Changes in Microbial Community and Activities in Continuous Pepper Cropping Soil After a Trichoderma Hamatum MHT1134 Application

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Research Article

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Changes in microbial community and activities in continuous pepper cropping soil after a *Trichoderma hamatum* MHT1134 application

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Abstract To clarify the control effects of strain MHT1134 on *Fusarium* wilt in continuous pepper cropping fields and its regulatory effects on soil microecology, the physical and chemical properties, enzyme activities, community structures of soil samples from five field types were analysed. Samples were taken from fields in their 1st, 5th and 9th planting years, and at 1 and 2 years after the application of strain MHT1134 in 9th planting year field. The MHT1134 control effects on pepper wilt after 1 and 2 years were 63.03% and 70.21%, respectively. 4 kinds of physical and chemical indexes and 6 kinds of enzyme activities in soil were increased. With the continuous cropping years increased, the microbial abundance and diversity decreasing significantly. The relative abundances of *Fusarium*, *Gibberella* increased along with the planting years, but decreased after the MHT11134 application. However, the relative abundances of *Trichoderma* and *Chaetomium* significantly increased. Additionally, as the cropping years increased, the soil abundance of Actinobacteria gradually decreased, but it significantly increased from 17.56% to 22.44% after the MHT1134 application. Thus, strain MHT1134 effectively improved the microbial community structure of the soil, and it also positively affected soil quality. A continuous application may improve the control effect.

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Introduction

Pepper *Fusarium* wilt is an important and frequent disease, which is caused by *Fusarium oxysporum* Schlecht, in pepper (*Capsicum annuum* L.) production. It is a worldwide soil-borne disease[1] and is especially common in continuous cropping fields, in which *F. oxysporum* is more likely to occur and cause serious yield losses. *Fusarium oxysporum* sporifera have a wide host range and can live in the soil for a long time, especially during the continuous cultivation of pepper plots. It can accumulate and spread in the field year after year, leading to widespread disease occurrences, resulting in more than 80% yield losses in severe cases[2,3]. It is difficult to control this soil-borne disease. At present, pepper *Fusarium* wilt is still mainly controlled by chemical fungicides. However, pesticide applications not only fail to control the disease effectively, they also destroy the soil microecology, inhibit the reproduction of beneficial microorganisms in the soil, and allow pathogenic microorganisms to evolve and multiply. The agricultural soil environment is polluted

by residual harmful substances, which alter the balance of soil microbial population structures and negatively affect soil health[4].

Biological control is environmentally friendly and can overcome the disadvantages caused by pesticides. In particular, the exploitation and utilisation of microbial resources have become in plant disease control[5]. For instance, antagonistic fungi, such as *Trichoderma harzianum* strain CH1, *Trichoderma asperellum* strain MC1, *Trichoderma brevicompactum* MF1[6], *Chaetomium globosum* LJ-S2L1[2] and the mutant of *Trichoderma viride* TvM2-UV/60 show activities against *F. oxysporum*[7]. Additionally, some antagonistic bacteria, such as isolates BR6 and BR9[8], *Bacillus* sp. MB015 and *Pseudomonas* sp. MB108[9] have obvious inhibitory effects on *F. oxysporum*. *Bacillus subtilis* and *Pseudomonas fluorescens* show superior inhibitory effects against *F. oxysporum* growth[10]. Moreover, many biocontrol agents regulate microbial community structures when applied to soil. Zhong *et al.*[11] found that applying bio-organic fertilizer for two consecutive years not only significantly reduces the *F. oxysporum* level and makes the soil culturable microbial community structure more balanced, but it also improves the control effects on *F. oxysporum*, resulting in improved yield and fruit quality. Shen *et al.*[12] found that by applying microbial agents for two consecutive years, soil bacterial diversity and microbial community structure became optimised, thus reducing the plant disease incidence. At present, research on pepper *Fusarium* wilt mainly focuses on the differences in soil microbial community diversity between diseased and healthy plants. There are few studies on the changes in soil microecology, the physical and chemical properties, and enzyme activity levels after the application of biocontrol agents in the diseased plots of continuous cropping systems. This study used a field group test. The Illumina MiSeq 2500 high-throughput sequencing platform was used to study the effects of a continuous 2-year application of the biocontrol bacterium MHT1134 fermentation broth on the microbial community structure and physicochemical properties of pepper rhizosphere soils in continuous cropping plots. The aims were to explore the soil microbiological mechanisms used by the biocontrol bacterium MHT1134 against pepper *Fusarium* wilt and to provide a theoretical reference for the development of antimicrobial control agents against this disease.

Materials and methods

Fungal strain and fermentation broth preparation

Trichoderma hamatum strain MHT1134 was originally isolated from pepper soil by Mao and colleagues from Guizhou University and Guizhou Institute of Plant Protection, China. It was stored in the China Center for Typical Microbiology (CCTCC 2018709). The strain can not only achieve 81.80% inhibition of *F. oxysporum*, but also has good inhibitory effects on seven other pathogens. In addition, the application of the MHT1134 fermentation broth increases the pepper yield after one year[13]. To prepare the MHT1134 inocula, the strain was placed on potato dextrose (PD) agar plates and incubated in the dark at 28°C for 7 days. Then, the culture was made into cakes and inoculated into PD liquid medium with shaking at 160 rpm at 25°C for 7 days. Afterward, the inocula was filtered using double-layered gauze. To

increase the spore content of the MHT1134 fermentation broth, it was allowed to grow for another 5 days. Finally, sterile distilled water was added to adjust the inoculation density to 1×10^6 cfu/mL.

Profile of the test site and field assays

Field tests were conducted in pepper bases from May 2017 to November 2017 and from May 2018 to November 2018. The test site is a project test site of the Institute of Plant Protection, Guizhou Academy of Agricultural Sciences, China, where field tests and investigations can be carried out. The experimental site was located in the pepper planting base of Huangnitang Town, Dafang County, Bijie City, Guizhou Province, China (27° 10′ 24″ N, 105° 42′ 17″ E, elevation 1,314.0 ± 3 m). The flat plots in their 1st, 5th and 9th continuous planting years were selected from the experimental area. These plots were in the same location and shared the same soil quality and terrain. The soil type was yellow, and the climate was subtropical humid monsoon. The average temperature in the hottest month (July) was 20.7°C. In addition, over the last 3 years, pepper wilt had occurred in the plots in their 5th and 9th continuous cropping years. The experimental site for MHT1134 inocula application was the plot in which pepper had been planted for 9 consecutive years.

Experimental design

The purpose of this experiment was to collect five kinds of soil samples from pepper fields that had been continuously planted for 1, 5 and 9 years and apply the biocontrol bacteria for 1 and 2 years in the latter. There were five experimental treatments, and each treatment was applied to three plots, which were randomly arranged in groups. There were 15 plots in total, and each plot had an area of 8 $0m^2$, which held 308 peppers. The plant and row spacings of each plot were 30 cm and 80 cm, respectively. The five treatments were as follows: plots undergoing continuous pepper cultivation for 1 year (CC1); plots undergoing continuous pepper cultivation for 5 years (CC5); plots undergoing continuous pepper cultivation for 9 years (CC9); plots in which the MHT1134 fermentation broth was applied 1 year in advance (2018) to pepper fields that had been continuously cropped for 9 years (TR1); and plots in which the MHT1134 fermentation broth was applied 2 years in advance (2017 and 2018) to pepper fields that had been continuously cropped for 9 years (TR2).

