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Research Article

Effects of Garlic Rotation and Organic Fertilizer Application on Bacterial Community Structure in Rhizosphere Soil of Continuous Cropping Tomato

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Abstract: 16S/18S/ITS's amplicon and metagenome sequencing had been used extensively to investigate the root microbial community structure of crops and model plants. In this study, the rhizosphere soils of the continuous cropping (Control), garlic rotation and organic fertilizer application tomato plants were taken as the test samples, respectively, to measure the microbial community structure and soil enzyme activities. The results showed that both planting modes had significant effects on the microecology of continuous cropping tomato rhizosphere soil. After garlic rotation, the diversity (Shannon index and Simpson index) and relative abundance (Ace index and Chao1 index) of soil bacterial community decreased significantly in the early stage, but increased significantly in the later stage. These changes could be significantly alleviated by the application of organic fertilizer. During the whole growth period of tomato plants, the activities the six soil enzymes (sucrase, catalase, urease, protease, amylase and alkaline phosphatase) were all the highest in the organic fertilizer application plot, followed by the rotation plot and the continuous cropping plot. With the extension of planting time, the activities of soil protease, urease and alkaline phosphatase increased gradually, while that of sucrase decreased gradually. Catalase activity initially increased and then decreased, while amylase activity did not change significantly with time. After garlic rotation, mortality rate of tomato plants decreased significantly, and all growth indexes were significantly better than those of the control treatment. To sum up, garlic/tomato rotation can effectively improve the soil environment and alleviate the continuous cropping obstacle of tomatoes in greenhouse. Moreover, rotation combining with organic fertilizer application can acquire the most obvious mitigation effect.

***Keywords*:** tomato, continuous cropping obstacle, garlic rotation, organic fertilizer application, soil microorganisms

1. Introduction

Soil microorganisms play an important role in plant productivity, nutrient absorption and resistance to soil-borne diseases [1]. Resistance of plant to diseases, especially soil-borne diseases, is closely related to the structure of rhizosphere soil microbial community [2]. The number and proportion of rhizosphere soil microbial population can affect plant growth, yield, soil health, incidence of soil-borne diseases and damage degree [3]. The soil microecological environment of facility cultivation is destroyed due to annual production, intensive fertilizer application and other reasons,

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and soil-borne diseases are easy to occur. Xun et al. [4] found that in their latest research the diversity of soil bacteria community would decrease with the lessening of biodiversity in the environment, and the enrichment of specific bacteria species leaded to the limitation of community function and the destruction of soil ecological environment. In soils with reduced bacterial abundance, microbial community structure was more susceptible to environmental factors such as pH value and salt ion concentration [5]. The results showed that the addition of leguminous green fertilizer could significantly improve the soil fertility and change the soil microbial community structure [6], and Fusarium oxysporum was significantly positively correlated with the total number of fungi and bacteria. Marton et al. [7] found that adding straw to the soil could alleviate the effect of salt stress on the rhizosphere microbial diversity of wheat. They believed that adding straw promoted plant growth mainly by improving the soil aggregate structure, therewith increased the root exudates. On the other hand, the rhizosphere microorganisms cannot get nutrients from straw but from the root exudates, thus adding straw could affect the structure and diversity of microbial community indirectly by improving wheat growth.

With the maturity of modern high-throughput sequencing technology, some rare and partially unknown species in the soil can be detected [8]. Many scholars use this technique to detect the soil environment. Effects of root exudates on soil microorganisms have been partially reported [9-10]. However, it is mainly concentrated in field crops or plants in the original ecological environment [11], and there is still a lack of targeted studies on the effects of garlic rotation on soil microbial community of facility continuous cropping. Soil borne diseases become more and more serious in the cultivation of facility agriculture.This study focused on the continuous cropping obstacles existing in tomato production. Comparative study on tomato plant rhizosphere bacteria community structure was established between garlic rotation and organic fertilizer application, using continuous cropping tomato as control. We aimed to develop a solution, a host of crop rotation combining the application of organic fertilizer, for prevention and control of soil continuous cropping obstacles.

2. Materials and methods

2.1 *Materials and processing*

The experiment was conducted from July 2018 to July 2019 in a solar greenhouse of Kaifeng Agricultural Park in Qinghai Province, China (East longitude 101°53′24 ~ 101°53′36, Northern latitude 36°42′20 ~ 36°42′48). Three treatments were set for pot cultivation, using soil that continuous cropped tomato for 5 years: FF (tomato continuous cropping), SF (garlic-tomato rotation) and SYF (garlic-tomato rotation with organic fertilizer application). In SF and SYF districts, garlic was rotated and garlic seedlings were harvested from July to November 2018. In FF districts, soil was left idle. In November 2018, all garlic seedlings were harvested, then the soil was turned over again, tomato plants were transplanted (Reifen: Ningxia Jufeng Seed and Seedling Co., LTD.), and 1/6 (soil volume ratio) organic fertilizer was applied in SYF districts, which was made of local cattle manure and rape straw. 40 plants were colonized for each plot, with plant row spacing 35 × 40 cm, and all treatments were repeated for 3 times. During the period of growth, no root and leaf pesticide was used, and other conventional field management was performed.

