Original Research

Functional Distribution of Bacterial Community under Different Land Use Patterns Based on FaProTax Function Prediction

Shichu Liang[1#], Jiaojiao Deng[2#], Yong Jiang[1], Shijun Wu[2], Yongbin Zhou[2],Wenxu Zhu[2]*

[1]Laboratory of Ecology of Rare and Endangered Species and Environmental Protection (Guangxi Normal University), Ministry of Education, Guilin, Guangxi, China
[2]College of Forestry, Shenyang Agriculture University, Shenyang, China

Abstract

Soil microorganisms play an important role in biogeochemical cycling. The bacterial community functional community profiles across different land use types in the mountainous region of eastern Liaoning Province, China, such as natural secondary forest (*Quercus mongolica* (QM)), shrub wood (SW), coniferous planation (*Larix gmelini* (LG), and *Pinus koraiensis* (PK)), and agricultural land (*Zea mays* (ZM)) were evaluated using high-throughput sequencing of the bacterial 16S rDNA gene, and predicted based on the FaProTax database. Bacterial functional groups varied across samples under different land use patterns. A total of 53 functional groups were acquired using FaProTaxin accordance with the classification annotation results of 16S rDNA sequences. These functional groups contained 5809 OTUs, accounting for 66.25% of all OTUs. The dominant functions were chemoheterotrophs and aerobicchemoheterotrophy with average relative abundance of 21.69% and 21.01%, respectively. In addition, we detected 15 and 12 ecological function groups related to the carbon and nitrogen cycles, respectively. The bacterial functional groups of ZM were clearly separated from those of PK, LG, QM, and SW, indicating that functional groups of agriculture soil differed from those of forest soils. Furthermore, the bacterial functional groups of LG and PK were obviously separated from those of QM and SW, suggesting that functional groups of broad leaf forest differed from those of coniferous forests. Also, we analyzed the main factors that influence soil bacterial community functional groups under different land use patterns. It was found that soil environmental conditions, especially soil pH, NH_4^+-N, and NO_3^--N content,and strongly influenced the distribution of functional groups about carbon

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[#]Authors who contributed equally to this work.
*e-mail: zhuwenxu.315@163.com

and nitrogen cycles in bacterial communities. It is of great significance to provide a scientific basis for the sustainable utilization of soil in northeastern China.

Keywords: forest soils, agriculture soil, FaProTax function prediction, carbon cycles, nitrogen cycles

Introduction

Global climate, biodiversity and ecosystem services are strongly influenced by land-use conversion [1-2]. And the rational land use types play an important role in improving soil structure and promoting regional ecological restoration. In recent years, an increasing number of natural forests have been converted to planted forests and arable landin order to fulfill needs for timber, agricultural production, and other economic forest products [3-5]. So far, more than 38% of natural landscapes around the world have been converted to managed systems [6]. The conversion of forests to farmland has been studied most frequently worldwide, with more research in tropical environments [7], which not only bring about great challenges for maintaining forest ecosystems, but also have a potential impact on terrestrial ecosystems. It has long been recognized that changes in land use types alter a soil's physical and chemical properties, the structure and function of an ecosystem, and soil quality [8-9]. Also, land-use change significantly affects the dynamics and stability of soil organic carbon (SOC) and enzyme activities [10]. In addition, land use is a crucial factor affecting soil microbial communities under the same climatic conditions [11-12]. Conversion of forest to cultivated land typically leads to a decline of soil organic matter (SOM) [13], which was linked to decreases in soil microbial biomass and activity [14]. Soil microorganisms, as the most active component of a soil ecosystem, can sensitively reflect the changes of soil ecological environment [15]. Therefore, analyzing soil microbial characteristics is a valuable approach for assessing the effectiveness of different land use types, and to evaluate the suitability of different land use types [7].

Microbial communities are incredibly active and diverse, which drive global biogeochemical cycling, such as C and N cycling [16], playing key roles in the terrestrial ecosystem [17], such as maintaining soil structure, conservation of soil fertility, forming and developing of soil, influencing soil ecological processes, and ensuring system stability [18-19]. In addition, soil microbes also play a crucial role in plant growth and crop production [20]. Researchers report that soil microorganisms are very sensitive to environmental stressors, and can respond quickly to the changes of physicochemical conditions, and abiotic and biotic environmental stressors [21]. So, changes in soil microbial community diversity, composition, and function can be used to rapidly reflect soil quality and vegetation changes, and accurately evaluate sustainable ecosystem development of soil [22-23].

Recently, the influence of different land use patterns on soil microbiological indicators has been reported both in China and abroad [24-25]. Surveys targeting the effects of land use change on soil microbial communities have been extensively studied in grassland, jungle, and forest ecosystems, frequently contrasting between natural forests, jungles, and plantations [26,10], natural grassland and agricultural fields [27], forest, grassland and no-till cropping [28]. Overall, changes in land use patterns can significantly affect soil microbial community diversity and composition [29],as well as their metabolic activity [9] – especially between wooded and agricultural soils [30]. In addition, land use change shifts the decomposer community structure and negatively affects litter decomposition [26]. Land use has strong effects on soil microbes, with consequences pertaining to microbial community diversity and composition [31]. However, the ecological function of this variation remains largely unknown. Microbial communities of different functions are involved in various processes of C and N cycle and play an important role in maintaining ecosystem stability. Even fewer studies exist targeting soil microbial community function relevant to Cand N cycling under different land use types. In light of this, it is necessary to deeply study the soil C and N process in different land use types, which will help to elucidate the microbiological mechanisms that are related to the nutrient cycling in the land-use change.

Monitoring soil bacterial community function changes in different land use patterns can provide a theoretical basis for the restoration of degraded soil ecosystems and sustainable management of forests. High-throughput sequencing of 16S rDNA is a commonly used technique for investigating bacterial community structure [32-34]. With the development of bioinformatics tools and databases [35-36], metabolome-encoded data have been widely used for not only describing the composition of a soil microbial community, but also for predicting the soil microbial community function in various environmental conditions. And many functional prediction software programs have been developed based on 16S rDNA sequencing data, such as PICRUST [37], Tax4Fun [38], FAProTax [35], have been widely used in soil bacterial function prediction [39-40].

In northeastern China, due to the increasing demand for economic demand, part of the secondary forests have been converted into plantation and cultivated land. Although the change in land use pattern has brought about a substantial economic increase, there is a lack of research on changes in soil ecosystems – especially

soil microbial communities. Our study focused on the changes of the soil bacterial community functional groups under the impacts of different land use types in the mountainous region of Liaodong, including *Quercus mongolica*, shrub wood, *Larixgmelini*, *Pinuskoraiensis*, and *Zeamays*. Here, soil bacterial community functional groups were analyzed via the functional annotation of prokaryotic taxa (FAPROTAX) database on the basis of experimental literature through 16S rDNA high-throughput sequencing. We then explored soil bacterial ecological functional groups related to C and N cycles in different land use patterns, and functional groups in correlation with soil environmental factors. Specifically, the following hypotheses were tested: (1) converting broadleaf forests to plantations and farmland decreasing concentrations of total C and total N; (2) the soil bacterial community functional groups related to C and N cycling shows a difference between broadleaf forests, plantations, and farmland; and (3) change in the soil bacterial community functional groups caused by land-use change are linked to changes in soil environment factors. The ultimate objective was to investigate which of these soil environment factors may play a central role in determining the soil microbial function under different land use types. It can provide scientific basis for rational utilization and sustainable management of agricultural and forestry land resources in the region, as well as in northeastern China.

