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A Meta-Analysis in Nine Different Continuous Cropping Fields to Find the Relationship between Plant Species and Rhizosphere Fungal Community

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Abstract: Plant species and cropping systems influence rhizospheric fungal communities' composition, diversity, and structure. The fungus community is one of the main factors behind soil health and quality. Yet, there is insufficient evidence and research on the effect of plant species with continuous cropping histories on the rhizospheric fungal community. In order to investigate how the fungal community responds to the various plant species and cropping systems, we have chosen one field that is left fallow along with eight continuously farmed areas to research. Among the eight phyla, the relative abundance of *Ascomycota* was significantly higher in *Polygonum multiflorum,* which was continuously cropped in fields for two years (P2). *Basidiomycota* was considerably higher in the fallow field (CK). Among the 1063 genera, the relative abundance of *Fusarium* was significantly higher in maize continuous-cropped fields for six years (M6), followed by *Fritillaria thunbergii* continuous-cropped fields for two years (F2), and found lower *Fusarium* abundance in CK. The alpha diversity observed in taxa, Chao1, and phylogenetic diversity indices were significantly higher in M2. β-diversity found that the fungal communities in the samples clustered from the fields in the same year were quite similar. In all the soil samples, the saprotrophic trophic type was the most common among the OTUs that had been given a function. Our studies have proved that continuous cropping and plant species changed the fungal community's composition, diversity, and structure. This research may serve as a guide for overcoming significant agricultural challenges and advancing the industry's sustainable growth.

Keywords: fungal community; composition; diversity; structure; cropping system; next generation sequencing

Citation: Pang, Q.; Alami, M.M.; Yu, W.; Ouyang, Z.; Shu, S.; Tu, D.; Alami, M.J.; Wang, X. A Meta-Analysis in Nine Different Continuous Cropping Fields to Find the Relationship between Plant Species and Rhizosphere Fungal Community. *Agronomy* **2023**, *13*, 1827. https://doi.org/10.3390/agronomy13071827

Academic Editor: Nikolaos Monokrousos

Received: 1 June 2023
Revised: 1 July 2023
Accepted: 4 July 2023
Published: 10 July 2023

1. Introduction

Due to limited land and rising market demand for food and cash crops, producers typically adopt continuous cropping systems [1]. Continuous cropping produced considerable plant production and quality changes, as well as the occurrence of different root rot diseases, for a long period of time [2]. In addition to plants and soil, soil microbial community composition and diversity are also altered by the continuous cropping system, as previously reported by the researcher [1–3]. However, in several studies, rotational

diversity has been shown to provide below-ground advantages, such as increases in soil organic matter stocks. The advantages of plant diversity below ground have been connected to microbial community shifts in natural systems. This is also true for agroecosystems, which may function at many geographical and temporal dimensions. Microbial community composition, diversity, and structure vary significantly in fallow or continuous cropping soils [4–6]. Fallow or crop rotation has recently been used to fix continuous farming difficulties, lowering the presence of dangerous bacteria. By enhancing the soil microenvironment, technologies have been enhanced to reduce ongoing agricultural challenges [1,7–11].

Soil fungal communities are an integral factor in the soil ecosystem's processes of the material cycle and the transformation of energy, and they are different in bulk and rhizosphere soils due to a variety of biotic and abiotic variables. Changes in the composition, diversity, and organization of the soil fungus community directly impact soil health and quality. Fungal populations in the rhizosphere are linked to plant development, nutrient absorption, and soil-borne illnesses [8,11,12]. Soil fungi often vary in response to changing plant species, altering the composition and productivity of plant communities. Plant roots also create complex chemicals, generating resource-rich hotspots with unique properties from the bulk soil and recruiting microbial communities in the rhizosphere preferentially [1,7,9–11,13]. Other soil physical and chemical indices are less susceptible to changes in external variables such as plant rotation, land management, and cultivation methods than soil microorganisms. As a result, the biomass, composition, and diversity of microbial communities are often utilized as markers of changes in soil quality [14–16].

Previous studies have reported the effect of cropping systems on soil fungal communities in some specific crops and cash crops. However, less attention has been paid to comparing plant species with cropping histories, and it is still unclear whether plant species affect the composition and diversity of the fungal community or not. In this study, we have selected eight fields that have been continuously cropped with different plant species, such as *Coptis chinensis* Franch for six years, maize for 2 and 6 years, *P. multiflorum* for 2 and 6 years, sweet potato for 2 years, *F. thunbergia* for 2 years, cabbage for 2 years, and fallow fields as controls. This study aims to find the relationship between plant species and fungal community composition and diversity, find a suitable crop rotation for *C. chinensis* (one of the most important medicinal plants in the area) and other cash crops, and mitigate the continuous cropping obstacles.

2. Material and Methods

2.1. Soil Sampling and Soil Physicochemical Property Analysis

Soil samples were collected from eight distinct fields situated in Lichuan City, Hubei Province, China. These fields exhibited variations in terms of plant types, cropping years, and fallow areas. The predominant soil types encountered were sandy and clay, and the region experienced an average annual rainfall ranging from 1198 to 1650 mm with a yearly temperature of 12.7 °C. This particular research project focused on different types of fields: maize continuous-cropped fields referred to as M2 and M6, continuous-cropped *C. chinensis* fields known as C6, sweet potato continuous-cropped fields labeled as S2, *P. multiflorum* continuous-cropped fields termed P2 and P6, cabbage continuous-cropped fields denoted as CB2, *F. thunbergii* continuous-cropped fields identified as F2 and fallow fields simply called CK. Within each field, four soil samples were collected from the rhizospheres of ten randomly selected plants: *C. chinensis*, maize, *P. multiflorum*, sweet potato, *F. thunbergii*, and cabbage. Carefully, the roots of these plants were shaken to eliminate loosely attached soil particles. Additionally, four soil samples were obtained from the fallow fields, each consisting of five cores. All soil samples were then transported to the laboratory in ice boxes. To ensure the removal of debris and rocky components, a 2 mm sieve was employed to sieve each soil sample. Following the homogenization of the samples, 10 g of soil was placed in sterile tubes and preserved at −80 °C for subsequent DNA extraction [17].

In the analysis of soil physicochemical properties, the pH of the soil was measured using a Mettler-Toledo TE 20 instrument (Mettler-Toledo, Columbus, OH, USA). A soil

suspension was prepared by mixing the soil with deionized distilled water in a ratio of 1:20 (weight/volume). The determination of soil organic matter (OM) was carried out using the potassium dichromate internal heating technique. For the assessment of total nitrogen (TN) and phosphorus (TP) levels, a Smartchem 200 discrete analyzer (Unity Scientific, Milford, MA, USA) was employed. The total potassium (TK) content was determined using an FP series multielement flame photometer (Xiang Yi, Hunan, China). To analyze the soil-available nutrients, specific extraction and measurement methods were utilized. The alkali hydrolyzed diffusion method was employed for determining soil-available nitrogen (AN). The sodium bicarbonate extraction molybdenum antimony anti-colorimetry method was used to assess soil-available phosphorus (OP). The ammonium acetate extraction flame photometer was utilized to measure soil-available potassium (AK). Lastly, the boiling water extraction curcumin colorimetry technique was employed to determine the soil-available boron (AB). These analytical techniques and instruments allowed for the comprehensive assessment of soil physicochemical properties, enabling the understanding of important nutrient levels and pH conditions in the soil [17].

2.2. Soil Enzyme Activities

Sucrase activity (S_SC) was assessed using the 3,5-Dinitrosalicylic acid colorimetry method, and the results were reported as the amount of glucose produced per gram of soil after 1 h. Urease activity (S_UE) was determined through indigo blue colorimetry, and the values were expressed as the quantity of NH3-N generated per gram of soil after 1 h. To measure phosphatase activity (S_ACP), disodium phenyl phosphate was utilized, and the activity was quantified accordingly [5].

2.3. 18S rRNA Gene Amplification and Sequencing

The PowerSoil Kit (MO BIO Laboratories, Carlsbad, CA, USA) was able to extract DNA from 0.5 g of dry soil samples (weight). The concentration of the DNA was measured using a Thermo Scientific Nanodrop 2000C Spectrophotometer (which is located in Wilmington, DE, USA). Before it was put to use, the soil DNA was kept at a temperature of −80 °C.