2.2 *Sampling and analysis methods*

The samples were randomly sampled at 0 d (November 2018, 3 d after garlic sprout harvest), 60 d (January 2019, the initial flowering period), 120 d (April 2019, the initial fruiting period) and 180 d (July 2019, the later fruiting period) after tomato planting. On 0 d, mixed soil samples were collected directly. On 60 d, 120 d and 180 d, the rhizosphere soils of each treated tomato plant were collected by shaking root method [12]. Three mixed soil samples were collected from each treated plant, sealed in sterile bags and brought back to the laboratory. The samples were divided into two parts: One part was passed through a 40-mesh sieve and used for soil biological shape and bacterial community structure analyses, and 2 g of soils were stored at -80°C for Total DNA extraction of each sample; The other part was sieved through a 100-mesh sieve and used for soil physical and chemical properties analyses after natural air drying [13].

The number of culturable microorganisms in soil was determined by dilution plate method [14]. The bacteria were cultured by beef extract peptone AGAR medium, the fungi were cultured by Madin AGAR medium, and the actinomycetes were cultured by modified Gaos No. 1 AGAR medium [15].

Soil enzyme activities were determined by referring to the method of Guan Songyin (1986). Sucrase and amylase activities were determined by 3, 5-dinitrosalicylic acid colorimetric method. The indophenol colorimetric method was used for soil urease. Indene colorimetry was used for soil protease. The soil alkaline phosphatase activity was colorimetric by phenyldisodium phosphate. Soil catalase activity was titrated with potassium permanganate [15].

The microbial community structure analyses of the soil samples were prformed by Shanghai Meiji Biomedical Technology Co., LTD, based on high-throughput sequencing technology. OMEGA Kit E.z.n.TM MAg-BIND Soil DNA Kit was used to extract Total DNA, and Qubit 3.0 DNA detection Kit was used to accurately quantify genomic DNA, so as to determine the amount of DNA to be added in PCR reaction. Bacteria and archaea PCR primers used in the blend of the Miseq sequencing platform V3-V4 universal primers, the sequences are as follows:

341F primer: CCCTACACGACGCTCTTCCGATCTG;

805R primer: GACTGGAGTTCCTTGGCACCCGAGAATTCCA.

Duncan's new complex range test was used for multiple comparison of data. The cloud data analysis platform I-Sanger provided by the biotechnology company was used to analyze the bacterial diversity of the rhizosphere soils of the plants, and the community map of species composition was constructed. Ace index [16], Chao1 index, Shannon index [17] and Simpson index were selected for bacterial diversity index [18]. Ace index and Chao1 index are indexes measuring species richness. The size of the numerical values indicates the level of species richness. The larger the index is, the higher the species richness will be. Species diversity are usually measured by Shannon index. While Simpson index represents the probability that individuals randomly sampled in the community belong to the same species, it is also used to measure species diversity.

3. Results

3.1 *Effects of crop rotation on rhizosphere soil microecological environment*

3.1.1 *Quantity of culturable microorganisms*

Table 1. Effects of crop rotation and application of organic fertilizer on the quantity of culturable microorganisms in rhizosphere soil

Treatment		Bacteria ($\times 10^6$ CFU · g^{-1})	Fungi ($\times 10^4$ CFU · g^{-1})	Actinomycetes ($\times 10^5$ CFU · g^{-1})
0 d	FF	28.33 ± 0.77b	53.33 ± 0.23b	26.00 ± 0.57b
	SF	24.00 ± 0.37c	45.67 ± 0.62c	33.33 ± 0.63a
	SYF	23.00 ± 0.98c	45.67 ± 1.03c	29.67 ± 0.79ab
120 d	FF	28.57 ± 1.44b	43.69 ± 3.56c	20.38 ± 1.25c
	SF	32.14 ± 1.78b	36.71 ± 3.22d	31.46 ± 4.37a
	SYF	81.33 ± 2.95a	125.41 ± 16.90a	32.08 ± 2.62a

Note: The data in the table are means ± SD. Different letters in the same column mean significant difference between treatments at $P < 0.05$. The same below.

As can be seen from Table 1, the number of culturable bacteria, fungi and actinomycetes in the rhizosphere soil of tomato continuous cropping was significantly affected by galic rotation and organic fertilizer application. In the initial planting stage of tomato, the number of bacteria and fungi in SF and SYF treatments was significantly reduced compared with the control treatment (FF), while that of actinomycetes was increased. At the later stage of cultivation, there was no significant difference in the number of bacteria in the SF treatment compared with the FF treatment, and the number of fungi significantly decreased, while the number of actinomyces was significantly higher than that of the FF treatment ($P < 0.05$). The number of culturable actinomycetes in the SYF treatment was not significantly different from that in the SF treatment, but the number of culturable bacteria and fungi was significantly higher than that in the SF and FF treatments ($P < 0.05$). It can be seen that applying organic fertilizer after garlic rotation can significantly increase the number of culturable microorganisms.

3.1.2 *Soil enzyme activity*

As shown in Figure 1, the activities of soil protease, urease and alkaline phosphatase increased with the extension of planting time, which might be related to the rising soil temperature at the late time of the cultivation in greenhouse. At the lowest temperature in January, protease activity in SYF was significantly higher than other treatments. Moreover, the soil urease and alkaline phosphatase activities in the SF and SYF treatment were always higher than the FF treatment.