Materials and Methods

Research Area

Our research was carried out at the experimental forest farm of the Liaoning Institute of Forest Management, Liaoning Province, China (40°52′31″N-123°56′43″E), which lies in the hilly land of the mountainous region of eastern Liaoning Province with an average altitude of 645 m. The research area has a typical temperate continental monsoon climate with an annual average temperature of 6.5ºC and mean annual precipitation of 926.3 mm. The lowest temperature is -33ºC and the highest is 32ºC. The soil type is brown soil with soil freezing depth of 150 cm. Due to excessive harvesting and utilization over the past 100 years, the original vegetation has been seriously damaged. A large number of secondary forests have been transformed into *Pinus koraiensis* and *Larix gmelinii* plantations due to the increasing demand for timber, some of which were reclaimed as farmland. Land use patterns mainly include natural secondary forests, plantations, and agricultural lands (*Zea mays* and *Glycine max*). Prior to the cultivation of *Zea mays,*the forest vegetation type was shrub wood, whichwas cultivated for 5 years. Field management in the area is extensive, no irrigation measures were used, and harvested corn straw were not returned to the field.

Soil Sample Collection

In our study, five sites with uniform conditions were selected in July 2017, including *Quercus mongolica* (QM), shrub wood (SW), *Larix gmelini* (LG), *Pinus koraiensis* (PK), and *Zea mays* (ZM). Soil samples were collected from 3 plots (20 m×20 m) as three independent replicates, using a soil auger (8 cm in diameter, 10 cm deep) at 10 cm depths from each plot. To guarantee the representativeness of soil samples in each forest, "S" multi-point sampling was applied. Soil from 15-20 points were mixed together and placed in sterile plastic bags as a replicate sample from each plot, with marks after the samples had been well mixed, and then samples were stored in ice boxes. Soil samples were well mixed, and screened using 2 mm sieves to remove residual plant roots and debris. A portion of the prepared soil samples was stored at -80ºC for high-throughput sequencing, and the rest was air-dried at room temperature for determining soil chemical characteristics.

Determining Soil Properties

Soil pH was measured using a pH meter after shaking a soil-water (1:5w/v) suspension for 30 min. Soil total carbon (C) and total nitrogen (N) contents were measured with an elemental analyzer (Elementar, Germany). Available nitrogen (N) was determined by the alkali diffusion method. Total phosphorus (P) was measured by spectrophotometry after wet digestion with $HClO_4$-H_2SO_4. Contents of soil KCl extraction nitrate nitrogen (NO_3^--N) and ammonium nitrogen (NH_4^+-N) and were determined using an AA3 (Germany) continuous flow analytical system.

Soil DNA Extraction

Total genomic DNA was extracted from 0.5 g of soil using a Fast DNA SPIN extraction kit (MP Biomedicals, Santa Ana, CA, USA), in accordance with the manufacturer's protocol, and stored at -20ºC until further analysis. A NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure the amount of DNA, and the DNA quality was measured using agarose gel electrophoresis.

16S rDNA Amplicon Pyrosequencing

PCR amplification in the bacterial V3-V4 region of 16S rDNA was carriedout using the forward primer 338F (5'-ACTCCTACGGGAGGCAGCA-3') and the reverse primer 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The sample-specific 7-bp barcodes were incorporated in the primers for multiplex sequencing. Each of three independent 25 μL reactions per DNA sample contained 5 μL of Q5 reaction buffer (5×), 5 μL of Q5 High-Fidelity GC buffer (5×), 1 μL (10 uM) of

forward primer,1 μL (10 uM) of reverse primer, 2 μL (2.5 mM) of dNTPs, 0.25 μL of Q5 High-Fidelity DNA Polymerase (5 U/μl), 2 μL (40-50 ng)of DNA template, and 8.75 μL of ddH$_2$O. Cycling conditions were 98ºC for 5 min; 25 cycles of 98ºC for 15 s, 55ºC for 30 s, 72ºC for 30 s, and 72ºC for 5 min. Agencourt AMPure Beads (Beckman Coulter, Indianapolis, IN) was used to purify the PCR amplicons, and a PicoGreen dsDNA assay kit (Invitrogen, Carlsbad, CA, USA) was applied to quantify the PCR amplicons. After the individual quantification step, amplicons were pooled in equal amounts and pair-end 2×300 bp sequencing was performed using the Illlumina MiSeq platform with a MiSeq Reagent Kit v3 (Shanghai Personal Biotechnology Co., Ltd, Shanghai, China).

FAProTax Function Prediction

Functional annotation of prokaryotic taxa (FAPROTAX), a database that extrapolates functions of cultured prokaryotes to estimate metabolic or other ecologically relevant functions, is more suitable for the functional annotation and prediction of the biogeochemical cycle (especially the cycle of carbon, hydrogen, nitrogen, phosphorus and sulfur) of environmental samples [35]. The FAPROTAX database based on the current manual collation of culturable bacteriacontains more than 7,600 functional annotations from more than 80 functional groups (e.g., nitrate respiration, methanogenesis, fermentation, plant pathogens, etc.) collected from more than 4,600 prokaryotic microorganisms. Based on the mentioned functional information in the existing microbiology literature, the names of related species in the database are summarized from functional classification and annotation information. When used, read an annotated OTU table of Greengenes or Silva database, match the annotated information of OTU with the species information in the database through python program, and output the predicted result of function (http://www.zoology.ubc.ca/louca/FAPROTAX/lib/php/index.php?section=Home).

Data Analysis

A one-way analysis of variance (ANOVA) was conducted to analyze soil chemical conditions and the relative abundance of bacterial functional groups in different soil samples using SPSS 19.0 software (SPSS Inc., USA) with an LSD test [41]. The heat map representation of bacterial functional groups in different samples was established using RStudio packages with the "pheatmap" [42]. The bacterial community functional variation across samples was built via beta diversity analysis, and visualized through nonmetric multidimensional scaling (NMDS) on account of unweighted UniFrac distance metrics [43]. Differences in relative abundance of bacterial ecological functional groups about C cycle were measured at the 95%

Table 1. Soil physical and chemical characteristics under different land use patterns. Data are means ± standard error (n = 3). Different lowercase letters within rows indicate significant difference at P<0.05 level.

Land use pattern	pH	Total C (g·kg⁻¹)	Total N (g·kg⁻¹)	C/N	Available N (mg·kg⁻¹)	NH$_4^+$-N (mg·kg⁻¹)	NO$_3^-$-N (mg·kg⁻¹)	Total P (g·kg⁻¹)
Quercus mongolica	5.99±0.15ab	57.74±15.68a	4.40±1.11a	13.09±0.39a	33.63±7.19a	4.42±0.50b	17.09±3.69ab	0.59±0.25ab
Shrub wood	6.29±0.16a	38.27±5.49b	2.79±0.42b	13.71±0.31a	23.50±5.87b	3.78±0.15b	9.58±0.68b	0.54±0.25ab
Larix gmelini	5.57±0.24c	52.24±3.36ab	3.89±0.27a	13.43±0.20a	32.05±3.61a	6.33±1.44a	26.44±9.88a	0.47±0.13ab
Pinus koraiensis	5.54±0.11c	20.08±4.01c	1.53±0.32c	13.24±1.88a	12.96±2.56c	7.68±1.45a	24.17±2.81a	0.30±0.02b
Zea mays	5.85±0.16bc	17.46±2.33c	1.31±0.20c	13.39±0.28a	11.90±1.33c	3.94±0.77b	19.53±6.47ab	0.73±0.23a

confidence level, and multiple test corrections with Storey's false discovery rate (FDR) was used [44]. The potential relationships between soil environmental factors and the relative abundances of functional groups related to C and N cycles were built via Pearson correlation analysis and showed through the heatmap, which was built using RStudio packages with "psych" and "corrplot".

