RESEARCH ARTICLE

Soil microbiome mediated nutrients decline during forest degradation process

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ABSTRACT

Degradation succession in forests is an important and serious land use/cover change problem in ecology, and during these processes soil microbial communities mediate the recycling of most important nutrients. To reveal the effect of degradation succession processes on soil microbial community diversity, structure, and species interrelationships, we collected abundant samples (21 per vegetation type) in broad-leaved forest, coniferous forest, and meadow to observe the microbial community dynamics. The results showed that diversity and structure of soil prokaryotic and fungal communities responded differently to different forest degradation processes, diversity of soil microbial communities increased during degradation processes. Soil microbial communities abundance changes may indicate that prokaryotic communities showed a living strategies change as an ecological adaption to harsh conditions during forest degradation process. While for fungal communities, their abundance changes may indicate that environmental selection pressure and plant selectivity during forest degradation process. Changes in soil prokaryotic communities and fungal communities were both correlated with soil carbon and nitrogen loss. The soil microbial interaction network analysis indicated more complex species interrelationships formed due to the loss of soil nutrients during degradation succession processes, suggesting soil microbial communities might form more complex and stable networks to resist the external disturbance of soil nutrient loss. All results suggested soil microorganisms, including bacteria, archaea and fungi, all involved in the soil nutrient decline during the forest degradation process.

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1 Introduction

Due to the global climate change, local disasters (i.e., fire, drought, pest and disease, exotic species invasion) and/or artificial intervention, forest degradation processes occur in many forest ecosystems worldwide (Cashore et al., 2006;

Zhang et al., 2007). The conversion process from forests to other land-cover types, such as shrubs or grassland, and major changes in tree species composition, all these function-damaged conversion processes are both considered to be forest degradation (Sasaki and Putz, 2009; Ghazoul et al., 2015; Celentano et al., 2017). These forest alterations could, in turn, damage the capacity of a forest to provide goods and services, leading to soil nutrients release, decrease in soil water storage, and loss of soil ecological services, particularly in mountainous forest regions (Yan et al., 2005; Cao, 2011; Ghazoul et al., 2015; Feng et al., 2017a).

In the soil, bacteria, archaea and fungi represent considerable fractions of the living biomass, and they have formed a complex community with high diversity and diverse metabolic capabilities (Falkowski et al., 2008; Zhao et al., 2014; Zhou et al., 2016). Meanwhile, they also played important roles in biological nutrient transformation processes (e.g. carbon and nitrogen cycles) across various ecosystems (Guo and Gifford, 2002; Marin-Spiotta et al., 2009; Powers et al., 2011). Forest soils could be one of the most important habitats for various microorganisms which perform critical roles in the decomposition of organic matter and the transformation of nitrogen containing compounds (Stursova and Baldrian, 2011; Baldrian, 2017; Fierer, 2017). Additionally, symbiotic bacteria and mycorrhizal fungi (Lang et al., 2011) provide nutrients to their host plants (van der Heijden, 2008). Saprotrophic fungi and some bacteria are responsible for the decomposition of labile and recalcitrant organic matter (Baldrian, 2017). Archaeal ammonia oxidizers are considered to be the most important contributors to aerobic ammonia oxidation in soil (Leininger et al., 2006). Taken together, these showed that both prokaryotic (i.e. bacterial and archaeal) and fungal communities played important roles in forest ecosystems. According to previous studies, deforestation caused significant microbial population differences (Borneman and Triplett, 1997). Generally, soil biomass C declined during deforestation process (Sahani and Behera, 2001), while surprisingly, many studies both found diversity of soil microbial communities increased during forest degradation process (Rodrigues et al., 2013; Crowther et al., 2014; Bastida et al., 2015; Navarrete et al., 2015). Specifically, consistent with expectation, different microbial groups responded differently during forest degradation process, for example, relative abundances of Acidobacteria (bacteria) and Basidiomycetes (fungi) were lower after deforestation (Crowther et al., 2014), but there was little effect of deforestation on the diversity of AM fungi (Zhang et al., 2004). Theoretical models predict that via the formation of long-lived networks of hyphae, fungal communities are more resistant (but not resilient) than bacteria to land-use change (de Vries et al., 2012). However, since previous empirical studies have focused on only bacterial communities, or only on fungal biomass, specific groups of mycorrhizal fungi (Öpik et al., 2013), while the susceptibility of both prokaryotic and fungal to forest degradation change remains unresolved.