Application of MHT1134 fermentation broth

The inocula was prepared in accordance with the preparation method of strain MHT1134[13]. During the transplanting of pepper seedlings (May 10, 2017 and May 6, 2018), 50 mL MHT1134 fermentation liquid was irrigated into the hole dug for each plant, covered with a little soil, and then the pepper seedlings were transplanted. Before the pepper flowering period (July 9, 2017 and July 5, 2018), 50 mL of MHT1134 strain fermentation broth at the same concentration was irrigated into the pepper rhizosphere. The peppers in the plots of CC1, CC5 and CC9 were planted and managed using normal practices.

Soil sample collection

Five treated soil samples were collected on day 60 (September 3, 2018), day 90 (October 3, 2018) and day 120 (November 2, 2018) after the second biocontrol strain application. The five-point sampling method was used to take the soil from approximately 10 cm below the rhizosphere soil of the pepper, and the rhizosphere soil of three pepper trees was taken from each point. The collected rhizosphere soil of each plot was mixed evenly as a sub-sample and taken to the laboratory for preservation at −80°C.

Control effect investigation

The investigation was conducted when *Fusarium* wilt was present in the field. The numbers of diseased plants at all levels were recorded, the Percent Disease Index (PDI) and control effects were calculated, and the chili pepper yield was measured and converted into quintals/hectare during the pepper harvesting period. The survey methods were published previously in Mao *et al.*[13].

Soil physical and chemical properties

Soil, with and without the biocontrol bacterium MHT1134 fermentation broth, was collected for physical and chemical property detection from CC9. Alkali-hydrolysable nitrogen levels were determined using the alkali-hydrolysable diffusion method[14]. Soil available phosphorus and potassium levels were determined by the sodium bicarbonate extraction of molybdenum and antimony anticolourimetry and by ammonium acetate extraction of flame photometry, respectively[15]. The organic matter content was determined using the potassium dichromate volumetric method[16].

Enzymatic activity

The urease, dehydrogenase, invertase, acid phosphatase, catalase and acid protease activities in the pepper soil were measured. The soil urease activity was determined using indophenol blue colorimetry, and the acid phosphatase activity was determined using the disodium phenyl phosphate colorimetry method. The catalase activity was determined using the KMnO4 titration method, invertase activity was determined using 3,5-dinitrosalicylic acid colorimetry, and both dehydrogenase and acid protease activities were determined using the double-antibody sandwich method[17].

Analysis of the microbial community

Microbial community genomic DNA was extracted from each of the samples using an E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) following the manufacturer's recommendations. The DNA extract was checked on a 1% agarose gel, and DNA concentration and purity were determined using a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

The hypervariable region V3-V4 of the bacterial 16S rRNA gene were amplified with the primer pair 338F/806R[18] on an ABI GeneAmp® 9700 PCR thermocycler (Applied Biosystems, Foster City, CA, USA). The ITS regions of fungi were used as the target sequences. The PCR amplification was carried out with the universal

primers ITS1F and ITS2R[1]. PCR reactions were performed in triplicate. The PCR product was extracted from a 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) in accordance with the manufacturer's instructions and quantified using Quantus™ Fluorometer (Promega, USA). Finally, the Illumina paired-end library preparation, cluster generation and Illumina MiSeq PE300 paired-end sequencing were performed by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

The raw gene sequencing reads were demultiplexed, quality-filtered by fastp version 0.20.0[19] and merged using FLASH version 1.2.7[20]. Operational taxonomic units (OTUs) with 97% similarity cut-offs were clustered using UPARSE version 7.1 [21,22], and chimeric sequences were identified and removed. The taxonomy of each OTU representative sequence was analysed by RDP Classifier version 2.2[23] against the DNA database using a confidence threshold of 0.7.

Statistical analyses.

The experiments were organised in a completely randomised design. Data were subjected to an analysis of variance using SPSS sofware (IBM Corporation, Armonk, NY, USA). Treatment means were separated using Duncan's multiple range test ($P < 0.05$ and $P < 0.01$).

Results

Field control effect of strain MHT1134 on Fusarium wilt of pepper

In TR1 and TR2CC9, the disease rate and disease index of pepper wilt decreased compared with the plots not receiving the biocontrol MHT1134 strain. In TR1, the disease rate and disease index of pepper wilt decreased by 8.44% and 3.76%, respectively, , whereas in TR2, the disease rate and disease index of pepper wilt decreased by 57.69% and 63.02%, respectively, compared with CC9. However, in the TR2 plots over 2018 and 2019, the disease rate and disease index decreased to 7.13% and 3.03%, which were 64.26% and 70.20%, respectively, less than in the CC9 plots. The control effect of MHT11341 on pepper wilt was 63.03% and 70.21% after one and two years of continuous cropping field, respectively (Table 1). The results indicated that the continuous application of a biocontrol bacteria further consolidated and improved the control effect.

Effects of strain MHT1134 on the physical and chemical properties of pepper rhizosphere soil

Soil samples from different planting years showed significant differences in their physical and chemical properties. With the increase in planting years, the organic matter and alkali-hydrolysable nitrogen contents in the soil showed decreasing trends. The organic matter content in the CC9 soil samples was 23.64% less than in the CC1 soil samples, and the alkali-hydrolysable nitrogen content was 45.2% less. The available phosphorus and available potassium levels did not show regular change trends, but the available potassium content in the CC9 soil was lower than in the CC1 soil.

Compared with the CC9 soil samples, the alkali-hydrolysed nitrogen, organic matter, available phosphorus and available potassium contents in TR1 soil samples increased by 46.82%, 6.26%, 5.09% and 47.06%, respectively. The available potassium content increased most obviously, followed by alkali-hydrolysable nitrogen. The alkali-hydrolysable nitrogen, organic matter and available phosphorus contents decreased slightly in TR2, but were still higher than those in the CC9 soil samples. In addition, the available potassium content continued to increase by 20% after the application of biocontrol bacterium MHT1134 in the second year (Table 2).

Effects of strain MHT1134 on enzymatic activities in pepper rhizosphere soil

By comparing the activities of six kinds of enzymes in the five groups of soil samples, we found that all the activities, except for that of acid phosphatase, in the CC9 soil were lower than those in the CC1 soil. In TR1 and TR2, the activities of the six enzymes in the soil increased. The urease, dehydrogenase, acid phosphatase, catalase, invertase and acid protease activities increased by 9.04%, 4.42%, 29.02%, 9.35%, 17.83% and 6.83% in TR1, respectively, and by 18.60%, 20.26%, 22.86%, 18.87%, 16.59% and 14.30% in TR2, respectively (Fig. 1A–F). The results indicated that MHT1134 applications could improve the enzyme activities in the soil to different degrees. Moreover, the urease, dehydrogenase, catalase and acid protease activities in soil significantly increased after the continuous application of MHT1134.

Microbial diversity and richness

The sample dilution curve tended to be flat, and the fungal and bacterial diversity index table (Table 3) shows that the library coverage levels were greater than 99% and 98%, respectively. Together, they indicate that the OTU coverage of the soil samples is basically saturated; therefore, the OTUs reflect the species and structures of the fungal and bacterial communities in the samples. High-throughput sequencing results showed that 765,747 16S rRNA sequences and 1,012,237 ITS sequences were obtained from 15 samples of pepper rhizosphere soil subjected to five treatments. After data quality control, there were 35,362–72,498 bacterial 16S rRNA sequences and 54,007–74,562 fungal ITS sequences. In addition, using the 97% standard, the bacterial and fungal OTU numbers were 17,444–47,775 and 50,876–71,236, respectively.

