

# EFFECTS OF DIFFERENT FUNGAL AGENTS ON THE BACTERIAL COMMUNITY STRUCTURE AND DIVERSITY IN THE RHIZOSPHERE SOIL OF WHEAT INFECTED WITH CYST NEMATODES

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**Abstract.** The effects of different fungal agents on the bacterial community structure and biodiversity in the rhizosphere soil of wheat (*Triticum aestivum*) infected with cereal cyst nematodes (*Heterodera avenae*) were analyzed. Field experiments were conducted on plots with severe wheat cyst nematode disease. The control group (X1) was not treated with bacteria. The fungi *Trichoderma viride*, *Paecilomyces lilacinus*, *Aspergillus niger* and *T. spinosum* were applied to the experimental treatments (X2~X4). The experimental group (X6) was treated with the nematicide abamectin. Illumina MiSeq high-throughput sequencing technology was used to monitor the changes in bacterial community structure and metabolic types in the wheat rhizosphere soil in the six experimental groups. The results of high-throughput sequencing showed that the wheat rhizosphere soil bacteria that are pathogens were primarily concentrated in 13 phyla, 26 classes and 98 genera. Among them, the dominant bacteria primarily included *Proteus*, *Actinomyces*, *Acetobacter* and *Bacteroides*. The analysis of bacterial community metabolic type showed that the bacteria in the wheat rhizosphere soil could fix nitrogen, perform nitrification, reduce nitrate, and photosynthesize when the four groups of fungal agents were added at much higher levels than those of the control group. These metabolic activities contributed to promoting the cycle between nitrate nitrogen and ammonium nitrogen to increase soil fertility.

**Keywords:** cereal cyst nematode, biological control, soil improvement, biological diversity, microbial metabolism

## Introduction

Cereal cyst nematode (*Heterodera avenae*) is a soilborne disease, which propagates by infecting wheat (*Triticum aestivum*) root tissue and absorbing nutrients from the host (Li et al., 2010). The infected wheat plants are yellow, stunted, thin, and deficient in fertilizer with reduced tillers and sparse growth (Wang, 1992). Cereal cyst nematode disease was first found in Germany. It was subsequently found in Spain, Australia, and other countries (Liu, 2012). The annual reduction in wheat yield caused by cereal cyst nematode disease can be as high as 23%~89%, and it can even reach more than 90% in areas that have severe infections (Brown, 1982; Philis, 1997). It causes economic losses

of up to 78 billion USD per year (Zhang et al., 2018). In China, cereal cyst nematode was first found in Hubei in 1989. Currently, cereal crops in Hebei, Jiangsu, Shandong, and other places are plagued by infections with cereal cyst nematode (Chen et al., 1991; Yu et al., 2012). It has been reported that cereal cyst nematode disease has been found in Shangqiu, Yuzhou, Weihui, Luoyang, and other areas in Henan. In addition, wheat yield reduction caused by pests and diseases accounts for 35% of the total (Peng et al., 2010; Yuan et al., 2012). At most, there are also 10%~28% yield reductions in wheat (Kong et al., 2021) in Hebei, Beijing, Qinghai, and other regions. Currently, the diseases of cereal crops caused by cyst nematodes in China are primarily caused by cereal cyst nematodes and Philip cyst nematodes (Peng et al., 2010; Cui et al., 2017). The occurrence of the disease and pest has seriously threatened the production of wheat and therefore, food security in China.

The prevention and control of cereal cyst nematode diseases and pests primarily includes several methods, such as breeding tolerant varieties (Cheng et al., 2015), fallowing the field and rotating crops, spraying chemical agents (Liu, 2012) and applying biological controls (Ismail et al., 2001; Cui et al., 2012). Planting wheat varieties that are resistant to cyst nematode is one of the most effective methods. Australia and other countries have curbed cyst nematode disease by planting disease resistant wheat (Eastwood et al., 1991; Rathjen et al., 1998). Currently, Yangzhou No. 5 and Zhengzhou No. 831 have some resistance to cyst nematodes in China (Zheng et al., 1999; Cui et al., 2020). The resistance of other wheat varieties to cyst nematode disease is weak, thus they are very susceptible to cyst nematode disease (Andersen, 1959; Andersen and Andersen, 1982). Additionally, the breeding cycle of resistant varieties tends to be long, and different anti-disease genes exhibit complete or partial resistance to different cyst nematodes. Nicol and Rivoal (2007) reported that some *Cre* genes are resistant to specific races and pathotypes of the cereal cyst nematode. Modern biological control methods primarily use parasitic bacteria and fungi to induce plant resistance through parasitism, competition or by changing the microbial community structure of rhizosphere soil microorganisms to achieve control, which is a safe and green control method (Zhang et al., 2014). Wheat roots secrete proteins, lipids, amino acids, ketones, and other nutrients during growth and development; these chemicals will not only stimulate cereal cyst nematode eggs to hatch and increase its rate of incidence but also affect the structure of microbial flora in the rhizosphere soil (Wang et al., 2021; Jiang et al., 2021). Rhizosphere soil is affected by plants and microorganisms, and its characteristics, nutrition, and functions are completely different from those of other soils. The diversity of rhizosphere soil microorganisms can reflect the functional evolution of its ecosystem and changes after environmental stress, and it can accurately reflect the relationship between the change in soil microbial flora and the external environment (Edgar, 2013; Wang et al., 2019). Analyzing the change of microbial colony structure in the soil can not only reflect the difference of bacterial population between diseased wheat and normal wheat but also enhance knowledge of how the bacterial population changes during the production of plant resistance, which can enhance stable yields and promote the growth of wheat. This experiment utilized Illumina MiSeq sequencing technology to analyze the bacterial colony structure of wheat rhizosphere soil microorganisms following treatment with different fungal agents. They were compared in terms of bacterial structure, diversity composition, and macrometabolic type. This study provides a theoretical basis for the development of new agents to control cereal cyst nematodes.

