Effects of Continuous Cropping of Lilium Lancifolium on Rhizosphere Soil Physical and Chemical Properties and Fungal Community Structure

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Abstract

Lilium lancifolium is an important economic crop in Huoshan county of Anhui province, China. Continuous cropping obstacles serious affect the yield and quality of *L. lancifolium*. At present, the effect of the continuous cropping of *L. lancifolium* on soil fungal community structure is not clear. In this study, Illumina MiSeq was used to study the fungi of the rhizosphere soil associated with *L. lancifolium* subjected to three treatments: no continuous cropping, continuous cropping for 3 years, and continuous cropping for 5 years. The results showed that continuous cropping of *L. lancifolium* could increase the fungal richness and diversity in the rhizosphere to different degrees. *Ascomycota* was the dominant phylum, and its abundance increased after continuous cropping. In addition, the abundance of beneficial fungi, such as *Chaetomium*, decreased, and the abundance of harmful fungi, such as *Fusarium* and *Colletotrichum,* greatly increased with the duration of continuous cropping. Overall, continuous cropping changed the composition of soil fungal communities, reduced the abundance of beneficial fungi, and increased the abundance of harmful fungi. Thus, continuous cropping increased the potential for soil-borne diseases and endangered the bulb growth of *L. lancifolium*.

Highlights

- Rhizosphere soil was sampled after 1, 3, and 5 years of *Lilium lancifolium* cropping
- Continuous cropping impacted soil organic matter content and enzyme activity
- Continuous cropping significantly changed rhizosphere fungal richness and diversity
- Continuous cropping promoted pathogenic fungi and reduced beneficial fungi
- Continuous cropping endangers *Lilium lancifolium* bulb growth

1. Introduction

Lilium lancifolium is a perennial herb that grows from bulbs and has a long history of being planted in Huoshan County, Anhui Province, China. *Lilium lancifolium* is one of the traditional and dominant agricultural pillar industries in Huoshan County. However, this plant is extremely vulnerable to disease throughout the growth period, from sowing to harvesting. Further, continuous cropping obstacles severely affect *L. lancifolium* growth. According to previous studies, continuous cropping obstacles can reduce the yield of *L. lancifolium* by 20–30% and can even lead to plant death in severe cases (Murphy et al.2006; Zhou et al. 2018; Gao et al.2019). Thus, continuous cropping obstacles seriously restrict the healthy development of the lily industry in China (Murphy et al.2006).

Soil microorganisms are mostly beneficial to crop growth and development, and they have significant impacts on soil formation and development, material circulation, and soil fertility. Therefore, the quantity and species of microorganisms in the rhizosphere can directly affect the healthy growth of plants (Gyeryeong et al. 2019; Yan et al. 2020). Many papers have shown that imbalance of the rhizosphere micro-ecological system is the most fundamental cause of continuous cropping obstacles. Furthermore, continuous cropping leads to the deterioration of the structure and functional diversity of the rhizospheric microbial community. Fungi are one of the main groups of decomposers in soil. Fungal community structure and diversity are very important to the balance of the soil ecosystem (Yan et al. 2020; Lindsay et al. 2021). Some fungi can cause plant diseases. For example, root rot of *L. lancifolium* caused by *Fusarium oxysporum* is a devastating soil-borne disease that has seriously affected the yield and quality of *L. lancifolium* (Dilip et al. 2017). On the contrary, some beneficial fungi can inhibit pathogenic bacteria and reduce the occurrence of rhizosphere-based plant diseases (Yan et al. 2020). Therefore, continuous cropping obstacles are closely related to the imbalance of fungal communities in the soil, and this has attracted extensive research attention in China and internationally.

With the rapid development of biotechnology, a new generation of sequencing technology has been widely used to determine the composition of microbial communities. These new technologies help to obtain more accurate and high-quality biological information (Zhao et al. 2020). Liu et al. applied a high-throughput sequencing technique to study the changes in the rhizosphere fungal community after the continuous cropping of ginger *(Zingiber officinale*). The results showed that the proportion of harmful fungi increased significantly, and that of beneficial fungi decreased dramatically in the soil. This presented a serious obstacle for continuous cropping in ginger planting areas (Liu et al. 2019).

At present, the edible and medicinal values of *L. lancifolium* are being increasingly recognized. However, there have been few reports on the obstacles facing the continuous cropping of *L. lancifolium*. In this paper, the soil associated with *L. lancifolium* plants that had been subjected to different numbers of continuous cropping years was selected for study. The differences in fungal community

structure in the root soil samples after continuous cropping were analyzed in depth using high-throughput sequencing technology. The aim was to analyze the mechanisms underlying the continuous cropping obstacles of *L. lancifolium* from the perspective of microbial community changes. The results could provide a theoretical basis for developing effective production technologies for *L. lancifolium* to overcome continuous cropping obstacles.

2. Materials And Methods

2.1 Sampling area

The experimental site was located at Manshui River Lily Cultivation Base in Huoshan County, Lu 'an City, Anhui Province (31°11'23.54"N, 116°0'31.42" E; average altitude of approximately 500 m). This region is located in the hinterland of the Dabie Mountains, which belongs to the northern subtropical humid monsoon climate zone. The climate in the county is generally humid, with four distinct seasons, a long frost-free period, an average temperature of approximately 15°C, and an annual rainfall of 1,391 mm. *Lilium lancifolium* is mainly planted in the middle mountain, low mountain, and basin areas at altitudes of under 700 m. These planting areas have fertile soil and high organic matter content, which is very suitable for lily growth. However, long-term planting for successive years has seriously restricted the growth and development of *L. lancifolium*, and soil-borne diseases such as anthrax, fusarium wilt, and root rot occur frequently (Wu et al. 2015). Thus, continuous cropping obstacles have become an agricultural production problem that urgently needs to be solved.

2.2 Sample collection

Fields with a continuous cropping duration of 1 year, 3 years, and 5 years were selected from within the sampling area. Three *L. lancifolium* plants were randomly selected from each field. The whole *L. lancifolium* bulb and the soil under it were completely removed from the ground with a sampling shovel. The loose soil that was adhered to the periphery of the root system was shaken off. The soil that was still adhered to the surface of the root system was collected together with the root system by cutting. The rhizosphere soil collected from multiple points was fully mixed, and then placed in a sterile self-sealing bag. The soil samples were taken back to the laboratory, and a part of each soil sample was stored in a refrigerator at − 80°C in preparation for the microbial diversity analysis. The other part of the soil sample was stored at − 4°C. Part of this subsample was naturally air-dried and sieved with a 1 mm sieve to determine the soil enzyme activity, soil water content, alkali-hydrolyzable nitrogen, available phosphorus, pH, and organic matter content (Li et al. 2015).

