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Bacterial Diversity Change after Soil Fumigation and its Effect on the Population of Root-Knot Nematode

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Abstract: To explore the effects of soil fumigation on its bacterial diversity and root-knot nematode populations, the greenhouse tomato soil with root-knot nematode disease was fumigated during the summer idle period. This experiment used the high-efficiency organic sulfur soil fumigation treatment "Wofengkang"300 kg/hm^2 to fumigate the greenhouse tomato soil with a root-knot nematode incidence rate of 64.37% and a disease index of 60.18. Soil samples were collected before and after fumigation to determine the index of bacterial diversity and the populations of root-knot nematodes. The results showed that a total of 2 kingdoms, 36 phyla, 117 classes, 227 orders, 368 families, and 614 genera were detected after soil fumigation and the archaea were significantly reduced by 98.42%. There was no significant difference in all phyla before and after soil fumigation ($p>0.05$). After soil fumigation, Gammaproteo bacteria containing multiple pathogenic genera significantly decreased by 34.36%; Xanthomonadales, the pathogens of plant fusarium wilt and canker, significantly decreased by 34.21%; Xanthomonidaceae that can infect more than 400 plant varieties significantly decreased by 41.52%; Burkholderia, which is a rhizosphere microorganism for biodegradation, biological control and promotion of plant growth in agriculture, significantly decreased by 89.62%. Generally, the high-efficiency organic sulfur soil fumigant "Wofengkang"300 kg/hm^2 in plots with a more serious incidence of root-knot nematode disease can significantly reduce the diversity of pathogenic microorganisms and the populations of root-knot nematodes. This can provide new measures for the effective prevention and control of greenhouse root-knot nematodes in the future and has great practical guiding significance.

Keywords: Soil Fumigation, Bacteria Diversity, Root-Knot Nematode, Nematode Decline Rate

Introduction

Meloidogyne is the main cause of continuous cropping obstacles for many economic crops, such as vegetables, fruit trees, oil crops, tobacco, and Chinese herbal medicine. In recent years, vegetable cultivation in China has continued to expand and root-knot nematode disease has become more and more serious. According to preliminary estimates, the current domestic facility vegetable disease area exceeds 20 million mu, causing direct economic losses of tens of billions of yuan. Root-knot nematode disease is a soil-borne disease, which is difficult to prevent and treat. The prevention and control of it mainly depend on improving agricultural measures, using chemical pesticides, and biological control (Detrey *et al*., 2022; Liu *et al*., 2021; Talavera *et al*., 2021; Bao *et al*., 2021). But they all have different degrees of limitations or disadvantages and the effect is poor.

How find a convenient and effective method has become the primary problem to be solved in the production of greenhouse agriculture. Soil fumigation is an effective measure and means to control root-knot nematode disease in recent years. Jin *et al*. (2021) reported the vegetable root-knot nematode and soil nematode communities by using the method of cabbage biological fumigation. Zhu *et al*. (2021) reported the control effect of calcium cyanamide and dazomet on pepper root-knot nematode. The above studies focused on the control effect

of root-knot nematode, but there were few reports on the changes in soil microbial community after soil fumigation.

Based on the previous research of our group, this experiment selected a greenhouse with a serious incidence of tomato root-knot nematodes in Dachengzi, Ningcheng County, Chifeng City, Inner Mongolia, with an incidence rate of 64.37% and a disease index of 60.18. The soil in the summer idle period was fumigated by the high-efficiency organic sulfur soil fumigant “Aofengkang”. The soil samples were collected before and after fumigation and the bacterial diversity was determined by the 16S rDNA high-throughput sequencing method (Hasan *et al*., 2021). At the same time, the changes in root-knot nematode populations before and after fumigation were also measured. Through the analysis of soil bacterial kingdoms, phyla, class, order, family, and genera before and after fumigation, the changes in bacterial flora richness and the influence on root-knot nematode populations were compared to provide new methods and practical guidance for the effective prevention and control of greenhouse root-knot nematode disease in the future.

