Effects of living cover on the soil microbial communities and ecosystem functions of hazelnut orchard

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Abstract

Background: Living covers are an important management measure for orchards in China, which has a certain influence on soil properties, microorganisms and micro-ecological environment. However, there are few studies on the effects of living covers on the soil changes in hazelnut orchard. In this study, we compared living cover treatment by *Vulpia myuros* and no cover treatment, and analyzed the changes on the soil properties, microorganisms and microbial function by using high-throughput ITS rDNA and 16S rRNA gene Illumina sequencing.

Results: The consequences demonstrated that the total organic carbon content of living cover treatment in the 20-40 cm soils increased by 32.87 % and 14.82 % respectively in May and July compared with no cover treatment, and living cover treatment by *Vulpia myuros* can also significantly increased the contents of total phosphorus (TP), total nitrogen (TN), available phosphorus (AP) and available potassium (AK) in the soil samples. Moreover, the influence of seasons is not as significant as that of soil depth. The living cover treatment significantly improved the soil enzymes activity levels. Among the four kind of soil enzymes, the soil invertase activity of living cover treatment was 50.94 % greater than that of no cover treatment in 0-20 cm soils and 52.17 % in 20-40 cm soils in May. The consequences demonstrated that Ascomycota, Mortierellomycota and Basidiomycota were the dominant fungal phylum in all samples, while Proteobacteria, Actinobacteria, Acidobacteria, Firmicutes and Chloroflexi were the dominant bacterial phylum, but different treatments impacted the composition of fungal and bacterial communities. Principal component analysis (PCA) showed that living cover with *Vulpia myuros* significantly changed the soil fungal community structure whereas the bacterial community structure may be more sensitive to seasonal changes. At the microbial functional level, the pathotrophs, saprotrophs and symbiotrophs accounted for approximately 36.69 %, 49.80 % and 2.59 % of no cover treatment fungal OTUs respectively whereas in living cover treatment was 19.76 %, 41.51 % and 6.89 %.

Conclusions: According to this study, we believe that the living cover with *Vulpia myuros* has a favorable regulating influence on soil properties, microbial community and microbial function, and can be considered as a management measure for hazelnut orchards management.

Background

Hazelnut, a shrub or small tree belonging to *Corylus* L. Betulaceae, is one of the four largest nuts in the world, together with walnut, almond and cashew. It has high ecological and economic value. *Corylus heterophylla* Fisch. × *Corylus avellane*, which is mainly cultivated in China, is an interspecific hybrid selected and bred by the cross-breeding of *Corylus heterophylla* and *Corylus avellane*. It concentrated *Corylus heterophylla*'s strong cold adaptability, the characteristics of good kernel flavor and *Corylus avellane*'s advantages of high yield and kernel rate [1]. In the breeding process of hazelnut is usually to the clean tillage of orchard, the clean tillage can reduce weeds compete with the nutrition of fruit trees, but also can make the soil directly exposed, so that the rainy season is prone to surface runoff, reduce the

soil nutrient supply capacity, soil harden and soil and water loss, organic matter, orchard ecological environment destruction and so on [2–4].

The interplanting grass in orchard is a kind of agroforestry system which has developed rapidly in recent years[5, 6]. Previous studies try to use *Trifolium repens* and *Lolium perenne* as interplanting grass in orchard materials, which can effectively inhibit soil and water loss. However, due to its close growing period to deciduous fruit trees, it will inevitably lead to the problem of water and fertilizer competition [7–10]. *Vulpia myuros* as conservation grass in deciduous orchards has potential advantages which is a perennial grass with a plant height of about 50 cm and dense growth. It sprouts in September every year and dies in June the following year. The remaining seeds can germinate in September of the same year. In summer and autumn, the dead but fixed murine thatch can keep soil moisture and prevent soil erosion [6, 11, 12]. *Vulpia myuros* decay can not only inhibit the growth of other weeds [13], but also replenish organic matter in the soil and improve the soil properties [14, 15]. In addition, *Vulpia myuros* and hazelnuts have different fertilizer period needed for growth, which can meet the requirements for green fertilizer in winter. Yang et al. found that July to October is the main period for *Vulpia myuros* decomposition, which can provide nutrients for the growth of fruit trees instead of competing for nutrients [16]. At present, *Vulpia myuros* is mostly used in the planting of fruit trees and green fertilizers, and the application of *Vulpia myuros* intercropping on hazelnut orchards has not been reported. Therefore, we use *Vulpia myuros* as the living cover material.

