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Effects of grassland afforestation on structure and function of soil bacterial and fungal communities

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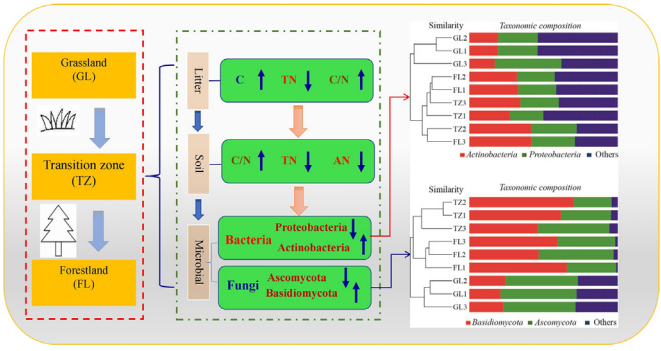
HIGHLIGHTS

- Soil fungal diversity decreases significantly after afforestation.
- ECM fungus abundance increases significantly due to pine plantation on grasslands.
- Soil microbial diversity is similar between forest-grassland transitions & forestland.
- Soil nitrogen plays critical roles in driving soil microbial community changes.

GRAPHICAL ABSTRACT



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ABSTRACT

Grassland afforestation strongly influences the structure and function of soil microorganisms. Yet the mechanisms of how afforestation could simultaneously alter both the soil fungal and bacterial communities and its implications for ecosystem management are poorly understood, especially in nitrogen-limited ecosystems. Using high-throughput sequencing of 16S rRNA and ITS rRNA genes, the present study investigated the changes in soil properties and soil microorganisms after afforestation of natural grasslands with Chinese pine (*Pinus tabuliformis*) on the Loess Plateau in China. Results showed that soil bacterial diversity had no significant differences among the grassland (GL), forest–grassland transition zone (TZ), and forestland (FL), while soil fungal diversity in the GL was significantly higher than that in the FL and TZ ($P < 0.05$). The proportion of shared OTUs in the soil bacterial community was higher than that in the soil fungal community among the three land use types. The dominant bacterial phylum shifted from Proteobacteria to Actinobacteria, while the dominant fungal phylum shifted from Ascomycota to Basidiomycota after the GL conversion to the FL. The functional groups of ECM fungi increased significantly while biotrophic fungi decreased significantly after grassland afforestation. Both the soil

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Soil bacteria
Soil nitrogen

bacterial and fungal communities in the TZ showed great similarity with those in the FL. In addition, among all examined soil properties, soil nitrogen (N) showed a more significant effect on the soil microbial communities. The reduction of soil N after grassland afforestation resulted in both the structure and function changes in soil microbial communities. Our results demonstrated simultaneously differential changes in the composition and diversity of both soil bacterial and fungal communities after afforestation from grasslands to planted forests.

1. Introduction

Soil microorganisms are primary drivers of ecosystem processes, accomplishing soil organic matter and plant litter decomposition, as well as mediating carbon (C) and nitrogen (N) biogeochemical cycles in terrestrial ecosystems (de Menezes et al., 2017; Wang et al., 2017). Therefore, understanding soil microbial composition and diversity can reveal interrelations between soil microorganisms and local environments, and how these communities respond to anthropogenic disturbances (Jangid et al., 2011; Mendes et al., 2015).

Land use conversion has significantly affected on the composition and structure of soil microbial community (Guo et al., 2018; Mendes et al., 2015; Tian et al., 2017). On the one hand, both the quality and quantity of aboveground litter and belowground roots supplied to soil microorganisms differ among land types (Jangid et al., 2011). On the other hand, changes in soil properties such as pH, moisture, clay, C, N and phosphorus availability, under different land types, have significant impacts on soil microbial communities (Fierer, 2017; Guo et al., 2018). For example, Mendes et al. (2015) reported a higher abundance of Acidobacteria and Chlamydiae in the forest soil, Actinobacteria in deforested site, Nitrospira and Deinococcus-Thermus in agriculture, and Firmicutes in pasture. Wood et al. (2017) found an increased bacterial diversity after the conversion of tropical forests to oil palm plantation, whereas only slight differences in bacterial diversity and community composition between the regenerating and primary forests. Although studies have documented the response of soil microbial communities to land use conversions, most of them have focused only on either bacterial or fungal community (Bachelot et al., 2016; Cao et al., 2017; Yang et al., 2017, C. Zhang et al., 2016). Both bacteria and fungi are major players in soil biogeochemical cycles, and the effects of land use conversion on the composition and structure of soil bacterial and fungal communities remain poorly understood (Gunina et al., 2017; Tian et al., 2017). A more comprehensive understanding of the interplay among land use types, soil properties, and soil bacterial and fungal communities will improve the prediction and management of terrestrial ecosystem.

Afforestation has been widely implemented all over the world for the purpose of soil conservation, ecological improvement and C sequestration (Hiltbrunner et al., 2013; Cavagnaro et al., 2016; Deng et al., 2017; Deng and Shangguan, 2017; Ren et al., 2018). Because of the critical role of bacteria and fungi in the soil C and N cycle, the effects of afforestation on soil microbial communities have been already studied (Kageyama et al., 2008; Macdonald et al., 2009; Bachelot et al., 2016; Gunina et al., 2017; Zhao et al., 2018). Studies have shown that soil microbial communities respond differently to afforestation since the difference in site conditions, soil types, land use history, tree species and age (Jangid et al., 2011; Mendes et al., 2015; Kang et al., 2018). However, the use of the modern high-throughput sequencing approach to study the effects of afforestation on soil microbial communities is limited, particularly how afforestation could concurrently affect soil bacterial and fungal communities.

The transition zones between grassland to forestland experience the combined effects of forest and grass ecosystems, where the soil properties, such as moisture, pH, organic matter, extractable NH_4^+, etc., are intermediate between grassland and forest soils (Griffiths et al., 2005). Changes in soil properties in transition zone are inevitably associated with changes in soil microorganisms. For example, Kageyama et al. (2008) found that bacterial communities near the meadow–forest transition zone reflected current vegetation, and fungal communities under meadow vegetation near the forest edge were intermediate between those found in meadow and forest soils. Thus, the study of soil microbial communities in transitional zones could help to promote our understanding of the effects of grassland afforestation on soil C and N cycles (Wei et al., 2010). However, a simultaneous analysis of the composition and structure of soil bacterial and fungal communities in transitional zones from grassland to forestland has been largely ignored.

In the present study, the changes in the diversity, composition, and relative abundance of the dominant taxa of both soil bacterial and fungal communities in grasslands (GL), forestlands (FL), and their transitional zones (TZ) after grassland conversion to Chinese pine plantation were addressed, and the relationships of specific microbial phylum with soil physical and chemical properties were also analyzed. We hypothesized that the soil bacterial and fungal communities could differentially respond to land use conversion, and that their different responses are related to soil physicochemical alterations that have been resulted from grassland afforestation. We also reasoned that an increase in the symbiotic ectomycorrhizal (ECM) fungi and arbuscular mycorrhizal (AM) fungi were likely respectively with pine trees and grasses, and an increased in the diversity of microbial communities as a whole could be based on more recalcitrant pine litter. In three contrasting land use types of GL, FL and TZ, the main objectives of this study were thus to identify (1) their differential changes in the composition and diversity of both soil bacterial and fungal communities; and (2) the potential key soil properties that could affect the structure and diversity of bacterial and fungal communities.

