Distribution diversity of fungi and bacteria communities in the pure plantation of *Larix gmelinii* and *Fraxinus mandshurica*

Qing Yu Wei

Northeast Forestry University School of Forestry <https://orcid.org/0000-0002-0409-2462>

Wei Li

Northeast Forestry University

Yulong Zhang

Northeast Forestry University

Xing Wei (✉ weixing94@163.com)

Northeast Forestry University <https://orcid.org/0000-0001-6491-9006>

Research

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**Distribution diversity of fungi and bacteria communities in
the pure plantation of *Larix gmelinii* and *Fraxinus
mandshurica***

Qing-Yu Wei^{1,2}, Wei Li^{1,2}, Yun-Long Zhang^{1,2}, Xing Wei^{1,2*}

Qing-Yu Wei: 2595251322@qq.com;

Wei Li: liwe930201@163.com;

Yun-Long Zhang: 840263726@qq.com;

Xing Wei*: weixing94@163.com.

*Correspondence: weixing94@163.com

¹ School of Forestry, Northeast Forestry University, Harbin 150040, Heilongjiang, P. R. China;

² Key Laboratory of Sustainable Forest Ecosystem Management-Ministry of Education, Northeast Forestry University, Harbin 150040, P. R. China.

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Abstract

Background: *Larix gmelinii* (larch) and *Fraxinus mandshurica* (ash) are two important tree species in northeast China and are infected by Ectomycorrhizal fungi and arbuscular mycorrhizal fungi, respectively.

Method: Using the high-throughput sequencing method, we analyzed the composition of Fungi and bacterial communities in the roots, Rhizosphere, and Non-rhizosphere soil of 21-year-old larch and ash pure plantations. Furthermore, we also analyzed the impact of soil environmental factors on the Fungi and bacterial community diversity according to soil nutrition.

Results: There were differences in the fungal community diversity between larch and ash. *Ascomycota* increased gradually from the larch root to non-rhizosphere soil, whereas *Streptophyta* decreased sharply from the larch root to non-rhizosphere soil. However, the trend of *Basidiomycota* and *Streptophyta* under the ash forest was opposite to that of the larch. At the same time, it was found that *Larix*, *Pyronemataceae*_Unclassified, *Cenococcum*, and *Ulmus* were endemic to larch, whereas *Anemone* and *Monographella* were endemic to ash. The bacteria were similar under larch and ash forest. *Proteobacteria* decreased gradually from rhizosphere to non-rhizosphere soil, and the relative abundance of *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Rokubacteria*, *Gemmatimonadetes*, *Firmicutes*, and *Nitrospirae* were the lowest in the roots of the two species. *Pseudomonas*, one of the Plant Growth-Promoting Rhizobacteria (PGPR), had high relative abundance in the roots of the two tree species. The fungal and bacterial communities in the root, rhizosphere soil, and non-rhizosphere soil of the same tree species were different. The distribution diversity of the fungal and bacterial community of larch was non-rhizosphere soil > rhizosphere soil > root. The bacterial community diversity of the ash rhizosphere soil was the highest, whereas the fungal community diversity in the root was the highest. The *Larix*, the special fungus in the larch, were mainly distributed in the root and decreased sharply outside the root. The *Pyronemataceae*_Unclassified, *Cenococcum*, and *Ulmus* were mainly distributed in the rhizosphere soil. The special fungi of ash were mainly distributed in the rhizosphere. *Burkholderiaceae*-Unclassified, one of the PGPR, was mainly distributed in the roots of larch, but it was the opposite in the ash. *Bacillus* and *Paenibacillus* existed widely in the rhizosphere soil of ash. However, the abundance of *Paenibacillus* in larch was low, and it gradually increased from the root to the outside. The relative abundance of *Streptomyetaceae*_Unclassified was slightly high in the larch non-rhizosphere soil and ash rhizosphere soil. There was a correlation between PGPR and some fungi under the two tree species. Among them, *Bacillus* had a significant synergistic effect with *Mortierella* and *Mucor* under larch forest. There was a positive correlation between total nitrogen and bacteria in rhizosphere soil under larch forest, and its content was significantly higher than that of other treatments. There was a positive correlation between total phosphorus and fungi in ash rhizosphere soil, and the content was significantly lower than that in non-rhizosphere soil. However, the relationship between soil fungi and bacteria to soil nutrients was not significant.

Conclusion: Therefore, compared with the bacterial community, endomycorrhizal tree species have greater differences in the fungal community. The diversity of fungal and bacterial communities in ectomycorrhizal trees increase from rhizosphere soil to non-rhizosphere soil, while the diversity of fungal communities in endomycorrhizal trees is the highest in roots.

Key Words: *Larix gmelinii*; *Fraxinus mandshurica*; Rhizosphere soil; Soil Fungi; Soil bacteria

Background

After long-term continuous growth of the pure artificial forest, the decline of soil fertility in most woodlands is easy to cause the slow growth of trees^[1]. Mycorrhiza can absorb more nutrients by promoting the absorption area of tree roots in the forest^[2,3], to promote the growth of trees. The formation of mycorrhiza is related to the type, quantity, and distribution of fungi. Moreover, Plant Growth-Promoting Rhizobacteria (PGPR) in soil^[4] contributes to the growth and development of some mycorrhizal fungi by promoting mycorrhizal colonization^[5-7].

