Different revegetation types alter soil physical-chemical characteristics and fungal community in the Baishilazi Nature Reserve (#30722)

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

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The effects of different revegetation types on soil physical-chemical characteristics and fungal community have been established in the Baishilazi Nature Reserve. We studied the 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. Soil fungal communities were assessed employing ITS rRNA Illunima Miseq high-throughput sequencing. Response of soil fungi community to soil environmental factors was assessed through canonical correspondence analysis (CCA) and Person's rank correlations. Our results suggested that coniferous forest (LG, PK) and conifer-broad forest (CB) had reduced soil total C, total N, and Available N compared with broad-leaved forest (JM, QM). The average fungus diversity according to Shannon index, ACE index, Chao1 index and Simpson index were increased in the JM. Basidiomycota, Ascomycota, Zygomycota and Rozellomycota were the predominant fungal community in this region. The most predominant member of fungal communities was the phylum Basidiomycota in the QM, CB, LG, and PK. On the contrary, the relative abundances of Ascomycota was most predominant group in the JM. The clear differentiation of fungal communities and the clustering in the heatmap and in NMDS plots showed that broad-leaved forests, conifer-broad forest and coniferous forests each owned different fungal community. The results of canonical correspondence analysis (CCA) shown that the soil environmental factors, such as soil pH, total C, total N, available N and available P had greatly influenced on the fungal community structure. Our results suggested that differential responses of soil fungal community composition to the different revegetation types largely dependent on soil physicochemical characteristic in Baishilazi Nature Reserve.

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Abstract

The effects of different revegetation types on soil physical-chemical characteristics and fungal community have been established in the Baishilazi Nature Reserve. We studied the 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. Soil fungal communities were assessed employing ITS rRNA Illunima Miseq high-throughput sequencing. Response of soil fungi community to soil environmental factors was assessed through canonical correspondence analysis (CCA) and Person's rank correlations. Our results suggested that coniferous forest (LG, PK) and conifer-broad forest (CB) had reduced soil total C, total N, and Available N compared with broad-leaved forest (JM, QM). The average fungus diversity according to Shannon index, ACE index, Chao1 index and Simpson index were increased in the JM. Basidiomycota, Ascomycota, Zygomycota and Rozellomycota were the predominant fungal community in this region. The most predominant member of fungal communities was the phylum Basidiomycota in the QM, CB, LG, and PK. On the contrary, the relative abundances of Ascomycota was most predominant group in the JM. The clear differentiation of fungal communities and the clustering in the heatmap and in NMDS plots showed that broad-leaved forests, conifer-broad forest and coniferous forests each owned different fungal community. The results of canonical correspondence analysis (CCA) shown that the soil environmental factors, such as soil pH, total C, total N, available N and available P had greatly influenced on the fungal community structure. Our results suggested that differential responses of soil fungal community composition to the different revegetation types largely dependent on soil physicochemical characteristic in Baishilazi Nature Reserve.

Key words: Different revegetation types, Soil physical-chemical characteristics, Fungal community, The Baishilazi Nature Reserve

Introduction

Due to long-term human disturbance and intensive land use, native vegetation temperate zone in

China have been severely damaged, resulting in the reduction of biodiversity and the deterioration of ecological functions, threating the safety and sustainable development of regional ecolo Thus it can be seen that vegetation restoration is an important measure of ecological environment restoration due to its multifarious ecological benefits umerous researches have illustrated that revegetation had an effect on soil physicochemical characteristics, such as soil bulk density, field capacity (Zhang et al., 2018), infiltration rate (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 microbial communities (Herrera Paredes et al., 2016). Soil microbial community, which is a key bridge to connect the advantage plant community aboveground with ecological process underground, is one of the most important regulator of soil nutrient transformation (Cheng et al., 2013). Soil microorganism can not only directly affect the storage of soil nutrients by its own biomass, but also can indirectly effect on soil nutrient transformation through the metabolic activity (Jangid et al., 2013; You et al., 2014). However, fewer researches have followed changes in soil microbial community dynamics, despite the important role of microorganisms in biogeochemical cycling (Guo et al., 2018).

Fungal community and diversity have important influence 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 cycle in continental ecosystem (Tedersoo et al., 2014). Fungal diversity and community composition have been indicated to be closely related to numerous abiotic and biotic factors, such as elevation (Kernaghan & Harper, 2010; Bahram et al., 2012), soil environment (Peter et al., 2001; Dickie et al., 2002), plant species (Lovett et al., 2004; Weand et al., 2010), plant diversity (Dickie. 2007; Waldrop et al., 2006), and stand age (Zhu et al., 2010; Wallander et al., 2010). An increasing number of work have shown that a number of soil properties including soil pH (Fierer & Jackson, 2006), soil texture (Girvan et al., 2003), and soil nitrogen availability (Frey et al., 2004) can be associated with changes fungal communities structure. Unfortunately, few researchers have addressed the connection between the Different revegetation types and the fungi community

structure in natural secondary forest and plantation forest. Directly, the relationship between the soil nutrient and the fungi community is not explicit, however, previous work has concentrated on the effect of grassland and leguminous species (Harrison & Bardgett, 2010). But there remains a need for interpreting which of these factors was the dominant influence on the soil fungal communities in different revegetation types.

As the national nature reserves, the Baishilazi Nature Reserve is located in the Montainous Region of the Eastern Liaoning Province, China. The Baishilazi Nature Reserve was established in 1988, which belongs to the Changbai Mountain system. The original vegetation was broad-leaved *Pinus koraiensis* forests, which was severely damaged due to the over-exploitation of the past 100 years. At present, vegetation mainly consist of natural secondary forests and conifer plantations, which provides the unique opportunity to investigate the soil fungal community among different revegetation ecosystems under the same climatic conditions. Luxuriant researches have investigated the changes of soil microbial biomass (Fan et al., 2014), soil organic carbon contents (Qi et al., 2017) in different revegetation types, however it is acquainted scarcely about the how the different revegetated forests determined the soils fungal community diversity and structure in this area. For that matter, we applied pyrosequencing of the ITS rRNA gene to explore both diversity and composition of soil fungal community responses to different revegetation types from five sites in the Baishilazi Nature Reserve in Liaoning Province, China. Our objective was to use five different revegetation types to examine how soil fungi may respond to different revegetation types and, more specifically, how shifts in the abundance and composition of soil fungal communities response to changes in soil 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° 50′ 00″ ~40° 57′ 12″ N, 124° 44′ 07″ ~124° 57′ 30″ E). It is a comprehensive nature reserve with forest ecosystem as the main protection object. The total area of the Baishilazi Nature Reserve is 7407hm^2, which

belongs to the mountain range of Changbai Mountain. This area is characterized by continental monsoon climate, with long cold winters, warm wet summers, and higher diurnal temperature variation. The annual mean amount of evaporation is 885 mm. The annual average temperature is 6.4℃, and the average annual precipitation amount is 1158 mm. The region has a relatively rich and unique biodiversity, possessing significant ecological status and scientific value both in China and the world. The characteristics of the five selected study samples are listed in Table 1.

Soil sampling

In July, 2017, we sampled from three plots per forest type after removal of the litter layer, including *Juglans mandshurica* (JM), *Quercus mongolica* (QM), conifer-broadleaf forest (CB), *Larix gmelinii* (LG), and *Pinus koraiensis* (PK). Soil samples were collected with use of a soil auger (8 cm in diameter, 10 cm deep) from a 20 m×20 m plot with each forest type replicate. A strip sampling method was used to ensure the representativeness of soil samples in each forest. The soils of 15-20 points were mixed together and placed in sterilized ziplock bags as a replicate sample. Identifically, in each vegetation form, three subsamples were collected. Immediately arrival to the laboratory the sample in cooled boxes were sieved (2 mm mesh) to undock roots and dopant, and divided into two sub-samples, one of which was air-dried and used for physical and chemical analyses, and the other was stored at -80 ℃ until DNA extraction and used for microbial analyses.

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 set: ITS1F (50-CTTGGTCATTTAGAGGAAGTAA-30) (Gardes &Bruns, 1993) and ITS2 (50-GCTGCGTTCTTCATCGATGC-30) (White et al., 1990) was selected to target the fungal ITS1 region. Sample-specific 7-bp barcodes were incorporated into the primers for multiplex

sequencing. PCR amplification were requiresd 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 (5U/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. 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.