Understanding the interaction among different species within a community and their responses to environmental changes is important and may help to ascertain the functional roles or environmental niches occupied by certain microorganisms (Faust and Raes, 2012). Ecological association networks named molecular ecological networks (MENs) through Random Matrix Theory (RMT)-based methods are widely used to explore inter-species interactions within a community. This method calculate correlations of OTU abundance across different samples, positive or negative interactions among different species could be identified, and this interactions between two OTUs describes the co-occurrence of these two OTUs across different samples. In addition, through eigenvalue analysis, key populations in community could be identified. Although molecular ecological networks (MENs) were widely used, change of soil microbial interactions among different specie during degradation process are not well studied.

Due to the presence of various cohorts of plant roots and wood debris (Saetre, 1999; Stursova and Baldrian, 2011; Baldrian, 2017), the spatial heterogeneity of forest topsoil is fairly high, which could lead to large variations in microbial communities (Heijnen et al., 1993; Grundmann and Debouzie, 2000; Ranjard and Richaume, 2001). Furthermore, the fine scale physical and chemical heterogeneity within soil particle aggregates creates micro-niches that can support a large diversity of microorganisms living within close proximity to each other and capable of a wide range of environmental functions and interactions (Dighton et al., 1997; Bird et al., 2002; Kang and Mills, 2006). Therefore, sampling strategy is critical to the investigation of soil microbiomes in such a highly heterogeneous ecosystem, so, by increasing the number of samples taken it may be feasible to capture a more complete picture of the microbial communities in these systems (Kang and Mills, 2006; Zhou et al., 2017).

In this study, we extensively sampled a temperate forest located in the Songshan Forest Reserve area. In this area, *Quercus mongolica* used to be the dominant broad-leaved tree mixed with coniferous tree *Pinus tabulaeformis*. However, due to the climate change and anthropogenic disturbance, *Quercus mongolica* suffered area loss and was gradually replaced by *Pinus tabulaeformis* or meadow (Yue et al., 2009). To investigate the belowground mechanism of forest degradation, three vegetation types, 21 soil samples per type, were chose to represent the three typical stages of this degradation (broad-leaved forest, coniferous forest and meadow). High-throughput sequencing of both 16S rRNA gene and internal transcribed spacer (ITS) were used to analyze the soil prokaryotic and fungal communities, respectively. The aim of this study is to determine (i) the effect of degradation succession on soil prokaryotic and fungal community diversity and composition; (ii) variations in the soil prokaryotic and fungal interaction networks during the degradation process and (iii) the major environmental factors driving soil prokaryotic and fungal communities.

2 Material and method

2.1 Sample site description and soil sampling method

The studied sites were located in the Songshan Forest Reserve area (115°43'44"–115°50'22"E, 40°29'9"–40°33'35"N) in Yanqing, Beijing, China. Soil samples were collected from three adjacent vegetation types, including broad-leaved forest (BF), coniferous forest (CF), and meadow (BG).

A nested sampling design was utilized to examine the microbial diversity at each of three sites (BF, CF and BG) (Fig. S1). At each site we collected and homogenized surface soil cores from 21 individual square meter plots (5–10 cm depth) in October 2015. The 1 m² plots were laid out in a cross pattern with plots adjacent to 1, 5, 20, 50 and 70 m in each cardinal direction from a center point. In total, 63 samples of three forest soils were collected for the study. After collection, all samples were stored on ice until they could be shipped back to the laboratory where they were kept at –20°C until DNA extraction.

2.2 Soil physiochemical measurement

Soil water content was measured by putting 2 g soil into 50°C oven until reaching constant weight. The percentage of weight loss after oven dry to the original weight was calculated as soil water content (%). Soil pH was measured in soil suspension with a soil:water ratio of 1:2.5 (weight:volume) by using pH meter (Sartorius Basic pH meter PB-10). Soil organic carbon (SOC) was measured by potassium dichromate oxidation-ferrous sulfate titrimetry, total nitrogen (TN) was measured by the potassium persulfate-wet oxidation method with 0.02 g soil per sample. Ammonium (NH₄-N) and nitrate (NO₃-N) concentrations were determined by potassium chloride leaching indophenol blue colorimetric method, available phosphorus was determined by sodium bicarbonate extraction-molybdenum antimony anti-coloring method (olsen method).

2.3 DNA extraction, amplification and sequencing of soil microorganisms

DNA was extracted from 0.5 g of soil using the PowerSoil DNA isolation kit (MP Biomedical, Carlsbad, CA, USA) according to the manufacturer's directions. After DNA extraction, quality and concentration of the extracted DNA were assessed based on absorbance ratios of 260/280 nm (~1.8) and 260/230 nm (>1.7) by using a NanoDrop 2000 Spectrophotometer (ThermoFisher, USA).

