

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

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Abstract

Background—The purpose of this study was to study determine 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, to a certain extent. It had a positive impact on microbial structure and function, improved the

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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

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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~~As intensive production ~~increases~~ and ~~the development of~~ facility agriculture ~~develops~~, continuous cropping obstacles such as serious soil-borne diseases have become increasingly prominent ~~and~~, resulting in reduced quality and yield, which seriously restricts the sustainable development of ~~the~~ 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 ~~conducted~~ by Fu *et al.* (2017) 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 (AK) and soil microbial community functional diversity index.

65 Microbial inoculants are ~~microbial inoculants~~those based on one or more functional or
 66 bacterial strains applied to the soil as a substitute for traditional inorganic fertilizers
 67 (-bio-fertilizers-), which can ~~exert biological control of pests and diseases (Ji. et al., 2023), or be~~
 68 used for bioremediation (Yang et al., 2023), and enhancement of soil properties (Jiang et al.,
 69 2022), and plant resistance (Xiao et al., 2024). While some soil inoculants (e.g., rhizobia) have a
 70 long and fruitful history of use (Abdurashytova et al., 2022), other soil inoculants have not
 71 performed consistently in the field (Chen et al., 2024;). (Letícia Lde Paula et al., 2024). Many
 72 scholars have learned about Bacillus amyloliquefaciens (Bouček Jiří et al., 2023), Bacillus subtilis
 73 Penicillium purpureum (Sedhupathi et al., 2022), Trichoderma harzianum (Shalaby Tarek A et al.,
 74 2022), and Bacillus amyloliquefaciens (Liu et al., 2018) through inoculation. The results show
 75 that it has a certain effect on improving tomato fruit quality, reducing tomato root knot
 76 nematodes, reducing seedling wilt disease, and inhibiting tomato disease occurrence Bouček Jiří
 77 et al. (Bouček Jiří et al., 2023) found that inoculation of tomato plants with Bacillus
 78 amyloliquefaciens had a favorable effect on fruit quality; Kamaraj S et al. (Sedhupathi et al.,
 79 2022) concluded that the fungi of the cushion mycorrhizal fungus (Rhizobium irregularis) and
 80 the biocontrol agents (BCA), Bacillus subtilis, and Penicillium violetum, the Biocontrol agents
 81 (BCA) were able to produce extracellular proteases and form maximal biofilms, which
 82 significantly reduced the number of egg masses and root knot index against tomato root knot
 83 nematodes; Shalaby Tarek A et al. (Shalaby Tarek A et al., 2022) found that Trichoderma
 84 harzianum in combination with polybutazole (PBZ) had a significant biochemical control of
 85 Trichoderma verticillioides in tomato seedlings. Liu et al. (Liu et al., 2018) found that Bacillus
 86 amyloliquefaciens SQR9 and amino acid-containing organic fertilizer (chicken manure)
 87 inhibited tomato diseases by changing the composition of rhizosphere bacterial communities. A
 88 more accurate understanding of the ecology and mode of action of inoculant strains is key to
 89 optimizing their efficacy and guiding their targeted use to address key constraints to crop
 90 production (Gu et al., 2024).

91 At present, there are few studies on the characteristics and function prediction of bacterial
 92 community in rhizosphere soil of tomato under continuous cropping for two years under and
 93 different microbial agents, which is very important for predicting the role of rhizosphere
 94 microorganisms in regulating plant-microorganism ecosystem function. This study focuses on
 95 tomatoes that have been continuously cultivated for two years. Different microbial agents were

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used, and 16S rRNA and ITS high-throughput sequencing techniques were employed to investigate the effects of microbial agents on the physicochemical properties and bacterial community composition of tomato rhizosphere soil. The aim was to explore the impact of several different microbial agents on the rhizosphere soil conditions of tomatoes that have been continuously cultivated for two years. This study helps to reveal the effects of different microbial agents on the rhizosphere soil layer of tomatoes, evaluate the practical application effects of different carrier agents in tomato agricultural production after two years of continuous cropping, provide theoretical support for the application of microbial agents in tomato continuous cropping obstacle reduction measures, and serve as a preliminary test screening, providing a good starting point for further exploring the screening of excellent strains and the preparation of subsequent microbial agents. In this study, tomatoes with different microbial agents for across 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 using 16S rRNA and ITS high throughput sequencing technology, in order to explore the mechanism of rhizosphere bacterial community construction. In a word, the This research is helpful to reveal in determining the soil ecological stability mechanism of microbial agents for promoting growth and disease resistance, and to evaluating the practical application effect of different carrier agents in agricultural production, so as to and providing theoretical support for the sustainable development of the tomato industry.

