

Interactive effects between soil properties and bacterial communities in tomato rhizosphere under the application of microbial agents (#110350)

1

First submission

Guidance from your Editor

Please submit by **15 Feb 2025** for the benefit of the authors (and your token reward) .



Structure and Criteria

Please read the 'Structure and Criteria' page for guidance.



Raw data check

Review the raw data.



Image check

Check that figures and images have not been inappropriately manipulated.

If this article is published your review will be made public. You can choose whether to sign your review. If uploading a PDF please remove any identifiable information (if you want to remain anonymous).

Files

Download and review all files from the [materials page](#).

6 Figure file(s)

1 Table file(s)

7 Raw data file(s)

12 Other file(s)



Structure and Criteria

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

 You can also annotate this PDF and upload it as part of your review

When ready [submit online](#).

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your [guidance page](#).

BASIC REPORTING

-  Clear, unambiguous, professional English language used throughout.
-  Intro & background to show context. Literature well referenced & relevant.
-  Structure conforms to [Peerj standards](#), discipline norm, or improved for clarity.
-  Figures are relevant, high quality, well labelled & described.
-  Raw data supplied (see [Peerj policy](#)).

EXPERIMENTAL DESIGN

-  Original primary research within [Scope of the journal](#).
-  Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
-  Rigorous investigation performed to a high technical & ethical standard.
-  Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

-  **Impact and novelty is not assessed.** Meaningful replication encouraged where rationale & benefit to literature is clearly stated.
-  All underlying data have been provided; they are robust, statistically sound, & controlled.
-  Conclusions are well stated, linked to original research question & limited to supporting results.



The best reviewers use these techniques

Tip

Example

Support criticisms with evidence from the text or from other sources

Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.

Give specific suggestions on how to improve the manuscript

Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).

Comment on language and grammar issues

The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult. I suggest you have a colleague who is proficient in English and familiar with the subject matter review your manuscript, or contact a professional editing service.

Organize by importance of the issues, and number your points

1. Your most important issue
2. The next most important item
3. ...
4. The least important points

Please provide constructive criticism, and avoid personal opinions

I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC

Comment on strengths (as well as weaknesses) of the manuscript

I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.

Interactive effects between soil properties and bacterial communities in tomato rhizosphere under the application of microbial agents

Longxue Wei¹, Dongbo Zhao¹, Lianghai Guo¹, Jianjun Guo¹, Jinying Zhu¹, Yanting Pei¹, Peiyan Guan², Zhihui Guo¹, Huini Cui¹, Jiansheng Gao¹, Yongjun Li¹, Liang Zhang¹, Fuyan Wang¹, Peng Liu^{Corresp. 1}

¹ Dezhou Institute of Agricultural Science, Dezhou, Shandong, China

² Dezhou College, Dezhou, Shandong, China

Corresponding Author: Peng Liu
Email address: wheatpb@163.com

Background To study the impact of microbial agents on the physicochemical properties , enzyme activities and bacterial communities in tomato (*Solanum lycopersicum* L.) rhizosphere soil, in order to provide a theoretical foundation for the stability of tomato rhizosphere soil microecology and the ecological preservation of farmland soil. **Methods** Tomato rhizosphere soils treated with various microbial agents including *Bacillus subtilis* (FQ_T1), *Trichoderma harzianum* (FQ_T2), *Bacillus amyloliquefaciens* (FQ_T3), *Verticillium thuringiensis* (FQ_T4), and *Verticillium purpureum* (FQ_T5), as well as an untreated control (FQ_CK), were analyzed. The effects of different microbial agents on the physicochemical properties, enzyme activities and bacterial community structure of tomato rhizosphere soil after two years of continuous cropping were analyzed by using 16S rRNA and ITS high-throughput sequencing techniques. **Results** With the application of microbial agents, microbial agent treatment was beneficial for improving the physicochemical properties and enzyme activity of tomato rhizosphere soil after two years of continuous cropping. Compared with the control FQ_CK, to a certain extent, bacterial treatment increased the richness of bacterial communities, the total number of species, and the overall relative abundance of beneficial bacterial phylum and genus . It had a positive impact on microbial structure and function, improved the interaction and stability of species ecological networks, and was conducive to the stability and sustainability of tomato rhizosphere soil microbial ecosystem functions.

Interactive effects between soil properties and bacterial communities in tomato rhizosphere under the application of microbial agents

Longxue Wei¹, Dongbo Zhao¹, Lianghai Guo¹, JianjunGuo¹,Jinying Zhu¹, Yanting Pei¹,
PeiyanGuan²,ZhihuiGuo¹,HuiniCui¹,JianshengGao¹,YongjunLi¹,LiangZhang¹,FuyanWang¹,Peng
Liu¹

¹Dezhou Institute of Agricultural Science, Dezhou, Shandong, China

²Dezhou College, Dezhou, Shandong, China

Corresponding Author:

PengLiu¹

No. 1245 University West Road, Dezhou, Shandong, 253000, China

Email address: wheatpb@163.com

Abstract

【Background】 To study the impact of microbial agents on the physicochemical properties, enzyme activities and bacterial communities in tomato (*Solanum lycopersicum* L.) rhizosphere soil, in order to provide a theoretical foundation for the stability of tomato rhizosphere soil microecology and the ecological preservation of farmland soil.

【Methods】 Tomato rhizosphere soils treated with various microbial agents including *Bacillus subtilis* (FQ_T1), *Trichoderma harzianum* (FQ_T2), *Bacillus amyloliquefaciens* (FQ_T3), *Verticillium thuringiensis* (FQ_T4), and *Verticillium purpureum* (FQ_T5), as well as an untreated control (FQ_CK), were analyzed. The effects of different microbial agents on the physicochemical properties, enzyme activities and bacterial community structure of tomato rhizosphere soil after two years of continuous cropping were analyzed by using 16S rRNA and ITS high-throughput sequencing techniques.

【Results】 With the application of microbial agents, microbial agent treatment was beneficial for improving the physicochemical properties and enzyme activity of tomato rhizosphere soil after two years of continuous cropping. Compared with the control FQ_CK, to a certain extent, bacterial treatment increased the richness of bacterial communities, the total number of species, and the overall relative abundance of beneficial bacterial phylum and genus. It had a positive impact on microbial structure and function, improved the interaction and stability of species ecological networks, and was conducive to the stability and sustainability of tomato rhizosphere soil microbial ecosystem functions.

Keywords: microbial agents; tomato; bacteria; soil properties; continuous cropping

Introduction

The tomato (*Solanum lycopersicum* L.) stands as one of the most significant vegetable crops cultivated globally (Singh et al., 2015). With the intensification of intensive production and the development of facility agriculture, continuous cropping obstacles such as serious soil-borne diseases have become increasingly prominent, resulting in reduced quality and yield, which seriously restricts the sustainable development of tomato industry (Su et al., 2023). Soil is the carrier of plant production and also a fundamental element of the ecological environment. Soil microorganisms act as "regulators" of soil nutrient cycling in the process of nutrient transformation, controlling the direction of soil nutrient cycling, the types of nutrient element compounds, and exchange fluxes (Adomako et al., 2022) (Luo et al., 2019). Soil microbial communities and diversity regulate the multifunctionality of soil ecosystems, thereby influencing the soil's resistance and resilience, which are essential for maintaining soil fertility and sustainable production (Hemkemeyer et al., 2021). Understanding the ecological processes involved in microbial community assembly helps to determine how microbial community composition responds to environmental changes (Oksana Coban et al., 2022). (Xu et al., 2015). Continuous cropping can disrupt the microbial community structure in the rhizosphere, impair the survival of beneficial organisms, foster the growth of pathogenic bacteria, intensify the occurrence of soil-borne diseases, and subsequently impact crop growth, resulting in yield reduction (Feng et al., 2023). (Xiao et al., 2024). Studies have found that rhizosphere microorganisms are crucial in the onset and control of soil-borne diseases. Plants can recruit beneficial microorganisms in the rhizosphere via root exudates, thereby enhancing disease resistance through the antagonism, competition, and induction of systemic resistance by these beneficial microorganisms (Su et al., 2023). Research by Fu et al. indicates that soil-related issues stemming from continuous monoculture have hindered the sustainable development of China's tomato industry (Fu et al., 2017). Tomato yield was significantly positively correlated with soil available potassium and soil microbial community functional diversity index.

Microbial inoculants are microbial inoculants based on one or more functional or bacterial strains applied to the soil as a substitute for traditional inorganic fertilizers (bio-fertilizers), which can exert biological control of pests and diseases (Ji. et al., 2023), or be used for bioremediation (Yang et al., 2023), and enhancement of soil properties (Jiang et al., 2022), and

plant resistance (Xiao *et al.*, 2024). While some soil inoculants (e.g. rhizobia) have a long and fruitful history of use (Abdurashytova *et al.*, 2022), other soil inoculants have not performed consistently in the field (Chen *et al.*, 2024). (Leticia L *et al.*, 2024). Bouček Jiří *et al.* (Bouček Jiří *et al.*, 2023) found that inoculation of tomato plants with *Bacillus amyloliquefaciens* had a favorable effect on fruit quality; Kamaraj S *et al.* (Sedhupathi *et al.*, 2022) concluded that the fungi of the cushion mycorrhizal fungus (*Rhizobium irregularis*), *Bacillus subtilis*, and *Penicillium violetum*, the Biocontrol agents (BCA) were able to produce extracellular proteases and form maximal biofilms, which significantly reduced the number of egg masses and root-knot index against tomato root-knot nematodes; Shalaby Tarek A *et al.* (Shalaby Tarek A *et al.*, 2022) found that *Trichoderma harzianum* in combination with polybutazole (PBZ) had a significant biochemical control of *Trichoderma verticillioides* in tomato seedlings. Liu *et al.* (Liu *et al.*, 2018) found that *Bacillus amyloliquefaciens* SQR9 and amino acid-containing organic fertilizer (chicken manure) inhibited tomato diseases by changing the composition of rhizosphere bacterial communities. A more accurate understanding of the ecology and mode of action of inoculant strains is key to optimizing their efficacy and guiding their targeted use to address key constraints to crop production (Gu *et al.*, 2024).

