Variations in Soil Functional Fungal Community Structure Associated With Pure and Mixed Plantations in Typical Temperate Forests of China

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Forest plants are in constant contact with the soil fungal community, which plays an important role in the circulation of nutrients through forest ecosystems. The objective of this study was to evaluate the fungal diversity in soil and elucidate the ecological role of functional fungal communities in forest ecosystems using soil samples from seven different plantations in northeastern China. Our results showed that the fungal communities were dominated by the phyla Ascomycota, Basidiomycota, and Mortierellomycota, and the mixed plantation of Fraxinus mandshurica and Pinus koraiensis had a soil fungal population clearly divergent from those in the other plantations. Additionally, the mixed plantation of F. mandshurica and P. koraiensis, which was low in soil nutrients, contained a highly diverse and abundant population of ectomycorrhizal fungi, whereas saprophytic fungi were more abundant in plantations with high soil nutrients. Redundancy analysis demonstrated a strong correlation between saprophytic fungi and the level of soil nutrients, whereas ectomycorrhizal fungi were mainly distributed in soils with low nutrient. Our findings provide insights into the importance of functional fungi and the mediation of soil nutrients in mixed plantations and reveal the effect of biodiversity on temperate forests.

Keywords: forest ecosystems, pure and mixed plantations, ectomycorrhizal fungi, saprotrophic fungi, high-throughput sequencing

INTRODUCTION

Soil fungi play a crucial role in the ecosystem diversity and functional reconstruction and represent an essential functional component of soil and ecological systems as decomposers, symbionts, and pathogens. Furthermore, fungi are closely involved in the energy flow, nutrient cycle, and transformation of organic substances in soil (Cromack et al., 1977; Buee et al., 2009; Li et al., 2018). Their biomass and structure could provide an early indication of changes in soil characteristics, thereby making fungi more credible than organic matter as indicators of environmental changes (Powlson et al., 1987; Blagodatskaya and Kuzyakov, 2008). Spatial and temporal variations in soil microorganisms are affected by differences in dominant tree species, which can ultimately alter the availability and dynamics of soil nutrients, and cause shifts in the composition of microbial communities as they adapt to new environmental conditions (Priha and Smolander, 1997; White et al., 2005; Saetre and Bååth, 2009; Nagati et al., 2018).
Ectomycorrhizal (ECM) and saprophytic fungi are important as two major fungal guilds in forest ecosystems, especially in temperate regions (Liu Y.P. et al., 2018). ECM fungi play a determinant role in the absorption of nutrients and water by plants through establishing mutualistic interactions with plant roots (Beccquer et al., 2018; Clasen et al., 2018). Saprophytic fungi are mainly responsible for the decomposition of complex organic matter and conversion of nutrients (Talbot et al., 2013; Nagati et al., 2018). Plant diversity can greatly affect the composition of saprotrophic fungi and increase their community diversity by providing a wide variety of substrates to establish facilitative interactions with them (Gessner et al., 2010; Zhang et al., 2018).

Forest plantation or reforestation represents a significant silviculture and forest management practice worldwide (Evans, 1992; Paquette and Messier, 2010); however, the establishment of large-scale and continuous pure plantations can cause many problems, including the biodiversity loss, soil degradation, and a decline in ecosystem stability. To improve the ecological functions and economic value of reforestation, a planting model of a mixed forest, with various different tree species, has been gradually adopted when establishing new plantations. Recently, many studies have described the most important afforestation tree species, based on their effects on microbial biomass and community structure (Chen et al., 2005; Busse et al., 2006; Wang W.X. et al., 2013).

The effects of pure and mixed plantations on microbial communities have been reported; however, there are relatively few studies on functional soil fungal communities in forest plantations (Ushio et al., 2010; Flores-Rentería et al., 2016; Žižčáková et al., 2016). *Juglans mandshurica* and *Fraxinus mandshurica* are important hardwood broad-leaved tree species in Northeast China (Sun and Liu, 2015). *Pinus koraiensis* is a constrictive tree species of the top vegetation community during natural succession in northeastern China; it is an important component of coniferous-broad leaf mixed forests in the cold temperate zone, and its dynamic change is related to the stability of forest ecosystems in northeast China (Liu et al., 2016; Wang et al., 2019). In this study, we determined the fungal diversity and characteristics of functional fungal communities in soil under three important afforestation tree species (*P. koraiensis*, *J. mandshurica*, and *F. mandshurica*) in northeastern China, as well as the relative roles of ECM and saprotrophic fungal communities in pure and mixed forests. These results provide a better understanding of the ecological functions of the soil–fungal community between mixed and pure plantations, and reveal preliminarily underlying mechanisms between soil nutrients and soil–fungal functional community, especially for those tree species used in forest plantation.