Data Analysis

Operational taxonomic units (OTU)-level alpha diversity indices, such as Chao1 index, ACE index, Shannon index, and Simpson index, were computed using the OTU table in QIIME. The shared and unique OTUs among samples were used to venn diagrams using the R software package. The heatmap representation of the relative abundance of fungal OTUs among samples was built using R. NMDS analysis was also conducted based on the genus-level compositional profiles.

One-way analysis of variance (ANOVA) was conducted using SPSS 19.0 software. Soil physicochemical characteristics, fungal total abundances, alpha diversity indices, and the taxa (phyla and genus) different forest soils were compared using LSD tests. Pearson correlation analysis was used to evaluate the correlations between soil fungal community diversity and structure and soil characteristics. Canonical correspondence analysis (CCA) which was performed via Canoco 4.5, was used to evaluate the linkages between dominant fungal groups related to soil environmental factors.

Results

Soil physicochemical properties

As seen in Table 2, the soil pH value ranged from 4.89 to 5.70. Soil pH value under QM was the

most acidic with 4.89, compared to others, followed by CB, and JM contained the highest soil pH value. There were significant difference among different forest types regarding soil total C and total N. Interestingly, both total C and total N exhibited highest in the soil of JM, which was 100.53 g/kg and 7800.00mg/kg, but only 31.76g/kg and 2476.67 mg/kg in the PK, respectively. Soil C/N in all the treatments were less than 25:1, among which CB had the highest C/N. Available N was found in ranked order of JM> QM >CB >LG > PK (Table 2). There were also significant differences in available P and total P among different forest types, with the highest values under PK and CB, respectively (Table 2).

Fungal community diversity responses to different revegetation types

Fungal a-diversity varied greatly across our samples. The Shannon index, ACE index, Chao1 index and Simpson index were the highest in the JM, followed by the CB (Table 3). Person's correlation coefficients indicated that the Simpson indices (r=0.680, $P<0.01$) and Shannon index (r=0.659, $P<0.01$) were positively correlated with pH, and Shannon index was positively correlated with C/N (r=0.528, $P<0.05$). In addition, ACE index and Chao1 index were significantly positively correlated with total C ($P<0.05$), C/N and total P ($P<0.01$) (Table 4).

Fungal community structure responses to different revegetation types

A total of 640,914 high quality ITS sequences were obtained after the elimination of chimeras and sequence of low quality, with an average of 42,727 sequences being acquired in each soil sample. At the phylum, we found 8875 fungal operational taxonomic units (OTUs) after quality filtering. On average, 592 OTUs were found in each sample. A maximum of 743 OTUs were detected in the CB, however, only 455 OTUs were obtained in the LG (Table 3). In order to determine rarefaction curves, richness, and diversity, 1,000 reads were randomly selected from each sample. At the 3% dissimilarity level (Fig. 1), the curve tended to flatten with the number of measured sequences increases, indicating that the experiment had obtained most of the sample information and had been able to reflect the fungal community composition of the forest soil.

The obtained sequences were affiliated with 15 phyla (including unknown). The dominant phyla accounted for more than 1% of the overall communities were Basidiomycota, Ascomycota,

Zygomycota and Rozellomycota, with relative abundances 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. 2). Phyla included Cercozoa, Chytridiomycota, Glomeromycota, Ciliophora, which were less abundant (<1 % of all classified sequences) still were found in all of the soils examined. The relative abundance of these most abundant fungal phyla varied significantly among different forest types. The relative abundances of Ascomycota was significantly higher in JM than others, while the relative abundances of Basidiomycota was the lowest. The relative abundances of Basidiomycota was the highest in the CB, while the relative abundances of Ascomycota and Rozellomycota were lowest (Fig. 2).

At the genus level, the dominant genus accounted 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 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. 3). *Sebacina* was the most abundant genus at PK of 21.17%. The relative abundances of *Russula* showed highest in the CB than others (Fig. 3).

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 five forest types (Fig. 4). 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 number of shared OTUs were 514 (JM vs. QM), 400 (LG vs. PK), 384 (JM vs. PK), 314 (JM vs. LG), 343 (QM vs. PK), 329 (QM vs. LG) (Fig. 4).

To show the fungal community structures of JM, QM, CB, LG, and PK, the heatmap analysis based on the top 50 most abundant fungal community using R software was used to intuitively display the differences in relative abundances of fungal OTUs among samples (Fig. 5), which can reflect the compositions and relative abundance of soil fungus differences under different forest

types were quite different. *Tomentella*, *Piloderma*, *Suillus*, *Oidiodendron*, *Inocybe*, *Entoloma*, *Cortinarius*, *Helvellosebacina*, and *Phaeoacremonium* dominated in LG. *Russula*, *Trichoderma*, *Leucoagaricus*, *Amphinema*, *Umbelopsis*, and *Thelephora* dominated in CB. Cladophialophora, Byssocorticium, Trichoderma, Hygrocybe, Exophiala, Leotia, and Knufia dominated in QM. Correspondingly, NMDS based Unifrac distance was carried out to show significant separation among treatments (Fig. 6). With both analyses indicating that different revegetation types had a great effect on fungal community.

The LEfSe analysis was documented to determine the classified fungal taxa with significant abundance differences among the different sampling sites. As presented in Fig.7, 63 fungal taxa were showed significantly different with LDA effect size scores were > 4 (Fig. 7A), and 10 fungal taxa were showed significantly different with LDA effect size scores were > 5 (Fig. 7B). At the phylum level, the biomarkers were affiliated with Basidiomycota, and Ascomycota, respectively.

Fungal community distribution relate to the soil properties

Canonical correspondence analysis (CCA) was used to analyze the relative abundance of dominant fungal phyla constrained by soil properties variables (Fig. 8). The results showed that the cumulative interpretation variations of the first and second axes were 93.5%, indicating that soil environmental factors had greatly influenced the fungal community structure. At the phylum level (Fig. 8), 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%. C/N (r=-0.7322) and total P (r=-0.8094) were significantly related with axis2.

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

243 P<0.01) (Table 5).

244 At the genus level, the abundance of *Sebacina* was significantly negatively correlated with total
245 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
246 significantly positively correlated with C/N (r=0.637, P<0.05) and total P (r=0.751, P<0.01).
247 *Humicola* was significantly positively with total C (r=0.745, P<0.01), total N (r=0.735, P<0.01)
248 available N(r=0.705, P<0.01). The relative abundance of *Geminibasidium* was significantly
249 negatively correlated with total C (r=-0.530, P<0.05), total N (r=-0.516, P<0.05) and available
250 N(r=-0.569, P<0.05). *Archaeorhizomyces* was exhibited a positive correlation with C/N (r=0.672,
251 P<0.01) and total P (r=0.591, P<0.05). *Cryptococcus* abundance was existed a significantly
252 negative correlation with total C (r=-0.655, P<0.01), total N (r=-0.655, P<0.01) available N(r=-
253 0.698, P<0.01). *Simplicillium* (r=0.540, P<0.05) and *Bullera* (r=0.596, P<0.05) were significantly
254 negatively correlated with pH.

255 **Discussion**

256 **Distinct soil characteristics among the different revegetation types**

257 The soil physicochemical conditions we observed nutrient concentrations (C, N and P) varied
258 significantly among different revegetation types (Table 2). According to our findings, coniferous
259 forest (LG, PK) and confer-broad forest (CB) had reduced soil total C, total N, and Available N
260 compared with broad-leaved forest (JM, QM), which was consistent with study of Rahimabady et
261 al. (2015). This influence may be attributed to different tree species which have differences in litter
262 quality, and root exudates (Grayston & Prescott, 2005). Compared to others, the soil under QM
263 was more alkaline can be related with the quality of litter. Compared to other broadleaf forests, the
264 QM litter leaf quality is low, which has low nitrogen content, high C/N ratio, higher lignin content,
265 and higher lignin/N. Therefore, the decomposition rate of QM litter and the release rate of plant
266 nutrients are gradually slowed down. These are several reasons contributing to the effect that soil
267 under QM was lowest.

