

SOIL BACTERIAL COMMUNITY STRUCTURE AND DIVERSITY OF BROWN CONIFEROUS FOREST IN DAXING'ANLING MOUNTAIN, CHINA

LIBIN YANG¹, XIN SUI^{2*}, TONG ZHANG², DAOGUANG ZHU¹, FUXING CUI¹,
CHUNRONG CHAI¹ AND HONGWEI NI¹

Institute of Nature and Ecology, Heilongjiang Academy of Sciences, Harbin, 150040, China

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Abstract

In order to understand the changes in soil habitats and soil bacterial diversity of three different forest types in Daxing'anling mountain in northeastern China, three typical forest types were selected for study. The results showed that there were differences in soil nutrients between three forest types. The bacterial diversity of three forest types showed that brown grass coniferous forest was the highest. The result showed that the main bacteria were Acidobacteria (39.53%), Proteobacteria (34.99%), Actinobacteria (6.08%), Bacteroidetes (3.88%), Gemmatimonadetes (2.77%) and Verrucomicrobia (2.46%). Redundant analysis showed that the OTU (97%) composition of soil bacteria in the three forest types and the contribution rates of the two axes were 53.39 and 27.81%, respectively. TN, AN, AP, TK, AK, OC, pH are related with the bacterial community of brown coniferous forest, MC and TP related with the bacterial diversity of brown grass forest coniferous and typical brown coniferous forest.

Introduction

Soil microorganisms are not only the decomposers of forest ecosystems, but also consumers. They are involved in the circulation of materials, the flow of energy and the transmission of information in forest ecosystems. The species composition and community structure of soil microorganisms largely determine the biological activity of the soil (Karabi *et al.* 2016). The structure and function of the forest ecosystem can be predicted by the structural characteristics of the soil microorganisms (Lagomarsino *et al.* 2009). The amount and type of soil microorganisms in soil bacteria are mostly involved in soil organic matter decomposition and mineralization, circulation of chemical pollution, degradation and repair the ecological environment (Huo *et al.* 2013). The community structure and composition of soil bacteria can reflect the influence of soil nutrient changes and environmental changes, and can directly affect the function of soil (Guo *et al.* 2014). Studies using traditional microbiological culture methods and identification cannot accurately characterize a large number of microorganisms that cannot be cultured, and therefore it is difficult to accurately reveal the community structure and function (Wright *et al.* 2009, Sui *et al.* 2016).

The mountain, Daxing'anling is located at the high latitude of the northern hemisphere and is one of the most sensitive regions in response to global climate change (Gao *et al.* 2016). The brown coniferous forest soil is the zonal soil type of the north end of daxinganling (Li 1997). Forest soil is the direct source of nutrients needed for forest growth, and it restricts the composition of forest communities, stand structure, forest productivity, and the stability of forest ecosystems (Lu *et al.* 2015). Many scholars reported that different types of forest soil organic carbon (LYU *et al.* 2016, Xiong *et al.* 2017), nitrogen, phosphorus (Cheng *et al.* 2018), potassium, magnesium, iron, sodium (Liu *et al.* 2009), such as content of spatial heterogeneity, in contrast to the study of soil microbial community structure characteristics are relatively few.

*Author for corresponding: <xinsui_cool@126.com>. ¹Institute of Advanced Technology, Heilongjiang Academy of Sciences, Harbin, 150020, China. ²College of Life Science, Heilongjiang University, Harbin, 150080, China. ³College of Life Science, Northeast Forestry University, Harbin, 150040, China.

In 2014, the research team established an international monitoring sample of 25 ha in Xingan deciduous pine forest in Huzhong national nature reserve in China. Three types of brown coniferous soils via light brown coniferous forest soils, grass brown coniferous forest soils and typical brown coniferous forest soils were observed in Daxingan mountain pine forest in Huzhong (Wang 2011). In the present study the method of high throughput sequencing to study the soil bacterial diversity and community structure of three types of brown coniferous forest was adopted. Besides the analysis of relative abundance of soil bacterial species, and evolutionary relationship was done. The diversity of soil bacteria and the intrinsic relationship between soil environmental factors was carried out. In order to reveal the soil environment in the construction of forest ecological system and the interaction mechanism of microbial communities was attempted to explore.

