Different Stand types Alter Soil Properties and Microbial Community in Warm Temperate Forest

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Research Article

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Abstract

Soil microorganism play an important role in maintaining the structure and function in warm temperate forest ecosystem. In order to explore the characteristics of soil microbial community under different stand types in in warm temperate zone, Illunima Miseq High-throughput Sequencing was used to assess the soil bacteria (16S rRNA) and fungi (ITS rRNA) communities of five forest stands (*Pinus tabulaeformis* [PT], *Juglans mandshurica* [JM], *Betula platyphylla* [BP], *Betula dahurica* [BD] and *Quercus mongolica* [QM]) in Songshan Nature Reserve. The results showed that the bacterial diversity under *Juglans mandshurica* forest was higher than other types, the fungal diversity under *Pinus tabulaeformis* forest was higher than other types. The dominant phyla and gene of soil bacteria were similar in different stand types, but there were significant differences in abundance and dominant gene of fungal community. VPA analysis showed that soil explained 49.1% of the variance in bacterial community composition and 70.6% of the variance in fungal community composition. RDA analysis showed that the dominant phyla were significantly correlated with soil pH, SOM, TN and AN. Based on our results, there are significant differences in soil microbial community structure among different stand types. Consequently, our results have important implications for understanding the driving mechanisms that control the soil microbial community during warm temperate forest.

1 Introduction

Soil microbiotas play important roles in forest ecosystem such as promoting nutrient circling and energy flow, adjusting the dynamic state of vegetation community and maintaining ecosystem equilibrium [1, 2]. Soil microbial diversity and community structure are important indicators to measure the stability of forest ecosystem [3] (Banning et al.,2011). Methods of studying soil microbiotas from Biolog to Sequencing. At present, the main method of studying soil microbiotas is High-throughput Sequencing, as it has high integrity and veracity [4]. This technology is used to obtain the characteristics of microbial community, and further explain the factors shaping or controlling the diversity and composition of soil microbial community in forest.

Soil microbial diversity and community structure are closely related to various factors, they have different effects on soil microorganisms, such as the aboveground vegetation, soil environment [5], climate [6], litter [7] and so on. But according to the study, which shows that only individual factors will mainly control soil microorganism. Soil microorganisms are forest stands-specific [8], different forest stands attract specific rhizosphere microorganisms by using their specific root exudates as substrates and signal molecules [9], resulting in significant changes in bacterial and fungal communities [10], thus reflecting plant productivity [11]. The decomposition rate and chemical composition of litter of different forest stands are significantly different, which will change the soil physical and chemical properties under different forest types, soil physicochemical properties can directly affect soil microbial community composition. These factors interact with each other, but their relative importance of microbial community needs to be further elucidated.

Songshan Natural Reserve is the most representative mountain ecosystem of warm temperature zone in North China. The reserve have various vegetation types, which mainly formed by coniferous forest and natural secondary forest. In this study, we studied the differences of soil properties under different forest stands, and analyzed the diversity and community composition of soil bacteria and fungi by Illunima Miseq High-throughput sequencing. Here, we aim to answer how different forest stand-types affect the soil properties and microbial community in the warm temperate forest.

2 Materials And Methods

2.1 Experimental design and sampling

The study area is located in Songshan Nature Reserve (115°43'44"-115°50'22" E,40°29'9"- 40°33'35" N), the southern foot of Haituo Mountain in Beijing. The region belongs to the Temperate continental climate, the annual mean amount of evaporation is 1772 mm, with an annual average temperature of 8.9℃, and an average annual precipitation of 493 mm. The soil types are Brown soil and Meadow soil. The region has a relatively rich biodiversity, including meadows, shrubs and forests, which the forest coverage rate is 87.6%. The main trees in the reserve are *Juglans mandshurica*, *Betula platyphylla*, *Quercus mongolica*, *Pinus tabuliformis*, *Betula dahurica*, etc.

Under each stand types, three 20*20 m^2 plots were set up according to the actual situation. There were 15 sample plots in total. Table 1 shows the overview of the different stand types. After removing litter and stone, collected 0-20 cm soil layers from every plot (from four corners and center points), and then fully mixed and packed in plastic bags. After arriving laboratory, the samples were stored in a cooling box, divided into two sub samples, one of which was air dried and used for soil characteristic analysis, and the other was stored at -60 ℃ for microbial analysis.

Table 1
Forest stands information

Stand types	Elevation/m	Slope/°	Aspect	Density/hm^{-2}	Canopy/%	Associated tree species
Betula platyphylla	1400	10	N	1400	85	*Syringa oblata, Spiraea salicifolia*
Pinus tabuliformis	1210	18	EN	1210	80	*Syringa amurensis, Corylus mandshurica*
Betula dahurica	1020	30	ES	1020	80	*Ulmus laciniata, Rhamnus leptophylla Schneid*
Juglans mandshurica	760	25	WN	760	85	*Syzygium aromaticum, Fraxinus chinensis*
Quercus mongolica	670	23	WN	670	80	*Spiraea trilobata, Corylus heterophylla*
Note: Data of each stand are mean of three sampling plots. The same below.						