268 **Fungal community diversity and structure response to different revegetation types**

269 We documented that different forest revegetation types had distinct soil fungal community

diversity and composition (Table 3, Fig. 2, Fig. 3), as reported by Myers et al. (2001). 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). The composition of fungi phylum and in different revegetation types were similar to each other, but the relative abundance of fungi phylum was various, which may be related to different root residues and secretions produced by different revegetation types (Degrune et al., 2015). The results of our comparison of soil fungal communities among different revegetation types revealed that the predominant members of fungal communities were the phylum Basidiomycota in the QM, CB, LG, and PK, followed by Ascomycota, Zygomycota and Rozellomycota (Fig. 2), which was consistent with the results from Gutianshan National Nature Reserve (Yu et al., 2013) and Mount Nadu (Liu et al., 2018). And other previous studies have also supported the conclusion (Leff et al., 2015; Yu et al., 2013). Basidiomycota tended to live in dry and cooler environments due to their history of evolution (Treseder et al., 2014). The relative abundance of Basidiomycete in soils might be related to their ability of degradation of lignocellulose (Lundell et al., 2010), which were affected by dynamics of soil organic matter (Hannula et al., 2012).

On the contrary, in our study, we observed that the relative abundances of Ascomycota, over Basidiomycota, Zygomycota, and Rozellomycota, was predominant group in the JM, which was similar to the previous research (Curlevski et al., 2010). Moreover, these results proved that the higher abundance of Ascomycota suggest the enrichment of saprotrophic species, which might be related to organic matter input, given that Ascomycota tend to use the easily degradable residues (Lundell et al., 2010). However, the finding from Yarraman natural forest and the hoop pine plantation 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. Our finding indicated that in the broadleaf forests (QM, JM), Ascomycota were the most predominant phylum, which was similar to the findings from other tropical regions (Kerfahi et al., 2014; McGuire et al., 2014).

The dominant fungal genera (*Sebacina*, *Russula*, *Tomentella*) were representative of dominant

297 genera found in our study (Fig.3), which was accordant with previous researches (Welc et al.
298 2014). *Sebacina* was the most common genus in our study, and previous studies have put forward
299 that *Sebacina* could help their host plant overcome biotic and abiotic stresses by supplying it with
300 water and nutrients (Gao & Yang, 2016). *Tomentella* has been reported to be distributed
301 throughout the world (Kõljalg et al., 2000), which was also the common genus in our study. The
302 influence of different revegetation types on soil fungal community is often related to the nature
303 and quantity of organic matter returned by plant litter and provides major resources for soil
304 microorganisms (Saetre & Bååth, 2000; Wardle et al., 2004).

305 The clear differentiation of fungal communities and the clustering in the heatmap (Fig. 5) and
306 in NMDS (Fig. 6) plots suggested that broad-leaved forests and coniferous forests each owned
307 different fungal community.

308 **Relationship between fungal communities and soil environmental factors**

309 Soil environmental factors variables demonstrated remarkable relationship with fungal diversity.
310 The Simpson index and Shannon index were positively correlated with pH (Table 4). Similar
311 results have been reported (Djukic et al., 2014; Liu et al., 2018; Wang et al., 2015) that the
312 diversity of the fungal community increased with soil pH value. In our study, soil Chao1 index,
313 ACE index, and Shannon index significantly increased with the increasing C/N ratio (Table 4),
314 which was not agreement with previous study. In addition, ACE index and Chao1 index were
315 significantly positively correlated with total P (Table 4), which was accordance with previous work
316 reported that fungal diversity was significantly affected by soil P-related factors (Liu et al., 2018).

317 Just as the soil fungal diversity, soil environmental factors had greatly influenced on the fungal
318 community structure. Previous studies have shown that soil physicochemical properties, such as
319 soil moisture (Brockett et al., 2012), soil pH (Rousk et al., 2010), available soil nutrients (Lauber
320 et al., 2008), soil total C (Yang et al., 2014), and C/N ratio (Christianl et al., 2008) more strongly
321 affected fungal communities. Moreover, our study also confirmed that the abundances of the most
322 dominant fungal communities correlated significantly with soil pH value. What's more, total C,
323 total N, available N and available P were also closely linked to the fungal community structure

(Fig. 8, Table 5), which was consistent with other researches (Sun et al., 2016; Zhang et al., 2017). Basidiomycota are generally sensitive to physic-chemical characteristic disturbance (Osono, 2007). In our study, the relative abundances of Basidiomycota was significantly negatively correlated with pH and available P, which was not agreement with previous studies (Tedersoo et al., 2014; Tian et al., 2017). Soil with higher relative abundance of Ascomycetes has higher pH values (Lauber et al., 2008). However, in our study, Ascomycota was not correlated with soil pH value. A relatively small pH rang (4.89 to 5.70) existed in our study, which might be difficult to ascertain the correlation. Interestingly, the relative abundance of Ascomycota was positively correlated with total C, total N and available N. It is supported by recent research that showed that Ascomycota were associated with the content of soil organic matter (Sterkenburg et al., 2015).The abundance of Zygomycota was positively correlated with available P in our study. Our results ulteriorly proved that soil available P was considered an important regulator of fungal communities in the soil, consistent with 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 characteristic. And the decisive role of soil physicochemical variables in altering fungal communities during vegetation restoration, in consistent with previous studies (Kuramae *et al*., 2010)

Conclusions

Our results here showed that different revegetation types were the main driver of the soil fungal diversity and community composition, which generated shifts in soil chemical characteristics, controlling the composition of fungal community in Baishilazi Nature Reserve. Basidiomycota, Ascomycota, Zygomycota and Rozellomycota were the predominant fungal community in this region, and the relative abundance of these abundant fungal phyla varied significantly among different revegetation types. The average Shannon index, ACE index, Chao1 index and Simpson index were predicated for JM. The abundances of the most dominant fungal communities correlated significantly with soil pH, total C, total N, available N and available P.

Acknowledgment

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Figure 1

Rarefaction curves.

JM: *Juglans mandshurica*; QM: *Quercus mongolica*; CB: Conifer-broadleaf forest; LG: *Larix gmelinii*; PK: *Pinus koraiensis*.