Materials & Methods

Test materials and test site

This experiment was conducted from between January 19, 2023 and 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 "Chun Li" 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 (OM) 13.28g·kg⁻¹, total nitrogen (TN) 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* (Biovox Razen, wettable powder, effective live bacterial count was 1.00×10⁹ CFU·mL⁻¹); FQ_T2: *Trichoderma harzianum* (Biovox Rootshield, wettable powder, effective live bacterial count was 3.00×10⁷ CFU·mL⁻¹); FQ_T3:

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You can format it into 3 sentences

However, you can't conclude your introduction in this manner. You can add the study hypothesis and I expected you to add how this study contributes to scientific knowledge.

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127 *Bacillus amyloliquefaciens* (Shanxi Xiannong Biotechnology Co. wettable powder, the effective
 128 number of live bacteria ~~was~~ 1.00×10^9 CFU·mL⁻¹); FQ_T4: ~~verticillium~~ *chlamydosporium* sp.
 129 (~~Yunnan Microstructure Source, micronutrient~~), the effective number of live bacteria ~~was~~
 130 2.50×10^8 CFU·mL⁻¹); FQ_T5: *Paecilomyces lilacinus* (~~Chongqing micro-nuclear biological~~
 131 ~~purple warrior, wettable powder~~), the effective number of live bacteria ~~was~~ 1.00×10^8 CFU·mL⁻¹);
 132 Data were collected as previously described in [Wei \(2024\)](#). Five microbial fungicides were
 133 purchased from the biological plant protection station of the Fruit Yau Agricultural Resources
 134 and Beihai Qunlin biological factory store; The test was conducted in a plastic greenhouse with
 135 double-layer thermal insulation cultivation facilities—cultivation; The greenhouse was set up
 136 with a control (FQ_CK) and five microbial agents treatments (FQ_T1, FQ_T2, FQ_T3, FQ_T4,
 137 FQ_T5); pPlanted on March 20, April 20, and May 19 via inter-root furrow application of five
 138 agents (dosage of 150 times the liquid, each time 100 mL per plant); control conventional
 139 management, no agents. Each experimental plot covered an area of 50 m², and experiments
 140 were repeated three times, randomly arranged, and the field management measures were the
 141 same as those of local high-yield greenhouses ([Wei et al., 2024](#)).

142 Soil sample collection

143 Soil samples were collected on June 27th, 2023.

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

153 Determination of soil physicochemical properties and enzyme activities

154 Soil organic matter (OM) content was titrated using the by redox method after high temperature
 155 digestion; —Total nitrogen (TN) content was titrated by acid standard solution using the semi-trace
 156 Kjeldahl method; alkali nitrogen content was titrated by acid standard solution using the alkali
 157 diffusion method; available phosphorus (AP) content was extracted by 0.5 mol·L⁻¹ NaHCO₃ and

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capital letter.

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with the font, font size used in the manuscript

Comment [AA15]: You are mixing a lot of
information together. From what I observed
here, you are discussing your results in
methodology when you supposed to explain
how you conducted your research starting from
soil sampling, how you isolate your microbial
isolates (pour plate or spread plate), DNA
extraction from the microbial isolates,
sequencing, and data and statistical analysis.
I implore you to go over this section again
removing all result and discussion.

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soil sample at this temperature for DNA
extraction.
Moreover, did you carried out extraction of
DNA from soil samples directly or you isolated
bacteria and fungi from the soil before DNA
extraction from the isolates

158 then determined ~~using the~~by molybdenum antimony antimonimony ~~anti~~-colorimetric method ([Xu](#)
159 [et al., 2001](#)), and then detected by TU-1810 UV-Vis Spectrophotometer (Beijing Pudian General
160 Instrument Co., Ltd., [Beijing, China](#)); and quick-acting potassium content was detected by
161 TU-1810 UV-Vis Spectrophotometer. The content of ~~available~~potassium~~AK~~ was detected by
162 CH₃COONH₄ extraction and then ~~detected by~~BWB-1 flame spectrophotometer (BWB_
163 Technologies, [Newbury, UK](#)) ([Wei et al., 2024](#)).