Results and Discussion

Soil Chemical Characteristics under Different Land Use Patterns

The vegetation types and soil management of the land surface were determined by different land use types, which in turn affected soil physical and chemical characteristics [45, 21]. Soil basic chemical properties reflect soil nutrient levels, and the ANOVA for soil properties showed that there were significant differences among different land use patterns. The soil pH value ranged from 5.54 to 6.29 in samples from all land use types. Soil pH was the highest in SW with 6.29, and the QM and SW had higher pH value than LG, PK, and ZM. Compared to SW, the ZM land reduced soil pH, which may be mainly due to the increase in NO_3^--N contents in soil after increasing nitrogen input leads to a decrease in soil pH [46]. In our study, the NO_3^--N content in different land use types decreased in the following order LG>PK>ZM>QM>SW (Table 1). NH_4^+-N content in LG and PK was 6.33 mg·kg^{-1} and 7.68 mg·kg^{-1}, respectively – significantly higher than the NH_4^+-N contents in QM, SW, and ZM. The natural

Table S1. OTUs per functional group. Number of OTUs assigned to each functional group, compared to the total number of taxonomically annotated OTUs.

Functional group	OTUs	Fraction (%)
Chemoheterotrophy	1426	24.55
Aerobic chemoheterotrophy	1257	21.64
Nitrification	263	4.53
Predatory or exoparasitic	198	3.41
Aerobic ammoniao xidation	168	2.89
Cellulolysis	143	2.46
Nitrate reduction	142	2.44
Respiration of sulfur compounds	131	2.26
Sulfur respiration	126	2.17
Intracellular parasites	125	2.15
Nitrogen fixation	121	2.08
Animal parasites or symbionts	120	2.07
Phototrophy	115	1.98
Fermentation	98	1.69
Ureolysis	98	1.69
Photoautotrophy	96	1.65
Aerobic nitrite oxidation	95	1.64
Nitrogen respiration	95	1.64
Nitrate respiration	94	1.62
Human pathogens all	83	1.43
Photoheterotrophy	80	1.38
Nitrite respiration	63	1.08
Nitrate denitrification	61	1.05
Nitrite denitrification	61	1.05
Nitrous oxide denitrification	61	1.05
Denitrification	61	1.05
Human pathogens pneumonia	61	1.05
Anoxygenic photoautotrophy S oxidizing	61	1.05
Anoxygenic photoautotrophy	61	1.05
Aromatic compound degradation	40	0.69
Cyanobacteria	35	0.60
Oxygenic photoautotrophy	35	0.60
Chitinolysis	24	0.41
Manganese oxidation	23	0.40
Methylotrophy	16	0.28
Chloroplasts	13	0.22
Hydrocarbon degradation	10	0.17
Methanotrophy	8	0.14
Methanol oxidation	8	0.14
Sulfate respiration	5	0.09
Iron respiration	5	0.09
Human gut	4	0.07
Mammal gut	4	0.07
Dark oxidation of sulfur compounds	3	0.05
Nitrite ammonification	2	0.03
Invertebrate parasites	2	0.03
Thiosulfate respiration	1	0.02
Dark hydrogen oxidation	1	0.02
Nitrate ammonification	1	0.02
Xylanolysis	1	0.02
Dark sulfide oxidation	1	0.02
Aromatic hydrocarbon degradation	1	0.02

secondary forest (QM) had the highest content of total C, total N, and available N with 57.74 g·kg⁻¹, 4.40 g·kg⁻¹, and 33.63 mg·kg⁻¹, respectively, followed by LG, while in our study the content of total C, total N, and available N were lowest in the ZM with 17.46 g·kg⁻¹, 1.31 g·kg⁻¹, and 11.90 mg·kg⁻¹, respectively. These results support our first hypothesis and are consistent with other research [47]. Numerous studies have also found that soil carbon reserves differ according to different land use patterns [48], and the conversion from forests into farmland reduces soil carbon storage [49-50]. Generally, soils under agricultural land uses showed reduced nutrient concentrations, especially for the contents of total C and total N [51]. Deep tillage after the application of fertilizer in the farmland significantly alter the structure of soil by breaking up soil aggregates, and protecting soil physical structure consequently acceleratesthe release of soil organic carbon into the atmosphere [52-53]. Moreover, the litter accumulation in agricultural land was less and the nutrient return was lower. Both of these processes would reduce the input of organic C into the soil environment and may also explain the reduction of soil total C and total N, suggesting the loss of soil carbon and nitrogen in the process of transforming forests into other land use patterns. Similarly, previous research hasreported that the concentration of soil organic carbon decreased after conversion of natural forests to *Pinus* plantations [5, 10, 54], which were similar to our study results.The input of carbon, nitrogen and organic matter in soil is mainly due to the return of nutrients and biological nitrogen fixation in the litter [55]. The biomass allocation strategies between coniferous forests and broad-leaved forests are different. Broad-leaved forests can allocate more biomass to roots, especially fine roots, which in turn fix more carbon

into the soil [56]. Meanwhile,the litter of coniferous forests decomposes more slowly compared with broad-leaved trees [57], leading to litter accumulation [58-59]. Although LG and PK were coniferous forests, the content of soil total C, total N, available N in LG land were significantly higher than in PK land (*P*<0.05) (Table 1). A previous study demonstrated that LG belongs to cold-temperate deciduous coniferous forests with a large amount of litter that contains lignin, resin, tannins, and waxy substances that are difficult to decompose. However, the coniferous litter covered on the surface of the soil could promote the formation of an air-free environment and contribute to the accumulation of soil nutrients [60]. On the contrary, PK is a temperate evergreen coniferous forest with less litter, resulting in poor soil nutrients. While no significant differences in soil C/N among the samples were observed, and the C/N varied from 13.09 to 13.71. Overall, our results demonstrated that converting secondary forest to pinus plantations and farmland caused a reduction in both the concentration of soil total C and total N. These findings illustrated that plantations (LG and PK) and farmland (ZM) did not improve soil conditions to the same degree as did QM and SW, and the potentially positive effects of QM on soil nutrient conditions.

Bacterial Functional Annotation and Distribution under Different Land Use Patterns

FAProTax is more suitable for functional annotation prediction of biogeochemical circulation processes [35]. The relative abundances of bacterial community functional groups in per sample were calculated as the OTUs cumulative abundance allocated to each functional group. OTUs without any functional
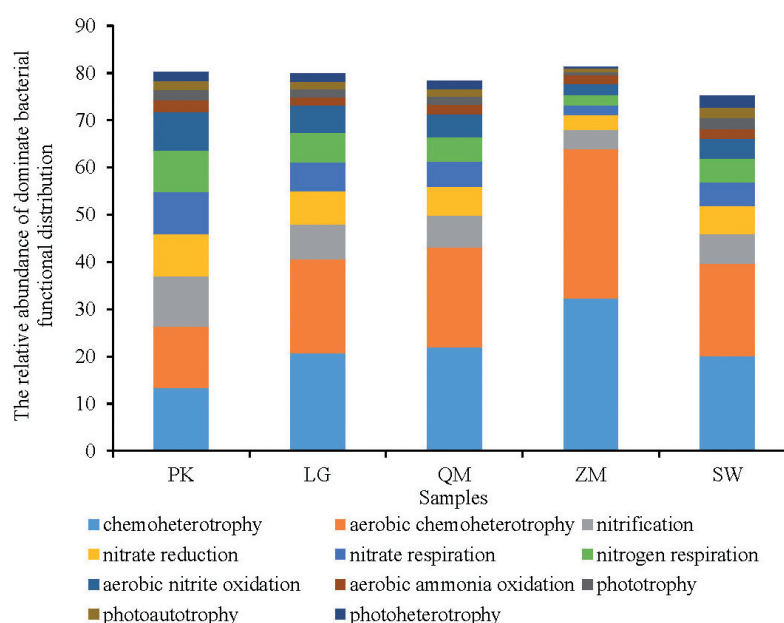

Fig. 1. The relative abundance of dominate bacterial functional groups distribution. QM: Quercus mongolica; SW: Shrub wood; LG: Larix gmelini; PK: Pinus koraiensis; ZM: Zea mays.

annotation were excluded from the analysis. Then, we inquired about potential functional groups involved in soil environmental conditions. According to the classification annotation results of 16S rDNA sequences, a total of 53 functional groups were obtained using FAProTax. These functional groups contained 5809 OTUs, accounting for 66.25% of all OTUs. OTUs per functional group are in Table S1.

