

# Impact of *Robinia pseudoacacia* stand conversion on soil properties and bacterial community composition in Mount Tai, China

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## Research

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## Abstract

**Background:** *Robinia pseudoacacia* is a widely planted pioneer tree species in reforestations on barren mountains in northern China. Because of its nitrogen-fixing ability, it can play a positive role in soil and forest restoration. After clear-cutting of planted stands, *R. pseudoacacia* stands become coppice plantations. The impacts of shifting from seedling to coppice stands on soil bacterial community and soil properties have not been well described. This study aims to quantify how soil properties and bacterial community composition vary between planted seedling versus coppice stands.

**Methods:** Nine 20×20 m plots were randomly selected in seedling and coppice stands. The bulk soil and rhizosphere soil were sampled in summer 2017. Bulk soil was sampled at 10 cm from the soil surface using a soil auger. Rhizosphere soil samples were collected using a brush. The soil samples were transported to the laboratory for chemical analysis, and bacterial community composition and diversity was obtained through DNA extraction, 16S rRNA gene amplification and high-throughput sequencing.

**Results:** The results showed that, compared to seedling plantations, soil quality decreased significantly in coppice stands, but without affecting soil exchangeable  $Mg^{2+}$  and  $K^+$ . Total carbon (C) and nitrogen (N) were lower in the rhizosphere than in bulk soil, whereas nutrient availability showed an opposite trend. The conversion from seedling to coppice plantations was also related to significant differences in soil bacterial community structure and to the reduction of soil bacterial  $\alpha$ -diversity. Principal component analysis (PCA) showed that bacterial community composition was similar in both bulk and rhizosphere soils in second generation coppice plantations. Specially, the conversion from seedling to coppice stands increased the relative abundance of *Proteobacteria* and *Rhizobium*, but reduced that of *Actinobacteria*, which may result in a decline of soil nutrient availability. Mantel tests revealed that C, N, Soil organic matter (SOM), nitrate nitrogen ( $NO_3^-$ -N) and available phosphorus positively correlated with bacterial community composition, while a variation partition analysis (VPA) showed that  $NO_3^-$ -N explained a relatively greater proportion of bacterial distribution (15.12%), compared with C and SOM. Surprisingly, N showed no relationship with bacterial community composition, which may be related to nitrogen transportation.

**Conclusions:** The conversion from seedling to coppice stands reduced soil quality and led to spatial-temporal homogenization of the soil bacterial community structure in both the rhizosphere and bulk soils. Such imbalance in microbial structure can accelerate the decline of *R. pseudoacacia*. This may affect the role of *R. pseudoacacia* coppice stands in soil and forest restoration of barren lands in mountain areas.

## Background

*Robinia pseudoacacia* (Black locust) is a leguminous tree which can rapidly fix nitrogen (N) from the atmosphere via *Rhizobium* (Zhang et al. 2019) and further alter soil properties by increasing mineral N (Medina-Villar et al. 2016). *R. pseudoacacia* is able to disperse quickly and colonize a broad range of xeric habitats, including steep rocks or toxic man-made substrata (Cierjacks et al. 2013), and has been extensively naturalized in the temperate regions of North America, Europe, and Asia (Sabo, 2000; Lee et al. 2004; Vítková et al. 2017; Yang et al. 2019). Natural reproduction of *R. pseudoacacia* plantations is primarily vegetative through root suckering and stump sprouting, allowing vigorous regeneration after coppicing and disturbance (Peng et al. 2003). However, after two or three rotations, the productivity of *R. pseudoacacia* coppice plantations tends to decline (Cierjacks et al. 2013), which may further jeopardize its ecological role in soil and forest restoration.

