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Effect of Different Types of Continuous Cropping on Microbial Communities and Physicochemical Properties of Black Soils

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Abstract: The barriers caused by continuous tillage have had a negative impact on the crop and soil environment. Black soils are economically important as a valuable natural resource in Northeast China, but limited soil resources have led to continuous planting of major food crops and medicinal plants. At present, the extent to which two different types of plants—grains and medicinal plants that are successively grown on the same soil—have an impact on soil quality and microbiology is not known. In this study, we investigated the effects of different types of long-term continuous cropping on soil and soil microbial communities by determining the physicochemical properties, the soil community composition and function of grain crops and medicinal-plant soils with more than five years of continuous cropping, as well as fallow soils. The results showed that long-term continuous cropping reduced the pH of different types of soils, but there was no significant difference in the content of AK. The relative abundance of beneficial dominant phyla, such as Actinomycetes, Acidobacteria, and Green Campylobacter decreased and the relative abundance of pathogenic genera such as Alternaria and Didymellaceae, increased after the long-term continuous cropping of DM (grain crops) and DG (medicinal plants). Specifically, continuous cropping increased the relative abundance of fungi with pathogenic potential, such as Sordariomycetes, Dothideomycetes, Saccharomycetes, and Mucoromycetes in grain soils and Agaricostilbomycetes in herb soils. Among the soil physicochemical properties, NH_4^+-N and pH were the most important factors contributing to changes in the composition of bacterial and fungal communities, respectively. Continuous cropping of different types of plants altered the diversity of the microbial communities, with the most significant effect from the continuous cropping of food crops. Our findings provide a scientific and theoretical basis for future agricultural research to improve soil microbial activity, mitigate continuous-cropping barriers, and increase productivity.

Keywords: continuous cropping; soil microbial community; soil physicochemical

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1. Introduction

As one of the four major black-soil regions in the world, the Northeast China black-soil region plays an irreplaceable and important role in ensuring regional ecological and environmental security, national food security, and sustainable agricultural development. The northeast black-soil region is an important commercial base for grain in China [1], and since 1949, the share of grain production in the northeast has increased from 11.5% to 16.5% in the country. It provides up to 30–35 million tons of commercial grain per year, with maize accounting for 90% of the grain commodities [2]. In addition to producing food crops, the region's farmland has been cultivated with medicinal plants for the past 40 years [3]. However, the high-intensity, continuous use of black soil resources due to the pursuit of economic yield has led to increased soil erosion, declined soil organic matter, thinning

of the black-soil cultivation layer, low soil-nutrient reservoir capacity, and increased soil acidification. This has led to the serious degradation of black soils, turning Northeast China from an "ecologically functional area" to an "ecologically fragile area", and severely limiting the sustainable development of agriculture in Northeast China [4–6].

In order to preserve the viability of soil ecosystems and plant health, soil microbes are crucial [7]. The composition of the microbial community can be considerably altered by various farming strategies [8]. The type of cropping system, the soil type, and the crop species all have a role in these changes. For example, Tang et al. and Zhu et al. found that the abundance of Actinomycetes was significantly reduced under continuous cropping in soybeans compared to rotational systems [9]. According to research by Garbeva et al., long-term continuous-cropping causes a buildup of soil-borne plant diseases and lowers crop yields. Continuous cropping, according to Xu et al., decreased Penicillium abundance while increasing Fusarium abundance. In contrast to crop rotation, a different study found that continuous soybean cultivation did not alter the composition of the microbial population. The inconsistency between these studies was mostly caused by variations in the types of soil, research techniques, and continuous-cropping years. As a result, the mechanisms behind plant continuous cropping are intricate and require further investigation.

Previous studies on continuous cropping have focused on nutrient disorders and autotoxicity caused by chemosensitizers in root secretions [10,11]. However, recent studies have found that inter-root microorganisms play an important role in plant growth as a second genome of the plant along with the plant-associated microbiome. Hu found that potato continuous cropping reduced soil quality, growth, and development and increased the relative abundance of harmful microorganisms [12]. Wu found that the replantation disease of *P. heterophylla* was associated with changes in the bacterial community structure and potential [13]. Gao found that continuous cropping altered the fungal community structure of sweet potato inter-root soil, disrupting the balance between beneficial and harmful microorganisms and reducing the yield and quality of the sweet potatoes [14]. Although plant–microbial community interactions have been reported to play a crucial role in crop health, the effects of different continuous cropping on soils are different. Several studies have shown that changes in the soil microbiota are associated with changes in the plant species and soil environment types [15–17]. Therefore, it is hypothesized here that the continuous cropping of food crops and medicinal plants may directly affect soil chemical properties and soil microbial communities, and may further negatively affect plant growth. It is important to compare the differences in the specific soil microbiomes and the changes in soil physicochemical properties under the long-term continuous cropping of different types of crops to understand the importance of the effects of long-term continuous cropping on the soil.

In this study, sampling studies were conducted on the continuous cropping of food-crop (soybean and corn) soil, medicinal-plant (perilla and mint) soil, and uncultivated soils. The aims of this study were to (i) analyze and compare the composition and structures of the bacterial and fungal community structures of two continuous-cropping soils and uncultivated soil; (ii) elucidate the coupling relationship between changes in the physicochemical properties and changes in the soil microbial communities of two continuous-cropping soils and uncultivated soil; (iii) explore the relevant communities and their potential disease capacity under the continuous cropping of food crops and medicinal plants, and provide a theoretical basis for the control of disorders of continuous cropping and soil nutrient management. The results of this study may enable scientists to develop guidelines for good agricultural practices and give recommendations for nutrient management on farmland under continuous cropping to ensure the sustainable production of food crops and medicinal plants in Northeast China.

2. Materials and Methods

2.1. Sampling

Soil samples were collected in October 2021 at the experimental field of the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun, Jilin Province (125°406′554″ E, 44°000′361″ N). The area is located at an altitude of 250 m in the northeastern part of the country, with an average annual temperature of 6.5 °C, a frost-free period of about 150 days, an annual precipitation of 800–1000 mm, and a brown loam soil type [18]. Soil samples were randomly collected from soils that had not been plowed for many years (CK), soils with five consecutive years of the medicinal plant, perilla (DG1); soils with five consecutive years of the medicinal plant, mint (DG2); soils with five consecutive years of the food crop, maize (DM1); and soils with five consecutive years of the food crop, soybean (DM2). Five-point sampling was used to gather samples from each area in order to acquire representative samples. For a total of fifteen samples, five soil samples from each group were taken, pooled into one sample, and then reproduced three times. Samples were promptly stored at 4 °C for subsequent analysis and at −80 °C for sequencing after being aseptically extracted from the soil surface-growth layer (0–20 cm depth), sieved to 2 mm into 50 mL centrifuge tubes for homogenization, and then stored.