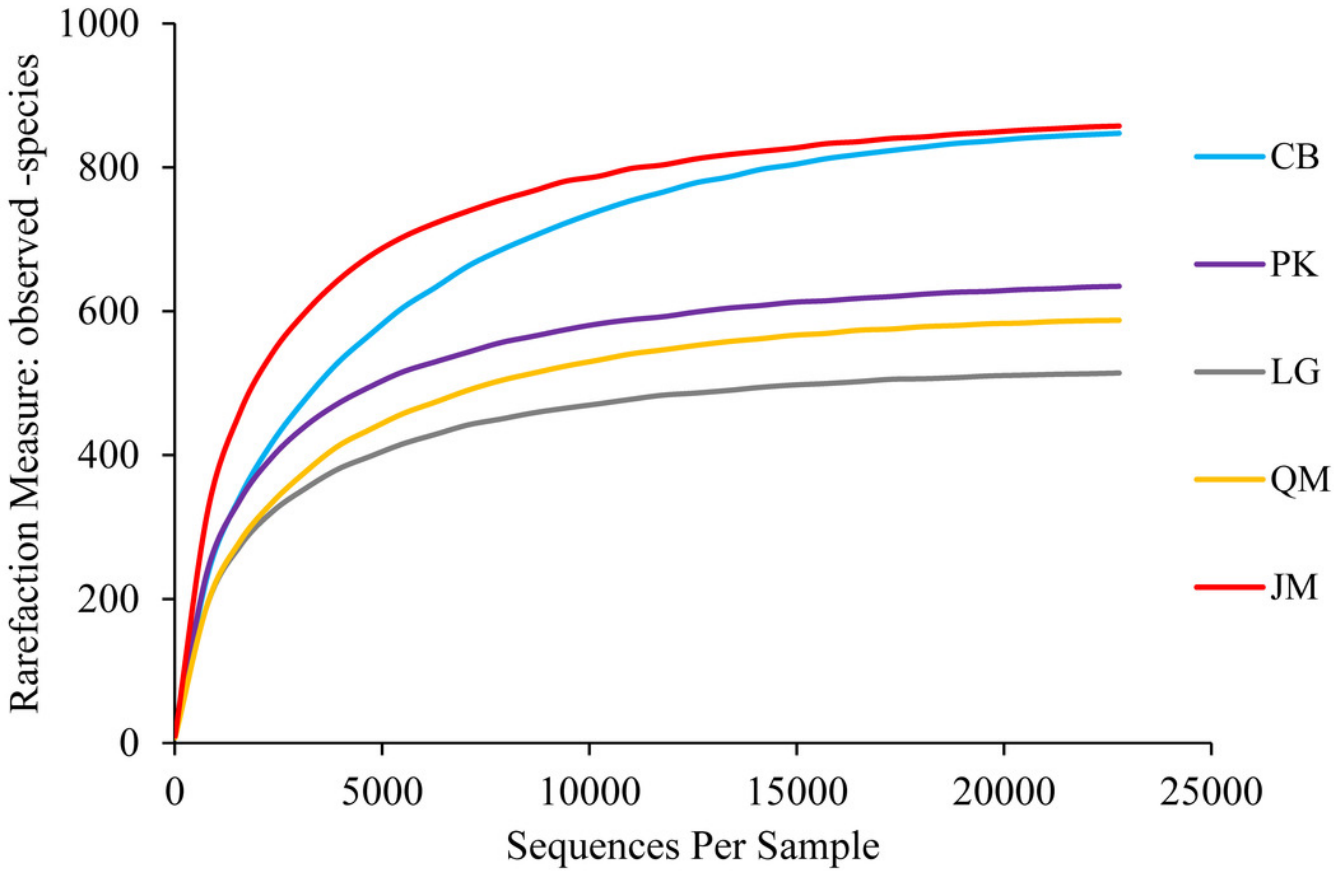


Figure 2

Relative abundance of fungus phyla present in five different revegetation types.

JM: *Juglans mandshurica*; QM: *Quercus mongolica*; CB: Conifer-broadleaf forest; LG: *Larix gmelinii*; PK: *Pinus koraiensis*.

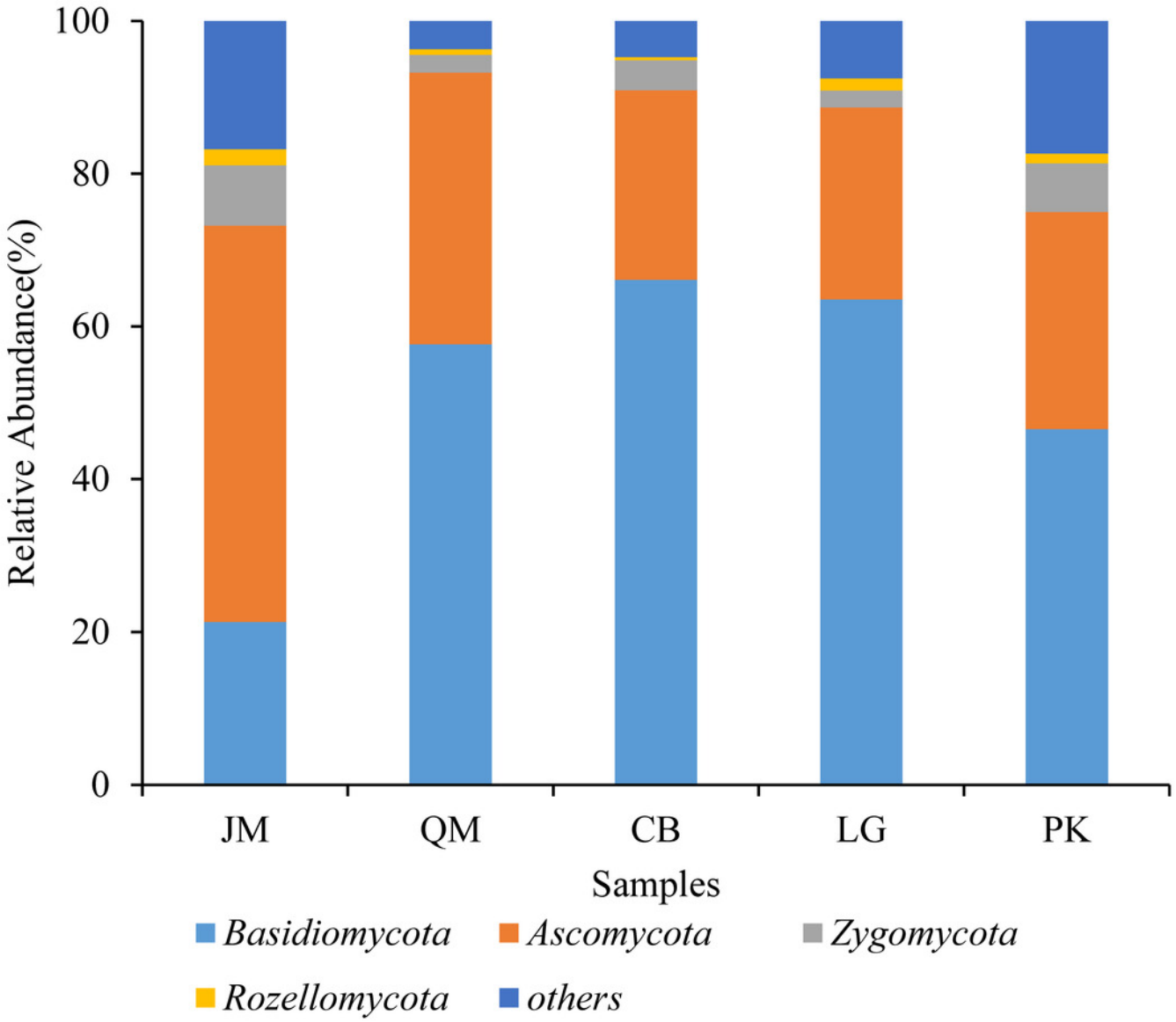


Figure 3

The distribution of partial sequences of fungal ITS gene at genus level.

JM: *Juglans mandshurica*; QM: *Quercus mongolica*; CB: Conifer-broadleaf forest; LG: *Larix gmelinii*; PK: *Pinus koraiensis*.

