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Sod Culture with *Vicia villosa* Alters the Diversity of Fungal Communities in Walnut Orchards for Sustainability Development

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Abstract: Monoculture frequently causes loss of soil nutrients and the emergence of soil-borne diseases in walnut orchards, whereas it is unknown whether sod culture with *Vicia villosa* (a popular agroforestry system) in walnut orchards impacts the structural composition and diversity of soil fungal communities. Fungal communities in walnut orchards with the cover plant *V. villosa* were investigated in this work utilizing high-throughput sequencing of ITS, as well as examination of root arbuscular mycorrhizal colonization and hyphal length of soil fungi. The monoculture and interplanted walnut models generated 33,511 and 34,620 effective tags with sequence similarity of 97%, respectively annotating 245 and 236 operational taxonomic units (OTUs). Among these, a total of 158 OTUs were found to be shared across monoculture and interplanted orchards. Walnuts grown in monoculture had a total of 245 species, belonging to 245 genera and 36 phyla, while walnuts with *V. villosa* as cover crops had 236 species, belonging to 236 genera and 19 phyla. The application of *V. villosa* as a cover plant significantly increased 1-Simpson and Shannon indices of soil fungi, indicating that interplanting *V. villosa* promoted soil fungal community diversity. Three dominant fungal phyla were detected in the soil, with Glosseromycota being the most dominant phylum. *V. villosa* as a cover plant significantly reduced the abundance of *Funneliformis* and *Densospora* in the soil, while it significantly increased the colonization of native arbuscular mycorrhizal fungi in roots by 94%, along with a 39% significant decrease in mycorrhizal hyphal length, as compared with the monoculture. Overall, *V. villosa* as a cover plant alters the composition and diversity of the soil fungal community, with reduced *Funneliformis* (*F. geosporum*) and *Densospora* abundance, and increased mycorrhizal colonization rate in roots, contributing to the sustainable and high-quality development of walnuts.

Keywords: fungal communities; high-throughput sequencing of ITS; beneficial microbes; monoculture

Citation: He, W.-X.; Sun, Q.-F.; Hashem, A.; Abd_Allah, E.F.; Wu, Q.-S.; Xu, Y.-J. Sod Culture with *Vicia villosa* Alters the Diversity of Fungal Communities in Walnut Orchards for Sustainability Development. *Sustainability* **2023**, *15*, 731. https://doi.org/10.3390/su151310731

Academic Editor: Silvia Tabacchioni

Received: 23 May 2023
Revised: 30 June 2023
Accepted: 5 July 2023
Published: 7 July 2023

1. Introduction

Walnut is a globally important economic tree species [1,2], rich in proteins, vitamins, and unsaturated fatty acids that are favourable to human health [3,4]. Walnut is frequently monocultured, with excessive use of chemical fertilizers and herbicides, resulting in poor soil environments that suppress soil microbial communities and yields [5]. Compared to monocultures, agroforestry systems can capture more resources like light and nutrients, as well as improve soil microbial populations. Bai et al. [6] reported that walnut–tea

intercropping significantly and positively affected the abundance of fungal operational taxonomic units (OTUs) and thus increased host adaptability and growth. Mwakilili et al. [7] documented that maize–*Desmodium* intercropping increased the diversity of beneficial fungi in the soil and resulted in strong heterogeneity of the fungal microbiome. The intercropping of *Morus alba* and *Lespedeza bicolor* altered soil nitrogen levels as well as the homogeneity and diversity of fungal communities in plantation forests, thereby affecting soil carbon and nitrogen cycles [8].

Plants and soil environments interact with each other, and the number and species of rhizospheric soil microorganisms affect the uptake and transformation of soil nutrients by plants, thereby regulating plant growth, development, and health [9]. Successive monocultures increase soil-borne pathogen accumulation, along with reduced beneficial microorganisms [10,11], resulting in a severe reduction in plant productivity [12,13]. Arafat et al. [14] discovered that beneficial fungal species decreased and soil pathogenic microorganisms increased following years of long-term tea monoculture, leading to reduced tea yield. Fungi are an abundant and important group of soil microorganisms [15] that can function as plant symbionts, pathogens, and decomposers [16,17]. Among soil symbiotic fungi, many plant roots establish reciprocal relationships with arbuscular mycorrhizal fungi (AMF) and ectomycorrhizal fungi [18], which aid in plant nutrient and water uptake [19]. Therefore, the composition and diversity of fungal communities in the soil can be important indicators of soil health and sustainability [20–22]. Walnut plants can form ectomycorrhizae as well as arbuscular mycorrhizae in their roots [23]. Mycorrhizae have been demonstrated to play critical roles in nutrient absorption and growth promotion of walnuts [24,25]. Moreover, walnut as a deep-rooted plant serves as a reservoir of AMF propagules for the restoration of surrounding vegetation [26]. In addition, in walnut agroforestry ecosystems, allelopathic substrates, such as juglone, can be transferred from walnut trees to their neighbouring plants via the common mycorrhizal network, thus inhibiting the growth of adjacent plants in the same ecosystem [27]. The variability of soil mycorrhizal fungi in walnut agroforestry systems is an important indicator of soil health in walnut orchards.