2.2. Soil Physicochemical Analysis

Soil pH was determined by the leaching potential method (soil–water ratio of 1:5) [18]; soil total phosphorus (TP) was determined by H_2SO_4 ablation—molybdenum sulfate inverse colorimetric method; total nitrogen (TN) was determined by the Kjeldahl method—continuous-flow analyzer; soil organic matter (SOM) was determined by the external heating method—potassium dichromate volumetric method [19]. Soil effective phosphorus (AP) was determined by 0.5 mol-L^{-1} $NaHCO_3$ extraction—molybdenum sulfate inverse colorimetric method; fast-acting potassium (AK) was determined by 1 mol-L^{-1} CH3COONH4 extraction—inductively coupled plasma-emission spectroscopy (ICPS-7500) ICP-AES method [20]. Soil fast-acting nitrogen (NH_4^+-N) was determined by 2 mol·L^{-1} KCl extraction with a flow analyzer.

2.3. DNA Extraction, PCR, and Sequencing

DNA was extracted, and the concentration was assayed and purified as described by Ji [18]. The V3–V4 hypervariable region of the bacterial 16S rRNA gene was amplified using primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTW TCTAAT-3′); the primers used to amplify the fungal ITS1 region were 5-GGAAGTAAAAGT CGTAACAAGG-3 and 5-GCTGCGTTCTTCA TCGA TGC-3. The 50 μL PCR reaction system had the following components all included in the kit: dNTPs (2.5 mmol/L), forward and reverse primers (10 μmol/L each), 10 Pyrobest buffer (5 μL), Pyrobest DNA polymerase (2 U/μL), and dissolved DNA in ddH_2O (36.7 μL, 1 ng/μL). Temperature was set to 95 °C for five minutes, followed by 25 cycles at 95 °C for 30 s, 56 °C for 30 s, and 72 °C for 40 s in the PCR process [21]. Paired-end sequencing was performed on the Illumina-MiSeq platform, and the raw data were filtered by truncating the end sequences of reads with quality values lower than 20, removing junction-contaminated reads, removing N-containing reads, and removing low-complexity reads (Adjustment of Short reads, v1.2.11). The paired reads obtained by double-end sequencing were assembled into one sequence using the overlap relationship to obtain high-variation region tags (minimum match length of 15 bp, allowable mismatch rate of 0.1 in the overlap region, removed reads without overlap relationship), and used QIIME2 classify-sklearn algorithm (https://github.com/QIIME2/q2-feature-classifier, accessed on 29 December 2021). The QIIME2 program was used to annotate the species for each ASV feature sequence using a pretrained simple Bayesian classifier with default settings. Using the Greengenes database (Release 13.8, http://greengenes.secondgenome.com, accessed on 29 December 2021), species classification annotation of bacterial 16S rRNA sequences was carried out; for species taxonomic annotation of fungal ITS sequences, the UNITE database (Release 8.0,

https://unite.ut.ee, accessed on 29 December 2021) was used. At 0.6, the confidence level was established.

2.4. Bioinformatics and Statistical Analysis

To determine whether the sample sequencing depth reached saturation, sparsity curves and taxonomic composition were plotted by using QIIME2 (2019.4). To determine alpha diversity indices, including Chao1 and Shannon indices, the "phyloseq" package of Microbiome Analyst1 was used for export. To determine the number of shared and unique OTUs between different samples, the "VennDiagram" package in R (v3.6.1) was used. To determine differences in microbial structures, principal coordinate analysis (PCoA) based on Bray–Curtis distances was used. To determine differences in relative abundance at the phylum level, ANOVA was calculated. R's "vegan" package was used to conduct redundancy analysis (RDA) to show the connection between bacterial and fungal communities (v3.6.1). In order to examine the differences in relative abundance of bacteria and fungi between different soil types, i.e., long-term continuous-cropping soils and fallow soils, a statistical-significance test was performed using the STAMP analysis software (version 2.1.3). Correlation coefficients based on total OTU and Spearman's rank correlation coefficient ($|p| > 0.6$, $p < 0.05$) were adjusted for multiple testing to see if changes in microbial communities were connected to the soil environment. Functional prediction analysis was carried out utilizing the FAPROTAX software to look into the bacterial function of various samples, and the FUNGuild software to look into the fungal function of various samples. With the aid of SPSS, Welch's *t*-test and one-way ANOVA (Tukey's multiple-comparison test) were performed to examine mean differences between samples (v20.0). All results were deemed statistically significant if the *p*-value for differences was less than 0.05.

3. Results

3.1. Soil Physicochemical Properties

Changes in the physicochemical properties of inter-root soil samples from different plants in continuous cropping are shown in Table 1. Compared with CK, long-term continuous cropping significantly reduced soil pH in DG and DM. In addition, DG and DM were significantly different, not only between groups, but also within groups. NH_4^+-N, TP, TN, and SOM were significantly lower in DG1 compared to DG2; NH_4^+-N, TN, and SOM were also significantly lower in DM2 compared to DM1, while TP was significantly higher ($p < 0.05$) than in either group (Table 1).

Table 1. Soil physicochemical data under different treatments. "a, b, c, d, e" indicates the significance of differences between data.

Treat	CK	DG1	DG2	DM1	DM2
pH	7.12 ± 0.01 a	6.69 ± 0.02 c	6.73 ± 0.01 c	6.83 ± 0.02 b	6.26 ± 0.03 d
AP (mg/kg)	10.42 ± 0.10 bc	6.02 ± 1.83 c	7.57 ± 0.45 c	13.06 ± 1.42 b	30.08 ± 4.16 a
NH_4^+-N (mg/kg)	32.16 ± 2.23 d	18.43 ± 0.88 e	44.21 ± 0.68 c	94.71 ± 1.34 a	73.81 ± 0.79 b
AK (mg/kg)	176.52 ± 3.16 a	174.77 ± 5.79 a	170.03 ± 8.61 a	177.34 ± 8.94 a	164.51 ± 38.18 a
TP (g/kg)	0.60 ± 0.01 d	0.59 ± 0.02 e	0.63 ± 0.01 c	0.69 ± 0.02 b	0.92 ± 0.01 a
TN (g/kg)	1.93 ± 0.01 c	1.60 ± 0.02 e	1.95 ± 0.02 b	2.95 ± 0.02 a	1.66 ± 0.01 d
SOM (mg/kg)	15.88 ± 0.20 b	16.15 ± 0.29 b	17.05 ± 0.14 a	17.09 ± 0.56 a	13.76 ± 0.11 c

Data in the table are mean ± standard deviation ($n = 3$). Different lowercase letters indicate one-way ANOVA (Tukey's multiple-comparison test). pH—pH; AP—effective phosphorus; NH_4^+-N—soil ammonium nitrogen; AK—effective potassium; TP—total phosphorus; TN—total nitrogen; SOM—soil organic matter; CK—soil with many years of unopened stalks; DG1—soil planted with many years of volatile medicinal plant, perilla; DG2—soil planted with many years of volatile medicinal plant, mint; DM1—soil planted with many years of oilseed grain crop, soybean; DM2—soil planted with many years of oilseed grain crop, maize.

