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Paddy-*Lilium* Crop Rotation Improves Potential Beneficial Soil Fungi and Alleviates Soil Acidification in *Lilium* Cropping Soil

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Abstract: *Lilium* growth is severely impeded by continuous cropping, and crop rotation is essential to reducing the detrimental effects of monocultures. Soil (0–20 cm) was collected in three *Lilium* cropping patterns in Longshan County, Hunan Province, including continuous *Lilium* cropping (Lilium), corn upland rotation with *Lilium* (Corn), and paddy rotation with *Lilium* (Rice). Using Illumina high-throughput sequencing technology, the fungal ribosomal DNA internal-transcribed spacer 1 (ITS1) was examined to evaluate the features of soil fungi communities among three cropping patterns. Crop rotation has an impact on soil properties and the microbial community. Rice soil has a significantly higher pH than *Lilium* and corn soil, while corn and rice soil have a greater total nitrogen and total phosphorus content than *Lilium* soil. Rotation cropping clearly shifted the fungi community diversity based on the results of principal coordinate analysis (PCoA) and nonmetric multidimensional scaling (NMDS). *Ascomycota* was the most prevalent phylum, with the highest levels in *Lilium* soil. Genetic analysis revealed that paddy rotation led to a clear reduction in or non-detection of eight potentially pathogenic fungal genera and a noticeable accumulation of eight beneficial fungal genera compared to *Lilium* continuous cropping. Fungi communities and their abundant taxa were correlated with soil pH and nutrients. Altogether, we propose that rice rotation, with its ability to mitigate soil acidification, reducing pathogenic and accumulating beneficial communities, may be an effective strategy for alleviating the continuous cropping barrier.

Keywords: rotation cropping; fungi diversity; beneficial community; soil pH; high-throughput sequencing

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1. Introduction

Continuous or monoculture cropping, which is driven by production demands, involves cultivating the same crop over a long period of time [1,2]. This practice often causes soil nutrient imbalances, stunted plant growth, and increased crop disease and pest [3,4]. In addition, with the continuous cropping, crop roots secrete certain identical exudates, such as phenolic acids, which may alter the soil microbial community and enhance the prevalence of the pathogenic microbiome [5]. As a result, continuous or monoculture cultivation ultimately poses a serious threat to yield reductions and the healthy advancement of agriculture [5,6]. Previous studies have shown that continual cropping with a single crop can diminish crop yields by 20 to 50 percent [7,8]. Therefore, it is imperative to look into feasible approaches for alleviating the continuous cropping barrier and achieving green and sustainable development.

Soil microbes play a vital role in agroecosystems, involving nutrient cycling, organic matter decomposition, and the promotion or suppression of crop diseases [1,3]. The modification of soil microbe abundance and species can be used to predict soil health and fertility in agroecosystems [9]. It has been extensively demonstrated that continuous cropping can readily disrupt the balance of soil microbial communities, resulting in a decline in microbial diversity and homogenization of microbial community structure [1,4,5,10]. In soils, loss

and simplification of soil community composition impair soil ecosystem function, further affecting plant growth [11]. Generally, soil microorganisms are frequently classified into pathogenic and beneficial groups based on their function in the agroecosystem [5,12]. Continuous cropping tends to cause a detectable increase in populations of pathogenic fungi and a decrease in beneficial fungi. Li et al. [5] and Gao et al. [10], for instance, demonstrated that continuous cropping of peanuts or potatoes significantly inhabited the populations of *Fusarium*, *Phoma*, *Verticillium*, *Colletotrichum,* and *Bionectria* and decreased some plant-beneficial fungal groups (like *Trichoderma* and *Mortierella*). The accumulation of a load of pathogenic fungi in the soil at the expense of plant-beneficial fungi appears to be a likely explanation for the decline in yield and quality as a consequence of continuous cropping [5]. Thus, knowledge of the variability in important functional microbial communities might shed light on the way certain cultivation strategies perform to eliminate barriers to continuous cropping.

Rotational cropping has been gradually implemented in agriculture to boost crop yields and eliminate the continuous cropping barrier [13,14]. A meta-analysis found that, in China, rotation cultivation increased crop yields by an average of 15–25% more than continuous monoculture [8,15]. In particular, the corn yield from the corn–soybean–wheat rotation was 11.8 Mg ha^{-1}, which was 15% greater than the yield obtained from continuous corn rotation [15]. However, the quantitative synthesis of magnitude and variability in yields has not been reported. This is mostly caused by the broad variation in climate factor, crop species, field management, and soil nutrient delivery associated with various rotation patterns [1,8,15–17]. In addition, research shows that crop rotation has a variety of consequences on the soil microbial population, including positive, negative, and neutral effects [6,18–22]. Therefore, it is necessary to evaluate the impact of various rotation cropping systems on the variation in soil microbial community structure.

Lilium tigrinum (*Lilium lancifolium* Ker Gawl.) is a valuable medicinal herb of the *Liliaceae* family and is distributed in Eastern and Central China. In addition, *L. tigrinum* is an ornamental horticultural crop and food with high economic value [4]. In China, the herb has been cultivated commercially for over 1400 years, and its bulbs are widely used in a variety of Chinese medicinal products due to their lung-moistening and cough-relieving properties [23]. As a result, there has been a growing push for farmers to plant *Lilium* continuously in growing areas. Nonetheless, continuous cropping with *Lilium* leads to severe consecutive replant problems, including extreme soil degradation, crop yield and quality decline, and frequent soil-borne diseases, e.g., wilt disease and black spot disease, which has become an important factor limiting the sustainable development of the *L. tigrinum* industry [4]. A data survey indicates that constant replanting around 6–9 years results in a decline in the dry weight of *Lilium* bulb of 13.25–15.54%, losing farmers an estimated USD 50 million [4]. Thus, it is imperative to investigate the most efficient ways to mitigate the barriers to continuous cropping. To avoid these issues, it is common practice to plant *Lilium* in previously uncultivated fields. Despite this, there is a restricted quantity of land available for producing *Lilium*, and it is anticipated that such detrimental planting strategies would pose a severe danger to the *Lilium* industry in the near future. Recently, certain rotational patterns, such as paddy–*Lilium* rotation, soybean–*Lilium* rotation, and potato–*Lilium* rotation, have been conducted to replace continuous cropping regions with a single crop [24–26]. However, there are distinct differences between the land management practices, crop species employed, and associated root quantity or quality, and other soil parameters among these types that may impact soil quality and fertility [6]. Additionally, soil microorganisms may be affected by these factors. Thus, the objectives of this study were to (1) assess how different patterns of *Lilium* cropping affect the abundance and composition of soil community; (2) compare the pattern of fungal diversity and structure among three different *Lilium* cropping patterns; and (3) examine how potentially pathogenic and beneficial fungal communities change when *Lilium* monoculture cropping is converted into corn–*Lilium* cropping and paddy–*Lilium* cropping. Understanding the influence of