For each DNA extraction, we performed PCR amplification of the prokaryotic 16S rRNA gene V4 hypervariable region with primer set 515F/806R (515F, 5'-GTGCCAGCMGC-CGCGGTAA-3' and 806R, 5'-GGACTACHVGGGTWTC-TAAT-3') and fungal ITS2 region with primer set gITS-7F/4R (7F, 5'-GTGARTCATCGARTCTTG-3' and 4R, 5'-TCCTCCGCTTATTGATATGC-3') in a 50 µL reaction using 0.5µL of DNA ExTaq polymerase (TaKaRa), 5 µL 10 × PCR buffer, 1.5 µL 2.5 mM dNTP, 1.5 µL 10 mM of both forward and reverse primer, 20 µL 30ng·µL⁻¹ DNA template. Cycling

parameters were as follows: 1 cycle of 94°C for 1 min, 30 cycles of 94°C for 20 s, 57°C for 25 s and 68°C for 45s followed by a final extension at 68°C for 10 min. Both forward and reverse primers contained 12 base pair barcodes that were unique to each sample.

After amplification, the PCR amplicons were examined by agarose gel electrophoresis and purified by Gel Extraction Kit (D2500-02, OMEGA BioTek). The purified DNA was quantified based on absorbance ratios of 260/280 nm (~1.8) and 260/230 nm (>1.7) through NanoDrop 2000 Spectrophotometer (ThermoFisher, USA), and the optical density of the gel was confirmed by Gel Image System (Taxon-1600). Thereafter, a standard regression model was used to fit the net optical density and DNA concentration. A reference value that was the closest value to the regression curve was selected to obtain the required volume of 150 ng DNA, and others were referred to this value to obtain their volume based on their net optical reference. All purified DNA were pooled together and pooled DNA mixture was used to construct a library by ligating to Illumina adapters, following the protocol of VAHTSTM Nano DNA Library Prep Kit from Illumina®. Library products were quantified using Qubit assay with Qubit 2.0 Fluorometer (Life Technologies). Finally, sample library for sequencing was prepared according to the MiSeq Reagent Kit Preparation Guide (Illumina). The sequencing run was conducted on Miseq sequencing machine (Illumina) at Central South University, China, for 251, 12 and 251 cycles for forward, index and reverse reads, respectively (Feng et al., 2017b).

The 16S rRNA gene and ITS DNA sequences were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under study accession numbers SRP127999 and SRP128072, respectively.

2.4 Sequence processing

Initial sequence processing and sample assignment were done in galaxy pipeline (<http://mem.rcees.ac.cn:8080/>). First, the quality of the raw sequence data was evaluated with FastQC. Then, raw data was assigned to corresponding samples according to the unique barcodes, allowing for one mismatch. Both forward and reverse of primers were trimmed with one mismatch. After that, paired end sequences were merged into full length sequences (average 253 bp for 16S V4 hypervariable region and average 265 bp for ITS2 region) with 30 bp overlap by FLASH program (Magoc and Salzberg, 2011). Then quality control was performed by trim N and Btrim program (Kong, 2011). Trim N was used to remove sequences that contained ambiguous bases. While the Btrim program, with threshold of Quality Score >20 and 5 as window size, was used to filter out unqualified sequences. Sequences after quality filtering were clustered by using UPARSE (Edgar, 2013) at 97% similarity to produce OTUs, and representative sequences of each OTU were generated simultaneously. RDP Classifier method (Wang et al., 2007) was employed to assign prokaryotic and fungal sequences to different taxa against the Greengene and Unite database respectively.

2.5 Statistical analysis

We calculated α diversity (Shannon index, chao 1) and β diversity (Bray-Curtis Index) to describe abundance and richness of prokaryotic and fungal communities by resampled OTU Table (25000 resample size for 16S and 8000 resample size for ITS).

Soil microorganisms spatial pattern was displayed by nonmetric multidimensional scaling analysis (NMDS) based on Bray-Curtis distance. To evaluate dissimilarity of both prokaryotic and fungal communities of different terrestrial ecosystem, MRPP, ANOSIM, PERMANOVA (global test and pairwise comparison) methods were utilized to compare dissimilarity of soil microorganisms (prokaryotic and fungal) communities across studied sites. Mantel test was used to determine the correlation of soil characteristics with soil organisms community spatial pattern based on Bray-Curtis Index. The significance of the test was assessed by 999 times permutation. All above-mentioned analysis were performed by R packages *vegan* (v.2.3.5) in R (v3.2.5). The correlation between relative abundance of dominant genera and soil SOC or TN were assessed by Pearson method. The significance of comparing soil properties of three sample vegetation was determined by one-way analysis of variance (ANOVA).

