sustainability

MDPI

Article

Alfalfa Cover Crops Influence the Soil Fungal Community and Function in Apple Orchards in Arid Desert Oases in Northwest China

Zhenlei Wang [1], Weiliang Zhao [1] and Linqiao Xi [2,*]

[1] The National-Local Joint Engineering Laboratory of High Efficiency and Superior-Quality Cultivation and Fruit Deep Processing Technology on Characteristic Fruit Trees, College of Plant Science, Tarim University, Alar 843300, China
[2] College of Animal Science, Tarim University, Alar 843300, China
* Correspondence: gsxlq666@163.com; Tel./Fax: +86-09974680332

Abstract: The present study investigated the effects of alfalfa cover crops on soil fungal communities and function in apple orchards in arid desert oases. A five-year apple orchard was subjected to two treatments: Intercropping with an alfalfa cover crop (A) and clean tillage (QG). The soil fungal ITS (internal transcribed spacer) region was analyzed using Illumina MiSeq high-throughput sequencing technology, and fungal function was determined using FUNGuild. Changes in the fungal community structure, diversity, and metabolic function in the 0–60 cm soil layer of the apple orchard were compared. The results showed that the alfalfa cover crops enhanced fungal richness but reduced diversity. The alfalfa cover crops improved fungal copy numbers but reduced the relative abundance of the dominant phylum, Ascomycota. Correlations between soil fungi and soil factors revealed that total nitrogen and total carbon were the most important nutrient factors in positively regulating the fungal community. The main negative factors were soil total salts and pH. The FUNGuild functional prediction showed that Ectomycorrhizal-Wood Saprotroph and Endophyte-Undefined Saprotroph only appeared in the alfalfa cover crops. The abundance of endophytes was enhanced ($p < 0.05$), but the abundance of plant pathogens and wood saprotrophs decreased ($p < 0.01$). Alfalfa cover crops could increase the copy numbers and richness in arid oasis apple orchards.

Keywords: alfalfa cover crop; fungi; apple orchards; arid desert oasis; community and function

check for updates

Citation: Wang, Z.; Zhao, W.; Xi, L. Alfalfa Cover Crops Influence the Soil Fungal Community and Function in Apple Orchards in Arid Desert Oases in Northwest China. *Sustainability* **2022**, *14*, 11816. https://doi.org/10.3390/su141911816

Academic Editors: Wu Xiong, Nan Zhang, Zhihui Xu and Jia Li

Received: 13 August 2022
Accepted: 14 September 2022
Published: 20 September 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

The Tarim Basin is one of China's most important fruit-growing regions. In Xinjiang, the planting area and fruit yield exceed 75%, with crops primarily consisting of Ziziphus jujuba, *Juglans regia* L., *Malus pumila* L., and Pyrus brestschneideri Rehd [1]. The soil organic matter (SOM) is less than 1.0% in the Tarim Basin [1]. However, the long-term utilization of chemical fertilizers led to a decrease in SOM in this region, which negatively affects improvements in fruit quality and limits the sustainable development of the fruit industry in the area.

Cover crops are used in orchard management [2–4]. Cover crops were mowed, and the residues were returned to the soil in a timely manner, which released nutrients via soil microbes [5]. Cover crops play an important role in improving soil fertility [6], increasing soil coverage, reducing surface runoff [7], protecting soil physical conditions, improving soil aeration [8], and also increasing apple orchard biodiversity, maintaining apple orchard ecological balance [9], promoting fruit tree growth, and improving apple quality and yield [10]. The microbes involved in enzymatic hydrolysis processes are the essence of cover crop degradation, and soil microbes play an important role. The decomposition of residues that affect the metabolism of nitrogen, carbon, and other elements in apple orchards is driven by the structures and functions of microbial communities [11,12]. The structure and

diversity of fungal communities in cover crops have been extensively discussed, but little is known about the dynamics of fungal community function in cover crops in orchards. Therefore, more studies are needed on different soil types. Microbial functional diversity was not examined using high-throughput sequencing (NGS) in arid desert oases with cover crops.

Intercropping legumes in orchards is an effective way to increase nutrient content [13,14]. Alfalfa (*Medicago sativa* L.) is an excellent green manure and cover crop. It is high in nitrogen and has a high capacity for enriching P, K, and other mineral elements. Alfalfa is a perennial leguminous plant that fixes nitrogen and has a large mass [13]. The mowing and return of the crop to the orchard rapidly improves soil-available nutrients and soil structure.

Although the diversity of fungi and bacteria has been studied, fungi are better at degrading complex compounds than bacteria, and bacterial diversity was discussed in other articles. The current study investigated and analyzed the potential functions of the fungal community in alfalfa cover crops in an apple orchard. NGS technique is a novel approach to studying soil microbes [15]. Therefore, the fungal community and function were examined using NGS in alfalfa cover crops apple orchards in arid desert oases.

2. Materials and Methods

2.1. Experimental Design and Sampling Collection

The experiment was carried out in an old orchard (40°56′ N latitude and 81°04′ E longitude) in the city of Alar, Xinjiang, China, in 2019. Red love (Switzerland119-06) was planted in a 4.0 × 1.5 m rowing space in 2015. The irrigation methods included flood irrigation in spring and autumn and growth period-specific drip irrigation. The treatments included orchard clean tillage (QG) and an alfalfa cover crop (A). The row spacing for the alfalfa was 15 cm, and the sowing rate was 7.5 kg hm^{-1}. Eight rows were sown between the apple tree rows. There was no fertilizer applied to the alfalfa between the rows of fruit trees. Ditches were opened at a distance of 50 cm from the trunks of fruit trees at the beginning of October each year to facilitate the application of basal fertilizer for fruit management during the growing season. At the beginning of November, the fruit was irrigated during the winter. Watering was also done at the end of May, June, and the beginning of August. A water-soluble humic acid fertilizer was applied approximately 10 days before each watering, and foliar fertilizer was sprayed 4–5 times per year. Soil samples were collected in May, July, and September 2019. The results of soil physical and chemical properties are listed in Table 1.

Table 1. Physical and chemical properties of the soil in an apple orchard under alfalfa cover crop treatment.

Treatment	QG5	A5	QG7	A7	QG9	A9
TC (gkg^{-1})	2.97 ± 0.31 a	3.1 ± 0.3 a	2.72 ± 0.4 a	2.9 ± 0.39 a	2.78 ± 0.24 a	2.88 ± 0.25 a
TN (gkg^{-1})	0.85 ± 0.06 ab	0.91 ± 0.07 ab	0.8 ± 0.05 b	0.91 ± 0.04 a	0.81 ± 0.06 ab	0.91 ± 0.07 ab
NH_{4^+}-N ($mgkg^{-1}$)	38.92 ± 9.82 a	30.33 ± 6.92 a	4.78 ± 4.77 b	5.4 ± 2.4 b	1.15 ± 0.19 b	1.58 ± 0.7 b
AP ($mgkg^{-1}$)	9.91 ± 3.97 b	14.57 ± 2.32 ab	24.2 ± 7.87 a	20.65 ± 3.58 ab	23.72 ± 2.49 a	21.95 ± 0.59 a
AK ($mgkg^{-1}$)	136.77 ± 38.5 a	155.03 ± 42.8 a	123.67 ± 6.28 a	99.4 ± 46.35 a	120.27 ± 45.91 a	83.7 ± 22.4 a
Cu ($mgkg^{-1}$)	21.59 ± 0.78 c	21.47 ± 0.57 c	23.32 ± 0.99 ab	23.39 ± 0.93 a	22.74 ± 0.31 abc	21.81 ± 1.26 bc
Zn ($mgkg^{-1}$)	72.36 ± 1.29 a	69.69 ± 2.05 a	74.45 ± 4.78 a	72.34 ± 2.72 a	74.68 ± 1.06 a	73.14 ± 3.12 a
Fe ($mgkg^{-1}$)	25.9 ± 0.56 a	25.98 ± 0.36 a	25.29 ± 0.77 a	25.84 ± 0.95 a	27.01 ± 0.04 a	26.74 ± 0.71 a
Mn ($mgkg^{-1}$)	0.58 ± 0.02 a	0.58 ± 0.01 a	0.58 ± 0.02 a	0.59 ± 0.02 a	0.61 ± 0.02 a	0.59 ± 0.02 a
Ca ($mgkg^{-1}$)	0.56 ± 0.29 b	2.57 ± 0.56 a	2.1 ± 0.27 a	2.61 ± 0.46 a	1.75 ± 0.46 a	2.5 ± 1.13 a
Mg ($mgkg^{-1}$)	0.05 ± 0.02 d	0.13 ± 0.02 ab	0.17 ± 0.03 a	0.14 ± 0.04 ab	0.12 ± 0.04 bc	0.07 ± 0.01 cd
pH	8.18 ± 0.08 a	7.91 ± 0.05 c	8.03 ± 0.01 b	7.94 ± 0.05 c	7.91 ± 0.05 c	7.81 ± 0.02 d
TS(%)	0.35 ± 0.15 b	0.86 ± 0.18 a	0.86 ± 0.18 a	0.93 ± 0.16 a	0.67 ± 0.19 ab	0.86 ± 0.33 a

