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Effects of Continuous Tomato Monoculture on Soil Microbial Properties and Enzyme Activities in a Solar Greenhouse

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Abstract: Soil-related obstacles resulting from continuous monoculture have limited the sustainable development of the tomato industry in China. An experiment on tomatoes with seven continuous monoculture treatments (the 1st, 3rd, 5th, 7th, 9th, 11th, and 13th crops, respectively) was conducted in a solar greenhouse, to investigate the influence of monoculture on soil quality. Most soil quality indicators first increased and then decreased with increasing continuous monoculture crops, and significant differences among crops were observed. Indicators at the 13th crop were significantly lower than those at the other crops in terms of average well color development (AWCD), substrate richness (S), the Shannon diversity index (H), and the McIntosh index (U) of the soil microbial community (SMC), soil urease (UR), and neutral phosphatase (N-PHO) activities, and available nitrogen (AN) and potassium (AK). However, fungal abundance (FUN) at the 13th crop was significantly higher than that at the other crops. As principal component analysis (PCA) revealed, SMC functional diversity at the 1st, 11th, and 13th crops were similar, and were obviously distinguished from those at the other crops. Moreover, the tomato yield was significantly and positively correlated with soil-available potassium and SMC functional diversity indexes. Our findings indicated that short-term continuous monoculture, e.g., for fewer than seven or nine crops, was beneficial for soil quality improvement. However, continuous monoculture for greater than 11 crops had adverse effects on soil enzyme activities, soil microbial abundances, soil chemical properties, soil SMC functional diversity, and the tomato yield, particularly at the 13th crop.

Keywords: continuous tomato monoculture; soil microbial properties; soil enzyme; tomato yield

1. Introduction

Solar greenhouses offer advantages such as a high land utilization rate, short production cycle, and high technical content. They are therefore suitable for crop production in the cold regions of northern China [1,2], and provide much higher yields and incomes than open field cultivation [3,4]. However, microclimate factors in greenhouses, such as the temperature, lightintensity, humidity, and aeration, and management methods, including tillage and fertilization, can give rise to soil acidification, secondary salinization, and nutrient enrichment and unbalance [5–8].

The tomato, a vegetable cultivar native to western South America, has been widely cultivated in China since the 1950s, due to its high yield and rich nutrition [9,10]. In 2014, the tomato cultivated area in the Liaoning Province was 850 million square meters, of which 75% was protected cultivation area. Continuous monoculture of tomato is more common in protected cultivation than in non-protected cultivation [11]. However, the continuous cropping of a single species leads to several soil-related obstacles [12,13], manifesting as retarded plant growth, serious pest and disease damage, low crop productivity, and soil degradation [14,15]. Numerous studies have asserted that the mechanisms of these obstacles mainly include changes in soil physicochemical properties, destruction of the ecological environment, and plant autotoxicity [16,17]. Over the past several years, many researchers have attempted to determine how to eliminate these obstacles through improving cultivation and management methods. Most research has focused on screening superior varieties to alleviate pests and disease, introducing grafting techniques to enhance plant resistance, applying organic fertilizer to improve soil quality, or selecting alternative rotations to balance soil nutrition [18–20]. However, the changes in soil quality due to continuous monoculture over several years or crops have been rarely reported. In addition, the relationships between soil microbial properties, soil enzyme activities, and continuous tomato monoculture in solar greenhouses, remain poorly understood.

Soil microorganisms play vital roles in soil ecosystems, dominating the cycling of nutrients, the decomposition of organic matter, and the maintenance of soil fertility [7,21–23]. The total microbial abundance is a basal indicator of soil quality [24]. Over years of continuous cropping, the abundance of soil bacteria first decreases and then increases, whereas the abundance of fungi increases [25]. Increasing evidence indicates that an appropriate community population, abundant diversity, and high microbial activity, are all important factors for maintaining the sustainability and productivity of soil ecosystems [26,27]. In addition, a long-term field experiment revealed that the level of SMC functional diversity was significantly enhanced in plots treated with both chemical fertilizer and compost, compared with that in plots treated with only chemical fertilizer or in untreated control plots [28].

The enzyme activity in the soil is another potentially sensitive indicator of soil quality. It is indicative of soil quality changes that occur due to management practices, and it can also be used to monitor soil microbial activity that is related to nutrient transformation [29,30]. Xiao et al. concluded that the activities of soil invertase, urease, and alkaline phosphatase were promoted in an intercropping system, when compared with those in a monoculture cropping system, and that the stimulation of urease and alkaline phosphatase activity from intercropped garlic was maintained until the garlic harvest [31].