**MATERIALS AND METHODS**

**Study Site and Sampling**

The experimental site was located in the Maoershan Forestry Experimental Station of Northeast Forestry University, Heilongjiang Province, China, at a latitude of 45°21′–45°25′N, a longitude of 127°30′–127°34′E, and an altitude of 390 m. The area is characterized by a temperate continental monsoon climate with the mean annual precipitation of 723 mm and the mean annual air temperature of 2.8°C. The zonal soil is a dark brown earth (Gu et al., 2010).

The test stands were reforested with pure or mixed plantations in 1986. Five different plantations were selected, including (1) pure *P. koraiensis* forest (PK), (2) pure *J. mandshurica* forest (JM), (3) pure *F. mandshurica* forest (FM), (4) mixed forest of *P. koraiensis* and *J. mandshurica* (P × J), and (5) mixed forest of *P. koraiensis* and *F. mandshurica* (P × F). The coniferous forest (PK), broad-leaved forests (JM and FM), and mixed coniferous and broad-leaved forests (P × J and P × F) were established at the same site by strips (each pure or mixed forest of ~0.5 ha), with a planting row space of 2 m × 1.5 m. Each plantation was separated by an interval zone.

Soil samples were collected in July 2014 from the soil in the root zone under the different forests. In the two mixed plantations, samples were collected from the soil under each tree species. Therefore, there were seven treatments, namely, PK, JM, FM, *J. mandshurica* from P × J [JM(P × J), P. koraiensis from P × J [PK(P × J)], *F. mandshurica* from P × F [FM(P × F)], and *P. koraiensis* from P × F [PK(P × F)]. Three plots (10 m × 20 m each) were set per treatment, and the soil samples from each plot were collected at a depth of 0–10 cm using a 10-spot sampling method and mixed. We removed impurities such as rocks, plant roots, and other objects. Chemical properties were determined for all soil samples, and the samples were stored at −80°C until DNA extraction for fungal community analysis.

**Determination of Soil Chemical Properties**

To analyze its chemical properties, soil was air-dried at room temperature and subsequently sieved. Total carbon (TC) content was determined using a total organic carbon analyzer (Vario, Elementar, Langenselbold, Germany). Total nitrogen (TN) content was determined using a Kjeldahl apparatus (BUCHI, Ltd., Flawil, Switzerland). Total phosphorus (TP), available phosphorus (AP), and alkali-hydrolyzable nitrogen (AHN) content were determined as reported previously (Olsen and Sommers, 1982; Lu, 2000; Khan et al., 2001). Soil pH was measured in a 1:2.5 soil/water suspension using a pH meter (PHS-3C; INESA Scientific Instrument Co., Ltd., Shanghai, China) (Dong et al., 2014). The potential nitrification (PN) rates were measured according to ISO 15685 (2012). Graphs were generated using the SigmaPlot software (v.11.0; Systat Software, Inc., San Jose, CA, United States).

**High-Throughput Sequencing of Internal Transcribed Spacer (ITS) Regions**

Genomic DNA was extracted from 250 mg of fresh soil using a PowerSoil DNA isolation kit (Mobio Laboratories, Inc., Carlsbad, CA, United States) according to the manufacturer instructions. Polymerase chain reaction (PCR) amplification was conducted using the primer set ITS1F (5′-CTTGGTCAATTAGGAAAGTAA-3′) and ITS2R (5′-GCTGCGTCTTTTCGATGC-3′) (Adams et al., 2013). Each
primer contained a barcode unique to each sample. The PCR conditions included initial denaturation at 95°C for 5 min, followed by 29 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 45 s, and a final extension at 72°C for 5 min. Amplon sequencing was performed on the Illumina HiSeq platform according to standard protocols.