Functional community function varied across samples in different land use types. The bacterial core functional groups (higher relative abundance>1.5%) are shown in Fig. 1, including chemoheterotrophy, aerobic chemoheterotrophy, nitrification, nitratereduction, nitrogenrespiration, aerobic nitrite oxidation, aerobic ammonia oxidation, phototrophy, photoautotrophy, and photoheterotrophy with average relative abundance of 21.69%, 21.01%, 7.10%, 6.16%, 5.49%, 5.49%, 5.10%, 2.00%, 1.74%, 1.62%, and 1.60%, respectively (Fig. 1). In our study, the predominant functional groups were chemoheterotrophy and aerobic chemoheterotrophy.

Abundant chemoheterotrophy in soil indicated that a large number of microbes cannot fix carbon and have to obtaincarbon and energy from the oxidation of preformed organic compounds [61]. The bacterial community functional groups from soil differed from those of mudflat, and previous study from the mangrove-inhabited mudflat revealed that photoautotrophy and nitrate respiration were a rich repertoire of metabolic functional groups [61]. Bacterial functional distribution under different land use patterns changed significantly. A hierarchically clustered heatmap was eatablished based on all the bacterial functional groups, and the differences of relative abundances of soil bacterial functional groups among samples from different land use types were reflected by the heatmap. The results showed that the five soil samples were divided into two large groups. One group contained the QM, SW, LG, and PK, and the other group was ZM (Fig. 2). The NMDS plot also indicated that the bacterial functional groups of ZM were separated from those of PK, LG,
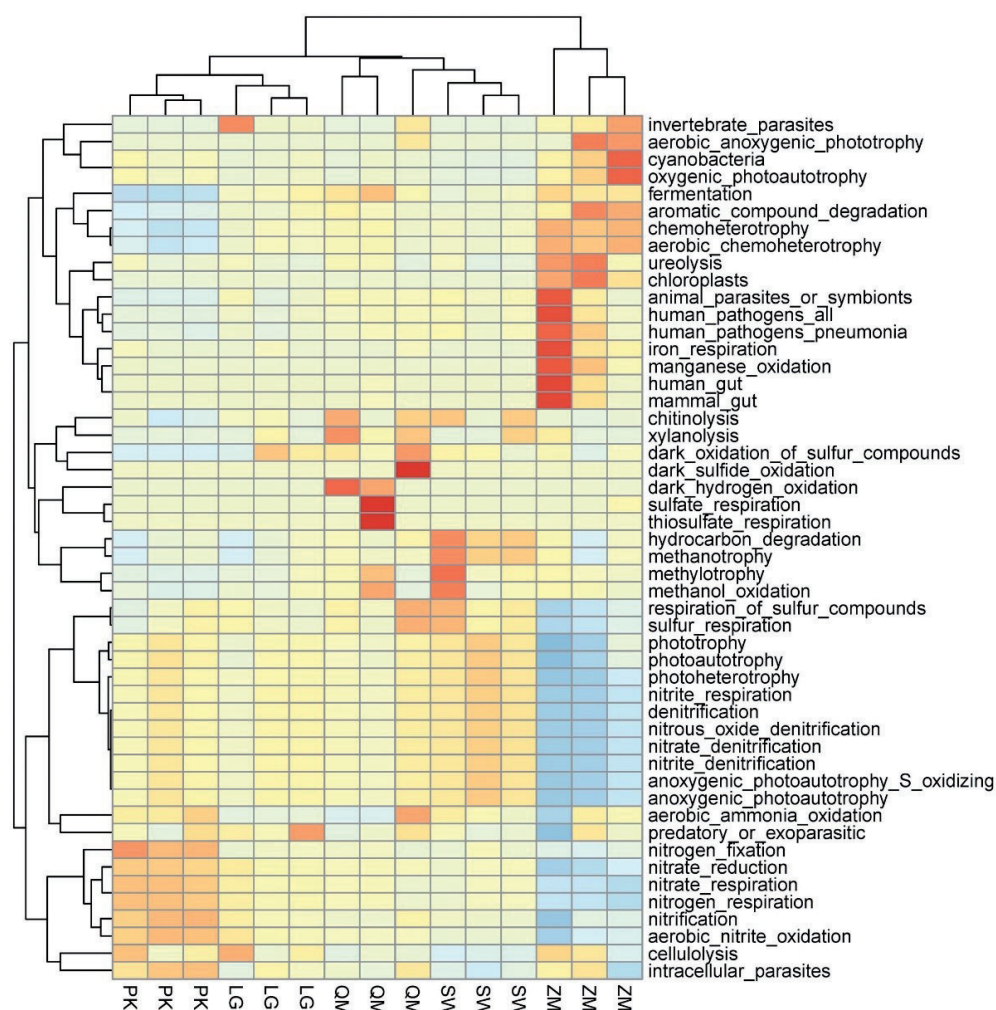

Fig. 2. Distribution of bacterial function in the FaProTax database for different land use patterns. Different colors indicate the relative abundance of groups in the individual samples. The samples are grouped according to the similarity of each other, and the clustering results are arranged horizontally according to the clustering results. In the figure, red represents the function with higher abundance in the corresponding sample, and blue represents the function with lower abundance. QM: Quercus mongolica; SW: Shrub wood; LG: Larix gmelini; PK: Pinus koraiensis; ZM: Zea mays.
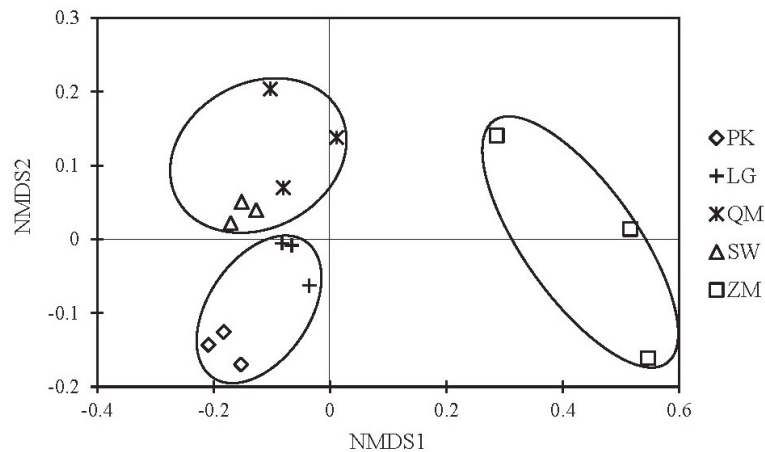
Fig. 3. Results of nonmetric multidimensional scaling analysis-Biplot of the all investigated bacterial functional distribution. QM: *Quercus mongolica*; SW: Shrub wood; LG: *Larix gmelini*; PK: *Pinus koraiensis*; ZM: *Zea mays*.

