Soil inoculation of Trichoderma asperellum M45a regulates rhizosphere microbes and triggers watermelon resistance to Fusarium wilt

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Abstract

Fusarium wilt (FW) caused by Fusarium oxysporum f. sp. niveum (FON) is a soil-borne disease that seriously limits watermelon production. In the present study, the Trichoderma asperellum (T. asperellum) M45a was shown to be an effective biocontrol agent against Fusarium wilt (FW). In a pot experiment, the application of $10^5$ cfu/g of T. asperellum M45a granules had better control effect on FW at the blooming period (up to 67.44%) from soils subjected to five years of continuous cropping with watermelon, while the average length of watermelon vine was also significantly improved ($P < 0.05$). Additionally, the acid phosphatase (ACP), cellulase (CL), catalase (CAT) and sucrase (SC) activities in the M45a-inoculation group were significantly higher than in the control (CK) group, and the soil nutrients (total N, NO3-N, and available P) transformation was significantly increased. Moreover, T. asperellum M45a inoculation reduced fungal diversity and increased bacterial diversity, especially enhancing the relative abundance of PGPR (plant growth promoting rhizobacteria), such as Trichoderma, Sphingomonas, Pseudomonas, Actinomadura and Rhodanobacter. Through functional prediction, the relative abundance of Ectomycorrhizal, Endophyte, Animal pathotroph and saprotroph in fungal community was determined to be significantly lower than observed in the M45a-treated soil. Correlation analysis revealed that Sphingomonas, Pseudomonas and Trichoderma had the most differences in microorganisms abundance, positively correlated with ACP, CL, CAT and SC. These findings will provide ecological fungicide advice for microecological control of FW in continuous cropping watermelon.

Introduction

Continuous cropping obstacles result in stunted crop growth, aggravation of disease and decreased quality, and become the bottleneck restricting the sustainable development of agriculture (Wu et al., 2009). However, vegetable planting in a facility with single and continuous cropping phenomenon is widespread in China, due to the restriction of production and cultivation conditions and economic interests (Gao et al., 2019). The soil sterilization is the main measure to overcome the continuous cropping obstacle of crops in production. Although it alleviates the crops continuous cropping obstacle, it produces huge and far-reaching harm to environment in the long run. Therefore, people are also exploring some environmentally friendly methods to alleviate crop continuous cropping obstacles by regulating soil microbial communities (Umadevi et al., 2019).

Soil microorganisms and soil enzymes are important components of soil ecosystems and are important indexes to evaluate soil ecological quality and soil fertility (Li et al., 2018). Moreover, continuous cropping often leads to changes in nutrient element content, soil enzyme activity and other physical and chemical properties, as well as soil microbial environment, which restricts the absorption of nutrients in soil and even causes soil-borne disease (An et al., 2011). The plant rhizosphere, which is the region of the soil that adheres to plant roots., is the site of complex ecological and biological processes, and affected by root exudates (Fang et al., 2013; Philippot et al., 2013). The plant rhizosphere microorganisms play a key role in maintaining ecosystems, and the abundance and diversity of microbial communities are sensitive to fertilization, irrigation, and plant health (Mazzola, 2004; Raaijmakers et al., 2009; Zhao et al., 2018; Zhou
et al., 2017). For watermelon, changes in the rhizosphere microbial community may affect the plant resistance to FON (García-Ruiz et al., 2008). In addition, soil enzyme activity plays an important role in nutrient cycling and reflects the total soil biological activity, which may be associated with plant diseases. Additionally, soil microorganisms are the promoters and participants of soil biochemical reactions and have a direct correlation with soil enzyme activities (Wang et al., 2018).

Watermelon (*Citrullus lanatus*) is one of the most popular summer fruit crops worldwide (Guo et al., 2013). However, watermelon growth and yield have been significantly reduced by several soil-borne diseases, such as Fusarium wilt (FW) (Zhang et al., 2005) and blight disease (Zhang et al., 2016). FW, one of the most harmful diseases, is caused by *Fusarium oxysporum f. sp. niveum* (FON) and leads to decreased yields (An et al., 2011). This fungal pathogen population could reside in the soil for a long time and rapidly restored after the host plant roots, which leads to necrosis and induces wilting of the plants at later stages of infection, reducing the yield and in turn limiting overall economic productivity (Liu et al., 2019a).

