

Response of Microbial Diversity in Soil under Plastic Shed to Different Years of Continuous Cropping

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Abstract—This study aimed to investigate the changes in bacterial and fungal community composition and its relationship with environmental factors in the red soil (Ferralsols) under different continuous cropping years. Changes in composition and diversity of bacterial and fungal communities in lily red soil from continuous cropping for 4 years (C4), 5 years (C5), and 7 years (C7) were studied by MiSeq high-throughput sequencing. Alpha diversity analysis showed that the Shannon and Simpson diversity indexes were significantly increased for bacteria and significantly decreased for fungi, respectively, in the red soil under protected lily cultivation with the extension of continuous cropping. In the analysis of microbial community composition, the dominant bacterial phyla were *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, and *Chloroflexi*. Those dominant bacterial phyla in C4, C5, and C7 accounted for 85.17, 81.04, and 81.64% of the total bacterial community, respectively. Among the fungi, only *Ascomycota* was the dominant taxa, and the dominant taxa in C4, C5, and C7 accounted for 93.69, 92.20, and 84.31% of the total fungi community, respectively. The abundance of *Fusarium ascomycetes* was 44.02–58.83% in these three soils. Correlation analysis showed that soil pH, organic carbon, and total nitrogen were significantly related to the abundance of *Proteobacteria*, *Actinobacteria*, and *Acidobacteria*. The pH value was positively correlated with *Penicillium* and negatively correlated with *Fusarium*. Environmental factors such as pH, total nitrogen (TN), soil organic carbon (SOC), available phosphorus, and available potassium affect the composition of the soil microbial community by influencing the dominant bacteria phyla. The decrease in pH after continuous cropping leads to soil acidification, which affects the soil microbial community, which in turn reduces soil stability. The results of this study showed that the diversity index of soil bacterial microbial community increased significantly after continuous cropping, and the diversity of the fungal microbial community decreased significantly. Soil pH is the key factor to predict the structure of soil microbial community and to regulate the continuous cropping obstacle reasonably. Meanwhile, the dominant bacterial phyla were *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, and *Chloroflexi*. *Fusarium* dominated in the Ascomycota after continuous cropping, which might be one of the main reasons for the continuous cropping obstacle in facility lily red soil.

Keywords: High-throughput sequencing, Dominant taxa, Community composition, microbial diversity, *Fusarium*

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INTRODUCTION

Facility planting has the characteristics of high output and high efficiency, which is favored by growers. In recent years, with the rapid development of facility planting technology, the area of facility planting is increasing. The extension of years of facility planting can easily cause soil degradation, crop quality degradation, and even serious diseases. Existing research shows [10] that with the increase of continuous cropping years, the soil is prone to acidification, nutrient accumulation, and increase in organic matter content. In addition, growers blindly pursue benefits, and blind

fertilization is also a problem that cannot be ignored. Soil acidification and salinization can be caused by the large-scale application of long-term nitrogen fertilizer and no rainfall leaching in the greenhouse [56]. A large number of studies have shown that continuous cropping has become an important factor affecting crop growth [51]. After long-term continuous cropping, the absorption and transformation of nutrients in the soil are affected by microorganisms [9], and the imbalance of microbial population composition will reduce soil quality and crop yields [11, 24]. Also, reduced microbial diversity in the plant rhizosphere affects negatively plant growth and health [33]. Soil

nutrient deficiency, harmful substances produced by plants, changes in microbial community structure and imbalance of microbial ecosystem [19, 29] are all the causes of crop continuous cropping obstacles. Specifically, with the increase of continuous cropping years, the diversity of soil microbial community decreases and the community structure is simplified [55, 14]. It has also been found that after continuous cropping, pathogenic fungi will be enriched in the soil and inhibit crop growth [3, 37].

At present, the changes in microbial taxa after continuous cropping and the factors that cause microbial taxa changes after continuous cropping are hot issues that people have been studying. Compared with other microbial assays (such as T-RFLP, PLFA), the advantage of high-throughput sequencing technology is that it can distinguish the differentiation of microbial taxa, the relative abundance and the evolutionary relationship in the natural environment, without separation and the laboratory culture of individual taxa [12]. Therefore, high-throughput sequencing (metabarcoding) has been applied more and more widely in the research of soil microorganisms [22, 34].

Lily (Latin scientific name: *Lilium*) is a perennial herbaceous bulbous plant of the lily family, which is mainly grown in Yunnan, Sichuan, Shaanxi, and Gansu provinces in China. Relying on the rich natural resources and inherent advantages of Yunnan Province, fresh-cut lily is one of the characteristic industries on the Yunnan Plateau. It has the largest planting area and the largest output. However, because of continuous cropping management, time, and shortage of land resources, there is a continuous cropping obstacle in cut lily, which leads to a decrease in the yield and quality of cut lily, it also restricts the sustainable development of facility planting of cut lily. Previous studies have found that The main pathogenic fungi that cause lily wilt under field conditions are *Fusarium* [39]. Therefore, this study uses high-throughput sequencing technology to study the changes in the microbial community of lily red soil in different continuous cropping years, and further reveals the obstacle mechanism of lily continuous cropping in red soil, thereby contributing to the cultivation and sustainable development of flowers.