Note: QG, orchard clearing tillage. A, Ground cover with alfalfa green manure in apple orchards. The numbers 5, 7, and 9 indicate the month of sampling. Data followed by lowercase letters in each line were separated using one-way ANOVA (Tukey HSD multiple range test, $p = 0.05$). TC, total carbon. TN, total nitrogen. NH_{4^+}-N, alkali-hydrolyzable nitrogen. AP, available phosphorus. AK, available potassium. TS, total salt.

2.2. Collection and Treatment of Soil Samples

Soil samples were collected 15 days after each mowing in May, July, and September 2019. Five points between the rows and three rows were randomly collected using a soil borer with a diameter of 4.0 cm. The soil was collected at a 0–60 cm depth, which was divided into three layers. Following the removal of roots, animals, and residues, the five sampling points were evenly mixed and placed in self-sealing bags for temporary storage. The mixed soil samples were divided into two parts. One part was air dried to determine chemical properties. The other part was kept in a refrigerator for fungal NGS.

2.3. Determination of Soil Chemical Analysis

The contents of total carbon, total nitrogen, and available nitrogen, phosphorus and potassium in soil are determined by conventional methods [16]: The total carbon content in soil is determined by external heating with potassium dichromate. Determination of total nitrogen content in soil was carried out using the Kjeldahl method. The alkaline hydrolysis diffusion method was used to determine the content of alkaline hydrolysis nitrogen. The content of available phosphorus was determined by sodium bicarbonate extraction-molybdenum antimony anti-colorimetry. The content of available potassium was determined by ammonium acetate extraction-flame spectrophotometry. The soil micronutrients were determined by ICP-OES 730. Make the calibration curve of the standard solution, input the sample mass and volume, and test the elements, such as Ca, Mg, Cu, Fe, Mn, Zn, and B dissolved in the solution. The potential method [16] was used to measure the pH value of soil (the ratio of water to soil is 2.5:1). The content of water-soluble salt was determined by the mass method [16].

In order to ensure the authenticity and credibility of the data, all the above experiments on soil properties were repeatedly analyzed and tested 3 times, and the average value was taken. The values of soil micro-nutrients and secondary nutrients reflect the total amount of environmental soil nutrients at that time.

2.4. Fungal DNA Extraction and NGS

Total soil fungal DNA was extracted using the FastDNA® SPIN Kit for Soil. The primers were ITS3F (5′-GCATCGATGAAGAACGCAGC-3′) and ITS4R (5′-TCCTCCGC TTATTGATATGC-3′). The soils were sent to Genesky Biotechnologies, Inc. (Shanghai, China) for sequencing on an Illumina NovaSeq 6000 sequencer [13–15].

2.5. Data Processing and Analysis

The raw sequence reads were processed using QIIME2 [17,18]. The OTUs (operational taxonomic units) were classified [19]. The taxonomy of the fungal gene sequence was contrasted with the RDP Classifier (Michigan State University, USA) (v2.12) [20]. R-vegan (v2.5.6) was used to analyze the alpha diversity (Chao, ACE, Simpson and Shannon). R (Ross Ihaka and Robert Gentleman were founded in Auckland University, New Zealand) (v3.5.1) was used to compare the FUNGuild database (An open annotation tool for parsing fungal community datasets by ecological guild.University of Minnesota)(v1.1) based on the fungal functional groups. The PLS-DA (Partial Least Squares Discriminant Analysis) was performed using R (v6.6.2) [21,22]. Other statistical analyses were performed using SPSS (International Business Machines Corporation (IBM) for short. Thomas watson was founded in the United States.) 18.0.

3. Results

3.1. Diversity and Richness of the Soil Fungal Community

The alpha diversity indexes (Shannon and Simpson) and richness (OTUs, Chao and ACE) were used to evaluate the soil fungal community. A Venn analysis of specific soil fungal OTUs from the clean tillage and alfalfa cover crop treatments in the apple orchard was performed (Figure 1). There were 145 soil fungal OTUs under the different treatments. At 5, 7, and 9 months, the treatment QG contained 285, 265, and 237 specific soil fungal

OTUs, respectively, while treatment A contained 245, 301, and 321 OTUs. Specific OTUs in treatment A gradually increased from May to July until September, whereas OTUs in treatment QG decreased. From July to September, the OTUs were higher in the A treatment than in the QG treatment.

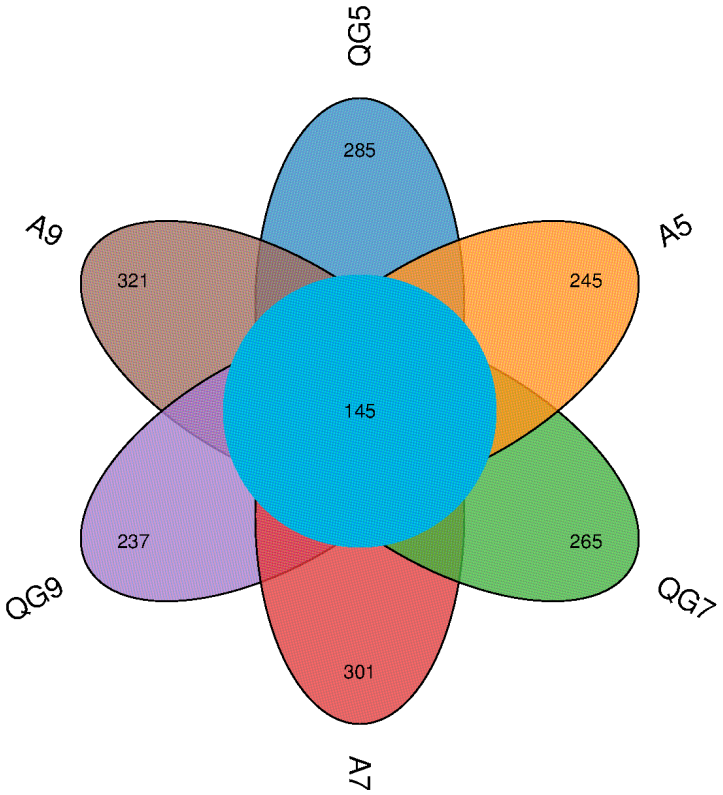

Figure 1. Venn diagram analysis of soil fungal OTUs in apple orchards planted with alfalfa cover crops. Note: QG, Orchard clean tillage. A, Ground cover with alfalfa green manure in orchards. The numbers 5, 7, and 9 indicate the month of sampling.

Compared to the QG treatment, the fungal richness (Chao1, ACE, observed) was enhanced significantly, but the diversity decreased in the A treatment (Table 2). The alfalfa cover crop had a positive effect on fungal richness. The richness under the alfalfa cover crop increased gradually from May to July, but the richness under clean tillage decreased. In July and September, the Shannon index for fungal diversity was low, while the Simpson index was high in the alfalfa cover crop treatment. One reason for this phenomenon may be that the litter releases nutrients, and root exudates change the rhizosphere soil microenvironment. The inhibition of the propagation of dominant fungi may have decreased the Shannon index and increased the evenness index (Simpson index).

Table 2. Mean alpha diversity indexes of the fungal communities (n = 3).