2.3 Determination of soil physicochemical properties and enzyme activity

The soil pH was determined using a Hanna HI-8314 portable pH meter with a soil to water ratio of 1:5. The water content was determined by adopting a drying method. The phosphatase activity was determined using a disodium phosphate colorimetric method expressed as milligrams of phenol released in 1.0 g of soil after 24 h; The sucrase activity was determined using a 3,5- dinitrosalicylic acid colorimetric method expressed as milligrams of 1.0 g soil glucose 24 h later; The urease activity was determined using indophenol blue colorimetric method, expressed as the milligrams of NH_3-N in 1.0 g of soil after 24 h; Dehydrogenase is determined by TTC reduction method. The organic matter, phosphorus, and nitrogen contents were determined using the ignition method, alkali fusion-Mo-Sb Anti spectrophotometric method, and Kjeldahl method, respectively (Alladassi et al. 2020).

2.4 DNA extraction and high-throughput sequencing

To identify and analyze fungi in the soil, the collected soil samples were subjected to high-throughput sequencing by Shanghai Parsons Nuo Biotechnology Co., Ltd. using an Illumina MiSeq sequencing platform. The specific method was as follows. The DNA of each sample was quantified using a Nanodrop spectrophotometer, and the extraction quality of the DNA was detected via 1.2% agarose gel electrophoresis. A target sequence, such as microbial ribosomal RNA or specific gene fragments, can reflect the composition and diversity of the flora, and can thus be used as a target. The corresponding primers were designed according to the conserved regions in the sequence, and a sample-specific barcode sequence was added. The internal transcribed spacer 1 (ITS1) region of the fungal DNA was amplified using the primers ITS5-1737F and ITS2-2043R.

2.5 Data analysis

Data were analyzed using Excel 2010 and SPSS statistical software version 22.0 (IBM SPSS Inc, Armonk, NY). The comparisons of multiple means were performed using one-way analysis of variance (ANOVA), and $p \leq 0.05$ indicated a statistically significant

difference. The alpha diversity analysis was performed using the QIIME2 (2019.4) software package for sequence analysis. The abundance was characterized using Chao1 and Observed species indexes, and the diversity was characterized using Shannon and Simpson indexes. The evolution-based diversity was characterized based on Faith's PD index. The evenness was determined using Pielou's evenness index, and the coverage was characterized using Good's coverage index. The genera in each sample were clustered using R software, and the top 50 most abundant genera were clustered and a heat map was drawn.

3. Results

3.1 Physicochemical properties and enzyme activity in the rhizosphere soil

With the increasing duration of the continuous cropping of *Lilium lancifolium*, soil water content and pH gradually decreased and soil organic matter content first increased and then decreased (Table 1). This indicated that continuous cropping affects the organic matter content in rhizosphere soils. The alkali-hydrolyzable nitrogen and available phosphorus contents in the soil increased with the number of continuous cropping years. In terms of soil enzyme activities, the dehydrogenase, sucrase, and alkaline phosphatase activities gradually decreased with increasing duration of continuous cropping. Meanwhile, the urease activity reached a maximum after continuous cropping for 3 years, and then decreased (Table 1). The urease and sucrase activities were significantly lower in the soil samples from 3 and 5 years of continuous cropping than in samples from fields without continuous cropping.

The dehydrogenase activities of the soils after three and five years of continuous cropping were significantly lower than those of the soils without continuous cropping, by 27.53% and 41.3%, respectively. The activities of soil alkaline phosphatase under continuous cropping for 3 and 5 years were 52.38% and 59.37% lower than those under non-continuous cropping. The activities of soil sucrase in 3 and 5 years of continuous cropping were 20.30% and 77.75% lower than those in the same cropping. The urease activity of continuous cropping for 3 and 5 years was 87.68% and 106.90% higher than that of the same cropping.

The pH values of continuous cropping for 3 and 5 years were significantly reduced by 17.32% and 33.18% compared with those of non-continuous cropping. The water content of continuous cropping 3 years and 5 years was 6.30% and 12.78% lower than that of non-continuous cropping soil. The contents of soil organic matter, available N and available P in the three and five years of continuous cropping were 9.43%, 20.10%, 15.01%, 16.58%, 30.37% and 32.94% higher than those in the soil without continuous cropping.

3.2 Pearson correlation analysis between soil physical and chemical properties and enzyme activity

PH has a significant negative effect on urease activity, while organic matter, alkali-hydrolyzable nitrogen and available phosphorus have a significant positive effect on urease activity PH has a significant positive effect on the activities of sucrase and dehydrogenase, while organic matter has the opposite effect; Organic matter and available phosphorus have a significant negative correlation with dehydrogenase; Organic matter, available nitrogen and available phosphorus have significant negative effects on sucrase. Pearson correlation was used to get the correlation between them. In order to further understand the interaction between soil physical and chemical properties and enzyme activities, path analysis was carried out.

3.4 Path analysis of soil enzyme activity and physical and chemical properties.

After obtaining correlation analysis, the data were analyzed by multiple linear stepwise regression (Table 3). The multiple linear regression equations between soil physical and chemical properties and enzyme activities are all $p < 0.05$, which is significant. The independent variables x1-x5 can explain 91.6%, 91.6%, 85.1% and 83.6% of y1-y4 changes respectively. The coefficient of multivariate linear equation obtained is the direct path coefficient, multiplied by the correlation coefficient among the factors (Table 2) is the indirect path coefficient (Table 4).

Path coefficient (Table 4) showed that organic matter, available nitrogen and available phosphorus had great influence on urease activity, with coefficients of -0.648, 0.478 and 0.357. The change of urease activity in soil was negatively correlated with the content of organic matter in soil, and the correlation coefficient was − 0.648. Soil water content, phosphorus content and soil pH value affect urease activity by regulating the content of organic matter in soil. Soil alkaline phosphatase activity is directly affected by water content, organic matter and available phosphorus content, with coefficients of -0.271, 0.203 and 0.105. Phosphatase activity is positively correlated with water content and available phosphorus, and has a promoting effect, while organic matter has a significant

negative effect on it. PH, available nitrogen and available phosphorus have great direct negative effects on sucrase activity, with coefficients of -0.404, -0.357 and-0.379. Water content and organic matter mainly affect sucrase activity by affecting pH, available nitrogen and available phosphorus. PH and alkali-hydrolyzable nitrogen have great direct effects on dehydrogenase activity, with coefficients of -0.56 and 0.73. Water content, organic matter and available phosphorus affect dehydrogenase activity by affecting pH and alkaline hydrolysis of nitrogen.

