agriculture

MDPI

Article

Analysis of Soil Fungal Community in Aged Apple Orchards in Luochuan County, Shaanxi Province

Xin Xu [1,2], Weitao Jiang [2], Gongshuai Wang [1,3], Fengxia Ding [4], Qianjin Li [5], Ruolan Wang [2], Xuesen Chen [2], Xiang Shen [2], Chengmiao Yin [2,*] and Zhiquan Mao [2,*]

1 Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (Germplasm Resources Utilization), Ministry of Agriculture, Huludao 125100, China
2 State Key Laboratory of Crop Biology, College of Horticultural Science and Engineering, Shandong Agricultural University, Tai'an 271018, China
3 College Agriculture Science and Technology, Shandong Agriculture and Engineering University, Jinan 251100, China
4 Agricultural Comprehensive Service Center of Jieshishan Town, Wudi County, Binzhou 251910, China
5 Apple Industry R&D Center, Luochuan County, Yan'an 727406, China
* Correspondence: cmyin@sdau.edu.cn (C.Y.); mzhiquan@sdau.edu.cn (Z.M.)

Abstract: The Luochuan area is an important area for apple production in China. With the renewal and transformation of aged apple orchards, the occurrence of apple replant disease (ARD) was inevitable and has seriously affected the sustainable development of apples. Therefore, we randomly selected 14 soil samples from aged apple orchards in the Luochuan area to study the structural changes in the soil fungal community. The results showed that there were significant differences in the diversity of fungal communities between different aged apple orchards. The harmful fungi *Gibberella*, *Fusarium*, and *Cryptococcus* existed in 14 aged apple orchards in the Luochuan area, but their abundances were different in different aged apple orchards. A FUN Guild analysis showed that fungi were mainly present in the aged apple orchards in Luochuan in the saprotroph and pathotroph nutrition modes. Pathogenic fungi were widely present, which increased the risk of disease and seriously affected the growth and development of fruit trees. To sum up, there was a strong correlation between the ages of orchards and the unbalanced microbial community structure. Therefore, pathogenic fungi could be prevented and controlled during the renewal and transformation of aged orchards to reduce the impact of ARD on the apple industry.

Keywords: Luochuan; aged; apple orchard; fungal community

Citation: Xu, X.; Jiang, W.; Wang, G.; Ding, F.; Li, Q.; Wang, R.; Chen, X.; Shen, X.; Yin, C.; Mao, Z. Analysis of Soil Fungal Community in Aged Apple Orchards in Luochuan County, Shaanxi Province. *Agriculture* **2023**, *13*, 63. https://doi.org/10.3390/agriculture13010063

Academic Editors: José Antonio Pascual and Margarita Ros

Received: 6 December 2022
Accepted: 19 December 2022
Published: 25 December 2022

1. Introduction

Luochuan County, Shaanxi Province, is in the gully area of the Loess Plateau. It is one of the most suitable areas for planting and producing apples because of its high altitude, sufficient light, large temperature difference between day and night, and season of rain and heat [1,2]. With these natural geographical advantages, apples had been widely grown throughout the county [3]. Through nearly 80 years of development, the apple industry had become the leading industry in Luochuan County, Shaanxi Province, and the main source of income for local farmers [4]. However, with the long-term planting of fruit trees, Luochuan orchards gradually entered the aging stage [5]. During this stage, the growth and development of the replanted trees were poor and the rot disease was serious, even leading to death, which severely restricted the sustainable development of the apple industry of Luochuan County [6].

Fungi are an important type of soil microorganism and play an important role in material circulation and soil-borne diseases [7]. However, fungi are generally considered to be an important pathogen in soil; about 70% of plant infectious diseases are caused by fungi [8]. They can also be used as decomposers of mycorrhizal symbionts [9] and

several refractories [10] compounds in soil, such as cellulose, hemicellulose, and lignin. The long-term cultivation of one crop can damage the soil microbial community structure and species diversity [11]. This phenomenon not only promotes the growth and accumulation of pathogenic fungi but also inhibits the reproduction of beneficial microorganisms [12], finally leading to an imbalance in the soil microbial community structure [13]. It can further damage or disrupt the normal physiological activities of fruit trees, thus reducing plant resistance [14]. Studies have shown that soil pasteurization [15] or fumigation [16,17] can improve the growth of trees in orchard soil with a long history. After replanting fruit trees with sterilized soil in aged orchards, researchers found that the fruit trees planted after soil sterilization grew better than those in the aged orchard [18]. Therefore, it is very important to manage aged orchards by investigating the structures of soil microbial communities. The fungal community compositions of soil ecosystems in apple orchards are usually dominated by *Ascomycota*. *Ascomycetes* are important decomposers in the nutrient cycle [19]. Their dominant genera are *Mortierella*, *Fusarium*, *Chaetomium*, *Rhizoctonia*, *Cylindrocarpon*, and so on [20]. A recent study found that *Fusarium* is the main pathogen causing ARD in China [21]. *Mortierella* and *Chaetomium* are common beneficial microorganisms that not only promote the growth of plants but also improve the level of plant resistance [22].

Soil physical and chemical properties are generally considered as the basic elements of sustainable agricultural production [23]. The types and contents of nutrient elements contained in soil are relatively stable, while the demand types and absorption proportions of soil nutrients by the same crop are basically fixed [24]. Therefore, the long-term planting of a single crop, such as cotton [25], cucumber [26], or strawberry [27], on the same land will cause the deficiency of some elements that are needed by the crop and cause an imbalance in soil nutrients [28]. Soil physical and chemical properties are closely related to microbial community composition and functional composition [29], and microbial communities are greatly affected by soil physical and chemical properties [30]. Therefore, it is of great significance to study the relationship between soil physical and chemical properties and microbial community compositions.

The response of a microbial community to habitat change can be predicted by changes in community function [31]. Therefore, this study used high-throughput technology to further explore the impacts of soil fungal groups in aged orchards on the basis of studying the soil fungal community structure of aged orchards. The results provided a theoretical basis for the regulation of soil microorganisms in aged apple orchards.

2. Materials and Methods

2.1. Soil Sample Collection and Processing

The test soil samples were collected from 14 representative aged apple orchards in Luochuan County, Shaanxi Province (Table S1). They were Gao Bao Village (T1), Lu Bai Village (T2), Bei Anshan Village (T3), Lunar Eclipse Village (T4), Bei Gu Village (T5), Yang Wu Village (T6), Bed Guang Rong Village (T7), Bai Jia Zui Village (T8), Northwest Ding Village (T9), Shang Cao Di Village (T10), Shang Huang Zhang Village (T11), Gu Xian Village (T12), Jing Yao Ke Village (T13), and A Si Village (T14). The specific sampling point distribution is shown in Figure 1. All soil samples were selected from 25-year-old orchards of "Yanfu 3" fruit trees. When taking soil samples, the top layer of soil was removed from 0 to 10 cm, and the rhizosphere soil was collected at 0–60 cm from the position of the tree trays. Each orchard was repeated three times. The soil samples taken from each orchard were mixed thoroughly to form a composite soil sample. The soil sample of each orchard was equally divided into 2 portions. One portion was frozen with liquid nitrogen, while the other portion was kept in a sterile self-sealing bag and brought back to the laboratory for storage.

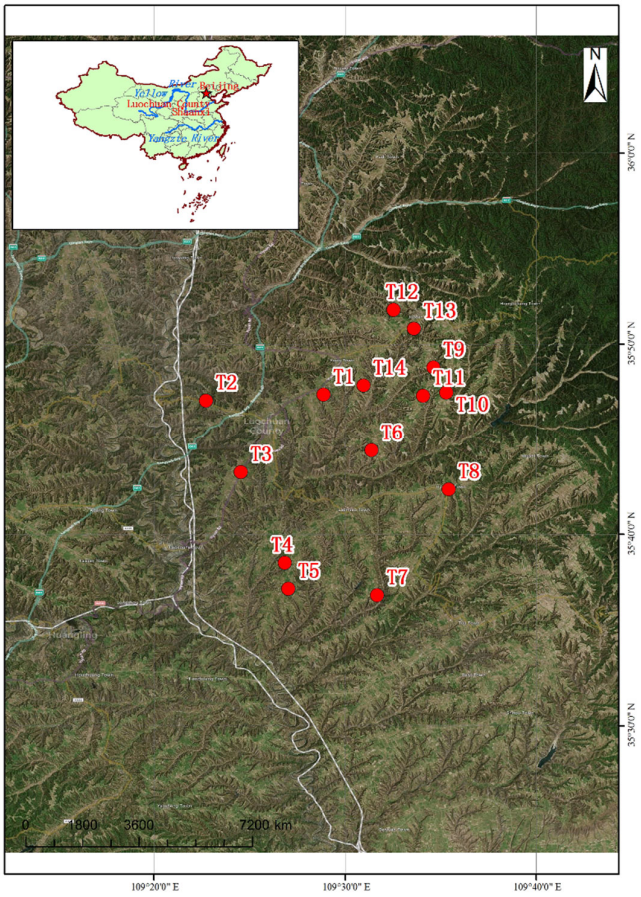

Figure 1. Collection sources of soil samples from 14 aged apple orchards in Luochuan County. Note: the red dot represents the sampling location, and the map was generated by ArcMap. T1: Gao Bao Village; T2: Lu Bai Village; T3: Bei Anshan Village; T4: Lunar Eclipse Village; T5: Bei Gu Village; T6: Yang Wu Village; T7: Bed Guang Rong Village; T8: Bai Jia Zui Village; T9: Northwest Ding Village; T10: Shang Cao Di Village; T11: Shang Huang Zhang Village; T12: Gu Xian Village; T13: Jing Yao Ke Village; T14: A Si Village.