It is critical to maintain soil fertility and limit weed growth in orchards [28]. Orchard mulching has numerous ecological and economic benefits, including improved soil microbial abundance, decreased incidence of plant diseases and pests, reduced usage of pesticides and chemical fertilizers, and improved fruit quality and yield [29,30]. Leguminous crops are commonly used as cover crops in orchards to improve soil fertility, maintain soil moisture, and improve soil physicochemical properties [31,32], which may indirectly alter the diversity, activity, and community of microorganisms in the soil [32]. The intercropping of three green-manure crops changed the fungal community in apple orchards on the Loess Plateau, assisting in the optimisation of soil fungal community composition, thereby suppressing soil pathogens and promoting nutrient uptake by plants [33]. Many walnut orchards have successfully introduced cover crops, in which legumes and grasses can be employed as annuals or perennials, producing a positive impact on the orchard soil and eco-environment [34]. For example, sod culture in orchards can suppress rank weeds, prevent water and fertilizer loss, reduce the occurrence of pests and diseases, and improve soil physical and chemical properties and ecological environment, thereby achieving sustainable development of orchards [28,29,33].

Vicia villosa Roth. is a high-quality legume forage grass that has been widely used in sod culture of orchards because of its adaptability, nitrogen fixation, rapid decomposition and return to the soil as green manure, and the enrichment of beneficial microbes [35]. Jiang et al. [36] used *V. villosa* as a cover plant in apple orchards and found that the relative abundance of beneficial bacteria in the soil was increased, and the relative abundance of pathogenic fungi was decreased, thus improving the soil microenvironment in orchards. In China, the cover plant *V. villosa* has been used in walnut orchards, with positive impacts on soil fertility [35,37]. However, it is unclear how soil fungal populations change in the interplanted model, which is critical for understanding the high quality and productivity

of walnuts grown in this manner. High-throughput sequencing of ITS was used in this study to analyse changes in the soil fungal community structure of walnut orchards after *V. villosa* was planted, as well as changes in root mycorrhizal colonization and soil mycelium length. The results are expected to provide a reference for future fungal (particularly AMF) management in walnut orchards with sod culture.

2. Materials and Methods

2.1. Experimental Location

This study was conducted at the Hubei Walnut Germplasm Repository (31°21′ N, 111°23′ E) in Yantang village, Chengguan town, Baokang county, Xiangyang, Hubei, China. The walnut variety 'Qingxiang' was used. The average annual rainfall was 934.6 mm and the average annual frost-free period was 240 days. The soil parent material was mostly mudstone type and carbonate type, and the soil was yellow-brown loam. The soil organic carbon content in the 0–10 cm layer of the study site was 14.62 g/kg, the alkaline nitrogen was 97.41 mg/kg, the Olsen-P was 22.20 mg/kg, and the available potassium was 169.01 mg/kg [35]. *V. villosa* was sown in rows in 2014.

2.2. Soil Sampling and Experimental Design

On 15 April 2019, four 'Qingxiang' walnut trees were chosen as experimental materials from the orchard planted with the cover crop *V. villosa*. In four directions under the canopy (1 m distant from the tree trunk) of each walnut, the top 0–5 cm of soil was removed and 5–10 cm of soil was collected and mixed well in all four directions for one sample. At the same time, four walnut trees without the cover crop treatment were employed as the controls. All samples were divided into two groups: one without any treatment was brought back to the laboratory for the analysis of root AMF colonization rate and soil hyphal length; the other was preserved on dry ice and used for fungal sequencing. Therefore, there were two treatments in this experiment: one for walnuts covered with *V. villosa* between rows (interplanted walnut), and the other for walnuts not covered with any crops as the control (monoculture walnut).

2.3. Determinations of Root AMF Colonization Rate and Soil Mycelial Length

Root mycorrhizal colonization rate was assessed according to the method described by Huang et al. [24]. The sampled roots were sliced into 1-cm segments and placed in 10% KOH solution at 95 °C for 2.5 h, incubated with 10% H_2O_2 solution for 15 min, acidified with 0.2 mol/L HCl for 15 min, stained with 0.05% trypan blue in lactophenol, and microscopically examined for mycorrhizal colonization. Here, 12 root segments were observed per tree, with a total of 96 root segments. Mycorrhizal colonization rate (%) = 100 × lengths of colonized root segments / total lengths of root segments detected. Soil mycelial length analysis was carried out in accordance with the method described by Bethlenfalvay and Ames [38].