## Materials and Methods

### Overview of the experimental site

The experimental site is located at the Luyou Family Farm, Runan County, Zhumadian City, Henan Province (33°00'46.1"N, 114°36'13.8"E). The surrounding area is convenient for transportation and management. The area of the field is more than 100 hm<sup>2</sup>; the contour is square; the terrain is flat, and the soil is evenly fertile. The average altitude of the experimental site is 84 m. The climate belongs to the humid semi-humid monsoon climate with abundant sunshine and four distinct seasons. The annual average temperature is 15°C; the annual precipitation is approximately 890 mm, the annual average sunshine is more than 1,854.1 hours, and the frost-free period is 230 days.

The measurements of the organic matter, alkali-hydrolyzable N, and available K contents in soil have been clarified: "Soil samples were collected 15 cm below the surface in the plot using a five-point sampling method, with three replicates for each point. After drying, the contents of organic matter, alkali-hydrolyzable N, available K, and available P were measured to evaluate the nutrient abundance in the original soil.". The content of organic matter, alkali hydrolyzable nitrogen, available potassium, and available phosphorus in the topsoil of the experimental plot were 90 g/kg, 109 mg/kg, 208 mg/kg and 101 mg/kg, respectively.

In this study, the density and distribution of *Heterodera avenae* in the plot were investigated during soil sample collection, and the results have been discussed in the manuscript: "The density of *Heterodera avenae* in the plot was investigated by random sampling. Specifically, 10 plots (30 m<sup>2</sup>) were randomly selected, and a sampling area that exceeded 50% of the total area was used to guarantee data validity. Soil samples were collected from each plot using a five-point sampling method, and sporangia of *Heterodera avenae* were isolated. As indicated, 147, 153, 143, 147, 141, 150, 144, 139, 151, and 147 sporangia/500 g soil were identified in the 10 plots, respectively, suggesting a uniform distribution of *Heterodera avenae* sporangia in the plot, and allowing field tests to be conducted.

### Experimental materials

New types of biocontrol agents against cereal cyst nematodes include *Trichoderma viride*, *T. echinococcum*, *Aspergillus niger*, and *Paecilomyces lilacinus* with effective live spores  $\geq 5 \times 10^8$  CFU/g, which were provided by Institute of Biology, Henan Academy of Sciences Co., Ltd. (Zhengzhou, China). Avermectin, with an effective component of 0.5%, was produced by Shenzhen Novozymes Agrochemical Co., Ltd.

### Experimental methods

The experiment was designed with six treatments. Each treatment was repeated in three groups, and each treatment was randomly arranged with each test area of 30 m<sup>2</sup>. Treatment 1 was the control group with severe disease, and no fungal agent was applied. Treatments 2 and 3 were treated with *T. viride* and *P. lilacinus*, respectively. Treatments 4 and 5 were treated with *A. niger* and *T. spinosum*, and treatment 6 was 0.5% of avermectin. Treatments 1 to 6 were designated X1~X6. Before the wheat was planted, the treatments were sprayed on the soil surface and ploughed under the soil layer. Other fertilizers were applied normally.

### ***Soil sample collection***

The soil samples were taken when the wheat had matured. A five-point sampling method was used to collect the soil samples. The stones and gravel were removed, and the five samples were mixed evenly. They were then sealed with self-sealing bags and stored in ice boxes. The collected soil sample was divided into two parts after passage through a 20-mesh sieve. One part was stored in a sterile centrifuge tube at -80 °C for future use, which was used to extract the soil genome, and the other part was used to determine the soil physical and chemical properties after air drying.

### ***Determination of soil physical and chemical properties***

The content of hydrolyzed nitrogen in the soil was determined by alkali hydrolysis diffusion. The content of available phosphorus in the soil was determined by sodium bicarbonate extraction molybdenum antimony resistance colorimetry. The content of available potassium in the soil was determined by ammonium acetate extraction flame photometric method, and the content of organic matter in soil was determined by oil bath heating potassium dichromate volumetric method.

### ***DNA extraction and amplification of the 16S rRNAV3-V4 region***

The DNA of soil samples was extracted with a soil genome extraction kit. The concentration and purity of the extracted genomic DNA were detected by 1% agarose gel electrophoresis. The qualified samples were used as templates to amplify the 16S rRNAV3-V4 region with primers 338F and 806R.