	OTUs	Chao1	ACE	Shannon	Simpson	Coverage
QG5	376 ± 92 a	391 ± 79 a	391 ± 79 a	4.0708 ± 0.2631 a	0.0534 ± 0.0270 b	1.00 ± 0.0 a
A5	367 ± 85 a	383 ± 64 a	383 ± 64 a	3.9907 ± 0.1239 a	0.0504 ± 0.0078 b	1.00 ± 0.0 a
QG7	363 ± 106 a	370 ± 103 a	370 ± 103 a	4.0163 ± 0.3887 a	0.0544 ± 0.0409 b	1.00 ± 0.0 a
A7	390 ± 71 a	390 ± 71 a	390 ± 71 a	3.4343 ± 0.6231 b	0.1056 ± 0.0636 a	1.00 ± 0.0 a
QG9	346 ± 4 a	359 ± 7 a	359 ± 7 a	3.8754 ± 0.3665 a	0.0478 ± 0.0132 b	1.00 ± 0.0 a
A9	420 ± 45 a	424 ± 45 a	424 ± 45 a	3.7333 ± 0.3552 ab	0.0831 ± 0.0436 ab	1.00 ± 0.0 a

Note: QG, orchard clearing tillage. A, Ground cover with alfalfa green manure in apple orchards. The numbers 5, 7, and 9 indicate the month of sampling. Kruskal–Wallis test, $p < 0.05$. The same letter "a", "b" and "ab" means that there is no significant difference, but different letters mean that there are significant differences.

3.2. Distribution of the Soil Fungal Community

The PLS-DA showed significant differences in the fungal community between treatment QG and treatment A (Figure 2), and the relative distances were close between May, July, and September in treatment QG but were far apart in treatment A. These results suggest that the fungal community was more sensitive to alfalfa cover crops than clean tillage.

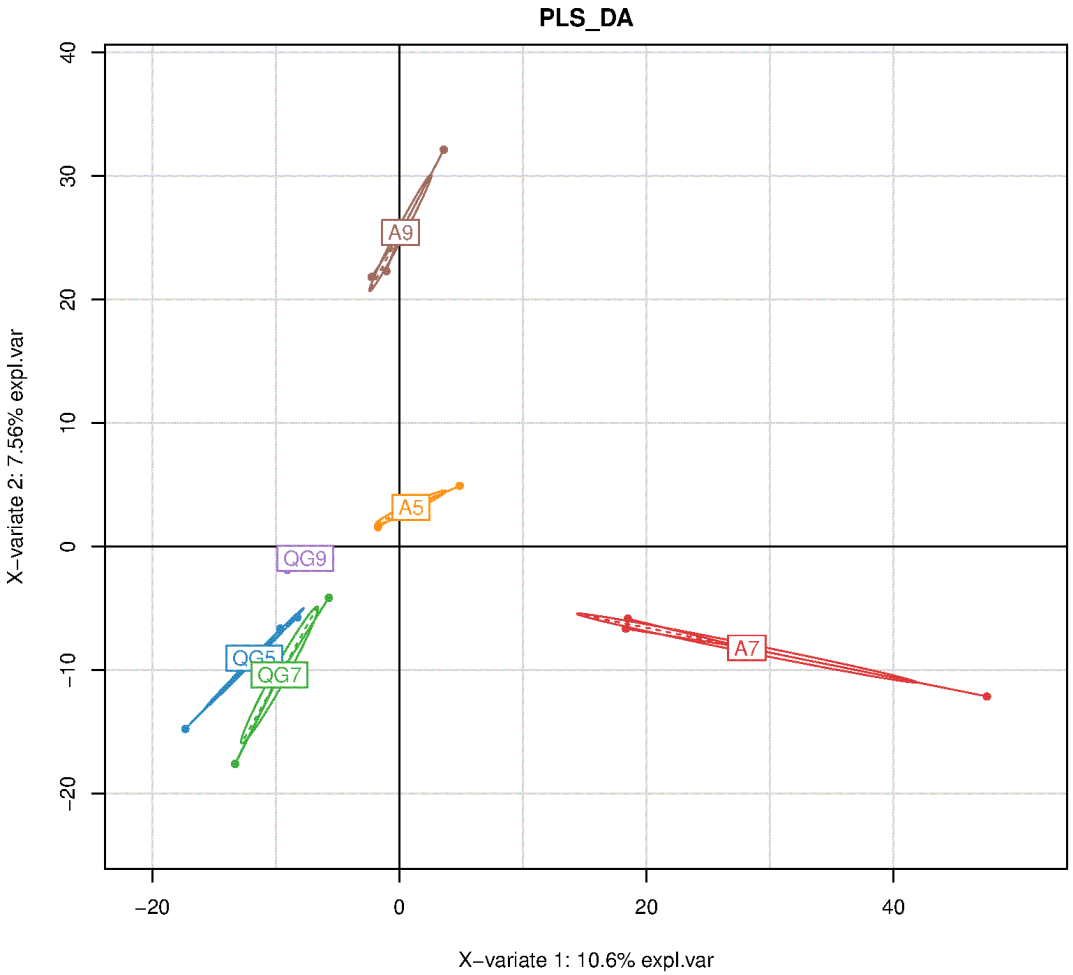

Figure 2. PLS-DA (partial least squares discriminant analysis) of fungi in apple orchards planted with alfalfa cover crops. Note: QG, Orchard clean tillage. A, Ground cover with alfalfa green manure in apple orchards. The numbers 5, 7, and 9 indicate the month of sampling.

3.3. Composition and Structure of the Fungal Community

Nine fungal phyla were discovered in the soil samples (Figure 3). The top three most relative abundant phyla were *Ascomycota* (42.62–71.81%), *Basidiomycota* (2.42–13.2%), and *Mortierellomycota* (2.21–7.83%). *Ascomycota* was the most dominant phylum. In May, treatment A had a higher relative abundance of Ascomycota than treatment QG, but it had a lower relative abundance in July and September ($p < 0.05$). The dominant phyla in terms of absolute abundances of Ascomycota were A5 > QG5, A7 > QG7, and A9 > QG9. The absolute abundance of *Ascomycota* in the A treatment was higher than the QG treatment at the 0–20 cm, 20–40 cm, and 40–60 cm soil depths and increased 187.60%, 130.18%, and 22.71%, respectively. *Ascomycota* showed the highest abundance at 1.74×10^6 in treatment A7. The absolute abundance of *Mortierellomycota* was higher in treatment A than treatment QG. Alfalfa cover crops increased the copy numbers of fungi in the 0–20 cm, 20–40 cm, and 40–60 cm soil layers, particularly in July. The alfalfa cover crop inhibited the dominant phylum *Ascomycota*.