The primers SSU0817F (5′-TTAGCATGGAATAATRRAATAGGA-3′) and 1196R (5′-TCTGGACCTGGTGAGTTTCC-3′) of the V5–V7 regions of the 18S rRNA gene of fungus were used in this study. The following procedures were used to conduct the PCR reaction: 3 min of denaturation at 95 °C, followed by 30 cycles of 30 s at 95 °C, 30 s of annealing at 55 °C, 45 s of elongation at 72 °C, and then extension at 72 °C for 10 min and triplicated in a 20 µL mixture that included FastPfu Buffer 4 µL, dNTPs2.5 mM 2 µL, each primer (5M) 0.8 µL, FastPfu Polymerase 0.4 µL, BSA 0.2 µL, and template DNA 10 µg. Purified PCR products were measured using QuantiFluorTM-ST (Promega, Madison, WI, USA) and purified using the DNA gel extraction kit AxyPrep (Axygen Biosciences, Union City, CA, USA) [6].

We sequenced the pooled pure amplicons using an Illumina MiSeq (Illumina, San Diego, CA, USA) according to Illumina's standard procedures. The proportions of the purified amplicons in the pool were equal. The quality of the raw FASTQ was improved by using Trimomatic [18] and FLASH [19] to filter them according to the following criteria: (1) Any site that had an average quality score of 20 or below over a sliding window of 50 base pairs was used to truncate the 300 base pair reads, and any truncated reads that were shorter than 50 base pairs were removed from the analysis. Additionally, reads that included N-bases were thrown out. (2) Sequences were merged according to their overlaps, regardless of whether there was a mismatch of more than 2 base pairs, or if the overlaps were longer than 10 base pairs. (3) The sequences of each sample were identified by using barcodes (exactly matching), primers (allowing for two nucleotide mismatching), and readings with ambiguous bases. Barcodes and primers were both used to ensure that each sample's sequences were identical. Quantitative insights into microbial ecology (QIIME) [20] were used to develop operational taxonomic units (OTUs) by similarity using Mothur version 1.31.1 [21] with 97 percent cutoff points, and chimeric sequences were

removed from the dataset. A degree of confidence of 70% was assigned to the results of comparing the taxonomy of each gene sequence to the Silva (SSU123) database.

For fungal functional prediction, the fungal OTUs were formatted into text and sent to the FUNGuild (https://github.com/UMNFuN/FUNGuild, (accessed on 10 December 2021)) [22].

2.4. Statistical Analyses

For the purpose of conducting multiple comparisons, a one-way analysis of variance (ANOVA) was done, followed by Tukey's (honestly significant difference) multiple-range tests. The Pearson correlation coefficients between soil parameters and fungal phylum abundances were computed using SPSS v20.0 (provided by SPSS Inc., Chicago, IL, USA). The OTU was used as a basis for establishing all of the other alpha diversity evaluations. The maximum amount of dissimilarity that may exist across clusters is 3%. Calculations of Chao, Simpson, Shannon, and phylogenetic diversity were performed on each sample in order to quantify its inherent richness and diversity. The Mothur Version 1.35.1 program was used to construct rarefaction curves by comparing the relative quantities of fungal OTUs based on the average number of OTUs that were found. The nine distinct field soils contributed to the overall variety. In order to create PCoA plots, both the weighted and unweighted UniFrac distance metrics were used, both of which were determined by the phylogenetic structure. Finally, Venn diagrams were used to illustrate species that were shared by more than one sample.

3. Results

3.1. Composition of Fungal Community

The rarefaction curves were generated by picking sequences at random from among the altered sequences and then graphing the sequences against the OTUs that each sequence represented (Figure S1). The curves became more uniform in shape as the number of sequences and OTUs increased. In addition to this, the number of reads found in M2 was the greatest. The nine soil samples included OTUs that belonged to 16 phyla, 70 classes, 179 orders, 432, 1063, 1963, and 9443 OTUs. Eight phyla and one unclassified phylum were classified among the phyla, which accounted for over 99.32% of the fungal sequences. The nine most dominant phyla were *Ascomycota* (46.50%), *Basidiomycota* (22.67%), *Mortierellomycota* (15.84%), unclassified fungi (9.41%), *Rozellomycota* (2.74%), *Chytridiomycota* (1.44%), *Glomeromycota* (0.72%), and *Olpidiomycota* (0.7%) (Figure 1).

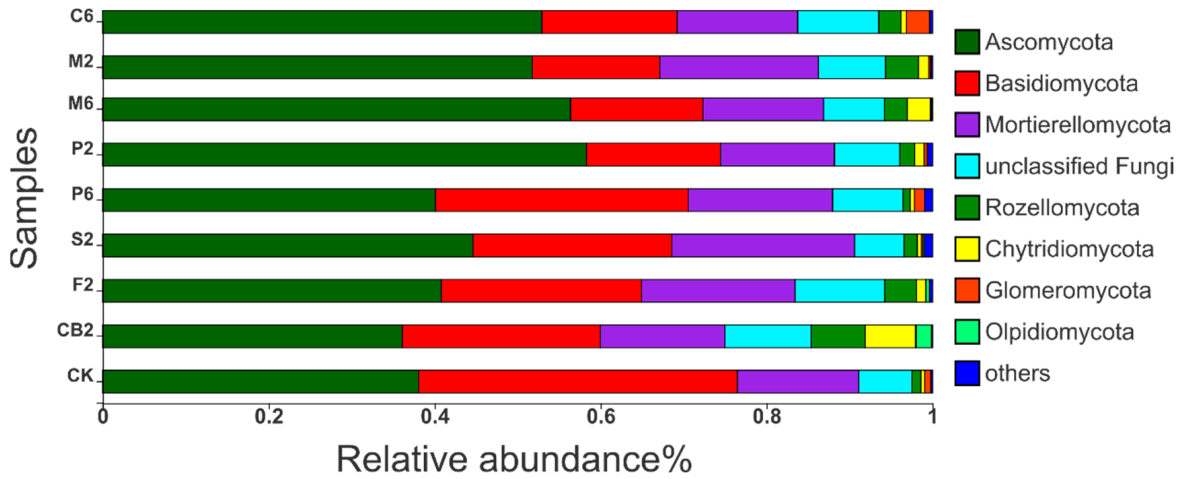

Figure 1. Fungal community composition at the phylum taxonomic level under different cropping systems C6; continuous cropping of *Coptis chinensis* Franch for 6 years, M2 and M6; maize for 2 and 6 years, P2 and P6; *Polygonum multiflorum* for 2 and 6 years, S2; sweet potato for 2 years, F2; *Fritillaria thunbergia* for 2 years, CB2; cabbage for 2 years, and CK; fallow field.

Mortierellaceae, *Piskurozymaceae*, *Nectriaceae*, and *Trichosporonaceae* were the most abundant fungal families among the 432 fungal families. The relative abundance of *Russulaceae* was higher in CK (23.50%), followed by CB2 (0.62%). At the genus taxonomic level, *Mortierella*, *Solicoccozyma*, *Saitozyma*, *Trichoderma*, *Exophiala*, *Apiotrichum*, *Penicillium*, *Trichocladium*, and *Fusarium* were identified in the soil samples. Among the 1063 genera, the relative abundance of *Fusarium* was significantly higher in M6 (4.67%), followed by F2 (2.96%), and lower in CK (0.01%) (Table S1).

The findings of several comparisons revealed that the mean percentage of *Ascomycota* was substantially more significant in (P2) (58.31%). The relative abundance of *Basidiomycota* was markedly higher in (CK) (38.37%). The *Mortierellomycota* phylum was significantly higher in S2 (22.04%) and lower in P2 (13.74%). *Rozellomycota and Chytridiomycota* were considerably more abundant in CB2 (6.03% and 6.03%, respectively). C6 had the highest mean proportion of *Glomeromycota* (2.80%), followed by P6 (1.22%) and CK (0.74%). The relative abundance of *Olpidiomycota* was considerably higher in CB2 (1.87%) than in the other fields (Figure 2 and Table S2).

