum relative abundance changes in rhizosphere soil samples. (d) Bar graph of top 10 genus relative abundance changes in rhizosphere soil samples. Statistical significance was assessed using the Kruskal-Wallis test. Groups with the same letters are not significantly different at an adjusted p value < 0.05.

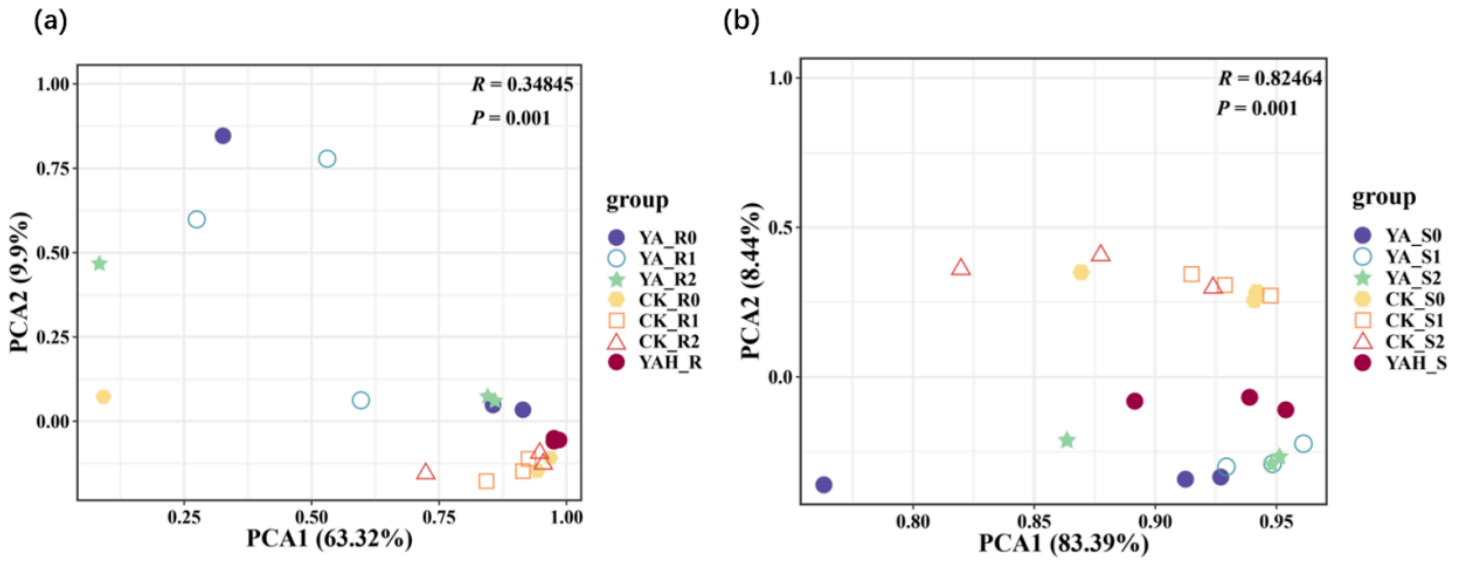

Figure 7

PCA analysis of the *P. edulis* samples. **(a)**Rhizome root samples. **(b)** Rhizosphere soil samples.

