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Differences in Soil Microbial Diversity between Long-term Continuous Cropping and Rotation of Cherry Tomato (*Lycopersicon esulentum* Mill)

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Abstract

Continuous cropping had negative effects on soil microbial community, while rotation was beneficial to the formation of soil microbial community diversity. However, the difference in composition and diversity of microbial communities are still unclear under two cultivation patterns of long-term cherry tomato (*Lycopersicon esulentum* Mill) continuous cropping and cherry tomato - rice (*Oryza sativa* L.) rotation. soils from rice-cherry tomato rotation for 10 years (R10) and continuous cropping cherry tomato for 10 years (C10) were selected to as study objects, and high-throughput sequencing technology was conducted to study the difference under two cultivation patterns. The main objective is to provide a theoretical basis for applying rotation measures to reduce the continuous cropping obstacles of cherry tomato from the perspective of microbial ecology. The Chao1 and ACE indices of soil fungi in C10 were significantly higher than those in R10. The Shannon index of soil bacterial community was significantly greater in C10 than in R10, but that of fungal community was significantly lower in C10 than in R10.The relative abundance of beneficial microorganisms was in the order R10 > C10. the relative abundance of Acidobacteria, Actinobacteria, *Candidatus_Solibacter*, *Bryobacter*, *Bacillus*, *Mortierella*, *Trichoderma*, and *Penicillium etc.* was significantly higher in R10 than in C10. alkali-hydrolyzed nitrogen (AN) and available P (AP) were important factors affecting the bacterial community structure, AP was an important factor affecting the fungal community structure, as indicated by significant positive correlations between the important environmental factors and bacterial and fungal community structure.

Keywords

Cherry tomato, continuous cropping, rice-cherry rotation, microbial diversity, soil factors

1. Introduction

Cherry tomato (*Lycopersicon esulentum* Mill), also known as small tomato, has been listed as one of the "four fruits" promoted by the Food and Agriculture Organization of the United Nations because of its rich nutritional value. However, the

long-term single cultivation mode leads to the serious continuous cropping obstacles, which including imbalance of soil nutrient, high incidence of diseases and pests and the deterioration of soil physicochemical properties, such as soil acidification, reduction of organic matter content and so on [1]. Many studies had shown that the occurrence of continuous cropping obstacles was not only related to the change of soil physicochemical properties, but also related to soil microbial community composition [2, 3]. Rotation can not only coordinate the limitations of nutrient absorption among different crops, avoid imbalance of nutrient, improve soil nutrient availability and enzyme activities, but also adjust the composition of rhizosphere microbial community through root exudates, reduce the occurrence of soil-borne diseases and pests, and improve economic benefits [4]. However, the differences in community composition and diversity of soil microbial between long-term cherry tomato (*Lycopersicon esulentum* Mill) continuous cropping and cherry tomato - rice (*Oryza sativa* L.) rotation have not been reported. The main objective is to provide a theoretical basis for applying rotation measures to reduce the continuous cropping obstacles of cherry tomato from the perspective of microbial ecology.

2. Materials and methods

2.1 Description of study area

On the basis of full investigation, three fields of rice-cherry tomato rotation for 10 years (R10) and three fields of continuous cropping cherry tomato for 10 years (C10) were selected to as study area in Wushan and Junchang Villages of Guangpo Town, Lingshui County, Hainan Province. The cherry tomato variety, cultivation conditions and management methods of each field was the same. The study area has a typical tropical maritime monsoon climate with sufficient light and heat conditions. and the annual precipitation is approximately 1,718 mm, and the annual average sunshine duration is approximately 2262 hours. The mean annual air temperature is 25.4°C, with a minimum temperature 20.6°C in January and a maximum temperature 28.6°C in June. Geographic location and soil physicochemical properties of the six study area were presented in **Table 1**.

