Different revegetation types alter soil physical-chemical characteristics and fungal community in the Baishilazi Nature Reserve

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Abstract

The effects of different revegetation types on soil physical-chemical characteristics and fungal community diversity and composition of soils sampled from five different revegetation types (JM, *Juglans mandshurica*; QM, *Quercus mongolica*; CB, conifer-broadleaf forest; LG, *Larix gmelinii*; PK, *Pinus koraiensis*) in the Baishilazi Nature Reserve were determined. Soil fungal communities were assessed employing ITS rRNA Illunima Miseq high-throughput sequencing. Responses of the soil fungi community to soil environmental factors were assessed through canonical correspondence analysis (CCA) and Pearson's rank correlations. The coniferous forests (LG, PK) and conifer-broadleaf forest (CB) had reduced soil total Carbon (C), total Nitrogen (N), and available N values compared with the broadleaf forest (JM, QM). The average fungus diversity according to the Shannon, ACE, Chao1 and Simpson index were increased in the JM site. Basidiomycota, Ascomycota, Zygomycota, and Rozellomycota were the dominant fungal taxa in this region. The phylum Basidiomycota was dominant in the QM, CB, LG, and PK sites, while, Ascomycota was the dominant phylum in the JM site. The clear differentiation of fungal communities and the clustering in the heatmap and in NMDS plot showed that broadleaf forests, conifer-broadleaf forest, and coniferous forests harboured different fungal communities. The results of the canonical correspondence analysis (CCA) showed that soil environmental factors, such as soil pH, total C, total N, available N and available Phosphorus (P) greatly influenced the fungal community structure. Based on our results, the different responses of the soil fungal communities to the different revegetation types largely dependent on different forest types and soil physicochemical characteristic in Baishilazi Nature Reserve.

Introduction

Due to long-term human disturbances and intensive land use, the native vegetation of temperate zones in China is severely damaged, with reduced biodiversity and deteriorated ~~of~~ ecological functions (Liu & Diamond, 2005). Numerous researches have established that vegetation restoration is an important measure to obtain ecological benefits (Gao et al., 2002; Nunezmir et al., 2015; Xiao et al., 2015), such as enhancing biodiversity (Dosskey et al. 2012), the restoration of damaged natural ecological system (Fule´ et al. 2012), and the recovery of ecosystem services (Benayas and Bullock, 2009). Revegetation also has numerous positive effects on soil physicochemical characteristics, such as soil bulk density, field capacity (Zhang et al., 2018), infiltration rates (Wu et al., 2016), soil organic carbon (Georgiadis et al., 2017), and soil nitrogen (Fu et al., 2010). Feedback processes of plant-soil play crucial roles in altering the structure and dynamics of soil microorganisms (Herrera Paredes et al., 2016). The soil microbial community, which is a key bridge that connects the plant community with soil processes, is one of the most important regulators of soil nutrient transformation (Cheng et al., 2013). Soil microorganisms can not only directly affect the storage of soil nutrients via microbial biomass, but also can indirectly effect soil nutrient transformation through the metabolic activity (Jangid et al., 2013; You et al., 2014). In this sense, different revegetation types, combined with accurate biological monitoring, can achieve effective and targeted restoration goals (Collen & Nicholson, 2014). However, studies on the changes in soil microbial community dynamics, despite the important role of microorganisms in biogeochemical cycling, are still scarce (Guo et al., 2018).

Fungal community and diversity have important influences on plant communities and ecosystems (van der Heijden et al., 2008; Devi et al., 2012). Furthermore, fungi play crucial roles in many respects of ecosystem development (Chen et al., 2010; Geml et al., 2014), determining biochemical cycles in continental ecosystems (Tedersoo et al., 2014). Fungal diversity and community composition are closely related to numerous abiotic and biotic factors, such as elevation (Kernaghan & Harper, 2010; Bahram et al., 2012), soil environment (Peter et al., 2001;

79 Dickie et al., 2002), plant species (Lovett et al., 2004; Weand et al., 2010), plant diversity (Dickie.
80 2007; Waldrop et al., 2006), and stand age (Zhu et al., 2010; Wallander et al., 2010). An
81 increasing number of studies have shown that a number of soil properties, including soil pH
82 (Fierer & Jackson, 2006), soil texture (Girvan et al., 2003), and soil nitrogen availability (Frey et
83 al., 2004), can be associated with changes in fungal community structure; these soil
84 characteristics are often influenced by vegetation cover at the same area. Any changes in the
85 fungal community in the process of ecological restoration are key indicators of restoration
86 success (Harris, 2009). A previous study has reported that replanting native vegetation can result
87 in dramatic shifts in the fungal community towards that of the natural fungal community (Yan et
88 al., 2018). Unfortunately, few researchers have addressed the connection between the different
89 revegetation types and the fungal community structure in broadleaf forests, coniferous forests,
90 and conifer-broadleaf forest. Specifically, it is unclear which soil environmental factors play the
91 principal roles in driving fungal dynamics. Previous work has concentrated on the relationships
92 between soil characteristics and soil fungi under grassland and leguminous species (Harrison &
93 Bardgett, 2010), but there remains a need for interpreting which of these factors were the
94 dominant influence on the soil fungal communities in different revegetation types.
95 As the national nature reserves, the Baishilazi Nature Reserve is located in the mountainous
96 region of the eastern Liaoning Province, China. The reserve was established in 1988 and is part
97 of the Changbai Mountain system. The original vegetation was broadleaf *Pinus koraiensis* forests,
98 which were severely damaged due to the over-exploitation of the past 100 years. At present, the
99 vegetation mainly consists of natural secondary forests and coniferous forests, which provides a
100 unique opportunity to investigate the soil fungal communities of different revegetation
101 ecosystems under the same climatic conditions. Numerous researches have investigated the
102 changes in soil microbial biomass (Fan et al., 2014), and soil organic carbon contents (Qi et al.,
103 2017) in different revegetation types; however, studies on the impacts of different reforestation
104 pathways on the soil fungal community are scarce. In this context, we applied pyrosequencing of

the ITS rRNA gene to explore both the diversity and composition of soil fungal communities in responses to different revegetation types from five sites in the Baishilazi Nature Reserve, Liaoning Province, China. Our objective was to examine how soil fungi may respond to different revegetation types and, more specifically, how the abundance and composition of soil fungal communities respond to changes in soil physicochemical properties.

Material and methods

Site description

The field study was carried out at the Baishilazi Nature Reserve (approval number# 20170628-7), the eastern mountainous areas of Liaoning Province (40.83° ~40.95° N, 124.74° ~124.96° E) (; Fig. 1). It is a comprehensive nature reserve with forest ecosystem as the main protection object. The reserve covers an area of 7407 ha and belongs to the Changbai mountain range. This area is characterized by a continental monsoon climate, with long cold winters, warm wet summers, and a higher diurnal temperature variation. The annual mean amount of evaporation is 885 mm, with a annual average temperature of 6.4 ℃, and a average annual precipitation of 1158 mm. The soil type is Brown soil (REF?). The region has a relatively rich and unique biodiversity, possessing significant ecological status and scientific value both in China and on a global level. The characteristics of the five selected study samples are listed in Table 1.

