Land-Use Types Combined with Plant Species Alter Soil Fungal Community and Functional Guilds in the Eastern Mountainous Region of Liaoning Province, China

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Received: 26 February 2020
Accepted: 26 April 2020

Abstract

Land-use changes or plant species have a profound impact on soil chemical properties, but under their combined action, little is known about the changes in soil fungal communities and functional guilds. We investigated the effects of different land-use types and plant species on the fungal community and functional guilds in the eastern mountainous area of Liaoning Province, including natural secondary forests (Quercus mongolica, shrub wood), plantation forests (Larix gmelini, Pinus koraiensis), and farmland (Zea mays). Fungal community diversity and composition were analyzed using Illumina Miseq high-throughput sequencing. Responses of soil fungal communities to environmental factors were assessed through canonical correlation analysis (CCA) and Pearson correlation analysis. The results illustrated that LG, and PK did not improve soil conditions to the same degree as QM. In addition, compared to SW, ZM decreased soil fertility. The dominant phyla were Ascomycota and Basidiomycota, with average relative abundances of 55.3% and 31.78%, respectively. The phylum Ascomycota was the dominant group in QM, SW, and ZM, while, Basidiomycota dominated in LG, and PK. Heatmap (P<0.05) and NMDS (stress = 0.07) plots showed that soil fungal communities from LG, PK, and ZM tended to be separated from those of QM and SW. Additionally, soil fungal community functions from QM, SW, and LG were separated from those of PK and ZM. Both analyses demonstrated that different land-use types and plant species had significantly different impacts on the soil fungal communities. Canonical correlation analysis suggested that soil pH value, NH4+-N, and NO3--N contents were the main factors affecting the soil fungal community diversity and composition. Our
results demonstrated that fungal community diversity, composition, and functional groups significantly differed among the three different land use types, so were differences in different plant species under the same land-use type.

**Keywords**: the eastern mountainous region of Liaoning Province, land use types, plant species, fungal community, functional guilds

**Introduction**

Forest biodiversity and ecosystem services are of global importance [1]. However, during the past 100 years, an increasing number of natural forests have been under constant threat of deforestation as a consequence of economic development and agricultural expansion in China and other countries [2-4]. There are growing evidences that that land use conversion is a key component of global changes and strongly affects many ecosystem services and functions in terrestrial ecosystems [5-9], such as a noticeable loss of plant and animal biodiversity [10-11], erosion and nutrient leaching [12], reduction in soil carbon stocks and soil nitrogen availability [13-14], soil degradation [15], and increases in greenhouse gas emissions [16]. Converting natural and semi-natural vegetation into agricultural fields is currently the most significant land use change on a global scale [17]. So far, over 38% of all pristine forests worldwide have been converted to managed systems, such as agricultural land and plantation forests [18], a phenomenon that is particularly prevalent in South America, Africa, and Asia [19]. Land-use conversion drastically modifies vegetation type, which is one of the major drivers that influence soil environmental conditions [20-21], mainly in terms of soil physical-chemical properties [22], such as soil temperature, water balance, pH, carbon and nutrient dynamics [23-24], as well as soil enzymatic activities [25], which can directly affect soil microbial communities [26-28]. Soil microbial community diversity and structure are sensitive indicators of soil health and quality and hence rapidly respond to land-use conversions [29]. Any changes in soil nutrients and environmental quality can be predicted through the diversity and variability of soil microorganisms [30].

Microorganisms occupy an important position in terrestrial ecosystems, regulating biogeochemical cycling of nutrients [31-33], and affecting vegetation growth [34] and ecosystem stability [35]. Fungi, as indicators of ecosystem health and important decomposers, are ubiquitous inhabitants of soil ecosystems; they are crucial for ecosystem functioning [36] and strongly drive abiotic soil conditions [37-38]. Soil fungi have a high plasticity and can take various forms to respond to adverse or unfavorable conditions [39]. They can decompose plant residues and complex compounds such as lignin, hemicellulose, and cellulose, thereby releasing nutrients for the vegetation [40]. Fungi show a particularly high sensitivity to shifts in vegetation because of their close associations with plants [41]. Previous studies have suggested that land use change can affect the microbial decomposition of litter and SOM, and against this background, changes in soil fungal diversity can influence soil ecological processes such as nutrient cycling [42-43] and gas release [44-46] in terrestrial ecosystems. In this sense, the impact of land use conversion on soil fungal communities is receiving increasing attention [47], and it is crucial to research the fungal diversity and community structure to increase our understanding of the functions and processes of ecosystems and to evaluate ecosystem services.

The effects of conversion from natural forests into plantation forests [48-49] and agriculture [50] on soil physicochemical characteristics in the tropical forests have been comprehensively investigated. In addition, previous studies have demonstrated that microbial biomass, microbial activity [51], and microbial communities can differ between forested and agricultural lands [52-54]. Further, numerous studies have quantified the responses of bacterial communities to land use changes in tropical systems [55-58]. However, the changes in the soil fungal community diversity and structure as a consequence of converting natural secondary forests into plantations and farmland have been less studied, especially in temperate ecosystems. In particular, the effects of land use conversion on soil fungal community functional guilds have not yet been sufficiently assessed. Nevertheless, the obtain relevant information on fungal responses to changes in the soil environment and to adequately predict ecosystem stability and services, the consequences of land use conversion for soil fungal community diversity, structure, and functional guilds should be investigated. In addition, elucidating the changes of soil nutrient and fungal community structure and function in different land use types and vegetation types is of great significance for selecting suitable vegetation for restoration.