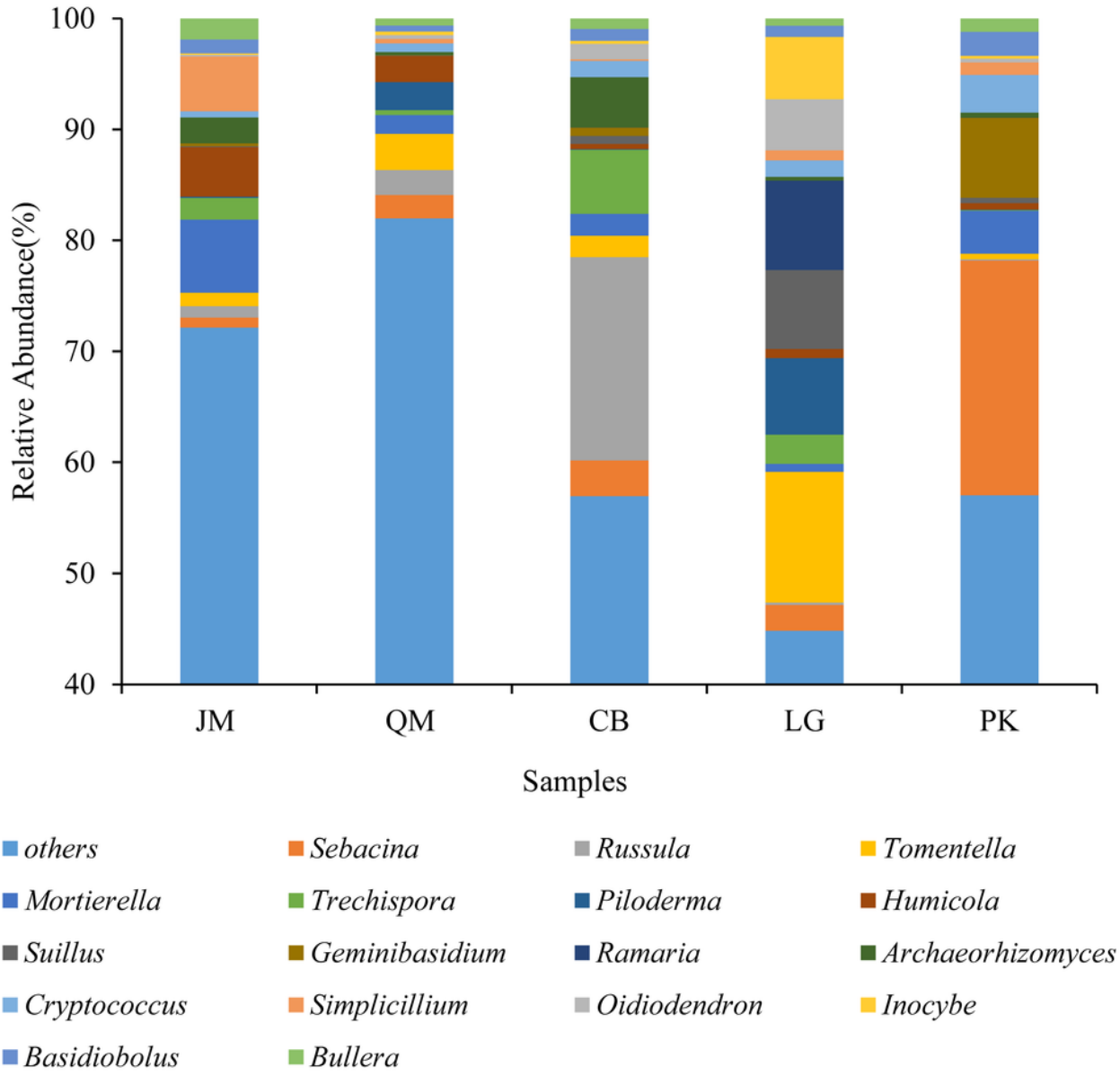


Figure 4

Venn diagrams of OUT richness.

JM: *Juglans mandshurica*; QM: *Quercus mongolica*; CB: Conifer-broadleaf forest; LG: *Larix gmelinii*; PK: *Pinus koraiensis*.

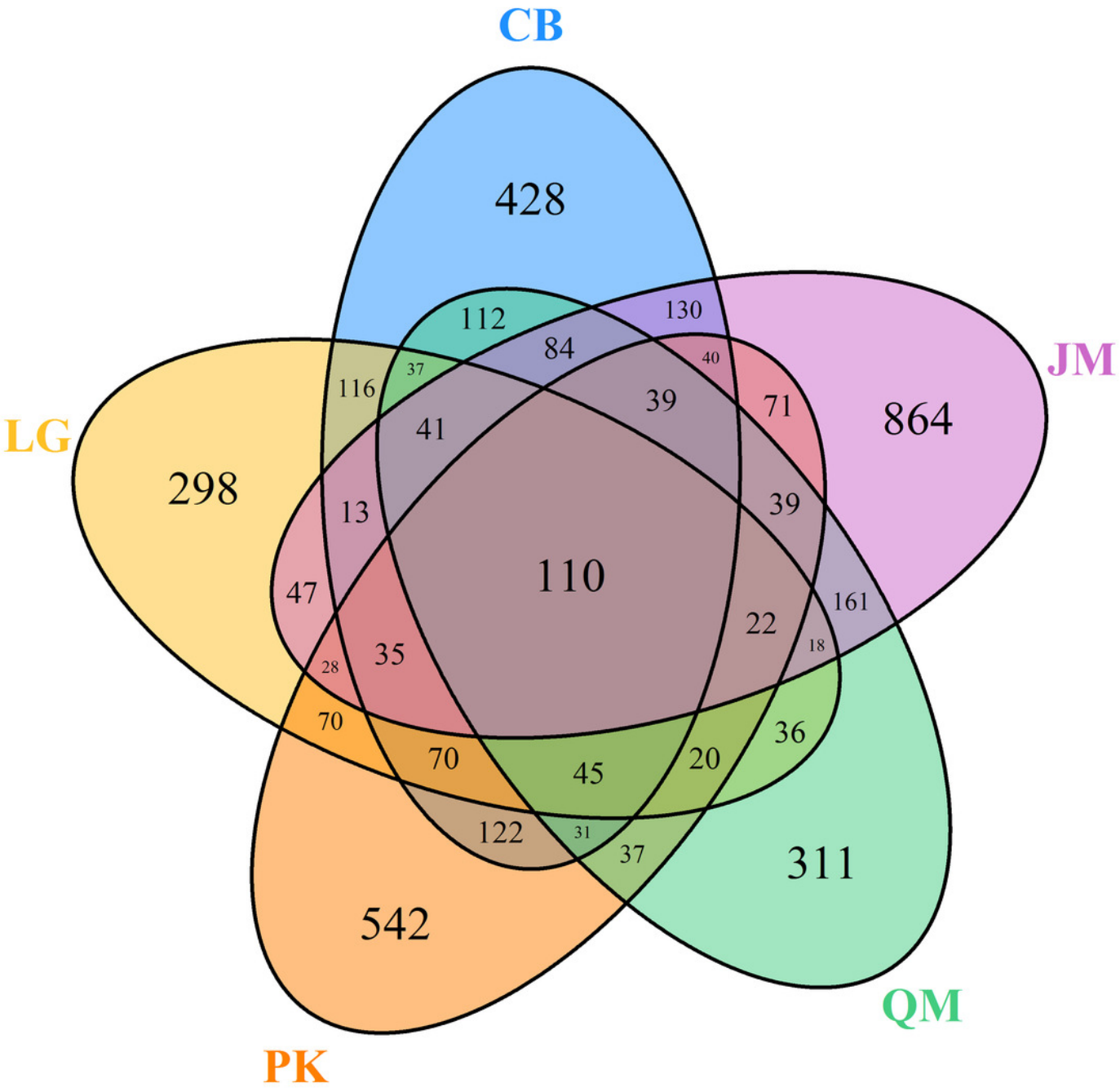


Figure 5

Heat map and hierarchical cluster analysis based on the relative abundances of the top 50 genera identified in the bacterial communities of the soils.

JM: *Juglans mandshurica*; QM: *Quercus mongolica*; CB: Conifer-broadleaf forest; LG: *Larix gmelinii*; PK: *Pinus koraiensis*.

