Community structure and functional group of root-associated Fungi of *Pinus sylvestris* var. *mongolica* across stand ages in the Mu Us Desert

Pei-shan Zhao\(^{1,2,3}\) | Mi-shan Guo\(^{1,2,3}\) | Guang-lei Gao\(^{1,2,3}\) | Ying Zhang\(^{1,2,3}\) | Guo-dong Ding\(^{1,2,3}\) | Yue Ren\(^{1,2,3}\) | Mobeen Akhtar\(^{1,2,3}\)

\(^{1}\)Yanchi Research Station, School of Soil and Water Conservation, Beijing Forestry University, Beijing, China
\(^{2}\)Key Laboratory of State Forestry and Grassland Administration on Soil and Water Conservation, Beijing, China
\(^{3}\)Engineering Research Center of Forestry Ecological Engineering, Ministry of Education, Beijing Forestry University, Beijing, China

Correspondence
Guang-lei Gao, Yanchi Research Station, School of Soil and Water Conservation, Beijing Forestry University, Beijing 100083, China. Email: gaoguanglei@bjfu.edu.cn

Funding information
Fundamental Research Funds for the Central Universities, Grant/Award Number: 2017PT03 and 2015SZCQ-SB-02; National Natural Science Foundation of China, Grant/Award Number: 31600583; National Key Research and Development Program of China, Grant/Award Number: 2018YFC0507010

Abstract
Root-associated fungi (RAF) are an important factor affecting the host's growth, and their contribution to *Pinus sylvestris* var. *mongolica* plantation decline is substantial. Therefore, we selected three age groups of *P. sylvestris* plantations (26, 33, and 43 years), in the Mu Us Desert, to characterize the community structure and functional groups of RAF, identified by Illumina high-throughput sequencing and FUNGuild platform, respectively. The effects of soil properties and enzyme activities on fungal diversity and functional groups were also examined. The results indicated that (a) 805 operational taxonomic units of RAF associated with *P. sylvestris* belonged to six phyla and 163 genera. Diversity and richness were not significantly different in the three age groups, but community composition showed significant differences. Ascomycota and Basidiomycota dominated the fungal community, while *Rhizopogon* dominated in each plot. (b) The proportion of pathotrophs decreased with increasing age, while that of symbiotrophs increased sharply, which were mainly represented by ectomycorrhizal fungi. (c) Stand age and soil enzyme activity had a greater influence on fungal community composition than did soil properties, whereas environmental variables were not significantly correlated with fungal diversity and richness. Dynamics of fungal community composition and functional groups with the aging plantations reflected the growth state of *P. sylvestris* and were related to plantation degradation.

Keywords
ectomycorrhizal fungi, pathogenic fungi, *Pinus sylvestris* var. *mongolica*, saprotrophic fungi, soil property, stand age, the Mu Us Desert
1 | INTRODUCTION

Pinus sylvestris var. mongolica plantations are diminishing in the desertified Northern China.

As an important evergreen species with strong environmental resistance, millions of P. sylvestris have been planted in the desertified Northern China, to protect meadows, farmlands, oases, etc. Presently, P. sylvestris plantations occupy at least 3.0 \times 10^5 \text{ ha} in the desertified land (Song et al., 2018). P. sylvestris alleviates the effects of desertification and sandstorms, mainly by reducing the wind speed and enhancing sand fixation. Both the ecosystems and citizens benefit from these green guards. However, these P. sylvestris plantations have been experiencing leaf etiolation, disease outbreaks, growth declines, and regeneration barriers since the 1990s (Song, Zhu, Yan, Li, & Yu, 2015). The life span of P. sylvestris plantations is about 60 years, which is much shorter than that of natural forests, and the quality of these plantations has significantly declined in the past few years (Zhu, Fan, Zeng, Jiang, & Matsuzaki, 2003). The degradation of these plantations is continuing and is spreading to other maturing P. sylvestris plantations in the desertified lands of Northern China. Therefore, there is an urgent need to identify the causes underlying this deterioration.

Due to the aridity and water shortage in the desertified regions, water stress severely restricts plant growth (Liu, Bao, Song, Cai, & Sun, 2009). Hence, it has been suggested that soil moisture is the critical factor determining P. sylvestris plantation degradation, because variations in precipitation and available groundwater result in an unsustainable water balance (Song, Zhu, Li, & Zhang, 2016; Zheng, Zhu, Yan, & Song, 2012). However, in practice, although the increasing rainfall and careful irrigation have greatly improved the P. sylvestris plantation quality, forest degradation still restricts the development and benefits of P. sylvestris plantations, especially their regeneration. Previous studies have proven that rainfall exhibits significant secular trends in the semi-arid regions of Northern China (Gong, Shi, & Wang, 2004; Wei & Wang, 2013); however, the plantations continue to recede, indicating that some unheeded and invisible factors are driving this process.

Root-associated fungi (RAF), which form a large invisible world beneath the soil, connect the aboveground biomass with the underground ecosystem. They play important roles in ecosystem functions, by participating in a series of crucial ecological processes in terrestrial ecosystems (Fisher et al., 2012; Wehner et al., 2014). In early studies, scholars mainly focused on RAF diversity and community composition in specific habitats or host plants (Barnes, Maldonado, Froslev, Antonelli, & Ronsted, 2016a; David, Seabloom, & May, 2016). The identification of fungal functional groups was then explored, based on RAF community taxonomy, to accurately reflect the growth, development, and fitness of host plants (Nguyen et al., 2016). The connection between RAF of different functional groups and hosts can be both beneficial and detrimental. Pathogenic fungi can cause diseases in host plants, while symbiotic fungi promote the absorption of water and mineral nutrients by the host plants, and improve resistance and adaptability (Kolaříková et al., 2017). Compared to saprotrophic fungi, the productivity and diversity of plant communities respond more strongly to the richness of ectomycorrhizal (ECM) and plant pathogenic fungi (Peay, Baraloto, & Fine, 2013). Therefore, functional groups provide a better and direct understanding of the correlations between visible vegetation and invisible soil microbes, suggesting that these fungi may be potential decisive factors for P. sylvestris plantation degradation.