### ***Analysis of the high-throughput sequencing data***

The PE reads obtained were first spliced according to the overlay relationship. Quality control and filtering of the sequences was performed after the samples had been distinguished; after this, operational taxonomic unit (OTU) cluster and species taxonomy analyses were performed (Wang et al., 2007; Edgar, 2013). Based on the results of OTU clustering analysis, the diversity index of OTU was analyzed, and the sequencing depth was detected. The taxonomic information entailed statistical analyses of the community structure at various classification levels, and the relevant information mining and mapping were completed by R software (Jaime, 2016; Emmanuel et al., 2018).

### ***Data processing***

The results of physiological and biochemical determination of soil samples were collated by Microsoft Excel (Redmond, WA, USA) and analyzed by DPS 7.05. The data between the samples and sample groups were analyzed by a Tukey test in a one-way analysis of variance (ANOVA).

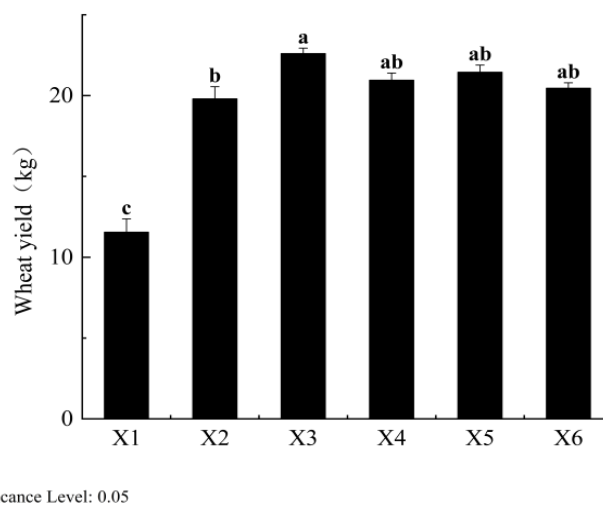
After PCR amplification, the samples were sent to Shanghai Sangong Biotechnology Co., Ltd. (Shanghai, China) for sequencing using an Illumina MiSeq sequencing platform.

## **Results and analysis**

### ***Statistics of wheat yield***

As shown in *Figure 1*, the yield of wheat in the control group infected with cereal cyst nematode was 11.5 kg, which was the lowest, while that of the *P. lilacinus* group was

22.6 kg, which was the highest. The yield of other groups was more than 19 kg. The difference between the control group and other experimental groups was significant ( $P \leq 0.001$ ), which indicated that the other experimental groups successfully controlled infection with cereal cyst nematodes. There was some difference between the *T. viride* and *P. lilacinus* groups ( $P \leq 0.05$ ), but there was no significant difference between the other experimental groups ( $P > 0.05$ ). This indicated that *P. lilacinus* was the most effective at controlling this disease based on the yield of wheat. There was no significant difference between the abamectin group and the experimental group that applied the fungal agents ( $P > 0.05$ ). There was a very significant difference between the control and abamectin groups ( $P \leq 0.001$ ), which indicated that the fungal agent and abamectin could play a significant role in controlling cereal cyst nematodes.



**Figure 1.** Statistical significance analysis of wheat yield in different product groups. \* $P \leq 0.05$ . \*\*\* $P \leq 0.001$ . (X1: control group, X2: *T. viride*, X3 *P. lilacinus*, X4: *A. niger*, X5 *T. spinosum*, X6: 0.5% of avermectin)

### Soil physical and chemical properties

Based on the analysis of soil physical and chemical properties of different fungal agents in the experimental field of Runan Luyou Family Farm (Table 1), the contents of organic matter, alkali hydrolyzable nitrogen, available potassium and available phosphorus in the soil samples treated with fungal agents were higher than those in the control group ( $P < 0.05$ ). The organic matter and effective phosphorus content were the highest at 114 mg/kg and 141 mg/kg, respectively, and the highest was 129 mg/kg in *K. A. niger* and the others were both above 265 mg/kg in the control group ( $P > 0.05$ ) and the experimental group with fungal agent ( $P < 0.05$ ). In general, the use of avermectin can achieve some control. However, the use of biocontrol agents can not only control cereal cyst nematodes but also improve the soil nutritional structure.

### Results of high-throughput sequencing data analysis

A total of 267,479 raw sequences with a total base number of 120,918,592 bp were obtained in the 190s from the six samples after high-throughput sequencing by Illumina MiSeq. After optimization and quality control filtering, 265,829 effective sequences were obtained; the number of effective bases was 110,352,175 bp, and the average length was

451 bp. The six soil samples produced 2,747 OTUs. X4 had the highest number of OTUs treated with 599, while X6 had the lowest number of OTUs at 261. The remaining treatments produced approximately 330 OTU numbers.