2.4. DNA Extraction, PCR Amplification, and Illumina Sequencing

After genomic DNA extraction using the Dneasy PowerSoil kit (Qiagen, USA), the DNA was visualized using 0.8% agarose gel electrophoresis. PCR amplification was carried out using the diluted genomic DNA as a template, with specific primers of ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) with barcode based on the fungal ITS fragment sequencing region. The PCR procedure was as follows: 94 °C for 1 min, 30 cycles; 94 °C for 20 s, 48 °C for 30 s, and 72 °C for 30 s; and 72 °C for 5 min. The PCR products were examined by 2% agarose gel electrophoresis. The TruSeq DNA PCR-Free Sample Prep kit (FC-121-3001/3003) was used to create the library. After the library was constructed and qualified through quantification and library testing, sequencing was performed on the Illumina Hiseq 2500 platform in PE250 mode with the Illumina Hiseq Rapid SBS kit v2 (FC-402-4023 500 Cycle).

2.5. Data Analysis

To splice the double-ended sequences, FLASH was used for sequence processing. Each sample sequence was split from the reads based on Barcode. The raw data were achieved by truncating the Barcode sequences, and then quality control was performed using Trimmomatic to obtain valid Clean Reads. Based on Usearch (v7.1), the UPARSE algorithm was used to cluster OTUs at 97% consistency levels, selecting the sequence with the highest frequency of occurrence in each OTU as the representative sequence of the OTU. The UCLUST classification and the UNITE database were used for annotation analysis. Multiple alignment of representative sequences was performed using the FFT-NS-2 algorithm of MAFFT (v7). Community composition analysis, alpha diversity, beta diversity, and differential species analysis were analysed using R software (v2.15.3).

The data regarding root mycorrhizal colonization rate and soil mycelial length were analysed by analysis of variance using SAS software (v9.1.3), and significant differences between treatments were analysed using Duncan's multiple range test ($p < 0.05$), where root mycorrhizal colonization rate was arcsine-transformed prior to the analysis.

3. Results

3.1. Changes in Sequencing Data and OTUs

According to the ITS sequencing data, the numbers of spliced sequences obtained from monoculture walnuts and interplanted walnuts were 35,184 and 36,315, respectively (Table 1). After filtering the chimeras, the final number of valid sequences obtained were 33,511 and 34,620, respectively, in the monoculture walnut and interplanted walnut, with effective rates of 92.01% and 90.28%, respectively. The average length of valid sequences was 258 bp for both treatments.

Table 1. Data statistics of fungus communities in different treatment walnut fields.

Treatments	Raw Tags	Effective Tags	Effective Rates (%)	Average Length (bp)
Mw	35,184	33,511	92.01	258
Iw	36,315	34,620	90.28	258

Abbreviations: Mw, monoculture walnut; Iw, interplanted walnut.

Rarefaction curves can reflect the abundance of species in a sample in an indirect way. When the rarefaction curve of the samples levelled off, it suggested that the amount of sequencing data was sufficient and the sequencing results basically reflected all species in the walnut rhizosphere. The rarefaction curve (Figure 1a) clearly showed that after 12,500 sequences, the number of OTUs for ITS stabilized at 97% similarity. There were 245 OTUs in the monoculture walnut sample and 236 OTUs in the interplanted walnut, for a total of 323 OTUs in the two treatment samples, including 158 OTUs in both monoculture walnut and interplanted walnut (Figure 1b). The use of *V. villosa* as a cover crop in the walnut orchard reduced the number of fungal OTUs, resulting in a shift in the distribution of soil fungal community composition.

Based on the annotated OTUs, the monoculture mode had 245 species, belonging to 245 genera and 36 phyla; the interplanted mode had 236 species, affiliated with 236 genera and 19 phyla (Table 2).