Experimental Design and Research Methods

Test Materials

The high-efficiency organic sulfur soil fumigant "Aofengkang" was provided by Beijing Qigao Biotechnology Co., Ltd. Its composition is 98% of Dazomet and the molecular formula is C5H10N2S2. It is efficient, safe, and has no residue. This product was researched by the Inner Mongolia 2060901 major science and technology project (zdzx2018009). The appropriate amount of fine soil was added to the fumigant to mix evenly and then spread evenly on the soil surface. After application, mechanical rotary tillage (control the soil depth as 15-20 cm) was carried out to ensure that the medicine was evenly mixed into the cultivated layer and then covers with mulching film after watering. The soil was loosened after uncovering the film and transplanted or sowed after 5-7 days.

Field Treatment Method

On August 23, 2020, the high-efficiency organic sulfur soil fumigant "Aofengkang" was used for soil fumigation. The field dosage was 300 kg/hm^2. The soil fumigant was mixed with fine sand and evenly sprinkled into the shed and then rotated the ground, watered, and covered with film. After the high-temperature closed shed for 15 days, the shed film was removed for ventilation. The mulching film was removed on September 10 and waited for the soil to dry. After that, the fumigation was completed on September 21 and soil samples were taken to prepare for the colonization of the next crop of tomatoes. Soil samples were collected before and after fumigation and 100 g soil, bacterial diversity index, and populations of root-knot nematodes were measured.

Determination Index and Method

Soil Sample Collection Method

Before soil fumigation, three sampling points were determined according to the incidence of root-knot nematodes during the last tomato planting. Each point adopted a "W"-shaped 5-point sampling method, the 20 cm soil layer on the surface was drilled, fully mixed in the field, put into plastic bags, marked, and taken back to the laboratory for treatment. The fresh soil samples were collected and packed in 5 mm centrifuge tubes, labeled, stored in a low-temperature refrigerator at -80°C, and then sent to Shenzhen Weikemeng Technology Group Co., Ltd. to detect bacterial diversity. The collected fresh soil samples were tested for root-knot nematode populations within 3 days. The soil sample before and after fumigation was marked as bf (before the fumigation) and af (after the fumigation), respectively.

Separation of Soil Nematodes-Baermann Shallow Disc Method

(1) Put the sieve in a small basin and then spread a layer of tissue paper on the sieve
(2) Crush the soil and mix well, take 100 g of soil and place it on the facial tissues
(3) Add water from the gap between the sieve and the small pot. The water should cover the soil, but not the tissues
(4) After standing for 24-48 h at room temperature, the water in the small basin was passed through a 500 mesh screen. At this time, the nematodes remained on the sieve and then they were rinsed in a glass dish with a small amount of water, standing for 1-2 min. After that, they were counted under a stereoscope (or a microscope)

Nematode decline rate = Number of soil root-knot nematodes treated with 100 g -Number of root-knot nematodes in 100 g control soil/Number of root-knot nematodes in 100 g control soil.

Grading Standard of Disease Index of Root-Knot Nematode Disease

Grading 0: Healthy root system, no root knots.

Grading 1: Small root nodules appeared and the incidence of root nodules was less than 20%.

Grading 2: Some adjacent small root knots on the lateral roots were connected to form larger root knots and the incidence of root knots was 20-40%.

Grading 3: Large root knots appeared on part of the tap roots, the incidence of root knots was 40-60%.

Grading 4: Lateral roots were underdeveloped, large root knots appeared on the main roots, and the incidence of root knots was 60-80%.

Grading 5: The entire root system was thick, deformed, and rotted without roots and the incidence of root knots was greater than 80%.

Classification Standards for the Incidence of Greenhouse Root-Knot Nematodes

Class I: The incidence rate, the disease grade, and the disease index were all 0; the root-knot nematodes in 100 g soil were no more than 100 and there was no incidence.

Class II: The incidence rate was 0-30%; the disease grade was 1-2 and the disease index was 0-40. The root-knot nematodes in 100 g soil were 101-499 and the incidence was light.

Class III: The incidence rate was 31-59%; the disease grade was 2-3 and the disease index was 40-60. The root-knot nematodes in 100 g soil were 500-999 and the incidence was medium.

Class IV: The incidence rate was 60-79%; the disease grade was 3-4 and the disease index was 60-80. The root-knot nematodes in 100 g soil were 1000-1999 and the incidence was a little serious.

