

# Long-term Effects of Mixed Management on Arbuscular Mycorrhizal Fungal Community of Root and Soil in *Juglans mandshurica* Plantation

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## Research article

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# Abstract

**Background:** The establishment of mixed plantations is an effective way to improve soil fertility and increase forest productivity. Arbuscular mycorrhiza (AM) fungi are a kind of obligated symbiotic fungi, which can promote plants absorption of mineral nutrients and regulate intraspecific and interspecific competition. However, the effects of mixture on the community structure and abundance of AM fungi are still unclear. The Illumina MiSeq sequencing technique was used to determine the AM fungal community in the roots and soils of pure and mixed plantations (*Juglans mandshurica* × *Larix gmelinii*). The objective is to compare the response from root and rhizosphere soil AM fungal communities of *Juglans mandshurica* to long-term mixed plantation management.

**Results:** *Glomus* and *Paraglomus* were the dominant genus in root samples, which accounted for more than 80% of the sequences. Compared with the pure plantation, the relative abundance of *Glomus* was higher in mixed plantation. *Glomus*, *Diversispora* and *Paraglomus* accounted for more than 85% of the sequences in soil samples. The relative abundance of *Diversispora* and Unclassified\_c\_Glomeromycetes were higher and lower in pure plantation, respectively. Samples of the Root\_P (roots in pure plantation) had the highest number of unique OTUs (Operational Taxonomic Units), which were mainly composed of sequences belonging to unclassified\_c\_Glomeromycetes, *Paraglomus*, *Glomus* and *Acaulospora*. The amount of unique OTU detected in soil in pure and mixed plantation was relatively close. In the same types of sample (whether root or soil), the forest type did not have significant effect on AM fungal diversity, but the Sobs, Shannon, Chao1 and Ace indices of AM fungi in the roots were significantly higher than those in the soil.

**Conclusions:** The mixed forest management had little effect on the AM fungal community in the root of *Juglans mandshurica*, but significantly changed the community composition of the soil AM fungi rather than diversity.

## Background

Arbuscular mycorrhizal (AM) are widely spread and form symbiotic associations with about 80% of terrestrial plant species [1]. AM fungi can not only promote plant absorption of mineral nutrients (C, N, P) [2–4], provide resistance to environmental stress [5, 6] and regulate intraspecific and interspecific competition [7], but also directly and indirectly improve soil structure and affect the circulation of matter and flow of energy in the ecosystem [8]. Some studies have found that host plants preferentially distribute carbohydrates to more beneficial symbionts while they provide photosynthetic products to AM fungi [9–11], and the preference/selection of different plants species leads to the difference in the growth rate of AM fungi, thus forming different AM community richness, composition and diversity [12].

Previous studies have indicated that AM fungal taxonomic groups differ in terms of the main propagule form for colonizing new roots and the allocation of biomass to the compartments of roots and soil [13]. To a certain extent, the composition and community structure of AM fungi depends on their propagation

forms (spores, infected root segments, extensive extraradical mycelium), and the difference between the propagation of AM fungi in root (intraradical) and soil (extraradical) samples may be related to the location and density of AM fungal propagules [14]. In addition, host plant selection for AM fungi exhibited strong selection pressure for AM fungi in the root system [15]. Moreover, the response of different AM fungal groups to host plants or the rhizosphere microenvironment may also lead to host plant selection preferences for some AM fungal species [16]. There are differences in the distribution patterns of root carbon and the pathway to secondary metabolites or secretions, which may cause changes of soil environmental conditions [9, 17]. A recent study found that AM fungal structures in root and soil have different responses to biotic and abiotic factors. The AM fungal community structure in roots is mainly affected by host plants and disturbances (grazing), while AM fungi in soil is greatly affected by environmental factors [18].

AM fungi could form a huge mycorrhizal network and connect individual plants within the community which facilitate the transport of nutrient resources between plants [19]. Therefore, neighboring plants could change the impact on host plants on AM fungi or form specific AM fungal communities when multiple plants are mixed planting in terrestrial ecosystems [12, 20]. Previous studies have found that the construction of AM fungal communities is influenced by the identity of adjacent plants which mostly occur to greenhouses and invasive systems [21]. However, it is not clear how coexistence of multiple plants affects arbuscular mycorrhizal fungal communities in natural ecosystems, especially in forest ecosystems. Forests play an important role in the production of wood and fuel, controlling soil erosion and maintaining ecosystem functions [22]. Monoculture area accounts for 80% of the total planting area in China. The establishment and management of monoculture is relatively easier compared with mixed plantation, but it will reduce the ecological function of the forest, and long-term planting of monoculture will cause litter quality and soil fertility decline and other problems [23]. Mixed plantations building is an effective way to improve soil fertility and increase forest productivity [22, 24]. Numerous studies have shown that rational mixed plantations can improve soil fertility [25], nutrient cycle [25], stand productivity [26, 27], tree nutrition and resistance to pests and diseases [28]. However, the mixed effects vary from tree species [29, 30]. Therefore, comparing the differences of AM fungal communities in different plantation types is conducive to a profound understanding of the stimulation mechanism of mixed plantations.