164 Soil sucrase was determined ~~by using~~the colorimetric method of 3,5-dinitrosalicylic acid;
165 soil catalase was determined ~~using~~by the titrimetric method of potassium permanganate; ~~and~~;
166 alkaline phosphatase was determined ~~by using~~the Sodium Benzene Phosphate colorimetric
167 method. All ~~the~~ steps were performed by the China Rice Research Institute according to the
168 instructions of the corresponding enzyme activity kits provided by Suzhou Keming
169 Biotechnology Co. Ltd. [Jiangsu, China](#), and the samples were colorimetrically determined using
170 a multifunctional enzyme marker (TECAN-Spark 20M) ([Wei et al., 2024](#)).

171 **Analysis of microbial diversity measurements of rhizosphere soils of tomatoes**

172 High-throughput sequencing analyses were performed ~~by using the~~ Shanghai Meiji
173 Biomedical Technology Co. Ltd. ~~the~~ labelled bacterial universal primer 338F
174 (5'-ACTCCTACGGGAGGCAGCA-3')-806R (5'-GGACTACHVGGGTWCTTAAT-3') to
175 amplify the soil bacterial ~~16S~~rRNA gene region. Afterwards, the raw data were quality filtered
176 and merged on the Illumina MiSeq platform (Illumina, [San Diego, CA, USA](#)) ([Chen et al.,](#)
177 [2018](#)), according to the standard method provided by Majorbio ~~((Majorbio-Shanghai, China))~~,
178 and then clustered at 97% similarity level to obtain the ~~OTU~~(operational taxonomic unit ([OTU](#))
179 ([DeveFrancioli et al., 2020](#)). Species annotation were performed on the OTU sequences
180 ([BolyenEvan et al., 2019](#)) and the community composition, alpha diversity, and relative
181 abundance of the samples were counted at each taxonomic level, while heatmap, Mantel Test
182 network analyses, ~~and Faprotax-FAPROTAX~~ function prediction analysis were performed within
183 the platform on the correlation between the soil environmental factors and microbial
184 communities to determine the effects of soil environmental factors on the composition of
185 microbial communities([Gu et al., 2024](#)).

186 **Data processing and analysis of high-throughput sequencing results**

187 Trimmomatic software was used for quality control of the sequenced raw sequences, and
188 FLASH1.2.11 software was used for double-ended sequence splicing, and UPARSE7.1 software

Comment [AA18]: In case you extracted your DNA samples from the soil, how did you differentiate DNA to be used for 16S rRNA from ITS when sequencing? Although, you did not mention ITS (18s) here, but I could see *Verticillium*, *Paecilomyces lilacinus*, *Chlamydosporium* spp in your write up which are fungi

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 performance of 16S gene function prediction analysis (COG database).

The relevant indicators are represented by the mean and standard error (SEM) of three biological replicates. The data were statistically analyzed using Excel 2007 and SPSS Statistics 2026.0 software (IBM, Inc., Armonk, NY, USA), and the soil microbial diversity analysis and visual mapping were performed using Origin2019b and MajorBIO cloud platform (www.majorbio.com, Shanghai, China), 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'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 (AK) and organic matter (OM) 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 (AK) 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 (OM) was 13.82% higher than with the control FQ CK treatment. Except for the FQ T2 treatment, there was a significant difference in total nitrogen (TN) content between tomato rhizosphere soil treated with microbial agents and the control FQ CK treatment. The total nitrogen (TN) 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 (AP) content of tomato rhizosphere soil after microbial agent treatment compared to the control FQ CK treatment. The available phosphorus (AP) content of tomato rhizosphere soil treated with microbial agent was on average 30.81% higher than that of the control FQ CK treatment.