Soil sampling

In July 2017, we sampled soils from three randomly selected 20 m × 20 m plots per forest type after removal of the litter layer. The sampled forest types were including *Juglans mandshurica* (JM), *Quercus mongolica* (QM), conifer-broadleaf forest (CB), *Larix gmelinii* (LG), and *Pinus koraiensis* (PK), giving a total of 15 plots. Soil samples were collected use of a soil auger with 8 cm in diameter, and 10 cm deep. The soils of 15-20 points were collected at a depth of 0-10 cm along an "S" shaped path to ensure the representativeness of soil samples in each forest, mixed together and placed in sterilized ziplock bags as a replicate sample. Identically, in each vegetation form, three subsamples were collected. Immediately arrival to the laboratory the samples were

stored cooled boxes, sieved (2 mm mesh) to undock roots and dopant, and divided into two sub-samples, of which one was air-dried and used for physical and chemical analyses, and the other one was stored at -80 ℃ prior to DNA extraction and used for microbial analyses.

Determination of the physical and chemical properties

Soil pH was measured using a pH meter after shaking a soil-water (1:5w/v) suspension for 30 min (Bao, 2000; Ren et al., 2017). Soil total C and total N were measured with an Elemental Analyzer (Elementar, Germany) (Schrumpf et al., 2011). Available nitrogen (AN) was determined by the alkali diffusion method (Bao, 2000). Total phosphorus (TP) was measured by spectrophotometry after wet digestion with $HClO_4$-H_2SO_4 (Parkinson and Allen, 2009). Available phosphorus (AP) was measured using the colorimetric method with extraction via 0.5 M $NaHCO_3$ (Emteryd, 1989).

DNA extraction and PCR amplification sequencing

Total fungal genomic DNA samples were extracted from 0.5 g of soil using the Fast DNA SPIN extraction kits (MP Biomedicals, Santa Ana, CA, USA), according to the manufacturer's instructions. The quantity and quality of extracted DNAs were measured using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The primer sets: ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') (Gardes & Bruns, 1993) and ITS2F (5'-GCTGCGTTCTTCATCGATGC-3') (White et al., 1990) were selected to target the fungal ITS1 region. Sample-specific 7-bp barcodes were incorporated into the primers for multiplex sequencing. The PCR amplification required two steps. During the first step, each of three independent 25 μl reactions per DNA sample included 5 μl of Q5 reaction buffer (5×), 5 μl of Q5 High-Fidelity GC buffer (5×), 1 μl (10 uM) of each forward and reverse primer, 2 μl (2.5 mM) of dNTPs, 0.25 μl of Q5 High-Fidelity DNA Polymerase (5 U/μl), 2 μl of DNA Template, and 8.75 μl of ddH_2O. Cycling conditions were 98 °C for 5 min; 25 cycles of 98 °C for 15 s, 55 °C for 30 s, 72 °C for 30 s, followed by 72 °C for 5 min. The PCR amplicons were purified with Agencourt AMPure Beads (Beckman Coulter, Indianapolis, IN) and quantified using the PicoGreen dsDNA

Assay Kit (Invitrogen, Carlsbad, CA, USA). After the individual quantification step, amplicons were pooled at equal amounts, and pair-end 2×300 bp sequencing was performed using the Illlumina MiSeq platform with the MiSeq Reagent Kit v3.

Sequence data analysis

The raw data yielded from Illumina sequencing were analyzed using QIIME software (v1.9.0) and the UPARSE pipeline (Zhong et al., 2015). The UPARSE pipeline was performed for taxonomic assignment with similarities >97% (Edgar, 2013). Taxonomic classification was conducted with Unite databases for fungi. The raw data of fungi were submitted to the NCBI with the SRA accession number: PRJNA494279.

The operational taxonomic identity was annotated using a BLAST algorithm against sequences within the Unite Database, using the QIIME software (Kõljalg et al., 2013). An OTU table was further generated to record the abundance of each OTU in each sample and the taxonomy of these OTUs. Operational taxonomic units (OTU)-level alpha diversity indices, such as Chao1 index (Chao, 2002), ACE index, Shannon index, and Simpson index, were computed using the OTU table in QIIME (Caporaso et al., 2010). The shared and unique OTUs among samples were used to generate Venn diagrams using the R software package with "VennDiagram", based on the occurrence of OTUs across sample (Zaura et al., 2009). The heatmap representation of the top 50 classified genera in per sample was built using the R (R v.3.4.4) software package with "gplot" and "pheatmap" (Team, 2009). Non-metric multidimensional scaling (NMDS) with the dissimilarity matrices was conducted the soil fungal community composition using the 'metaMDS' function in the Vegan package (Yang et al., 2017). The linear discriminant analysis (LDA) effect size (LEfSe) method ~~was~~were performed to detect potential biomarkers of abundant taxa based on a normalized relative abundance matrix across groups, using the default parameters, which was built using Galaxy based on the online interactive analysis of microflora data (Segata et al., 2011).

Statistical analysis

Differences in soil physicochemical characteristics, fungal alpha diversity indices, and the relative abundance of taxa (phyla and genus) of different forest soils were compared using one-way analysis of variance (ANOVA), which was followed by the least significant difference (LSD) test performed in IBM SPSS (version 19.0, Chicago, IL, USA) (Banerjee, 2016). Pearson's correlation analysis was used to evaluate the correlations between soil fungal community diversity, fungal community composition and soil characteristics. Canonical correspondence analysis (CCA), which was performed via Canoco 4.5, was used to evaluate the linkages between dominant fungal groups and soil measured environmental factors (Braak and Smilauer, 2002).

Results

Soil physicochemical properties

As seen in Table 2, soil pH value ranged from 4.89 to 5.70. Soil pH value under QM was the most acidic with 4.89, followed by CB, while, JM contained the highest soil pH value. There were significant differences among different forest types regarding soil total C and total N contents ($P < 0.05$). Interestingly, both total C and total N exhibited highest value –in the soil of JM, with values of 100.53 g/kg and 7.80 g/kg, but only 41.70 g/kg and 3.58 g/kg in the PK site, respectively. Soil C/N values in all the treatments were below 25:1, among which CB had the highest C/N with 13.81. Available N was found in ranked order of JM > QM > CB >LG > PK. There were also significant differences in available P and total P among different forest types ($P < 0.05$), with the highest values under PK and CB, respectively (Table 2).

The first two axes of the principal components analysis accounted for 96.9% of the total variance. The biplot showed a clear spatial separation among different revegetation types. In fact, the axis 1 discriminated for the PK situated in the first quadrant and JM, QM, and LG in third and fourth quadrant, while the axis 2 discriminated for PK and LG situated in the first and second quadrants and the JM, QM, and CM soils in the second and third quadrants. Also, the investigated soil characteristics were clearly separated in the quadrants (Fig. 2). The C/N was situated in the first quadrant, soil total C and available P concentrations in the second quadrant, soil total P and

209 total N in the third quadrant, and all other parameters were located in the fourth quadrant (Fig. 2).

210 **Fungal community diversity responses to different revegetation types**

211 Fungal α-diversity varied greatly across the samples. The Shannon index, ACE index, Chao1
212 index, and Simpson index showed the highest values in the JM site, with 8.18, 879.57, 879.08,
213 and 0.99, respectively, followed by the CB site. The ACE index and Chao1 index were the lowest
214 in the LG site, with 522.15 and 521.58, respectively, while, the Shannon index and Simpson
215 index were the lowest in the QM site, with 5.79 and 0.89, respectively (Table 3). Pearson's rank
216 correlation coefficients indicated that the Simpson index ($r = 0.680$, $P < 0.01$) and Shannon index
217 ($r = 0.659$, $P < 0.01$) were positively correlated with soil pH, while the Shannon index was
218 positively correlated with C/N ($r = 0.528$, $P < 0.05$). In addition, ACE index and Chao1 index
219 were significantly positively correlated with total C ($P < 0.05$), C/N and total P ($P < 0.01$) (Table
220 4).

