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Change in composition and potential functional genes of soil bacterial and fungal communities with secondary succession in *Quercus liaotungensis* forests of the Loess Plateau, western China

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ABSTRACT

Vegetation succession is one of the most important factors driving changes in microorganisms. It is unclear, however, how the microbial composition and the potential function of C and N cycling change with forest secondary succession. Using soil metagenomic sequencing methods, we studied these changes in bacterial and fungal communities with secondary succession from cropland to a *Quercus liaotungensis* forest over approximately 120 years on the Loess Plateau of China. The results revealed the following. (1) Soil microbial biomass C, N, and P increased significantly in topsoil (0–20 cm) with vegetation succession. (2) The abundances of bacteria increased initially and then decreased slightly, whereas an increase in fungal abundances and the ratio of fungi to bacteria was detected along a successional gradient. Microbial communities tended to shift from r- to K-strategists, both at the phylum and genus levels. (3) With vegetation succession, the abundances of C and N cycle-related potential functional genes first significantly increased and then stabilized. Among them, the relative abundances of recalcitrant C degradation-, N fixation-, and ammonification-related genes increased, whereas labile C degradation-, N reduction-, and denitrification-related genes tended to decrease. (4) Redundancy analysis indicated that bacterial communities were influenced by available phosphorus contents and soil C: N ratios, and that fungal communities were mainly affected by ammonium N contents and root biomass. (5) Predicted microbial functional genes were affected by ammonium N and activated C contents. Our study showed that with vegetation succession, microbe communities tended to shift from r- to K-strategists both at the phylum and genus levels, which increased the abundance of organisms expressing C- and N-cycle related genes.

1. Introduction

Secondary succession is an effective way to restore degraded ecosystems and has attracted increased attention due to the increased amount of abandoned cropland (Finegan, 1984; Zhong et al., 2018). At present, changes in plant community composition and soil properties during post-agricultural succession are well- documented (Carney and Matson, 2006; Noah and Jackson, 2006; Lozupone, 2007; Wu et al., 2008). The microbial communities can decompose litter and provide mineral nutrients for the growth of plants during secondary succession (Zhang et al., 2016). However, microorganisms, as the main participants in the process of succession, have been rarely studied. Therefore, understanding changes in microbial composition and potential functional genes could provide knowledge regarding the mechanisms that drive successional dynamics.

Regarding plant community succession, Odum (1969) reported that r-strategy species with a faster growth rate and higher turnover rate predominate in early successional stages, whereas K-strategy species with a slower growth rate and higher competitive capacity predominate in late succession. However, it is unclear whether soil microbes, which are affected by vegetation succession, tend to shift from r- to K- strategists during succession. Previous meta-analysis and individual studies

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have proven the significant increase in the fungi-to-bacteria ratio in a microbial community during natural succession of abandoned cropland (Jia et al., 2010; Zhou et al., 2017). Those studies indicated a possible shift from r- to K-strategists because bacteria tend to be r-strategists and fungi tend to be K-strategists (Bardgett et al., 2005; Kaiser et al., 2014; Zechmeister-Boltenstern et al., 2015; Chen et al., 2016). Therefore, we expect that the microbial community would also shift from dominant r-strategists to dominant K-strategists as the ratio of fungi to bacteria increases with the succession of plant communities.

Furthermore, the newest generation sequencing technologies offer new insight for studying belowground communities by profiling microbial composition at a finer taxonomic level. Some studies have addressed the changes in bacterial communities, with the common phyla Acidobacteria and Actinobacteria regarded as K-strategists and the phyla Proteobacteria and Bacteroidetes considered r-strategists (Fierer et al., 2007; Zechmeister-Boltenstern et al., 2015). Moreover, in a meta-analysis, the abundances of Proteobacteria and Bacteroidetes were found to significantly decrease and that of Acidobacteria was found to increase during secondary succession (Fierer et al., 2007). Zechmeister-Boltenstern et al. (2015) also reported that the bacterial phyla Acidobacteria and Actinobacteria dominate the late successional stage. To some extent, this phenomenon suggests that vegetation succession causes bacterial communities to shift from dominant r-strategists to dominant K-strategists. In addition, numerous studies have indicated a similar successional strategy in fungal communities. For example, Zumsteg et al. (2012) investigated changes in a microbial community in exposed rock substrates of the Damma glacier forefield and found that the most abundant fungal phyla shifted from Ascomycota (r-strategists) to Basidiomycota (K-strategists). Similarly, Bastian et al. (2009) observed an increase in Basidiomycota abundance in wheat straw soil as incubation proceeded. However, previous studies have focused mainly on microbes at the phylum level, failing to demonstrate finer taxonomic resolution. We hypothesized that as the secondary forest succession of *Quercus liaotungensis* proceeds, abundances of the phyla Acidobacteria, Actinobacteria, and Basidiomycota would increase, whereas abundances of the phyla Proteobacteria, Bacteroidetes, and Ascomycota would decrease; moreover, at the genus level, bacterial and fungal communities might shift from r- to K-strategists.

Soil microorganisms are pivotal players in soil C and N cycles (Palomo et al., 2016; Allison and Goulden, 2017). Understanding the changes in potential functional genes during secondary succession can provide more direct insight into how environmental changes influence microbial processes that relate to ecosystem functions. However, how the secondary succession of vegetation changes the microbial composition and soil C and N cycle functions is disputed. Previous studies that have focused on related functional genes relied on quantitative polymerase chain reaction (qPCR) to quantify microbial communities in soil samples. For example, Liu et al. (2018) found that C degradation genes and N cycle-related genes increase in biological soil crusts along a revegetation chronosequence in the Tengger Desert. Similarly, Zeng et al. (2016) described an increase in N cycle-related genes along deglaciated forelands, and Blaud et al. (2018) reported that the abundances N cycle-related genes increase during secondary succession from cropland to forest. Overall, it appears that with vegetation succession, the abundances of C and N cycle-related genes in soil microbes increase. However, Zhong et al. (2018) observed that changes in predicted microbial functional genes relied on shotgun DNA sequencing methods. They found that the relative abundance of C cycle-related genes decreased and that the relative abundance of N cycle-related genes first increased and then decreased during secondary succession from grassland to forest. That may be because of differences in vegetation types in different ecosystems. Furthermore, metagenomics have only been used in a few studies of ecosystem restoration and succession. In addition, several studies have shown that microbial composition and potential function are determined mainly by the dominant species in an ecosystem (Urbanová et al., 2015). If the dominant plants are unchanged with succession, potential microbial C and N cycle functions might either increase or decrease. However, many researchers have found that soil microbe composition and functions shift because the soil properties and composition of plant species change with forest secondary succession even when the dominant plant does not change (Sun and Badgley, 2019). Overall, the relationship between microbial function and dominant plant species needs to be verified by additional field experiments. In this study, we hypothesized that the abundances of C and N cycle-related genes would increase as succession proceeds with the same dominant tree species.

To test our two hypotheses, we investigated shifts in microbial composition and potential function during the conversion of land from cropland to *Q. liaotungensis* forest at 30, 60, and 120 years of succession. We also determined factors underlying these changes.

2. Materials and methods

2.1. Description of the study area

This study was conducted in a *Q. liaotungensis* natural secondary forest located in the Renjiatai forest region of Fuxian county (36°04′–36°05′N, 109°08′–109°11′E), which is located on the southern Loess Plateau, Northwest China. This region of the Loess Plateau has hills and valleys at an elevation of 1157–1396 m. It has a warm, temperate, continental monsoon climate with a mean annual temperature of 9 °C with 576.7 mm of precipitation, 2671.0 h of sunshine, and a frost-free period 150 days each year. The soil type is calcareous cinnamon soil and classified as Ustalfs (FAO Soil Taxonomy).