Soil fungi and bacteria, as the most important functional components in soil biota, play an important role in the ecosystem. They are mainly affected by tree root exudates and litter decomposition products^[8]. Because the root exudates and litter decomposition products of different tree species are different, different soil microbial groups are formed, and these microorganisms directly or indirectly participate in the process of soil nutrient transformation^[9,9]. Mycorrhiza is a symbiont formed by soil fungi infecting vegetative roots of plants. It can form hyphae to extend outward to absorb nutrients and water to supply plants, and mycorrhizal fungi can adjust their activities by absorbing carbohydrates from plants through this symbiotic relationship^[11]. Mycorrhizal types have a certain regulatory effect on root exudates and litter decomposition of different tree species, and also affect nutrient absorption of trees^[8]. Compared with bacteria, fungi can coexist with plants, which makes them more sensitive to different vegetation types^[12]. Among them, the community structure of AM fungi is greatly influenced by host species^[13]. At the same time, mycorrhizal fungi play an important role in material and energy conversion in the root microenvironment^[14]. As the main part of soil microorganisms, soil bacteria can promote the growth of mycorrhizal fungi by improving the nutrient status and inhibiting pathogenic bacteria^[14]. Xu Tan et al. found that the soil nutrient availability under poplar plantation was significantly related to the evolution of the soil bacterial community. They think that soil bacteria in poplar plantation have been affected by overground vegetation for a long time, which changes their properties, thus affecting forest soil, resulting in a gradual downward trend of soil nutrient content with the increase of continuous cropping time, and finally affecting overground vegetation in turn^[16]. It can be seen that bacteria play a key role in the growth of aboveground vegetation and soil fertility. The contents of soil available phosphorus and organic matter have a very significant positive correlation with the number of soil bacteria, and the number of bacteria in rhizosphere soil is more than that in non-rhizosphere soil^[17]. Therefore, it is of great significance to study the relationship between soil environment and diversity of fungi and bacteria communities in plantations for rational utilization of soil, prevention of soil fertility decline, and improvement of the ecological environment.

To further explore the response of soil fungi and bacteria communities under different mycorrhizal tree species and the interaction between them and nutrient acquisition in forest land. We took pure artificial forests of larch and ash as research objects, which were ectotrophic mycorrhizas (EM) species and arbuscular mycorrhizas (AM) species, respectively. The community structure of fungi and bacteria in the root, rhizosphere soil, and non-rhizosphere soil under 21-year-old larch and ash plantations was measured and the relationship between fungi and soil nutrients was analyzed. The purpose of this study is to understand how soil nutrients of larch and ash plantations affect the diversity and function of soil fungi and bacteria communities under the same stand age and site conditions. And which nutrient elements play a leading role in it? It also reveals the internal mechanism of soil fertility after the natural thinning of larch and ash forests, the interaction effect

between soil fungi and bacteria communities, and the relationship between the diversity of fungi and bacteria communities and the soil environment. It is beneficial to further optimize the stand structure of larch and ash, prevent the decline of soil fertility, and improve the ecological environment. To provide a reference for rational planting and management of larch and ash plantations in Northeast China.

Results

Sequencing results of fungal and bacterial samples

The fungi and bacteria in the root, rhizosphere, and non-rhizosphere soil of larch and ash were sequenced by the Illumina Miseq sequencing platform. The effective sequences of fungi in larch and ash were clustered into 605 and 611 OTUs; respectively under 97% similarity. The effective bacterial sequences were clustered into 3824 and 3846 OTUs with 97% similarity. The number of bacteria was greater than that of fungi, and the number of microbes in ash was greater than that of larch. The rarefaction curve of the vast majority of samples tended to be flat, which indicated that the sequencing quantity of the measured samples was reasonable and could truly reflect the fungal community structure in different areas under two tree species (Figure 1). However, the rarefaction curve of bacteria still had an upward trend and had not yet reached saturation, indicating that new groups may be found with the increase of sequencing depth.

Composition and diversity of fungal and bacterial community structure

Composition of fungal and bacterial community structure

Among the 15 fungi phylum obtained from 18 soil samples (Figure 2a), *Ascomycota*, *Streptophyta*, *Mucoromycota*, and *Basidiomycota* were the important groups with higher relative abundance. *Ascomycota* and *Mucoromycota* increased from root to non-rhizosphere soil. The relative abundance of *Basidiomycota* in larch roots was less. The abundance of *Streptophyta* decreased sharply from larch root to non-rhizosphere soil. *Basidiomycota* and *Streptophyta* showed an opposite trend to larch under ash. *Larix*, *Pyrenomataceae*_Unclassified, *Cenococcum*, and *Ulmus* were the special fungi of larch. *Larix* was the most dominant in larch roots. *Pyrenomataceae*_Unclassified, *Cenococcum*, and *Ulmus* were distributed in the rhizosphere soil. *Anemone* and *Monographella* were special fungi of ash, which were distributed most in rhizosphere soil. A large number of *Gagea* were found at individual sampling points in the soil outside the root of ash, but the abundance at other sampling points was small or non-existent (Figure 2b). *Mortierellaceae*-Unclassified had little abundance in the roots of the two species, but more in the rhizosphere soil of the two species. *Fraxinus* was only found in the roots of two species.

A total of 26 bacterial groups were obtained at phylum level (Figure 3a), the dominant bacteria are *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Verrucomicrobia*, *Chloroflexi*, *Bacteroidetes*, *Rokubacteria*, *Gemmatimonadetes*, *Firmicutes*, and *Nitrospirae*. *Proteobacteria* had the largest abundance in the roots of the two tree species and showed a decreasing trend from the roots to the non-rhizosphere soil. *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Rokubacteria*, *Gemmatimonadetes*, *Firmicutes*, and *Nitrospirae* all showed that the abundance in the roots of the two tree species was lower than that in the outer soil. *Verrucomicrobia* was the most abundant in FR and decreases from FR to the outside, but it did not exist in larch (Figure 3b). There were some PGPR in the obtained bacterial genus, including *Pseudomonas*, *Burkholderiaceae*-Unclassified, *Bacillus*, *Paenibacillus*, and *Streptomyces*_Unclassified. The relative abundance of *Pseudomonas* in the roots of the two species was higher. *Burkholderiaceae*-Unclassified was more

in LR than in other sampling positions, but the opposite was true in ash. *Bacillus* had the highest relative abundance in FRS. *Paenibacillus* widely existed in FRS, and its abundance was low at every sampling point of larch, and it gradually increased from the root to the outside. The relative abundance of *Streptomyetaceae*_Unclassified in LNS and FRS was relatively high. In larch forest (Table 1), *Pseudomonas* and *Burkholderiaceae* were positively correlated with *Larix*, and *Bacillus* had a significant synergistic effect with *Mortierella* and *Mucor*. Under ash forest (Table 2), *Burkholderiaceae* had a very significant positive correlation with *Gagea* and *Colletotrichum*. *Pseudomonas* had a significant synergistic effect with *Fraxinus*. And *Bacillus*, *Paenibacillus*, and *Streptomyetaceae* had a very significant positive correlation with *Anemone*.