221 **Fungal community structure responses to different revegetation types**

222 A total of 640,914 high-quality ITS sequences were obtained after the elimination of chimeras
223 and sequences of low quality, with an average of 42,727 sequences being obtained in each soil
224 sample. At the phylum level, we found 8,875 fungal operational taxonomic units (OTUs) after
225 quality filtering. On average, 592 OTUs were found in each sample. A maximum of 743 OTUs
226 were detected in the CB site, however, only 455 OTUs were obtained in the LG site (Table 3). In
227 order to determine rarefaction curves, richness, and diversity, 22, 716 reads were randomly
228 selected from each sample. At the 3% dissimilarity level (Fig. 3), the curve tended to flatten with
229 the number of measured sequences increases, indicating that the experiment had obtained most of
230 the sample information and had been able to reflect the fungal community composition of the
231 forest soil.

232 The obtained sequences were affiliated with 15 phyla (including unknown). The dominant
233 phyla, accounting for more than 1% of the overall communities, were Basidiomycota,
234 Ascomycota, Zygomycota and Rozellomycota, with relative abundance values ranging from

21.31 to 66.08%, 24.82 to 51.88%, 2.21 to 6.37%, and 0.42 to 2.09%, respectively (Fig. 4). Phyla included Cercozoa, Chytridiomycota, Glomeromycota, and Ciliophora, which were less abundant (< 1% of all classified sequences), but still were found in all of the examined soils. The relative abundances of these most abundant fungal phyla varied significantly among different forest types. The relative abundance of Ascomycota in JM was significantly higher than that of the other phyla, while the relative abundances of Basidiomycota was the lowest in JM. In the CB site, the relative abundance of Basidiomycota was highest, while, the relative abundances of Ascomycota and Rozellomycota were the lowest (Fig. 4).

At the genus level, the dominant genus, accounting for more than 1% of the overall communities, were *Sebacina*, *Russula*, *Tomentella*, *Mortierella*, *Trechispora*, *Piloderma*, *Humicola*, *Suillus*, *Geminibasidium*, *Ramaria*, *Archaeorhizomyces*, *Cryptococcus*, *Simplicillium*, *Oidiodendron*, *Inocybe*, *Basidiobolus*, and *Bullera*. Their average relative frequencies differed, respectively, as 5.95%, 4.38%, 3.74%, 2.97%, 2.17%, 1.92%, 1.75%, 1.69%, 1.65%, 1.62%, 1.59%, 1.54%, 1.50%, 1.37%, 1.32%, 1.20%, and 1.07% (Fig. 5). *Sebacina* was the most abundant genus at PK, accounting for 21.17%. The relative abundances of *Russula* showed highest in the CB than others (Fig. 5).

Venn diagrams were used to compare the fungal communities based on shared and unique OTUs among the samples. At the genus level, the Venn diagram showed 110 OTUs among the five forest types (Fig. 6). A total of 1,453, 1,006, 1,321, 1,143, and 1,742 OTUs were observed in the CB, LG, PK, QM and JM. JM harbored 864 unique OTUs. QM harbored 311 unique OTUs. CB harbored 428 unique OTUs. LG harbored 298 unique OTUs. PK harbored 542 unique OTUs. The numbers of shared OTUs were 514 (JM vs. QM), 400 (LG vs. PK), 384 (JM vs. PK), 314 (JM vs. LG), 343 (QM vs. PK), and 329 (QM vs. LG) (Fig. 6).

To illustrate the fungal community structures of JM, QM, CB, LG, and PK, the heatmap analysis based on the top 50 most abundant fungal communities ~~, using R software,~~ was used to intuitively display the differences in relative abundances of fungal OTUs among the samples (Fig.

7). The relative abundance of the soil fungal community from high to low is represented by red through black to green, reflecting the different compositions and relative abundances of soil fungi under different forest types. The genera *Tomentella*, *Piloderma*, *Suillus*, *Oidiodendron*, *Inocybe*, *Entoloma*, *Cortinarius*, *Helvellosebacina*, and *Phaeoacremonium* dominated in LG. While in CB, *Russula*, *Trichoderma*, *Leucoagaricus*, *Amphinema*, *Umbelopsis*, and *Thelephora* were the most dominated genera. *Cladophialophora*, *Byssocorticium*, *Trichoderma*, *Hygrocybe*, *Exophiala*, *Leotia*, and *Knufia* dominated in QM. Correspondingly, NMDS based Bray distance was carried out to show the significant separation among different forest types (Fig. 8). By considering the phylogenetic relationship, both analyses elucidated that significant variations of fungal community structure occurred among the different revegetation types, demonstrating the significant effects of tree species on the fungal community composition following different revegetation types.

The LEfSe analysis was documented to determine the classified fungal taxa with significant abundance differences among the different sampling sites. As presented in Fig. 9, 16 fungal taxa were significantly different among the sites with LDA effect size scores were > 4.8 (Fig. 9A), and 5 fungal taxa were showed significantly different with LDA effect size scores were > 5.2 (Fig. 9B). At the phylum level, the biomarkers were affiliated with Basidiomycota, and Ascomycota, respectively.

Fungal community distribution as related to the soil properties

Canonical correspondence analysis (CCA) was used to analyze the relative abundances of dominant fungal phyla constrained by soil properties (Fig. 10). The results showed that the cumulative interpretation variations of the first and second axes were 93.5%, indicating that soil environmental factors greatly influenced the fungal community structure. At the phylum level (Fig. 10), soil pH (r = 0.9104) and available P (r = 0.6891) were significantly correlated with axis1, and the first axial interpretation rate was 69.1%. The parameters C/N (r = -0.7322) and total P (r = -0.8094) were significantly related with axis2.

Pearson's rank correlation analyses were used to explore the relationships between soil properties and the relative abundances of the 4 most abundant fungal phyla and 15 most abundant fungal genera. At the phylum level, the relative abundance of Basidiomycota was significantly negatively correlated with soil pH (r = -0.680, $P < 0.01$) and available P (r = -0.611, $P < 0.01$). Ascomycota relative abundance was positively correlated with total C (r = 0.608, $P < 0.05$), total N (r = 0.655, $P < 0.01$) and available N (r = 0.693, $P < 0.01$). Zygomycota relative abundance was positively correlated with pH (r = 0.530, $P < 0.05$) and available P (r = 0.665, $P < 0.01$). Rozellomycota relative abundance was significantly positively correlated with pH (r = 0.716, $P < 0.01$;) (Table 5).