The activity of amylase did not change significantly with the planting time, but its activity in SYF treatment was significantly higher than in other treatments. The activity of sucrase decreased with planting timing, which might be due to the decrease of organic nutrients at the later stage of growth in the soil. SF treatment showed the highest sucrase activity at the initial stage of planting, while declined notably in April and July 2019. Catalase activity increased firstly and then decreased, and exhibited the highest level at the lowest temperature stage, with no significant difference among three treatments.

As shown in Figure 2, soil enzyme activities in rhizosphere soils of different treatments were significantly different. Among them, soil urease, amylase and alkaline phosphatase activities of SYF treatment were significantly higher than those of other treatments. Soil protease activity of SYF treatment was also significantly higher than SF and FF before April 2019, nevertheless the difference became less between SYF and other treatments in July. The highest sucrase activity was detected in SF treatment, followed by SYF and FF treatments, in addition, the total activity of sucrase in SYF was expectably higher than FF.

The above data indicated that the activity of enzymes involved in soil carbon, nitrogen and phosphorus cycle in the rhizosphere soil of tomato could be significantly improved by applying organic fertilizer after rotation, thus promoting the material cycle in the soil micro-environment of the plant rhizosphere and improving the soil ecological status.

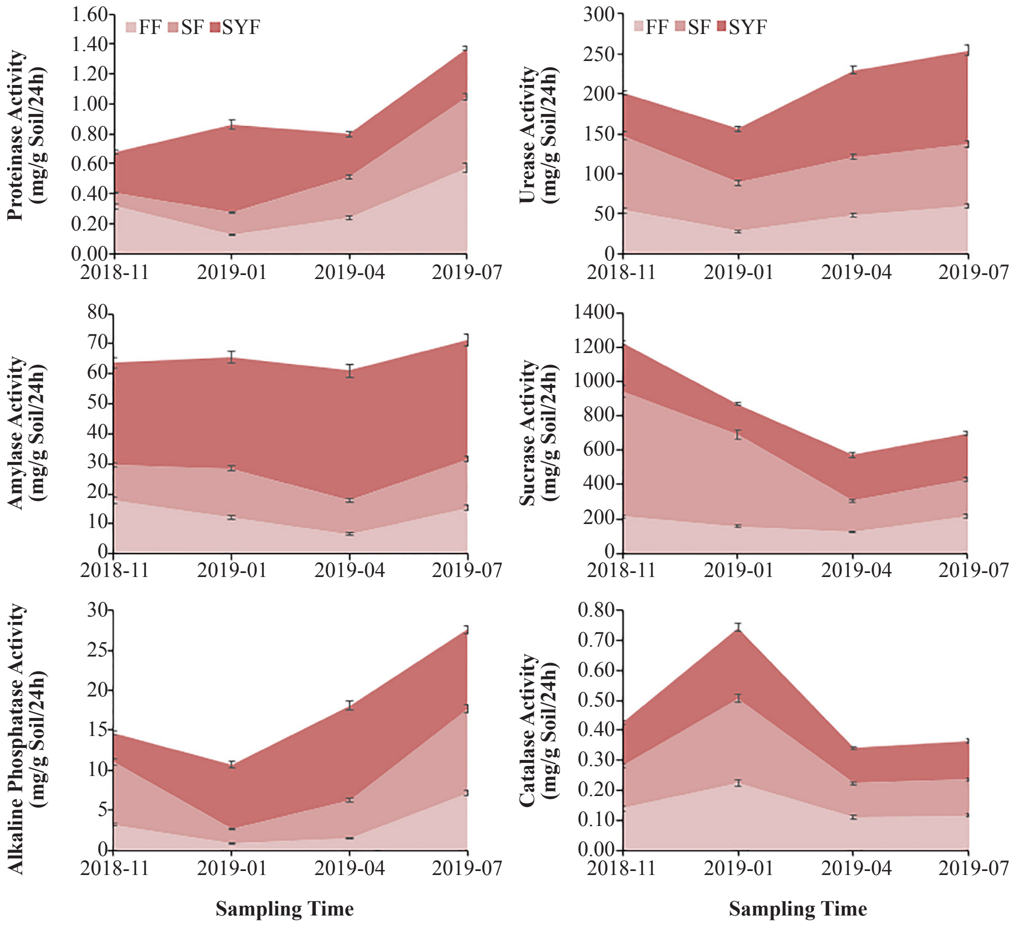

Figure 1. The change trend of enzyme activity of tomato rhizosphere soil under different treatments with time