Table 1. Soil physicochemical properties at different sample area

Sample area	Geographic location	Soil texture	pH	OM(g/kg)	AN (mg/kg)	AP(mg/kg)	AK(mg/kg)
R10-1	110°04′27.5″E, 18°33′23.9″N	sandy loam	4.67	8.75	63.9	74.8	227
R10-2	110°04′35.4″E, 18°33′17.6″N	sandy loam	5.34	13.7	79.0	89.5	137
R10-3	110°04′37.7″E, 18°33′59.8″N	sandy loam	5.36	12.1	66.6	45.9	155
C10-1	109°58′01.9″E, 18°36′14.1″N	sandy loam	5.65	13.4	95.9	259	220
C10-2	109°56′40.0″E, 18°37′17.7″N	sandy loam	5.69	19.6	118	185	331
C10-3	109°56′40.6″E, 18°37′19.8″N	sandy loam	5.46	12.7	128	306	81.9

R10, Rice-cherry tomato rotation for 10 years; C10, continuous cropping cherry tomato for 10 years; OM, organic matter; AN, alkali-hydrolyzed nitrogen; AP, available phosphorus; AK, available potassium.

2.2 Soil sample collection and analysis of physicochemical properties

Soil samples were taken from 0-20-cm tillage layer by applying five point sampling method in November 2021. The samples were well mixed into one composite sample about 1000g per site, sealed in sterile bags, and transported in an ice cube-filled box to the laboratory within two days. Each soil sample was divided into three parts. One part was air-dried, passed through a 2-mm sieve to analyze soil pH, the subsample was then sieved again (< 0.15 mm) and used for other physicochemical analyses. The second part was stored at -80°C and then used for microbial diversity. Soil pH, organic matter (OM), alkali-hydrolyzed nitrogen (AN), available P (AP), and available K (AK) were measured using routine methods described by Lu [5]. Soil pH was measured using a pH electrode (Leici, Shanghai, China) at a soil: water ratio of 1:2.5. The OM content was measured using a potassium dichromate volumetric method. The AN, AP, and AK contents were analyzed using a diffusion method, the Olsen method, and ammonium acetate extraction flame photometry, respectively.

2.3 Soil DNA extraction, PCR amplification and sequencing

Total soil DNA was extracted using a Power Soil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, United States) according to the manufacturer's protocol.16S rRNA and ITS gene sequencing were used for bacterial and fungal profiling, in which the bacterial 16S gene V3-V4 region and the fungal ITS1 region was amplified using the universal primers (319F-ACTCCTACGGGAGGCAGCA /806R-GGACTACHVGGGTWTCTAAT) and ITS1F/ITS2R(ITS1F-CTTGGTCATTTAGA GGAAGTAA /ITS2R-GCTGCGTTCTTCATCGATGC), respectively, with adapter and bar code sequences. The PCR was conducted under the conditions described by Wu et al. [6]. The libraries were build and sequenced by Biomarker Biotechnolo-

gy Co., Ltd. (Beijing, China) using the Illumina Novaseq 6000 sequencing system (Illumina, Santiago, CA, United States).

2.4 Sequence processing and statistical analysis

QIIME 1.8.0 was used to splice and filter the high-throughput original sequences. UCHIME v4.2 was used to identify and remove chimeras to obtain optimized sequences. Then, UPARSE (USEARCH, version 10.0) was used to cluster the optimized sequences at the 97% similarity level to obtain Operational taxonomic units (OTUs). All the analysis, including alpha diversity, beta diversity, NMDS, ANOSIM, RDA and Mantel Test, were performed using BMKCloud (www. biocloud.net). Richness index Chao1 and diversity index Shannon were used to evaluate α - diversity. NMDS and ANOSIM were used to evaluate β - diversity. RDA was used to analyze the correlation between soil environmental factors and microbial community, and Mantel Test was used to detect the significant factors affecting microbial community. Data from replicates are expressed as the mean ± standard deviation (SD). SPSS v.17.0 software package (IBM Corp., Armonk, NY, United States) was used to perform the calculations and compare the treatment means for each experiment. Significant differences between the means were assessed using Duncan's tests. Statistical significance was set at $p \leq 0.05$.

3. Results and Discussion

3.1 Dissimilarity between microbial community diversity in soils from long-term continuous cropping and rotation of cherry tomato

Chao1 and ACE indices of bacterial communities were not significantly different between in soils from C10 and R10, Shannon index of bacterial communities was significantly greater in soils from C10 than in R10 (**Table 2**).However, the Chao1 and ACE indices of fungal communities were significantly greater in soils from C10 than in R10, Shannon index of fungal communities was significantly lower in soils from C10 than in R10. In the NMDS analysis, the bacterial and fungal communities were all clearly separated in soils from C10 and R10 **(Fig. 1)**, indicating there were obvious differences in composition of microbial communities between in soils from long-term continuous cropping and rotation of cherry tomato.