This ecosystem is very fragile because of soil erosion caused by inappropriate agricultural practices (Wu et al., 2004). To address this problem, since 2000, the Chinese government has implemented policies aimed at returning agricultural land on the Loess Plateau to forests or grasslands. In addition, in some areas close to our study site, cropland has been abandoned and restored to its native vegetation since 1842. Thus, forests (*Q. liaotungensis*) of various ages, from young stands to mature stands, have been observed in this region over the past approximately 160 years (Wang et al., 2010). Here, we used a method of space-for-time substitution to study the effects of secondary succession from cropland to forest. Our group investigated four sites as follows: cropland (CL) and forests in which the predominant species was 30-, 60-, and 120-year-old *Q. liaotungensis* (QL30, QL60, and QL120). Four replicate plots (20 m × 20 m) were established at each site in 2014, and then two 5 m × 5 m and three 1 m × 1 m quadrats were randomly selected from each plot to investigate the composition of trees, shrubs, and herbs, respectively. The site characteristics are shown in Table S1 and were provided by Sun and Badgley (2019).

2.2. Soil, plant sampling, and soil property measurements

The soil was sampled in September 2018. Soil cores with a 38-mm inner diameter were collected at a depth of 20 cm from eight randomly selected locations in each plot and combined into one composite sample. Samples were sieved through a 2-mm screen to remove roots and other debris, and at each forest site, the humus layer was removed. Each sieved sample was divided into two subsamples. One subsample was air-dried and then divided into two parts, with one part sieved through a 0.25-mm mesh and used to determine soil organic C (SOC), total N (TN), total phosphorus (TP), and activated C (EC) contents and the other part sieved through a 1-mm mesh and used to determine pH, soil nitrate N (NN), soil ammonium N (AMN), and soil available phosphorus (AP) content. The other subsample was also divided into two parts. One part was stored at 4 °C until dissolved organic C (DOC), soil microbial biomass C (MBC), soil microbial biomass N (MBN), and soil microbial biomass P (MBP) were measured. The other part was stored at −80 °C until soil DNA was extracted. Additionally, using metal cylinders, soil cores 50 mm in height and 50 mm in diameter

were obtained at a depth of 200 mm, with two replicates, to determine the bulk density and water content of soils.

Root biomass at different soil layers was collected using a root auger with a diameter of 10 cm, and subsequently oven-dried at 70 °C for 36 h to obtain its weight. Furthermore, 20 samples were collected along an S-shaped pattern from each plot, and every sample was collected as far away from the trees as possible to ensure that they represent the roots of the entire ecosystem. Litter biomass was determined by drying branches and leaf litter at 70 °C for 36 h. Sample plots in the forest, shrub, and herbaceous communities were 20 m × 20 m, 5 m × 5 m, and 1 m × 1 m, respectively, and used to assess plant diversity. In each quadrat, the number of plant species was counted. The Shannon diversity index of the plant community (SI) was calculated using the equation of Cheng et al. (2016), where P_i is the relative abundance of each species in total and n is the number of species.

The chemical and physical properties of soil samples were determined using standard procedures. SOC was measured using the H_2SO_4-$K_2Cr_2O_7$ method. TN was measured using the Kjeldahl method (Bremner and Mulvaney, 1982). Soil TP was determined colorimetrically after digestion with H_2SO_4 and $HClO_4$. Soil EC was determined using the 333 mM $KMnO_4$ oxidized method (Chan et al., 2001). DOC was extracted with deionized water after shaking for 1 h and then filtering the soil through prewashed cellulose acetate filters (0.45-μm pore size). DOC concentrations were determined using a TOC analyzer (Liqui TOC II, elementar, Germany). NH_4^+-N and NO_3^--N from filtered 2.0 mol l^{-1} extracts of fresh soil were measured with a flow injection autoanalyzer (OI Analytical, College Station, USA). Soil AP was measured by molybdenum - antimony colorimetry using Na $(HCO_3)_2$ extracts (Olsen and Sommers, 1982). Soil pH was determined in 1:2.5 (w: v) solutions. C: N was represented by the SOC: TN ratio of soils. Soil microbial biomass was measured by chloroform fumigation, MBC was determined using a TOC analyzer (Liqui TOC II, elementar, Germany), and MBN was determined by ultraviolet spectrophotometric colorimetry (Hitachi UV2300). MBP was measured by molybdenum - antimony colorimetry using $Na(HCO_3)_2$ extracts.

2.3. DNA extraction, library construction, and metagenomic sequencing

Total genomic DNA was extracted from soil samples using an E.Z.N.A. DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's instructions. The concentration and purity of extracted DNA were determined using a TBS-380 and NanoDrop 2000, respectively. DNA extract quality was evaluated on a 1% agarose gel.

DNA extracts were fragmented into an average size of approximately 300 bp using a Covaris M220 ultrasonicator (Gene Company Limited, China). A paired-end library was constructed using the TruSeqTM DNA Sample Prep Kit (Illumina, San Diego, CA, USA). Adapters containing the full complement of sequencing primer hybridization sites were ligated to the blunt ends of fragments. Paired-end sequencing was performed on an Illumina HiSeq4000 platform (Illumina Inc., San Diego, CA, USA) at Majorbio Bio-Pharm Technology Co., Ltd., (Shanghai, China) using a HiSeq 3000/4000 PE Cluster Kit and HiSeq 3000/4000 SBS Kit according to the manufacturer's instructions (www.illumina.com). Sequence data associated with this project have been deposited into the NCBI Short Read Archive database (accession number: PRJNA561480).

Adapter sequences were stripped from the 3′ and 5′ ends of paired-end Illumina reads using SeqPrep (https://github.com/jstjohn/SeqPrep). Low-quality reads (length < 50 bp, quality value < 20, or N bases) were removed in Sickle (https://github.com/najoshi/sickle). Therefore, an average of 14.9 Gb of paired-end reads were obtained for each sample, totaling 238.4 Gb of high-quality data. Metagenomics data were assembled using MEGAHIT (Li et al., 2015) (https://github.com/voutcn/megahit), which makes use of succinct de Bruijn graphs. Contigs with lengths ≥300 bp were selected as the final assembling result, and then the contigs were used for further gene prediction and annotation (Table S2).

Open reading frames (ORFs) from each assembled contig were predicted using MetaGene (Hideki et al., 2006) (http://metagene.cb.k.u-tokyo.ac.jp/) (Table S2). The predicted ORFs with lengths greater than 100 bp were retrieved and translated into amino acid sequences using the NCBI translation table (https://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/index.cgi?chapter). All predicted genes with a 95% sequence identity (90% coverage) were assigned to clusters using CD-HIT (Fu et al., 2012) (http://www.bioinformatics.org/cd-hit/), and the longest sequence from each cluster was selected as a representative sequence to assemble a non-redundant gene catalog. Reads remaining after quality control were mapped to these representative sequences with 95% identity using SOAP aligner (Li et al., 2008) (http://soap.genomics.org.cn/), and the abundances of genes in each sample were evaluated.

Taxonomic assignment of non-redundant genes was carried out using BLASTP (Version 2.2.28+, http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul et al., 1997) against the NCBI-NR database with an e-value $\leq 10^{-5}$. The BLASTP results were annotated using MEGAN 5.2.3 (Huson et al. 2011) for the assignment of taxonomic genera. The abundance of a taxonomic group was calculated by summing the abundance of genes annotated to a feature. At the phylum and genus level, the abundance of a taxon below 1% was combined into others as shown in. Functional annotation of all ORFs was conducted using hmmscan (http://hmmer.janelia.org/search/hmmscan) and BLASTP against Carbohydrate-active enzymes (CAZy) version 5.0 (http://www.cazy.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases (Chen et al., 2011) (http://www.genome.jp/keeg/), also with an e-value $\leq 10^{-5}$.