Processing of ITS Sequencing Data
Data generated by sequencing were processed and analyzed using the QIIME pipeline (version 1.8.0) (Caporaso et al., 2010). Forward and reverse reads were merged using Flash (version 1.2.11). Sequences shorter than 200 bp were truncated with a quality score <20 over a 50-bp sliding window (Xu et al., 2017). Exact barcode matching was used, allowing a two-nucleotide mismatch. Reads containing ambiguous bases were removed. Only sequences that overlapped by >10 bp were assembled. Chimeras were screened and removed using the UCHIME algorithm. Sequences were clustered into operational taxonomic units (OTUs) at a 97% identity threshold using the UPARSE software package in the USEARCH platform (version 7.1). A representative sequence for each OTU was assigned to annotate taxonomic information using the UNITE community database. To equalize read sizes for their comparison among soil samples, random subsampling was conducted with the lowest value (50,328 reads) for further standardization analysis. Rarefaction analysis was performed for each sample. The Mothur software (version v1.35.0) was used to estimate coverage according to Good’s estimator; community richness and diversity were estimated, using the Chao index, abundance coverage estimator (ACE), and Shannon index. The H-cluster of each sample was analyzed at the OTU level using the R software’s Vegan package based on the Bray Curtis dissimilarity distance matrix and pairwise comparison of each sample and assessed by multivariate permutational analysis of variance (PERMANOVA). The R software (Venn diagram package) was used to depict a Venn diagram. The obtained OTUs were assigned into fungal functional guilds using the FUNGuild database (Nguyen et al., 2016).

Statistical Analyses
Differences in soil chemical properties, read numbers of dominant OTUs, and α-diversity indices (all fungi, ECM, and saprotrophic fungi) were assessed using ANOVA in SPSS 19.0 (SPSS Inc., Chicago, IL, United States), and a p-value of 0.05 was considered statistically significant (Shen et al., 2016). Redundancy analysis (RDA) was performed using the CANOCO software (Canoco for Windows 4.5, Microcomputer Power Inc., Willis, TX, United States) to test the relationships among genera and soil chemical properties according to the method described by Van Den Wollenberg (1977). Statistical significance of the relative abundance data at the phylum and class levels was analyzed using the STAMP software (Parks et al., 2014). Co-occurrence network analysis was performed using relative abundance values of OTUs with a Spearman correlation coefficient (r) >0.7 and p < 0.05, and the data were visualized using Cytoscape (version 3.4.0), according to a previous study (Shannon et al., 2003).

Data Access
All the fungal raw sequences have been deposited to GenBank Short Read Archive (No. SRP191732).

RESULTS
Soil Chemical Properties
The properties of the different soils are shown in Table 1. TN and TC contents, pH, and the PN rates of the soil in the mixed plantation of P. koraiensis and J. mandshurica were much higher than those in the corresponding pure plantations (p < 0.05); and although not significantly different, the soil AP levels were also higher in P × J than in the pure plantations. Conversely, TN and TC contents and the PN rates of the soil in the mixed plantation of P. koraiensis and F. mandshurica were lower than those in the corresponding pure plantations, with some values showing significant differences (p < 0.05). Soil TP content, AHN level, and C/N ratio mainly showed downward trends in both mixed plantations, although no significant differences were found. Generally, the levels of soil nutrients in the mixed plantation of P. koraiensis and J. mandshurica were higher than those in the mixed plantation of P. koraiensis and F. mandshurica. These results indicated that the mixture of different plant species could alter soil nutrient levels.

Fungal Diversity and Richness
The number of OTUs increased with the number of reads in each sample, and the number of ITS sequences reached a saturation plateau in all soil types, indicating that new fungal phylotypes would not be detected with the increase in the number of reads (Supplementary Figure S1). Redundancy analysis for all plantation types was >99%, demonstrating that the number of sequences was adequate to reveal the fungal diversity in different forest types.

The richness indices (ACE and Chao) for the mixed forest plantation types, except for the FM(P × F) sample, showed increasing trends compared with those for the corresponding pure forests, although the differences were not statistically significant (Table 2). Additionally, the OTU numbers and fungal diversity (Shannon index) in the soil varied among the different plantation types, showing increases in the mixed plantation of P. koraiensis and J. mandshurica and decrease in the mixed plantations of P. koraiensis and F. mandshurica relative to the values obtained for the corresponding pure plantations, with no significant differences.