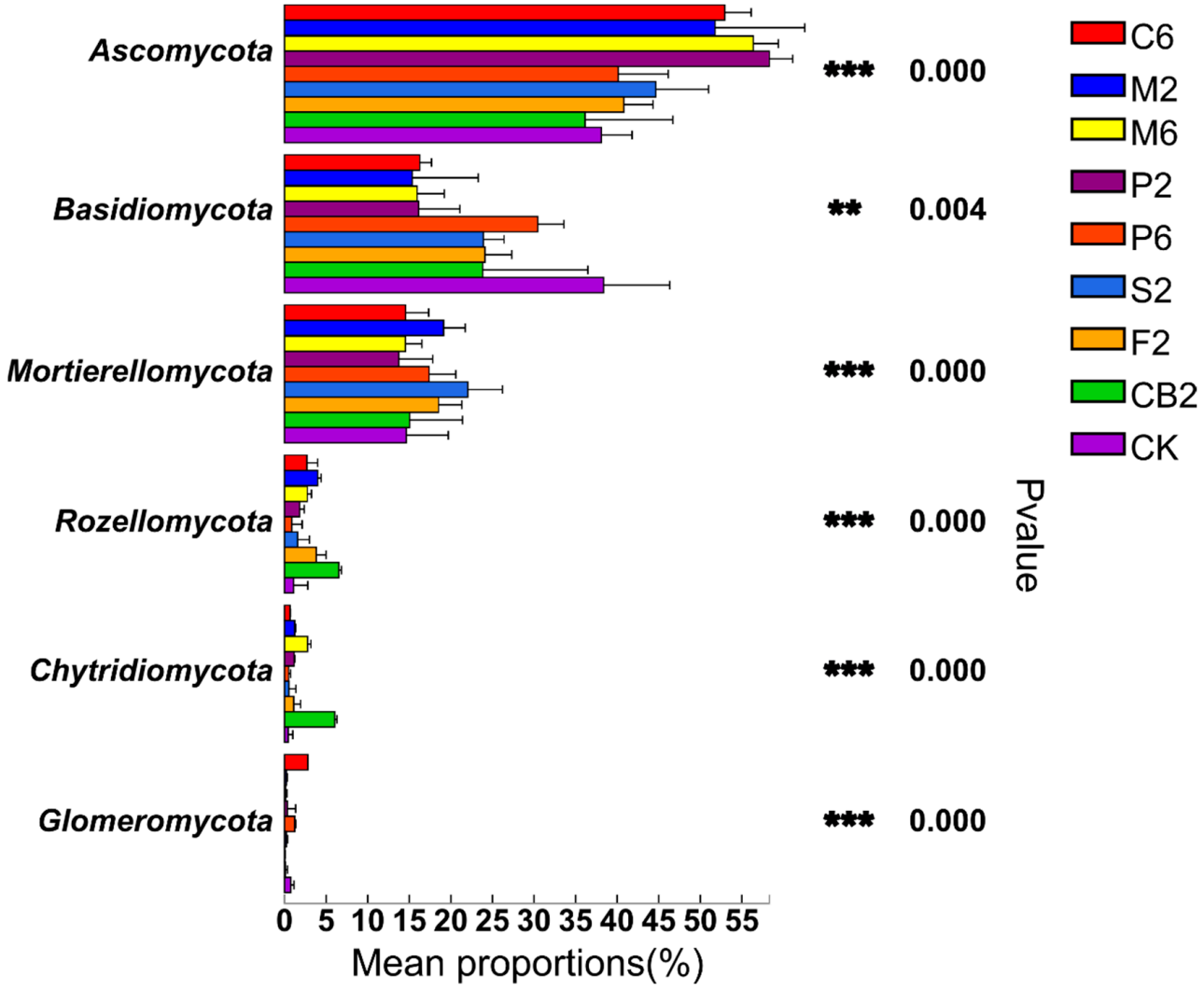

Figure 2. Multiple comparisons of soil fungal phyla in different cropping fields C6; continuous cropping of *Coptis chinensis* Franch for 6 years, M2 and M6; maize for 2 and 6 years, P2 and P6; *Polygonum multiflorum* for 2 and 6 years, S2; sweet potato for 2 years, F2; *Fritillaria thunbergia* for 2 years, CB2; cabbage for 2 years, and CK; fallow field. Asterisks (** and ***) show significant differences as *p*-values < 0.01, and 0.001, respectively.

Venn diagrams illustrate the degree of similarity and overlap between several samples of a species. There were 56, 46, 40, 40, 40, 37, 39, 26, and 111 unique species that were present in the C6, M2, M6, P2, P6, S2, F2, CB2, and CK fields, respectively. 148 (7.66%) species were common among the nine treatments, with CK (111) having the highest species number (Figure 3).

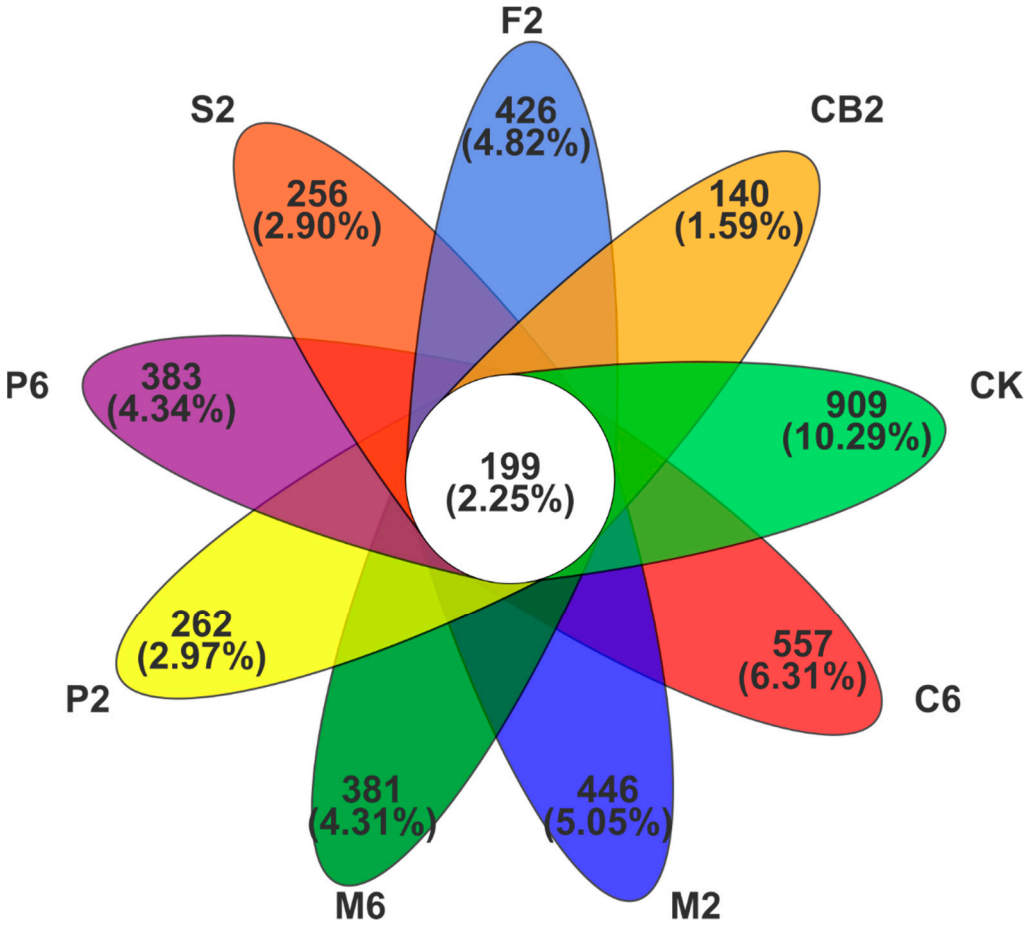

Figure 3. The Venn diagram shows the unique and shared fungal species numbers in different fields with different plant species. C6; continuous cropping of *Coptis chinensis* Franch for 6 years, M2 and M6; maize for 2 and 6 years, P2 and P6; *Polygonum multiflorum* for 2 and 6 years, S2; sweet potato for 2 years, F2; *Fritillaria thunbergia* for 2 years, CB2; cabbage for 2 years, and CK; fallow field.