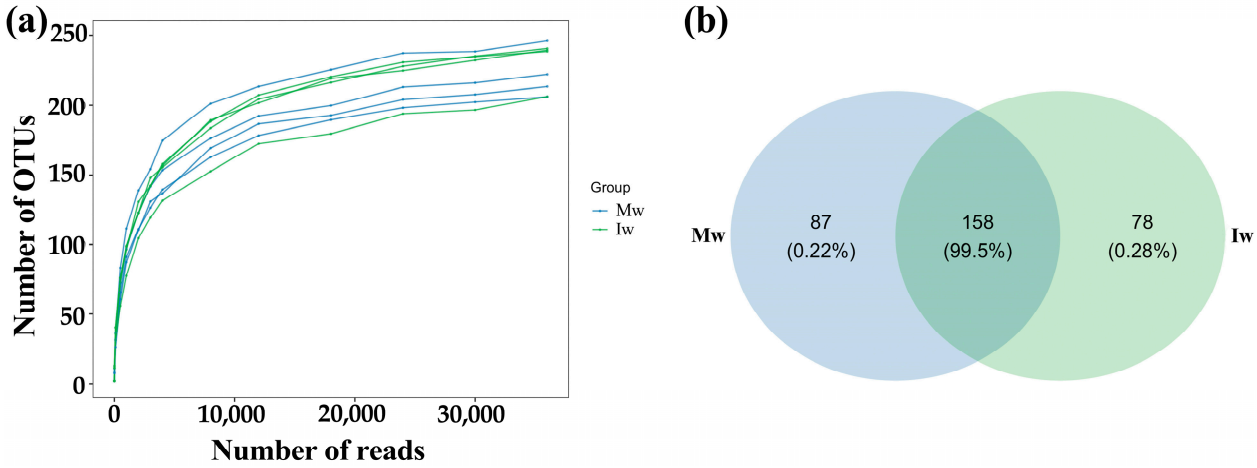

Figure 1. Rarefaction curves (**a**) and Venn diagram depicting the numbers and percentages of fungi (**b**) in different walnut orchards. See Table 1 for abbreviations.

Table 2. Results of OTU annotation of fungi in different walnut orchards.

Treatments	Kingdom	Phylum	Class	Order	Family	Genus	Species
Mw	34	36	244	245	245	245	245
Iw	18	19	234	236	236	236	236

See Table 1 for abbreviations.

3.2. Changes in the Structural Composition of Soil Fungal Communities at the Phylum, Genus, and Species Level

The dominant species in two treatments of walnut soil samples belonged to the Glomeromycota, Mucoromycota, and Ascomycota, according to phylum taxonomic analysis of soil fungal community composition (Figure 2a). Glomeromycota had average relative abundance of nearly 100% in all groups of soil and was the most dominant phylum in the interplanted walnut. In addition, the cover plant *V. villosa* in the walnut orchard reduced the abundance of Mucoromycota by 99%, compared to the monoculture walnut.

Similarly, the structural composition of soil fungal communities changed significantly at the class, order, family, genus, and species level (Figure 2b,c; Supplementary Material Figure S1). The community composition of soil fungi in the walnut orchard was analysed at the genus level, with *Funneliformis*, *Densospora*, *Symbiotaphrina*, and *Preussia* being the dominant fungi in the two groups of soil samples (Figure 2b). Compared with monoculture walnut, the interplanted walnut significantly reduced the abundance of *Funneliformis* and *Densospora*, with *Funneliformis* decreasing by 96%. In *Funneliformis*, *F. geosporum* abundance was obviously reduced by 96% under intercropped versus monoculture walnut conditions (Figure 2c).

Cluster analysis of the dominant fungi in both monoculture walnut and interplanted walnut at the genus level showed that the distribution of fungal community structure was similar within the same group, while there were significant differences in the distribution of fungal community structure between the groups. *Preussia* and *Symbiotaphrina* were more abundant in the interplanted walnut sample and less abundant than in the monoculture walnut sample, but *Funneliformis* and *Densospora* were more abundant in the monoculture walnut than in the interplanted walnut (Figure 2d).