The frozen soils were stored in the refrigerator at −80 °C. Then, 2 g of each of the 14 soil samples were added to clean centrifuge tubes. Each plot was repeated three times and then entrusted to Shanghai Meiji Biomedical Technology Co., Ltd. for high-throughput sequencing analysis. One part of the soil sample was passed through a 1.70 mm (18 mesh) sieve, and the screened soil sample was placed in a clean, non-polluting site to air dry naturally. During the drying period, the soil samples could be turned over to increase the drying speed. The air-dried soil samples were placed in clean Ziplock bags for soil physical and chemical property analyses.

2.2. Determination of Soil Physical and Chemical Properties

The soil physical and chemical properties were determined as described in Xiang et al. (2021) [32]. The physical and chemical properties of soil mainly include the soil available nitrogen, soil available phosphorus, soil available potassium, and soil organic matter content. Among them, the content of soil available nitrogen (AN) was determined with an alkaline dissolution diffusion method. First, 2 g air-dried soil samples were placed in a digestive tube and hydrolyzed with sodium hydroxide and a zinc–ferric sulfate reductant to easily reduce the hydrolyzed nitrogen and nitrate nitrogen to ammonia under alkaline conditions. The distilled ammonia was absorbed in a 2% solution of boric acid and then titrated with a standard acid. The available phosphorus (AP) was determined with a 0.5 mol·L^{-1} sodium bicarbonate extraction that used the Mo antimony colorimetric

method on a UV spectrophotometer (UV-2600, Shimadzu, Japan). The sieved air-dried soil sample was used and shaken with 0.5 mol·L^{-1} NaOH for 30 min. It was then filtered with phosphorus-free filter paper, and the filter liquor was removed by suction. Mo-sb-vc was added after dilution with distilled water, mixed well, and incubated for 30 min. The absorbance was measured at 700 nm. A sample without soil was used as a blank control. Please refer to the Supplementary Material 1.1.1 for the specific determination method. The content of soil available potassium (AK) was determined by a 1 mol·L^{-1} ammonium acetate extraction-flame photometer. Potassium ions were extracted from the soil with 1 mol·L^{-1} NH_4OAc, shaken for 30 min, filtered, and measured on a flame photometer with the standard series. Please refer to the Supplementary Material 1.1.2 for the specific determination method. The content of soil organic matter was determined with a dilution heat method. A total of 0.5 g of soil was added to 1 mol·L^{-1} 1/6 $K_2Cr_2O_7$ and mixed, and 20 mL of concentrated sulfuric acid was added. The mixture was incubated on an asbestos sheet for 30 min after mixing and diluted to 250 mL with water. A phenanthroline indicator was added, and the solution was titrated with 0.5 mol·L^{-1} $FeSO_4$. A sample without soil was used as a blank control. Based on the difference in the mass of the oxidant before and after oxidation, the content of soil organic matter was calculated.

2.3. High-Throughput Sequencing of Soil Fungi

2.3.1. DNA Extraction and PCR Amplification

DNA from soil samples was extracted according to the instructions of a FastDNA® Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA). After genomic DNA was extracted, it was detected by 1% agarose gel electrophoresis, and the DNA concentration and purity were determined by Nanodrop 2000 and finally diluted to 0.5 ng/μL with ddH_2O.

Using the diluted genomic DNA as the template, PCR amplification was carried out with the ITS1F (5′-CTTGGTCATTTAGGAAGTAA-3′) and ITS2F (5′-GCTG CGTTCTTCATCGATGC-3′) PCR primers. Each 20 μL reaction system contained 0.8 μL of DNA (final concentration: 10 ng), 4 μL of 5× FastPfu buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu polymerase, 0.2 μL of BSA, and sterile double-distilled water to a final volume of 20 μL. The thermal cycling conditions were as follows: pre-denaturation at 95 °C for 3 min; 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s; and a final extension at 72 °C for 10 min.

2.3.2. Library Establishment and Illumina Sequencing

The recovered product was purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), detected by 2% agarose gel electrophoresis, and quantified with a Quantus™ Fluorometer (Promega, Madison, WI, USA). Next, a flextm rapid DNA SEQ Kit (Bio Scientific, Avondale, AZ, USA) was used to build the database, and the Miseq PE300 platform from the Illumina company was used for sequencing (Shanghai Meiji Biomedical Technology Co., Ltd., Shanghai, China).

2.4. Bioinformatics Analysis

The original sequences were first quality-controlled using fastp software (https://github.com/OpenGene/fastp, 0.19.6, accessed on 16 October 2018). The 300 bp reads were truncated at any site that received an average quality score of <20 over a 50 bp sliding window, and truncated reads shorter than 50 bp were discarded. Exact barcode matching was required; reads with a 2-nucleotide mismatch in primer matching and reads that contained ambiguous characters were removed. Double-ended sequences were spliced using flash (https://ccb.jhu.edu/software/FLASH/index.shtml, 1.2.11) (accessed on 5 December 2022), and only sequences that overlapped by more than 10 bp were assembled based on their overlapping sequences. Sequences with 97% similarity were grouped into an operational taxonomic unit (OTU) after removing chimeras using uparse software (http://www.drive5.com/uparse/, 7.0.1090) (accessed on 5 December 2022). A total of 2,426,088 valid sequences were obtained from 42 soil samples (14 treatments in

three replicates), with an average sequence length of 258.78 (Table S2). These sequences were distributed among 8029 fungal OTUs. Each sequence was annotated for species classification using RDP classifier (http://rdp.cme.msu.edu/, version 2.2) (accessed on 5 December 2022) and compared to Unite (Release 8.0 http://unite.utee/index.php) (accessed on 5 December 2022), and the comparison threshold was set to 70%. The samples were evaluated using rarefaction curves (Figure S1), and the dilution curve tended to be saturated, justifying the depth of this sequencing and the data indicating that no new species would be generated by continuing sequencing.

2.5. Statistical Analysis

An alpha diversity index analysis was performed using Mothur software (version v.1.30.2, https://mothur.org/wiki/calculators/) (accessed on 5 December 2022) and plotted using origin 2018 software (Origin Lab Cor-poration, Northampton, MA, USA). Venn plots and a PCoA analysis were generated using R language (version 3.3.1), and a microbial abundance heatmap and an RDA analysis were constructed using the vegan package of R language (version 3.3.1). Network software was used to calculate the node degree distribution of the network, the diameter of the network, the average shortest path of the network, and properties such as the degree, closeness centrality, and betweenness centrality to obtain intra- or intergroup correlation information of species and samples. The functional annotation and prediction of fungal communities used the FUN Guild program. The experimental data were expressed as the means ± standard deviations of three replicates, and a one-way ANOVA was performed using SPSS 26.0 software (SPSS Inc., Chicago, IL, USA) to compare significant differences in soil physicochemical properties ($p < 0.05$). Origin 2018 was used to construct graphs.

3. Results

3.1. Soil Physical and Chemical Properties

The content of soil organic matter was between 1.05 and 2.33 g/kg, and the soil organic matter and soil available nitrogen (AK) contents of orchards in T10 were higher than those in the other areas, while the soil organic matter content and soil available nitrogen content of orchards in T5 were the lowest among all orchards (Table 1). The content of available phosphorus in T11 soil was higher than that in the other orchards, and there were significant differences between the T1–T8 area and T14 orchards, while there were no significant differences among T1–T6. The soil potassium content of the T6 orchard was significantly higher than that of other orchards, with 121.19 mg/kg. The soil potassium content of T14 was significantly lower than those of orchards in other areas.

Table 1. Physical and chemical properties of soil samples from different aged apple orchards.