3.2. Fungal Community Diversity

The alpha diversity provides a measurement of the variety that exists within the fungal community and may be used in the process of comparing the diversity that exists among fungal communities found in various fields that practice continuous cropping. Alpha diversity indicators such as Chao and Shannon, as well as observed species (Sobs) and Phylogenetic diversity (Pd), were used in order to conduct an analysis of the richness and diversity of the community contained within the samples. The alpha diversity of Sobs, Chao, and PD was significantly higher in M2. Compared with C6, the Chao and observed species' (Sobs) diversity was more elevated in M2, M6, and F2 and lower in S2, CB2, CK, P2, and P6. The Shannon index was higher in M2, P2, P6, and F2 and lower in M6, S2, CB2, and CK. It indicates that the plant's type and continuous cropping were the major factors affecting the fungal community's diversity (Figure 4).

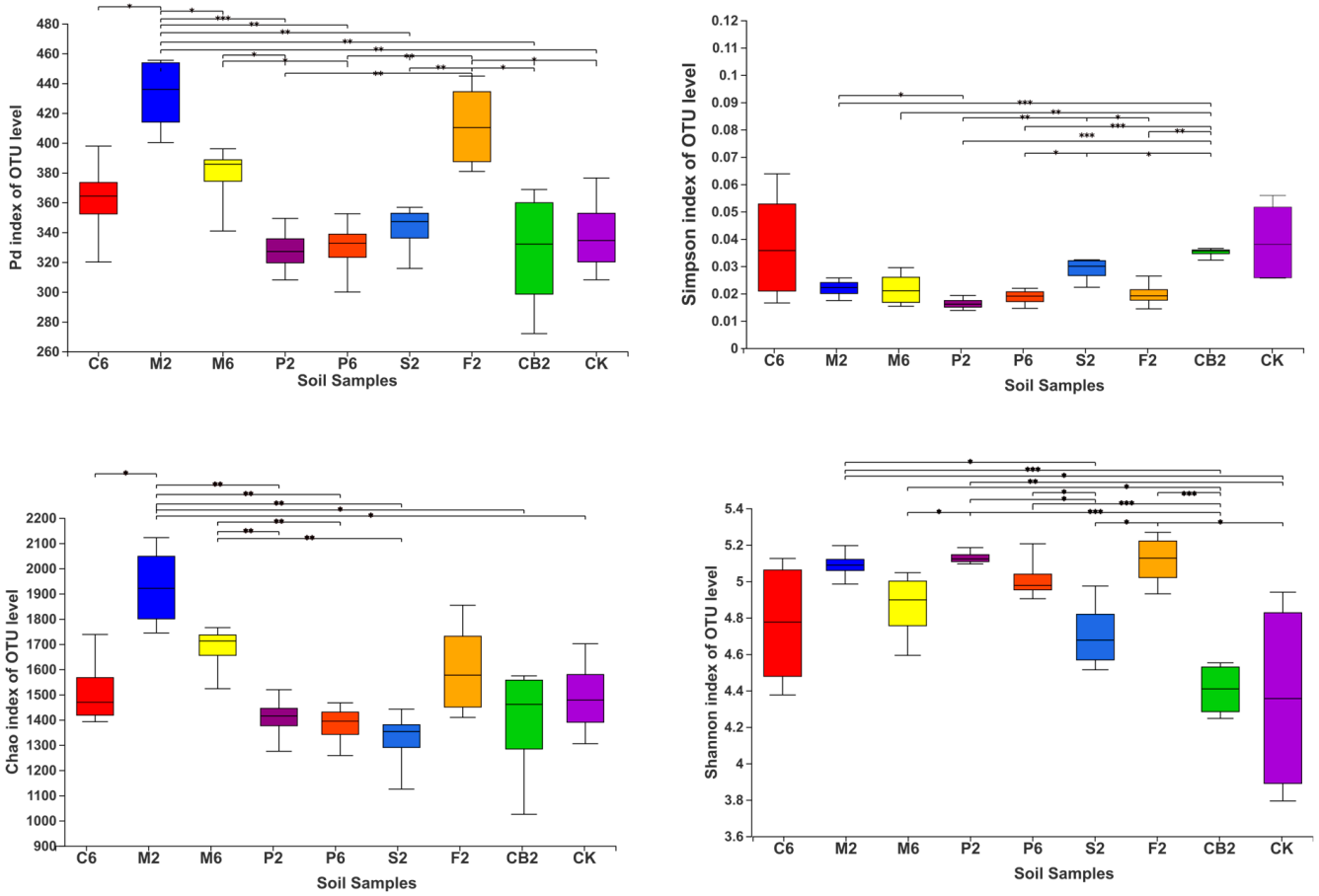

Figure 4. Alpha diversity of the rhizosphere fungal community under different cropping systems with different plant species C6; continuous cropping of *Coptis chinensis* Franch for 6 years, M2 and M6; maize for 2 and 6 years, P2 and P6; *Polygonum multiflorum* for 2 and 6 years, S2; sweet potato for 2 years, F2; *Fritillaria thunbergia* for 2 years, CB2; cabbage for 2 years, and CK; fallow field. Asterisks (*, **, and ***) show significant differences as *p*-values < 0.05, 0.01, and 0.001, respectively.

The fungal ß-diversity was evaluated based on an unweighted UniFrace matrix in order to provide a more accurate assessment of the distance connection between a variety of soil samples and plant species. The principal coordinate analysis (PCoA) provides a graphical representation of the fact that the fungal community compositions differed significantly across the various soil samples. The biological replicates clustered together; samples from the C6 soil clustered with M2, M6, and CK soil samples, which showed that the fungal species in these soil samples were more similar than other samples, and samples from the other sites (P2, P6, CB2, F2, and S2) clustered together. The first two axes explain 40.9% and 18.11% of the total variation. Importantly, significant divergences in β-diversity were identified between maize-cropped fields and other fields. Moreover, the plant types affected the community composition and were marginally influenced by the sampling sites (Figure 5).

β-diversity indicated that fungal communities in soil samples gathered from fields in the same year were more comparable to plant kinds in soil samples collected from different years. For instance, the fungal communities in soil from *C. chinensis* continuous cropping fields (C6), maize cropped fields (M2, M6), and fallow fields (CK) were more comparable within fields than between fields.