2.2 Determination of the physical and chemical properties

Soil pH was measured using a pH meter after shaking a soil-water (1:5 w/v) suspension for 30 min [12]. Soil organic material (SOM) was measured by external heating method [12]. Total nitrogen (TN) content was assessed via the Kjeldahl method. Available nitrogen (AN) was determined by the alkali diffusion method (Bao., 2000). Available phosphorus (AP) was measured using the colorimetric method with extraction via 0.5M $NaHCO_3$ (Emteryd, 1989). Available potassium (AK) was determined by flame photometry [12].

2.3 DNA extraction and PCR amplification sequencing

Total DNA of Soil microorganisms was extracted using the Mobo Power Soil DNA Isolation Kit. The primer sets: ITS1F(5'-CTTGGTCATTTAGAGGAAGTAA-3') [13] and ITS2F(5'-GCTGCGTTCTTCATCGATGC-3') [14] were selected to target the fungal ITS1 region, 338F (5'-ACTCCTACGGGAGGCAGCA 3') and 806R (5'- GGACTACHVGGGTWTCTAAT -3')

[15] were selected to target the bacterial V3-V4 region. Sample-specific seven-bp barcodes were incorporated into the primers for multiplex sequencing. PCRs were performed in triplicate using a 20 μL mixture containing. 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase and 10 ng of template DNA. The resulting PCR products were extracted from a 2% agarose gel, further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA) in accordance with the manufacturer's protocol.

2.4 Sequence data and Statistical analysis

The original offline data of High-throughput sequencing was preliminarily screened according to the sequence quality. The original sequences passed the preliminary quality screening were divided into libraries and samples according to Index and Barcode information. Barcode sequences were removed, and OTU clustering was performed according to the QIIME2 DADA2 analysis process. The CD-HIT method was used to classify OTU according to 97% similarity, and the bacteria and fungi were compared with Silva and Unite databases respectively to obtain the species classification information corresponding to each OTU. Using QIIME to analyze the microbial diversity index, including Shannon index and Chao1 index, to compare the community composition of soil microbial communities in different forest types at the taxonomic level of phylum and genus.

Variations in the soil properties were assessed by one-way analysis of variance (ANOVA), and comparisons between the means were performed using the least significant difference (LSD) method ($P < 0.05$) with SPSS software (version 19.0; Chicago, IL, USA) (Banerjee et al., 2016). R was used to conduct principal coordinate analysis based on Unifrac distance algorithm, and Pearson correlation analysis and Redundancy analysis (RDA) were used to test the relationship between soil microbial communities and soil environmental factors. We also used variation partition analysis (VPA) to estimate the relative contributions of soil using the following variations in species composition.

3 Result

3.1 Soil properties

There were significant differences in soil properties under different stand types (Table 2). Soil pH value ranged from 5.87 to 6.51. Soil organic material and available N exhibited highest value in the soil of PT forest, but only 33.16 g/kg and 68 mg/kg in the QM forest, respectively. Total N was found in ranked order of BP > PT > JM > QM > BD. JM was the highest values in available P, but was the lowest values in available K. PT was the highest values in AK, but was the lowest values in AP.

Table 2
Soil properties

Stand types	pH	SOM (g/kg)	TN (g/kg)	AN (mg/kg)	AP (mg/kg)	AK (mg/kg)
BP	6.09±0.25 ab	66.93±2.74 a	1.61±0.07 a	236±9.63 a	12.71±0.52 b	214±8.74 b
PT	6.25±0.26 ab	70.28±2.75 a	1.57±0.07 a	247±10.08 a	2.76±0.11 e	378±15.43 a
BD	6.33±0.26 ab	33.08±1.35 c	0.82±0.03 c	147±6.00 b	4.64±0.19 c	178±7.27 c
JM	6.51±0.27 b	42.29±1.72 b	1.09±0.04 b	77±3.14 c	14.25±0.58 a	142±5.80 d
QM	5.87±0.24 a	33.16±1.36 c	0.88±0.03 c	68±2.78 c	3.78±0.16 d	174±7.10 c
sig	P=0.198	**	**	**	**	**

Note: JM, Juglans mandshurica; **BP**, Betula platyphylla; **QM**, Quercus mongolica; **PT**, Pinus tabuliformis; **BD**, Betula dahurica. **The data in the table is the mean ± standard deviation; Different lowercase letters indicate that the properties indexes of soil samples of different forest stands are significantly different at 0.05 level.**

3.2 Microbial community diversity

Bacterial and fungal α-diversity varied greatly across the samples. The Chao1 index and Shannon index of bacteria showed the highest values in the JM forest. The Chao1 index, Shannon index of fungi, the value of PT were significantly higher than other species (Table 3). Pearson correlation analysis indicated that the α-diversity of bacteria and fungi were related to soil properties.