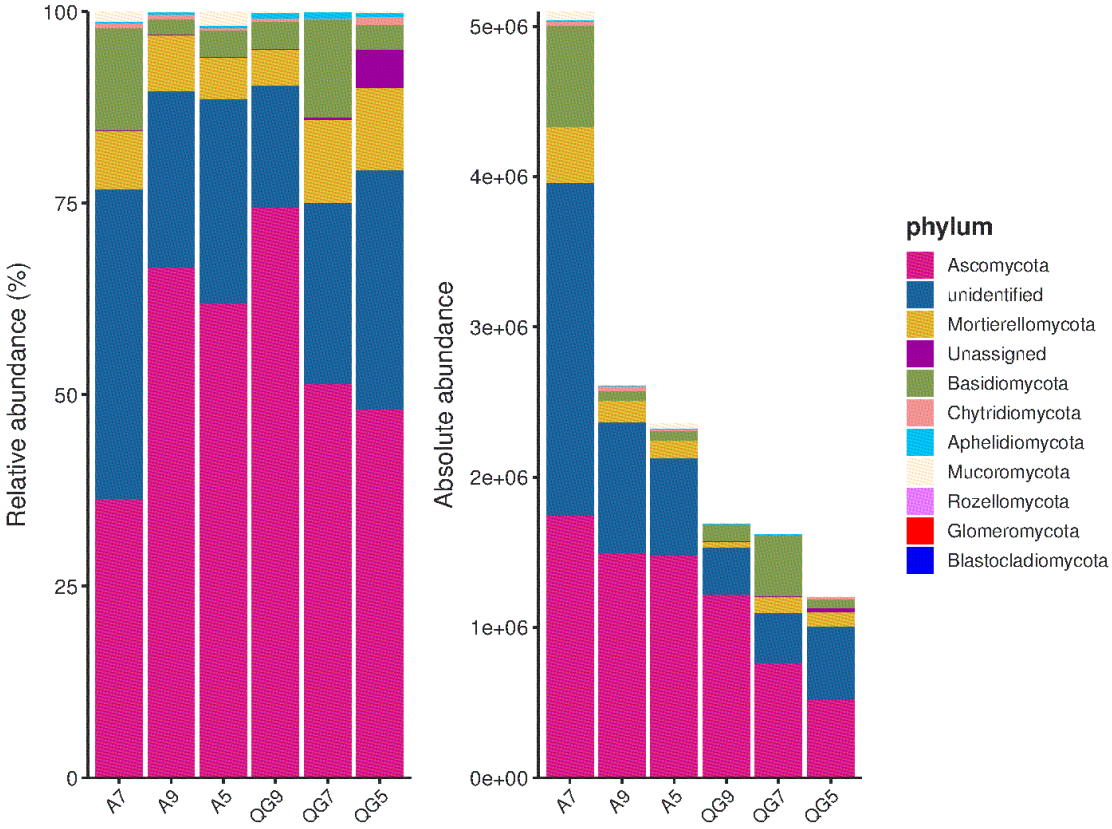

Figure 3. Combined analysis of the soil community composition and relative abundance differences at the phylum level. Note: QG, orchard clean tillage. A, Ground cover with alfalfa green manure in apple orchards. The numbers 5, 7, and 9 indicate the month of sampling.

Twenty-five fungal classes were found, and nine classes showed a relative abundance greater than 1.0%, including *Tremellomycetes*, *Dothideomycetes*, *Pezizomycetes*, *Sordariomycetes*, *Agaricomycetes*, *Mucoromycetes*, *Eurotiomycetes*, *Leotiomycetes* and *Mortierellomycetes*. *Sordariomycetes*, and *Dothideomycetes* were the dominant classes in the *Ascomycota* phylum, and the absolute abundance of *Dothideomycetes* was higher in treatment A than treatment QG. *Sordariomycetes* was more abundant in treatment A than treatment QG, except in September. The alfalfa litter is easily degraded and may be released first and absorbed by plants and microorganisms, while the remaining macromolecular substances that are not easily degraded inhibited *Sordariomycetes* growth. In July, the absolute abundance of Spizellomycetes was significantly higher in treatment A ($p < 0.05$). Dothideomycetes had the lowest relative abundance in July (Figure 4). In different months, the relative abundances of Dothideomycetes, Tremellomycetes, Orbiliomycetes, and Spizellomycetes were significantly higher in the A treatment than the QG treatment ($p < 0.05$). Rhizophlyctidomycetes had a significantly higher relative abundance in September, while Laboulbeniomycetes had a higher relative abundance in May. According to the combined analysis, the alfalfa cover crop may have inhibited the dominant class, but it improved subdominant class growth. A total of 175 fungal genera were found across all samples. Nineteen genera were significantly different ($p < 0.05$) and more abundant in the A treatment than the QG treatment, except for the absolute abundance of *Hydropisphaera* (Figure 5). Twenty-eight genera showed a relative abundance greater than 1.0%. *Fusarium* (3.57–22.15%), *Coprinellus* (0.20–24.04%), *Cladosporium* (0.01–18.17%), *and Mortierella* (2.21–7.83%) were the dominant genera. The relative abundances of *Cladosporium* and *Mortierella* increased, while *Fusarium* and *Coprinellus* decreased under the alfalfa cover crop. Brachyphos, Acrotalagmus, Geastrum, Scedosporium, Gaertneriomyces, and Lecanicillium genera were only found in the alfalfa cover crop treatment ($p < 0.05$). *Hydropisphaera* was detected in the QG treatment but not in the alfalfa cover crop treatment ($p < 0.05$). The fungal communities in treatments A

and QG clustered together, according to cluster analysis. The fungal communities in the 0–40 cm soil layer were altered by alfalfa cover crops.

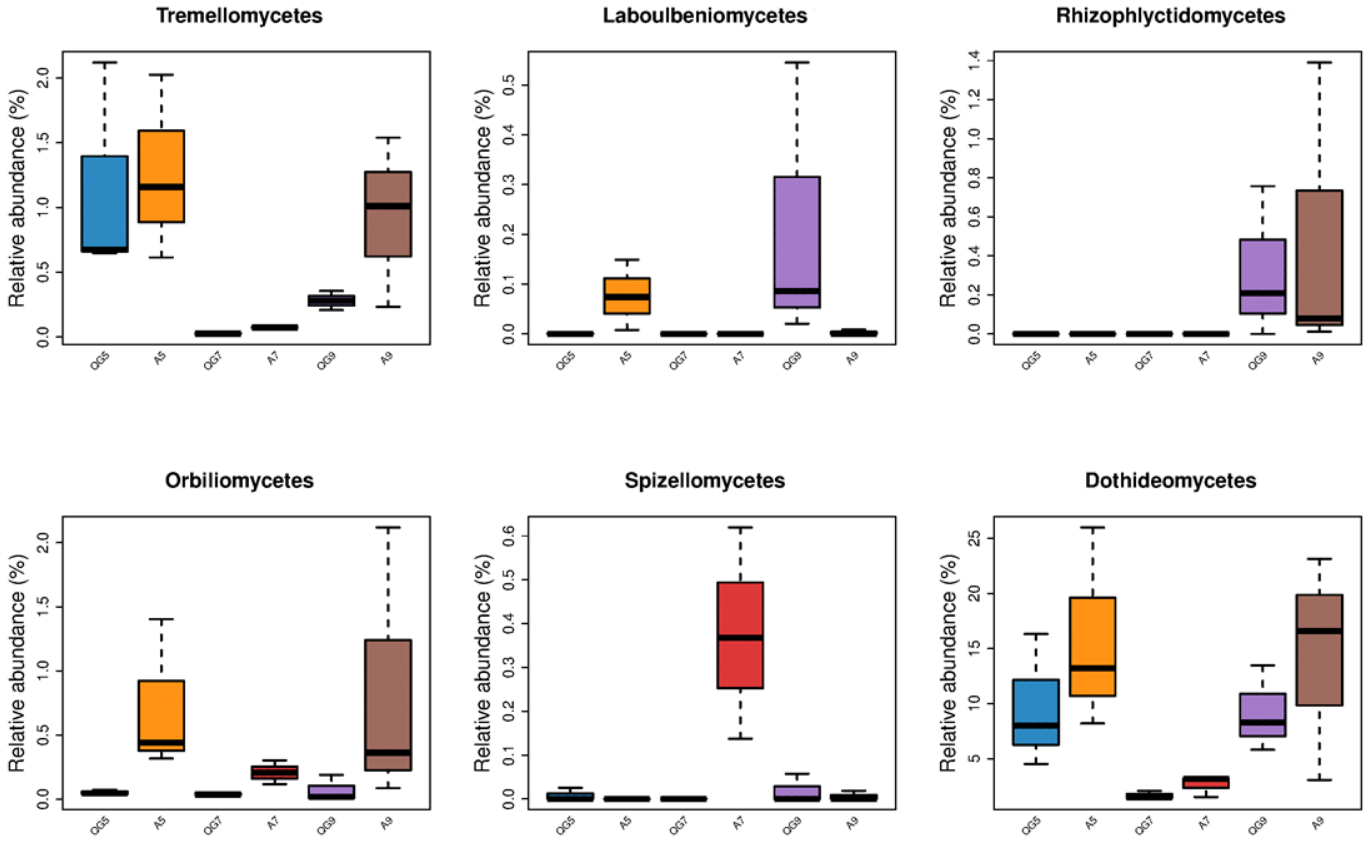

Figure 4. Different analyses of fungal relative abundance at the class level in apple orchards planted with alfalfa cover crops. Note: QG, Orchard clean tillage. A, Ground cover with alfalfa green manure in apple orchards. The numbers 5, 7, and 9 indicate the month of sampling.