Fungal Taxonomic Differences Between Mixed and Pure Forests
We constructed a Venn diagram to analyze the common and unique fungal OTUs among pure and mixed plantations (Figure 1). Figure 1A shows that 456 OTUs were common among the pure and mixed plantation types of P. koraiensis,
TABLE 1 | Soil chemical properties in seven different plantation types.

| Type        | pH       | Total nitrogen (mg g⁻¹) | Total phosphorus (mg g⁻¹) | Total carbon (mg g⁻¹) | C/N ratio | Alkaline hydrolyzable nitrogen (mg kg⁻¹) | Available phosphorus (mg kg⁻¹) | Potential nitrification rate (µg NO₂-N g⁻¹ h⁻¹) |
|-------------|----------|-------------------------|---------------------------|-----------------------|-----------|----------------------------------------|------------------------------|---------------------------------------------|
| PK          | 5.09 ± 0.07a | 4.48 ± 0.52ab            | 1.01 ± 0.04abc            | 84.0 ± 1.7b           | 18.92 ± 1.97a | 369.7 ± 48.4a                          | 21.0 ± 0.7a                  | 0.93 ± 0.10d                               |
| JM          | 5.46 ± 0.05cd | 4.44 ± 0.72ab            | 1.13 ± 0.04c              | 110.1 ± 9.9c          | 25.01 ± 2.35b  | 348.6 ± 103.9a                         | 19.4 ± 1.9a                  | 0.84 ± 0.03c                               |
| JM(P × J)   | 5.51 ± 0.06d | 6.88 ± 0.36d             | 1.04 ± 0.13c              | 136.1 ± 1.6d          | 19.82 ± 1.27a  | 412.9 ± 52.5a                          | 29.6 ± 0.7b                  | 1.93 ± 0.03f                               |
| PK(P × J)   | 5.42 ± 0.03c | 5.55 ± 0.27c             | 0.98 ± 0.03ab             | 105.2 ± 1.4c          | 18.97 ± 1.09a  | 309.9 ± 68.9a                          | 22.4 ± 8.1a                  | 1.71 ± 0.04e                               |
| FM          | 5.40 ± 0.05c | 4.75 ± 0.05b             | 1.03 ± 0.08b              | 85.8 ± 3.1b           | 18.06 ± 0.57a  | 383.7 ± 21.9a                          | 21.7 ± 2.7a                  | 1.93 ± 0.03c                               |
| FM(P × F)   | 5.27 ± 0.02b | 3.89 ± 0.14a             | 0.96 ± 0.03ab             | 80.8 ± 3.7b           | 20.78 ± 1.08a  | 284.8 ± 13.2a                          | 25.0 ± 3.5ab                 | 0.37 ± 0.02a                               |
| PK(P × F)   | 5.22 ± 0.04b | 3.82 ± 0.06a             | 0.89 ± 0.01a              | 71.5 ± 2.8a           | 18.73 ± 0.49a  | 290.6 ± 17.1a                          | 19.2 ± 2.3a                  | 0.58 ± 0.01b                               |

The different letters indicate a significant difference among the seven plantations, Duncan’s multiple range test. TC, total carbon; TN, total nitrogen; PN, the potential nitrification rate; TP, total phosphorus; AP, available phosphorus; AHN, alkaline hydrolyzable nitrogen.

TABLE 2 | Richness and diversity estimators of soil–fungal community in the seven different types of plantation.

| Sample | OTUs Mean | SE | ACE Mean | SE | Chao Mean | SE | Shannon Mean | SE |
|--------|-----------|----|----------|----|-----------|----|-------------|----|
| PK     | 775       | 46.5 | 806      | 38.3 | 813       | 43.0 | 4.57        | 0.46 |
| JM     | 890       | 76.2 | 931      | 59.4 | 937       | 59.5 | 4.77        | 0.35 |
| JM(P × J) | 927       | 98.1 | 955      | 86.3 | 969       | 84.2 | 4.99        | 0.52 |
| PK(P × J) | 869       | 135.2 | 911     | 119.6 | 926       | 112.1 | 4.48        | 0.79 |
| FM     | 804       | 114.5 | 843     | 73.0  | 846       | 75.3  | 4.47        | 1.04 |
| FM(P × F) | 725       | 130.7 | 810     | 130.7 | 818       | 138.5 | 3.89        | 0.80 |
| PK(P × F) | 755       | 159.5 | 833     | 147.5  | 840       | 156.4 | 4.39        | 0.60 |

F, 1.149; P, 0.385; SE, standard error.

FIGURE 1 | Venn diagram showing the number of unique and shared species in soil samples from pure and mixed plantations of (A) *P. koraiensis* and (B) *J. mandshurica* or *F. mandshurica*. 
The mixed plantation of *P. koraiensis* revealed a distinct distribution of the predominant fungi. The majority of OTUs were found in all soil samples, and comparative analysis revealed that in the mixed plantation of *P. koraiensis* and *F. mandshurica* than in the pure plantation of *F. mandshurica*, whereas there was a significantly greater number of reads for OTU1421 in the mixed plantation of *P. koraiensis* and *F. mandshurica*.