Diversity analysis of fungi and bacteria communities

By comparing and analyzing the number of OTU and four kinds of α diversity indices, we found that the richness and diversity of ash fungal community gradually decreased from inside to outside the root, while that of larch fungal community gradually increased from inside to outside the root (Table 3). The richness and diversity of the larch bacterial community were consistent with the change of its fungal community, while the richness and diversity of the ash bacterial community were in the order of rhizosphere soil > root > non-rhizosphere soil. Based on the weighted unifracs distance (Table 4), the differences between fungal and bacterial communities between the two tree species in the root, rhizosphere soil, and non-rhizosphere soil were compared at OTU classification level (Figure 4). The bacterial communities of the two tree species at different sampling points in the root, rhizosphere soil, and non-rhizosphere soil were similar ($d < 0.3$).

However, the differences in different areas and sampling points under the two tree species had a great influence on the diversity of fungal communities. There were differences between LR fungal community and LRS, FR, FRS, and FNS fungal community ($d > 0.5$). Except for individual sampling points of ash, there were differences between the FNS fungal community and other sampling points of ash, and there were also great differences among the three sampling points of FNS. There were similarities among different sampling points in the same area under some same tree species, especially in LR, FR, and FRS ($d < 0.5$).

Based on the Bray-Curtis distance matrix, the UPGMA (unweighted pair group method with arithmetic mean) cluster tree was constructed by the unweighted group average method in hierarchical clustering (Figure 5). F₁NS, F₃NS, L₁R, and L₂R were different from other samples in species composition and relative abundance, and they were separated first. Three samples of FNS were in different branches. In contrast, LRS, LNS, and FRS were closely related. According to the analysis, it could be roughly classified into four categories, among which LR was classified as Class I; FR was class II; FNT was classified as class III; LRS, LNS, and FRS were grouped into class IV.

Soil nutrients and their effects on the diversity of fungi and bacteria communities

Soil nutrients

The contents of total nitrogen and total potassium in the soil under larch forest were higher than that of ash. The highest content of total phosphorus in FNS was 1555.00mg/kg, but the lowest content of total phosphorus in FRS was 765.50mg/kg. The highest total nitrogen content in LRS was 0.32g/kg, which was significantly higher than other treatments ($P < 0.05$). Total potassium content in LNS was significantly higher than that in FRS and FNS, and there was no significant difference between LRS, FRS, and FNS. The total phosphorus content of FNS was significantly higher than that of LRS and FRS, but there was no significant difference between FNS and LNS, while there was a significant difference between FRS and LRS ($P < 0.05$) (Table 5).

Relationship between soil nutrients and diversity of fungi and bacteria communities

According to the relative abundance matrix of bacteria and fungi OTU, the gradient length in the first axis of DCA results was 0.55 and 2.59, respectively, so RDA was selected in this study (Figure 6). The first and second sorting axes of soil fungal community diversity and soil nutrient index RDA explained 39.42% and 44.10% of total species variables, respectively (Figure 6a). TK value had a positive correlation with the second-ranking axis, which had a high degree of influence on the diversity of LNS and FNS fungal communities. TN and TP mainly affect the diversity of other fungal communities, among which TN and TP had a positive correlation with fungal community diversity of FRS.

The first and second sorting axes of soil bacterial community diversity and soil nutrient index RDA explained 16.52% and 21.01% of total species variables, respectively (Figure 6b). FNS and LNS were separated from other treatments along the first sorting axis and negatively correlated with TN. Bacterial community diversity of LNS and LRS is greatly influenced by TP and TN, and TP had a higher degree of influence on bacterial community diversity of FRS. Different samples in FNS were scattered, which reflected the diversity of soil fungal community in FNS to a certain extent. The influence degree of each soil nutrient index did not reach a significant level ($p>0.05$), which indicated that microbial diversity in rhizosphere soil and non-rhizosphere soil of larch and ash was less sensitive to soil nutrients.

Discussion

Community structure characteristics of fungi and bacteria

Soil fungi are the source of most plant residue degrading enzymes. The vast majority of known fungi discovered so far belong to *Ascomycota* and *Basidiomycota*, which are closely related and dominate the degradation process of plant residues^[18], and there are a large number of unclassified species^[18]. According to the high-throughput sequencing analysis, we found that the relative abundance of *Ascomycota*, *Streptomyces*, *Mucoromycota*, and *Basidiomycota* in larch and ash was relatively high. Among them, *Ascomycota* was dominant in all samples, and its relative abundance in the non-rhizosphere soil of ash was as high as 39.34%. It indicated that ash, as an arbuscular mycorrhizal tree species, had a stronger ability to decompose litter, promoted the formation of soil organic matter, and provided more powerful conditions for the survival of fungi and bacteria in the soil. This is consistent with the research results of Wang Xinqi et al^[20]. *Proteobacteria*, *Actinobacteria*, and *Firmicutes* are the dominant bacterial groups in the rhizosphere, and some pieces of literature also record *Verruciformis* and *Nitrosporum*^[20-24]. In this study, *Proteobacteria*, *Acidobacteria*, and *Actinobacteria* were relatively abundant in the root and undergrowth soil of larch and ash (average relative abundance value > 15%). *Proteobacteria* was the dominant bacterial group in all sampling sites (relative abundance value > 20%). *Proteobacteria* is considered to consume a large number of organic compounds released by roots^[23,25]. *Proteobacteria* might grow rapidly in the rhizosphere microflora of various plants^[26], which led to the relative abundance of *Proteobacteria* in larch and ash forests in the order of rhizosphere soil > non-rhizosphere soil.