Materials and Methods

The research area is located within the Huzhong National Nature Reserve in Daxinganling, and the sample plot is located in the international monitoring site of 25 ha of *Larix gmelini* plantation in Daxinganling in 2014 (Fig. 1). The plot is flat, with an elevation of 847~974 m, an average annual temperature of -4°C , an average annual rainfall of 458.3 mm, an average relative humidity of 71%, and an annual evaporation of 911 mm. Community structure is simple, in order to utilize modern larch as the main dominant species of the northern bright coniferous forest, the formation of duchamp sing-an larch forest, grass sing-an larch forest and the cuckoo - sing-an larch forest are three main community types. There are three types of soil subtypes: surface light brown coniferous forest soils, grass brown coniferous forest soils and typical brown coniferous forest soils.



Fig. 1. Location of sampling sites in the Daxinganling mountain-Huzhong national nature reserve.

There are 41 species of woody plants with DBH > 1 cm in the sample area, including 4 species of trees, 37 species of shrubs and 127 species of herbs, belonging to 21 families and 39 genera (Yang *et al.* 2017). The light brown coniferous forest soil is located in the lower part of the plot, with a small slope (5°). The trees are *Leymus chinensis* (Trin.) Tzvel. and *Betula platyphylla* suk. The main shrubs include *Ledum palustre* L. and *Vaccinium uliginosum* Linn. The main herbs include the

Maiethehemum bifolium (L.) FW Schmidt and *Sanguisorba officinalis* L. Grass brown coniferous forest soil in the sample area is upper slope which is bigger (30°), steep terrain, utilize modern trees for larch and birch *Betula platyphylla* Suk., shrubs, mainly for the sing-an azalea *Rhododendron dauricum* L. and meadow sweet *Spiraea salicifolia* L., herbaceous plants are more.

The northern wild pea *Vicia ramuliflora* Linn, the small white flower *Sanguisorba tenuifolia* var. *alba*, the two-leaf *Maianthemum bifolium* (L.) FW Schmidt, the longjiang wind, *Saussurea amurensis* Turcz. Ex DC., *Geranium platyanthum* Duthie, the *Calamagrostis angustifolia* Kom. were recorded.

Typical brown coniferous forest soil is located at the top of the plot hill with a relatively flat topography and a small slope (10° ~ 15°). The trees are *L. gmelinii* and *Betula platyphylla* Suk. and the main shrubs are *Rhododendron dauricum* L and *Bilberry Vaccinium* spp. The main herbs are saffron *Pyrola incarnata* Fisch. ex DC. and *Saussurea amurensis* Turcz.

Three 20 m × 20 m standard plots were set for each sub-category soil, each plot being 100 m apart in the experimental plots. Samples were collected from four corners and center setting five sampling points in July 2017 removing the litter and humus layer, with a diameter of 10 cm soil layer soil auger acquisition 0 ~ 20 cm soil, pick up litter, fine root, and small stones and other debris after 2 mm nylon screen and into 2 portions, one was used for the determination of soil microorganisms; another was used to determine the physical and chemical parameters.

Soil moisture content was determined by drying method. The pH value was determined by potentiometric method; Soil total nitrogen, alkali-nitrogen and organic carbon mass fraction were determined by CN analyzer. The total phosphorus mass fraction of soil was determined by molybdenum antimony colorimetry. Soil available phosphorus fraction was determined by NaHCO₃ Extraction Colorimetry. The quality fraction of total potassium and instant potassium in soil were determined by flame photometry.