Table 3
Soil microbial Alpha diversity

Microbial category	Bacterial		Fungal	
diversity index	Chao1	Shannon	Chao1	Shannon
BP	24132.37±2090.38bc	11.70±0.28b	264.97±38.12a	5.66±0.31c
PT	20678.23±5507.72ab	10.68±0.48a	600.57±82.87b	5.92±0.46c
BD	23575.17±912.58bc	11.50±0.26b	255.82±50.02a	4.23±0.56b
JM	26627.80±519.60c	12.07±0.04b	225.09±1.81a	5.90±0.29c
QM	17007.67±525.20a	10.73±0.32a	154.24±31.32a	3.00±0.22a

Note: The data in the table is the mean ± standard deviation; Different lowercase letters indicate that the properties indexes of soil samples of different forest stands are significantly different at 0.05 level.

3.3 The composition of the microbial communities

At the phylum level, the dominant bacteria in soil of all stand types were Actinobacteria (40.54%-19.75%), Proteobacteria (25.78%-13.04%) and Acidobacteria (26.83-13.59%). The total relative abundance reached more than 67.17%, but there were great differences content among different stand types. The abundance of Verrucomicrobia, Gemmationadetes and Rokubacteria were also the main soil bacteria groups (Fig. 1A). Basidiomycota

(16.64%-77.63%) and Ascomycota (19.94%-49.84%) were the dominant fungi in soil. Except QM, Mortierellomtcota (9.61%-1.30%) was the main soil fungi group in other stand types (Fig. 1B).

At the genus level, the compositions of bacterial were similar but the contents were different under different stand types (Fig. 2A). But the difference of fungal composition were obvious. On the whole, the dominant genera were Mortierella and Russula (Fig. 2B).

In order to further clarify the differences in community species composition among samples, PCoA analysis was used to measure the similarity of community species composition of bacteria and fungi in 5 stand types. The results showed that the three repeats of each forest type were very close, indicating that the repeatability of the sample was good and the variation within the group was relatively small. As shown in Figure 3A, PCo1 and PCo2 cumulative contribution rate is 63%. As can be seen from Figure 3B, the cumulative contribution rate of PC1 and PC2 is 44.6%. The differences of soil bacterial and fungal communities among the 5 stand types were much greater than those within the group.

The result of variation partition showed that soil and environment explained 49% and 22% of the variance in bacterial community composition (Fig. 4A) and 71% and 29% of the variance in fungal community composition, respectively (Fig. 4B).

3.4 Relationship between soil microbial community and soil properties

Redundancy analysis was performed on dominant soil microbial communities and soil properties. RDA 1 (62.68%) and RDA 2 (11.89%) accounted for 77.54% of the variation of soil bacterial community structure. Among them, TN, AN and SOM were significantly positively correlated with Actinobacteria, and pH was significantly positively correlated with Chloreflexi (Fig. 5A). Fig. 5B shows the RDA sequence diagram of fungi and properties. RDA 1 was 75.97%, and RDA 2 was 14.93%, accounting for 90.90% of the variation of soil fungal community structure, indicating that soil environmental factors greatly influenced the fungal community structure,the results showed that AN was positively correlated with Mortierellomycota and pH was positively correlated with Ascomycota.

4 Discussion

4.1 Soil characteristics

Due to different root exudates of different stand types and different chemical components and decomposition rates of litter, the organic nutrients input into the soil are different [16, 17, 18], lead to significant differences in understory soil characteristics. This study showed that there were significant differences in soil properties under 5 different stand types. Previous studies have shown that the litter of deciduous coniferous forest contains some undecomposable organics, such as lignin, tannin and wax, which cause the litter decomposition is relatively slow [19] and the amount of the accumulated litters is relatively large, the massive litters covered the soil surface, thus declined the utilized rate of soil nutrients and sped up the accumulation of soil nutrients. This conclusion confirmed that the soil nutrient content under *Pinus tabuliformis* forest was higher than other forest stand. The highest soil acidity of *Quercus mongolica* forest undergrowth was 5.87. Compared with other broad-leaved forests, the litter of *Quercus mongolica* forest was relatively low, and in this litters, the N content is relatively few, the content of C/N and lignin is relatively high, its nutrient content is relatively less, which cause the pH value of undergrowth is relatively

little. Under the same climatic conditions, there are significant differences in soil characteristics under different stand types, which has a certain impact on soil microbial community.