QM, and SW, especially along NMDS1, indicating that bacterial functional groups of agriculture soil differed from those of forests. This indicated that the conversion of forest land to cultivated land and the application of long-term chemical fertilizers have changed the main metabolic groups of soil microorganisms. Compared with forest land, cultivated land has obvious changes in its surface vegetation, which has led to changes in root exudates. Studies have shown that root exudates play an important role in the distribution of soil microbes, directly affecting the quantity, activity and community structure of soil microbes [62], and its soil physical and chemical properties have also changed significantly. The combined effects of these differences have led to significant changes in the bacterial community functional groups of farmland. In addition, the bacterial functional groups of LG, and PK were separated from those of QM and SW, especially along NMDS2 (Fig. 3), indicating that functional groups of broadleaf forest differed from those of coniferous forests. Both of them illustrated that similar land-use patterns usually have similar soil bacterial community functional groups in a specific area. Also, a finding from the semiarid Loess Plateau region in China established that similar land-use types in a specific area generally have similar soil chemical properties, and similar microbial communities [63], which supported our research results to some extent. Previous study documented that planted forests change bacterial community composition compared to natural broad leaf forests, and soil bacterial physiological characteristics indicated the potential ecological role for nutrient cycles in the ecosystem [64], which proved our research conclusions to some degree. Under the same climatic conditions, the differences between broad-leaved forests and coniferous forests serve as an important factor affecting bacterial community functional groups. Thus it can be seen that different land use patterns could distinctly influence the decomposer community composition owing to the different vegetation types [65].

Due to the existence of functional gene redundancy, these functional profiles were observed among bacterial communities [66].

Functional Groups Related to the C and N Cycles under Different Land Use Patterns

The C and N cycles of the terrestrial ecosystem and its regulation mechanism are the hot topics in the science research of soil ecology and global change ecology [67]. As is known to all, soil bacterial communities provide an important role in biogeochemical cycles [68]. Under the background of global climate change, the diversity of soil microorganism is bound to be affected, and the change of biological diversity affects the change of soil carbon and nitrogen. Land use and land cover change impact plant litter on the soil surface, and environmental conditions, such as water and heat, the transformation and flow of soil nutrients, and the quantity, composition, and activity of soil microorganisms [69]. The decomposition of specific carbon substrates of different land use patterns could determine the different community composition and functional group of soil bacteria [70-71]. In our study, we detected 15 ecological function groups related to the C cycles, including chemoheterotrophy, aerobic chemoheterotrophy, phototrophy, photoautotrophy, photoheterotrophy, anoxygenic photoautotrophy S oxidizing, anoxygenic photoautotrophy, cellulolysis, aromatic compound degradation, oxygenic photoautotrophy, methylotrophy, methanol oxidation, hydrocarbon degradation, methanotrophy, and aromatic hydrocarbon degradation (Table S2). However, previous studies from a temperate deciduous broad leaved forest and a tropical mountain rainforest detected eight ecological function groups connected with the carbon cycles [71]. In our research, after the conversion from SW to ZM, soil bacterial function for aerobic chemoheterotrophy ($P = 0.0005$) and chemoheterotrophy ($P = 0.0016$) significantly increased (Fig. 4), while,

Table S2. Functional groups related to the carbon cycle. QM: *Quercus mongolica*; SW: Shrub wood; LG: *Larix gmelini*; PK: *Pinus koraiensis*; ZM: *Zea mays*.

Samples	Chemoheterotrophy	Aerobic chemoheterotrophy	Phototrophy	Photoautotrophy	Photoheterotrophy	Anoxygenic photoautotrophy S oxidizing	Anoxygenic photoautotrophy
PK	13.34±1.31cC	13.00±1.19cC	2.05±0.18abA	1.93±0.21abA	1.90±0.22bAB	1.78±0.25abA	1.78±0.25abA
LG	20.66±0.82bB	19.94±0.82bB	1.77±0.24bA	1.65±0.28bA	1.70±0.22bB	1.58±0.26bA	1.58±0.26bA
QM	22.05±2.18bB	21.01±2.07bB	1.78±.25bA	1.63±0.26bA	1.72±0.26bAB	1.57±0.27bA	1.57±0.27bA
ZM	32.25±1.04aA	31.63±0.99aA	0.75±0.55cB	0.68±0.45cB	0.31±0.32cC	0.24±0.21cB	0.24±0.21cB
SW	20.16±0.79bB	19.46±0.51bB	2.38±0.23aA	2.20±0.23aA	2.37±0.24aA	2.19±0.23aA	2.19±0.23aA

Samples	Cellulolysis	Aromatic compound degradation	Oxygenic photoautotrophy	Methylotrophy	Methanol oxidation	Hydrocarbon degradation	Methanotrophy
PK	0.86±0.35aA	0.27±0.06cB	0.15±0.05bB	0.02±0.00bA	0.01±0.01aA	0.01±0.01bB	0.01±0.01bB
LG	0.85±0.43aA	0.68±0.13bcB	0.07±0.02bB	0.05±0.02abA	0.04±0.02aA	0.01±0.01bB	0.01±0.01bB
QM	0.38±0.08abA	0.80±0.19bB	0.06±0.03bB	0.11±0.08abA	0.09±0.08aA	0.02±0.01bB	0.02±0.00bB
ZM	0.74±0.47abA	1.54±0.48aA	0.44±0.24aA	0.08±0.02abA	0.07±0.02aA	0.02±0.01bB	0.02±0.01bB
SW	0.23±0.03bA	0.59±0.03bcB	0.01±0.01bB	0.15±0.11aA	0.10±0.10aA	0.05±0.01aA	0.05±0.01aA

soil bacterial function for photoheterotrophy ($P = 0.0004$), photoautotrophy ($P = 0.0023$), phototrophy ($P = 0.0044$), anoxygenic photoautotrophy S oxidizing ($P = 0.0006$), and anoxygenic photoautotrophy ($P = 0.0006$) significantly decreased (Fig. 4). The functional groups of the bacterial community related to C cycling in long-term agricultural soil is significantly altered from the original forest ecosystem, which was consistent with a previous study [62]. This may be due to the fact that fertilization in farmland can directly provide a large amount of carbon sources for microorganisms and promote the assimilation, utilization of carbon by microorganisms, thereby promoting the increase of chemical heterotrophic microbes. Different vegetation types could cause changes in bacterial community function in the soil. In our study, comparedwith coniferous forests, the soil bacterial functional abundance of aerobic chemoheterotrophy and chemoheterotrophy in QM were the highest with 22.05%, and 21.01%, respectively (Table S2). However, we found no significant differences with LG. Compared to the LG, soil bacterial function for aerobic chemoheterotrophy ($P = 0.0223$) and chemoheterotrophy ($P = 0.0303$) in PK significantly decreased, while phototrophy ($P = 0.0242$) and photoautotrophy ($P = 0.0306$) significantly increased (Fig. 5). Accordingly, the soil bacterial community function groups were closely related to the species of aboveground plants, which could provide various nutrients through the litters and root exudates, altering the growth environment of soil bacteria and eventually leading to the changes in the soil bacterial community functional groups [72].