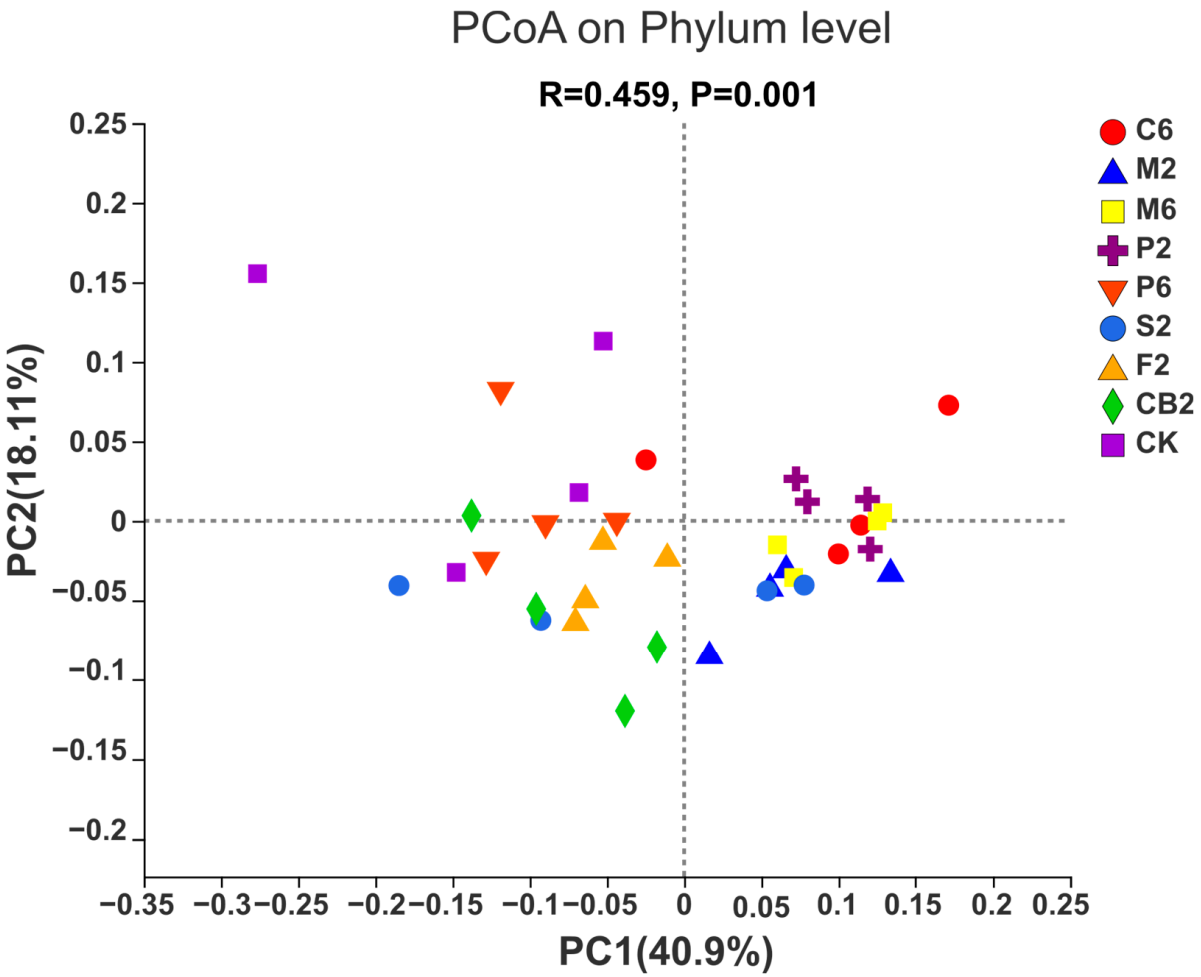

Figure 5. Principal Coordinate Analysis (PCoA) shows soil fungal community structure. C6; continuous cropping of *Coptis chinensis* Franch for 6 years, M2 and M6; maize for 2 and 6 years, P2 and P6; *Polygonum multiflorum* for 2 and 6 years, S2; sweet potato for 2 years, F2; *Fritillaria thunbergia* for 2 years, CB2; cabbage for 2 years, and CK; fallow field.

3.3. The Correlation Coefficient Analysis of Environmental Factors and Soil Fungal Community

The link between soil physicochemical parameters, soil enzymatic activities, and fungal phyla relative abundance was evaluated using Spearman's correlation coefficient. The *Mucoromycota* relative abundance showed a negative correlation with soil physicochemical properties, and the correlation coefficient was obtained in pH, TP, AK, TK, and AB. *Glomeromycota* was negatively correlated with TK, TP, OP, and AB. *Chytridiomycota* showed a positive correlation with TK, AK, and AB. *Rozellomycota* phyla relative abundances were positively correlated with TK, AK, TP, OP, and AB (p-values $\leq$ 0.01, <0.01, <0.01, <0.01, and <0.01, respectively) and not significantly negatively correlated with OM (p-value = 0.385). *Olpidiomycota* phyla had a positively significant correlation with TK, TP, OP, and AB. *Mortierellomycota* phyla are abundant and showed a significant positive correlation with TP, OP, AB, TN, AN, and OM and a negative correlation with TK, AK, and P.H. *Zoopagomycota* phyla showed a positive relationship with TP and OP and a negatively correlated relationship with TK and AB (Figure 6 and Tables S3 and S4).

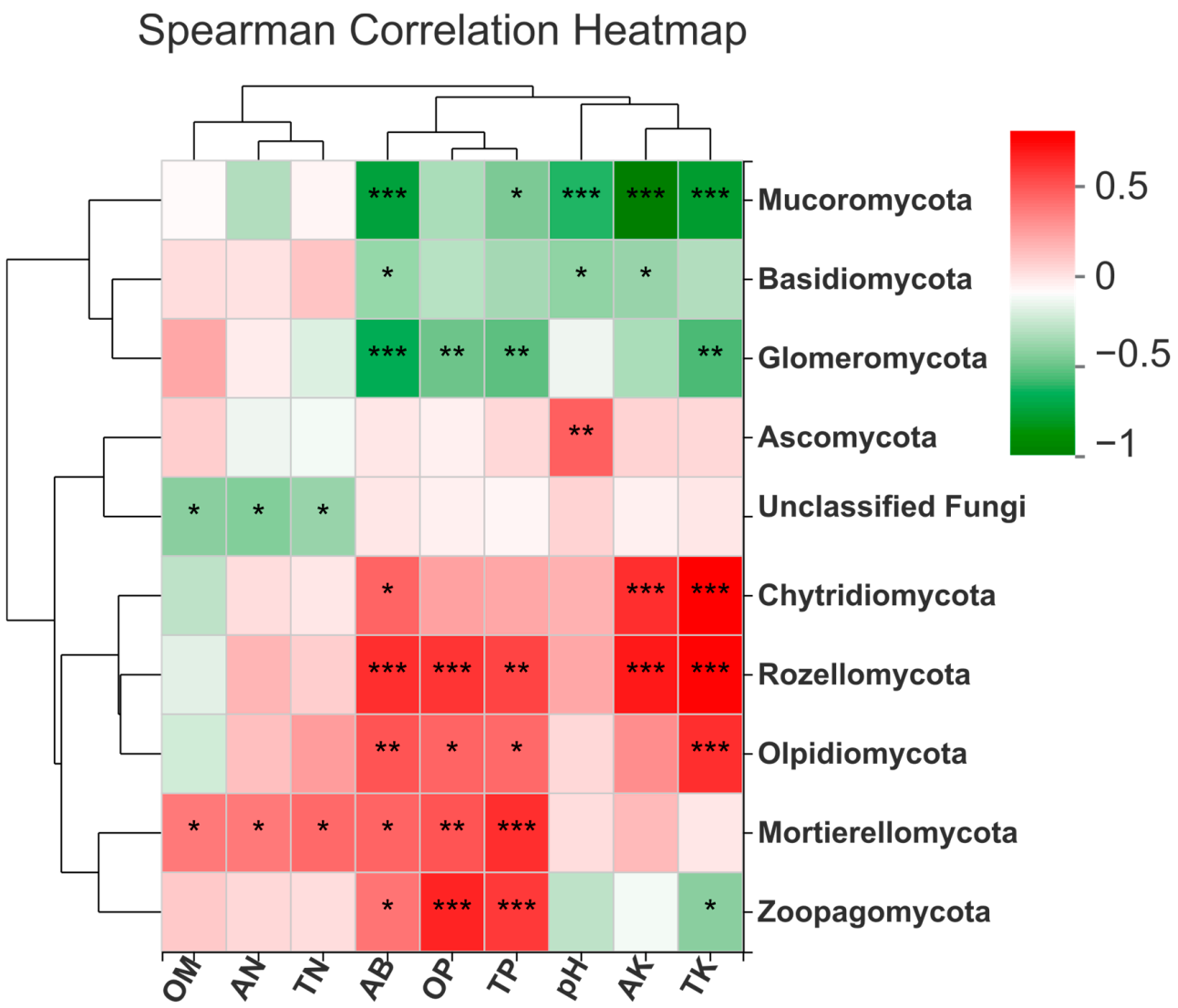

Figure 6. Spearman correlation analysis of soil fungal phyla and soil physicochemical properties under different cropping fields OM; soil organic matter, AN; soil-available nitrogen, TN; total nitrogen, AB; soil-available boron, OP; soil-available phosphorus, TP; total phosphorus, pH; the potential of hydrogen, AK; soil-available potassium, TK; total potassium. Asterisks (*, **, and ***) show significant differences as *p*-values < 0.05, 0.01, and 0.001, respectively.