4.2 Diversity of soil microbial communities

Different stand types are key factors determining of forest soil microbial community [20, 21, 22, 23]. Our results showed that the soil bacterial diversity under *Juglans mandshurica* forest was the highest, and the bacterial diversity under broad-leaved forest were significantly higher than soil under *Pinus tabuliformis* forest and *Quercus mongolica* forest. Generally, the quality and the decomposition rate of litter produced by broad-leaved forest is more than that of coniferous forest [24], which has provided enough substance conditions for rapid growth and breed of bacteria. The decomposition of broad-leaved forest litter is mainly based on catalase and polyphenol oxidase, the activity of polyphenol oxidase is positively correlated with bacteria diversity, and catalase activity is positively correlated with bacteria amount [25]. In conclusion, the differences of soil bacteria diversity of different forest stand of the same broad-leaved forests are not clear, the soil bacteria diversity of broad-leaved forests were greater than coniferous forests. In the present study, the diversity of soil fungus is that the soil under *Pinus tabuliformis* forests was more than the rest stand types, the differences of soil fungus diversity of the rest broad-leaved forests were not so obvious. The significant differences in soil microbial communities between coniferous and broad-leaved trees has been previously reported [8]. Compared with broad-leaved forests, the litter layers of coniferous trees are thicker, the lignin content more higher [26], but most complex organics in soil need to be decomposed by fungus [27], and some soil fungus could coexist with plants or form mycorrhiza fungi, this interspecies cooperation approach improved the competitiveness of cooperative soil fungus, thus it can improve the diversity of fungus community with limited resources and limited space. Our result showed the undergrowth soil nutrient content of *Quercus mongolica* forest is relatively low, and its diversity indexes of undergrowth soil microbial communities are also relatively low, which indicates that soil microbial diversity has prominent relations with soil nutrient content, the rich soil nutrient content will promote the richness of soil microbial communities.

4.3 Soil microbial community structure

Soil microbial community structure play an important role in soil nutrient cycling, and the dominant species of soil microbial play an important role in soil genesis [28]. Soil microbial community has obvious stand type specificity [8]. The dominant bacteria of soil are Proteobacteria, Acidobacteria and Actinobacteria, which is similar to the results of dominant bacteria detected in other forest ecosystem environments, it explained that the ecological amplitude of the three kinds of microflora is relatively wide [11, 29, 30], and they have a good adaptability to forest environment. However, the relative abundance of different bacteria phylum were significantly different in the soil of different stand types. The average abundance of Actinobacteria (19.75%) in soil under *Quercus mongolica* forest was significantly lower than other, and the average abundance of Acidobacteria (26.83%) was significantly higher than other. Actinobacteria belongs to G+ bacteria, and it is able to decompose cellulose and lignin [31]. Acidobacteria are acidophilic and oligotrophic [32]. This study showed that the soil pH value under *Quercus mongolica* is the lowest and the soil nutrient content is significantly lower than that of other stand types. This condition is more suitable for Acidobacteria to exist and breed, which is consistent with the results of Peng’s and Naether’s study [33, 34]. The composition of bacterial dominant genera under the 5 stand types were similar, but the content of individual dominant genera is different, Subgroup-2 only existed in the soil under *Quercus mongolica* forest, which may have a great relationship with environment.

In this study, the dominant microflora of soil fungus were Basidiomycota and Ascomycota, the contents of them have difference with significance. The results showed that the dominant phyla of fungus were different under different stand types, which is consistent with the results of Sheng's study [35]. The dominant soil fungus under the

soil of *Betula dahurica*, *Pinus tabuliformis* and *Juglans mandshurica* forest are Ascomycota, followed by Basidiomycota, the same situation appeared in tropical forest soil, subtropical forest soil in Australia, Guandi mountain forest soil and aeolian sand area in Northwest Liaoning. However, Basidiomycota was the dominant fungus in the soil under *Betula platyphylla* and *Quercus mongolica* forest, followed by Ascomycota, the same results also appeared in European forest soil, temperate forest soil and subtropical forest soil in China. Other research results showed that Ascomycota was the most abundant fungi in broad-leaved forest. However, the results of this study didn't confirm this conclusion, which showed the complexity and difference of soil environment under different stand types. Other research founded that there was usually one phylum with the highest fungal abundance in different stand types in the same area, but this study founded that the phylum with the highest fungal abundance were difference in different stand types. This result showed that stand types and soil properties have significant impact on soil community, while other conditions such as climate have little impact on soil community. Another possibility was that the results were related to the scale or the methods used to access soil microbial community. In this study, only 3 groups of repetitions were set up, which may have a small number of repetitions, which had an impact on the research results. In this study, the species of soil fungus of different forest stand were rich and significant different at the genus level. Many fungi are specific root symbionts and pathogens, their growth and reproduction are more directly dependent on the biological nutritional interaction between litter and trees [36/37]. The dominant plants in each sample plots were different, which will also leaded to great differences in fungal communities [38,,39, 40].