3.4. Correlation between Soil Fungi and Soil Factors

The soil factors were divided into two clusters (Figure 6): The first cluster included total carbon (TC), total nitrogen (TN), alkali-hydrolyzable nitrogen (NH_{4^+}-N), available phosphorus (AP), available potassium (AK), Fe, Mn, Cu, and Zn, and the other cluster included pH, total salt (TS), Ca and Mg. The first cluster promoted the growth of the dominant fungal class and limited the second-most abundant class. Soil physical and chemical properties regulate the diversity of the soil fungal community. TC and TN positively correlated with fungal classes. *Tremellomycetes* positively correlated with TN, TC, and Fe and negatively correlated with Mg. *Agaricomycetes* positively correlated with TC and TN. *Mucoromycetes* positively correlated with TN, NH_{4^+}-N, and TC. *Pezizomycetes* and *Orbiiomycetes* positively correlated with TC and TN. *Leotiomycetes* positively correlated with TN, TC, AP, and Mn. *Eurotiomycetes* positively correlated with TC, TN, Zn, Cu, and AP. *Aphelidiomycetes* positively correlated with TC, Zn, and AP. *Cystobasidiomycetes* positively correlated with TC and TN. *Mortierellomycetes* and *Saccharomycetes* positively correlated with TC and TN. *Dothideomycetes* positively correlated with TC, TN and Fe and negatively correlated with Mn. *Sordariomycetes* positively correlated with TC and TN and negatively correlated with Zn, Mn, Fe, and AP. *Exobasidiomycetes* positively correlated with Mg and negatively correlated with Mn and Fe. *Spizellomycetes* positively correlated with Cu. There was a positive correlation between *Glomeromycetes* and Ca. *Rhizophlytidomycetes* positively correlated with Fe and negatively correlated with NH_{4^+}-N and pH. *Laboulbeniomycetes* positively correlated with Fe.

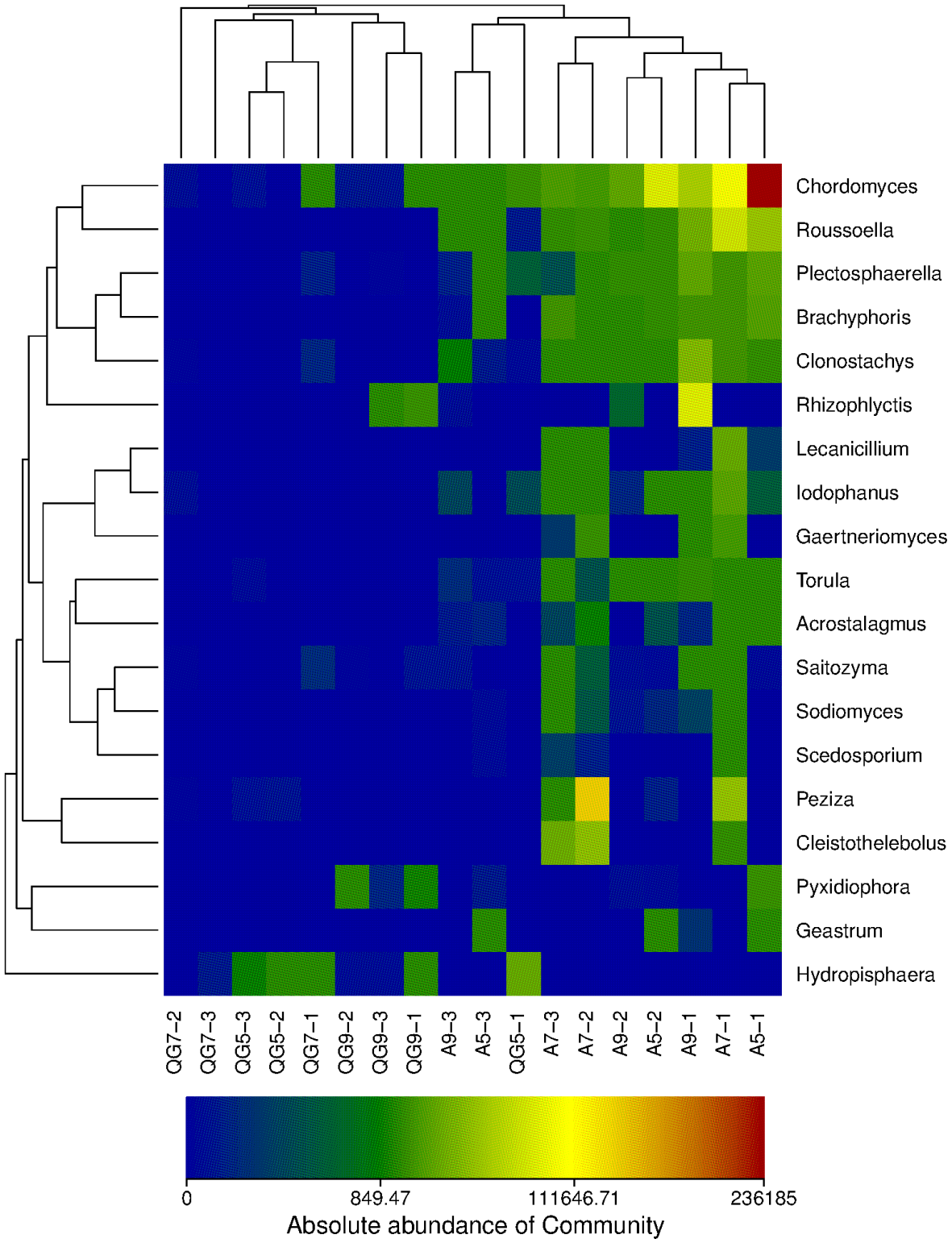

Figure 5. Heatmap analyses of the top 10 fungi in apple orchards planted with alfalfa cover crops at the genus level. Note: QG, Orchard clean tillage. A, Ground cover with alfalfa green manure in apple orchards. The numbers 5, 7, and 9 indicate the month of sampling. The numbers −1, −2, and −3 indicate the depth of the soil samples.

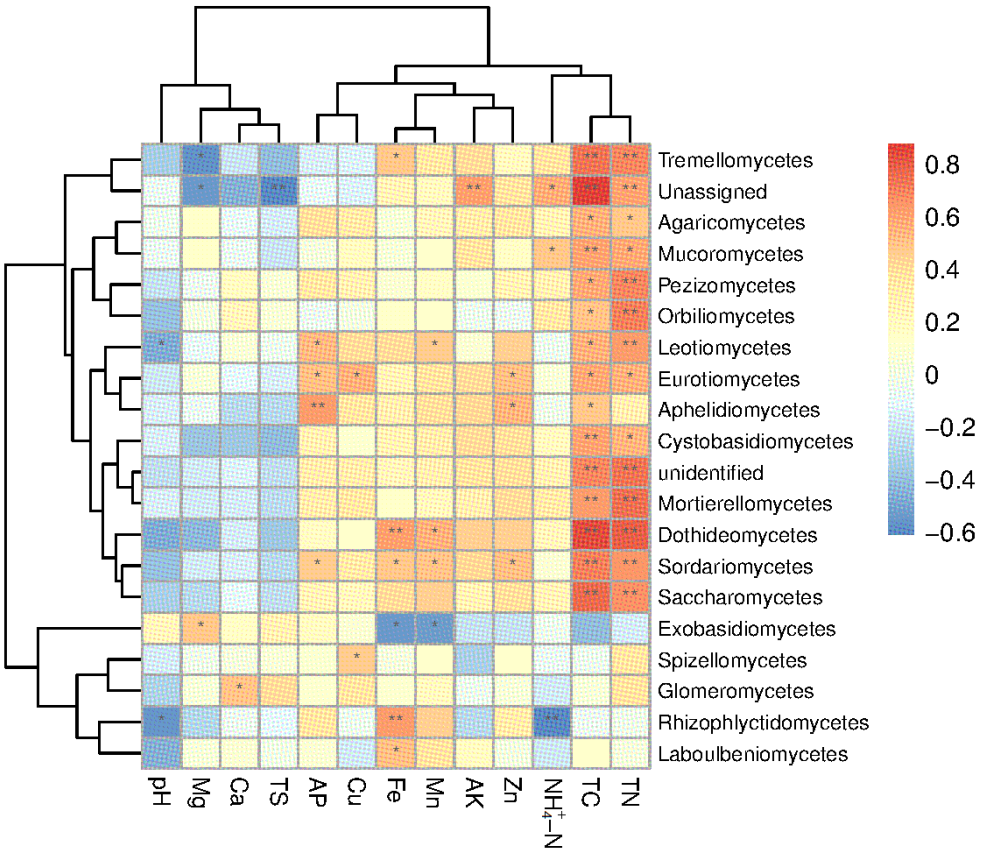

Figure 6. Heatmap of the Pearson correlation analyses between the fungal community at the class level and soil factors. Note: TN, total nitrogen. TC, total carbon. AP, available phosphorous. AK, available potassium. TS, total salt. NH_{4^+}-N, alkali-hydrolyzable nitrogen. "*" indicates significant difference, and "**" indicates extremely significant difference.