Sample	Organic Matter (SOM) (g/kg)	Available Nitrogen (AN) (mg/kg)	Available Phosphorus (AP) (mg/kg)	Available Potassium (AK) (mg/kg)
T1	1.48 ± 0.08 bcde	39.32 ± 5.75 cde	131.15 ± 31.23 d	56.90 ± 10.02 b
T2	1.21 ± 0.29 e	30.57 ± 4.08 ef	4.55 ± 1.89 d	53.14 ± 16.58 bcd
T3	1.25 ± 0.14 de	34.65 ± 2.02 de	2.03 ± 0.21 d	55.18 ± 4.78 bc
T4	1.47 ± 0.07 cde	31.15 ± 2.02 ef	8.84 ± 6.07 d	46.92 ± 5.28 bcd
T5	1.05 ± 0.26 e	24.15 ± 2.02 f	4.02 ± 2.96 d	28.62 ± 5.72 bcd
T6	1.37 ± 0.15 de	32.32 ± 4.67 ef	18.52 ± 11.24 d	121.19 ± 38.89 a
T7	1.24 ± 0.03 de	32.32 ± 1.17 ef	167.72 ± 1.39 bc	15.14 ± 0.11 cd
T8	1.66 ± 0.01 bcd	35.23 ± 0.58 de	151.65 ± 0.98 cd	14.50 ± 0.12 cd
T9	1.92 ± 0.02 ab	47.48 ± 0.58 abc	177.84 ± 3.58 abc	16.33 ± 0.30 bcd

Table 1. *Cont.*

Sample	Organic Matter (SOM) (g/kg)	Available Nitrogen (AN) (mg/kg)	Available Phosphorus (AP) (mg/kg)	Available Potassium (AK) (mg/kg)
T10	2.33 ± 0.01 a	51.57 ± 0.58 a	199.47 ± 0.70 ab	20.66 ± 0.53 bcd
T11	2.33 ± 0.02 a	49.23 ± 0.58 ab	205.68 ± 1.48 a	20.42 ± 0.13 bcd
T12	1.88 ± 0.08 bc	45.73 ± 0.58 abc	196.22 ± 3.47 ab	19.87 ± 0.05 bcd
T13	1.85 ± 0.06 de	42.23 ± 0.58 bcd	198.42 ± 2.17 ab	17.44 ± 0.38 bcd
T14	1.37 ± 0.05 de	35.23 ± 0.58 de	157.56 ± 2.87 cd	12.45 ± 0.12 d

Note: different letters in the same column indicate significant differences between aged apple orchards ($p < 0.05$). T1: Gao Bao Village; T2: Lu Bai Village; T3: Bei An Shan Village; T4: Lunar Eclipse Village; T5: Bei Gu Village; T6: Yang Wu Village; T7: Bed Guang Rong Village; T8: Bai Jia Zui Village; T9: Northwest Ding Village; T10: Shang Cao Di Village; T11: Shang Huang Zhang Village; T12: Gu Xian Village; T13: Jing Yao Ke Village; T14: A Si Village.

3.2. Analysis of Soil Fungal Community Structures in Different Aged Apple Orchards

3.2.1. OTU Analysis of Soil Fungal Communities

There were obvious differences in the proportions of OTUs in different aged orchards, among which T12 had the largest proportion, followed by T3 (Figure 2a). The soil samples of all aged apple orchards shared 91 OTUs, that is, 91 common species (Figure 2b). In addition, the numbers of unique fungi in different aged apple orchard soil samples showed certain differences. Among them, T5 had the largest number of unique OTUs, T11 had the lowest number of unique OTUs, and T1 and T6 had the same number of unique OTUs.

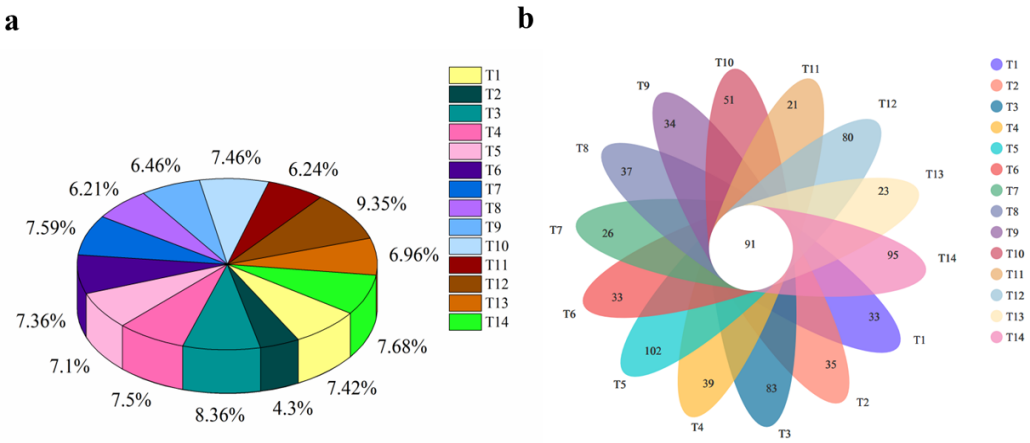

Figure 2. Number of fungal OTUs in soil samples from different aged apple orchards. (**a**) Proportion of total OTUs in different aged orchards; (**b**) Number of unique OTUs in different aged orchards. T1: Gao Bao Village; T2: Lu Bai Village; T3: Bei Anshan Village; T4: Lunar Eclipse Village; T5: Bei Gu Village; T6: Yang Wu Village; T7: Bed Guang Rong Village; T8: Bai Jia Zui Village; T9: Northwest Ding Village; T10: Shang Cao Di Village; T11: Shang Huang Zhang Village; T12: Gu Xian Village; T13: Jing Yao Ke Village; T14: A Si Village.

3.2.2. Soil Fungal Community Composition and Relative Abundance

At the phylum level, the soil fungal community compositions of the 14 aged apple orchards were similar, but there were differences in the relative abundances of fungal phyla among different apple orchards (Figure 3a). The dominant phylum was *Ascomycota*, and the relative abundance of *Ascomycota* was significantly higher in T2 aged apple orchards than in other aged apple orchards (Figure S2a). The second was *Zygomycota*, with the lowest relative abundance in T5 and the highest relative abundance in T14. The relative abundance of *Basidiomycota* was 1.24–35.99% in the 14 apple orchards, and the highest relative abundance of *Chytridiomycota* was found in the T11 orchard. *Chytridiomycota* was present in higher relative abundances in T5 and T8.

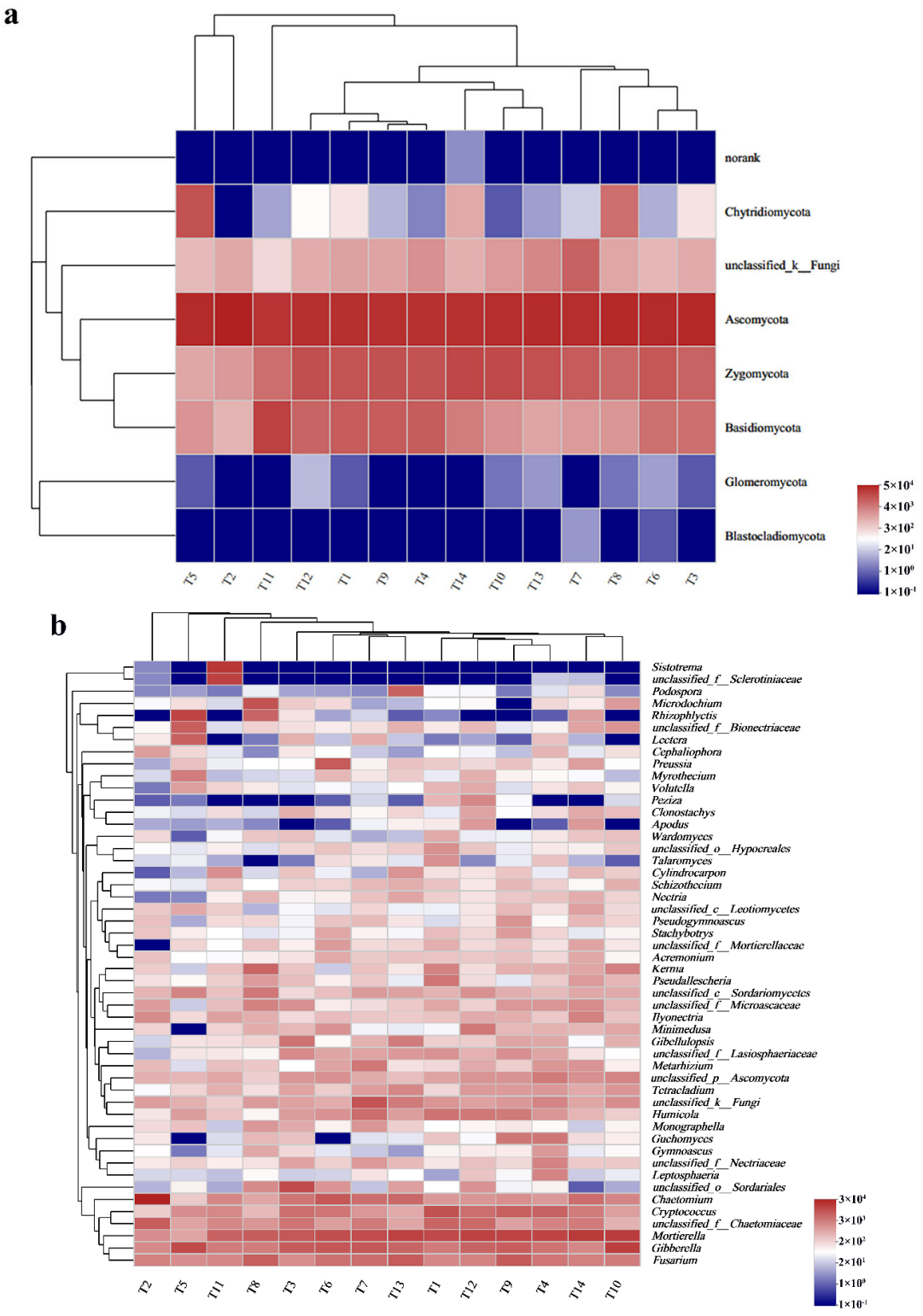

Figure 3. Differences in fungal community structures and compositions in soil samples of different aged apple orchards. (**a**) The compositions of fungal community structures characterized by the phylum level; (**b**) The compositions of fungal community structures characterized by the genus level. T1: Gao Bao Village; T2: Lu Bai Village; T3: Bei An Shan Village; T4: Lunar Eclipse Village; T5: Bei Gu Village; T6: Yang Wu Village; T7: Bed Guang Rong Village; T8: Bai Jia Zui Village; T9: Northwest Ding Village; T10: Shang Cao Di Village; T11: Shang Huang Zhang Village; T12: Gu Xian Village; T13: Jing Yao Ke Village; T14: A Si Village.