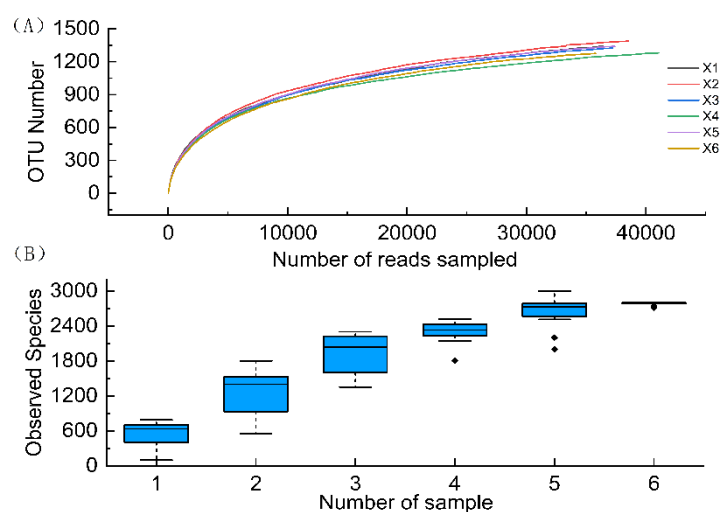
**Table 1.** Physical and chemical properties of the soil in plots with different fungal agents

| Processing group | Organic matter (g*kg <sup>-1</sup> ) | Alkali hydrolyzable nitrogen (mg*kg <sup>-1</sup> ) | Available potassium (mg*kg <sup>-1</sup> ) | Available phosphorus (mg*kg <sup>-1</sup> ) |
|------------------|--------------------------------------|-----------------------------------------------------|--------------------------------------------|---------------------------------------------|
| X1               | 90c                                  | 109c                                                | 208a                                       | 101d                                        |
| X2               | 107b                                 | 129b                                                | 221b                                       | 141b                                        |
| X3               | 114a                                 | 124a                                                | 236c                                       | 108c                                        |
| X4               | 106b                                 | 136d                                                | 265d                                       | 118a                                        |
| X5               | 103b                                 | 187e                                                | 268d                                       | 136e                                        |
| X6               | 92c                                  | 107c                                                | 202a                                       | 103d                                        |

Note: Different lowercase letters in the same column indicate a significant difference at the 0.05 level. (X1: control group; X2: *T. viride*; X3: *P. lilacinus*; X4: *A. niger*; X5: *T. spinosum*; X6: 0.5% of avermectin)

The dilution curve involved randomly selecting a certain number of sequences from the samples, and the  $\alpha$ -diversity index of the samples that corresponded to these sequences was counted. The data was drawn on the horizontal axis, with the  $\alpha$ -diversity index value as the vertical axis. The amount of sequencing data was sufficient based on whether the curve is flat.

The dilution curve (Figure 2A) showed that new OTUs still appeared when the sample read length exceeded 30,000. The dilution curve had flattened at a read length of approximately 35,000. The library coverage had exceeded 98% (Table 2), indicating that the OTU coverage of the sample had reached the assessment criteria when the sampling depth can truly reflect the diversity of bacterial communities in the soil. The species accumulation bin plots indicated that the rate of new observed species that emerged under continuous sampling will flatten out as the number of samples increases (Figure 2B), indicating that the number of bacterial species in the environment did not increase significantly with the number of samples, and the sequenced samples were sufficient to cover the bacterial population of the test sample.



**Figure 2.** High flux sequencing dilution curve (A) and species accumulation box diagram (B)

**Table 2.**  $\alpha$ -Diversity index of rhizosphere soil bacterial community of cereal cyst nematode with different bacterial agents

| Group | OTUs | ACE index | Shannon index | Simpson index | Coverage (%) |
|-------|------|-----------|---------------|---------------|--------------|
| X1    | 214  | 1277b     | 3.986b        | 0.013b        | 99.00a       |
| X2    | 592  | 1613a     | 5.538a        | 0.123a        | 98.94a       |
| X3    | 567  | 1664a     | 5.593a        | 0.132a        | 98.82a       |
| X4    | 514  | 1569a     | 5.475a        | 0.097a        | 99.14a       |
| X5    | 599  | 1592a     | 5.606a        | 0.135a        | 98.84a       |
| X6    | 261  | 1336b     | 4.379b        | 0.014b        | 98.88a       |

Note: Different lowercase letters in the same column indicate a significant difference at the 0.05 level. (X1: control group; X2: *T. viride*; X3: *P. lilacinus*; X4: *A. niger*; X5: *T. spinosum*; X6: 0.5% of avermectin)

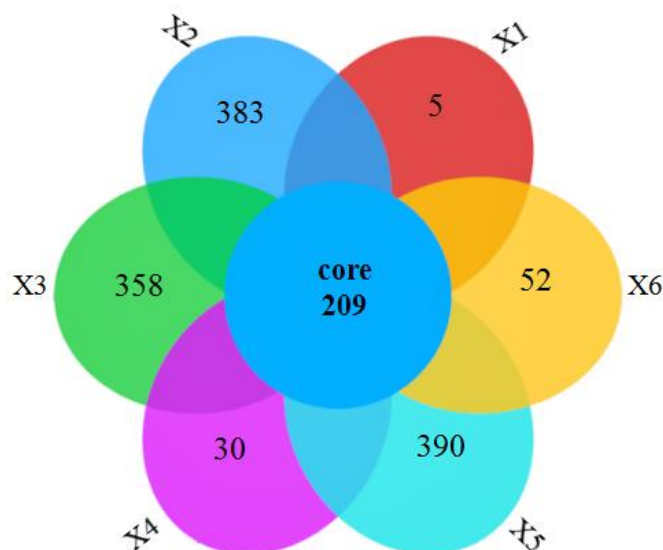
### Analysis of bacterial structural diversity among the samples