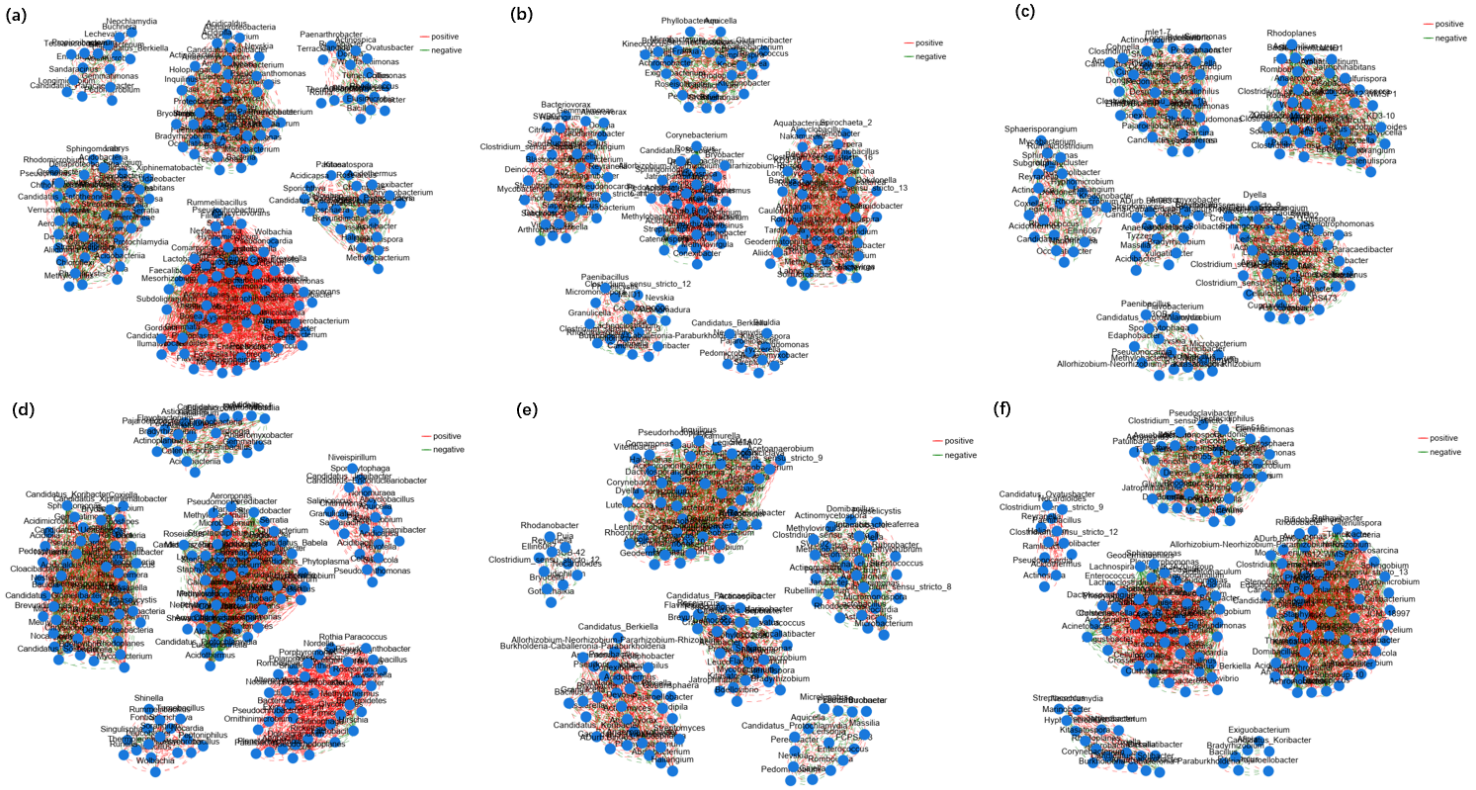

Figure 8

Inter-generic correlation network diagram. (a): CK_R2, (b): YA_R2, (c): YAH_R, (d): CK_S2, (e): YA_S2, (f): YAH_S.