2. Materials and methods

2.1. Study sites

The study site is located in the Lianjiabian Forest Farm (108°10′–109°18′E; 35°03′–36°07′N) in Heshui County, Ganshu Province, China (Fig. 1). The Lianjiabian Forest Farm lies in the hinterland of Ziwuling forest region of Chinese Loess Plateau. This site has a typical landform of loess hilly topography with an altitude of 1211–1453 m. This region has a mean annual temperature of 10 °C and a mean annual rainfall of 587 mm (Wang et al., 2016). The soils at the study site are classified as Inceptisols (USDA Soil Taxonomical System), i.e. loess, which has developed from primary or secondary loessial parent materials. The main types of natural vegetation are *Quercus liaotungensis* and *Populus davidiana* forest with a canopy density typically in the range of 80%–90%, whereas *Pinus tabulaeformis* (Chinese pine) is the most widely planted tree throughout the region (Deng et al., 2016; Zhu et al., 2017).

In the early 1980s, the Chinese government launched a reform of rural land contracting system. Since then, numerous croplands have been abandoned for natural succession in the Ziwuling forest region owing to the land being transferred from the village to the state-owned forestry farm. During 1999–2010, a large-scale ecological restoration program named "Grain for Green Program" has been launched by the Chinese government, and numerous natural types of grassland in the Lianjiabian Forest Farm have been converted to Chinese pine plantation for the purpose of soil conservation. Nevertheless, some natural grassland still exists.

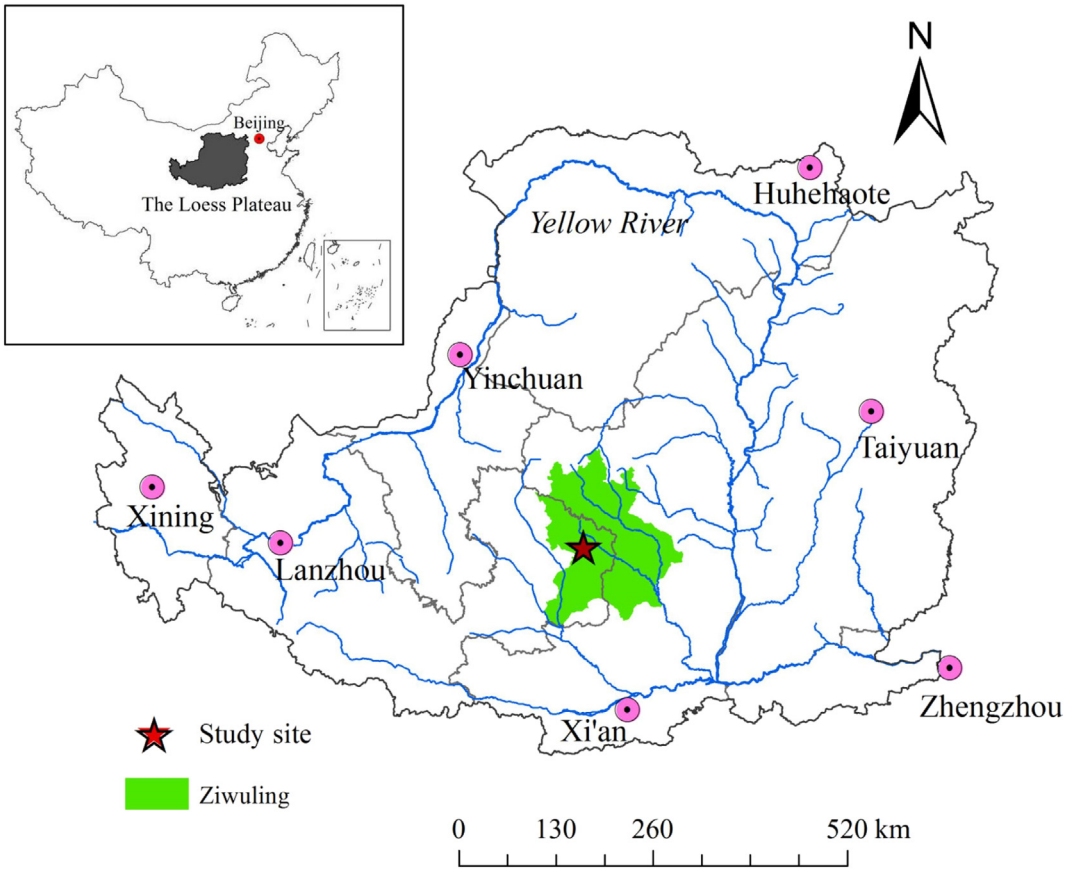

Fig. 1. Study area located in the Loess Plateau, China.

2.2. Experimental design and soil sampling

Soil microbial community is sensitive to climate, soil properties, vegetation types, etc., and has a high spatial and temporal variability (Tedersoo et al., 2014). Moreover, land use history also has an important influence on the soil microbial community (Jangid et al., 2011). Therefore, appropriate experimental sites are the prerequisites for successful investigation of soil microbial community. In the present study, all the sampling plots had similar soil and climate conditions, as well as land use history (originated from farmland >50 years ago). Thus, the sampling site provides an ideal location for studying the effects of grassland afforestation on soil biotic and abiotic factors. In addition, as soil microorganisms in the surface soil are the most sensitive to land use conversion, only the surface soils at 0–5 cm depth were collected.

Three paired plots with distance of about 1 km from each other were randomly selected in the study site. In each plot, a transect (200 m × 50 m) perpendicular to the forest–grass boundary was established. In the study site, the natural grasslands were formed after cropland abandonment for about 30 years, and then the Chinese pine were planted on these natural grasslands 15 years ago. In the forestland, the Chinese pine had a canopy density about 90%, and the surface soil of the forestland was almost completely covered by needle litters of about 6-cm thickness; thus, little grass existed on the ground. In the transition zones, the canopy density of Chinese pine was about 40% and grass grew in the forest gaps. And in the grassland, it exhibited about 90% coverage with the main species of *Bothriochloa ischaemum*, *Carex lanceolata*, and *Potentilla chinensis*. Soils were separately sampled in three paired Chinese pine forestlands (under tree canopies), their transition zones from grasslands to forestlands (in the forest gaps) and adjacent natural grasslands (under vegetation) in late May 2017. Leaf litter in the forestland and grassland was collected in late November 2017 to measure their carbon and nitrogen content.

Along each transect, one sampling subplot (10 m × 10 m) in the center of each land use type was set and each plot had divided into 5 subplots. Five soil cores (5 cm inner diameter, 0–5 cm depths) were collected from the center and four corners of each subplot, and then pooled into a composite sample. Litter and humus were removed before soil sampling, and the soil temperature (ST) and soil moisture (SM) at 5 cm depth were measured using a portable soil moisture and temperature meter (Takeme-20, Dalian Endeavour Technology Co. Ltd., China) in each sampling subplot. All the samples were sieved through a 2-mm sieve to remove the roots, and then divided into two subsamples. One subsample was air-dried for measuring the basic physical and chemical parameters, while another was stored at −80 °C for DNA extractions and molecular analyses.