The bacterial DNA isolated from the soil was extracted using the American Strong Soil Kit (MOBIO12888) following the instructions in the manual. Using 50 ng DNA as a template, universal primers (338F/806R) (Yang *et al.* 2018) were used to amplify in the V4-V5 highly variable region. The PCR amplification system consisted of 5 × Fast Pfu Buffer 4 µl, 2.5 mmol/l dNTP 2 µl, 5 µmol/l Bar-PCR Primer F 0.8 µL, 5 µmol/l Primer R 0.8 µl, 5U/µl Polymerase 0.4 µl, DNA template 10 ng, ddH₂O to 20µl. Amplification conditions were pre-denaturation at 95°C for 3 min, denaturation at 95°C for 30 s, annealing at 45°C for 20 s, extension at 65°C for 30 s, 5 cycles, secondary amplification at 95°C for 20 s, annealing at 55°C for 20 s, 72°C Extend 30 s, 20 cycles, and finally extend for 5 min at 72°C. The mixed PCR product of the same sample was detected by 2% agarose electrophoresis, and the PCR product was recovered by cutting the gel using AxyPrep DNA gel recovery kit. The purified amplification product was equimolarly mixed to construct the Miseq library and sequenced using the Illumina MiSeq platform (Yang *et al.* 2017).

Data were processed using Qiime (version 1.17 <http://qiime.org/>) software. Operational taxonomic units (OTUs) were divided into standards based on 97% similarity using Usearch (version 7.1, <http://drive5.com/parse/>). Using the RDP classifier Bayesian algorithm for taxonomic analysis of OTU representative sequence, the confidence threshold is 0.7. The mothur software was used for alpha diversity index analysis and rarefaction analysis (Yang *et al.* 2017), and the sequencing depth index was expressed using the coverage index (<https://www.mothur.org/wiki/Coverage>), using Ace (<https://www.mothur.org/wiki/Ace>) and Chao1 (<https://www.mothur.org/wiki/Chao>) indices indicate community abundance using Shannon (<https://www.mothur.org/wiki/Shannon>) and Simpson (<https://www.mothur.org/wiki/Simpson>) index indicates the richness and diversity of bacterial community species. The above index was analyzed with mothur; correlation analysis (level 0.05) using Pearson method using SPSS 17.0

software; Redundancy analysis using R language vegan package (RDA) and ggplot package mapping for redundancy analysis (Yang *et al.* 2018). Correlation analysis was followed by the Pearson method.

Results and Discussion

From Table 1 it is apparent that the soil moisture content of the three sub-categories decreased with increasing altitude, and the soil water content of the light brown coniferous forest was the largest, 2.5 times that of the soil moisture of the other two sub-class soils. The result showed that the soils of all sub-categories are acidic, and the pH values of light brown coniferous forest soils were the strongest. There were differences in soil nutrient elements. Among them, the contents of alkali-hydrolyzable nitrogen, total phosphorus, available phosphorus, total potassium, and available potassium of brown grass of coniferous forest of soil were higher than others.

Table 1. Comparison of soil physicochemical properties in different sub-coniferous forests.

Soil sub-categories	Water content %	pH	Total nitrogen (g/kg)	Alkaline nitrogen (mg/kg)	Total phosphorus (g/kg)	Available phosphorus (mg/kg)	Total potassium (g/kg)	Available potassium (mg/kg)	Organic carbon (g/kg)
B	40.1 ± 2.2a	4.4 ± 0.1c	4.1 ± 0.1a	99.8 ± 0.7a	2.5 ± 0.1b	36.9 ± 0.6b	36.2 ± 0.2c	329.8 ± 9.1b	42.2 ± 2.5c
C	16.5 ± 0.7b	5.0 ± 0.1b	3.0 ± 0.1c	81.6 ± 0.5b	3.1 ± 0.1a	41.9 ± 0.4a	49.7 ± 0.1a	362.9 ± 5.4a	21.5 ± 1.3a
D	14.3 ± 0.9b	5.4 ± 0.3a	3.2 ± 0.1b	38.9 ± 0.1c	2.0 ± 0.0c	26.7 ± 0.3c	41.2 ± 0.4b	317.9 ± 7.2c	27.5 ± 1.1b

There is a significant difference in delivery of different lowercase letters in the same column ($p < 0.05$), the same below.

The original sequence number of soil samples was 654196, and the total number of effective sequences was 553971 after optimizing the filtration of low-quality sequences. The effective sequence was about 84.64%, and the average length of the effective sequence was 448.29. The samples of soil samples were decomposed, and the OTU clustering was performed at 97% of the similarity, and 700 OTU were obtained. The sequence obtained by sequencing was randomly sampled, and the number of OTUs represented by the number of sequences extracted was used to construct a dilution curve. The dilution curves of soil samples of different subtypes tend to be flat and tend to be saturated. This showed that the reasonable selected samples (Fig. 2), test data volume was reasonable, the actual soil environmental fungal community structure confidence level was higher, which reflect the real soil samples of fungal community, increase the quantity of sequencing to discover new OTU contribution rate which is small.

