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13 Cycles of Consecutive Tomato Monoculture Cropping Alter Soil Chemical Properties and Soil Fungal Community in Solar Greenhouse

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Abstract: Consecutive tomato monoculture cropping (CTM) obstacles severely restrict the development of facility tomato industry in China. However, the effect of CTM on the soil fungal community in greenhouses is still unclear. Here, we aim to identify the variation of soil chemical properties and soil fungal community associated with CTM for 1, 3, 5, 9 and 13 cycles. The results indicated that CTM led to a significant increase in soil total phosphorus (TP) and soil electrical conductivity (EC) value. CTM, though, significantly increased soil fungal community diversity, yet also led to the imbalance of soil fungal community compositions. Specifically, a beneficial soil fungus, *Chaetomiaceae*, decreased significantly at CTM13, while several soil pathogenic fungi, including *Fusarium* and *Cladosporium*, increased significantly at CTM13. A redundancy analysis (RDA) indicated that soil EC value, pH and TP had a greater impact on soil fungal community structure. Structural-equation-model (SEM) analysis indicated that, when compared with CTM3–CTM9, the decline of tomato fruit fresh weight per plant (TFFW) at CTM13 might be related to the significant increase in soil EC value, soil *Fusarium* and *Cladosporium*. Thus, appropriately decreasing soil EC and soil pathogenic fungi and enhancing soil beneficial fungi under a CTM system is crucially important for sustainable tomato production in greenhouses.

Keywords: consecutive tomato monoculture cropping (CTM); soil fungal community; soil chemical properties; solar greenhouse

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1. Introduction

Tomato (Lycopersicon esculentum Mill.) is a world-famous fruit vegetable because of its high yield and rich nutrition [1,2]. Facility cultivation is one of the main forms of tomato production in Liaoning Province, China, and the cultivation area of facility tomatoes increased year by year [3]. Consecutive monoculture cropping of tomatoes in facility is a widespread cultivation pattern in northern China [4]. At present, the research on consecutive tomato monoculture cropping mainly focus on how to repair soil restriction through rotation, intercropping and returning organic materials to CTM fields. However, there is little research on the generation rule of soil obstacles under consecutive tomato monoculture cropping [4,5].

The main reason for consecutive monoculture cropping is that growers want to plant suitable crops in suitable soil and climate conditions. Moreover, compared with rotation and intercropping, consecutive monoculture cropping often needs simpler technical requirements for agricultural producers and is conducive to the formation of stable production

and marketing channels [6]. However, consecutive monoculture cropping for several cycles usually had a negative impact on plant growth and the formation of crop yield. For example, compared with non-continuous cropping sugar beet, the height, fresh weight and dry root weight of continuous sugar beetroot were all significantly decreased [7]. Zhao et al. also found that, with the increase of continuous cropping years, the fruit yield of cucumber significantly decreased [8]. In addition, consecutive monoculture cropping also led to the occurrence of some plant diseases which were caused by the accumulation of soil-borne pathogens. Hu et al. indicated that higher root disease incidences were detected in continuous monoculture soils than in non-continuous monoculture soil [9]. It is generally believed that the detrimental effects of consecutive monoculture cropping on crops are mainly related to the degradation of soil quality [10,11]. Therefore, we believe that it is important to clarify the soil degradation rule under the consecutive tomato monoculture cropping system for targeted remediation of soil continuous cropping obstacles in facility.

Soil microbes are a vital pointer for soil health and function because of their sensibility to any slight soil environmental changes [12,13]. Several studies have revealed the change rule of soil bacteria at consecutive tomato monoculture cropping cultivation patterns. Fu et al. [14] and Zhao et al. [15] found, respectively, that long-term (13-cycles or 20-years) tomato continuous cropping significantly decreased the number of soil bacteria. Min et al. [16], using T-RFLP combined with 16S rRNA gene clone library technology, reported that continuous tomato cropping decreased soil bacterial diversity when compared with rotation cropping, and *Proteobacteria* were predominant in continuous tomato cropping rhizosphere soils, followed by *Bacteroidetes* and *Acidobacteria*. As another kind of plenteous and diverse group of soil microbes, fungi not only serve as decomposers in soil ecosystems, but also participate in soil nutrient cycling [17,18]. Some recent studies have concentrated on the shifts of soil fungal communities under consecutive monoculture cropping systems of many crops. Li et al. found that soil-borne disease pathogens, including *Fusarium* and *Guehomyces*, were significantly increased, while the soil-beneficial species *nematicidal* decreased after continuous strawberry cropping. These genera might be the key fungi associated with the strawberry continuous cropping obstacle [19]. Zhao et al. [20] reported that soil fungal community diversity significantly decreased when cucumber mono-cropping took place for more than 8 years, and soil pH, OM and NO_3^--N markedly responded to the shifts of soil fungal community. Nevertheless, there are few studies, or still at an early stage if any, about the effect of different consecutive tomato monoculture crops on dynamic changes of soil fungal community composition.

Therefore, the objective of this study was to explore the variation of soil fungal community diversity under CTM systems in a solar greenhouse using Illumina pyrosequencing technology and then to clarify the relation between soil fungal community and soil chemical properties. On this basis, we expected to assess the effect of soil fungal community and soil chemical properties on TFFW in solar greenhouses and offer theoretical support to control consecutive tomato monoculture cropping obstacles in solar greenhouses.