2.3. Physical and chemical analyses

Soil bulk density (BD) for the top 5 cm soil layer was measured using a 100 cm^3 soil bulk sampler. The soil particle sizes (Clay, Silt, and Sand content) were determined by laser granulometry (Mastersizer 2000, Malvern Instruments Ltd., UK), and the soil pH was measured at a soil/water ratio of 1:2.5 (PHSJ-4A pH meter, Zhangqiu Meihua International Trading Co., China). The soil inorganic carbon (SIC) was analyzed using the CM140 Total Inorganic Carbon Analyzer (UIC Inc., Rockdale, Illinois, USA), and the soil organic carbon (SOC) and leaf litter carbon (LC) were measured using the dichromate oxidation method (Nelson and Sommers, 1996). The soil total nitrogen (TN) and leaf litter nitrogen (LN) were assayed using the Kjeldahl method (Bremner, 1996), soil available nitrogen (SAN) was calculated as the sum of soil ammonium nitrogen (NH_4^+) and nitrate nitrogen (NO_3^-). Soil NH_4^+ and NO_3^- were

extracted with 2 M KCl for 18 h and colorimetrically determined using an Alpkem Autoanalyzer (OI Analytical, College Station, USA).

2.4. DNA extraction, PCR amplification, and Illumina HiSeq 2500 sequencing

DNA was extracted from 0.5 g of fresh soil sample using E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA), according to the manufacturer's protocols. The DNA concentration and purity were monitored on 1% agarose gels. For bacteria, the V3-V4 region in the 16S rRNA gene was amplified by PCR using the primers 341F (5′-barcode-GTGCCAGCMGCCGCGG-3′) and 806R (5′-CCGTCAATTCMTTTRAGTT T-3′). For fungi, the ITS1 region in the rRNA gene was amplified by PCR using the primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′). The PCR was performed in triplicate using 20 μL of reaction mixture containing 4 μL of 5 × FastPfu buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu polymerase, and 10 ng of template DNA. The PCR for both bacteria and fungi were performed using a Veriti Thermal Cycler (Applied Biosystems) with initial denaturation at 95 °C for 2 min, followed by 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min. The amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions, and quantified using QuantiFluor™-ST (Promega, USA). The purified PCR products of all samples were mixed in equal mole amounts and paired-end sequenced (2 × 250) on an Illumina HiSeq 2500 platform (Illumina Int., San Diego, CA, USA) according to the standard protocols. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SRP150160).

2.5. Processing of sequencing data

The raw sequence files were analyzed and quality-filtered using QIIME (version 1.9.1) with the following criteria: (i) the 250-bp reads were truncated at any site receiving an average quality score of <20 over a 50-bp sliding window; (ii) the exact barcode matching two-nucleotide mismatch in primer matching reads containing ambiguous characters were removed; and (iii) only sequences with >10 bp overlap were assembled according to their overlap sequence. Reads that could not be assembled were discarded. The chimeric sequences were identified and removed using UCHIME software (http://drive5.com/uchime/). The operational taxonomic units (OTUs) with 97% similarity cutoff were clustered using the UPARSE software (http://drive5.com/uparse/). The representative sequence of each OTU was taxonomically classified by the Ribosomal Database Project (RDP) Classifier (http://rdp.cme.msu.edu/) against the SILVA (SSU123) database for 16S rRNA and the UNITE database for ITS rRNA using a confidence threshold of 70% (Amato et al., 2013).

The sequencing depth of the soil bacteria and fungi in all samples was >98%, indicating that they were reliable sequencing results. The average values were 39,934 high-quality 16S sequences for bacteria and 38,325 high-quality ITS sequences for fungi of all samples. A normalized sequences dataset was used to assess the microbial diversity among the samples in a comparable manner. To normalize the data, a subset of the lowest number of sequences across all samples (33,459 for bacteria and 31,787 for fungi) was randomly selected using the mothur software package. The rarefaction curves for the bacteria and fungi demonstrated that our sequencing data were representative of most of their compositions (Supplementary Fig. S1). Indices (Chao, Shannon) reflecting community diversity and Good's coverage were also analyzed based on MOTHUR (v.1.21.1, http://www.mothur.org/). In addition, the fungi were divided into four functional types of biotroph, saprotroph, symbiotroph, and unidentified according to their trophic status based on the Index Fungorum database (www.indexfungorum.org) (Supplementary Table S1).

2.6. Statistical analyses

Venn diagrams were constructed to count the number of common and unique OTUs in different land use types using the R VennDiagram package (Chen and Boutros, 2011). Principal coordinate analysis (PCoA) was conducted to compare and visualize the similarities among soil samples using the R vegan package (Oksanen et al., 2018). Similarities in the samples based on the microbial taxon (bacteria and fungi) were measured using the weighted UniFrac distance with the QIIME version 1.9.1 for phylogenetic relationships. Cluster analysis on phylum was performed with the RDP Classifier using the complete linkage hierarchical clustering technique from the R cluster package (Maechler et al., 2018). Canonical Correlation Analysis (CCA) was used to elucidate the relationships between the soil properties and microbial groups using the R vegan package (Oksanen et al., 2018). The relations between the soil properties and microbial groups were examined using the Monte Carlo permutation (999 repetitions). The data sets were analyzed prior to the CCA assay using the detrended correspondence analysis to confirm that the gradient lengths fit a hump model. All statistical analyses were conducted using the R software package v.3.5.0 (R Core Team, 2018).

One-way analysis of variance (ANOVA) was used to analyze the differences in the diversity index of both the soil bacterial and fungal communities among the FL, GL, and TZ sites. Tukey's HSD (honestly significant difference) test was used for multiple comparisons when a test for homogeneity of variance was successful, and significance was observed at $P < 0.05$. Stepwise regressions were performed to identify the best independent soil factors affecting soil bacterial and fungal diversity. One-way ANOVA, Tukey's HSD test and stepwise regressions were conducted using SPSS 16.0.

3. Results

3.1. Changes in soil and litter properties

A significant decrease was observed in ST, SM, Clay, Silt, SIC, SOC, TN, SAN, and LN; yet Sand, C/N, LC and L-C/N increased after Chinese pine planted on the grasslands ($P < 0.05$) (Table 1). In addition, some soil properties in TZ also showed significant differences from GL and FL ($P < 0.05$) (Table 1). However, soil physical properties such as ST, Silt, Sand in TZ were more close to those in GL, while soil chemical

Table 1
Properties of soil at 0–5 cm depth and litter in the three land use types.