The total number of OTU of soil bacteria in different subgroups was $C > D > B$, 264, 222 and 214 respectively, (Fig. 3). The number of OTU of soil bacteria was higher than that of B and D, respectively 49, 2 and 2. The total number of OTU in 3 subsoil bacteria was 199, BC is 203, BD is 208 and CD is 211. The total number of OTU indicated that there were similarities between each other. As a result, it could be seen that B, C and D have the same number of OTU, indicating that the similarity was larger.

The ANOVA analysis of soil 16s rDNA diversity index of different subspecies brown coniferous forests is presented in Table 2. The coverage index of soil environmental sample sequencing of different subgroups approaches 1 and there was no significant difference, indicating that the sequencing results can be accurate. The true conditions of the soil samples reflect the test. Ace, Chao, Shannon and Simpson index $C > D > B$, are indicating that the total bacterial count, bacterial population abundance, bacterial information content and complexity of the soil in the brown grass of coniferous forest were the highest.

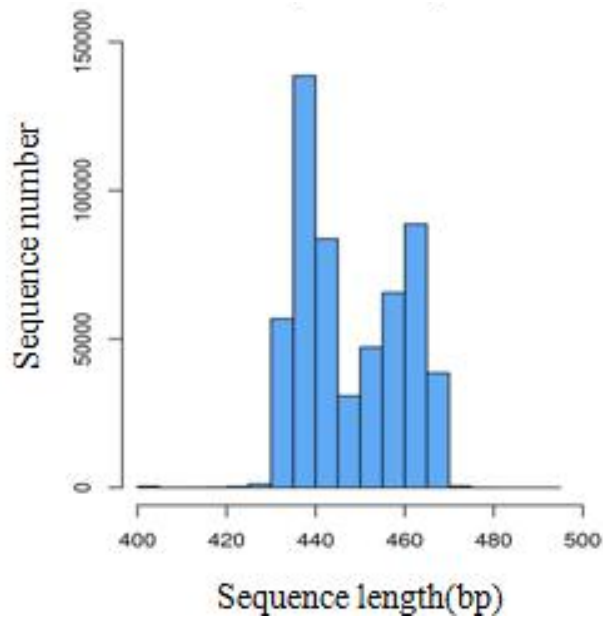


Fig. 2. Effective sequence length distribution.

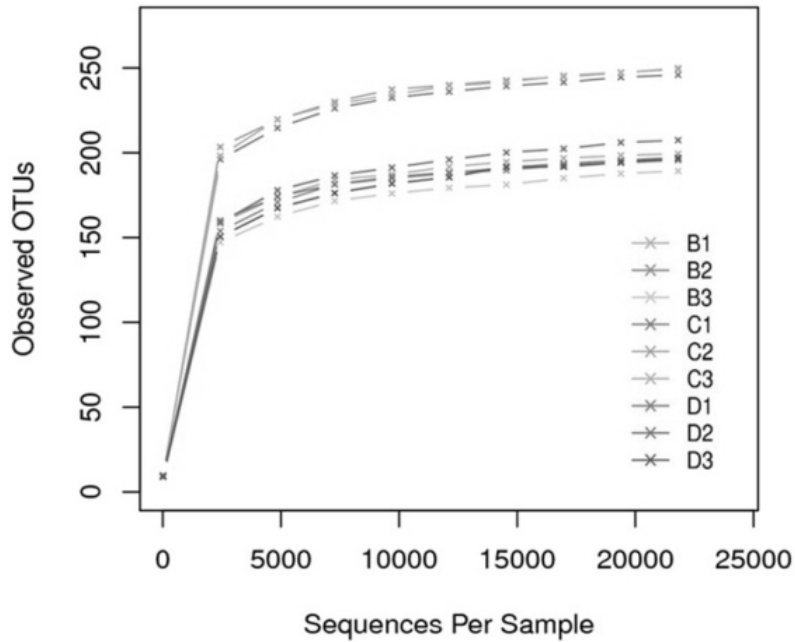


Fig. 3. Rarefaction curve of different soil samples.