2.6 Network construction with RMT-based approach and topological analysis

To elucidate soil prokaryotic and fungal interactions in response to forest degradation process, soil prokaryotic and fungal molecular ecological networks were constructed based on random matrix theory (RMT) method (Deng et al., 2012) in molecular ecological network analysis pipeline (MENA, <http://ieg2.ou.edu/MENA/>) (Deng et al., 2012). The whole process was described in a previous study comprehensively (Deng et al., 2012) and we briefly introduce the procedure used here. First, the prokaryotic OTUs appeared in more than 16 samples (21 samples totally for each group) and fungal OTUs appeared in more than 14 samples (21 samples totally for each group) were kept without log-transferring prior to obtaining Spearman rank correlation matrix (r value). A series of thresholds from 0.01 to 0.95 with 0.01 intervals were obtained and applied to the matrix, and the correlations above a specific threshold were kept for calculating the network eigenvalues. The finest threshold was selected when the nearest-neighbor spacing distribution followed Poisson distribution well, which is related to specific and nonrandom properties of a complex system (Luo et al., 2007). In addition, to compare these three networks under same conditions, a uniform threshold (0.84–0.86 for prokaryotic networks, 0.55 for fungal networks) was determined to generate three networks.

Fast greedy modularity optimization was used to separate modules (a module is a group of OTUs that are highly connected among themselves but with very few connections outside the group). Module eigenvalue is a centroid of a

module and determined by analysis of singular value decomposition (SVD), which provides the best summary of variation in relative abundance of OTUs within a module. Furthermore, the correlations between module eigenvalues and soil physiochemical properties were used to detect the modules' response to environmental changes.

Network properties were calculated to describe network differences, such as R^2 of power law, average connectivity (the number of links of one node to other nodes), modularity (measures the degree to which the network was organized into clearly delimited modules), geodesic distance (the shortest path between two nodes), and clustering coefficient (indicates how well a node is connected with its neighbors). The networks were visualized using Cytoscape 3.3.0 (Shannon et al., 2003).

3 Results

3.1 General characteristics of forest soil samples

Soil physiochemical properties were systematically measured in all 63 samples collected from the three typical vegetation types (Table S1). Among the measured soil variables, TN, $\text{NO}_3\text{-N}$, SOC, water content, pH, C/N ratio were significantly different among the three vegetation types ($P < 0.05$) (Table S2). SOC gradually, but significantly (ANOVA, $F = 4.025$, $P = 0.02$), decreased from 6.97 ± 2.85 (%) in broad-leaved forests (BF) to 5.88 ± 3.59 in coniferous forest (CF) and finally to 3.73 ± 0.82 in meadow (BG). TN also followed the same trend as SOC (ANOVA, $F = 3.09$, $P = 0.05$). In addition, $\text{NO}_3\text{-N}$, moisture content, and C/N ratio were significantly higher in coniferous and broad-leaved forests than meadow (ANOVA, $P < 0.05$). These results indicated that the soil nutrients were significantly decreased when the vegetation changed from broad-leaved to coniferous forest and finally to meadow.

3.2 Soil prokaryotic and fungal diversity changes during degradation process

The Shannon indexes for soil prokaryotic and fungal communities were calculated based on 16S and ITS Miseq sequences, respectively. The result showed that α diversity of soil prokaryotic and fungal community exhibited different trends in the three vegetation types (Figure S2A, B). For example, prokaryotic Shannon index in CF (7.44 ± 0.21) was significantly lower ($P < 0.05$) than BF (7.62 ± 0.27) and BG (7.63 ± 0.28). While no significant difference was found in fungal α diversity between these three vegetation types (Figure S2A, B). These results suggest prokaryotic α diversity significantly ($P < 0.05$) decreased during the forest degradation from broad-leaved forest (BF) to coniferous forest (CF), then may increase ($P < 0.05$) from coniferous forest (CF) to meadow (BG). However, for fungal community, both of these vegetation conversion processes may have less effect on α diversity.

To investigate the response of both prokaryotic and fungal β diversity to vegetation conversion processes, NMDS plots

were used to visualize the Bray-Curtis dissimilarity among vegetation types (Fig. 1A, B). The results showed that samples from different vegetation sites were clearly separated from each other for both prokaryotic and fungal communities, this was confirmed by three dissimilarity tests under both global tests and pairwise comparison tests ($P \leq 0.001$) (Table S3). The results from these tests showed that community structures were significantly altered during forest degradation.