At present, there are few studies on the characteristics and function prediction of bacterial community in rhizosphere soil of tomato under continuous cropping for two years under different microbial agents, which is very important for predicting the role of rhizosphere microorganisms in regulating plant-microorganism ecosystem function. In this study, tomatoes with different microbial agents for two years were used as the research object. The effects of microbial agents on the physical and chemical properties of tomato rhizosphere soil and the composition of soil bacterial community structure were investigated by 16S rRNA and ITS high-throughput sequencing technology, in order to explore the mechanism of rhizosphere bacterial community construction. In a word, the research is helpful to reveal the soil ecological stability mechanism of microbial agents for promoting growth and disease resistance, and to evaluate the practical application effect of different carrier agents in agricultural production, so as to provide theoretical support for the sustainable development of tomato industry.

Materials & Methods

Test materials and test site

This experiment was conducted from January 19, 2023 to February 17, 2023 at Xin Sheng

Seed Industry, Pingyuan County, Dezhou City, and from February 19, 2023 to September 2023 at the greenhouse of Dazhuang Village, En Township, Pingyuan County, Dezhou City (120.4114°E,30.4406°N), with tomato varieties of " Chun Li " as the test crop, and the previous crop in the test site was tomato. The basic physicochemical properties of the soil were organic matter 13.28g·kg⁻¹, total nitrogen 1.12g·kg⁻¹, effective phosphorus 125.55mg·kg⁻¹, and fast-acting potassium 116.37mg·kg⁻¹.

Microbial agents for testing: FQ_T1: *Bacillus subtilis* (effective live bacterial count is 1.00×10⁹ CFU·mL⁻¹); FQ_T2: *Trichoderma harzianum* (effective live bacterial count is 3.00×10⁷ CFU·mL⁻¹); FQ_T3: *Bacillus amyloliquefaciens* (the effective number of live bacteria is 1.00×10⁹ CFU·mL⁻¹); FQ_T4: *verticillium chlamydosporium sp.* (the effective number of live bacteria is 2.50×10⁸ CFU·mL⁻¹); FQ_T5: *Paecilomyces lilacinus* (the effective number of live bacteria is 1.00 × 10⁸CFU·mL⁻¹); five microbial fungicides purchased from the biological plant protection station of the Fruit Yau Agricultural Resources and Beihai Qunlin biological factory store; the test was conducted in a plastic greenhouse double-layer thermal insulation facilities cultivation, the greenhouse set up a control (FQ_CK) and five microbial agents treatments (FQ_T1, FQ_T2, FQ_T3, FQ_T4, FQ_T5). Planted March 20, April 20 and May 19 inter-root furrow application of five agents (dosage of 150 times the liquid, each time 100mL per plant); control conventional management, no agents. Each experimental plot covers an area of 50m², repeated 3 times, randomly arranged, and the field management measures are the same as those of local high-yield greenhouses.

soil sample collection

Soil samples were collected on June 27th, 2023.

Firstly, remove the 0-3cm soil on the surface layer, and then take out the plants together with the surrounding soil to keep the root system intact; Secondly, fully shake off the soil around the root system, gently brush the soil close to the root system with a sterile brush to remove foreign matters; Then, the rhizosphere soil of three tomato plants was randomly selected, mixed and put into sterile plastic bags, which was one soil sample. The soil samples were divided into two parts after passing a 2mm mesh sieve: one part was stored in an ultra-low temperature freezer at -80 °C for DNA extraction, and the other part was placed in a cool and dry place for natural air drying for the determination of soil physicochemical properties and enzyme activities.

Determination of soil physicochemical properties and enzyme activities

Soil organicmatter content was titrated by redox method after high temperature digestion, Totalnitrogen content was titrated by acid standard solution using semi-trace Kjeldahl method, alkali nitrogen content was titrated by acid standard solution using alkali diffusion method, availablephosphorus content was extracted by $0.5\text{mol}\cdot\text{L}^{-1}\text{NaHCO}_3$, and then determined by molybdenum antimony antimonimony colorimetric method, and then detected by TU-1810 UV-Vis Spectrophotometer (Beijing Pudian General Instrument Co., Ltd.), and quick-acting potassium content was detected by TU-1810 UV-Vis Spectrophotometer. The content of availablepotassium was detected by $\text{CH}_3\text{COONH}_4$ extraction and then detected by BWB-1 flame spectrophotometer (BWBTechnologies, UK).

Soil sucrase was determined by the colourimetric method of 3,5-dinitrosalicylic acid; soil catalase was determined by the titrimetric method of potassium permanganate; alkaline phosphatase was determined by the Sodium Benzene Phosphate colourimetric method. All the steps were performed by the China Rice Research Institute according to the instructions of the corresponding enzyme activity kits provided by Suzhou Keming Biotechnology Co. Ltd, and the samples were colourimetrically determined using a multifunctional enzyme marker (TECAN-Spark 20M).

Analysis of microbial diversity measurements of rhizosphere soils of tomatos

High-throughput sequencing analyses were performed by Shanghai Meiji Biomedical Technology Co. Ltd: the labelled bacterial universal primer 338F (5'-ACTCCTACGGGGAGGCAGCA-3')-806R (5'-GGACTACHVGGGTWTCTAAT-3') to amplify the soil bacterial 16SrRNA gene region. Afterwards, the raw data were quality filtered and merged on the Illumina MiSeq platform (Illumina, USA) (*Chen et al., 2018*), according to the standard method provided by Majorbio((Majorbio Shanghai, China)), and then clustered at 97% similarity level to obtain the OTU (operational taxonomic unit) (*DavideFrancioli et al., 2020*). Species annotation were performed on the OTU sequences (*BolyenEvan et al., 2019*) and the community composition, alpha diversity and relative abundance of the samples were counted at each taxonomic level, while heatmap, Mantel Test network analyses, and Faprotax function prediction analysis were performed within the platform on the correlation between the soil environmental factors and microbial communities to determine the effects of soil environmental factors on the composition of microbial communities.

Data processing and analysis of high-throughput sequencing results

Trimmomatic software was used for quality control of the sequenced raw sequences, and FLASH1.2.11 software was used for double-ended sequence splicing, and UPARSE7.1 software was used for OTU clustering of the sequences based on 97% similarity. Based on the OTU clustering results, the Majorbio Cloud platform was used for further data analysis and information mining, including species community composition analysis, a diversity analysis (including Sobs index, Simpson index, Shannon-Wiener index, Chao1 richness, Abundance-based Coverage Estimator (ACE) and coverage and other indices and performed 16S gene function prediction analysis (COG database).

The data were statistically analysed using Excel 2007 and SPSS statistics 20.0 software, and the soil microbial diversity analysis and visual mapping were performed using Origin2019b and MajorBIO cloud platform, and the OTUs were subjected to multiple sequence comparison. Analysis of variance and multiple comparisons ($P < 0.05$) were performed using one-way ANOVA and Duncan (Duncan's Multiple Range Test, DMRT) method.

Results

Effects of different microbial agents on soil physicochemical properties and enzyme activities of Tomato

Different microbial agents have varying degrees of effects on the physicochemical properties and enzyme activities of tomato rhizosphere soil.

The content of available potassium and organic matter in tomato rhizosphere soil treated with microbial agents showed significant differences relative compared to the control FQ_CK (Fig.1): on average, the content of available potassium in tomato rhizosphere soil treated with five microbial agents was 50.56% higher than the control FQ_CK treatment, and the content of organic matter was 13.82% higher than the control FQ_CK treatment; Except for the FQ_T2 treatment, there was a significant difference in total nitrogen content between tomato rhizosphere soil treated with microbial agents and the control FQ_CK treatment. The total nitrogen content of tomato rhizosphere soil treated with microbial agents was on average 22.83% higher than that of the control FQ_CK treatment; Except for the FQ_T1 treatment, there was a significant difference in the available phosphorus content of tomato rhizosphere soil after microbial agent treatment compared to the control FQ_CK treatment. The available phosphorus content of tomato rhizosphere soil treated with microbial agent was on average 30.81% higher than that of the control FQ_CK treatment.