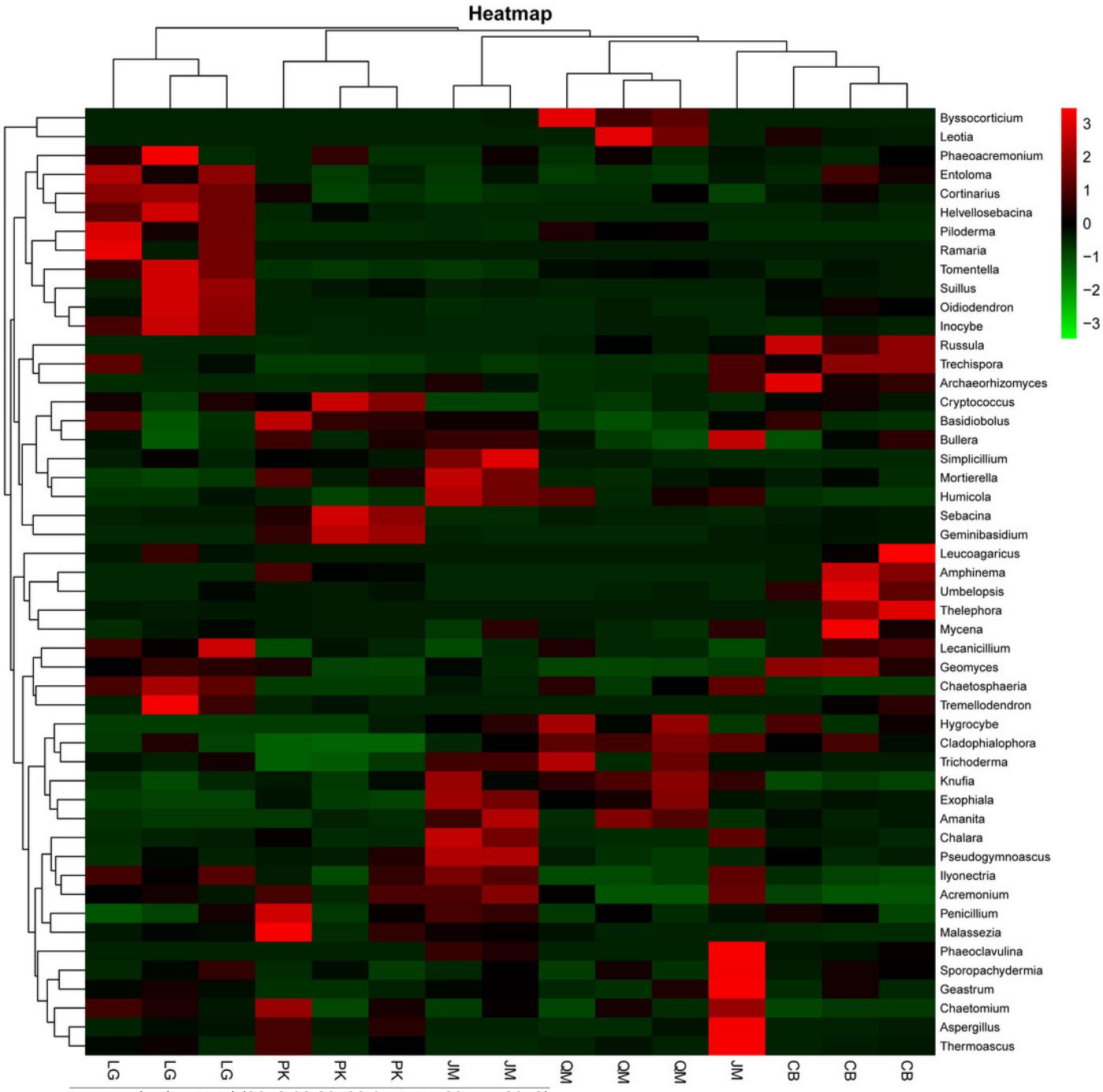


Figure 6

Weighted UniFrac NMDS analysis of the composition of fungal communities in the soil of forests with different dominant trees.

JM: *Juglans mandshurica*; QM: *Quercus mongolica*; CB: Conifer-broadleaf forest; LG: *Larix gmelinii*; PK: *Pinus koraiensis*.

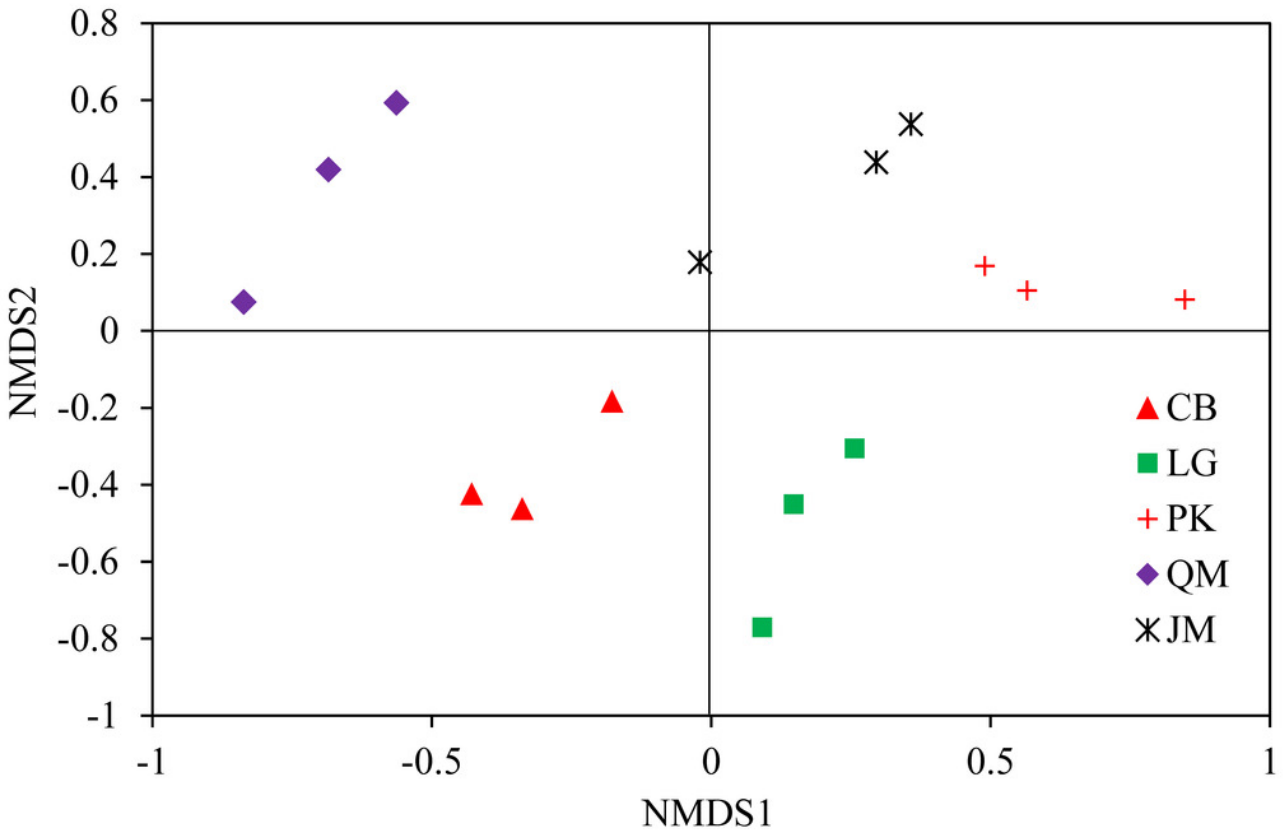
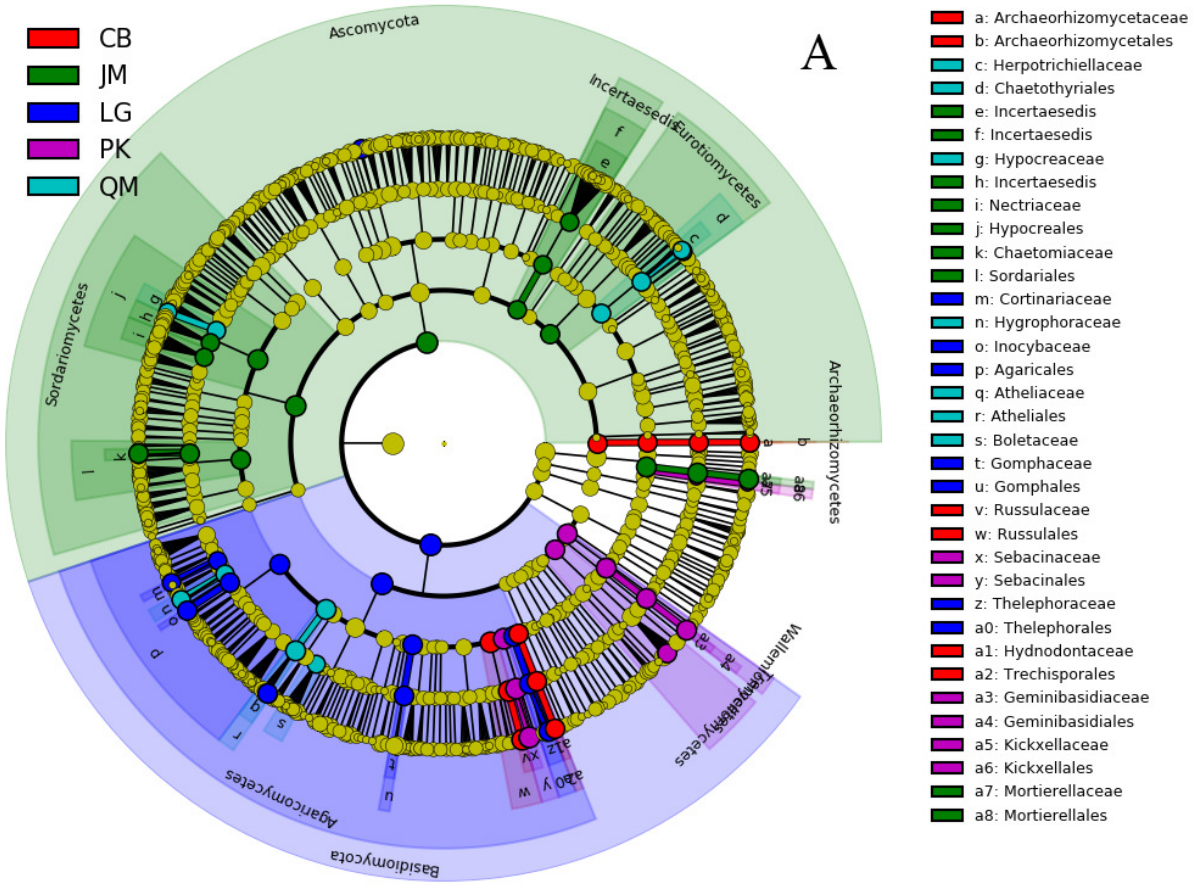


Figure 7

The cladogram of fungal communities among different sampling sites.