The growth and reproduction ability of soil bacteria is more vigorous than that of fungi^[27]. In this study, there were more bacteria than fungi in the root and soil of larch and ash plantations. The bacterial richness of the ash forest was greater than that of the larch forest, but its fungal richness was less than that of the larch forest. It might be related to different mycorrhizal infection types of two tree species. The difference in a litter between broad-leaved trees and conifers may also affect

the richness of fungi and bacteria. A large number of soil bacteria are related to fungal hyphae^[28], among which *Burkholderia* and *Pseudomonas* are the most abundant^[14,29]. *Pseudomonas* in the mycorrhizal rhizosphere can significantly promote mycorrhizal synthesis^[30]. In this study, *Pseudomonas* was distributed most in the root and root zone soil of larch, and more in the rhizosphere soil. However, it was rarely found in the rhizosphere soil and non-rhizosphere soil of ash, which might make the root system of larch have a larger nutrient absorption area than ash. Studies had found that the abundance and diversity index of bacteria and fungi in the root of the ectomycorrhizal tree *Quercus mongolica* were lower than those in the surrounding soil^[31]. In this study, the richness and diversity of fungi and bacteria in larch undergrowth were non-rhizosphere soil > rhizosphere soil > root, which was consistent with its conclusion. In this study, the richness and diversity of bacteria in the rhizosphere soil of ash were the highest, but the richness and diversity of fungi in its roots were the highest. Ash is an endomycorrhizal tree species, and fungal hyphae in roots can penetrate root cells for reproduction, which may lead to higher richness and diversity of fungi in roots.

Effects of soil nutrients on the diversity of fungi and bacteria communities

Soil fungi and bacteria have an impact on soil nutrient utilization and plant growth^[32]. The abundance of samples in winter is significantly higher than that in summer^[33-35], which may be related to temperature^[36]. Studies have shown that autumn is the most period for the growth and development of fungi^[37]. In this study, the influence of soil nutrients on soil microorganisms is small, which may be related to the inactivity of soil microorganisms in June. Nitrogen is an essential nutrient element for plants. We found that the total nitrogen content in the rhizosphere soil of larch was significantly higher than other treatments, and it was positively correlated with the diversity of soil bacterial community. We know that PGPR can promote the acquisition of nitrogen through non-symbiotic and symbiotic ways, including *Burkholderiacepacia* and *Pseudomonas*^[38]. And TN in the rhizosphere soil of larch was higher than that of ash. *Cenococcum*, as a special fungus of larch, may slow down the decomposition of fungal necrosis and root tissue^[39,40], thus affecting the storage of TN under larch forest. At the same time, *Burkholderiacepacia* and *Pseudomonas* were found to have the highest relative abundance in larch roots. *Burkholderiacepacia* has a certain hyphal transmission ability^[41], and *Pseudomonas* strains show ACC deaminase activity. This enzyme can decompose ACC and promote root elongation^[38]. Thus *Burkholderiacepacia* and *Pseudomonas* in larch roots help trees absorb nitrogen in the surrounding soil more widely.

Phosphorus is a key element for plant growth, but it is difficult for plants to obtain it^[42]. Most phosphorus in the soil exists in the form of a phytic acid inorganic compound, and phytic acid can't be directly obtained by roots^[43]. Phytase degrades phytic acid to lower phosphate ester, which has been found in *Bacillus*, *Burkholderia*, and *Pseudomonas*, among which *Bacillus* and *Pseudomonas* are the main contributors to help plants obtain phosphorus from the soil due to their strong solubility characteristics^[44]. In this study, total phosphorus was positively correlated with fungal and bacterial communities in the rhizosphere soil of ash. *Pseudomonas*, *Bacillus*, and *Burkholderia* were the most abundant in the rhizosphere soil of ash (relative abundance > 1%). It indicated that there was more soluble phosphorus in the rhizosphere soil of ash for plants to absorb. In this study, *Mortierella*-unclassified was relatively abundant in the rhizosphere soil of ash. *Mortierella*-unclassified can dissolve mineral phosphorus in various types of soil by synthesizing and secreting oxalic acid, which is released into soil solution^[45,46] and absorbed by plants. *Mortierella* and *Bacillus* had a remarkable synergistic effect under larch forest, which widely existed in rhizosphere soil and non-rhizosphere

soil of larch. *Mortierella* can promote soil organic matter storage, maintain soil health, and promote plant growth by stimulating primary metabolism-related genes and reshaping the rhizosphere bacterial community^[47]. Compared with larch, ash, as a broad-leaved tree species, needs to absorb more phosphorus for photosynthesis. In this study, there were great differences among the three sampling points of ash non-rhizosphere soil, which might be related to the spatial heterogeneity of nutrients in forest land. However, tree species with thicker roots like ash have little or no response to nutrient heterogeneity^[48]. Moreover, different types of mycorrhizal tree species have different ways to obtain nutrients, that is, AM tree species ash obtains nutrients by producing more roots, while EM tree species larch produces more mycorrhizal fungi hyphae^[49]. Compared with the root system or AM fungi, EM fungi can decompose some organic matter and obtain nutrition from it^[50]. The mobility of phosphorus itself is very poor, so ash can't directly obtain nutrients in further areas by producing hyphae as root extension like larch. This may result in the lowest total phosphorus content in rhizosphere soil and the highest total phosphorus content in non-rhizosphere soil of ash.

Conclusions

Under the same site conditions, the diversity and distribution of fungi and bacteria in larch and ash plantations were quite different. The bacterial richness of ash pure forest was greater than that of larch pure forest, whereas the fungal richness was less than that of larch pure forest. The diversity of fungal and bacterial communities in larch roots was the smallest. However, the bacterial diversity in the root of ash was the smallest and the fungal diversity was the largest, which showed the characteristics of ash as an endomycorrhizal tree species. Moreover, larch and ash showed differences in fungal community diversity. Bacteria were similar in larch and ash forests. There were many PGPR in LR and FRS that could promote the mycorrhizal growth and plant nutrient absorption, including *Pseudomonas*, *Burkholderiaceae*-Unclassified, *Streptomyces*, *Bacillus*, and *Paenibacillus*. these PGPR had a significant synergistic effect with some mycorrhizal fungi. There was more total phosphorus in the non-rhizosphere soil of the two tree species. The total phosphorus content in the rhizosphere soil of ash was significantly lower than that in non-rhizosphere soil. The soil under larch forest was rich in total nitrogen. Soil fungi and bacteria were less sensitive to soil nutrients, but there was a positive correlation trend between soil total nitrogen and fungi and bacteria.