*Trichoderma* spp. are opportunistic, act as biocontrol agents and have the ability to induce plant resistance and promote plant growth; thus, some *Trichoderma* species has been used as effective biofertilizers and biocontrol agents for plants grown in greenhouses and fields (Hermosa et al., 2012; Pandey et al., 2016). For example, Yuan et al., (2018) has demonstrated that five *Trichoderma* strains substantially facilitate the growth and improve the nutritional quality of orchard grass. Yang et al., (2011) also showed that *Trichoderma harzianum* (SQR-T307) is efficient biological control agents against *Fusarium oxysporum*. He et al., (2019) has found that *Trichoderma asperellum* GDFS1009 granules can promote growth and resistance to *Fusarium graminearum* in maize. In recent years, most studies on the soil rhizosphere microbial community mainly focused on the bacterial communities, while there is little information available on the fungal communities (Yu et al., 2013; Zhao et al., 2014; Shen et al., 2015; Shen et al., 2015). However, there have been few studies on the effect of *Trichoderma asperellum* on continuous cropping watermelon growth, particularly the microecological regulation mechanism in rhizosphere soil during the pathogenesis of FW. The hypothesis is that the application of *Trichoderma asperellum* strain M45a (*T. asperellum* M45a) can change the bacterial and fungal community compositions in watermelon continuous cropping rhizosphere soil. In this study, we used high-throughput sequencing to compare the rhizosphere microbial communities before and after treatment with *T. asperellum* M45a and explored the underlying mechanisms on plant growth promotion and disease (FW) suppression.

**Materials And Methods**

**Trichoderma strains and plant material**

*Trichoderma asperellum* M45a (CCTCC NO: M2019513, China Center for Type Culture Collection), which was provided by the Hunan Agricultural Biotechnology Research Institute, Changsha, China, was used throughout our study. *T. asperellum* M45a was cultured in potato dextrose agar (PDA) broth at 28 °C. The
T. asperellum M45a conidia suspension was prepared according to Zhang et al., (2013b), and the determined concentration was 7.5 × 10^6 colony-forming units (cfu)/ml. The watermelon cultivar ‘Zaojia 8424’, which is susceptible to FON, was used for investigation of FW disease incidence (Faheem et al., 2015) and length of watermelon vine.

Greenhouse Experimental Design And Soil Sample Collection

In the greenhouse experiment, the potting test soil was collected from a field at the Hunan Academy of Agricultural Sciences in Hunan, China (lat. 28°28′55″ N and long. 113°20′58″ E), which was subjected to five years of continuous cropping with watermelon for a pot experiment. The clay soil contained 2.7% organic matter, 1818.68 mg/kg total N, 438.13 mg/kg available K, 73.26 mg/kg available P, and pH = 4.6. The soil enzyme activity levels were as follows: 0.7 u/g ACP, 9.44 u·g-1 CL, 26.30 u/g CAT, 6.545 u/g SC and 683.18 u/g UE. The number of FON in soil was 5 × 10^3 cfu/g, and the content of Trichoderma spp. was 0.03 ng/g. Two treatments were performed in the greenhouse experiment in May 2018: Trichoderma (add 200 ml of Trichoderma asperellum M45a (7.5 × 10^6 cfu/ml) to 15 kg of soil, and compound to 10^5 cfu/g test soil) and Control (add 200 ml of sterile water to 15 kg of soil, and compound to 10^5 cfu/g test soil). Each treatment had five plastic pots and 30 seeds per pot, and all the plastic pots were laid out randomly. All watermelon plants were grown in a greenhouse with temperatures from 28 to 35 °C, natural light (day)/22 to 25 °C, (night).

In addition, soil samples were collected from two treatments in June and July of 2018. Each mixing rhizosphere soil sample was collected from five watermelon plants at the germination period (S1), seedling period (S2), smoke trailing period (S3) and the blooming period (S4), respectively. All the soil samples were sieved (2-mm mesh) and divided into two subsamples: one subsamples was stored at 4 °C for soil enzyme activity and property analysis, and the other subsamples were stored at -60 °C for DNA extraction.