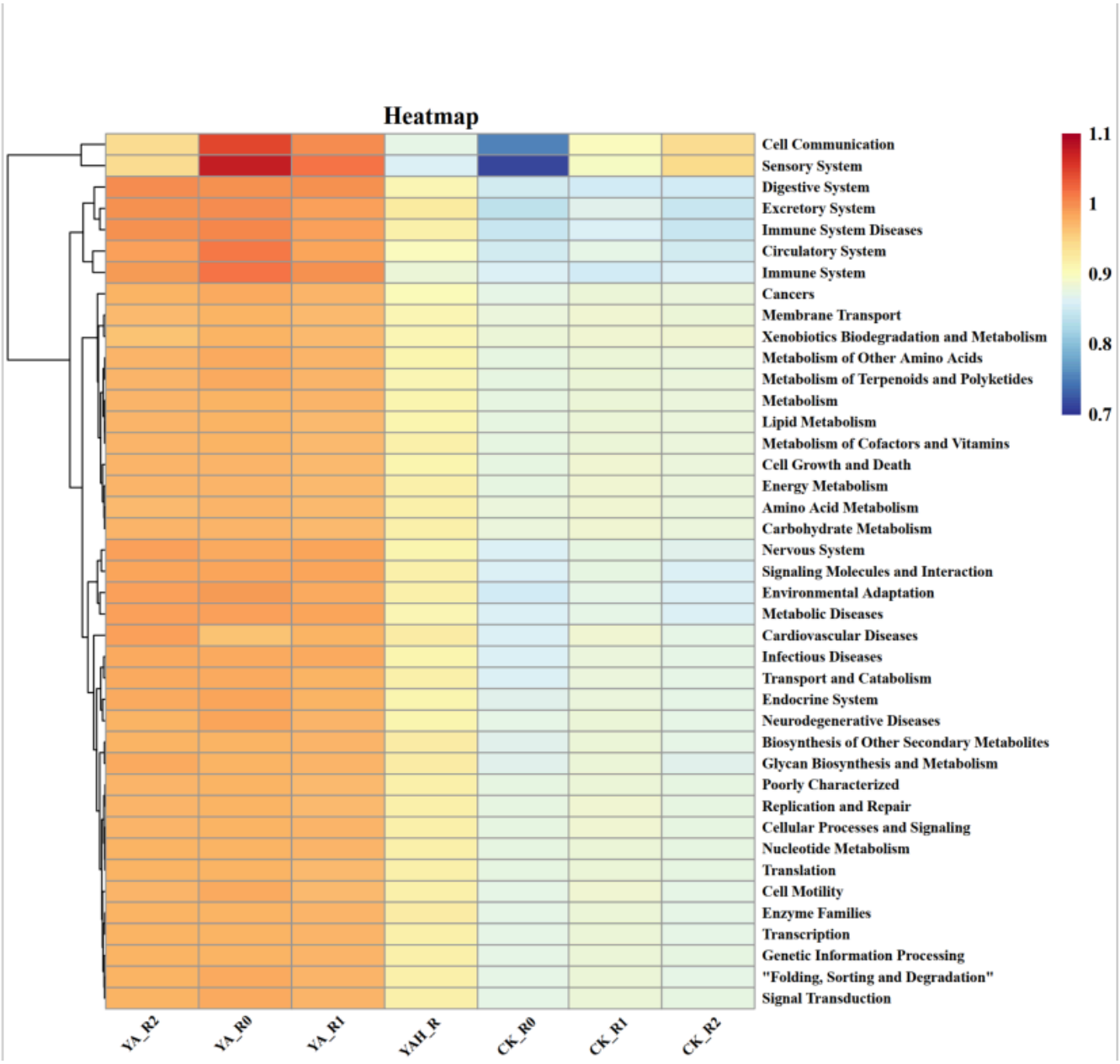

Figure 9

Functional expression abundance of bacterial communities in the *P. edulis* rhizome roots samples