Table 2. Soil bacterial diversity index of different sub-coniferous forests.

Sample	Coverage	Ace	Chao	Shannon	Simpson
B	0.9997 ± 0.0003a	203 ± 1b	205 ± 1b	5.84 ± 0.20b	0.965 ± 0.009bc
C	0.9993 ± 0.0003a	258 ± 1a	261 ± 7a	6.84 ± 0.29a	0.986 ± 0.006a
D	0.9993 ± 0.0003a	213 ± 6b	217 ± 7b	6.26 ± 0.88b	0.979 ± 0.002ab

With the similar level of 97%, taxonomic analysis was performed on the representative sequences of OTUs, and 14 soil bacteria, 49 orders, 49 families, and 437 genera were obtained. From Fig. 5, it is apparent that Acidobacteria, Proteobacteria, Actinobacteria, Bacteroidetes, and Verrucomicrobia are common dominance gate (relative abundance $\geq 1\%$) bacteria at the level of phylum classification. In addition, Gemmatimonadetes and FCPU426 are, the dominant bacteria phylum in the samples C and B, respectively. From Fig. 6, it can be seen that the soil bacteria in the light brown coniferous forest at the generic classification level was 31 genera, in the typical brown coniferous forest was 30 genera, and in the brown coniferous forest grassland was 37 genera. *Edaphobacter*, *Afipia*, *Acidothermus* sp., *Mycobacterium* sp. and *Candidatus solibacter* are common dominating genus of several subtypes of soil bacteria. It could be seen from the clustering that the soil of the brown-brown coniferous forest was similar to that of the typical brown coniferous forest soil and was different from the bacterial flora of the brown coniferous forest soil.

Redundant analysis was performed on the OTU (97%) composition of soil bacteria in the three sub-categories (Fig. 7). The contribution rates of the two axes were 53.39 and 27.81%, respectively. TN, AN, AP, TK, AK, OC and pH were positively correlated with first axis and MC and TP are negatively correlated with the first sorting axis. The light brown coniferous forest soil is located in the second quadrant, the brown grass forest coniferous soil is located in the fourth quadrant, and the typical brown coniferous forest soil is located in the third quadrant, which indicate that the soil bacteria differences of the three sub-categories of coniferous forest soil are relatively high.

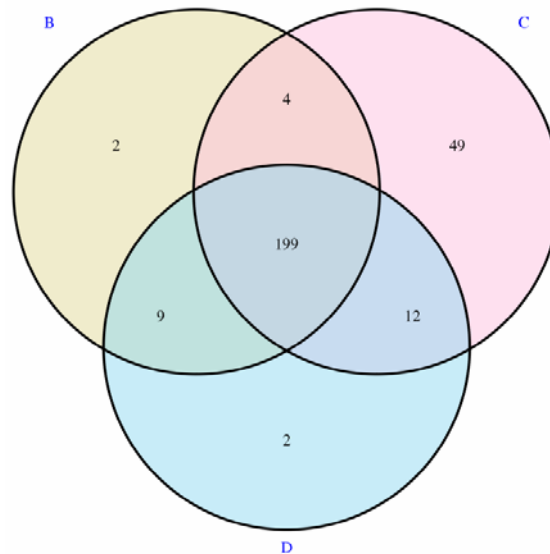


Fig. 4. Venn analyses of different soil samples.