At the genus level, the abundance of *Sebacina* was significantly negatively correlated with total C (r = -0.563, $P < 0.05$), total N (r = -0.533, $P < 0.05$) and available N (r = -0.604, $P < 0.05$). *Russula* was significantly positively correlated with C/N (r = 0.637, $P < 0.05$) and total P (r = 0.751, $P < 0.01$). While, *Humicola* was significantly positively correlated with total C (r = 0.745, $P < 0.01$), total N (r = 0.735, $P < 0.01$), and available N (r = 0.705, $P < 0.01$). The relative abundance of *Geminibasidium* was significantly negatively correlated with total C (r = -0.530, $P < 0.05$), total N (r = -0.516, $P < 0.05$), and available N (r = -0.569, $P < 0.05$). *Archaeorhizomyces* exhibited a positive correlation with C/N (r = 0.672, $P < 0.01$) and total P (r = 0.591, $P < 0.05$). *Cryptococcus* abundance showed a significantly negative correlation with total C (r = -0.655, $P < 0.01$), total N (r = -0.655, $P < 0.01$), and available N (r = -0.698, $P < 0.01$). While, *Simplicillium* (r = 0.540, $P < 0.05$) and *Bullera* (r = 0.596, $P < 0.05$) were significantly negatively correlated with pH.

Discussion

Soil characteristics of the different revegetation types

The soil nutrient concentrations (C, N and P) we observed varied significantly among the different revegetation types (Table 2). According to our findings, coniferous forests (LG, PK) and the conifer-broadleaf forest (CB) had reduced soil total C, total N, and available N value

compared with broadleaf forests (JM, QM), which was consistent with the study of Rahimabady et al. (2015). This influence may be attributed to different tree species with different litter quality and root exudates (Grayston & Prescott, 2005). In our study, soil total C, total N, and available N in LG were higher than those of PK. A previous study has indicated that LG is a cold temperate deciduous coniferous forest with a higher litter amount. Despite the numerous recalcitrant substances found in the litter from LG site, such as lignin, resin, tannin, and wax, the dense coniferous litter covering the soil surface impedes air circulation, and accelerates the accumulation of soil nutrients. On the contrary, PK is often a component of warm evergreen coniferous forests; it produces relatively little litter, and its nutrient content in the soil is relatively low (Yang & Han, 2001). Soil pH in this area ranged from 4.89 to 5.70, and compared to others, the soil under QM was most acidity, which might be associated with the litter quality. Compared to other broadleaf forests, the QM litter leaf quality is low, which has low nitrogen content, high C/N ratio, higher lignin content, and higher lignin/N (Gao et al., 2016). Therefore, these are several factors contributing to the effect that soil pH under QM was lowest. In fact, it can be said that different tree species result in different soil characteristics under the same climatic conditions because of differences in litter quality and quantity (Jahed & Hosseini, 2014), which was also observed in our study that the separation of the soils into four groups could depend on the fact that each one is mainly affected by certain soil characteristics (Fig. 2). Anyway, the forest types would seem to play an important role in regulating soil characteristics inside the same soil climate, and in particular, the difference between between broadleaf forest and coniferous forest should be noted.

Fungal community diversity and structure response to different revegetation types

We documented that different forest revegetation types had distinct soil fungal community diversity and composition (Table 3, Fig. 4, Fig. 5), as reported by Myers et al. (2001). In our study, we observed that the average fungal Shannon index, ACE index, Chao1 index and Simpson index were the highest in JM, followed by CB (Table 3), indicating that soil fungal

richness and evenness indices in JM were the highest. The ACE index and Chao1 index were the lowest in the LG while, the Shannon index and Simpson index were the lowest in the QM. This finding may be attributed to the differences in the chemical composition and decomposition rate of litter (Bray et al., 2012), which modify soil physical and chemical properties and, consequently, alter the soil fungal diversity indices. These findings verified that the soil fungal diversity indices were affected by tree species following revegetation.

The composition of fungal community in terms of phyla and genera did not significantly differ among the different revegetation pathways, although the relative abundance values varied, probably as a result of different root residues and secretions produced by different tree species (Degrune et al., 2015). Although our research is limited due to the low number of replicates (three for each kind of revegetation forest), significant differences in fungal communities among different revegetation types were observed. The results of our comparison of soil fungal communities among different revegetation types revealed that the predominant taxa of fungal communities were the phylum Basidiomycota in the QM, CB, LG, and PK sites, followed by Ascomycota, Zygomycota and Rozellomycota (Fig. 4), which was consistent with the results from Gutianshan National Nature Reserve (Yu et al., 2013), and from Mount Nadu, southwestern China (Liu et al., 2018). Similar studies have also found such results (Leff et al., 2015; Yu et al., 2013). Basidiomycota tended to live in dry and cooler environments (Treseder et al., 2014), and the relative abundance of Basidiomycete in soils might be related to their ability to degrade of lignocellulose (Lundell et al., 2010), which were affected by dynamics of soil organic matter (Hannula et al., 2012). On the contrary, we observed that the relative abundance of Ascomycota, over Basidiomycota, Zygomycota, and Rozellomycota, was predominant group in the JM, which was in agreement with previous research (Curlevski et al., 2010). Findings from other tropical regions indicated that in the broadleaf forests, Ascomycota was the most predominant phylum (Kerfahi et al., 2014; McGuire et al., 2014). In our study, the higher abundance of Ascomycota in JM suggested the enrichment of saprotrophic species, proving that Ascomycota tend to use the

easily degradable residues (Lundell et al., 2010), which might be related to organic matter input (Lundell et al., 2010). However, the finding from Yarraman showed that Zygomycota was the dominant phylum (He et al., 2010). These disparate results may indicate there is a lack of global common distribution and major fungal phyla in forest soils (Wu et al., 2013).

The dominant fungal genera (*Sebacina*, *Russula*, *Tomentella*) were representative of the dominant genera found in our study (Fig. 5), which was accordant with previous research (Welc et al. 2014). *Sebacina* was the most common genus in our study, and previous studies have put forward that *Sebacina* could help its host plant to overcome biotic and abiotic stresses by supplying it with water and nutrients (Gao & Yang, 2016). Based on previous research, *Tomentella* has been reported to be distributed throughout the world (Kõljalg et al., 2000), which was also the common genus in our study. The influence of different revegetation types on the soil fungal community is often related to the nature and quantity of organic matter returned by plant litter, which provides major resources for soil microorganisms (Saetre & Bååth, 2000; Wardle et al., 2004).

The results of clear differentiation in the heatmap (Fig. 7) and NMDS (Fig. 8) plots illustrated that significant differences in the fungal communities were observed in different revegetation types, suggesting that broad-leaved forests and coniferous forests each owned different fungal community. Our results were agreement with previous study which have established that the composition of the soil fungal community in natural forest differed from those in the hoop pine plantation (Lin et al., 2011). These findings confirmed that revegetation with different tree species altered the soil fungal community diversity and composition. The significant contribution of different forest types for shaping the soil fungal community has been established by previous findings (Sun et al., 2016).