3.3 Vegetation conversions alter soil prokaryotic and fungal compositions

For the prokaryotic community, the dominant bacterial OTUs were classified to Proteobacteria ($32.90 \pm 6.18\%$), Actinobacteria ($20.57 \pm 3.47\%$), Acidobacteria ($15.34 \pm 3.16\%$), Verrucomicrobia ($6.50 \pm 3.59\%$), Bacteroidetes ($6.68 \pm 2.07\%$), Chloroflexi ($3.93 \pm 0.84\%$), Gemmatimonadetes ($3.47 \pm 1.41\%$), Planctomycetes ($3.47 \pm 1.41\%$), Nitrospirae ($0.94 \pm 0.47\%$), Firmicutes ($0.73 \pm 0.65\%$) and the dominant archaeal phylum was Crenarchaeota ($2.92 \pm 2.25\%$) (Figure S3A). At finer taxonomic resolution, *DA101* ($4.84 \pm 3.76\%$), *Rhodoplanes* ($3.16 \pm 1.01\%$), *Kaistobacter* ($1.67 \pm 0.90\%$), *Bradyrhizobium* ($2.14 \pm 0.85\%$) and *Mycobacterium* ($0.92 \pm 0.36\%$) were the top five dominant genera (Fig. 2). Relative abundance of all five dominant genera changed significantly between stages of forest degradation ($P < 0.5$) (Fig. 2). Dominant phyla and genera responded differently to degradation processes (Figure S4A, Fig. 2).

The detected OTUs of fungal community were classified into 5 phyla (Figure S3B). The dominant genera included *Mortierella* ($44.57 \pm 25.36\%$), *Cryptococcus* ($2.86 \pm 2.87\%$), *Exophiala* ($1.92 \pm 5.28\%$), *Geminibasidium* ($1.37 \pm 3.58\%$), *Phoma* ($1.44 \pm 2.25\%$), *Minimedusa* ($1.10 \pm 1.80\%$), *Mucor* ($0.59 \pm 1.08\%$), *Cristinia* ($0.52 \pm 2.16\%$) and *Russula* ($0.34 \pm 0.97\%$) (Fig. 2). However, only the relative abundance of *Russula*, *Mucor*, *Geminibasidium*, *Mortierella* changed significantly during degradation processes ($P < 0.5$) (Figure S4).

3.4 The driven environmental factors for prokaryotic and fungal communities

To distinguish the driving environmental factors, Mantel test and CCA analysis were performed on both soil prokaryotic and fungal communities for all measured soil properties (Table 1, Figure S5). For soil prokaryotic community, soil water content ($r = 0.1916$, $P = 0.003$), TN ($r = 0.2291$, $P = 0.002$), SOC ($r = 0.1850$, $P = 0.011$), pH ($r = 0.2721$, $P = 0.001$) and C/N ratio ($r = 0.1826$, $P = 0.003$) were all significantly associated with alternations in the prokaryotic community, while for the fungal community, $\text{NO}_3\text{-N}$ ($r = 0.2129$, $P = 0.015$), TN ($r = 0.2028$, $P = 0.008$) and SOC ($r = 0.1589$, $P = 0.032$) were significantly correlated. This result indicated both prokaryotic and fungal communities are influenced by soil nutrients such as SOC and TN, while other factors could be divergent (i.e., pH with highest r value in prokaryotic community only).

3.5 Species interactions in prokaryotic and fungal communities during forest degradation

The phylogenetic molecular ecological network (pMEN) was performed to identify the species interactions within prokaryotic and fungal communities during forest degradation. Prokaryotic pMEN identified 276, 338 and 326 nodes (OTUs) that interacted with each other in BF, CF, and BG vegetation sites respectively (Fig. 3A, B; Table S4). Network topological indexes, such as average connectivity (avgK) and average clustering coefficient (avgCC), were higher in coniferous forest than the other two vegetation sites (Table S4), indicating that soil microbial species were more tightly linked in coniferous forest (CF) than other two vegetation sites. In pMENs, a module is a group of OTUs that are highly connected within the group, but has very few connections outside the group. A total of 10, 5, and 10 modules with greater than 5 nodes were present in BF, CF, and BG respectively (Fig. 3).

Fig. 1 Nonmetric multidimensional scaling plot of prokaryotic and fungal community (Bray-Curtis Index) across three sample sites. (A) NMDS plot of soil prokaryotic communities; (B) NMDS plot of soil fungal communities. Different land use type were separated clearly by different colors and indicated community structure changed during forest degenerative succession processes.

Fig. 2 Relative abundance of dominant phylum for soil prokaryotic and fungal communities across three sample sites. (A) Relative abundance of soil prokaryotic communities; (B) Relative abundance of soil fungal communities.

Table 1 Mantel test based on Bray-Curtis index to evaluate the relationships between various soil variables and microbial community structure.