**Fungal Community Composition**

The identified sequences from the different plantation types were affiliated with 15 fungal phyla (Figure 2A), among which Ascomycota, Basidiomycota, and Mortierellomycota were predominant in the soils. These dominant fungal phyla were found in all soil samples, and comparative analysis revealed a distinct distribution of the predominant fungi. The mixed plantation of *P. koraiensis* and *F. mandshurica* was characterized by a significantly higher abundance of Basidiomycota and significantly lower abundance of Ascomycota, whereas the other pure and mixed forest soils showed a lower abundance of Basidiomycota and higher abundance of Ascomycota (Supplementary Figure S2).

At the class level, 11 dominant classes were identified, which are shown in Figure 2B. The fungal communities had a similar taxonomic distribution at the class level across all plantation types, and were dominated by Agaricomycetes, Leotiomycetes, Sordariomycetes, and Tremellomycetes. Sordariomycetes, Tremellomycetes, and Leotiomycetes were predominant in the mixed plantation of *P. koraiensis* and *J. mandshurica*, with a much higher abundance than that in the mixed plantation of *P. koraiensis* and *F. mandshurica*. However, Agaricomycetes showed a significantly higher abundance in the mixed plantation of *P. koraiensis* and *F. mandshurica* than in most of the other forest types (*p* < 0.01 or *p* < 0.001) (Supplementary Figure S3).

The dominant fungal OTUs are shown in Supplementary Table S1. Most of the dominant fungal OTUs reached significant levels in the different plantations. The numbers of reads for OTU1432, OTU2963, and OTU3597 were significantly higher in the pure plantations of *P. koraiensis*, *J. mandshurica*, and *F. mandshurica*, and in the mixed plantation of *P. koraiensis* and *J. mandshurica*, whereas there was a significantly greater number of reads for OTU1421 in the mixed plantation of *P. koraiensis* and *F. mandshurica*.

We then performed cluster analysis based on the relative abundance of OTUs in soils from the different plantations (Supplementary Figure S4). The results revealed separate clusters for the PK(P × J) and FM(P × F) samples and JM(P × J), JM, PK, FM, and PK(P × J) samples. The PERMANOVA analysis indicated significant differences between the two clusters (*r*² = 0.25, *p* = 0.001), suggesting differences in the fungal communities between the two groups.

We used co-occurrence analysis to evaluate the relationships among the microbial communities at the species level (Xiao et al., 2016). A co-occurring network of these dominant fungal OTUs was plotted to show positive relationships, with 45 OTUs (nodes) displaying positive associations based on the correlation analysis (Figure 3) and connections (91 lines) in the network representing strong (*r* > 0.7) and significant (*p* < 0.05) correlations. These dominant OTUs in the network belonged to 33 genera, with *Mortierella* being the most abundant. This finding suggested a considerable connection of *Mortierella* with other OTUs or genera based on the presence of 32 edges and high *r*-values.

**Functional Fungal Populations**

Most of the fungi were mainly divided into ECM and saprotrophic taxa (including the ECM–saprotroph type). The ECM and saprotrophic fungal communities were compared...
among the different plantation soils according to their total abundance (Figure 4A) and α-diversity indices (Supplementary Tables S2, S3). The total abundance of ECM fungi was significantly higher in the soil under the mixed plantation of *P. koraiensis* and *F. mandshurica* than in that under the other plantations. Additionally, saprotrophic fungi showed significantly higher total abundance in the soils under the mixed and pure plantations of *P. koraiensis* and *J. mandshurica* relative to that in the mixed plantation of *P. koraiensis* and *F. mandshurica* (Supplementary Figure S5). A similar pattern was observed for the α-diversity of saprotrophic fungi, whose greater richness and diversity indices (ACE and Chao) in the mixed plantation of *P. koraiensis* and *J. mandshurica* [PK(P × J)] and JM(P × J)] significantly differed from those of other samples. Meanwhile, the ECM fungi from the mixed plantation of *P. koraiensis* and *F. mandshurica* showed greater richness and diversity indices relative to those in the other plantations (Supplementary Tables S2, S3).

Regarding functional genus-based analysis, 52 and 290 identified genera were assigned to ECM and saprotrophic genera, respectively; however, the relative proportions of the ECM and saprotrophic genera differed among the different plantation types (Figures 4B,C). Although the biodiversity was quite stable, changes in the relative abundance of some specific genera were obvious. The most abundant saprotrophic genus was *Mortierella*, whereas *Tomentella*, *Sebacina*, and *Inocybe* were predominant ECM genera, according to their total abundance, especially in the mixed plantation of *P. koraiensis* and *F. mandshurica*.