4.4 Relationship between soil and microbial community

We found that soil respectively explained 49.1% of the variance in bacterial community composition and explained 70.6% of the variance in fungal community. These results indicated that soil played considerable roles in changing the composition of microbial community, especially in fungal community. However, some variances in bacterial and fungal community compositions have not been explained, therefore, other factors such as climate and season may also drive the composition of soil microbial communities, which needs to be further studied. Soil pH can change bacteria towards its nutrient utilization rate, the physiological metabolism activity, and the competition among populations, which can directly or indirectly affect soil bacteria diversity, the appropriate soil pH value will promote microbial growth [41]. RDA analysis showed that TN, AN and SOM were positively correlated with the abundance of Actinobacteria, which proved that C and N in soil provided material basis for the growth of Actinobacterial [42], growth and development of Actinobacterial are closely related to soil carbon and nitrogen cycle [43]. Some studies showed that soil pH and soil available nutrients have strong impact on fungal community, but this study founded fungi have no significant correlation with soil pH, which may be related to the small range of soil pH under 5 stand types. RDA analysis showed that Basidiomycota negatively correlated with AP, which conforms to the research results of Deng J [44], but contrary to some previous studies [45, 46]. Because soil microorganisms contribute to different soil nutrient element cycles and play different roles in different nutrient element cycles, the chord diagrams could prove that soil microorganisms are closely related to soil nutrients.

Conclusion

In this study, the effects of different stand types on soil properties and soil microbial community in warm temperate forest were discussed, and the driving factors of soil microbial community change in warm temperate forest were determined. We observed that different stand types on the ground changed soil properties, which are the main factors to change soil microbial community, especially for fungal community. Other factors such as climate will also have a certain impact on soil microorganisms, so further research is needed. This study provides some insights for soil microbial community to study nutrient cycling and maintain ecosystem function in warm temperate forests.