There were significant differences in fungal species and relative abundance among the 14 aged apple orchard soils (Figure 3b). *Motierella*, *Gibberella*, *Chaetomium*, *Fusarium*, and *Crptococococcus* fungi were widely present in various orchards in Luochuan. Among them, *Motierella*, *Gibberella*, and *Fusarium* were higher in all orchards of Luochuan (Figure S2b). However, the relative abundances of *Motierella* and *Gibberella* in the T2 area were lower than those in the other orchards, while the relative abundance of *Chaetomium* was significantly higher than those in the other orchards.

3.3. Analysis of Soil Fungal Community Diversity in Different Aged Apple Orchards

3.3.1. Alpha Diversity Analysis of Soil Fungal Communities

The alpha diversity index is a measure of the abundance and diversity of the microbial communities within a sample. Specifically, the Chao index values in the T1, T3, and T12 areas were significantly higher than those in the other orchards (Figure 4a), indicating that the species richness of fungi in the T1, T3, and T12 areas were higher, while the Simpson index had the opposite trend (Figure 4b). The Simpson index is often used to estimate the species diversity in samples. The species diversity in the T12 area was the highest, and that in the T2 area was the lowest.

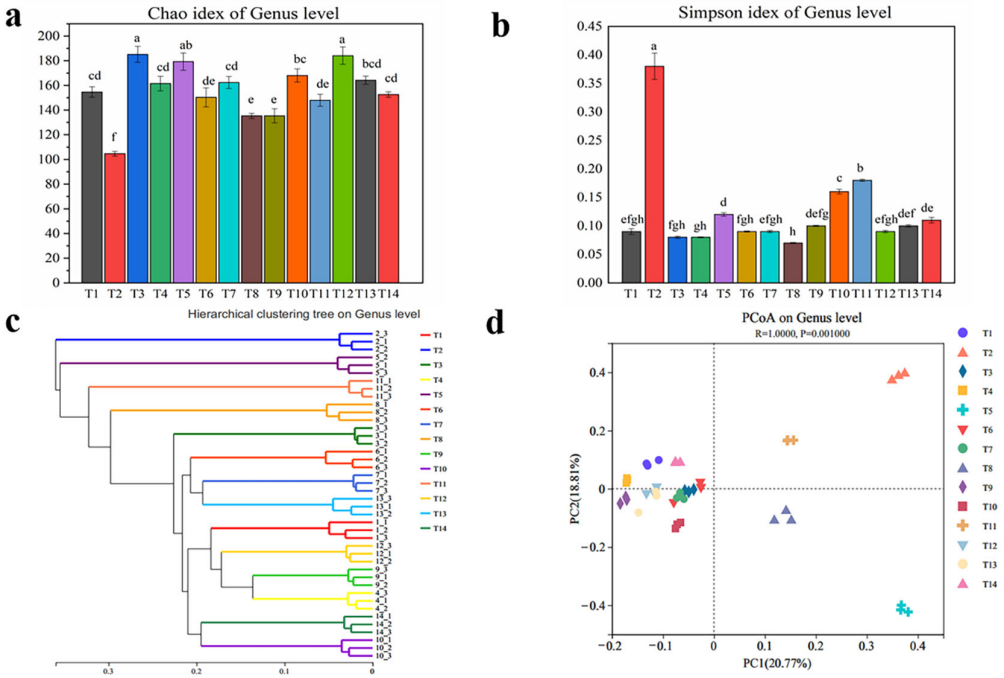

Figure 4. Alpha diversity analysis, cluster analysis, and PCoA analysis of fungal genera in soil samples from different aged apple orchards. (**a**) Chao index of fungi; (**b**) Simpson index of fungi; (**c**) Hierarchical cluster analysis of fungi; (**d**) PCoA analysis of fungi. A single-factor ANOVA was used to test the differences in alpha and beta diversity of soil samples from different aged apple orchards, and the *y*-axis shows the average value of each index. Values followed by different letters indicate significant difference among treatments ($p < 0.05$) T1: Gao Bao Village; T2: Lu Bai Village; T3: Bei An Shan Village; T4: Lunar Eclipse Village; T5: Bei Gu Village; T6: Yang Wu Village; T7: Bed Guang Rong Village; T8: Bai Jia Zui Village; T9: Northwest Ding Village; T10: Shang Cao Di Village; T11: Shang Huang Zhang Village; T12: Gu Xian Village; T13: Jing Yao Ke Village; T14: A Si Village.

3.3.2. Cluster Analysis and PCoA Analysis of Soil Fungal Community

A hierarchical clustering analysis found that the fungal communities in the T4 and T9, T7 and T13, and T10 and T14 regions were the most similar, followed by the T1 and T12 regions, T6 and T13 regions, and T4, T9, and T4 regions (Figure 5). A principal coordinate analysis (PCoA) is a non-constrained data downscaling analysis method that can be used to study similarities or differences in the composition of sample communities. The analysis

results showed that the structures of the fungal communities of the replanted soil in the T2 and T5 areas were more distant from the other orchards (Figure 5), followed by the T8 and T11 areas, which formed independent community structures, while the structures of the soil fungal communities in the other orchards were more similar.

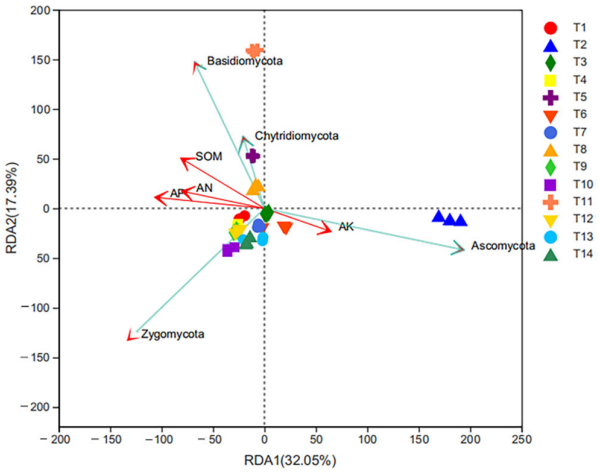

Figure 5. Correlation between fungal community composition and soil physical and chemical properties of soil samples from different aged apple orchards. SOM: soil organic matter; AN: soil available nitrogen; AP: soil available phosphorus; AK: soil available potassium. The values on axis 1 and axis 2 represent the percentage interpreted for each axis. The red arrow represents environmental factors, and the green arrow represents dominant bacteria. Patterns with different colors and shapes refer to soil samples from different aged apple orchards. T1: Gao Bao Village; T2: Lu Bai Village; T3: Bei An Shan Village; T4: Lunar Eclipse Village; T5: Bei Gu Village; T6: Yang Wu Village; T7: Bed Guang Rong Village; T8: Bai Jia Zui Village; T9: Northwest Ding Village; T10: Shang Cao Di Village; T11: Shang Huang Zhang Village; T12: Gu Xian Village; T13: Jing Yao Ke Village; T14: A Si Village.

3.4. Correlation Analysis between Soil Fungal Community Structure and Soil Physicochemical Properties

The results of a redundancy analysis (RDA) explained the relationship between soil physical and chemical properties and soil community composition. *Zygomycota*, *Basidiomycota*, and *Chytriomycota* showed opposite correlations with the AK content (Figure 5) and positive correlations with SOM, AP, and AN.

3.5. Soil Fungal Community Association Network Analysis

The spearman correlation coefficient was used to analyze the two-way correlation network between the top 120 fungal genera and the soil physical and chemical properties ($p < 0.05$). The results showed that the correlations between different fungal genera and soil physicochemical properties were somewhat different. *Cylindrocarpon* was positively correlated with SOM and AN; *Lectera* was negatively correlated with SOM, AN, and AP; and *Cephaliophora* was positively correlated with AK (Figure 6a). A univariate network analysis showed that there were antagonistic or synergistic effects among different fungal genera. For example, *Cryptococcus*, *Humicola*, and *Unclassified—f-lasiosphaeriaceae* showed synergistic relationships with each other, but *Gibberella* had antagonistic effect with *Ilyonectria* (Figure 6b). In addition, it was also found that the eight most abundant fungal genera showed less correlation.