Root-associated fungi community structure and functional groups are influenced by host characteristics and the abiotic environment. In secondary forests with various plant species, host specificity or preference is a key driver (Horn, Hempel, Verbruggen, Rillig, & Caruso, 2017), but in case of a single target plant species, stand age has shown to be an important determinant of the fungal community composition (Gao et al., 2014; Spake et al., 2016). Moreover, soil properties are also regarded as crucial environmental variables. The communities and trophic modes of RAF may fluctuate with the pH (Li et al., 2018), water content (Barnes, Gast, Burns, McNamara, & Bending, 2016b), elemental composition (Liu et al., 2014), and soil enzyme activities (Kyaschenko, Clemmensen, Hagenbo, Karlton, & Lindahl, 2017). However, it is challenging to determine the causality between soil factors and fungi assembly. A comprehensive and reasonable interpretation and understanding of the RAF community and functional groups need to be fully combined with a variety of driving factors.

The Mu Us Desert, located in Northern China, is one of the twelve largest deserts in China, occupying a total area of 3.2 km² across the Shaanxi Province and the Inner Mongolia and Ningxia Hui Autonomous Regions. As an optimal species for conifer afforestation, P. sylvestris was successfully introduced into Yulin City, Shaanxi Province, from the Hulunbuir Desert, in 1964. Since then, P. sylvestris plantations have continued to increase, albeit accompanied by forest degradation. In this paper, we hypothesized that P. sylvestris plantation degradation is a consequence of variations in RAF and their functions, resulting from stand aging. The fungal community structure was examined across a chronosequence of stand age, for 26 (half-mature forest), 32 (nearly mature forest), and 43 (mature forest) years, and functional groups were analyzed using the FUNGuild platform. The objectives of this study were as follows: (a) to explore the variations in community structure and functional groups of RAF of P. sylvestris, (b) to identify how the soil factors and stand age affect these variations. This information will improve our knowledge of P. sylvestris plantation degradation, and provide a firm basis for drafting and implementing policies for efficiently restoring degraded plantations.

2 | MATERIALS AND METHODS

2.1 | Study site

The study site was located in the Hongshixia Sandy Botanical Garden, on the southern edge of the Mu Us Desert (38°26′N, 109°12′E; 1,080 m elevation), Shaanxi Province. The site had a warm...
temperate continental monsoon climate, characterized by four distinct seasons, adequate light and heat, and great temperature variations. The annual average temperature was 9.1°C, and the annual average precipitation and evaporation were 385.5 and 2,502 mm, respectively. The soil was classified as azonal eolian sandy soil, with loose topsoil structure and low water retention (Gao et al., 2014).

We selected three 50 × 50 m plots in *P. sylvestris* plantations under different stand ages: (a) half-mature forest (MUh), (b) nearly mature forest (MUn), and (c) mature forest (MUm). All plots were established without tending measures and visible disturbance by an mature forest (MUn), and (c) mature forest (MUm). All plots were under different stand ages: (a) half-mature forest (MUh), (b) nearly mature forest (MUn), and (c) mature forest (MUm). All plots were established without tending measures and visible disturbance by anthropogenic activities. Information regarding the plots, including a substantial range of basic plot investigation, soil enzyme activities, and soil properties, was assessed (Table 1; Table S1).

### 2.2 Sample collection

Samples were collected in August 2017, which is the peak of the growth season. Within each age group, five standard trees, at least 10 m apart, were sampled. At each sampling position, litter, herbs, and the undergrowth humus layer were removed, and terminal root samples were dug out, careful not to include any miscellaneous roots. Then, the three repeated samples in the same tree were mixed to create one composite sample. In addition, soil around the root system (about 0–20 cm deep), corresponding to each root sample, was collected independently to evaluate soil properties. A total of 15 fine roots samples (three plots × 5 standard trees) were collected and prepared for subsequent assays. All samples were placed in sealed bags and preserved at −4°C.

### 2.3 Soil analysis

The soil water content (SWC) used to be determined gravimetrically after drying samples in an oven at 105°C for 12 hr. Soil pH was measured using a pH-3E pH meter (INESA, Shanghai, China). Total nitrogen (TN) and total phosphorus (TP) content were measured with the indophenol blue spectrophotometric method and Mo-Sh anti-colorimetric analysis method, respectively, with SmartChem Discrete Auto Analyzer (AMS). The total soil organic carbon (SOC) was determined using the dichromate oxidation method. Soil ammonium (NH$_4^+$-N) and nitrate (NO$_3^-$-N) were extracted with 2 M KCl solution and quantified colorimetrically from the supernatant using TU-1810 UV-Vis spectrophotometer (Pgeneral). Invertase, urease, and acid phosphatases activity levels were measured by 3,5-dinitrosalicylic acid colorimetry, sodium phenol-sodium hypochlorite colorimetry, and phenyl phosphate disodium salt colorimetry, respectively.

### 2.4 Sequencing data and fungal functional groups

DNA was extracted using the PowerSoil® DNA Kit (MO BIO), according to the manufacturer’s protocol. Polymerase chain reaction (PCR) amplification of RAF rDNA internal transcribed spacer (ITS) was performed, using the primers ITS1F (5’-CTTGGGTCAATTAGACGAAAT-3’) and ITS2 (5’-GCTGCGTTCTTCTCATGC-3’). The PCR amplification products were then detected and quantified using AXYGEN Gel Extraction Kit (Qiagen) and QPCR, respectively. An equimolar mix of all three amplicon libraries was used for sequencing, at the Allwegen Company. Each sequence was separately used to perform individual nucleotide–nucleotide searches, using the NCBI BLAST algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi), against the fungal taxa. Sequences with 97% similarity were classified as an operational taxonomic unit (OTU).