Lilium planting on the soil fungus community would give theoretical guidelines for rational farming mode adoption and sustainable farmland usage.

2. Material and Methods

2.1. Field Description and Sampling

The study sites were located in Longshan County (109°13′−109°48′ E, 28°46′−29°38′ N) of Hunan Province. This region has a typical subtropical monsoon climate with mean annual temperature of 15.8 °C and annual precipitation of 1400 mm. The soil type is categorized as Gleysol in Chinese soil classification, which is derived from sand and shale [27]. At the beginning of this field experiment, soil chemical characteristics at 0–20 cm layer were as follows: pH 5.26, soil organic carbon (SOC) 10.38 g kg^{-1}, total nitrogen (TN) 1.36 g kg^{-1}, total phosphorous (TP) 0.88 g kg^{-1}, total potassium (TK) 23.90 g kg^{-1}, available P 126.7 mg kg^{-1}, available K 139 mg kg^{-1}, NH_4^+-N 0.90 mg kg^{-1}, and NO_3^--N 8.01 mg kg^{-1}. The experiment had three treatments, including continuous cropping of *Lilium* (Lilium), corn upland rotation with *Lilium* (corn), and paddy rotation with *Lilium* (rice). Each treatment set triple plots (20 m × 15 m). The agricultural land management was practiced with typical management of the region. Detailed information, including the crop species, tillage frequency, planting time, harvest frequency, fertilization rate, and replanting frequency, is presented in Table S1.

Lanceleaf *Lilium* bulb was grown in these research region for more than 40 years. The *Lilium* was generally managed as follows: the soil was tilled to a depth of 25 cm, and the plant was fertilized three times throughout the growth period. The experimental bulbs were uniformly sized, second-generation, and planted 7–10 cm deep. The soil was kept moist but not soggy during the planting procedure. The bulbs weighed roughly 100 g and were spaced 15–20 cm apart, with a planting density of roughly 100,000 plants/hm^2.

2.2. Soil Sampling and Determination of Soil Physiochemical Properties

Soil sampling (0–20 cm) was conducted in 2022, at the crop-harvesting stage for *Lilium*, corn, and rice, respectively. A random collection of 5 points were chosen in each plot and then mixed as one sample. After removing stones and roots, a portion of fresh soil sample was stored at −80 °C for DNA extraction; the remaining portion of the soil sample was air-dried and passed through a 0.15 mm mesh to measure soil physicochemical properties. Soil pH was measured using a pH meter (FE20K, Mettler Toledo, Greifensee, Switzerland) at a soil/water ratio of 1:2.5 [28]. The SOC and TN contents were analyzed by K_2CrO_7-H_2SO_4 digestion and semi-micro Kjeldahl digestion, respectively [28]. Soil TP was measured using the molybdenum blue colorimetric method, and soil TK was determined using inductively coupled plasma-atomic emission spectrometry [28]. In addition, methods for detecting soil-based properties have also been performed previously [28].

2.3. DNA Extraction, PCR Amplification, and Amplicon High-Throughput Sequencing

ITS rRNA amplification sequencing was performed by Genesky Biotechnologies Inc. (Shanghai, China). Briefly, DNA was isolated from 0.25 g soil using a FastDNA® SPIN Kit for soil (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions. The integrity of genomic DNA was detected through agarose gel electrophoresis, and the concentration and purity of genomic DNA were detected through the NanoDrop 2000 and Qubit 3.0 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). DNA from the soil samples was used as a template for PCR amplification. As positive control, standard genomic DNA of fungi was amplified in triplicate. The PCR products were purified with Agencourt AMPure XPPCR Purification Beads (Beckman Coulter, Brea, CA, USA) to assess their specificity. The primers used for the amplification of the ITS1 region were ITS1F(5′-CTTGGTCATTTAGAGGAAGTAA−3′)/ITS2R(5′-GCTGCGTTCTTCATCGATGC−3′) and then sequenced using Illumina NovaSeq 6000 sequencing (Illumina, San Diego, CA, USA) [29–32]. The PCR mixture consisted of 1 uL of 10×Toptaq buffer, 0.8uL of 2.5 mM dNTPs, 0.3 uL of F/R primer (10 uM), 0.2 uL of

Toptaq DNA polymerase (Transgen, Beijing, China), 3 uL template DNA, and up to 10 uL ddH_2O. The thermal cycling parameters for PCR amplification were as follows: initial denaturation at 94 °C for 3 min, 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min; 25~27 cycles of denaturing at 72 °C for 10 min, and hold at 4 °C. The purified products were then indexed in the 16S V4–V5 library. The library quality was assessed on the Qubit@3.0 Fluorometer (Thermo Scientific, Wilmington, DE, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) systems. Finally, the purified amplifications from all the samples were pooled in equimolar concentrations, and then the pooled library was sequenced on a NovaSeq 6000 platform (Illumina, San Diego, CA, USA), SP-Xp (PE250) using a 2 × 250-paired-end sequencing kit (250 bp).