Relationship between fungal communities and soil environmental factors

Soil environmental factors demonstrated remarkable relationships with fungal diversity. The Simpson index and Shannon index were positively correlated with pH (Table 4). Similar results

391 have been reported previously (Djukic et al., 2014; Liu et al., 2018; Wang et al., 2015) that the
392 diversity of the fungal community increased with soil pH value. In our study, soil Chao1 index,
393 ACE index, and Shannon index significantly increased with the increasing C/N ratios (Table 4).
394 In addition, ACE index and Chao1 index were significantly positively correlated with total P
395 (Table 4), which was in accordance with a previous study reporting that fungal diversity is
396 significantly affected by soil P-related factors (Liu et al., 2018).
397 Just as the soil fungal diversity, soil environmental factors had greatly influenced on the fungal
398 community composition. Previous studies have shown that soil physicochemical properties, such
399 as soil moisture (Brockett et al., 2012), soil pH (Rousk et al., 2010), available soil nutrients
400 (Lauber et al., 2008), soil total C (Yang et al., 2014), and C/N ratio (Christianl et al., 2008),
401 strongly affected fungal communities. Moreover, our study also confirmed that the abundances of
402 the most dominant fungal communities were significantly correlated with soil pH value. In
403 addition, total C, total N, available N, and available P were also closely linked to the fungal
404 community composition (Fig. 10, Table 5), which was consistent with other researches (Sun et al.,
405 2016; Zhang et al., 2017). Basidiomycota are generally sensitive to physic-chemical characteristic
406 disturbance (Osono, 2007). In our study, the relative abundances of Basidiomycota was
407 significantly negatively correlated with pH and available P, which was in contrast to the findings
408 of previous studies (Tedersoo et al., 2014; Tian et al., 2017). In a previous study, soil with higher
409 relative abundance of Ascomycetes has a higher pH value (Lauber et al., 2008). However, in our
410 study, Ascomycota was not correlated with soil pH value. A relatively small pH range (4.89 to
411 5.70) was observed in our study, which might be difficult to ascertain such a correlation.
412 Interestingly, the relative abundance of Ascomycota was positively correlated with total C, total
413 N and available N. In a recent study, Ascomycota abundance was associated with the content of
414 soil organic matter (Sterkenburg et al., 2015). In our research, the abundance of Zygomycota was
415 positively correlated with available P. This leads us to infer that soil available P was an important
416 regulator of fungal communities, which is consistent with the findings of Dang et al. (2017).

These results indicated that differential responses of soil fungal community composition to the different revegetation types largely dependent on soil physicochemical characteristics, highlighting the decisive role of soil physicochemical variables in altering fungal communities during vegetation restoration, which has also been stated previously (Kuramae *et al*., 2010)

Conclusions

Our results here showed that the different revegetation types would seem to play an important role in regulating soil characteristics in the same climate, especially between broadleaf forest and coniferous forest, which generated shifts in soil fungal community diversity and composition. Basidiomycota, Ascomycota, Zygomycota and Rozellomycota were the predominant fungal community in Baishilazi Nature Reserve, and the relative abundances of these abundant fungal phyla varied significantly among the different revegetation types. The average Shannon index, ACE index, Chao1 index and Simpson index were highest in JM. The abundances of the most dominant fungal communities correlated significantly with soil pH, total C, total N, available N, and available P.

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References

Bahram M, Põlme S, Kõljalg U, Zarre S, Tedersoo L. 2012. Regional and local patterns of ectomycorrhizal fungal diversity and community structure along an altitudinal gradient in the Hyrcanian forests of northern Iran. *New Phytologist* **193**(2): 465-473 DOI 10.1111/j.1469-8137.2011.03927.x.

Banerjee S. 2016. Network analysis reveals functional redundancy and keystone taxa amongst bacterial and fungal communities during organic matter decomposition in an arable soil. *Soil*

443 *Biology and Biochemistry* **97**:188-198 DOI org/10.1016/j.soilbio.2016.03.017.
444 Bao SD. 2000. Soil and Agricultural Chemistry Analysis. Beijing: China Agriculture Press.
445 Benayas JMR, Bullock JM. 2009. Enhancement of biodiversity and ecosystem services by
446 ecological restoration: a meta-analysis. *Science* **325**(5944): 1121-1124 DOI
447 10.1126/science.1172460
448 Braak CJFT, Smilauer P. 2002. CANOCO Reference Manual and CanoDraw for Windows User's
449 Guide: Software for Canonical Community Ordination (version 4.5). Ithaca Ny Usa Www.
450 Bray SR, Kitajima K, Mack MC. 2012. Temporal dynamics of microbial communities on
451 decomposing leaf litter of 10 plant species in relation to decomposition rate. *Soil Biology*
452 *and Biochemistry* **49**(1): 30-37 DOI 10.1016/j.soilbio.2012.02.009.
453 Brockett BFT, Prescott CE, Grayston SJ. 2012. Soil moisture is the major factor influencing
454 microbial community structure and enzyme activities across seven biogeoclimatic zones in
455 western Canada. *Soil Biology and Biochemistry* **44**(1): 9-20 DOI
456 org/10.1016/j.soilbio.2011.09.003.
457 Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña
458 AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE,
459 Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ,
460 Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows analysis
461 of highthroughput community sequencing data. *Nat Methods* **7**(5): 335-336 DOI
462 10.1038/nmeth.f.303.
463 Chao A, Bunge J. 2002. Estimating the number of species in a stochastic abundance model.
464 *Biometrics* **58**(3):531-539 DOI 10.1111/j.0006-341X.2002.00531.x.
465 Chen X, Sun XD, Bi SY, Lu GZ. 2010. Fungi diversity of ginseng rhizosphere soil in
466 northeastern China. *Agricultural Science and Technology-Hunan* **11**(2): 132-136.
467 Cheng F, Peng X, Zhao P, Zhong C, Cheng Y, Cui C, Zhang S. 2013. Soil Microbial Biomass,
468 Basal respiration and enzyme activity of main forest types in the Qinling Mountains. *Plos*

One **8**(6):e67353 DOI 10.1371/journal.pone.0067353.

Christianl L, Michaels S, Marka B, Fierer N. 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biology and Biochemistry*, **40**(9):2407-2415 DOI org/10.1016/j.soilbio.2008.05.021.

Collen B, Nicholson E. 2014. Conservation. Taking the measure of change. *Science* **346**(6206):166-167 DOI 10.1126/science.1255772.

Curlevski NJA, Xu ZH, Anderson IC, Cairney JWG. 2010. Soil fungal communities differ in native mixed forest and adjacent *Araucaria cunninghamii* plantations in subtropical Australia. *Journal of Soils and Sediments* **10**(7):1278-1288 DOI org/10.1007/s11368-010-0239-x.

Dang P, Yu X, Le H, Liu J, Shen Z, Zhao Z. 2017. Effects of stand age and soil properties on soil bacterial and fungal community composition in Chinese pine plantations on the Loess Plateau. *Plos One* **12**(10): e0186501 DOI 10.1371/journal.pone.0186501.

Degrune F, Dufrêne M, Colinet G, Massart S, Taminiau B, Bodson B, Hiel MP, Daube G, Carine N, Vandenbol M. 2015. A novel sub-phylum method discriminates better the impact of crop management on soil microbial community. *Agronomy for Sustainable Development* **35**(3):1157-1166 DOI 10.1007/s13593-015-0291-4.

Devi LS, Khaund P, Nongkhlaw FMW, Joshi SR. 2012. Diversity of culturable soil micro-fungi along altitudinal gradients of Eastern Himalayas. *Mycobiology* **40**(3): 151-158 DOI 10.5941/MYCO.2012.40.3.151.

Dickie IA, Xu B, Koide RT. 2002. Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T-RFLP analysis. *New Phytologist* **156**(3): 527-535 DOI 10.1046/j.1469-8137.2002.00535.x.

Dickie IA. 2007. Host preference, niches and fungal diversity. *New Phytologist* **174**(2): 230-233 DOI 10.1111/j.1469-8137.2007.02055.x.

Djukic I, Zehetner F, Mentler A, Gerzabek MH. 2010. Microbial community composition and

activity in different Alpine vegetation zones. *Soil Biology and Biochemistry* **42**(2): 155-161 DOI org/10.1016/j.soilbio.2009.10.006.