2. Materials and Methods

2.1. Site Description and Soil Sampling

Our experiment was carried out in a solar greenhouse (60 m length,10 m span and area total 600 m^2) at a horticulture college at Shenyang Agriculture University, China (41°31′ N, 123°24′ E). The original soil for experimental purposes was collected from a plot outside the greenhouse, where no solanaceous vegetables had been planted before. The soil type was Hapli-Udic Cambisol with the following basic properties measured in 2009: pH 7.04, EC 290.30 μs·cm^{-1}, available N 95.81 mg·kg^{-1}, available P 94.65 mg·kg^{-1}, available K 255.70 mg·kg^{-1}. In our solar greenhouse, there are 39 vacant cultivation pools, 1.5 m long, 1.0 m wide and 0.8 m deep each, with surrounding cement walls and soil bottom, used for consecutive tomato monoculture cropping. In the spring of 2009, we filled three vacant pools with the original soil. Then, consecutive tomato monoculture was performed in these three pools as two cycles in one year, including the spring cycle (from March to July) and

the autumn cycle (from August to January of the following year), lasting from the spring of 2009 to the spring of 2015. Therefore, by the end of the spring cycle of 2015, CTM13 was obtained. Then, in the autumn of 2009, another three vacant pools were selected and filled with the original soil for consecutive tomato monoculture in both autumn and spring every year until the spring cycle of 2015. Therefore, by the end of the spring crop of 2015, CTM12 was obtained. Just in this way, every three new cultivation pools were added with the original soil, which was used to plant tomato seedlings consecutively each season until the spring cycle of 2015. Finally, we acquired consecutive monoculture tomato plant samples and soil samples in July 2015, including the CTM13, CTM12, CTM11, CTM10, CTM9, CTM8, CTM7, CTM6, CTM5, CTM4, CTM3, CTM2 and CTM1 cycles. We selected CTM1, CTM3, CTM5, CTM9 and CTM13, respectively, for analysis. In this study, seedlings of "Liaoyuanduoli", a common tomato variety of large fruit type, were planted with conventional management, with three ears of fruit reserved before harvesting. Before tomato planting for each cropping season, each cultivation pool was applied about 4.0 kg of puffed chicken manures (25.50 g N kg^{-1}, 39.00 g P_2O_5 kg^{-1} and 27.30 g K_2O kg^{-1}) and 0.12 kg of nitrogen-phosphorus-potassium mixed fertilizer (16:16:16). Eight tomato seedlings were planted in two rows per cultivation pool, with plant spacing of 35cm for each seedling. The soil surface of each cultivation pool was covered with black plastic film. The plants were irrigated by drip irrigation to ensure uniform water content in each pool during the growth period.

2.2. Measure of Tomato Fruit Fresh per Plant

For each treatment, three tomato plants were selected to measure tomato fruit fresh weight per plant (TFFW). The weight of 12 fruits per tomato plant (3 ears per tomato plant, 4 fruits per ear) was used to represent the tomato fruit fresh weight per plant. Tomato fruit fresh weight was measured continuously using an electronic scale as the fruit ripened, and the average tomato fruit fresh weight per plant (TFFW) was calculated for each treatment.

2.3. Soil Sampling

Soil samples were collected on the 105th day after spring planting in 2015. For each cultivation pool, the soil sample was a mixed one from 5 points, as randomly collected with an auger (D = 2.5 cm) drilling down for 20 cm each within a radius of 20 cm away from the tomato main stem. A total of 15 soil samples (5 treatments × 3 pools per treatment) were placed into sterilized plastic bags, respectively, and transported in ice boxes to the lab. In the laboratory, we separated each soil sample into two parts: One part was stored at −80 °C for analyzing the soil fungal community, while the other part was air-dried for the analysis of soil chemical properties.

2.4. Analysis of Soil Chemical Properties

Soil pH and soil organic matter content (OM) had been measured and published (14). Soil EC was measured with a Thunder Magnetic DDS-307 conductivity meter (INESA, Shanghai, China) using a soil-to-water ratio of 1:5 [21]. Soil total nitrogen content (TN) was digested with 5 mL concentrated H_2SO_4 and mixed catalyst, and then analyzed by an automatic Kjeldahl nitrogen analyzer (BUCHI, Flaville, Switzerland) [22]. Soil total phosphorus content (TP) was extracted using the $HClO_4$-H_2SO_4 method and then determined by the molybdenum blue colorimetric method [23]. Soil total potassium content (TK) was extracted via the NaOH digestion and then analyzed using a flame photometer (iCE3000, Thermo Fisher Scientific, Waltham, MA, USA) [23].

2.5. Soil DNA Extraction, PCR and Sequencing Analysis of Soil Fungal Community

DNA was extracted from a 0.5 g soil sample using the Fast DNA Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions [24]. Fungal ITS2 was amplified by PCR with primers adopted as ITS2F: GCATCGATGAAGAACGCAGC and ITS2R: TCCTCCGCTTATTGATATGC [24]. PCR reactions were con-

ducted in a 30 μL mixture, containing 15 μL of 2 × KAPA Library Amplification ReadyMix, 1 μL of 10 μM each primer, 50 ng of template DNA and ddH_2O. Amplicons were purified by using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified by Qubit® 2.0 (Invitrogen, USA). After building the sequencing library, we sequenced on the HiSeq PE250 platform (Illumina, Inc., CA, USA) at Realbio Genomics Institute (Shanghai, China). Raw fastq files were quality-filtered by Trimmomatic and merged by FLASH. Operational taxonomic units (OTUs) were clustered with a 97% similarity cutoff by using Uparse (version 7.0.1090 http://drive5.com/uparse/; accessed on 1 June 2021). The taxonomy of each ITS2 rRNA gene sequence was analyzed by the RDP Classifier algorithm (http://rdp.cme.msu.edu/; accessed on 1 June 2021) against the UNITE database (Release 8.0 https://unite.ut.ee/index.php; accessed on 1 June 2021). The sequencing data were submitted to the NCBI Sequence Read Archive database (accession number: PRJNA523068). The Abbreviations were all listed in the back matter.