In this study, we studied the soil properties (soil pH, water content, nutrient contents and enzyme activities), microbial diversity, community structure and functional prediction of different depth (0–20 cm, 20–40 cm) under two different treatments (no cover, living cover) in spring and autumn. Soil microbial communities with obvious differences in two treatments were recognized by Linear discriminant analysis Effect Size tool (LEfSe). And the relationships between soil microbial community composition and soil properties were discussed by the spearman correlation analysis. We supposed that (1) living cover treatment can affect soil properties and microbial community composition and function; (2) living cover treatment is beneficial to the development of soil micro-ecology. This study could provide theoretical support for scientific management of hazelnut orchards.

Results

Soil nutrient contents

As shown in supplementary Table S1, the water content and pH of the living cover was considerably ($p < 0.05$) higher than the no cover treatment. In the upper soil samples, the water content of living cover treatment was 11.59% ($17.23 \pm 0.36\%$; $15.44 \pm 0.36\%$) and 19.43% ($22.50 \pm 1.75\%$, $17.45 \pm 0.95\%$) higher than that of no cover treatment in May and July respectively. While in the deeper soil, they are 28.94% ($19.36 \pm 0.98\%$, $16.21 \pm 0.69\%$) and 48.05% ($28.78 \pm 0.98\%$, $19.44 \pm 0.58\%$), respectively. In May, the pH of living cover treatment was 14.53% (6.07 ± 0.06 , 5.30 ± 0.10) and 9.88% (6.23 ± 0.06 , 5.67 ± 0.12) higher than that of no cover treatment in the upper soil and deeper soil respectively. And in July, it was 6.37%

(6.17 ± 0.06 , 5.80 ± 0.10) and 10.70% (6.83 ± 0.06 , 6.17 ± 0.06) respectively. The soil TOC content under living cover treatment was higher than before (11.37 g/kg), but had no significant ($p > 0.05$) effect under no cover treatment. Compared with no cover treatment, the TOC content of living cover treatment in the upper soil increased by 18.02% ($14.08 \pm 0.59 \text{ g/kg}$, $11.93 \pm 0.74 \text{ g/kg}$) and 17.21% ($13.35 \pm 0.51 \text{ g/kg}$, $11.39 \pm 0.89 \text{ g/kg}$) respectively in May and July, while that in the deeper soil increased by 32.87% ($12.37 \pm 0.33 \text{ g/kg}$, $9.31 \pm 0.88 \text{ g/kg}$) and 14.82% ($10.15 \pm 0.39 \text{ g/kg}$, $8.84 \pm 0.30 \text{ g/kg}$) respectively. In May, the levels of TP, TN, AP and AK increased by 13.20%, 8.89%, 13.84% and 13.79%, respectively, while in July, they were 12.17%, 12.12%, 16.82% and 23.10%. And with the change of seasons, TN content decreased in two soil layers, but TP, AP and AK contents increased.