In this context, we elucidated the responses of the soil fungal communities relevant to land-use conversion and compared, soil fungal community diversity, structure, and functional guilds from three different land use types, including natural secondary forests (QM: *Quercus mongolica*, SW: shurwood), plantation forests (LG: *Larix gmelini*, PK: *Pinus koraiensis*), and farmland (ZM: *Zea mays*) in the eastern mountainous region of Liaoning Province. For this, we used internal transcribed spacer (ITS) ribosomal DNA (rDNA)
via Illumina Miseq high-throughput sequencing and analyzed soil characteristics, fungal community diversity, composition, and functional guilds across three different land use types. We further tested the hypotheses that

1) the conversion of secondary forests to different plantation forests and agricultural land significantly alter soil characteristics;

2) the conversion of secondary forests to different plantation forests and agricultural land would significantly alter the fungal community diversity, structure, and function;

3) land use types and plant species changes generated changes in soil chemical properties, influencing the composition of the soil fungal communities. Monitoring soil fungal community composition and function changes under different land use types could provide a theoretical basis for the restoration of degraded soil ecosystems and for the sustainable management of plantations.

Materials and Method Sites

Description

The research area is located in the experimental forest farm of the Liaoning Institute of Forest Management (40°52′31″N, 123°56′43″E), in the hilly land of the eastern mountainous region of Liaoning Province, China. The region is typically a temperate continental monsoon climate with a mean annual temperature of 6.5ºC, an annual average precipitation of 926.3 mm, and an annual average evaporation of 1,056 mm. The soil type is characterized as Eutrochrepts, according to the USDA system [59], with a thickness of 50 cm and frozen to a depth of 150 cm. To date, numerous secondary forests have been transformed into plantation forests due to the increasing demand for timber, some of which were reclaimed as farmland. Currently, land

Table 1. Sample information.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Forest age</th>
<th>Stand density (ind·hm⁻¹)</th>
<th>Crown density</th>
<th>Mean DBH (cm)</th>
<th>Herb coverage</th>
<th>Main herb under the forest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercus mongolica</td>
<td>61</td>
<td>2357</td>
<td>90%</td>
<td>20.18</td>
<td>60%</td>
<td>Vicia unijuga, Gueldenstaedtia verna, Atractylodes Lancea, Schisandra chinensis, Asparagus oligoclonos, Corylus mandshurica, Celastrus orbiculatus, Lespedeza bicolor</td>
</tr>
<tr>
<td>Larix gmelini</td>
<td>40</td>
<td>2100</td>
<td>80%</td>
<td>12.68</td>
<td>90%</td>
<td>Rubus crataegifolius, Rubus idaeus, Asparagus oligoclonos, Schisandra chinensis, Athyrium brevifrons, Menispermum dauricum</td>
</tr>
<tr>
<td>Pinus koraiensis</td>
<td>61</td>
<td>1800</td>
<td>70%</td>
<td>21.94</td>
<td>30%</td>
<td>Polygonatum odoratum, Vitis amurensis, Athyrium brevifrons, Menispermum dauricum, Vicia unijuga, Asparagus oligoclonos, Gueldenstaedtia verna, Atractylodes Lancea, Schisandra chinensis, Asparagus oligoclonos</td>
</tr>
</tbody>
</table>
use patterns mainly include natural secondary forests, plantations, and agricultural lands (Fig. 1; Table 1). *Zea mays* was cultivated for 5 years, before which the dominant vegetation type was shrubwood. The field management in the area is extensive, with no irrigation and straw returning [60].

**Sample Collection**

In July 2017, we sampled among five sites with uniform conditions (same slope, slope aspect and altitude), including shrub wood, *Quercus mongolica*, *Pinus koraiensis*, *Larix gmelini*, and *Zea mays*. Soil samples were collected from three districts (20 m × 20 m) with three independent replicates in each site. An “S” strip sampling method was used to ensure the representativeness of soil samples in each district. Sampling was performed with a soil auger (10 cm deep, 8 cm in diameter) at a depth of 10 cm, with a total number of 15 samples. All soil samples were well mixed, and sieved to pass a 2-mm sieve; roots and other debris were removed. The prepared soil samples for the determination of soil properties was air-dried at room temperature, and the rest for high-throughput sequencing was stored at –80°C.

**Determination of Soil Chemical Properties**

Soil pH was measured in a soil-water (1:5w/v) suspension for 30 min using a pH meter after shaking [61]. The concentrations of soil total C and total N were determined by an Elemental Analyzer (Elementar, Germany) [62]. Alkaline diffusion method was adopted for the determination of available nitrogen (AN) [63]. Additionally, the contents of ammonium nitrogen (NH₄⁺-N) and nitrate nitrogen (NO₃⁻-N) were measured using an AA3 continuous flow analytical system with 1M KCl extraction (AA3, Germany) [63].

**Soil DNA Extraction**

The FastDNA SPIN extraction kits (MP Biomedicals, Santa Ana, CA, USA) was used to extract total genomic DNA from 0.5 g soil, following the manufacturer’s instructions. The quantity and quality of the extracted DNAs were measured using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively.

**ITS rDNA High Throughput Sequencing**

The PCR amplification of the fungal ITS rDNA region was performed using the primers ITS1F (5’-CTTGTGGTCATTAGAGGAATTA-3’) and ITS2 (5’-GCTGCGTTCTTCATCGATGC-3’) [64]. A total of 25 μl of PCR amplification reaction for each DNA sample contained 5 μl of Q5 reaction buffer and Q5 High-Fidelity GC buffer, respectively, 1 μl of ITS1F and ITS2 primer (10 μM), respectively, DNA template (40-50 ng) 2 μl, dNTPs (2.5 mM)2 μl, Q5 high-fidelity DNA polymerase (5 U/μl) 0.25 μl, and ddH₂O 8.75 μl. Cycling conditions were pre-denaturation at 98°C for 5 min, 25 cycles of denaturation at 98°C for 15 s, annealing at 52°C for 30 s, extend at 72°C for 30 s, followed by extending at 72°C for 5 min. PCR amplicons were purified using Agencourt AMPure Beads (Beckman Coulter, Indianapolis, IN) and quantified with the PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA) [64-65]. Pair-end 2×300 bp sequencing was performed using the Illumina MiSeq platform with MiSeq Reagent Kit v3 (Shanghai Personal Biotechnology Co., Ltd, Shanghai, China).