Soil Enzyme Activities And Properties

For each rhizosphere soil sample, the activities of extracted soil enzymes were determined fluorometrically using methylumbelliferone (MUB)-linked model substrates with a TECAN Infinite M200 spectrophotometer (G10S UV-Vis, Thermo Fisher, USA), including soil acid phosphatase activities, soil urease (Emami et al., 2013) and soil cellulase (Kizilkaya et al., 2012), soil sucrase and soil catalase activities (Allison and Jastrow, 2006), which were detected at the following wavelengths: 450 nm for S-CAT and 365 nm for the other enzymes. Then, the determination of the content of soil organic matter (OM) (method for determination of soil organic matter, GB9834-1988), soil total nitrogen (TN) and available nitrogen (AN) (modified Kjeidahl method, HJ/T 707–2014), soil available kalium (AK) (flame atomic absorption spectrophotometry method, GB 9836 – 1998), and soil available phosphorus (AP)
determination (sodium hydrogen carbonate solution-Mo-Sb anti spectrophotometric method, HJ/T 704–2014), were measured by the Institute of Soil Science, Chinese Academy of Sciences (Nanjing, China).

Quantitative PCR analysis of *Trichoderma* spp. and FON

The abundances of *Trichoderma* spp. and *F. oxysporum f. sp. niveum* (FON) in the rhizosphere soil and root tissue of watermelon were estimated by real-time PCR assays with an IQ5 Real-time PCR system (Bio-Rad Lab, LA, United States). The *Trichoderma* spp. was quantified by quantitative PCR with DG/DT primers (5'-CTGGCATCGATGAAGAACG-3'/5'-ATGCGAGTGTGCAAACACTCTG-3') (Han et al., 2013). The FON species was preamplified by PCR for 18 cycles by Fonq-F/Fonq-R (5'-GTTGCTTACGTTCTAACTGTGC-3'/5'-GGTACTTGGAAGGAATTGTGGG-3'), and then Fluorescence quantitative PCR was performed using 1 µl of PCR product as template in our laboratory (Xiao et al., 2018). The initial copy number of the target gene was calculated by comparing the threshold cycle (Ct) values of each sample with the calibration curve. The calibration curve was fixed according to the methods suggested by Wei et al., (2013) and Xiao et al., (2018). Sterile water was used as a negative control. All amplifications were conducted three times.

Rhizosphere Soil DNA Extraction And Sequencing

Each rhizosphere soil sample DNA was extracted from 100 mg of soil using a MoBio kit (MO BIO Laboratories, Inc., USA) according to the manufacturer's instructions. DNA sample quality was measured using a NanoDrop spectrophotometer (2000/2000C, Thermo Scientific, USA), and 2 µL of each sample was subjected to electrophoresis on a 0.8% agarose gel using 1 × TAE buffer. Three replicates for each treatment were obtained in our experiment.

For bacterial community analysis, the bacterial 16S rRNA gene was amplified using the following primers: 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTAC HVGGGTWTCTAAT-3') (Zhao et al., 2014). For fungal community analysis, the internal transcribed spacer (ITS) regions were amplified using the following primers: ITS 5F (5'-GGA AGTAAAAGTCGTAACAAGG-3') and ITS 1R (5'-GCTGCGTTCTTCTACGT AGC-3') (Lopez-Mondejar et al., 2010). The DNA library was prepared utilizing the TruSeq Nano DNA LT Library Prep Kit for Illumina. Sequencing of the paired-end library was performed using the Illumina MiSeq PE250 sequencing platform. The all raw sequence data have been submitted to the NCBI sequence Read Archive (SRA) under the accession numbers SRP265681 and SRP265697.

Data Analysis And Statistical Analyses

Raw bacterial and fungal sequences were assigned to each sample based on the corresponding unique barcodes. The sequences were clustered into operational taxonomic units (OTUs) at 97% sequence similarity by utilizing UCLUST (Edgar, 2010) after quality control and elimination of short reads, chimaeras, ambiguous sequences, and low-quality sequences using QIIME (Caporaso et al., 2010). The
OTUs were classified using the UNITE and Greengenes databases for fungi and bacteria, respectively (Desantis et al., 2006; Urmas et al., 2013). To analyse the differences in the bacterial and fungal community structures among the different treatments, principal co-ordinates analysis (PCoA) (Jiang et al., 2013) and redundancy analysis (RDA) (Jongman et al., 1995) were applied. Differences in parameters among treatments were compared by performing a one-way analysis of variance (ANOVA) at the end of each bioassay followed by Duncan's multiple range tests in IBM SPSS Statistics 25.0 (P < 0.05). P < 0.05 was regarded as significant.