Paired-end reads were assembled using FLASH to obtain raw tags. The raw read sequences were processed in the Quantitative Insights Into Microbial Ecology (QIIME) toolkit [29]. The adaptor and primer sequences were trimmed using the cutadapt plugin. High-throughput sequencing data were clustered into operational taxonomic units (OTUs) at a 97% similarity level using UPARSE pipeline, and the chimeric sequences were identified and removed. DADA2 plugin was used for quality control and to identify amplicon sequence variants (ASVs) [33]. Taxonomic assignments of ASV representative sequences were performed with confidence threshold 0.6 by a pre-trained Naive Bayes classifier, which was trained on the UNITE (version 8.2).

2.4. Data Analysis

Before statistical analyses, all the dependent variables were tested for normality and natural log-transformed when necessary. One-way ANOVA with LSD test was conducted using SPSS ver.16.0 for Windows (SPSS Inc., Chicago, IL, USA) to determine significant differences among three cropping types ($p < 0.05$). The relative abundance was determined by the number of sequences affiliated with the same phylogenetic groups divided by the total number of the target phyla or genera per sample. The Shannon, ACE, Chao 1, and Simpson index were calculated to compare the fungi community alpha diversity for each sample. The Venn analysis was performed to compare the fungal composition. Principal coordinate analysis (PCoA) and nonmetric multidimensional scaling (NMDS) were performed to detect the beta diversity between individual samples. Additionally, the similarity of community composition in the abovementioned communities was evaluated sequentially using the "Adonis" and "AOSIMN" functions (999 permutations) of the R package "phyloseq" (version 1.26.1) and "Vegan" (version 2.5.6). Linear discriminant analysis (LDA) effect size (LEfSe) was used to elucidate the biomarkers in each treatment. Those with an LDA $\geq$ 2.0 were considered to be important biomarkers in each treatment. Redundancy analysis (RDA) with a forward selection option and Monte Carlo permutation test (999 permutations) was conducted to select the significant explanatory variables (soil properties) contributing significantly ($p < 0.05$) to soil fungi microbial community variation. The RDA was performed using the R package "vegan" (version 2.4.5).

3. Results

3.1. Changes in Soil Properties Underlie Three Land Types

Except soil TK, soil pH, SOC, TN, TP, C:N, C:P, and N:P significantly differed among three cropping patterns (Table 1). The soil pH was significantly higher in rice soil than in *Lilium* and corn soils (Table 1). The content of TN and TP was higher in corn and rice soil than that in *Lilium* soil, and the highest content of SOC was observed in corn soil (Table 1). Inversely, the ratio of soil C:N and C:P was significantly higher in *Lilium* soil than that in the other two rotation patterns (Table 1, $p < 0.05$).

Table 1. Soil properties under three cropping patterns.

	Lilium	Corn	Rice
pH	5.12 (0.06) ^{b}	5.08 (0.02) ^{b}	5.51 (0.01) ^{a}
SOC, g/kg	11.57 (0.22) ^{b}	14.00 (0.06) ^{a}	10.71 (0.21) ^{c}
TN, g/kg	1.24 (0.02) ^{c}	1.73 (0.06) ^{a}	1.45 (0.02) ^{b}
TP, g/kg	0.72 (0.02) ^{c}	1.71 (0.01) ^{a}	0.91 (0.00) ^{b}
TK, g/kg	21.37 (0.03)	21.77 (0.15)	21.80 (0.17)
C:N	9.36 (0.20) ^{a}	8.13 (0.32) ^{b}	7.39 (0.20) ^{b}
C:P	16.19 (0.78) ^{a}	8.17 (0.02) ^{c}	11.73(0.25) ^{b}
N:P	1.59 (0.01) ^{a}	1.01 (0.04) ^{b}	1.73 (0.08) ^{a}

Values are presented as means (standard error). Lilium: continuous cropping with *Lilium*, Corn: corn upland rotation with *Lilium*, Rice: paddy rotation with *Lilium*. Different low letters present significant difference among three cropping patterns at $p < 0.05$.

3.2. Fungal Abundance and Diversity

Fungal composition and diversity were measured based on OTUs. The OTU distribution Venn analysis demonstrated that there were a total of 2412 OTUs, and 610, 453 and 608 unique OTUs in *Lilium*, corn and rice soil, respectively (Figure S1). These unique OTUs accounted for about 63.36–73.36% for all OTUs in three cropping patterns (Figure S1). In addition, there were 91 shared fungal OTUs, accounting for about 10.66–12.73% among three cropping patterns (Figure S1).

The alpha diversity indices were evaluated based on the OTUs. The ACE, Chao1, Shannon, and Simpson diversity reveal an depletion of OTU similarity in *Lilium* and rice soil compared to *Lilium* and corn soil (Table 2). The beta diversity of fungal community over all samples is illustrated using a PCoA and NMDS plot (Figure 1). At the OTU level, the distribution pattern revealed that the fungal community structure was distinctly different among three cropping patterns (Figure 1). The first two principal components explained approximately 76.58% of the variance in the fungal composition based on the result PCoA (Figure 1A). The aggregation degrees of the sample points from the rice soil were distant from those of the sample points from corn soil, and both soils were extremely far away from the sample points in *Lilium* soil (Adonis: $R^2 = 0.762$, $p < 0.01$; ANOSIM: statistic R = 1, $p < 0.01$; Figure 1B). Based on cluster tree analysis, all samples are grouped into two main groups: samples of rice soil are separated from samples of the other two soils, and samples of *Lilium* and corn soils are grouped into one group (Figure S2).