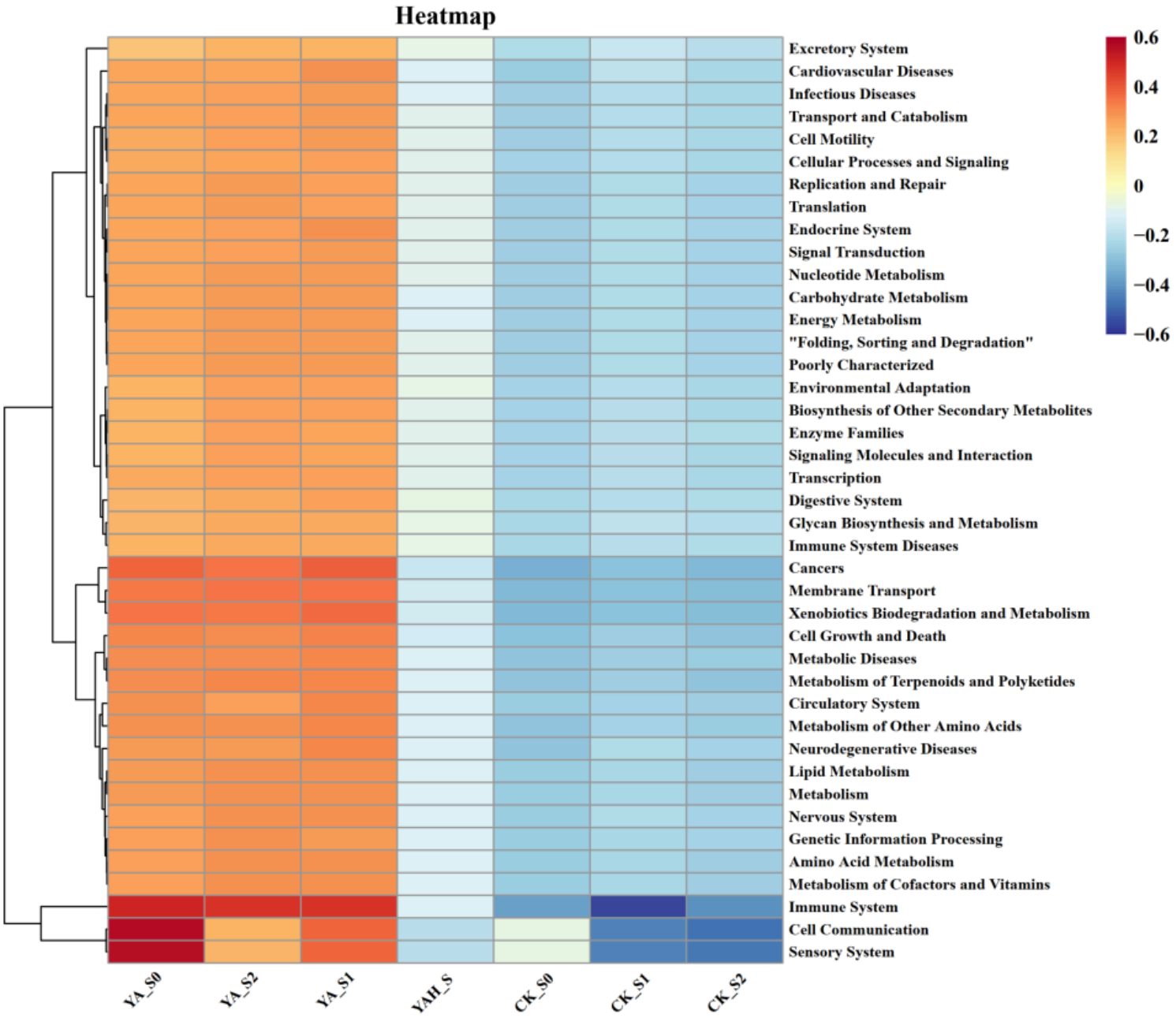

Figure 10

Functional expression abundance of bacterial communities in the *P. edulis* rhizosphere soil samples