	Land types	GL	TZ	FL
Soil	ST (°C)	25.7 ± 0.8a	26.7 ± 1.9a	21.1 ± 0.7b
	SM (%)	9.0 ± 1.1a	5.8 ± 0.9c	7.2 ± 1.0b
	BD (g/cm^3)	1.16 ± 0.03a	1.13 ± 0.04a	1.14 ± 0.04a
	Clay (%)	11.6 ± 0.9a	10.0 ± 0.8b	9.1 ± 0.4b
	Silt (%)	44.2 ± 3.0a	44.5 ± 2.9a	39.9 ± 2.5b
	Sand (%)	44.1 ± 3.8b	45.5 ± 3.6b	51.0 ± 2.9a
	pH	8.23 ± 0.03b	8.35 ± 0.08a	8.21 ± 0.08b
	SIC (g/kg)	18.1 ± 0.7a	16.2 ± 1.0b	15.9 ± 0.5b
	SOC (g/kg)	20.3 ± 0.7a	16.2 ± 0.5c	18.9 ± 0.9b
	TN (g/kg)	1.71 ± 0.05a	1.23 ± 0.10b	1.36 ± 0.04b
	C/N	12.3 ± 0.4b	14.2 ± 1.4a	14.1 ± 0.4a
	SAN (mg/kg)	10.2 ± 0.5a	5.1 ± 0.8b	3.7 ± 1.1b
Litter	LC (g/kg)	397.2 ± 30.3b	/	481.5 ± 21.6a
	LN (g/kg)	13.4 ± 0.1a	/	8.4 ± 0.2b
	L-C/N	29.6 ± 2.2b	/	57.3 ± 2.8a

Note: GL, FL, and TZ indicate in natural grassland, Chinese pine forest, and the transition zone from natural grassland to Chinese pine forest, respectively. ST (soil temperature); SM (soil moisture); BD (soil bulk density); Clay (soil clay content); Silt (soil s ilt content); Sand (soil sand content); SIC (soil inorganic carbon); SOC (soil organic carbon); TN (soil total nitrogen); C/N (ratio of soil organic carbon to soil total nitrogen); SAN (soil avaible nitrogen); LC (litter carbon); LN (litter Nitrogen); L-C/N (ratio of litter carbon to litter nitrogen); / (not measured). Different lower-case letters mean significant differences among different sites ($P < 0.05$). The same as below.

Table 2
HiSeq 2500 sequencing results and diversity index of soil bacterial and fungal communities in the three land use types. Different lower-case letters mean significant differences among different land use types ($P < 0.05$).

Soil microorganism	Sites	Sequences	0.97*			
			OTU	Chao	Shannon	Coverage
Bacteria	GL	34,982 ± 1730b	1991 ± 172a	2254 ± 198a	5.9 ± 0.5a	0.989 ± 0.0
	TZ	38,173 ± 1003a	2010 ± 32a	2306 ± 39a	6.2 ± 0.1a	0.989 ± 0.0
	FL	31,098 ± 1618c	1865 ± 77a	2228 ± 27a	6.1 ± 0.2a	0.987 ± 0.0
Fungi	GL	38,168 ± 4954a	592 ± 63a	629 ± 30a	4.5 ± 0.4a	0.998 ± 0.0
	TZ	34,857 ± 4403a	410 ± 23b	487 ± 11b	3.0 ± 0.1b	0.997 ± 0.0
	FL	40,511 ± 1606a	338 ± 69b	415 ± 48b	2.6 ± 0.6b	0.998 ± 0.0

Note: GL, FL, and TZ indicate study sites in natural grassland, Chinese pine forest, and the transition zone from natural grassland to Chinese pine forest, respectively. *The operational taxonomic units (OTUs) with 97% similarity cutoff were clustered using UPARSE software (version 7.1 http://drive5.com/uparse/).

properties such SIC, TN, C/N and SAN were similar with those in FL ($P > 0.05$) (Table 1).

3.2. Diversity of soil bacterial and fungal communities

The differences were insignificant with respect to the OTUs, Chao index, and Shannon index of the soil bacterial community among the GL, FL and TZ ($P > 0.05$) (Table 2). In contrast, the OTUs, Chao index, and Shannon index of the soil fungal community in the GL were significantly higher than those in the FL and TZ ($P < 0.05$), whereas there were no significant differences in the soil fungal diversity between the FL and TZ (Table 2). A total of 1991 soil bacterial OTU sequences were common among the FL, GL, and TZ, accounting for a range of 81.8%–86.2% of the total bacterial OTUs (Fig. 2a). In contrast, only 266 soil fungal OTU sequences were common among the three land use types, accounting for 30.2%–49.9% of the total fungal OTUs (Fig. 2b).

3.3. Composition of soil bacterial and fungal communities

The average number of bacterial OTUs in GL, FL and TZ were 1991 ± 172, 1865 ± 77, and 2010 ± 32, respectively (Table 2). The average number of fungal OTUs in GL, FL and TZ were 592 ± 63, 338 ± 69, and 410 ± 23, respectively (Table 2).

At the level of phylum, the dominant bacterial groups were Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, and Firmicutes with a relative abundance of >5% in all three land use types (Fig. 3a). After grassland afforestation for 15 years, the most dominant bacterial phylum changed from Proteobacteria (32.8%) to Actinobacteria (35.7%) (Fig. 3b). When compared with the GL, the mean relative abundance of Actinobacteria and Chloroflexi increased by 17.0% and 1.6%, whereas that of Proteobacteria, Acidobacteria, and Firmicutes decreased by 6.1%, 9.6%, and 2.6% in the FL, respectively (Fig. 3b). A similar variation in the dominant bacterial phylum was observed in the TZ where the relative abundance of Actinobacteria and Chloroflexi increased by 15.8% and 0.9%, but the relative abundance of Proteobacteria, Acidobacteria, and Firmicutes decreased by 6.4%, 8.1%, and 2.0%, respectively, compared with the GL.

With regard to the fungal community, the dominant soil fungal groups at the phylum level were Basidiomycota and Ascomycota in all three land use types (Fig. 3c). In the FL and TZ, Basidiomycota and Ascomycota accounted for 98% and 95% of the soil fungal community, respectively (Fig. 3d). When compared with the GL, the mean relative abundance of Basidiomycota increased by 34.5% and 36.7%, whereas that of Ascomycota, Fungi_Unclassified, and Zygomycota decreased by 8.7% and 13.7%, 17.0% and 13.9%, and 8.6% and 8.8% in the FL and TZ, respectively (Fig. 3d).

At the level of genus, the composition of high abundance bacterial genera was generally consistent across GL, FL and TZ, but there was an obviously difference in both the composition and abundance of fungal genera between GL and other two land use types (Fig. 4). The most dominating assigned bacterial genera in all sites were *Bacillus*, *Lactococcus* and *Sphingomonas* (Supplementary Fig. S2). The most dominating assigned fungal genera were *Mortierella*, and *Clavaria* in the GL, while they changed to *Sebacina* and *Trechispora* in FL and TZ (Supplementary Fig. S3).

The cumulative loads of the first two axes of the PCoA of both the bacterial and fungal communities were 78.7% and 78.2%, respectively, which distinctly separated with the three land types (Supplementary Fig. S4). When compared with bacterial PCoA, the fungal PCoA separated the three land types more clearly. On the first major axis, GL was well separated from FL and TZ, whereas on the second major axis, FL and TZ were well separated (Supplementary Fig. S4). Similarly, the cluster analysis also distinguished soil bacteria and fugal communities in the GL from those in the FL and TZ (Fig. 3a, c). These results also indicated that the soil microbial composition was close to each other in the TZ and the FL.