Table 1
Alpha diversity index of rhizosphere soil fungi

Sample	pH	Water content (%)	Organic matter ($g \cdot kg^{-1}$)	Aikali-hydrolyzable N ($mg \cdot kg^{-1}$)	Available P ($mg \cdot kg^{-1}$)	Urease ($mg \cdot g^{-1}$)	Phosphatase ($mg \cdot g^{-1}$)	Sucrase ($mg \cdot g^{-1}$)	Dehydrogenase ($mg \cdot g^{-1}$)
1	5.52 ± 0.007^{a}	15.41 ± 0.08^{a}	$15.08 \pm 0.28b$	71.41 ± 1.5^{b}	146.18 ± 10.35^{b}	4.06 ± 0.001^{b}	1.07 ± 0.56^{a}	37.84 ± 0.11^{a}	2.59 ± 0.502^{a}
3	4.56 ± 0.39^{b}	14.44 ± 0.21^{b}	16.55 ± 0.14^{a}	82.13 ± 2.43^{a}	194.33 ± 5.79^{a}	7.62 ± 0.32^{a}	0.51 ± 0.14^{b}	30.16 ± 1.95^{b}	1.87 ± 0.168^{ab}
5	3.69 ± 0.26^{c}	13.44 ± 0.13^{c}	$13.78 \pm 0.27c$	83.25 ± 1.17^{a}	209.95 ± 7.5^{a}	0.40 ± 0.47^{c}	0.44 ± 0.05^{b}	8.42 ± 0.11^{c}	1.52 ± 0.002^{b}

Note: Three replications were used for each treatment. The lower case letters in the same column represent the difference between rhizosphere soils of different growth years;There were significant differences between the different letters($P < 0.05$).

Table 2
Correlation between Soil Enzyme Activity and Physical and Chemical Properties in Long-term Lianjuandan Lily Field

Index	pH	Water content	organic matter	Alkaline hydrolyzable N	Available P	Urease	Phosphatase	Sucrase	Dehydrogenase
pH	1	0.861*	-0.979**	-0.948**	-0.948*	-0.910*	0.730	0.862*	0.828*
Water content		1	– .824*	-0.781	-0.729	-0.673	0.579	0.711	0.693
organic matter			1	0.901	0.964**	0.893*	-0.594	-0.854*	-0.899*
Alkaline hydrolyzable N				1	0.948**	0.971**	-0.855*	-0.953**	-0.810
Available P					1	0.973**	-0.662	-0.942**	-0.929**
Urease						1	-0.764	-0.960**	-0.871*
Phosphatase							1	0.729	0.393
Sucrase								1	0.811*
Dehydrogenase									1

Notes: * and * * indicated significant correlation at $P < 0.05$ level and extremely significant correlation at $P < 0.01$ level, respectively, the same below

Table 3
Multiple linear stepwise regression equations of soil enzyme activity on soil physical and chemical properties

Regressive Equation	F	R^2	P
$Y1 = 0.316 + 0.126x_1 + 0.078x_2 - 0.648x_3 + 0.478x_4 + 0.357x_5$	70.941	0.916	P < 0.05
$Y2 = 9.058 - 0.090x_1 - 0.271x_2 + 0.203x_3 - 0.074x_4 + 0.105x_5$	28.218	0.916	P < 0.05
$Y3 = 181.075 - 0.185x_1 - 0.066x_2 - 10.269x_3 + 0.11x_3 + 0.131x_4$	29.643	0.851	P < 0.05
$Y4 = 3.581 - 0.560x_1 + 0.28x_2 + 0.004x_3 + 0.730x_4 - 0.089x_5$	26.522	0.836	P < 0.05

Table 4
path coefficient between soil enzyme activity and soil physical and chemical properties

Index	pH	Water content	organic matter	Alkaline hydrolyzable N	Available P	Urease	Phosphatase	Sucrase	Dehydrogenase
pH	1	0.861*	-0.979**	-0.948**	-0.948*	-0.910*	0.730	0.862*	0.828*
Water content		1	– .824*	-0.781	-0.729	-0.673	0.579	0.711	0.693
organic matter			1	0.901	0.964**	0.893*	-0.594	-0.854*	-0.899*
Alkaline hydrolyzable N				1	0.948**	0.971**	-0.855*	-0.953**	-0.810
Available P					1	0.973**	-0.662	-0.942**	-0.929**
Urease						1	-0.764	-0.960**	-0.871*
Phosphatase							1	0.729	0.393
Sucrase								1	0.811*
Dehydrogenase									1

3.5 Fungal community diversity and richness in the rhizosphere soil

As shown in Table 2, the coverage rate of each sample library was approximately 0.99. This indicated that there was a low probability that a sequence in the sample was not detected. Thus, the sequencing results could be considered to represent the real situation of each sample. Using a similarity threshold of 97%, the fungal sequences obtained from high-throughput sequencing were clustered into operational taxonomic units (OTUs). A total of 1651 OTUs were obtained. Among them, 395, 952, and 647 OTUs were found in the rhizosphere samples of *L. lancifolium* planted for 1 year, 3 years, and 5 years, respectively. Moreover, the fungal species in the rhizosphere soil differed significantly between the three treatments, with the total number of shared OTUs being only 55. Most OTUs were unique to each treatment. Moreover, the fungal species in the rhizosphere soil differed significantly between the three treatments, with the total number of shared OTUs being only 55 (Fig. 1). Most OTUs were unique to each treatment. In the rhizosphere soil of L. lancifolium under the 1-year, 3-year, and 5-year continuous cropping treatments, there were 298, 684, and 381 unique OTUs, respectively. With the increased duration of continuous cropping, the number of unique OTUs first increased, and then decreased. This was consistent with the trends observed for the total number of OTUs.