2.4. Calculations and statistical analyses

The relative abundance of bacterial phyla was the ratio of the abundance each bacterial phylum to bacterial total abundance. Furthermore, the relative abundance of fungal taxa and functional categories and taxa were determined in the same manner. The relative abundance of C cycle genes was calculated based on the ratio of the abundance of C cycle genes to total KEGG pathway level gene abundance. The relative abundance of N cycle genes was calculated based on the ratio of N cycle gene abundance to total KEGG Orthology gene abundance. The relative abundances of nitrogen fixation genes, nitrate reduction genes, denitrification genes, and ammonification genes were calculated based on the ratios of nitrogen fixation genes, nitrate reduction genes, denitrification genes, and ammonification genes to the abundance of N cycle genes, respectively. The relative abundances of cellulose degradation genes, cellulose transport genes, hemicellulose degradation genes, chitin degradation genes, sugar utilization genes, and sugar transporter genes were calculated based on the ratios of cellulose degradation genes, cellulose transport genes, hemicellulose degradation genes, chitin degradation genes, sugar utilization genes, and sugar transporter genes to C degradation gene abundance. An analysis of variance (ANOVA) and the least significant difference (LSD) multiple comparisons test ($P < 0.05$) were used to determine the significance of differences in microbial diversity indexes (Shannon-Wiener), abundances, and soil and plant factors among various successional stages. Statistical analyses of the data were conducted in SPSS 22 for Windows (SPSS Inc, Chicago, USA). A principal component analysis (PCA) was used to test differences in plant factors (i.e. Shannon-Wiener index, roots biomass, litter biomass) and soil physicochemical characteristics (e.g. total SOC, TN, TP, pH) between the successional stages. A redundancy analysis (RDA) was implemented to elucidate the relationships between microbial genes and soil factors during succession. Monte Carlo permutation (999 repetitions) tests were used to identify significant associations between litter and soil factors and gene changes. An analysis of similarity (ANOSIM) in the R vegan package was used to test for differences in microbial taxa and

gene families within a group and between different groups. Similarities based on microbial taxa (bacteria and fungi) and abundances of genes (KEGG and CAZy databases) were evaluated by Bray-Curtis distance testing of phylogenetic relationships and the Euclidean dissimilarity index for gene families, respectively. Principal coordinates analysis (PCoA) was used to evaluate overall differences in the structures of bacterial and fungal communities based on Bray-Curtis distances between successional stages. PCoA, ANOSIM, RDA, and PCA were conducted using R software package v.3.6.0. (R Core Team, 2014).

3. Results

3.1. Changes in plant community characteristics and soil properties with secondary succession

The contents of SOC, TN, DOC, and EC at the QL30 site increased markedly compared to those in CL, whereas NN, AP, and MOI contents significantly decreased. During the forest succession process, AMN and DOC contents and soil moisture significantly increased, whereas pH significantly decreased. Furthermore, DOC content was the highest at the QL60 site. Other soil physicochemical characteristics showed no significant changes (Table 1). The Shannon-Wiener index of plant communities significantly increased from CL to QL30; there was no difference between the QL30 and QL120 sites, but the index was significantly higher in these samples than in the QL60 site. In addition, litter biomass and root biomass significantly increased from CL to QL30, and thereafter showed no significant change with forest succession (Table 1). The PCA analysis base on the soil physicochemical characteristics (Fig. S1a) and plant characteristics (Fig. S1b) showed that the soil properties of QL30 and QL60 were similar to each other but differed from those of CL and QL120, whereas the plant characteristics of forest were similar to each other but differed from those of cropland.

3.2. Changes in soil microbial biomass and gene abundances during secondary succession

Fig. 1 shows that MBC, MBN, and MBP increased significantly with secondary succession. The lowest and highest microbial biomasses were observed in CL and QL120, respectively. The minimum MBC, MBN, and MBP were 132.20 (mg/kg), 17.82 (mg/kg), and 44.15 (mg/kg), respectively, and the maximum MBC, MBN, and MBP were 415.64 (mg/kg), 123.70 (mg/kg), and 90.74 (mg/kg), respectively.

Abundance profiles were obtained by comparing sequences from samples with those in the NCBI-NR database. In total, 64,830,374 total genes, 62,500,588 bacterial genes, and 36,692 fungal genes were screened in all metagenomes. Furthermore, the annotation results showed that total gene abundance, bacterial abundance, and fungal abundance significantly increased when the cropland transformed into the forest and then did not differ significantly among forest succession (Table S2). The ratio of fungal to bacterial gene abundance slightly increased with successional stages (Fig. 2b). The Shannon index indicated that diversity of the soil bacterial community of QL30 decreased markedly compared to that of CL and then remained stable with time. Conversely, the Shannon index showed that the diversity of soil fungal communities of QL30 increased markedly compared to the that of CL and then remained stable with time (Fig. 2a).

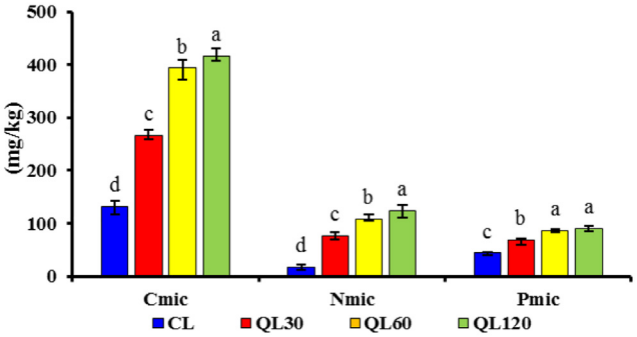

Fig. 1. The trend of microbial biomass among four different successional stages. Cmic, Nmic and Pmic represent microbial biomass carbon, microbial biomass nitrogen, microbial biomass phosphorus. Different letters indicate significant differences ($p < 0.05$) among the four different succession period based on a one-way ANOVA followed by a LSD test.

3.3. Changes in bacterial and fungal composition during secondary succession

Based on similarities to entries in the NCBI-NR database, 95.43% of sequences were classified as Bacteria, 4.24% were classified as Archaea, 0.15% were classified as Eukaryota, and 0.02% were classified as viruses. The phyla Proteobacteria (36.00%) and Actinobacteria (31.15%) dominated soil bacterial communities (Table 2, Fig. S2), and the dominant fungal phyla at all four successional stages were Ascomycota (51.19%) and Basidiomycota (35.73%) (Table 3, Fig. S3). With

Table 1
Variations of soil properties and plant biomass among four different successional stage.

	CL	QL30	QL60	QL120
SOC (g/kg)	4.1 ± 0.09 b	22.27 ± 2.61 a	24.28 ± 1.51 a	25.94 ± 2.86 a
TN (g/kg)	0.79 ± 0.02 b	1.84 ± 0.18 a	1.93 ± 0.09 a	1.95 ± 0.15 a
TP (g/kg)	0.58 ± 0.01 a	0.62 ± 0.03 a	0.60 ± 0.02 a	0.61 ± 0.02 a
Soil C/N	5.21 ± 0.11 c	12.05 ± 0.29 b	12.57 ± 0.22 a	13.18 ± 0.50 a
DOC (mg/kg)	130.08 ± 5.39 c	150.90 ± 14.27 b	184.11 ± 10.37 a	174.00 ± 6.04 a
NN (mg/kg)	12.95 ± 2.06 a	8.83 ± 0.97 b	9.47 ± 1.72 b	8.16 ± 0.63 b
AMN (mg/kg)	16.20 ± 0.75 b	16.53 ± 0.30 b	17.63 ± 0.80 b	20.63 ± 0.27 a
AP (mg/kg)	16.62 ± 1.48 a	4.31 ± 0.67 b	5.11 ± 0.48 b	4.68 ± 0.63 b
pH	8.54 ± 0.03 a	8.45 ± 0.02 a	8.48 ± 0.04 a	8.39 ± 0.05 b
MOI (%)	0.17 ± 0.00 b	0.12 ± 0.00 d	0.13 ± 0.01 c	0.19 ± 0.00 a
BD (g/cm^3)	1.25 ± 0.10 a	1.54 ± 0.15 a	1.5 ± 0.11 a	1.38 ± 0.05 a
EC (g/kg)	1.39 ± 0.03 b	5.11 ± 1.16 a	5.79 ± 0.42 a	8.01 ± 2.49 a
SI	1.41 ± 0.06 c	2.22 ± 0.10 a	1.88 ± 0.05 b	2.16 ± 0.16 a
RB (kg/m^2)	0.03 ± 0.00 b	346.49 ± 64.04 a	358.95 ± 48.39 a	405.80 ± 77.07 a
LB (kg/m^2)	1.44 ± 0.10 b	70.05 ± 2.09 a	64.78 ± 8.52 a	62.53 ± 3.42 a