3.4. Functional groups of soil fungal communities

A total of 245 genera of fungi were detected in the GL, including 105, 37, 10, and 72 genera of biotroph, saprotroph, symbiotroph, and

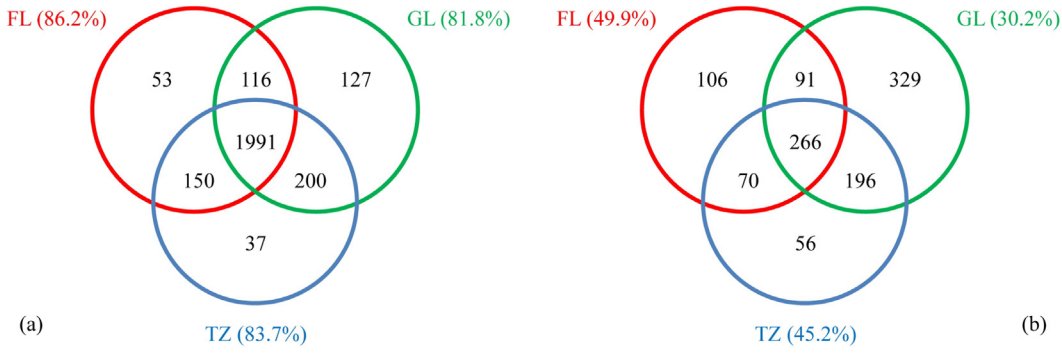

Fig. 2. OTU Venn analyses of the soil bacterial (a) and fungal communities (b) in the three land use types. The proportion of the common OTUs in each land use type was indicated in the in parentheses. GL, FL, and TZ indicate natural grassland, Chinese pine forest, and the transition zone from natural grassland to Chinese pine forest, respectively.

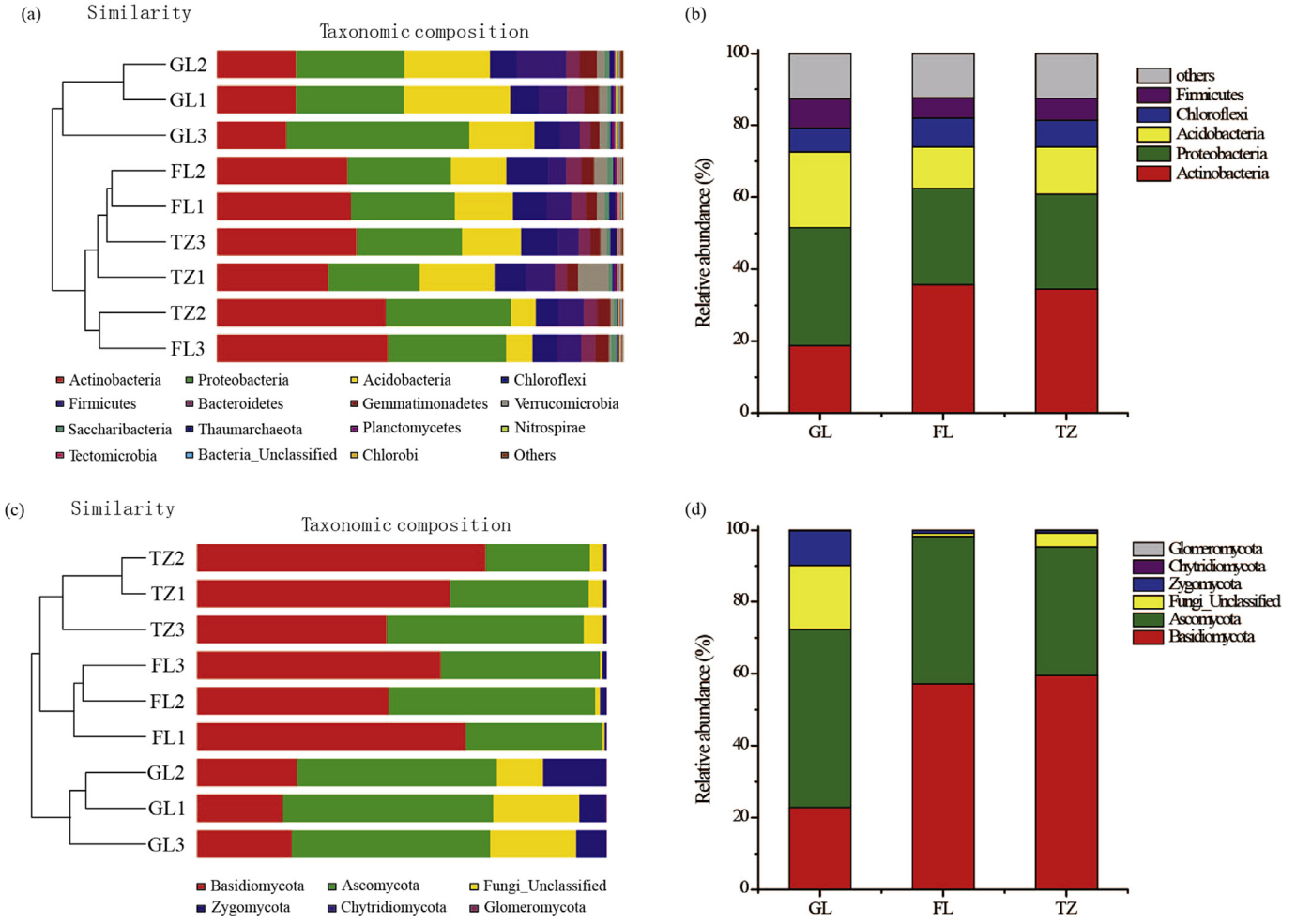

Fig. 3. Cluster and taxonomic composition of the soil bacterial (a) and fungal (c) communities at the levels of phylum. Figures in the right show the relative abundance of the major bacterial (b) and fungal (d) communities in the three land use types. GL, FL, and TZ indicate natural grassland, Chinese pine forest, and the transition zone from natural grassland to Chinese pine forest, respectively.

unclassified fungi, respectively (Supplementary Table S1). The relative abundance of the functional groups of biotroph, saprotroph, symbiotroph, and unclassified fungi were 5.8%, 30.3%, 3.4%, and 60.6%, respectively (Fig. 5). When compared with the GL, the number of fungal genera in the FL and TZ was lower (186 and 187 fungal genera, respectively) (Supplementary Table S1). Furthermore, the relative abundance of biotroph, saprotroph, symbiotroph and unclassified fungi decreased to 0.7% and 0.9%, 15.7% and 16.1%, 58.1% and 66.0% and 25.5% and 17%, in the FL and TZ, respectively (Fig. 5). Moreover, almost all of the symbiotroph fungi in the FL and TZ were ECM fungi.

3.5. Relationship between microbial community composition and soil environmental factors

The CCA explained 76.4% and 92.8% of the relationship between bacterial and fungal phylum and soil factorsacross the first two canonical axes, respectively (Fig. 6). The CCA also showed that SAN and TN were the predominant factors that both significantly affected the soil bacterial and fungal communities ($P < 0.05$). SM, clay, and SOC also had substantially, but not significantly, effects on the soil bacterial and fungal communities ($P < 0.1$) (Supplementary Table S2). In addition, the stepwise regressions models indicated that SAN was the determining factor in prediction of soil fungal diversity (Supplementary Table S3).