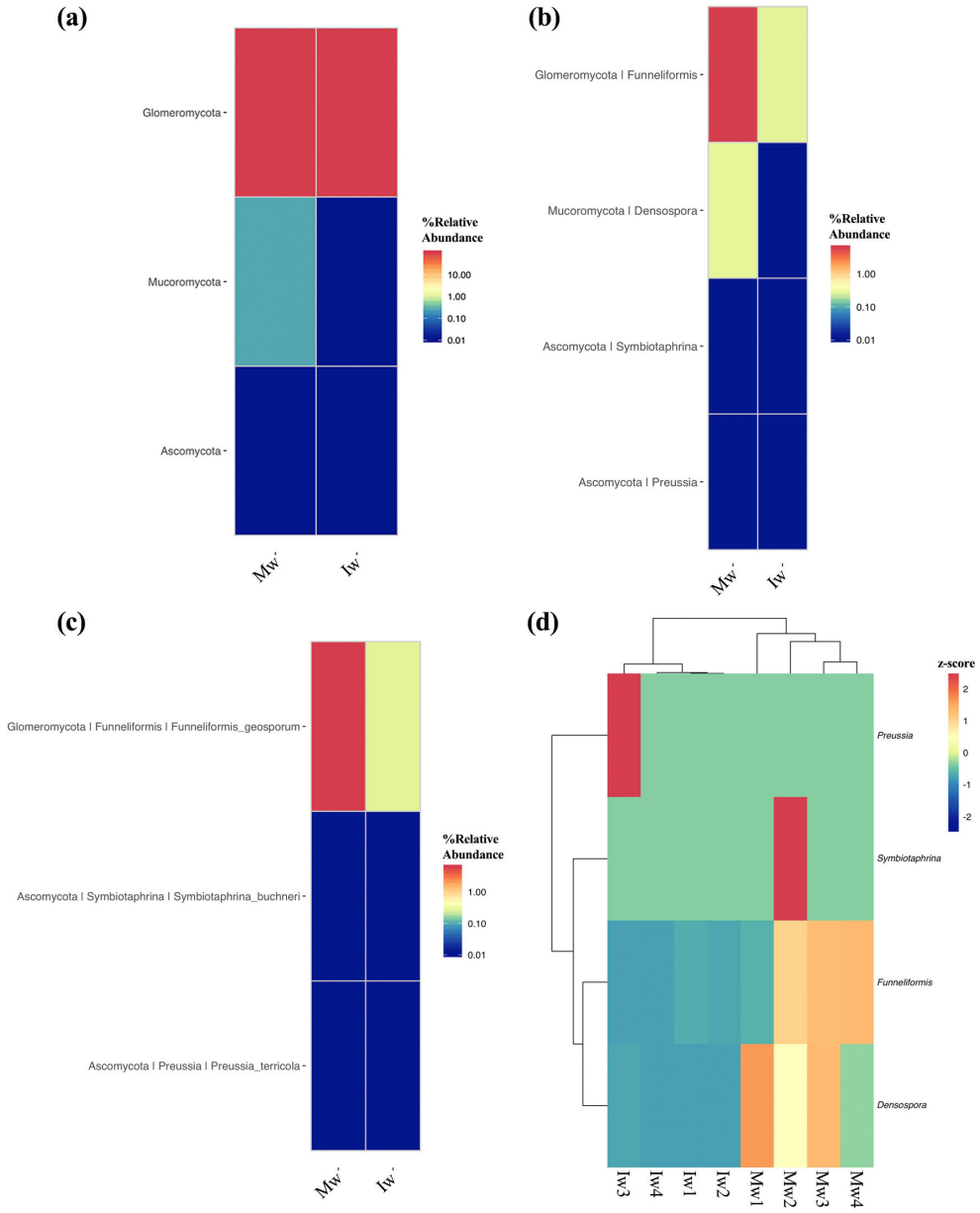

Figure 2. Relative abundance of fungal communities with high abundance at the phylum (**a**), genus (**b**) and species (**c**) level in different walnut orchards, along with a heatmap (**d**) of soil fungal community composition at the genus level. See Table 1 for abbreviations.

3.3. Changes in the Diversity of Soil Fungal Communities

Alpha diversity of fungi can reflect the abundance, homogeneity, and diversity of fungal communities. The results showed that the 1-Simpson (Figure 3b) and Shannon (Figure 3c) index of the soil fungi was higher in the interplanted walnuts than in the monoculture walnuts, with no difference in the Chao1 (Figure 3a) index between the two treatments. This indicated that the cover plant *V. villosa* increased the alpha diversity of fungi in the walnut orchard.

Beta diversity of fungi gives an indication of fungal community structure. A principal component analysis (PCA) of the fungal composition of walnut soils showed that PC1 and PC2 contributed 63.7% and 26.9%, respectively, with a total contribution of 90.6% (Figure 3d). PC1 clearly distinguished between the monoculture walnut and the interplanted walnut groups. The four samples from the monoculture walnut treatment clustered together, indicating that the fungal community composition within the group was similar. In contrast, the high dispersion between the four samples from the interplanted walnut

indicated significant changes in fungal community structure (Figure 3e). These results suggest that *V. villosa* as a cover crop had a high degree of variability on the soil fungal community structure of walnut orchards. At the taxonomic unit level of genus, a random forest analysis of soil fungi revealed that *Densospora* and *Funneliformis* differed significantly between the two groups, with a greater influence of *Densospora* on the grouping (Figure 3f).