In the present study, to further understand soil microbial properties under different tomato monoculture crops, a Biolog EcoPlate was adopted to study the SMC functional diversity. The EcoPlate is based on the carbon substrate utilization by microbial communities, and the resulting data are analyzed via multivariate statistics, including principal component analysis (PCA) and the analysis of microbial community dynamics [32,33]. In this study, soil was analyzed to determine the microbial parameters, chemical properties, and enzyme activity under different continuous monoculture crops in a solar greenhouse. This study increases our understanding of the changes in soil enzyme activity, soil chemical properties, SMC functional diversity, and tomato yields under continuous tomato monoculture crops in a solar greenhouse, and it provides insight into the crop at which the continuous cropping obstacles emerge due to continuous cropping. This study also provides a theoretical basis for the remediation of soil and fertilization during tomato cultivation under continuous monoculture in solar greenhouses.

2. Materials and Methods

2.1. Site Description and Experimental Design

Our experiment was performed in a solar greenhouse at Shenyang Agriculture University, Liaoning Province, China (41°31′N–123°24′E). A conventional, large-fruited tomato variety, "Liaoyuanduoli", was adopted as the test material. Tomato plants were maintained under regular irrigation and fertilization management, with a single branch after pruning, and three clusters of fruits after pinching. Naïve soil for the continuous monoculture experiment was selected from a plot in which solanaceous vegetables had never been planted. The basic chemical properties of the soil are provided in Table 1, and were determined by the Bao's Method [34]. In the greenhouse, 39 culture pots were established, and each was 1.5 m long × 1.0 m wide × 0.8 m deep (with cement walls but not a cement bottom). Prior to planting tomatoes in the spring of 2009, naïve soil was placed into three pots. Then, continuous monoculture of tomatoes in both the spring (from March to July) and autumn (from August to January of the following year) was conducted in these three pots, such that soil samples of the 13th crop were available by the end of the spring in 2015. In the autumn of 2009, three additional pots were filled with naïve soil, and continuous monoculture of tomatoes in both the spring and autumn was performed until the end of the spring of 2015, allowing soil samples of the 12th crop under continuous monoculture to be sampled. In this manner, three new culture pots and tomato plants were successively added each spring and autumn from 2010 to 2015, ultimately providing soil samples for the 11th, 10th, 9th, 8th, 7th, 6th, 5th, 4th, 3rd, 2nd, and 1st continuous crops. Prior to the tomato planting for each crop each season, 4.0 kg of decomposed chicken manure was applied to the arable layer (approximately 0–20 cm thick) of each culture pot. Then, the manure was mixed thoroughly and evenly in each culture pot, and 0.12 kg of compound fertilizer (N:P:K = 16:16:16) was applied to each pot via ridging. Two rows of tomato plants were planted with a spacing of 35 cm, with eight plants per culture pot. Tomato plant samples and soil samples from the 1st, 3rd, 5th, 7th, 9th, 11th, and 13th crops under continuous monoculture were acquired at the end of the spring of 2015 and were subjected to analysis.

Table 1. Chemical properties of the experimental soil.

pH	EC ($\mu s \cdot cm^{-1}$)	AvailableNitrogen (AN, $mg \cdot kg^{-1}$)	Available Phosphorus (AP, $mg \cdot kg^{-1}$)	Available Potassium (AK, $mg \cdot kg^{-1}$)	Organic Matter (OM, $g \cdot kg^{-1}$)
7.04	290.30	95.81	94.65	255.70	20.30

2.2. Soil Sampling and Analysis

2.2.1. Soil Sampling

The soil samples were all collected on the last day of the spring crop in 2015. For each culture pot, the soil sample was a composite of five soil cores randomly collected approximately 20 cm from a tomato trunk (2.5 cm in diameter, 0–20 cm in depth). All 21 soil samples (seven crops × three pots per crop) were placed into sterile plastic bags and then into ice bags, before being transported to the laboratory. In the lab, each soil sample was divided into two portions. One portion was passed through a 2-mm sieve and stored at 4 °C for a subsequent analysis of the microbial parameters. The other portion was air-dried and then passed through a 1-mm sieve for a subsequent analysis of the soil enzyme activities and soil chemical properties. Microbial analysis should be performed within one week.

2.2.2. Analysis of Soil Microbial Abundance

Soil microbial abundance was determined using the traditional culture method described by Zhou et al. [35]. The abundance of bacteria (BAT), fungi (FUN), and actinomycetes (ACT) was

determined by soil dilution plating on beef extract peptone medium, Martin's medium, and Gause's No. 1 synthetic medium, respectively. All media were prepared according to the methods proposed by Dong et al. [36].The bacteria, fungi, and actinomycetes were cultivated in a 28 °C incubator for two, three, and six days, respectively. The sum of bacteria (BAT), fungi (FUN), and actinomycetes (ACT), represented the total microbial abundance (TMA) of the soil.