2.6. Statistical Analysis

All significant differences among the treatments, including TFFW, soil chemical properties, soil fungal community diversity indexes and composition, were all performed using a one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests ($p < 0.05$). A Venn diagram at the OTU level was performed with R (version 3.3.1). Principle coordinates analysis (PCoA) using R (version 3.3.1), along with ANOSIM analysis using QIIME (Version 1.9.1), were both performed with Bray–Curtis distance matrices based on OTU level. A redundancy analysis (RDA) and Heatmap analysis, which both compared with the Spearman correlation test, were performed with R (version 3.3.1) to examine the relationships between soil fungal genera and soil chemical properties.

A structural equation model (SEM) was applied to test the effects of soil chemical properties and soil fungal genera on TFFW, using the Amos 21.0 software (AMOS IBM, USA). The fitness of the model was evaluated via a non-significant chi-square test ($p > 0.05$), low $\chi 2$ /df (<2), the comparative fit index (GFI > 0.9) and the low root square mean errors of approximation (RMSEA < 0.05).

3. Results

3.1. Tomato Fruit Fresh per Plant and Soil Chemical Properties

TFFW first increased and then decreased gradually with the increasing of consecutive tomato monoculture cycles (Table 1). Compared with CTM1, the TFFW of CTM3, CTM5 and CTM9 were all significantly increased ($p < 0.05$). TFFW of CTM13 had no significant difference with CTM1, however, it was significantly lower than those in CTM3, CTM5 and CTM9 ($p < 0.05$).

Table 1. Tomato Fruit fresh per plant and soil chemical properties among different CTM cycles.

	TFFW (g·Plant^{-1})	TN (g·kg^{-1})	TP (g·kg^{-1})	TK (g·kg^{-1})	EC (μs·cm^{-1})
CTM1	1495.60 ± 42.85 b	1.13 ± 0.08 b	2.34 ± 0.17 c	14.12 ± 0.64 a	520.30 ± 17.49 c
CTM3	2014.87 ± 33.58 a	1.17 ± 0. 03 b	2.51 ± 0.18 c	14.54 ± 0.45 a	556.97 ± 7.53 c
CTM5	1969.55 ± 14.22 a	1.43 ± 0.23 ab	2.98 ± 0.26 bc	14.91 ± 0.31 a	673.47 ± 57.50 b
CTM9	1936.78 ± 63.68 a	1.67 ± 0.08 a	3.84 ± 0.29 a	15.48 ± 0.34 a	795.37 ± 12.76 a
CTM13	1466.24 ± 32.00 b	1.20 ± 0.08 b	3.61 ± 0.08 ab	16.61 ± 1.99 a	887.30 ± 42.66 a

TFFW = Tomato fruit fresh weight per plant; TN = soil total nitrogen content; TP = soil total phosphorus content; TK = soil total potassium content; EC = electrical conductivity; different letters in the same column represent the significant difference ($p < 0.05$).

Compared with CTM1, soil TN exhibited an increasing trend with the increase of CTM cycles, but only soil TN at CTM9 was significantly higher than that of CTM1 ($p < 0.05$). Soil TP and soil EC also showed an increasing trend with the increase of CTM cycles. Soil TP of CTM9 and CTM13 were significantly higher than those of CTM1 and CTM3 ($p < 0.05$). Soil EC of CTM5, CTM9 and CTM13 appeared significantly higher than that of CTM1 by 29.44,

52.87 and 70.54%, respectively ($p < 0.05$). Soil TK showed no significant difference among all treatments (Table 1).

3.2. Soil Fungal Community Diversity

A total of 887,656 fungal sequences with an average length of 307 bp were obtained using Illumine Hiseq pyrosequencing technology (Table S1). The coverage index of all samples was greater than 0.99, and there was no significant difference among all treatments ($p < 0.05$) (Figure 1A). With the increase of consecutive tomato monoculture cycles, Sobs and PD indexes decreased at first, and then increased, while the Simpson index opposed the change rule (Figure 1B–D). Compared to CTM1, the Sobs and PD index of CTM13 were significantly increased ($p < 0.05$) (Figure 1B,D). The lowest Simpson index was at CTM13, which was significantly lower than that of other treatments ($p < 0.05$) (Figure 1C). The Venn diagram indicated that the unique OTUs number of CTM1, CTM3, CTM5, CTM9 and CTM13 for soil fungi were 38, 45, 31, 41 and 325, respectively, while the common OTUs number of all treatments was 169 (Figure S1).