A total of 2,747 OTU was obtained from six soil samples. As shown in Table 2, the ACE index was 1,592; the Shannon index was 5.606, and the Simpson index was 0.135. The control group had an OTU number of at least 214, whose ACE, Shannon and Simpson indices were 12,773, 986, and 0.013, respectively. The index of the avermectin group was similar to that of the control group, and the difference was not significant ( $P > 0.05$ ). The results of OTU number, ACE index, and the Shannon and Simpson indices of the *T. viride*, *P. lilacinus* and *A. niger* groups were similar to those of the *T. echinococcus* group, but it was significantly different from the control group ( $P < 0.05$ ). The results showed that the species diversity and richness in the fungal-treated soil samples were significantly higher than those in the control and avermectin groups. In terms of species abundance, *P. lilacinus* group > *T. viride* group > *T. spinosum* group > *A. niger* group > avermectin group > control group. The species diversity was shown to be the following: *T. spinosum* group > *P. lilacinus* group > *T. viride* group > *A. niger* group > avermectin group > control group. The difference in the bacterial colony structure of the avermectin group and fungal agents illustrates the different mechanisms of action in wheat.

### Wayne diagram of bacterial distribution between samples

The Wayne diagram can be used to count the number of total and unique OTUs of the samples. It visually shows the similarity and overlap of the OTU number composition of the environmental samples. As shown in Figure 3, 2,747 OTUs were produced in the wheat rhizosphere soil of the six treatments, including 214, 592, 567, 514, 599 and 261 OTUs in X1~X6. Among them, there were 209 OTU numbers. In the unique OTU numbers, the least five control OTU numbers accounted for 0.18% of the total number of OTUs. The *T. echinococcus* group had the largest number of OTUs, with 599 accounting for 21.8% of the total OTUs. The OTU numbers of the six soil samples from large to small were as follows: X5 > X2 > X3 > X4 > X1 > X6. The bacterial community composition of the whole Venn diagram showed that the OTU numbers produced by the control group were significantly lower than those of the applied fungal agent groups, which indicates that the number of bacterial communities in the soil was reduced compared with the treatments in which avermectin was not applied. This was consistent with the data of the soil bacterial diversity index, and to some extent, indicates that the decrease of bacterial species in the soil will increase the risk of wheat infection. There was no significant

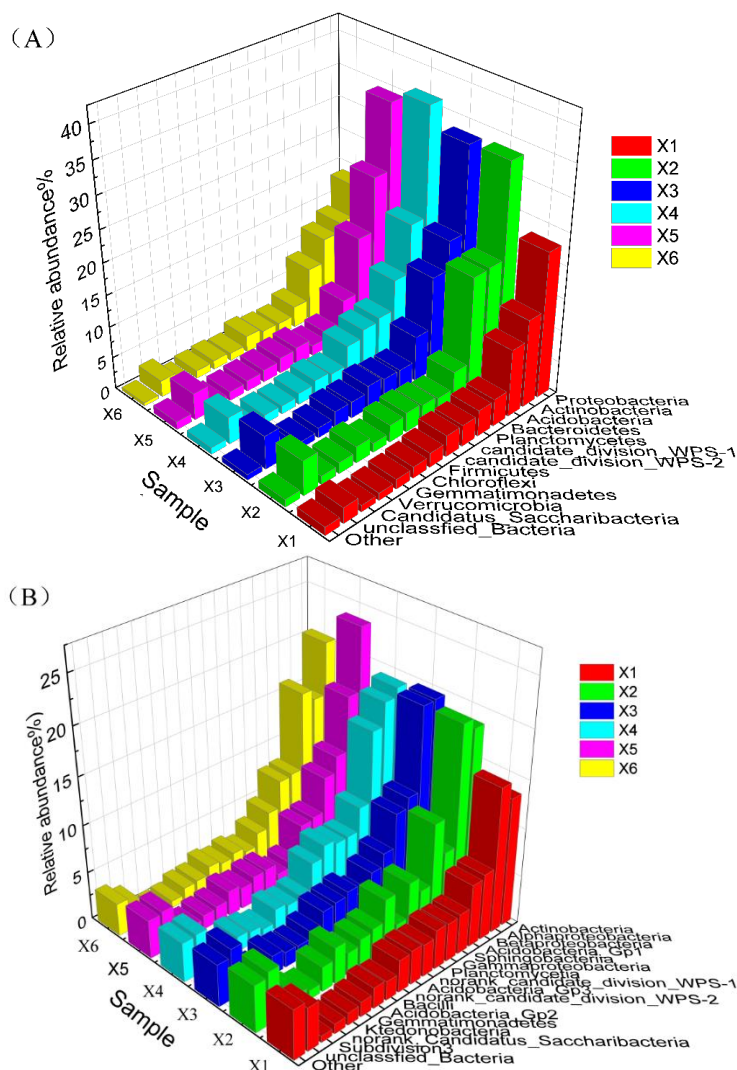
difference in the OTU number between the control and avermectin groups, which indicates that the chemical did not improve the wheat rhizosphere soil and also shows the differences in the mode of action between the biological and chemical agents.