Results

Effect of *T. asperellum* M45a on watermelon health properties and FON content

The effect of *T. asperellum* M45a on watermelon health parameters was assessed using the FW disease incidence (DI) and the length of watermelon vine. FW disease occurs in the seedling stage and then erupts rapidly until it becomes stable in the flowering stage. Compared to CK, the biocontrol effects of *T. asperellum* M45a on watermelon FW disease were 89.65%, 72.62% and 66.72% at the seedling period (S2), the smoke trailing period (S3) and the blooming period (S4), respectively, which was significantly different from the CK group in the same period (P < 0.01) (Fig. 1a). Additionally, the vine lengths of watermelon inoculated with strain M45a also increased significantly by 29.44%, 26.43% and 49.15% in the S2, S3 and S4 periods, respectively (Fig. 1b).

To understand the dynamic colonization relationship between *Trichoderma* spp. and *Fusarium oxysporum f. sp. niveum* (FON) in the rhizosphere soil, qPCR technique was applied. In the study, we found that the number of FON in CK rapidly increased to 9543.66 cfu/g in the germination period (S1), which was significantly higher than *T. asperellum* M45a treatment. Except for the smoke trailing period (S2), FON in the control rhizosphere soil was significantly higher than that in M45a treatment (Fig. 1a). In addition, the contents of *Trichoderma* spp. in the rhizosphere soil of the M45a treatment were 13.89, 10.96, 8.47 and 15.4 times higher than CK treatment in the same stage, respectively, and remained at 2.32–4.25 ng/g for the onset of FW (Fig. 1b).

Effect of *T. asperellum* M45a on soil enzyme activities and properties in rhizosphere soil

In the study, the enzyme activities of CL, ACP, CAT and SC in rhizosphere soil were increased following treatment with M45a, except to the activities of SC in the seedling period (S2) (Fig. 2). For example, the maximum increases in the activities of SC (224.15%) and ACP (95.80%) were obtained in the smoke trailing period (S3). Additionally, the activities of UE in the control group decreased gradually with the occurrence of watermelon FW. In contrast, M45a treatment could effectively enhance the UE enzyme activity, and the highest activity (501.478 U/g) was observed in the S4 period. In addition, no significant difference was observed in OM and the available K, while the TN and available nutrients (NO3-N, and P) were under significantly different treatments (Table 1). Compared with the control (CK), treatments with inoculated M45a significantly increased the contents of TN, NO3-N and AP, and decreased the AK content.
Effect of inoculating M45a on soil enzyme activities in the rhizosphere of watermelon. The application of *Trichoderma asperellum* M45a in continuous cropping soil (Trichoderma). The non-inoculated control (CK). Trichoderma1, Trichoderma2, Trichoderma3, Trichoderma4: the treatment with *Trichoderma asperellum* M45a at S1, S2, S3 and S4 period, respectively; CK1, CK2, CK3, CK4: the CK treatments at S1, S2, S3 and S4 period, respectively. S1: the germination period; S2: the seedling period; S3: the smoke trailing period; S4: the blooming period.