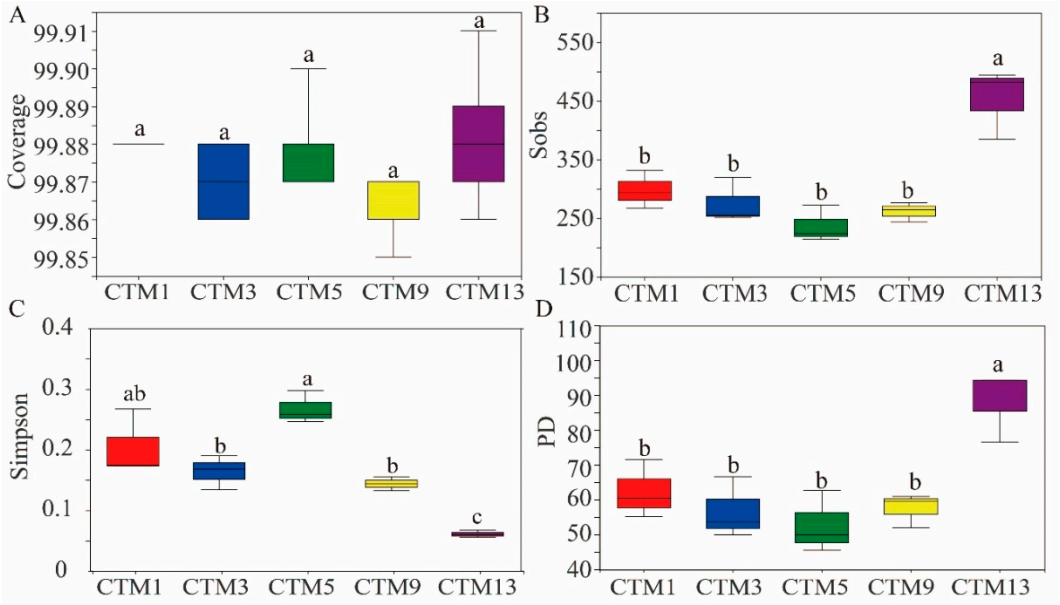

Figure 1. Soil fungal alpha-diversity indexes among different CTM cycles. (**A**) Coverage at the OTU level; (**B**) Sobs index at the OTU level; (**C**) Simpson index at the OTU level; (**D**) Phylogenetic diversity (PD) index at the OTU level; different letters above boxplots indicated a significant difference between treatments based on Student's *t*-test ($p < 0.05$).

3.3. Soil Fungal Community Composition

Principal coordinates analysis (PCoA) at the OTU level was used to demonstrate the different compositions of the fungal community. The two first components (PC1 and PC2) represented 48.61% and 14.62% variation of soil fungal community composition, respectively (Figure 2). CTM1, CTM3, CTM5 and CTM9 were clustered together and were separated from CTM13. Moreover, ANOSIM analysis also revealed a significant difference (ANOSIM, r = 0.5985, $p = 0.001$) in soil fungal community composition among all treatments (Figure 2).

As shown in Figure S2, the dominant fungal phyla (relative abundance > 1%) were *Ascomycota* (94.33–99.74%), *Basidiomycota* (0.08–2.81%) and *Mortierellomycota* (0.07–2.39%) in CTM systems (Figure S2). The relative abundance of *Ascomycota* gradually decreased with the increase of consecutive tomato monoculture cropping cycles, while the relative abundance of *Basidiomycota* and *Mortierellomycota* at CTM3, CTM9 and CTM13 were all higher than those at CTM1. A one-way ANOVA analysis revealed that there were no

significant differences among all treatments for phylum *Ascomycota*, *Basidiomycota* and *Mortierellomycota* ($p < 0.05$ or $p < 0.01$).

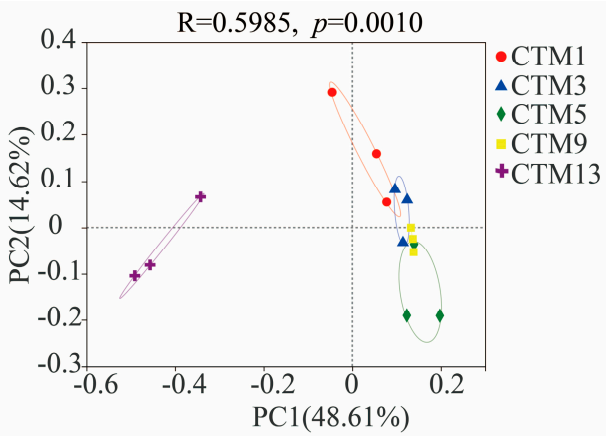

Figure 2. Principle coordinates analysis (PCoA) and ANOSIM analysis of soil fungal community composition at the OTU level with Bray–Curtis distance matrices among different CTM cycles.

For the family level, the OTUs classified from all soil samples were mainly affiliated with 11 fungal families with a relative abundance higher than 1% (Figure S3). No significant differences in the relative abundance of *Chaetomiaceae* were found among CTM1, CTM3, CTM5 and CTM9. Interestingly, the relative abundance of *Chaetomiaceae* at CTM13 was significantly lower than that of CTM1, CTM3 and CTM5 ($p < 0.05$). Conversely, although the relative abundance of *Nectriaceae*, *Cladosporiaceae* and *Plectosphaerellaceae* at CTM3, CTM5 and CTM9 all had no significant difference when compared with CTM1, those at CTM13 were increased significantly when compared with CTM1 ($p < 0.05$).

Among 16 fungal genera whose relative abundance was greater than 1%, 5 fungal genera showed a significant change with the increase of consecutive tomato monoculture cropping cycles. The relative abundance of *Fusarium*, *Cercophora* and *Cladosporium* among CTM1, CTM3, CTM5 and CTM9 did not exhibit significant differences, but those at CTM13 were significantly higher than at CTM1, CTM3, CTM5 and CTM9 ($p < 0.05$ or $p < 0.01$). The relative abundance of *Pseudaleuria* at CTM13 was significantly higher when compared with CTM3 and CTM5 ($p < 0.05$). In contrast, the relative abundance of *Trichocladium* at CTM13 was significantly lower than that at CTM3 and CTM5 ($p < 0.01$) (Figure 3).