Table 5
Alpha diversity index of rhizosphere soil fungi

Sample	OUT number	α-Diversity index				
		Simpson	Shannon	Chao1	Observed_species	Good's coverage
1	395	0.8693 ± 0.060^{b}	4.2141 ± 0.66^{b}	156.86 ± 58.72^{c}	156.67 ± 58.80^{c}	0.9999
3	952	0.9485 ± 0.005^{a}	5.7822 ± 0.78^{a}	448.84 ± 5303^{a}	446.9 ± 54.04^{a}	0.9998
5	647	0.8369 ± 0.018^{b}	3.899 ± 0.33^{b}	331.59 ± 37.80^{b}	326.8 ± 36.01^{b}	0.9996

Note: Three replications were used for each treatment. The lower case letters in the same column represent the difference between rhizosphere soils of different growth years;There were significant differences between the different letters($P < 0.05$).

The alpha diversity can reflect the abundance and diversity of microbial communities. The diversity indexes of the fungal communities in the rhizosphere soil of *L. lancifolium* subjected to different numbers of continuous cropping years are shown in Table 1. The Shannon and Simpson indexes represent the species diversity of the community. The results showed that the fungal community diversity in the different treatments was in the order of 3 years > 1 year > 5 years. The Chaol and Observed species indexes represent the species richness of the community. The species richness of the fungal communities of the different treatments was in the order of 3 years > 5 years > 1 year. The Pielou's evenness index was used to characterize the uniformity, and the Good's coverage index was used to characterize the coverage of the fungal communities. It could be clearly seen from the alpha diversity index values that long-term continuous cropping of *L. lancifolium* could change the composition and distribution of fungal communities in the rhizosphere. With the increasing duration of continuous cropping, the fungal community diversity first increased and then decreased. The species diversity and abundance indexes in the rhizosphere soil were higher after 3 years of continuous cropping than after 1 year and 5 years of planting. The continuous cropping time had a marked effect on the diversity and richness of the soil fungal community.

To visualize these effects of continuous cropping on fungal community structure and diversity, the top 50 most abundant genera were selected for clustering, and a heat map was drawn (Fig. 2). At the genus level, the diversity of fungi in the rhizosphere soil of L. lancifolium planted for 1 year was low. After continuous cropping, the fungal diversity in the rhizosphere soil increased.

3.6 Fungal phyla and genera in the rhizosphere soil

The fungal sequencing results showed that the fungal diversity in the rhizosphere soil of *L. lancifolium* significantly differed between the different continuous cropping treatments. At the phylum level (Fig. 3A), *Ascomycota* was the most abundant phylum among the soil fungi of the different treatments. *Basidiomycota* and *Mortierellomycota* were the next two most dominant fungal phyla. Together, these three phyla accounted for approximately 70% of the total fungal phyla in each type of soil. After continuous cropping for 3 years and 5 years, the content of *Ascomycota* was 11.24% and 21.93% higher ($p < 0.05$), respectively, than after 1 year. At the genus level (Fig. 3B), the dominant fungi in the *L. lancifolium* rhizospheric soil samples were *Talaromyces*, *Fusarium*, *Cladosporium*, *Clavulina*, *Mortierella*, *Humicola*, and *Colletotrichum*. The abundance of *Fusarium* and *Colletotrichum gloeosporioides* increased with the extension of continuous cropping time. These genera are characterized by plant pathogens, which can cause diseases of *L. lancifolium*. Meanwhile, the abundance of *Chaetomium* spp. gradually decreased with increasing duration of continuous cropping. *Chaetomium* spp. are beneficial fungi that can be used to prevent and treat various plant diseases and inhibit the activity of pathogenic microorganisms (Yue et al. 2018). This indicated that the continuous cropping of *L. lancifolium* reduced the content of beneficial microorganisms and increased the number of pathogenic microorganisms in the soil.

3.7 Principal coordinates analysis (PCoA) and cluster analysis of samples

To further understand the succession of changes in the fungal community structure of the rhizosphere of *L. lancifolium* under continuous cropping, PCoA was performed on different samples at the OTU level using weighted UniFrac distances. The first and second axes of the PCoA plot accounted for 24.2% and 15.5% of the difference in fungal community structure, respectively (Fig. 4). Thus, in total, the first and second axes accounted for 39.7% of the cumulative change in fungal community composition among the samples. Samples from the 3-year (sample group A in Fig. 4) and 1-year (sample group C in Fig. 4) continuous cropping treatments were distributed in the positive region, while samples from the 5-year treatment (group B) were distributed in the negative region of the

PCoA plot. Additionally, groups A, B, and C were distributed far apart from each other. This further indicated that the structure of rhizospheric fungal communities significantly differed between the different *L. lancifolium* continuous cropping treatments.

In the corresponding cluster analysis (Fig. 5), all the samples were clustered into two groups; continuous cropping samples (3 and 5 years) were clustered into one group, and the non-continuous cropping soil samples (1 year) separated into the other group. Thus, the rhizospheric fungal community structure was similar in the 3-year and 5-year continuous cropping treatments. However, the rhizospheric fungal community structure differed between the non-continuous cropping and continuous cropping treatments. Overall, these results indicate that the continuous cropping of *L. lancifolium* had an obvious effect on the fungal community in the associated rhizosphere soil.

4. Discussion

Soil type, planting pattern, and soil microbial community structure are the key factors affecting the healthy growth of crops in agricultural production (Chen et al. 2014). After long-term continuous cropping of *Lilium lancifolium*, organic acids are enriched in the rhizosphere, resulting in the reduction of soil pH (the pH changes from neutral to acidic). In more acidic soils, the physiological activity of microorganisms is more inhibited and fewer nutrients are available for plants to absorb from the soil. Acidic environments also promote the proliferation of fungal pathogens and cause lily diseases. Moreover, long-term continuous cropping reduces the soil organic matter content and enzyme activity (Acosta-Martínez et al. 2006;Yin et al. 2009; Li et al. 2019).

The path analysis coefficient between soil physical and chemical properties and enzyme activity indicated that the soil performance was not determined by a single factor, but was the result of synergistic effect of multiple factors. The rise and fall of various indicators of soil physical and chemical properties affect the activities of various enzymes in the soil, which are related to and restrict each other:

Plants maintain energy transport by absorbing water in soil for their own growth and development. Through correlation analysis, it was found that soil water content had a significant impact on the activities of alkaline phosphatase and dehydrogenase in soil. Chrost et al. showed that soil water content is the key factor of soil enzyme activity, and soil with proper water content has higher enzyme activity(Chrost et al.1991).