Soil nitrogen fixation, nitrification, denitrification, ammonification, and other major nitrogen transformation processes are mainly mediated by soil bacteria [73]. And the soil N cycles,especially biological nitrification and denitrification processes, can affect the production and emission of such greenhouse gases as CO_2, CH_4, and N_2O [74]. And the problem of land degradation and ecological function change caused by the change of land use mode has been widely concerned [75-77]. Therefore, it is particularly important to clarify the microbiological mechanism of the nitrogen transformation process in response to different land use types. In our study, we examined 12 ecological function groups connected to the N cycles, including nitrification, aerobic ammonia oxidation, nitrate reduction, nitrogen fixation, aerobic nitrite oxidation, nitrogen respiration, nitrate respiration, nitrite respiration, nitrate denitrification, nitrite denitrification, nitrous oxide denitrification, and denitrification (Table S3), which was similar to [71]. Bacterial community function for nitrification, nitrate reduction, nitrate respiration, nitrogen respiration, aerobic nitrite oxidation,aerobic ammonia oxidation, nitrate denitrification, nitrite denitrification, nitrous oxide denitrification, denitrification, nitrite respiration, and nitrogen fixation in ZM were 4.18%, 3.01%,2.14%, 2.14%, 2.38%, 1.80%, 0.27%, 0.27%, 0.27%, 0.27%, 0.27%, and 0.11%, respectively – significantly decreased
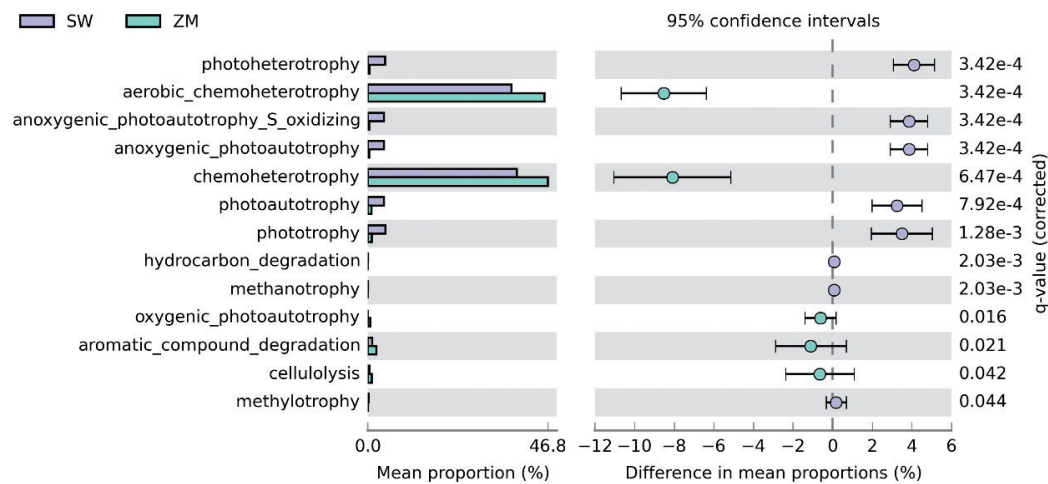
Fig. 4. Differences in relative abundance of function between SW and ZM sites. Only significantly different function are shown, with corrected P-values calculated using Storey's FDR approach (Two-sided) (P<0.05). Error bars show 95% confidence intervals. ZM, *Zea mays*; SW, *Shrub wood*.

compared to forest soils (Table S3). And nitrate denitrification, nitrite denitrification, and denitrification related to the nitrogen cycle showed a significant difference between ZM and forest soil (*P*<0.01) (Table S3), and significantly higher in the forests. The reason for this difference might be due to the existence of the denitrifying genes (nirS, nirK, and nosZ) in soil microorganisms. Previous research from Lammel et al. [78] reported that the variation pattern of functional gene abundance of microorganisms related to N cycling during the transition from forest to farmland, and the results showed that the abundance of functional genes in forest soil were significantly higher than that in farmland, which confirmed our results to some extent. Conversion of forests to agricultural land uses reduces litter inputs [79], and forest vegetation showed more refractory substances, and carbon and nitrogen contents [80]. With the increase of litter and plant root exudates

in forest ecosystems, soil nitrogen content increased, which stimulated the growth and reproduction of denitrifying microorganisms, significantly increased microbial activity, and altered N cycles.

To our knowledge, this is the first attempt to clarify the differences of bacterial community functional groups between forest soils (QM, SW, LG, and PK) and agricultural soil (ZM) in the mountainous region of eastern Liaoning Province, China. These conclusions might be explained by the soils at the forest and agriculture sites maybe having different functional groups for the C and N cycles. And LG and PK lands had similar bacterial function compared to the QM land. However, FAPROTAX is not a substitute for a whole metagenome profiling, which may pose a problem when the analysis of the microbiome contains large amounts of uncultured bacteria and does not involve the function of the ancient community.
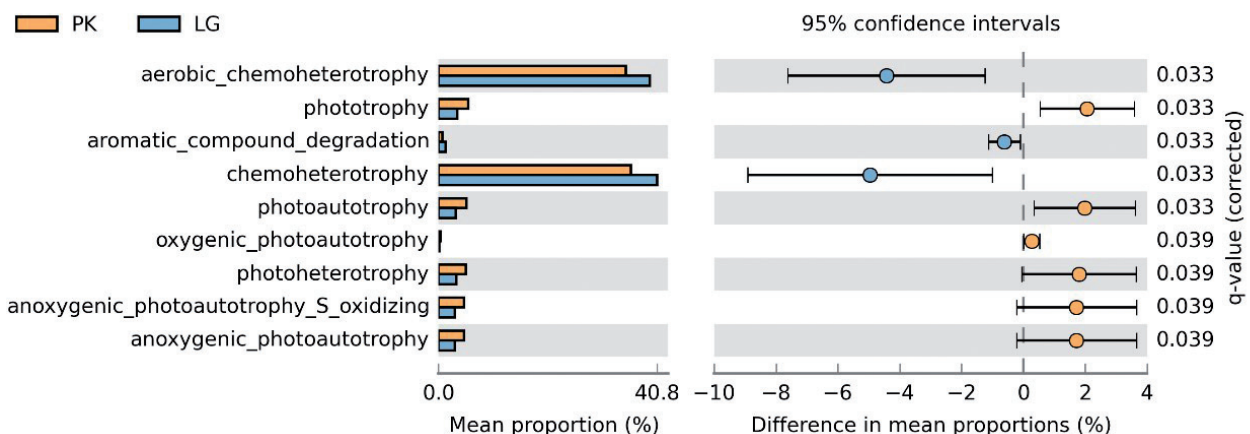
Fig. 5. Differences in relative abundance of function between PK and LG. Only significantly different function are shown, with corrected P-values calculated using Storey's FDR approach (Two-sided) (P<0.05). Error bars show 95% confidence intervals. LG: *Larix gmelini*; PK, *Pinus koraiensis*.

Table S3. Functional groups related to the nitrogen cycle. QM: *Quercus mongolica*; SW: Shrub wood; LG: *Larix gmelini*; PK: *Pinus koraiensis*; ZM: *Zea mays*.