Overall, the results clearly showed significantly higher proportions of ECM taxa and lower proportions of saprotrophic taxa in the mixed plantation of *P. koraiensis* and *F. mandshurica*, whereas no significant differences in the total abundance of ECM and saprotrophic taxa were observed in the mixed plantation of *P. koraiensis* and *J. mandshurica* relative to those in the pure plantations.

### Relationships Between Soil Chemical Properties and Functional Fungal Genera

The relationships between functional fungal communities and soil chemical properties were visualized through RDA ordination (Figure 5). The first two principal components explained 46.6 and 10.7% of variance, respectively, and the ordination diagram shows a distinct distribution between saprotrophic and ECM fungal communities and soil nutrients along the RDA1 axis. Thus, saprotrophic fungi were positively correlated with most of the soil nutrient factors, which indicated that the relative abundance of saprotrophic genera, such as *Mortierella* and *Inocybe*, was higher in soils with higher nutrient content.

*FIGURE 3* | Network analysis showing fungal co-occurrence patterns in soil samples from different plantations. All OTUs with an average abundance >0.2% in all samples were included. The size of each node is proportional to the relative abundance of the fungal OTUs, and nodes in the network are marked with identical colors for the same genera. The thickness of each solid line is proportional to the $r$-value.
fungi was higher when soil was nutrients rich. Meanwhile, ECM fungi were negatively correlated with most of the soil nutrient factors, implying that a soil low in nutrients might induce an increase in the abundance of ECM fungi. Furthermore, saprotrophic fungi were mainly distributed in higher-pH areas, whereas ECM fungi were mainly distributed in lower-pH areas.

**DISCUSSION**

Microorganisms play a key role in forest soil ecosystems, and maintain an ecosystem balance in forest soils through decomposition of soil organic matter and processes of nutrient mineralization (Comerford et al., 2013; Richter et al., 2018). Additionally, microorganisms present in soil directly or indirectly influence soil nutrient transformations (Ingham et al., 1985; Van Der Heijden et al., 2008). Previous studies have demonstrated that pure forest plantations or long-term continuous cropping systems decrease soil nutrient consumption and fertility, thereby increasing nutrient imbalances or decreasing soil–microbial activity and diversity by altering the population structure (Reeves, 1997; Wang H. et al., 2013; Xiong et al., 2015). In the present study, we found that variations in the soil nutrient content and functional fungal communities under the mixed plantation of *P. koraiensis* and two broad-leaved tree species were different from those observed in pure-forest stands.
Most forests are multi-species and heterogeneous (mixed forests). The positive effects of mixing species have been widely recognized in silviculture, especially for certain tree species with different functional traits (Cannell et al., 1992). Compared with monocultures, mixed forests may exhibit greater levels of ecosystem functions and services, including enhanced productivity, improved soil nutrients, and increased soil microbial diversity and enzyme activity (Singh et al., 2012; Seidel et al., 2013; Forrester, 2014). A previous study has indicated that soil microorganisms in mixed forests are better than those in pure forests (Jiang et al., 2012). In the present study, richness indices (ACE and Chao) were higher in soil samples from mixed plantations than in those from corresponding pure forests, with the exception of the FM(P x F) sample. Weber et al. (2014) proposed that the elevation in richness tends to weaken the dominance of the most competitive species in an ecosystem and promote the harmonious coexistence of microbial taxa. Similarly, Chauvat et al. (2011) demonstrated that the biodiversity of the soil faunal community could increase during conversion from pure stands to mixed forest stands. These findings suggest that establishing plantations not only changes soil nutrients but also directly affects soil fungal community and may provide more fungus-mediated benefits to plant and soil ecosystems.