| Treatment   | TC (%)  | TN (mg/kg-1) | NH4+ (mg/kg-1) | NO3- (mg/kg-1) | AP (mg/kg-1) | AK (mg/kg-1) |
|-------------|---------|-------------|---------------|---------------|--------------|--------------|
| CK 1        | 2.72 ± 0.09a | 2000.60 ± 6.86a | 25.24 ± 0.60a | 364.14 ± 15.50a | 67.43 ± 1.99a | 490.54 ± 9.46a |
| Trichoderma 1 | 2.65 ± 0.05a | 2015.34 ± 3.40b | 24.88 ± 1.12a | 367.69 ± 4.67a | 76.85 ± 0.77b | 358.29 ± 6.64b |
| CK 2        | 2.21 ± 0.09a | 1816.65 ± 4.10a | 38.66 ± 0.51a | 338.55 ± 11.20a | 65.44 ± 1.87a | 511.52 ± 4.64a |
| Trichoderma 2 | 2.33 ± 0.12a | 2058.45 ± 5.76b | 48.43 ± 2.01b | 365.94 ± 6.55b | 68.46 ± 1.35a | 483.30 ± 8.31b |
| CK 3        | 2.51 ± 0.09a | 1807.73 ± 7.46a | 14.60 ± 1.13a | 268.75 ± 8.21a | 69.88 ± 1.53a | 420.91 ± 5.63a |
| Trichoderma 3 | 2.27 ± 0.08ab| 1920.03 ± 9.10b | 30.38 ± 1.17b | 283.62 ± 5.54ab | 63.26 ± 2.18b | 417.80 ± 5.93a |
| CK 4        | 2.74 ± 0.09a | 1797.65 ± 13.09a | 10.08 ± 0.80a | 258.40 ± 10.04a | 56.85 ± 0.88a | 323.62 ± 17.40a |
| Trichoderma 4 | 2.58 ± 0.10a | 1837.18 ± 6.58ab | 9.38 ± 0.78a | 287.23 ± 8.80ab | 71.84 ± 2.21b | 266.37 ± 6.75b |
| Trichoderma 1 | 2.65 ± 0.05a | 2015.34 ± 3.40b | 24.88 ± 1.12a | 367.69 ± 4.67a | 76.85 ± 0.77b | 358.29 ± 6.64b |

**Effect of T. asperellum M45a on microbial community structure in rhizosphere soil**

In total, 3,607,435 and 3,417,512 high-quality 16S and ITS sequences were obtained from the rhizosphere soil samples in the four stages, respectively. In the present study, the significant difference of alpha diversity between the two groups is shown in Fig. 3 and Table S1. There was significant difference in Chao1 between the M45a-treated group and the CK group (ANOVA, *p* < 0.05), and the fungal Shannon index in the M45a group was significantly lower than those of the CK group at all stages (ANOVA, *p* < 0.01). With the occurrence of watermelon wilt, the fungal Chao1 values in the S1 and S2 groups were significantly higher than those of the S3 and S4 groups (Table S1, *p* < 0.05). Additionally, the principal coordinate analysis (PCoA) ordinations showed that M45a(T) had significantly effect on bacterial and fungal community composition (Fig. 3). The first coordinate (PCoA1) showed 41.1% and 50.4% difference in community variation, and PCoA2 explained 17.6% and 25.8% dissimilarity, Respectively. In
addition, it was further verified by the PERANOVA dissimilarity tests based on Bray-Curtis distance among the two groups (Bacterial: $R = 0.05787, p = 0.001$; Fungal: $R = 0.27598, p = 0.001$).

**Effect of** T. asperellum **M45a on the microbial community composition**

In general, the dominant bacterial phyla between two groups are visualized in the Fig. 4a and Fig. S1. In brief, the most abundant bacterial phyla were Proteobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, Saccharibacteria and Acidobacteria, which contributed almost 86.7–91.8% of the bacterial sequences. While the rhizosphere communities were compared at different growth stages, Saccharibacteria (2.0-14.1%) was present at a significantly increased proportion in the rhizosphere with the onset of FW. At the genus level, among the top 20 genera, the relative abundance levels of *Pseudomonas, Sphingomonas, Actinomadura* and *Rhodanobacter* in the M45a group were significantly higher than in the CK group (Fig. S1, $p < 0.05$).

To investigate the difference in the fungal classes between the two groups, as illustrated in Fig. 4b, we found that the most relative abundant class was Sordariomycetes (60.12–78.67%) in the M45a group, which was significantly higher than in the CK group. However, the second relative abundance of Eurotiomycetes was consistently lower in M45a treatments (0.89–24.71%) than in the CK group (32.26–55.62%). For the fungal genera, the relative abundance levels of *Penicillium, Chaetomium, Aspergillus* and *Acremonium* in the M45a-treated rhizosphere soil were significantly lower than in the CK group (Fig. S2, $p < 0.05$). However, *Trichoderma* was present at a significantly increased proportion during the growth stages in M45a treatment, consistent with the real-time PCR results.