Dosskey MG, Bentrup G, Schoeneberger M. 2012. A role for agroforestry in forest restoration in the lower Mississippi Alluvial Valley. *Journal of Forestry* **110**(1): 48-55 DOI 10.5849/jof.10-061.

Edgar RC. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods* **10**(10): 996-998 DOI 10.1038/nmeth.2604.

Emteryd O. 1989. Chemical and physical analysis of inorganic nutrients in plant, soil, water and air. Swedish University of Agricultural Sciences. Department of Forest Site Research.

Fan A, Liu F. 2014. Seasonal Variations of Soil Microbial Biomass and Its Influence on Soil Microbial Respiration in Secondary Forest Communities in Montane Region of Eastern Liaoning Province. *Journal of Northeast Forestry University* (3):99-102 DOI 10.13759/j.cnki.dlxb.2014.03.023.

Fierer N, Jackson JA, Vilgalys R, Jackson RB. 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Applied and Environmental Microbiology* **71**(7):4117-4120 DOI 10.1128/AEM.71.7.4117-4120.2005.

Frey SD, Knorr M, Parrent JL, Simpson RT. 2004. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. *Forest Ecology and Management* **196**(1):159-171 DOI org/10.1016/j.foreco.2004.03.018.

Fulé PZ, Crouse JE, Roccaforte JP, Kalies EL. 2012. Do thinning and/or burning treatments in western USA ponderosa or Jeffrey pine-dominated forests help restore natural fire behavior? *Forest Ecology and Management*, **269**(269): 68-81 DOI 10.1016/j.foreco.2011.12.025.

Gao Q, Yang ZL. 2016. Diversity and distribution patterns of root-associated fungi on herbaceous plants in alpine meadows of southwestern China. *Mycologia* **108**(2):281-291.

Gao Y, Qiu GY, Shimizu H, Tobe K, Sun B, Wang J. 2002. A 10-Year study on techniques for

vegetation restoration in a desertified salt Lake Area. *Journal of Arid Environments* **52**(4): 483-497 DOI org/10.1006/jare.2002.1013.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular ecology* **2**(2): 113-118 DOI 10.1111/j.1365-294X.1993.tb00005.x.

Geml J, Pastor N, Fernandez L, Pacheco S, Semenova TA, Becerra AG, Wicaksono CY, Nouhra ER. 2014. Large-scale fungal diversity assessment in the Andean Yungas forests reveals strong community turnover among forest types along an altitudinal gradient. *Molecular ecology* **23**(10): 2452-2472 DOI 10.1111/mec.12765.

Georgiadis P, Vesterdal L, Stupak I, Raulund-Rasmussen K. 2017. Accumulation of soil organic carbon after cropland conversion to short-rotation willow and poplar. *GCB Bioenergy* **9**(8): 1390-1401 DOI 10.1111/gcbb.12416

Girvan MS, Bullimore J, Pretty JN, Osborn AM, Ball AS. 2003. Soil Type Is the Primary Determinant of the Composition of the Total and Active Bacterial Communities in Arable Soils. *Applied and Environmental Microbiology* **69**(3):1800-1809 DOI 10.1128/AEM.69.3.1800-1809.2003.

Grayston SJ, Prescott CE. 2005. Microbial communities in forest floors under four tree species in coastal British Columbia. *Soil Biology and Biochemistry* **37**(6):1157-1167 DOI 10.1016/j.soilbio.2004.11.014.

Guo Y, Chen X, Wu Y, Zhang L, Cheng J, Wei G, Lin Y. 2018. Natural revegetation of a semiarid habitat alters taxonomic and functional diversity of soil microbial communities. *Science of the Total Environment* **635**: 598-606 DOI 10.1016/j.scitotenv.

Hannula SE, Boschker HTS, Boer WD, Veen JAV. 2012. ^{13}C pulse-labeling assessment of the community structure of active fungi in the rhizosphere of a genetically starch-modified potato (Solanum tuberosum) cultivar and its parental isoline. *New Phytologist* **194**(3):784-799 DOI 10.1111/j.1469-8137.2012.04089.x.

547 Harris J. 2009. Soil microbial communities and restoration ecology: facilitators or followers?
548 *Science* **325**(5940): 573-574 DOI 10.1126/science.1172975.
549 Harrison KA, Bardgett RD. 2010. Influence of plant species and soil conditions on plant–soil
550 feedback in mixed grassland communities. *Journal of Ecology* **98**(2): 384-395 DOI
551 10.1111/j.1365-2745.2009.01614.x.
552 Hatakka A. 2010. Lignin-modifying enzymes from selected white-rot fungi: production and role
553 from in lignin degradation. *Fems Microbiology Reviews* **13**(2-3):125-135 DOI
554 10.1111/j.1574-6976.1994.tb00039.x.
555 He J, Xu Z, Hughes J. 2005. Analyses of soil fungal communities in adjacent natural forest and
556 hoop pine plantation ecosystems of subtropical Australia using molecular approaches based
557 on 18S rRNA genes. *Fems Microbiology Letters*, **247**(1): 91-100 DOI
558 10.1016/j.femsle.2005.04.033.
559 Herrera Paredes S, Lebeis SL. 2016. Giving back to the community: microbial mechanisms of
560 plant–soil interactions. *Functional Ecology* **30**(7):1043-1052 DOI
561 10.1111/j.1365-2435.12684.
562 Jahed RR, Hosseini SM. 2014. The effect of natural and planted forest stands on soil fertility in
563 the Hyrcanian region, Iran. *Biodiversitas Journal of Biological Diversity*, **15**(2): 206-214.
564 DOI 10.13057/biodiv/d150213.
565 Jangid K, Whitman WB, Condron LM, Turner BL, Williams MA. 2013. Soil bacterial community
566 succession during long-term ecosystem development. *Molecular Ecology* **22**(12):3415-3424
567 DOI 10.1111/mec.12325.
568 Jin Z, Li X, Wang Y, Wang K, Cui B. 2016. Comparing watershed black locust afforestation and
569 natural revegetation impacts on soil nitrogen on the Loess Plateau of China. *Scientific*
570 *Reports* **6**(25048):25048 DOI 10.1038/srep25048.
571 Kerfahi D, Tripathi BM, Lee J, Edwards DP, Adams JM. 2014. The Impact of Selective-Logging
572 and Forest Clearance for Oil Palm on Fungal Communities in Borneo. *Plos One*