**Bioinformatics and Statistical Analysis**

This project used the Illumina MiSeq platform for paired-by-end sequencing of community DNA fragments. Sequencing raw data saved in FASTQ format and the raw Q30 rate in our study was 91.97%. The raw data obtained from Illumina sequencing were merged and analyzed using the QIIME software (Quantitative Insights Into Microbial Ecology, v1.8.0, http://qiime.org/) [66] and the UPARSE (v5.2.236, http://www.drive5.com/usearch/) pipeline [67]. In order to integrate the original double-end sequencing data, the double-ended sequence of FASTQ format was first screened by mass using the sliding window method with the window size of 10 bp and the step size of 1 bp. Moving from the first base position at the 5’ end, the average mass of the base in the window should be great than Q20 (ie, the average base sequencing accuracy≥99%). The sequence with an average quality value below Q20 in the first window need to be truncated, and the truncated sequence length was greater than or equal to 150 bp and no ambiguous base N was allowed. Subsequently, the FLASH software (v1.2.7, http://ccb.jhu.edu/software/FLASH/) [68] was used to pair and connect the double-ended sequences that pass through the initial mass screening according to the overlapping bases. The overlapping base length of Read 1 and Read 2 sequences was ≥10 bp. Finally, the concatenated sequence recognition was completely assigned to the corresponding sample according to the barcode sequence corresponding to each sample, thereby obtaining the effective sequence of each sample. We uploaded all raw sequences to the NCBI Sequence Read Archive under submission number SUB4745583 and BioProject number PRJNA503702. OTU divided by 97% sequences similarity using the UCLUST sequence alignment tool [69]. Then the most abundant sequence in each OTU was selected as the representative sequence of the OTU. Rare OTUs and the OTUs with abundance values below 0.001% of the total sequencing of the entire sample were removed for subsequent analysis [70]. We classified each OTU into
an ecological guild using FUNGuild [71] to determine specific functional groups of fungi among different samples.

Venn diagrams were used to visualize the shared and unique OTUs among samples using the R package with the “VennDiagram”, based on the occurrence of OTUs across samples. The heatmap representation of the top 50 relative abundance of fungal genera, and the fungal functions among samples were performed using the R packages (v 3.4.4) with “gplot” and “pheatmap”. Beta diversity analysis was performed to investigate the structural variation of fungal communities across samples, using nonmetric multidimensional scaling (NMDS) based on unweighted Unifrac distance metrics. The linear discriminant analysis (LDA) effect size (LEfSe) was fulfilled to detect differentially abundant taxa across groups using the default parameters. One-way analysis of variance (ANOVA) was conducted using the software package SPSS 19.0 (SPSS Inc., USA). Soil chemical characteristics, fungal total relative abundances, and alpha diversity indices under different land-use types were compared using the LSD test. Pearson correlation analysis was used to calculate the correlations between alpha diversity indices and soil characteristics. Canonical correspondence analysis (CCA), which was performed by functions in the Cannon 4.5, was used to evaluate the linkages between dominant fungal groups related to the measured soil environmental factors.

### Results

#### Soil Chemical Characteristics in Different Sites

Soil nutrient levels can be reflected by basic soil chemical properties, and significant differences in soil chemical characteristics among sites were observed (Table 2). The soil pH value ranged from 5.54 to 6.29 in all sites, with the highest level in SW with 6.29, followed by QM, LG, PK, and ZM (P<0.05). No significant differences in soil C/N among the different samples were observed, and soil C/N varied from 13.09 to 13.71. In terms of natural secondary forest, the contents of total C, total N, and available N in SW were 38.27 g·kg⁻¹, 2.79 g·kg⁻¹, and 23.50 mg·kg⁻¹, respectively. While, the QM stand had the highest total C, total N and available N values with 57.74 g·kg⁻¹, 4.40 g·kg⁻¹, and 33.63 mg·kg⁻¹, respectively, significantly higher than those of SW, surprisingly, without a distinct difference to LG; however, the values were significantly higher than in PK (P<0.05). Compared to the site SW, the agricultural soils exhibited a reduction in soil total C, total N, and available N. The contents of NH₄⁺-N in LG and PK were 6.33 mg·kg⁻¹ and 7.68 mg·kg⁻¹, respectively, significantly higher than in QM, SW, and ZM. The contents of NO₃⁻-N in the different sites decreased in the order of LG>PK>ZM>QM>SW, with the lowest level of 9.58 mg·kg⁻¹ (Table 2).

#### Table 2. Soil chemical characteristics under different sites.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>pH value</th>
<th>Total C (g·kg⁻¹)</th>
<th>Total N (g·kg⁻¹)</th>
<th>C/N ratio</th>
<th>Available N (mg·kg⁻¹)</th>
<th>NH₄⁺-N (mg·kg⁻¹)</th>
<th>NO₃⁻-N (mg·kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QM</td>
<td>5.99±0.15ab</td>
<td>57.74±15.68a</td>
<td>4.40±1.11a</td>
<td>13.09±0.39a</td>
<td>33.63±7.19a</td>
<td>4.42±0.50b</td>
<td>17.09±3.69ab</td>
</tr>
<tr>
<td>SW</td>
<td>6.29±0.16a</td>
<td>38.27±5.49b</td>
<td>2.79±0.42b</td>
<td>13.71±0.31a</td>
<td>23.50±5.87b</td>
<td>3.78±0.15b</td>
<td>9.58±0.68b</td>
</tr>
<tr>
<td>LG</td>
<td>5.57±0.24c</td>
<td>52.24±3.36ab</td>
<td>3.89±0.27a</td>
<td>13.43±0.20a</td>
<td>32.05±3.61a</td>
<td>6.33±1.44a</td>
<td>26.44±9.88a</td>
</tr>
<tr>
<td>PK</td>
<td>5.54±0.11c</td>
<td>20.08±4.01c</td>
<td>1.53±0.32c</td>
<td>13.24±1.88a</td>
<td>12.96±2.56c</td>
<td>7.68±1.45a</td>
<td>24.17±2.81a</td>
</tr>
<tr>
<td>ZM</td>
<td>5.85±0.16bc</td>
<td>17.46±2.33c</td>
<td>1.31±0.20c</td>
<td>13.39±0.28a</td>
<td>11.90±1.33c</td>
<td>3.94±0.77b</td>
<td>19.53±6.47ab</td>
</tr>
</tbody>
</table>