*Larix gmelinii* is a major afforestation and fast-growing species in north China [31]. There always had some problems, such as biodiversity loss, soil degradation occurs to rapid development of larch monoculture [32], which seriously affect the sustainable management. *Juglans mandshurica* (arbuscular mycorrhizal species) is one of the precious timber trees species in northeast China and has important economic value. It has been reported that the mixed management of *Juglans mandshurica* and *Larix gmelinii* can improve the soil fertility and stand productivity [33], however, until now, the synergistic mechanism of mixed management, especially the interaction between the host plant and soil AM fungal community structure is still obscure. For this purpose, we compared the AM fungal community composition, structure and diversity of root and soil of *Juglans mandshurica* of pure and mixed plantations in order to provide theoretical basis for the stimulation mechanism of temperate mixed plantations. We hypothesized that (1) mixed management significantly changed the AM fungal

community structure and composition in the root and soil of *Juglans mandshurica*, and have higher AM fungal diversity and richness were higher in mixed plantation compare with pure plantation; (2) in all two forest types, the diversity and richness of AM fungi in the soil are higher than those in the roots.

## Results

### Soil properties and AM fungal colonization

Soil pH and total phenol content was significantly higher in pure plantation than in the mixed plantation (Table 1;  $P < 0.05$ ). The  $\text{NH}_4^+$ -N content was significantly higher in mixed plantation than in the pure plantation. Compared with mixed plantation, soil moisture, P, C/N ratio,  $N_{\text{mic}}$ , CPh, WSPh and colonization mainly showed downward trends in pure plantations, although no significant differences were found ( $P > 0.05$ ).

Table 1  
Soil variables and AM fungal colonization in pure and mixed plantations

Environmental variables	Pure plantation	Mixed plantation
pH	5.89 ± 0.06a	5.63 ± 0.04b
Moisture (%)	84.60 ± 2.23a	91.83 ± 10.59a
C (g·kg <sup>-1</sup> )	105.97 ± 10.12a	105.02 ± 20.26a
N (g·kg <sup>-1</sup> )	9.36 ± 0.8a	9.07 ± 1.72a
P (g·kg <sup>-1</sup> )	1.24 ± 0.07a	1.29 ± 0.16a
C/N	11.31 ± 0.16a	11.56 ± 0.06a
C <sub>mic</sub> (mg·kg <sup>-1</sup> )	2299.94 ± 142.23a	1590.32 ± 372.04a
N <sub>mic</sub> (mg·kg <sup>-1</sup> )	351.32 ± 21.99a	402.67 ± 16.29a
NH <sub>4</sub> <sup>+</sup> -N (mg·kg <sup>-1</sup> )	6.01 ± 0.28b	8.20 ± 0.57a
NO <sub>3</sub> <sup>-</sup> -N (mg·kg <sup>-1</sup> )	31.73 ± 1.23a	28.62 ± 2.37a
TPh (mg·kg <sup>-1</sup> )	690.17 ± 8.54a	536.37 ± 37.86b
CPh (mg·kg <sup>-1</sup> )	188.21 ± 6.93a	230.47 ± 13.81a
WSPH (mg·kg <sup>-1</sup> )	4.37 ± 0.19a	4.63 ± 0.33a
Colonization (%)	95.56 ± 2.94a	96.70 ± 2.41a
C <sub>mic</sub> : soil microbial biomass carbon. N <sub>mic</sub> : soil microbial biomass nitrogen. TPh: soil total phenol. CPh: soil complex phenol. WSPH: soil water-soluble phenol.		

## Sequence Summary

Across all soil and root samples analyzed, 190,433 quality AM fungal sequences were yielded by Illumina Miseq sequencing, with 11,388 – 19,701 AM fungal sequences (mean = 15,869). The average read lengths were 238 bp for the 18S rRNA genes regions and larger than 99% of the Good's coverage for the 18S rRNA genes regions. The rarefaction curves of genes tended to approach the saturation plateau at 97% sequence similarity for all samples (Supplementary Fig. S1), which also indicated that the sequencing depth was adequate for assessing the diversity of AM fungal communities of all samples.

## Am Fungal Community Composition

Across all root samples, nine AM fungal genera were detected. *Glomus* and *Paraglomus* were the dominant genus, which accounted for more than 80% of the sequences (Fig. 1A). Compared with the pure plantation, the relative abundance of *Glomus* in root sample was higher in mixed plantation (Fig. 1A). Across all soil samples, ten AM fungal genera were detected. *Glomus*, *Diversispora* and *Paraglomus* accounted for more than 85% of the sequences (Fig. 1B). The relative abundance of *Diversispora* and Unclassified\_c\_Glomeromycetes were higher and lower in pure plantation, respectively. At the OTU level, 178 AM fungal taxa were detected. OTU79 and OTU76 were the dominant OTU and had mean relative abundances of 12.15% and 6.54%, respectively (Supplementary Table S2, Fig. 2).

## Am Fungal Community Structure And Diversity

The number of Sobs, Shannon, Chao1, Ace and Faith PD indices of root samples were higher in pure plantation (Fig. 3A-3F;  $P > 0.05$ ), while the Sobs, Shannon, Chao1 and Faith PD indices of soil samples were higher in mixed plantation ( $P > 0.05$ ). The Simpson index of soil sample was significant higher in pure plantation than in mixed plantation ( $P < 0.05$ ). In the same types of sample (whether root or soil), the forest type did not have significant effect on AM fungal diversity, but the Sobs, Shannon, Chao1 and Ace indices of AM fungi in the roots were significantly higher than those in the soil ( $P < 0.05$ ).