3.2. Diversity of Microbial Communities

Shannon indices of soil bacterial communities were significantly lower in DM and DG compared to CK ($p < 0.05$) (Figure 1B). Shannon indices of soil fungal communities were

similarly significantly lower in the DM group ($p < 0.05$) (Figure 1D), while there were no significant differences in bacterial and fungal Chao1 indices in DG and DM compared to CK ($p < 0.05$) (Figure 1A,C).

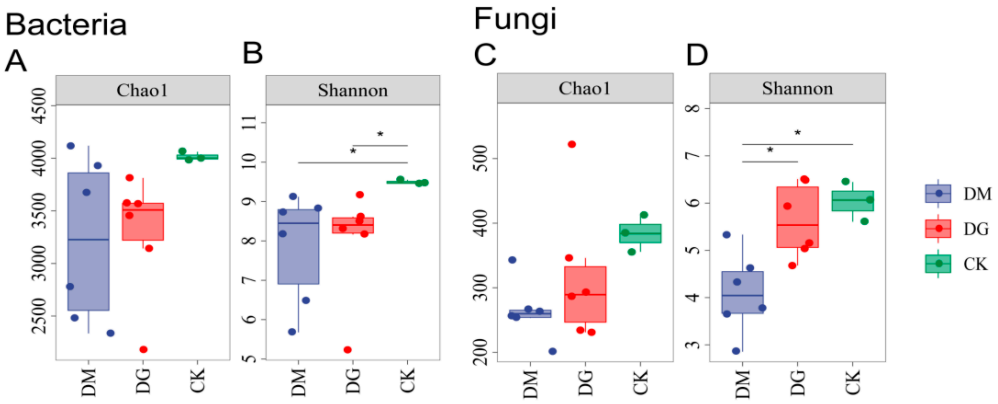

Figure 1. For bacterial (**A**,**B**) and fungal (**C**,**D**) communities, Chao1 and Shannon indices. The symbol * above the bar indicates significant differences between samples ($p < 0.05$).

Additionally, a PCoA ranking method based on the the Bray–Curtis distance matrix was used to assess the general pattern of the bacterial and fungal communities of DM, DG, and CK (Figure 2). The PCoA analysis of bacterial communities revealed that the first principal component (PCo1) and the second principal component (PCo2) could explain 31.1% and 19.41% of all variables, respectively (Figure 2A). The PCo1 and PCo2 could explain 30.1% and 16.47% of all variables, respectively, in the PCoA analysis of fungal communities (Figure 2B). Taken together, the soil microbial composition of DM, DG, and CK groups differed (Figure 2).

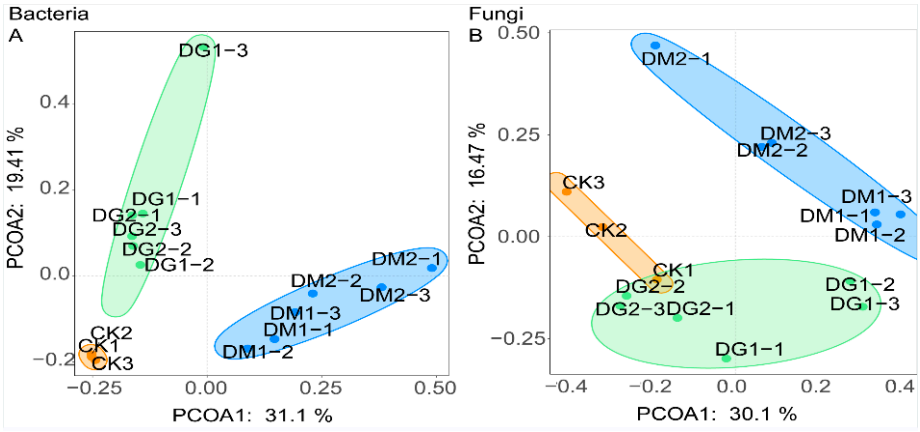

Figure 2. Communities of bacteria (**A**) and fungi (**B**) were analyzed using principal coordinate analysis (PCoA). Each dot stands for a sample, and variously colored dots denote various categories. Long-term fallow soils in CK, long-term continuous cropping of the volatile medicinal plant, perilla, in DG1, long-term continuous cropping of the volatile medicinal plant, mint, in DG2, long-term continuous cropping of the grain crop, corn, in DM1, and long-term continuous cropping of the grain crop, soybean, in DM2 are examples of long-term continuous-cropping soils.

3.3. Bacterial and Fungal Populations in Soil Composition

In order to investigate how soil microbial taxa respond to various agricultural continuous crops, the fluctuations in the relative abundance of microbial communities at various stages was evaluated. The top 10 clades, in terms of average relative abundance, are shown in Figure 3. Among them, Proteobacteria (27.24~51.60%), Actinobacteria (18.65~39.01%), Acidobacteria (7.82~15.21%), Bacteroidetes (2.53~11.13%), and Chloroflexi

(3.92~6.43%) were the most dominant bacterial phyla in the three groups of black-soil samples (Figure 3A); Ascomycota (73.64~89.87%) and Basidiomycota (5.41~18.32%) were the most abundant fungal phyla in the three groups of black-soil samples (Figure 3B). When compared to CK soil, the relative abundance of Proteobacteria increased in the bacterial population, while Actinobacteria, Acidobacteria, and Chloroflexi declined (Figure 3A). When compared to CK, the relative abundance of Ascomycota in DG soil and Basidiomycota in DM soil decreased in the fungal community (Figure 3B).