The functional groups of RAF in our study were assessed using the FUNGuild platform (http://www.states.org/guilds/app.php) (Nguyen et al., 2016), and only results with guild assignments that were "highly probable" or "probable" were accepted (Table 2). It was identified that fungi with combined trophic mode and combined guild were incorporated into "other fungi" and "other pathotrophic/saprotrophic fungi," respectively.

### 2.5 Statistical analyses

Shannon, Pielou, and Simpson indices were calculated, based on the relative abundance of OTUs, using the Vegan package in R-3.6.0. One-way analysis of variance (ANOVA) with least-significant difference (LSD) was used to compare the diversity indices and soil properties among the three age groups. All statistical analyses were performed in SPSS 20.0, and p < .05 was considered statistically significant.

For the convenience of description, the RAF genera are divided into dominant genus (>10.00%), common genus (10.00% ~ 1.00%), and rare genus (<1.00%) according to relative abundance. Groups of RAF (relative abundance > 1.00%) and intergroup similarity are

### Table 1 Basic characteristics of different age groups

| Plot | Stand age (a) | Average height (m) | Average DBH (cm) | Stand density (N/ha) | Canopy density |
|------|--------------|-------------------|-----------------|---------------------|---------------|
| MUh  | 26           | 12.48 ± 3.69      | 11.76 ± 3.72    | 2,500               | 0.79          |
| MUn  | 32           | 13.96 ± 2.38      | 13.58 ± 2.44    | 2,500               | 0.86          |
| MUm  | 43           | 14.14 ± 1.84      | 19.95 ± 3.03    | 1,650               | 0.73          |

Note: Values are mean ± SE. Abbreviation: DBH, diameter at breast height.
TABLE 2 Classification of fungal trophic modes and guilds

| Trophic mode | Guild                          |
|--------------|-------------------------------|
| Pathotroph   | Animal pathogens              |
|              | Plant pathogens               |
|              | Other pathotrophic fungi      |
| Saprotroph   | Dung saprotrophs              |
|              | Plant saprotrophs             |
|              | Soil saprotrophs              |
|              | Wood saprotrophs              |
|              | Undefined saprotrophs         |
|              | Other saprotrophic fungi      |
| Symbiotroph  | Arbuscular mycorrhizal fungi  |
|              | Ectomycorrhizal fungi         |
|              | Endophytes                    |
|              | Lichenized fungi              |
| Other fungi  | –                             |

3 | RESULTS

3.1 | Fungal diversity and community structure

We obtained a total of 361,548 high-quality sequences after processing and comparing against our criteria, which were clustered into 805 OTUs. The alpha diversity indices were not significantly different between the stand ages (p < .05) (Table 3). Shannon, Simpson, and Pielou indices exhibited maximum values in the half-mature forest, which were 3.10, 0.90 and 0.55, respectively.

Operational taxonomic units were assigned to six phyla, 17 classes, 54 orders, 87 families, and 163 genera. Across all samples, not only the relative abundance of Ascomycota (69.00%–73.50%) was higher than Basidiomycota (17.92%–22.60%) (Figure 1(a)), but Ascomycota also contain more genera. Among all the dominant and common genera, Chalara, Ilyonectria, Tuber, Xenopolsyclatum, Penicillium, Hypocreæ, Oidiodendron, Fusarium, and Geopora belong to Ascomycota, while only three genera were assigned to Basidiomycota (Rhizopogon, Inocybe, and Tomentella). Other phyla included Rozellomycota, Chytridiomycota, and Glomeromycota. There was no significant difference of relative abundance in phylum-level between age groups (p > .05).

In genus-level, the dominant fungi were different in each plot (Figure 1(b)). There were no dominant genera in the half-mature forest, and the common genera, such as Rhizopogon (7.24%), Chalara (6.84%), Inocybe (5.58%), and Hypocreæ (5.24%), were distributed evenly. Conversely, in nearly mature forests, Chalara (12.28%), Ilyonectria (11.27%), and Rhizopogon (10.96%) occupied a dominant position, followed by Penicillium (4.22%) and Tuber (4.05%). In mature forests, the genus with the highest relative abundance was Rhizopogon (17.05%), followed by Xenopolsyclatum (7.44%), Mortierella (4.67%), and Tuber (4.42%). The RAF community structure, based on genera, was more similar between the half-mature and nearly mature forests.

3.2 | Functional groups

The relative abundances of different RAF functional groups showed varying trends with increasing stand age (Table S2). The major functional group was the symbiotroph, mainly comprised of ECM fungi. In mature forests, the relative abundance of saprotrophs increased sharply, while that of pathotrophs declined, with increasing forest age. The patterns of fungal communities in three stand age were showed in a ternary plot (Figure 2), which were obtained analyzing of composition of OTUs and their abundances. The dominant genera among all plots were mostly ECM fungi (e.g., Rhizopogon, Tuber, Inocybe,
Tomentella, Geopora, Amphinema, and Hebeloma), particularly in nearly mature and mature forests. Furthermore, it showed that several fungi with combined trophic modes (e.g., Chalara and Oidiodendron) and saprotrophs (e.g., Xenopolyscytalum and Talaromyces), and only one fungus, belonged to pathotroph (Hypocrea).

3.3 | Factors affecting fungal diversity and functional group

Soil properties and soil enzyme activities did not exhibit any significant correlations with fungal diversity and richness (Table 4). However, the Mantel analysis revealed that the RAF community was significantly correlated with soil enzyme activities, rather than with soil properties (Table S3). In the RDA of RAF and environmental factors, all soil indicators explained 71.4% of the variation in functional groups. Of these, urease (19.7%, \( p < .05 \)), \( \text{NH}_4^+ \)-N (19.5%, \( p < .05 \)), age (17.9%, \( p < .05 \)), and invertase (17.2%, \( p < .05 \)) were shown to have a greater impact on the functional group composition, compared to other variables (Figure 3). Saprotrophs were chiefly affected by SOC and invertase, while pathotrophs were mainly affected by urease and \( \text{NH}_4^+ \)-N.