Principal coordinates analysis (PCoA) analysis at the OTU level showed that samples from the Root\_P, Root\_M, Soil\_P and Soil\_M were separated from each other (Fig. 4A). The two principal component axes explained more than 50% of the total variation (PCoA 1 = 33.07%, PCoA 2 = 17.17%). Analysis of similarities (ANOSIM), permutation multivariate analysis of variance (PERMANOVA) demonstrated that AM fungal community structure differed among types of samples ( $P < 0.001$ ) (Fig. 4B, Table S3).

## Shared And Unique Otus

Venn diagram analysis of OTUs at 97% sequence similarity showed that all samples shared 22 OTUs, which accounted for 7.86% of the total OTUs observed (Fig. 5). At the genus level, these shared OTUs were mainly composed of sequences belonging to *Glomus*, *Paraglomus* and *Diversispora* (Table S4). Samples from the Root\_P had the highest number of unique OTUs, which were mainly composed of sequences belonging to unclassified\_c\_Glomeromycetes, *Paraglomus*, *Glomus* and *Acaulospora* (Table S5). The amount of unique OTU detected in soil in pure and mixed plantation was relatively close. At the genus levels, the Soil\_P had significantly higher relative abundances of *Diversispora* than other treatments (Fig. 6,  $P < 0.05$ ).

## Relationships Between Am Fungal Communities And Soil Characteristics

The RDA analysis and Mantel test were conducted to identify the key drivers of AM fungal community structure. In the RDA plots of both AM fungal community structure, soil  $\text{NO}_3^-$ -N,  $C_{\text{mic}}$  and pH appeared to be the most important soil characteristics in controlling the root AM fungal community structure, which represented major variations among microbial communities (Fig. 7A). Mantel test demonstrated that root AM fungal community structure was significantly correlated to  $\text{NO}_3^-$ -N ( $R^2 = 0.906$ ,  $P = 0.029$ ) and  $C_{\text{mic}}$  ( $R^2 = 0.881$ ,  $P = 0.044$ ) (Table 2). The soil complex phenol (CPh), C/N, pH,  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N had longer arrows than the others (Fig. 7B). It indicates that these variables have a greater impact on the AM fungal community. Of all the soil characteristics tested, C/N ( $R^2 = 0.864$ ,  $P = 0.032$ ) and CPh ( $R^2 = 0.994$ ,  $P = 0.019$ ) were correlated with community composition significantly (Table 2).

Table 2

Mantel analysis on the relationship between the relative abundance of OTUs and soil or plant variables.

Environmental variables	Root		Soil	
	$R^2$	$P$	$R^2$	$P$
pH	0.647	0.213	0.695	0.158
Moisture (%)	0.61	0.257	0.178	0.743
C ( $\text{g}\cdot\text{kg}^{-1}$ )	0.543	0.29	0.148	0.788
N ( $\text{g}\cdot\text{kg}^{-1}$ )	0.565	0.254	0.116	0.821
P ( $\text{g}\cdot\text{kg}^{-1}$ )	0.311	0.594	0.047	0.922
C/N	0.386	0.472	<b>0.864</b>	<b>0.032*</b>
$C_{\text{mic}}$ ( $\text{mg}\cdot\text{kg}^{-1}$ )	<b>0.881</b>	<b>0.044*</b>	0.455	0.415
$N_{\text{mic}}$ ( $\text{mg}\cdot\text{kg}^{-1}$ )	0.170	0.749	0.439	0.411
$\text{NH}_4^+$ -N ( $\text{mg}\cdot\text{kg}^{-1}$ )	0.454	0.394	0.688	0.165
$\text{NO}_3^-$ -N ( $\text{mg}\cdot\text{kg}^{-1}$ )	<b>0.906</b>	<b>0.029*</b>	0.656	0.208
TPh ( $\text{mg}\cdot\text{kg}^{-1}$ )	0.542	0.336	0.442	0.419
CPh ( $\text{mg}\cdot\text{kg}^{-1}$ )	0.603	0.257	<b>0.944</b>	<b>0.019*</b>
WSPH ( $\text{mg}\cdot\text{kg}^{-1}$ )	0.601	0.233	0.676	0.179
Colonization (%)	0.521	0.306	—	—

$C_{\text{mic}}$ : soil microbial biomass carbon.  $N_{\text{mic}}$ : soil microbial biomass nitrogen. TPh: soil total phenol. CPh: soil complex phenol. WSPH: soil water-soluble phenol.