SOC, total soil organic carbon; TN, total nitrogen; TP, total phosphorus; Soil C/N, the ratio of SOC: TN of the soil; DOC, dissolved organic carbon; EC, activated carbon; NN, soil nitrate nitrogen; AMN, soil ammonium nitrogen; AP, soil available phosphorus. BD, soil bulk density; SI, vegetal Shannon-Wiener index; LB, litter biomass; RB, roots biomass; MOI: soil moisture. The treatments CL, QL30, QL60, and QL120 represent cropland and forests in which the predominant species was 30-, 60-, and 120-year-old *Q. liaotungensis*, respectively. All data are presented as mean ± SD (n = 4). Different lowercase letters in the same line indicate statistically significant differences among the different successional stage ($p < 0.05$).

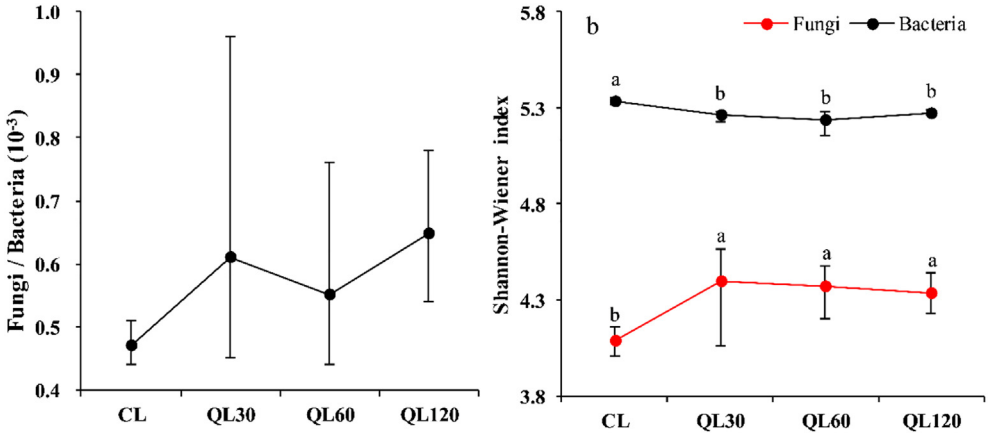

Fig. 2. The trend of the ratio of fungal abundance to bacterial abundance (a) and the trend of Shannon-Wiener index of bacteria and fungi among four different successional stages (b). Different letters indicate significant differences ($p < 0.05$) among the four different succession period based on a one-way ANOVA followed by a LSD test.

succession, the relative abundance of genes from the phyla Proteobacteria, Firmicutes, Nitrospirae, Planctomycetes, and Ascomycota tended to decrease, whereas that of Actinobacteria, Verrucomicrobia, and Basidiomycota increased across the four succession stages (Tables 2 and 3).

At the genus level (Fig. S3a), in bacterial communities, the relative abundances of *Bradyrhizobium* (Proteobacteria) increased with succession. Relative abundances of *Solirubrobacter* and *Streptomyces* (Actinobacteria) increased with succession, whereas that of *Nocardioides* decreased. The relative abundance of *Nitrospira* (Nitrospirae) decreased, whereas that of *Terrimicrobium* (Verrucomicrobia) increased with succession (Table S3). In fungal communities, the relative abundances of *Aspergillus*, *Talaromyces*, *Fonsecaea*, *Trichoderma*, and *Fusarium* (Ascomycota) decreased with succession. Relative abundances of *Moniliophthora* (Basidiomycota) increased with succession (Table S3, Fig. S3b).

In addition, PCoA showed that the bacterial composition of microbial communities differed between CL and QL120 and that the composition at both of these stages differed from those at QL30 and QL60, which were similar (Fig. 3a). The ANOSIM results showed a similar result (Table S4). A difference in fungal community composition was detected between cropland and forest, whereas fungal composition was similar across forest succession (Fig. 3b).

3.4. Potential functional pathways in microbes during secondary succession

The ORFs annotated using the KEGG database and the CAZy database were then used to compare the relative abundances of potential functional genes among the successional stages. In total, 55,837,634 KEGG pathway-associated genes and 1,534,876 CAZy genes were screened from all metagenomes. The dominant categories were carbon metabolism, biosynthesis of amino acids, ABC transporters, quorum sensing, and purine metabolism, representing 5.26%, 4.54%, 3.59%, 3.12%, and 3.02% of the total KEGG pathway Level3 annotated genes, respectively. Among all categories, the relative abundances of genes related to ABC transporters, quorum sensing, and two-component systems increased with successional stages (Table 4). However, the relative abundances of genes associated with carbon metabolism, biosynthesis of amino acids, purine metabolism, and pyrimidine metabolism

Table 3
The variations of bacterial phyla over successional stages (relative abundance > 1%) (CL: cropland; QL30: *Q. liaotungensis* forest that had been restored for 30 years; QL60: *Q. liaotungensis* forest that had been restored for 60 years; QL120: *Q. liaotungensis* forest that had been restored for 120 years). The relative abundances of fungal phyla were averaged for each site (n = 16). The variance explained (R2), regression slope and p value of the linear regression with successional stages were shown in the table (* indicates $p < 0.05$, ** indicates $p < 0.01$ and ***indicates $p < 0.001$).

Phylum	Relative abundance % (mean abundance)	R^2	Slope ± SE	p value
Ascomycota	51.19% (1053)	0.57	−9.53 ± 1.97	0.001**
Basidiomycota	35.73% (960)	0.48	9.03 ± 2.49	0.003**
Chytridiomycota	6.62% (142)	0.005	−0.14 ± 0.52	0.79
Blastocladiomycota	3.60% (77)	0.07	−0.25 ± 0.25	0.34
Glomeromycota	1.23% (44)	0.29	0.13 ± 0.11	0.28
Entomophthoromycota	1.11% (27)	0.03	0.1 ± 0.15	0.53

Table 2
The variations of bacterial phyla over successional stages (relative abundance > 1%) (CL: cropland; QL30: *Q. liaotungensis* forest that had been restored for 30 years; QL60: *Q. liaotungensis* forest that had been restored for 60 years; QL120: *Q. liaotungensis* forest that had been restored for 120 years). The relative abundances of bacterial phyla were averaged for each site (n = 16). The variance explained (R^2), regression slope and p value of the linear regression with successional stages were shown in the table (* indicates $p < 0.05$, ** indicates $p < 0.01$ and ***indicates $p < 0.001$).