2.2.3. Analysis of the Functional Diversity of the Soil Microbial Community

The functional diversity of the SMC was assessed using Biolog Eco microplates (Biolog Inc., Hayward, CA, USA), which contained 31 different carbon substrates, allowing the arrangement of triplicate samples on a single 96-well plate. Each soil sample (equivalent to 5 g dry weight) was added to 45 mL sterilized NaCl solution, which was shaken at room temperature at 220 rpm for 30 min and left undisturbed for an additional 30 min. The soil suspension was then diluted to 10^{-3}, and 150 µL was added to each well of the Biolog EcoPlates. The microplates were incubated at 25 °C in the dark and measured at 590 nm every 24 h, for up to 192 h. The soil microbial activity, expressed as the average well color development (AWCD), was determined by the method described by Garland et al. [32]. Functional diversity indexes, including species richness (S), Shannon's diversity index (H), Simpson's dominance index (D) and the McIntosh index (U), and principal component analysis (PCA) were applied, to evaluate differences in the microbial communities at the 144 h AWCD value.

AWCD is calculated as follows:

$$\mathrm{AWCD} = \sum(\mathrm{C}_i - \mathrm{R})/\mathrm{n}$$

Substrate richness (S) is the sum of all of the positive wells. A well with an optical density value greater $\geq$0.2 is identified as a positive well.

Shannon's diversity index (H) is used to evaluate richness and is calculated as follows:

$$\mathrm{H} = -\sum(\mathrm{P}_i \times \ln \mathrm{P}_i)$$

Simpson's dominance (D) indicates the dominance of species and is calculated as follows:

$$\mathrm{D} = 1 - \sum \mathrm{P}_i^2$$

McIntosh index (U) is a diversity index and a consistency measurement, and it is based on the distances of community species in multidimensional space. It is calculated as follows:

$$\mathrm{U} = \sqrt{(\sum \mathrm{n}_i^2)}$$

In the above equations, C_i is the optical density value of each well, R is the absorbance value of the control well, n is the number of carbon substrates (n = 31), P_i is the ratio of the relative absorbance value to the total relative absorbance value for the whole plate, and n_i is the relative absorbance value of well *i*.

2.2.4. Determination of Soil Chemical Properties

The soil chemical properties were determined according to Bao [34]. Soil pH was determined in water suspensions at a soil/water ratio of 1:2.5 with a glass electrode. Organic matter (OM) was measured according to the potassium dichromate external heating method, and available nitrogen (AN) was measured using the alkaline-hydrolysable diffusion method. Soil-available phosphorus (AP) was extracted with sodium bicarbonate and determined by the molybdenum blue spectrophotometry method. Soil-available potassium (AK) was extracted with ammoniumacetate and measured using flame photometry.

2.2.5. Determination of Soil Enzyme Activities

Four enzymatic activities were determined using air-dried soil, according to Yan [37]. Neutral phosphatase (N-PHO) was analyzed with nitrophenyl phosphate disodium (PhOH, $mg \cdot g^{-1}$, 37 °C, 24 h), and catalase (CAT) was analyzed with $KMnO_4$ (0.1 $mol \cdot L^{-1}$ $KMnO_4$, $mg \cdot g^{-1}$, 37 °C, 20 min). Invertase (INV) was analyzed with glucose (0.5 $mol \cdot L^{-1}$ glucose, $mg \cdot g^{-1}$, 37 °C, 24 h), and urease (URE) was analyzed with a pH 6.7 citrate acid buffer solution (NH_3–N, $mg \cdot g^{-1}$, 37 °C, 24 h). The soil enzyme activities were examined in triplicate.

2.2.6. Measurement of Average Plant Yield

We randomly selected six tomato plants (two tomato plants per pot × three pots) per treatment. Upon the ripening of fruit, the yield per plant was measured by the weighing method, and the average value was calculated. The average plant yield was then converted into yield per square meter.

2.3. Statistical Analysis

One-way ANOVA and Duncan's multiple range tests ($p < 0.05$) were performed with SPSS software version 18 (SPSS, Chicago, IL, USA) to test for differences in soil enzyme activities, soil chemical properties, AWCD, and microbial functional diversity indexes, among the different continuous monoculture crops. The correlation analysis was also performed with SPSS 18. All graphs were produced using Origin8.0 software (Origin Lab, Northampton, MA, USA).

3. Results

3.1. Soil Microbial Abundance

The dynamic changes in soil total microbial abundance (TMA), bacterial abundance (BAC), fungal abundance (FUN), actinomycetes abundance (ACT), and B/F value over the different continuous monoculture crops, are shown in Table 2. Soil total microbial abundance (TMA) and bacterial abundance (BAC) increased from the 1st to the 7th monoculture crop, and then gradually decreased thereafter. B/F value exhibited a similar pattern assoil bacterial abundance. The soil total microbial abundance (TMA), bacteria abundance (BAC), and B/F value at the 13th crop, were all significantly lower than those at the 5th, 7th, and 9th crops ($p < 0.05$). Actinomycetes abundance (ACT) did not obviously differ among the crops. Soil fungal abundance (FUN) first increased, then decreased, and finally increased again as the continuous monoculture crops increased, with fungal abundance (FUN) being significantly higher at the 13th crop than at the other crops ($p < 0.05$).

Table 2. Soil microbial abundances of the different continuous monoculture crops (CFU/g dry weight soil).