This paper reveals the differences in the distribution and diversity of fungal and bacterial communities between ectomycorrhizal and endomycorrhizal artificial pure forests. The reasons for this difference are analyzed from the aspects of the interaction between fungi and bacteria and soil nutrients. To provide a theoretical basis for exploring the interaction between fungi and bacteria under larch and ash forests and the relationship between fungi and soil nutrient acquisition in forest land.

Materials and methods

Study sites description

This research was conducted on woodlands at Maoershan Experimental Forest Farm (127°-127°33'E, 45°19'-45°24'N) of Northeast Forestry University in Shangzhi City, Heilongjiang Province. It is located on the west slope of Zhangguangcai Range in the north of Changbai Mountains and is the transition zone from Songnen Plain to Zhangguangcai Range. It is a low hilly area with an average elevation of 350m and a general slope of 10°~15° and has a temperate continental monsoon climate, with an annual average temperature of 2.75°C, the effective

accumulated temperature of 2530°C, annual sunshine duration of 2470h, and a frost-free period of 120-140 days. Shangzhi City receives an average of 723.5mm of precipitation annually, with 31.1% and 21.2% occurring in July and August, respectively. The annual average evaporation is 1094.5mm. Woodlands were situated on a dark brown soil. The stands we studied were divided into larch and ash pure plantations (21year) afforested with seedlings in the clear-felling stands of secondary forest in the spring of 1998. The afforestation density of them was 2m×2m. The two stands are located in the middle and lower part of the southeast slope ($<10^{\circ}$), with flat slope and similar sit conditions, with an altitude of about 300m and a soil layer thickness of about 35cm. After afforestation, the stands have not been thinning, but the stand density has decreased due to natural sparse sampling.

Sample collection and processing

In mid-June, 2019, three 20 m×20 m sample plots were randomly selected from larch and ash pure plantations, with a total of 6 sample plots. According to the principle of the "five-point sampling method", a target tree was randomly selected within a 1m radius of each sampling point. We removed litter on the surface of the soil and collected Fine roots, rhizosphere soil, and non-rhizosphere soil with a soil drill (inner diameter of 4 cm) at 1m from the trunk and 20-40 cm deep, respectively. The soil passed through a 20-mesh sieve, and the samples at the same sampling position in the same place were mixed equally (each treatment weight was not less than 500 g). There were 18 mixed samples of larch and ash, including the root of larch (LR), rhizosphere soil of larch (LRS), non-rhizosphere soil of larch (LNS), root of ash (FR), rhizosphere soil of ash (FRS) and non-rhizosphere soil of ash (FNS). All samples were transported back to the laboratory by icebox. Soak the root sample with 0.1% Tween-20 for about 1 hour, thoroughly clean the adhered soil particles in running water. Dry the root sample, pulverize and mix well, and put it in a sterile 50mL centrifuge tube for DNA extraction and high-throughput sequencing. 5 g of soil samples were stored at -80°C for DNA extraction and high-throughput sequencing. The rest was used for the determination of soil physical-chemical characteristics after air drying.

Test methods

Determination of soil nutrients

soil nutrients of rhizosphere and non-rhizosphere soil samples of larch and ash were determined, and each sample was repeated three times. We mixed air-dried soil samples Evenly and took some soil samples by quartering. After grinding the soil sample by 0.25 mm sieve, we determined the total nitrogen, total phosphorus, and total potassium. Total nitrogen was determined by the semi-micro Kjeldahl method; Total phosphorus was determined by sodium hydroxide melting-molybdenum antimony colorimetric method; Total potassium was determined by sodium hydroxide fusion-flame spectrophotometry^[51].

Determination of community structure of fungi and bacteria

20-30 ng DNA was used to generate amplicons using a panel of primers designed by GENEWIZ (GENEWIZ, Inc, South Plainfield, NJ, USA). We used primers containing the sequence "CCTACGRRBGCASCAGKVRVGAAT/GGACTACNVGGGTWTCTAATCC" to amplify the V3 and V4 hypervariable regions of bacteria and Archaea 16S rDNA, primers containing the sequence "CGWTAACGAACGAG/AICCATTCATCGG" to amplify the V7 and V8 hypervariable regions of fungi 18S rDNA, and primers containing the sequence "GTGAATCATCGARTC/TCCTCCGCTTATTGAT" to amplify the ITS2 region of fungi ITS. At the same time, indexed adapters were added to the ends of the 16S rDNA amplicons to generate

indexed libraries ready for downstream NGS sequencing on Illumina. Besides the 18S and ITS target-specific sequences, the primers also contain adaptor sequences allowing uniform amplification of the library with high complexity ready for downstream NGS sequencing on the Illumina Miseq platform. PCR reactions were performed in triplicate 25 μ L mixture containing 2.5 μ L of TransStart Buffer, 2 μ L of dNTPs, 1 μ L of each primer, and 20 ng of template DNA. DNA libraries' concentration was validated by Qubit3.0 Fluorometer. Quantify the library to 10nM, DNA libraries were multiplexed and loaded on an Illumina MiSeq or NovaSeq instrument according to manufacturer's instructions (Illumina, San Diego, CA, USA) . Sequencing was performed using paired-end; image analysis and base calling were conducted by the Control Software embedded in the instrument.

Statistical Analyses

The QIIME data analysis package was used for 16s rRNA, 18S rRNA, and ITS rRNA data analysis. The forward and reverse reads were joined and assigned to samples based on barcode and truncated by cutting off the barcode and primer sequence. Quality filtering on joined sequences was performed and sequence which did not fulfill the following criteria were discarded: sequence length <200bp, no ambiguous bases, mean quality score ≥ 20 . Then the sequences were compared with the reference database (RDP Gold database) using the UCHIME algorithm to detect chimeric sequence, and then the chimeric sequences were removed. The effective sequences were used in the final analysis. Sequences were grouped into operational taxonomic units (OTUs) using the clustering program VSEARCH (1.9.6) against the Silva 132 database (16s rRNA; 18S rRNA) and the UNITE ITS database (ITS rRNA) pre-clustered at 97% sequence identity. The Ribosomal Database Program (RDP) classifier was used to assign a taxonomic category to all OTUs at a confidence threshold of 0.8. The RDP classifier uses Silva 132 database (16s rRNA; 18S rRNA) and the UNITE ITS database (ITS rRNA) which have taxonomic categories predicted to the species level.