**Effect of** T. asperellum **M45a on potential functional composition diversities**

For the bacterial community, amino acid metabolism (10.81%-10.98%), carbohydrate metabolism (10.45%-10.81%), and energy metabolism (5.53%-5.81%) were the main bacterial metabolic pathways in all treatments (Fig. S3). In this study, the enzyme families (2.02%-2.11%) were different between the M45a treatment and the control (CK). Compared with the control soil (CK), functional profiles with lower abundance were related to enzyme families in M45a-treated soil at different stages, but there were no significant differences in response to disease (Fig. 5a).

For the fungal community, the different functional profiles of trophic mode (symbiotroph, saprotroph, pathotroph) and guild (plant pathogen) of fungal communities were compared between M45a and CK treated soil at different stages. Compared with the CK treated soil, the relative abundance levels of Ectomycorrhizal, Endophyte, Animal pathotroph and saprotroph (Dung Saprotroph, Plant Saprotroph, Soil Saprotroph and Wood Saprotroph) in the fungal community were significantly lower in M45a-treated soil (Fig. 5b). Additionally, the relative abundance levels of pathogens and other trophic modes showed no significant differences in these treatments.

**Relationships among soil enzyme activities, soil properties and microbial communities**
The RDA analysis showed that enzyme activities (CL, ACP and CAT) and the properties (AP) could greatly affect the microbial community composition in rhizosphere soil (Fig. 6). In addition, a significant positive correlation was observed between the FW disease incidence (DI) and soil cellulase activities (CL). However, TN, NH4+-N and NO3-N were negatively correlated with the DI. Likewise, there were significant positive correlation between Sphingomonas, Rhodanobacter, Pseudomonas, Gemmatimonsa, Streptomyces and S-CL activities, and a significant negative correlation between Nocardioides and S-CL activities (p < 0.05) (Table S2). The correlations between the major fungal genera and the rhizosphere soil enzyme activities were then observed (Table S3). Fungi are likely to be more sensitive to ACP activities than bacteria; thus, the increased Trichoderma spp. in the M45a treatment soil are sufficient to impose a stress on fungi and thereby likely influence fungi species, such as Penicillium, Chaetomium, Aspergillus and Dendroclathra, which were significantly negatively related to the ACP and SC activities in the rhizosphere soil, while the opposite trend was observed for the UE activities (p < 0.05).

Discussion

In the present study, our results showed that the treatment with Trichoderma-inoculation significantly reduced the incidence of watermelon FW and increased the length of watermelon vines under continuous cropping. Many studies have reported that Trichoderma spp. exhibits significant effects against watermelon FW (Wu et al., 2009), tomato late blight (Bahramisharif and Rose, 2018), rice sheath blight (Jambhulkar et al., 2018), maize stalk rot (Li et al., 2016) and banana wilt (Taribuka et al., 2017). Additionally, Trichoderma spp. have significant promoting effects on plants, seedlings and crop yields in some crops, such as those of cucumber, tomato and alfalfa (Zhang et al., 2013; Liu et al., 2017; Zhang et al., 2019). Trichoderma spp. are adept at promoting plant growth, which is consistent with our result that the length of watermelon vines in the T. asperellum M45a treatment (T) was significantly higher than that in control (CK). Several studies have demonstrated that Trichoderma spp. could increase the solubilization of soil nutrients (Yedidia et al., 2001; Harman et al., 2004). Trichoderma spp. can produce a large amount of organic acids, which release the delayed nitrogen, available nutrients (N, P, and K) in the soil for plant growth (Zhang et al., 2019). That well explains our result that M45a treatment had the greater contents of available N and P.

Enzyme activities have been proposed as biological indicators of soil quality, and are closely related to soil function (Kandeler et al., 1996; Xian et al., 2015). The activities of catalase (CAT), cellulase (CL), sucrase (SC), urease (UE) and acid phosphatase (ACP) are closely related to the cycling of soil organic matter, carbon, nitrogen and phosphorus, respectively (Trasar-cepeda et al., 2007). In our study, it was found that M45a significantly increased the activities of CL and ACP, with significant differences observed at the spreading and flowering stages. Compared with the control group (CK), the activities of CL, ACP, CAT and SC in soil treated with M45a were all increased, which indicated that T. asperellum M45a could promote the transformation of related enzyme activities such as nitrogen, carbon and phosphorus in soil, thus promoting the decomposition of delayed nutrients in soil into available nutrients absorbed and utilized by plants. These findings are consistent with the studies of some scholars, and
indicated that *Trichoderma spp* could effectively increase the soil enzyme activity and improve plant growth (Zhang et al. 2018; Laur et al. 2018).