Urease specifically catalyzes the hydrolysis of urea to produce ammonia and carbonic acid for absorption by plants, so nitrogen is closely related to urease activity. The researchers found that urease had a significant positive effect on the content of alkali-hydrolyzable nitrogen in soil. Similarly, alkaline phosphatase also affected the decomposition rate of available phosphorus in soil. Huang et al. found a strong correlation and positive effect between phosphatase and available phosphorus.

The results of this study showed that soil pH was significantly reduced after long-term continuous cropping of *L. lancifolium*, and pH not only affected urease activity, but also had a greater direct effect on dehydrogenase activity. In addition, we have also found that the optimum pH for the hyphal growth of the two pathogenic bacteria causing the root rot of *L. lancifolium*—*Fusarium solani* and *Fusarium oxysporum*—is within the range of 4–8, and the reduction of pH value is beneficial to the proliferation of soil harmful microorganisms. Furthermore, the soil from the continuous cropping of *L. lancifolium* gradually changes from high fertility soil of "bacterial type" to low fertility soil of "fungal type" (Wu et al. 2015). This significantly increases the incidence of *L. lancifolium* root rot. Lily root rot is mainly caused by mixed infection with the soil-inhabiting fungi *Fusarium oxysporum* and *Fusarium solani*. The abundance of *Fusarium* in the rhizosphere soil significantly increases after continuous cropping (Arafat et al. 2019). Long-term continuous cropping of *L. lancifolium* leads to substantial changes in the soil microbial community diversity and species abundance, and causes soil acidification, abnormal soil enzyme activity, and soil nutrient imbalance (Qin et al. 2019). Thus, overall, continuous cropping eventually leads to frequent plant diseases and promotes insect pests of *L. lancifolium*, which leads to significant reductions in plant yield and quality.

Illumina Miseq high-throughput sequencing was used to analyze the fungal community structure of the rhizosphere of *L. lancifolium* subjected to different numbers of continuous cropping years. The analysis showed that after continuous planting of *L. lancifolium* for many years, the OTU number and Chao, Ace, and Shannon index values increased. The results indicated that the diversity and richness of soil fungi increased after continuous cropping. At the same time, the sequencing results showed that the dominant phyla of the *L. lancifolium* rhizosphere soil were *Ascomycota* and *Basidiomycota*. The relative abundance of *Basidiomycota* decreased

significantly after continuous cropping ($p < 0.05$) (Li et al. 2015). Basidiomycetes can degrade soil organic matter, and thus, this reduction in their content is associated with reduced soil fertility.

The dominant genera in the *L. lancifolium* rhizosphere soils were *Talaromyces*, *Fusarium*, *Cladosporium*, *Clavulina*, *Mortierella*, *Humicola*, and *Colletotrichum*. *Mortierella* is a beneficial fungus that possesses biological control functions. *Mortierella* species can dissolve insoluble phosphorus and potassium fixed in the soil and transform them into effective phosphorus and potassium, providing nutrition for and improving the disease resistance of plants (Yue et al. 2018; Song et al. 2020). However, the abundance of *Mortierella* in the rhizosphere soil decreased when *L. lancifolium* was planted continuously for many years. In contrast, the abundance of *Fusarium*, which can cause root rot (Li et al. 2015), increased with continuous cropping duration. The abundance of *Colletotrichum*, which is another important soil fungus that can cause a variety of plant diseases and is difficult to effectively control in production, with the continuous cropping of *L. lancifolium*, *Colletotrichum gloeosporioides* proliferated in the soil (Gu et al. 2020). The mass reproduction of pathogenic fungi in the rhizosphere can lead to a variety of diseases, which in turn lead to crop failure and yield reduction.

Continuous cropping obstacles are caused by interactions within the soil–crop–microorganism system. In this study, it was found that the fungal diversity and richness in *L. lancifolium* rhizosphere soils differed significantly between the continuous cropping treatments and the treatment without continuous cropping. However, the specific reasons for these differences are still unclear. It is speculated that the continuous cropping of *L. lancifolium* will lead to changes in the living environment of fungi in the rhizosphere (Zhang et al. 2015; Shen et al. 2020; Qiao et al. 2020). For example, the enrichment of root exudates changes the soil pH and microbial community structure to a certain extent. Therefore, the mechanism underlying the formation of continuous cropping obstacles is not regulated by a single factor (Zhao et al. 2020). The next step should be to comprehensively analyze the dynamic changes in soil physical and chemical properties, nutrient contents, enzyme activities, and microorganisms in response to the continuous cropping of *L. lancifolium*. Such analyses will enable us to gain a deep understanding of the interactions between these various factors. This resulting information will provide a theoretical basis, on the level of soil microbial ecology, for developing a beneficial cropping pattern that will help to improve the quality and yield of *L. lancifolium*.

5. Conclusions

There are many complex reasons underlying the emergence of continuous cropping obstacles. Changes in soil microbial richness and diversity comprise one of the main reasons. In the present study, continuous cropping changed the rhizosphere microbial environment of *L. lancifolium*. As the number of planting stubble increased, the abundance of the dominant fungal group, *Ascomycota*, increased dramatically, and the diversity and abundance of fungi in the rhizosphere soil increased markedly. At the same time, the abundance of beneficial fungi in the soil decreased, and the abundance of harmful fungi increased. These changes increase the potential for soil-borne diseases (Wang et al. 2020). Studying the richness and diversity of rhizosphere microorganisms is helpful for better understanding the relationship between microorganisms and crop growth and development. The results of such studies provide support for overcoming the obstacles that face continuous cropping (Mazzola et al. 2007; Ding et al. 2020; Xiao et al. 2020).

Declarations

Declaration of competing interest

The authors report no conflicts of interest.