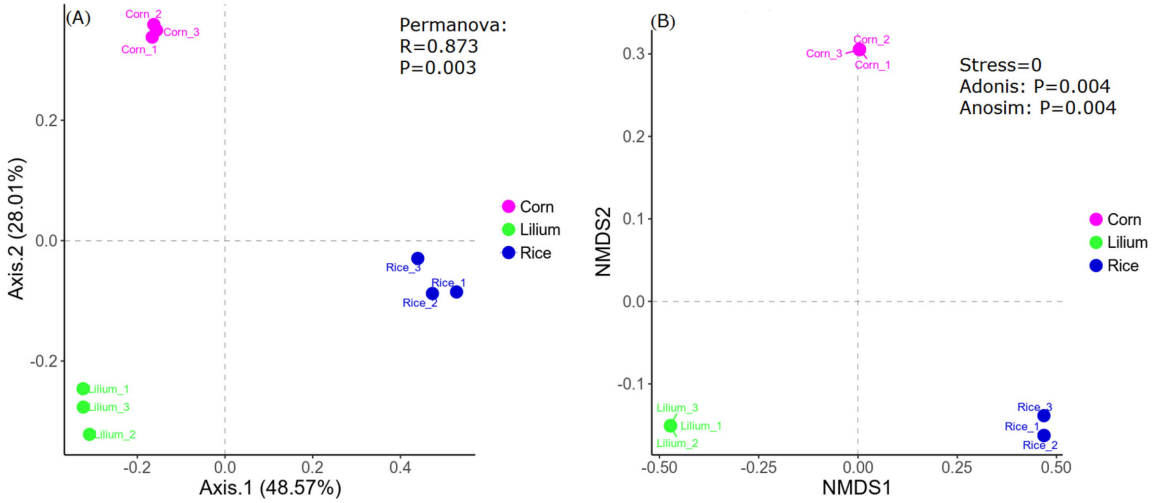

Figure 1. Beta diversity of soil fungal communities under three cropping patterns. PCoA (**A**) and NMDS (**B**) based on the Bray–Curtis distance dissimilarity. PCoA: principal coordinate analysis, NMDS: nonmetric multidimensional scaling. Lilium: continuous cropping with *Lilium*, Corn: corn upland rotation with *Lilium*, Rice: paddy rotation with *Lilium*.

Table 2. Soil fungi diversity under three rotation patterns.

	Alpha Diversity			
	ACE	**Chao 1**	**Shannon**	**Simpson**
Lilium	445.74 (26.70)	444.88 (26.38)	4.25 (0.09)	0.044 (0.00)
Corn	395.38 (31.76)	394.52 (31.44)	3.99 (0.05)	0.055 (0.01)
Rice	440.27 (26.01)	439.09 (26.28)	4.11 (0.18)	0.046 (0.01)

Value are presented as means (standard error). ACE: abundance-based coverage estimator metric. Lilium: continuous cropping with *Lilium*, Corn: corn upland rotation with *Lilium*, Rice: paddy rotation with *Lilium*.

3.3. Fungal Community Structure and Composition

The OTUs identified in all samples were divided into eight phyla, 18 classes, 40 orders, 64 families, and 72 genes. Among three cropping types, the overall fungal community was dominated by the phyla (relative abundance > 1% all samples) *Ascomycota* (average relative abundance > 65%), followed by *Basidiomycota* (14.82%), *Mortierellomycota* (7.26%), and *unassigned* fungi (6.72%) (Figure S3A). The relative abundance of these dominant fungal phyla responded differently to cropping patterns. For detail, the relative abundance of *Ascomycota* was highest in *Lilium* soil, followed by rice soil, in contrast to the corn soil (Figures 2A and S3B). Nevertheless, the relative abundance of *Basidiomycota* and *Mortierellomycota* was significantly higher in corn soil and lowest in rice or *Lilium* soil ($p < 0.05$, Figures 2A and S3B). Further taxonomic classification at the gene level revealed that 258 genera were detected, 79 of which differed between the three cropping patterns ($p < 0.05$). *Fusarium*, *Mortierella*, *Gibberella*, *Trichobolus*, and *Humicola* were the predominant fungal genera in *Lilium* and corn soils, while *Mortierella*, *Fusarium*, *Talaromyces*, *Lecanicillium*, and *Colletotrichum* were the dominant fungal genera in rice soil (Figure 2B). The relative abundances of *Fusarium*, *Gibberella*, *Humicola*, and *Trichobolus* were significantly reduced or not detected, while the relative abundances of *Mortierella*, *Pencillium*, *Talaromyces*, *Lecanicillium*, *Trichoderma*, and *Colletotrichum* increased in rice soil compared to the *Lilium* soil (Figure 2B).

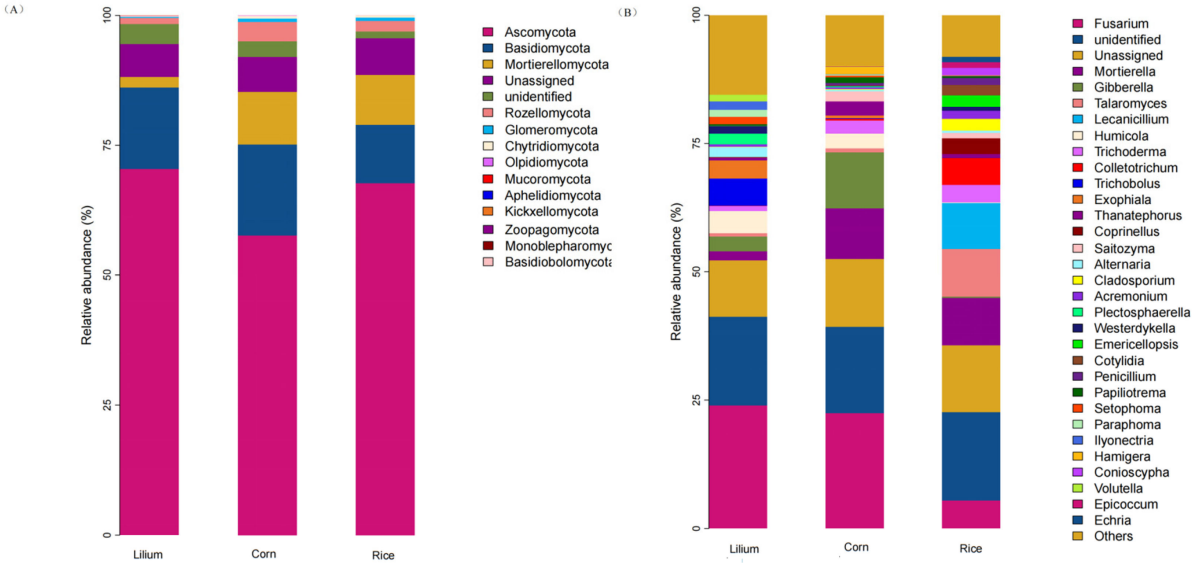

Figure 2. Relative abundance of dominant fungal phyla (**A**,**B**) among three cropping patterns. Lilium: continuous cropping with *Lilium*, Corn: corn upland rotation with *Lilium*, Rice: paddy rotation with *Lilium*.