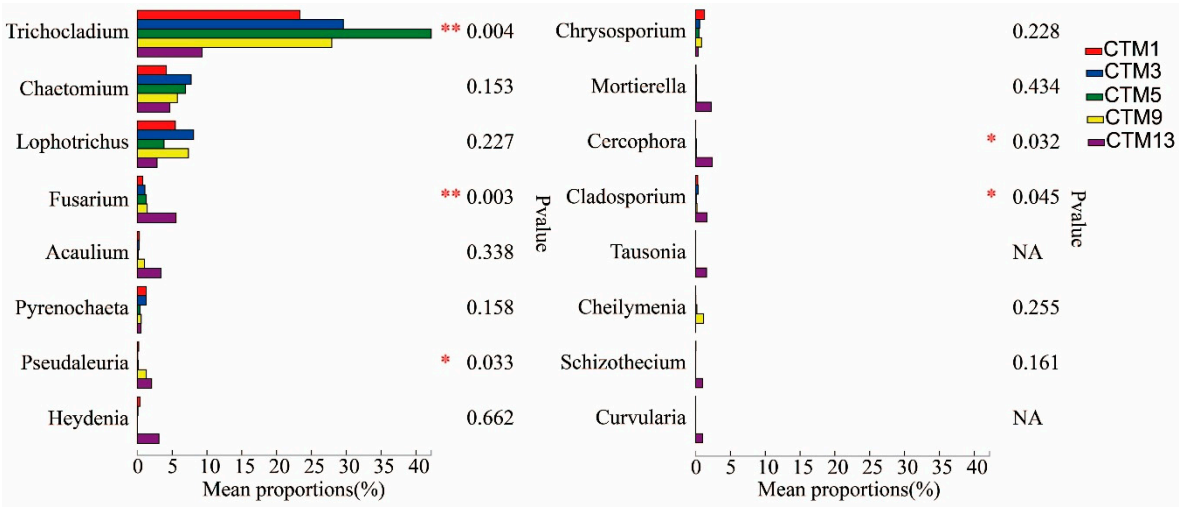

Figure 3. Difference analysis of soil fungal genera among different CTM cycles. Significances between different treatments were compared using One−way ANOVA, with the results indicated by red asterisks (** $p < 0.01$; * $p < 0.05$).

3.4. Effects of Soil Chemical Properties on Soil Fungal Community Composition

RDA analysis indicated that soil EC, pH, TP, TN, TK and OM explained 53.69% of the variation of soil fungal community composition at the genus level and the influence of soil chemical properties on soil fungal genera community composition could be ranked as EC > pH > TP > TN > TK > OM (Figure 4, Tables S2 and S3). A Spearman correlation and Heatmap analysis between soil fungal genera and soil chemical properties indicated that soil *Trichocladium* exhibited a significantly positive correlation with soil pH ($p < 0.01$). Soil *Fusarium* showed a significant positive correlation with soil TK, TP and soil EC ($p < 0.05$). Soil *Acaulium*, *Pseudaleuria* and *Mortierella* showed a significant positive correlation with soil EC and a significant negative correlation with soil pH ($p < 0.05$ or $p < 0.01$). Soil *Tausonia* exhibited a significant positive correlation with soil EC ($p < 0.05$ or $p < 0.01$), while Soil *Pyrenochaeta* exhibited a significant negative correlation with soil EC ($p < 0.01$). Soil *Cercophora* and *Pseudaleuria* both exhibited a significant positive correlation with soil TP ($p < 0.05$ or $p < 0.01$). Soil *Cladosporium* exhibited a significant negative correlation with soil pH ($p < 0.01$) (Figure 5, Table S4).

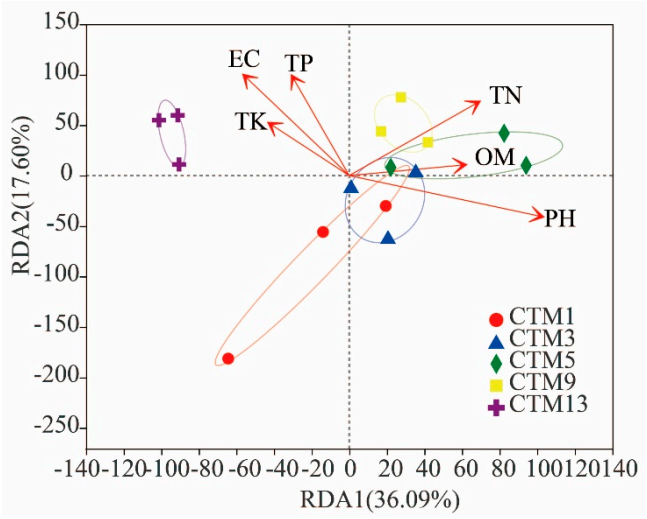

Figure 4. Redundancy analysis (RDA) between soil fungal genus and soil chemical properties among different CTM cycles. TN = soil total nitrogen content; TP = soil total Phosphorus content; TK = soil total potassium content; EC = electrical conductivity; OM = soil organic matter content.