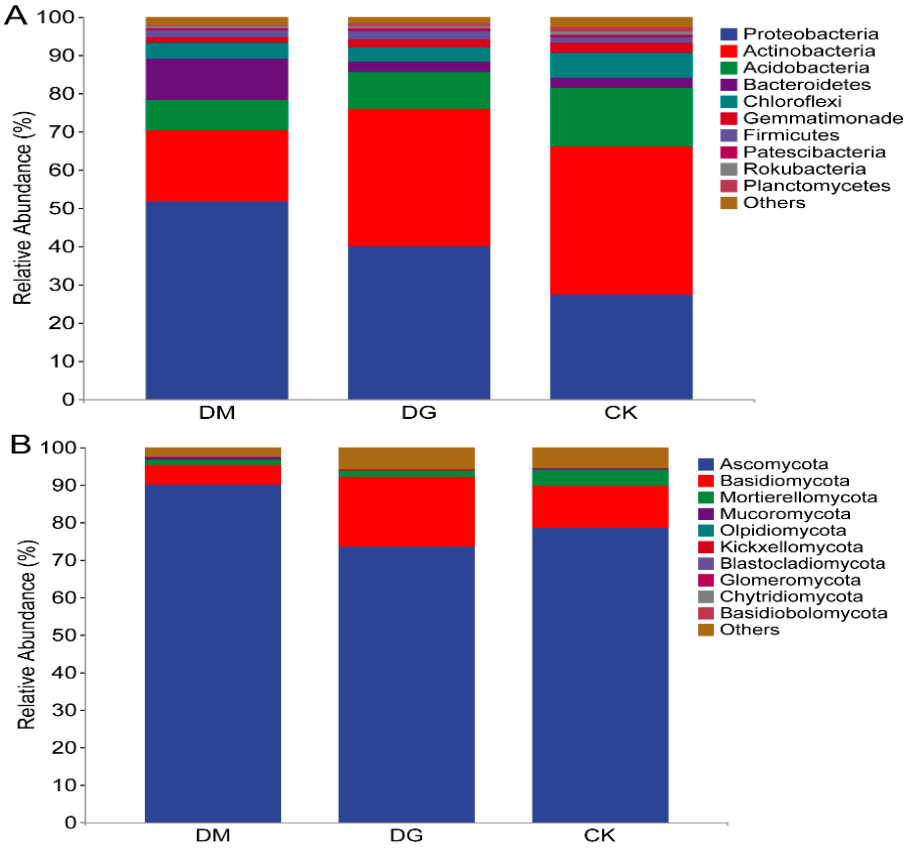

Figure 3. Phylum levels of the top 10 bacteria (**A**) and fungi (**B**) in terms of average relative abundance.

The Venn diagram also illustrated that the proportion of unique microbial taxa increased with prolonged cultivation of medicinal and grain compared to CK. The proportion of unique bacterial and fungal taxa was higher in the DM and DG soil samples than in the CK soil samples, and the proportion of unique bacterial and fungal taxa was higher in the DG soil samples than in the DM soil samples (Figure 4). To further determine the microbial response to different types of soils, STAMP analysis ($p < 0.05$) was performed at the level of the bacterial and fungal genera (Figure 5). It was found that at the level of the bacterial genera, there were 106 differential taxa in CK compared to DM ($p < 0.05$), and the top 10 genera with the greatest differences were found to be Blastococcus, Marmoricola, Krasilnikovia, Rubrobctaer, Modestobacter in soils of the DM group. In addition, Azospirillaceae, Skermanella, Geodermatophilus, and Solirubrobacter were significantly lower in relative abundance, while the relative abundance of Rhizobacter was significantly higher (Figure 5A). Ninety-five taxa were found to differ between CK and DG ($p < 0.05$), and the top 10 genera with the greatest differences were found to be Skermanella, Solirubrobacter, Marmoricola, Rubrobacter, Nocardioides, Blastococcus, Haliangium, Jatrophihabitans, and Geodermatophilus, which were significantly decreased in relative abundance, and Pelomonas, which was significantly increased in relative abundance (Figure 5B). At the fungal genus level, there were 10 differential taxa in CK compared to DM ($p < 0.05$), with significantly lower relative abundances of Solicoccozyma, Tetracladium, Cladophialophora,

Preussia, Dokmaia, and Monodictys in the DM group soils, while Alternaria, Olpidium, Didymellaceae, and Hymenoscyphus were significantly higher in relative abundance (Figure 5D); for CK compared to DG ($p < 0.05$), there were Monodictys, Myrmecridium, Dokmaia, and Nectriaceae in soils of the DG group. The relative abundance of Monodictys, Myrmecridium, Dokmaia, and Nectriaceae was significantly reduced, while the relative abundance of Basidiomycota and Filobasidium was significantly increased in the soil of DG group (Figure 5E).

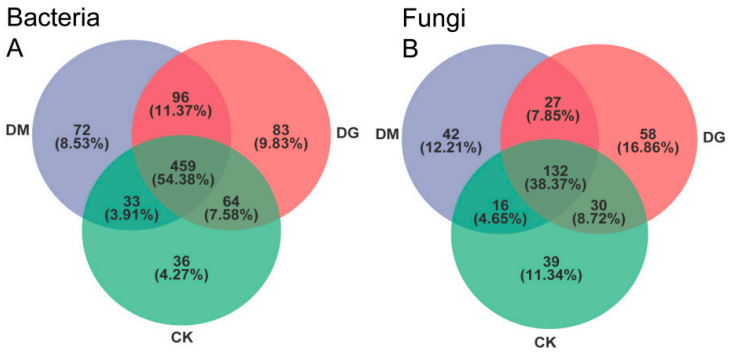

Figure 4. Venn diagrams of bacteria (**A**) and fungi (**B**) from different soil types at the genus level in the soil.