The Pearson correlation analysis showed that the fungal diversity and soil factors, including the Chao1, ACE, observed, and Simpson indexes, positively correlated with TC, TN, NH_{4^+}-N, AP, AK, Fe, Mn, Cu, and Zn, but the Shannon index negatively correlated with these factors. Fungal diversity negatively correlated with Mg, pH, TS, and Ca. Fungal richness positively correlated with TC ($p < 0.01$) and TN, Zn, Cu, and Mn ($p < 0.05$).

3.5. Functional Groups of Soil Fungi

FUNGuild was used to annotate the functions of fungi in the rhizosphere soil of apple orchards under different treatments (Figure 7). The nutrient patterns of the fungal community were saprotrophic, symbiotic, and pathotrophic. Alfalfa cover crops enriched the diversity of fungi, including arbuscular mycorrhizae, dung saprotrophes–endophytes–plant pathogens, undefined saprotrophes, and endophytes-undefined saprotrophes. Alfalfa cover crops increased the absolute abundance of endophytes ($p < 0.001$), endophytes–letter saprotrophs–soil saprotrophs–undefined saprotrophs ($p < 0.01$), endophytes–undefined saprotrophs ($p < 0.01$), undefined saprotrophs ($p < 0.05$), and endophytes–lichen parasites–plant pathogens–undefined saprotrophs ($p < 0.05$). The absolute abundance of plant pathogens decreased from May to September in treatment A but increased in treatment QG. The abundance of plant pathogens was significantly lower in treatment A than in treatment QG ($p < 0.05$).

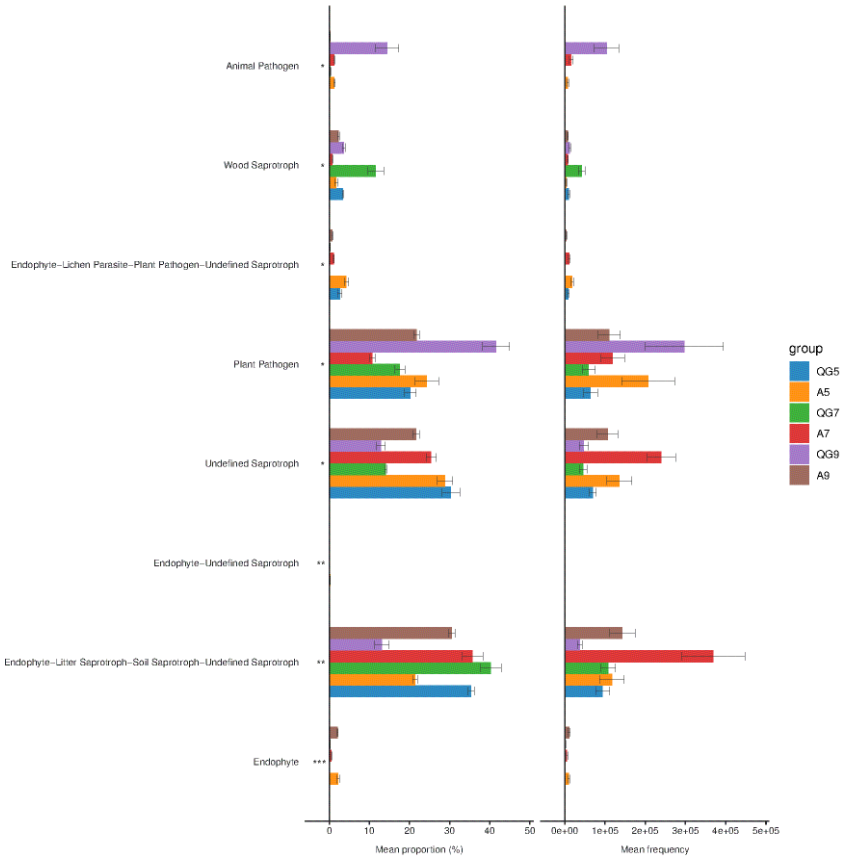

Figure 7. Comparison of fungal functional groups based on their relative abundance and absolute abundance. Note: QG, Orchard clean tillage. A, Ground cover with alfalfa green manure in apple orchards. The numbers 5, 7 and 9 indicate the month of sampling. ANOVA, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4. Discussion

4.1. Impact of Cover Crops on Microbial Diversity

The richness and diversity of soil fungi are important indicators of soil ecological function, and changes in community diversity and richness affect fungal community function. Agricultural management (fertilization, crop rotation, cover crops, etc.) and soil environmental factors [23] affect the structure and diversity of soil fungal communities. Cover crops planted in orchards increase the number of soil microorganisms and enzymatic activity [24,25]. Compared with no grass mulching, grass growing in orchard increased the number of microorganisms in soil, and the number of microorganisms increased significantly with the extension of grass growing time [26]. The numbers of soil bacteria, fungi, and actinomycetes increased by 49.0%, 47.8%, and 35.4%, respectively, for three consecutive years in tobacco-planted soil [27]. The alfalfa cover crops increased the copy numbers of the fungi in the 0–60 cm soil layers compared to the clean tillage treatment, which is consistent with the above results. Most research reported that fungi were affected in the 0–20 cm soil layer, but fungi were affected in the 0–40 cm soil layer in the present study, possibly due to the deep root system of alfalfa cover crops and rotary tillage.

The Chao1 and ACE diversity indexes are commonly used to estimate total microbial richness, and the Shannon and Simpson indexes are generally used to estimate the diversity and evenness of species, respectively. The effects of cover crops on fungal diversity were not consistent across the orchard soil. Microbial richness and diversity were affected by soil basic fertility and the planting of cover crops. Covering with white clover in apple orchard can significantly improve the metabolic activity of soil microbial community, diversity, and richness of soil microbial community, and reduce evenness [28]. The Shannon index and Chao index of rhizosphere soil fungi were different with different cover crops in tea plants,

and the order was TJ (smooth vetch-tea manure No. 1) > CK (control) > JM (*Vicia sativa* No. 1-*Sesbania sativa*) > CF (Feitian radish *Cassia rotundifolia*) [29]. Planting alfalfa cover crops in apple orchards can improve the richness index while decreasing the diversity index when compared to clean tillage. This phenomenon may be caused by the release of nutrients by litter, and root exudates change the rhizosphere soil micro-environment. Inhibiting dominant fungi propagation may have reduced the Shannon index while increasing the evenness index [30]. After the alfalfa cover crop was broken down in May, July, and September, a mixed litter system was formed.

4.2. Impact of Cover Crops on Fungal Community Structure

The alfalfa cover crop increased the copy numbers of Ascomycota and Mortierellomycota in the apple orchards. The absolute abundance was found to be positively correlated with TN and TC ($p < 0.01$). Plant litter decomposition was aided by saprotrophic Ascomycota. Ascomycota is an important soil decomposer that degrades refractory organic matter and contributes significantly to nutrient cycling [31,32]. Therefore, the alfalfa cover crop may have increased the TN and TC after annual mowing (Table 1), which would support the growth of *Ascomycota*. The abundance of *Mortierellomycota* is an indicator of the soil organic matter and nutrient content. These fungi dissolve phosphorus and decompose cellulose, hemicellulose, and lignin in soil and use sugars in soil for growth and metabolism to increase soil organic matter and nutrient contents [33]. *Basidiomycota* are mostly saprophytic fungi with a strong ability to decompose macromolecular compounds, such as cellulose and lignin [33]. The number of fungi in the 0–20 cm soil layer was higher than in the 20–40 cm soil layer. Soil temperature and moisture are the main factors affecting the decomposition of cover crops. The results showed that the alfalfa cover crop significantly increased the absolute abundance of *Basidiomycota* in the 20–40 cm soil layer in July. Most residues may have been distributed in the 20–40 cm soil layer after the cover crop was mowed, which was beneficial to the growth of *Basidiomycota*.