phylum	Relative abundance % (mean abundance)	R^2	Slope ± SE	p value
Proteobacteria	36.00% (1392398)	0.55	−1.35 ± 0.32	0.001**
Actinobacteria	31.15% (1231298)	0.62	1.87 ± 0.39	< 0.001***
Acidobacteria	6.95% (272511)	0.01	0.08 ± 0.27	0.77
Chloroflexi	4.55% (178895)	0.05	−0.11 ± 0.13	0.40
Gemmatimonadetes	3.47% (138610)	0.06	−0.18 ± 0.2	0.38
Firmicutes	2.51% (96936)	0.25	−0.07 ± 0.03	0.05*
Verrucomicrobia	2.29% (97399)	0.86	0.77 ± 0.08	< 0.001***
Candidatus_Rokubacteria	1.85% (70834)	0.09	−0.06 ± 0.05	0.27
Candidatus_Tectomicrobia	1.79% (70693)	0.11	0.06 ± 0.04	0.21
Cyanobacteria	1.49% (58404)	0.001	−0.003 ± 0.02	0.89
Nitrospirae	1.28% (40390)	0.60	−0.74 ± 0.16	< 0.001***
Planctomycetes	1.15% (44081)	0.39	−0.06 ± 0.02	0.01*
Bacteroidetes	0.95% (36679)	0.37	−0.02 ± 0.02	0.16

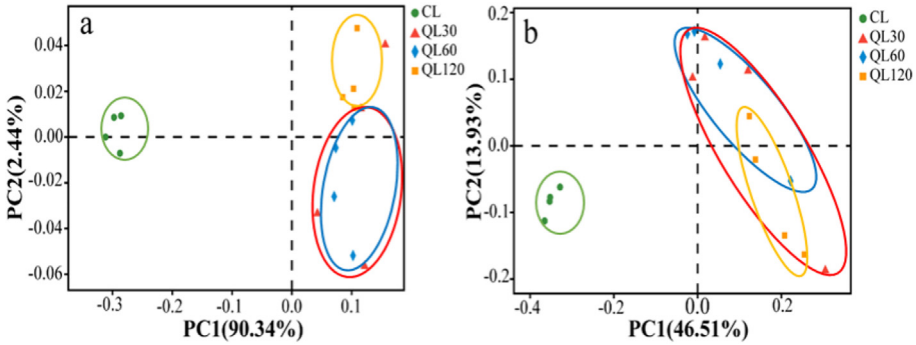

Fig. 3. Principal coordinate analysis (PCoA) plot based on the Bary-curits distance of samples for the bacterial (a) and fungal communities (b) at all successional stages.

decreased with time (Table 4). Carbohydrate-active enzymes (CAZy) function in degradation, modification, and glycosidic bond formation, which can reveal the metabolism of microbial carbohydrates. Auxiliary activities (AA, 5.64%), carbohydrate-binding modules (7.24%), carbohydrate esterases (17.26%), glycoside hydrolases (GH, 35.64%), glycosyl transferases (32.51%), and polysaccharide lyases (1.70%) were related to CAZy among the successional stages in this study. The abundance of all CAZy categories was higher in the later stages (Table S5). Furthermore, ANOSIM indicated that microbial metabolic potential functional pathway Level3 and CAZy genes changed significantly as cropland turned into forest, whereas potential microbial functions were similar across forest stages (Table S4). RDA showed that AP and the ratio of C to N significantly affected potential functional genes and CAZy genes (Fig. S4). The results of species and functional regression analyses showed that changes in diversity in microbial composition were positively associated with differences in microbial function (Fig. 4). The diversity of potential functional genes significantly increased with successional stages.

3.5. Carbon and nitrogen cycling in microbial communities during secondary succession

Genes related to C and N cycling were identified based on the KEGG database (Table S6). In total, 9,228,656 genes related to the C cycle, 50,468 genes related to the N cycle, and 466,042 genes related to C degradation were identified in all metagenomes. With cropland transformation into forest, we found that the abundance of genes related to C and N cycling increased, whereas the abundance of genes related to C and N cycling tended to remain stable across forest successional stages (Fig. 5). However, the relative abundances of genes related to C (from 16.88% to 16.62%) and N cycling (from 0.22% to 0.14%) significantly decreased with time (Table 5). Among C degradation genes, the relative abundances of cellulose transport (from 0.36% to 0.43%) and cellulose degradation genes (from 16.77% to 19.48%) both increased, whereas the relative abundance of hemicellulose degradation (from 3.60% to 2.68%) and chitin degradation genes (from 1.09% to 0.96%) decreased (Fig. 6). For N cycle-related genes, the relative abundance of N fixation (from 12.67% to 19.90%) and ammonification genes (from 24.56% to 50.82%) gradually increased, whereas the relative abundance of nitrate

Table 4
The variations of the Level3 of KEGG over successional stages (relative abundance > 1%) (CL: cropland; QL30: *Q. liaotungensis* forest that had been restored for 30 years; QL60: *Q. liaotungensis* forest that had been restored for 60 years; QL120: *Q. liaotungensis* forest that had been restored for 120 years). The relative abundances of functional genes were averaged for each site (n = 16). The variance explained (R^2), regression slope and p value of the linear regression with successional stages were shown in the table (* indicates p < 0.05, ** indicates p < 0.01 and ***indicates p < 0.001).

Level3	Relative abundance % (mean abundance)	R^2	Slope ± SE	p value
Carbon metabolism	5.26% (182855)	0.32	−0.05 ± 0.02	0.02*
Biosynthesis of amino acids	4.54% (157545)	0.62	−0.09 ± 0.02	< 0.001***
ABC transporters	3.59% (127174)	0.60	0.17 ± 0.04	< 0.001***
Quorum sensing	3.12% (110193)	0.47	0.12 ± 0.04	0.003**
Purine metabolism	3.02% (104536)	0.58	−0.08 ± 0.02	0.001**
Pyrimidine metabolism	2.29% (78610)	0.67	−0.12 ± 0.02	< 0.001***
Carbon fixation pathways in prokaryotes	2.05% (70599)	0.55	−0.08 ± 0.02	0.001**
Two-component system	2.04% (71919)	0.49	0.05 ± 0.01	0.002**
Oxidative phosphorylation	2.00% (68987)	0.47	−0.06 ± 0.02	0.003**
Glyoxylate and dicarboxylate metabolism	1.98% (68876)	0.35	−0.02 ± 0.01	0.02*
Pyruvate metabolism	1.91% (66318)	0.17	−0.01 ± 0.01	0.11
Methane metabolism	1.63% (56598)	0.38	−0.02 ± 0.01	0.01*
Alanine, aspartate and glutamate metabolism	1.61% (55678)	0.56	−0.04 ± 0.01	0.001**
Aminoacyl-tRNA biosynthesis	1.59% (54871)	0.70	−0.07 ± 0.01	< 0.001***
Glycolysis/Gluconeogenesis	1.58% (55120)	0.13	0.01 ± 0.004	0.17
Citrate cycle (TCA cycle)	1.47% (50676)	0.48	−0.06 ± 0.02	0.003**
Glycine, serine and threonine metabolism	1.46% (50955)	0.07	−0.01 ± 0.01	0.32
2-Oxocarboxylic acid metabolism	1.37% (47212)	0.66	−0.07 ± 0.01	< 0.001***
Propanoate metabolism	1.36% (47411)	0.26	−0.01 ± 0.004	0.04*
Butanoate metabolism	1.33% (46026)	0.51	−0.04 ± 0.01	0.002**
Valine, leucine and isoleucine degradation	1.22% (42689)	0.09	0.01 ± 0.01	0.27
Cysteine and methionine metabolism	1.13% (38922)	0.73	−0.05 ± 0.01	< 0.001***
Fatty acid metabolism	1.12% (39628)	0.46	0.03 ± 0.01	0.004**
Amino sugar and nucleotide sugar metabolism	1.09% (38394)	0.57	0.04 ± 0.01	0.001**
Ribosome	1.00% (34185)	0.61	−0.08 ± 0.02	< 0.001***
Starch and sucrose metabolism	1.00% (35314)	0.79	0.05 ± 0.01	< 0.001***