The linear discriminant analysis effects size (LEfSe) was analyzed for discerning the abundant taxa among three cropping types (Figure 3). There are a total of one, seven, and five biomarkers in *Lilium*, corn, and rice soil, with all LDA values over 3.0. Furthermore, all

biomarkers were completely different amongst soils, demonstrating that rotation cultivation induced alterations in the fungal community structure of *Lilium* soil (Figure 3).

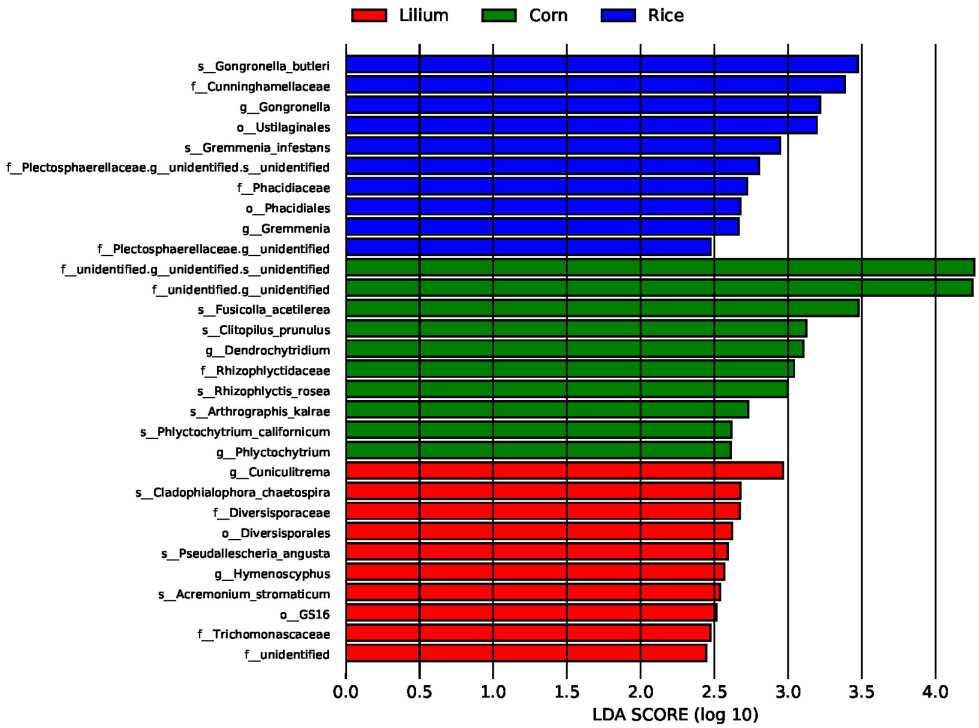

Figure 3. LDA (linear discriminant analysis) discriminant results. Taxa enriched in *Lilium* are shown in red with a positive LDA score, corn in green with positive LDA score and rice in blue with a positive LDA score ($p < 0.05$; LDA score ≥ 2.0).

3.4. Changes in Potential Pathogenic and Beneficial Fungi

Based on a subsequent fungal guild analysis, eight potentially pathogenic fungi and eight beneficial fungi were found to be considerably distinct in corn and rice soils in comparison to *Lilium* soils (Table 3). The relative abundance of potentially pathogenic fungus with names, like *Fusarium*, *Gibberella*, *Volutella*, *Alternaria*, *Humicola*, *Aspergillus*, *Trichobolus,* and *Exophiala,* was substantially reduced or not observed in rice soil compared to *Lilium* soil (Table 3). Meanwhile, certain plant-beneficial fungi, such as *Mortierella*, *Lecanicilium*, *Trichodema*, *Acremonium*, *Clonostachys*, *Metarhizium*, *Metacordyceps,* and *Penicillium*, expanded more in rice soil than that in *Lilium* soil (Table 3).

Table 3. The relative abundance of potentially pathogenic fungi and potentially beneficial fungi.

	Lilium	**Corn**	**Rice**
Potentially pathogenic fungi			
Fusarium	23.97(2.09) ^{a}	22.46(2.37) ^{a}	5.47(2.01) ^{b}
Gibberella	2.83(0.49) ^{b}	10.88(1.43) ^{a}	0.25(0.11) ^{c}
Volutella	1.30(0.24)	0.02(0.01)	0
Humicola	4.28(1.18) ^{a}	2.90 (0.15) ^{a}	0.07 (0.02) ^{b}
Alternaria	1.91 (0.31) ^{a}	0.35 (0.07) ^{b}	0.44 (0.04) ^{b}
Aspergillus	0.48 (0.28)	0.03 (0.01)	0.01(0.01)
Trichobolus	5.28 (1.34) ^{a}	0.15(0.07) ^{b}	0
Exophiala	3.55(0.64) ^{a}	0.50(0.10) ^{b}	0.06(0.01) ^{b}

Table 3. *Cont.*

	Lilium	Corn	Rice
	Potentially beneficial fungi		
Mortierella	1.80(0.38) ^{b}	9.89(1.10) ^{a}	9.18(0.66) ^{a}
Lecanicillium	0	0	8.97(3.82)
Trichoderma	1.01(0.20)	2.19(0.89)	3.46(1.34)
Acremonium	0.43(0.14) ^{b}	0.34(0.07) ^{b}	1.58(0.20) ^{a}
Clonostachys	0.77(0.16) ^{a}	0.08(0.03) ^{ab}	0.35(0.19) ^{b}
Metarhizium	0.08(0.00) ^{b}	0.71(0.25) ^{a}	0.12(0.07) ^{b}
Penicillium	0.04(0.01) ^{b}	0.50(0.38) ^{b}	1.39(0.06) ^{a}
Metacordyceps	0	0	0.07

Values are presented as means (standard error). Different low letters present significant difference among three patterns at $p < 0.05$. Lilium: continuous cropping with *Lilium*, Corn: corn upland rotation with *Lilium*, Rice: paddy rotation with *Lilium*.