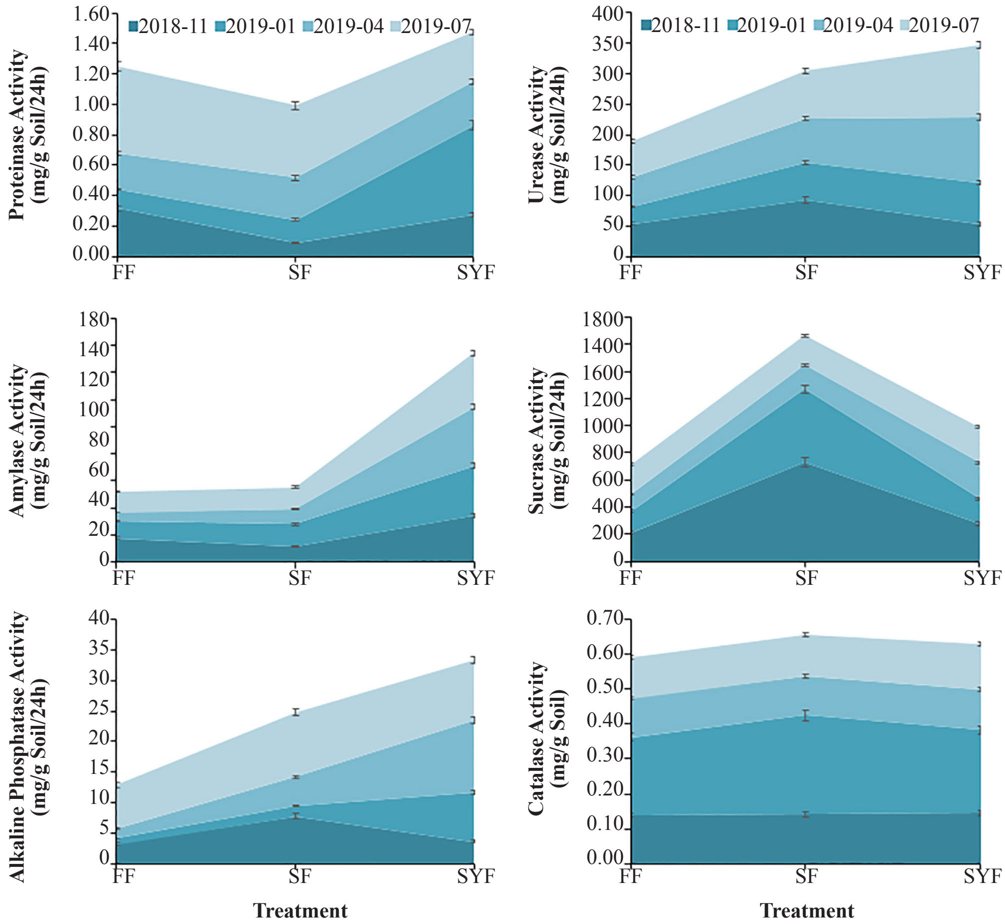

Figure 2. The enzyme activity of tomato rhizosphere soil was different among different treatments

3.2 *Effects of crop rotation on bacterial community structure in rhizosphere soil*

3.2.1 *Bacterial community Alpha diversity in rhizosphere soil*

As shown in Table 2 and Figure 3, compared with FF treatment, the Shannon index of microbial community in rhizosphere soils of SF treatment decreased by 15.99%, and the Simpson index increased by 229.25%, indicating that garlic rotation significantly reduced the microbial community diversity ($P \leq 0.05$). Meanwhile the Ace index decreased by 25.15%, and the Chao1 index decreased by 26.74%, indicating that garlic rotation significantly reduced the microbial relative abundance of tomato rhizosphere soils. After 120 days of cultivation, Shannon index, Ace index and Chao1 index of SF treatment all increased remarkably, and Simpson index decreased distinctly, suggesting that the diversity and richness of soil microbial community increased. It can be seen that with the growth of plants, the inhibitory effect of garlic rotation on the diversity of rhizosphere microbial community was weakened and even lost.

According to the Shannon index and Simpson index, the bacterial species diversity in SYF treatment at 0 d was significantly increased compared with that in SF treatment, but decreased remarkably at 120 d (Figure 3, $P \leq 0.05$). Based on the Ace index and Chao1 index, the relative abundance of bacterial communities in SYF treatment at the early stage of cultivation (0 d) was notably higher than that in SF treatment, but there is no difference between this two treatments at the later stage of growth (120 d). From these data we speculated the effect of garlic rotation on the soil bacterial community structure could be alleviated at the early stage of tomato cultivation by adding organic fertilizer, and the remission effect got weakened with tomato growth.

The soil microorganisms in SYF and FF treatment had no prominent difference in the rhizosphere soil of 0 d and 120 d. This indicated the application of organic fertilizer could, significantly relieve garlic root secretion inhibition of tomato rhizosphere microbes, but did not strikingly increase the diversity of rhizosphere microorganisms and relative abundance during these two periods. These results were inconsistent with the culturable microorganisms (Table 1). This might because the number of culturable microorganisms in soil only accounts for 1% of the total microorganisms.