Soil Enzymes

The living cover treatment significantly ($p < 0.05$) improved the soil urease (URE), catalase (CAT), alkaline phosphatase (ALP), invertase (INV) activity contents (Additional file 2: Table S2). In the upper soil, the soil urease activity of living cover treatment ($11.60 \pm 0.13 \text{ IU/g}$) was 22.88% more than that of no cover treatment ($9.44 \pm 0.07 \text{ IU/g}$) in May and 49.15% in July ($12.35 \pm 0.20 \text{ IU/g}$, $8.28 \pm 0.04 \text{ IU/g}$). And the soil catalase activity of living cover treatment was 12.28% ($187.50 \pm 2.78 \text{ IU/g}$, $167.00 \pm 2.02 \text{ IU/g}$) higher than that of no cover treatment soil in May and 33.38% ($194.48 \pm 2.84 \text{ IU/g}$, $145.81 \pm 2.35 \text{ IU/g}$) in July. And the soil alkaline phosphatase activity of living cover treatment was 5.33% ($0.79 \pm 0.01 \text{ IU/g}$, $0.75 \pm 0.02 \text{ IU/g}$) higher than that of no cover treatment soil in May and 11.76% ($0.76 \pm 0.01 \text{ IU/g}$; $0.68 \pm 0.02 \text{ IU/g}$) in July. The soil invertase activity of living cover treatment was 50.94% ($0.80 \pm 0.03 \text{ IU/g}$, $0.53 \pm 0.02 \text{ IU/g}$) higher than that of no cover treatment soil in May and 21.43% ($0.85 \pm 0.04 \text{ IU/g}$, $0.70 \pm 0.01 \text{ IU/g}$) in July. In the deeper soil, the soil urease activity of living cover treatment was 1.05 times ($8.37 \pm 0.10 \text{ IU/g}$, $7.90 \pm 0.17 \text{ IU/g}$) that of no cover treatment soil in May and 1.10 times ($8.11 \pm 0.15 \text{ IU/g}$, $7.36 \pm 0.17 \text{ IU/g}$) in July. And the soil catalase activity of living cover treatment was 1.30 times ($166.87 \pm 1.28 \text{ IU/g}$, $128.33 \pm 3.84 \text{ IU/g}$) that of no cover treatment soil in May and 1.11 times (151.76 ± 1.38 , $136.66 \pm 1.50 \text{ IU/g}$) in July. The soil alkaline phosphatase activity of living cover treatment was 1.22 times ($0.73 \pm 0.01 \text{ IU/g}$, $0.60 \pm 0.02 \text{ IU/g}$) that of no cover treatment soil in May and 1.17 times ($0.56 \pm 0.03 \text{ IU/g}$, $0.48 \pm 0.02 \text{ IU/g}$) in July. The soil invertase activity of living cover treatment was 1.52 times ($0.70 \pm 0.02 \text{ IU/g}$, $0.46 \pm 0.03 \text{ IU/g}$) that of no cover treatment soil in May and 1.13 times ($0.72 \pm 0.02 \text{ IU/g}$, $0.64 \pm 0.01 \text{ IU/g}$) in July. The enzyme activities of each treatments were higher in the upper soil than that in the deeper soil.

Fungal Community Diversity

The Shannon index (H'), Richness index (S) and Evenness index (E') of fungal community were evaluated with the Illumina Miseq high-throughout sequencing data (Additional file 3: Table S3). Most living cover treatments remarkably increased the levels of H' , S , and E' in the soil. And living cover treatment can significantly ($p < 0.05$) increase the richness index (S). Living treatment (189.7 ± 6.4 , 188.7 ± 18.9) was 38.91% higher than no cover treatment (136.7 ± 8.5 , 135.7 ± 16.2) in spring while it was 38.80% in summer (171.0 ± 8.5 , 161.7 ± 13.4 ; 115.0 ± 5.6 , 124.7 ± 10.8). Among the no cover treatments and living cover treatments, there was no remarkably ($p > 0.05$) difference in the three diversity indexes between the

upper and deeper soil. And the season had no obvious influence on the three indexes. The S level of living cover treatment was the highest in the upper soil in May (189.7 ± 6.4), which was considerably ($p < 0.05$) higher than the no cover treatments.

Bacterial Community Diversity

The Shannon index (H'), Richness index (S) and Evenness index (E') of bacterial community were evaluated by the Illumina Miseq high-throughout sequencing data (Additional file 4: Table S4). All living cover treatments improved the H', S, and E' in the soil but there was no obvious ($p > 0.05$) difference in these three diversity indexes between the no cover treatments and living cover treatments except NB_May (392.7 ± 24.8) and LB_May (440.7 ± 33.8). And the season and the soil depth have no significant ($p > 0.05$) influence on the three indexes. The S level of living cover treatment (440.7 ± 33.8) was the highest in the deeper soil in May.