**Figure 3.** Venn diagram of species distribution under different fungal treatments. (X1: control group; X2: *T. viride*; X3: *P. lilacinus*; X4: *A. niger*; X5: *T. spinosum*; X6: 0.5% of avermectin)

#### Structure of dominant bacterial communities at different treatment gate levels and class levels

At the phylum level, 13 bacterial phyla were identified from the six samples (Figure 4A). Excluding the undetermined genera, they were as follows: Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, Planctomycetes, Candidate\_division\_WPS-1, Candidate\_division\_WPS-2, Firmicutes, Chloroflexi, Gemmatimonadetes, Verrucomicrobia and Candidatus Saccharibacteria. Among them, the dominant bacterial families (with a relative abundance of more than 5%) were Proteobacteria, Actinobacteria, Acidobacterium, and Bacteroidetes. The relative abundance was as follows: 18.6%~36.2%, 13.7%~19.8% and 10.6%~18.5%, 5.1%~8.4%, respectively. As shown in the phylum-level species abundance map, the species abundance of dominant bacteria in the diseased soil was significantly lower than that where the fungal agents were applied. Compared with the control, the species abundance of *Proteus* in the soil treated with *T. viride*, *P. lilacinus*, *A. niger* and *T. spinosum* increased by 10.2%, 11.1%, 14.2% and 12.3%, respectively. The species abundance of *Acidobacterium* increased by 4.8%, 5.9%, 5.4% and 10.5%, respectively. The relative abundance of species from the other phyla also increased. The species content of the avermectin group was not significantly different from those of the control group. This also revealed the drawbacks of chemical pesticides in improving the structure of soil microbial communities. The four fungal agent groups significantly improved the bacterial community structure in the diseased soil and increased the number of dominant bacterial groups, thus, effectively inhibiting the occurrence of disease.

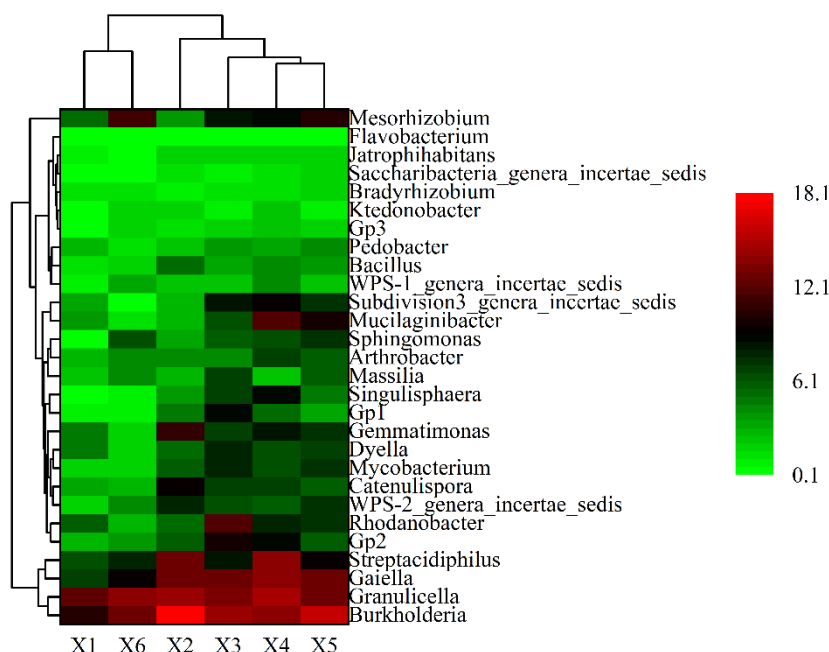


**Figure 4.** Species abundance of different species at the level of phylum (A) and at the level of class (B). (X1: control group; X2: *T. viride*; X3: *P. lilacinus*; X4: *A. niger*; X5: *T. spinosum*; X6: 0.5% of avermectin)

The bacterial communities were annotated in the six soil samples at the class level (Figure 4B), and the seven dominant bacteria were as follows: Actinobacteria, Alphaproteobacteria, Betaproteobacteria, Acidobacteria\_Gp1Sphingobacteria, Gammaproteobacteria, and Planctomycetia. The relative abundances of these dominant bacterial classes in the control group were 13.4%, 15.1%, 7%, 6.6%, 4.2%, 4%, and 4.1%, respectively. After the application of the four biological agents, the relative abundance of Actinobacteria and  $\beta$ -Proteobacteria in the *A. niger* group was 23.6% and 16.1%, which was 10.2% and 9.1% higher than that in the control group. The *P. lilacinus* group of  $\alpha$ -Proteobacteria increased by 4.4% over the control group. In the other experimental groups, the dominant bacteria were improved compared with those in the control group. From the relative abundance map of the species at the class level, the fungal treatments significantly increased the number of the dominant microflora in the wheat base soil, which is consistent with the findings of the phylum-level analysis.