Alpha-diversity analysis of fungi and bacteria

The changes in fungal and bacteria diversity are shown in Table 3. According to the Shannon index analysis, the species richness of fungi in CC1 was the highest (2.88). As the planting years increased, the Shannon index decreased gradually (2.71 in CC5 and 2.69 in CC9). Although ACE and Chao indexes, representing the species abundance of the community, did not show obvious increasing trends, in CC9, the values of the two indexes were significantly higher than in CC1, which indicated that as the planting years increased, the diversity of fungi in the pepper soil decreased, while the species abundance increased. As shown in Table 3, in TR1, the Simpson index, representing species dominance, and the Sobs index, representing species

richness, increased significantly, and the Shannon index also increased. In TR2, the Shannon index increased significantly, while the values of other indexes decreased slightly. We hypothesised that after the first year of application, the strain MHT1134 colonised in large numbers, resulting in it being the dominant community species. After continuous application, the soil ecology had adjusted, and the diversity of soil fungi continued to increase. In general, the application of the biocontrol bacterium MHT1134 increased the diversity of fungi in the pepper rhizosphere soil and decreased the dominance of some species.

The changes in bacterial diversity and abundance in the pepper rhizosphere soil after different periods of continuous cropping are shown by the decreases in the Shannon and Sobs indexes decreased as the planting years increased, indicating that bacterial diversity and bacterial community richness decreased. Although ACE and Chao indexes representing the species abundance of the community did not show regular decreasing trends, in CC9, the values of the two indexes were significantly lower than in CC1, indicating that as the planting years increased, the diversity and richness of bacteria in the pepper soil decreased. Strain MHT1134 had no significant effect on the alpha-diversity index of soil bacteria in TR1, but Simpson, ACE and Chao indexes increased in TR2.

Effects of MHT1134 on the microbial community structure in pepper rhizosphere soil

All the bacteria were classified into 352 genera and 23 phyla according to their 16S rRNA sequences, and all the fungi were classified into 6 phyla and 194 genera according to their ITS sequences. The top five phyla in terms of bacterial abundance were Actinobacteria, Acidobacteria, Chloroflexi, Gemmatimonadetes and Nitrospirae. The top six phyla in terms of fungal abundance were Ascomycota, Zygomycota, Basidiomycota, Glomeromycota, Chytridiomycota and Rozellomycota.

Effects of MHT1134 on fungal community structure in pepper rhizosphere soil

The effects of the biocontrol treatment on fungal phyla are shown in Fig. 2A. After treatment with MHT1134, the relative abundance of Ascomycota decreased significantly from 77.9% to 70.99%. The abundance of Basidiomycota increased significantly after the treatment, whereas it decreased with the continuous cropping time before the MHT1134 application. However, Zygomycota increased in abundance with the continuous cropping time. The abundance of strain MHT1134 increased significantly and then decreased by 1 year after treatment.

By analysing the relative abundance of fungi of different genera in the soil, it was found that the fungi of several genera showed similar change trends in different soil treatments. The relative abundances of *Fusarium*, *Gibberella* and the alkali-resistant fungus *Pseudallescheria* in the soil increased along with continuous cultivation years (CC1 < CC5 < CC9). The relative abundance levels of fungi in these three genera decreased in TR1 and TR2 (CC9 > TR1 > TR2). In addition, the trend was found for *Trichoderma*, *Chaetomium* and *Mortierella*, which declined as the planting years

increased, but their relative abundance levels significantly increased in TR1 and significantly increased again in TR2 (Fig. 2B).

Using *Fusarium* as the control, we analysed the variation trends of microorganisms in CC9, TR1 and TR2 soil samples. As shown in Figure 3, the levels of three genera were positively correlated with the *Fusarium* change trend, *Gibellulopsis*, *Giberella* and *Pseudallescheria*, while three genera, *Trichoderma*, *Chaetomium* and *Mortierella*, were negatively correlated with *Fusarium*. Thus, the abundance levels of fungi in *Gibellulopsis*, *Gibberella* and *Pseudallescheria* were reduced after the MHT1134 application. Some species of *Gibellulopsis* are the pathogenic bacteria that cause *Verticillium* wilt, and some species of *Gibberella* are the pathogenic bacteria that cause gibberellic diseases. The abundance levels of *Trichoderma*, *Chaetomium* and *Mortierella* significantly increased after the application of strain MHT1134.

Effects of MHT1134 on bacterial community structure in pepper rhizosphere soil

At the phylum level, the species abundance analysis of the five soil samples showed that the abundance of Actinobacteria in the soil decreased gradually as the planting years increased (CC1 > CC5 > CC9), whereas the abundance of Actinobacteria in the soil increased significantly after the application of MHT1134 fermentation broth (CC9 < TR1 < TR2). The proportion increased from 17.56% (CC9) to 22.44% (TR2). On the contrary, the abundance of Acidobacteria in the pepper rhizosphere soil increased along with the planting year. However, in TR2, the abundance of Acidobacteria significantly decreased. The proportion of Acidobacteria decreased from 17.45% to 14.47% (Fig. 4A). At the genus level, the abundance levels of *Phingomonas*, *Streptomyces*, *Bryobacter*, *Mizugakiibacte* and *Gemmatimonas* increased significantly after the application of the MHT1134 fermentation broth (Fig. 4B).

Effects of MHT1134 on the relationship between soil environmental factors and microbial community structure

According to the redundancy analysis of the community soil bacterial and fungal genera and soil environmental factors, the contribution of bacterial community composition was 14.93% and 46.48% in principal coordinates one and two, respectively, of the Figure 5A. In the Figure 5B, fungal community composition contributed 18.97% and 28.63% to principal coordinates one and two, respectively. The bacterial and fungal community compositions of the five soil samples were each located in four quadrants, and the three replicates of each treatment were similar, which indicated that each treatment had good repeatability. Soil enzyme activities (phosphoacidic enzyme, dehydrogenase, urease and invertase) and physicochemical characteristics (total nitrogen, available phosphorus, available potassium and organic matter) had great effects on the bacterial community composition of the five pepper soil samples. Among the physical and chemical factors, available phosphorus had the greatest effect. Acid phosphatase, invertase and acid protease activities, as well as soil total nitrogen content, were positively correlated with soil bacterial community after

the strain MHT1134 biocontrol application (Fig. 5A). Soil enzyme activities (urease, dehydrogenase, acid phosphatase, catalase, sucrase and acid protease) and physical and chemical characteristics (available phosphorus, available potassium and organic matter) had great effects on the fungal community composition of the five pepper soil samples. Among them, acid phosphatase radiation was the greatest, having the strongest effect on the pepper fungal community, and among the physical and chemical factors, effective sulphur had no obvious increasing effect on the soil fungal community composition (Fig. 5B).

Discussion

In recent years, soil-borne diseases have become more common owing to continuous cropping practices and improper applications of pesticides and fertilizers. They have resulted in great agricultural production losses. The occurrences of soil-borne diseases are mainly correlated with an imbalance in the soil microbial community structure and the deterioration of the ecological environment[25]. The microbial diversity and uniform structure of crop rhizosphere soil decreases in plots in which soil-borne diseases occur. Deng *et al*.[26] found in a comparison of the diversity of bacterial rhizosphere communities between healthy and diseased banana plants that the latter had less bacterial diversity. Xiao *et al*.[27] compared the bacterial community diversity levels in the rhizosphere soils of anthrax-infected and healthy strawberry using high-throughput sequencing and found that the richness and diversity of the former were lower than the latter. He *et al*.[28] found that the diversity and richness of bacteria and fungi in the soil during the peak of a watermelon *Fusarium* wilt disease incidence decreased compared with in the non-diseased control. Li *et al*.[29] studied the changes in microbial community structure and diversity of cucumber rhizosphere soil in a greenhouse at 1, 3, 5 and 7 years after planting and found that the relative abundance of most bacterial genera increased slightly in the 3rd year and then decreased gradually, while the community diversity of soil samples under continuous cropping for 7 years was the lowest. Moreover, as the planting years increased, the soil bacterial function showed a downward trend.