Microbial agent treatment had a certain degree of impact on the enzyme activity of tomato rhizosphere soil. The combination of microbial agent treatment FQ_T2 and FQ_T5 showed a significant difference in the sucrose enzyme activity of tomato rhizosphere soil compared to the control FQ_CK treatment; The catalase activity in tomato rhizosphere soil treated with microbial agents FQ_T2, FQ_T3, FQ_T4, and FQ_T5 showed significant differences compared to the control FQ_CK treatment; The alkaline phosphatase activity in tomato rhizosphere soil treated with microbial agents FQ_T1, FQ_T2, and FQ_T3 showed significant differences compared to the control FQ_CK treatment; After treatment with microbial agents, the average activities of sucrose enzyme, catalase, and alkaline phosphatase in tomato rhizosphere soil were 7.59%, 4.39%, and 15.82% higher than those in the control FQ_CK treatment, respectively. From this, it can be seen that although microbial agents have different effects on the physicochemical properties and enzyme activities of tomato rhizosphere soil, overall, microbial agent treatment was beneficial to improve the physicochemical properties and enzyme activities of tomato rhizosphere soil in two years of continuous cropping(Fig.1).

Effects of different microbial agents on the microbial community in tomato rhizosphere soil

Evaluation of Sample Sequencing Depth and OTU Cluster Analysis

The dilution curve reached the plateau period represents that the sequencing data can cover the microbial community diversity, and the dilution curve can also indirectly reflect the species diversity Richness. Sequencing of bacterial samples yielded a total of 248,652,103,952,463 bases (Fig. 2), with an average sequence length of 418 bp, and OTU sequence similarity was 0.97. and the dilution curve for the USEARCH11-uparse clustered OTU gradually plateaus after 25,000 sequences, suggesting an adequate supply of sequencing data and reliable outcomes, suitable for further analysis.

Alpha diversity of soil bacteria treated with different microbial agents

Alpha diversity denotes the number and diversity of species within a local habitat or ecosystem, typically assessed through Sobs, Simpson, Shannon, Chao1, and ACE indices. The Sobs and Chao1 indices reflect community richness, with higher indices indicating more species, while the Simpson index assesses community diversity, with a higher Simpson value indicating lower diversity. As shown in Table 1, the coverage of soil bacterial samples reached over 98.74%, confirming that the study's findings accurately represent the bacterial community diversity in the samples, and the sequencing results are deemed reliable. There were significant

differences in ACE index, Chao1 index and Sobs index between FQ _ T1, FQ _ T5 microbial agent treatment and FQ _ CK treatment. There was no significant difference in Simpson and Shannon index between the microbial agent treatment and the control FQ _ CK. Overall, compared with the control FQ _ CK, the application of microbial agents increased the community Sobs and Chao1 index at different levels, indicating that the treatment of microbial agents increased the richness of bacterial communities and the total number of species to a certain extent.

Analysis of soil bacterial community structure after treatment with different microbial agents

Following the application of different microbial agents, significant changes were observed in the relative abundance of bacterial communities in the soil across all treatment groups (Fig. 3).

Rhizosphere soil samples contained bacteria from 40 phylum, 130 classes, 309 orders, 490 families, and 877 genus, totaling 1598 species. As illustrated in Fig. 3, at the phylum level, 11 rhizosphere soil bacterial phylum exhibited relatively high abundances (>1%), with *Proteobacteria* and *Firmicutes* as the predominant phylum, averaging 44.55% across all treatments. In comparison to the FQ_ CK treatment, the average relative abundance of *Proteobacteria* across the five microbial treatments decreased by 2.42%, with the FQ_ T1 treatment showing the largest reduction at 14.61%. The FQ_ CK treatment displayed a higher relative abundance of *Proteobacteria* at 28.03%; Relative to the FQ_ CK treatment, the average relative abundance of *Firmicutes* across the five microbial treatments increased by 2.22%, with the FQ_ T4 treatment showing the largest increase at 4.25%; In comparison to the FQ_ CK treatment, the relative abundances of *Chloroflexi* and *Bacteroideta* across the five microbial treatments decreased by an average of 4.69% and 1.79%, respectively. Meanwhile, the relative abundances of *Actinobacteria* and *Acidobacteria* increased by an average of 1.54% and 2.94%, respectively; Compared with FQ_ CK treatment, the relative abundance changes of *Myxococcata*, *Gemmatimonadota*, *Nitrospira*, *Verrucomicrobiota*, and *Entotheonellaeota* treated with five bacterial agents were slightly different, but the overall results showed an average increase of 0.21%, 0.16%, 0.05%, 0.02%, and 0.34%, respectively.

Fig.S1 shows the dominant genus with relative abundance>1% at genus level and ranking among the top 10. The 10 genus account for 27.03% on average among the treatments. Among them, *Bacillus* and *Paenisporosarcina* are the dominant genus with relative abundance of top2,

and the unnamed genus account for 42.97% of the 10 genera. Compared with FQ_CK treatment, the relative abundance of *Bacillus* increased by 52.52% on average; The relative abundance of *paenisporsarcina* was 0.19 times higher than that of the treatment without application of fungicides for two years; The average relative abundance of *Nitrospira* in five microbial agents treatment was 2.91% higher than that in FQ_CK treatment. In general, the application of microbial agents increased the relative abundance of *Bacillus*, *Paenisporsarcina* and *Nitrospira* in tomato rhizosphere soil in two years of continuous cropping, and had a certain impact on the composition of bacterial community in tomato rhizosphere soil in two years of continuous cropping

Correlation between soil environmental factors and microbial community

Mantel Test network heatmap analysis is a correlation analysis of two matrices, which is commonly used in microbiome to analyze the correlation between environmental factors and microbial community structure.

In Fig.4, Mantel 'r (absolute value of R) was used to plot the correlation between bacterial community and environmental factors in tomato rhizosphere soil. The Mantel 'r (absolute value of R) of bacterial community and AP (available phosphorus) and TN (total nitrogen) content was between 0.4-0.6, Mantel 'p < 0.05, and there was a positive correlation. There was a positive correlation between the bacterial community and the Mantel 'r (the absolute value of R) of CA (catalase activity) and AK (available potassium) content between 0.4 and 0.6, Mantel 'p ≥ 0.05. There was a positive correlation between bacterial community and OM (organic matter content), APA (alkaline phosphatase activity) Mantel 'r (absolute value of R) < 0.4, Mantel 'p ≥ 0.05;The bacterial community was negatively correlated with Mantel 'r (absolute value of R) < 0.4 and Mantel 'p ≥ 0.05 of SA (sucrase activity). There were different correlations among the seven environmental factors, and only there was a significant positive correlation between CA (catalase activity) and AP (available phosphorus) content.

Analysis of Microbial Function Prediction in Tomato Rhizosphere Soil

FAPROTAX software maintains a functional classification database based on species information, which includes more than 80 functional classifications of carbon, nitrogen, phosphorus, sulfur and other element cycles, as well as plant and animal pathogens, methane generation, fermentation, etc., covering more than 4,600 different prokaryotic species. It has good predictive effects on the biochemical cycle processes of environmental samples (*LoucaS et*

al., 2016). At present, many scholars have used FAPROTAX to carry out functional prediction of microorganisms (Li *et al.*, 2024). This study also used FAPROTAX to analyze and predict the functions of bacteria in the rhizosphere soil of tomato treated with different microbial agents in the second year of continuous cropping, and obtained 53 functional groups (Fig. 5). The analysis of functional bacteria with a relative abundance of >1% (average total bacterial proportion of 87.74%) showed that chemolithoautotrophic functional bacteria dominated in tomato rhizosphere soil (accounting for 24.98%–33.39% of total bacteria), including Chemoheterotrophy and Aerobic Chemoheterotrophy bacteria; The relative abundance of phototrophic bacteria was relatively low (0.45%–1.91% of total bacteria), and the average relative abundance of phototrophy bacteria treated with the bacterial agent was 7.37% higher than that of the control FQ_CK treatment, which may be related to the promotion of the growth of phototrophic bacteria by the application of the bacterial agent.

The total relative abundance of functional microorganisms related to the nitrogen cycle (Nitrate Respiration, Nitrate Reduction, Nitrogen Respiration) was relatively high (averaging 8.02% of the total bacteria), and the average relative abundance of nitrogen cycle-related functional bacteria treated with microbial agents was 45.09% higher than that of the control FQ_CK treatment. This might mainly be due to the fact that the application of microbial agents offered a favorable growth environment for these bacteria. The relative abundance of communities with fermentation functional genes (Fermentation) ranged from 3.53% to 6.96%. The total relative abundance of functional microorganisms related to parasites (animal parasites or symbionts, intracellular parasites) averaged 2.08% of the total bacteria. The total relative abundance of functional microorganisms related to decomposition (xylanolysis, cellulolysis, chitinolysis, ureolysis) ranged from 3.30% to 5.51%. Among them, the total relative abundance of decomposition-related functional bacteria in FQ_T1 was the highest, 23.38% higher than that of the control FQ_CK treatment. Functional microorganisms related to human pathogenic bacteria (human pathogens pneumonia, human pathogens all, an average total relative abundance accounting for 2.11% of the total bacteria) and those related to sulfur and manganese oxidation cycles (dark thiosulfate oxidation, dark oxidation of sulfur compounds, manganese oxidation, an average total relative abundance accounting for 3.80% of the total bacteria) were also detected at the sampling sites.