3.5. Relation between Fungal Community and Soil Properties

A mantel test analysis revealed a strong correlation between fungal community structures and soil pH, SOC, TP, TN, C:P, and N:P, and soil pH was the most substantial explanatory variable (r = 0.7863, $p < 0.01$, Table S2). Likewise, RDA was applied to analyze the relationships between the fungal community compositions and soil properties (Figure 4). RDA1 and RDA2 contributed to 60.93% of the total variances at the phylum level (Figure 4). The RDA plot of the fungal community composition rendered it abundantly evident that the samples from *Lilium*, corn, and rice varied considerably from one another (Figure 4). The first axis was positively correlated with soil pH, SOC, TN, TK, and negatively correlated with soil pH, C:P, and N:P. The second axis was positively correlated with soil TK, C:N, C:P, and N:P and negatively correlated with soil pH, SOC, TN, TK, and TP. Soil pH was the most crucial variable in shaping the relative abundance of *Basidiomycota* (r = −0.95, $p < 0.001$, Table S3) at the phylum level.

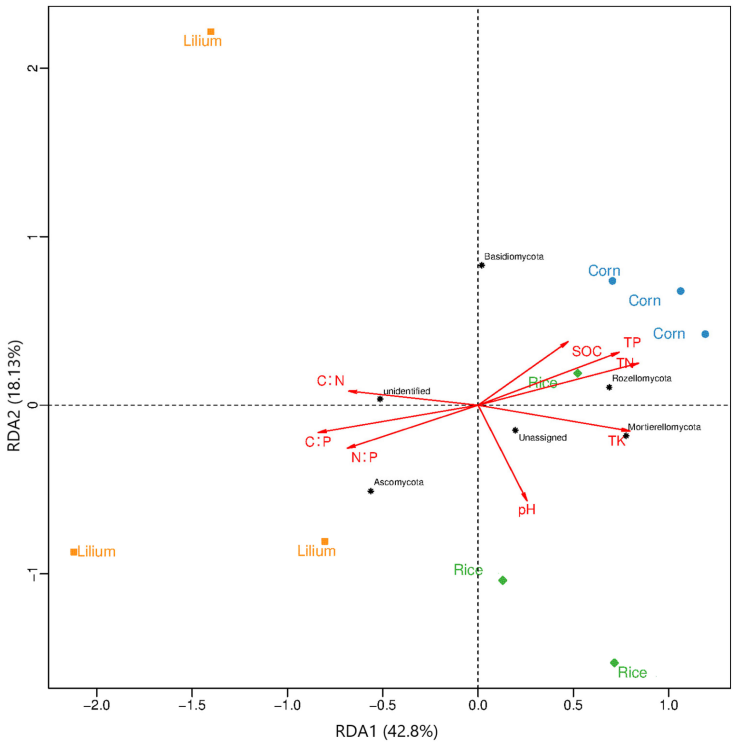

Figure 4. Redundancy analysis (RDA) diagram illustrating the relationship between the soil fungal community composition at the phyla-level from different sampling sites and environmental variables.

The explanatory variables are showed by different red arrows, soil fungal community composition by black star circle. pH: salinity, SOC: soil organic carbon, TN: soil total nitrogen, TP: soil total phosphorus, TK: soil total potassium, C:N: the ratio of SOC to TN, C:P: the ratio of SOC to TP, N:P: the ratio of TN to TP. The orange squares represent *Lilium* cropping (Lilium), the blue squares represent corn upland rotation with *Lilium* (Corn), the green squares present paddy rotation with *Lilium* (Rice).

We also used Spearman's rank correlation to evaluate the associations between the abundance of fungus phyla and soil physicochemistry properties (Figure 5). *Ascomycoto* had a negative correlation with SOC, TN, and TP but a substantial positive correlation with soil C:P and N:P. There was a significant and positive correlation observed within *Mortierellomycota* and *Mucoromycota* and SOC, TP, and/or TN. While *Olpidiomycota*, *Aphelidiomycota*, and *Kickxellomycota* were significantly and positively associated with pH and negatively correlated with TK, *Glomeromycota* and *Rozellomycota* were considerably and positively correlated with TK (Figure 5).