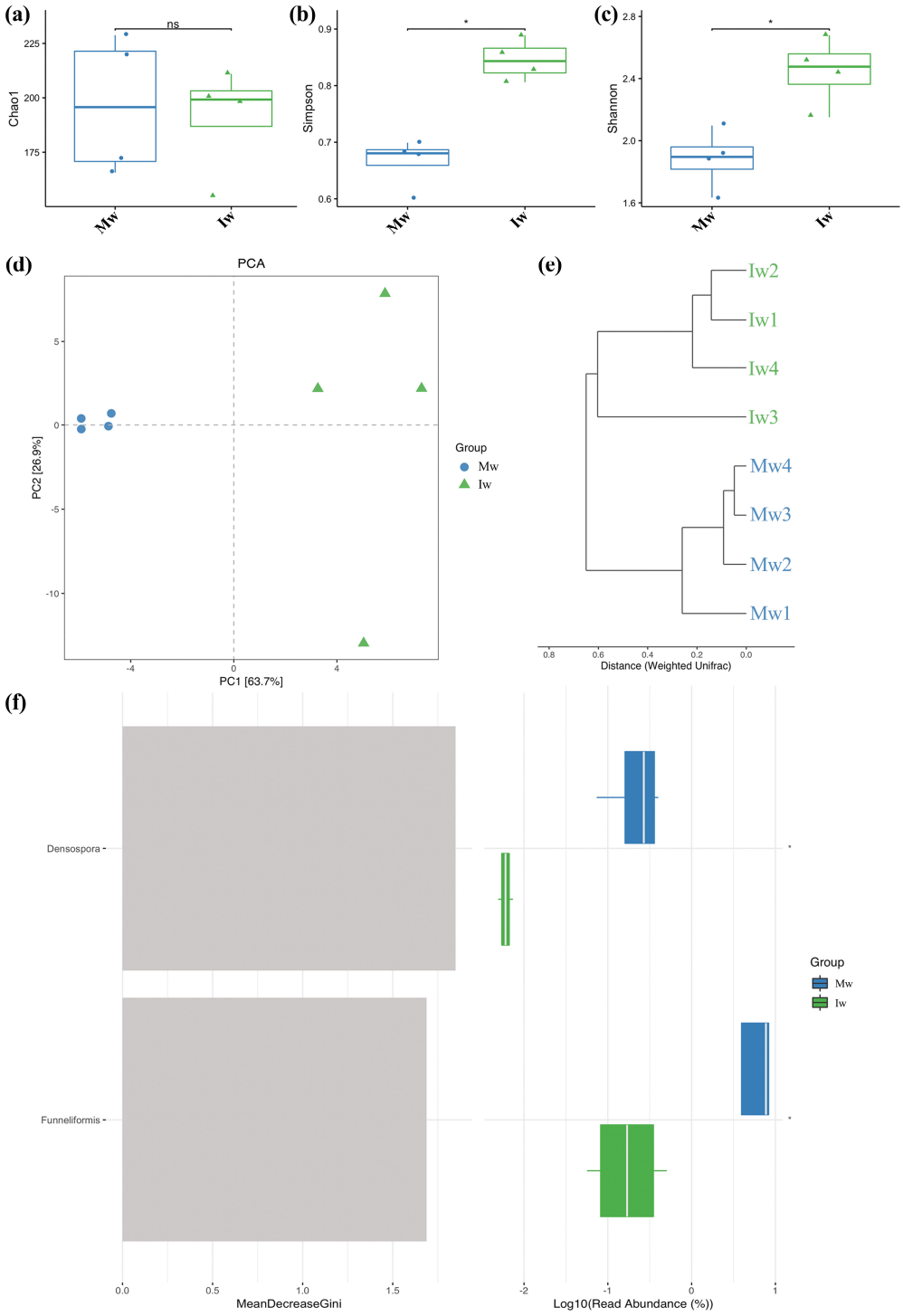

Figure 3. Alpha diversity index of fungal community richness (Chao1) (**a**) and diversity (1-Simpson and Shannon) (**b**,**c**) in different walnut orchards, as well as principal component analysis (**d**), weighted unifrac clustering analysis (**e**), and random forest analysis (**f**). *: $p < 0.05$, ns: $p > 0.05$. See Table 1 for abbreviations.

3.4. Changes in Mycorrhizal Fungal Growth in Soil and Roots

A good symbiotic association could be developed between walnut roots and native AMF (Figure 4a), with root AMF colonization rate of 38.67% and 75.12% in monoculture and interplanted walnut groups, respectively (Figure 4b). The cover plant *V. villosa* significantly promoted walnut root AMF colonization rate by 94%, compared to the monoculture. A large number of mycorrhizal hyphae existed in the rhizosphere of walnuts (Figure 4c), with soil hyphal length ranging from 3.41–5.60 cm/g (Figure 4d). Soil hyphal length was significantly reduced by 39%, after planting the cover plant *V. villosa* compared to the monoculture walnut.