Discussion

Microbial agents are beneficial for improving the physicochemical properties and enzyme activity of tomato rhizosphere soil

The contents of AK (available potassium) and OM (organic matter) in the rhizosphere soil of tomatoes treated with microbial agents showed significant differences compared with the control FQ_CK treatment: The contents of AK (available potassium), OM (organic matter), TN (total nitrogen), AP (available phosphorus) and the activities of sucrase, catalase and alkaline phosphatase in the rhizosphere soil treated with microbial agents were all higher than those in the control FQ_CK. Changes in soil microbial characteristics that may result from changes in soil chemical properties under two years of continuous cropping (*Wang et al., 2013*). In contrast, the application of microbial agents could improve the physicochemical properties and enzyme activities in the rhizosphere soil, thereby improving the soil nutrient environment, increasing the abundance of beneficial microorganisms, and exerting microscopic regulatory effects on the ecosystem. This might be that some environmental variables have potential influences on the assembly of rhizosphere microbial communities and play a key role in the construction of rhizosphere microbial communities. The deterministic and stochastic selection of microbial agent treatments regulates the physicochemical properties, enzyme activities and the construction of microbial communities in the rhizosphere soil (*CHEN et al., 2021*).

Microbial agents can enhance the diversity of bacterial microorganisms in tomato rhizosphere soil

Soil microbial diversity and network complexity are two important factors affecting ecosystem multifunctionality, and soil microbial diversity plays a positive role in promoting soil multifunctionality (*Gong et al., 2024*). Compared to the control FQ_CK, the bacteriophage treatment led to an increase in bacterial community richness and total number of species to some extent. This is consistent with the research conclusion of Wang et al. (*Wang et al., 2020*) who conducted pot experiments on Hubei Haitang with *Bacillus FKM10*, which showed an increase in bacterial abundance and diversity and a change in the structure of the soil microbial community in the treatment compared to the control. *Proteobacteria* and *Firmicutes* were the common dominant phyla. Compared with the FQ_CK, the relative abundances of *Actinobacteriota*, *Acidobacteriota*, *Gemmatimonadota* and *Nitrospirota* all increased. Previous studies have shown that *Actinobacteriota* can inhibit the activity of some pathogenic fungi,

promote the activity of microorganisms in the soil that are beneficial to crops, and release a variety of substances in metabolic activities to stimulate the growth and division of plant cells, play an important role in the decomposition and utilisation of organic matter, carbon and nutrient cycling, and have a better salt tolerance to promote the decomposition of plant residues, soil structure formation, seed germination and root growth (HenningSM *et al.*, 2017)(SathyaA *et al.*, 2017).The abundance of *Acidobacteriota* is highly correlated with soil nutrition and plays an important role in carbon and nitrogen metabolism (HOUL *et al.*, 2018),and is an important flora promoting denitrification (CHEND *et al.*, 2018). *Acidobacteriota* can also degrade plant residues, participate in the iron cycle and produce active soil metabolites (GLICKBR *et al.*, 2012). Previous studies have pointed out that the relative abundance of *Acidobacteriota* is closely related to soil pH, and the abundance of *Acidobacteriota* shows an increasing trend with the decrease of soil pH (Lauber *et al.*, 2009).Changes in the relative abundance of *Acidobacteriota* can indicate different nutritional strategies of soil bacterial communities; *Nitrospirae* is greatly related to the oxidation and reduction of nitrate. Among them, *Nitrospira* as a nitrifying bacterium can oxidize nitrite to nitrate (Feng *et al.*, 2008), and can play a key role in the decomposition of particulate organic matter (García-LópezM.etal *et al.*, 2019); *Chloroflexi* has strong tolerance to barren and harsh external growth environments and is suitable for growth and reproduction in low-nutrient environments (Zhou *et al.*, 2015). (Huang *et al.*, 2019)(EPELDE L *et al.*, 2015);*Acidobacteriota* has a significant inhibitory effect on soil organic matter mineralization (Guo *et al.*, 2017). On the contrary, *Bacteroidetes* is the main participant in soil organic matter mineralization (Guo *et al.*, 2015). This article found that the microbial agent treatments increased the abundance of *Acidobacteriota* , decreased the relative abundances of *Bacteroidetes* and *Chloroflexi* .The reason is that *Acidobacteriota* can achieve neutralization and regulation of alkaline soil through metabolic acid production (Schmalenberger *et al.*, 2013),is a beneficial bacterium in the plant salt resistance mechanism (Xu *et al.*, 2020).Some Acidobacterial microorganisms, as plant growth promoting rhizobacteria (PGPR), can produce plant growth promoting hormones (auxin IAA) and have the ability to produce iron carriers and dissolve phosphate, interact with plants, thereby promoting plant growth (SadafKALAM *et al.*, 2022).Therefore, the increase in the abundances of these beneficial phyla and the decrease in the abundances of harmful phyla after the application of microbial agents can promote the development of soil bacterial communities in a beneficial direction and increase soil activity.

There is an interactive relationship between the bacterial microbial community in tomato rhizosphere soil and soil physicochemical properties and enzyme activity

The bacterial community was positively correlated with AP (available phosphorus) and TN (total nitrogen) contents; the bacterial community was positively correlated with CA (catalase activity) and AK (available potassium) contents; the bacterial community was positively correlated with OM content (organic matter) and APA (alkaline phosphatase activity); the bacterial community was negatively correlated with SA (sucrase activity); the seven environmental factors had different correlation relationships among each other, and only catalase activity (CA) and available phosphorus (AP) content had a significant positive correlation relationship. A large number of studies have reported that different microbial agents can also change soil physicochemical properties and soil enzyme activities and promote crop growth. Abdelraouf et al. (*Abdelraouf Ahmed et al., 2023*) greatly improved the soil enzyme activity in the rhizosphere of tomatoes and reduced the infection of tomato Fusarium wilt by exogenous application of nanochitosan encapsulated *Pseudomonas*. Jia et al. (*Jia et al., 2023*). cocultured *Bacillus subtilis* and *Pseudomonas fluorescens* to synthesize microorganisms for the synergistic biological control of tomato early blight, which could reduce the incidence of diseases, increase biomass, increase the number of potential beneficial bacteria, and significantly change the rhizosphere microbial community.

Microbial agents promote beneficial bacterial functions in the rhizosphere soil of tomato after two years of continuous cropping

Ecological network analysis, as an important means to clarify the interactions among microorganisms, have great significance in exploring the assembly, construction and stability of rhizosphere microorganisms during grassland degradation. The use of microbial agents increased the diversity of fungi and the interaction and stability of species ecological networks, enhanced the ability of microorganisms to resist external environmental disturbances, and contributed to maintaining the stability and sustainability of farmland ecosystem functions (*Yu et al., 2022*). Using FAPROTAX to analyze and predict the functions of tomato rhizosphere soil bacteria, after different microbial agent treatments in the second consecutive year, a total of 53 functional population groups were obtained. Functional bacteria with a relative abundance > 1% (average total bacteria accounted for 87.74%) were selected for analysis. The results showed that chemoheterotrophic functional bacteria in the tomato rhizosphere soil were dominant

(accounting for 24.98% - 33.39% of the total bacteria), including Chemoheterotrophy and Aerobic Chemoheterotrophy bacteria; the average relative abundances of functional bacteria related to the nitrogen cycle and phototrophic bacteria were 45.09% and 7.37% higher than those of the control FQ_CK. The total relative abundances of functional bacteria related to fermentation and decomposition ranged from 7.28% to 11.43%.

The types and effective bacterial concentrations of microbial agents, soil characteristics, planting patterns, different varieties and application techniques all affect the field application effects and sustainability of microbial agents. At present, there are various types of single microbial fertilizers and compound microbial agents in production. The synergy of multiple microorganisms applied in the soil is also an important scientific issue. This study only conducted field experiments on five single microbial agents in the soil of 'Chunli' tomatoes. Whether it has the same effect on other varieties of tomatoes and more than two consecutive years of continuous cropping still needs further study. The persistence of microbial agents in the soil and the relationship between microbial agents and organic fertilizers and chemical fertilizers in plant fruit growth and quality improvement were not involved in this article and will be explored in subsequent experiments.

Conclusions

This study showed that the application of different microbial agents had significant improvement effects on soil physicochemical properties and enzyme activities. At the same time, the composition of the microbial community in the tomato rhizosphere soil changed with the application of microbial agents, and the increase in microbial diversity and the enrichment of beneficial bacteria in the tomato rhizosphere had certain effects on the interaction, stability of the microbial ecological network and resistance to environmental changes. Therefore, in actual production, attention should be paid to maintaining the stability of the tomato rhizosphere soil microecology by applying microbial agents, which may have a certain alleviating effect on continuous cropping obstacles. The findings of this study offered a comprehensive demonstration of the relationship between microbial fertilizers and the diversity of the tomato root microbial community. This was valuable for further understanding the construction process and potential mechanisms of the tomato root microbial community when different microbial fertilizers were used. Additionally, it provided a theoretical foundation for the development of microbial fertilizers, particularly composite microbial fertilizers, for soil improvement. Ultimately, this

research contributed to stabilizing the microecology of root soil.

Acknowledgements

Thanks to everyone who helped with the experiment and the completion of the manuscript.