Table 2. Alpha diversity indices of bacterial and fungal communities in soils from long-term cherry tomato continuous cropping and rice-cherry tomato rotation

	Bacterial community			Fungal community		
	Chao1	ACE	Shannon	Chao1	ACE	Shannon
C10	2133.7±0.60 a	2100.2±8.60 a	9.4250±0.1700 a	778.5±42.0 a	941.4±82.0 a	6.0825±0.3690 b
R10	2052.4±65.2 a	2016.1±52.5 a	9.2806±0.1781 b	701.9±41.0 b	862.4±121 b	6.5542±0.1045 a

C10, continuous cropping cherry tomato for 10 years; R10, Rice-cherry tomato rotation for 10 years; Values are the mean ± standard error (n = 3). Values with different lowercase letters in each column are significantly different at P ≤ 0.05.

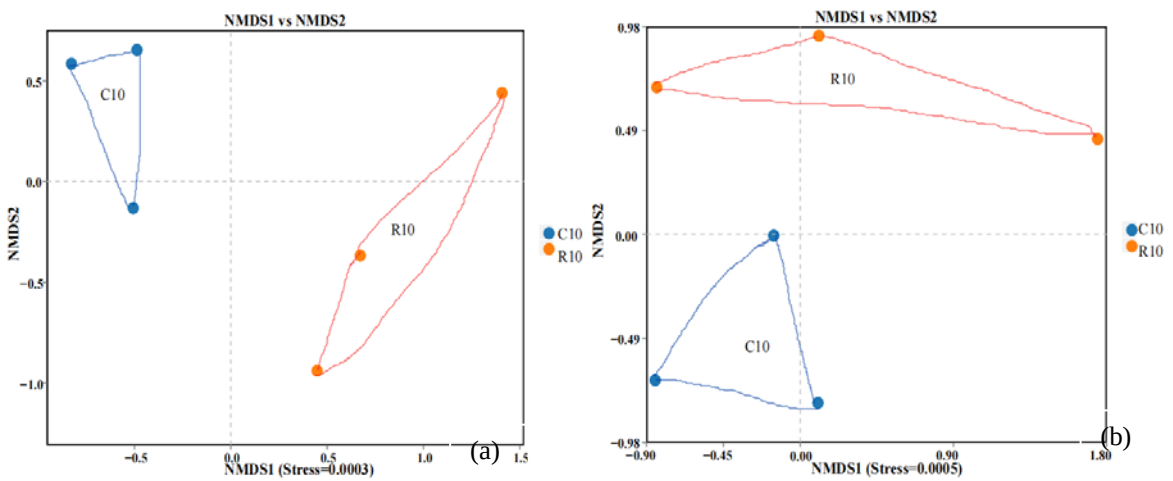

Fig.1. Non-metric multidimensional scaling (NMDS) ordination based on operational taxonomic units for (a) bacteria, (b) fungi. C10, cherry tomato continuous cropping for 10 years; R10, Rice-cherry tomato rotation for 10 years.

3.2 Dissimilarity between microbial community compositions in soils from long-term continuous cropping and rotation of cherry tomato