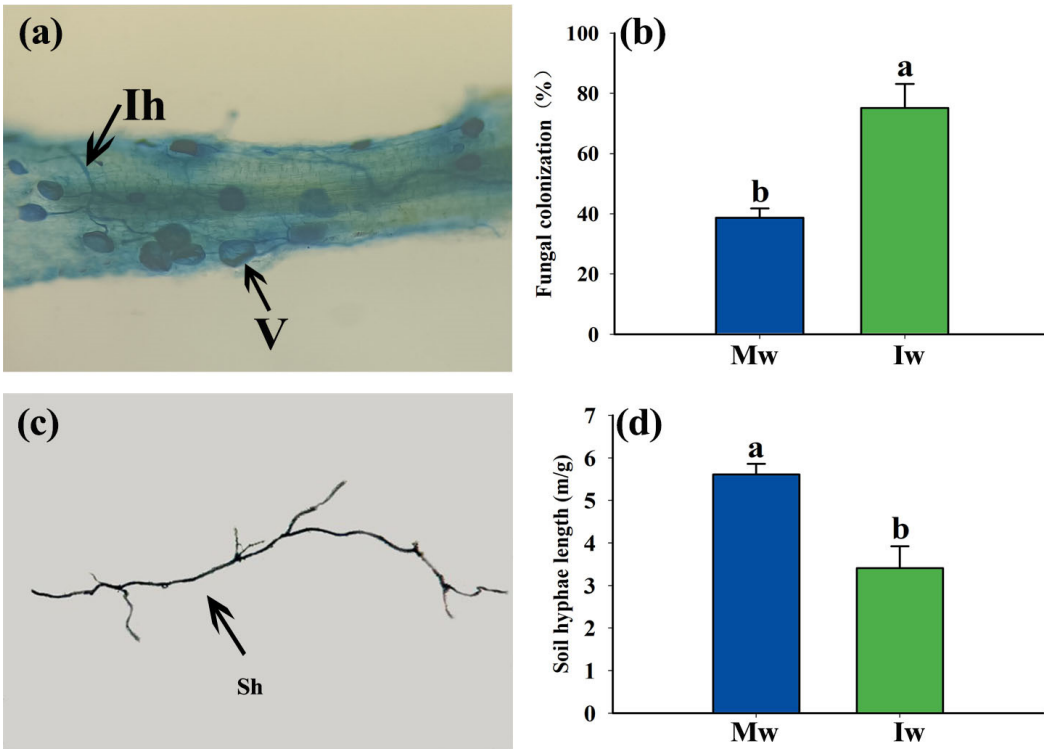

Figure 4. Changes in AMF colonization and soil hyphae of walnuts after planting *V. villosa*. (**a**) AMF colonization of walnut roots; (**b**) changes in AMF colonization rate; (**c**) soil mycorrhizal hyphae of walnut; (**d**) changes in rhizosphere soil hyphal length. Data (means ± SE, $n = 4$) with different letters above the bars indicate significant ($p < 0.05$) differences. Abbreviations: Ih, intraradical hyphae; Iw, interplanted walnut; Mw, monoculture walnut; V, vesicle; Sh, soil hyphae.

4. Discussion

High-throughput sequencing techniques can provide detailed insights into the composition and dynamics of soil microbial communities and have become a prominent way for studying microbial community diversity [39], with ITS sequencing being widely used for fungal taxonomy [40]. In this study, high-throughput sequencing of ITS was performed on soils from walnut orchards in monoculture and from walnut orchards interplanted with the cover crop *V. villosa* to elucidate the effect of agroforestry systems on soil fungal diversity in walnut orchards. The sequencing demonstrated that *V. villosa* as a cover plant altered the fungal community composition of walnut orchards and enriched the fungal community diversity of soils, which was consistent with the results of Qin et al. [41] in the soil fungal community of cucumber intercropped with watercress. Studies have demonstrated that *V. villosa* promoted soil fungal diversity and improved soil quality [42]. According to the findings of Qian et al. [33], white clover as a cover plant in apple orchards improved the diversity, richness, and relative abundance of soil beneficial fungi and specific fungal genera. In our study, sod culture dramatically reduced soil fungi at the genus and species level, as compared with monoculture, which may be due to the toxic effect of juglone released by

walnut roots on soil fungi [43]. The results of PCA showed that the fungal communities in the interplanted walnut differed significantly from those in monoculture forests. The fungal diversity of the four samples in the interplanted walnut was quite discrete, indicating that the effect of *V. villosa* as a cover plant on soil fungi in walnut orchards was variable.