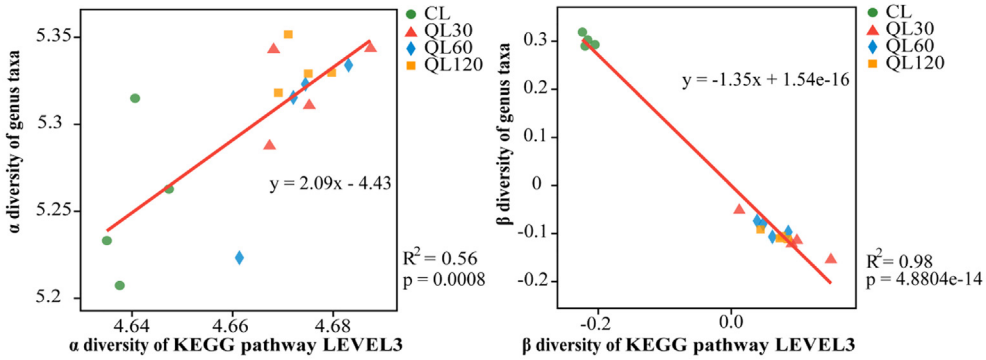

Fig. 4. Species and functional regression analysis of Species (α diversity (a), β diversity (b)) and functional (KEGG pathway LEVEL3). The α diversity and β diversity of species was calculated at the genus level.

reduction (from 52.42% to 27.51%) and denitrification genes (from 10.35% to 1.78%) decreased (Fig. 7).

4. Discussion

4.1. Changes in bacterial and fungal community composition during Q. liaotungensis secondary succession

In this study, the abundances of fungi and bacteria were found to increase significantly with succession from cropland to forest. However, the abundance of fungi increased and that of bacteria decreased with forest secondary succession. Thus, a slight increase in the ratio of fungi to bacteria abundance was detected with secondary succession (Fig. 1). The abundances of the phyla Acidobacteria, Actinobacteria, and Basidiomycota (K-strategists) gradually increased and abundances of the phyla Proteobacteria and Bacteroidetes decreased during succession (Fig. 3). These results supported our first hypothesis that microbial communities might shift from predominantly r-strategists to K-strategists with secondary succession.

Previous studies have shown that bacterial biomass increases as SOC and TN contents increase in soils (Jia et al., 2010; Nielsen et al., 2010). However, in this study, the abundance of bacteria first increased and then slightly decreased with succession, which might be partially attributable to the remarkable change in soil properties such as SOC and TN when cropland changed into forests. Nevertheless, in later forest successional stages, those soil properties showed no significant changes (Table 1). Furthermore, the development of fungal communities resulted in partial niche loss for bacterial communities during the forest stages of succession. This process also resulted in a decrease in abundances of bacteria. The abundance of fungi increased and the ratio of fungi to bacteria increased (Fig. 2), which is consistent with the changes noted in earlier reports (Kaiser et al., 2014; Zechmeister-Boltenstern et al., 2015). Hu et al. (2019) observed symbiotic relationships between most communities of vegetation and fungi at different latitudes in terrestrial ecosystems. This phenomenon might explain the changes in fungal trends observed in this study; specifically, we found that changes in fungal community diversity and plant community diversity were similar. In addition, the ratio of fungi to bacteria correlated positively

Table 5
The variations of functional genes involved in C, N cycle and Carbon degradation over successional stages. (CL: cropland; QL30: *Q. liaotungensis* forest that had been restored for 30 years; QL60: *Q. liaotungensis* forest that had been restored for 60 years; QL120: *Q. liaotungensis* forest that had been restored for 120 years). The relative abundances of functional genes were averaged for each site (n = 16). The variance explained (R^2), regression slope and p value of the linear regression with successional stages were shown in the table (* indicates $p < 0.05$, ** indicates $p < 0.01$ and ***indicates $p < 0.001$).

Functional gene	Relative abundance % (mean abundance)	R^2	Slope ± SE	p value
C cycle	15.17% (576791)	0.55	−0.99 ± 0.24	0.001**
N cycle	0.16% (3154)	0.55	−0.03 ± 0.01	0.001**
Nitrogen Fixation	17.6% (561)	0.59	2.12 ± 0.48	0.001**
Nitrate Reduction	34.95% (1085)	0.65	−7.43 ± 1.47	< 0.001***
Denitrification	3.76% (112)	0.52	−2.52 ± 0.64	0.002**
Ammonification	43.68% (1396)	0.62	7.83 ± 1.65	< 0.001***
C Degradation	1.32% (29128)	0.70	0.15 ± 0.03	< 0.001***
Cellulose Degradation	18.8% (5594)	0.33	0.83 ± 0.32	0.02*
Cellulose Transport	0.42% (126)	0.02	0.01 ± 0.02	0.57
Hemi-cellulose Degradation	2.74% (758)	0.31	−0.25 ± 0.1	0.03*
Chitin Degradation	1.03% (298)	0.18	−0.06 ± 0.03	0.11
Sugar Utilization	17.9% (5284)	0.65	0.76 ± 0.15	< 0.001***
Sugar Transporters	59.10% (17069)	0.47	−1.29 ± 0.37	0.003**

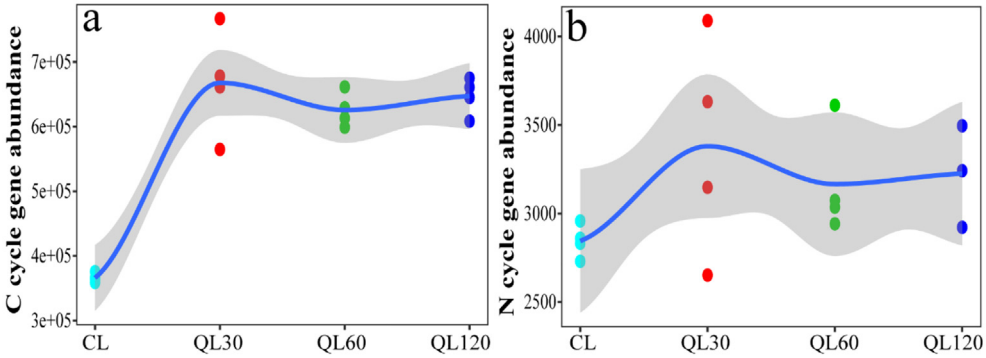

Fig. 5. Regression analysis of the association between years of succession and the abundance of selected functional genes from the KEGG database C cycle genes (a), and N cycle genes (b). All regressions were significant at the $P < 0.01$ level.

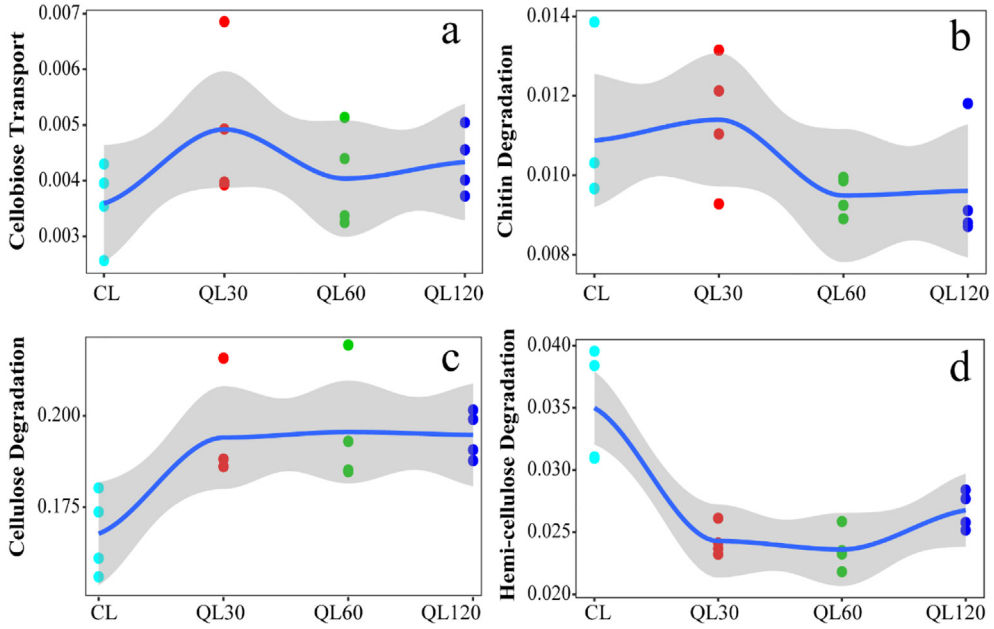

Fig. 6. Regression analysis of the association between years of succession and the relative abundance of selected C cycle functional genes: Cellulose Transport (a), Chitin Degradation (b), Cellulose Degradation (c), Hemi-cellulose Degradation (d).