Plant community structure and productivity in natural environments depend, among other factors, on soil nutrient availability and soil microbial communities (Reynolds, H. L., & Haubensak, K. A. 2009; Vitkova et al. 2015; Liu et al. 2018; Chen et al. 2020). Soil nutrient availability can alter soil processes catalyzed by soil microbial communities (Yang et al. 2016). Therefore, changes in soil microbial community composition can affect the plant community (Balota et al. 2013; Ma et al. 2018) and nutrient absorption by plants (Weidner et al. 2015; Zhang et al. 2018b). In turn, plants can directly and indirectly influence soil microbial communities by the effect of root exudation and litters (Sasse et al. 2018). So plant-soil feedback may play a key role in plant species coexistence and phylogeny of plant community (Crawford et al. 2019).

Rhizosphere is a critical interface supporting the exchange of resources between plants and the surrounding soil environment, which provides microhabitats and niches for diverse microorganisms and microbial species (Philippot et al. 2013; Mendes et al. 2013). Rhizosphere microorganisms play a key role in plant growth and soil properties, especially in the rhizosphere niche (Philippot et al. 2013; Zhang et al. 2018a), which influences several plant physiological processes such as growth and energy metabolism affecting

overall plant health (Fonseca et al. 2018). Generally, there are significant differences between rhizosphere and bulk soil microenvironments, the most obvious of which is that the higher nutrient content and root exudates in the rhizosphere contribute to improving soil carbon and nitrogen concentrations (Yin et al. 2018). Such differences may affect the composition of the rhizosphere microbial community (Neumann et al. 2014). Soil properties and their ecological processes provide a scientific basis for understanding the interaction between root physiological activity and soil physical and biological environments. At the same time, rhizosphere dynamics may be a key driver for understanding tree growth mechanisms.

Previous research has reported the high capacity of *R. pseudoacacia* for nitrogen fixation (Buzhdygan et al. 2016), and higher N mineralization and nitrification rates in *R. pseudoacacia* plantations compared to surrounding soils (Williard et al. 2005). Moreover, the excess of N can accumulate in the soil (Berthold et al. 2009) by means of root exudates, contributing to increasing soil fertility (Joëlle et al. 2010). The main nitrogen form uptaken by plants is inorganic nitrogen including nitrate and ammonium. *R. pseudoacacia* benefits from nitrogen fixation associated with symbiotic rhizobia in root nodules (Cierjacks et al. 2013). The reduction of soil N availability induces nodulation and biological nitrogen fixing of *R. pseudoacacia* in order to sustain the required nitrogen amounts for plant growth (Mantovani et al. 2015). Therefore, both bacteria and N play an important role in the growth and development of *R. pseudoacacia* plantations.

With the development of *R. pseudoacacia* coppice plantations through stand conversion, unexpected problems have arisen in Mount Tai (China) forest ecosystems, including the decline of landscape quality, soil erosion and plant dwarfing, in line with previous research suggesting tree growth decline and trunk shape worsening (Geng et al. 2013). However, to date, most studies have attempted to investigate the effects of conversion from natural forests to plantations on soil properties, soil microbes and their community structure (Zhang et al. 2017; Yang et al. 2018). In consequence, there is a gap in knowledge concerning the effects of the conversion from seedling plantations to coppice stands. Radtke et al. (2013) showed that repeated clear-cuttings every 20-30 years favored the spread of *R. pseudoacacia*. Yet, the effects of shifting from seedling to coppice plantations on soil properties and soil microbes are not yet well understood, and information is scarce. We hypothesized that (1) the changes caused by the conversion of seedling to coppice stands lead to decline of soil quality, and to alterations in soil bacterial community composition, (2) nutrient availability plays an important role in shaping the bacterial community, and (3) the relative abundance of *Rhizobium* decreases in coppice plantations. The aims of this study were to (1) shed light on the effects of shifting from seedling to coppice stands in *R. pseudoacacia* plantations on soil properties and soil bacterial community composition, especially on *Rhizobium*, and (2) investigate the relationships between soil properties and bacterial community composition in seedling and coppice plantations, respectively.