Sod culture in orchards increases total and soluble organic carbon concentrations of the soil, thus altering the abundance and diversity of fungi in the soil [44]. Mulching in hazelnut orchards improved soil physicochemical conditions, resulting in reduced pathogenic fungi and increased symbiotic fungi [45]. Similarly, long-term sod culture also changed the soil fungal community composition of Chinese hickory orchards, with increasing the relative abundance of Ascomycota, lowering the relative abundance of Basidiomycota, and changing the dominant genus in the soil [46]. The results of the high-throughput ITS sequencing data also showed that *V. villosa*, as a cover plant, had an effect on soil fungi of walnut orchards at different taxonomic levels. At the phylum level, Glomeromycota exhibited the highest relative abundance in the interplanted walnut, while Mucoromycota had a lower abundance. Glomeromycota are known to be associated with mycorrhizal fungi in the soil, and they can colonize about 80% of terrestrial plants to establish mycorrhizae that help plants obtain nutrients and water [47]. Mucoromycota can establish a variety of beneficial or pathogenic associations with their hosts [48]. The use of *V. villosa* as a cover plant in walnut orchards lowered the similarity in soil fungal community composition and considerably reduced the abundance of *Funneliformis* (a group of arbuscular mycorrhizal fungi) and *Densospora* (a group of ectomycorrhizal fungi) in the soil. In addition, native AMF could colonize roots of walnut, with fungal colonization rates of 38.67% and 75.12% in monoculture and intercropped walnut trees, respectively. The colonization of indigenous AMF was observed in walnuts in Linxiang, Yunnan, China, with an AMF colonization rate of 80.67% [49]. The variability in mycorrhizal colonization rates between our study and the study by Mao et al. [49] could be attributed to the fact that root AMF colonization rate is influenced by a variety of factors such as soil conditions, environmental factors, management practices, and tree age [50]. Interplanting *V. villosa* promoted the colonization of beneficial AMF on walnut roots. Similarly, Wang et al. [51] also reported an increase in root mycorrhizal colonization of citrus trees after sod culture with white clover. However, interplanting *V. villosa* also dramatically reduced soil hyphal length, as compared with the monoculture walnuts, which may be related to the high dispersion of soil fungi induced by *V. villosa*. In addition, interplanting *V. villosa* also reduced the relative abundance of *Funneliformis*. Both *Funneliformis* and *Densospora* are beneficial root-associated fungi that form mycorrhizae with plant roots. They may form a dense underground common mycorrhizal network in the soil between plants that contribute to plant water and nutrient uptake [52]. Indeed, legumes (e.g., *V. villosa*) require large amounts of phosphorus for nitrogen fixation, and mycorrhizal fungi are prominent in promoting P uptake by the host [30]. Thus, in walnut intercropped with *V. villosa*, more mycorrhizal fungi were enriched in the intercropped plants than in the walnut rhizosphere. Another study conducted by He et al. [53] also showed that *V. villosa* could be colonized by AMF, with a higher root colonization rate in *V. villosa* (78%) than in walnuts (57%). Therefore, mycorrhizal fungi such as *Funneliformis* and *Densospora* may heavily colonize roots of *V. villosa*, resulting in a reduction in the walnut rhizosphere. Draghi et al. [54] also confirmed that in sustainable agricultural systems, *V. villosa* as a cover plant was able to recruit root-associated beneficial microorganisms, such as *Burkholderia* spp., in its roots, contributing to the dominance of beneficial microorganisms for subsequent crop growth. In such intercropping pattern of walnut, mycorrhizal fungi also increased root colonization rate of walnut, thus reducing the abundance of mycorrhizal fungi in the soil. The present study only analysed the changes in soil AMF, and more research is needed in conjunction with mycorrhizal fungal diversity in walnut roots as well as in roots of intercrops. Mycorrhizal fungi from the roots of *V. villosa* can be released into the walnut root environment after the senescence of *V. villosa* in summer, which is helpful for walnut growth and hence the sustainability of walnuts.

Interplanted walnuts reduce abundance of soil *Funneliformis*, resulting in a decrease in soil mycorrhizal hyphae. In turn, this appears to cause more *Funneliformis* to colonize walnut or *V. villosa* roots, leading to an increased rate of root mycorrhizal colonization, which benefits the growth and nutrient absorption of walnut [23–25], thus encouraging sustainable production of walnut. Furthermore, sod culture in orchards also improved soil fertility, thereby reducing nitrogen fertilizer inputs [55]. However, the effect of sod culture on soil microbes would exhibit distinct differences with the growth period of fruit trees [36]. Therefore, an in-depth study of the composition and diversity of soil fungal communities in the interplanted model is useful for elucidating the *V. villosa*–AMF–walnut interactions and assessing soil productivity, health, and sustainability in walnut orchards.

5. Conclusions

Interplanting *V. villosa* in walnut orchards increased the diversity and changed community composition of soil fungal populations in walnut orchards, but reduced the abundance of *Funneliformis* and *Densospora*, with *F. geosporum* reducing rapidly. One of the reasons for the decrease in mycorrhizal fungi in the walnut rhizosphere is the increased accumulation of mycorrhizal fungi in roots, which ensures the long-term sustainable production of walnut. The changes in the soil fungal population and diversity caused more AMF to colonize roots after sod culture with *V. villosa*, which is beneficial to the growth of walnut and the reduction of fertilizer inputs in orchards, as well as building a good microenvironment for the soil to achieve sustainable development of the orchard.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su15130731/s1, Figure S1. The relative abundance of fungal communities at the class (a), order (b), and family (c) level in different walnut orchards.

Author Contributions: Conceptualization, Q.-S.W.; data curation, W.-X.H. and Q.-F.S.; methodology, W.-X.H.; resources, Q.-S.W. and Y.-J.X.; supervision, Q.-S.W.; writing—original draft preparation, W.-X.H.; writing—review and editing, A.H., E.F.A., Y.-J.X. and Q.-S.W. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Hubei Province '14th Five-Year' Major Science and Technology Aid Tibet project (SCXX-XZCG-22016). The authors are grateful to their sincere appreciation to the Researchers Supporting Project Number (RSP2023R356), King Saud University, Riyadh, Saudi Arabia.

Data Availability Statement: All data supporting the findings of this study are included in this article.

Acknowledgments: The authors are grateful to their sincere appreciation to the Researchers Supporting Project Number (RSP2023R356), King Saud University, Riyadh, Saudi Arabia.

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

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