In this study, soil samples were collected from plots having pepper wilt disease that had been continuously cropped for 1, 5 and 9 years, and the changes in the microbial community structure of the soil were analysed. For fungi, as the planting years increased, the Shannon index, representing species richness, decreased gradually, whereas the ACE and Chao indexes, representing species abundance, did not show obvious decreasing trends, but in CC9, the values of the two indexes were also significantly lower than in CC1. Thus, with an increase in planting years, the diversity and abundance of fungi in pepper soil decreased. For bacteria, as the planting years increased, the Shannon and Sobs indexes decreased. In CC9, the ACE and Chao indexes were significantly lower than in CC1. Thus, as the planting years increased, the diversity and richness of bacteria in the pepper soil decreased.

In addition, this study showed that as the planting years increased, the abundance of *Fusarium* fungi in the soil increased, as did the abundance levels of *Gibellulopsis*, *Giberella* and *Pseudallescheria*. Some species in the genus *Gibellulopsis* are

responsible for causing sugar beet[30], spinach[31] and potato[32,33] *Verticillium* wilt. *Gibberella zeae* of the *Gibberella* genus is the pathogen responsible for wheat scab[34]. Some species of *Pseudallescheria* are alkaline tolerant. For example, Wang *et al.*[35] reported isolating the alkali-tolerant fungus *Pseudallescheria* sp. JSM-2 from alkaline papermaking wastewater. As the continuous cropping time increases, the soil gradually becomes salinised[36], and the abundance levels of alkaline-tolerant fungi may also increase. In addition, the abundance of Acidobacteria increased along with the planting years. Jones *et al*.[37] reported that Acidobacteria are usually more prevalent in acidic environments. Therefore, it can be inferred that the increased abundance of Acidobacteria after continuous pepper cultivation indicates that this cultivation system affected the acidity and alkalinity of the soil, which further affected the fungal community structure, especially increasing the abundance of some pathogenic fungi. This indicates that some harmful microorganisms in the soil will become more abundant as the continuous cropping time increases .

Biological control is a popular green and safe measure for the prevention and control of soil-borne diseases. Its principle is to use biocontrol microorganisms or their metabolites to inhibit the reproduction and growth of pathogenic microorganisms and restore the soil microbiological ecosystem to a healthy and balanced state[38]. Inoculations of microorganisms can influence, even temporarily, the native microbial communities[39].

In this study, samples were collected from plots in which pepper had been continuously cropped for different numbers of years, and the plots that had been used the longest were selected for the application of the biocontrol strain *Trichoderma* MHT1134. On the one hand, it is clear that the longer the continuous cropping time, the greater the decreases in the diversity and abundance of microorganisms in soil, while the abundance levels of some harmful microorganisms increases. On the other hand, compared with CC9, the TR1 soil samples had significantly increased Simpson and Sobs indexes, but the Shannon index was not significantly increased. For TR2, the Shannon index increased significantly, while the values of the other indexes slightly decreased. We speculated that the dominant species in the community was influenced by the massive colonisation of strain MHT1134 after the first application year. After continuous application, the soil ecology re-adjusted, and the soil fungal diversity level continued to increase. Moreover, the effect on the bacterial community structure in the soil was also very obvious. After the continuous application of strain MHT1134, the Simpson index increased significantly, and the dominance of bacteria in the soil increased. This is consistent with reports by Bonanomi *et al*.[40] and Cordier *et al*.[41] In general, the application of the biocontrol bacterium MHT1134 improved the diversity and richness of microorganisms in the pepper rhizosphere soil and, to a large extent, prevented the decreasing trend in species richness caused by continuous cropping.

In addition, we found that the application of strain MHT1134 to continuous cropping fields effectively improved the microecology of the soil. On the one hand, the abundances of the fungal genera *Gibellulopsis*, *Gibberella* and *Pseudallescheria* decreased significantly, while the abundances of *Trichoderma*, *Chaetomium* and

Mortierella increased significantly. Many varieties of *Trichoderma* are excellent sources of biocontrol bacteria for plant diseases[42,43], and many species of *Chaetomium* also have good biocontrol effects on soil-borne plant diseases[44]. Thus, the application of strain MHT1134 increased the number of beneficial fungi in the pepper rhizosphere soil and reduced the number of harmful fungi. On the other hand, the abundance of Acidobacteria decreased significantly, while the abundance of Actinobacteria increased significantly. The abundances of five bacterial genera, *Sphingomonas*, *Streptomyces*, *Bryobacter*, *Mizugakiibacte* and *Gemmatimonas*, increased significantly. Du *et al*.[24] reported that *Bryobacter* species can promote soil carbon cycling, and *Sphingomonas* is a rich new microbial resource with strong degradative functions that has great application potential in environmental protection. Many species of *Streptomyces* can produce antibiotic metabolites. Therefore, the application of MHT1134 could increase the number of beneficial bacteria, to a certain extent, in the pepper rhizosphere soil. Overall, the application of *Trichoderma* spp. to continuous cropping pepper fields reduced the number of harmful microbial species and increased the number of beneficial microbial species.

Additionally, we determined that the biocontrol *Trichoderma* MHT1134 strain had a positive effect on the physical and chemical properties, as well as enzyme activities, of the soil. The organic matter and alkali-hydrolysable nitrogen contents in the CC9 soil samples were lower than those in the CC1 soil samples, while the alkali-hydrolysable nitrogen, organic matter, available phosphorus and available potassium contents in the soil increased after the application of strain MHT1134. Yin *et al*.[45] reported that after applying biocontrol agents to the soil, the damage to the continuous cropping soil was repaired, to some extent, and the physical and chemical properties of the soil improved. Huang *et al*.[46] applied biocontrol agents to plots in which banana *Fusarium* wilt occurred and found that the numbers of banana rhizosphere bacteria and Actinomycetes significantly increased, as did the soil organic matter, organic carbon, total nitrogen and rapidly available potassium contents. This is consistent with our experimental results.

Plant protection is achieved through enzymatic catalytic activities. Acid proteases, acid phosphatases and invertases are all hydrolases, which are involved in the hydrolysis and lysis of the molecular bonds of various compounds in soil. Acid proteases are involved in the transformation of nitrogen-containing compounds in soil, and hydrolysates are also a nitrogen source for plants. Acid phosphatase catalyses the conversion of organic phosphorus in soil to inorganic phosphorus for plant absorption. Invertase plays an important role in increasing soluble nutrients in soil, and its activity is also correlated with the number of microorganisms and rate of soil respiration. The higher the soil fertility, the higher the invertase activity. In this experiment, after the application of MHT1134, soil enzyme activity levels and physicochemical properties improved to varying degrees. The application of strain MHT1134 effectively improved the rhizosphere soil environment, enhanced the enzyme activities in the soil, and then promoted the mass reproduction of microorganisms, which is conducive to the absorption of nutrients from the soil and enhances the disease resistance of the plants.

In general, the biocontrol *Trichoderma* MHT1134 strain can reduce the disease incidence and regulate the soil microecological structure. Long-term applications effectively alleviate the harmful effects of continuous pepper cropping on the soil; consequently, it is a biocontrol resource worth developing and applying.