n = 3. Different lowercase letters in each column indicate a significant difference (P<0.05). QM: Quercus mongolica; SW: Shrub wood; LG: Larix gmelini; PK: Pinus koraiensis; ZM: Zea mays.

#### Table 3. Soil fungal diversity indices of different sites.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>No. of sequences</th>
<th>Shannon index</th>
<th>Chao1 index</th>
<th>ACE index</th>
<th>Simpson index</th>
</tr>
</thead>
<tbody>
<tr>
<td>QM</td>
<td>49878</td>
<td>7.30±0.13a</td>
<td>870.63±170.47a</td>
<td>882.80±181.64a</td>
<td>0.968±0.011a</td>
</tr>
<tr>
<td>SW</td>
<td>36900</td>
<td>7.33±0.76a</td>
<td>792.29±19.79ab</td>
<td>791.63±18.81ab</td>
<td>0.964±0.024a</td>
</tr>
<tr>
<td>LG</td>
<td>62011</td>
<td>5.82±0.93c</td>
<td>529.62±128.40c</td>
<td>531.75±126.12c</td>
<td>0.918±0.044b</td>
</tr>
<tr>
<td>PK</td>
<td>58994</td>
<td>6.09±0.47bc</td>
<td>611.29±50.08bc</td>
<td>621.30±45.22bc</td>
<td>0.954±0.012ab</td>
</tr>
<tr>
<td>ZM</td>
<td>58934</td>
<td>6.94±0.41ab</td>
<td>652.87±95.52bc</td>
<td>655.14±99.62bc</td>
<td>0.973±0.009a</td>
</tr>
</tbody>
</table>

Different lowercase letters within same column indicate significant difference at P<0.05 level (n = 3). QM: Quercus mongolica; SW: Shrub wood; LG: Larix gmelini; PK: Pinus koraiensis; ZM: Zea mays.
Soil Fungal Diversity in Different Sites

A total of 800,155 sequences, targeting the ITS gene, were obtained from the 15 soil samples using Illumina MiSeq sequencing, ranging from 36,900 to 62,011 reads per sample (Table 3). At the 3% dissimilarity level (Supplement Fig. 1), the curve tended to flatten with an increasing number of measured sequences, indicating that most of the sample information was obtained and that the information adequately reflected the fungal community composition of the soil. Venn diagrams were used to compare the shared and unique OTUs between fungal communities among the samples. PK, LG, QM, SW, and ZM fungal communities had 107 shared OTUs and 457, 410, 591, 641, and 801 unique OTUs, respectively (Fig. 2). The unique OTUs accounted for 41.7%, 35.1%, 32.4%, 40.1%, and 50.1% of the total detected OTUs in PK, LG, QM, SW and ZM, respectively.

Fungal richness and evenness were estimated by Shannon, Simpson, Chao1, and ACE indices. Among the different sites, the average values of Shannon index, Chao1 index, ACE index, and Simpson index in QM were 7.30, 870.63, 882.80, and 0.968, respectively, significantly higher than in PK, and LG. Additionally, it is found that PK exhibited higher Shannon, Chao1, ACE, and Simpson indices than LG. While, compared to the SW, the agricultural soils exhibited a reduction in soil fungal Shannon index, Chao1 index, and ACE index, with 6.94, 652.87, and 655.14, respectively. Pearson correlation analysis showed that the soil pH had a positive relationship with Chao1 index ($r = 0.56$, $P < 0.05$), ACE index ($r = 0.54$, $P < 0.05$), and Shannon index ($r = 0.64$, $P < 0.05$). While, the content of NO$_3^-$-N had a negative relationship with Chao1 index ($r = -0.58$, $P < 0.05$), ACE index ($r = -0.57$, $P < 0.05$), and Shannon index ($r = -0.53$, $P < 0.05$). The Shannon index ($r = -0.84$, $P < 0.01$), Chao1 index ($r = -0.58$, $P < 0.05$), ACE index ($r = -0.55$, $P < 0.05$), and Simpson index ($r = -0.61$, $P < 0.05$) exhibited a negative correlation with the content of NH$_4^+$-N (Table 4).

Soil Fungal Community Composition under Different Sites

Based on the RDP database, all efficient sequences from the five samples were classified from phylum to genus. The obtained 800,155 fungal ITS sequences were categorized as 8 phyla, 295 families, and over 300 genera, demonstrating abundant fungal communities in this ecosystem. There were significant differences in
fungal community abundance at different phylogenetic levels. The majority of dominant fungal phylum across all soil samples belonged to Ascomycota, with a mean relative abundance ranging from 38.73% to 73.40%, followed by Basidiomycota (14.30% to 50.91%), and Zygomycota (1.56% to 5.84%) (Fig. 3). The relative abundances of the minor phyla Cercozoa, Rozellomycota, Chytridiomycota, Glomeromycota, and Ciliophora were all below 1%. In addition, numerous sequences could not be classified into known fungi, with relative abundances varying from 3.02% to 12.82%. The QM had the highest abundance of Ascomycota (73.40%), and the lowest abundance of Basidiomycota (14.30%). The phylum Ascomycota was the dominant group in QM, SW, and ZM, while, Basidiomycota dominated in LG and PK (Fig. 3).