Fungal Community Structure

To standardize the different sequencing depths, each sample made random selection of 44,412 reads to analysis (Additional file 5: Table S5). Ascomycota (60.16%) and Basidiomycota (25.36%) were the main fungal communities (Fig. 1). Unclassified_k_Fungi was represented in 3.14% of all sequences. The relative abundances of Ascomycota and Mortierellomycota of the living cover treatments decreased compared with that in no cover treatments, but relative abundances of Basidiomycota increased. Principal component analysis (PCA) also showed the difference between no cover and living cover treatments, and the soil samples in different treatments were obviously separated. Two principal components were determined, which clarified the total variance of 99.29% in the dataset (Fig. 2). Sequences from the Ascomycota dominated in upper soil samples (63.31%) and deeper soil samples (57.01%) collected from both two treatments (no cover and living cover) (Fig. 1a). With the change of seasons, the relative abundances Ascomycota decreased, whereas that of the Basidiomycota increased under two treatments (no cover and living cover) in phylum level, but there was no obvious change in Mortierellomycota (Fig. 1a). The relative abundance of Ascomycota in spring was higher than that in summer, but the relative abundance of Basidiomycota was opposite.

The overall fungal community was dominated by Sordariomycetes (40.92%) and Agaricomycetes (14.43%) (Fig. 1b) under the class level. In the both all treatments, the class Agaricomycetes and Tremellomycetes were represented by 18.89% and 18.95% of the sequences in living cover sampling, whereas the class represented only 9.98% and 2.70% in no cover (Fig. 1b). And the the class Mortierellomycetes and Eurotiomycetes were represented by 15.93% and 10.07% of the sequences in no cover sampling, whereas the class represented only 4.96% and 4.02% in living cover (Fig. 1b). With the seasons change, the relative abundances of Sordariomycetes of the living cover treatments decreased while in deeper soil increased. The relative abundances of Agaricomycetes, Tremellomycetes and Leotiomycetes of the living cover treatments increased compared with that in no cover treatments in both upper soil and deeper soil, while the situation of Mortierellomycetes, Eurotiomycetes and Pezizomycetes were the opposite (Fig. 1b).

Bacterial Community Structure

To standardize the different sequencing depths, each sample made random selection of 14,275 reads to analysis (Additional file 6: Table S6). The dominant fungi are Proteobacteria (44.78%), Actinobacteria (16.48%) and Acidobacteria (15.22%) among all samples (Fig. 3). The living cover was not obviously affected the relative abundances of the phylum except Elusimicrobia (Additional file 7: Figure S1). The relative abundances of Acidobacteria of the living cover treatments decreased compared with that in no cover treatments while relative abundances of Actinobacteria increased but all of these two phylum were not demonstrated a significant correlation. And there was no significant ($p > 0.05$) difference between deeper soil and upper soil while there were significant differences between seasons in phylum and class level (Additional file 8,9: Figure S2, Figure S3).

The result of PCA showed the obvious difference between soil samples in spring and summer. And two principal components were determined, which can explain 88.38% of the total variance in the dataset (Fig. 4). Sequences from Proteobacteria dominated in Spring soil samples (37.79%) and Summer soil samples (51.77%) collected from both two seasons (Fig. 3). The relative abundance of Proteobacteria and Acidobacteria were the highest in Summer, and the lowest in Spring while Actinobacteria and Firmicutes reached its highest abundance in Spring.

Relationship Between Microbial Community Structures And Soil Properties

Living cover treatment changed microbial community structures and soil properties. After removal of the redundant variables, ten soil properties were chosen for RDA. As shown in Fig. 5a,b, pH, TOC, TP, TN, AK, URE and INV significantly affected the fungal community structure in upper soil, while soil properties except AK and URE all obviously ($p < 0.05$) impacted the fungal community composition in the deeper soil. Soil properties except TOC, TN and ALP all remarkably ($p < 0.05$) impacted the bacterial community composition in the upper soil, while SWC, pH, AP and ALP dramatically ($p < 0.05$) impacted the bacterial community composition in the deeper soil (The relevant p value was in Additional file 10: Table S7). From the angle between arrow connecting lines representing different soil properties, TP, pH, INV, AK, AP, and SWC were always small, which showed that they had a good correlation and were positively correlated in each treatment. In the same way, TN, TOC, ALP, and URE were also positively correlated.

Prediction Of Community Functions Of Soil Fungi And Bacteria

The micro-ecological functions of fungi and bacteria in the soil of hazelnut orchard under no cover and living cover treatments were studied by analyzing the fungal and bacterial communities by FUNGuild and PICRUST1. The guilds identified in present study were listed in Fig. 6, the consequences demonstrated that the functional prediction results of fungi were related to different treatments. And three nutritional patterns (pathotrophs, saprotrophs and symbiotrophs) accounted for approximately 36.69%, 49.80% and 2.59% of no cover treatment fungal OTUs respectively whereas in living cover treatment was 19.76%, 41.51% and 6.89%. There was no obvious divergence between no cover treatment and living cover treatment according to analyzing the bacterial communities in the Cluster of Orthologous Groups (COG) database by PICRUST1 (Additional file 11: Figure S4).