The composition of bacterial communities was basically the same, but the relative abundance of individual bacteria was significantly different in C10 and R10 (**Fig.2**). At the phylum level (**Fig.2**(a)), The 7 phyla of Proteobacteria, Acidobacteria, Chloroflexi, Gemmatimonadetes, Actinobacteria, Verrucomicrobia, and Bacteroidetes were the dominant taxa in two cultivation patterns, accounting for 91.5% and 88.5% of the total relative abundance in the soils from C10 and R10, respectively. Relative abundance of beneficial bacteria Acidobacteria and Actinobacteria were increased by 10.6% and 58.7%, respectively, in R10 compared with C10. Acidobacteria plays an important role in the improvement of soil nitrogen nutrition level by take part in cycling of nitrogen [7]. Actinobacteria are important sources of antibiotics, biocides and antifungal agents in agricultural production activities, and some actinomycetes are also rhizosphere bacteria, symbiotic bacteria and endophytes that promote the growth of crops [8]. At the genus level (**Fig.2**(b)), the 14 genera of *uncultured_bacterium_o_Acidobacteriales*, *uncultured_bacterium_c_Subgroup_6*, *Candidatus_Solibacter*, *uncultured_bacterium_f_Gemmatimonadaceae*, *uncultured_ bacterium_f_SC-I-84*, *Sphingomonas*, *Haliangium*, *uncultured_bacterium_o_Gaiellales*, *Bryobacter*, *uncultured_bacterium_ c_KD4-96*, *Bacillus*, *uncultured_bacterium_o_Saccharimonadales*, *uncultured_bacterium_f_Xanthobacteraceae*, and *uncultured_bacterium_o_Elsterales* were the dominant taxa in two cultivation patterns. Relative abundance of beneficial microorganisms *Candidatus_Solibacter*, *Bryobacter*, and *Bacillus* were increased by 77.6%, 36.8% and 8.8%, respectively, in R10 compared with C10. *Candidatus Solibacter* and *Bryobacter* can decompose organic matter and promote the cycling of soil carbon [9]. Bacillus is a potentially beneficial bacterium that increases plant disease resistance and promotes plant growth [10].

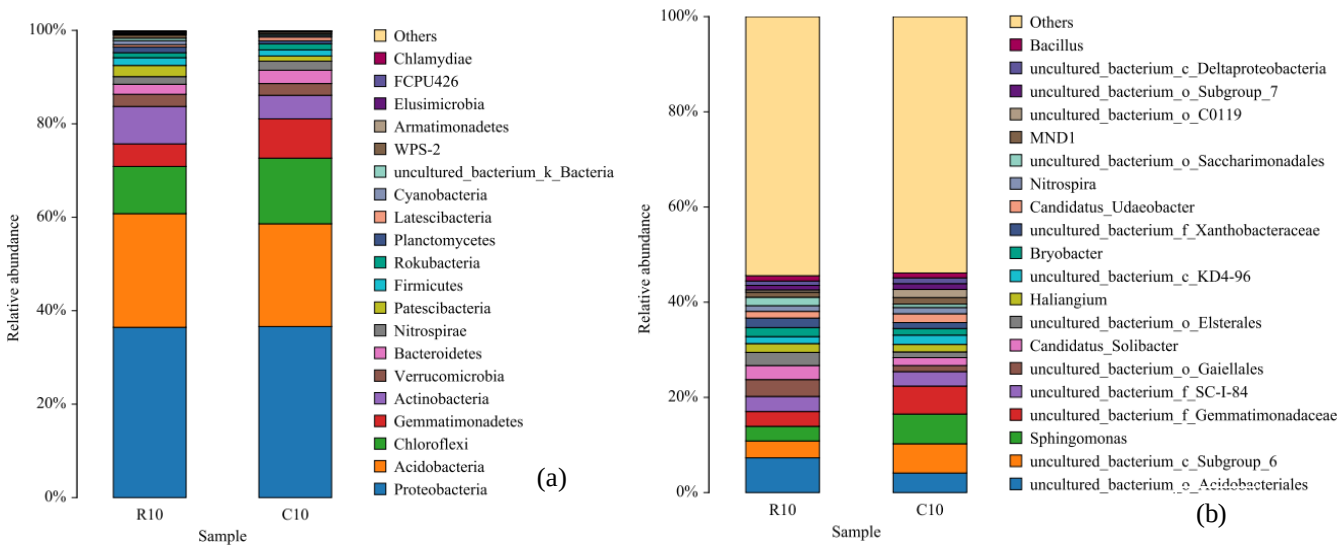

Fig. 2. Composition of bacterial communities in soils from long-term cherry tomato continuous cropping and rice-cherry tomato rotation (C10, cherry tomato continuous cropping for 10 years; R10, Rice-cherry tomato rotation for 10 years.). (a)Phyla; (b) genera.