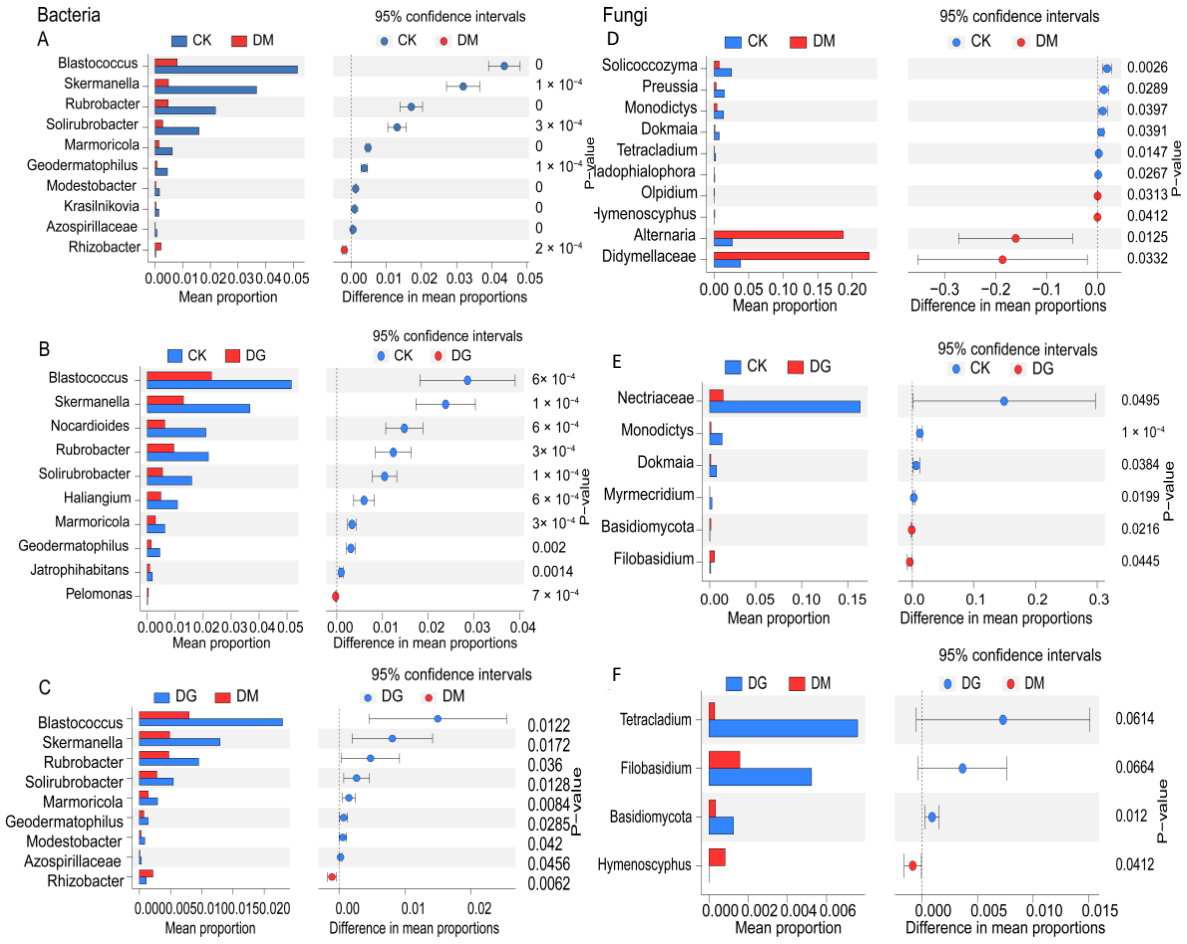

Figure 5. STAMP analysis of different soil samples with differing taxa (95% confidence intervals, $p < 0.05$). Bacteria (**A**,**B**) and fungi (**D**,**E**) indicate significant responses of genera between CK and DM and between CK and DG, and bacteria (**C**) and fungi (**F**) indicate significant responses of differential genera (**A**–**D**) between DM and DG.

The proportions of unique bacterial and fungal groups in the DG soil samples were higher than the proportions of unique bacterial and fungal groups in the DM soil samples, as known from the Venn diagram. To understand the differences between long-term cultivation of food crops and medicinal plants, we performed further comparative analyses of the above differential taxa obtained from CK compared to DM and CK compared to DG. It was found that, at the bacterial genus level, there were nine differential taxa ($p < 0.05$) in DM compared to DG ($p < 0.05$). Marmoricola, Blastococcus, Solirubrobacter, Skermanella, Geodermatophilus, Rubrobacter Modestobacter, and Azospirillaceae significantly increased in relative abundance, while Rhizobacter significantly decreased in relative abundance (Figure 5C). At the fungal genus level, there were four differential taxa in DM compared to DG ($p < 0.05$), with Basidiomycota, Tetracladium, and Filobasidium significantly increased in relative abundance in DG soils and Hymenoscyphus significantly decreased in relative abundance in DG soils (Figure 5F).

3.4. Network of Soil Bacterial and Soil Fungal Communities That Co-Occur

To track changes in microbial interactions during various plant continuous-cropping types, fungal and bacterial networks based on the class level were built. The information demonstrated that the number of nodes of DM, DG, and CK soil bacterial community networks were 97, 104, and 113, and the number of edges were 360, 268, and 1355, respectively, with positive correlations of 81.11%, 85.07%, and 52.47% and negative correlations of 18.89%, 14.93%, and 47.53%, respectively. The number of nodes in the DM, DG, and CK soil fungal community networks were 19, 21, and 30, and the number of edges were 21, 27, and 90, respectively, with positive correlation ratios of 57.14%, 51.85%, and 62.22% and negative correlation ratios of 42.86%, 48.15%, and 37.78%, respectively (Table 2). Based on the size of the circles in the symbiotic network, i.e., degree, Subgroup_6, Gemmatimonadetes was found to be the core bacteria in the soil community of DM (Figure 6A), and Sordariomycetes, Dothideomycetes, Pezizomycetes, and Cystobasidiomycetes, Saccharomycetes, and Mucoromycetes were the core fungi in the soil community of DM (Figure 6B); Subgroup_20 was the core bacteria in the soil community of DG (Figure 6C), and Dothideomycetes and Agaricostilbomycetes were core fungi in the soil community of DG (Figure 6D). Acidobacteriia, Acidimicrobiia, Thermoleophilia, and Fimbriimonadia were core bacteria in CK (Figure 6E), and Leotiomycetes, Dothideomycetes, Pezizomycetes, Pezizomycotina_cls_Incertae_sedis, Sordariomycetes, Agaricomycetes, Exobasidiomycetes, Microbotryomycetes, and Tremellomycetes were the core fungi in CK (Figure 6F).

Table 2. Analysis of the bacterial and fungal populations' network of co-occurrences.

Sample	Bacteria				Fungus			
	Nodes	Edges	Positive	Negative	Nodes	Edges	Positive	Negative
DM	97	360	81.11%	18.89%	19	21	57.14%	42.86%
DG	104	268	85.07%	14.93%	21	27	51.85%	48.15%
CK	113	1355	52.47%	47.53%	30	90	62.22%	37.78%

3.5. Relationships between Soil Physicochemical Characteristics and Microbial Populations

The results of the redundancy analysis (RDA) of the top 10 genera and the environmental factors of the inter-rhizosphere soil of different plants in continuous cropping are shown in Figure 7. The contributions of bacterial RDA1 and RDA2 were 35.07% and 23.15%, respectively (Figure 7); the contributions of fungal RDA1 and RDA2 were 38.31% and 18.35%, respectively (Figure 7B). The order of effect on the bacterial community structure of soil properties was: soil NH_4^+-N > TP > pH > AP > SOM > TN > AK (Figure 7A); the order of effect on the fungal community structure was: soil pH > NH_4^+-N > TP > TP > AP > TN > SOM > AK (Figure 7B). The results showed that soil NH_4^+-N ($r^2 = 0.8329$, Pr = 0.001) was significantly correlated with the bacterial community; soil pH ($r^2 = 0.6821$, Pr = 0.001) was significantly correlated with the fungal community. This indicates that

in inter-rhizosphere soils with various plant continuous cropping, NH_4^+-N is a strong predictor of the makeup of the bacterial population and pH is a strong predictor of the composition of the fungus community. Additionally, the outcomes of the examination of the Spearman's correlation coefficient were as follows (Figure 8): NH_4^+-N was positively correlated with bacteria Subgroup_6 (R = 0.86), KD4-96 (R = 0.75), Skermanella (R = 0.66), and Blastococcus (R = 0.60), but was negatively correlated with Bradyrhizobium (R = −0.47) and Pseudomonas (R = −0.53) (Figure 8A). pH was positively correlated with the fungi Didymellaceae (R = 0.61) and Alternaria (R = 0.62), but was negatively correlated with Fusarium (R = −0.54) (Figure 8B).