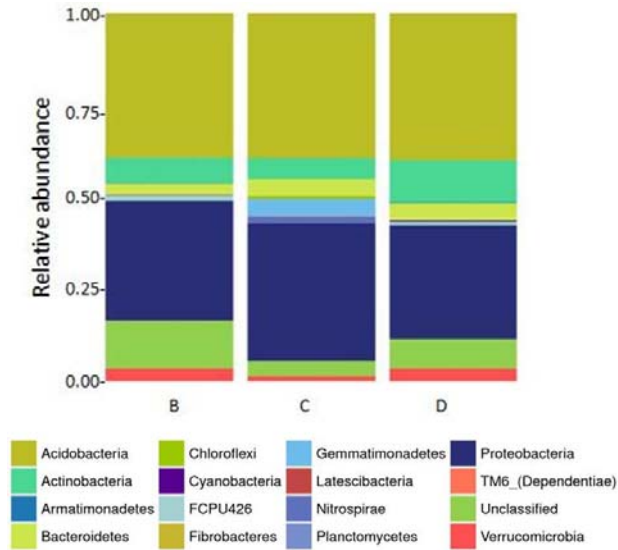


Fig. 5. Histogram of soil bacteria in different soil samples.

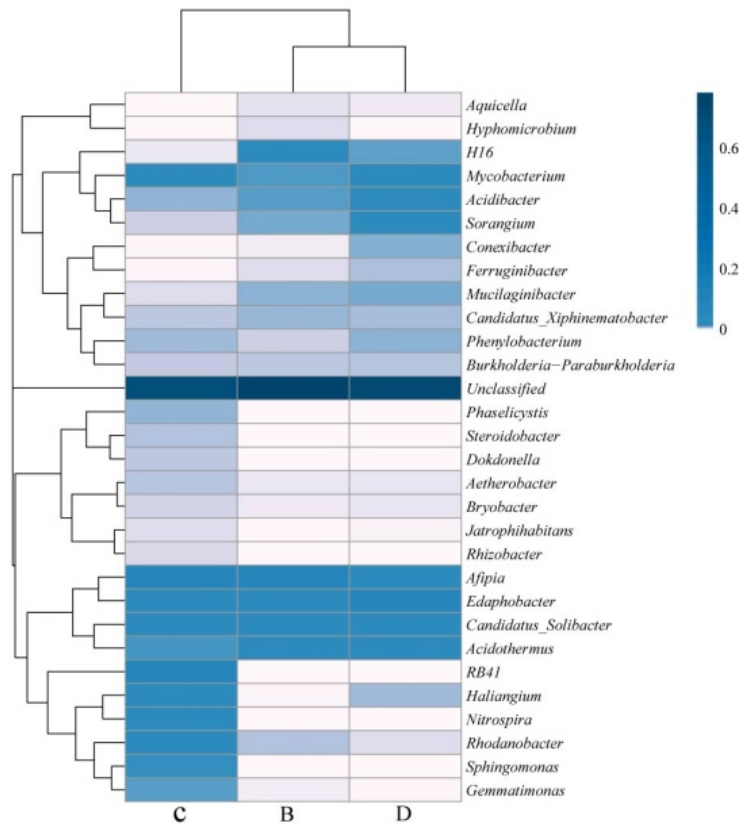


Fig. 6. Heatmap of different soil samples.

According to the analysis of multiple metastats (Fig. 8) and FDR (Fig. 9), the five bacterial phyla with the greatest differences in soil bacteria among the three sub-categories were Actinobacteria, Chloroflexi, Gemmatimonadetes, Nitrospirae, and Proteobacteria, in which the *Chloroflexi* phylum bacteria only exist in the soil of the brown grass of coniferous forests. Non-parametric tests were performed by ANOSIM analysis. It can be seen from the Fig. 9 that the differences among the soil bacterial groups in the three sub-categories were significantly higher than the intra-group differences ($R = 0.844$), and the differences were significant ($p < 0.01$).

Choosing Shan and Shannon index as the representative of the alpha diversity index, using the Pearson method the correlation between soil physico-chemical properties and the soil bacterial alpha diversity index was calculated. Table 3 it is apparent that that the Chao index is significantly positively correlated with AN, Shannon. The index is significantly and positively correlated with OC.

Table 3. Correlation coefficients between soil bacterial α -diversity indices and soil physicochemical properties.

Diversity index	MC	Ph	TN	AN	TP	AP	TK	AK	OC
Chao	-0.606	0.561	0.313	0.997*	-0.513	0.603	0.828	0.823	0.921
Shannon	-0.879	0.851	-0.103	0.941	-0.820	0.223	0.985	0.983	1.000*

* $p < 0.05$.