Declarations

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Figures

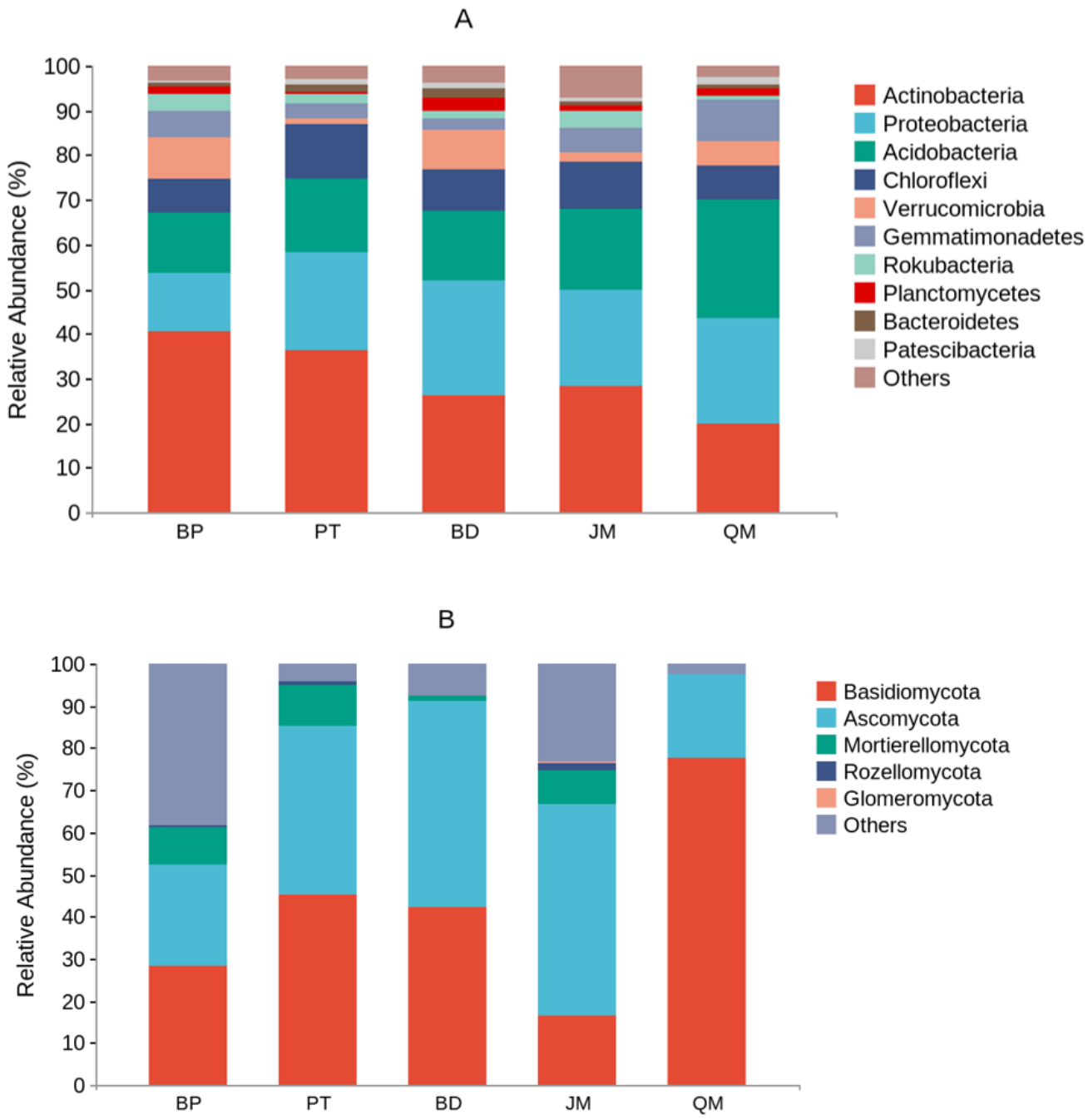

Figure 1

Relative abundance of bacteria and fungus phyla present in 5 different stand types (n=3).

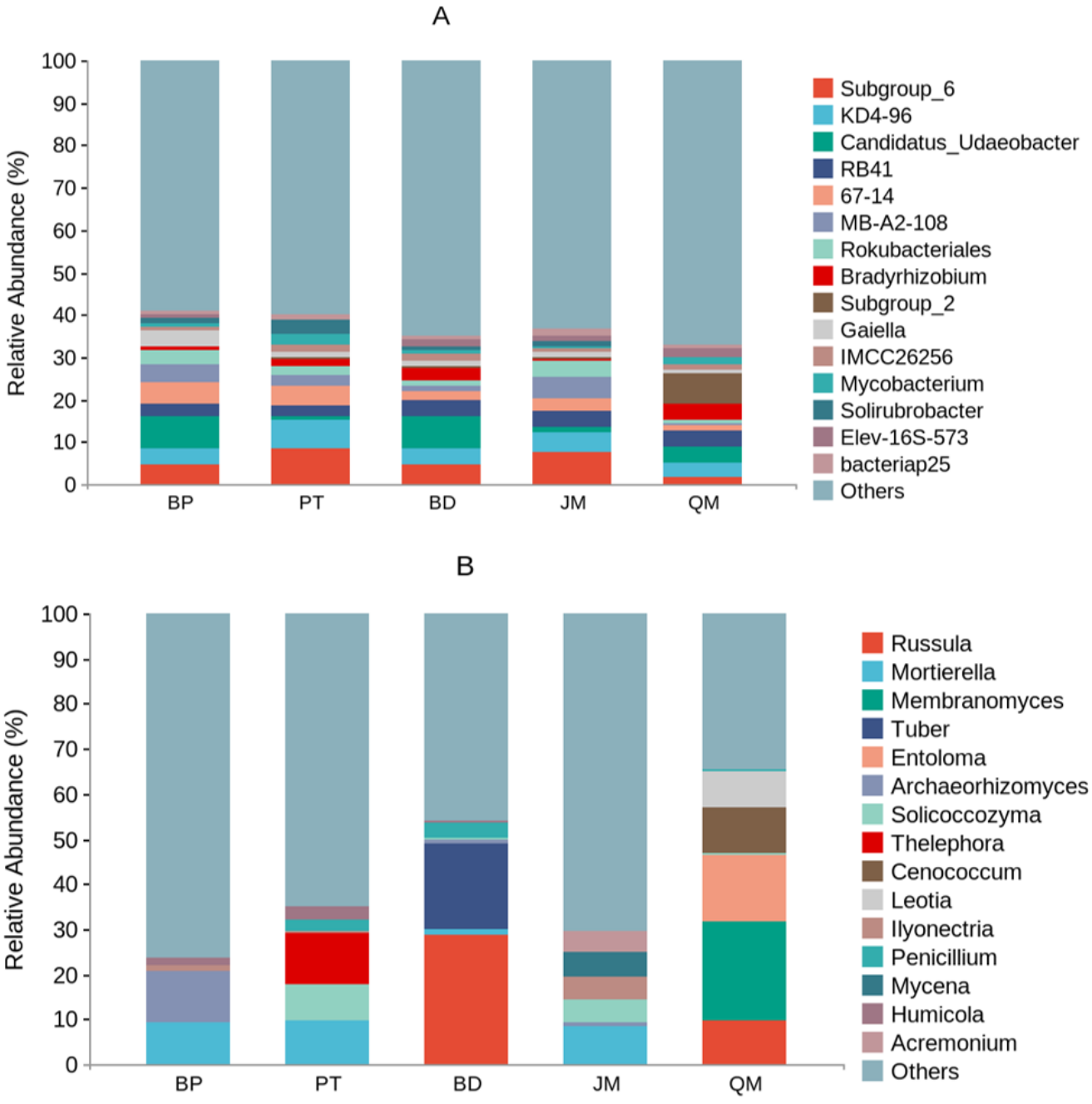

Figure 2

Relative abundance of bacteria and fungus gene present in five different stand types. (n=3).