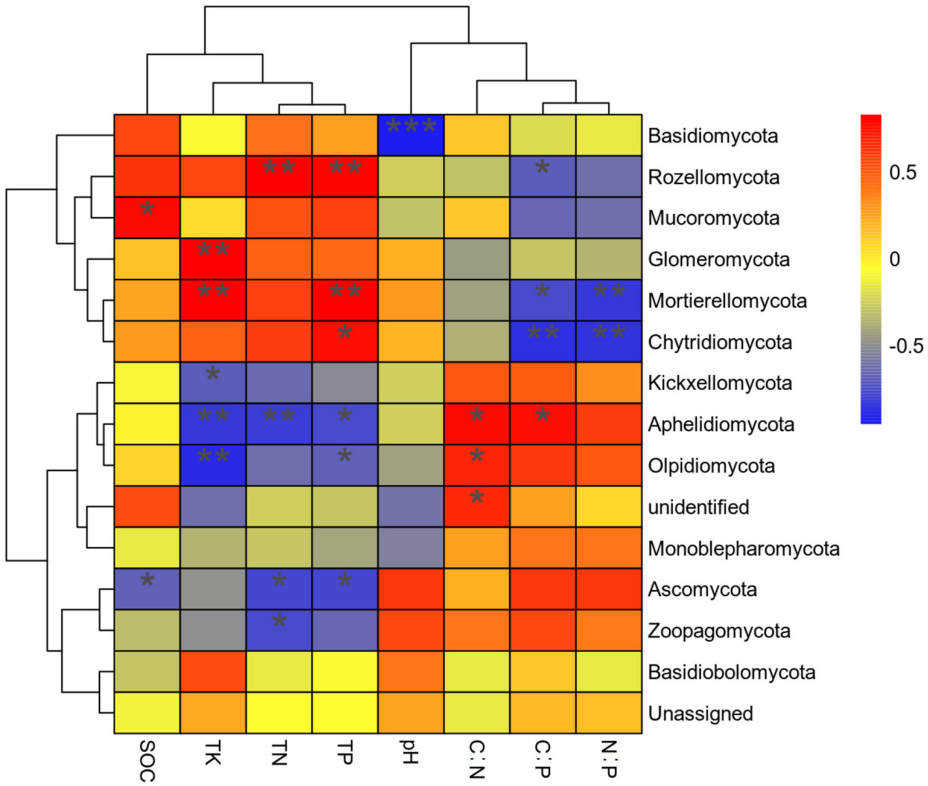

Figure 5. Spearman's rank correlations between soil nutrients and the relative abundances of the different fungal phyla. pH: salinity, SOC: soil organic carbon, TN: soil total nitrogen, TP: soil total phosphorus, TK: soil total potassium, C:N: the ratio of SOC to TN, C:P: the ratio of SOC to TP, N:P: the ratio of TN to TP. "*", "**" and "***"present significant difference at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

4. Discussion

4.1. The Influence of Rotation Cropping Patterns on Slowing Soil Acidification and Improving Soil Nutrients

Long-term continuous cultivation with a single crop can deteriorate the soil environment, causing nutrient imbalances and acidity [34–36]. Rotation cropping, which effectively improves soil physical and chemical properties, regulates soil fertility and increases crop yields by altering the soil microenvironment, and it has been recognized as a suitable practice to protect crop production [6,17]. Among these practices, lowland paddy and upland crop rotations are accompanied by an anaerobic and aerobic microenvironment,

which jointly modify soil physicochemical features and nutrient conversion, most likely facilitating the growth of microorganisms and plants [14,37]. In acidic soils, pH tends to move towards neutral after inundation [38]. Consequently, paddy-upland rotation has a noticeable effect on slowing soil acidification. The results of this study indicate that paddy-upland rotation may help to mitigate soil acidification since the average pH of rice soil increased significantly overall, as compared to Lilium soil by 0.39 units. The increased value (0.25 unit) of soil pH in rice soil, as compared to base soil (mean value = 5.26), further supports this result. This finding is in line with that of Zhang et al. [14], who demonstrated that the paddy-upland rotation can produce comparatively higher soil pH and diminish the tendency of soil acidification than continuous cultivation with a single upland crop. Different rotation types also regulated the soil nutrient content. Compared with *Lilium* monoculture cultivation, the content of SOC, TN, and TP increased by 21.00%, 16.94%, and 137.5%, respectively, in corn soil. Meanwhile, the TN and TP contents of rice soil were comparatively greater, suggesting that these two crop rotation strategies could improve the potential for sequestering soil organic matter. In contrast, the paddy-upland rotation model distinctly reduced SOC content, which is consistent with the results published by Wang et al. [16]. This is presumably due to water leaching reducing the excess SOC during the rice rotation phase. As taken together, rice–*Lilium* rotation cropping can minimize soil acidity, improve soil nutrients, and possibly modify the soil microbial community.

4.2. Impact of Different Cropping Patterns on Fungi Community Diversity

An indicator of soil microbial community diversity is critical for the productivity and stability of agricultural soil ecosystems [39]. The findings of the alpha diversity analysis showed that the fungal community's richness in rice soil was comparable to that in *Lilium* soil; however, it decreased substantially in corn soil, as compared to that in *Lilium* soil. Additional data indicated that fungi in *Lilium* and rice soils possessed more variety, as revealed by the Ace and Shannon index. Gao et al. [10] and Liu et al. [2] also proposed that the diversity of fungi was markedly increased by the continuous cropping of sweet potato and soybean. This observation, however, was contrary to the findings published by Shi et al. [4], who found that continuous cropping with *Lilium* reduced fungal richness. This might be due to inconsistencies in detection methodologies or differences in crop and soil conditions, as various crops have diverse root exudates that alter microbial communities [40]. For beta diversity, both PCoA and NMDS analysis indicated that crop rotation clearly had a significant impact on the matrix fungal community structure [22,41,42], which was confirmed by the hierarchical clustering (Figure S2).

It is widely acknowledged that the structure and composition of soil microbial community are determined by the soil physicochemical properties [1,41,43]. Similarly, this discovery showed that the structure and diversity of soil fungus are greatly affected by soil pH, TN, SOC, TP, and TK. The findings can be attributed to differences in soil properties brought about by various agricultural practices, which affect the availability of microbial nutritional elements. Consequently, these elements serve as cofactors in the synthesis of numerous organic compounds, including acids, proteins, and carbohydrates. Of these, soil pH appears to be one of the key factors regulating fungal community structure under different cropping patterns [35,41,43–45]. Shen et al. [46] and Shi et al. [47] showed that soil pH can alter the bioavailability of soil nutrients, substrates, and hazardous metals, which can alter the composition of the microbial community. Furthermore, the overly acidic soil may limit most soil fungal growth and propagation, and these fungal communities will thrive when the soil pH limitation is removed [45]. Thus, soil fungi growth improved in rice soil with relatively high pH.

4.3. Effects of Cropping Pattern on Fungal Community Taxonomic Classification and Structure

The alternation in soil fungal community structure and composition under continuous cropping has been well documented [1,2,44]. When OTUs are grouped at the phylum level, *Ascomycota*, *Basidiomycetes,* and *Mortiomycota* were the most dominant phyla that appear