OBJECTS AND METHODS

Field description and experimental design. The study area was selected in Hong-shan (E 102°12'–102°52', N 24°23'–24°48'), the main producing area of facility cut lilies, Jin-ning County, Kunming City, with an altitude of 2000 m, and the climate is free from severe cold in winter and heat in summer, with four seasons like spring and distinct dry and wet seasons. It belongs to the low-latitude plateau northern subtropical monsoon climate zone. The soil type is red soil. The annual average temperature is 15°C, the annual

rainfall is 1450 mm, and the frost-free period is more than 240 days.

In October 2017, 3 plots of typical facility soil for lily cropping for 4 years (C4), 5 years (C5), and 7 years (C7) were selected with the same parent material, soil type, and field management measures, and there were 9 experimental plots in total (each plot area is $5.6 \times 42 \text{ m}^2$). Fertilization is carried out through the integrated water-fertilizer irrigation technology (water-fertilizer ratio 1000 : 1). The annual fertilization amount is: nitrogen fertilizer 104.3 kg hm^{-2} , P_2O_5 104.3 kg hm^{-2} , K_2O 141.8 kg hm^{-2} , and organic fertilizer (rapeseed cake fertilizer) $3000.0 \text{ kg hm}^{-2}$.

Collection and preparation of soil samples. The soil samples were collected in March 2018 and randomly collected at multiple points in each block. After removing the oily residue on the surface of the soil, the soil of 0–20 cm was collected with a soil drill, and the soil samples collected at multiple points were mixed evenly and collected in a ziplock bag. After being brought back to the laboratory, the animal and plant residues and roots are removed. After passing through a 2 mm sieve, soil samples were divided into two parts: one stored in a -80°C refrigerator for soil DNA extraction, and the remaining part is air-dried and subsequently passed through a 100-mesh sieve for determination of soil physicochemical properties.

The detections of soil physicochemical properties. The basic physical and chemical properties of the soil were measured by LU [40]: the pH value of the soil was measured by a pH meter, with the water-soil ratio of 2.5 : 1; the total organic carbon of the soil was determined by the conventional $\text{K}_2\text{Cr}_2\text{O}_7\text{--H}_2\text{SO}_4$ oxidation method, and the N content determined by Semimicro-Kjeldahl determination using Se, CuSO_4 , and K_2SO_4 as catalysts. Phosphorus was determined by using molybdenum antimony colorimetric method, and available potassium uses flame photometer method.

DNA extraction and high-throughput sequencing. Soil microbial DNA was extracted in triplicates with 0.500 g for each soil sample by using the FastDNA Spin Kit for Soil kit (MPBiomedicals). The bacterial 16S rDNAV4 + V5 region amplification of all samples was amplified with primer 515F (5'-GTGCCAGC-MGCCGCGG-3') and primer 907R (5'-CCGT-CAATTCMTTTRAGTTT-3'). The fungal ITS1F-ITS2R region of all samples was amplified with primers ITS1F (5'-CTTGGTCATTTAGAGGAAG-TAA-3') and primers ITS2R (5'-GCTGC-GTTCTTCATCGATGC-3') [28]. After successful amplification was achieved, the PCR products were sent to Genesky Biotechnologies, Inc. (Shanghai, China) for Illumina MiSeq sequencing.

Data processing. High-throughput sequencing of soil samples based on the bacterial 16S rDNAV4 + V5 region and the fungal ITS1F-ITS2R region was performed on all samples from three different succes-

Table 1. Effects of different continuous cropping years on soil physical and chemical properties of facility lily

Treatment	pH	AP	AK	TN	SOC	C/N
		mg/kg		g/kg		
C4	4.92 ± 0.04a	66.19 ± 2.31b	424.23 ± 9.02a	1.33 ± 0.13b	18.84 ± 1.78b	14.19 ± 0.11a
C5	4.72 ± 0.05c	117.61 ± 4.66a	451.83 ± 8.34a	1.74 ± 0.09a	24.12 ± 0.73a	12.70 ± 0.43b
C7	4.80 ± 0.02b	118.00 ± 4.19a	460.74 ± 4.00a	1.84 ± 0.07a	20.45 ± 0.69b	11.11 ± 0.68c

Different small letters meant significant difference among treatments at 0.05 level, the same below. (AP is short for quick-acting phosphorus, AK is available potassium, TN is total nitrogen, and SOC is organic carbon).