Class V: The incidence rate was ≥80%; the disease grade was 4-5 and the disease index was more than 80. The root-knot nematodes in 100 g soil were ≥2000 and the incidence was serious.

Calculation Formula of Disease Index and Nematode Decline Rate

Disease index = ∑(Diseased plants at all levels × Representative values at all levels)/(Total investigated plants × The highest representative value) × 100.

Nematode decline rate = Number of control nematodes - Number of nematodes treated/Number of control nematodes.

16S rRNA Sequencing Technology Process

Sample preparation → DNA extraction and detection → PCRamplification → Product purification → Library preparation and library inspection → Novaseq online sequencing.

Data Processing

IBM SPSS26.0 was used to calculate the mean ± standard deviation of the data and test the paired data *t*.

Results and Analysis

Venn Diagram of Bacterial out Distribution before and after Soil Fumigation

In this experiment, the average effective sequence count of the soil fumigation obtained after the barcode splitting of the sequencing data was 84681 and 69776.33 before the soil fumigation. The Demultiplexed sequence counts summary reached 463372. The DADA2 plug-in in Qiime 2 software was used for quality control, denoising, and chimerism removing all original sequences in all samples to form OTU. Based on the absolute abundance and species annotation information of OTU, the proportion of the sequences in each sample at a total of 7 classification levels, kingdom, phylum, class, order, family, genera, and species to the total sequences can effectively evaluate the resolution of the species annotation of the sample.

It can be seen from Fig. 1 that the two have a total OTU of 938. Bacterial OTU after fumigation was more than before fumigation. The total OTU after fumigation reached 3122, increased by 11.62% compared with bf, indicating that the soil bacterial species diversity was increased after soil fumigation. A paired-sample t-test was carried out on the obtained OTU data and they did not reach a significant difference ($p>0.05$). However, the specific increase in species and number still needs to be further analyzed for its kingdoms, phylums, classes, orders, families, genera, and species.

Comparison of Bacterial Kingdom Differences BEFORE and AFTER Soil Fumigation

Table 1 showed that using the paired sample t for test, two levels named bacteria and archaea were detected in the kingdom classification. The bacteria before and after fumigation accounted for 99.53 and 99.99%, respectively. The bacteria increased, but there was no significant difference between the bacteria after soil and before fumigation ($p>0.05$). Archaea before and after fumigation accounted for 0.47 and 0.005%, respectively. There was a significant difference between bf and af. After fumigation, it was significantly reduced by 98.42% compared with bf ($p<0.05$).

Comparison of Bacterial Phyla Differences Before and After Soil Fumigation

Table 2 shows that the phylum horizontal flora before and after soil fumigation has changed. After fumigation, Proteobacteria, Gemmatimonadetes, Acidobacteria, Acidobacteria, Chloroflexi, Verrucomicrobia, Chlorobi, Armatimonadetes and Cyanobacteria increased and others decreased. A total of 36 phyla were detected. Proteobacteria was the dominant bacterial flora, accounting for 53.17 and 44.22% of the total detected bacteria before and after fumigation. After fumigation, it was 16.83% lower than that before fumigation. The second was Actinobacteria, Gemmatimonadetes, Acidobacteria, Firmicutes, Bacteroidetes, and Chloroflexi. The t-test results showed that the differences of bacteriaphyla before and after soil fumigation did not reach a significant difference ($p>0.05$).

Comparison of Bacteria Class Differences before and after Soil Fumigation

Table 3 shows that before and after fumigation, the bacteria class flora has changed. After fumigation, Alphaproteobacteria, Gemmatimonadetes, Bacilli, Deltaproteobacteria, Acidobacteria, Acidimicrobiia, Bacteroidia, Thermomicrobia, Verrucomicrobiae, Anaerolineae, Chloroflexi, and Deinococci increased, but none of them was significantly different from before fumigation ($p>0.05$). Other bacteria classes decreased. Gammaproteobacteria reached a significant difference

(p>0.05) before and after fumigation and the difference after fumigation was reduced by 34.36% compared with bf. There were 117 bacteria orders. Alphaproteobacteria was the dominant bacteria community and accounted for 23.61 and 24.66%, respectively before and after fumigation. After fumigation, it increased by 1.05% compared with before fumigation. Unspecified_Bacteria and others accounted for 0.57 and 0.74, 18.84 and 26.17%, respectively.