References

1. Adams, R. I., Miletto, M., Taylor, J. W. & Bruns, T. D. Dispersal in microbes: fungi in indoor air are dominated by outdoor air and show dispersal limitation at short distances. *ISME J.* **7**(7), 1262–1273. https://doi.org/10.1038/ismej.2013.28 (2013).
2. Mao, T. T. *et al.* Isolation and identification of a strain of coprophilous Chaetomium and its biocontrol effect against pepper Fusarium wilt. *Chin. J. Biol. Control* **33**(4), 552–560. https://doi.org/10.3969/j.issn.2095-1191.2019.08.14 (2017).
3. Sixto, V. F., Jose, A. G. T., Sergio, H. V., Carlos, A. L. O., & Jesus, E. R. M. Occurrence of *Fusarium oxysporum* causing wilt on pepper in Mexico. *Canadian J. Plant Pathol.* **40**(2), 238–247. https://doi.org/10.1080/07060661.2017.1420693 (2018).
4. Rieke, E. & Cappellazzi, S. Assessing soil health: measuring the soil microbiome. *Crops Soils.* **54**(2), 32–35. https://doi.org/10.1002/crso.20099 (2021).
5. Tariq, M. *et al*. Siddiqui. Biological control: a sustainable and practical approach for plant disease management. *Acta Agr Scand Section B: Soil Plant Sci.* **70**(6), 507–524 (2020).
6. Mousumi-Das, M., Harida, M., Sabu, A. Biological control of black pepper and ginger pathogens, *Fusarium oxysporum*, *Rhizoctonia solani* and *Phytophthora capsici*, using *Trichoderma* spp. *Biocatalysis and Agricultural Biotechnology*. **17**, 177-183 (2019).
7. Saini, K. K. & Kshirsagar, C. R. Strain improvement of *Trichoderma viride* (Pers.) by UV mutagenesis for enhancing bio-control potential against *Fusarium oxysporum*. *Ann. Plant Protect. Sci.* **23**(2), 342–346. (2015).
8. Sinulingga, S., Munir, E. & Nurtjahja, K. Potential of *capsicum annum* rhizosphere bacteria in inhibiting the growth of *Fusarium oxysporum* in-vitro. *IOP Conf. Ser.: Earth Environ. Sci.* **305**(1), 012060 (7pp). https://doi.org/10.1088/1755-1315/305/1/ 012060 (2019).
9. Toloza-Moreno, D. L., Lizarazo-Forero, L. M. & Uribe-Vélez, D. Antagonist capacity of bacteria isolated from cape gooseberry cultures (*Physalis peruviana* L.) for biological control of *Fusarium oxysporum*. *Trop. Plant Pathol.* **45**(1), 1–12. https://doi.org/10.1007/s40858-019-00313-z (2020).
10. Ragab, M. M. M., Ashour, A. M. A., Abdel-Kader, M. M., El-Mohamady, R. & Abdel-Aziz, A. In vitro evaluation of some fungicides alternatives against *Fusarium oxysporum* the causal of wilt disease of pepper (*Capsicum annum* L.). *Int. J. Agric. Forest.* **2**(2), 70–77. https://doi.org/10.5923/j.ijaf.20120202.11 (2012).
11. Zhong S. T. *et al*. Effects of continuous application of bio-organic fertilizer on

banana production and cultural microflora of bulk soil in orchard with serious disease incidence. *Chin. J. Appl. Ecol.* **26**(2), 481–489. https://doi.org/10.13287/j.1001-9332.20141203.009 (2015).
12. Shen Z. Z., Ruan Y. Z., Chao X., Zhang J., Li R. & Shen Q. R. Rhizosphere microbial community manipulated by 2 years of consecutive biofertilizer application associated with banana *Fusarium* wilt disease suppression. *Biol. Fertil. Soils* **51**(5), 553–562. https://doi.org/10.1007 /s00374-015-1002-7 (2015).
13. Mao, T. T., Chen, X. J., Ding, H. X., Chen, X. Y. L. & Jiang, X. L. Pepper growth promotion and *Fusarium* wilt biocontrol by *Trichoderma hamatum* MHT1134. *Biocontrol Sci. Technol*. **33**(4), 552–560. https://doi.org/10.1080/09583157.2020.1803212 (2020).
14. Yang, Z. M., Zhou, W. Z. & Shi, R. H. Comprehensive evaluation on potassium supplying potential of main soils in China. *Acta Peoologica Sinica.* **36**(3), 377–385 (1999).
15. Zhang, G. L. & Gong, Z. T. Soil survey laboratory analysis methods. 38-80 (Beijing, 2012).
16. Bao, S.D. Soil and agricultrual chemistry analysis. 30-33 (Beijing, 1999).
17. Hu, H., Zhou, H., Zhou, S., Li, Z. & Hay, A. G. Fomesafen impacts bacterial communities and enzyme activities in the rhizosphere. *Environ. Pollut.* **253**, 302–311. https://doi.org/10.1016/j.envpol.2019.07.018 (2019).
18. Xu, N., Tan, G., Wang, H. & Gai, X. P. Effect of biochar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure. *Eur. J. Soil Biol.* **74**, 1–8. https://doi.org/10.1016/ j.ejsobi.2016.02.004 (2016).
19. Chen, S., Zhou, Y., Chen, Y. & Gu, J. Fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **34**(17), 1884–1890. https://doi.org/10.1093/bioinformatics/bty560 (2018).
20. Magoč, T. & Salzberg, S. L. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **27**(21), 2957–2963. https://doi.org/10.1093/bioinformatics/btr507 (2011).
21. Edgar, R. C. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods*. **10**(10), 996–998. https://doi.org/10.1038/nmeth.2604 (2013)
22. Stackebrandt E. & Goebel B. M. Taxonomic Note: A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol.* **44**(4), 846–849. https://doi.org/10.1099/00207713-44-4-846 (1994).
23. Wang, Q., Garrity, G. M., Tiedje, J. M. & Cole, J. R. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol*. **73**(16), 5261–5267. https://doi.org/10.1128/AEM.00062-07 (2007).
24. Du, S. Y. *et al*. Effect of facility management regimes on soil bacterial diversity and community structure. *Chin. J. Eco-Agric.* **25**(11), 1615–1625. https://doi.org/10.13930/j.cnki.cjea.170291 (2017).
25. Van Bruggen A. H. C. & Semenov A. M. In search of biological indicators for soil health and disease suppression. *Appl. Soil Ecol.* **15**(1), 13–24.