3.5. Linking TFFW to Soil Chemical Properties and Soil Fungal Genera

SEM was used to assess the effects of soil chemical properties and soil fungal genera on TFFW (Figure 6). As shown in Figure 6A, the fitted models explained 77% of the variance in TFFW among CTM1, CTM3, CTM5 and CTM9. Soil TP and the relative abundance of *Chaetomium* both had a positive direct effect ($p < 0.01$ and $p < 0.001$) on TFFW, and the abundance of *Fusarium* had a negative direct effect ($p < 0.001$) on TFFW. The abundance of *Fusarium* had a positive direct effect on *Chaetomium* ($p < 0.001$) and *Trichocladium* ($p < 0.01$). Standardized total effects showed that TP, EC, *Chaetomium*, *Fusarium* and *Trichocladium* all had positive total effects on TFFW among CTM1, CTM3, CTM5 and CTM9 (Figure 6C). As shown in Figure 6B, the fitted models explained 97% of the variance in TFFW among CTM3, CTM5, CTM9 and CTM13. Soil TP had a positive direct effect on soil EC ($p < 0.001$). Soil EC directly induced changes in the abundance of *Cercophora* ($p < 0.01$) and *Cladosporium* ($p < 0.05$), and further caused positive effects on the abundance of *Fusarium* ($p < 0.01$ and $p < 0.001$). The abundance of *Fusarium* ($p < 0.001$) and *Cladosporium* ($p < 0.01$) were identified as significant negative drivers of TFFW. Standardized total effects showed that TP, EC, *Chaetomium*, *Fusarium* and *Trichocladium* all had negative total effects on TFFW among CTM3, CTM5, CTM9 and CTM13 (Figure 6D).

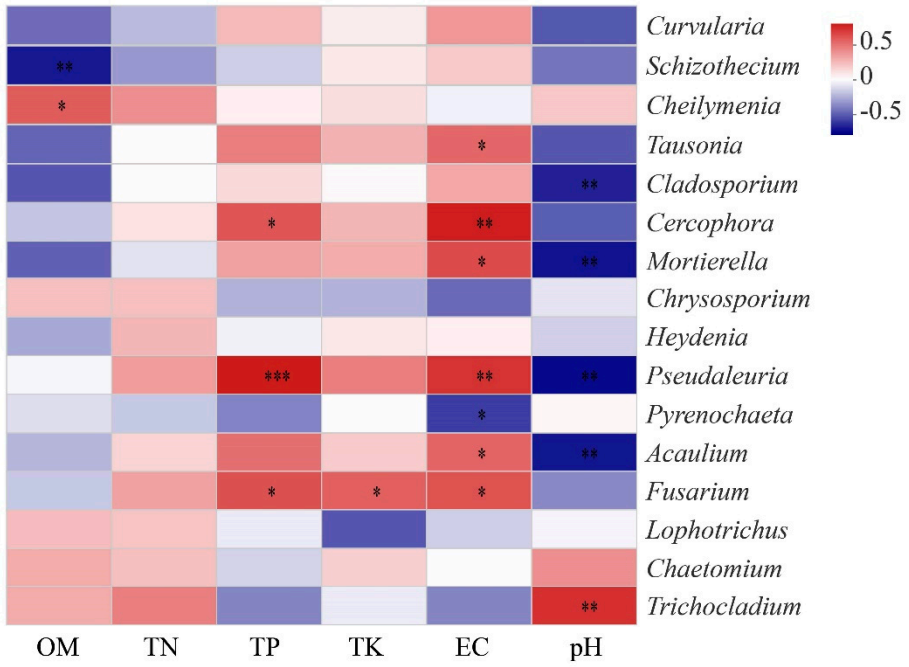

Figure 5. Spearman correlation heatmap analysis between soil fungal genera and soil chemical properties among different CTM cycles. TN = soil total nitrogen content; TP = soil total Phosphorus content; TK = soil total potassium content; EC = electrical conductivity; OM = soil organic matter content. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