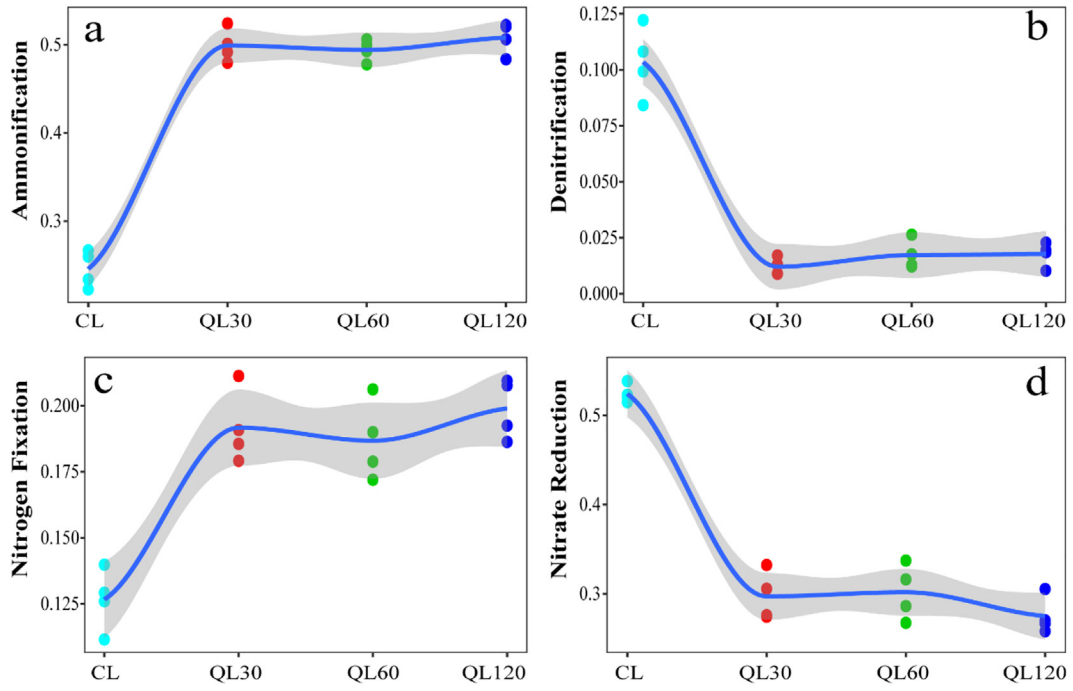

Fig. 7. Regression analysis of the association between years of succession and the relative abundance of selected N cycle functional genes: Ammonification (a), Denitrification (b), Nitrogen Fixation (c), Nitrate Reduction (d).

with the soil C:N ratio in this study, supporting the findings of Jia et al. (2010), Zhou et al. (2017), and Liu et al. (2018). This correlation might exist because bacteria require more N per unit of accumulated C biomass than fungi (Fierer et al., 2009; Deyn et al., 2010).

Previous studies have shown that copiotrophic and oligotrophic microbes strongly correspond with r- and K-strategists, respectively (Bastian et al., 2009; Zhou et al., 2017). These changes among Actinobacteria, Acidobacteria, Bacteroidetes, and Proteobacteria during secondary succession in the present study are consistent with those found by Zumsteg et al. (2012), Cline and Zak (2015), and Williams et al. (2013). That might suggest that bacterial communities transitioned from r- to K-strategists with vegetation succession. In addition, we found that the tendency for this shift in bacteria was induced by the change in soil C to N ratios during *Q. liaotungensis* secondary succession. Furthermore, in the present study, the relative abundance of Actinobacteria increased during vegetation succession, and the main functions of these bacteria include the decomposition of organic materials (Heuer et al., 1997; Zumsteg et al., 2012). That might because of the increase in litter biomass and because the process of litter decomposition might increase soil C availability (e.g. EC) (Fierer et al., 2007). Furthermore, increases in soil available nutrients and decreases in pH caused by an increase in litter biomass are associated with an increase

Table 6
Pearson's correlation coefficients between the environmental factors and the bacterial and fungal phyla.

	SOC	TN	TP	C/N	DOC	NN	AMN	AP	pH	MOI	EC	BD	SI	RB	LB
Proteobacteria	−0.63**	−0.58*		−0.67**		0.53*		0.53*							−0.59*
Actinobacteria	0.64**	0.60*		0.68**	0.51*	−0.52*		−0.52*					0.52*		0.62*
Acidobacteria											−0.55*				
Chloroflexi							−0.54*			−0.74**					
Nitrospirae	−0.91**	−0.91**		−0.98**	−0.63**	0.55*		0.94**	0.50*		−0.63**		−0.80**	−0.85**	−0.95**
Gemmatimonadetes										−0.87**					
Bacteroidetes		−0.50*		−0.51*				0.58*		0.87**					−0.64**
Verrucomicrobia	0.84**	0.81**		0.87**	0.63**		0.74**	−0.75**			0.69**		0.66**	0.72**	0.71**
Ascomycota	−0.66**	−0.65**		−0.74**				0.73**					−0.77**	−0.71**	−0.81**
Basidiomycota	0.63**	0.62*		0.69**				−0.67**					0.75**	0.70**	0.76**
C degradation	0.84***	0.82***		0.93***	0.56*			−0.93***	−0.55*		0.64**		0.79***	0.83***	0.91***
C cycle	0.82***	0.79***		0.91***				−0.92***	−0.57*		0.61*		0.78***	0.78***	0.88***
N cycle								−0.5*							

** $p < 0.01$; *$p < 0.05$; SOC, total soil organic carbon; TN, total nitrogen; TP, total phosphorus; Soil C/N, the ratio of SOC: TN of the soil; DOC, dissolved organic carbon; NN, soil nitrate nitrogen; AMN, soil ammonium nitrogen; AP, soil available phosphorus. MOI, soil moisture; EC, activated carbon; BD, soil bulk density; SI, vegetal Shannon-Wiener index; RB, roots biomass; LB, litter biomass.

in the abundances of genes from acidophilic organisms (Acidobacteria; Table 1) (Jones et al., 2009; Cline and Zak, 2015).

An interesting finding of this study was that the relative abundances of the phyla Nitrospirae and Verrucomicrobia in the bacterial community were increased in the cropland and forest stages, respectively. This phenomenon might reflect changes in soil microenvironments with changes in vegetation type. However, Zhang et al. (2018) found that the abundance of Nitrospirae correlates positively with soil TN, which differed from the results of this study. This relationship might exist because Nitrospirae is dominant in acidic soils and can be affected by small soil pH changes such as a change from pH 8.39 to 8.54 observed in this study (Table 1) (Kowalchuk et al., 2010). Moreover, results of the correlation analysis (Table 6) showed a higher correlation coefficient for the abundance of Nitrospirae and soil NN contents, which suggests that a greater abundance of genes related to nitrification could enrich soil NN contents. For the phylum Verrucomicrobia, we found that abundance gradually increased with forest succession. This finding is consistent with those of other studies on secondary succession (Nemergut et al., 2007; Zhang et al., 2018). Similarly, results of the correlation analysis supported the view that Verrucomicrobia abundance is associated with C, DOC, and N contents in soils and with plant factors (e.g. LB and RB). Changes in Nitrospirae and Verrucomicrobia might also reflect the succession of bacterial communities from r- to K-strategists.