https://doi.org/10.1016/S0929-1393(00)00068-8 (2000).
26. Deng, X., Li, Q. F., Wu, C. Y., Li, Y. & Liu, J. K. Comparison of soil bacterial genetic diversity in root zone of banana (*Musa paradisiaca*) infected with fusarium wilt and noninfected plants. *Ecol. Environ. Sci.* **24**(3), 402–408. https://doi.org/CNKI:SUN:TRYJ.0.2015-03-006 (2015).
27. Xiao, R. *et al*. A comparative study on rhizosphere soil bacterial communities of healthy strawberry and infected strawberry with anthracnose by high-throughput sequencing. *Chin. Agric. Sci. Bull*. **33**(11), 14–20. https://doi.org/CNKI:SUN: ZNTB.0.2017-11-004 (2017).
28. He *et al*. Analysis of rhizosphere microbial community structure in different pathogenesis stages of watermelon *Fusarium* Wilt. *Agricultural Science & Technology*. **21**(2), 19-25 (2020).
29. Li, Y. *et al*. Effects of different continuous cropping years on bacterial community and diversity of cucumber rhizosphere soil in solar-greenhouse. *Curr Microbiol*. 7**8**, 2380-2390. https://doi.org/10.1007/s00284-021-02485-x (2021).
30. Yang, Z., Zhao, Z., Guo, Q. & Lei, B. First report of wilt of sugar beet caused by *Gibellulopsis nigrescens* in the Xinjiang region of China. *Plant Dis.* **101**(7), 1318. https://doi.org/10.1094/PDIS-01-17-0087-PDN (2017).
31. Igesias-Garcia, A. M., Villarroel-Zeballos, M. L., Feng, C., Toit, L. J. D. & Correll, J. C. Pathogenicity, virulence, and vegetative compatibility grouping of *verticillium* isolates from spinach seed. *Plant Dis*. **97**, 1457–1469. https://doi.org/10.1094/PDIS-01-13-0016-RE (2013).
32. Tirado, O. M. Effect of green manures and organic amendments on *Verticillium* wilt of potato in Manitoba. (Doctoral dissertation, University of Manitoba (Canada).; 2010).
33. Zare, R., Gams, W., Starink-Willemse, M. & Summerbell, R. C. Gibellulopsis, a suitable genus for *verticillium nigrescens*, and musicillium, a new genus for *V. theobromae*. *Nova Hedwigia* **85**(3), 463–489. https://doi.org/10.1127/0029-5035/2007/0085-0463 (2007).
34. Shude, S., Yobo, K. S. & Mbili, N. C. Progress in the management of *Fusarium* head blight of wheat: an overview. *South Afr. J. Sci.* **116**(11/12), 60-66. https://doi.org/10.17159/sajs.2020/7854 (2020).
35. Wang, K., Luo, H. Y., Peng-Jun, S., Wang, Y. R., Yang, P. L. & Yao, B. Cloning, expression and characterization of an alkaline xylanase from alkali-tolerant fungus *pseudallescheria* sp. JSM-2. *Curr. Biotechnol.* **1**(1), 61–67. https://doi.org/10.3969/j.issn.2095-2341.2011.01.10 (2011).
36. Song, X. L., Zhu, Y. H., & Chen, W. F. Dynamics of the soil respiration response to soil reclamation in a coastal wetland. *Sci. Rep.* **11**(1), 1-14. https://doi.org/10.1038/s41598-021-82376-0 (2021).
37. Jones, R. T., Robeson, M. S., Lauber, C. L., Hamady, M., Knight, R. & Fierer, N. A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. *ISME J.* **3**(4), 442-453. https://doi.org/10.1038/ismej.2008.127 (2009).
38. Kexiang, G., Xiaoguang, L., Yonghong, L., Tianbo, Z., & Shuliang, W. Potential

of *Trichoderma harzianum* and *T. atroviride* to control *Botryosphaeria berengeriana* f. sp. *piricola*, the cause of apple ring rot. *J. Phytopathol.* **150**(4-5), 271-276. https://doi.org/10.1046/j.1439-0434.2002.00754.x (2002).
39. Trabelsi, D. & Mhamdi, R. Microbial inoculants and their impact on soil microbial communities: a review. *Biomed. Res. Int*. **2013**, 1-11. https://doi.org/10.1155/2013/863240 (2013).
40. Bonanomi, G., Capodilupo, M., Incerti, G., Gaglione, S. A. & Scala, F. Fungal diversity increases soil fungistasis and resistance to microbial invasion by a non resident species. *Biol. Control* **72**(Complete), 38-45. https://doi.org/10.1016/j.biocontrol.2014.02.005 (2014).
41. Cordier, C. & Alabouvette, C. Effects of the introduction of a biocontrol strain of *Trichoderma atroviride* on non target soil micro-organisms. *Eur. J. Soil Biol.* **45**(3), 267-274. https://doi.org/ 10.1016/j.ejsobi.2008.12.004 (2009).
42. de Oliveira, C. M. *et al*. Biological control of *Pratylenchus brachyurus* with isolates of *Trichoderma* spp. on soybean. *Biol. Control* **152**, 104425. https://doi.org/10.1016/j.biocontrol.2020.104425 (2021).
43. Ferreira, F. V. & Musumeci, M. A. Trichoderma as biological control agent: scope and prospects to improve efficacy. *World J. Microbiol. Biotechnol.* **37**(5), 90. https://doi.org/ 10.1007/s11274-021-03058-7 (2021).
44. Silva-Valderrama, I., Toapanta, D., Miccono, M., Lolas, M. & Castro, A. Biocontrol potential of grapevine endophytic and rhizospheric fungi against trunk pathogens. *Front Microbiol.* **11**, 614620 https://doi.org/10.3389/fmicb.2020.614620 (2021).
45. Yin, J. Z. & Cai, B. Y. Continuous cropping obstacle repair by bio-control bacterium. *Chin. Agric. Sci. Bull.* **33**(021), 101-107. https://doi.org/CNKI:SUN:ZNTB.0.2017-21-018 (2017).
46. Huang, J. F. *et al*. Biocontrol efficacy on *Fusarium* wilt of banana by the collaborative of acid soil ameliorant and biocontrol materials. *Chin. J. Trop. Crops* **38**(003), 545–550. https://doi.org/10.3969/j.issn.1000-2561.2017.03.025 (2017).

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Figure Legends

Figure 1. Changes in the enzyme activities in the continuously cropped pepper rhizosphere soil after the application of strain MHT1134. Activity levels of (**a**) urease; (**b**) dehydrogenase; (**c**) acid phosphatase; (**d**) catalase; (**e**) invertase; and (**f**) acid protease.

Figure 2. Fungal clustering accumulation map in pepper rhizosphere soil at the phylum (**a**) and genus (**b**) levels.

Figure 3. The relative abundances of the first 15 genera after the MHT1134

application .* $0.01 < P \leq 0.05$

Figure 4. Bacterial clustering accumulation map and distribution heatmap in pepper rhizosphere soil samples at the phylum (**a**) and genus (**b**) levels. * $0.01 < P \leq 0.05$

Figure 5. Compositions of pepper soil bacterial (**a**) and fungal (**b**) communities, and analyses of soil physical and chemical characteristics and enzyme activities using RDA. The RDA mapping data came from soil samples of the five continuous pepper cropping systems. Different coloured points or shapes in the figures represent sample groups from different environments or conditions. Red arrows indicate quantitative environmental factors. The lengths of the arrows represent the interpreted degree of influence of the environmental factors on species data. The included angles between environmental factor arrows represent positive and negative correlations (acute angle, positive correlation; obtuse angle, negative correlation; right Angle, no correlation). The distance from the projection point to the origin represents the relative influence of environmental factors on the sample community distribution. P: available phosphorus; K: available potassium; S: effective sulphur; TN: Total Nitrogen; O: organic matter; UE: urease; DHO: dehydrogenase;invertase; AP: acid phosphatase; CAT: catalase; ACP: acid protease.

Tables

Table 1. Control effects of strain MHT1134 on *Fusarium* wilt in continuous pepper cropping fields.

Treatment	Disease incidence(%)	Disease index	Control efficacy(%)
MHT1134 was applied for 1 year	8.44 ± 0.27b	3.76 ± 0.20b	63.03 ± 1.99b(A)
MHT1134 was applied for 2 year	7.13 ± 0.46b	3.03 ± 0.10b	70.21 ± 1.03a(A)
Continuous cropping 9 years without MHT1134	19.95 ± 0.40a	10.17 ± 0.10a	—

Table 2. Effects of MHT1134 on physical and chemical properties of the pepper rhizosphere soil.

Treatment	Organic matter content/(g/kg)	Alkaline hydrolysis nitrogen content/(mg/kg)	Available phosphorus content/(mg/kg)	Available potassium content/(mg/kg)
CC1	26.57 ± 30.79 a	304.63 ± 17.28 a	26.69 ± 7.65 d	0.35 ± 0.02 a
CC5	22.85 ± 2.46 b	172.45 ± 84.05 cd	46.79 ± 5.70 a	0.35 ± 0.04 a
CC9	20.29 ± 1.79 d	166.91 ± 38.70 d	29.67 ± 4.77 bc	0.17 ± 0.08 d
TR1	21.56 ± 0.56 c	245.05 ± 73.76 b	31.18 ± 1.03 b	0.25 ± 0.05 c
TR2	21.28 ± 0.57 c	184.45 ± 32.44 c	28.44 ± 3.53 bc	0.30 ± 0.05 b

CC1, CC5 and CC9, represent the plots where pepper had been continuously planted for 1, 5 and 9 years, respectively, and TR1 and TR2 represent CC9 plots in which the MHT1134 biocontrol fermentation broth had been applied 1 and 2 years in advance, respectively.