Environmental factors	Prokaryote		Fungi	
	r_m	P	r_m	P
Water Content	0.1916	0.003**	0.1255	0.059
NH ₄ -N	0.0254	0.598	0.0361	0.258
NO ₃ -N	-0.0221	0.345	0.2129	0.015*
TN	0.2291	0.002**	0.2028	0.008**
SOC	0.1850	0.011*	0.1589	0.032*
Available P	-0.00271	0.442	-0.0264	0.599
pH	0.2721	0.001**	0.1123	0.052
C/N ratio	0.1826	0.003**	-0.1071	0.913

Significant results are indicated by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Fig. 3 Networks interactions of the soil microbial OTUs across three sample sites. The network based on RMT and fast greedy modularity optimization method. Colors of the nodes indicate different major phyla. Colors of edges indicate different interactions between OTUs, red edges indicate positive interactions and blue edges indicate negative edges. (A) Networks interactions of the soil prokaryotic OTUs across three sample sites; (B) Networks interactions of the soil fungal OTUs across three sample sites.

Moreover, soil fungal pMENs for all three vegetation sites were substantially different. A total of 51, 56, and 97 nodes, respectively, were identified in BF, CF, and BG fungal networks (Fig. 3B, Table S4). Other network indexes, including average connectivity (avgK) and average clustering coefficient (avgCC), gradually increased from BF to CF and BG. These consistent trends indicated that soil fungal community networks gradually became more complex and tighter during forest degradation process. A total of 3, 3, 4 modules were identified in fungal BF, CF and BG pMENs respectively (Fig. 3).

To further distinguish center species in the interaction networks, all nodes were divided into four categories (peripherals, connectors, module hubs and network hubs) according to the z_i (within-module connectivity) and P_i values (among-module connectivity) in ZP-plot (Figure S6). The majority of nodes could be classified into peripherals with most of their links inside their modules which accounting for 98.91%, 93.49% and 98.77% of BF, CF, and BG prokaryotic nodes respectively. Module hubs represent nodes highly connected within their own modules that could be regarded as center species in each unit. Only a few nodes were identified as module hubs in BF (3 nodes), CF (3 nodes) and BG (4 nodes). Among these module hubs, four were from Proteobacteria, while others were from Acidobacteria, Planctomycetes, Nitrospirae and Verrucomicrobia. Connectors represented nodes were highly linked to other modules acting

as bridges. Nineteen nodes were identified as connectors in CF but none in either BF or BG. These connectors including ten Acidobacteria, six Proteobacteria, and three Actinobacteria (Table S5). For soil fungal community, most of nodes were also peripherals, only one module hub from Ascomycota was found in BG (Table S5).

3.6 The associations between network topologies and environmental variables

The correlation between modular eigenvalue and environmental factors could be used to measure the responses of a group of species to different environmental variables. Most modules were significantly ($P \leq 0.05$) correlated with one or more soil variables, indicating species in those modules could respond to certain environmental factors (Fig. 4A, B). However, it seems that modules in soil prokaryotic networks were more significantly influenced by soil properties than those of fungal networks during degradation processes. For example, in BF, CF and BG prokaryotic networks, more than half of modules were correlated with TN, SOC and pH, while none of the environmental factors could affect more than one third of all modules in fungal networks. In addition, in three prokaryotic networks, no modules were significantly correlated with available P, but there were three modules in fungal networks were significantly correlated with available P, indicating available P had more impacts on shaping fungal community than prokaryotic community.

Although above results revealed that the majority of prokaryotes were more sensitive to soil properties than fungi, the network analysis demonstrated soil nutrients (e.g. SOC, TN, $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$) had large impacts on microbial interactions (Fig. 4), indicating soil carbon and nitrogen nutrients significantly influenced microbial compositions and species interactions during vegetation degradation processes.

4 Discussion

Due to more tremendous global and local environmental changes, as well as anthropogenic disturbance, forest degradation occurs everywhere (Cashore et al. 2006) including our sampling site Songshan forest reserve area (Li et al., 2008). In our studied temperate forest, two sequential conversions of the dominant plant species, from broad-leaved trees to coniferous trees, and further from coniferous trees to shrubs and/or grasses, have been regarded as common symptoms for forest degradation. To investigate the changes in soil prokaryotic and fungal communities during these two typical conversional processes, we used high-throughput sequencing technology to compare the soil microbial diversity, composition, and molecular ecological networks in broad-leaved forest (BF), coniferous forest (CF) and meadow (BG). We found the diversity of microbial communities in these forest soils were significantly altered when the vegetation types converted from BF to CF and BG, and that both conversional processes were highly correlated to soil nutrients (including both carbon and nitrogen) loss.