The control effect of *Trichoderma* spp. on watermelon FW was also reflected in the interaction between *Trichoderma* spp. and *Fusarium oxysporum*. After the application of *Trichoderma asperellum* to the field, the *Trichoderma* spp. spores can rapidly colonize and reproduce itself, further inhibiting the reproduction of *Fusarium oxysporum* (He et al., 2018). Thus, we further used real-time PCR to quantify *Trichoderma* spp. and *Fusarium oxysporum* f. sp. *niveum* (FON) in rhizosphere soil. The quantitative determination results showed that the number of *Trichoderma* spp. in rhizosphere soil treated with M45a was 10-fold higher than the control and remained stable during the whole process, while the amount of FON was greater than that in the control treatment only during the smoke trailing period (S3). The most possible reason for this difference is a large number of FON could cause the occurrence of FW by infecting watermelon roots, while the M45a treatment can induce rapid proliferation of *Trichoderma* spp., around the rhizosphere of watermelon to effectively prevent FON infection of watermelon roots.

As previously reported, soil microbial diversity is a key factor affecting soil quality and health (Zhao et al., 2018). Liu et al., (2019a) found that continuous cropping led to increases in the diversity and richness of fungi in the rhizosphere soil. Therefore, the balance of rhizosphere soil microecology is crucial for healthy growth. Many studies have demonstrated that low fungal diversity and high bacterial diversity were observed after application of Trichoderma bio-organic fertilizer and Fen-Daqu (Zhang et al. 2016; Zhao et al. 2018). These results are consistent with our study, which showed that a significant difference was observed in the fungal diversity of watermelon rhizosphere soil between the M45a and control treatments. M45a treatment reduced the fungal diversity in the rhizosphere soil. To compare the different populations between the two groups at the genus level, we found that the relative abundance levels of *Penicillium*, *Chaetomium*, *Aspergillus* and *Acremonium* in M45a group were significantly lower than in the CK group. We hypothesized that *T. asperellum* M45a could rapidly proliferate after inoculation, and reduce the relative abundance levels of *Penicillium*, *Chaetomium*, *Aspergillus* and *Acremonium* through nutrient competition, thus regulating the structure and composition of soil fungal community.

In addition, the soil bacterial and fungal community compositions vary in different treatments, where the antagonism may take place between the inoculated-*Trichoderma* and some bacteria (Pan and Jash, 2011). In our study, the abundances of *Sphingomonas*, *Pseudomonas*, *Actinomadura* and *Rhodanobacter* increased significantly in the M45a treatment, which could potentially promote plants growth and health among the top 20 abundant dominant bacterial genera. Therefore, we hypothesized that the application of M45a may stimulate the proliferation of *Pseudomonas*, *Sphingomonas* and *Rhodanobacter*, which are widely recognized as beneficial to plant growth and disease resistance (Cordier and Alabouvette, 2009; Laur et al., 2018; Carlson H et al., 2019). Functional prediction showed that there was no significant difference in the bacterial metabolic pathways between the two treatments, which was similar to the results described by Benitez et al., (2017). However, some fungi such as *Chaetomium* and *Acremonium*, as Saprotrophic or Pathotrophic fungi, which obtain nutrients by attacking host cells, lacking in the M45a treated soil, so, they exert negative effects on other organisms (Nguyen et al 2016). And that has been
reported that *Trichoderma* (Hu et al., 2016) and *Pseudomonas* (Wang et al., 2015) could efficiently control FW, they might play a synthetic role in promoting the plant resistance.