Based on OTU analysis results are obtained, using the method of random sampling sample sequences is flat, calculate Ace, Shannon, Chao1, Simpson alpha diversity index, community species Abundance and diversity of Rarefaction curves, and We made LSD multiple comparative analysis (IBM SPSS Statistics19) of Ace, Shannon, Chao1, Simpson alpha diversity index of 18 samples, Through the weighted Unifrac analysis and comparison between samples whether there are significant differences of the microbial community. With the Vegan package in R software, we calculate the weighted Unifrac distance between 18 samples at the OTU level. Through the weighted clustering hierarchy and the group average method to construct UPGMA (Unweighted pair group method with arithmetic mean) clustering tree.

We added the environmental factors, including total nitrogen, total phosphorus, and total potassium, and made LSD multiple comparative analysis (IBM SPSS Statistics19) , and we cleared the relationship between soil fungi, bacteria, and soil nutrients by making redundancy analysis (RDA) with Canoco for windows 5.

Abbreviations

OTU: Operational Taxonomic Units; TN: total nitrogen; TP: total phosphorus; TK: total potassium.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

Please contact author for data requests

Competing interests

The authors declare that they have no competing interests.

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Competing interest

Authors' contributions

QY and YL designed the experiments. QY and WL analyzed the data. QY wrote the paper.

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Figure and table legend

Table 1 Correlation analysis between five beneficial bacteria and Top30 fungi in the larch forest

	<i>Pseudomonas</i>	<i>Burkholderiaceae</i>	<i>Bacillus</i>	<i>Paenibacillus</i>	<i>Streptomycetaceae</i>
<i>Gagea</i>	-0.311	-0.352	-0.323	-0.333	-0.264
<i>Larix</i>	0.970**	0.918**	-0.525	-0.398	-0.282
<i>Mortierellaceae</i> _Unclassified	-0.415	-0.413	0.868**	0.429	0.426
<i>Pseudogymnoascus</i>	-0.369	-0.57	-0.241	-0.343	-0.29
<i>Rubus</i>	-0.186	-0.162	-0.193	-0.208	-0.164
<i>Fraxinus</i>	0.11	-0.205	0.148	-0.184	-0.193
<i>Mortierella</i>	-0.523	-0.593	0.822**	0.378	0.474
<i>Trimorphomycetaceae</i> _Unclassified	-0.513	-0.553	-0.206	-0.065	-0.149
<i>Anemone</i>	\	\	\	\	\
<i>Pyronemataceae</i> _Unclassified	-0.145	-0.229	-0.385	-0.069	-0.13
<i>Cortinarius</i>	0.123	-0.189	0.151	-0.177	-0.19
<i>Phialocephala</i>	0.278	0.099	0.112	-0.102	-0.029
<i>Tricholomataceae</i> _Unclassified	-0.205	-0.186	-0.312	-0.057	-0.101
<i>Vishniacozyma</i>	-0.37	-0.585	-0.096	-0.103	-0.215
<i>Mucor</i>	-0.342	-0.331	0.761*	0.279	0.266
<i>Cenococcum</i>	-0.148	-0.194	-0.308	-0.209	-0.207
<i>Ascochyta</i>	-0.128	-0.369	0.169	-0.057	-0.067
<i>Humicola</i>	-0.513	-0.529	0.521	0.31	0.33
<i>Ulmus</i>	-0.222	-0.243	-0.136	-0.206	-0.138
<i>Monographella</i>	\	\	\	\	\
<i>Papiliotrema</i>	-0.267	-0.336	0.027	0.485	0.226

<i>Fusarium</i>	-0.254	-0.248	0.05	0.505	0.216
<i>Penicillium</i>	-0.417	-0.547	-0.246	-0.153	-0.134
<i>Metarhizium</i>	-0.437	-0.455	-0.178	-0.197	-0.101
<i>Colletotrichum</i>	-0.223	-0.253	-0.16	-0.213	-0.15
<i>Phoma</i>	-0.344	-0.52	0.102	0.334	0.167
<i>Trichoderma</i>	-0.436	-0.459	0.48	-0.028	-0.109
<i>Ilyonectria</i>	0.073	-0.228	-0.095	-0.269	-0.27

* indicates a significant correlation between bacteria and fungi ($P < 0.05$), ** indicates an extremely significant correlation between bacteria and fungi ($P < 0.01$).

Table 2 Correlation analysis between five beneficial bacteria and Top30 fungi in ash forest