In order to better illustrate the difference between natural secondary forests and managed systems, a cluster tree was adopted. The results clarified that soil fungal community at the phylum could be divided into two clusters, including QM, SW, plus ZM, and PK, plus LG.

At the family level, 17 groups with average relative abundances higher than 1% were obtained in QM, SW, LG, PK, and ZM, including Chaetomiaceae (14.96%, 3.59%, 1.23%, 5.11%, 6.05%), Suillusaceae (0.14%, 23.63%, 3.33%, 0.06%, 0.00%), Cordycipitaceae (5.89%, 12.26%, 2.38%, 0.36%, 1.31%), Trichocomaceae (1.91%, 1.25%, 5.49%, 3.29%, 8.56%), Sebacinaeae (2.56%, 2.44%, 8.87%, 0.97%, 5.17%), Thelephoraceae (1.68%, 4.07%, 6.53%, 1.71%, 0.55%), Geminibasidiaceae (1.35%, 0.02%, 10.96%, 0.62%, 0.18%), Mortierellaceae (2.45%, 2.12%, 2.40%, 1.11%, 5.01%), Atheliaceae (0.07%, 6.43%, 4.00%, 0.04%, 0.08%), Dermateaceae (0.29%, 0.02%, 8.95%, 0.04%, 0.58%), Nectriaceae (1.70%, 0.44%, 0.22%, 1.26%, 5.19%), Hygrophoraceae (0.15%, 0.16%, 0.01%, 7.31%, 0.06%), Myxotrichaceae (0.56%, 1.32%, 3.90%, 0.23%, 0.68%), Herpotrichiellaceae (2.21%, 0.58%, 0.69%, 2.16%, 0.93%), Russulaceae (0.27%, 0.02%, 2.26%, 0.78%, 3.24%), Lasiosphaeriaceae (1.44%, 0.74%, 0.32%, 0.67%, 2.72%), and Gnomoniaceae (0.02%, 0.00%, 5.20%, 0.10%) (Supplement Fig. 2).

At the genus level, 17 groups obtained higher than 1% in QM, SW, LG, PK, and ZM, including Suillus, Humicola, Sebacina, Simplicillium, Penicillium, Geminibasidium, Mortierella, Piloderma, and Tomentella.

**Table 4. Pearson correlations between the fungal community diversity and soil chemical characteristics.**

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>Total C</th>
<th>Total N</th>
<th>C/N ratio</th>
<th>Available N</th>
<th>NH₄⁺-N</th>
<th>NO₃⁻-N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simpson index</td>
<td>0.39</td>
<td>-0.23</td>
<td>-0.22</td>
<td>-0.10</td>
<td>-0.26</td>
<td>-0.61</td>
<td>-0.26</td>
</tr>
<tr>
<td>Chao1 index</td>
<td>0.56*</td>
<td>0.12</td>
<td>0.12</td>
<td>-0.02</td>
<td>0.09</td>
<td>-0.58*</td>
<td>-0.58*</td>
</tr>
<tr>
<td>ACE index</td>
<td>0.54*</td>
<td>0.11</td>
<td>0.12</td>
<td>-0.04</td>
<td>0.09</td>
<td>-0.55*</td>
<td>-0.57*</td>
</tr>
<tr>
<td>Shannon index</td>
<td>0.64*</td>
<td>0.11</td>
<td>0.10</td>
<td>0.01</td>
<td>0.08</td>
<td>-0.84*</td>
<td>-0.53*</td>
</tr>
</tbody>
</table>

**correlation significant at 0.01 level (two-tailed); *correlation significant at 0.05 level (one-tailed).**
Mortierella, Cryptococcus, Knufia, Hygrocybe, Piloderma, Chaetomium, Staphylotruchum, Russula, Tomentella, Bullera, and Gnomonia. There was a strong influence of different land-use types on the composition of the fungal communities. The genera Suillus and Simplicillium were more abundant in LG, whereas Geominasidium and Cryptococcus were more abundant in PK. The relative abundance of Humicola was significantly higher in QM compared to the other sites, while Piloderma was only observed in LG, PK, and ZM, and the genera Suillus was only present in QM, SW, LG, and PK. The genera Hygrocybe and Gnomonia were not found in PK (Fig. 4).

We used the linear discriminant analysis (LDA) effect size (LEfSe) to search for biomarkers with the soil fungal community phylogenetic tree in different classification levels under different sites with an LDA score of 2.0 (Fig. 5, Fig. S3). In total, 1 phylum, 2 classes, 10 orders, 12 families, and 18 genera showed significant differences under the different sites. At the phylum level, Chytridiomycota was enriched under QM. At the extremely significant difference with the LDA score of 4.5, the relative abundances of Sordariales, Chaetomiaceae, Suillus, Suillaceae, Wallemiomycetes, Geominasidiales, Geminisidium, Geominasidiaeae and Dermateaceae differed significantly, which were found in PK, LG, and QM. The most significant differences were found for Sillus and Suillaceae, which showed the highest abundances in LG (Fig. S3).

A hierarchically clustered heatmap also illustrated that the relative abundances and distributions of soil fungi under different sites differed significantly. Based on these results, the five sites could be divided into two groups: one group included the QM and SW, and the other group included LG, PK, and ZM (Fig. 6).

The NMDS plot based on unweighted unifrac distances showed that samples from LG and PK tended to be separated from the QM and SW, especially along NMDS1. Both analyses demonstrated that land use types had significant impacts on the soil fungal communities.