## Materials And Methods

### Study area

This study was conducted in Mount Tai region of Shandong Province, eastern China. The region is characterized by a typical temperate climate. The mean annual temperature is 12.8 °C, and the mean annual precipitation is 1124.6 mm. In the 1920s, *R. pseudoacacia* was introduced to Mount Tai because of its potential for soil and forest restoration. Afforestation was mainly conducted between 1956 and 1958 through seedling planting. However, with increasing timber demand for use in construction, seedling plantations were gradually harvested leading to naturally-regenerated coppice stands. As a result, nowadays, most *R. pseudoacacia* stands are coppice plantations, mainly distributed along an altitudinal gradient from 500 to 1000 meters above sea level, and southern aspects.

The study was performed in a: i) first generation seedling plantation stand (hereinafter referred to as "First", "F", 36°16'45"N, 117°3'26"E), ii) first regeneration coppice stand generated after clear-cutting of a seedling stand (hereinafter referred to as "Second", "S", 36°16'40"N, 117°3' 21"E) and iii) second generation coppice stand generated after clear-cutting of a first generation coppice stand (hereinafter referred to as "Third", "T", 36°16'40"N, 117°3' 22"E) (Figure S1). The understory vegetation is mainly composed of *Vitex negundo*, *Oplismenus undulatifolius*, *Digitaria sanguinalis*, *Paspalum thunbergii*, *Rubia cordifolia* and *Oxalis corniculata*. The three forest stands were close to each other as shown in Figure S1, and therefore represented homogeneous conditions in terms of topography (i.e., slope 25° and southwestern aspect), previous land use (i.e., stands developed from barren land) and initial soil characteristics and taxonomy (i.e., classified as Alfisols). Soil moisture was about 10%. The microbial biomass of carbon in the three stand development stages were 247 mg/kg, 200 mg/kg, and 190 mg/kg, respectively, and those of nitrogen were 41 mg/kg, 28 mg/kg, and 25 mg/kg, respectively (unpublished data).

## Sampling

Three 20×20 m plots were randomly selected in each seedling and coppice stand (i.e., a total of nine plots). The bulk soil and rhizosphere soil were sampled in the nine above-mentioned sample plots in August 2017. Bulk soil was sampled at 10 cm from the soil surface by using a soil auger (length 50 cm, diameter 5 cm, volume 100 cm<sup>3</sup>). Rhizosphere soil samples were collected by brush (5 samples per plot). The soil samples were transported on ice to the laboratory, where they were sieved (mesh size 2 mm) and divided into two parts, one was air-dried and stored at room temperature prior to chemical analysis and the other was stored at -80°C for further analysis. Hereafter in this manuscript, FR, SR and TR refer to the rhizosphere of F, S and T, respectively; and FNR, SNR and TNR refer to bulk soil of F, S and T, respectively.

## Analysis of soil physicochemical properties

Total soil carbon (C) and nitrogen (N) contents were measured by dry combustion in an Elemental Analyzer (Costech ECS4010, Italy). The soil nitrate (NO<sub>3</sub><sup>-</sup>-N) and ammonium (NH<sub>4</sub><sup>+</sup>-N) were extracted by shaking 20g of fresh soil in 100 ml of 2M KCl solution for 1h and were analyzed with continuous flow analytical system (AA3, German), available N (A.N) was a sum of NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N, available P in the soil was measured using the colorimetric method with 0.5M NaHCO<sub>3</sub> extraction, the total soil phosphorus (P) and available P (A.P) were measured with a continuous flow analytical system (AA3, German), and the soil organic matter (SOM) was measured via the standard Mebius method (Nelson and Sommers 1982). The exchangeable cations (Ca<sup>2+</sup>, Mg<sup>2+</sup> and K<sup>+</sup>) were measured using titration and atomic absorption spectroscopy (AAS, TAS-990MFG, China). Soil moisture was determined using the soil core method, and obtained by calculating the ratio of soil mass to total volume (g·cm<sup>-3</sup>) after oven-drying to a constant weight at 105°C (Zhang et al. 2019). To better describe changes in soil properties, soil quality index (*SQI*) (Guo, 2019) was calculated.

$$SQI = \sum_{i=1}^n W_i Y_i$$

Where *W* is the weighting factor for the indicator selected and *Y* is the score. The final *SQI* can be used to evaluate soil quality following vegetation restoration, with a high *SQI* value indicating a high-quality soil.