Table 2. Notes and assessment of soil samples in different continuous cropping years

Treatment	Bacteria				Fungi			
	sequence number	H-index	simpson index	coverage %	sequence number	H-index	simpson index	coverage %
C4	35636.00	996a	0.49b	99.10	65347.00	301a	0.036a	99.89
C5	42513.33	1200.33b	0.56a	99.14	63380.33	376.67b	0.019b	99.85
C7	43948.67	1325.33b	0.58a	99.19	73962.00	371.67b	0.021b	99.86

Different small letters meant significant difference among treatments at 0.05 level, the same below.

sional years to obtain a total of bacterial valid sequences, and OTU clustering of non-repetitive sequences (excluding single sequences) according to 97% similarity to obtain the total bacterial OTUs, and similarly non-repetitive sequences (excluding single sequences) according to 97% similarity to obtain the total fungal OTUs. The total number of fungal OTUs was obtained by OTU clustering of non-repeated sequences (excluding single sequences) according to 97% similarity. The results showed that the effective bacterial and fungal sequences obtained after sequencing of all samples were 249003 and 451044, respectively, and the total number of bacterial and fungal OTUs was 906 and 298, respectively.

All data used Excel and SPSS software for statistical testing and correlation analysis, and the Duncan method was used to test the significance of differences between different continuous cropping years ($P < 0.05$), and the Mothur software was used to analyze the Alpha diversity. Using RDP classifier Bayesian algorithm to perform taxonomic analysis on 97% similar OTU representative sequences, The R Programming Language for statistics and graphing, Use Canoco for Windows 4.5 to perform redundancy analysis on soil physical and chemical properties, soil microbial communities and Principal Component Analysis.

Bioinformatic analyses. Sequencing data were processed and analyzed using the QIIME software package (version 1.9.1) [23]. NGS uses Fastp software package (version 0.19.6). Use “multiple_join_paired_ends.py”, “multiple_extract_bars_codes.py”, and “multiple_split_libraries_fastq.py” scripts to merge end-to-end reads, remove barcodes, and control quality, respec-

tively. The resulting high-quality sequences were then clustered into operational taxonomic units (OTUs) using the ‘pick_open_reference_otus.py’ script with a threshold of 97% identity against UNITE database [6]. Fungal ITS uses the UNITE 8 database, bacteria use databases such as SILVA 138, RDP 11.5 and Green-Genes 135 [43], all analysed by Mothur 1.30.2.

RESULTS

Soil physical and chemical properties of different continuous cropping years. Soil pH was firstly significantly ($P < 0.05$) decreased and then considerably ($P < 0.05$) increased with the continuous cropping years, whereas the ratio of C/N was remarkably ($P < 0.05$) decreased with the increase of cropping years.

It can be seen from Table 1 that with the increase of continuous cropping years, soil pH showed a downward trend and the content of available phosphorus and total nitrogen increased significantly, the content of available potassium gradually increased and the organic carbon content increased first and then decreased, and it was significantly higher than other continuous cropping years after five years of continuous cropping, and the ratio of carbon to nitrogen decreased significantly.

OTU annotation and evaluation of soil samples with different continuous cropping years. It was found that the library coverage of 16S rRNA sequencing of soil bacteria with different continuous cropping years was above 99%, and the coverage of the fungal gene library reached 99.80% (Table 2). This also means that the soil sequencing library has reached saturation, and deeper sequencing will not result in a large increase in

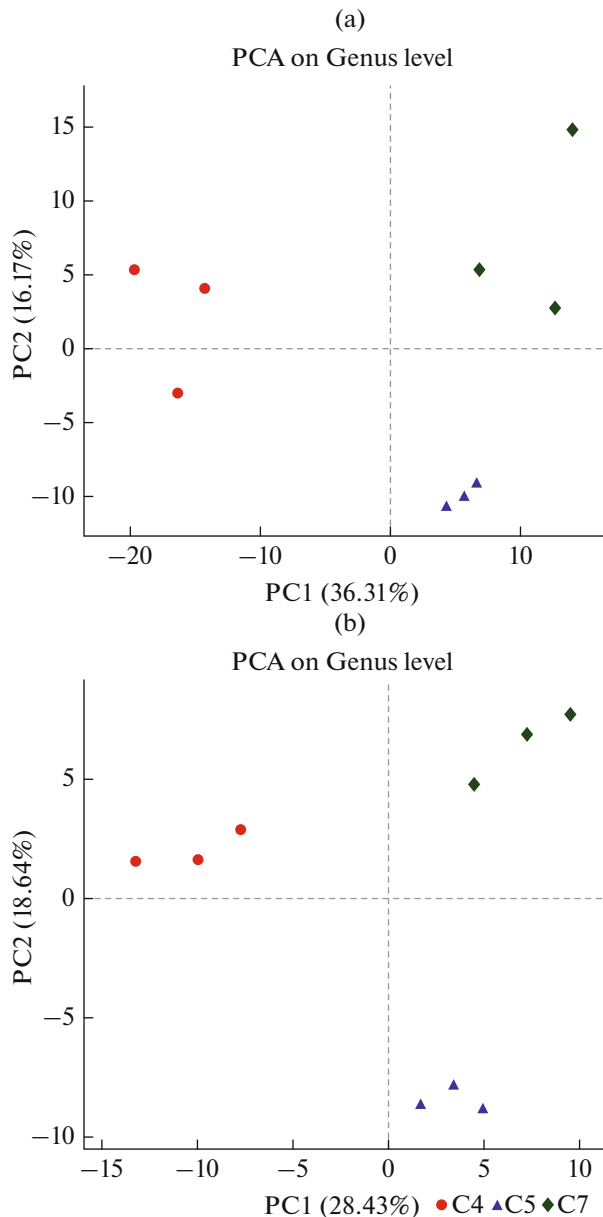


Fig. 1. Principal component analysis of the major changes of bacterial (a) and fungal (b) communities in different continuous cropping soils.

the number of OTU. The changes of the microbial community structure in the soil of different planting years are shown in Table 2. The H-index and Simpson Index represent the richness and diversity of microbial communities respectively. With the extension of planting years, the H-index of bacteria and fungi increased significantly; the bacterial Simpson index increased significantly, and the fungal Simpson index decreased significantly.