573 **9**(11):e111525 DOI 10.1371/journal.pone.0111525.
574 Kernaghan G, Harper KA. 2001. Community structure of ectomycorrhizal fungi across an
575 alpine/subalpine ecotone. *Ecography* **24**(2): 181-188 DOI
576 10.1034/j.1600-0587.2001.240208.x.
577 Koljalg U, Afs T, Larsson EHN, Larsson E, Hallenberg N, Stenlid J, Larsson KH, Fransson PM,
578 Kårén O, Jonsson L. 2010. Diversity and abundance of resupinate thelephoroid fungi as
579 ectomycorrhizal symbionts in Swedish boreal forests. *Molecular Ecology* **9**(12):1985-1996
580 DOI org/10.1046/j.1365-294X.2000.01105.x.
581 Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AF, Bahram M, Bates ST, Bruns TD,
582 Bengtsson-Palme J, Callaghan TM, Douglas B, Drenkhan T, Eberhardt U, Dueñas M,
583 Grebenc T, Griffith GW, Hartmann M, Kirk PM, Kohout P, Larsson E, Lindahl BD, Lücking
584 R, Martín MP, Matheny PB, Nguyen NH, Niskanen T, Oja J, Peay KG, Peintner U, Peterson
585 M, Põldmaa K, Saag L, Saar I, Schüßler A, Scott JA, Senés C, Smith ME, Suija A, Taylor
586 DL, Telleria MT, Weiss M, Larsson KH. 2013. Towards a unified paradigm for
587 sequence-based identification of fungi. *Molecular Ecology* **22**(21):5271-5277 DOI
588 10.1111/mec.12481.
589 Kuramae EE, Gamper HA, Yergeau E, Piceno YM, Brodie EL, Desantis TZ, Andersen GL, van
590 Veen JA, Kowalchuk GA. 2010. Microbial secondary succession in a chronosequence of
591 chalk grasslands. *Isme Journal* **4**(5):711 DOI org/10.1038/ismej.2010.11.
592 Lauber CL, Strickland MS, Bradford MA, Fierer N. 2008. The influence of soil properties on the
593 structure of bacterial and fungal communities across land-use types. *Soil Biology and*
594 *Biochemistry* **40**(9):2407-2415 DOI org/10.1016/j.soilbio.2008.05.021.
595 Leff JW, Jones SE, Prober SM, Barberán A, Borer ET, Firn JL, Harpole WS, Hobbie SE,
596 Hofmockel KS, Knops JM, McCulley RL, La Pierre K, Risch AC, Seabloom EW, Schütz M,
597 Steenbock C, Stevens CJ, Fierer N. 2015. Consistent responses of soil microbial
598 communities to elevated nutrient inputs in grasslands across the globe. *Proceedings of the*

National Academy of Sciences of the United States of America **112**(35):10967-10972 DOI 10.1073/pnas.1508382112.

Liu D, Liu G, Li C, Wang J, Zhang L. 2018. Soil pH determines fungal diversity along an elevation gradient in Southwestern China. *Science China Life Sciences* (6):1-9 DOI 10.1007/s11427-017-9200-1.

Liu M, Liu J, Chen X, Jiang C, Wu M, Li ZP. 2018 Shifts in bacterial and fungal diversity in a paddy soil faced with phosphorus surplus. *Biology and Fertility of Soils* **54**(2):259-267 DOI 10.1007/s00374-017-1258-1.

Liu J, Diamond J. 2005. China's environment in a globalizing world. *Nature*, **435**(7046):1179-1186 DOI 10.1038/4351179a.

Lovett GM, Weathers KC, Arthur MA, Schultz JC. 2004. Nitrogen cycling in a northern hardwood forest: do species matter. *Biogeochemistry* **67**(3): 289-308 DOI 10.1023/B:BIOG.0000015786.65466.f5.

Lundell TK, Makela MK. 2010. Lignin-modifying enzymes in filamentous basidiomycetes--ecological, functional and phylogenetic review. *Journal of Basic Microbiology* **50**(1):5-20 DOI 10.1002/jobm.200900338.

Mcguire KL, D'Angelo H, Brearley FQ, Gedallovich SM, Babar N, Yang N, Gillikin CM, Gradoville R, Bateman C, Turner BL, Mansor P, Leff JW, Fierer N. 2015. Responses of soil fungi to logging and oil palm agriculture in Southeast Asian tropical forests. *Microbial Ecology* **69**(4):733-747 DOI 10.1007/s00248-014-0468-4.

Myers RT, Zak DR, White DC, Peacock A. 2001. Landscape-Level Patterns of Microbial Community Composition and Substrate Use in Upland Forest Ecosystems. *Soil Science Society of America Journal* **65**(2):359-367 DOI 10.2136/sssaj2001.652359x.

Ni Y, Yang T, Zhang K, Shen C, Chu H. 2018. Fungal Communities Along a Small-Scale Elevational Gradient in an Alpine Tundra Are Determined by Soil Carbon Nitrogen Ratios. *Frontiers in Microbiology* **9**: 01815 DOI org/10.3389/fmicb.2018.01815.

Nunezmir GC, Iannone BVI, Curtis K, Fei S. 2015. Evaluating the evolution of forest restoration research in a changing world: a "big literature" review. *New Forests* **46**(5-6): 669-682 DOI 10.1007/s11056-015-9503-7.

Osono T. 2007. Ecology of ligninolytic fungi associated with leaf litter decomposition. *Ecological Research* **22**(6):955-974 DOI 10.1007/s11284-007-0390-z.

Parkinson JA, Allen SE. 2009. A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. *Communications in Soil Science and Plant Analysis* **6**(1):1-11 DOI org/10.1080/00103627509366539.

Peter M, Ayer F, Egli S. 2001. Nitrogen addition in a Norway spruce stand altered macromycete sporocarp production and below-ground ectomycorrhizal species composition. *New Phytologist* **149**(2): 311-325 DOI 10.1046/j.1469-8137.2001.00030.x.

Qi JH. 2017. Contents of soil organic carbon and its relations with physicochemical properties of secondary natural oak forests in Eastern Mountain Area of Liaoning Province. *Journal of Soil and Water Conservation* **31**(4):135-140 DOI 10.13870/j.cnki.stbcxb.2017.04.022.

Rahimabady MS, Akbarinia M, Kooch Y. 2015. The effect of land covers on soil quality properties in the Hyrcanian regions of Iran. *Journal of Bioscience and Biotechnology* **4**(1) 73-79.

Ren C, Zhao F, Kang D, Yang G, Han X, Tong X, Feng Y, Ren GX. Linkages of C:N:P stoichiometry and bacterial community in soil following afforestation of former farmland. Forest Ecology and Management 2016, **376**:59-66 DOI 10.1016/j.foreco.2016.06.004.

Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, Knight R, Fierer N. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. *Isme Journal Multidisciplinary Journal of Microbial Ecology* **4**(10):1340-1351 DOI10.1038/ismej.2010.58.

Saetre P, Bååth E. 2000. Spatial variation and patterns of soil microbial community structure in a mixed spruce–birch stand. *Soil Biology and Biochemistry* **32**(7):909-917 DOI

10.1016/S0038-0717(99)00215-1.

Schrumpf M, Schulze ED, Kaiser K, Schumacher, J. 2011. How accurately can soil organic carbon stocks and stock changes be quantified by soil inventories? *Biogeosciences Discussions* **8**(1): 1193-1212 DOI org/10.5194/bg-8-1193-2011.

Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C. 2011. Metagenomic biomarker discovery and explanation. *Genome Biology* **12**(6):R60 DOI 10.1186/gb-2011-12-6-r60.

Sterkenburg E, Bahr A, Clemmensen KE, Clemmensen KE, Lindahl BD. 2015. Changes in fungal communities along a boreal forest soil fertility gradient. *New Phytologist* **207**(4):1145-1158 DOI 10.1111/nph.13426.

Sun H, Terhonen E, Kovalchuk A, Tuovila H, Chen H, Oghenekaro AO, Heinonsalo J, Kohler A, Kasanen R, Vasander H, Asiegbu FO. 2016. Dominant tree species and soil type affect fungal community structure in a boreal peatland forest. *Applied and Environmental Microbiology* **82**(9):2632 DOI 10.1128/AEM.03858-15.

Team RDC. 2009. R: a language and environment for statistical computing. **14**:12-21.