Crop	TMA ($\times 10^6$)	BAC ($\times 10^6$)	FUN ($\times 10^3$)	ACT ($\times 10^5$)	B/F Value
1	3.19 ± 0.23 bc	3.01 ± 0.21 bc	8.84 ± 0.14 b	1.65 ± 0.28 a	341.71 bc
3	3.23 ± 0.41 bc	3.09 ± 0.39 bc	9.41 ± 0.42 b	1.43 ± 0.17 a	330.89 bc
5	4.82 ± 1.13 ab	4.72 ± 1.10 ab	9.80 ± 0.58 b	1.25 ± 0.38 a	489.44 ab
7	6.24 ± 0.40 a	6.12 ± 0.42 a	9.30 ± 0.82 b	1.15 ± 0.27 a	641.73 a
9	5.97 ± 0.82 a	5.81 ± 0.84 a	8.81 ± 0.34 b	1.52 ± 0.31 a	667.63 a
11	4.83 ± 0.61 ab	4.67 ± 0.58 ab	10.15 ± 0.60 b	1.55 ± 0.62 a	468.47 abc
13	2.45 ± 0.42 c	2.18 ± 0.38 c	12.08 ± 0.75 a	2.51 ± 0.55 a	183.69 c

Note: TMA: total microbial abundance; BAC: bacterial abundance; FUN: fungal abundance; ACT: actinomycetes abundance; Values are means ± standard deviation (n = 3). Means followed by the same letter for a given factor are not significantly different (LSD test, $p < 0.05$).

3.2. Functional Diversity of the Soil Microbial Community

As shown in Figure 1, soil microbial activity (measured as AWCD) increased steadily with incubation time for all crops. Soil microbial activity at the same incubation time increased from the 1st to the 7th crop, and then decreased gradually thereafter. The highest AWCD value was observed at the 7th crop. At all incubation times except 24 h, the lowest AWCD value was observed at the 13th crop, which was significantly lower than the values at the other crops ($p < 0.05$).

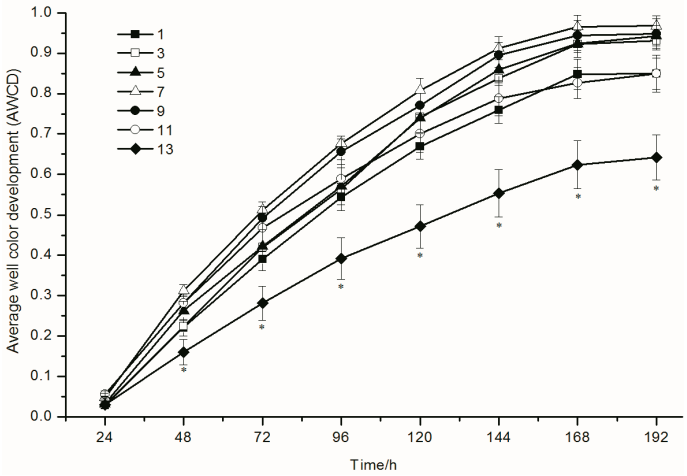

Figure 1. Change of soil microbial community AWCD with incubation time of the different continuous monoculture crops. Note: Values are means ± standard deviation (n = 3). * $p < 0.05$, significant difference.

Except for amines and polymers, carbon source utilization, including carbohydrates, amino acids, carboxylic acids, and phenolic compounds, initially increased and then decreased (Figure 2). The values of the six categories of carbon sources at the 13th crop were all lower than those at the other crops, and significantly lower than those at the 7th and 9th crops.

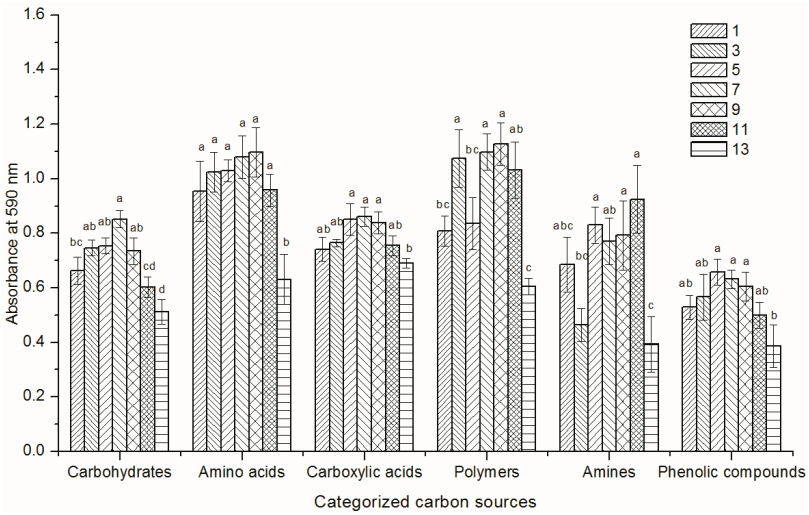

Figure 2. Carbon source utilization of the soil microbial community of the different continuous monoculture crops. Means followed by the same letter for a given factor are not significantly different (LSD test, $p < 0.05$).