The soil properties have been considered as an important factor for changing plant rhizosphere microorganisms in previous studies (Zhou et al., 2017). In this study, the RDA results showed that M45a-inoculation greatly influenced watermelon growth and FW disease incidence in the pot experiment. It could increase soil available N and P, which are directly beneficial to plant growth. These findings support our results that the soil available N and P contents were significantly higher in the M45a treatment than in the control treatment and that M45a treatments significantly increased the length of watermelon vine. The M45a treatment had a negative effect on the soil fungal community, and the soil fungal community was most closely associated with changes in soil nutrients and enzyme activities. For example, *Trichoderma, Pseudomonas, Sphingomonas* and *Rhodanobacter* were positively correlated with ACP, CL, CAT and SC activities, which may indicate that soil enzyme activities are closely associated with living organisms (Govarthanan et al., 2018; Cruz et al., 2018). For species and functional analysis, metagenomic and transcriptomic techniques are required in a future study.

Therefore, M45a-inoculation regulated available soil nutrients, rhizosphere soil enzyme activities and soil microbial community. Additionally, the inoculation indirectly improved crop growth and reduced the FW disease incidence in the continuous cropping watermelon. These results indicate that improving the plant rhizosphere microbiota can help control soil-borne diseases and promote plant growth. In future studies, we intend to study the microecology mechanisms in different soil environments by exploring *Trichoderma* responses to different soil environments. This will provide valuable information regarding *Trichoderma* application in different regions.

**Abbreviations**

FW: Fusarium wilt; DI: disease incidence; ACP: acid phosphatase; CAT: catalase; CL: cellulase; UE: urease; SC: sucrase; OM: organic matter; TN: total nitrogen; AK: available kalium; AP: available phosphorus; PCoA: the principal coordinate analysis; ANOSIM: analysis of similarities;

**Declarations**

**Authors’ Contribution**

ZHL designed the experiments. YZ and ZHL performed the experiments, CT and LJX analyzed the data, CT prepared figures and/or table, LW, YT and YZ revised this manuscript language. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and material

The strains were available upon request. All data obtained have been included into the manuscript and its additional files.

Consent for publication

Not applicable.

Ethics approval and consent participate

Not applicable.

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Figures

Figure 1

Effect of inoculating M45a on watermelon health properties and FON content. The inoculation of T. asperellum M45a in continuous cropping soil (T). The non-inoculated control (CK). S1: the germination period; S2: the seedling period; S3: the smoke trailing period; S4: the blooming period.

Figure 2

Effect of inoculating M45a on soil enzyme activities in the rhizosphere of watermelon. The inoculation of T. asperellum M45a in continuous cropping soil (Trichoderma). The non-inoculated control (CK). S1: the germination period; S2: the seedling period; S3: the smoke trailing period; S4: the blooming period.

Figure 3

Richness (Chao1) and shannon diversity indexes for each treatment. The application of T. asperellum M45a in continuous cropping soil (Trichoderma). The non-inoculated control (CK). S1: the germination period; S2: the seedling period; S3: the smoke trailing period; S4: the blooming period.

Figure 4

The PCoA result of the bacterial and fungal communities structure among the different treatment. The application of T. asperellum M45a in continuous cropping soil (Trichoderma). The non-inoculated control (CK). S1: the germination period; S2: the seedling period; S3: the smoke trailing period; S4: the blooming period.

Figure 5

Relative abundances of bacterial phyla and fungal class under each treatment. The application of T. asperellum M45a in continuous cropping soil (Trichoderma). The non-inoculated control (CK). Trichoderma1, Trichoderma2, Trichoderma3, Trichoderma4: the treatment with T. asperellum M45a at S1, S2, S3 and S4 period, respectively; CK1, CK2, CK3, CK4: the CK treatments at S1, S2, S3 and S4 period, respectively.
Figure 6

Relative abundances of bacterial metabolic pathways (a) and fungal trophic modes (b) identified from each treatment. The application of T. asperellum M45a in continuous cropping soil (Trichoderma). The non-inoculated control (CK). S1: the germination period; S2: the seedling period; S3: the smoke trailing period; S4: the blooming period.

Figure 7

Redundancy analysis (RDA) of bacterial (a) and fungal (b) genera datasets and measured soil properties in rhizosphere soils after interactive forward selection (p < 0.05, VIFs > 20). The application of T. asperellum M45a in continuous cropping soil (Trichoderma). The non-inoculated control (CK). S1: the germination period; S2: the seedling period; S3: the smoke trailing period; S4: the blooming period.

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