References

Abdelraouf Ahmed Mohamed Nabil,Hussain Akram Abdelmonem&Naguib

DeyalaM.(2023). Nano-chitosan encapsulated *Pseudomonas fluorescens* greatly reduces *Fusarium* wilt infection in tomato.*Rhizosphere*, **25**,100676.DOI:10.1016/j.rhisph.2023.100676

Abdurashytova, E. R., Melnichuk, T. N., Abdurashytov, S. F., Egovtseva, A. Y., Turin, E. N., & Gongalo, A. A. (2022). Adaptability of the Sorghum Bicolor Rhizosphere Microbiocoenosis Inoculated by Microbial Agents in Southern Chernozem Soils.*Russian Agricultural Sciences*,**48**(3), 212-218. DOI : 10.3103/S1068367422030028

Adomako, M. O., Roiloa, S., & Yu, F. H. (2022). Potential Roles of Soil Microorganisms in Regulating the Effect of Soil Nutrient Heterogeneity on Plant Performance. *Microorganisms*, **10**(12), 2399. DOI:10.3390/microorganisms10122399

Applied Soil Ecology.*AppliedSoilEcology*, **144**: 60-67.DOI: 10.1016/j.apsoil.2019.07.009

BolyenEvan,RideoutJaiRam,DillonMatthewR,BokulichNicholasA,AbnetChristianC,Al-GhalithGabrielA...CaporasoJGregory.(2019).Reproducible,interactive,scalableandextensible microbiomedatascienceusingQIIME2.*Naturebiotechnology*,**37**(8),852-857.DOI : 10.1038/s41587-019-0209-9

Bouček Jiří,Kulháněk Martin,Košnář Zdeněk,Podhorecká Klára,Obergruber

Michal,Hönig Vladimír... Trakal Lukáš.(2023).Is;Bacillus amyloliquefaciens;inoculation effective for the enhancement of soil and plant nutrient status and fruit quality of;Solanum lycopersicum;L. in the presence of composted organic fertilisers?.*Archives of Agronomy and Soil Science*(**2**),182-196. DOI : 10.1080/03650340.2021.1970747

Chen Shifu;Zhou Yanqing;Chen Yaru;Gu Jia(2018). fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, **34**(17), i884-i890. DOI : 10.1093/bioinformatics/bty560

Chen, Y., Zang, H., Bai, L., Lv, C., Chen, X., Li, S., & Dai, J. (2024). Repeated inoculations improve wheat yield through modifying the rhizobacterial communities and nitrogen and phosphorus fractions.*Applied Soil Ecology*,**196**, 105287. DOI :

10.1016/J.APSOIL.2024.105287

CHEND,WANGH,YANGK,etal.(2018). Performance and microbial communities in a combined bioelectrochemical and sulfur autotrophic denitrification system at low temperature.*Chemosphere*, **193**: 337-342. DOI:10.1016/j.chemosphere.2017.11.017

CHENQL,HUHW,YANZZ,LICY,NGUYENBAT,SUNAQ,ZHUYG,HEJZ.(2021).Deterministic s election dominates microbial community assembly in termitemounds.*Soil Biology and Biochemistry*,152:108073.DOI: 10.1016/j.soilbio.2020.108073

DavideFrancioli,SophieQ.vanRijssel,JaspervanRuijven,AadJ.Termorshuizen,T.E.AnneCotton,AlexJ.Dumbrell...LiesjeMommer.(2020).Plant functional group drives the community structure of saprophytic fungi in a grassland biodiversity experiment.*Plantand Soil*,**461**, 91–105.DOI : 10.1007/s11104-020-04454-y

EPELDE L,LANZEN A,BLANCO F, Urich T, Garbisu C.(2015). Adaptation of soil microbial community structure and function to chronic metal contamination at an abandoned Pb-Zn mine.*FEMS Microbiology Ecology*,**91** (1) : 1-11. DOI:10.1093/femsec/fiu007

Feng, C., Yi, Z., Qian, W., Liu, H., & Jiang, X. (2023). Rotations improve the diversity of rhizosphere soil bacterial communities, enzyme activities and tomato yield.*PloS one*,**18**(1), e0270944. DOI:10.1371/journal.pone.0270944

FengDL, WuZC, XuSH. (2008). Nitrification of human urine for its stabilization and nutrient recycling.*BioresourTechnol*, **99** (14) : 6299-6304.DOI: 10.1016/j.biortech.2007.12.007

Fu H, Zhang G, Zhang F, Sun Z, Geng G, Li T.(2017). Effects of Continuous Tomato Monoculture on Soil Microbial Properties and Enzyme Activities in a Solar Greenhouse.*Sustainability*.**9**(2):317. DOI:10.3390/su9020317

García-LópezM, Meier-KolthoffJP, TindallBJ, etal.(2019). Analysis of 1,000 Type-Strain Genomes Improves Taxonomic Classification of Bacteroidetes.*Front Microbiol*, **10**: 2083.DOI: 10.3389/fmicb.2019.02083

GLICKBR(2012).Plantgrowth-promotingbacteria:mechanismsandapplications [J].*Scientifica*, **2012**:963401. DOI:10.6064/2012/963401

GuoW,AndersenMN,QiXB,LiP,LiZY,FanXY,ZhouY.(2017). Effects of reclaimed water irrigation and nitrogen fertilization on the chemical properties and microbial community of soil . *Journal of Integrative Agriculture*, **16**(3):679-690. DOI:10.1016/S2095-3119(16)61391-6

- 496 **GuoYH,GongHL,GuoXY.(2015).** Rhizosphere bacterial community of Typha angustifolia L.
497 and water quality in a river wetland supplied with reclaimed water. Applied Microbiology and
498 Biotechnology, **99**(6):2883-2893. DOI:10.1007/s00253-014-6182-9
- 499 **Hemkemeyer, M., Schwalb, S. A., Heinze, S., Joergensen, R. G., & Wichern, F. (2021).**
500 Functions of elements in soil microorganisms.Microbiological research,252, 126832.
501 DOI:10.1016/j.micres.2021.126832
- 502 **HenningSM,YangJP,ShaoP,etal.(2017).**Health benefit of vegetable / fruitjuice-baseddiet:role
503 of microbiome.Scientific Reports, **7**,2167, DOI:10.1038/s41598-017-02200-6
- 504 **HOUL,ZHOUQ,WUQ,etal.(2018).**Spatiotemporal changes in bacterial community and
505 microbial activity in a full-scale drinking watertreat-mentplant[J]. Science of The Total
506 Environment,**625**:449-459. DOI:10.1016/j.scitotenv.2017.12.301
- 507 **Ji, C., Liang, Z., Cao, H., Chen, Z., Kong, X., Xin, Z., ... & Li, K. (2023).** Transcriptome-
508 based analysis of the effects of compound microbial agents on gene expression in wheat roots
509 and leaves under salt stress.*Frontiers in Plant Science*,**14**, 1109077-1109077.
510 DOI:10.3389/fpls.2023.1109077
- 511 **Jia,Y.,Niu,H.,Zhao,P.,Li,X.,Yan,F.,Wang,C.,&Qiu,Z.(2023).** Synergistic biocontrol of
512 Bacillus subtilis and Pseudomonas fluorescens against early blight disease in tomato.Applied
513 Microbiology and Biotechnology,**107**(19),6071-6083. DOI:10.1007/s00253-023-12642-w
- 514 **Jiancai Xiao,Meiyu Cao,Kunyang Lai,Kai Sun,Lin Zhang,Pengchao Gao... Lanping**
515 **Guo.(2024).**Unveiling key metabolic pathways in Bacillus subtilis-mediated salt tolerance
516 enhancement in Glycyrrhiza uralensis Fisch. through multi-omics analysis.Environmental and
517 Experimental Botany,**219**,105631.DOI : 10.1016/J.ENVEXPBOT.2023.105631
- 518 **Jiang, X., Fang, W., Tong, J., Liu, S., Wu, H., & Shi, J. (2022).** Metarhizium robertsii as a
519 promising microbial agent for rice in situ cadmium reduction and plant growth
520 promotion.*Chemosphere*,**305**, 135427-135427. DOI: 10.1016/j.chemosphere.2022.135427
- 521 **Jing Zhou, Dawei Guan, Baoku Zhou, Baisuo Zhao, Mingchao Ma, Jie Qin, Xin Jiang,**
522 **Sanfeng Chen, Fengming Cao, Delong Shen, Jun Li.(2015).** Influence of 34-years of
523 fertilization on bacterial communities in an intensively cultivated black soil in northeast China
524 .SoilBiologyandBiochemistry,**90**: 42-51. DOI:10.1016/j.soilbio.2015.07.005
- 525 **Lauber CLHamady M, Knight R, Fierer N.(2009).**Pyrosequencing-Based Assessment of Soil

pH as a Predictor of Soil Bacterial Community Structure at the Continental Scale. Appl Environ Microbiol, **75**(15): 5111-5120. DOI:10.1128/AEM.00335-09

Leticia L. de Paula, Vicente P. Campos, Willian C. Terra, Daniele de Brum, Dustin C. Jacobs, Hung Xuan Bui Johan Desaegeer.(2024).The combination of *Bacillus amyloliquefaciens* and *Purpureocillium lilacinum* in the control of *Meloidogyne enterolobii*. Biological Control, **189**, 105438. DOI : 10.1016/J.BIOCONTROL.2023.105438