## Discussion

# Response of AM fungal diversity, composition and community structure on mixed management

The core species composition of AM fungal communities was very similar and conservative in pure and mixed plantations (*Glomus* mainly, Fig. 1), which is consistent with the result of Senés-Guerrero and Schüßler [34]. They found that there is a conservative core species AM fungal community structure both at different stages of plant development and under different environmental conditions in the Andean ecosystem. Plant community diversity and AM fungal community interact to improve the diversity of AMF community [15]. Van der Heijden, et al. [35] believed that the change of host plants caused by the infection of a single mycorrhizal species was an important factor determining the species composition and diversity of plant community. However, some researchers have reported that there is a negative correlation between plant diversity and AM fungal communities [36]. In this study, the mixture reduced AM fungal community diversity and OTU number in root of Manchurian walnut (Fig. 3,  $P > 0.05$ ). The lower diversity of AM fungi might have been attributable to a lower “carrying capacity” of fewer walnut roots than in the pure plantation. In mixture, larch roots possessed a greater plasticity in traits related to resource uptake than walnut roots [37]. In addition, larch root exudates could alleviate autotoxic effect caused by juglone, which secreted by Manchurian walnut after mixture. Our previous studies have found that larch root exudates promoted the variation of soil microbial communities of Manchurian walnut and improved soil invertase and urease activities [38]. Salahuddin, et al. [37] indicated that the ratio of root tip tissue of Manchurian walnut significantly increased after mixed with larch, but the mycorrhizal infection rate was inhibited. Achatz, et al. [39] found that mycorrhizal mycelia could promote the migration and transport of juglone secreted by plant roots and mycorrhizal could enhance interspecific interaction thought the study on the species of the genus *Juglans* (Juglandaceae). AM fungi has certain host specificity [40]. Mummey and Rillig [20] found that spotted knapweed as an invasive plant could significantly change the AM fungal community in the invasive site.

AM fungal community composition and abundance were obviously different between pure and mixed plantations (Fig. 2 and Fig. 6), and there was more OTUs in the soil of mixed plantation (Fig. 5). The reasons may be as follows: (1) Compared with pure plantation, mixed plantation has more advantages in terms of litter quantity and quality, soil nutrients and stand structure, etc. The change of soil microenvironment leads to the variation of AM fungal community; (2) larch affects the root system of Manchurian walnut after mixture, which reshapes the mycorrhizal network. [41] found that abiotic factors (soil moisture content, nitrate and soil enzymes, etc.) had a greater impact on the soil AM fungal community when *Robinia pseudoacacia* mixed with *Platycladus orientalis*. The growth of some plants may change soil quality or other abiotic characteristics, which may lead to changes in rhizosphere AM fungal communities [42].

## Comparison of AM fungal communities in root and soil samples

Our study found that the AM fungal community in the root system of Manchurian walnut was significantly different from that in rhizosphere soil, which is consistent with previous results on temperate steppe [43], temperate farmland [44] and Mediterranean shrub community [45]. Due to plants will have different degrees of specific selection on AM fungi according to their own nutritional requirements in different growth and development stages, which will inevitably lead to differences in the relative abundance, species and quantity of AM fungi in soil and roots. In addition, there are great differences between the environmental conditions of AM fungi in plant roots and soil. The living environment of AM fungi in roots is mainly regulated by the physiological activities of individual plants, while AM fungi in soil are mainly affected by external environmental conditions.

Many scholars believe that the AM fungal community in soil represents a species pool, which plants can freely recruit some species, that is, the AM fungal richness in soil is the highest [46–49]. However, some scholars have found that the abundance of AM fungi in roots and soil is the same [50–52] or the AM fungi in the root system is more abundant than the soil [53, 54]. In this study, the amount of OTU in roots was higher than that in soil samples (whether in pure or mixed plantations), and a large proportion of AM fungi were detected in roots. There are many potential methodological and biological explanations for differences in AM fungal community, especially the small amount of OTU detected in soil. Firstly, the biomass of AM fungi in soil is an order of magnitude lower than that of roots [55]. Therefore, the concentration of soil AM fungi DNA is relatively low. The AM fungi in root samples have higher sequencing depth than soil samples, and low DNA template quantity may lead to the underestimation of species richness.

Secondly, there are differences in the distribution of AM fungi from different taxa to the root (intraradical) and soil (extraradical) samples [56]. Compared with the internal structure, Glomeraceae spend less on external structure [57]. In this study, the *Glomus* of the roots showed a higher abundance than the soil, which was consistent with previous research results. Some studies have found that Gigasporaceae and Acaulosporaceae could produce a large number of external hyphae compared with internal structures. In this study, Diversisporaceae and Gigasporaceae were mainly found in soil, with few sequence numbers in the root system which is consistent with the early findings that Diversisporaceae and Gigasporaceae are poor root colonizers [43, 54, 57]. Paraglomeraceae was mainly found in soil samples, with only few in the roots, which is consistent with previous studies [43, 54].

## Conclusions

Our study showed that long-term (almost 30 years) mixed management had little effect on the AM fungal community in the root of *Juglans mandshurica*, but significantly changed the community composition of AM fungi in soils rather than diversity. Samples from the root in pure plantation had the highest number of unique OTUs. The core species composition of AM fungal communities was very similar and conservative in pure and mixed plantations. In the future, the combination of root traits and mycorrhizal symbiosis should be considered to comprehensively evaluate the mechanism of nutrient absorption and utilization of mixed tree species, in order to lay a foundation for sustainable management of plantations.

## Methods

### Study area and sample design

The study site was located in Maoershan Forest Research Station (127°30'–127°34'E, 45°21'–45°25'N) of Northeast Forestry University, Heilongjiang province, China. This area is characterized by a continental monsoon climate of a windy spring, a warm and humid summer, and a dry and cold winter. Mean annual temperature is 2.8°C, with the minimum temperature in January (-40.9°C) and maximum temperature in July (34.2°C). The frost-free period fluctuates between 120 and 140 days. Annual precipitation ranges from 600 to 800 mm. Soils are Hap-Boric Luvisols [58] with high organic matter content and well-developed horizons, and are well drained.