Table 2. Effects of different treatments on Alpha diversity of tomato rhizosphere soil bacterial community

Sample		Nseqs	Shannon	Simpson	Ace	Chao1	Coverage
0 d	FF	24222	6.35	5.87E-03	3479	3265	0.96
	SF	29009	5.34	1.93E-02	2604	2392	0.98
	SYF	22347	6.44	5.24E-03	3335	3287	0.96
120 d	FF	20957	6.56	3.97E-03	3965	3802	0.95
	SF	33345	6.66	3.00E-03	3953	3964	0.97
	SYF	31365	6.15	1.64E-02	3887	3850	0.97

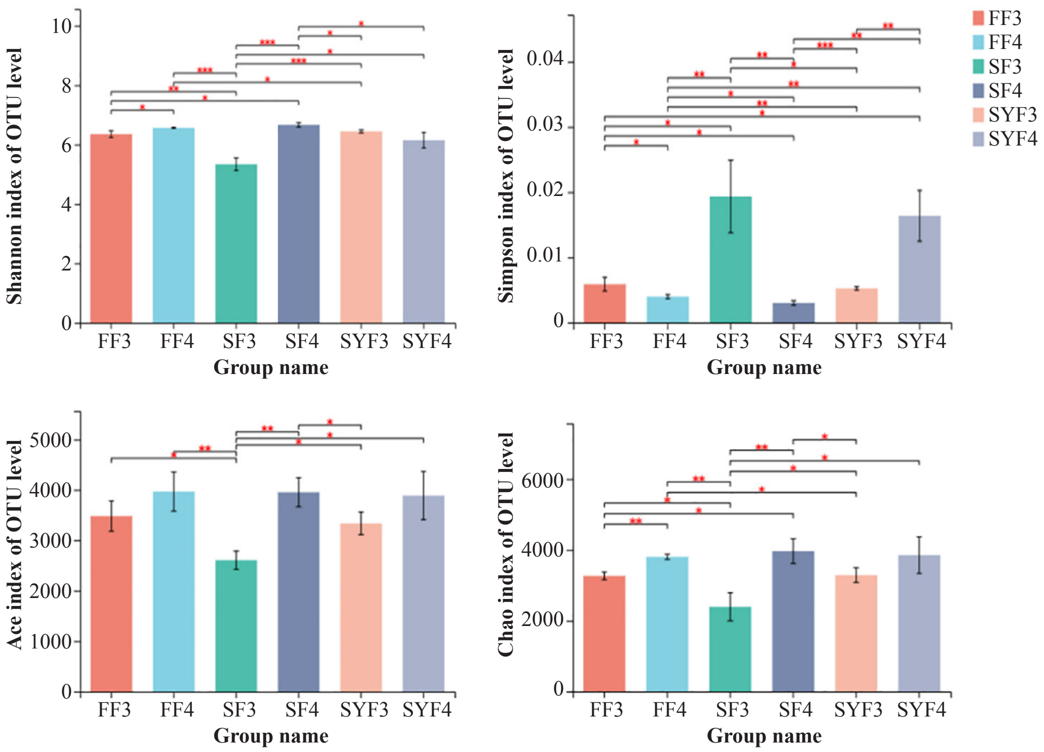

Figure 3. Stuedent's-test of soil bacterial community Alpha diversity

Note: The FF3, SF3, SYF3 in figure means 0 d samples; FF4, SF4 and SYF4 are 120 d samples.
The * indicates significant difference between treatments, Student 's-test at $P \leq 0.05$, same as below.

3.2.2 *Cluster analysis based on OTU level*

As shown in Figure 4, there were 1360 OTUs shared by all treatment treatments, and the number of total and unique OTUs in each treatment increased significantly in later growth period compared with early. The number of bacteria OTUs in SF plot (2439) was significantly lower than that in FF plot (3334) on day 0 d, but SYF plot (3468) was basically the same as FF plot, indicating that garlic had a significant inhibitory effect (bactericidal effect) on bacteria in soils and organic fertilizer application alleviated this effect. After 120 days growth, the number of bacterial OTUs in SF (4148) and SYF (4120) plots was higher than that in FF plots (3732). It is speculated that the root secretion antibacterial substances of garlic would be degraded gradually with tomato plant growth, while the beneficial substances produced by garlic rotation would significantly promote the bacterial community reconstruction [19].

The unique OTU numbers of 0 d and 120 d in SYF increased from 141 to 439, and in SF from 86 to 190, which meant both organic fertilizer and garlic rotation had remarkable influence on bacteria species. In consideration of these increasement all happened along with the growth of tomato plants, we guessed the growth of plants also had a great impact on the rhizosphere bacteria community. Meanwhile the higher the soil temperature at later growth stage, environmental factors could also be important factors affecting bacteria community structure.