4. Discussions

4.1. Effects of land use change on soil microbial community

Our results refused the hypothesis that soil microbial diversity increased due to more recalcitrant litter after afforestation with Chinese pine plantation. Differences in the soil bacterial diversity were insignificant among the GL, FL, and TZ ($P > 0.05$), whereas the soil fungal diversity was significantly decreased after grassland afforestation ($P < 0.05$) (Table 2). Moreover, the proportion of shared OTUs in soil bacterial community was much higher than that in soil fungal community among the three land types (Fig. 2). These results indicated that soil fungi responded stronger to Chinese pine planted on grassland than bacteria did. This finding is in agreement with previous studies in which afforestation has been noted to typically stimulate the development of soil fungal communities (Gunina et al., 2017; Jangid et al., 2011), whereas soil bacteria appeared to be less sensitive to land use changes (Xue et al., 2016; Zhong et al., 2018). It is reported that bacterial communities are more resistant and resilient to environmental perturbations than fungi in terms of structure, diversity and biomass (Uroz et al., 2016). Bacteria are capable of metabolizing a wider range of compounds, which may explain their relative stability. In contrast, fungi depend strongly on the existing of their tree hosts (Hartmann et al., 2013), thus they would greatly response to the land use change.

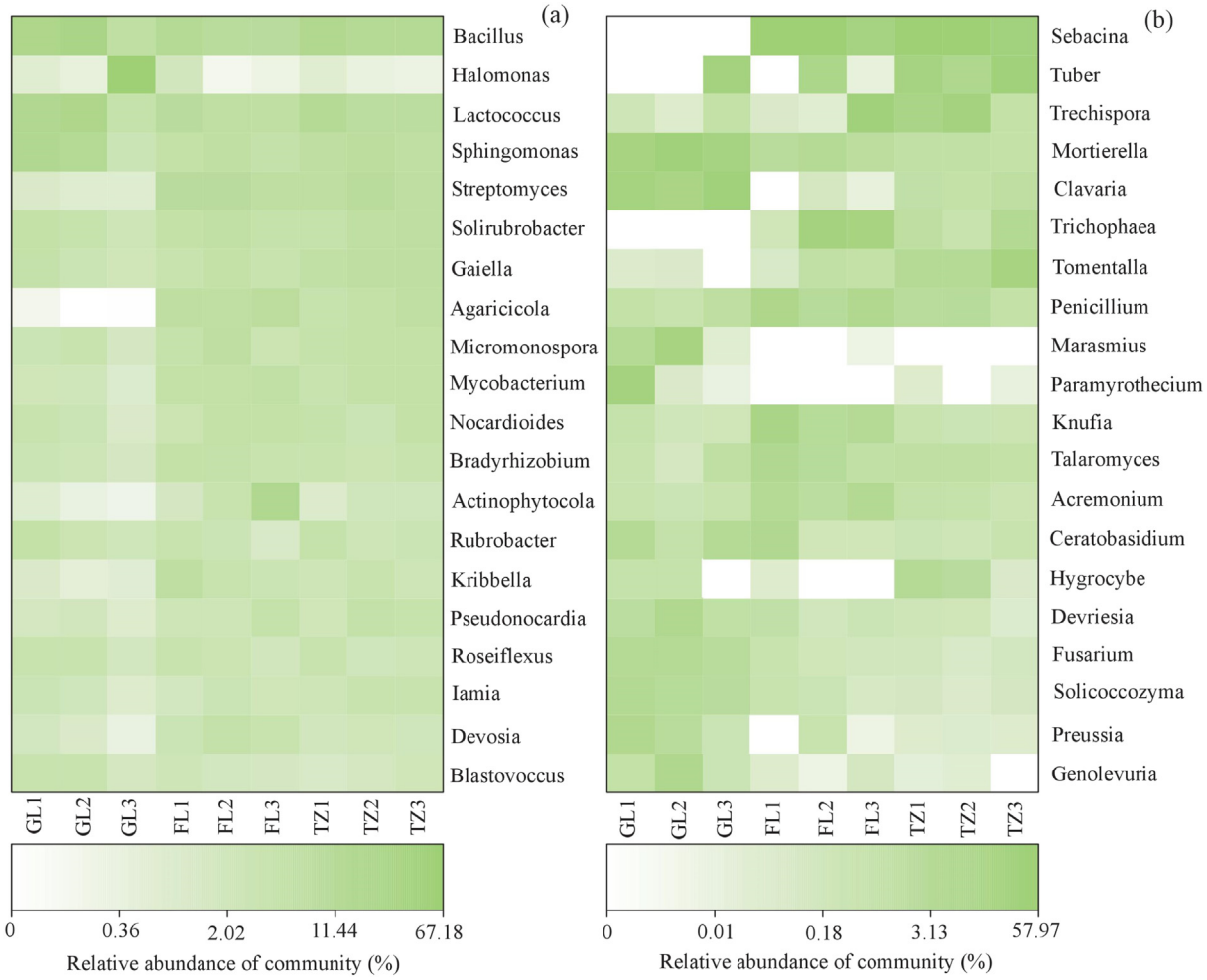

Fig. 4. Heatmap displaying the relative abundance of top 20 assigned bacterial (a) and fungal (b) genera across all the land use types. GL, FL, and TZ indicate natural grassland, Chinese pine forest, and the transition zone from natural grassland to Chinese pine forest, respectively.

In the present study, the variation patterns of soil bacterial and fungal community at the genera level across the GL, FL and TZ were generally consistent with those changes at the phylum level (Figs. 3 and 4).

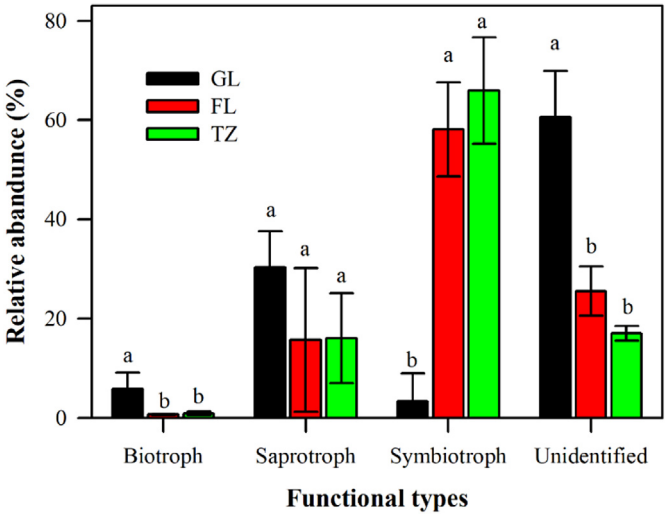

Fig. 5. Relative abundance of the fungal functional types in the three land use types. Since the coverage for AMF is incomplete using ITS primers, only ECM fungi were included in the symbiotroph group. GL, FL, and TZ indicate natural grassland, Chinese pine forest, and the transition zone from natural grassland to Chinese pine forest, respectively.

Since the low relative abundance and the high variations within group of soil bacterial and fungal community at the genera level, only the composition and relative abundance at the phylum level were discussed in the study.