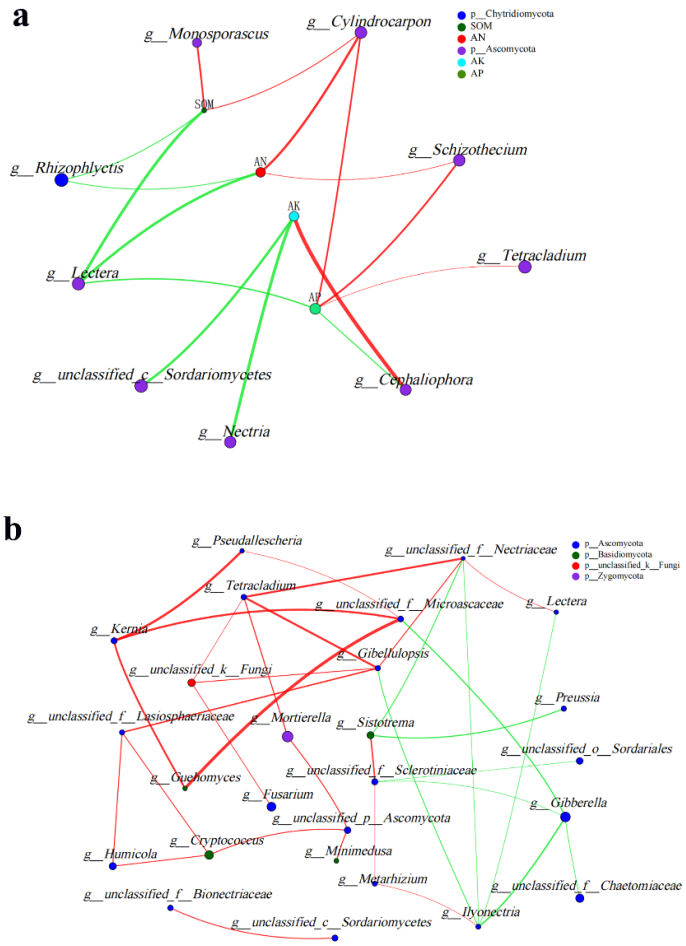

Figure 6. Correlation network analysis of fungal communities in soil samples of aged apple orchards. (**a**) Two-way network correlation analysis of environmental factors and total species abundance of the top 120 fungal genera; (**b**) Univariate network analysis among the top 30 fungal genera at the taxonomic level. The node size is directly proportional to environmental factors and species abundance. Nodes with different colors represent different species. The connecting line color indicates a positive or negative correlation: red indicates a positive correlation, and green indicates a negative correlation. The thickness of the connecting line indicates the magnitude of the correlation coefficient. The thicker the connecting line, the higher the correlation between species; more lines indicate a closer connection between the nodes.

3.6. Functional Prediction Analysis of Soil Fungal Community Taxa

Information on the functional classification of the fungi in the samples and the abundance of each functional classification in the different aged apple orchards could be obtained based on the functional prediction of FUN Guild (Figure 7). There were mainly 12 guilds in the 14 aged apple orchards, and five functional groups had high abundance. They were endophyte—litter saprotroph—soil saprotroph—undefined saprotroph, plant pathogen, undefined saprotroph, animal pathogen—dung saprotroph—endophyte—epiphyte—plant saprotroph—wood saprotroph, and animal pathogen–endophyte—lichen parasite—plant pathogen—soil saprotroph—wood saprotroph. These groups belonged to the saprotroph trophic type, the pathotroph basic type, the saprotroph—symbiotroph cross-trophic type, and the pathotroph—saprotroph—symbiotroph cross-trophic type. In addition, the endophytes—litter saprotroph—soil saprotroph—undefined saprotroph group only corresponded to *Mortierella*. The plant pathogen group corresponded to *Gibberella*, *leptospharia*, and *lectera*; the animal pathogen—dung saprotroph—endophyte—epiphyte—plant saprotroph—wood saprotroph group only corresponded to *Chaetomium*; the animal pathogen–endophyte—lichen parasite—plant pathogen—soil saprotroph—wood sapro-

troph group only corresponded to *Fusarium*; and the fungal parasite–undefined saprotroph group only corresponded to *Cryptococcus* (Table S3).

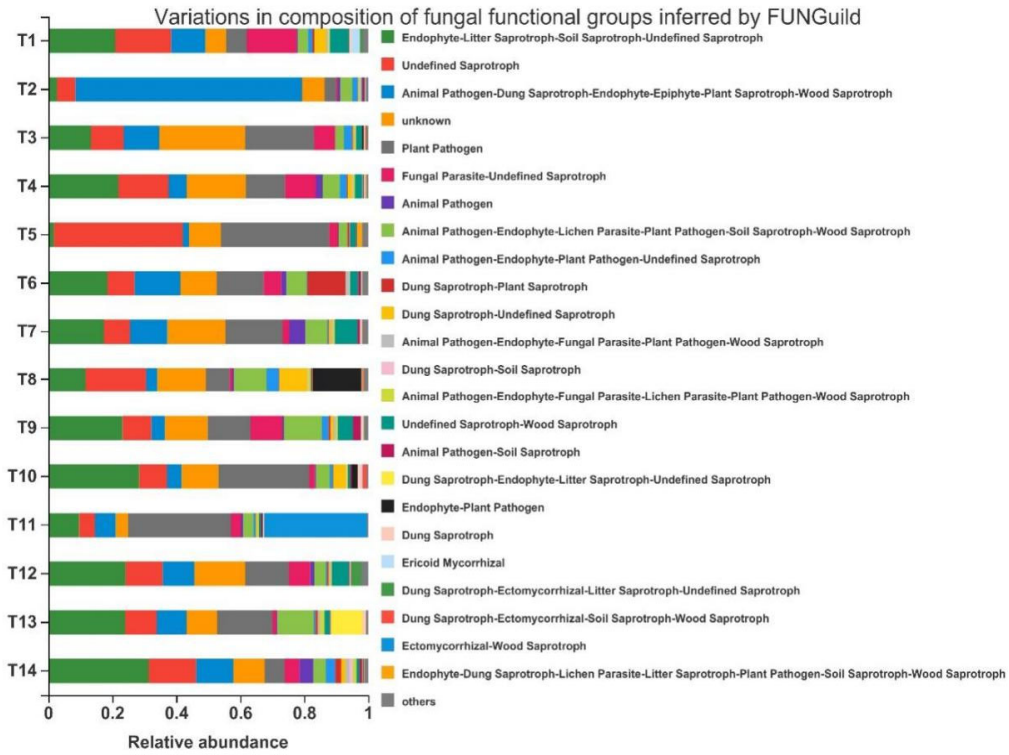

Figure 7. Relative abundance of FUN Guild functional groups of soil fungal communities in different aged apple orchards. T1: Gao Bao Village; T2: Lu Bai Village; T3: Bei An Shan Village; T4: Lunar Eclipse Village; T5: Bei Gu Village; T6: Yang Wu Village; T7: Bed Guang Rong Village; T8: Bai Jia Zui Village; T9: Northwest Ding Village; T10: Shang Cao Di Village; T11: Shang Huang Zhang Village; T12: Gu Xian Village; T13: Jing Yao Ke Village; T14: A Si Village.

4. Discussion

The contents of nitrogen, phosphorus, and potassium in different orchards were also affected by the fertilization management mode and agronomic practices of the growers [13]. The results showed that the soil organic matter in the 14 aged apple orchards in Luochuan was generally low. There were significant differences in the contents of nitrogen, phosphorus, and potassium in different aged apple orchards, which might be related to the years of planting fruit trees and unreasonable fertilization management [33]. In the main apple planting area of the Weibei dryland (including Luochuan), the application amount of organic fertilizer is not high, as 68.3% of orchards do not exceed 500 kg per 667 m^2, and only 6.1% of orchards exceed 1500 kg [34]. In addition, crops had certain selectivity for soil nutrients. Planting the same crop on the same plot year after year could cause a large uptake of soil nutrients, which could lead to reductions in the organic matter, nitrogen, phosphorus, and potassium contents in the soil. The imbalance in sprouting affected the growth and development of plants [35,36].