Comparison of Bacterial Order Differences before and after Soil Fumigation

Table 4 shows that after fumigation, the bacteria order group has changed. Before and after fumigation, Sphingomonadales, Rhodospirillales, Rhodobacterale, Bacillales, Bacteroidales, Myxococcales, Acidimicrobiales, Clostridiales, Lactobacillales, and Syntrophobacterales increased, but none of them was significantly different from before fumigation (p>0.05). After fumigation, the soil was significantly reduced by 34.21, 53.35, and 69.77% compared with before fumigation. A total of 227 orders of bacteria were detected. Actinomycetales, Xanthomonadales, Burkholderiales, Rhizobiales and Sphingomonadales were the dominant flora. Before and after fumigation, soil fumigation accounted for 10.52 and 7.40, 8.29 and 6.29, 7.74 and 4.07, 7.54 and 4.69, 6.29 and 8.00%, respectively of the total detected bacteria. After fumigation, Actinomycetales, Xanthomonadales, Burkholderiales, and Rhizobiales decreased by 9.20, 34.21, 38.95, and 24.63%, respectively compared with before fumigation. Among them, Xanthomonadales reached a significant difference before and after soil fumigation (p<0.05). Sphingomonadales increased after soil fumigation but did not reach a significant difference before fumigation (p>0.05). Unspecified_Bacteria and others accounted for 12.27 and 17.95, 24.11 and 22.32%, respectively before and after soil fumigation.

Comparison of Bacterial Family Differences before and after Soil Fumigation

Table 5 shows that before and after fumigation, the bacteria order flora has changed. After fumigation, Sphingomonadaceae, Rhodospirillaceae, Rhodobacteracea, Sinobacteraceae, Micromonosporaceae, Erythrobacteraceae, Bradyrhizobiaceae, and Caulobacteraceae increased, but none of them was significantly different from before fumigation (p>0.05). Others decreased and Xanthomonadaceae, Hyphomicrobiaceae, Micrococcaceae, Microbacteriaceae, Burkholderiaceae, Rhizobiaceae, Streptomycetaceae, and Cytophagaceae reached a significant difference before and after fumigation (p<0.05). After fumigation, the soil was significantly reduced by 41.52, 18.70, 39.10, 46.45, 85.80, 81.38, 48.47, and 56.16%, respectively compared with before fumigation. A total of 368 bacteria orders were detected. Xanthomonadaceae, Sphingomonadaceae, Rhodospirillaceae, Hyphomicrobiaceae, Micrococcacea, and Comamonadaceae are the dominant bacteria communities. Before and after fumigation, they accounted for 6.92 and 3.52, 5.10 and 6.66, 4.23 and 4.29, 3.83 and 2.68, 3.45 and 1.72, 3.31 and 2.17%, respectively. Unspecified_Bacteria and others accounted for 31.37 and 39.76,20.02 and 24.47%, respectively.

Comparison of Bacteria Genus Differences Before and After Soil Fumigation

Table 6 shows that before and after fumigation, the bacteria genus flora has changed. After soil fumigation, *Rhodobacter*a and *Alicyclobacillus* increased and *Rhodobacter* did not reach a significant difference before fumigation(p>0.05). *Alicyclobacillus* reached a significant difference after fumigation(p<0.05). Others all decreased. Among them, *are Arthrobacter*, *Rhodoferax*, *Pseudomonas*, *Burkholderia*, *Streptomyces*, Microbacterium, *Achromobacter*, *Rhodanobacter*, *Hyphomicrobium*, *Mesorhizobium*, *Rhizobium*, *Pimelobacter*, and *Comamonas* reached significant differences before and after fumigation(p<0.05). A total of 614 bacteria genera were detected. *Arthrobacter, Rhodoplanes, Rhodoferax, Rhodobacter, Bacillus, and Pseudomonas* were dominant bacteria genera. Before and after fumigation, the soil accounted for 3.16 and 1.68, 2.26 and 3.15, 2.04 and 0.94, 1.95 and 3.15, 1.70 and 0.74, 1.53 and 0.95% of the total detected bacteria, respectively. Unspecified_Bacteria and others accounted for 59.69 and 64.54,20.77 and 24.66%, respectively.