Changes in soil physical and chemical properties and other factors affect the composition of the soil fungal community, but the regulatory mechanisms are different for these factors [34]. The correlation analysis between fungi and soil factors showed that most fungi positively correlated with TC and TN, and alfalfa cover crops promoted the growth of fungi. However, the fungal communities negatively correlated with pH and total salt, which inhibited their growth. Six classes of fungi positively correlated with pH and TS, such as *Exobasidiomycetes*, *Spizellomycetes*, and *Glomeromycetes*, which may be halofungi. *Exobasidiomycetes* positively correlated with Mg ($p < 0.05$). *Glomeromycetes* positively correlated with Ca ($p < 0.05$). The soil pH value was the main factor that affected the fungal community structure in cinnamon soil [34,35], but it was not the main factor regulating the composition of the tea rhizosphere soil community [29,36]. The present experiment found that the soil pH value had a great influence on the fungal community composition. The key factors affecting the fungal community composition in the arid area of Northwest China were soil TC, TN, TS, and pH in apple orchards. Therefore, increasing soil nutrients and reducing soil pH and total soluble salt increase the diversity and stability of soil fungi in apple orchards. Therefore, the function and structure of fungal communities play an important role in apple orchards.

4.3. Impact of Cover Crops on Fungal Function

Fungi are classified into three types based on their mode of nutrition: Pathogens, saprophytes, and symbionts [37]. Pathotrophic fungi obtain nutrients from host cells, and pathotrophic fungi in soil can inhibit plant growth [38]. The change of pathotrophic fungi was different in soil in May, July, and September, which may be caused by complex organic matter formed by cover crop residues. In the current study, Treatment A resulted in significantly lower pathogen numbers than the QG treatment in September, and it has the potential to reduce the risk of infection to apple and fruit trees. It can reduce the chance of infection to apple and fruit trees. The mowing of the cover crop and

rotary tillage inhibited the growth of pathotrophic fungi in the soil. The endophytic fungi were mainly Ascomycetes, including pyrenomyetes, discomyetes, and loculoascomyetes. Ectomycorrhizal-Wood Saprotroph and Endophyte-Undefined Saprotroph function appeared in the alfalfa cover crop treatment but not in the clean tillage treatment. The absolute abundance of arbuscular mycorrhizal (AM) cover crops was greater than the absence of cover crops. AM form large mycelia in soil and exhibit symbiotic relationships with host plants. AM promotes host absorption and the utilization of nitrogen and phosphorus [39]. AM EndophyteAM improves drought and disease resistance in host plants [40,41]. The FUNGuild functional comparison was based on existing literature and data, and only some of the fungi's functions were analyzed. More research is needed to understand how the complex soil fungal community functions. Analyses of relative and absolute abundance revealed that certain functional roles, such as plant pathogens, changed differently. The absolute abundance of plant pathogens was A7 > QG7, but the relative abundance was A7 < QG7 in July. Therefore, joint analysis is required to better understand the variations in functions.

The number of fungi in the arid desert oasis orchard was lower than in the semi-arid Loess Plateau, and the diversity of fungi was also lower than other areas. The indicator fungi were found to tolerate salt and alkali in an arid desert oasis orchard. The soil pH value and organic matter content had a significant impact on the composition of the fungal community in the Loess Plateau, but TN, TC, pH, and TS were dominant in an arid desert oasis orchard. It could be caused by poor soil and secondary salinization in arid desert oasis. Therefore, more attention should be paid to the soil health of orchards in arid desert oasis.

5. Conclusions

Alfalfa cover crops could increase the copy numbers and richness in arid oasis apple orchards. Alfalfa cover crops and soil environmental factors jointly regulate the fungal community. Alfalfa cover crops have the potential to alter and increase the functional diversity of soil fungi. It increased the relative abundance of beneficial endophyte fungi while decreasing pathogens. When compared to clean tillage, alfalfa cover crops can improve biological soil health by increasing the abundance of microbial communities.

Therefore, the orchard in the Xinjiang desert area of China or in arid and windy areas of the world can be tried with grass mulching to increase soil nutrients, maintain the ecological balance of the orchard, promote the growth of fruit trees, improve the quality and yield of fruits, and then promote the optimization and upgrading of the world's fruit industry and the healthy and sustainable development.

Author Contributions: Conceptualization, L.X.; Project administration, L.X.; Writing—original draft, Z.W. and W.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Science and Technology Project for planting green manure, China (2018AB037).

Informed Consent Statement: Get the informed consent of all the subjects who have participated in the study.

Data Availability Statement: Not applicable. This study reports only preliminary research, and further study is in progress.

Acknowledgments: The authors acknowledge Genesky Biotechnologies Inc. for technical assistance with DNA sequencing and the cloud platform.