Samples	Nitrification	Nitrate reduction	Nitrate respiration	Nitrite respiration	Nitrogen respiration	Aerobic nitrite oxidation	Aerobic ammonia oxidation
PK	10.66±0.44aA	8.92±0.20aA	8.88±0.21aA	8.88±0.21aA	8.88±0.21aA	8.12±0.31aA	2.53±0.24aA
LG	7.45±0.83bB	6.91±0.5bB	6.21±0.43bB	6.21±0.43bB	6.21±0.43bB	5.85±0.87bB	1.59±0.03aA
QM	6.85±1.04bB	6.12±0.11cB	5.20±0.49cC	5.20±0.49cC	5.20±0.49cC	4.89±0.08bcBC	1.96±0.99aA
ZM	4.18±1.59cC	3.01±0.68dC	2.14±0.26dD	2.14±0.26dD	2.14±0.26dD	2.38±0.86dD	1.80±0.74aA
SW	6.35±0.21bBC	5.84±0.31cB	5.02±0.31cC	5.02±0.31cC	5.02±0.31cC	4.25±0.07cC	2.11±0.28aA

Samples	Nitrate denitrification	Nitrite denitrification	Nitrous oxide denitrification	Denitrification	Nitrite respiration	Nitrogen fixation
PK	1.77±0.26abA	1.77±0.26abA	1.77±0.26abA	1.77±0.26abA	1.77±0.26abA	1.00±0.10aA
LG	1.62±0.26bA	1.62±0.26bA	1.62±0.26bA	1.62±0.26bA	1.62±0.26bA	0.31±0.05bB
QM	1.61±0.30bA	1.61±0.30bA	1.61±0.30bA	1.61±0.30bA	1.61±0.30bA	0.30±0.07bB
ZM	0.27±0.19cB	0.27±0.19cB	0.27±0.19cB	0.27±0.19cB	0.27±0.19cB	0.11±0.05cB
SW	2.21±0.22aA	2.21±0.22aA	2.21±0.22aA	2.21±0.22aA	2.21±0.22aA	0.31±0.11bB

Relationships between Bacterial Community Functional Groups and Soil Environmental Factors

Canonical correspondence analysis (CCA) was applied to explore the soil environmental factors and functional groups related to the C and N cycles. The first and second axes accounted for 94.2% and 93.7% of the variation, respectively, indicating that soil environmental factors significantly influence the bacterial community functional groups (Fig. 6). In terms of C cycles, soil pH (r = 0.7451), NH_4^+-N (r = -0.5606), and NO_3^--N (r = -0.6784) were significantly correlated with axis1, and the first axial interpretation rate was 88.0% (Fig. 6a). In terms of N cycles, soil pH (r = 0.6093), and NH_4^+-N (r = -0.6293) were significantly correlated with axis2, and the second axial interpretation rate was 40.7% (Fig. 6b).

To further explore the relative importance of individual environmental variables, we performed the Pearson correlation analysis of the relative abundances of functional groups within each functional group, using eight key environmental variables as shown in Fig. 7. Soil pH was significantly negatively correlated with phototrophy (r = -0.57, *P*<0.05), photoautotrophy (r = -0.61, *P*<0.05), photoheterotrophy (r =- 0.61, *P*<0.05), anoxygenic photoautotrophy S oxidizing (r = -0.65, *P*<0.05), and anoxygenic photoautotrophy (r = -0.65, *P*<0.05) (Fig. 7a). Our results were consistent with a previous study that indicated that (oxygenic) photoautotrophy was negatively correlated to soil pH value [61]. In our study, the content of NO_3^--N was negatively correlated to chemoheterotrophy (r = -0.72, *P*<0.01), aerobic chemoheterotrophy (r = -0.71, *P* < 0.01), and aromatic compound degradation (r = -0.64, *P*<0.01), which haspositive correlation with photoheterotrophy (r = 0.55, *P*<0.05), anoxygenic photoautotrophy S oxidizing (r = 0.55, *P*<0.05), and anoxygenic photoautotrophy (r = 0.55, *P*<0.05). Also, a past study documented that the content of NO_3^--N was the dominating environmental factor related to C cycle [71]. The decomposition potential of soil microbes to refractory carbon is greatly affected by soil N content.

The processes of soil microorganism nitrification and denitrification are affected by various physical, and chemical factors, such as soil moisture condition, NH_4^+ -N and NO_3^-- N contents, soil organic matter, and so on [81]. We found that soil environmental conditions (especially soil pH, NH_4^+-N, and NO_3^--N contents) strongly influenced the distribution of functional groups about C and N cycles in bacterial communities. In our study, soil pH was significantly negatively correlated with nitrification (r = -0.68, *P*<0.01), nitrate reduction (r = -0.78, *P*<0.01), nitrate respiration (r = -0.81, *P*<0.01), nitrogen respiration (r = -0.81, *P*<0.01), aerobic nitrite oxidation (r = -0.74, *P*<0.01), nitrate denitrification (r = -0.64, *P*<0.01), nitrite denitrification (r = -0.64, *P*<0.01), nitrous oxide denitrification (r = -0.64, *P*<0.01), denitrification (r = -0.64, *P*<0.01), nitrite respiration (r = -0.64, *P*<0.01), and nitrogen fixation (r = -0.61,

P<0.05). A previous study suggested that nitrification is negatively correlated to soil pH [61], which was similar to our study results. Soil pH not only affects the process of soil nitrification via the activity and composition of nitrifying microorganisms, but it also can directly affect the activity of denitrifying microorganisms and

the denitrification process [82]. The reason may be that pH changes the chemical form, concentration and effectiveness of substrates in forest soil, thus affecting the nitrifying and denitrifying bacterial community and metabolic activity. Enwall et al. used nosZ and narG genes as functional markers of denitrifying
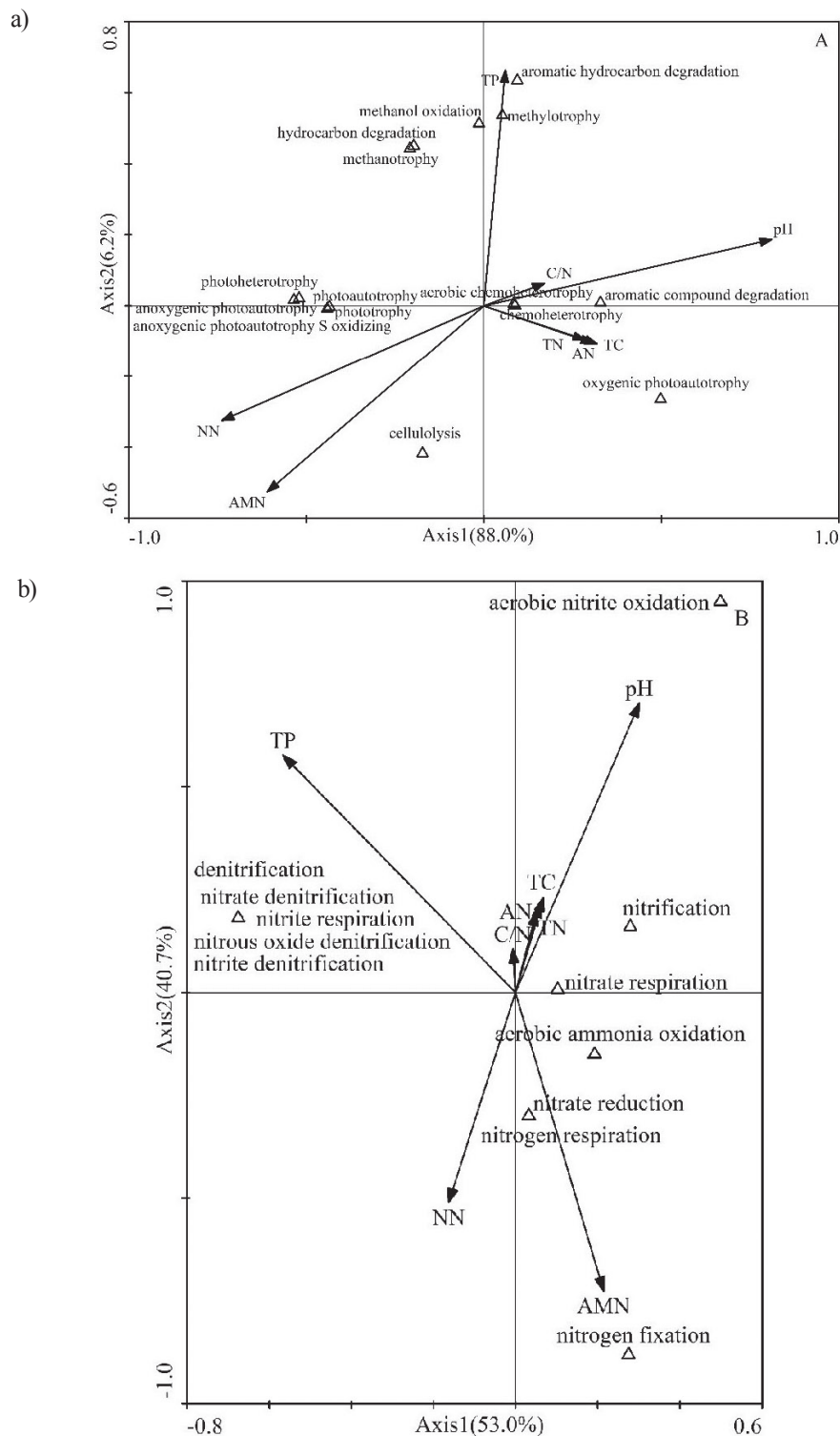