Discussion

The effects of the living cover on soil properties

One of the main considerations in the technology of living cover is to consider the water distribution between living cover and fruit trees. Some living covers will compete with fruit trees for water, which will adversely affect the growth of fruit trees [17–19]. In the present study, because of the relatively abundant rainfall before the two sampling, the water competition between live cover with *Vulpia myuros* and hazelnut was very weak. On the contrary, as shown in Table S1, live covering with *Vulpia myuros* may also be beneficial to water collection and storage.

Studies on apples and teas showed that pH decreased with the increase of planting years in orchards [20, 21]. Under acidic conditions, the ability of plant roots to absorb water and nutrients was limited, and the growth and development of plants are inhibited. The accumulation of H^+ in soil will accelerate soil acidification, which will destroy the plasma membrane of root cells and lead to nutrient loss [22]. It can be clearly seen from Fig. 1 that the value of pH in upper layer of the uncovered soil after planting for three years was lower than that before planting, but the pH value can be significantly increased after planting *Vulpia myuros*, and the living environment of living cover can better meet the pH requirements (5.5–8.0) in the technical regulations for cultivation of Ping'ou hybrid hazelnuts [23]. In the living cover treatments, the reason for the increase of pH value may be attributed to ion uptake by living cover to reducing the salt level in the soil solution all the time. The use of nutrient ions by plentiful microbial communities in the rhizosphere of living cover roots may also contribute to this trend [24].

Plant litter covers the base of trees or turns into soil, thus providing nutrient resources for soil through microbial decomposition [25–27]. And fertilization every year was also an important way to improve soil nutrients. Studies had proved that living cover can improve the soil nutrient level by improving the microbial activity in the soil [27–29]. In this study, all of the living cover treatments remarkably enhanced the TOC, TP and TN content of the soil samples. The total nutrient level of the soil shows the storage of soil nutrients, while the available nutrient level of soil reflects the dynamic balance between plant absorption and soil mineralization [30]. In our study, all the living cover treatments obviously enhanced the AP and AK contents in the soil, revealing the positive effect of living cover plants on soil P and K. In summary, *Vulpia myuros* had no competitive effect on hazelnut.

The effects of the living cover on soil enzyme activity levels

The activities of soil urease, soil alkaline phosphatase activity and soil invertase activity have a very good correlation with the content of soil TOC and TN.

Enzyme activity in soil can be regarded as one of the important indicators of soil fertility, and played a crucial role in maintaining and improving soil fertility [31–36]. Different from the Zhu et al.'s result [37, 38] that the covers with Leguminosae in vineyards obviously raised the activities of soil INV, URE and ALP, whereas the covers with Gramineae had no substantially effect on the activities of these three enzymes.

In this study, the consequences showed that the positive effects of living cover treatments on the soil enzyme activity contents was better than that of no cover treatment. The different expressions of soil enzyme activity may be related to the influence of living cover treatment on the composition of soil microbial community [29, 39]. The results obtained in this study were similar to the research of Xu et al., that the cover with gramineae had obvious influences on the soil enzyme activity contents compared with no cover treatment. The possible reason for the improvement of soil enzyme activity level was that living cover improved the soil properties, thus raising the number and activity of microorganisms [40–42].

As shown in Table S2, the enzyme activity of upper soil was remarkably higher than that of lower soil samples in all treatments. Many research results showed that soil enzyme activity decreases with the deepening of soil layer thickness. There may be two reasons: on the one hand, the content of soil organic matter and other nutrients which had great influence on enzyme activity decreased with the increase of soil profile depth; on the other hand, fine roots were mainly distributed in the surface soil, while *Vulpia myuros* had the characteristics of fibrous roots, and its roots were mainly concentrated in the upper soil. So it gradually decreased from top to bottom in soil, so soil enzyme activity also showed a trend of decreasing [43–45].