Beta diversity analysis of bacteria and fungi in soils with different continuous cropping years. PCA showed that both the bacterial and fungal communities of dif-

ferent continuous cropping years were significantly distinct (Fig. 1). explanatory variable variances of principal component 1 (PC1) and principal component 2 (PC2) related to the soil bacterial community are 36.31 and 16.17%, respectively; As shown in Fig. 2b, the explanatory variable variances of principal component 1 (PC1) and principal component 2 (PC2) related to the soil fungal community are 28.43 and 18.64%, respectively.

Composition and differences of soil microbial community in different continuous cropping years. It can be concluded from Fig. 2a that a total of 10 phyla have been detected in the bacterial phyla level classification in continuous cropping soil samples, namely *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Chloroflexus*, *Chloroflexi*, *Bacteroidetes*, *Gemmatimonadetes*, *Planctomycetes*, *Firmicutes*, *Cyanobacteria*, and *Nitrospirae*. The *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, and *Chloroflexi* belong to the dominant strains among these phyla. The ratios of dominant strains in C4, C5, and C7 to the total number of bacteria were 85.17, 81.04, and 81.64% respectively. According to the proportion of the bacterial phyla in the figure, it can be seen that the continuous cropping years have a significant impact on the composition of the bacterial community. As shown in Fig. 2b, the abundance of *Acidobacteria* and *Gemmatimonadetes* increased significantly, and the abundances of *Proteobacteria* and *Cyanobacteria* decreased significantly with the increase of continuous cropping years. The abundances of *Chloroflexi*, *Nitrospirae*, and *Planctomycetes* have an upward trend with continuous cropping years, while the abundances of *Actinobacteria*, *Bacteroidetes* and *Firmicutes* decreased; Among them, the *Proteobacteria*, *Acidobacteria*, *Chloroflexi* and *Gemmatimonadetes* in soils of different continuous cropping years are extremely different. There are significant differences between the phylum *Planctomycetes* and *Nitrospira* (Fig. 3a). From the level of genus, it can be concluded that the composition of the bacterial community is significantly different for different continuous cropping years. The abundance of *Mizugakiibacter*, *Pseudolabrys*, *Bryobacter*, *Candidatus-Solibacter*, and *Bacillus* sp. increases significantly with the increase of continuous cropping years, while *Burkholderia-Paraburkholderia*, *Pseudolabrys*, *Bryobacter*, *Acidibacter*, *Granulicella* and *Flexivirga* decreased significantly; and it can be seen that *Mizugakiibacter*, *Bryobacter*, *Gemmatimonas*, *Candidatus_Solibacter* and *Pseudolabrys* have extremely significant differences in different continuous cropping years, and *Acidibacter* and *Mucilagibacter* have significant differences (Fig. 3c).

From the sequencing results in Fig. 2c, a total of 3 phyla were detected in the fungal phyla horizontal classification in continuous cropping soil samples, namely *Ascomycota*, *Zygomycota* and *Basidiomycota*. Among these phyla, only *Ascomycota* is a dominant taxon, and the dominant taxa in C4, C5, and C7 accounted for 93.69, 92.20, and 84.31% of the total