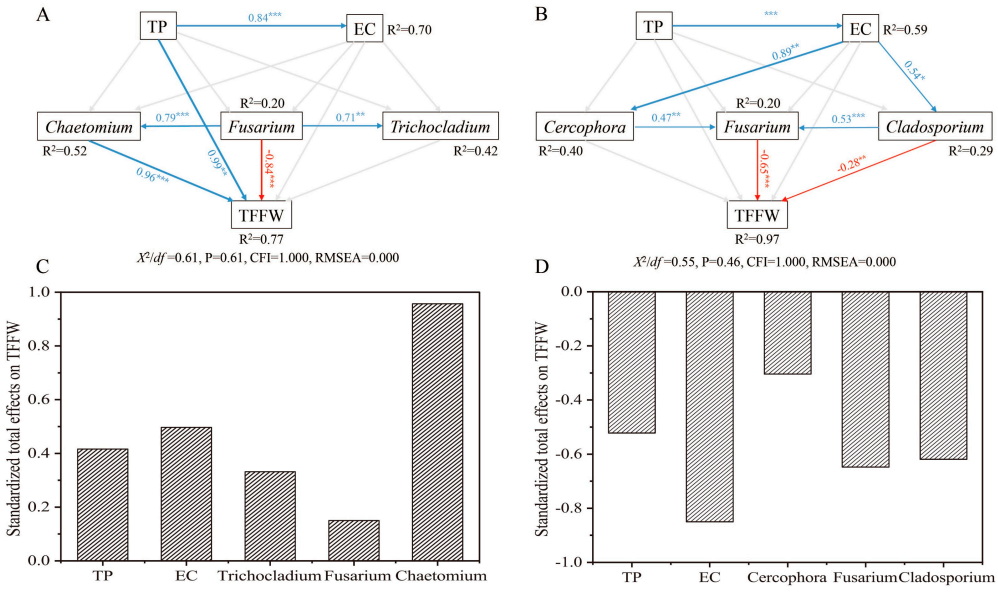

Figure 6. Structural equation models (SEM) based on the effect of soil chemical properties (EC, TP), soil fungal genera (*Fusarium*, *Chaetomium*, *Trichocladium*, *Cercophora* and *Cladosporium*) on TFFW under different CTM cycles. (**A**) SEM among CTM1, CTM3, CTM5 and CTM9. (**B**) SEM among CTM3, CTM5, CTM9 and CTM13. (**C**) Standardized total effects among CTM1, CTM3, CTM5 and CTM9. (**D**) Standardized total effects among CTM3, CTM5, CTM9 and CTM13. Blue and red arrows indicate significant positive and negative correlations, respectively. Gray arrows indicate no significant correlations. The width of the arrows indicates the strength of the correlations. Numbers at the arrows represent standardized path coefficients. R^2 indicates the proportion of variance explained by the model. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

4. Discussion

4.1. Effects of 13 Cycles of Consecutive Tomato Monoculture on Soil Chemical Properties

Soil nutrient levels are generally regarded as fundamental factors for maintaining crop yields [25,26]. Several studies indicated that long-term consecutive monoculture cropping often resulted in soil nutrient imbalance, which mainly had a close relationship with long-term partial absorption of the same crop. Studies, such as that by Li et al. [27], carried out that long-term continuous cropping of tea trees resulted in the lack of soil TK and the excess of soil TN and TP. Our study showed that, compared to CTM1, long-term consecutive tomato monoculture cropping led to the increase of soil TN, TP and TK, especially the significant increase of soil TP. Similarly, studies on tomato [28] or cucumber [20] also stated that soil TN, TP and TK were significantly higher in consecutive monoculture cropping soils than in the initial soils in the greenhouse. Thus, it can be seen that the imbalance or accumulation of soil nitrogen, phosphorus and potassium nutrients probably occurred under consecutive monoculture cropping systems of different crops. Compared with open fields [26], soil nutrient accumulation under consecutive monoculture cropping systems is more common in facility cultivation [28,29]. The main reason is that the amount of fertilizer application for facility vegetables during one cultivation cycle is 4.1 times when compared with open field, and the application amount of nitrogen fertilizer, phosphorus fertilizer and potassium fertilizer is 1.9 times, 5.4 times and 1.6 times the recommended number of vegetables [30]. Therefore, we speculated that the accumulation of nitrogen, phosphorus and potassium after consecutive tomato cropping in this study could be excessive.

Some reports have also pointed out that long-term accumulation of fertilization year by year could lead to soil acidification or soil salinization [31,32]. Our previous report indicated that the soil pH significantly decreased by about 0.25 units from CTM1 (6.88) to CTM13 (6.63) under consecutive tomato monoculture cropping systems, yet they were all in the proper growth and development range of tomatoes [14]. However, soil EC, in this test, appeared an obvious increase with the increase of consecutive monoculture cropping cycles, especially at CTM13, with a significant increase up to 887.30 $\mu s \cdot cm^{-1}$. Li et al. reported that the growth of tomatoes is suppressed when soil EC exceeds 800 $\mu s \cdot cm^{-1}$ [33]. Soil EC at CTM13 has exceeded the suitable range of tomato suitable growth and development range, which might further cause tomato growth disorder.

4.2. Effects of 13 Cycles of Consecutive Tomato Monoculture Cropping on Soil Fungal Community

Most studies realized that long-term consecutive monoculture cropping always led to a decrease in soil microbial richness and diversity, and the decrease in fungal diversity always resulted in a decrease in soil disease suppression. However, the change rule of soil fungal diversity in our study appeared inconsistent with these studies. We indicated that CTM13 appeared a significant increase in Sobs and PD indexes and a significant decrease in Simpson indexes when compared with CTM1. That is, we concluded that long-term CTM (CTM13) indeed led to the increase of soil fungal richness and diversity. Similarly, Li et al. also reported that with the increasing of continuous strawberry cropping years in the greenhouse, soil fungal diversity at the genus level was increased [19]. Gao et al. [34] showed that the fungal diversity and richness significantly increased in soil under a continuous sweet potato cropping system. Pervaiz et al., who concluded the results of [19] and [34], indicated that relatively higher fungal diversity was not necessarily a positive role in soil disease suppression, they felt that fungal species identity rather than diversity may be important for soil health [35].

Based on the PCoA analysis, continuous sweet potato cropping led to a significant difference in soil fungus community composition [34]. Appearing to be the same as the above study, our results revealed that the soil fungal community at CTM13 appeared significant separation from all the other treatments. The same as the report of the study on continuous cucumber cropping [36], we found the difference in soil fungus community composition in our study also mainly manifests in fungus family and genus level rather than phyla level. The relative abundance of *Chaetomiaceae* at CTM13 was significantly