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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 (CA) 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 (APA) 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, respectively, 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 overall beneficial to improving 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, representing indicating 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 plateaued after 25,000 sequences, suggesting an adequate supply of sequencing data and reliable outcomes, which was 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

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

258 | **Analysis of soil bacterial community structure after treatment with different microbial** 259 | **agents**

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

262 | Rhizosphere soil samples contained bacteria from 40 phyla, 130 classes, 309 orders, 490
263 | families, and 877 genera, totaling 1,598 species. As illustrated in Fig. 3, at the phylum level, 11
264 | rhizosphere soil bacterial phylum exhibited relatively high abundances (>1%), with

265 | *Proteobacteria* and *Firmicutes* as the predominant phyla, averaging 44.55% across all
266 | treatments. In comparison to the FQ_CK treatment, the average relative abundance of
267 | *Proteobacteria* across the five microbial treatments decreased by 2.42%, with the FQ_T1
268 | treatment showing the largest reduction at 14.61%. The FQ_CK treatment displayed a higher

269 | relative abundance of *Proteobacteria* at 28.03%. Relative to the FQ_CK treatment, the average
270 | relative abundance of *Firmicutes* across the five microbial treatments increased by 2.22%, with
271 | the FQ_T4 treatment showing the largest increase at 4.25%. In comparison to the FQ_CK
272 | treatment, the relative abundances of *Chloroflexi* and *Bacteroideta* across the five microbial
273 | treatments decreased by an average of 4.69% and 1.79%, respectively. Meanwhile, the relative
274 | abundances of *Actinobacteria* and *Acidobacteria* increased by an average of 1.54% and 2.94%,

275 | respectively. Compared with the FQ_CK treatment, the relative abundance changes of
276 | *Myxococcata*, *Gemmatimonadota*, *Nitrospira*, *Verrucomicrobiota*, and *Entotheonellaeota* treated
277 | with five bacterial agents were slightly different, but the overall results showed an average
278 | increase of 0.21%, 0.16%, 0.05%, 0.02%, and 0.34%, respectively.

279 | Fig_S1 shows the dominant genus with relative abundance>1% at the genus level and
280 | ranking among the top 10. The 10 genera accounted for 27.03% on average among the
281 | treatments. Among them, *Bacillus* and *Paenisporsarcina* were the top two most dominant

genera with relative abundance of top 2, and the unnamed genera accounted for 42.97% of the 10 genera. Compared with the 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 the 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 that, which is commonly used in microbiomes to analyze the correlation between environmental factors and microbial community structure.—

In Fig. 4, Mantel 'r' (the absolute value of R (Mantel 'r')) was used to plot the correlation between the bacterial community and environmental factors in tomato rhizosphere soil. The Mantel 'r' (absolute value of R (Mantel 'r')) of the 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, and 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, and 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 (SA)). There were different correlations among the seven environmental factors, and only there was a significant positive correlations 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, and fermentation, etc., covering more than 4,600 different prokaryotic species. It has

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313 | good predictive effects on the biochemical cycle processes of environmental samples (LoucaS et
314 | al., 2016). At present, many scholars have used FAPROTAX to carry out functional prediction
315 | of microorganisms (Li et al., 2024). This study also used FAPROTAX to analyze and predict the
316 | functions of bacteria in the rhizosphere soil of tomato treated with different microbial agents in
317 | the second year of continuous cropping, and obtained 53 functional groups (Fig. 5). The analysis
318 | of functional bacteria with a relative abundance of >1% (average total bacterial proportion of
319 | 87.74%) showed that chemolithoautotrophic functional bacteria dominated in tomato rhizosphere
320 | soil (accounting for 24.98%–33.39% of total bacteria), including cChemoheterotrophicy and
321 | aAerobic cChemoheterotrophicy bacteria. The relative abundance of phototrophic bacteria was
322 | relatively low (0.45%–1.91% of total bacteria), and the average relative abundance of
323 | phototrophicy bacteria treated with the bacterial agent was 7.37% higher than that of the control
324 | FQ_CK treatment, which may be related to the promotion of the growth of phototrophic bacteria
325 | by the application of the bacterial agent.—