frequently in soil. The phylum *Mortierellomycota* was more conducive to enrichment in healthy soil, whereas *Ascomycota* was more likely to be formed in diseased soil, according to the findings presented by Yuan et al. [48]. *Ascomycota* has been found in association with a wide range of crop monoculture systems [3,10,44] and the phyla *Ascomycota* was detected in *Lilium* soil with higher relative abundance (average 70.45%) related to that in corn and rice soil. Moreover, we discovered that the phylum *Mortrellomycota* was considerably more prevalent in rice soil compared to *Lilium* soil, indicating that crop rotation is an improved strategy to defend crops against specific diseases like *Fusarium* wilt disease. Additionally, the *Ascomycota* class *Sordariomycetes* is the most dominant (top 1 class, average RA = 43.65%), which is consistent with numerous studies, showing *Sordariomycetes* to be the most prevalent fungal class in a variety of agricultural systems [2]. Members of this class, including *Fusarium*, *Gibberlla*, *Volutella,* and *Humicola* gene, have a broad distribution as plant endophytes and pathogens in almost all ecosystems [2]. At the genus level, *Fusarium*, *Mortierella*, *Gibberella*, *Humicola*, *Trichobolus,* and *Exophiala* were the dominating genes in *Lilium* soil at the genus level. One of them, *Mortierella,* has been proven to be a beneficial fungus with biocontrol characteristics [2,12,49], and its abundance increased when *Lilium* continuous cropping was converted into rice–*Lilium* rotation cropping. In addition, the existence of putative pathogenic fungi, such as *Fusarium*, *Gibberella*, *Humicola*, *Trichobolus,* and *Exophiala,* can cause soil-borne disease, affecting crop growth and production [16,50–52].

4.4. Changes in Potentially Pathogenic and Beneficial Fungi in Response to Different Crop Rotations

The emergence of crop diseases caused by the formation or expansion of pathogenic communities, such as *Fusarium*, *Alternaria*, *Gibberella,* and *Colletotrichum*, seems to be the main reason for the decline in crop output and the stunted growth of monoculture crops [5,44,50,51,53]. These pathogenic fungal communities, including *Fusarium*, *Alternaria*, and *Gibberella,* were the most prevalent in the current study. Their relative abundance was, likewise, significantly higher in Lilium monocultures and lower in rice–*Lilium* rotations, demonstrating that certain pathogenic fungi thrive in *Lilium* monocultures. Specifically, *Fusarium* is generally recognized as a pathogenic fungus that causes devastating crop diseases, such as *Lilium* wilt disease, *Fusarium* root rot, and banana *Fusarium* wilt disease [4,10,50,54,55]. As the largest genus, the relative abundance of *Fusarium* was distinctly significantly higher in *Lilium* soil and lower in rice soil (Table 3). *Alternaria* and *Gibberella* can infect various crops and cause foolish seeding disease of rice, soybean *Altenaris* leaf spot, citrus *Alternaria* brown spot, and Sanqing ginseng root rot [51,53]. In our study, other certain fungal genera, such as *Humicola*, *Trichobolus*, *Exophiala*, *Volutella,* and *Aspergillus,* were found to be more abundant in *Lilium* soil and reduced or not detected in rice soil (Table 3). These five fungal genera have been identified as potentially pathogenic fungi based on other research [52,56,57]. Thus, our findings imply that a rise in the abundance of the aforementioned pathogenic fungal community is a potential cause of the continuous cropping barrier, which restricts the establishment of *Lilium* monocultures.

The reduction in pathogenic and the accumulation of potential beneficial communities might help to alleviate the continuous cropping barrier [4,5,13]. Our findings also revealed that rotation cropping cultivation increased or some potential beneficial fungi species appeared, e.g., *Mortierella*, *Penicillium*, *Trichoderma*, *Lecanicillium,* and *Acremonium*. Certain species of *Mortierella* have the ability to shield crops from *Fusarium* wilt disease and *Fusarium* root rot [2,49]. As the major dominant genus, the relative abundance of *Mortierella* was four-times more abundant in rice soil (9.18% on average) than in *Lilium* soil (1.80% on average). Previous studies have illustrated substantial correlation between higher *Mortierella* abundance and good soil health during continuous replanting [1]. *Penicillium* can act as a fungal antagonist and plant growth promoter due to its function in producing a vast variety of physiologically active chemicals [58]. *Trichoderma*, *Lecanicillium,* and *Acremonium* also have biocontrol or plant growth-promoting ability based on their

significant and positive correlation with crop physiological characteristics [14]. Here, the relative abundance of *Trichoderma*, *Lecanicillium,* and *Acremonium* was distinctly higher in rice soil compared with in *Lilium* soil. Meanwhile, other certain genera, i.e., *Metarhizium* and *Metacordyceps,* have been identified as parasitic fungi of insects and susceptible to rotation cropping [59,60]. In conclusion, compared to *Lilium* monoculture cropping, rice–*Lilium* rotation can improve the quantify of beneficial fungi and lessen the abundance of pathogenic fungi. As a consequence, it may be extremely crucial in alleviating the barrier associated with continuous cropping in agricultural production.

5. Conclusions

In this study, different cropping patterns had divergent effects on soil physicochemical properties and microbial community structure. Rice–*Lilium* rotation cropping considerably reduced the development of soil acidification and increased soil nutrient content, as compared to *Lilium* continuous cropping. Soil fungal communities in rice and corn soil were noticeably distinct from those in *Lilium* soil. *Ascomycota* was found to be the dominant group, with the highest abundance in *Lilium* soil. Additionally, we observed a higher relative abundance of eight beneficial fungi and a decline in or non-detection of eight potentially pathogenic fungi in rice soil compared to *Lilium* soil. Overall, this study provides evidence that paddy-upland rotation cropping can improve soil fungi structure by reducing soil acidity and improving some beneficial fungi communities.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14010161/s1, Table S1: Management information for the three cropping patterns. Table S2: Mantel test result for the correlation between soil fungal community composition and environmental variables. Table S3: The correlation between fungal phyla and soil properties variables. Figure S1: Venn of soil fungi under different cropping patterns. Lilium: continuous cropping with Lilium, Corn: corn upland rotation with Lilium, Rice: paddy rotation with Lilium. Figure S2: Hierarchical clustering of fungal communities underlying three different cropping patterns. Lilium: continuous cropping with *Lilium*, Corn: corn upland rotation with *Lilium*, Rice: paddy rotation with *Lilium*. Figure S3: The barplot showed the relative abundance of the main fungal phyla(A) and the heatmap showed their variations among soil samples at phylum level (B) present in three cropping soils. Lilium: continuous cropping with *Lilium*, Corn: corn upland rotation with *Lilium*, Rice: paddy rotation with *Lilium*.

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