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Figures

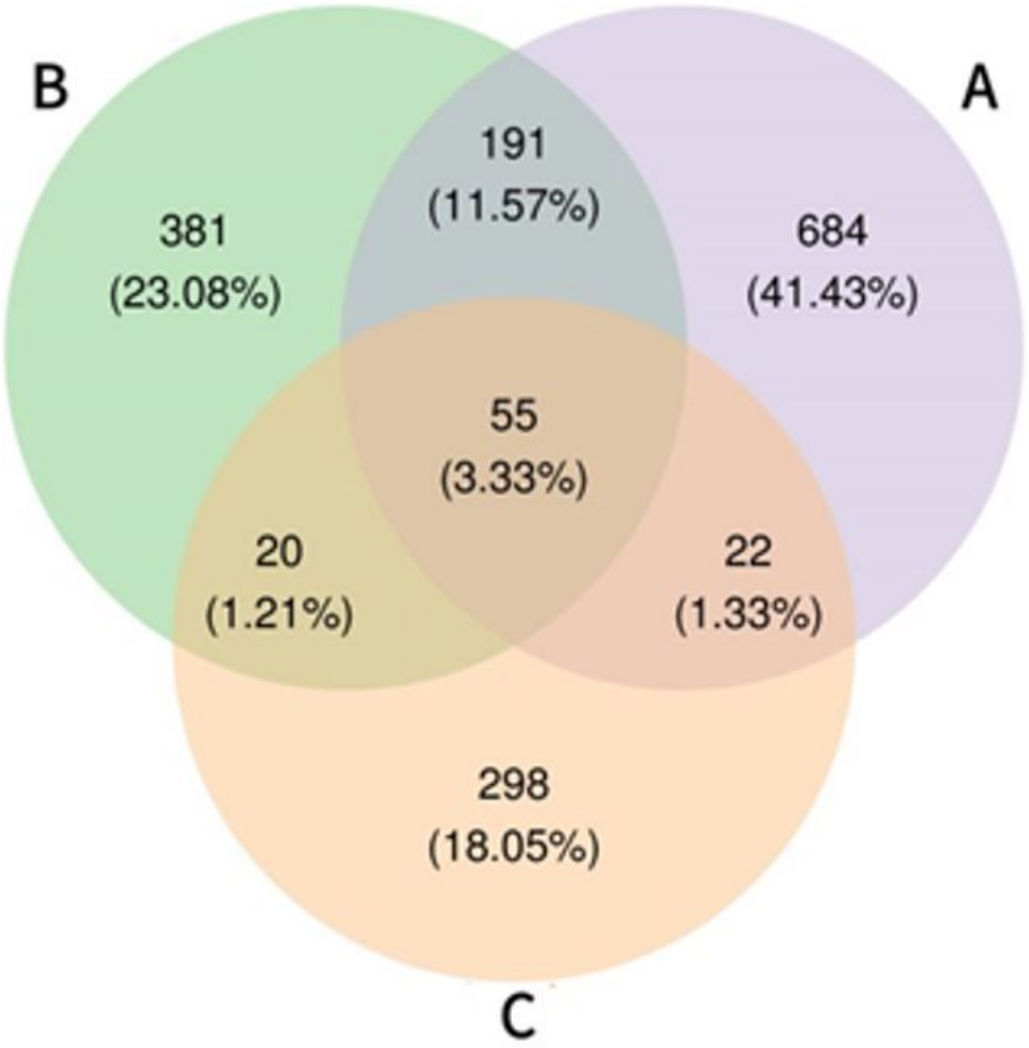

Figure 1

Venn diagram analysis showing the number of unique and shared fungal operational taxonomic units (OTUs) among treatment groups. Groups A, B, and C represent the samples from the treatments of 3 years of continuous cropping, 5 years of continuous cropping, and without continuous cropping (1 year), respectively.

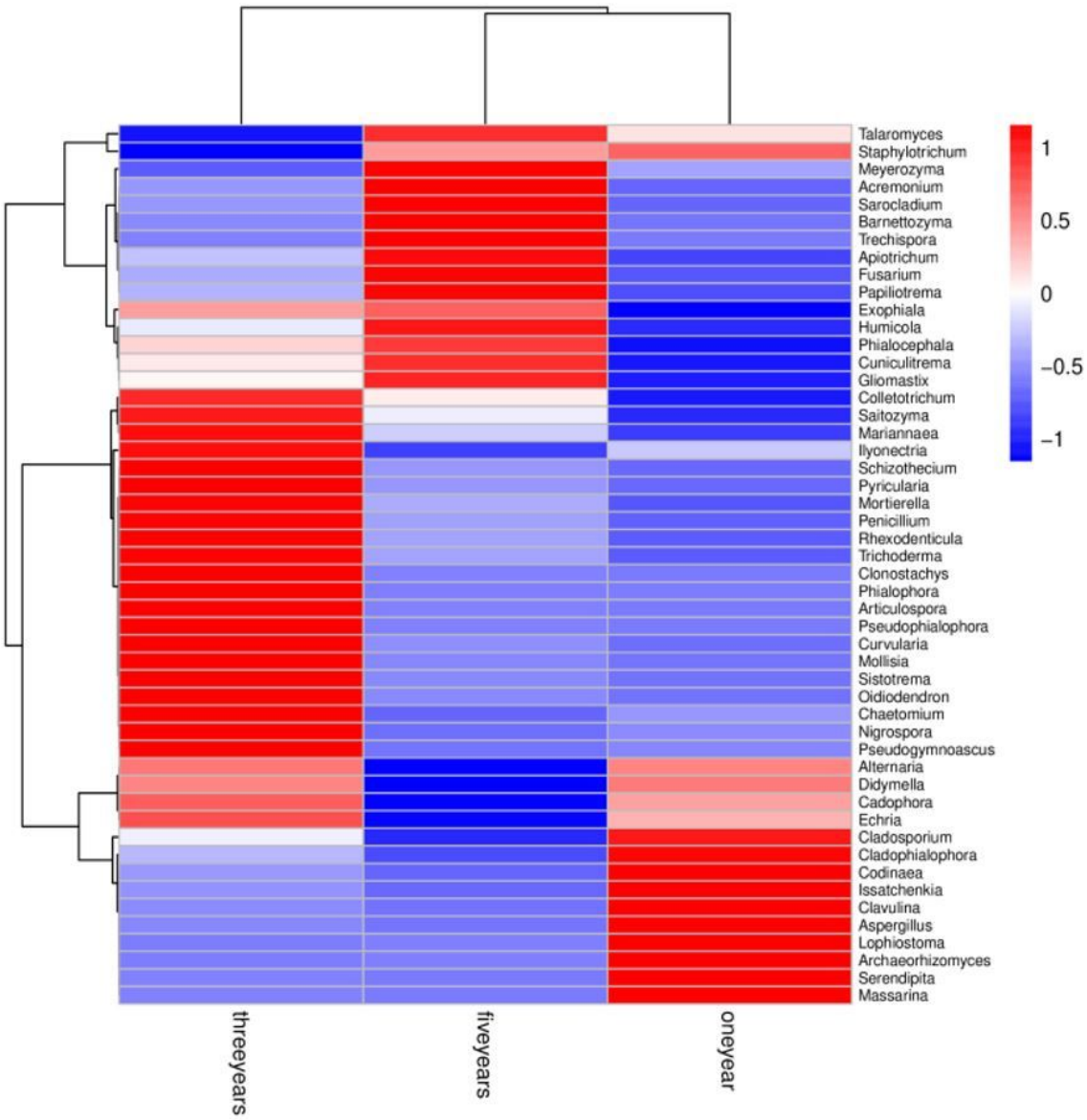

Figure 2

Microbial community heatmap analysis of the fungal genus detected across all samples. A□B and C represent the sampling for three years of continuous cropping , five years of continuous cropping and without continuous cropping, respectively. The relative values for fungal genus are indicated by color intensity with the legend at the bottom of the picture.