	<i>Pseudomonas</i>	<i>Burkholderiaceae</i>	<i>Bacillus</i>	<i>Paenibacillus</i>	<i>Streptomycetaceae</i>
<i>Gagea</i>	-0.277	0.883**	-0.154	-0.163	-0.182
<i>Larix</i>	\	\	\	\	\
<i>Mortierellaceae</i> _Unclassified	-0.134	0.143	0.446	0.501	0.607
<i>Pseudogymnoascus</i>	-0.009	-0.318	-0.175	-0.162	-0.158
<i>Rubus</i>	0.272	-0.191	-0.032	-0.163	-0.2
<i>Fraxinus</i>	0.879**	-0.339	-0.289	-0.307	-0.364
<i>Mortierella</i>	0.102	-0.048	0.331	0.349	0.408
<i>Trimorphomycetaceae</i> _Unclassified	-0.147	-0.268	-0.113	-0.128	-0.12
<i>Anemone</i>	-0.002	-0.252	0.990**	0.988**	0.949**
<i>Pyronemataceae</i> _Unclassified	\	\	\	\	\
<i>Cortinarius</i>	-0.229	-0.31	-0.223	-0.178	-0.229
<i>Phialocephala</i>	-0.208	-0.265	-0.094	-0.057	0.043
<i>Tricholomataceae</i> _Unclassified	-0.144	-0.174	-0.174	-0.171	-0.187
<i>Vishniacozyma</i>	0.167	-0.342	-0.194	-0.301	-0.336
<i>Mucor</i>	-0.12	-0.25	0.096	0.097	0.067
<i>Cenococcum</i>	\	\	\	\	\
<i>Ascochyta</i>	-0.194	0.241	-0.11	-0.123	-0.063
<i>Humicola</i>	-0.178	-0.429	0.132	0.172	0.237
<i>Ulmus</i>	\	\	\	\	\
<i>Monographella</i>	-0.191	0.265	-0.098	-0.107	-0.047
<i>Papiliotrema</i>	0.097	-0.332	-0.187	-0.275	-0.31
<i>Fusarium</i>	0.437	-0.354	-0.203	-0.304	-0.348
<i>Penicillium</i>	-0.237	-0.29	0.054	0.046	0.058
<i>Metarhizium</i>	-0.179	-0.301	-0.062	-0.015	0.104
<i>Colletotrichum</i>	-0.245	0.829**	-0.164	-0.194	-0.236
<i>Phoma</i>	-0.06	-0.135	-0.224	-0.226	-0.228
<i>Trichoderma</i>	-0.189	-0.336	-0.14	-0.1	-0.008
<i>Ilyonectria</i>	0.186	-0.245	-0.223	-0.224	-0.26

* indicates a significant correlation between bacteria and fungi ($P < 0.05$), ** indicates an extremely significant correlation between bacteria and fungi ($P < 0.01$).

Table 3 Richness and community diversity indexes of fungi and bacteria of the root, rhizosphere soil, and non-rhizosphere soil of larch and ash.

Sample	OTUs	Ace index	Chao1 index	Shannon index	Simpson index
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	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi
L ₁ R	1273	197	1588.751	305.042	1634.829	329.143	8.166	2.131	0.983	0.616
L ₂ R	1279	224	1584.575	282.491	1603.154	278.486	7.974	2.157	0.985	0.605
L ₃ R	1343	433	1627.275	498.408	1636.572	494.742	8.294	4.951	0.988	0.929
LR Mean	1298c	285b	1600.200c	361.980bc	1624.852b	367.457cd	8.145b	3.080ab	0.985b	0.717ab
L ₁ RS	1386	334	1611.682	336.347	1624.564	335.000	8.699	6.806	0.993	0.983
L ₂ RS	1505	477	1753.708	486.916	1786.269	493.667	9.076	6.377	0.996	0.971
L ₃ RS	1484	357	1721.925	446.526	1718.433	431.417	8.960	3.947	0.995	0.841
LRS Mean	1458ab	389ab	1695.772abc	423.263ab	1709.755ab	420.028bcd	8.912a	5.710a	0.995a	0.932a
L ₁ NS	1460	540	1698.125	508.503	1689.163	512.125	8.943	5.310	0.995	0.883
L ₂ NS	1524	475	1763.274	638.706	1800.171	644.643	9.061	5.623	0.996	0.949
L ₃ NS	1548	559	1838.704	558.401	1838.390	559.524	8.949	6.348	0.995	0.971
LNS Mean	1511a	525a	1766.701a	568.537a	1775.908a	572.097ab	8.984a	5.760a	0.995a	0.934a
F ₁ R	1489	522	1722.662	616.291	1748.681	616.448	8.459	5.797	0.986	0.963
F ₂ R	1558	524	1776.682	590.090	1811.673	608.058	8.711	5.158	0.990	0.909
F ₃ R	1397	424	1669.070	533.853	1677.636	522.875	8.193	3.790	0.985	0.789
FR Mean	1481a	490a	1722.805abc	580.078a	1745.997ab	582.460a	8.454b	4.915ab	0.987b	0.887a
F ₁ RS	1515	489	1796.686	514.180	1824.631	512.694	9.096	4.024	0.996	0.848
F ₂ RS	1510	474	1744.278	523.691	1743.455	531.022	8.749	5.057	0.992	0.915
F ₃ RS	1483	463	1743.273	544.297	1750.414	530.289	9.016	4.577	0.996	0.908
FRS Mean	1503a	475a	1761.412ab	527.389a	1772.833a	524.668abc	8.954a	4.553ab	0.995a	0.890a
F ₁ NS	1354	125	1558.115	137.220	1553.613	131.333	8.949	0.366	0.996	0.061
F ₂ NS	1209	374	1513.641	380.780	1620.613	386.667	8.602	5.765	0.995	0.952
F ₃ NS	1446	221	1778.895	277.653	1744.650	280.000	8.820	0.847	0.995	0.149
FNS Mean	1336bc	240b	1616.884bc	265.218c	1639.625b	266.000d	8.790a	2.326b	0.995a	0.387b

The different lowercase letters represent the statistical difference of indexes between the samples ($P<0.05$) . OTU: Operational Taxonomic Units.

Table 4 weighted Unifrac distance matrix (OTU level) of fungi and bacteria of the root, rhizosphere soil, and non-rhizosphere soil of larch and ash.