The effects of the living cover on soil microbial communities

In this study, ITS and 16S rDNA Illumina Miseq high-throughout sequencing was used to measure the communities changes of fungi and bacteria in soil. The Illumina Miseq high-throughout sequencing can reflect the genetic diversity of communities of fungi and bacteria, and the Illumina Miseq high-throughout sequencing analysis included the whole microbial community. As shown in Table S3, living cover treatments obviously increased the fungal community diversity and functional structure in the soil samples. And the PCA results also showed a clear separation between soil samples collected in different treatments (no cover and living cover). Living cover treatment can significantly improve the abundance and diversity of soil microorganisms. This consequence was consistent with the previous research results[46, 47]. However, there was no significant correlation with soil depth and seasonal changes. This may be because the abundance and diversity of fungi were insensitive to the changes of environmental factors caused by soil depth and seasonal changes. And this may be the reason why many studies only analyze the changes in abundance and diversity of bacteria or nematodes caused by environmental changes [38, 42, 48].

Fungi are the main microbial group that decompose forest soil organic matter by producing enzymes [49]. Our results demonstrated that Sordariomycetes and Mortierellomycetes may have an important contribution to soil organic matter decomposition in the no cover treatments, while Agaricomycetes, Tremellomycetes and Leotiomyces might be more important in living cover treatments (Fig. 1) [50, 51]. According to the reported studies, some members of Agaricomycetes are related to most ectomycorrhiza, which has been widely agreed that ectomycorrhiza can promote the growth of trees and is very important to temperate forests. Other members as critical decomposers can effectively decompose wood polymers [52–56]. The most plentiful genus of Agaricomycetes in the living cover treatments soil were *Tomentella*,

Paxillus, *Inocybe* and *Hymenogaster*, which are known as ectomycorrhizal fungi in present study [57, 58]. Erik A et al. suggested that *Tomentella* was related to nitrogen deposition and was an important part of community structure under the condition of high overall nutrient availability [59]. Studies had shown that *Paxillus involutus* can degrade organic matter like plant litter by overexpressed a number of transcripts of oxidases [60]. And polyphenol oxidase and protease activities in plant litter inoculated with *Paxillus involutus* were significantly improved, which was 2–3 times higher than that without *Paxillus involutus* treatment [61]. *Inocybe* and *Hymenogaster* also play a very important role in the growth and ecology of trees by forming ectomycorrhizal associations [62–70]. According to the function of ectomycorrhizal fungi clarified in the above research, it was speculated that the increase of their content in living cover may lead to the change of soil nutrients enzyme activity levels. In the upper soil, the extracellular enzyme activities reached its peak in Summer because of the fresh grass litter accumulated. The results of RDA analysis (Fig. 5) also showed that there was a favorable correlation between extracellular enzyme activity and relative plentiful of Tremellomycetes. The change of fungal community composition in living cover treatments measured may be an important factor leading to the increase of organic matter available for recycling. Oligotrophic is a characteristic of members of Acidobacteria [69], which explains that the members of Acidobacteria could live in with low carbon and pH environments. This may demonstrate why the proportion of Acidobacteria had enhanced in no cover treatments, which had lower soil carbon and pH than living cover treatments. And this also may demonstrate the influence of sampling depth that deeper soil had lower soil carbon and pH than upper soil. Moreover, the ratio of Proteobacteria to Acidobacteria can be used to represent the nutritional status of the ecosystem, and if the ratio is low, it indicates the malnutrition status [70]. In the current study, the proportion of no cover treatments was low, which proved that no cover treatments could be regarded as malnutrition status compared with living cover treatments. And this was consistent with the redundancy analysis results of environmental factors in Fig. 5c, 5d. Seasonal changes have a significant impact on the community structure of bacteria, it may be that the sampling site belongs to Monsoon Climate of Medium Latitudes, bacterial structure was more sensitive in alternate periods of season [71].

The effects of the living cover on soil microbial functions

Previous studies had shown that reducing farming can protect some fungal plant pathogens from high temperature, limited water resources and interference [72, 73]. Some studies had also shown that reducing cultivation can limit the movement of plant pathogen spores, maintain more microbial communities, and inhibit the invasion and establishment of plant pathogens [72, 74, 75]. Interestingly, the results of FUNGuild Showed that living cover treatments decreased the number of pathotrophs and increased symbiotrophs in this study, which was consistent with the research results of the latter.