The SMC functional diversity indexes of the soil microbial community initially increased and then decreased with increasing continuous monoculture crops (Table 3). The Shannon's diversity index (H), substrate richness (S), and McIntosh index (U) values of the 13th crop, were significantly lower than those of the other crops. Simpson's index (D) at the 13th crop was not significantly different from that at the 1st or 11th crop, but it was obviously lower than the values at the other crops.

Table 3. SMC functional diversity indexes of the different continuous monoculture crops.

Crop	S	H	D	U
1	25.11 ± 0.77 a	3.13 ± 0.02 a	0.95 ± 0.00 ab	5.23 ± 0.17 b
3	26.14 ± 0.63 a	3.18 ± 0.02 a	0.96 ± 0.00 a	5.54 ± 0.11 ab
5	26.43 ± 0.57 a	3.21 ± 0.02 a	0.96 ± 0.00 a	5.60 ± 0.14 ab
7	26.57 ± 0.65 a	3.20 ± 0.02 a	0.96 ± 0.00 a	6.02 ± 0.13 a
9	26.67 ± 0.53 a	3.12 ± 0.02 a	0.96 ± 0.00 a	5.86 ± 0.17 a
11	24.11 ± 0.79 a	3.09 ± 0.03 a	0.95 ± 0.00 ab	5.48 ± 0.23 ab
13	19.89 ± 1.24 b	2.88 ± 0.13 b	0.95 ± 0.01 b	4.39 ± 0.23 c

Note: S: substrate richness; H: Shannon's diversity index; D: Simpson's index; U: McIntosh index; Values are means ± standard deviation (n = 3). Means followed by the same letter for a given factor are not significantly different (LSD test, $p < 0.05$).

The PCA of SMC functional diversity of the different continuous monoculture crops indicated that the first and second principal components (PC1 and PC2) accounted for 38.87% and 12.78% of the total variance, respectively (Figure 3). All soil samples were distinguished clearly along the PC1 axis, with the 1st, 11th, and 13th crops being situated on the left of the axis, and the remaining crops being situated on the right.

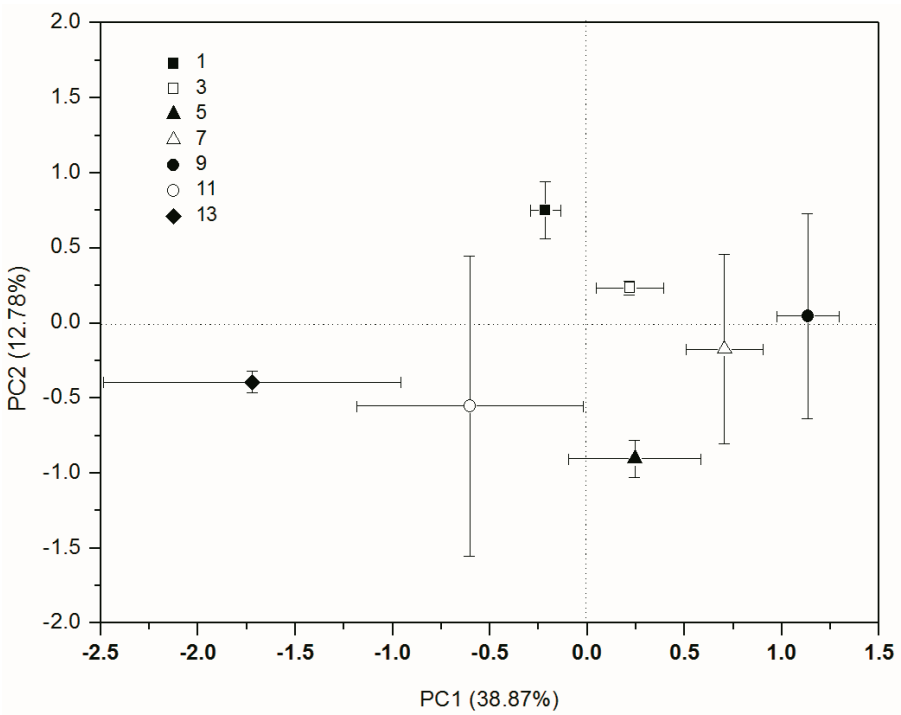

Figure 3. Principal component analysis (PCA) of SMC functional diversity of the different continuous monoculture crops.

3.3. Soil Chemical Properties

As shown in Table 4, the pH increased from the 1st to the 3rd crop and then gradually decreased thereafter. The pH at the 13th crop was only slightly different from that at the 11th, but was significantly lower than that at the other crops. The organic matter (OM), available nitrogen (AN), available phosphorus (AP), and available potassium (AK), initially increased and then decreased, as the continuous monoculture crops increased. The organic matter (OM), available nitrogen (AN), available phosphorus (AP), and available potassium (AK), were all significantly lower at the 13th crop than at the 5th, 7th, and 9th crops.