### Horizontal cluster heatmap analysis of different treated genera

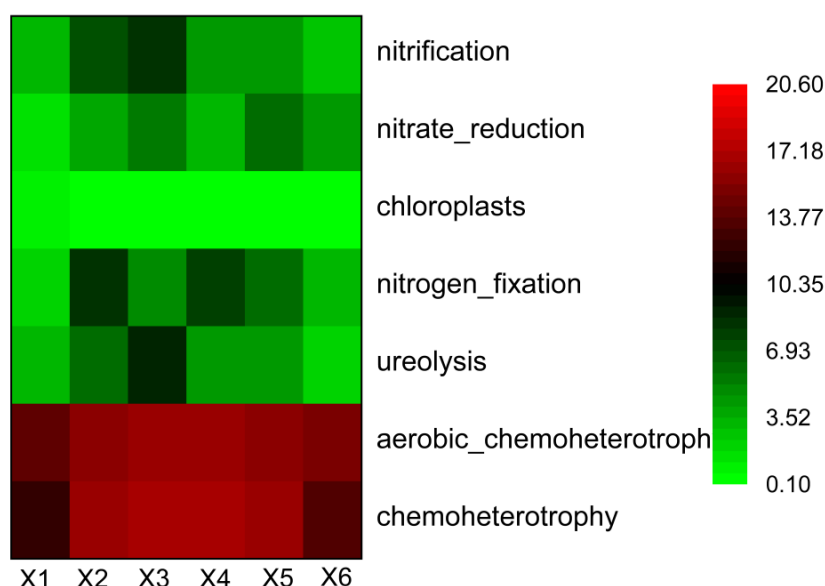
A horizontal cluster heat map of the different sample genera was established (Figure 5). As shown from the figure, 28 bacterial genera were pooled in the six experimental groups, including the controls. Among them, *Burkholderia*, *Gaiella*, *Granulicella*, *Massilia*, *GP1*, *GP2*, *Bacillus*, *Mesorhizobium* and *Sphingomonas* were highly abundant. In terms of species abundance, the abundances of the control and abamectin experimental groups were lower compared with the experimental groups that contained the fungal agents. A clustering analysis of the OTU abundance data of the bacterial genera described above showed that the six samples, including the control group, were divided into two major categories. The control group with severe disease and the experimental group with abamectin were classified into one category, and the other experimental groups that had been treated with fungal agents were classified into one category. From the perspective of the dominant genera of each treatment, the control group and the abamectin group were dominated by *Burkholderia*, *Marseilles*, *Gracilaria*, and *Mesorhizobium*. In the experimental group with fungal agents, in addition to the higher relative abundance of these genera, the relative abundances of *Gaiella*, *Rhodobacter*, and *Gemmatimonas* were also higher. The relative abundance of *Burketella* in the green *Trichoderma* group was 18.1%, which was 7.7% higher than that in the control group. The relative abundance of *Gaiella* in the *A. niger* group was 7.2% higher than that in the control group. In the *P. lilacinus* group, the relative abundance of *Marseilles* and *Mesorhizobium* increased by 4.8% and 2.9% compared with the control group, respectively. The dominant genus content in the *Spinosporum* group was also higher than that in the control group. In the four experimental groups that were treated with fungal agents, the species composition of the *A. niger* and *T. spinosum* groups were the most similar, while the relative abundance of *T. viride* group and the other three groups differed in their relative abundance, although these differences were not significant.



**Figure 5.** Cluster heatmap of the relative abundance of bacteria at the genus level. (X1: control group; X2: *T. viride*; X3: *P. lilacinus*; X4: *A. niger*; X5: *T. spinosum*; X6: 0.5% of avermectin)

### The metabolic function analysis of microbial communities in different treatments

In Figure 6, we selected seven bacterial community metabolic types closely related to soil properties and crops to create a microbial community metabolic function heatmap to reflect the impact of different treatments on plants and soil. The metabolic types of seven bacterial communities include chemoheterotrophic, aerobic-chemoheterotrophic, ureolytic, nitrogen-fixation, chloroplast photosynthesis, nitrate reduction, and nitrification. The first three metabolic types are very common in soil, and microorganisms of these metabolic types are found in most farmland soils. The latter four metabolic types are closely related to soil nutrition. As shown in Figure 6, the abundance of nitrogen fixing microorganisms in the four experimental groups with fungal agents was higher than those in the control and avermectin groups, which indicates that the application of fungal agents can effectively increase the species abundance of nitrogen fixing microorganisms. The abundance of microorganisms that promote the photosynthetic phosphorylation of crops also increased significantly, which facilitates crop growth. Bacteria that can reduce nitrate oxidize organic compounds with nitrate as the final receptor can grow using their energy. They produce ammonia and utilize crops at intermediate stages. In this experiment, such microorganisms were abundant in the *T. viride*, *P. lilacinus* and *A. niger* groups, which indicated that the content of ammonia nitrogen available for absorption by the plants was also high. Nitrification is the process of converting the oxidized ammonia nitrogen in the soil to nitric acid, and it is further oxidized to nitrate. This eventually reacted with the production of nitrate and is the primary source of nitrogen for dryland plants. According to the heatmap, among the seven major microbial metabolic types, the microbial species had the highest abundance in biological nitrogen fixation, nitrite reduction, and nitrification, while the remaining three groups also had more beneficial fungal agents than the control and avermectin groups. In conclusion, fungal agents can effectively improve the abundance of microbial species and thus, improve the soil fertility while controlling diseases.