Soil fungi are an important part of the soil ecosystem. They play an important role in the decomposition of organic matter, nutrient cycling and exchange, the biological control of soil-borne plant pests and diseases, and the promotion of plant growth and development [37]. Some soil fungi are pathogens that cause crop diseases, while other fungi are biocontrol factors that can inhibit or alleviate plant diseases [38]. This experiment found that the dominant phylum of the soil in the 14 aged apple orchards in Luochuan was *Ascomycota*, which was consistent with the research results of Franke-Whittle et al. [39]. *Ascomycota* had higher competitiveness and stress resistance and could use more resources to improve its dominant position in the soil [40]. At the genus level, *Mortierella*, *Gibberella*,

Chaetomium, *Fusarium*, and *Crptococcus* were common and abundant in the 14 aged apple orchards in the Luochuan area. Recent studies have shown that there are also differences in the roles of fungal communities in soil. *Mortierella* can dissolve the fixed insoluble phosphorus and potassium in the soil, convert them into bioactive phosphorus and potassium, provide nutrition for plants, and then improve the disease resistance of plants [22]. It has been proven that *Mortierella* had a significant negative correlation with the incidence rate of ARD [41]. Many members of *Gibberella* and *Fusarium* are pathogens that cause important plant root rot and *Fusarium wilt* [42,43]. *Gibberella zeae* was widely distributed on cereal crops and *Gibberella zeae* causes *Fusarium head blight* (FHB), which often leads to yield reduction and reduces the quality of wheat [44]. The abundance of *Fusarium* was significantly and positively correlated with the severity of apple replant disease (ARD) [45], and *Fusarium* was strongly pathogenic to young, replanted trees [46]. *Cryptococcus* can produce extracellular polymeric compounds that affect soil structure [47]. *Chaetomium* can produce cellobiose dehydrogenase (CDH) and improve the degradation of cellulose by cellulase [48]. The alpha diversity index is a measure of the abundance and diversity of microbial communities within a sample. The results showed that the Chao index values in the T1, T3, and T12 areas were significantly higher than those in the other orchards, indicating that the species richness of fungi in the T1, T3, and T12 areas was higher, while the Simpson index had the opposite trend. Principal coordinate analyses (PCoA) are often used to study similarities or differences in sample community composition. The results of this experiment showed that the community structures of soil fungi in continuous cropping areas T2 and T5 were far away from those in the other areas, forming independent community structures. Therefore, there were also significant differences in the composition and diversity of fungal communities in the soil of aged orchards in different regions, which might be related to the soil physical and chemical properties and the management methods of different aged apple orchards [49].

Changes in soil microbial communities are affected by soil physical and chemical properties [50]. SOM can provide nutrients for microbial growth [51]. The contents of nitrogen, phosphorus, and potassium in soil affect the compositions and structures of soil bacterial and fungal communities [52]. A redundancy analysis (RDA) mainly explained the relationships between the soil physical and chemical properties and the soil community composition. This study found that *Zygomycota*, *Basidiomycota*, and *Chytriomycota* showed opposite correlations with the AK content and positive correlations with SOM, AP, and AN. *Zygomycota* was positively correlated with SOM, AP, and AN. Different dominant fungi were closely related to soil physical and chemical properties, which was consistent with the research results of Zhao et al. [53]. Soil physical and chemical properties can affect the abundance and activities of microorganisms and soil animals and can indirectly affect the yield and quality of crops [54]. A network analysis was used to reveal the co-occurrence patterns or relationships of soil microbial communities [55]. The adaptability of complex fungal communities to soil nutrients might also affect the collinear network structures of soil fungi [56]. This study found correlations between fungal communities and soil physical and chemical properties in aged apple orchards. For example, *Cylindrocarpon* was positively correlated with SOM and AN. The availability of nutrient resources might affect the symbiotic patterns of microorganisms in soil [57,58]. The correlation network analysis showed that *Cryptococcus*, *Humicola*, and *Unclassified—f-lasiosphaeriaceae* showed a synergistic relationship, but *Gibberella* had an antagonistic effect with *Ilyonectric*. Complementary traits among microorganisms could promote microbial symbiosis [59]. This phenomenon indicated the existence of similar ecological niches among microorganisms [60]. Some studies have also shown that the similarity between microbial niches might cause microbial competition for nutrients [61], so the antagonism between species might be caused by competition for the same nutrient.

In this experiment, the FUN Guild method was used to predict the functions of the fungal communities in different aged apple orchards. The results showed that the functional abundances of endophyte—litter saprotroph—soil saprotroph—undefined saprotroph,

plant pathogen, undefined saprotroph, animal pathogen—dung saprotroph—endophyte—epiphyte—plant saprotroph—wood saprotroph, and animal pathogen—endophyte—lichen parasite—plant pathogen—soil saprotroph—wood saprotroph were higher, and the higher functional abundances of *Mortierella*, *Gibberella*, *Chaetomium*, and *Fusarium* corresponded to their higher abundances in the community compositions. The functional prediction further showed that *Gibberella* and *Fusarium* were the key pathogens that caused ARD. *Gibberella* is a sexual stage of *Fusarium* that produces specific toxins and affects the growth and development of plants [62]. Studies have shown that *Gibberella* is an important pathogen that causes plant root rot and *Fusarium Wilt* [42,43]. Wang et al. [63] detected *Fusarium*, which is a pathogenic fungus with a high abundance, in ten replanted orchard soils around the Bohai Sea. Xiang et al. [32] also emphasized that the degree of occurrence of apple replant disease (ARD) is directly or indirectly affected by *Fusarium*. *Fusarium* is also a harmful fungus for replant disease of lily [64], grape [10], passion fruit and other plants. *Mortierella* can decompose organic matter, such as plant residues and animal feces, so that they can be absorbed and utilized by plants [65]. The functional prediction showed that fungi took up nutrients in the soil in a variety of ways. When the soil environment changed, some fungi rapidly changed their nutritional patterns to resist the harmful effects of environmental changes [66]. Therefore, differences in the compositions and structures of fungi communities might have given rise to functional differences. In addition, FUN Guild predicted and analyzed the functions of fungi based on the existing literature. The classification of fungi was not comprehensive or perfect, so further research is still needed.

5. Conclusions

This study showed that the soil organic matter of 14 aged orchards in Luochuan was low; there were significant differences in the contents of nitrogen, phosphorus, and potassium; and the fungal community structures were rich. There were significant differences in the species and quantity of fungi in different aged apple orchards. *Motierella*, *Gibberella*, *Fusarium*, and *Cryptococcus* were common in the Luochuan aged apple orchards, and *Gibberella*, *Fusarium*, and *Cryptococcus* directly or indirectly affect the aged apple orchards. SOM, AN, AP, and AK were the main soil physical and chemical factors. These factors had significant effects on the diversity and structural compositions of the soil fungal communities. Pathotrophic fungi were widely present in the 14 aged apple orchards, which increased the risk of plant diseases, affected the growth and development of plants, and then caused the aging of orchards. Therefore, in the process of the renewal and transformation of the aged apple orchards in the future, we can prevent and control *Gibberella*, *Fusarium*, and *Cryptococcus* to further promote the sustainable development of the apple industry.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13010063/s1, Figure S1: Rarefaction curves in soil samples from different aged apple orchards; Figure S2: Analysis of soil fungal community composition in different aged apple orchards; Table S1: Basic information of 14 aged apple orchards; Table S2: Optimize sequence information. Table S3: OTUs of FUN Guild functional groups of soil fungal communities in different aged apple orchards.

Author Contributions: Conceptualization, X.X., W.J. and G.W.; Data curation, X.X.; Formal analysis, X.X.; Investigation, X.X., W.J., Q.L. and R.W.; Methodology, X.X., W.J. and G.W.; Project administration, X.X. and G.W.; Software, X.X. and F.D.; Supervision, X.C. and X.S.; Visualization, X.X.; Validation, C.Y. and Z.M.; Writing—original draft, X.X.; Writing—review and editing, C.Y. and Z.M. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (Germplasm Resources Utilization), Ministry of Agriculture of China (No. NYZS202001); the China Agriculture Research System of MOF and MARA (grant No. CARS-27); the Shandong Agricultural Major Applied Technology Innovation Project (grant No. SD2019ZZ008); the Taishan Scholar Funded Project (grant No. 20190923); the Qingchuang Science and Technology Support Project of Shandong Colleges and Universities (grant No. 2019KJF020); the Natural Science

Foundation of Shandong Province (grant No. ZR2020MC131); and the National Key Research and Development Program of China (grant No. 2020YFD1000201).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