Table 4. Soil chemical properties of the different continuous monoculture crops.

Crop	pH	OM ($g \cdot kg^{-1}$)	AN ($mg \cdot kg^{-1}$)	AP ($mg \cdot kg^{-1}$)	AK ($mg \cdot kg^{-1}$)
1	6.88 ± 0.01 abc	28.40 ± 3.93 bc	168.59 ± 2.26 d	150.48 ± 2.64 d	304.45 ± 6.17 d
3	6.94 ± 0.06 ab	28.57 ± 1.44 bc	198.64 ± 1.31 bc	186.53 ± 5.35 bc	363.65 ± 10.54 c
5	6.96 ± 0.03 a	33.35 ± 0.55 ab	192.87 ± 5.76 bc	206.52 ± 9.09 b	485.29 ± 5.98 b
7	6.87 ± 0.03 abc	37.62 ± 1.87 a	183.01 ± 2.60 cd	211.40 ± 7.87 b	484.36 ± 2.86 b
9	6.87 ± 0.01 abc	37.48 ± 1.33 a	220.75 ± 8.59 a	328.91 ± 15.13 a	521.38 ± 16.12 a
11	6.75 ± 0.08 cd	30.23 ± 1.64 bc	203.68 ± 5.41 ab	203.98 ± 10.42 b	357.73 ± 13.98 c
13	6.63 ± 0.09 d	25.52 ± 1.27 c	122.21 ± 9.25 e	170.45 ± 5.98 cd	236.03 ± 3.12 e

Note: OM: organic matter; AN: available nitrogen; AP: available phosphorus; AK: available potassium; Values are means ± standard deviation (n = 3); Means followed by the same letter for a given factor are not significantly different (LSD test, $p < 0.05$).

3.4. Soil Enzyme Activities

The soil enzyme activities of the different continuous monoculture crops are listed in Table 5. The activity of soil urease (URE) increased from the 1st to the 9th crop, and then gradually decreased thereafter. Soil urease (URE) activity was significantly lower at the 13th crop than at the other crops, and significantly higher at the 9th crop than at the other crops. Invertase (INV) activity exhibited a generally similar pattern as urease. However, the highest level of invertase (INV) was observed at the 7th crop, and was obviously higher than the levels at the 1st, 3rd, 11th, and 13th crops. The lowest level of neutral phosphatase (N-PHO) activity was observed at the 13th crop, and the highest occurred at the 9th crop. With the increase of continuous monoculture crops, catalase (CAT) activity gradually decreased. Catalase (CAT) activity at both the1st and 3rd crops was significantly higher than the activities at the 11th and 13th crops.

Table 5. Soil enzyme activities of the different continuous monoculture crops.

Crop	URE ($mg\ NH_3\text{-}N/g \cdot 24\ h$)	INV ($mg\ glucose/g \cdot 24\ h$)	N-PHO ($mg\ phenol/g \cdot 24\ h$)	CAT ($mg\ KMnO_4/g \cdot 20\ min$)
1	0.80 ± 0.03 b	12.12 ± 1.79 c	1.35 ± 0.04 ab	0.61 ± 0.01 a
3	0.92 ± 0.03 b	12.38 ± 0.20 c	1.34 ± 0.06 ab	0.59 ± 0.02 a
5	0.99 ± 0.09 b	17.27 ± 0.49 ab	1.30 ± 0.03 ab	0.56 ± 0.02 ab
7	1.00 ± 0.01 b	18.72 ± 1.32 a	1.34 ± 0.16 ab	0.53 ± 0.05 ab
9	1.45 ± 0.22 a	18.25 ± 1.96 a	1.51 ± 0.07 a	0.52 ± 0.03 ab
11	0.86 ± 0.09 b	13.12 ± 0.72 bc	1.17 ± 0.09 b	0.47 ± 0.04 b
13	0.38 ± 0.02 c	10.19 ± 1.63 c	0.57 ± 0.05 c	0.46±0.05 b

Notes: URE: urease; INV: invertase; N-PHO: neutral phosphatase; CAT: catalase; Values are means ± standard deviation (n = 3); Means followed by the same letter for a given factor are not significantly different (LSD test, $p < 0.05$).

3.5. Correlations of Soil Quality Indicators

Table 6 presents the relationships of soil quality indicators of different continuous monoculture crops. Under continuous monoculture, soil fungal abundance (FUN) was significantly negatively

correlated with urease (URE) and neutral phosphatase (N-PHO) activity. Soil actinomycetes (ACT) abundance also showed significantly negative correlations with soil pH value, neutral phosphatase (N-PHO) activity, and available potassium (AK) with the increase of the continuous monoculture crops. The soil AWCD at 144 h, total microbial abundance (TMA), and bacterial abundance (BAC), were all significantly positively correlated with soil urease (URE) activity, invertase (INV) activity, organic matter (OM), and available potassium (AK). The soil AWCD value at 144 h was strongly and positively correlated with soil neutral phosphatase activity (N-PHO) and available nitrogen (AN).