At the phylum level, grassland afforestation did not change the dominant phyla of the soil bacterial community, but their relative abundances were obviously altered (Fig. 3b). The dominant bacterial phyla, included Actinobacteria, Acidobacteria, Chloroflexi, Firmicutes, and Proteobacteria, were consistent with those in previous studies (Zeng et al., 2017; Y. Zhang et al., 2016, C. Zhang et al., 2016). When compared with the GL community, the relative abundance of Proteobacteria and Acidobacteria decreased by 6.1% and 9.6%, whereas that of Actinobacteria increased by 17.0% in the FL (Fig. 3a). Actinobacteria are spore-forming bacteria that are considered to be dominant under harsh and stressful soil conditions (Dang et al., 2017; Teixeira et al., 2010), whereas Proteobacteria have the ability to rapidly grow in soil with sufficient labile substrates (Fierer et al., 2007; Y. Zhang et al., 2016, C. Zhang et al., 2016). Besides, Actinobacteria are capable of decomposing more recalcitrant organic carbon by penetrating their hypha into bulky plant tissues (Dang et al., 2017). Thus, it can be concluded that the transition in the relative abundance of Proteobacteria and Actinobacteria after land use conversion could be a result of decrease in soil nutrients (SOC, TN and SAN) in Chinese pine plantation (Table 1, Fig. 6a). However, the abundance of Acidobacteria also declined in the FL and TZ, which is in contrast to the results of previous studies that reported that Acidobacteria are also groups preferring nutrient-poor environments (Fierer et al., 2007; Zeng et al., 2017; Y.

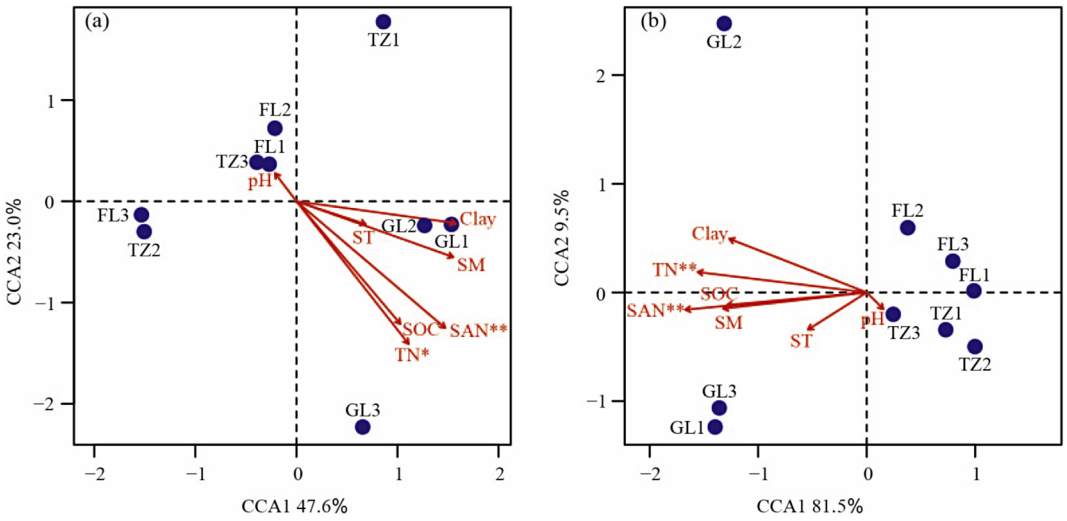

Fig. 6. CCA of the soil bacterial (a) and fungal (b) community structures and environmental parameters. Arrows indicate the direction and magnitude of the environmental parameters associated with bacterial and fungal community structures. GL, FL, and TZ indicate natural grassland, Chinese pine forest, and the transition zone from natural grassland to Chinese pine forest, respectively. TN: soil nitrogen, SOC: soil organic carbon, SAN: soil Available nitrogen, SM: soil moisture, ST: soil temperature. * indicates that the correlations are significant at $P < 0.05$. ** indicates that the correlations are significant at $P < 0.01$.

Zhang et al., 2016, C. Zhang et al., 2016). These inconsistent findings could be attributed to the numerous subdivisions in the Acidobacteria phylum that were found in a wide range of habitats in the terrestrial ecosystem (Kielak et al., 2016). It must be noted that different subdivisions show varied correlations with respect to soil nutrients. For example, while the subdivision 1 presented negative correlations with soil C, N, and phosphorous, the members of the subdivisions 5, 6, and 17 appeared to be highly abundant in more nutrient-rich soils (Kielak et al., 2016).

Basidiomycota and Ascomycota were the most abundant soil fungal phyla in all three land use types. However, the dominant fungal phylum changed from Ascomycota to Basidiomycota after grassland afforestation (Fig. 3d). This result was supported by previous studies that reported the relative abundance of Basidiomycota increased from 10.9% to 68.7% after pine planted in abandoned land for 29 years (Dang et al., 2017). As an important source of nutrients and energy for soil microorganisms, leaf litter plays an essential role in shaping the composition and structure of soil microbial community (Grosso et al., 2016). In the study, litter quality is significantly declined (lower LN, higher C/N) after grassland afforestation (Table 1), which would contribute to the shift of soil fungal community. Both the litter C/N ratio and N content were significantly correlated with bacterial and fungal communities (Purahong et al., 2016). For low-quality litter with high lignified and aromatic substrates, fungi may use extracellular peroxidases to oxidize lignin, obviously to obtain access to cellulose, N, and other nutrients that are physically or chemically protected by lignin in plant litter. However, only a fraction of fungal taxa has the ability to secrete enzymes that catalyse the degradation of complex macromolecules such as lignin (Floudas et al., 2012). And they are largely restricted to the class Agaricomycetes within the Basidiomycota (Treseder and Lennon, 2015). Meanwhile, a previous study reported a clear shift from Ascomycota to Basidiomycota with the decline of litter quality (Purahong et al., 2016). In contrast, vegetation restoration has been noted to cause a transition in the soil fungal community from Basidiomycota-dominant to Ascomycota-dominant due to an increase of available nutrient (Yang et al., 2017). In the present study, the shift from Ascomycota-dominant to Basidiomycota-dominant soil fungal community after grassland afforestation was associated with a significant decrease in SOC, TN, SAN and litter quality (Table 1).

In addition, the functional groups of the fungal community also significantly changed after land use conversion and the relative abundance of ECM fungi distinctly increased from the GL to FL and TZ (Fig. 5). It has been reported that the ECM fungi are the most widespread among trees of the temperate and boreal zones (Baldrian, 2017). As ECM fungi are strongly affected by their host, their richness is positively correlated with the proportion and species richness of ECM plants (Tedersoo et al., 2014). ECM fungi comprise 34.1% of all the taxa in the northern temperate deciduous forests, while they account for only 11.9% in grasslands, which reflecting the paucity of host plants in grassland ecosystems (Tedersoo et al., 2014). When compared with grassland, forestland provides wider niche for fungal infestation. The pine family, as a member of the well-known ECM plant taxon, that their dominance and therefore presence in greater density, provides more roots for the colonization by ECM fungi, thus yielding greater species density than grassland can (Tedersoo et al., 2014). Moreover, ECM fungi benefit from organic matter decomposition primarily through increased nitrogen mobilization (Lindahl and Tunlid, 2015), and the mycelia of ECM extend from tree roots into soil and supply trees with mineral nutrients, especially nitrogen (Baldrian, 2017). In the present study, the increase in ECM fungal abundance in the FL and TZ, when compared with that in the GL, corresponded to the decreases in the TN and SAN after land use conversion. We might conclude that the FL and TZ compensates for the decrease of available N by enhancing N uptake of functional microbial community in compared with GL.