For the fungal community, Ascomycota are known to be able to live in barren soil during the early succession stage (Jumpponen, 2003; Gleeson et al., 2006), whereas the fungal community of later succession is dominated by Basidiomycota, which are often associated with soil nutrients (e.g. SOC and TN) (Bastian et al., 2009; Zumsteg et al., 2012). These observations are consistent with the present results. Furthermore, the abundances of Basidiomycota were significantly correlated with litter biomass. In the present study, due to an increase in litter biomass with vegetation succession, there could be an increase in the content of recalcitrant organic matter in the composition of litters. That might cause an increase in the abundance of Basidiomycota, which can mainly degrade the recalcitrant organic matter. (Voříšková and Baldrian, 2013). Consequently, Basidiomycota and Ascomycota were closely positively and negatively associated with soil C:N ratios, respectively (Table 2). It could thus be concluded that fungal communities also show a succession from r- to K-strategists.

A recent study by Sauvadet et al. (2019) indicated, at a finer taxonomic scale, that microbial communities shift from r- to K-strategists. Only a few studies have examined whether the genus *Aspergillus* comprises r-strategists, and these found that the abundances of such species decrease along a successional gradient (Waring et al., 2013; Brabcova et al., 2016), which is consistent with results obtained in this study. In addition, in the present study, we found that abundances of the bacterial genus *Nitrospira* decreased, whereas those of *Terrimicrobium*, *Bradyrhizobium*, *Solirubrobacter*, *Streptomyces*, and *Nocardioides* increased during secondary succession. In fungal communities, the highest and lowest abundances of the genera *Aspergillus*, *Talaromyces*, *Fonsecaea*, *Trichoderma*, and *Fusarium* were observed in CL and QL120, respectively. In contrast, *Moniliophthora* increased with succession. RDA showed that variations in bacterial and fungal communities were associated with soil C: N ratios and root biomass (Fig. S4). This suggested that the bacterial and fungal communities shift from predominantly r-strategies to K- strategists with succession.

4.2. Changes in potential microbial functional genes during secondary succession in a Q. liaotungensis forest

In this study, we found that the abundance of microbial genes associated with soil C and N cycling increased with succession, which supported our second hypothesis (Fig. 6). We found that the abundance of C and N cycling-related genes increased significantly during succession. Our results were consistent with results from Blaud et al. (2018), Liu et al. (2018), Zeng et al. (2016), who also reported this phenomenon. In addition, our results suggest that a significant positive correlation between high EC and the abundances of soil C- and N-related genes might be the result of more readily available nutrients for microbes.

However, Blaud et al. (2018) found that the abundances of ammonia-oxidizing bacteria and archaea (AOB and AOA) in the soil during the farming stage of vegetation succession were significantly higher than those in grassland and forest stages due to fertilization. Similarly, we found that the relative abundance of C and N cycling-related genes was highest in farmland, which might reflect human disturbance (e.g. fertilization and cropping) in this ecosystem and promote C and N cycling at this stage. For example, the long-term application of fertilizer might increase the relative abundance of N cycling genes by increasing microorganisms such as Nitrospira. In contrast, we also found that the relative abundances of carbon metabolism, biosynthesis of amino acids, and purine metabolism pathways, which all belong to the term metabolism (of KEGG pathway Level1) decreased with time. Conversely, the secondary succession of abandoned farmland resulted in an increase in the relative abundances of ABC transporters and quorum sensing, which belong to the terms environmental information processing and cellular processes (of KEGG pathway Level1), respectively. Furthermore, the diversity of potential functional pathway Level3 genes increased with secondary succession. That might imply that microbes tend to increase the proportion of potential advanced functional genes and decrease the proportion of potential basic functional genes during the process of

secondary succession.

In terrestrial ecosystems, litter decomposition is a key process that connects plants and soil (Cusack et al., 2010). Litter not only serves as a source of SOC but also provides nutrients for plants and microorganisms to grow (García-Palacios et al., 2016). In this study, we found that the abundance of C degradation-associated genes increased with succession. However, among the C degradation genes, their relative abundances varied with vegetation type. For example, the relative abundance of recalcitrant C degradation (e.g. cellulose) genes tended to increase with succession, whereas the relative abundances of labile C degradation (e.g. hemicellulose, chitin) genes tended to decrease (Fig. 6). Liu et al. (2018) obtained similar results in a study of microbial communities in biological crusts. This might reflect the increase in abundance of microorganisms such as Basidiomycota, which degrade complex structures, resulting in an increase in the abundance of functional genes for the degradation of recalcitrant C to maintain nutrients available for plant growth, and this could be associated with the increase in complexity of plant litter with succession (Zhong et al., 2018). In addition, previous studies have shown that AA and GH in CAZy genes are closely related to litter (e.g. lignin and humic matter) degradation (Cardenas et al., 2015). In this study, the abundances of AA and GH increased significantly with succession (Table S5), supporting the view that changes in litter composition can cause changes in the abundance litter decomposition-related genes with succession.

Morales et al. (2010) found the highest and lowest abundances of N fixation genes in forest and cropland stages of succession. They also showed that changes in soil C and N significantly impacted microbial N fixation functions. This relationship was supported by the results of a study by Blaud et al. (2018), who showed that abundances of N fixation genes significantly increased when cropland changed to another vegetation type. These observations are consistent with those from the present study. In addition, we observed that potential ammonification genes, based on the relative abundance of genes associated with the N cycle, increased from CL to QL120 (Fig. 7). This finding might explain the relative increase in soil N availability with succession (Table 1). In addition, the abundance of N reduction and denitrification genes showed a decreasing trend with succession (Fig. 7). This might be associated with a decline in N reduction and denitrification substrate (NO_3^--N) availability. In contrast, NH_3 and AMN, produced by N fixation and ammonification processes, inhibit N reduction and denitrification, resulting in a decrease in the abundance of associated genes.

At the community level, Cline and Zak (2015) found an overlap in the potential functions of various microorganisms across 86 years of forest succession, which was consistent with our findings. First, we found that variation in the range of Euclidean differences in microbial potential functions was smaller than that in Brays-Curtis distances of microbial composition (especially for fungal communities; Table S7), which proved the phenomenon of functional overlap. This might be because different microorganisms play specific roles in different succession stages with the same dominant forest species. Second, ANOSIM results showed that the composition of microbial communities in the forest stage changed significantly over 120 years of succession; however, the potential functions associated with different microbial compositions did not change significantly (Table S4). This could be because of the similarity of root exudates and litter during the succession of a single dominant species. This hypothesis remains to be tested in future studies.

Overall, this study provides insight into how successional strategies of bacterial and fungal communities change with vegetation succession. The results showed that the composition of bacterial communities changed significantly with vegetation succession. However, the fungal composition was similar during the succession of a single dominant species. In addition, the results indicate that the structure associated with microbial composition might determine its primary functions (Song et al., 2017). However, potential functional genes became stable when the bacterial communities continued to shift, implying that potential microbial functions will overlap at later stages of succession. The abundances of C and N cycle-related genes first significantly increased and then stabilized and were closely associated with the nutrients available in the soil. Furthermore, microorganisms might reduce their investment in basic functions in the later successional stages, which were associated with higher functional diversity. Our study provides insight into the mechanisms underlying the coupling relationship between soil microbes and potential functional genes in nutrient cycles during vegetation succession.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2020.114199.

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