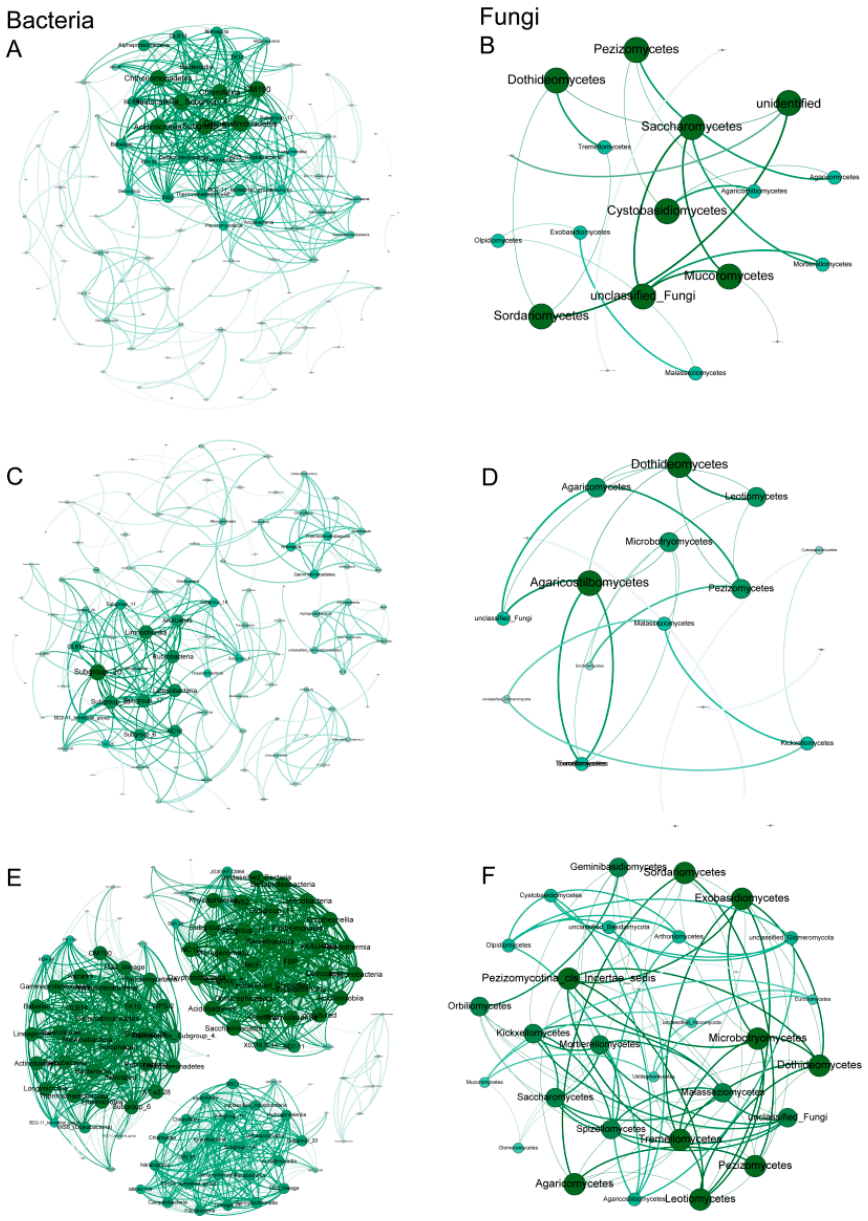

Figure 6. Network diagrams at the level of bacteria and fungi, where (**A**,**B**) are the network diagrams of soil bacteria and fungi at the level of DM long-term continuous crop of food crops, respectively; (**C**,**D**) are the network diagrams of soil bacteria and fungi at the level of DG long-term continuous crop of medicinal plants, respectively; (**E**,**F**) are the network diagrams of soil bacteria and fungi at the level of CK long-term fallow.

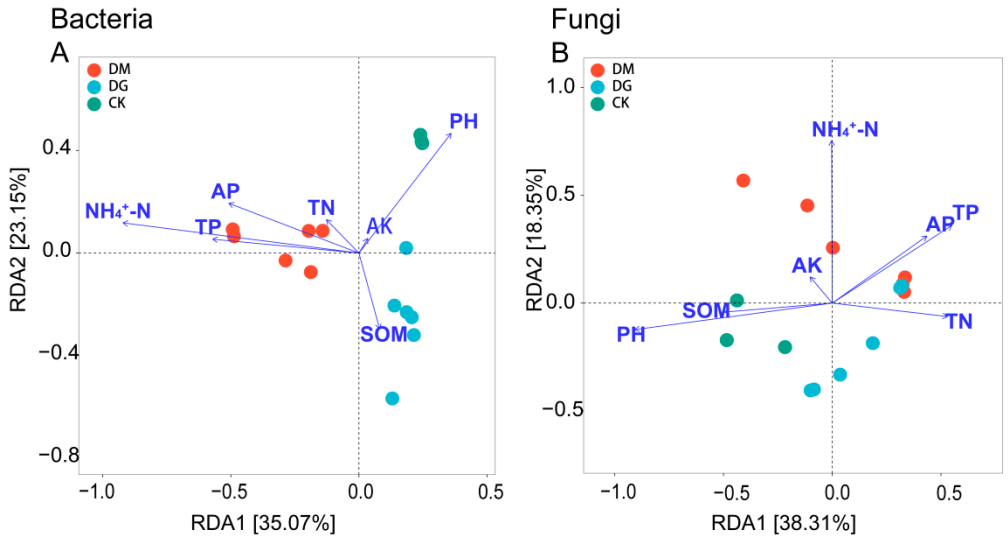

Figure 7. Redundancy analysis of differential bacterial (**A**) and fungal (**B**) and physicochemical factors for different plant continuous-cropping soil types.