4 | DISCUSSION

4.1 | Variations in RAF community structure, functional groups, and diversity

Fungal succession, a complex and unpredictable process, is likely to hold species richness, and diversity does not change significantly (Jumpponen, Brown, Trappe, Cázares, & Strömmer, 2012; Martínez-García, Richardson, Tylianakis, Peltzer, & Dickie, 2015). It can explain the relative stability in OTU richness in the roots of \( P. \) sylvestris through different age groups. RAF of \( P. \) sylvestris in the Mu Us Desert were diverse, and composed mainly of Ascomycota and Basidiomycota, while members of Zygomycota, Rozellomycota, Chytridiomycota, and Glomeromycota were not as numerous. This was consistent with previous observations made in most ECM-dependent trees, such as Quercus and Salix polaris (Kernaghan & Patriquin, 2011; Toju et al., 2013). In the present study, the species and OTUs assigned to Ascomycota were more numerous than those assigned to Basidiomycota, because the faster evolutionary rate and higher species diversity of Ascomycota render it more adaptable in an arid environment than Basidiomycota (Lutzoni et al., 2004).

The proportion of RAF genera showed strong heterogeneity in the different age groups, even though the top genera were similar in fungal community composition, and this evenness decreased with increasing stand age. In each age group, there were more than 130 genera which distributed unequally, confirming the results of previous research on intraspecific compositional diversity, even at fine-scales (Bahram, Polme, Koljalgi, & Tedersoo, 2011; Mundra et al., 2015). The nonuniformity in fungal distribution derived from stand age can be explained by vegetative growth, root growth, and root exudates. In different development stage, the utilization of nutrient substance would determine the recruitment of required fungi, and microbial growth rates must be seen in light of the root growth rates (Kuzyakov & Razavi, 2019). The components of root exudates are connected with stand age, with different root exudates directly influencing the proportion and community structure of rhizosphere microorganisms (Broeckling, Broz, Bergelson, Manter, & Vivanco, 2008; Llado, Lopez-Mondejar, & Baldrian, 2018).

The functional group structure of the RAF of \( P. \) sylvestris changed dynamically with the forest age, but the interaction between the different functional groups was not clear. A "down-up" trend was observed for both symbiotrophs and saprotrophs, while the proportion of pathotrophs continued to decline with the maturing of plantations. Most mycorrhizal fungi (especially ECM fungi) cannot decompose and utilize lignin and cellulose like saprophytes (Moore et al., 2004), and ECM fungi absorb a large amount of nutrients from the soil for the host plants (Martin, Uroz, & Barker, 2017). Therefore,
we believe that the sudden increase in saprotrophs is probably related to the premature maturation of *P. sylvestris*. The amount of litter and dead tissue increases gradually with maturation, providing an organic matter pool for the saprophytes. When the proportion of pathotrophs and saprotrophs is higher than symbiotrophs, it is likely to cause plant diseases (Chu, Wang, Wang, Chen, & Tang, 2016; Millberg, Boberg, & Stenlid, 2015). It might well be a potential driver to the decline of *P. sylvestris*.

The half-mature forest was more susceptible to external disturbance, which may occur a heavier recession. With the maturing of plantations and the increase of saprotrophs, ECM fungi will be driven to improve tolerance to desiccation and defense against root pathogens (Frew, Powell, Glauser, Bennett, & Johnson, 2018).

### 4.2 Predominance of ECM fungi in RAF communities

The dominant RAF of Pinaceae are the ECM fungi, providing "positive plant-soil feedbacks" (Tedersoo, May, & Smith, 2010). In the present study, the prominent fungal groups associated with roots of *P. sylvestris* were ECM fungi (notably *Rhizopogon*, *Tuber*, *Inocybe*, *Chalara*, *Geopora*, *Tomentella*, *Oidiodendron*, *Xenopopysrcatum*, *Hypocrea*, *Oidiodendron*).
Tomentella, and Geopora), which have also been confirmed previously by ECM ground surveys using molecular identification, at a global scale (Guo et al., 2020; Hayward, Horton, & Nunez, 2015; Long, Liu, Han, Wang, & Huang, 2016; Roy-Bolduc, Laliberte, & Hijri, 2016). Some ECM fungi have strong environmental adaptability and can establish relatively stable symbiotic relationships with hosts. At the same time, the interaction between stand age and ECM fungi was reflected in changes in tree health, owing to the reciprocity between hosts and ECM fungi. Undermining the health affects the diversity and community structure of fungi by changing the supply of ectomycorrhizal nutrients (Koide, Fernandez, & Petrprakob, 2011).

Rhizopogon, as a predominant member of ECM fungal communities in temperate coniferous forests (Grubisha, Trappe, Molina, & Spatafora, 2002), can improve survival and drought tolerance of conifer seedlings (Van Dorp, Beiler, & Durall, 2016). Inocybe and Tomentella species are good dispersers, as they can be easily established from spores (Nara, 2009; Peay, Schubert, Nguyen, & Bruns, 2012). Tomentella is an ECM fungi with saprotrophic ability, and identification using FUNGuild revealed that some symbiotroph fungi also exhibited free-living saprophytic life strategies. We speculated that these fungi may change their trophic mode with the death of plant root tissues and/or environmental changes, to maintain normal physiological activities (Baldrian, 2009).

Geopora, widely distributed fungus that can colonize a variety of host plant species (Long et al., 2016), has been reported to be one of the dominant genera associated with P. sylvestris roots in arid and alkaline conditions (Ishida, Nara, Ma, Takano, & Liu, 2009), by facilitating the resistance of host plants to stress conditions.