Fig. 6. Canonical correlation analysis (CCA) of the predicted FAPROTAX functions associated with soil physiochemical properties. a) Functional groups that are related to carbon cycle. b) Functional groups that are related to nitrogen cycle. QM: *Quercus mongolica*; SW: *Shrub wood*; LG: *Larix gmelini*; PK: *Pinus koraiensis*; ZM: *Zea mays*.
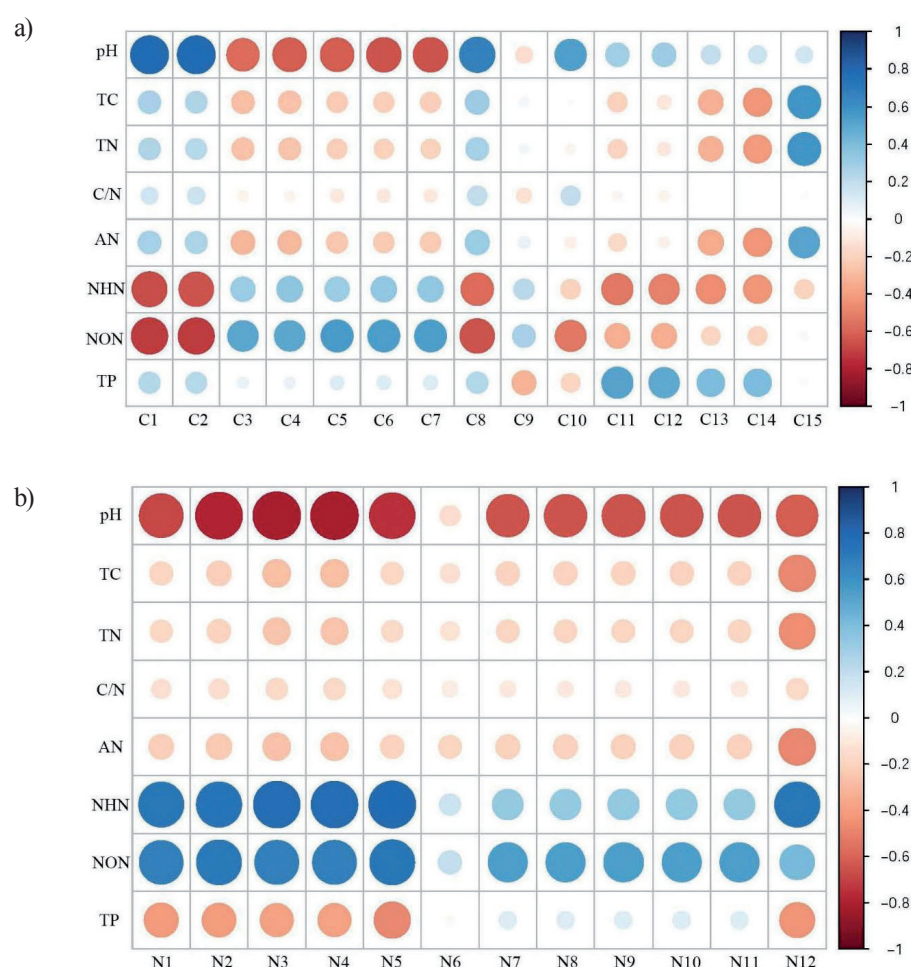
Fig. 7. Rank correlations between soil environmental variables and functional groups that are related to carbon cycle (A) and nitrogen cycle (B). Blue and red colors indicate positive and negative correlations, respectively. Functional groups related to the carbon cycle and nitrogen carcle in the Table S2.

bacteria to explore the effect of applying organic and inorganic fertilizers on the activity and composition of denitrifying bacteria in cultivated soil. It was found that the long-term application of $(NH_4)_2SO_4$ resulted in the changes of soil pH, resulting in changes in the composition of the denitrifying bacteria community [82]. NH_4^+-N was significantly positively correlated with nitrification (r = 0.73, *P*<0.01), nitrate reduction (r = 0.73, *P*<0.01), nitrate respiration (r = 0.78, *P*<0.01), nitrogen respiration (r = 0.78, *P*<0.01), aerobic nitrite oxidation (r = 0.79, *P*<0.01), and nitrogen fixation (r = 0.73, *P*<0.01). NO_3^--N was significantly positively correlated with nitrification (r = 0.68, *P*<0.01), nitrate reduction (r = 0.72, *P*<0.01), nitrate respiration (r = 0.69, *P*<0.01), nitrogen respiration (r = 0.69, *P*<0.01), aerobic nitrite oxidation (r = 0.73, *P*<0.01), nitrate denitrification (r = 0.55, *P*<0.05), nitrite denitrification (r = 0.55, *P*<0.05), nitrous oxide denitrification (r = 0.55, *P*<0.05), denitrification (r = 0.55, *P*<0.05), and nitrite respiration (r = 0.55, *P*<0.05) (Fig. 7b). In our study, the contents of NH_4^+-N showed significantly positive correlation with aerobic nitrite oxidation. As the first substrate of nitrification, a stable supply of NH_4^+-N is necessary

to maintain the population activity of ammonia oxides, and the availability of NH_4^+-N plays an important role in regulating the process of ammonia oxidation [84]. In addition, research by Petersen et al. found that the AOA community structure was significantly correlated with the content of NH_4^+-N [85]. A previous study indicated that nitrification rate was significantly positively correlated with soil NH_4^+-N content, and denitrification rate was significantly positive correlation with soil NO_3^--N content. However, some studies have reported that the higher the soil organic matter content, the greater the denitrification potential [86]. In consequence, the change in the soil bacterial community functional groups caused by land-use change is linked to changes in soil environment factors – especially soil pH, NH_4^+-N, and NO_3^--N contents.

Conclusions

In conclusion, bacterial functional groups varied across samples under different land use patterns. The dominant functions were chemoheterotrophs

and aerobic chemoheterotrophy with average relative abundance of 21.69% and 21.01%, respectively. In addition, we detected 15 and 12 ecological function groups related to the carbon cycles, and nitrogen cycles, respectively. It seems that similar land-use patterns usually have similar soil bacterial community functional groups in a specific area. The bacterial functional groups of ZM were clearly separated from those of PK, LG, QM, and SW, indicating that functional groups of agriculture soil differed from those of forest soils. Furthermore, the bacterial functional groups of LG and PK were obviously separated from those of QM and SW, suggesting that functional groups of broadleaf forest differed from those of coniferous forests. Soil environmental conditions, especially soil pH, NH_4^+-N, and NO_3^--N content, strongly influenced the distribution of functional groups about carbon and nitrogen cycles in bacterial communities.

Acknowledgements

Conflict of Interest

The authors declare no conflict of interest.

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