Ascomycota phyla abundance was positively correlated with S_SC (*p*-value < 0.01), and *Basidiomycota* phyla abundance was significantly negatively correlated with S_SC (*p*-value < 0.01). *Mortierellomycota*, *Zoopagomycota*, *and Mucoromycota* were significantly negatively correlated with S_ACP (*p*-values = 0.01, <0.01, and 0.05, respectively). *Chytridiomycota* was significantly correlated with S_UE (*p*-value < 0.01), while *Olpidiomycota* was positively correlated with S_SE and had a negative correlation with S_SC (*p*-values = 0.01 and 0.01, respectively). *Glomeromycota* phyla are significantly positively correlated with S_SC and significantly positively correlated with S_UE (Figure 7 and Table S5).

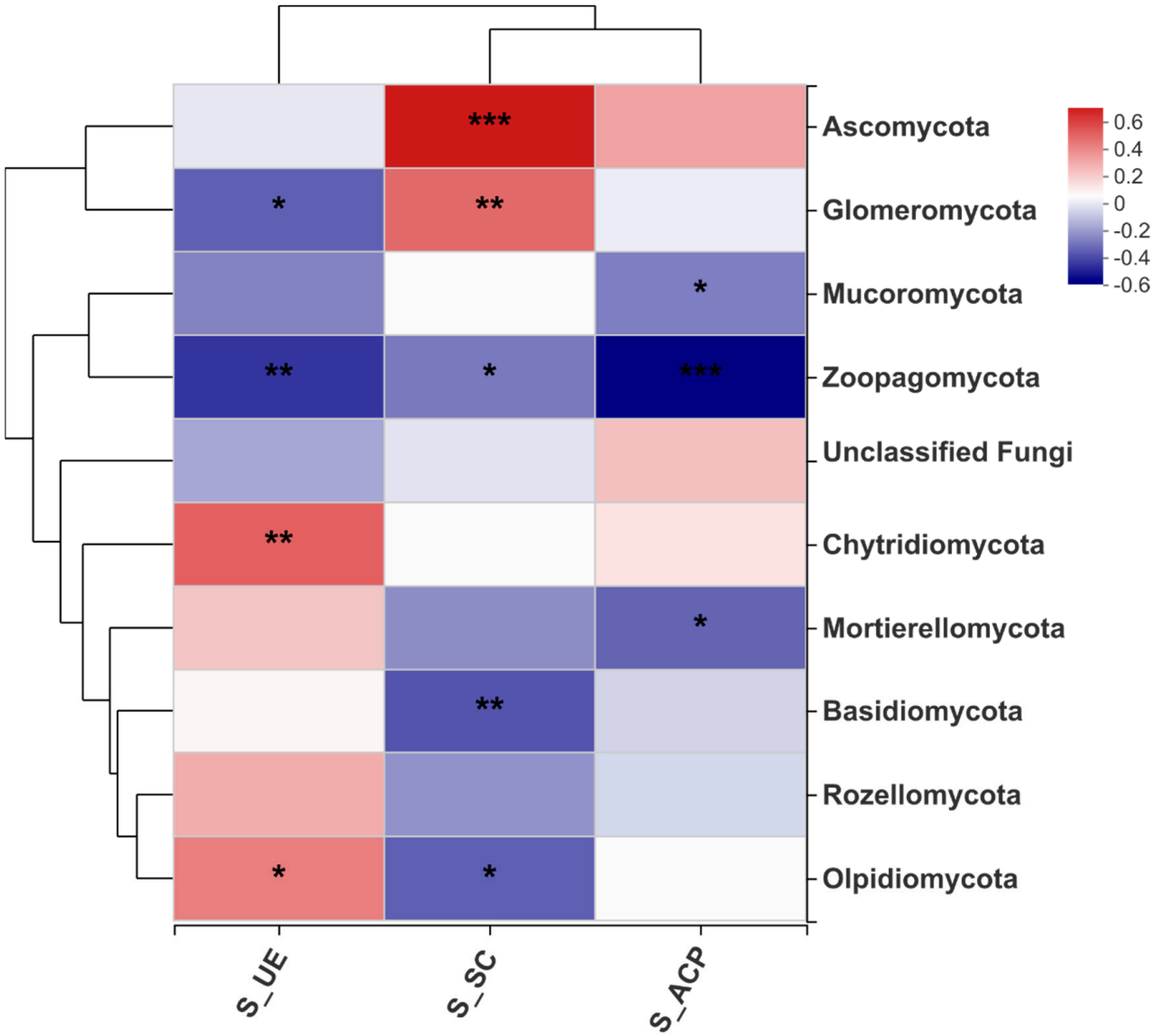

Figure 7. Spearman correlation analysis of soil fungal phyla and soil enzyme activities in different cropping fields S_UE: urease activity; S_SC: sucrase activity; S_ACP: phosphatase activity. Asterisks (*, **, and ***) show significant differences as *p*-values < 0.05, 0.01, and 0.001, respectively.

Different patterns of fungal community structure were found in different soil samples based on the RDA of the most dominant phylum and soil physico-chemical properties. The first two RDA components, which are referred to as RDA1 and RDA2, explain 37.35 and 12.27% of the total variation in the soil's physicochemical characteristics, respectively (Figure 8B), whereas they represent 21.85 and 6.69% (Figure 8A) of the total variance in the soil's enzymatic activities, respectively. We determined the r^2 and *p*-values in order to investigate the importance of environmental influences in the soil on the composition of the fungus community. The total concentration of phosphorus (TP) exhibited the greatest r^2 value among the soil physicochemical values (r^2 = 0.3507, *p*-value < 0.01), which indicates that TP had the biggest influence on the fungal community composition. Among the soil physico-chemical properties, TP showed the highest r^2 value. The S_SC soil enzymatic activity exhibited the greatest r^2 value (r^2 = 0.2885, *p*-value < 0.01) across all of the soil enzymatic activities.

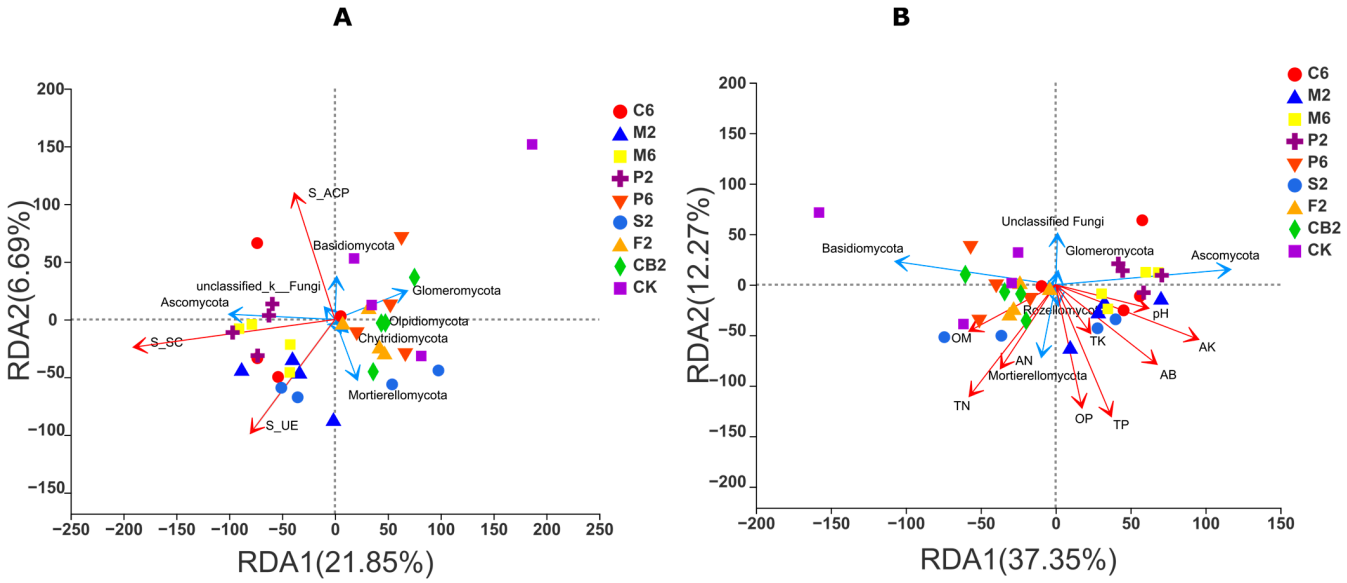

Figure 8. Redundancy analysis (RDA) shows the correlation between soil enzyme activities (**A**), physicochemical properties (**B**), and soil fungal phyla. C6; continuous cropping of *Coptis chinensis* Franch for 6 years, M2 and M6; maize for 2 and 6 years, P2 and P6; *Polygonum multiflorum* for 2 and 6 years, S2; sweet potato for 2 years, F2; *Fritillaria thunbergia* for 2 years, CB2; cabbage for 2 years, and CK; fallow field. OM; soil organic matter, AN; soil-available nitrogen, TN; total nitrogen, AB; soil-available boron, OP; soil-available phosphorus, TP; total phosphorus, pH; the potential of hydrogen, AK; soil-available potassium, TK; total potassium. S_UE; urease activity; S_SC; sucrase activity; S_ACP; phosphatase activity.