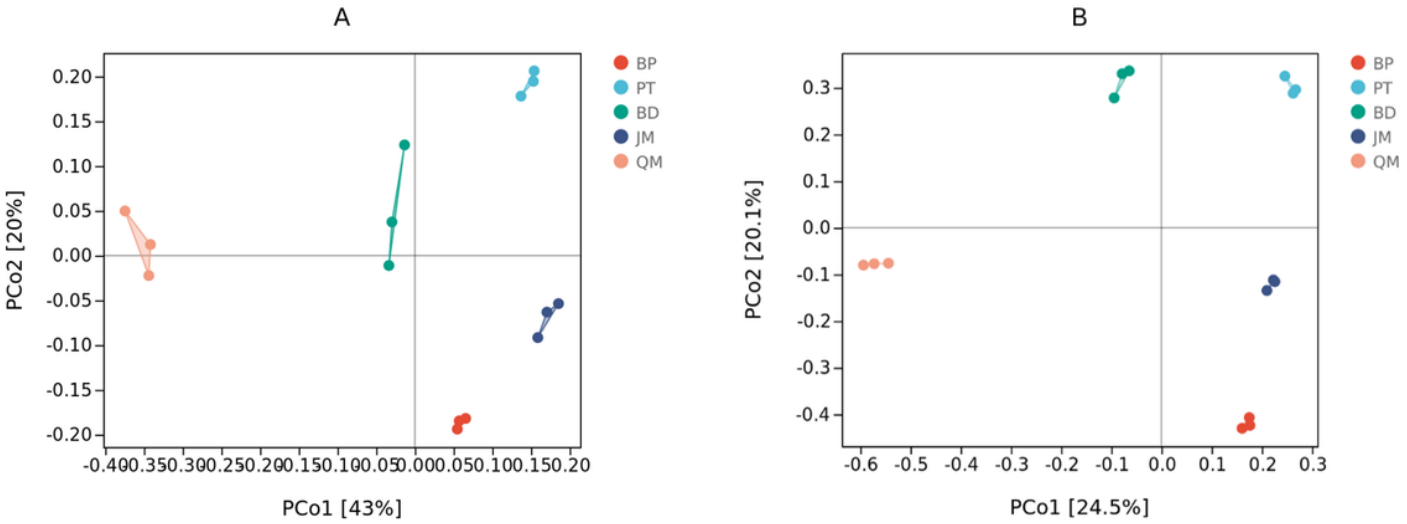

Figure 3

Principal coordinate analysis of soil bacterial (A) and fungal (B) community in different stand types (n=3).

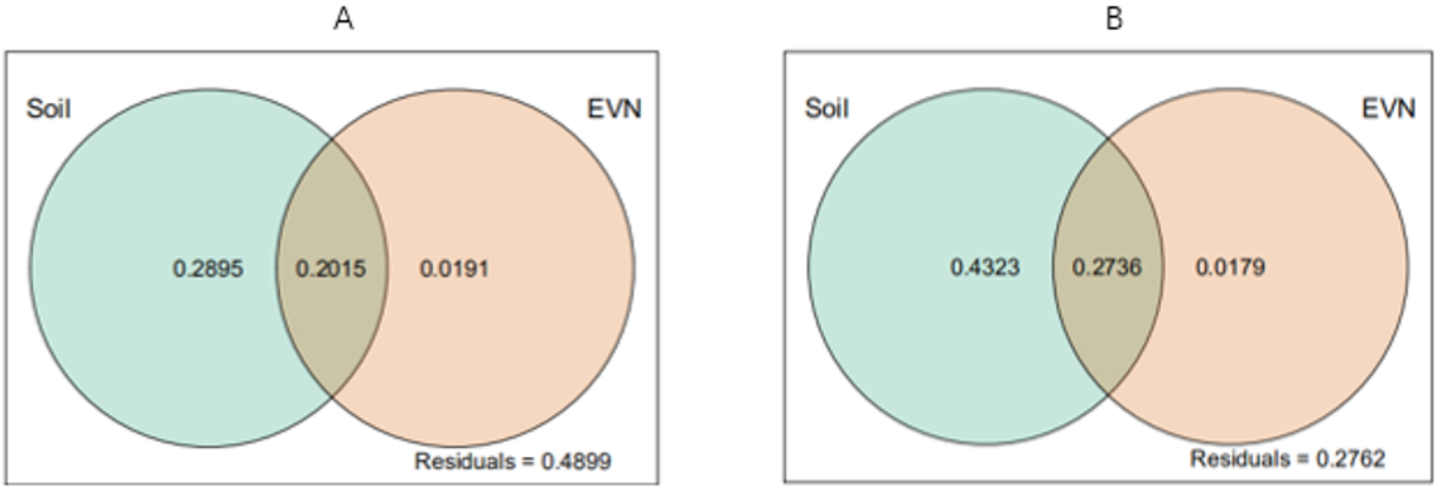

Figure 4

Variation partition analysis of soil for the community variances of bacteria (A) and fungi (B).