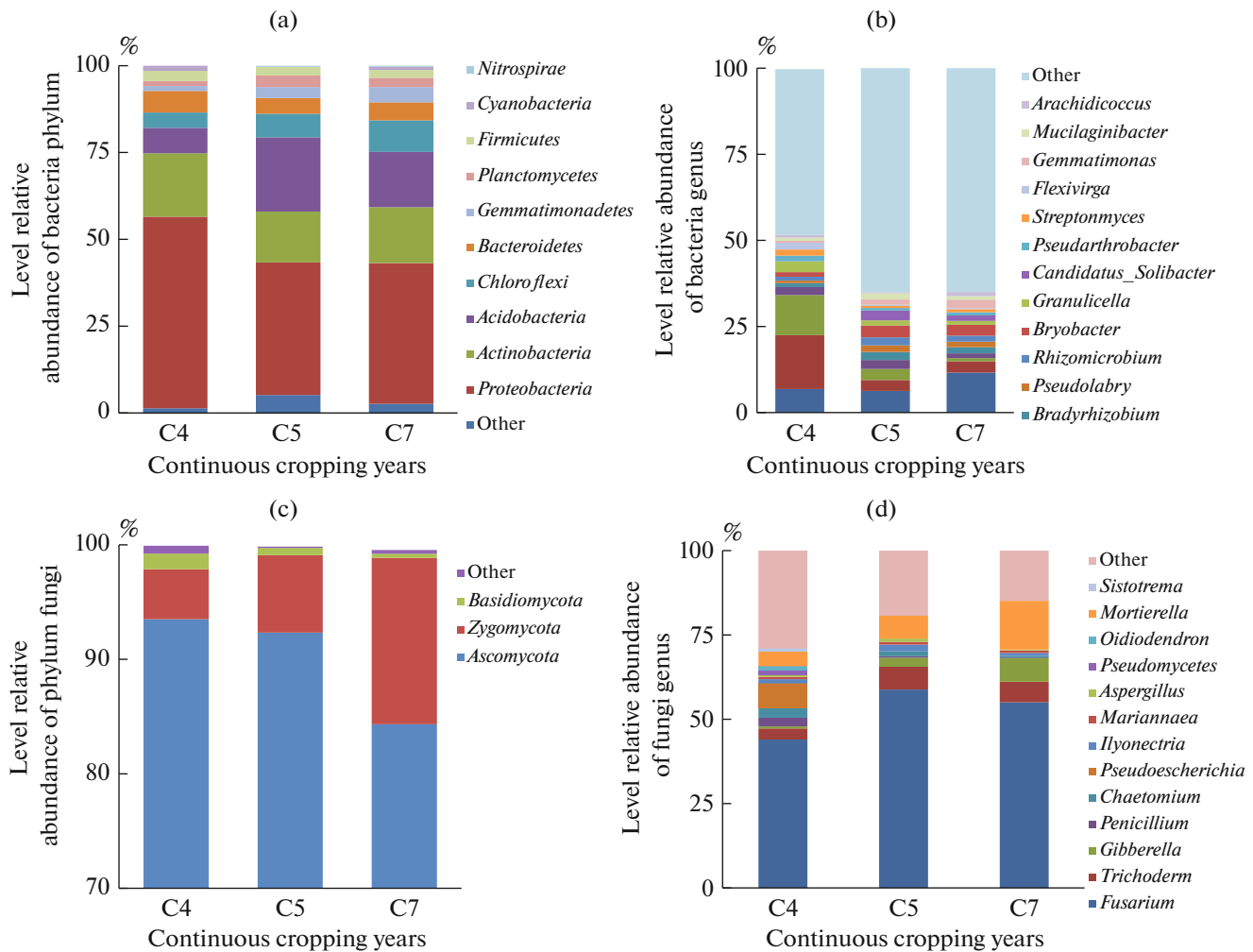


Fig. 2. Abundance changes of bacteria at phylum (a) and genus (b) levels under different continuous cropping years.

number of fungi, respectively. According to the proportion of bacteria in the table, it can be seen that the continuous cropping years also have a significant impact on the composition of the fungal community. In Fig. 2d, the abundance of *Ascomycota* and *Basidiomycota* gradually decreased with the continuous cropping years, while the abundance of *Zygomycota* significantly increased; Among them, there are significant differences between the *Ascomycota* and *Zygomycota*. Figure 3c shows the differences in the bacterial genus level community. It can be seen from the level of fungi that the abundance of *Fusarium*, *Trichoderma*, *Gibberella*, and *Mortierella* increased significantly with the extension of continuous cropping years, while the abundance of *Penicillium*, *Chaetomium*, *Pseudoescherichia*, *Pseudomyces*, *Oidiodendron* and *Sistotrema* decreased significantly; The difference can be seen from Fig. 3d. *Chaetomium* has extremely significant differences in three different continuous cropping years. *Fusarium*, *Mortierella*, *Trichoderma*, *Gibberella*, *Ilyonectria*, and *Penicillium* have significant differences.

Redundant analysis of soil environmental factors and microbial community composition in different continuous cropping years. It can be seen from Fig. 4 that pH is positively correlated with *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Penicillium*, and is significantly negatively correlated with *Acidobacteria* and *Fusarium*, indicating that pH affects microbial diversity by affecting the dominant bacterial phyla and fungal genera; Total nitrogen has a significant negative correlation with *Proteobacteria*, *Actinobacteria*, *Bacteroidetes* and *Penicillium*, and a significant positive correlation with *Acidobacteria* and *Fusarium*; Organic carbon has a significant negative correlation with *Actinobacteria* and a significant positive correlation with *Acidobacteria* and *Fusarium*. Available potassium content is significantly negatively correlated with *Proteobacteria* and *Penicillium*; Available phosphorus was significantly negatively correlated with *Proteobacteria*, *Bacteroidetes*, and *Penicillium*, and significantly positively correlated with *Acidobacteria* and *Fusarium*. It shows that pH, TN, and SOC can affect microbial