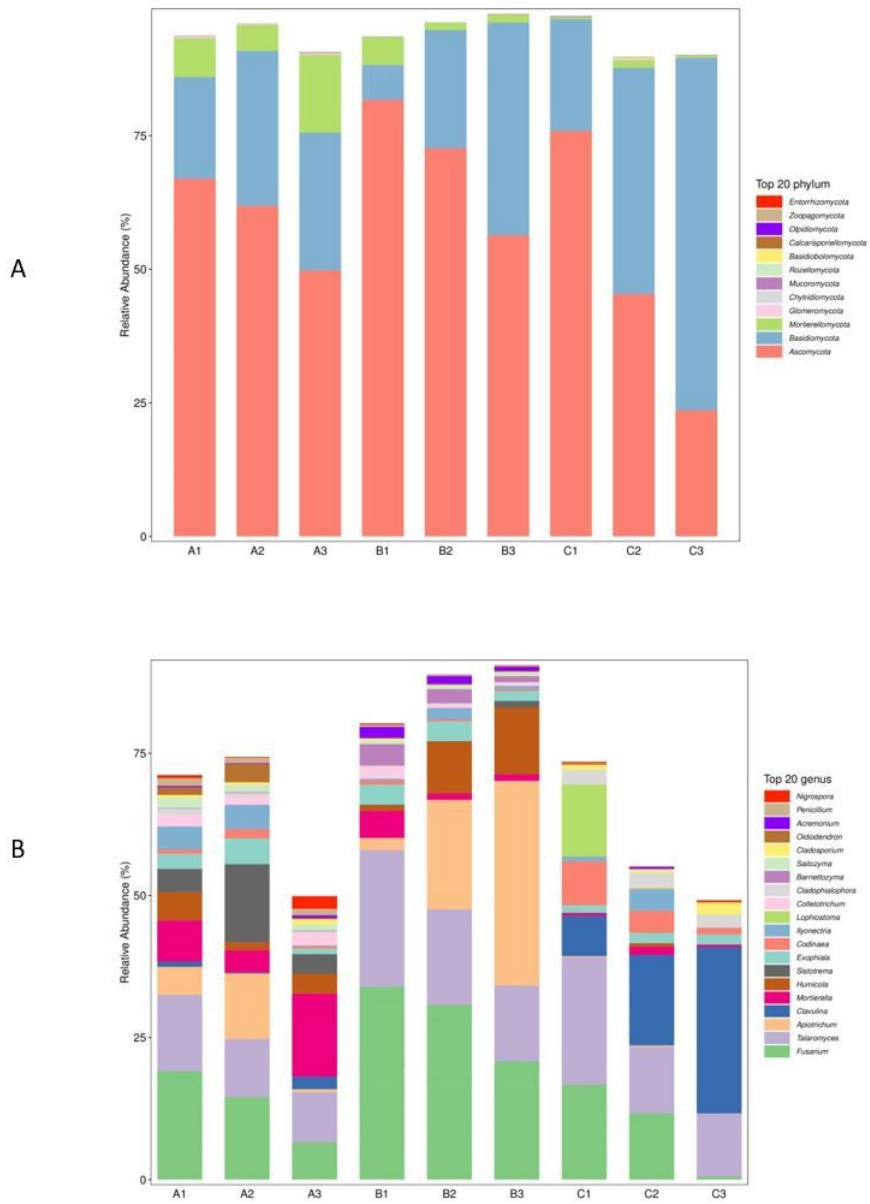

Figure 3

Relative abundance of the fungal phyla (A) and genera (B) in the rhizospheric soil of Lilium lancifolium subjected to continuous cropping. Groups A, B, and C represent the samples from the treatments of 3 years of continuous cropping, 5 years of continuous cropping, and without continuous cropping (1 year), respectively.