	L ₁ NS	L ₂ NS	L ₃ NS	L ₁ R	L ₂ R	L ₃ R	L ₁ RS	L ₂ RS	L ₃ RS	F ₁ NS	F ₂ NS	F ₃ NS	F ₁ R	F ₂ R	F ₃ R	F ₁ RS	F ₂ RS	F ₃ RS
L ₁ NS	0	0.331	0.399	0.705	0.635	0.402	0.247	0.233	0.678	0.865	0.329	0.674	0.392	0.440	0.629	0.491	0.530	0.643
L ₂ NS	0.073	0	0.534	0.705	0.618	0.518	0.370	0.332	0.802	0.863	0.402	0.565	0.354	0.470	0.606	0.586	0.656	0.752
L ₃ NS	0.065	0.068	0	0.741	0.718	0.490	0.528	0.446	0.389	0.921	0.508	0.795	0.600	0.587	0.742	0.303	0.374	0.509
L ₁ R	0.187	0.200	0.190	0	0.428	0.476	0.665	0.651	0.911	0.755	0.733	0.779	0.697	0.706	0.652	0.828	0.747	0.719
L ₂ R	0.218	0.221	0.210	0.100	0	0.428	0.594	0.597	0.910	0.661	0.683	0.758	0.634	0.671	0.563	0.763	0.705	0.711
L ₃ R	0.147	0.140	0.152	0.179	0.174	0	0.412	0.383	0.724	0.771	0.475	0.748	0.477	0.451	0.551	0.577	0.503	0.564
L ₁ RS	0.062	0.101	0.072	0.172	0.205	0.165	0	0.250	0.775	0.809	0.389	0.675	0.354	0.424	0.571	0.575	0.558	0.649
L ₂ RS	0.071	0.051	0.076	0.197	0.222	0.145	0.097	0	0.723	0.842	0.344	0.660	0.357	0.411	0.592	0.525	0.547	0.650
L ₃ RS	0.062	0.054	0.048	0.209	0.236	0.153	0.080	0.057	0	0.969	0.715	0.921	0.810	0.763	0.886	0.387	0.480	0.550
F ₁ NS	0.135	0.114	0.130	0.203	0.204	0.143	0.154	0.105	0.127	0	0.869	0.872	0.830	0.818	0.652	0.929	0.802	0.570
F ₂ NS	0.116	0.117	0.116	0.254	0.279	0.192	0.133	0.127	0.102	0.185	0	0.644	0.378	0.369	0.596	0.481	0.486	0.655
F ₃ NS	0.134	0.138	0.139	0.259	0.280	0.197	0.153	0.117	0.121	0.176	0.113	0	0.589	0.458	0.608	0.742	0.800	0.855
F ₁ R	0.166	0.155	0.179	0.225	0.229	0.102	0.194	0.163	0.176	0.131	0.216	0.217	0	0.344	0.469	0.544	0.605	0.708
F ₂ R	0.164	0.143	0.174	0.208	0.202	0.090	0.193	0.152	0.167	0.123	0.207	0.204	0.059	0	0.411	0.519	0.509	0.627

F ₃ R	0.191	0.185	0.202	0.216	0.208	0.090	0.209	0.195	0.206	0.161	0.238	0.243	0.060	0.072	0	0.683	0.624	0.597
F ₁ RS	0.173	0.140	0.160	0.230	0.217	0.157	0.192	0.137	0.156	0.074	0.204	0.180	0.143	0.136	0.168	0	0.336	0.496
F ₂ RS	0.114	0.091	0.102	0.225	0.236	0.164	0.143	0.102	0.097	0.129	0.133	0.144	0.178	0.165	0.201	0.140	0	0.347
F ₃ RS	0.192	0.162	0.185	0.250	0.236	0.172	0.217	0.158	0.178	0.099	0.221	0.189	0.155	0.145	0.167	0.059	0.164	0

Table 5 Physicochemical indexes of rhizosphere soil and rhizosphere soil of larch and ash

The data in the table were “mean±standard” deviation, the different lowercase letters represent the statistical difference of indexes between the samples ($P<0.05$). TN: total nitrogen; TP: total phosphorus; TK: Total potassium.

Figure. 1. Rarefaction curve of fungi and bacteria in the root, rhizosphere soil, and non-rhizosphere soil of larch and ash at a 97% level.

Figure. 2. Fungal phylum and genus community structure composition of the root, rhizosphere soil, and non-rhizosphere soil of larch and ash. Figure a shows the composition of the fungal community at the phylum level; Figure b shows the composition of the fungal community at the genus level.

Figure. 3 Bacterial phylum and genus community structure composition of the root, rhizosphere soil, and non-rhizosphere soil of larch and ash. Figure a shows the composition of the fungal community at the phylum level; Figure b shows the composition of the fungal community at the genus level.

Figure. 4 Difference analysis heatmap of Fungi and Bacteria Community diversity in the root, rhizosphere soil, and non-rhizosphere soil of larch and ash. The color depth represents the difference between the two samples. The lighter the color, the smaller the difference coefficient between two samples, and the smaller the difference of species diversity.

Figure 5 UPGMA (Unweighted pair group method with arithmetic mean) tree of bacterial and fungal communities in the root, rhizosphere soil and rhizosphere soil of larch and ash. Based on the Bray-Curtis distance matrix, the UPGMA tree is constructed by the unweighted group average method in hierarchical clustering.

Figure 6 RDA between soil physicochemical indexes and bacterial/fungal community diversity in different Sampling position of larch and ash. Figure a shows the RDA of soil nutrients and fungal community diversity; Figure b shows the RDA of soil nutrients and bacterial community diversity.

Figures

Figure 1

Rarefaction curve of fungi and bacteria in the root, rhizosphere soil, and non-rhizosphere soil of larch and ash at a 97% level.

Figure 2

Fungal phylum and genus community structure composition of the root, rhizosphere soil, and non-rhizosphere soil of larch and ash. Figure a shows the composition of the fungal community at the phylum level; Figure b shows the composition of the fungal community at the genus level.

Figure 3

Bacterial phylum and genus community structure composition of the root, rhizosphere soil, and non-rhizosphere soil of larch and ash. Figure a shows the composition of the fungal community at the phylum level; Figure b shows the composition of the fungal community at the genus level.

Figure 4

Difference analysis heatmap of Fungi and Bacteria Community diversity in the root, rhizosphere soil, and non-rhizosphere soil of larch and ash. The color depth represents the difference between the two samples. The lighter the color, the smaller the difference coefficient between two samples, and the smaller the difference of species diversity.

Figure 5

UPGMA—Unweighted pair group method with arithmetic mean—tree of bacterial and fungal communities in the root, rhizosphere soil and rhizosphere soil of larch and ash. Based on the Bray-Curtis distance matrix, the UPGMA tree is constructed by the unweighted group average method in hierarchical clustering.

Figure 6

RDA between soil physicochemical indexes and bacterial/fungal community diversity in different Sampling position of larch and ash. Figure a shows the RDA of soil nutrients and fungal community diversity; Figure b shows the RDA of soil nutrients and bacterial community diversity.