Tedersoo L, Bahram M, Põlme S, U Kõljalg, Yorou NS, Wijesundera R, Ruiz LV, Vasco-Palacios AM., Thu PQ, Suija A, Smith ME, Sharp C, Saluveer E, Saitta A, Rosas M, Riit T, Ratkowsky D, Pritsch K, Põldmaa K, Piepenbring M, Phosri C, Peterson M, Parts K, Pärtel K, Otsing E, Nouhra E, Njouonkou AL, Nilsson RH, Morgado LN, Mayor J, May TW, Majuakim L, Lodge DJ, Lee SS, Larsson KH, Kohout P, Hosaka K, Hiiesalu I, Henkel TW, Harend H, Guo LD, Greslebin A, Grelet G, Geml J, Gates G, Dunstan W, Dunk C, Drenkhan R, Dearnaley J, Kesel AD, Dang T, Chen X, Buegger F, Brearley FQ, Bonito G, Anslan S, Abell S, Abarenkov K. 2014. Global diversity and geography of soil fungi. *Science* **346**(6213):1-11 DOI 10.1126/science.1256688.

Tian Q, Taniguchi T, Shi WY, Li GQ, Yamanaka N, Du S. 2017. Land-use types and soil chemical properties influence soil microbial communities in the semiarid Loess Plateau region in

China. *Scientific Reports* **7**(7):45289 DOI 10.1038/srep45289.

Treseder KK, Maltz MR, Hawkins BA, Fierer N, Stajich JE, McGuire KL. 2014. Evolutionary histories of soil fungi are reflected in their large-scale biogeography. *Ecology Letters* **17**(9):1086-93 DOI 10.1111/ele.12311.

Van DHMGA, Bardgett RD, Van Straalen NM. 2010. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* **11**(3):296-310 DOI 10.1111/j.1461-0248.2007.01139.x.

Waldrop MP, Zak DR, Blackwood CB, Curtis CD, Tilman D. 2006. Resource availability controls fungal diversity across a plant diversity gradient. *Ecology Letters* **9**(10): 1127-1135 DOI 10.1111/j.1461-0248.2006.00965.x.

Wallander H, Johansson U, Sterkenburg E, Brandstrcem DM, Lindahl BD. 2010. Production of ectomycorrhizal mycelium peaks during canopy closure in Norway spruce forests. *New Phytologist* **187**(4): 1124-1134 DOI 10.1111/j.1469-8137.2010.03324.x.

Wang JT, Zheng YM, Hu HW, Zhang LM, Li J, He JZ. 2015. Soil pH determines the alpha diversity but not beta diversity of soil fungal community along altitude in a typical Tibetan forest ecosystem. *Journal of Soils and Sediments* **15**(5):1224-1232 DOI 10.1007/s11368-015-1070-1.

Wardle DA; Bardgett RD; Klironomos JN, Setälä H, van der Putten WH, Wall DH. 2004. Ecological linkages between aboveground and belowground biota. *Science* **304**(5677):1629-1633 DOI 10.1126/science.1094875.

Weand MP, Arthur MA, Lovett GM, Sikora F, Weathers KC. 2010. The phosphorus status of northern hardwoods differs by species but is unaffected by nitrogen fertilization. *Biogeochemistry* **97**(2-3): 159-181 DOI 10.1007/s10533-009-9364-2.

Welc M, Frossard E, Egli S, Bünemann EK, Jansa J. 2014. Rhizosphere fungal assemblages and soil enzymatic activities in a 110-years alpine chronosequence. *Soil Biology and Biochemistry* **74**:21-30 DOI org/10.1016/j.soilbio.2014.02.014.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications **18**(1): 315-322.

Wu G, Liu Y, Fang N, Deng L, Shi Z. 2016. Soil physical properties response to grassland conversion from cropland on the semia arid area. *Ecohydrology* **9**(8):1471-1479 DOI 10.1002/eco.1740.

Wu YT, Wubet T, Trogisch S, Both S, Scholten T, Bruelheide H, Buscot F. 2013. Forest age and plant species composition determine the soil fungal community composition in a Chinese subtropical forest. *Plos One* **8**(6): e66829 DOI 10.1371/journal.pone.0066829.

Xiao X, Wei X, Liu Y, Ouyang X, Li Q, Ning J. 2015. Aerial Seeding: An Effective Forest Restoration Method in Highly Degraded Forest Landscapes of Sub-Tropic Regions. *Forests* **6**(6):1748-1762 DOI 10.3390/f6061748.

Yan DF, Mills JG, Gellie NJC, Bissett A, Lowe AJ, Breed MF. 2018. High-throughput eDNA monitoring of fungi to track functional recovery in ecological restoration. *Biological Conservation*, **217**:113-120 DOI 10.1016/j.biocon.2017.10.035.

Yang XQ, Han YZ. 2001. Spatial variations of soil organic carbon and nitrogen of forest land in Guandi Mountain. *Forest research* **24** (2) 223-229.

Yang W, Guo Y, Wang X, Pelletier F. 2017. Temporal variations of soil microbial community under compost addition in black soil of Northeast China. *Applied Soil Ecology* **121**:214-222 DOI org/10.1016/j.apsoil.2017.10.005.

Yang Y, Gao Y, Wang S, Xu D, Yu H, Wu L, Lin Q, Hu Y, Li X, He Z, Deng Y, Zhou J. 2014. The microbial gene diversity along an elevation gradient of the Tibetan grassland. *Isme Journal* **8**(2):430-440 DOI 10.1038/ismej.2013.146.

You Y, Wang J, Huang X, Tang Z, Liu S, Sun OJ. 2014. Relating microbial community structure to functioning in forest soil organic carbon transformation and turnover. *Ecology and Evolution* **4**(5):633-647 DOI 10.1002/ece3.969.

729 Yu TW, Wubet T, Trogisch S, Both S, Scholten T, Bruelheide H, Buscot F. 2013. Forest Age and
730 Plant Species Composition Determine the Soil Fungal Community Composition in a
731 Chinese Subtropical Forest. *Plos One* **8**(6):e66829 DOI 10.1371/journal.pone.0066829.
732 Zaura E, Keijser BJF, Huse SM, Crielaard W. 2009. Defining the healthy" core microbiome" of
733 oral microbial communities. *BMC microbiology* **9**(1): 259 DOI 10.1186/1471-2180-9-259.
734 Zhang Q, Jia X, Zhao C, Shao M. 2018. Revegetation with artificial plants improves topsoil
735 hydrological properties but intensifies deep-soil drying in northern Loess Plateau, China.
736 *Journal of Arid Land* (3):1-12 DOI org/10.1007/s40333-018-0007-0.
737 Zhang Z, Zhou X, Tian L, Ma L, Luo S, Zhang J, Li X, Tian C. 2017. Fungal communities in
738 ancient peatlands developed from different periods in the Sanjiang Plain, China. *Plos One*
739 **12**(12):e0187575 DOI 10.1371/journal.pone.0187575.
740 Zhong Y, Yan W, Shangguan Z. 2015. Impact of long-term N additions upon coupling between
741 soil microbial community structure and activity, and nutrient-use efficiencies. *Soil Biology*
742 *and Biochemistry*, **91**:151-159 DOI 10.1016/j.soilbio.2015.08.030.
743 Zhu W, Cai X, Liu X, Wang JX, Cheng S, Zhang XY, Li DY, Li MH. 2010. Soil microbial
744 population dynamics along a chronosequence of moist evergreen broad-leaved forest
745 succession in southwestern China. *Journal of Mountain Science* **7**(4): 327-338 DOI
746 10.1007/s11629-010-1098-z.