Proteobacteria and Actinobacteria are copiotrophs, which are characterized by preferential consumption of soil organic carbon pools and high nutritional demand [51, 52, 69]. It is possible that there was little difference in the total amount of these two bacteria in each treatment, which led to similar prediction functions in each treatment. Studies in soil microcosms had shown that the ectomycorrhizal fungi mycelium can reduce the activity of saprophytic bacteria [76]. However, the function of bacteria had not

changed significantly in this study, which may be related to the low OTUs content of ectomycorrhizal fungi.

Conclusions

In our study, we researched the influences of living cover, soil depth and seasonal change on microbial diversity, community composition and ecological functions in hazelnut orchard soil. The microbial composition and ecological function can be affected by living cover treatment with *Vulpia myuros* on hazelnut orchard management. The results showed that living cover treatment with *Vulpia myuros* improved the physical and chemical conditions of the soil in hazelnut orchard and caused great changes of the soil microbial diversity, community composition and ecological functions, especially in fungal community, which reduced the OTUs number of pathotrophs and increased symbiotrophs. The living cover treatment with *Vulpia myuros* in hazelnut orchard could have more beneficial and diverse micro-ecological environment compared with no cover treatment. Therefore, living cover with *Vulpia myuros* could be a good management of the hazelnut orchard compared with no cover management. Furthermore, study on the reasons why living cover treatment has no obvious effect on the difference of bacterial function is indispensable. And it is also needed to study on the effects of soil microorganisms under different cover treatments to looking for the most suitable cover plant for hazelnut orchard in the future.

Methods

Study site and soil sampling

Our study was conducted in Yingkou (40°11'24" N, 122°9'30" E), Liaoning Province, China. This place has a Continental temperate monsoon climate. The average annual rainfall is 700 mm, and the average annual temperature is 9.8°C. According to the World Reference Base for Soil Resources (2014), the orchard soil type was divided into clay loam[77]. The main properties of the hazelnut orchard soil before the experiment were as follows: pH 5.90, total organic carbon (TOC, 11.37 g/kg), total nitrogen (TN, 0.62 g/kg), total phosphorus (TP, 0.79 g/kg). The variety of hazelnut planted in this experiment was 'Dawei' (*Corylus heterophylla* Fisch. × *Corylus avellane*), and the tree age was 3 years, and the row spacing × plant spacing was 4 m × 3 m. There are two treatment methods in this experiment: no cover, living cover with *Vulpia myuros*. Each treatment consisted of three randomly arranged plots, and each of which was about 288 m² (18.0 m × 16.0 m). Samples were taken in May and July respectively, and the upper (0–20 cm) and deeper (20–40 cm) soils were collected each time. Planting a 1.6-meter-wide living cover between rows. Clean cultivation was carried out under the fruiters. The covered crops were seeded in October 2016, with a sowing rate of 20 kg per ha of *Vulpia myuros*. All treatments used the same fertilization method which included using 750 kg urea fertilizer and 1000 kg manure compost ha⁻¹ • year⁻¹. Orchard soil samples were collected on May 10th and July 25th in 2019 after covered for three years. Collect and mix six random soils at the depth of 0–20 cm and 20–40 cm between the rows in each plot. The subsequent treatment of samples was consistent with the previous studies[38, 47].

Soil Physical And Chemical Properties

Use the method in previous study to determine the water content and pH of soil samples[38]. K_2CrO_4 oxidation method is used to determine the TOC and the Kjeldahl method is used to measure the TN. The TP in the soil adopts NaOH alkali fusion-atomic absorption method. The Olsen method is used to measure the soil AP. Using flame photometer determines the soil AK after NH_4OAc extraction. The URE, CAT, ALP and INV activity of the soil were determined according to Qian et al. [78].

DNA extraction and polymerase chain reaction amplification

Extraction of microbial DNA from soil samples, concentration and purification of final DNA, and inspection of DNA quality were described in Zhang's previous research[47]. The DNA samples were amplified in V5-V7 hypervariable regions in bacteria and 16S rDNA was amplified with primers 799 F (5'-AACMGGATTAGATACCCKG-3') and 1193 R (5'-ACGTCATCCCCACCTTCC-3'), while DNA samples amplified in ITS1 region in fungi were amplified with primers IT1F (5'-CTTGGTCATTTAGAGGAAGTAA - 3') and IT2R (5'-GCTGCGTTCTTCATCGATGC-3') by PCR (GeneAmp 9700, ABI, USA). The steps used in PCR reaction had been described in detail in previous studies. The specific steps of PCA reaction were described by Li et al. [47].