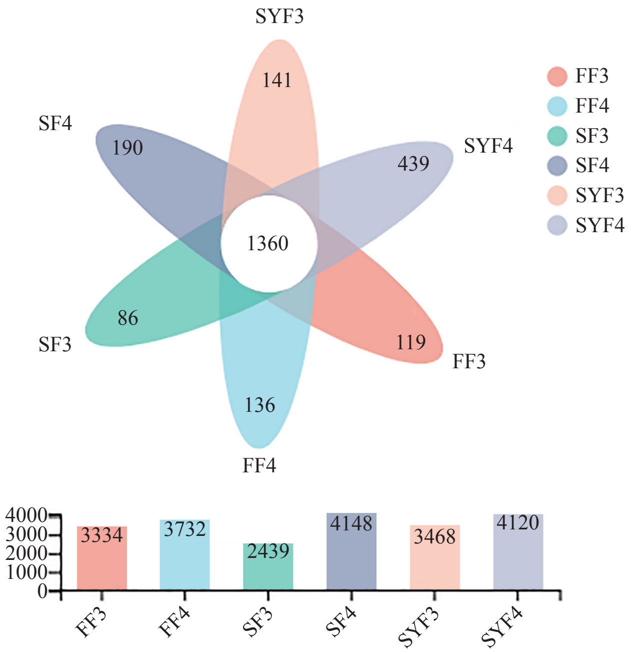

Figure 4. Wayne diagram analysis based on OTU

3.2.3 *Analysis of dominant microflora based on the phylum level*

Based on the phylum level analysis, it was found that there were 7 dominant bacterial phyla in the rhizosphere soil of tomato plants (Figure 5, left). The percentage of *Actinobacteria* was the highest, except for SF. In SF plot, *Proteobacteria* was the most. On the whole, Proteobacteria was the second large dominant bacteria. The proportion of *Proteobacteria* in FF treatment was the lowest at 0 d, while SYF became the lowest at 120 d. *Proteobacteria* of the other samples had no notable difference. However, garlic rotation (SF) and application of organic fertilizer (SYF) changed the proportion of *Planctomycetes*, *Firmicutes*, *Bacteroidetes*, *Chloreflexi* and *Gemmatimonadetes*. The rhizosphere soil bacterial composition of plants with garlic rotation and organic fertilizer application were significantly different from that of continuous plants (FF). The heat map (Figure 5, right) of relative abundance analysis of the top 25 dominant bacterial phyla showed that the abundance of bacterial species in SYF treatment was significantly higher than that in FF treatment after 120 days of cultivation.

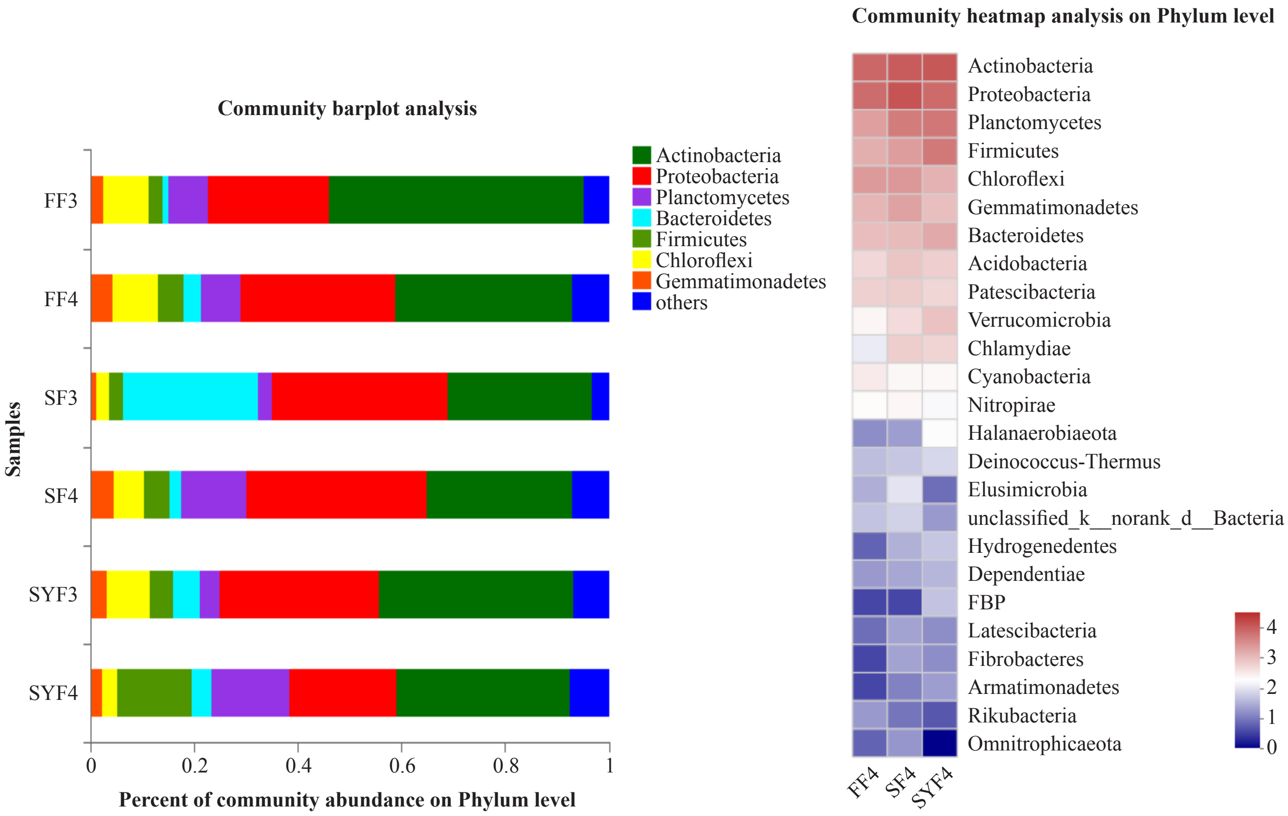

Figure 5. Results of classification level and relative abundance heat map of dominant phylum of bacteria under different treatments