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

References

1. Gao, W.H.; Yang, L. *Xinjiang Statistical Yearbook*; China Statistics Press: Urumqi, China, 2018; p. 358.
2. Daryanto, S.; Fu, B.; Wang, L.; Jacinthe, P.-A.; Zhao, W. Quantitative synthesis on the ecosystem services of cover crops. *Earth-Sci. Rev.* **2018**, *185*, 357–373. [CrossRef]
3. Du, S.; Bai, G.; Yu, J. Soil properties and apricot growth under intercropping and mulching with erect milk vetch in the loess hilly-gully region. *Plant Soil* **2015**, *390*, 431–442. [CrossRef]
4. Yao, S.R.; Merwin, I.A.; Bird, G.W.; Abawi, G.S.; Thies, J.E. Orchard floor management practices that maintain vegetative or biomass groundcover stimulate soil microbial activity and alter soil microbial community composition. *Plant Soil* **2005**, *271*, 377–389. [CrossRef]
5. Blesh, J. Functional traits in cover crop mixtures: Biological nitrogen fixation and multifunctionality. *J. Appl. Ecol.* **2018**, *55*, 38–48. [CrossRef]
6. Bénédicte, A.; Bruno, M.; Claire, C.; May, B.; Cyril, G.; Michel, B.; Gilles, G.; Nicolas, B. Alternative arable cropping systems: A key to increase soil organic carbon storage, Results from a 16 year field experiment. *Agric. Ecosyst. Environ.* **2016**, *232*, 150–164.
7. Prosdocimi, M.; Tarolli, P.; Cerdà, A. Mulching practices for reducing soil water erosion. *Earth-Sci. Rev.* **2016**, *161*, 191–203. [CrossRef]
8. Reeve, J.; Culumber, C.; Black, B.; Tebeau, A.; Ransom, C.; Alston, D.; Rowley, M.; Lindstrom, T. Establishing peach trees for organic production in Utah and the Intermountain West. *Sci. Hortic.* **2017**, *214*, 242–251. [CrossRef]
9. Pignataro, A.; Moscatelli, M.C.; Mocali, S.; Grego, S.; Benedetti, A. Assessment of soil microbial functional diversity in a coppiced forest system. *Appl. Soil Ecol.* **2012**, *62*, 115–123. [CrossRef]
10. Srivastava, A.; Huchche, A.; Ram, L.; Singh, S. Yield prediction in intercropped versus monocropped citrus orchards. *Sci. Hortic.* **2007**, *114*, 67–70. [CrossRef]
11. Zhong, Y.; Yan, W.; Wang, R.; Wang, W.; Shangguan, Z. Decreased occurrence of carbon cycle functions in microbial communities along with long-term secondary succession. *Soil Biol. Biochem.* **2018**, *123*, 207–217. [CrossRef]
12. Gabhane, J.; William, S.P.; Bidyadhar, R.; Bhilawe, P.; Anand, D.; Vaidya, A.N.; Wate, S.R. Additives aided composting of green waste: Effects on organic matter degradation, compost maturity, and quality of the finished compost. *Bioresour. Technol.* **2012**, *114*, 382–388. [CrossRef] [PubMed]
13. Duchene, O.; Vian, J.F.; Celette, F. Intercropping with legume for agroecological cropping systems: Complementarity and facilitation processes and the importance of soil microorganisms. A review. *Agric. Ecosyst. Environ.* **2017**, *240*, 148–161. [CrossRef]
14. Ramos, M.E.; Robles, A.B.; Navarro, A.S.; González-Rebollar, J.L. Soil responses to different management practices in rainfed orchards in semiarid environments. *Soil Tillage Res.* **2011**, *112*, 85–91. [CrossRef]
15. Knight, R.; Vrbanac, A.; Taylor, B.C.; Aksenov, A.; Callewaert, C.; Debelius, J.; Gonzalez, A.; Kosciolek, T.; McCall, L.-I.; McDonald, D.; et al. Best practices for analysing microbiomes. *Nat. Rev. Microbiol.* **2018**, *16*, 410–422. [CrossRef] [PubMed]
16. Bao, S.D. *Analysis of Soil Agrochemical*, 3rd ed.; China Agricultural Press: Beijing, China, 2000.
17. Bolyen, E.; Rideout, J.R.; Dillon, M.R.; Bokulich, N.A.; Abnet, C.C.; Al-Ghalith, G.A.; Alexander, H.; Alm, E.J.; Arumugam, M.; Asnicar, F.; et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME. *Nat. Biotechnol.* **2019**, *37*, 852–857. [CrossRef]
18. Lei, H.J.; Xiao, Z.Y.; Zhang, Z.H.; Jin, C.C.; Pan, H.W.; Sun, K.P.; Xi, H.P. Effects of Water-nitrogen coupled oxygen Irrigation on soil Fertility and bacterial Community of greenhouse Pepper. *Trans. Chin. Soc. Agric. Eng.* **2021**, *37*.
19. Spalding, E.P.; Miller, N.D. Image analysis is driving a renaissance in growth measurement. *Curr. Opin. Plant Biol.* **2013**, *16*, 100–104. [CrossRef]
20. Callahan, B.J.; Mcmurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.A.; Holmes, S.P. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **2016**, *13*, 581–583. [CrossRef]
21. Jiang, S.-Q.; Yu, Y.-N.; Gao, R.-W.; Wang, H.; Zhang, J.; Li, R.; Long, X.-H.; Shen, Q.-R.; Chen, W.; Cai, F. High-throughput absolute quantification sequencing reveals the effect of different fertilizer applications on bacterial community in a tomato cultivated coastal saline soil. *Sci. Total Environ.* **2019**, *687*, 601–609. [CrossRef]
22. Dang, A.T.N.; Nandy, S.; Srinet, R.; Luong, N.V.; Ghosh, S.; Kumar, A.S. Forest aboveground biomass estimation using machine learning regression algorithm in Yok Don National Park, Vietnam. *Ecol. Inform.* **2019**, *50*, 24–32. [CrossRef]
23. Yuan, S.; Zhang, N.; Wu, X.; Qian, Y.; Chen, X.; Raza, W.; Li, X. Effect of pruned material, extracts, and polyphenols of tea on enzyme activities and microbial community structure in soil. *Soil Sci. Plant Nutr.* **2017**, *63*, 607–615. [CrossRef]
24. Elfstrand, S.; Bath, B.; Martensson, A. Influence of various forms of green manure amendment on soil microbial community composition, enzyme activity and nutrient levels in leek. *Appl. Soil Ecol.* **2007**, *36*, 70–82. [CrossRef]
25. Caban, J.R.; Kuppusamy, S.; Kim, J.H.; Yoon, Y.-E.; Kim, S.Y.; Lee, Y.B. Green manure amendment enhances microbial activity and diversity in antibiotic-contaminated soil. *Appl. Soil Ecol.* **2018**, *129*, 72–76. [CrossRef]
26. Liu, Y.P.; Mao, Y.F.; Hu, Y.L.; Zhang, L.L.; Yin, Y.J.; Pang, H.L.; Su, X.F.; Yang, L.; Shen, X. Effects of grass planting in apple orchard on soil microbial diversity, enzyme activities and carbon components. *J. Plant Nutr. Fertil.* **2021**, *10*, 1792–1805.
27. Si, G.H.; Zhao, S.J.; Wang, R.; Xu, D.B.; Qin, X.C.; Tan, J.; Xiang, B.K.; Yuan, J.F. Effects of consecutive overturning of green manure on soil physical and biological characteristics in tobacco-planting fields. *J. Plant Nutr. Fertil.* **2014**, *20*, 905–912.
28. Wei, Q.Q.; Han, M.Y.; Mu, X.Q. Influences of white clover returning to apple orchard on soil microbial community. *Pratacultural Sci.* **2016**, *33*, 385–392.

29. Fu, H.P.; Zhou, P.Q.; Wang, Y.J.; Mo, Z.D.; Li, Z.H.; Ma, Q.P.; Hoang, H.N.; Li, X.H. Effects of intercropping different green manures on fungal community characteristics in rhizosphere soil of tea plant. *J. Tea Commun.* **2020**, *47*, 406–415.
30. Qian, Y.L.; Wang, X.Z.; Lai, X.F. Effects of perennial forage planting on soil fungal community characteristics in apple orchard. *J. Grass Ind.* **2019**, *28*, 124.
31. Glass, N.L.; Schmoll, M.; Cate, J.H.; Coradetti, S. Plant cell wall deconstruction by ascomycete fungi. *Annu. Rev. Microbiol.* **2013**, *67*, 477–498. [CrossRef]
32. Li, Y.; Li, Y.; Chang, S.X.; Liang, X.; Qin, H.; Chen, J.; Xu, Q. Linking soil fungal community structure and function to soil organic carbon chemical composition in intensively managed subtropical bamboo forests. *Soil Biol. Biochem.* **2017**, *107*, 19–31. [CrossRef]
33. Chen, L.L.; Liu, J.; Li, M.D.; Zhang, L.Z.; Zou, Y.B.; Huang, M. Diversity of Filamentous Fungus Community in Paddy Fields with Different Tillage Methods. *Acta Laser Biol. Sin.* **2018**, *27*, 60–68.
34. Lauber, C.L.; Strickland, M.S.; Bradford, M.A.; Fierer, N. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biol. Biochem.* **2008**, *40*, 2407–2415. [CrossRef]
35. Yan, H.-T.; Yin, Q.-Y.; Ding, S.-S.; Ren, T.-B.; Xu, J.-L.; Zong, H.; Gao, Q.; Liu, G.-S. Effect of biochar amendment on physicochemical properties and fungal community structures of cinnamon soil. *Environ. Sci.* **2018**, *39*, 2412–2419.
36. Jiang, Y.; Arafat, Y.; Letuma, P.; Ali, L.; Tayyab, M.; Waqas, M.; Li, Y.; Lin, W.; Lin, S.; Lin, W. Restoration of long-term monoculture degraded tea orchard by green and goat manures applications system. *Sustainability* **2019**, *11*, 1011. [CrossRef]
37. Wang, J.; Rhodes, G.; Huang, Q.; Shen, Q. Plant growth stages and fertilization regimes drive soil fungal community compositions in a wheat-rice rotation system. *Biol. Fertil. Soils* **2018**, *54*, 731–742. [CrossRef]
38. Anthony, M.A.; Frey, S.D.; Stinson, K.A. Fungal community homogenization, shift in dominant trophic guild, and appearance of novel taxa with biotic invasion. *Ecosphere* **2017**, *8*, e01951. [CrossRef]
39. Sanon, A.; Beguiristain, T.; Cébron, A.; Berthelin, J.; Duponnois, R. Towards the influence of plant-soil-microbes feedbacks on plant biodiversity, grassland variability and productivity. In *Grassland Biodiversity: Habitat Types, Ecological Processes and Environmental Impacts*; Runas, J., Dahlgren, T., Eds.; Nova Science Publishers: New York, NY, USA, 2011; pp. 267–301.
40. Li, T.; Hu, Y.; Hao, Z.; Li, H.; Wang, Y.; Chen, B. First cloning and characterization of two functional aquaporin genes from an arbuscular mycorrhizal fungus Glomus intraradices. *New Phytol.* **2013**, *197*, 617–630. [CrossRef]
41. De, L.; Pena, E.; Echeverría, S.R.; Van, D.; Putten, W.H.; Freitas, H.; Moens, M. Mechanism of control of root-feeding nematodes by mycorrhizal fungi in the dune grass Ammophila arenaria. *New Phytol.* **2006**, *169*, 829–840.