In spring 1987, one-year-old seedlings of *Juglans mandshurica* and *Larix gmelinii* were obtained from the Maoershan Forest Farm and transplanted to the experimental station for establishing the monoculture (*Juglans mandshurica*, JM) and mixed plantation (*Juglans mandshurica* × *Larix gmelinii*, J × L) of Manchurian walnut. The seedlings were designed by 1.5 m × 1.5 m in each plantation, and mixed by lining (three rows of JM × five rows of larch) for mixed plantation. These plantations have similar site conditions with an average gradient of 7°. Detailed tree growth for each plantation were shown in Table S1. Voucher specimen of *J. mandshurica* and *L. gmelinii* were not deposited in this study since they are the most common trees in Northeastern China.

### Sample Collection

In April 2016, three random sampling plots (20 m × 30 m 0.06 ha) were respectively selected for pure and mixed plantation described above, which were identified to serve as replicates. Rhizosphere soil and plant root samples were collected in July 2016. In each of these experimental plots, root samples were collected in 0–10 soil layers from nine individuals of *Juglans mandshurica* and mixed as a composite sample of each plot. The rhizosphere soils were sampled adjacent to the roots and brushed off from the plant root systems. The soil and root samples were packed in an ice box and transported to laboratory. Soil samples were sieved (1 mm mesh) to remove roots and debris, and subsamples were stored at -80°C for DNA extraction. The first three root orders of the roots of *Juglans mandshurica* are infected by mycorrhiza [59]. Root samples were washed using distilled water to remove soil particles and were stored at -80°C for DNA extraction.

### Dna Extraction And Pcr Amplification

Microbial DNA was extracted from root and soil samples using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to manufacturer's protocols. The final DNA concentration and purification were determined by NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was checked by 1% agarose gel electrophoresis. The fungal 18S rRNA

genes were amplified by a nested PCR reaction. According to the study of Lumini et al. 2010, AML1 (5'-ATCAACTTTCGATGGTAGGATAGA-3') and AML2 (5'-GAACCCAAACA CTTTGGTTTCC-3') where barcode is an eight-base sequence unique to each sample, were used in the first round PCR, and AMV4-5NF (5'-AAGCTCGTAGTTGAATTTTCG-3') and AMDGR (5'-CCCAACTATCCCTATTAATCAT-3') were used in the second round PCR by thermocycler PCR system (GeneAmp 9700, ABI, USA). The two rounds of PCR yielded amplicons of approximately 800 bp and 300 bp, respectively. PCR reactions were performed in triplicate 20 µL mixture containing 4 µL of 5 × FastPfu Buffer, 2 µL of 2.5 mM dNTPs, 0.8 µL of each primer (5 µM), 0.4 µL of FastPfu Polymerase and 10 ng of template DNA. The PCR reactions were conducted using the following program: 3 min of denaturation at 95 °C, 30 cycles of 30 s at 95 °C, 30 s for annealing at 55 °C, and 45 s for elongation at 72 °C, and a final extension at 72 °C for 10 min. The procedure of the second round PCR reaction was same as the first round, except for the cycle number, which was 35 [60].

## Illumina Miseq Sequencing

The resulted PCR products were extracted from a 2% agarose gel and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA) according to the manufacturer's protocol. Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 250) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SRP227587).

## Processing Of Sequencing Data

Raw fastq files were demultiplexed, quality filtered by Trimmomatic and merged by FLASH with the following criteria: (i) The reads were truncated at any site receiving an average quality score < 20 over a 50 bp sliding window. (ii) Primers were exactly matched allowing two nucleotide mismatching and reads containing ambiguous bases were removed. (iii) Sequences whose overlap longer than 10 bp were merged according to their overlap sequence.

Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1 <http://drive5.com/uparse/>) and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 18S rRNA gene sequence was analyzed by blast against the MaarjAM database using confidence threshold of 97%.

## Soil Physicochemical Analyses

Total carbon (C) and total nitrogen (N) were measured by a Macro Elemental Analyzer (vario MACRO, Elementar Co., Germany), and total phosphorus (P) was determined colorimetrically with a UV

spectrophotometer (TU-1901, Puxi Ltd., Beijing, China) after wet digestion with  $\text{HClO}_4\text{-H}_2\text{SO}_4$ . Soil pH was measured using a pH meter (MT-5000, Shanghai). Soil nitrate-N ( $\text{NO}_3^-$ -N) and ammonium-N ( $\text{NH}_4^+$ -N) were extracted in 2 M KCl and measured using a continuous-flow ion auto-analyzer (Scalar SANplus segmented flow analyzer, The Netherlands). Soil total phenol content was measured by the ultraviolet spectrophotometer method [61]. The Folin reagent colorimetric method was used to determine soil water-soluble phenol and complex phenol content [61]. Soil microbial biomass carbon (MBC) and nitrogen (MBN) was measured using a chloroform fumigation extraction method [62]. The mycorrhizal colonization rate of fine roots was determined according to method of Guo [59].