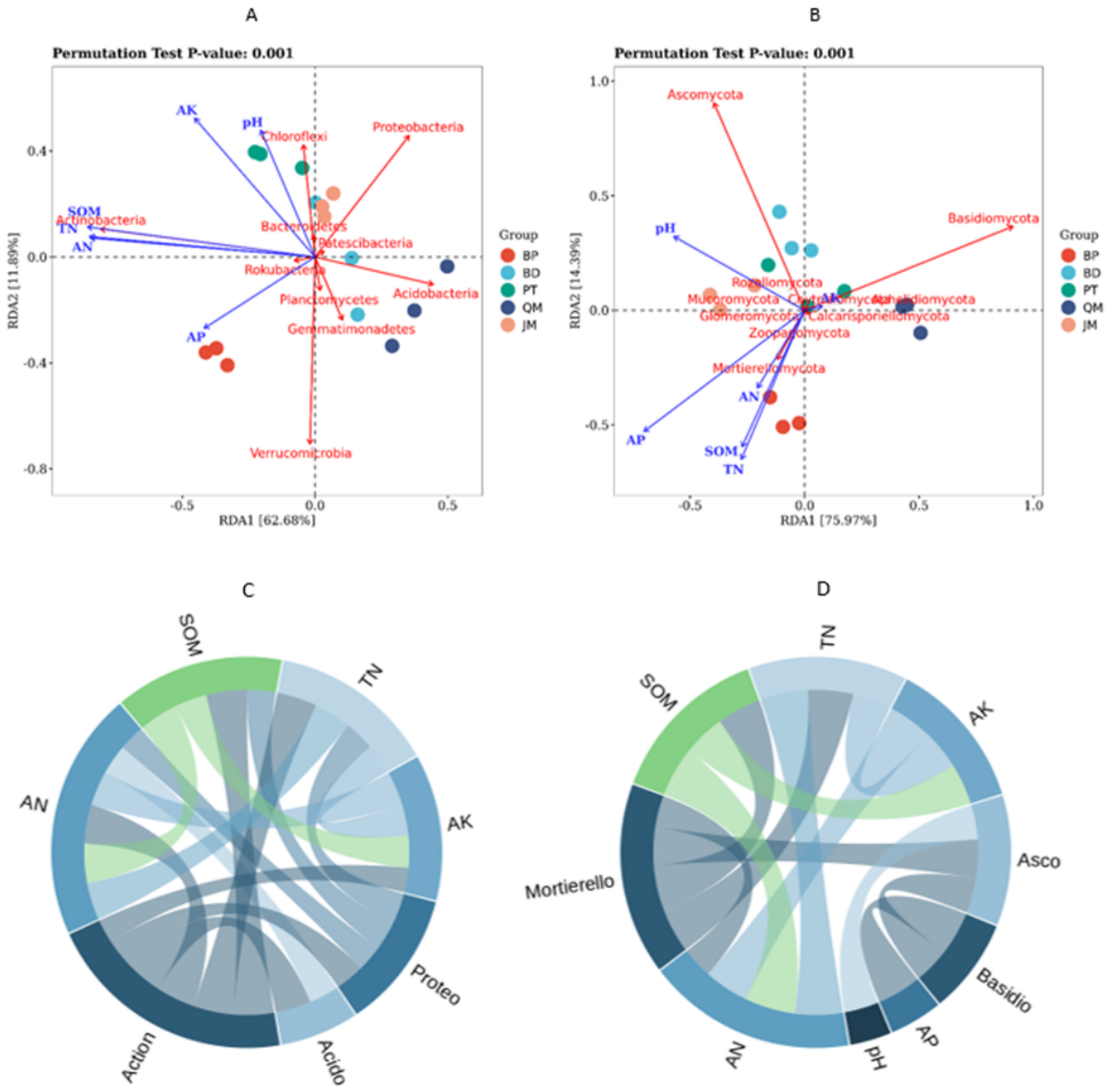

Figure 5

Redundancy analysis on soil dominant bacterial (A) and fungal (B) phyla constrained by soil variables. Chord plots showing the correlation between bacterial (C) and fungal (D) rich microbial groups and soil characteristics. Each sector of the circle represents one node of the network, and its width indicates the total amount of cooccurrence that connects a certain microbial or nutrient to the other microbial or nutrients, together with the total amount of cooccurrence that connects all the other microbial or nutrients to microbial or nutrient.