JM: *Juglans mandshurica*; QM: *Quercus mongolica*; CB: Conifer-broadleaf forest; LG: *Larix gmelinii*; PK: *Pinus koraiensis.*


A
CB
JM
LG
PK
QM
Ascomycota
Basidiomycota
Agaricomycetes
Sordariomycetes
Eurotiomycetes
Archaeorhizomycetes
Wallemiomycetes
Incertaesedis
a: Archaeorhizomycetaceae
b: Archaeorhizomycetales
c: Herpotrichiellaceae
d: Chaetothyriales
e: Incertaesedis
f: Incertaesedis
g: Hypocreaceae
h: Incertaesedis
i: Nectriaceae
j: Hypocreales
k: Chaetomiaceae
l: Sordariales
m: Cortinariaceae
n: Hygrophoraceae
o: Inocybaceae
p: Agaricales
q: Atheliaceae
r: Atheliales
s: Boletaceae
t: Gomphaceae
u: Gomphales
v: Russulaceae
w: Russulales
x: Sebacinaceae
y: Sebacinales
z: Thelephoraceae
a0: Thelephorales
a1: Hydnodontaceae
a2: Trechisporales
a3: Geminibasidiaceae
a4: Geminibasidiales
a5: Kickxellaceae
a6: Kickxellales
a7: Mortierellaceae
a8: Mortierellales

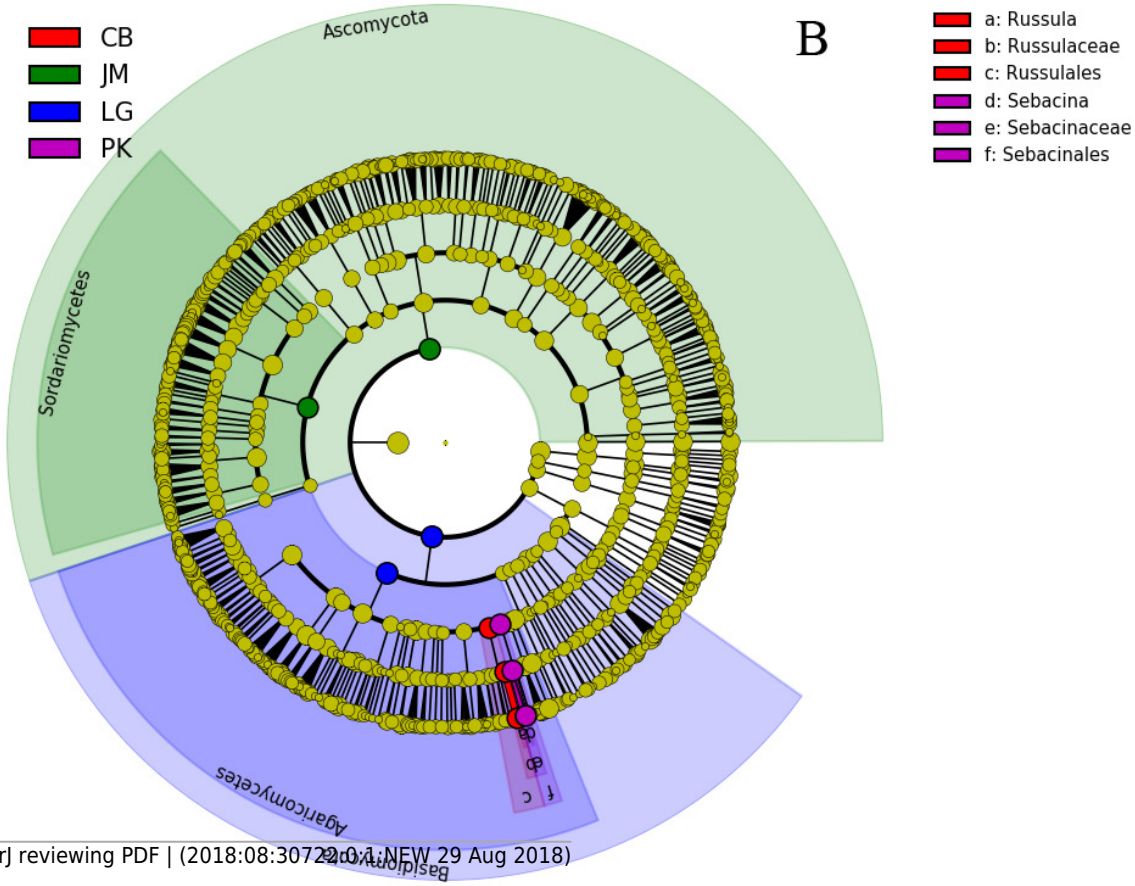

B
CB
JM
LG
PK
Ascomycota
Basidiomycota
Agaricomycetes
Sordariomycetes
a: Russula
b: Russulaceae
c: Russulales
d: Sebacina
e: Sebacinaceae
f: Sebacinales

Figure 8

Canonical correspondence analysis (CCA) on soil dominant fungal phyla constrained by soil variables.

TC: Total Carbon; TN: Total Nitrogen; C/N: C-N ration; AN: Available Nitrogen; TP: Total Phosphorus; AP: Available Phosphorus.

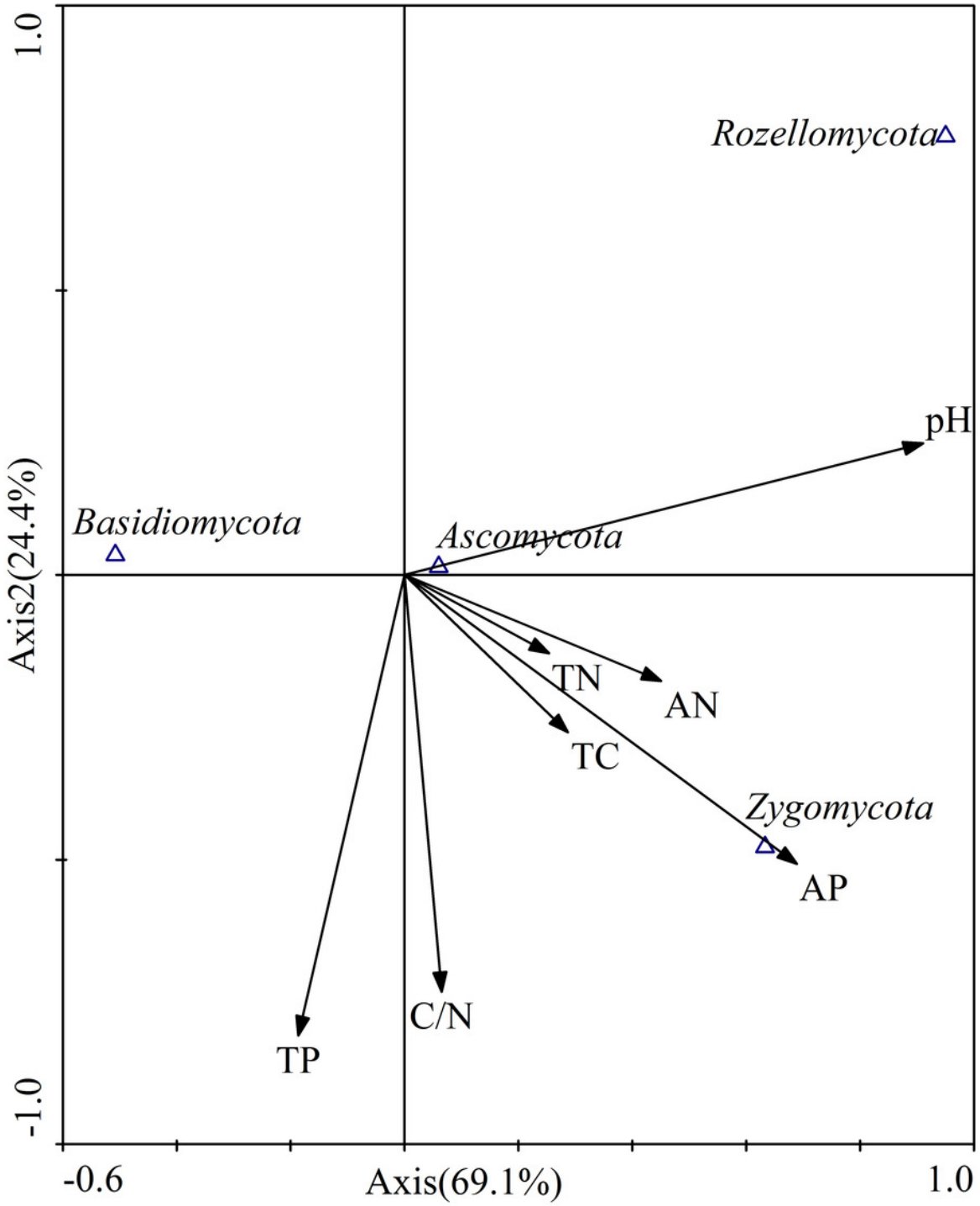

1.0
-1.0
Axis2(24.4%)
-0.6
Axis(69.1%)
1.0
Rozellomycota
pH
Basidiomycota
Ascomycota
TN
AN
TC
Zygomycota
AP
C/N
TP

Table 1(on next page)

Sites information of the Baishilazi Nature Reserve.