## Statistical analysis

For the Illumina MiSeq sequencing data, the Alpha diversity indices (OTU number of observed, Chao1, ACE, Faith's PD, Shannon and Simpson diversity indices) were generated using QIIME [63]. For beta diversity analysis, Bray-Curtis distances were calculated and Principal coordinates analysis (PCoA) was conducted to visualize the community similarity using by 'vegan' package in 'R' (Version 3.6.1) [64]. ANOSIM (Analysis of similarities) and PERMANOVA (Permutation multivariate analysis of variance) were carried out to test the differences in microbial communities with the Bray-Curtis distances and 999 permutations. Heat map analysis was used to compare the relative abundances of the top 50 most abundant classified AM fungal genera among treatments with 'pheatmap' package in 'R' (Version 3.6.1). The shared and unique OTUs among treatments were counted, and their distributions shown in a Venn diagram with the 'VennDiagram' package in 'R' (Version 3.6.1). Differences in relative abundances of microbial taxa between treatments were analyzed using Welch's *t* test and Tukey's honestly significant difference (HSD) test with Bonferroni correction in 'STAMP' [65]. The differences were considered statistically significant if  $P < 0.05$ . Redundancy analysis (RDA) was used to identify soil properties that predict the variation of AM fungal communities. Mantel test with a Monte Carlo simulation with 999 randomizations was used to assess the relationships between the Euclidean distance of AM fungal community and soil characteristic. RDA and Mantel test analyses were performed with the rda function in the 'vegan' package and the mantel.rtest function in the 'ade4' package in 'R' (Version 3.6.1), respectively.

### Supplementary information

Please see the supplementary files.

## Abbreviations

AM

Arbuscular mycorrhiza; OTUs:Operational Taxonomic Units;  $C_{\text{mic}}$ :soil microbial biomass carbon;  $N_{\text{mic}}$ :soil microbial biomass nitrogen; TPh:soil total phenol; CPh:soil complex phenol; WSPH:soil water-soluble phenol; Soil\_P:soil sample in pure plantation; Soil\_M:soil sample in mixed plantation; Root\_P:root sample in pure plantation; Root\_M:root sample in mixed plantation.

# Declarations

## Ethics approval and consent to participate

This study and the submission have been approved by the research ethics committee at Northeast Forestry University.

## Consent for publication

Not applicable

## Availability of data and materials

All datasets are presented in the main text and the additional file. The raw sequence data on 18S rRNA gene amplicons have been submitted to the NCBI Sequence Read Archive (SRA) database (Accession Number: SRP227587). The dataset analyzed during the current study is available from the corresponding author on reasonable request.

## Competing interests

The authors declare that they have no competing interests.

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## Author contributions

LJ, YY, and LY designed the work. LJ, NY and YZ performed the fieldwork, LJ wrote the manuscript. LJ and DZ conducted molecular experiments and analyzed the data. All authors have read and approved the manuscript.

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## Figures

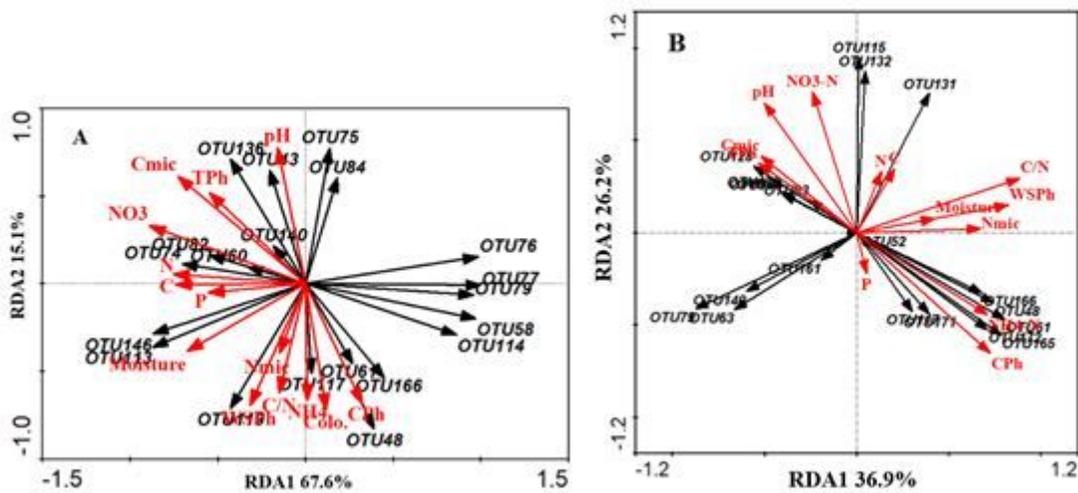
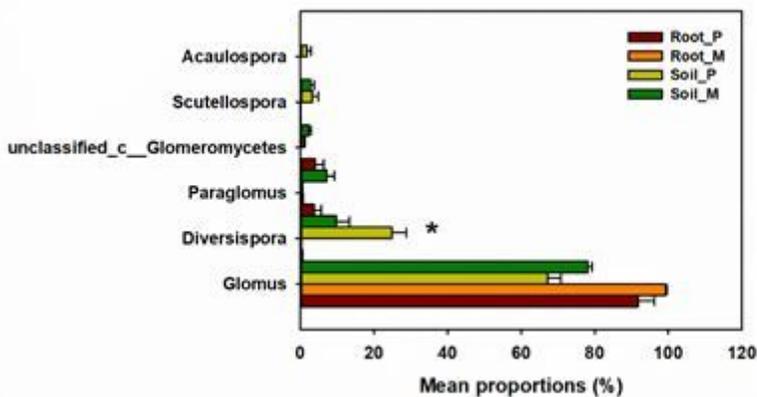