3.4. Prediction of Fungal Community Function

FUNGuild identified pathotroph, saprotroph, and symbiotroph major trophic modes and six functional guilds. The unassigned OTUs dominated sequence richness. In assigned OTUs with functions, the animal pathogen was the most dominant functional guild in all soil samples in the identified functional guild and was higher in M6 (22.50%). The lowest abundance was observed in CK (2.22%). The plant pathogen was detected in higher abundances in P6 (11.05%) and lower abundances in CK (1.15%). The Dung Saprotroph functional guild was most abundant in F2 (3.29%) and least abundant in CK (0.43%). C6, compared to other fields, had a lower number of functional guilds following the CK, which meant that cropping systems in *C. chinensis* and other species directly affected the abundance of functional fungi (Figure 9A).

The saprotroph dominated the trophic type in the samples, while the phototroph was the most dominant fungal trophic mode in CB2 (31.11%), followed by M6 (30.62%), and the least abundant was observed in CK (5.51%). Saprotroph trophic type was higher in S2 (48.48%), followed by F2 (42.08%), which was the least abundant in CK (28.48%). Moreover, the somatotrophic trophic mode was the most abundant in CK (30.72%) (Figure 9B).

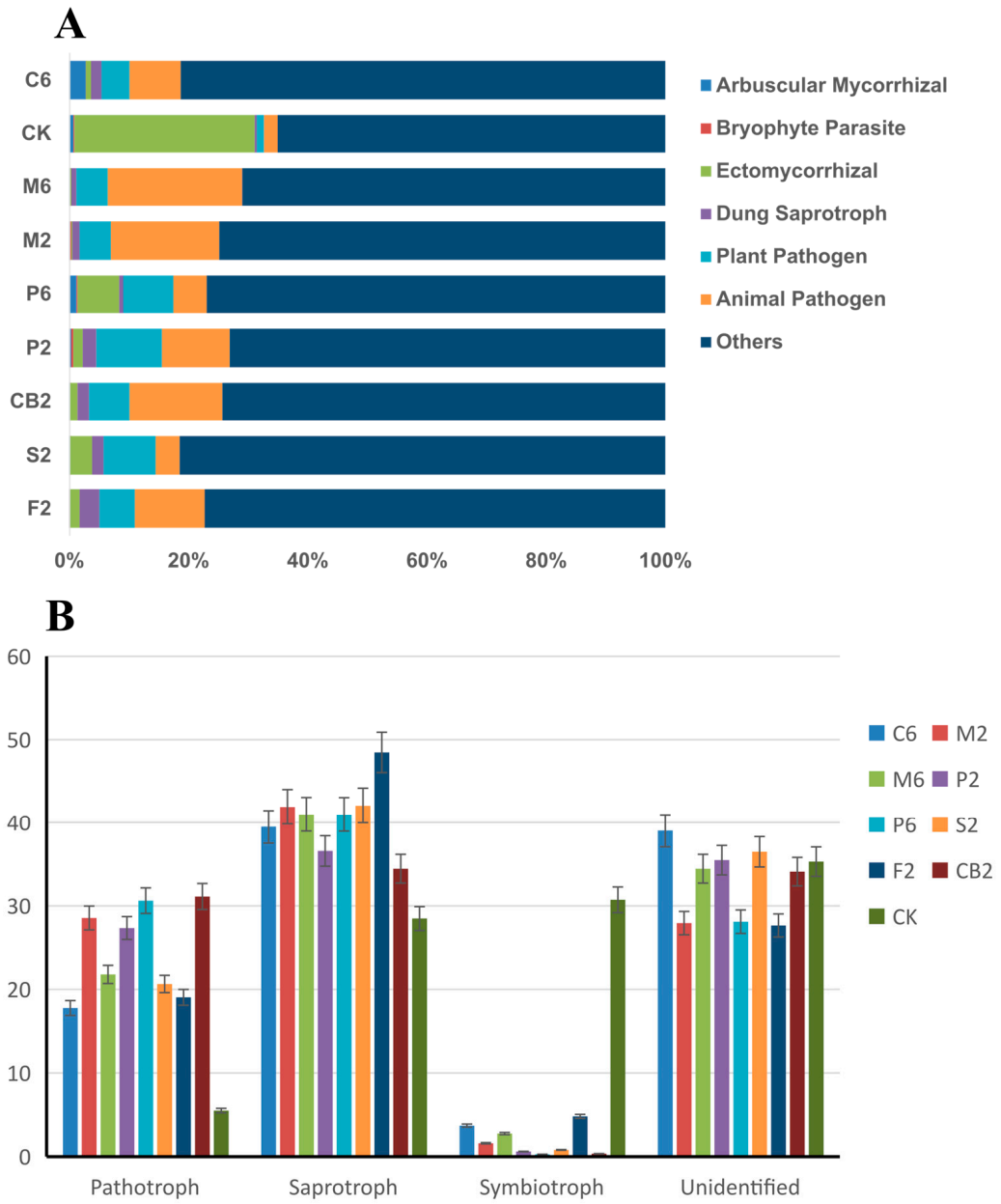

Figure 9. Functional guild (**A**) and trophic mode (**B**) of the fungal community under different rotational cropping patterns C6; continuous cropping of *Coptis chinensis* Franch for 6 years, M2 and M6; maize for 2 and 6 years, P2 and P6; *Polygonum multiflorum* for 2 and 6 years, S2; sweet potato for 2 years, F2; *Fritillaria thunbergia* for 2 years, CB2; cabbage for 2 years, and CK; fallow field.

4. Discussion

Continuous cropping significantly changed the diversity and richness of the fungal community, thereby affecting soil quality, function, and productivity [5,6,23]. Meanwhile, soil microorganisms are essential for plant health, and the microbial population surrounding them is added to the plant's second genome [24–28]. In this study, alpha diversity Sobs and Chao indices estimate species richness, which refers to the total number of different species present in a given area. M2, M6, and F2 plant species have higher alpha diversity indices, suggesting that these species have a greater number of unique species or higher species richness compared to other species mentioned. This could be due to specific ecological characteristics, habitat preferences, or historical factors that have led to a higher diversity of species in these particular plant species. The Shannon index takes into account both species richness and evenness, which refer to the relative abundance of different species.

Higher Shannon index values in M2, P2, P6, and F2 indicate that these species not only have a higher number of species but also a more even distribution of individuals among those species. This suggests that these plant species have a balanced representation of different species, contributing to higher diversity indices. Similarly, Alami et al. (2020) found that soil microbial diversity had almost disappeared after five years of continuous cropping of *C. chinensis*. One-year-old plants had a higher value than five-year-old plants [6]. Similar findings have also been recorded in cucumber and potato, thereby suggesting that plant species and cultivation systems significantly impact the diversity of fungal communities in the rhizosphere. Soil fungus diversity influences soil quality, health, and the soil ecosystem. Root rot diseases are related to a decline in soil fungal diversity [29,30]. A more comprehensive analysis of the specific characteristics, interactions, and environmental factors related to each plant species would be needed to ascertain the exact reasons behind the observed differences in alpha diversity indices.