3.2.4 *Analysis of dominant microflora based on the genus level*

Based on the genus level analysis (Figure 6), there were 12 dominant bacterial genera, each genus accounted for proportion more than 3% in the rhizosphere soils of tomato plants. The remained bacterial genera were represented by Others. Except for the SF3 and SYF4 treatments, the proportion of *Arthrobacter* in other samples was the highest. According to the proportion of the unique bacterial structure at the genus level , there were some special bacteria in the rhizosphere soil of tomato plant: *Nocardioides*, norank_o__*Actinomarinales*, *Pseudomonas*, norank_f__JG30-KF-CM45, *Marmoricola*, *Actinomadura*, *Saccharomonospora*, norank_f__*Gemmatimonadaceae*, norank_c__*Alphaproteobacteria* and *Longispora*. The distribution of dominant bacteria was significantly changed by garlic rotation and organic fertilizer application. In particular, the proportion of *Flavobacterium* in SF3 treatment reached 20.18%, which was significantly higher than other samples. Analysis of the relative abundance of the Top 25 dominant bacterial communities (Figure 6, right) showed that the relative abundance of bacterial genera in SF and SYF treatment was significantly higher than that in FF treatment after 120 days of cultivation.

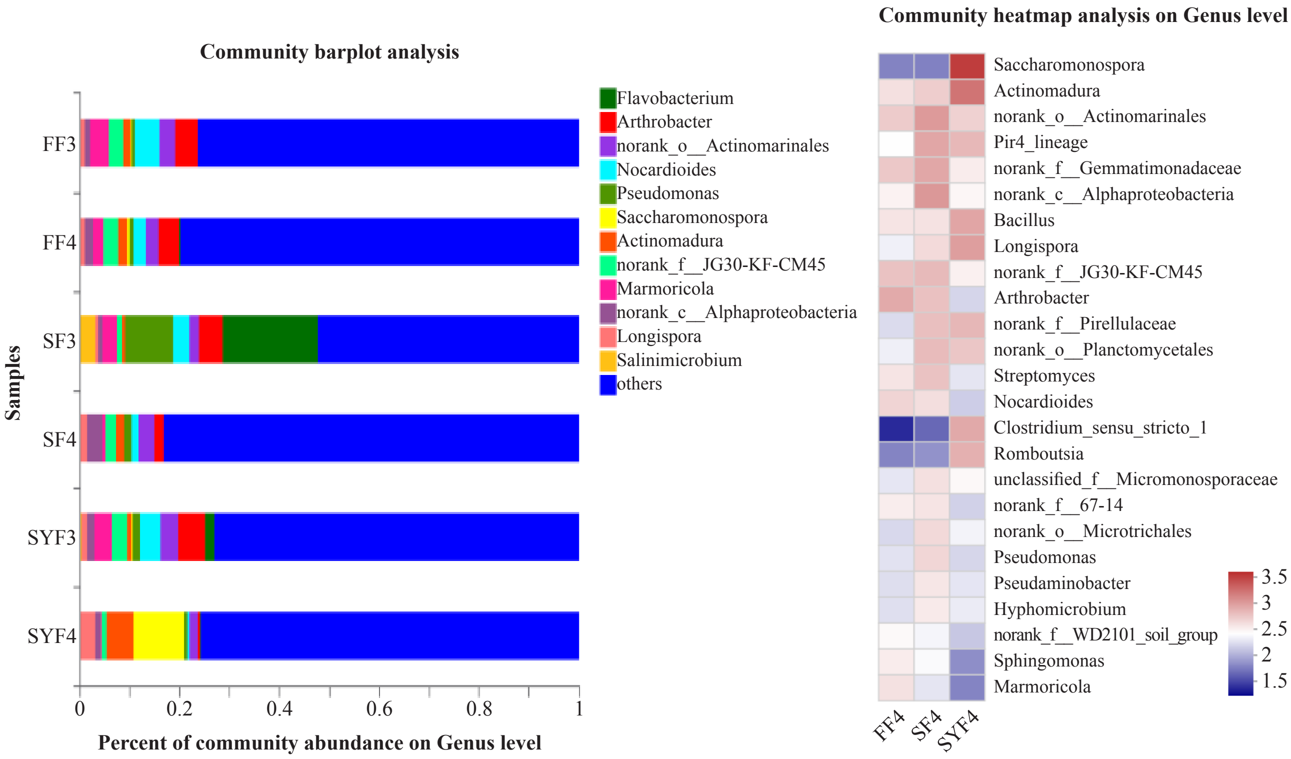

Figure 6. Composition and relative abundance analyses of the dominant bacterial genera in different treatments

3.3 *Effects of garlic rotation and organic fertilizer application on tomato plant growth*

Table 3. Growth status of tomato plants under different treatments in the early flowering period (January 2019)

Treatment	Plant Height (cm)	Stem Diameter (mm)	Number of Leaves (number)	Leaf Length (cm)	Leaf Width (cm)
FF	24.7 ± 5.519b	5.8 ± 0.99b	7.8 ± 0.92a	19.1 ± 3.479b	12.9 ± 2.73b
SF	19.9 ± 4.677c	5.9 ± 0.84ab	7.6 ± 0.84a	20.2 ± 4.10b	13.4 ± 3.03b
SYF	28.7 ± 1.947a	6.7 ± 0.66a	8.3 ± 1.16a	26.1 ± 1.66a	16.9 ± 1.85a

Data in Table 3 showed that the plant height, stem diameter, leaf length and leaf width of the SYF treatment were all remarkably higher than those in SF and FF treatments in the initial flowering period (60 d after cultivation) ($P \leq 0.05$). However the differences between FF and SF were not significant. In conclusion, the tomato growth status of the SYF treatment at the initial flowering period was the best.