Effects of Soil Fumigation on Root-Knot Nematode Populations in Greenhouse Soil

Table 7 shows that after soil fumigation, the population of root-knot nematodes in 100g soil was significantly reduced (p<0.05) and the nematode reduction rate reached 97.26%.

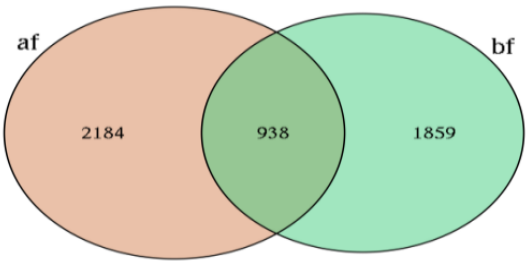

Fig. 1: Venn diagram of bacteria OUT distribution after soil fumigation

Table 1: T-test results of paired samples of bacteria kingdom after soil fumigation

Taxonomy	bf	af	t-value	p-value
Bacteria	32103.33±2332.64a	39343.00±6.69.06a		
Archaea	147.67±49.66a	2.33±2.33b	2.924	0.043

Table 2: T-test of paired samples of bacteria phyla after soil fumigation

Taxonomy	bf	af
Proteobacteria	17186.67±296.62a	16981.00±2329.40a
Actinobacteria	4813.00±650.33a	5239.00±1978.39a
Gemmatimonadetes	3623.67±263.49a	6440.33±1810.30a
Acidobacteria	1877.33±571.14a	2452.33±1063.35a
Firmicutes	1339.00±1174.93a	4233.67±5434.40a
Bacteroidetes	1334.33±293.38a	1332.00±731.54a
Chloroflexi	912.33±41.65a	1469.67±348.52a
Nitrospirae	282.67±134.29a	206.33±28.92a
Verrucomicrobia	226.00±122.10a	245.67±165.70a
Crenarchaeota	137.33±71.22a	2.33±4.04a
Chlorobi	62.00±40.85a	64.33±35.53a
Armatimonadetes	41.00±17.44a	43.00±28.16a
Planctomycetes	25.67±26.27a	23.00±9.54a
Fusobacteria	23.00±9.00a	13.33±23.09a
Cyanobacteria	18.33±5.51a	90.67±84.51a
Unspecified_bacteria	27.00±7.21a	13.33±23.09a
others	321.67±80.87a	442.33±65.69a

Table 3: T-test of paired samples of bacteria class after soil fumigation

Taxonomy	bf	af	t-value	p-value
Alphaproteobacteria	7596.33±983.13a	9736.33±2816.03a		
Gammaproteobacteria	4336.00±671.17a	2846.00±383.10b	7.428	0.018
Betaproteobacteria	4303.67±1071.68a	3130.33±67.28a		
Actinobacteria	3377.00±638.06a	3110.33±1539.52a		
Gemmatimonadetes	1772.67±245.66a	3504.67±1603.11a		
Bacilli	1025.67±877.41a	1796.67±2214.02a		
Deltaproteobacteria	924.33±262.39a	1179.00±250.30a		
Acidobacteria	819.67±239.65a	1215.33±583.99a		
Acidimicrobiia	561.00±161.28a	753..00±248.00a		
Sphingobacteriia	254.00±147.25a	23.33±17.61a		
Ktedonobacteria	196.67±145.88a	56.33±14.57a		
Bacteroidia	178.00±231.46a	712.33±681.17a		
Thermomicrobia	167.67±73.51a	303.00±109.42a		
Verrucomicrobiae	95.67±97.28a	159.33±163.99a		
Holophagae	35.00±19.15a	5.00±4.58a		
Anaerolineae	33.33±1.53a	63.67±44.46a		
Chloroflexi	22.67±3.51a	74.33±48.12a		
Deinococci	8.33±3.79a	48.67±25.42a		
Unspecified_bacteria	183.00±93.04a	294.00±25.36a		
Others	6360.33±1023.48a	10333.67±3219.00a		