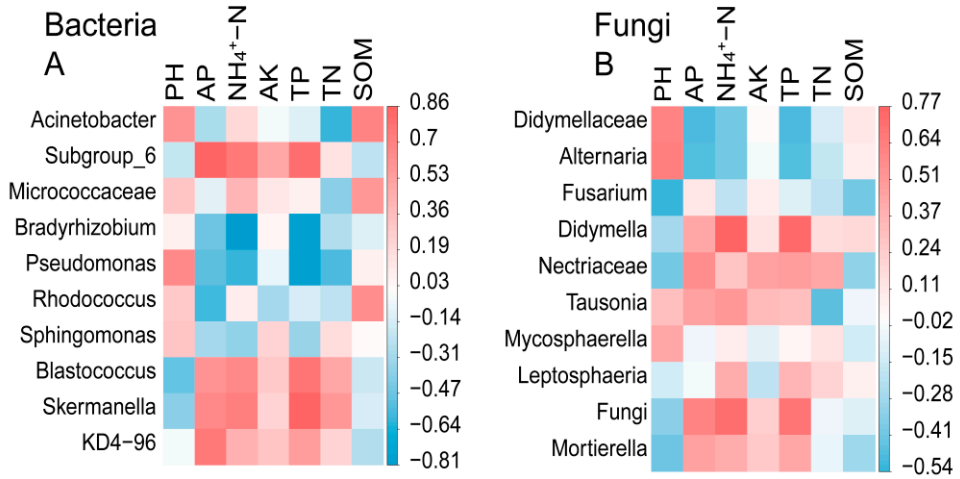

Figure 8. Correlation between genus level of variance and soil properties of differential bacterial (**A**) and fungal (**B**).

3.6. Prediction of Microbial Community Function

FAPROTAX software was used to conduct a functional prediction study to better understand the function of particular microorganisms in the soil of various soil types. According to the prediction outcomes, the primary functions of the various bacterial communities included aerobic chemoheterotrophy, chemoheterotrophy, fermentation, nitrate reduction, aromatic compound degradation, predatory or exoparasitic behavior, manganese oxidation, ureolysis, and nitrogen fixation (Figure 9A). This figure also demonstrated that, with the exception of ureolysis, the relative abundance of metabolic activities linked to chemical abnormalities was generally lower in the DM group than in the DG group. The same was true for metabolic functions connected to nitrogen.

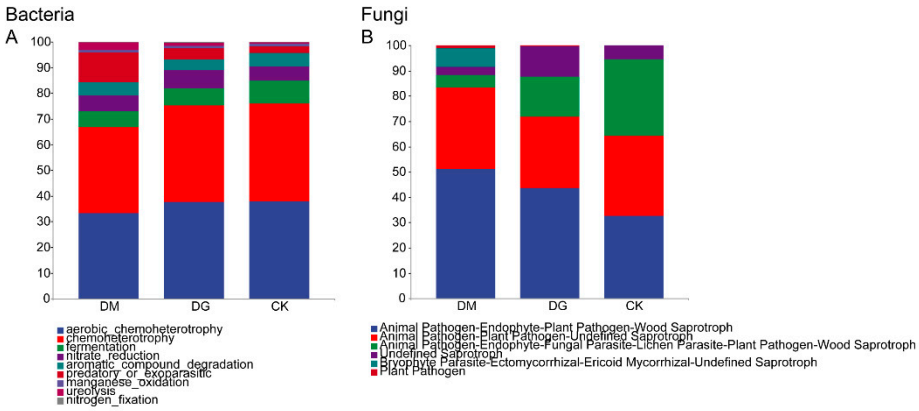

Figure 9. The top 10 bacterial genera in abundance as a function of (**A**) and the top 10 fungal genera in abundance as a function of (**B**).

FUNGuild was used to predict the trophic and functional groups of fungal communities across treatments (Figure 9B). FUNguild detected all two major trophic modes and 78 guilds. The most abundant was animal pathogen–endophyte–plant pathogen–wood saprotroph (32.67–51.22%), followed by animal pathogen–plant pathogen–undefined saprotroph (28.24–31.97%), animal pathogen–endophyte–fungal parasite–lichen parasite–plant pathogen–wood saprotroph (5.21–30.21%), undefined saprotroph (3.06–11.93%), bryophyte parasite–ectomycorrhizal–ericoid mycorrhizal–undefined saprotroph (0–7.38%), and plant pathogen (0–1.16%). The results indicated that the average relative abundance of plant pathogen was higher in DM and DG than in CK.

4. Discussion

Continuous cropping of different types of plants seriously affects the healthy growth and economic development of crops. The hazards include changes in factors of microbial community structure and abundance, accumulation of pathogenic bacteria, alteration of key core taxa, poor soil physicochemical properties, accumulation of root secretions and residual stubble decay, and enrichment of functions related to pathogenic potential. Soil microbes have been found to play an important role in maintaining plant health and associated microbial communities have been introduced as a second genome for plants [22–25]. However, our research indicates that continuous cropping significantly affects the makeup, diversity, and organization of microbial communities in soil [26,27]. In this study, on the same land and showed significant community changes (Figure 2). This result is consistent with the findings of Ji et al. [28], wherein the continuous cultivation of ginseng had a significant effect on the changes in the microbial community structure. Numerous other crops, including soybeans, were also affected by this event [29], as were panax ginseng [30], potato [31] and peanut [32] continuous-cropping systems. Therefore, we can speculate that continuous cropping affects soil the microbial community structure. Furthermore, our results found that continuous cropping reduced bacterial and fungal Chao1 and Shannon indices in soils of DM (for food crops) and DG (for medicinal plants) compared to CK (for long-term fallow soils) (Figure 1), and the Shannon index was significantly lower in DM than in CK soils. This is consistent with previous studies on panax ginseng [33], konjac [34], barley [35], and sorghum [36] under continuous-crop conditions. The differences between species were significant, implying that plant species and continuous cropping can alter the diversity of microbial communities compared to fallow soils [37].

In agroecosystems, the dynamics of organic matter and nutrient cycling are greatly influenced by the soil microbial communities [38]. Changes in the biomass or composition of the soil's microbial population affect the soil's quality [30]. Proteobacteria and Ascomycota phyla were, respectively, the most prevalent bacteria and fungi detected in the soils of the three groups in this investigation (Figure 3A,B). According to a previously published study, Proteobacteria phylum and Ascomycota phylum were the most prevalent phyla

in soybean and corn continuous-crop soils. The relative abundance of the Anaplasma phylum and Ascomycetes rose in the DM group compared to CK (Figure 3A,B) [39–41]. Zhao et al. found that the Proteobacteria phylum—including nitrogen-fixing bacteria, which have a role in crop nitrogen uptake in soil—is involved in various organic-matter carbon and nitrogen cycles in soil [42]. Wang et al. found that ascomycetes are saprophytes that cause mold and the decomposition of plant and animal residues, and speculated that increased ascomycetes might increase the risk of crop diseases [43]. In addition, long-term continuous cropping of grains and drugs reduced the relative abundance of beneficial dominant phyla such as Actinomycetes, Acidobacteria, and Green Curvularia (Figure 3A). In a recent study, Actinomycetes were found to play a key role in soil-borne diseases caused by Fusarium [44]. Subsequently, Trivedi et al. produced a disease-suppression model for Fusarium acnes and found that the abundance of Actinomycetes could be used as a major predictive marker for soil suppression at the continental scale [45]. Acidobacteria are significant soil microorganisms that may breakdown plant wastes, perform photosynthesis, take part in carbon metabolism, and play a critical role in the soil material cycle and the development of the ecological environment. Being a microbe that can use CO_2 as a source of carbon and create energy through photosynthesis, *P. aeruginosa* is also competitive in soils with low nutrient content such as organic matter, and medicine and grain are suitable for planting in areas with sufficient sunlight; therefore, *P. aeruginosa* may have an important role in the growth of medicine and grain. In conclusion, according to our results, the microbial community-composition structure in continuous-cropping soils was found to be significantly changed and altered the relative abundance of the dominant phyla. In addition, continuous cropping reduced the relative abundance of beneficial plant microorganisms that are important for crop production, which has important implications for crop production.