We found that 456 common OTUs were shared among soils from pure and mixed plantations of *P. koraiensis* (Figure 1A), with the dominant phyla mainly being Ascomycota, Basidiomycota, and Mortierellomycota. Similarly, 348 common OTUs from soils under the pure and mixed plantations of *J. mandshurica* and *F. mandshurica* were also primarily assigned to Ascomycota, Basidiomycota, and Mortierellomycota (Figure 1B). These common taxa can be deemed as the core microorganisms in forest soils and defined as the group of members shared among microbial communities (Turnbaugh et al., 2007; Xiao et al., 2016). Moreover, these core microorganisms may play vital roles in the function and stability of the microbiota (Xiao et al., 2016). Shade and Handelsman (2012) proposed that identifying core species or OTUs is essential for elucidating the ecology of microbial consortia and that microorganisms associated with a particular habitat are likely critical for the community function. Furthermore, we found that the number of unique OTUs was significantly higher in the mixed plantation of *P. koraiensis* and *J. mandshurica* than in the corresponding pure plantations. By contrast, the number of unique OTUs was lower in the mixed forest of *P. koraiensis* and *F. mandshurica* than in the corresponding pure plantations. These findings suggest differences in the interactions between *P. koraiensis* and other broad-leaf tree species, depending on the biological and chemical properties of the soil. Identification of the species represented by unique OTUs may better reflect changes introduced by different planting practices (Ma et al., 2016). Additionally, the proportion of unique OTUs representing unclassified fungi was higher in samples from the mixed forest of *P. koraiensis* and *J. mandshurica*. It is possible that some unknown functional fungi are represented by unclassified OTUs. In our future work, we will focus on these unknown species to elucidate their ecological functions.

In the present study, we found statistically significant differences in the fungal community structure regarding the dominant classes (Supplementary Figure S3). The abundance of the dominant classes distinctly varied among the different forest types, with Leotiomycetes, Tremellomycetes, and Sordariomycetes showing significant abundance in the mixed forest of *P. koraiensis* and *J. mandshurica*. The class Leotiomycetes comprises a variety of fungi with different ecological functions, which contribute to wood and plant litter decay (Boberg et al., 2011). Sordariomycetes represent well-characterized cellulolytic taxa of major degraders of cellulose (Wilhelm et al., 2017), and members of Tremellomycetes have a high potential for wood rot activity as saprotrophs (Millanes et al., 2011; Gusman et al., 2014). Members of cellulolytic taxonomic groups, as decomposers of plant litter, can critically influence soil nutrient cycling, and physicochemical characteristics (Song et al., 2015; Wilhelm et al., 2017). Notably, the only functional class displaying significant dominance in soil samples from the mixed forest of *P. koraiensis* and *F. mandshurica* was Agaricomycetes, representing ECM fungi, whereas the relative abundance of other functional classes was reduced, likely due to low soil nutrient levels. Low soil nutrients also explain the reduced diversity of functional fungal classes in the mixed plantation of *P. koraiensis* and *F. mandshurica*, which may indicate that Agaricomycetes are strong competitors under these conditions.

Fungal communities were further analyzed based on functional guilds to better understand their ecological specificity (Detheridge et al., 2018). Community differences among different plantations were largely attributable to differences in the relative abundance of major functional fungi. In this study, we focused on ECM and saprotrophic taxa and observed higher ECM fungal diversity.
diversity and abundance in the mixed plantation of *P. koraiensis* and *F. mandshurica*, with lower soil nutrients, which may indicate the existence of a mechanism enabling the regulation of soil low in nutrients. ECM fungi can excrete extracellular enzymes that degrade complex organic nitrogen compounds, thereby providing benefits to forest trees by enhancing the mobilization and uptake of soil nutrients (Nygren et al., 2007; Courty et al., 2010; Li et al., 2016; Luo et al., 2018). Additionally, ECM symbiosis is usually viewed as promoting nutritional mutualism and providing resources for host trees to withstand harsh conditions (Peay, 2016; Karst et al., 2018). Previous studies have shown that plants likely form different root symbioses via mycorrhizal networks, such as those associated with ECM fungi, to promote the growth of their neighbors (Simard et al., 2012; Teste et al., 2014; Luo et al., 2018). Therefore, if a mycorrhiza-mediated network mechanism does exist in forests, it may not only facilitate the assimilation of soil nutrients by mixed plant communities under poor nutrient conditions (Luo et al., 2018) but also greatly promote the co-existence of plant species during reforestation, especially in the cases of mixed ECM and non-ECM plant species.