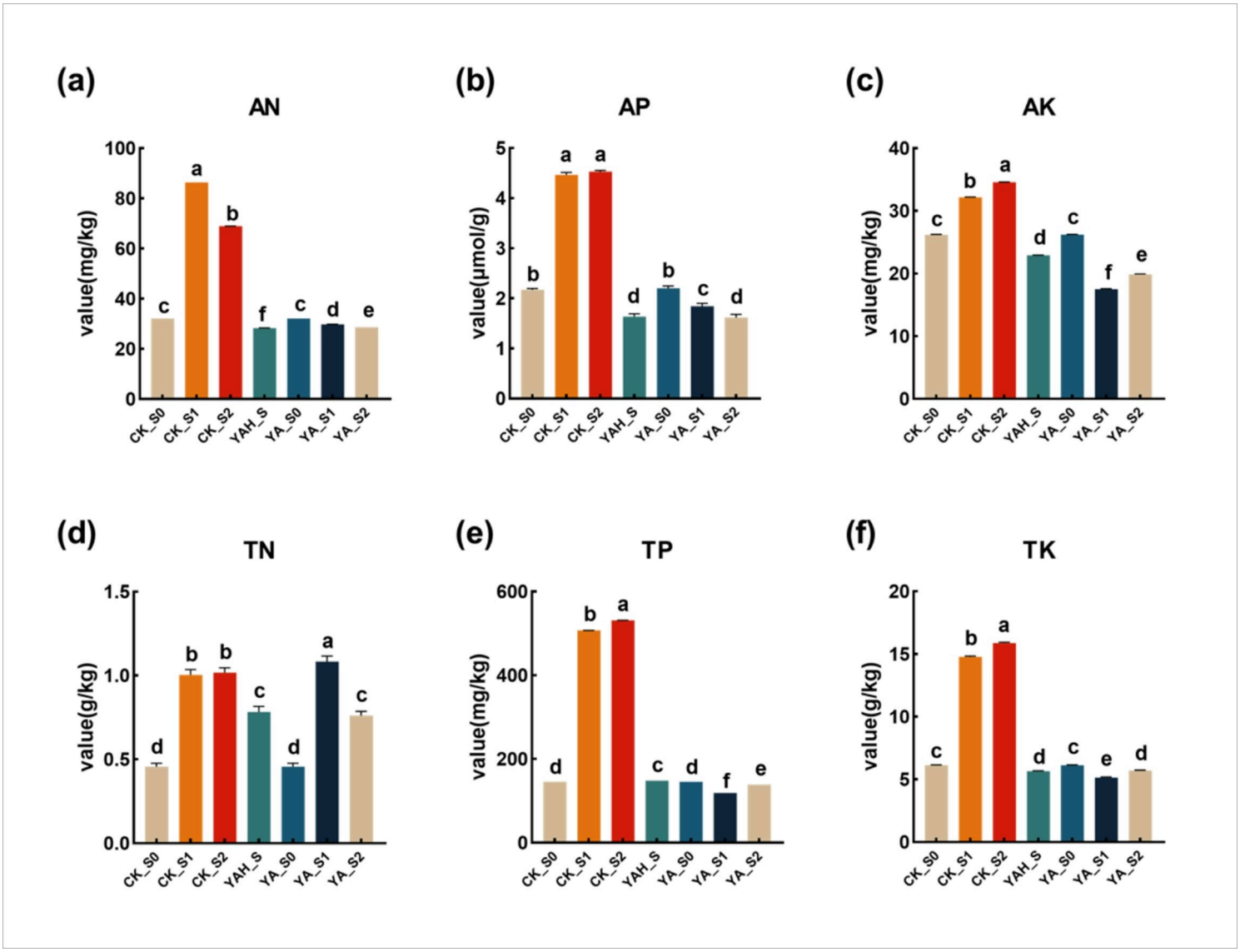

Figure 11

Chemical indicators of soil. **(a)** Available nitrogen (AN). **(b)** Available phosphorus (AP). **(c)** Available potassium (AK). **(d)** Total nitrogen (TN). **(e)** Total phosphorus (TP). **(f)** Total potassium (TK).