Fig. 5. The cladogram of soil fungal communities under five sites (LDA score = 2.0). QM: Quercus mongolica; SW: Shrub wood; LG: Larix gmelini; PK: Pinus koraiensis; ZM: Zea mays. In the cladogram, the circles radiating represent fungal taxon from phylum to genus from the inside out, and the diameter of the circle is proportional to the relative abundance of each taxon. A taxon with significant difference is marked with the same color as the sampling site where the taxon is ranked the highest, and the branch area is correspondingly shaded. A taxon without a significant difference is marked in yellow.
We used CCA to analyze the variation in fungal community composition and the associated with soil characteristics. The CCA plots based on the dominant fungal phyla and genera were nearly identical (Fig. 8). The overall composition of the dominant phyla or genera under different sites was significantly linked to the selected soil properties. At the phylum level, the eigenvalues of the first axis and the second axis were 0.947 and 0.053, respectively (Fig. 8a), and the parameters pH ($r = 0.79$), NH$_4^+$-N content ($r = -0.89$), and NO$_3^-$-N content ($r = -0.75$) had a more significant

Fig. S3 The significantly changed fungal taxon under different sites. A: Pinus koraiensis (PK); B: Larix gmelini (LG); C: Quercus mongolica (QM); D: Shrub wood (SW); E: Zea mays (ZM). In the significantly changed fungal taxon under different land use patterns, the ordinate is a taxonomic unit with significant differences between groups, and the abscissa visualizes the logarithmic scores of the LDA difference analysis corresponding to the taxon, and sorts them according to the size of the scores to describe them as different. The size of the difference in the grouped sample. The longer the length, the more significant the difference between the taxon units, and the different color of the bar chart indicates the higher abundance sample group corresponding to the taxon.

Relationship between Soil Fungal Communities and the Soil Properties

We used CCA to analyze the variation in fungal community composition and the associated with soil characteristics. The CCA plots based on the dominant fungal phyla and genera were nearly identical (Fig. 8). The overall composition of the dominant phyla or genera under different sites was significantly linked to the selected soil properties. At the phylum level, the eigenvalues of the first axis and the second axis were 0.947 and 0.053, respectively (Fig. 8a), and the parameters pH ($r = 0.79$), NH$_4^+$-N content ($r = -0.89$), and NO$_3^-$-N content ($r = -0.75$) had a more significant
A relationship with Axis1. Pearson correlation analysis of dominant fungal groups and soil environmental factors showed that the relative abundance of Basidiomycota was negatively correlated with pH ($r = -0.55$, $P < 0.05$) and significantly positively correlated with soil NH$_4^+$-N ($r = 0.68$, $P < 0.01$) (Table 5). At the genus level, the eigenvalue of Axis1 was 0.394, and this axis had a greater relationship with soil pH ($r = -0.74$) and NO$_3^-$-N content ($r = 0.84$), explaining 39.4% of the total microbial variance (Fig. 8b). Soil pH

Table 5. Correlation coefficients between dominant fungal groups and soil chemical characteristics.

<table>
<thead>
<tr>
<th>Fungal group</th>
<th>pH</th>
<th>Total C</th>
<th>Total N</th>
<th>C/N ratio</th>
<th>Available N</th>
<th>NH$_4^+$-N</th>
<th>NO$_3^-$-N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phylum</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Ascomycota</td>
<td>0.47</td>
<td>0.18</td>
<td>0.19</td>
<td>-0.17</td>
<td>0.20</td>
<td>-0.64*</td>
<td>-0.39</td>
</tr>
<tr>
<td>Basidiomycota</td>
<td>-0.55*</td>
<td>-0.10</td>
<td>-0.09</td>
<td>-0.04</td>
<td>-0.11</td>
<td>0.68**</td>
<td>0.42</td>
</tr>
<tr>
<td>Zygomycota</td>
<td>-0.08</td>
<td>-0.34</td>
<td>-0.35</td>
<td>0.08</td>
<td>-0.34</td>
<td>-0.29</td>
<td>0.30</td>
</tr>
</tbody>
</table>

**correlation significant at 0.01 level (two-tailed); *correlation significant at 0.05 level (two-tailed).
Land-Use Types Combined with Plant Species...  

Effects of Different Land-Use Types and Plant Species on Soil Chemical Characteristics

Vegetation type and soil management of the land surface are determined by the different land-use types, which in turn affects soil physical and chemical characteristics such as nutrient levels and abiotic conditions [72-74]. In our research, soil chemical properties varied among the different sites (Table 2). And the total C and total N levels were lowest in the ZM, which was consistent with other researches [75]. Land-use change, in particular the conversion of primary forest to agricultural ecosystems, is regarded as one of the large sources of soil C [14], in some cases, total nitrogen losses [23-24]. There is no exception to this principle in China [76-78], including the eastern mountainous region of Liaoning Province.
Fig. 9. Heatmap based on the relative abundances of the functional guilds. QM: *Quercus mongolica*; SW: Shrub wood; LG: *Larix gmelini*; PK: *Pinus koraiensis*; ZM: *Zea mays*.