Among other nonmycorrhizal fungi, Chalara, Ilyonectria, and Xenopolyscytalum were somewhat dominant, which was similar to the identification results of nonmycorrhizal RAF in Pinus wallichiana (Tyub et al., 2018). Chalara was identified as having a combined trophic mode (endophyte-plant pathogen-wood saprotroph) by FUNGuild. It is generally considered to be a litter saprotroph (Koukol, 2011), but certain Chalara species can become pathogenic, causing ash dieback (Pautasso, Aas, Queloz, & Holdenrieder, 2013). Some RAF in our study exhibited a similar phenomenon. The trophic and functional relationships between the RAF and the host plant are variable (Porras-Alfaro & Bayman, 2011), because

### TABLE 4

|                    | Shannon Coefficients | p     | Simpson Coefficients | p     | Pielou Coefficients | p     | Richness Coefficients | p     |
|--------------------|----------------------|-------|----------------------|-------|---------------------|-------|-----------------------|-------|
| Age                | 0.151                | .591  | 0.227                | .416  | 0.094               | .738  | −0.265                | .341  |
| pH                 | −0.089               | .752  | −0.082               | .771  | −0.111              | .694  | 0.175                 | .533  |
| SWC                | 0.214                | .443  | 0.089                | .752  | 0.189               | .499  | 0.154                 | .585  |
| TN                 | −0.018               | .950  | −0.079               | .781  | −0.086              | .761  | 0.079                 | .781  |
| TP                 | 0.179                | .524  | 0.250                | .369  | 0.136               | .630  | 0.064                 | .820  |
| SOC                | 0.111                | .694  | 0.218                | .435  | 0.154               | .585  | −0.254                | .362  |
| NH$_4^+$-N         | 0.179                | .524  | 0.111                | .694  | 0.075               | .791  | 0.214                 | .443  |
| NO$_3^-$-N         | 0.350                | .201  | 0.429                | .111  | 0.293               | .289  | −0.368                | .177  |
| Invertase          | −0.125               | .657  | −0.214               | .443  | −0.114              | .685  | 0.293                 | .289  |
| Urease             | 0.257                | .355  | 0.321                | .243  | 0.189               | .499  | −0.286                | .302  |
| Phosphatase        | −0.032               | .909  | −0.046               | .869  | −0.050              | .860  | 0.043                 | .879  |

Notes: **p < .01 and *p < .05.**

Abbreviations: NH$_4^+$-N, Nitrate nitrogen; NO$_3^-$-N, Ammonium nitrogen; SOC, soil organic carbon; SWC, soil water content; TN, total nitrogen; TP, total porosity.
the instability during the endophytic developmental stages causes them to constantly change ecological strategies and behavior in different hosts with diverse living conditions (Arnold & Lutzoni, 2007). Ilyonectria and Xenopolyctyalum were indicator genera in near-mature and mature forests (Table S4), both of which are saprotrophic fungi. Of these, Ilyonectria has been observed to be associated with plant disease and growth reduction in previous studies (Manici et al., 2018).

Remarkably, a few arbuscular mycorrhizal (AM) fungi were identified from the root tip samples of P. sylvestris in near-mature and mature forests, as also observed in Quercus rubra and Pinus densiflora, with the preference of ECM (Dickie, Koida, & Fayish, 2001; Toju & Sato, 2018). Furthermore, Oidiodendron, an ericoid mycorrhizal (ERM) fungus, was also identified in all plots, and ECM-ERM interactions have previously been reported in other nonericoid plants, such as Quercus ilex, Betula papyrifera, Abies balsamea, and Picea glauca (Bergero, Perotto, Girlanda, Vidano, & Luppi, 2000; Kernaghan & Patriquin, 2011). This spans the traditional classification model of mycorrhizal fungal colonization based on host plant species (Toju, Tanabe, & Sato, 2018), and it is more reasonable to explain the presence of AM or/and ERM fungi with fungal endophytic structures rather than the functional relationship of mycorrhizal symbiosis.

4.3 | Effects of soil enzymes and soil properties on RAF

In general, RAF diversity is affected by climatic and/or edaphic factors, especially soil pH, moisture, and SOC (Barnes, Gast, et al., 2016b; Tedersoo et al., 2014; Toljander, Eberhardt, Toljander, Paul, & Taylor, 2006). However, the correlation between the RAF diversity of P. sylvestris and environmental disturbance factors was poor, which has also been shown in previous studies (Entwistle, Zak, & Edwards, 2013; Jumpponen & Jones, 2014). We speculate that the shaping of fungal diversity by environmental factors depends on the scales and categories of the ecosystem, and fungi are tolerant and resilient to climate change in some ecosystems (Zheng, Hu, Guo, Anderson, & Powell, 2017).

A previous study suggested that fungi were more reactive to soil enzyme activity than were other microbial populations (Stursova, Barta, Santruckova, & Baldrian, 2016). We observed that the RAF community was strongly associated with soil enzyme activity, particularly to enzymes involved in the carbon (invertase), nitrogen (urease), and phosphorus (phosphatases) cycles (Baldrian, 2009) (Table S5). Invertase is related to the transformation and decomposition of SOC (Sotomayor-Ramirez, Espinoza, & Acosta-Martinez, 2009); however, with increasing SOC in the gradually maturing P. sylvestris plantation, the invertase activity was significantly reduced. This opposite relationship probably resulted from the increasing saprophytic fungi, which can also decompose the SOC. Urease plays a prominent role in nitrogen mineralization, making more nitrogen available to the plants (Song et al., 2012). In our study, urease activity tends to increase with the age of P. sylvestris, reflecting the tendency of an increase in soil nitrogen. Pathotrophs and soil nitrogen (e.g., TN, NH$_4$-N, and NO$_3$-N) were found to be negatively correlated, which confirmed that effective utilization of nitrogen can improve the resistance to pathogens (Dietrich, Ploss, Heil, & Cell & Environment, 2004); however, the interplay between the two cannot be easily determined (Dordas, 2008). Phosphatase activity in rhizosphere soil is higher than in the bulk soils, because it catalyzes the release of phosphate from organic compounds (Baldrian, 2014). Previous research has shown that organic phosphorus-targeting enzymes were of saprotrophic origin (Talbot et al., 2013; Zaviščič et al., 2016), thus saprophytic fungi in the roots of P. sylvestris were more intimately related to phosphatase.