Although we observed relatively similar levels of diversity and richness among fungal communities, the α-diversity indices and community compositions of the functional guilds (e.g., ECM and saprotrophic) differed significantly among the seven plantations (Supplementary Tables S2, S3). In particular, the mixed plantation of *P. koraiensis* and *F. mandshurica* showed higher ECM fungal diversity, whereas that of *P. koraiensis* and *J. mandshurica* showed higher saprophytic fungal diversity. We performed RDA to reveal potential relationships between functional genera and soil nutrients and found that saprotrophic fungi were most closely associated with higher soil nutrient levels, which might be attributable to their functional traits associated with the decomposition and nutrient cycling of leaf litter in soil (Ceci et al., 2018). However, ECM fungi were mainly distributed in low-nutrient areas. Marty et al. (2019) proposed that ECM fungi, as nutrient conduits, satisfy the need of host trees, whereas the role of saprotrophs is mainly to maintain nutritional homeostasis of the ecosystem. Furthermore, soils with a higher abundance of ECM fungi showed lower pH. Craig et al. (2018) reported that soils abundant in ECM fungi tended to be more acidic and that a low soil pH prevented the recession in ECM systems while influencing other soil microbial communities. Other studies that saprotrophic fungal biomass increased as the abundance of ECM fungal biomass declined (Fernandez and Kennedy, 2016). However, ECM fungi can exist in a state of competition with saprotrophic fungi by forming recalcitrant tissues to retard decomposition rates or inhibit the activities of saprotrophic fungi (Fernandez et al., 2013; Fernandez and Kennedy, 2016; Craig et al., 2018).

Network analysis can help identify interactions and provide a holistic view of microbial ecosystems to allow a better understanding of interactions within functional fungal communities (Banerjee et al., 2016). In this study, co-occurring network analysis suggested positive associations between functional genera, with significant positive associations among 45 OTUs (nodes) from 33 genera (p < 0.05; Figure 3). The resulting nodes were mainly divided into two networks, one of which comprised saprophytic fungi with 25 nodes, wherein Mortierella showed a high degree of connections with other genera and a higher relative abundance. The keystone species (high-degree species) in a co-occurrence network of soil microorganisms could have a large impact on the community composition (Berry and Widder, 2014; Chao et al., 2016). In the present study, the resulting network suggested that Mortierella, which is capable of transforming phosphorus from an insoluble to a soluble form that can then be directly utilized by plants, played a central role, with a significant impact on many other soil microbes, in both pure and mixed plantations (Osorio and Habte, 2013). The second network comprised ECM fungi with 18 nodes. ECM fungi provide multiple services to plants and ecosystems through their ability to convert and provide soil nutrients to plants (Liu Y.B. et al., 2018). Here, we found that *Tomentella* displayed a high degree of connections with other genera, followed by *Amphinema*, *Clavulina*, and *Russula*. Previous reports concerning the genus *Tomentella* demonstrated that *Tomentella* spp. were most frequently associated with ECM fungi in temperate forests (Jakucs et al., 2005) and were among the richest genera in a diverse ECM fungal community from *Abies religiosa* (also including *Inocybe*, *Russula*, and *Clavulina*) (Arguelles-Moyao et al., 2017). Moreover, the 91 interactions found in the network implied that close synergistic relationships could be formed within functional genera (e.g., ECM and saprotrophs) through direct or indirect interactions. Potential synergistic partnerships between functional genera in co-occurring networks would tend to strengthen the conversion of nutrients to promote ecological functions in a forest. Although ecological functions of soil microbial communities can influence plant development (Harris, 2009), selective actions of plants can also regulate the species composition and activity of soil microorganisms (Hartmann et al., 2009). It would be advantageous for plant species to broaden the niche and adapt to new environments by expanding the patterns and preferences of nutrient uptake with the help of symbiotic microbes (Wu et al., 2013; Afkhami et al., 2014). Thus, these results will facilitate effective forest management.

**CONCLUSION**

Our study assessed the influence of mixed conifer broadleaf plantations in typical temperate forests on soil characteristics and the composition of functional fungal communities. We found that soil fungal communities responded differently to changes associated with soil nutrient levels, indicating the existence of interdependent regulatory mechanisms between different forest types, soil nutrients, and soil microbial communities. Our results indicated higher saprotrophic fungal richness and diversity and soil nutrient levels in a mixed forest of *P. koraiensis* and *J. mandshurica* compared with those in a mixed forest of *P. koraiensis* and *F. mandshurica*, which showed a lower level of soil nutrients and higher abundance of ECM fungi. A positive

association between soil nutrients and soil fungi enhanced the ability of the mixed forests to withstand changes in the external environment. This study improves our understanding of the advantages of mixed forests (especially with ECM-associated tree species) by revealing the relationship between soil nutrients and functional fungal diversity.

**AUTHOR CONTRIBUTIONS**

GS contributed to conceiving and designing the experiments. DW, MZ, and WL performed the experiments and the data analysis. DW, MP, and XS drew the figures and wrote sections of the manuscript.

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**SUPPLEMENTARY MATERIAL**

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