lower than that of CTM1, CTM3 and CTM5. *Chaetomiaceae*, which belong to Ascomycota, were characterized as the producer of both antifungal compounds and cellulose and had the potential for soil disease suppression [37–39]. So, we speculated that the significant decrease of *Chaetomiaceae* at CTM13 might reduce soil disease suppression. In contrast, with the increase in CTM cycles, the relative abundance of some well-known soil pathogens increased significantly in our research. According to previous reports, *Cladosporium* has close relation with the occurrence of several crop leaf moulds [40]. Song et al. [41] indicated that the proportion of the genera *Cladosporium* was significantly higher in continuous 5 years of *Coptis chinensis* Franch monoculture cropping than that in 1 or 3 year(s). Similarly, the relative abundance of *Cladosporium* at CTM13 in our study was significantly higher than that of CTM1, CTM3, CTM5 and CTM9. Additionally, the fungus genus *Fusarium*, a soil pathogen, which often led to the occurrence of several plant diseases including tomato root rot [34,42–44], was significantly increased at CTM13 compared with that of CTM1, CTM3, CTM5 and CTM9. To sum up, the significant reduction of the relative abundance of *chaetomiaceae* and the significant increase of the relative abundance of both *Cladosporium* and *Fusarium* at CTM13 in our study indicated that long-term consecutive tomato monoculture cropping resulted in an imbalance of soil fungal community composition. The imbalance of soil fungal community composition might further lead to an increased risk of soil-borne disease [41,43,44]. Furthermore, soil chemical properties had been reported as one of the important influencing indicators in shifting microbial communities [45]. In this study, the RDA analysis indicated that soil EC, pH and TP were the most dominant factors in shaping soil fungal community composition. There was a significant negative correlation between soil pH and *Cladosporium*. Meanwhile, the significant positive correlation between soil EC and *Fusarium* in this study indicated that soil salinization and acidification might have a close relationship with the accumulation of soil fungal pathogens [46,47].

4.3. Effects of Soil Chemical Properties and Soil Fungal Community on TFFW

Compared with CTM1, TFFW at CTM3 had a significant increase. The most likely reason for this result was that short-term consecutive tomato monoculture cropping is beneficial for the enhancement of soil fertility and functions [48]. No significant difference in TFFW among CTM3, COM5 and CTM9 indicated that there was no obstacle in tomato consecutive cropping for 9 cycles. The possible reason we speculated was that when CTM had fewer than nine cycles, the raise in the relative abundance of soil *Fusarium* also promoted the increase in the relative abundance of beneficial fungal genera *Chaetomium* (Figure 6A). Similarly, the enrichment of beneficial fungal taxa (*Penicillium*) in diseased soils is also reported in some studies [49,50]. The reason for this may be that soil may control pathogenic microorganisms at a good level through feeding, competition and parasitism, thus readjusting the rhizosphere microbial colony and increasing the abundance of antagonistic beneficial bacteria in the community [51,52]. In combination, the positive effect of soil *Chaetomium* and soil TP on TFFW outweighed the negative effect of soil *Fusarium* on TFFW, which may be the reason why no significant decrease in TFFW appeared for fewer than nine cycles. TEEW at CTM13 decreased significantly when compared with CTM3, CTM5 and CTM9. We speculated that long-term consecutive monoculture might affect soil function and thus reduce crop yield [53]. Previous research on peanuts, sweet potatoes and strawberries also reported that the accumulation of soil fungal genus *Fusarium* might be the main explanation for yield declines as a consequence of consecutive crop cultivation [19,34,44]. Similarly, the significant decrease of TFFW at CTM13 still had a close relationship with the significant increase of soil pathogenic fungal genus *Fusarium* at CTM13 (Figure 6B). Moreover, we also found that, when CTM for 13 cycles, the increase of soil EC value which was beyond the suitable range for tomato growth and development had resulted in the occurrence of minor salt damage [33], which further led to the significant accumulation of soil pathogenic fungal genus *Cladosporium*. In the end, the increase of soil EC and the accumulation of *Cladosporium* and *Fusarium* together led to the decline of TFFW at CTM13. To sum up, we recommended that reducing the amount of fertilizer in each

cycle may be an effective method to alleviate tomato soil obstacles, because appropriate fertilizer reduction may alleviate the excessive accumulation of soil nutrients caused by fertilization, as well as the resulting soil salt damage and soil microflora imbalance [31,35].

5. Conclusions

In conclusion, CTM led to a significant increase in soil TP and soil EC. Though CTM significantly increased the soil fungal community diversity, it also resulted in the imbalance of soil fungal community compositions. When CTM for 13 cycles, soil pathogenic fungi *Fusarium* and *Cladosporium* increased significantly, while soil beneficial fungi *chaetomiaceae* decreased significantly. The decline of TFFW at CTM13 might have a close relationship with the significant increase in soil EC value and the imbalance of soil fungal community structure. To sum up, we recommended that appropriately decreasing the amount of fertilizer in each cycle and maintaining the balance of soil fungal community structure may be an effective method to alleviate soil obstacles of CTM.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9040505/s1, Table S1: Data output from ITS2 amplicon sequencing; Table S2: Variance Proportion of RDA analysis at OTU level; Table S3: Effect of soil chemical properties on soil fungal genera community composition using RDA analysis; Table S4: Spearman correlation heatmap analysis between soil fungal genera and soil chemical properties; Figure S1: Venn diagram of OTU level among different CTM cycles; Figure S2: Difference analysis of soil fungal phyla among different CTM cycles. Significances between different treatments were compared using One-way ANOVA, with the results indicated by red asterisks (** $p < 0.01$; * $p < 0.05$); Figure S3: Difference analysis of soil fungal family among different CTM cycles. Significances between different treatments were compared using One-way ANOVA, with the results indicated by red asterisks (** $p < 0.01$; * $p < 0.05$).

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Abbreviation

Abbreviation	Definition
CTM	Consecutive tomato monoculture cropping
TN	Soil total nitrogen
TP	soil total phosphorus
TK	Soil total potassium
EC	soil electrical conductivity
OM	soil organic matter content
TFFW	tomato fruit fresh weight per plant
RDA	Redundancy analysis
SEM	Structural-equation-model

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