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

References

1. Li, Q.J. Thoughts, and suggestions on the development of apple varieties in Luochuan, Shaanxi. *Northwest Hortic.* **2020**, *5*, 3–4. (In Chinese)
2. Sun, C. *Effects of Compound Microbial Fertilizer F25 on Soil Environment, Apple Yield and Quality in Weibei Apple Orchards*; Northwest University: Xi'an, China, 2017; p. 72.
3. Tian, J.; Sun, C.; Lu, P.P.; Li, F.; Shanguan, Y.Q.; Qi, F. Soil Biochemical Properties and Microbial Composition in Aged and Non-Aged Apple (*Malus domestica*) Orchards in Luochuan County, Loess Plateau, China. *Soil Use Manag.* **2020**, *37*, 879–890. [CrossRef]
4. Zhang, D.; Wang, C.; Li, L.X.; Yang, X.S.; Zhao, L.B.; Xia, S.J. Correlation of production constraints with the yield gap of apple cropping systems in Luochuan County, China. *ScienceDirect* **2019**, *18*, 1714–1725. [CrossRef]
5. Xue, C. *Manipulation of Microbial Community in Banana Rhizosphere to Suppress Fusarium wilt of Banana*; Nanjing Agricultural University: Nanjing, China, 2015. (In Chinese)
6. Yuan, J.J.; Zhao, Z.Y.; Zhang, L.S.; Hong, D.F.; Liu, W.L.; Shi, Q.F. Research on technique of inarching at bottom part of trunk with long shoot for renewal cultivar in old apple orchards of Loess Plateau. *J. Northwest A F Univ.* **2007**, *35*, 55–60.
7. Dabrowska, G.; Baum, C.; Trejgell, A.; Hrynkiewicz, K. Impact of arbuscular mycorrhizal fungi on the growth and expression of gene encoding stress protein—Metallothionein *BnMT2* in the non-host crop *Brassica napus* L. *J. Plant Nutr. Soil Sci.* **2014**, *177*, 459–467. [CrossRef]
8. Li, M.S.; Wang, Y.L.; Yang, B.; Liu, F.T.; Zhang, Y.J.; Ren, T.B.; Zhang, F.J. Effect of biochar on community structure and function prediction of rhizosphere fungi in flue-cured tobacco at maturity. *J. Agric. Resour. Environ.* **2022**, *39*, 1041–1048. (In Chinese)
9. Orgiazzi, A.; Lumini, E.; Nilsson, R.H.; Girlanda, M.; Vizzini, A.; Bonfante, P.; Bianciotto, V. Unravelling soil fungal communities from different mediterranean land-use backgrounds. *PLoS ONE* **2012**, *7*, e34847. [CrossRef]
10. Liang, H.B.; Wang, X.W.; Yan, J.W.; Luo, L.X. Corrigendum: Characterizing the Intra-Vineyard Variation of Soil Bacterial and Fungal Communities. *Front. Microbiol.* **2019**, *10*, 1551. [CrossRef]
11. Li, W.H.; Liu, Q.Z. Changes in fungal community and diversity in strawberry rhizosphere soil after 12 years in the greenhouse. *J. Integr. Agric.* **2019**, *18*, 199–209. [CrossRef]
12. Pervaiz, Z.H.; Iqbal, J.; Zhang, Q.M.; Chen, D.M.; Wei, H.; Saleem, M. Continuous cropping alters multiple biotic and abiotic indicators of soil health. *Soil Syst.* **2020**, *4*, 59. [CrossRef]
13. Kelderer, M.; Manici, L.M.; Caputo, F.; Thalheimer, M. Planting in the 'inter-row' to overcome replant disease in apple orchards: A study on the effectiveness of the practice based on microbial indicators. *Plant Soil* **2012**, *357*, 381–393. [CrossRef]
14. Ma, X.X.; Zhang, X.H.; Huang, M.; Su, X.G.; Dong, B.; Zhang, X.Q.; Liang, X.Q.; Lin, Y.Y.; Zhuang, P.Y. Effects of continuous cropping of fruit trees on soil microbial diversity. *Anhui Agric. Sci.* **2021**, *49*, 15–18. (In Chinese)
15. Yim, B.; Smalla, K.; Winkelmann, T. Evaluation of apple replant problems based on different soil disinfection treatments-links to soil microbial community structure? *Plant Soil* **2013**, *366*, 617–631. [CrossRef]
16. Chen, R.; Jiang, W.T.; Xu, S.Z.; Fan, H.; Chen, X.S.; Shen, X.; Yin, C.M.; Mao, Z.Q. An emerging chemical fumigant: Two-sided effects of dazomet on soil microbial environment and plant response. *Environ. Sci. Pollut. Res.* **2022**, *29*, 3022–3036. [CrossRef]
17. Slykhuis, J.T.; Thomas, S.C.L. Response of apple seedlings to biocides and phosphate fertilizers in orchard soils in British Columbia. *Can. J. Plant Pathol.* **1985**, *7*, 294–301. [CrossRef]
18. Munro, P.; Forge, T.A.; Jones, M.D.; Nelson, L.M. Soil biota from newly established orchards are more beneficial to early growth of cherry trees than biota from older orchards. *Appl. Soil Ecol.* **2020**, *155*, 103658. [CrossRef]
19. Egidi, E.; Delgadobaquerizo, M.; Plett, J.M.; Wang, J.; Eldridge, D.J.; Bardgett, R.D.; Maestre, F.T.; Singh, B.K. A few *Ascomycota taxa* dominate soil fungal communities worldwide. *Nat. Commun.* **2019**, *10*, 2369. [CrossRef]
20. Mazzola, M.; Manici, L.M. Apple Replant Disease: Role of Microbial Ecology in Cause and Control. *Annu. Rev. Phytopathpl.* **2012**, *50*, 45–65. [CrossRef]
21. Sheng, Y.F.; Wang, H.Y.; Wang, M.; Li, H.H.; Xiang, L.; Pan, F.B.; Chen, X.S.; Shen, X.; Yin, C.M.; Mao, Z.Q. Effects of Soil Texture on the Growth of Young Apple Trees and Soil Microbial Community Structure under Replanted Conditions. *Hortic. Plant J.* **2020**, *6*, 123–131. [CrossRef]
22. Ozimek, E.; Jaroszuk-Scisel, J.; Bohacz, J.; Kornillowicz-Kowalska, T.; Tyskiewicz, R.; Slomka, A.; Nowak, A.; Hanaka, A. Synthesis of Indoleacetic Acid, Gibberellic Acid and ACC-Deaminase by *Mortierella* Strains Promote Winter Wheat Seedlings Growth under Different Conditions. *Int. J. Mol. Sci.* **2018**, *19*, 3218. [CrossRef]
23. Arafat, Y.; Tayyab, M.; Khan, M.U.; Chen, T.; Amjad, H.; Awais, S.; Lin, X.M.; Lin, W.X.; Lin, S. Long-Term Monoculture Negatively Regulates Fungal Community Composition and Abundance of Tea Orchards. *Agronomy* **2019**, *9*, 466. [CrossRef]