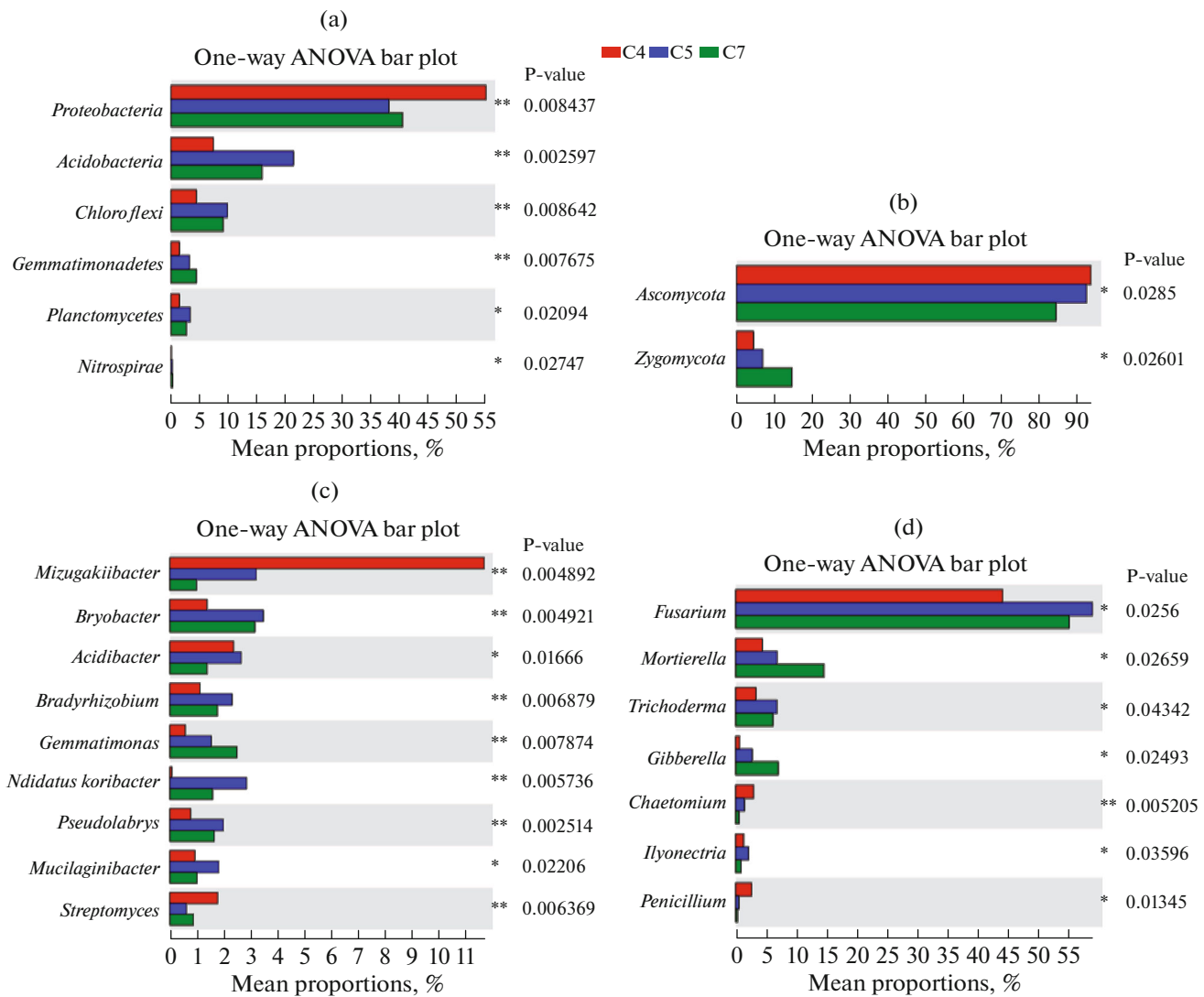


Fig. 3. Difference of microbial community composition in soil by one-way anova (a and b are phylum level anova of bacteria and fungi, and c and d are genus level anova of bacteria and fungi, respectively).

Note: * has significant difference at the level of $0.01 < P < 0.05$, ** has extremely significant difference at the level of $0.001 < P \leq 0.01$, $n = 9$.

diversity by affecting dominant bacterial taxa and fungal genera, and can also affect pathogenic microorganisms and beneficial microorganisms.

DISCUSSION

Effects of continuous cropping years on microbial diversity. In this study, the Miseq high-throughput sequencing of bacteria in the red soil of different continuous cropping facilities found that with the increase of continuous cropping years, the soil bacterial community diversity index and evenness index increased significantly. The diversity index can be used as an effective index to evaluate the diversity of soil microbial communities. The higher the diversity index, the higher the diversity of the microbial community [23].