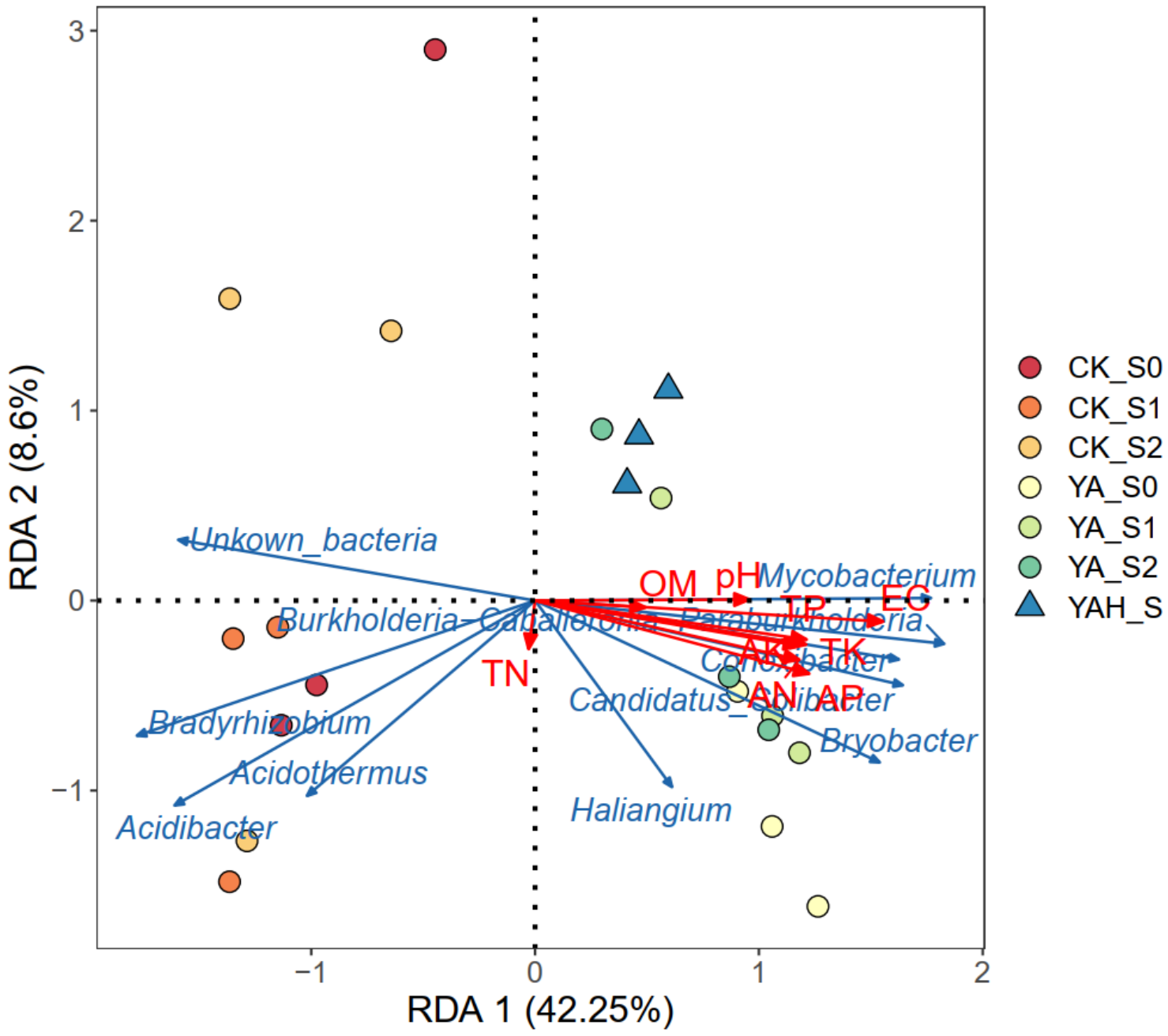

Figure 12

Redundancy analysis (RDA) showed correlations among different rhizosphere soil samples, environmental factors, and bacterial genera

Figure 13

Rhizome andrhizome roots of the 6th month. **(a)** No treatment sample. **(b)** Samples treated with microbial cultures. **(c)** Samples of high-yielding forests.