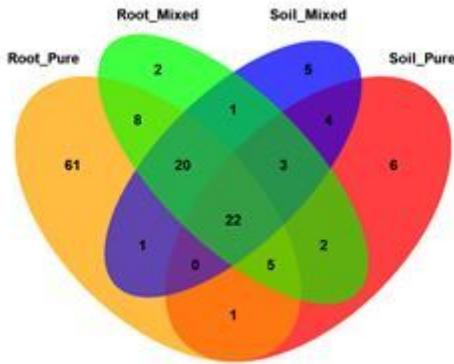
Figure 1

Ordination plots of the results from the redundancy analysis (RDA) to identify the relationships among the AM fungal taxa (Black arrows) and the soil characteristics (Red arrows). The top 20 most abundant classified AM fungal OTUs (97% sequence similarity) in the root (A) and soil (B) samples. Cmic: soil microbial biomass carbon. Nmic: soil microbial biomass nitrogen. TPH: soil total phenol. CPh: soil complex phenol.



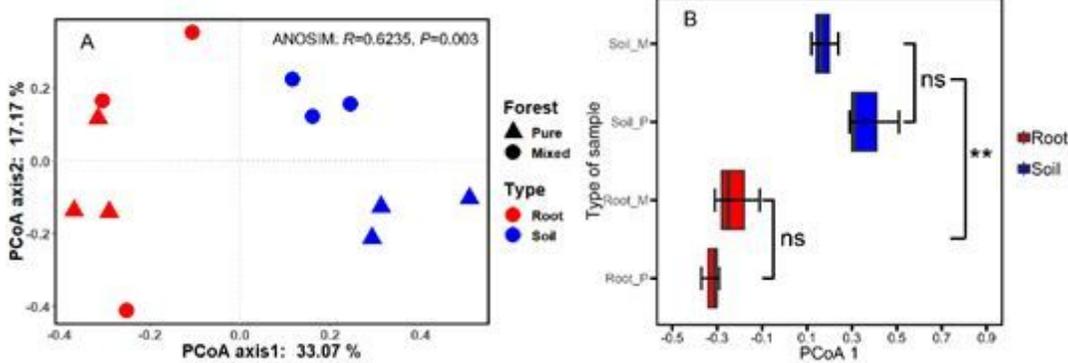
**Figure 2**

Relative abundances of main AM fungal genus in the root and soil samples. Genus and OTUs with average relative abundances > 1% were shown in at least one treatment. Values in the bar plot are expressed as mean  $\pm$  standard error. Asterisks indicate significant difference between treatments based on Tukey's test ( $P < 0.05$ )