Table 3. Alpha-diversity indexes of fungi and bacteria in different continuous pepper cropping soils.

	Treatments	Sobs	Shannon	Simpson	Ace	Chao	Coverage
Fungi	CC1	127.67 ± 4.78b	2.88 ± 0.13a	0.12 ± 0.02a	129.46 ± 4.27b	135.70 ± 4.48b	0.99 ± 0.00a
	CC5	127.00 ± 5.72b	2.69 ± 0.19c	0.13 ± 0.02b	130.70 ± 5.47c	130.90 ± 4.70c	0.99± 0.00a
	CC9	134.33 ± 3.86a	2.71 ± 0.16a	0.13 ± 0.02a	141.99 ± 2.05c	142.37 ± 2.58b	0.99 ± 0.00a
	TR1	136.33 ± 7.04c	2.72 ± 0.27ab	0.15 ± 0.02b	138.99 ± 4.76b	142.23 ± 1.52b	0.99 ± 0.00a
	TR2	134.67 ± 4.99ab	2.91 ± 0.24c	0.13 ± 0.04a	137.8 ± 5.05a	137.45 ± 4.56a	0.99 ± 0.00a
Bacteria	CC1	2083.67 ± 125.99	6.62 ± 0.05	2.94 ± 0.02	2319.21 ± 130.03	2364.43 ± 123.51	0.99 ± 0.00a
	CC5	1850.33 ±	6.59 ±	2.72 ±	2047.83 ±	2070.13 ±	0.99 ±

		125.39	0.03	0.1	160.36	150.62	0.00a
	CC9	1843.00 ± 71.01	6.57 ± 0.06	3.26 ± 0.8	2090.87 ± 66.11	2113.15 ± 86.55	0.98 ± 0.00a
	TR1	1667.67 ± 99.72	6.24 ± 0.09	4.69 ± 0.58	1906.17 ± 134.27	1931.39 ± 132.11	0.99 ± 0.00a
	TR2	1809.00 ± 157.92	6.17 ± 0.18	6.04 ± 0.12	2031.43 ± 170.75	2059.70 ± 159.25	0.99 ± 0.00a

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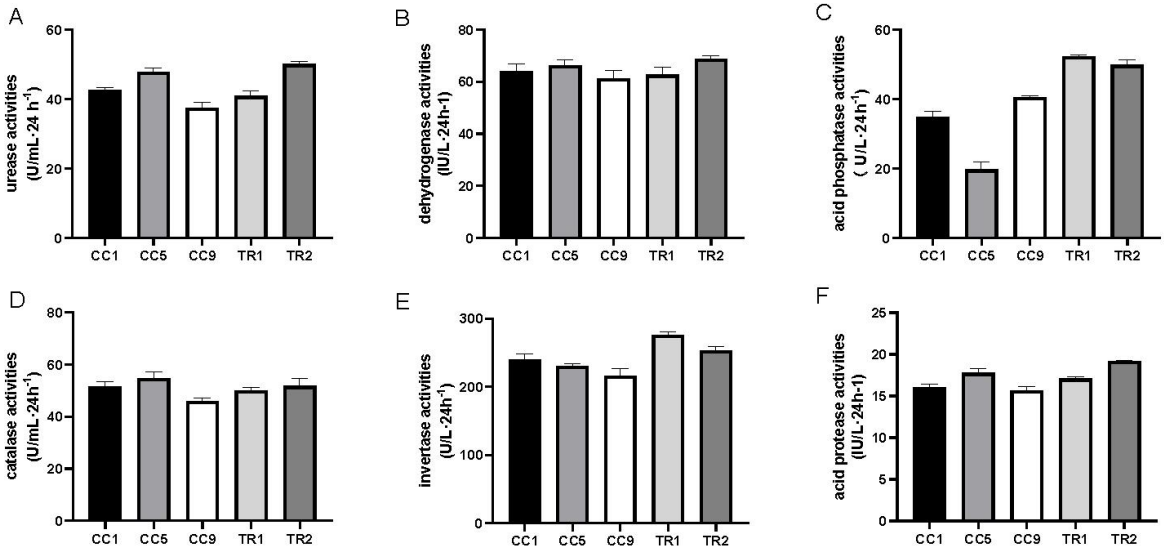
692 **Figures**

693 Figure 1



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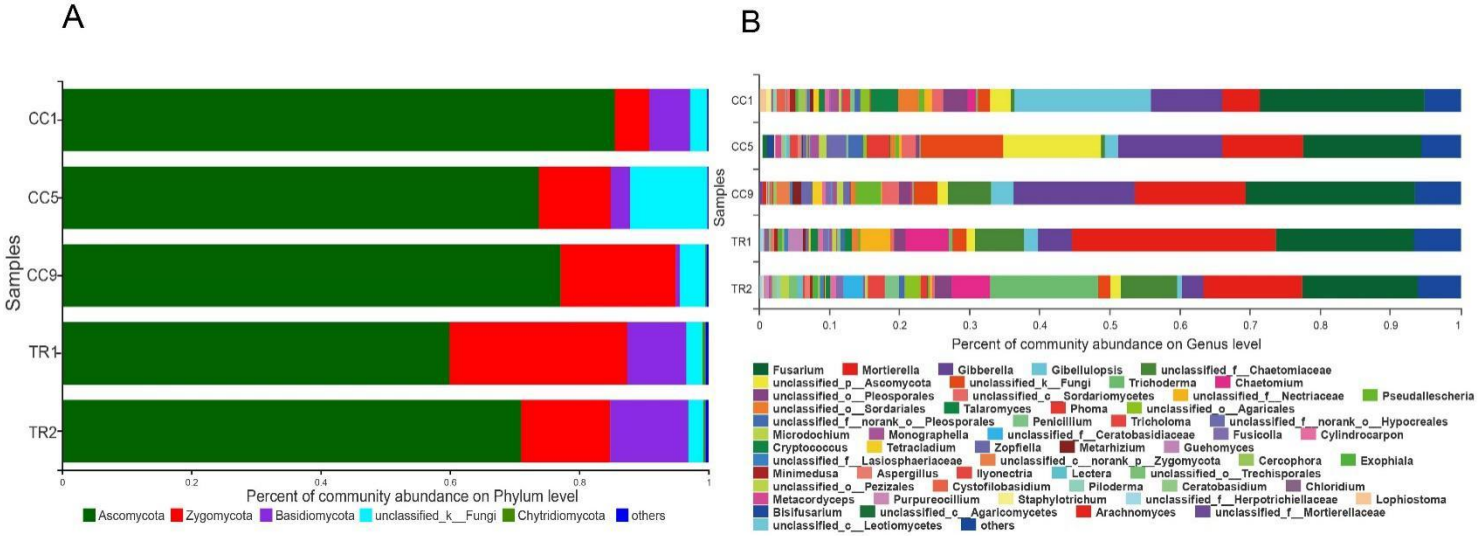
696 Figure 2