**Figure 6.** Metabolic function heatmap of different sample microbial communities. (X1: control group; X2: *T. viride*; X3: *P. lilacinus*; X4: *A. niger*; X5: *T. spinosum*; X6: 0.5% of avermectin)

## Discussion

In this study, the effects of fungal inoculants on the structure and diversity of bacterial colonies in the rhizosphere soil of wheat with cyst nematode disease were investigated by high-throughput sequencing. The results demonstrated that the microorganisms could protect plants from disease by parasitism, the secretion of metabolites inducing cyst-nematode death, and inducing the generation of anti-cyst nematode substances. Indeed, the wheat yield and organic matter, alkali-hydrolyzable N, available K, and available P contents in the soil of the experimental group were significantly higher than those of the control group. This is consistent with the previous study, wherein the contents of total nitrogen, alkali-hydrolyzable N, and available K in the soil were found to increase by 21.0, 57.3, and 63.3%, respectively, when beneficial inoculants were introduced to the rhizosphere soil of tobacco plants. In terms of the  $\alpha$ -diversity of the rhizosphere soil, the ACE and Shannon indices of the microbial flora in the wheat rhizosphere soil increased markedly after the application of inoculants, suggesting that the abundance and diversity of the bacterial colonies in the soil had increased. Jiang et al. (2021) reported that the abundance and diversity indices of bacteria in the rhizosphere soil of potato plants infected with *Meloidogyne* were lower than those in the rhizosphere soil of non-infected potato plants. In the present study, the structure, composition, and metabolic type of the microbial flora in wheat rhizosphere soils were investigated after the application of different inoculants. As indicated, the microbial flora in the wheat rhizosphere soil could be divided into 98 genera (e.g., *Streptococcus*, *Rhizobium*, and nitrifying bacteria), 26 classes, and 13 phyla, with the dominant phyla including Pseudomonadota, Actinomycetota, Acidobacteriota, and Bacteroidota. The microbial flora of the six samples showed consistent structures but varied in terms of species abundance. The Actinomycetota exhibited the highest species abundance in samples with fungal inoculant application. While these species were present in the control group, their abundance was extremely low but increased upon application of fungal inoculants, demonstrating that the application of fungal inoculants can effectively enhance the abundance of beneficial microorganisms in the soil, thereby inhibiting growth of cyst nematodes and improving crop yields. Zhang et al. (2023) reported that the structure of the microbial flora in the rhizosphere soil of winter wheat was improved by fertilizer application, with high-throughput sequencing results revealing that Pseudomonadota, Actinomycetota, and Acidobacteriota were the dominant phyla in these soils after fertilizer application. This is consistent with the conclusions of the present study.

Microorganisms in the soil participate in the metabolism and cycling of various substances in the soil, with variations seen in the functions of microorganisms of different metabolic types. Zhang et al. reported that the genus-level abundance of bacteria and fungi were significantly correlated with the physical and chemical properties of the soil, including pH, organic matter, NO<sub>x</sub>-N, available P, and total P. Changes in these parameters lead to changes in the structures of bacterial communities, thus facilitating the elucidation of the influence of the microorganisms in the wheat rhizosphere soil on plants, as well as the underlying mechanisms. In this study, 12 metabolic types of soil-related microorganisms were identified in the six samples, with seven of these generating abundant OTUs. In the control group, the abundance of microorganisms in the soil was reduced, with most of the microbial flora with beneficial metabolic functions having negligible influences. The abundance of nitrogen-fixing, nitrifying, photosynthesis-promoting, iron-respiration, and nitrate-reducing bacteria increased in the four soil samples after the application of fungal inoculants. These bacteria are involved in nitrogen

fixation, iron respiration, and degradation of nitrate nitrogen (into ammonium nitrogen), and some may be able to degrade hazardous aromatics. Thus, the metabolic activity of these bacteria can increase the contents of N, P, K, and organic matter in the soil.

In this study, four types of fungi, *T. viride*, *P. lilacinus*, *A. niger* and *T. spinosum*, were used as research objects to explore the mechanism of their role in controlling cereal cyst nematode in soil. The roles of different fungi were analyzed in terms of species diversity and the metabolism of bacteria. *P. lilacinus* achieved the best results in terms of actual wheat yield and soil physical and chemical properties. From the perspective of improving the biological community structure and increasing the species abundance, *T. viride*, *P. lilacinus*, *A. niger* and *T. spinosum* had obvious effects.

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