Forest soils are regarded as highly spatial heterogeneous environments (Baldrian, 2017), and this spatial heterogeneity had two major impacts on soil microbiomes. First, soil and litter physicochemical properties, such as organic matter content, pH value and other nutrients, could dramatically alter both prokaryotic and fungal communities (Fierer and Jackson, 2006; Lauber et al., 2008; Rousk et al., 2010). Second, vegetation composition, which is also important to microbial communities and may impact fungi to a greater degree than bacteria or archaea (Prescott and Grayston, 2013; Urbanova et al., 2015; Tedersoo et al., 2016). Microbial activity and biomass normally vary at the centimeter to meter scales but are often elevated in “hotspots,” for example, active microorganisms may be greater in the “hotspots” rhizosphere than in the bulk soil, in terms of respiration, growth rates, and RNA/DNA concentrations (Baldrian et al., 2010; Anderson et al., 2014). Thus sampling strategy is important to reveal the true state of soil microorganisms in forests. A large number of samples could be effective to cover microorganisms in environments with high spatial heterogeneity such as soil (Zhou et al., 2017). In our study, a high number of samples, within a nested design, were taken in each vegetation type. It was hoped that this sampling method would help to eliminate sampling errors and reflect the true composition of the soil microbiome during degradation processes.

Diversity of soil microbial communities are increased during forest degradation process, which are consistent with previous studies (Rodrigues et al., 2013; Crowther et al., 2014; Bastida et al., 2015; Navarrete et al., 2015). But soil microbial communities abundance changes may indicate that prokaryotic communities showed a living strategies change as

Fig. 4 The correlations between module eigenvalues and environmental traits in soil microbial community across three sample sites. The color of each plot indicates the correlation between corresponding module eigenvalue and environmental trait. (A) Heatmap of prokaryotic network module eigenvalues and environmental traits relationships. Red color means highly positive correlation and blue color means highly negative correlation. (B) Heatmap of fungal network module eigenvalues and environmental traits relationships. Significant results are indicated by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. These results indicate soil nutrient such as carbon and nitrogen both significantly influenced soil microbial interactions in degenerative succession processes.

an ecological adaption to harsh conditions during forest degradation process. For example, relative abundance of Proteobacteria and Verrucomicrobia both changed significantly during the process, but they responded differently to forest degradation, relative abundance of Proteobacteria decreased and Verrucomicrobia increased, these are reasonable because Proteobacteria are generally considered copiotrophic bacteria and Verrucomicrobia are generally considered oligotrophic bacteria (Borneman and Triplett, 1997; Bastida et al., 2015), suggests that higher soil C content might have selectively favored copiotrophic microorganisms with diverse carbohydrate metabolism. However, compared with bacterial communities, less change of archaea communities was detected, only crenarchaeota exhibited changes during forest degradation processes in our results. It might because less archaea existed in soil than bacteria and was more difficult to be detected by our universal 16S V3 primer than bacteria. For fungal communities, their abundance changes may indicate that environmental selection pressure and host specificity during forest degradation process. For example, *Mortierella* genus as the most common in soils of primary forests. *Mortierella alpina* is a common soil fungus that produces large amounts of arachidonic acid (Scupham et al., 2006), the frequency of these species is generally high in decomposing needles litter layer (Osono et al., 2006), both these results can be proved by our results. What's more, in BG grassland, relative abundance of Glomeromycota is significantly increased compared with CF, the phylum is composed almost entirely of AMF, although this phylum often appear to have little host specificity (Frac et al., 2018). abundance of the phylum were found decreased after N and P addition into the soil, which also can be confirmed by our results.

Understanding the changes in both prokaryotic and fungal communities with soil SOC is essential to reveal the mechanism under forest degradation. In our studied site, broad-leaved forest (BF) posed the highest SOC, while meadow (BG) held the lowest SOC. This SOC variation was closely related to the changes of soil microbial communities in three aspects. First, SOC was significantly correlated to both prokaryotic and fungal communities ($P < 0.05$) in Mantel tests (Table 1), which has been observed in a previous temperate forest study in Germany (Kaiser et al., 2016). Second, network eigenvalue analysis showed the majority of module eigenvalues in both prokaryotic and fungal networks were significantly ($P \leq 0.05$) correlated with soil nutrients, either positively or negatively (Fig. 4), indicating many species in those modules could sensitive to changes in SOC. Third, the relative abundance of multiple dominant prokaryotic and fungal taxa were consistent with SOC variation during forest degradation processes (Table S6), while their ecological functions may explain how they are involved in the SOC changes (Fig. 2). For example, *DA101* (Verrucomicrobia) was the most abundant genus across all three vegetation types, but its relative abundance was relatively lower in meadow than the two forests. This result was consistent with a recent study