Table 4: T-test of paired samples of bacterial orders after soil fumigation

Taxonomy	bf	af	t-value	p-value
Actinomycetales	3355.33±652.84a	3046.67±1562.67a		
Xanthomonadales	2668.67±118.78a	1755.67±234.03b	10.251	0.009
Burkholderiales	2522.67±734.89a	1540.00±343.23a		
Rhizobiales	2418.33±209.66a	1822.67±353.40a		
Sphingomonadales	2027.00±468.75a	3220.67±1311.64a		
Rhodospirillales	1432.67±299.90a	1866.33±491.88a		
Rhodobacterales	866.67±122.52a	1467.00±777.10a		
Bacillales	817.33±561.64a	1541.00±1855.36a		
Bacteroidales	178.00±231.47a	712.33±681.17a		
Myxococcales	608.00±117.15a	717.00±175.70a		

Table 4: Continue

Pseudomonadales	605.33±93.63a	510.00±200.58a		
Acidimicrobiales	561.00±161.28a	753.00±248.00a		
Alteromonadales	361.67±338.98a	226.00±112.06a		
Nitrospirales	282.67±134.29a	206.33±28.92a		
Clostridiales	281.67±263.62a	2385.00±3177.33a		
Cytophagales	263.67±64.47a	123.00±37.16b	6.764	0.021
Sphingobacteriales	254.00±147.25a	23.33±17.62a		
Legionellales	213.67±103.23a	60.00±6.00a		
Thiotrichales	204.00±51.39a	173.67±15.04a		
Lactobacillales	200.67±317.25a	245.33±346.56a		
Syntrophobacterales	187.33±70.73a	231.00±109.98a		
Acidobacteriales	176.33±6.03a	89.00±40.04a		
Enterobacteriales	161.00±5.58a	48.67±5.51b	19.234	0.003
Unspecified_Bacteria	3913.67±704.42a	7391.00±1831.92a		
Others	7689.67±954.89a	9190.67±1239.33a		

Table 5: T-test of paired samples of bacteria family after soil fumigation

Taxonomy	bf	af	t	p
Xanthomonadaceae	2215.67±222.59a	1295.67±318.50b	6.262	0.025
Sphingomonadaceae	1646.33±410.96a	2733.67±1377.27a		
Rhodospirillaceae	1366.33±285.39a	1664.00±345.31a		
Hyphomicrobiaceae	1230.00±100.85a	1000.00±144.38b	9.082	0.012
Micrococcaceae	1100.67±244.93a	670.33±181.78b	9.668	0.013
Comamonadaceae	1085.00±488.54a	752.33±360.15a		
Rhodobacteraceae	767.33±252.87a	1363.67±803.91a		
Oxalobacteraceae	668.33±248.25a	290.67±192.59a		
Pseudomonadaceae	562.33±106.00	450.67±189.10		
Bacillaceae	537.33±457.91a	328.00±264.18a		
Microbacteriaceae	507.33±43.73	271.67±104.04	5.713	0.029
Burkholderiaceae	460.00±36.51a	65.33±7.23b	23.062	0.002
Sinobacteraceae	453.00±248.57a	460.00±84.61a		
Micromonosporaceae	372.33±102.16a	586.33±302.37a		
Rhizobiaceae	361.67±87.84a	67.33±8.50b	6.415	0.023
Erythrobacteraceae	343.00±77.54a	460.00±140.15a		
Streptomycetaceae	326.67±102.57a	168.33±77.94b	10.737	0.009
Bradyrhizobiaceae	311.33±86.56a	428.33±367.58a		
Alcaligenaceae	300.00±60.26a	245.67±127.08a		
Caulobacteraceae	271.67±53.68a	1053.67±1068.27a		
Cytophagaceae	257.00±60.22a	112.67±28.01b	6.097	0.026
Unspecified_Bacteria	10044.33±1398.33	14636.33±2303.95		
Others	6409.33±910.24	9008.00±4501.60		