According to studies, each species of plant is inhabited by a distinct microbial community, and many bacteria and fungi that cause illness have co-evolved with plants and have a high host specificity [17,46]. Cardinale et al. found that strawberry inter-rhizosphere actinomycete communities differ from bulked soil in part due to plant species, which is a significant determinant [22]. Likewise, based on the Venn diagram's findings for this study, different types of plants have their own distinct genera (Figure 4). Therefore, the STAMP analysis at the genus level in soil samples of food crops and medicinal plants showed that long-term continuous cropping reduced the relative abundance of beneficial microorganisms such as Blastococcus, Marmoricola, Rubrobacter, Geodermatophilus, Solirubrobacter, and Dokmaia, and increased Alternaria and Didymellaceae. Soil pathogenic bacteria enrichment affects soil health and exacerbates the potential for soil-borne diseases (Figure 5). According to reports, the Alternaria genus contains phytopathogenic and saprophytic species that could harm field crops or lead to the postharvest degradation of plant products [47]. While Didymellaceae was found to be responsible for watermelon molluscum stem blight, this genus can also produce a wide range of secondary metabolites, including toxins, some of which are powerful mycotoxins (such as alternariol, alternariol methyl ether, tenuazonic acid, etc.) with mutagenic and teratogenic properties [48] as well as black rot of pumpkin [49] as the main causal agent. Thus, Alternaria and Didymellaceae have the potential to inhibit the growth of other microorganisms or organisms, leading to reduced metabolic capacity of microbial communities as well as crop diseases, resulting in field losses and yield losses.

Soil microorganisms are a complex network of relationships, with different interactions between different species. In this study, microbial network analysis showed that long-term food continuous cropping and medicinal-plant continuous cropping decreased negative and increased positive correlations among soil bacteria and increased negative and decreased positive correlations among soil fungi compared with multiyear fallow soils. This indicates that long-term food and medicinal crops decreased the competition between bacterial species and fungal species, and increased the cooperation between bacterial species and competition between fungal species, which affected the microbial community

structure (Table 2). In addition, long-term continuous cropping altered the core taxa in the soil, reducing the number of beneficial bacterial species in grain-cropped soils and increasing the number of Sordariomycetes [50], Dothideomycetes [51], Saccharomycetes, and Mucoromycetes [52] as fungi with pathogenic potential. Additionally, Agaricostilbomycetes [53], as core microorganisms in the soil of medicinal plants, greatly increased the risk of disease in medicinal plants.

According to studies, continual cropping causes an imbalance in the microbial community structure of the soil, which lowers soil quality and creates nutritional imbalances [54]. In the present study, total nitrogen, total phosphorus, and organic matter were increased in DM and DG soils compared to fallow soils (CK), which could be attributed to inorganic fertilizer abuse [55]. Tan et al. reported similar outcomes in a panax ginseng continuous crop, which could also be related to a protracted overuse of fertilizer [30]. Numerous studies have revealed that long-term continuous cropping increases the soil's direct or indirect buildup of organic and phenolic acids from inter-root secretions, which may be responsible for the significant reduction in soil pH in DM and DG. This is consistent with the results from Xiong et al., who reported that continuous cropping of black pepper reduced soil pH [56]. Other studies have reported similar results [57–59] (Table 1). It was discovered that the secretion of organic acids alters the microbial population of the soil and causes the creation of ammonia nitrogen in addition to lowering the pH of the inter-root soil [60]. This is also consistent with our results that NH_4^+-N significantly increases in mint, soybean, and maize continuous-cropping soils and that CCA also demonstrates that NH_4^+-N is an important bacterial change factor. The physiology of root and inter-root microbial populations, as well as the bioavailability of various toxic and nutritional elements, are all significantly influenced by soil pH, according to a number of studies, thus making root-mediated pH changes ecologically important, which is consistent with our results that soil pH is a key factor in fungal changes (Figure 7). In addition, continuous cropping of food crops increased the relative abundance of the pathogenic fungi Alternaria and Didymellaceae in soil, and changes in the abundance of Alternaria and Didymellaceae were positively correlated with soil pH and $NH4^+$-N (Figure 8). Thus, they may be influenced by NH_4^+-N and pH, causing changes in the correlation between microorganisms and a new balance in microbial composition.

To further understand the potential impact of microorganisms specific to different soil types in the soil, we performed FAPROTAX and FUNGuild functional analyses and found that long-term continuous cropping reduced the nitrate reduction function in bacteria. This leads to a large accumulation of nitrate in the soil, causing a continuous decrease in soil pH, which is consistent with our results. Secondly, grain and medicinal continuous cropping led to a decrease in chemoheterotrophy function in the soil, which seriously affected respiration and photosynthesis in perilla, mint, soybean, and corn. In addition, we found that continuous cropping increased guilds of plant pathogen and mixed-nutrient plant pathogen. therefore, we hypothesize that the endemic microorganisms in the soil of food crops, as well as medicinal plants in continuous cropping, are highly likely to increase crop morbidity and cause soil-borne diseases.

5. Conclusions

The objective of this study was to compare the differences in soil microbial composition and soil physicochemicals between different types of oil-producing crops, i.e., food crops and medicinal plants, grown continuously for more than 5 years. Our results showed that (1) continuous cultivation resulted in significant changes in soil physicochemical properties and a significant decrease in the soil pH of continuous-cropping soil; (2) the relative abundance of beneficial dominant phyla, such as Actinomycetes, Acidobacteria and Green Curvilinear phyla, decreased after the continuous cultivation of different types of plants; (3) continuous cultivation increased the abundance of food soils with Sordariomycetes, Dothideomycetes, Saccharomycetes, Mucoromycetes, and Agaricostilbomycetes and the relative abundance of fungi with pathogenic potential in herbal soils, increasing the risk of

disease in food crops and medicinal plants. Therefore, these results provide a platform for conducting sustainable agriculture research and a basis for future agricultural research to improve microbial activity and increase the yield of grain and medicinal crops in continuous cropping fields, which is critical for the production of grain and medicine in the northeast.

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