In contrast, many environmental factors frequently vary with changes in soil pH, which is considered to have the most definitive impact on soil fungal community composition (Barnes, Gast, McNamara, Rowe, & Bending, 2018). However, there were no significant correlations between the fungal communities in P. sylvestris roots and soil pH (Table S3), which may be because fungi are less sensitive to pH changes and have a wide optimum pH range, without significant inhibition of their growth in pH outside this range (Rousk et al., 2010). In addition, rhizosphere soil pH of the P. sylvestris plantation was around 7.4 with little pH gradient, which was not enough to affect the fungal community and function. Most soil physical and chemical properties were poorly correlated with RAF composition and trophic mode. Host roots maintain a relatively stable fend and feedback interaction with the RAF, while the rhizosphere of the individual host plant has buffering effects (Goldmann et al., 2016), so changing soil environment does not result in variations in the fungal community of roots.

Overall, we did not observe any decline in the soil fertility, but the degradation of P. sylvestris was evident, indicating that stand age was the chief factor. This study showed that RAF taxa and functional groups influenced the growth of P. sylvestris to some extent. Building the connection and circulation of the soil system, RAF and hosts are a new point to solving plantation decline. The half-mature forest was in a vigorous period of growth, but the near-mature forest showed signs of decline with a sharp downward trend of symbiotrophs. Furthermore, the maturing P. sylvestris lead to an increase in saprotrophs, and the RAF regulation mechanism required more symbiotrophs to sustain the growth of the decaying P. sylvestris. From a novel perspective of microbes, relevant studies are necessary to enhance our understanding of P. sylvestris plantation declines and provide theoretical support for P. sylvestris plantation management in China.

5 | CONCLUSIONS

In the P. sylvestris plantation, the RAF were abundant, with various functional groups. There was heterogeneity in the distribution of fungal communities and functional guilds in the different age groups, while Rhizopogon dominated in each plot. Among the symbiotrophs,
the ECM fungi represented a predominant RAF functional group, even if it fluctuated with the stand age. With the maturing of *P. sylvestris* plantation, the variation of RAF composition and structure caused by stand development and soil properties, especially soil enzyme activity, which can explain *P. sylvestris* degradation plantation in the Mu Us Desert. The host health status and the forest ecosystem stability can be indicated by community structure and functional groups of RAF.

**ACKNOWLEDGMENTS**

This study was supported by the Fundamental Research Funds for the Central Universities (no. 2017PT03 and 2015ZCQ-SB-02), the National Natural Science Foundation of China (no. 31600583), and the National Key Research and Development Program of China (no. 2018YFC0507101). We would like to thank the staff of the Yanchi Research Station and the Key Laboratory of State Forestry and Grassland Administration on Soil and Water Conservation for their assistance with the field investigation and laboratory analysis. We are also grateful to the anonymous reviewers for their constructive and valuable comments and suggestions that helped us improve this article.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**AUTHOR CONTRIBUTIONS**

Mi-shan Guo and Guang-lei Gao conceived and designed the experiments. Guang-lei Gao, Mi-shan Guo, and Yue Ren performed the experiments. Pei-shan Zhao analyzed the data. Pei-shan Zhao and Guang-lei Gao drafted the manuscript. Guang-lei Gao, Pei-shan Zhao, and Mobeen Akhtar reviewed and edited the manuscript. Guang-lei Gao, Ying Zhang, and Guo-dong Ding performed supervision and finalized the manuscript.

**DATA AVAILABILITY STATEMENT**

We uploaded all fungal raw sequencing data to NCBI database. The BioProject accession number was PRJNA587326 (BioSample accessions SAMN13186498, SAMN13186499 and SAMN13186500 for half-mature forest, nearly mature forest, and mature forest, respectively).

**ORCID**

Guang-lei Gao [https://orcid.org/0000-0002-0486-1532](https://orcid.org/0000-0002-0486-1532)

**REFERENCES**

Arnold, A. E., & Lutzoni, F. (2007). Diversity and host range of foliar fungal endophytes: Are tropical leaves biodiversity hotspots? *Ecology*, 88(3), 541–549. [https://doi.org/10.1890/05-1459](https://doi.org/10.1890/05-1459)

Bahram, M., Polme, S., Koljalg, U., & Tedersoo, L. (2011). A single European aspen (*Populus tremula*) tree individual may potentially harbour dozens of *Cenococcum geophilum* ITS genotypes and hundreds of species of ectomycorrhizal fungi. *FEMS Microbiology Ecology*, 75(2), 313–320. [https://doi.org/10.1111/j.1574-6941.2010.01000.x](https://doi.org/10.1111/j.1574-6941.2010.01000.x)

Baldrian, P. (2009). Ectomycorrhizal fungi and their enzymes in soils: Is there enough evidence for their role as facultative soil saprotrophs? *Oecologia*, 161(4), 657–660. [https://doi.org/10.1007/s00442-009-1433-7](https://doi.org/10.1007/s00442-009-1433-7)

Baldrian, P. (2014). Distribution of extracellular enzymes in soils: Spatial heterogeneity and determining factors at various scales. *Soil Science Society of America Journal*, 78, 11–18. [https://doi.org/10.2136/sssaj2013.04.0155dg](https://doi.org/10.2136/sssaj2013.04.0155dg)