Table 4. Growth status of tomato plants under different treatments in the fruiting period (April 2019)

Treatment	Plant Height (cm)	Stem Diameter (mm)	Number of Leaves (number)	Leaf Length (cm)	Leaf Width (cm)	Flower Panicle Number (number)	Fruit Number (number)
FF	91.02 ± 13.37b	7.56 ± 1.48c	13.67 ± 1.50a	34.56 ± 5.76b	25.63 ± 5.55a	1.56 ± 0.73a	2.33 ± 0.5a
SF	104.36 ± 9.84a	9.04 ± 1.56b	13.56 ± 2.07a	40.53 ± 3.90a	29.82 ± 4.35a	1.11 ± 0.33a	2.00 ± 1.22a
SYF	107.22 ± 7.93a	10.47 ± 0.86a	13.56 ± 2.07a	38.19 ± 5.01ab	26.70 ± 5.04a	2.00 ± 1.58a	2.78 ± 0.97a

As can be seen from Table 4 (120 days after cultivation), the plant height and stem diameter of SYF and SF treatments were significantly higher than those in FF treatment by 17.8%, 14.66%, and 38.49%, 20.82% ($P \leq 0.05$), respectively. In terms of leaf length and leaf width, SF treatment was larger than FF and SYF treatments. In terms of flower panicle number and fruit number, the SYF treatment was greater than the FF and SF treatments by 28.21%, 80.18%, and 19.31%, 39%, respectively, but the differences among these three treatments was not significant, because of the big deviation. In conclusion, the tomato growth index of SYF treatment at the initial stage of fruiting was the best.

4. Discussion and conclusion

Resistance of plant to soil-borne diseases is closely related to rhizosphere soil microecological environment, in which soil microbial community, soil microbial biomass and soil enzyme activity are important components [20]. The number and proportion of rhizosphere soil microbial population can affect plant growth, yield, soil health, incidence of soil-borne diseases and damage degree [21]. The studies on eggplant [22], chili [23], cucumber [24], and other crops found that, under the stress of soil borne pathogens, the balance of plants-soil-microorganism could be rebuilt by applying soil organic matter, thus raised resistance of plants to soil borne diseases [25]. The root secretion of plant released into rhizosphere soil will be changed after organic matters application as the interaction of soil and plant, which will result in the change of micro-ecological factors, including soil microbial population structure, soil microbial biomass and soil enzymes, finally breaking the soil environment suitable for the growth of soil-borne pathogens. These could be the reasons for organic fertilizers to alleviate continuous cropping barriers and improve crop disease resistance [25]. In this study, two consecutive years of rotation experiments were conducted to continuously measure the micro-ecological environment of the rhizosphere soil of tomato plants. It was found that garlic rotation and organic fertilizers had significant effects on the microbial community structure of rhizosphere soil, and soil enzyme activity, soil-borne diseases, as well as plant growth status, yield and quality, under continuous tomato cultivation.

According to our studies before, the disease mortality of tomato plants after garlic rotation decreased significantly, and all growth indexes were significantly better than those of the continuous cropping control treatment (the data is under publish), which was basically consistent with the results of previous studies [26]. Enzymes are the most active organic components in soil, and the enhancement of soil enzyme activity also represents the improvement of soil biological activity and plant resistance [27]. After garlic rotation, the related enzyme activities in the rhizosphere soils of plant were significantly affected [28], and the overall performance was inhibition firstly and then promotion. The enzyme activities related to protein and starch decomposition were significantly inhibited at the early stage of cultivation, while the other soil enzyme activities were not inhibited or the differences were not significant and presented a promoting trend all the time. This is similar to the results of Yang Ning et al. [29] on soil enzyme activity. After garlic rotation, activities of some soil enzymes were significantly increased by adding organic fertilizer, indicating that organic fertilizer could alleviate the inhibitory effect of root exudates of garlic in the early stage of cultivation and promote the soil microecological balance.

Through monitoring the rhizosphere soil microecological environment, we found that after garlic rotation, the microbial diversity and abundance of the rhizosphere soils of tomato plants decreased at the early stage of cultivation, and the inhibition effect disappeared with the growth of tomato plants. Applying organic fertilizer after garlic rotation can effectively alleviate the inhibitory effect of garlic root exudates on soil microbial community [30], and increase the microbial community diversity and relative abundance in rhizosphere soil. Crop rotation and application of organic fertilizer had important effects on bacterial composition at both phylum and genus level.

In this study, it was found that applying organic fertilizer after garlic rotation could further promote plant growth and significantly increase the yield. Moreover, the diversity and relative abundance of rhizosphere microbes, the number of unique OTUs, and the soil enzyme activity were all significantly increased after organic fertilizer application. Therefore, it is speculated that organic fertilizers might increase the relative abundance of beneficial microorganisms by ameliorating soil structure and improve soil porosity, which could increase the soil enzyme activity, thus accelerate the transformation of nutrients such as nitrogen, phosphorus and potassium, and finally promote plant growth and yield.

Competing interests

The authors declare that they have no competing interests.

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