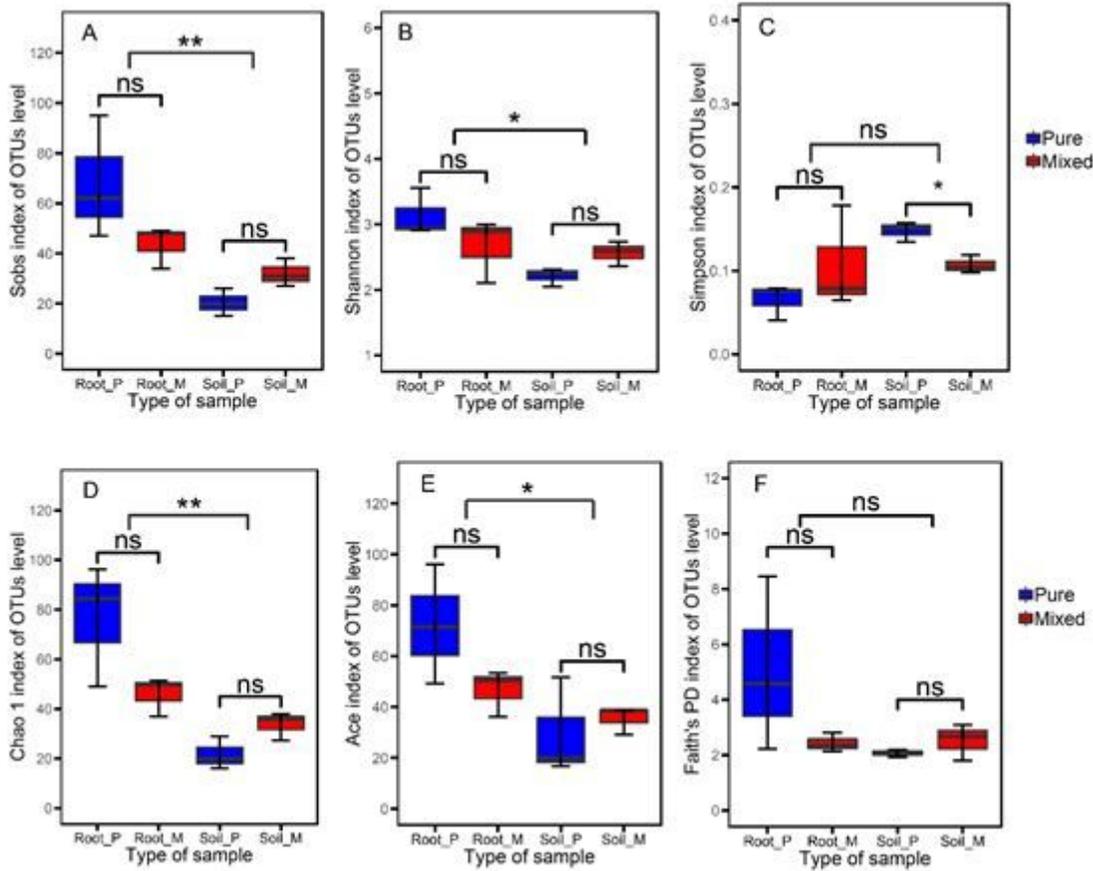
**Figure 3**

Venn diagram analyses of AM fungal communities in the root and soil samples. Venn diagram demonstrated the numbers of shared and unique observed OTUs at 97% similarity among treatments.



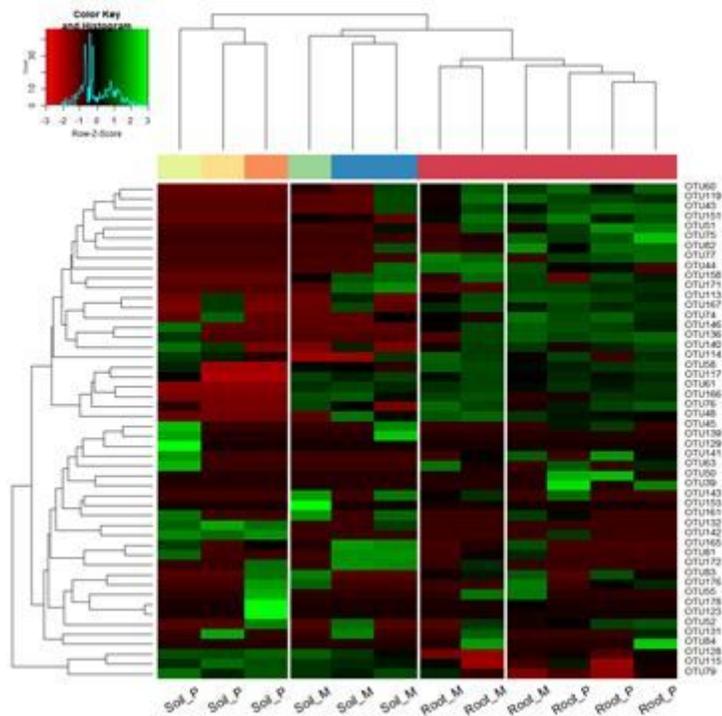
**Figure 4**

PCoA analyses of AM fungal communities in the root and soil samples. The PCoA plot was based on the Bray-Curtis distances at the OTU level (97% sequence similarity) of AM fungal communities.



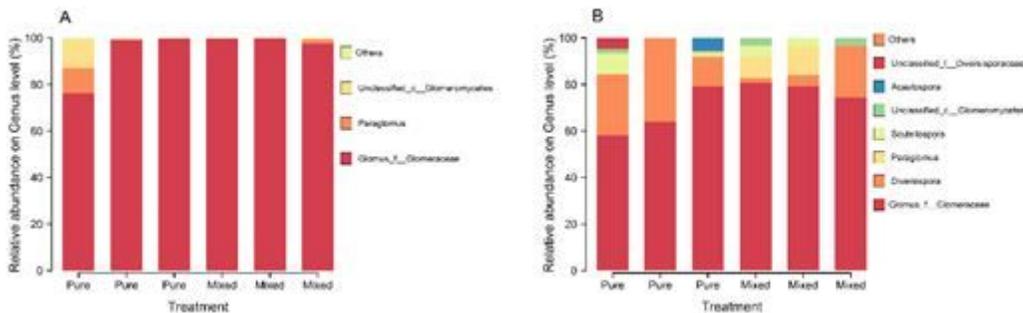
**Figure 5**

Alpha diversity indices of AM fungal communities in root and soil samples. For alpha diversity, number of OTUs observed (Sobs), Shannon, Simpson, Chao1, ACE and Faith's PD indices were calculated using random subsamples of 15,869 18S rRNA gene sequences per sample. OTUs were delineated at 97% sequence similarity. The box plot shows median (black line), first quartile–third quartile percentiles (box range) and 1.5× the interquartile range (whiskers). There were three independent replicates of each treatment.



**Figure 6**

Heat map analyses of AM fungal communities in the root and soil samples. The relative abundances of the top 50 most abundant classified AM fungal OTUs were identified in each sample by colors deduced from the raw Z-scores. Hierarchical clustering of all samples was performed using average clustering method with the Euclidean distances. Soil\_P represents soil sample in pure plantation; Soil\_M represents soil sample in mixed plantation; Root\_P represents root sample in pure plantation; Root\_M represents root sample in mixed plantation.



**Figure 7**

Relative abundances of main AM fungal genera in root (A) and soil (B) samples.

## Supplementary Files

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