326 | The total relative abundance of functional microorganisms related to the nitrogen cycle
327 | (nNitrate rRespiration, nNitrate rReduction, nNitrogen rRespiration) was relatively high
328 | (averaging 8.02% of the total bacteria), and the average relative abundance of nitrogen
329 | cycle-related functional bacteria treated with microbial agents was 45.09% higher than that of the
330 | control FQ_CK treatment. This might mainly be due to the fact that the application of microbial
331 | agents offered a favorable growth environment for these bacteria. The relative abundance of
332 | communities with fermentation functional genes (fFermentation) ranged from 3.53% to 6.96%.
333 | The total relative abundance of functional microorganisms related to parasites (animal parasites
334 | or symbionts, intracellular parasites) averaged 2.08% of the total bacteria. The total relative
335 | abundance of functional microorganisms related to decomposition (xylanolysis, cellulolysis,
336 | chitinolysis, ureolysis) ranged from 3.30% to 5.51%. Among them, the total relative abundance
337 | of decomposition-related functional bacteria in FQ_T1 was the highest, at 23.38% higher than
338 | that of the control FQ_CK treatment. Functional microorganisms related to human pathogenic
339 | bacteria (e.g., human pathogens linked to pneumonia, human pathogens al and other diseases, with
340 | —an average total relative abundance accounting for 2.11% of the total bacteria) and those
341 | related to sulfur and manganese oxidation cycles (e.g., dark thiosulfate oxidation, dark oxidation
342 | of sulfur compounds, and manganese oxidation, with —an average total relative abundance
343 | accounting for 3.80% of the total bacteria) were also detected at the sampling sites.

Discussion

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

The ~~contents of AK~~ ~~—(available potassium)—~~ and OM ~~contents~~ ~~—(organic matter)—~~ in the rhizosphere soil of tomatoes treated with microbial agents showed significant differences compared with those in the control FQ_CK treatment. The ~~contents of AK~~ ~~—(available potassium)—~~, OM ~~—(organic matter)—~~, TN ~~—(total nitrogen)—~~, AP ~~contents~~ ~~—(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 group. Changes in soil microbial characteristics ~~that~~ may have been the result ~~from~~of changes in soil chemical properties under two years of continuous cropping (Wang *et al.*, 2013). In contrast, the application of microbial agents ~~could~~may have improved 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 ~~because 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~~HEN~~ *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 most 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 utilization 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 in promoting denitrification (ChenHEND et al., 2018). Acidobacteriota can also degrade plant residues, participate in the iron cycle, and produce active soil metabolites (GlickLICKBR 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 matterOM (García-LópezM 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;)(EpeldePELDE-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-OM mineralization (Guo et al., 2015). This article found that the microbial agent treatments increased the abundance of Acidobacteriota, and 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) and, is a beneficial bacterium in the plant salt resistance mechanism (Xu et al., 2020). Some Acidobacterial microorganisms, acting 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, and 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_i (available phosphorus) and TN (total nitrogen) contents; the bacterial community was positively correlated with CA (catalase activity) and AK_i and (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_i (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, holds 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). When 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

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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 ~~c~~Chemoheterotrophic and ~~a~~Aerobic ~~c~~Chemoheterotrophic bacteria. The average relative abundances of functional bacteria related to the nitrogen cycle and phototrophic bacteria were 45.09% and 7.37% higher, respectively, 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.~~ The interaction between soil microbial communities and environmental factors reflects the complex adaptation mechanisms and feedback regulation processes of ecosystems. The spatiotemporal heterogeneity of environmental factors continuously screens and shapes the structure of microbial communities, thereby regulating the functionality and system stability of ecosystems. However, this study found that the correlation between some environmental factors and microbial community structure did not reach a significant level ($P > 0.05$). This may imply: 1) the presence of other key but undetected environmental drivers; 2) Microbial interspecific interactions may have a regulatory effect on the response of environmental factors; 3) There may be synergistic or antagonistic effects between different environmental factors. Therefore, future research should focus on reconstructing and building a multidimensional environmental factor detection system, integrating multi omics technologies such as metagenomics and metabolomics, and deeply analyzing the interaction network between microorganisms, environment, and ecological functions. On the one hand, it is

necessary to conduct in-depth research on the functional genomics of microorganisms, understand their specific metabolic pathways and ecological functions, in order to better utilize them for continuous cropping obstacle reduction. On the other hand, further exploration should be conducted on the stability and sustainability of these species differences and the resulting ecosystem changes on a long-term scale, providing a theoretical basis for evaluating the long-term effects of continuous cropping obstacle reduction.

Conclusions

This study showed that the application of different microbial agents had significant ~~improvement~~ positive 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.

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