The soil microbial communities in the TZ grouped together with those in the FL, were distinct from those in the GL, with the soil fungal communities being more distinguishable than the soil bacterial communities (Fig. 3 and S4). A previous study also reported that the soil fungal community in grasslands to forestlands transition zone was closer to that in the forest soil (Kageyama et al., 2008), which could be attributed to the similar soil nutrient conditions between the transition zone and forestland. In the present study, the TN, SAN, and C/N ratio showed similar between the TZ and FL, but were significantly higher in the GL (Table 1). It must be noted that these soil factors had the strongest influence on the composition of soil bacterial and fungal communities (Fig. 6). Plants influenced the soil microbial communities through their impact on the soil properties (Uroz et al., 2016). An earlier study demonstrated that trees were clearly dominating the soil biogeochemical cycle despite the presence of grass throughout the transition zone (Griffiths et al., 2005). In the forestland, the horizontal and vertical distribution of the root system underground is wider than that in the grassland (Schenk and Jackson, 2002), and although grass still covers the ground in the transition zone, the soil biological and abiotic processes are significantly affected by the encroachment of tree roots. A gradient

of ECM fungal inoculum radiating out from the forest edge has been reported, and the diversity of ECM fungi has been found to decline with distance from the trees. In particular, the effect of trees on soil fungi has been observed to reach as far as 20 m depending on the tree size and colonization age (Dickie and Reich, 2005).

4.2. Effects of soil environmental factors on soil microbial community

SAN and TN were the most important factors affecting the soil bacterial and fungal communities in the present study (Fig. 6; Supplementary Table S3). Previous studies also revealed that the composition of soil microbial community was significantly correlated with soil nutrients (Mendes et al., 2015; Tian et al., 2017; Yang et al., 2017; Y. Zhang et al., 2016, C. Zhang et al., 2016). Nitrogen limitation is common in most of the terrestrial ecosystems, often leading to strong competition between microorganisms and plants (Liu et al., 2016). With the increase in N availability, the taxonomic and functional traits of soil microbial communities had shift, which including decreases in the relative abundances of mycorrhizal fungi and also slow-growing, bacterial taxa (Leff et al., 2015). The increase in ECM fungi and decrease in Proteobacteria were consistent with the significant decrease of SAN and TN after grassland afforestation (Table 1). In general, N mineralization rates in grasslands are typically higher than those in forests, because grassland organic matter tends to be enriched in N than C due to their low litter C/N ratio (Griffiths et al., 2005). Owing to a low soil N content and litter quality in forestland, fungi appear to be the major decomposers of complex litter and soil organic matter and largely shape the associated bacterial communities and their activities (Baldrian, 2017). Besides, both fungal and bacterial groups contribute to improve N resource availability, which otherwise would tend to decrease during the decomposition process (Purahong et al., 2016).

Moreover, SOC, SM and Clay content also showed important effects on soil bacterial and fungal communities (Fig. 6; Supplementary Table S2). Carbon is the key resource supporting most terrestrial microbial communities. Proteobacteria require high nutrition and sufficient C substrate to keep their fast growing rate, while a low nutrition and available C substrate could only support slow growing microbial taxa (Sul et al., 2013; Tian et al., 2017). Soil moisture was another important limiting factor in arid and semiarid regions, which strongly influences soil microbial communities (C. Zhang et al., 2016; Zhao et al., 2016). An increased precipitation significantly reduced soil microbial diversity in native alpine grasslands (Y. Zhang et al., 2016); yet a decreased precipitation increased the relative mole percentage of fungal PLFAs and fungi/bacteria ratio in a semiarid steppe (Zhao et al., 2016). For soil clay, it is not only to provide protects for soil organic matter from decomposition and leaching by being bound in aggregates, but also has lager surface areas for soil microorganisms' growth (Crowther et al., 2014; Kotzé et al., 2017).

In addition, grassland afforestation had a significant impact on litter mass and quality, thus influenced soil physicochemical properties (Cavagnaro et al., 2016; Gunina et al., 2017). The increase in soil C/N ratio is likely due to larger litter inputs, and an increase in the C/N ratio of the litter produced by forest compared with that of grass species (Cavagnaro et al., 2016). A higher C/N ratio in pine forest, when compared with that in grassland, could exert selective pressure on the fungal species capable of degrading low-quality substrates, such as phenolic compounds that are commonly found in forest soils (Macdonald et al., 2009). For example, owing to the low-quality substrates in conifer forest, Basidiomycetes, including several lignin-degrading fungal species, were noted to become more abundant, when compared with those in the grassland soils with a lower C/N ratio (Allison et al., 2007; Dang et al., 2017). Similarly, in the present study, the proportion of Basidiomycetes obviously increased in the FL and TZ, when compared with that in the GL (Fig. 4d). Previous studies have reported that soil pH plays a key role in controlling the composition of microbial communities (Cao et al., 2017; Mendes et al., 2015; Tedersoo et al., 2012). However, in the present study, soil pH did not show significant effect on both soil bacterial and fungal communities due to its small gradient among land use types, similar to that reported by Macdonald et al. (2009), Tian et al. (2017) and Zhong et al. (2018).

4.3. Implications for ecological management

Owing to soil conservation and timber demand, numerous natural grasslands have been converted to artificial pine forest all over the world. Although the pine plantation has improved soil conservation of the ecosystem, when compared with natural grassland, litter quality and soil nutrients (SOC, TN and SAN etc.) have been found to decline in the pine forest (Table 1). Therefore, sustainable land use conversion from natural grassland to planted pine forest still needs to be addressed. In the present study, the soil bacterial and fungal communities were also significantly altered after planting Chinese pine in the grassland. It must be noted that the changes in the soil microbial communities are not only a response to the decline in soil nutrients, but also lay a biological foundation for further expansion of Chinese pine forest in grassland. The soil properties of the transition zone also support the above-mentioned assumption. Despite the presence of a large grassland cover in the TZ, the soil microbial community was found to be similar to that in the FL. Thus, it can be inferred that the grassland may gradually be replaced by pine forest even after discontinuation of the afforestation activity for several decades or longer. In addition, SAN was observed to play a vital role in determining the composition of both soil bacterial and fungal communities in the study area. In other words, the soil microbial community composition can be changed by altering the SAN content, thereby affecting the succession of the plant community on the ground. Therefore, in future, after the harvest of pine forest, N fertilizer should be applied to increase the SAN to regulate the composition of microbial communities and accelerate the restoration of natural grassland community.

5. Conclusions

Our results showed that the dominant soil bacterial phylum shifted from Proteobacteria to Actinobacteria, while the dominant fungal phylum transitioned from Ascomycota to Basidiomycota after conversion of grassland to Chinese pine forestland. Grassland afforestation had increased the ECM fungi but decreased the biotrophic fungi. Soil fungi were more sensitive to grassland afforestation when compared with soil bacteria. The composition and abundance of soil bacterial and fungal communities were remarkably similar between the TZ and the FL, but differed with the GL. Moreover, soil N exhibited the strongest effect in determining the soil bacterial and fungal communities. Further studies are needed to address if an increase soil N in planted pine forests could regulate the composition of microbial communities and improve soil quality at the same time and with forest history.

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