Table 6. Correlations of soil quality indicators of the different continuous monoculture crops.

	URE	INV	N-PHO	CAT	pH	OM	AN	AP	AK
AWCD	0.87 *	0.84 *	0.92 **	0.44	0.67	0.84 *	0.86 *	0.54	0.89 **
TMA	0.79 *	0.93 **	0.62	−0.11	0.18	0.96 **	0.68	0.72	0.90 **
BAT	0.79 *	0.94 **	0.63	−0.09	0.20	0.97 **	0.69	0.71	0.91 **
FUN	−0.79 *	−0.56	−0.98 **	−0.73	−0.66	−0.60	−0.75	−0.37	−0.62
ACT	−0.7	−0.74	−0.86 *	−0.51	−0.80 *	−0.71	−0.78 *	−0.28	−0.79 *

Note: URE: urease; INV: invertase; N-PHO: neutral phosphatase; CAT: catalase; OM: organic matter; AN: available nitrogen; AP: available phosphorus; AK: available potassium; * $p < 0.05$, significant correlation; ** $p < 0.01$, highly significant correlation.

3.6. Tomato Yield

To further analyze the effects of continuous monoculture, the tomato yield ($kg \cdot m^{-2}$) was measured. As shown in Figure 4, the tomato yield first increased and then decreased with increasing continuous monoculture crops. The 3rd crop presented the highest yield of 10.75 $kg \cdot m^{-2}$, and the 13th crop presented the lowest yield of 7.82 $kg \cdot m^{-2}$. The tomato yield did not significantly differ among the 1st, 11th, and 13th crops, and the tomato yield of these crops were all significantly lower than those of the other crops.

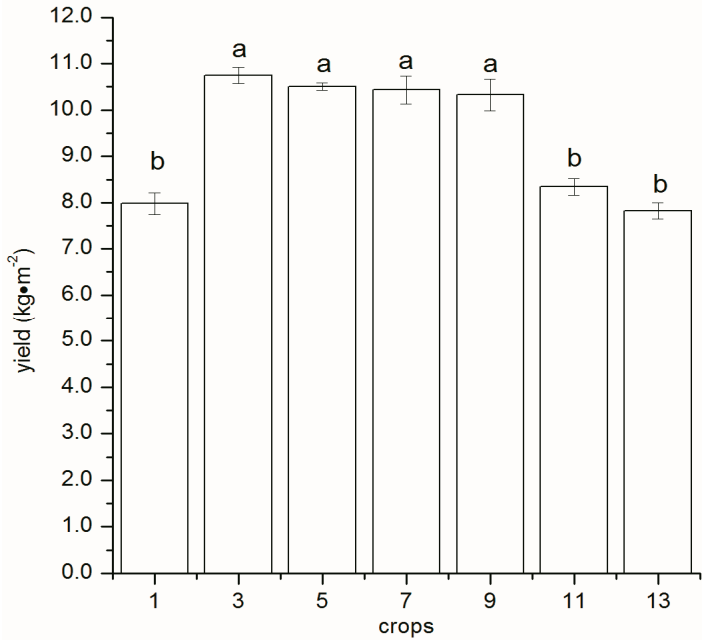

Figure 4. Tomato yield of the different continuous monoculture crops; Means followed by the same letter for a given factor are not significantly different (LSD test, $p < 0.05$).

3.7. Correlations between Tomato Yield and Soil Quality Indicators

The tomato yield had positive and significant relationships with the AWCD value at 144 h and the four SMC functional diversity indexes (data not shown). It also had a positive and significant relationship with the soil-available potassium (AK) (data not shown). The tomato yield had positive but non-significant relationships with the total microbial abundance, bacterial abundance, activities of the four soil enzymes, pH, organic matter content, available nitrogen, and available phosphorus (data not shown). It had negative but non-significant relationships with the fungal abundance and actinomycetes abundance (data not shown).

4. Discussion

Soil microbes play essential roles in crop growth. The seedling age was assumed to be an important influence on the microbial activity and diversity in the rhizosphere soil of plants [38]. Corneo et al. indicated that the most abundant and balanceable type of microflora in healthy soils was bacteria, followed by actinomycetes, and then fungi [39]. Our study demonstrated that the total soil microbial abundance, bacterial abundance, B/F value, and SMC functional diversity indicators, initially increased and then gradually decreased with the increase of continuous monoculture crops in a solar greenhouse. Fungal abundance followed an N-type trend with the increase in crop number. The changes in soil microbial abundance and SMC functional diversity indicators suggested that short-term (less than seven or nine crops) continuous monoculture was beneficial to soil microbial accumulation and SMC functional diversity promotion. However, long-term continuous monoculture decreased microbial accumulation and SMC functional diversity. Similar trends have been reported in other crop species. Recent studies found that the SMC diversity in rhizosphere soils increased under short-term (less than 5–10 years) continuous cotton cropping, but decreased under long-term (more than 15–20 years) cropping [40]. In addition, with increasing years of continuous cucumber cropping, the abundance of bacteria and total microbes followed a trend with an inverted saddle-shaped curve, and fungal abundance appeared to increase linearly [41]. The most likely reasons for the above results are as follows: short-term continuous monoculture is beneficial to soil ripening, and the process of soil ripening promotes an increase in SMC functional diversity. Furthermore, short-term continuous monoculture is less likely to have negative effects on soil and tomato yields than long-term continuous monoculture. Conversely, long-term continuous monoculture changes the soil predominant microbes which appear as a significant increase of fungi abundance, and this may lead to soil problems and limit continuous crops.