Barnes, C., Maldonado, C., Freslov, T. G., Antonelli, A., & Rosted, N. (2016a). Unexpectedly high beta-diversity of root-associat ed fungal communities in the Bolivian Andes. *Frontiers in Microbiology*, 7, 1377. [https://doi.org/10.3389/fmicb.2016.01377](https://doi.org/10.3389/fmicb.2016.01377)

Barnes, C. J., van der Gast, C. J., Burns, C. A., McNamara, N. P., & Bending, G. D. (2016b). Temporally variable geographical distance effects contribute to the assembly of root-associated fungal communities. *Frontiers in Microbiology*, 7, 195. [https://doi.org/10.3389/fmicb.2016.00195](https://doi.org/10.3389/fmicb.2016.00195)

Barnes, C. J., van der Gast, C. J., McNamara, N. P., Rowe, R., & Bending, G. D. (2018). Extreme rainfall affects assembly of the root-associated fungal community. *New Phytologist*, 220(4), 1172–1184. [https://doi.org/10.1111/nph.14990](https://doi.org/10.1111/nph.14990)

Bergero, R., Perotto, S., Giralda, M., Vidano, G., & Luppi, A. M. (2000). Ercidic mycorrhizal fungi are common root associates of a Mediterranean ectomycorrhizal plant (*Quercus ilex*). *Molecular Ecology*, 9(10), 1639–1649. [https://doi.org/10.1046/j.1365-294x.2000.01059.x](https://doi.org/10.1046/j.1365-294x.2000.01059.x)

Broeckling, C. D., Broz, A. K., Bergelson, J., Manter, D. K., & Vivanco, J. M. (2008). Root exudates regulate soil fungal community composition and diversity. *Applied and Environmental Microbiology*, 74(3), 738–744. [https://doi.org/10.1128/AEM.02188-07](https://doi.org/10.1128/AEM.02188-07)

Chu, H., Wang, C., Wang, H., Chen, H., & Tang, M. (2016). Pine wilt disease alters soils properties and root-associated fungal communities in *Pinus tabuliformis* forest. *Plant and Soil*, 404(1–2), 237–249. [https://doi.org/10.1007/s11104-016-2845-x](https://doi.org/10.1007/s11104-016-2845-x)

David, A. S., Seabloom, E. W., & May, G. (2016). Plant host species and geographic distance affect the structure of aboveground fungal symbiotic communities, and environmental filtering affects belowground communities in a coastal dune ecosystem. *Microbial Ecology*, 71(4), 912–926. [https://doi.org/10.1007/s00248-015-0712-6](https://doi.org/10.1007/s00248-015-0712-6)

Dickie, I. A., Koide, R. T., & Fayish, A. C. J. N. P. (2001). Vescicular-arbuscular mycorrhizal infection of *Quercus rubra* seedlings. *New Phytologist*, 151, 257–264.

Dietrich, R., Plass, K., Heil, M. J. P. (2004). Constitutive and induced resistance to pathogens in *Arabidopsis thaliana* depends on nitrogen supply. *Plant, Cell and Environment*, 27(7), 896–906. [https://doi.org/10.1111/j.1365-3040.2004.01195.x](https://doi.org/10.1111/j.1365-3040.2004.01195.x)

Dordas, C. (2008). Role of nutrients in controlling plant diseases in sustainable agriculture. A Review. *Agronomy for Sustainable Development*, 28(1), 33–46. [https://doi.org/10.1051/agro:2007051](https://doi.org/10.1051/agro:2007051)

Entwistle, E. M., Zak, D. R., & Edwards, I. P. (2013). Long-term experimental nitrogen deposition alters the composition of the active fungal community in the forest floor. *Soil Science Society of America Journal*, 77(5), 1648–1658. [https://doi.org/10.2136/sssaj2013.05.0179](https://doi.org/10.2136/sssaj2013.05.0179)

Fisher, M. C., Henk, D. A., Briggs, C. J., Brownstein, J. S., Madoff, L. C., McCraw, S. L., & Gurr, S. J. (2012). Emerging fungal threats to animal, plant and ecosystem health. *Nature*, 484(7393), 186–194. [https://doi.org/10.1038/nature10947](https://doi.org/10.1038/nature10947)

Frew, A., Powell, J. R., Glauser, G., Bennett, A. E., & Johnson, S. N. (2018). Mycorrhizal fungi enhance nutrient uptake but disarm defences in plant roots, promoting plant-parasitic nematode populations. *Soil Biology and Biochemistry*, 126, 123–132. [https://doi.org/10.1016/j.soilbio.2018.08.019](https://doi.org/10.1016/j.soilbio.2018.08.019)