Li, D., Duan, W., Guo, H., Zong, J., Chen, J., & Wang, J. (2024). High-nitrogen organic fertilizer promotes the growth of bermuda grass (*Cynodon dactylon*), zoysiagrass (*Zoysia japonica*) and paspalum grass (*Paspalum vaginatum*) by enhancing nitrogen use efficiency associated with *Bacillus*-stimulated bacterial community. *Scientia Horticulturae*, **329**, 113027. DOI:10.1016/j.scienta.2024.113027

Liu, H., Xiong, W., Zhang, R., Hang, X., Wang, D., Li, R., & Shen, Q. (2018). Continuous application of different organic additives can suppress tomato disease by inducing the healthy rhizospheric microbiota through alterations to the bulk soil microflora. *Plant and soil*, **423**, 229-240. DOI : 10.1007/s11104-017-3504-6

Louca S, Parfrey LW, Doebeli M. (2016). Decoupling function and taxonomy in the global ocean microbiome. *Science*, **353**, 1272-1277. DOI:10.1126/science.aaf4507

Luo, G., Wang, T., Li, K., Li, L., Zhang, J., Guo, S., Ling, N., & Shen, Q. (2019). Historical Nitrogen Deposition and Straw Addition Facilitate the Resistance of Soil Multifunctionality to Drying-Wetting Cycles. *Applied and environmental microbiology*, **85**(8), e02251-18. DOI:10.1128/AEM.02251-18

Oksana Coban, GERLINDE B. DE DEYN, MARTINE VAN DER PLOEG. (2022). Soil microbiota as game-changers in restoration of degraded lands. *Science*, **375**, 6584. DOI:10.1126/science.abe0725

Qiong Huang, Jianlei Wang, Cong Wang, Qing Wang. (2019). The 19-years inorganic fertilization increased bacterial diversity and altered bacterial community composition and potential functions in a paddy soil,

Sadaf KALAM, Anirban BASU & Appa Rao PODILE. (2022). Difficult-to-culture bacteria in the rhizosphere: The underexplored signature microbial groups. *Pedosphere*, **32**(01), 75-89. DOI:10.1016/S1002-0160(21)60062-0

- 556 **SathyaA, Vijayabharathi R, GopalakrishnanS. (2017). Plant growth-promoting**
- 557 **actinobacteria: a new** strategy for enhancing sustainable production and protection of grain
- 558 **legumes. 3Biotech, 7(2), DOI:10.1007/s13205-017-0736-3**
- 559 **Schmalenberger, A., O’Sullivan, O., Gahan, J., Cotter, P. D., & Courtney, R. (2013).**
- 560 **Bacterial communities established in bauxite residues with different restoration histories.**
- 561 **Environmental science & technology, 47(13), 7110-7119.DOI: 10.1021/es401124w**
- 562 **Sedhupathi Kamaraj,Kennedy Zackaria John,Shanthi Annaian,Akiyama K.,Matsuzaki**
- 563 **K.I.,Hayashi H.... Zhou Y..(2022).Interaction of arbuscular fungus (Rhizophagus irregularis),**
- 564 **Bacillus subtilis and Purpureocillium lilacinum against root-knot nematode (Meloidogyne**
- 565 **incognita) in tomato.Indian Journal of Nematology(1),92-103. DOI : 10.5958/0974-**
- 566 **4444.2022.00013.0**
- 567 **Shalaby Tarek A.,Taha Naglaa,ElBeltagi Hossam S. ElRamady Hassan.(2022).Combined**
- 568 **Application of Trichoderma harzianum and Paclobutrazol to Control Root Rot Disease Caused**
- 569 **by Rhizoctonia solani of Tomato Seedlings.Agronomy(12),3186-3186. DOI :**
- 570 **10.3390/AGRONOMY12123186**
- 571 **Singh N, Siddiqui ZA (2015) Effects of *Bacillus subtilis*,*Pseudomonas fluorescens* and**
- 572 ***Aspergillus awamori* on the wilt-leaf spot disease complex of tomato. Phytoparasitica 43:61–**
- 573 **75.DOI:10.1007/s12600-014-0427-0**
- 574 **Su Lv,Li Huatai,Wang Jing,Gao Wenting,Shu Xia,Sun Xiting... Shen**
- 575 **Biao.(2023).Composition, function and succession of bacterial communities in the tomato**
- 576 **rhizosphere during continuous cropping.Biology and Fertility of Soils 59, (7),723-732.**
- 577 **DOI:10.1007/s00374-023-01731-7**
- 578 **Su, L., Li, H., Wang, J., Gao, W., Shu, X.X., Sun, X., Wang, K., Duan, Y., Liu, Y.,**
- 579 **Kuramae, E.E., Zhang, R., & Shen, B. (2023). Composition, function and succession of**
- 580 **bacterial communities in the tomato rhizosphere during continuous cropping.*Biology and***
- 581 ***Fertility of Soils*, 59, 723 - 732. DOI:10.1007/s00374-023-01731-7**
- 582 **Wang,C.,Zhao,D.,Qi,G.,Mao,Z.,Hu,X.,Du,B.,...&Ding,Y.(2020).Effects of *Bacillus velezensis***
- 583 **FKM10 for promoting the growth of *Malushupehensis* Rehd.and inhibiting *Fusarium***
- 584 ***verticillioides*.*Frontiers in microbiology*,10,2889. DOI: 10.3389/fmicb.2019.02889**
- 585 **Wang,W.J.;Su,D.X.;Qiu,L.;Wang,H.Y.;An,J.;Zheng,G.Y.(2013).Concurrent changes in soil**

inorganic and organic carbon during the development of larch, *Larixgmelinii*, plant ations and their effects on soil physicochemical properties. *Environ. Earth Sci.* **69**, 1559–1570. DOI:10.1007/s12665-012-1990-7

Xiao, Z., Lu, C., Wu, Z., Li, X., Ding, K., Zhu, Z., Han, R., Zhao, J., Ge, T., Li, G., & Zhu, Y. G. (2024). Continuous cropping disorders of eggplants (*Solanum melongena* L.) and tomatoes (*Solanum lycopersicum* L.) in suburban agriculture: Microbial structure and assembly processes. *The Science of the total environment*, **909**, 168558. DOI:10.1016/j.scitotenv.2023.168558

XiaoqianGong, ScottJarvie, JiaWen, NierSu, YongzhiYan, QingfuLiuQingZhang.(2024). Compared with soil fungal diversity and microbial network complexity, soil bacterial diversity drives soil multifunctionality during the restoration process. *Journal of environmental management* **1,354**, 20379-120379. DOI:10.1016/J.JENVMAN.2024.120379

Xu, Z., Yu, G., Zhang, X., Ge, J., He, N., Wang, Q., & Wang, D. (2015). The variations in soil microbial communities, enzyme activities and their relationships with soil organic matter decomposition along the northern slope of Changbai Mountain. *Applied Soil Ecology*, **86**, 19-29. DOI:10.1016/j.apsoil.2014.09.015

Yang L, Shi Y, Yang X, Dong L, Lei F, Zheng C, Xie A, Zhang D, Sun L, Sun X. (2023). Analysis of Growth and Rhizosphere Soil Changes of Herbaceous Peony Treated with a Compound Microbial Agent under Contrasted Soil Conditions. *Horticulturae*. **9**(5):602. DOI:10.3390/horticulturae9050602

Yang Xu, Guanchu Zhang, Hong Ding, Dunwei Ci, Liangxiang Dai & Zhimeng Zhang. (2020). Influence of salt stress on the rhizosphere soil bacterial community structure and growth performance of groundnut (*Arachis hypogaea* L.). *International Microbiology* , **23**(3):453-465. DOI:10.1007/s10123-020-00118-0

YuJingjing, CongWei, DingYi, JinLixiao, CongJingZhangYuguang.(2022). Interkingdom Plant–Soil Microbial Ecological Network Analysis under Different Anthropogenic Impacts in a Tropical Rainforest. *Forests*. **13**(8), 1167. DOI : 10.3390/F13081167

Zechen Gu, Chengji Hu, Yuxin Gan, Jinyan Zhou, Guangli Tian Limin Gao.(2024). Role of Microbes in Alleviating Crop Drought Stress: A Review. *Plants (Basel, Switzerland)* **13**(3), 384, DOI : 10.3390/PLANTS13030384

Figure 1

Fig.1 Effects of different microbial agents on soil physicochemical properties and enzyme activities of Tomato

Note: Different lowercase letters above the bars indicate significant differences between treatments ($P < 0.05$).