The deterioration of soil chemical characters is considered a main cause of soil sickness [42]. In our study, the soil pH, organic matter, available nitrogen, and available phosphorus at the 13th crop, were all significantly lower than they were at the 5th, 7th, and 9th crops. These results show that long-term continuous monoculture accelerated the deterioration of soil chemical characters. Moreover, the relationship analysis showed that soil total microbial abundance and bacterial abundance were both significantly positively correlated with soil organic matter and available potassium content. The soil AWCD value at 144 h was significantly and positively correlated with available potassium and available nitrogen content after several continuous monoculture crops. Previous studies have indicated that soil nutrient content regulates soil microbial properties, and the importance of soil nutrient content in shaping microbial properties has been reported by several studies [43]. Therefore, we conclude that changes in soil chemical properties that occur under continuous cropping may lead to changes in soil microbial characteristics.

In our study, the activities of urease and invertase first increased and then gradually decreased under continuous monoculture. The neutral phosphatase activity observed at the 13th crop was significantly lower than the activities at the other crops. In addition, catalase activity decreased gradually with continuous monoculture crops. Our results are consistent with those of Lu et al., who concluded that continuous cotton monoculture initially enhanced soil urease and invertase activities, which peaked at 15 or 20 years, and then decreased as monoculture continued [44]. However,

our results of soil enzyme activity change under continuous cropping differ from those of other studies. For instance, Sun et al. found that the activities of soil phosphatase, saccharase, and urease decreased each year under continuous peanut cropping [45], and Xiong et al. found that long-term continuous monoculture led to obvious declines in soil enzymatic activities and bacterial abundance [17]. These among-study differences may be attributed to the following factors: different species or crops have different responses to continuous monoculture, such that changes in soil enzyme activity are inconsistent among studies [14]. In addition, the quality of the naïve soil used for continuous monoculture differs among studies. Several agricultural practices, such as fertilization and irrigation of poor- or medium-quality soil, could improve soil activity under short-term continuous monoculture. However, long-term irrigation, fertilization, and continuous monoculture patterns would cause secondary soil problems, which may indirectly lead to a decrease in enzyme activity [44].

Soil enzymes, which mainly originate from microorganisms and plants, are closely associated with soil microorganisms [46]. For example, the activities of soil catalase and alkaline phosphatase are highly correlated with the soil microbial biomass [47]. Mungai et al. concluded that SMC functional diversity had decisive effects on soil enzyme activity [48]. Soil invertase was associated with both soil microbial biomass and the soil microbial population [49]. In our study, the correlation analysis confirmed that the urease and invertase activities were strongly and positively correlated with the AWCD value at 144 h, in terms of SMC functional diversity, total soil microbial abundance, and bacterial abundance. Furthermore, the neutral phosphatase activity was strongly and positively correlated with SMC functional diversity, and the soil enzyme activities were negatively correlated with fungal abundance and actinomycete abundance. The correlation analysis demonstrated that the soil enzyme activities were closely associated with the soil microbial abundance and SMC functional diversity under the different continuous monoculture crops. Future studies will focus on the correlations between the diversity of the fungal or bacterial community structure and soil enzymes under different crops, under continuous monoculture in solar greenhouses.

5. Conclusions

In this study, traditional physiological and biochemical methods, and Biolog Eco microplates analysis technology, were adopted. Our findings indicated that continuous monoculture of tomato in a solar greenhouse led to changes in soil properties and affected the tomato yield. The results showed that short-term continuous monoculture of tomato for fewer than seven or nine crops, accelerated soil ripening and increased the tomato yield, whereas long-term continuous monoculture of tomato, i.e., for more than 11 crops, had adverse effects on the soil quality and tomato yield. These results were likely observed because the long-term continuous monoculture of tomato and additional fertilizer application decreased the availability of nutrient elements and soil enzyme activity, and led to the change in soil microorganism diversity and a decline in the tomato yield. Future research is required to evaluate the genetic and structural diversity of soil microorganisms for tomato crops under continuous monoculture, by performing high throughput sequencing analysis. Such analyses will provide theoretical support for the development of sustainable soils for protected crops under continuous monoculture.

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