Gao, G. L., Ding, G. D., Zhou, Y. Y., Wu, B., Zhang, Y. Q., Qin, S. G., ... Liu, Y. D. (2014). Fractal approach to estimating changes in soil properties following the establishment of *Caragana korshinskii* shelterbelts in Ningxia, NW China. *Ecological Indicators*, 43, 236–243. [http://dx.doi.org/10.1016/j.ecolind.2014.03.00](http://dx.doi.org/10.1016/j.ecolind.2014.03.00)
Porras-Alfaro, A., & Bayman, P. (2011). Hidden fungi, emergent properties: Endophytes and microbiomes. Annual Review of Phytopathology, 49, 291–315. https://doi.org/10.1146/annurev-phyto-080508-081831
Rousk, J., Bååth, E., Brookes, P. C., Lauber, C. L., Lozupone, C., Caporaso, J. G., ... Fierer, N. (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME Journal, 4(10), 1340–1351. https://doi.org/10.1038/ismej.2010.58
Roy-Bolduc, A., Laliberte, E., & Hijri, M. (2016). High richness of ectomycorrhizal and saprotrophic communities in soil organic matter decomposition. Soil Biology and Biochemistry, 92, 251–261. https://doi.org/10.1016/j.soilbio.2016.04.006
Sotomayor-Ramirez, D., Espinoza, Y., & Acosta-Martinez, V. (2009). Land use effects on microbial biomass C, β-glucosidase and β-glucosaminidase activities, and availability, storage, and age of organic C in soil. Biology and Fertility of Soils, 45(3), 487–497. https://doi.org/10.1007/s00374-009-0359-x
Spake, R., van der Linde, S., Newton, A. C., Suz, L. M., Bidartondo, M. I., & Doncaster, C. P. (2016). Similar biodiversity of ectomycorrhizal fungi in set-aside plantations and ancient old-growth broadleaved forests. Biological Conservation, 194, 71–79. https://doi.org/10.1016/j.biocon.2015.12.003
Stursova, M., Barta, J., Santruckova, H., & Baldrian, P. (2016). Small-scale spatial heterogeneity of ecosystem properties, microbial community composition and microbial activities in a temperate mountain forest soil. FEMS Microbiology Ecology, 92(12), fiw185. https://doi.org/10.1093/femsec/fiw185
Talbot, J. M., Bruns, T. D., Smith, D. P., Branco, S., Glassman, S. I., Erlandson, S., ... Peay, K. G. (2013). Independent roles of ectomycorrhizal and saprotrophic communities in soil organic matter decomposition. Soil Biology and Biochemistry, 57, 282–291. https://doi.org/10.1016/j.soilbio.2012.10.004
Pedersso, L., Bahram, M., Pölme, S., Köjalg, U., Yorou, N. S., Wijesundera, R., ... Abarenkov, K. (2014). Global diversity and geography of soil fungi. Science, 346(6213), 1256688. https://doi.org/10.1126/science.1256688
Pedersso, L., May, T. W., & Smith, M. E. (2010). Ectomycorrhizal lifestyle in fungi: Global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza, 20(4), 217–263. https://doi.org/10.1007/s00572-009-0274-x
Toju, H., & Sato, H. (2018). Root-Associated fungi shared between arbuscular mycorrhizal and ectomycorrhizal conifers in a temperate forest. Frontiers in Microbiology, 9, 433. https://doi.org/10.3389/fmicb.2018.00433
Toju, H., Tanabe, A. S., & Sato, H. (2018). Network hubs in root-associated fungal metacommunities. Microbiome, 6(1), 116. https://doi.org/10.1186/s40168-018-0497-1
Toju, H., Yamamoto, S., Sato, H., Tanabe, A. S., Gilbert, G. S., & Kadowaki, K. (2013). Community composition of root-associated fungi in a Quercus-dominated temperate forest: “coddominance” of mycorrhizal and root-endophytic fungi. Ecology and Evolution, 3(5), 1281–1293. https://doi.org/10.1002/ece3.546
Toljander, J. F., Eberhardt, U., Toljander, Y. K., Paul, L. R., & Taylor, A. F. (2006). Species composition of an ectomycorrhizal fungal community along a local nutrient gradient in a boreal forest. New Phytologist, 170(4), 873–883. https://doi.org/10.1111/j.1469-8137.2006.01718.x
Tyub, S., Kamili, A. N., Reshi, Z. A., Rashid, I., Mokhdomi, T. A., Bukhari, S., ... Qadri, R. A. (2018). Root-associated fungi of Pinus wallichiana in Kashmir Himalaya. Canadian Journal of Forest Research, 48(8), 923–929. https://doi.org/10.1139/cjfr-2018-0084
VanDorp, C. H., Beiler, K. J., & Durall, D. M. (2016). Dominance of a Rhizopogon sister species corresponds to forest age structure. Mycorrhiza, 26(2), 169–175. https://doi.org/10.1007/s00572-015-0660-5
Wehner, J., Powell, J. R., Muller, L. A. H., Caruso, T., Veresoglou, S. D., Hempel, S., ... van der Heijden, M. (2014). Determinants of root-associated fungal communities within Asteraceae in a semi-arid grassland. Journal of Ecology, 102(2), 425–436. https://doi.org/10.1111/1365-2745.12197
Wei, K., & Wang, L. (2013). Reexamination of the aridity conditions in arid northwestern China for the last decade. Journal of Climate, 26(23), 9594–9602. https://doi.org/10.1175/jcli-d-12-00605.1
Zavišič, A., Nassal, P., Yang, N., Heuck, C., Spohn, M., Marhan, S., ... Polle, A. (2016). Phosphorus availabilities in beech (Fagus sylvatica L.) forests impose habitat filtering on ectomycorrhizal communities and impact tree nutrition. Soil Biology and Biochemistry, 98, 127–137. https://doi.org/10.1016/j.soilbio.2016.04.006
Zheng, X., Zhu, J. J., Yan, Q. L., & Song, L. N. (2012). Effects of land use changes on the groundwater table and the decline of Pinus sylvestris var. mongolica plantations in southern Horqin Sandy Land, Northeast China. Agricultural Water Management, 109, 94–106. https://doi.org/10.1016/j.agwat.2012.02.010
Zheng, Y., Hu, H. W., Guo, L. D., Anderson, I. C., & Powell, J. R. (2017). Dryland forest management alters fungal community composition and decouples assembly of root- and soil-associated fungal communities. Soil Biology and Biochemistry, 109, 14–22. https://doi.org/10.1016/j.soilbio.2017.01.024
Zhu, J. J., Fan, Z. P., Zeng, D. H., Jiang, F. Q., & Matsuaki, T. (2003). Comparison of stand structure and growth between artificial and natural forests of Pinus sylvestris var. mongolica on sandy land. Journal of Forestry Research, 14(2), 103–111. https://doi.org/10.1007/bf02856774

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How to cite this article: Zhao P-S, Guo M-S, Gao G-L, et al. Community structure and functional group of root-associated Fungi of Pinus sylvestris var. mongolica across stand ages in the Mu Us Desert. Ecol Evol. 2020;10:3032–3042. https://doi.org/10.1002/ece3.6119