(Shen et al., 2017), which showed that genus *DA101* had an oligotrophic life-historic strategy in soil and was therefore more competitive in low carbon resource conditions found in meadows. Additionally, the relative abundance of another dominant genus *Kaistobacter* was lower in BF than either CF or BG. The species in this genus are all photosynthetic bacteria, which permits their survival in a nutrient-limited environments (Yang et al., 2016). These opposing trends in *DA101* and *Kaistobacter* might be highly related to soil SOC-loss during the forest degradation process. In the fungal community, SOC variation was significantly correlated with the change in the dominant genus *Mucor* (Table S6). *Mucor* is a fast growing saprotrophic fungus which is commonly found in many nutrient-rich environments (Osono, 2006; Sharma et al., 2011; Voriskova et al., 2014; Yao et al., 2017), specifically during the pioneering phases of cellulose colonization in forest soil (Pinzari et al., 2014). All of these results suggested that some dominant prokaryotic and fungal genera participate in SOC-loss during forest degradation process.

In addition to SOC, soil microorganisms involved in many N transition bioprocesses which could also mediate soil nutrient loss during forest degradation. In this temperate forest, soil TN gradually decreased from BF to CF, and then to BG, but $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ did not follow this trend (Table S1), indicating TN might be the key environmental variable to reflect forest degradation. This declining trend of TN was significantly related to soil microbial communities in terms of diversity, composition, and species interactions. First, TN was significantly correlated to both prokaryotic and fungal communities (Mantel, $P < 0.05$) (Table 1), indicating soil microbial β diversity was highly associated with nitrogen loss that was demonstrated by Kaiser et al. (Kaiser et al., 2016). Second, a number of module eigenvalues in both prokaryotic and fungal networks were significantly correlated ($P \leq 0.05$), either positively or negatively, with TN (Fig. 4), indicating soil nitrogen content might affect majority of microbial species in the networks. Third, the relative abundances of several N-related dominant genera co-varied with TN changes among the vegetation types. For example, the dominant genus *Rhodoplanes* (Proteobacteria) decreased significantly in accordance with TN decline during forest degradation process (Table S6). This can be explained by function of *Rhodoplanes*, which consists of a group of purple non-sulfur, denitrifying bacteria and carry out energy transformations and nitrogen cycling in soil (Lin et al., 2011). The dominant fungus *Russula* had positive linear correlations along the TN gradient (Table S6). Species in this genus favor richer soils with elevated concentrations of inorganic N (Kranabetter et al., 2009). In previous studies, some species of *Russula* are frequently found as ectomycorrhizal fungi and appear to be especially nitrophilic (Avis and Charvat, 2005; Cox et al., 2010; Avis, 2012). All of the above evidences suggested that prokaryotic and fungal communities are involved in N decline during forest degradation process, and some dominant genera may mediated this process directly.

Soil microbial networks results showed species interaction

networks were significantly altered and exhibited a overall trend of complexity. Although prokaryotic and fungal α -diversities showed different trends during those two conversions (Fig. 3), species interaction networks demonstrated both prokaryotic and fungal communities tended to become more complex in response the high stress from vegetation degradation. Both soil prokaryotic and fungal network properties such as total node number, average connectivity (avgK), and average clustering coefficient (avgCC) indicated species interactions became more complex during the vegetation shifting from BF to CF (Table S4, Fig. 3), while from CF to BG the fungal community become even more complex (Table S4, Fig. 3). Increasing community complexity is thought to be conducive for microbial community resistance to external disturbances such as the nutrient loss (Zhang et al., 2017).

The relationships between environmental factors and soil prokaryotic and fungal network networks dynamics, in some sense, might reflect the sensitivity of soil prokaryotic and fungal communities to soil physicochemical properties change during forest degradation, as well as the stability of interactions when soil physicochemical properties changes. According to our results, prokaryotic networks were more significantly influenced by soil properties than those of fungal networks during degradation processes, this may indicate that interactions between prokaryotic taxa within communities were more vulnerable and more likely to change with soil properties change such as soil nutrients and pH, water content than fungal communities. While for fungal communities, their inter-species interactions could be more stable and not easily influenced by soil properties change during forest degradation process. On the other hand, soil fungal taxa might have more tight relationships with plants instead of each other, consequently their interactions might be fewer and these fewer interactions should be more relevant to plants changes than soil properties during forest degradation (Mueller et al., 2014).

In summary, our results showed that soil prokaryotic and fungal communities were significantly altered during the forest degradation process, and these variations were highly associated with soil nutrient loss, especially soil organic carbon and total nitrogen contents. The species interaction networks indicated more complex networks could be formed under high degradation stress, suggesting soil microbial communities might form more complex and stable networks to resist the external disturbance of soil nutrients loss. All of our results suggested soil microbial communities, including bacteria, archaea and fungi, involved in the soil nutrient decline during the forest degradation process, and this belowground mechanism needs more attentions when we apply any strategies on forest protection and land conservation.

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Electronic supplementary material

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