Illumina MiSeq sequencing and data processing

The sequencing of purified amplicons were described in previous study[47]. The original readings were uploaded to the NCBI Sequence Read Archive (SRA) database (Study accession number: SRP278043; BioProject ID: PRJNA657994). Quality filtering of sequencing data, clustering, removal of chimeric sequences, control of joint database and other sequencing data processing are described in previous studies[47, 79].

Statistical analysis

One-way ANOVA of soil properties was carried out by SPSS (version 26.0; SPSS, Chicago, IL, USA). In all analyses, significance was evaluated by Tukey's test ($p < 0.05$). The largest axis length was 3.38 at OTU level and 1.62 at class level in Detrended correspondence analysis (DCA). Therefore, redundancy analysis (RDA) was carried out by using Monte Carlo permutations (permu = 999) to tested the significance of the soil properties. According to the functions of envfit (permu = 999) and vif.cca, soil properties were selected, and the soil properties with $p > 0.05$ or $vif > 20$ were eliminated from the following analysis. The vif values of CAT was higher than 20 and eliminated. The analyses of ANOSIM and RDA were carried out by R for statistical calculation [80]. The Canoco program for Windows 4.5 (Biometris, Wageningen, the Netherlands) was used for principal component analysis (PCA). Using FUNGuild which is used as a tool to classify and analyze fungal communities through microecological guides based on the published literature or authoritative website data, fungi are divided into pathotrophs, symbiotrophs and saprotrophs [81].

List Of Abbreviations

SWC: The water content of the soil; TOC: Total organic carbon; TN: Total nitrogen;

TP: Total phosphorus; AP: Available phosphorus; AK: Available potassium;

URE: Soil urease activity; CAT: Soil catalase activity;

ALP: Soil alkaline phosphatase activity; INV: Soil invertase activity

ITS: Internal transcribed spacer; LEfSe: Linear discriminant analysis Effect Size;

OTU: Operational Taxonomic Units; RDA: Redundancy analysis;

DCA: Detrended correspondence analysis; PCA: Principal component analysis

Declarations

Ethics approval and consent to participate

Not applicable. The soil samples used in this study were provided by Research Institute of Forestry, Chinese Academy of Forestry. It does not require ethical approval.

Consent for publication

Not applicable.

Availability of data and materials

The raw sequencing data have been deposited in NCBI Sequence Read Archive (SRA) under bioProject ID PRJNA657994 and study accession number SRP278043.

Competing interests

The authors declare that they have no competing interests.

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Author Contributions

WM carried out the experiments, collected and organized data, and wrote manuscript. YZ and SH participated in the data analysis. QM and LL reviewed the manuscript and gave constructive suggestions. GW participated in the design experiment and guided the research. CL helped to do the experiment. The corresponding author TZ, put forward the basic hypothesis of this work, designed experiments, and helped organize the structure of manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Relative abundances of fungal phylum and class in the no cover treatment and living cover treatment. a The structure of fungal community at phylum level. b The structure of fungal community at class level.

Figure 2

PCA of fungal communities. The values on axes 1 and 2 are the interpretable percentages of the corresponding principal components.

Figure 3

Relative abundances of bacterial phylum in the no cover treatment and living cover treatment.

Figure 4

PCA of bacterial communities. The values on axes 1 and 2 are the interpretable percentages of the corresponding principal components.

Figure 5

Redundancy analysis (RDA) of MiSeq data and soil properties. a RDA of fungal communities in 0-20cm. b RDA of fungal communities in 20-40cm. c RDA of bacterial communities in 0-20cm. d RDA of bacterial communities in 20-40cm. The red line with arrow indicated soil properties, and the blue line with arrow indicated top five fungi or bacteria class. The values on axes 1 and 2 are the interpretable percentages of the corresponding principal components.

Figure 6

Fungal community functional features inferred by FUNGuild.

Figure 7

OTUs average value of three kinds of nutrition modes of fungi

Figure 8

(no caption provided)

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