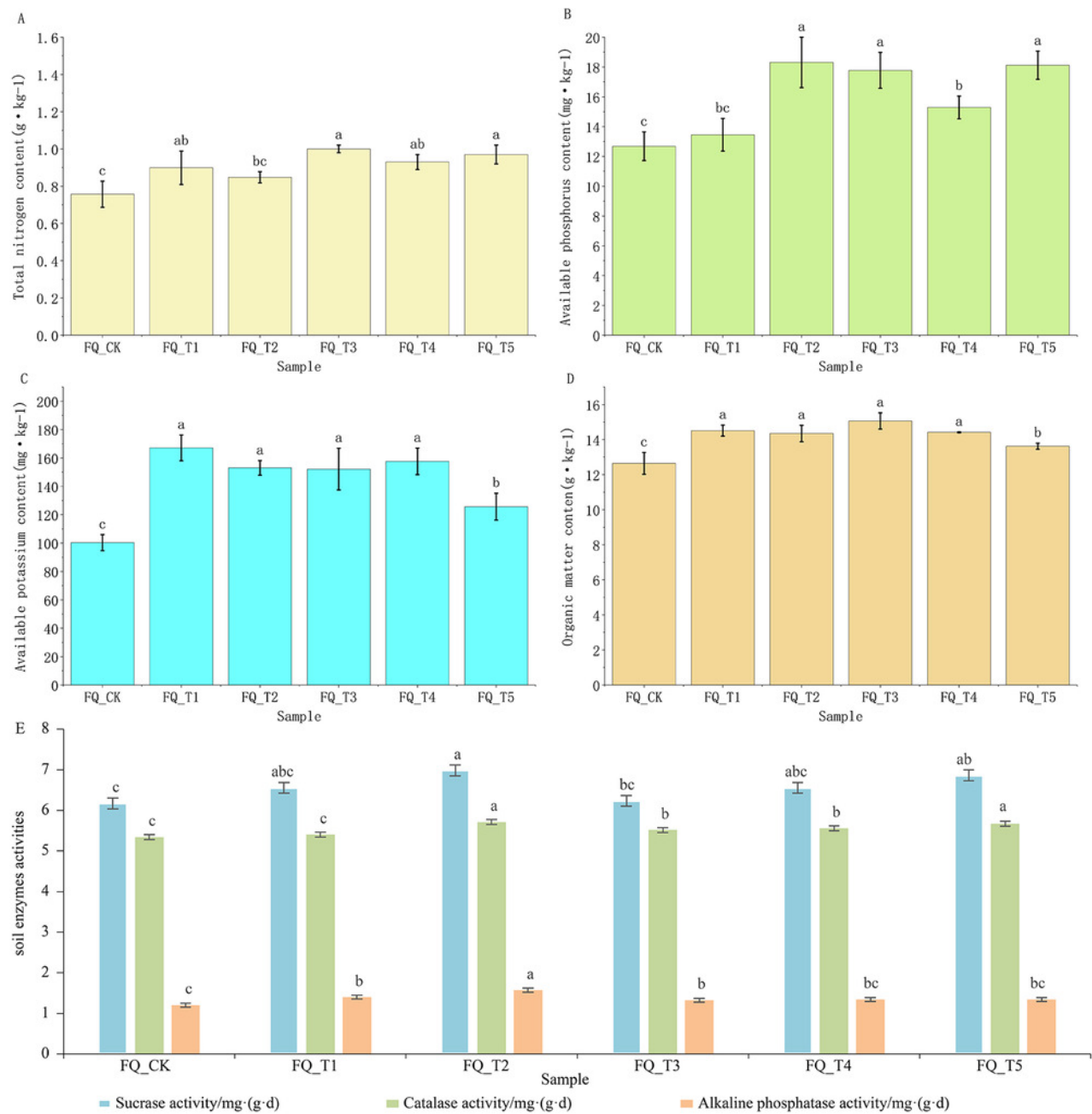


Figure 2

Fig.2 Rarefaction curve

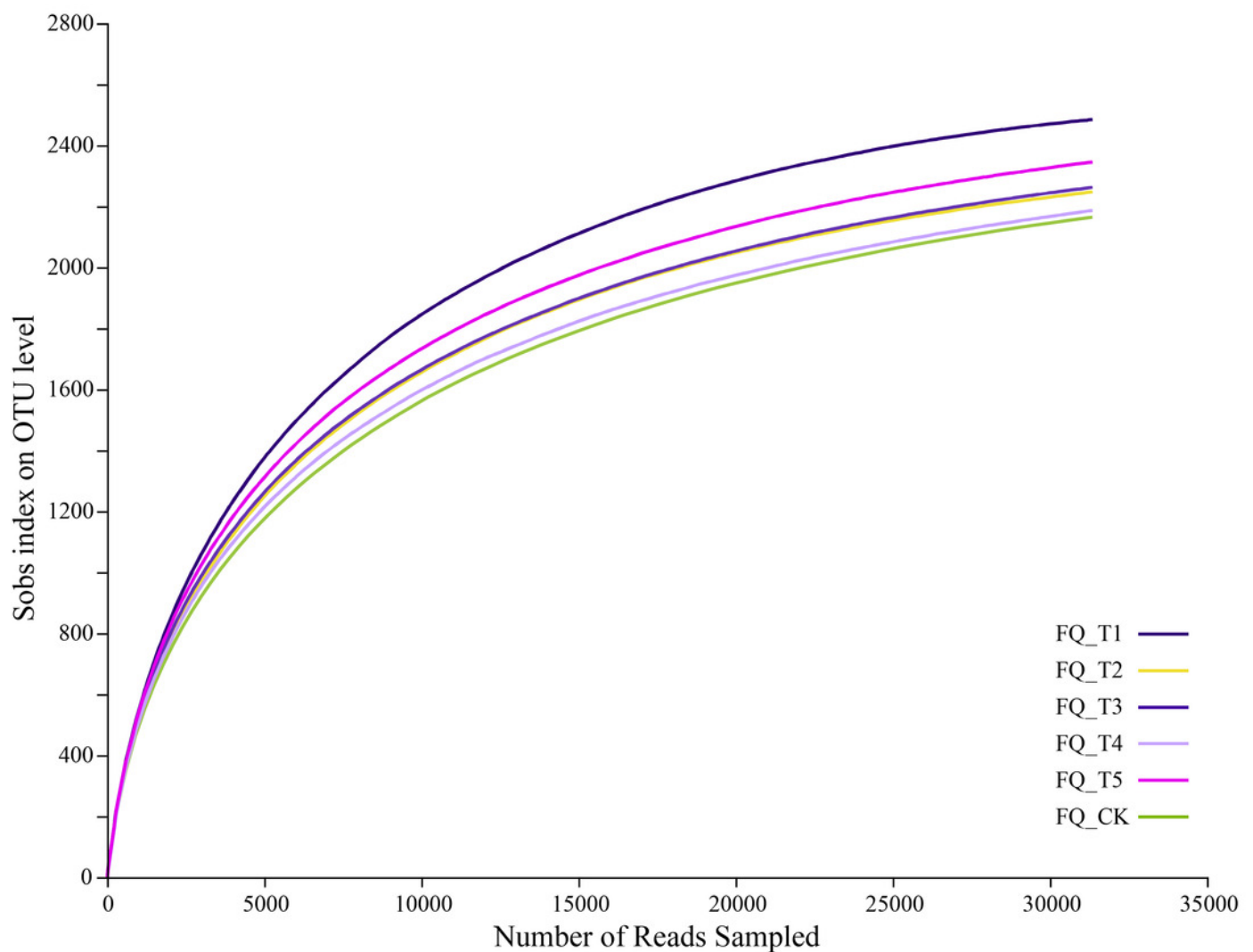


Figure 3

Fig.3 Relative abundance under different treatments in phylum level

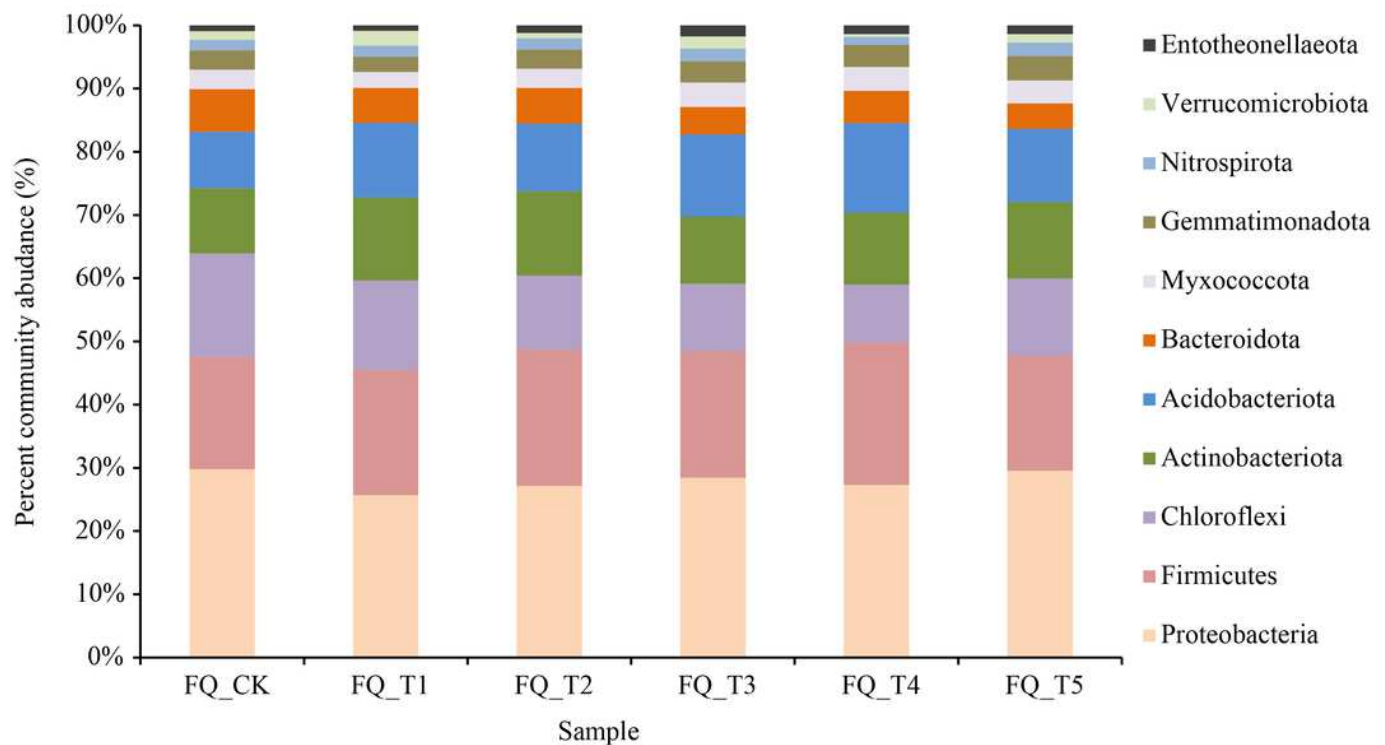


Figure 4

Fig.4 Heatmap of Mantel Test network analysis between tomato rhizosphere bacterial community and environmental factors