Table 6: T-test of paired samples of bacteria genus after soil fumigation

Taxonomy	/bf	/af	t-value	p-value
Arthrobacter	1010.33±150.67a	654.00±175.27a	11.944	0.007
Rhodoplanes	719.33±163.45a	553.00±118.70a		
Rhodoferax	672.33±335.29a	303.67±301.09a	7.243	0.019
Rhodobacter	617.33±225.52a	1197.00±812.38a		
Bacillus	532.33±460.78a	321.00±252.56a		
Pseudomonas	497.67±129.41a	332.33±151.32b	5.02	0.019
Burkholderia	382.00±45.92a	39.67±7.02b	14.897	0.004
Dokdonella	314.00±128.50a	178.33±90.67a		
Streptomyces	298.00±122.29a	167.33±79.19	4.943	0.039
Microbacterium	291.00±73.02a	110.67±70.89	6.332	0.024
Lysobacter	226.33±83.03a	182.33±16.26a		
Sphingomonas	219.67±154.48a	83.00±72.99a		
Achromobacter	140.00±53.56a	35.67±37.42b	4.826	0.04
Rhodanobacter	140.00±38.20a	22.00±8.19b	6.525	0.023
Hyphomicrobium	140.33±18.72a	104.33±13.32b	5.918	0.027

Table 6: Continue

Mesorhizobium	109.00±35.00a	16.00±14.42b	5.649	0.03
Rhizobium	105.33±14.15a	15.67±12.66b	7.714	0.016
Alicyclobacillus	47.33±10.41a	273.00±383.16b	-1.047	0.007
Pimelobacter	35.67±3.51a	5.67±9.81b	5.12	0.036
Comamonas	27.33±4.04a	0.00±0.00b	11.714	0.007
Unspecified_Bacteria	19084.00±1751.22a	25152.00±4579.10a		
Others	6641.67±867.83a	9598.67±5844.73a		

Table 7: T-test of root-knot nematode population after soil fumigation

Different treatment	Root-knot nematode population/(J2/100g)	t-value	*p*-value	95% Confidence interval		Root-knot nematode reduction rate/(%)
				Lower limit	Upper limit	
af	36.00±10.00b	- 8.311	0.014	616.57	1940.10	97.26
bf	1314.33±276.11a					—

Discussion

Plant-parasitic nematodes pose a serious threat to crop production. In China, *Meloidogyne spp*. is considered to be the limiting factor for most crop production. Root-knot nematode disease is a worldwide soil-borne disease and it is very difficult to control. Scholars have carried out many reports on it and screened out some safe and effective methods, such as crop rotation, and associated, chemical and biological control, but the effect is limited. The control effect of the watermelon-pepper crop rotation system reached 59.5% (Jiang and Li, 2021), that of different varieties of garlic and tomato was 41.57-77.92% (Liu *et al*., 2021) and that of 41.7% fluopyram suspension for root irrigation and medicine soil hole application were 70.4 and 66.2%, respectively. Soil fumigation technology is a new method that has emerged in recent years (Osman *et al*., 2021; El-Nagdi and Youssef, 2021). Studies have shown that the control effect of cabbage soil fumigation on tomato colonization for 90 days can reach 45.8% (Jin *et al*., 2021). However, biological control and biological fumigant (Cheng *et al*., 2021) have the disadvantages of low control effect and instability due to the complex field environment. In production, chemical control is still the main control means of root-knot nematode (Xi *et al*., 2021).

The study of soil microbial community structure and functional diversity can further analyze the changes in soil microecology and the occurrence of root-knot nematode disease is closely related to crop soil microorganisms. Research on the treatment of soil with chemical fumigant has been reported, but there are few reports on the changes in microbial diversity after soil fumigation. Zhang *et al*. (2021) analyzed the rhizosphere soil microbial communities of healthy and diseased tobacco fields at three levels of phylum, genus, and species and found that Proteobacteria, Gemmatimonadetes, and Ascomycoda were dominant in diseased tobacco fields; Actinoallomurus spadix and Arhrobacterramosus had significant differences between diseased fields and healthy soil species. Zhang *et al*. (2020) reported on the safety evaluation of chloropicrin, dazomet, methamphetamine, and their metabolites on cucumbers and tomatoes. The above fumigants were not detected and the soil microbial diversity after fumigation was not reported.