The composition of fungal communities was also basically the same, but the relative abundance of individual fungi was significantly different in soils from C10 and R10 (**Fig.3**). At the phylum level (**Fig.4(a)**), The five phyla of Ascomycota, Basidiomycota, Rozellomycota, Mortierellomycota, and Chytridiomycota were the dominant fungal phyla in the two cultivation patterns, accounting for 75.4% and 73.0% of the total relative abundance in C10 and R10, respectively. The relative abundance of Ascomycota, Mortierellomycota, and Chytridiomycota were increased by 7.37%, 31.9%, and 44.0%, respectively, in the soils from R10 compared with that from C10. At the genus level (**Fig.4**(b)), the eight genera of *Aspergillus*, *Acrophialophora*, *Mortierella*, *Byssochlamys*, *Trichoderma*, *Fusarium*, *Penicillium*, and *Curvularia* were the dominant genera shared by the two cultivation patterns. The relative abundance of beneficial fungi *Mortierella*, *Trichoderma*, and *Penicillium* were increased by 48.9%, 52.4%, and 99.8%, respectively, in R10 compared with C10. This result is basically consistent with the conclusion that continuous cropping inhibits the growth of beneficial microorganisms in soil [11-14]. And *Mortierella* can promote the transformation of soil carbon, nitrogen and phosphorus nutrients and the secretion of plant growth hormone, thus promoting plant growth and improving soil health [15]. *Trichoderma* is an important biocontrol fungi that can kill a variety of plant pathogens [16]. And the metabolites of *Penicillium* also generally have activities on antimicrobial and anti-insect [17].

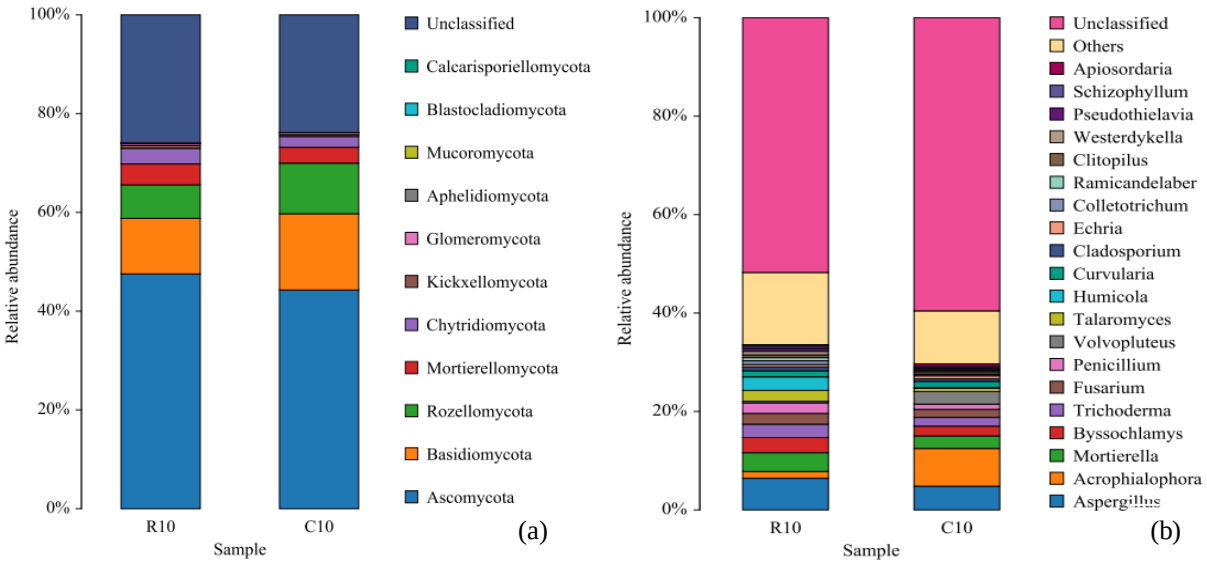

Fig. 3. Composition of fungal communities in soils from long-term cherry tomato continuous cropping and rice-cherry tomato rotation (C10, cherry tomato continuous cropping for 10 years; R10, Rice-cherry tomato rotation for 10 years). (a)Phyla; (b) genera.

3.3 Effects of soil environmental factors on microbial communities in soils from long-term continuous cropping and rotation of cherry tomato

The RDA (**Fig. 4**) and Mantel tests (**Table 3**) indicated that AN, and AP were two important environmental factors affecting bacterial community structure in soils from R10 and C10, and the *r* values were in the order were AP > AN. AP was an important environmental factor affecting fungal community structure in soils from R10 and C10. And it indicated by significant positive correlations between bacterial and fungal community structure and the environmental factors. Previous studies also found that soil nutrient contents were important factors affecting soil microbial communities in other studies [18, 19], and were significantly positively correlated with soil microbial diversity [20].