JM: *Juglans mandshurica*; QM: *Quercus mongolica*; CB: Conifer-broadleaf forest; LG: *Larix gmelinii*; PK: *Pinus koraiensis*.

Table 1:

Sites information of the Baishilazi Nature Reserve.

Types	Dominant Vegetation	Elevation (m)	Forest Type
JM	*Acanthopanax senticosus*, *Padus racemose*, *Magnolia sieboldii*, *Pimpinella brachycarpa*, *Puccinellia tenuiflora*	901.8	Natural secondary forest
QM	*Acer mono*, *Cerasus tomentosa*, *Carpinus cordata*	842.3	Natural secondary forest
CB	*Betula ermanii*, *Pinus koraiensis*, *Schisandra chinensis*, *Phryma leptostachya* L. subsp. *asiatica*	826.5	Natural secondary forest
LG	*Daemonorops margaritae*	552.7	Plantation forest
PK	*Daemonorops margaritae*, *Pteridium aquilinum*	552.7	Plantation forest

Note:

JM: *Juglans mandshurica*; QM: *Quercus mongolica*; CB: Conifer-broadleaf forest; LG: *Larix gmelinii*; PK: *Pinus koraiensis*.

Table 2(on next page)

Soil physical and chemical properties of different revegetation types.

JM: *Juglans mandshurica*; QM: *Quercus mongolica*; CB: Conifer-broadleaf forest; LG: *Larix gmelinii*; PK: *Pinus koraiensis*.

Table 2:

Soil physical and chemical properties of different revegetation types.

Types	pH	Total C (g/kg)	Total N (mg/kg)	C/N	Available N (mg/kg)	Total P (g/kg)	Available P (mg/kg)
JM	5.70a	100.53a	7800.00a	12.89ab	57.15a	0.93b	4.42ab
QM	4.89c	84.62b	7375.33a	11.64bc	41.25b	0.74bc	2.39ab
CB	4.99c	75.49c	5466.67b	13.81a	43.60b	1.46a	2.53ab
LG	5.40b	43.79d	3853.50c	11.36c	33.35c	0.62c	1.21b
PK	5.48b	41.70d	3580.50c	11.65bc	28.04c	0.77bc	5.65a

Note:

JM: *Juglans mandshurica*; QM: *Quercus mongolica*; CB: Conifer-broadleaf forest; LG: *Larix gmelinii*; PK: *Pinus koraiensis*.

Table 3(on next page)

Soil fungal diversity indexes of different revegetation types.

JM: *Juglans mandshurica*; QM: *Quercus mongolica*; CB: Conifer-broadleaf forest; LG: *Larix gmelinii*; PK: *Pinus koraiensis*.

Table 3:

Soil fungal diversity indexes of different revegetation types.

Types	No. of sequences	OTUs number (phylum)	Shannon Index	ACE Index	Chao1 Index	Simpson Index
JM	40811	715	8.18±0.23a	879.57±64.4767a	879.08±64.48a	0.99±0.00a
QM	33752	518	5.79±0.28c	597.78±98.62b	598.00±98.89b	0.89±0.03b
CB	37669	743	7.18±0.34b	870.95±192.83a	866.17±184.59a	0.98±0.01a
LG	37959	455	6.49±0.22bc	522.15±100.95b	521.58±101.48b	0.97±0.01a
PK	63447	525	7.06±0.89b	650.67±108.58b	649.10±109.27b	0.97±0.02a

Note:

JM: *Juglans mandshurica*; QM: *Quercus mongolica*; CB: Conifer-broadleaf forest; LG: *Larix gmelinii*; PK: *Pinus koraiensis*.

Table 4(on next page)

Person’s rank correlation coefficients between fungi diversity indices and measured soil characteristics.

*Correlation is significant at the 0.05 level (1-tailed). **Correlation is significant at the 0.01 level (2-tailed).

Table 4:

Person's rank correlation coefficients between fungi diversity indices and measured soil characteristics.

	pH	Total C	Total N	C/N	Available N	Total P	Available P
Simpson	**0.680****	-0.139	-0.337	0.472	-0.043	0.334	0.297
Chao1	0.089	**0.573***	0.389	**0.715****	0.397	**0.725****	0.238
ACE	0.085	**0.567***	0.383	**0.714****	0.389	**0.730****	0.234
Shannon	**0.659****	0.302	0.132	**0.528***	0.312	0.361	0.508

Note:

*Correlation is significant at the 0.05 level (1-tailed). **Correlation is significant at the 0.01 level (2-tailed).

Table 5(on next page)

Person’s rank correlations between the relative abundances of dominant bacteria groups and available edaphic factors.

**correlation significant at 0.01 level (two-tailed); *correlation significant at 0.05 level (two-tailed).

Table 5:

Person's rank correlations between the relative abundances of dominant bacteria groups and available edaphic factors.

Fungal group	pH	Total C	Total N	C/N	Available N	Total P	Available P
Phylun	--	--	--	--	--	--	--
Basidiomycota	**-0.680****	-0.455	-0.466	-0.010	-0.506	0.107	**-0.611****
Ascomycota	0.416	**0.608***	**0.655****	-0.004	**0.693****	-0.058	0.462
Zygomycota	**0.530***	0.274	0.179	0.284	0.245	0.146	**0.665****
Rozellomycota	**0.716****	-0.016	0.036	-0.278	0.052	-0.460	0.002
Genus	--	--	--	--	--	--	--
Sebacina	0.192	**-0.563***	**-0.533***	-0.282	**-0.604***	-0.132	0.171
Russula	-0.491	0.170	0.025	**0.637***	0.171	**0.751****	-0.121
Tomentella	0.009	-0.407	-0.339	-0.371	-0.247	-0.360	-0.473
Mortierella	**0.524***	0.456	0.383	0.264	0.447	0.123	**0.623***
Trechispora	-0.182	0.096	-0.039	0.490	0.071	**0.593***	-0.415
Piloderma	-0.066	-0.378	-0.278	-0.452	-0.316	**-0.534***	-0.482
Humicola	0.302	**0.745****	**0.735****	0.146	**0.705****	-0.098	0.152
Suillus	0.118	-0.438	-0.402	-0.287	-0.263	-0.236	-0.266
Geminibasidium	0.252	**-0.530***	**-0.516***	-0.226	**-0.569***	-0.105	0.271
Ramaria	0.116	-0.420	-0.378	-0.303	-0.353	-0.402	-0.368
Archaeorhizomyces	-0.111	0.300	0.132	**0.672****	0.334	**0.591***	-0.057
Cryptococcus	0.114	**-0.655****	**-0.655****	-0.212	**-0.698****	-0.069	-0.031
Simplicillium	**0.540***	0.415	0.380	0.110	0.387	-0.009	0.509
Oidiodendron	0.024	-0.417	-0.398	-0.211	-0.261	-0.098	-0.338
Inocybe	0.144	**-0.518***	-0.459	-0.397	-0.340	-0.383	-0.380
Basidiobolus	0.431	-0.378	-0.434	0.023	-0.406	-0.143	**0.578***
Bullera	**0.596***	0.340	0.273	0.231	0.313	0.091	0.357

Note:

**correlation significant at 0.01 level (two-tailed); *correlation significant at 0.05 level (two-tailed).