24. Yin, C.M.; Wang, G.S.; Li, Y.Y.; Chen, X.S.; Wu, S.J.; Mao, Z.Q. T-RFLP analysis of soil fungi in continuous cropping apple orchard. *J. Ecol.* **2014**, *3*, 4837–4846.
25. Wang, J.G.; Tian, T.; Wang, H.J.; Cui, J.; Shi, X.Y.; Song, J.H.; Li, T.S.; Li, W.D.; Zhong, M.T.; Zhang, W.X. Chitosan-coated compound fertilizer application and crop rotation alleviate continuouscotton cropping obstacles by modulating root exudates. *Rhizosphere* **2022**, *23*, 100581. [CrossRef]
26. Sun, K.N.; Fu, L.Y.; Song, Y.; Yuan, L.; Zhang, H.R.; Wen, D.; Yang, N.; Wang, X.; Yue, Y.Q.; Li, X.H.; et al. Effects of continuous cucumber cropping on crop quality and soil fungal community. *Environ. Monit. Assess.* **2021**, *193*, 7. [CrossRef] [PubMed]
27. Li, W.H.; Liu, Q.Z.; Chen, P. Effect of long-term continuous cropping of strawberry on soil bacterial community structure and diversity. *ScienceDirect* **2018**, *17*, 2570–2582. [CrossRef]
28. Li, X.Y.; Lewis, E.E.; Liu, Q.Z.; Li, H.Q.; Bai, C.Q.; Wang, Y.Z. Effects of long-term continuous cropping on soil nematode community and soil condition associated with replant problem in strawberry habitat. *Sci. Rep.* **2016**, *6*, 30466–30478. [CrossRef]
29. Cheng, R.; Liu, K.S.; Dou, P.P.; Li, J.H.; Wang, K. The Changes in Soil Microorganisms and Soil Chemical Properties Affect the Heterogeneity and Stability of Soil Aggregates before and after Grassland Conversion. *Agriculture* **2022**, *12*, 307.
30. Jiang, J.; Song, Z.; Yang, X.T.; Mao, Z.Q.; Nie, X.H.; Guo, H.; Peng, X.W. Microbial community analysis of apple rhizosphere around Bohai Gulf. *Sci. Rep.* **2017**, *7*, 8918. [CrossRef]
31. Tao, Y.; Li, X.F.; Zhang, Z.Q.; Zhang, Q.Z.; Li, X. Effects of soil sterilization methods on soil nutrients and microbial community structure at different depths. *J. Agric. Environ. Sci.* **2022**, *41*, 575–584.
32. Xiang, L.; Wang, M.; Jiang, W.T.; Wang, Y.F.; Chen, X.S.; Yin, C.M.; Mao, Z.Q. Key indicators for renewal and reconstruction of perennial trees soil: Microorganisms and phloridzin. *Ecotoxicol. Environ. Saf.* **2021**, *225*, 112723. [CrossRef]
33. Arrigoni, E.; Albanese, D.; Longa, C.M.O.; Angeli, D.; Donati, C.; Ioriatti, C.; Pertot, I.; Perazzolli, M. Tissue age, orchard location and disease management influence the composition of fungal and bacterial communities present on the bark of apple trees. *Environ. Microbiol.* **2020**, *22*, 2080–2093. [CrossRef]
34. Chen, X.; Zhang, X.Z.; Wang, H.T.; Ma, F.W.; Qu, F.; Li, C.Y. Research Report on Fertilization Status of Apple Orchards in Shaanxi Province. *China Fruit Tree* **2021**, *11*, 89–94.
35. Aparicio, V.; Costa, J.L. Soil quality indicators under continuous cropping systems in the Argentinean Pampas. *Soil Tillage Res.* **2007**, *96*, 155–165. [CrossRef]
36. Chen, M.N.; Li, X.; Yang, Q.L.; Chi, X.Y.; Pan, L.J.; Chen, N.; Zhen, Y.; Wang, T.; Wang, M.; Yu, S.L. Soil eukaryotic microorganism succession as affected by continuous cropping of peanut-pathogenic and beneficial fungi were selected. *PLoS ONE* **2012**, *7*, e40659. [CrossRef]
37. Finlay, B.J. Global dispersal of free-living microbial eukaryote species. *Science* **2002**, *296*, 1061–1063. [CrossRef]
38. Liu, H.; Pan, F.J.; Han, X.Z.; Song, F.B.; Zhang, Z.M.; Yan, J.; Xu, L. A comprehensive analysis of the response of the fungal community structure to long-term continuous cropping in three typical upland crops. *J. Integr. Agric.* **2020**, *19*, 866–880. [CrossRef]
39. Franke-Whittle, L.H.; Manici, L.M.; Insam, H.; Stres, B. Rhizosphere bacteria and fungi associated with plant growth in soils of three replanted apple orchards. *Plant Soil* **2015**, *395*, 317–333. [CrossRef]
40. Ding, S.; Zhou, D.P.; Wei, H.W.; Wu, S.H.; Xie, B. Alleviating soil degradation caused by watermelon continuous cropping obstacle: Application of urban waste compost. *Chemosphere* **2021**, *262*, 128387. [CrossRef]
41. Shen, Q.Q.; Yang, J.Y.; Su, D.F.; Li, Z.Y.; Xiao, W.; Wang, Y.X.; Cui, X.L. Comparative Analysis of Fungal Diversity in Rhizospheric Soil from Wild and Reintroduced *Magnolia sinica* Estimated via High-Throughput Sequencing. *Plants* **2020**, *9*, 600. [CrossRef]
42. Yun, S.H.; Arie, T.; Kaneko, L.; Yoder, O.C.; Turgeon, B.G. Molecular organization of mating type loci in heterothallic, homothallic and asexual *Gibberella/Fusarium* species. *Fungal Genet. Biol.* **2000**, *31*, 7–20. [CrossRef]
43. Zhou, Y.; Zhao, Z.; Guo, Q.; Lei, B. First report of wilt of sugar beet caused by *Gibellulopsis nigrescens* in the Xinjiang region of China. *Plant Dis.* **2017**, *101*, 1318–1319. [CrossRef]
44. Booth, C. *The Genus Fusarium*; Commonwealth Mycological Institute: Surrey, UK, 1971; pp. 89–101.
45. Wang, X.B.; Wang, G.S.; Liu, Y.S.; Chen, X.S.; Shen, X.; Yin, C.M.; Mao, Z.Q. Correlation analysis of apple replant disease and soil fungal community structure in the Northwest Loess Plateau area. *Acta Ecol. Sin.* **2018**, *45*, 855–864. (In Chinese)
46. Zhao, L.; Wang, G.S.; Liu, X.; Chen, X.S.; Shen, X.; Yin, C.M.; Mao, Z.Q. Control of Apple Replant Disease Using Mixed Cropping with *Brassica juncea* or *Allium fistulosum*. *Agriculture* **2022**, *12*, 68. [CrossRef]
47. Botha, A. The importance and ecology of yeasts in soil. *Soil Biol. Biochem.* **2010**, *43*, 1–8. [CrossRef]
48. Yao, Q.; Xu, Y.X.; Liu, X.F.; Liu, J.J.; Huang, X.Y.; Yang, W.G.; Yang, Z.; Lan, L.; Zhou, J.M.; Wang, G.H. Dynamics of soil properties and fungal community structure in continuous-cropped alfalfa fields in Northeast China. *PeerJ* **2019**, *7*, e7127. [CrossRef]
49. Jiao, Y.N.; Zhou, Z.C.; Chen, T.; Wei, Y.Y.; Zheng, J.; Gao, R.X.; Chen, H. Biomarkers of antibiotic resistance genes during seasonal changes in wastewater treatment systems. *Environ. Pollut.* **2018**, *234*, 79–87. [CrossRef]
50. Deng, L.T.; Tong, T.Q.; Ge, J.P. Redundancy analysis of soil microbial distribution and ecological factors in different orchards. *Chin. Agron. Bull.* **2017**, *33*, 41–47. (In Chinese)
51. Li, L.; Xu, M.; Mohammad, E.A.; Zhang, W.J.; Duan, Y.H.; Li, D.C. Factors affecting soil microbial biomass and functional diversity with the application of organic amendments in three contrasting cropland soils during a field experiment. *PLoS ONE* **2018**, *13*, 9. [CrossRef]

52. Zhao, Y.N.; Mao, X.X.; Zhang, M.S.; Yang, W.; Di, H.J.; Li, M.; Liu, W.J.; Li, B. The application of Bacillus Megaterium alters soil microbial community composition, bioavailability of soil phosphorus and potassium, and cucumber growth in the plastic shed system of North China. *Agric. Ecosyst. Environ.* **2021**, *307*, 107236. [CrossRef]
53. Zhao, F.; Zhao, M.Z.; Wang, Y.; Guan, L.; Pang, F.H. Study on microbial community structure and diversity in strawberry rhizosphere based on high throughput sequencing. *Soil* **2019**, *51*, 51–60.
54. Xie, X.F.; Pu, L.J.; Wang, Q.Q.; Zhu, M.; Xu, Y.; Zhang, M. Response of soil physicochemical properties and enzyme activities to long-term reclamation of coastal saline soil, Eastern China. *Sci. Total Environ.* **2017**, *607*, 1419–1427. [CrossRef]
55. Barberan, A.; Bates, S.T.; Casamayor, E.O.; Fierer, N. Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME J.* **2014**, *8*, 952. [CrossRef]
56. Wu, C.C.; Ma, Y.J.; Wang, D.; Shan, Y.P.; Song, X.P.; Hu, H.Y.; Ren, X.L.; Ma, X.Y.; Cui, J.J.; Ma, Y. Integrated microbiology and metabolomics analysis reveal plastic mulch film residue affects soil microorganisms and their metabolic functions. *J. Hazard. Mater.* **2022**, *423*, 127258. [CrossRef]
57. Lin, Q.; Li, L.J.; Adams, J.M.; Hedenec, P.; Tu, B.; Li, C.N.; Li, T.T.; Li, X.Z. Nutrient resource availability mediates niche differentiation and temporal co-occurrence of soil bacterial communities. *Appl. Soil Ecol.* **2021**, *163*, 103965. [CrossRef]
58. Liu, Z.S.; Cichocki, N.C.; Hubschmann, T.; Suring, C.; Ofiteru, I.D.; Sloan, W.T.; Grimm, V.; Muller, S. Neutral mechanisms and niche differentiation in steady-state insular microbial communities revealed by single cell analysis. *Environ. Microbiol.* **2019**, *21*, 164–181. [CrossRef]
59. Pozo, M.I.; Herrera, C.M.; Lachance, M.A.; Verstrepen, K.; Lievens, B.; Jacquemyn, H. Species coexistence in simple microbial communities: Unravelling the phenotypic landscape of co-occurring *Metschnikowia* species in floral nectar. *Environ. Microbiol.* **2016**, *18*, 1850–1862. [CrossRef]
60. Nannipieri, P.; Ascher-Jenull, J.; Ceccherini, M.T.; Pietramellara, G.; Renella, G.; Schloter, M. Beyond microbial diversity for predicting soil functions: A mini review. *Pedosphere* **2020**, *30*, 5–17. [CrossRef]
61. Tan, Z.T.; Li, A.D.; Yang, R.; Cai, G.J.; Wu, F.C.; Guo, C.Y. Effects of passion fruit continuous cropping on soil fungal community structure. *Mol. Plant Breed.* **2022**, *20*, 10. (In Chinese)
62. Cui, Y.F.; Huang, Y.; Jiang, L.H. Research progress of several important *Gibberella* fungi in agricultural production. *Chin. Agron. Bull.* **2007**, *23*, 441–446. (In Chinese)
63. Wang, G.S.; Yin, C.M.; Pan, F.B.; Wang, X.B.; Li, X.; Wang, Y.F.; Wang, J.Z.; Tian, C.P.; Chen, J.; Mao, Z.Q. Analysis of the Fungal Community in Apple Replanted Soil Around Bohai Gulf. *Hortic. Plant J.* **2018**, *4*, 175–181. [CrossRef]
64. Zhou, J.M.; Wang, X.E.; Song, R.; Cao, L.; Zhu, X.Q.; Xie, J.; Dai, Y.J. Review on biological mechanism and biological control technology of Lily continuous cropping obstacle. *Hunan Agric. Sci.* **2020**, *11*, 104–107. (In Chinese)
65. Li, F.; Chen, L.; Redmile-Gordon, M.; Zhang, J.B.; Zhang, C.Z.; Ning, Q.; Li, W. *Mortierella elongata*'s roles in organic agriculture and crop growth promotion in a mineral soil. *Land Degrad. Dev.* **2018**, *20*, 1642–1651. [CrossRef]
66. Peng, X.W.; Ding, L.; Zhang, L.; Li, Z.J.; Gu, M.Y. The Effect of antibiotic residue organic fertilizer on the community structure and functional groups of corn soil fungi. *China Resour. Compr. Util.* **2020**, *38*, 1–11. (In Chinese)