The results of this study showed that the bacterial community diversity index increased with the increase of planting years, indicating that continuous cropping may cause diversification of certain bacterial and microbial taxa in the soil, which is consistent with the results of Zhu [42]. The differences in the result of the study may be explained by differences in plant growth conditions, pathogenic factors, or microbial determination methods [14]. Compared with the bacterial community, the continuous cropping years had different effects on the fungal community. For fungi, with the extension of continuous cropping years, the richness and diversity of microbial communities in the soil decreased significantly, which is consistent with the results of Wu et al. [14], who indicated that continuous

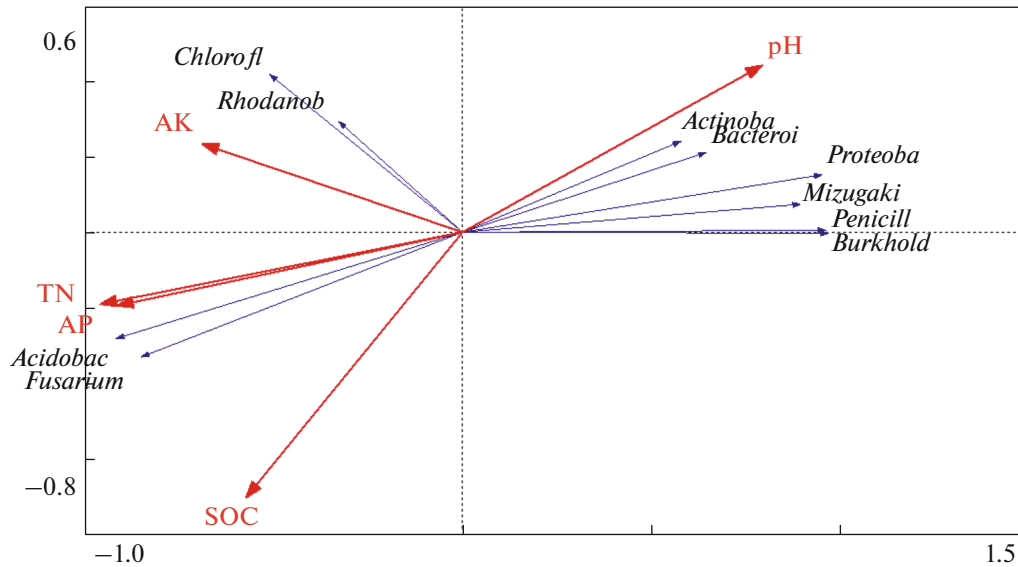


Fig. 4. RDA sequencing of soil environmental factors and microbial community structure.

cropping would have a significant impact on the microbial diversity index.

Effects of continuous cropping years on microbial community structure. In this study, the soil pH decreased significantly, and the accumulation of available nutrients and organic matter increased with the continuous cropping years (Table 2). Related studies claim that the direct or indirect factors affecting soil microbial bacterial communities include soil physical and chemical properties [1], soil types [46], crop types [21], soil fertility management [36], and so on. Among them, soil pH is considered to be one of the factors that can significantly affect the soil bacterial community [18, 53]. The comparative analysis of bacterial community abundance of different continuous cropping years found that different bacteria have obvious differences in different continuous cropping years. With the increase of continuous cropping years, the abundance of *Proteobacteria* and *Bacteroidetes* showed a downward trend. Studies have shown that most of the nitrogen-fixing bacteria, ammonia-oxidizing bacteria and denitrifying bacteria belong to the *Proteobacteria*, and the *Proteobacteria* play an important role in the nutrient cycle [57], and it is also related to soil enzymes [37]. The correlation analysis of this study showed that *Proteobacteria* was significantly positively correlated with soil pH, and significantly negatively correlated with organic carbon and nitrogen, and available phosphorus and potassium content. Studies have found that the *Proteobacteria* are suitable for living in soils with low pH and high carbon and high nitrogen, but it is at the expense of *Acidobacteria* [26]. Therefore, the increase in the abundance of *Acidobacteria* in this study may be one of the reasons for the decrease in the abundance of *Proteobacteria*.

The abundance of *Nitrospirae* showed an increasing trend, which may be related to the increase in the abundance of *Burkholderia-Paraburkholderia*. Studies have confirmed that the *Burkholderia* is diverse in phenotype, metabolism, and ecology, and most of its members have the potential to fix nitrogen [15]. That is, the increased nitrogen-fixing ability of *Burkholderia* bacteria promotes the nitrification of nitrifying bacteria, resulting in an increase in the abundance of *Nitrospirae*. Although the *Proteobacteria* is the most widely distributed bacterial group in terrestrial ecosystems [45], it is not very representative in culture, so there are many unknown phylotypes and genera that need to be further determined.

Studies have found that *Actinobacteria* with a variety of physiological and metabolic properties are one of the largest taxa in the bacterial domain [32], which is related to disease suppression [49]. In this study, the abundances of *Actinobacteria* showed a downward trend with the increase of continuous cropping years. Therefore, plants can use the microbial community in the soil to resist the decline of the infection ability when fungal pathogens are infested. Therefore, the decline in the abundance of *Actinobacteria* may increase the risk of soil diseases. Studies have shown that *Firmicutes* decompose organic matter while also secreting antifungal substances [49]. Therefore, the decrease in the abundance of *Firmicutes* may promote the growth of fungal pathogens.

The *Bacteroidetes* is recommended as a sensitive biological indicator for agricultural soil utilization [50], and it is dominant when the soil pH is high [26]. And the correlation analysis showed that the *Bacteroidetes* were significantly positively correlated with soil pH, which is consistent with the results of Lauber [4].