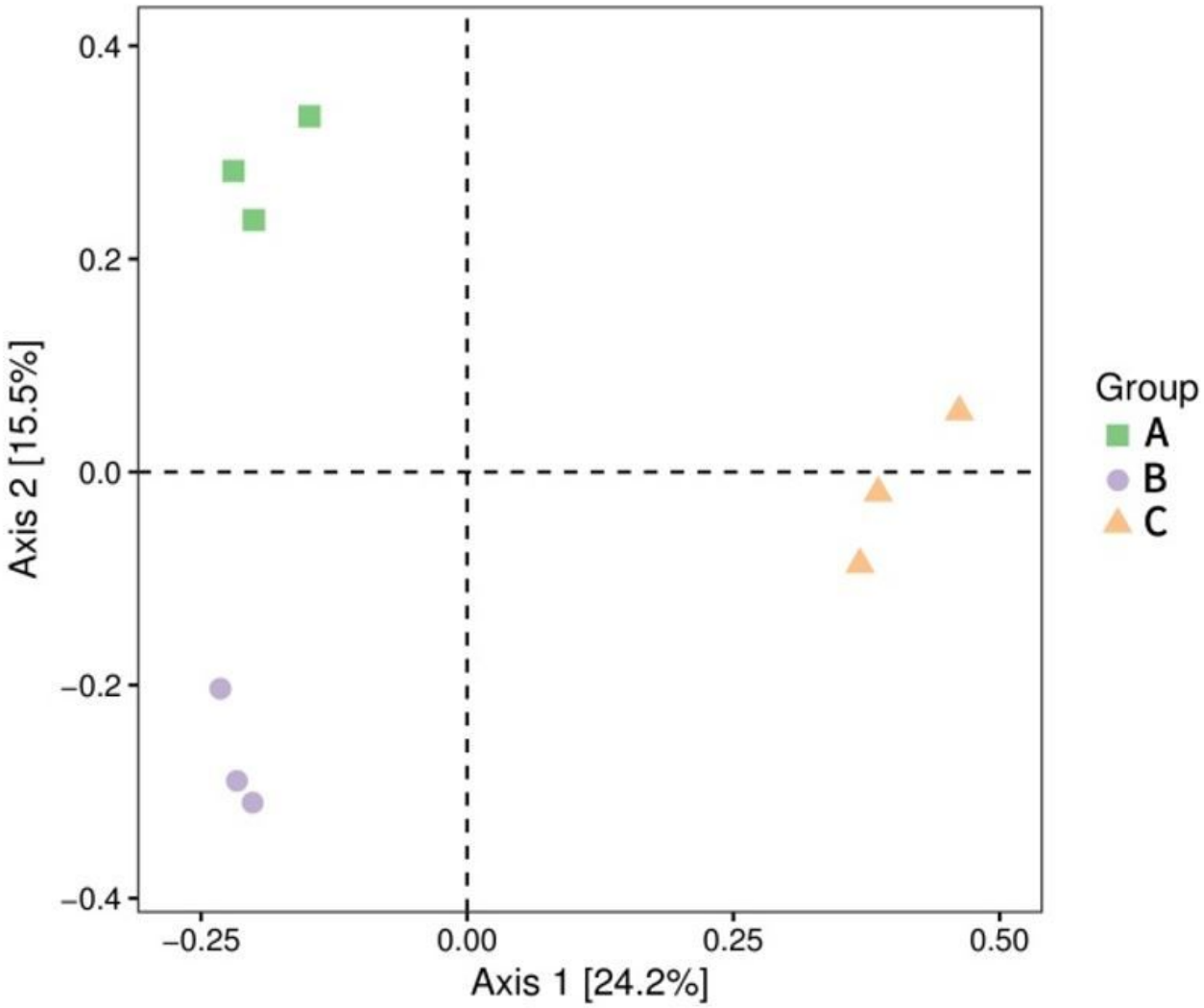

Figure 4

Principal coordinates analysis (PCoA) of the fungal operational taxonomic units (OTUs). Groups A, B, and C represent the samples from the treatments of 3 years of continuous cropping, 5 years of continuous cropping, and without continuous cropping (1 year), respectively.

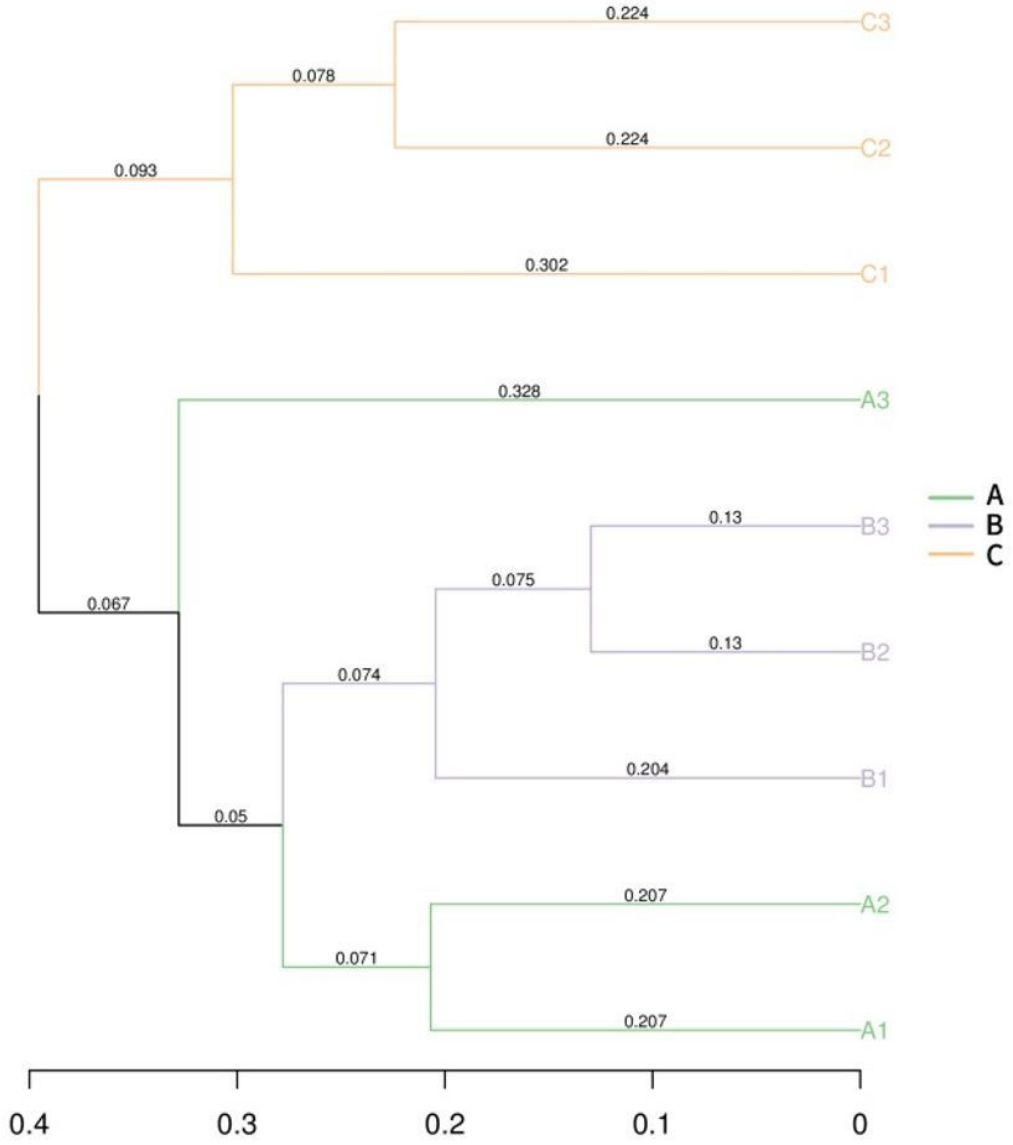

Figure 5

Results of weighted UniFrac cluster analysis of the fungal operational taxonomic units (OTUs) obtained from the rhizospheric soil of Lilium lancifolium. Groups A, B, and C represent the samples from the treatments of 3 years of continuous cropping, 5 years of continuous cropping, and without continuous cropping (1 year), respectively.