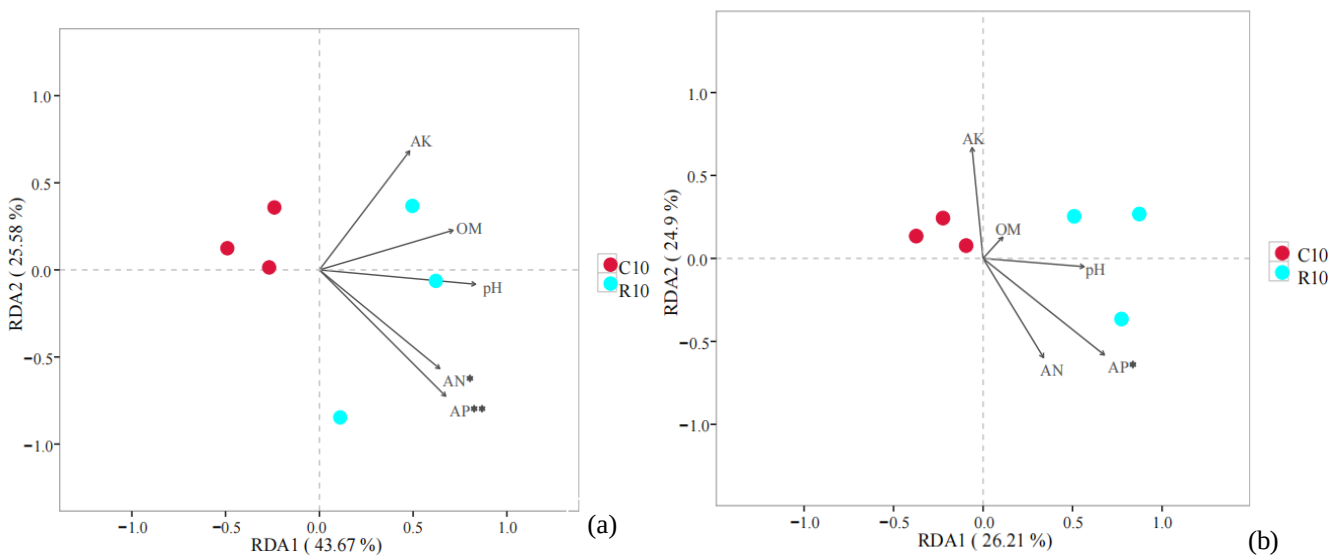

Fig. 4. Correlation between microbial community structure and environmental factors in soils from long-term cherry tomato continuous cropping and rice-cherry tomato rotation ((a) RDA analysis of bacteria; (b) RDA analysis of fungi)(C10, cherry tomato continuous cropping for 10 years; R10, Rice-cherry tomato rotation for 10 years; OM, organic matter; AN, alkali-hydrolyzed nitrogen; AP, available phosphorus; AK, available potassium).

Table 3. Correlation between microbial community structure and soil environmental factors tested by Mantel

Environmental factors	bacteria community structure		Fungi community structure	
	r	p	r	p
pH	0.1141	0.247	-0.2902	0.881
OM	-0.1005	0.560	-0.3035	0.779
AN	0.5914	0.011	0.1663	0.217
AP	0.7485	0.004	0.5817	0.038
AK	0.1911	0.293	-0.0426	0.492

OM, organic matter; AN, alkali-hydrolyzed nitrogen; AP, available phosphorus; AK, available potassium.

4. Conclusion

The composition and diversity of soil microbial community were significant different under two cultivation patterns of long-term cherry tomato continuous cropping and rice-cherry tomato rotation. The relative abundance of beneficial microorganisms such as Actinobacteria, *Bacillus*, *Mortierella*, *Trichoderma*, and *Penicillium* etc. were increased in soils from long-term rice-cherry tomato rotation. AP and AN were important factors affecting the bacterial community structure, AP was also important factor affecting the fungal community structure, as indicated by significant positive correlations between bacterial and fungal community structure and the environmental factors. It will provide a theoretical basis for applying rotation measures to reduce the obstacles of cherry tomato continuous cropping from the perspective of microbial ecology.

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