Fig. 10. Relative abundances of the different functional guilds under different sites. QM: *Quercus mongolica*; SW: Shrub wood; LG: *Larix gmelini*; PK: *Pinus koraiensis*; ZM: *Zea mays*.
Deep tillage activities after the application of fertilizer to farmland frequently leads to the disruption of the soil structure, an increased surface area of the contact between SOM and soil microbes, the physical release of SOM previously trapped/bound, the breakdown of plant residue, and increased aeration, consequently accelerating the release of soil organic carbon into the atmosphere [79-81]. Moreover, in agricultural land, litter accumulation, consequently, nutrient return to the soil, are lower. Both of these processes would reduce the input of organic C into the soil environment and may also explain the reduction in soil total C and total N, suggesting the loss of soil carbon and nitrogen in the process of transforming forests into farmland. It is found that QM owned higher levels of soil total C, total N, and available N than SW, LG, PK, demonstrating the potential positive effects of QM on soil nutrient conditions. Similarly, previous studies have reported that the concentration of soil organic carbon decreased after conversion of natural forests to Pinus plantations [82-84], which was similar to what was observed in our study. Inputs of carbon, nitrogen, and organic matter into soil are mainly due to the return of nutrients and biological nitrogen fixation in the litter [85]. Although the sites LG and PK were coniferous forests, the contents of soil total C, total N, and available N under LG land were significantly higher than those under PK land (P<0.05) (Table 2). LG belongs to cold-temperate deciduous coniferous forests with a large amount of litter than PK, which is as a temperate evergreen coniferous forest with less litter [65], which in turn enormously established that land use types and plant species affect soil property significantly. The soil in this region had a pH value ranging from 5.54 to 6.29, and the values were higher in QM and SW. Compared to SW, the ZM land reduced soil pH (Table 2), which may be mainly due to the increase in soil NO3--N content after increasing nitrogen input leads to a decrease in soil pH value. In our study, the conservation of secondary forests to plantation forests resulted in increased NH4+-N and NO3--N concentrations, which was in agreement with the findings of a study performed in Xishuangbanna [86]. These results illustrated that plantations (LG and PK) and SW did not improve soil conditions to the same degree as QM did, highlighting the potential positive effects of QM on soil nutrient conditions, and agriculture land use type could decrease the soil nutrients.

Table 6. The functional guilds of 14 dominant fungal genera under different sites.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Trophic Mode</th>
<th>Functional guild</th>
<th>Number of OTUs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>QB</td>
<td>SW</td>
</tr>
<tr>
<td>Suillus</td>
<td>Symbiotroph</td>
<td>Ectomycorrhizal</td>
<td>27</td>
</tr>
<tr>
<td>Humicola</td>
<td>Saprotroph</td>
<td>Undefined Saprotroph-Wood Saprotroph</td>
<td>2496</td>
</tr>
<tr>
<td>Sebacina</td>
<td>Symbiotroph</td>
<td>Ectomycorrhizal-Orchid Mycorrhizal-Root Associated Biotroph</td>
<td>269</td>
</tr>
<tr>
<td>Geminibasidium</td>
<td>Saprotroph</td>
<td>Undefined Saprotroph</td>
<td>255</td>
</tr>
<tr>
<td>Mortierella</td>
<td>Saprotroph</td>
<td>Undefined Saprotroph</td>
<td>462</td>
</tr>
<tr>
<td>Simplicillium</td>
<td>Pathotroph</td>
<td>Animal Pathogen</td>
<td>829</td>
</tr>
<tr>
<td>Knufia</td>
<td>Pathotroph-Saprotroph</td>
<td>Animal Pathogen-Plant Pathogen-Soil Saprotroph-Undefined Saprotroph</td>
<td>449</td>
</tr>
<tr>
<td>Piloderma</td>
<td>Symbiotroph</td>
<td>Ectomycorrhizal</td>
<td>3</td>
</tr>
<tr>
<td>Hygrocybe</td>
<td>Saprotroph-Symbiotroph</td>
<td>Undefined Saprotroph-Undefined Biotroph</td>
<td>28</td>
</tr>
<tr>
<td>Staphylotrichum</td>
<td>Saprotroph</td>
<td>Undefined Saprotroph</td>
<td>328</td>
</tr>
<tr>
<td>Russula</td>
<td>Symbiotroph</td>
<td>Ectomycorrhizal</td>
<td>36</td>
</tr>
<tr>
<td>Tomentella</td>
<td>Symbiotroph</td>
<td>Ectomycorrhizal</td>
<td>205</td>
</tr>
<tr>
<td>Gnomonia</td>
<td>Pathotroph</td>
<td>Plant Pathogen</td>
<td>3</td>
</tr>
</tbody>
</table>

QM: Quercus mongolica; SW: Shrub wood; LG: Larix gmelini; PK: Pinus koraiensis; ZM: Zea mays.

Response of the Soil Fungal Community and Functions to Different Sites

The fungal diversity index is an effective method to evaluate the diversity of different soil fungal communities. In our study, the Chaol index and ACE index in QM and SW were significantly higher than...
those in LG, PK, and ZM. With land use change from SW to ZM, the Chao1 index, and ACE index decreased dramatically, while, the Shannon index and Simpson index increased (Table 3). Our results did not completely agree with a previous report that the conversion from tropical rainforest to dryland agriculture brought about no differences in Shannon index and a decline in Chao1 index and Simpson index [58]. A previous study has suggested that long-term nitrogen application could reduce soil fungal diversity and fungal community composition [87]. And different nutrient levels result in differences in the microbial community structure and in functional diversity [88]. Fungi can live within wide pH and temperature ranges and are common in almost every environment [89]. In our study, pH was significantly positively related with the Chaol index, ACE index, and Shannon index (Table 4), which was in agreement with the previous research from Xishuangbanna Tropical Botanical, where soils with near-neutral pH had a higher fungal richness [58]. This might be explained by the application of high fertilizer doses to ZM, which decreased soil pH and weakened the diversity of the fungi [90]. In previous studies, long-term fertilizer application also adversely impacted microbe populations [91-92].