Note: Mantel test heatmap: The lines in the graph represent the correlation between communities and environmental factors, while the heatmap represents the correlation between environmental factors; Line thickness: The correlation between community and environmental factors, plotted using Mantel's r (absolute value of R); Relationship: Positive and Negative are positive and negative correlations between communities and environmental factors; In the heat map, different colors represent positive and negative correlations, color depth represents the magnitude of positive and negative correlations, and the asterisk in the color block represents significance, * $0.01 < P \leq 0.05$.

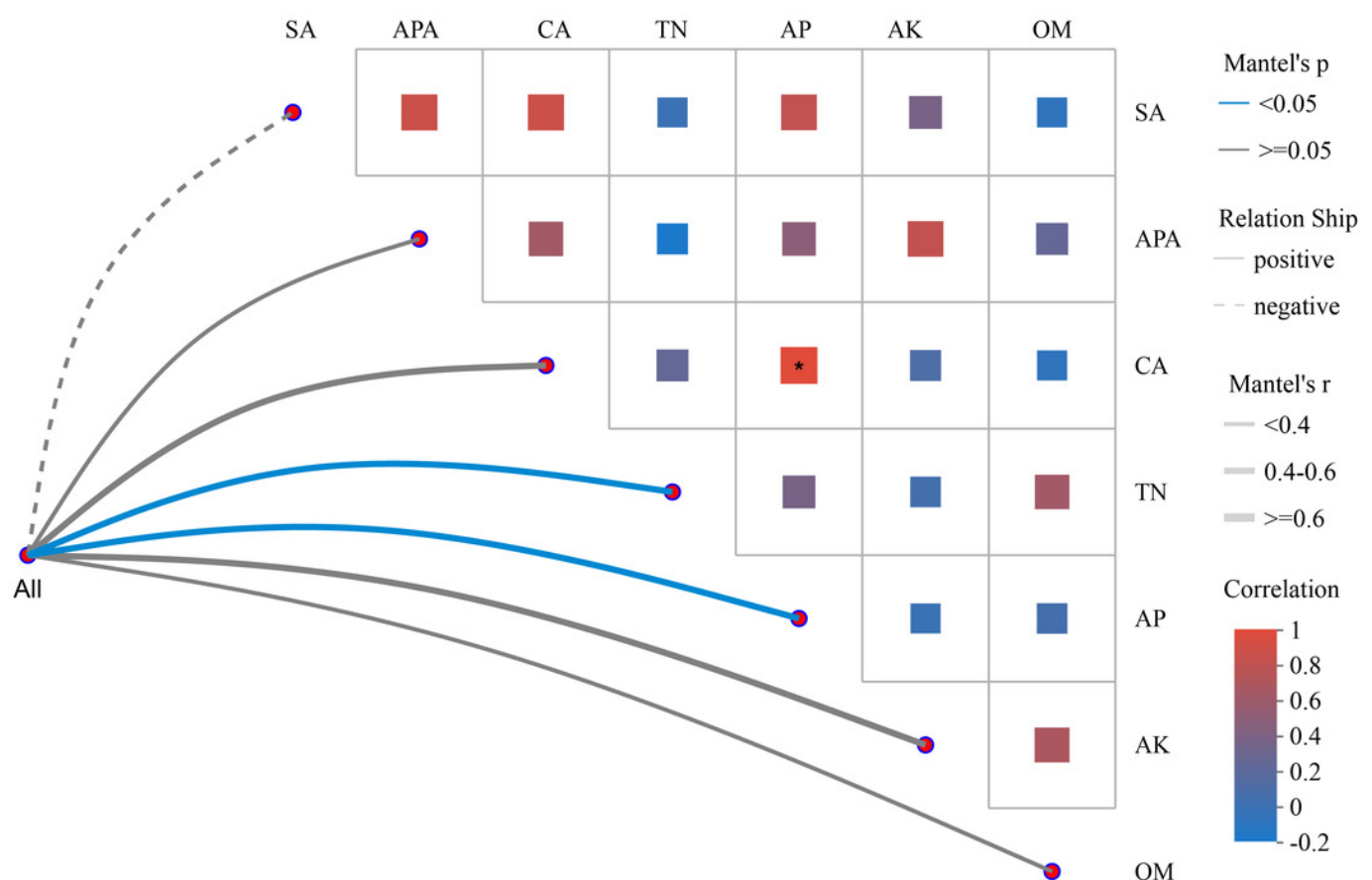


Figure 5

Fig.5 Analysis of Faporax functional prediction of tomato rhizosphere soil microorganisms

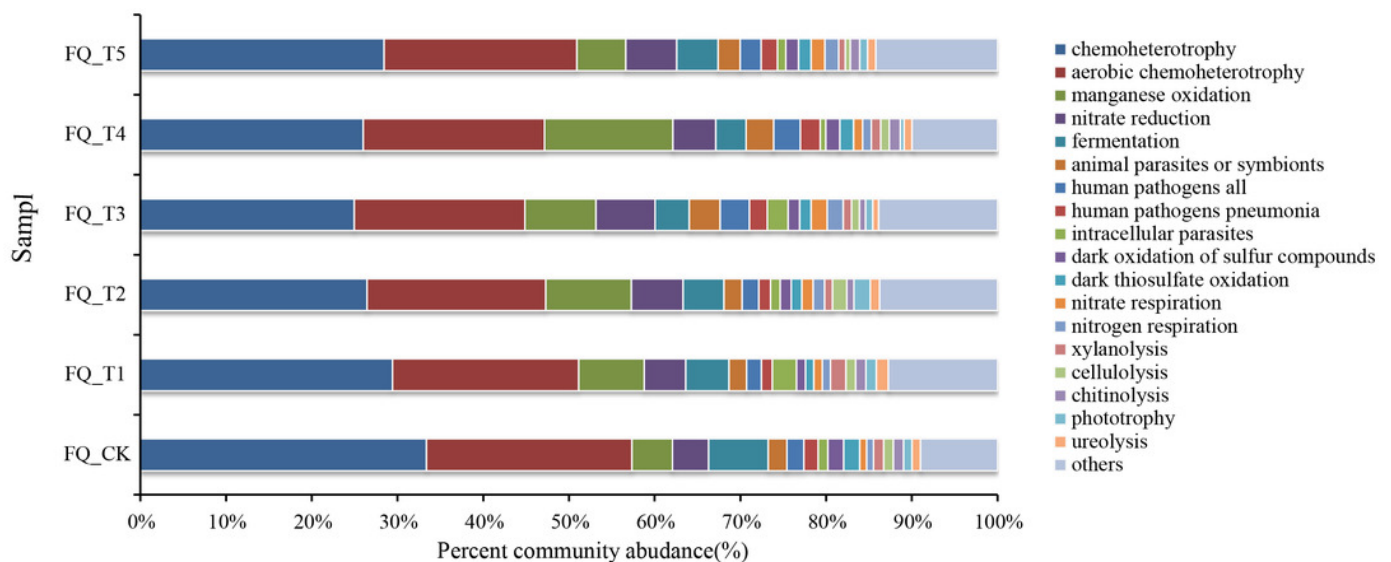


Table 1(on next page)

Table 1 Alpha diversity index of microbial community in rhizosphere soil of tomato

Note: Different lowercase letters indicate significant differences between treatments (P<0.05).

Table 1 Alpha diversity index of microbial community in rhizosphere soil of tomato

Sample	ACE index	Chao1 index	Shannon index	Simpson index	Sobs index	Cove
FQ_CK	2449.07±89.23c	2420.22±63.12b	6.49±0.83a	0.0036±0.0024a	2164±57.53c	
FQ_T1	2646.10±16.55a	2578.20±34.07a	6.70±0.26a	0.0035±0.0020a	2484±79.61a	
FQ_T2	2475.28±19.20c	2461.75±14.95ab	6.51±0.82a	0.0054±0.0051a	2247±6.94bc	
FQ_T3	2521.11±33.80bc	2517.96±39.22ab	6.59±0.22a	0.0039±0.0012a	2262±88.43bc	
FQ_T4	2478.76±60.89c	2475.56±92.17ab	6.42±0.20a	0.0078±0.0038a	2186±72.55c	
FQ_T5	2586.71±41.64ab	2564.73±79.42a	6.68±0.75a	0.0031±0.0011a	2345±83.56b	

Note: Different lowercase letters indicate significant differences between treatments (P<0.05).