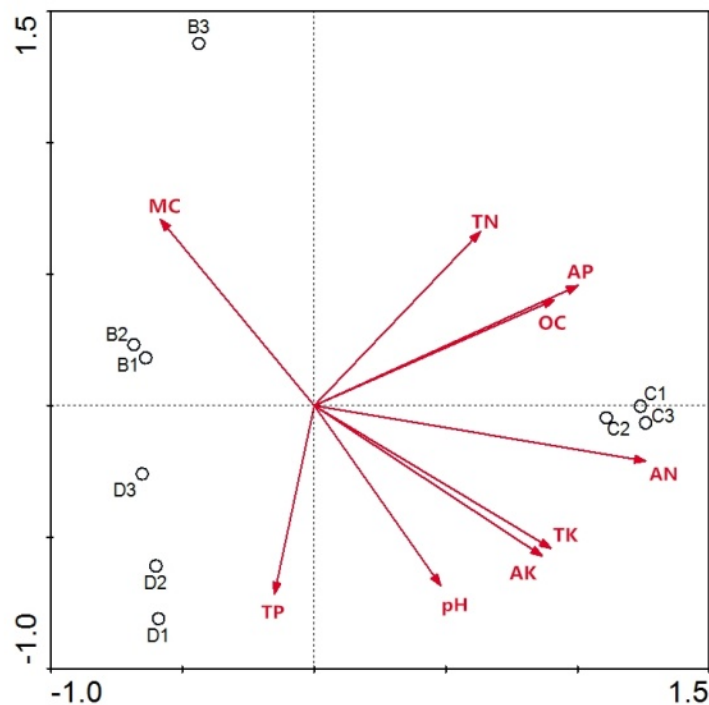


Fig. 7. CCA analyses of different soil samples.

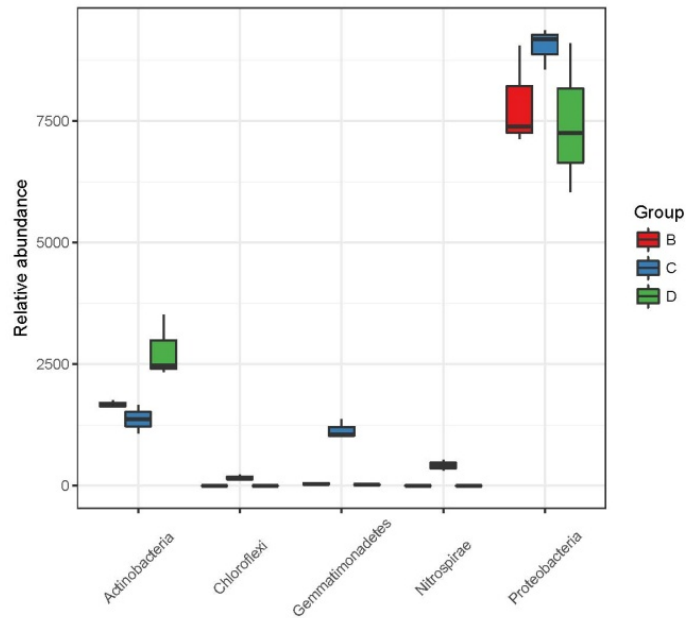


Fig. 8. Metastats analyses of different soil samples.

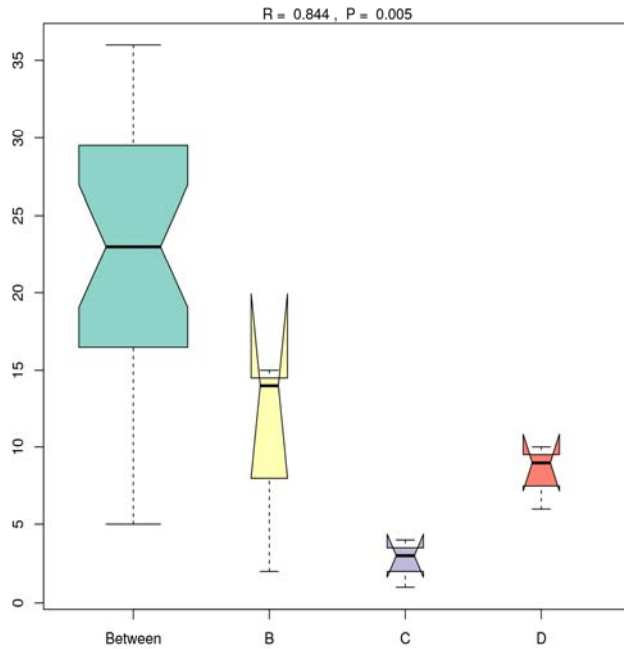


Fig. 9. FDR analyses of different soil samples.