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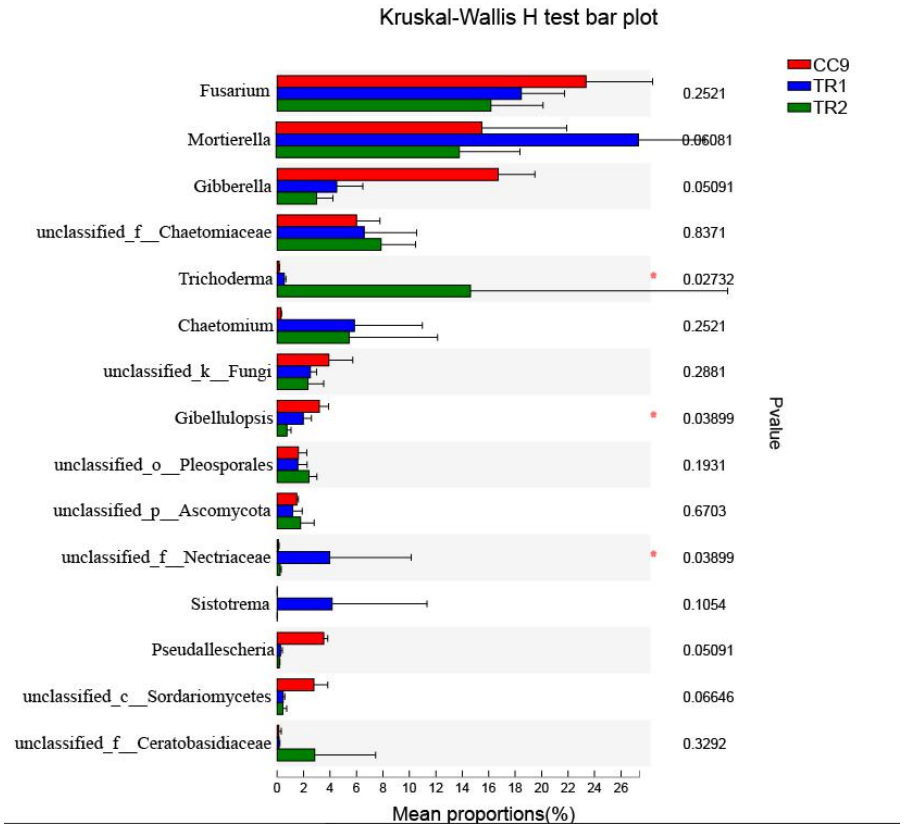
699 Figure 3



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701 Figure 4

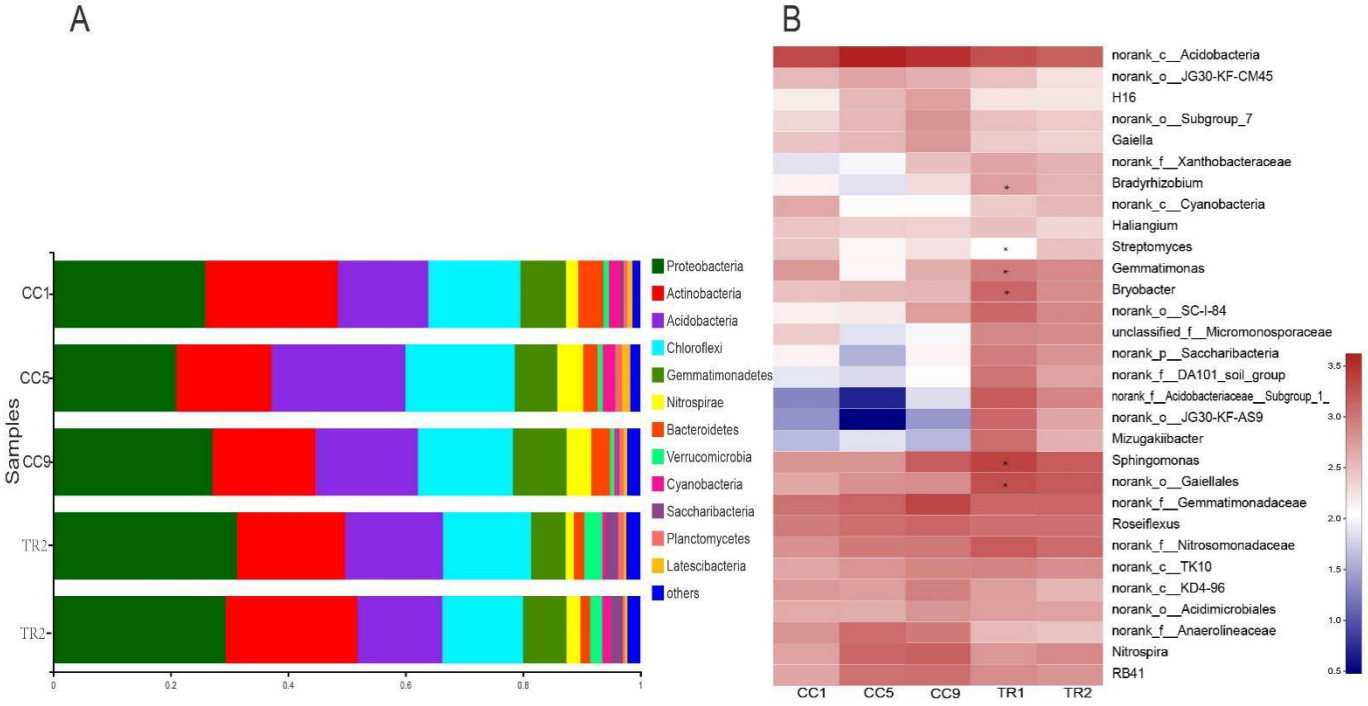

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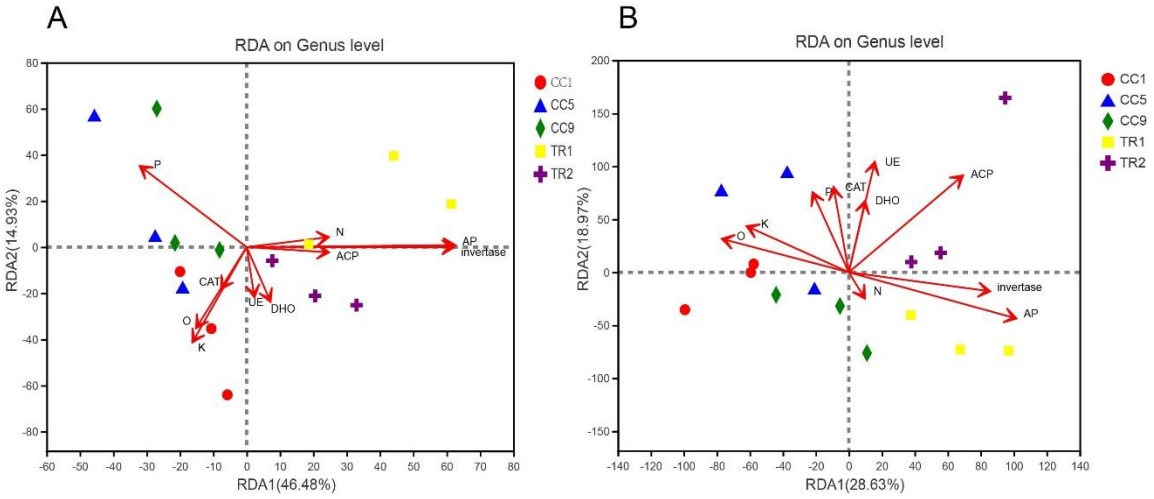
Figure 5


A
RDA on Genus level
RDA2(14.93%)
RDA1(46.48%)
P
N
AP
invertase
ACP
CAT
UE
DHO
O
K
CC1
CC5
CC9
TR1
TR2
B
RDA on Genus level
RDA2(18.97%)
RDA1(28.63%)
UE
ACP
P
CAT
DHO
K
O
N
invertase
AP
CC1
CC5
CC9
TR1
TR2