This study showed that the total OTUs before and after soil fumigation did not reach a significant difference. A total of 2 bacteria kingdoms, bacteria and archaea were detected and the Archaea significantly reduced by 98.42% after soil fumigation. Archaea were widely distributed in many natural environments. They are rich in shapes, have various metabolic types, harmless to other organisms and no pathogenic archaea are known. A total of 36 bacteria phyla were detected, each of which did not reach a significant difference before and after soil fumigation. This research result was consistent with that of Li *et al*. (2017), who fumigated the soil with chloropicrin and weibaimu. The results showed that the composition of the soil bacterial community did not change significantly at the phylum level, but several species with significant abundance differences at the genus and OTU levels.

117 bacteria classes were detected totally. Gammaproteobacteria significantly decreased by 34.36% after soil fumigation. Gammaproteobacteria is the most known diverse class of bacteria, such as Vibrionaceae and pseudomonadaceae. Many important pathogens belong to this class.

227 bacteria orders were detected in total. Xanthomonadales, Cytophagales, and Enterobacteriales significantly decreased by 34.21-69.77% after soil fumigation. Xanthomonas is a genus in the bacteria kingdom, Xanthomonas and Xanthomonaceae, and is the pathogen of plant fusarium wilt and canker. Sporocytophagais a gliding bacterium that degrades cellulose aerobicly and can degrade insoluble cellulose. Enterobacteriales have a wide distribution and a large host range. They live in soil or water and are the most abundant in plants and conditional pathogens.

368 bacteria families were detected in total. Xanthomonadaceae, Hyphomicrobiaceae, and Micrococcaceaesignificantly decreased by 18.70-0.85.80%. Xanthomonas of Xanthomonidaceae can infect more than

400 plant varieties; Micrococcaceae is gram-positive bacteria, which now include Micrococcus, Staphylococcus, and Kinetococcus. Micrococcus is mostly found in soil and water and is non-pathogenic bacteria. Rhizobiacea includes Rhizobium and Brachyrhizobium and they can be made into bacterial preparations and applied in the field as a means of increasing crop yields. Streptomycetaceae is a family of Actinomycetales, which is widely distributed in the soil and a few are plant pathogenic bacteria.

614 bacteria genera were detected in total. *Alicyclobacillus* significantly increased and others all decreased. Among them, *Arthrobacter, Rhodoferax, Pseudomonas,* and *Burkholderia* decreased by 3.22-100% than before fumigation. Alicyclobacillus usually exists in soil and adheres directly to the surface of the fruit through the soil or its special cell structure. 29 species of *Pseudomonas* have been confirmed. P. fluorescens is the most common microbial group in the rhizosphere of plants. It is widely distributed, large in number, and resistant to many plant diseases. *Burkholderia* is a phytopathogenic bacteria that is used in agriculture as a rhizosphere microorganism for biodegradation, biological control, and promotion of plant growth. Streptomyces is a large genus of Actinomycetes, which plays an important role in the mineralization of complex organic matter in the soil and is the most important antibiotic-producing bacteria. Microbacterium is a chemically heterotrophic bacteria, which is mainly respiration metabolism and may also be weakly fermented.

Conclusion

The results of this study showed that the soil fumigation reduced the pathogenic bacteria abundance and root-knot nematode populations of Gammaproteobacteria, Xanthomonadales, Xanthomonadaceae, and Burkholderia, indicating that the fumigation method adopted in this experiment has a significant control effect on tomato root-knot nematode disease. Since the high-efficiency organic sulfur fumigant is chemical, the dosage used should refer to the safe dosage range of the product and the incidence of nematodes in the field to increase or decrease appropriately, to ensure that the seedlings are not burned or harmful gases are generated to poison the plants. In addition, OTUs that cannot be classified and have high abundance account for a relatively high proportion, which provides a direction for the future exploration and development of unknown types of microbial information.

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Author's Contributions

Min Shan, Lihong Liu, and Jing Chen: Designed and performed the experiments, analyzed the data, and prepared the paper.

Min Hao, Lingling Zhang, Shuwen Xue, and Pushun Duan: Participated to collect the materials related to the experiment.

Lijin Qin: Designed the experiments and revised the manuscript.

Ethics

The authors declare their responsibility for any ethical issues that may arise after the publication of this manuscript.

Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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