Similar to the fungal diversity, the fungal community structure varied among different land-use types and plant species. Land-use type plays a key role in modulating the soil fungal community structure [93-94], even at a small geographical scale. Some studies have reported that the composition and distribution of soil fungal communities were affected by the different plants because of the differences in rhizosphere secretions [95]. We observed a relative abundance of the dominant fungal phylum Ascomycota, followed by Basidiomycota and Zygomyctota (Fig. 3), which was consistent with the findings of previous studies in tropical forests in Peruvian Amazonia and Panama [96-97]. In contrast, research in the Gutianshan National Nature Reserve has shown a dominance of Basidiomycota over Ascomycota and Zygomyctota [98], while Curlevski et al. [99] have reported that Zygomyctota was the dominant phylum. According to a previous study, Ascomycota and Basidiomycota can aerobically degrade cellulose, polyphenolic compounds, and other dissolved organic matter [100], and the preponderance of Ascomycota and Basidiomycota at the surface soil was consistent with this ability. The relative abundances of Ascomycota, Basidiomycota, and Zygomyctota varied among the different sites. Ascomycota showed the highest relative abundance under QM (Fig. 3), which was negatively correlated with soil NH$_4^+$-N. Previous studies have shown that Ascomycota play a key role in decomposition processes [101], and the relative abundance of Ascomycota increases with increasing soil nitrogen levels [102]. Compositional shifts in fungal communities, with a decrease of Basidiomycota and an increase of Ascomycota in agricultural transformation systems in comparison to secondary forests, as observed in our study, have also been shown elsewhere [103-104]. However, compared to Ascomycota and Basidiomycota, the relative abundance of Zygomyctota was considerably lower. Previous studies have illustrated that the Zygomyctota physiology may be distinct from that of Ascomycota and Basidiomycota [105]. By producing thick-walled and resistant spores, Zygomyctota can survive over long periods of dormancy [106]. Additionally, Zygomyctota members cannot use cellulose and sucrose degradation products [107], although they can use C substrates of animal and fungal origin, such as fungal hyphae [108]. We believe that, in the carbon cycling, Zygomyctota may play a key role, with a similar importance as Ascomycota and Basidiomycota.

The OTUs of the functional guilds of 14 dominant fungal genera differed under different land-use types. Ectomycorrhizal fungi represented the most abundant guild in the eastern mountainous region of Liaoning Province, and the dominant ectomycorrhizal genus in our study was the Suillus (Table 6). The lower OTUs number of Tomentella in the PK and ZM may be due to its nutritional requirements or lack of available arthropods and other animal vectors focusing on the dispersal of basidiospores [109]. In our study, after the conversion from SW to ZM, the abundances of Mortierella, Humicola, Piloderma, Hygrocybe, Staphylotrichum, Russula, and Gnomonia changed significantly. Mortierella groups have been suggested to be saprobes in forest ecosystems [110]. Mortierella may be tracking changes in nutrients driven by vegetation turnover because of their potentially endophytic trophic lifestyle [111].

At the scale of the five different sites, we found large changes in fungal community composition, particularly between the secondary forest and the plantation forests plus agricultural land (Fig. 6), which was consistent with the results of a previous study [93]. The NMDS plot showed that samples from LG and PK tended to be separated from those of QM and SW, especially along NMDS1 (Fig. 7). However, the fungal functional guilds in different land use types were divided into two groups, including QM, SW, plus LG, and PK plus ZM. Both analyses demonstrated that similar land use types usually have similar fungal communities [112], and significant difference were observed among different plant species under the same land use type. This is most likely because land-use change and plant species have led to drastic changes in plant biomass and litter inputs, severely affecting the soil fungal community composition [113-114].

**Different Land-Use Types and Plant Species Affect the Fungal Community through Influencing Soil Chemical Properties**

In this study, we provided, for the first time, insights into the contribution of land-use types, plant species and soil properties in shaping the composition of soil fungal communities in the eastern mountainous region of
Liaoning Province. Differences in microbial community composition following land use change and plant species also have been attributed to differences in soil properties [115-116]. Previous studies have established that changes in land use types could lead to changes in soil pH, soil moisture, and temperature [117-118], which in turn would inevitably affect microbial biomass and activity, as well as the microbial community structure [119]. The key role of soil properties in regulating the microbial community was clearly shown in this study. Fungal communities were correlated with soil chemical properties, such as soil pH, \( \text{NH}_4^+ \)-N content, and soil \( \text{NO}_3^- \)-N content (Fig. 8), and a previous study has indicated that the fungal community composition was correlated with \( \text{NO}_3^- \)-N, and soil pH [120]. Land use conversion-induced changes in the soil microbial community have also been related to soil pH and \( \text{NH}_4^+ \)-N content [121]. Basidiomycota was negatively correlated with pH, which was significantly positively correlated with soil \( \text{NH}_4^+ \)-N. In contrast to our findings, in a previous study, Basidiomycota was positively correlated with total N, C/N, and \( \text{NO}_3^- \)-N [122]. Soil pH, soil \( \text{NH}_4^+ \)-N content, and soil \( \text{NO}_3^- \)-N content were the main factors influencing the fungal community in our study area.

Conclusions

Forest plantations (LG and PK) did not improve soil conditions to the same degree as QM, and farmland (ZM) could decrease soil fertility compared to SW. Different land-use types, natural secondary forest (QM) conversion to plantation coniferous forest (LG, PK), and shrub wood (SW) conversion to agriculture (ZM) strongly affected fungal community diversity and structure, and the fungal communities from LG, PK, and ZM tended to be separated from those of QM and SW. When SW was changed into ZM, the Chaol index, and ACE index decreased dramatically, while, the Shannon index and Simpson index increased. And significant difference of soil characteristics and soil fungal communities were observed among different plant species under the same land use type. Land-use changes resulted in changes in the soil chemical properties, thereby controlling the composition of the soil fungal community. And soil pH, soil \( \text{NH}_4^+ \)-N content, and soil \( \text{NO}_3^- \)-N content were the main factors influencing the fungal community.

Acknowledgment

This research was financially supported by the National Science and Technology Support Program of China (2015BAD07B30103), the Sub-project of the National Key Research and Development Program (2017YFC050410501), the Special Fund for Forest Scientific Research in the Public Welfare (201304216), and Cfern and Beijing Techno Solutions Award Funds on Excellent Academic Achievements.

Conflict of Interest

The authors declare no conflict of interest.

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