The soil moisture content of three sub-categories of brown coniferous forests varies greatly, which might be due to the different slope positions of the plots. Three sub-categories of brown coniferous forest soils were distributed from the top of the mountain to the bottom of the mountain.

The soil of the light brown coniferous forest was at the bottom of the slope, the slope was gentle, and the gradient was 5°, which was conducive to the accumulation of soil moisture. The slope of soil in brown coniferous forest is relatively steep, with a slope of 30°, which leads to the loss of soil moisture and is not conducive to the accumulation of soil moisture. In addition, due to the steep slope of the plot, the majority of fallen trees formed forest windows, which led to increased solar radiation, resulting in lower soil moisture content. Due to the different soil moisture content, significant differences in soil pH might be resulted. It was observed that the pH value of the soil decreased with the increase of the soil moisture content, which might be one of the reasons causing the differences in the pH values of the three sub-categories. The use of diversity indices to analyze soil bacterial communities is a common and effective method (Sui *et al.* 2015). The soil environment (Berg *et al.* 2009) and soil nutrient status (Shen *et al.* 2013) of different soil types can affect soil microbes (Sui *et al.* 2016). In this study, in soils of different sub-groups, as the soil moisture content increased, the bacterial diversity index increased and decreased, which is similar to the results of Liu (Liu *et al.* 2014a) and Liu (Liu *et al.* 2014b). With the increase of pH value, the soil acidity weakened and the soil bacterial diversity index decreased. This observation is similar to the results reported by Guo *et al.* (2013). Pearson correlation analysis shows that diversity index and soil organic carbon in soil nutrient content and alkali solution nitrogen content. The results indicate that the soil moisture content, soil pH value, soil organic carbon, and alkali nitrogen content are all factors which affect the bacterial change in forest soils (Bai *et al.* 2015).

The soil bacteria in the present study were mainly Acidobacteria (39.53%), Proteobacteria (34.99%), Actinobacteria (6.08%), Bacteroidetes (3.88%), Verrucomicrobia (2.46%), and Gemmatimonadetes (2.77%), which were consistent with the finding of Janssen (2006). From the Metastats test and FDR analysis results, it was observed that the relative abundances of Proteobacteria, Actinobacteria, and Gemmatimonadetes in the three sub-groups of soil are maximum, with significant differences, especially Proteobacteria and Gemmatimonadetes (0.19, 4.72, 0.08%). The phylum of bacteria is different, and the main difference of Proteobacteria comes from the relative abundance of Betaproteobacteria (0.47, 4.14, 0.99%). This is similar to the results of Bai *et al.* (2015). The reason might be due to the fact that Betaproteobacteria contains a lot of Aerobic or facultative bacteria with variable biodegradability which are affected by environmental factors such as soil pH and nutrients (Nacke *et al.* 2011).

The research on soil bacteria is relatively extensive, but there are many uncertainties in soil bacteria known as “biological dark matter” (Jansson 2013). This study adopts a new generation of high-throughput sequencing technologies, to cool the three sub-types of coniferous forest where soil bacteria were determined. The results showed that different types of coniferous forest soil bacterial community structure which exists are significantly different. The difference of bacterial community influenced by soil moisture content, pH, organic carbon and alkaline hydrolysis. Zheng *et al.* (2004) mentioned that the soil environment of forest ecosystems is extremely complex, the distribution of soil microbial flora is affected by many factors such as topography, vegetation, soil, and human interference. On the other hand, Han *et al.* (2002) reported that soil microorganisms exhibit complex spatial heterogeneity. There are still many important Influencing factors which need further analysis.

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