Therefore, the abundance of *Bacteroidetes* will decrease with the increase of continuous cropping years.

The *Chloroflexi* is characterized by oxygen-loving thermophilicity. It can grow well under high-temperature conditions using oxygen, and can also use light for photosynthesis under conditions of anaerobic light nutrition. The correlation analysis of this study showed that there was no significant relationship between the *Chloroflexi* and soil pH, organic carbon and nitrogen, and available phosphorus and potassium. However, its abundance increases with the increase of continuous cropping years, and the reasons for this need to be further studied. Some researchers have also found that the organic carbon content also affects the bacterial community composition in the soil [5]. In Chaudhary's research, it was found that *Actinobacteria* are the main contributor to the biological buffer of the soil and can decompose organic matter [16]. However, the results of this study showed that the abundance of *Actinobacteria* under continuous cropping conditions was significantly negatively correlated with the content of organic carbon and total nitrogen (Fig. 4). It is possible that the organic carbon and total nitrogen accumulated in the soil will inhibit the activity of *Actinobacteria*, so continuous cropping may be an indirect reason for the decrease in the abundance of *Actinobacteria*.

Similarly, years of continuous cropping also significantly affected the fungal community structure. At all phyla levels, there are three known phyla of *Ascomycota*, *Zygomycota* and *Basidiomycota*, and the *Ascomycota* and *Zygomycota* are the dominant phyla. There is no significant difference in *Ascomycota* under different continuous cropping years, but there is a downward trend. The possible reason is that the increase in the number of *Zygomycota* reduces the abundance of the *Ascomycota* of the dominant phyla, which in turn leads to a decrease in the diversity of the *Ascomycota*, which promotes the proliferation of *Fusarium* spp., and its abundance reaches 44.02 to 58.83%. In Chávez's research, *Penicillium* is an active producer of xylan decomposing enzymes, the main component of lignocellulose [41]. Studies have shown that most of the strains contained in *Fusarium*, *Aspergillus*, and *Penicillium* are pathogenic fungi [2, 8]. Therefore, this result shows that continuous cropping will cause an increase in pathogenic fungi. Related studies have reported that the reduction of microbial diversity is the cause of soil-borne diseases [30]. And in this study, continuous cropping led to a decline in soil fungal diversity index, and a large number of bacterial genera disappeared after continuous cropping, which would make the microbial community in the soil unevenly distributed. Therefore, soil after continuous cropping has a higher disease potential than healthy farmland soil. Studies have shown that *Chaetomium* can decompose refractory organic carbon (cellulose and hemicellulose) [7] and produce antifungal compounds [20], and its existence can effectively prevent the reproduc-

tion of pathogenic microorganisms [27, 48]. Therefore, the significant decrease in the abundance of *Chaetomium* in this study may weaken the soil's ability to resist pathogens.

In Chen's research, it was found that the increase of fungal pathogens and the decrease of beneficial fungi may be important factors leading to the decline in the growth and yield of continuous cropping peanuts [31]. Therefore, the continuous cropping in this study may interfere with the interaction between the soil microorganisms of the facility lily, thereby affecting the balance between the beneficial bacteria and the pathogenic bacteria in the facility soil, and more likely to cause obstacles to the continuous cropping. Research shows the genera *Trichoderma* can serve as biocontrol agents against plant pathogens and opportunistic avirulent plant symbionts [35]. There are also research findings, that many antagonistic taxa of *Trichoderma harzianum* in the genus *Trichoderma* effectively inhibit pathogens and control soil-borne diseases [47]. The increase in the abundance of *Trichoderma* in this study can effectively inhibit the occurrence of diseases to a certain extent. Therefore, *Trichoderma* can be considered to control soil-borne diseases in continuous cropping.

Coupling relationship between environmental factors and community composition after continuous cropping. In this study, continuous cropping acidified the soil and also significantly affected the abundance of bacterial communities in the soil (Figs. 1a, 1b). After redundant analysis, it is concluded that the *Proteobacteria*, *Actinobacteria*, and *Acidobacteria* are significantly related to pH (Fig. 4). Among these bacterial phyla, *Proteobacteria* and *Actinobacteria* are the dominant phyla, so it is speculated that pH may affect the bacterial community in the soil by affecting the dominant phyla. Studies have confirmed that the relative abundance of *Acidobacteria* in soil was significantly negatively correlated with soil pH [17], which is consistent with the results of this study.

Some studies have pointed out that on the one hand, acid soil destroys the nutrient absorption of crops and inhibits their growth; On the other hand, acid soil stimulates the multiplication of fungi, which may seriously affect the quality and yield of crops and even aggravate the occurrence of diseases [54]. Therefore, soil acidification after continuous cropping may be the main reason for continuous cropping obstacles.

CONCLUSIONS

The research results showed that with the increase of continuous cropping years, the composition of bacterial community changed significantly, and the abundance of dominant bacteria showed a downward trend. Redundant analysis results show that pH is a key factor affecting changes in bacterial communities. In the composition of the fungal community, the increase in the abundance of *Fusarium* after continuous cropping may be the main cause of continuous cropping obstacles.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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