Continuous cropping and fallow soils had a significant effect on the composition and structure of the fungal population in the field. This study has found that the *Ascomycota* were significantly higher in P2. *Basidiomycota* was higher in CK, indicating that *Basidiomycota* include many important decomposers and mycorrhizal fungi, and their abundance might have been favored in the absence of continuous crop disturbance. The higher abundance of *Mortierellomycota* in S2, which likely had a history of fallow soil, suggests that the accumulation of organic material during the fallow period provided an ideal environment for the proliferation of these fungi. *Rozellomycota* and *Chytridiomycota* are both groups of zoosporic fungi that often inhabit aquatic or wetland environments. The higher presence of these fungal phyla in CB2 indicates that this area might have had high soil moisture content or experienced periods of water saturation, creating suitable conditions for the growth of these aquatic fungi. The dominance of *Glomeromycota* in C6 suggests that continuous cropping practices in this area likely promoted the establishment and colonization of mycorrhizal fungi, as these fungi form symbiotic relationships with plant roots and are commonly found in agricultural soils. *Olpidiomycota* is a recently discovered fungal phylum, and its ecological role is still being investigated. The higher presence of *Olpidiomycota* in CB2 suggests that this particular environment might have provided unique ecological niches or specific conditions that favored the growth and prevalence of these newly identified fungi. Overall, the results indicate that continuous cropping and plant species significantly changed the composition of fungal phyla in the field, which was consistent with the results of previous research [5,25,28,31].

The UniFrac-weighted principal coordinate analysis (PcoA) (Figure 5) indicated a significant disparity in the structural composition of the fungal communities present in the eight fields that alternated between fallow and continuous cropping. The C6 soil samples were grouped together and set apart from the other soil samples in their own little cluster. The distance between each field of continuous cropping was quite far apart, whereas the distance between individual plants of the same species was only somewhat spread out. The findings showed that continuous cropping had a considerable impact on the structure of the fungal population in the rhizosphere; Li et al. also reported findings that were comparable to these [32]. They conducted a 454-pyrosequencing study to get to the conclusion that the makeup of the soil microbial community and the architecture of that community altered dramatically across the three peanut fields due to the distinct monoculture histories. According to Venn diagrams (Figure 3), the number of distinct fungus species altered as one moved from one kind of continuous cropping area to another. According to research that was conducted similarly, the number of distinct fungal OTUs reduced with time in vanilla monoculture [33].

Soil environmental factors such as soil physicochemical properties and soil enzymatic activities closely correlate with certain rhizospheric fungi. The negative correlation between *Mucoromycota* and soil physicochemical properties suggests their ecological preferences and nutrient requirements. *Mucoromycota* prefers a slightly acidic to neutral pH, indicating a decrease in abundance with increasing alkalinity. Lower levels of total phosphorus favor

their abundance, indicating their adaptation to phosphorus-limited environments. *Mucoromycota* shows reduced abundance in potassium-rich soils, likely due to lower potassium requirements or competition. Similarly, elevated levels of available boron led to lower *Mucoromycota* abundance. *Glomeromycota* exhibits similar patterns, with reduced abundance in nutrient-rich soils, preferences for phosphorus-limited environments, and lower boron availability. *Chytridiomycota* exhibited a positive correlation with total potassium, soil-available potassium, and soil-available boron, indicating a potential adaptation to environments with higher levels of these nutrients. *Rozellomycota* showed positive correlations with total phosphorus, soil-available potassium, total potassium, soil-available phosphorus, and soil-available boron, suggesting a preference for nutrient-rich soils. *Olpidiomycota* displayed positive correlations with various nutrients such as total potassium, total phosphorus, soil-available phosphorus, and soil-available boron, indicating their reliance on these nutrients for growth. *Mortierellomycota* exhibited a positive correlation with several nutrients, including total phosphorus, soil-available phosphorus, soil-available boron, total nitrogen, soil-available nitrogen, and soil organic matter, while being negatively correlated with total potassium, soil-available potassium, and pH. Lastly, *Zoopagomycota* showed a positive relationship with total phosphorus and soil-available phosphorus while negatively correlating with total potassium and soil-available boron, suggesting specific nutrient preferences within this fungal group. This finding was consistent with previous studies, which showed that the composition and structure of the rhizospheric soil fungal population were significantly influenced by the number of continuous cropping years [34–40].

FUNGuild is a valuable online database for the functions of members within the fungal community. It may be possible to obtain data on the biological processes of fungi through trophic guilds instead of individual taxa [41,42]. The undefined saprotroph dominated the functional guilds (Figure 9B). After the fallow field (CK), the C6 had a less functional guild than other fields, thereby suggesting that continuous cropping of *C. chinensis* directly affected the abundance of functional fungi. OTUs with similar functions had different distributions in different continuous cropping fields and plant species, thereby indicating that the plant species significantly influenced the selection of the fungal community in the rhizosphere.

5. Conclusions

The consistent cultivation of *C. chinensis*, maize, and other plant species had a substantial impact on the diversity, composition, and organization of the fungal community. It was discovered that the fungal communities in the fallow fields were more diverse than those in the continuously cropped fields and that the plant species had a substantial impact on the selection of the fungal community. We have found that the richness and diversity of fungal communities were higher in maize continuous cropping fields; the *Ascomycota* genus is more accumulated in *P. multiflorum* continuous-cropped fields; and after the fallow field, the *C. chinensis* continuous cropping fields had less functional guild than other fields. Our results, therefore, provide a foundation for future agricultural research that aims to boost microbial activity and crop or cash crop output in continuous cropping areas, which are critical for *C. chinensis* and other cash crops.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13071827/s1. Figure S1: Rarefaction curves C6, M2, M6, P2, P6, S2, F2, CB2, and CK; Table S1: The composition of the fungal community at the family level; Table S2: The composition of the fungal community at the phylum level; Table S3: Spearmen correlation analysis of soil fungal phyla and soil physicochemical properties; Table S4: Spearmen correlation *p*-value for soil physicochemical properties and fungal phyla; Table S5. Spearmen correlation analysis soil physicochemical properties and fungal phyla.

Author Contributions: Conceptualization, Q.P. and X.W.; data curation, M.M.A., S.S. and X.W.; formal analysis, Q.P., M.M.A. and Z.O.; funding acquisition, X.W.; investigation, Q.P.; methodology, Q.P., M.M.A., D.T. and M.J.A.; project administration, X.W.; resources, W.Y., S.S., D.T. and M.J.A.; software, M.M.A., W.Y. and X.W.; supervision, X.W.; validation, S.S. and M.J.A.; visualization, W.Y., Z.O. and X.W.; writing—original draft, Q.P. and M.M.A.; writing—review and editing, Q.P. and M.M.A. All authors have read and agreed to the published version of the manuscript.

Funding: The study was made possible thanks to funding from the National Key R&D Program. A specialized study on the modernization of Traditional Chinese Medicine (TCM), the large-scale production of *Coptis chinensis* Franch, and other Chinese medicinal plants with high value and their involvement in the reduction of poverty (2017YFC1701000).

Informed Consent Statement: The plant that was used in this study was cultivated at Huazhong Agricultural University's experimental base located in Lichuan City, Hubei Province, China, with the cooperation of Zhuanzhuxi Huanglian Professional Cooperative Company. The plants were used with permission from the company and local residents. We confirm that all local, national, or international guidelines and legislation were adhered to in the production of this study.

Data Availability Statement: The datasets generated and/or analyzed during the current study are uploaded to the SRA repository of NCBI with the project number [SUB12266716]. The current status of SRA is under review, and the SRA accession number will be provided as soon as it is accepted by the NCBI. For the availability of data, contact the corresponding author, Xuekui Wang (wang-xuekui@mail.hzau.edu.cn).

Acknowledgments: We appreciate the help of Lichuan City Zhuanzhuxi Huanglian Professional Cooperative Company's Xu Zegang in preparing fields and cooperating with the collection of soil samples. We also thank the Chinese Scholarship Council (CSC) for supporting our studies.

Conflicts of Interest: The authors declare no conflict of interest.

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