## DNA extraction, 16S rRNA gene amplification, and high throughput sequencing

Total genomic DNA from soil samples (0.5g) was extracted using CTAB method. Bacterial 16S rRNA genes of distinct regions (V4-V5) was amplified with the primer pair 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') with single multiplex identifier (MID) and adaptors (Jiao et al. 2018). The initial enzyme activation was performed at 95 °C for 5 min, and then 35 cycles of the following program were used for amplification: 95 °C for 30 s, 58 °C for 30 s and 72 °C for 30 s (Chen et al. 2017). All PCR reactions were carried out with Phusion<sup>®</sup> High-Fidelity PCR Master Mix (New England Biolabs). The 16S rRNA genes were analyzed to evaluate bacterial diversity using IlluminaHiSeq (Novogene Bioinformatics Technology Co., Ltd., Beijing, China).

Sequences were analyzed using QIIME software package (Quantitative Insights Into Microbial Ecology) (Caporaso et al. 2017), and in-house Perl scripts were used to analyse alpha- (within samples) diversities. The low-quality sequences were filtered out using the following criteria: sequences with a length of < 150 bp, average Phred scores of < 20, containing ambiguous bases, and containing mononucleotide repeats of >8 bp (Ji et al. 2019). Following chimera detection, the remaining high-quality sequences were clustered into operational taxonomic units (OTUs) at 97% sequence identity using UCLUST. A representative sequence was selected from each OTU using default parameters. We picked a representative sequence for each OTU and used the RDP classifier to annotate taxonomic information for each representative sequence (Wang et al. 2007).

## Statistical analysis

Duncan's one-way ANOVA was conducted to examine differences in soil characteristics, *SQI* and relative abundance of *Rhizobium* between bulk and rhizosphere soils. A T-test was conducted to examine differences in Shannon and Simpson indices between bulk and rhizosphere soils. These analyses were performed using SPSS 24.0 (IBM, USA). Principal component analysis (PCA) was conducted to test for differences in the OUT-based community composition using Bray-Curtis distance. The relationships between soil properties and dominant bacterial community composition (TOP 10) were determined using Spearman correlation analysis. Mantel-tests and variation partition analysis (VPA) were used to determine the relative importance of the measured soil properties in shaping

soil bacterial community, which were calculated using the Bray-Curtis distance. These analyses were carried out using the “vegan” package of R software (Version 2.15.3). The graphics were drawn using Origin 2019.

## Results

### Impact of the conversion to coppice stands on soil quality

Soil nutrient contents diminished mostly from seedling to coppice plantations (Table 1). Soil characteristics varied considerably in both rhizosphere and bulk soil from F stands to T stands. Total C, N and  $\text{NO}_3^-$ -N concentration and SOM content in both the rhizosphere and bulk soil was significantly higher in seedling stands compared to first- and second-generation coppice stands. There were significant differences in P concentration in the rhizosphere and bulk soil. There was no statistically significant difference in available phosphorous (A.P) concentrations between FNR and SNR, but A.P concentration was significantly greater in FNR and SNR compared to TNR. No differences were found regarding exchangeable ions in bulk soil between seedling and coppice plantation, while significantly higher concentrations appeared in the rhizosphere of coppice plantations compared to seedling stands. The *SQI* of both bulk soil and rhizosphere was higher in seedling plantations than in coppice stands, i.e., the highest *SQI* value (29.14) was found in the rhizosphere of seedling stands whereas the lowest *SQI* (24.33) was found in the bulk soil of second generation coppice stands.

### Differences in soil bacterial abundance and diversity

In total, 2,562,381 sequences and 2,358,270 combined sequences were obtained. The read lengths ranged from 211 to 407 base pairs (bp), with an average of 373 bp. When grouped at the 97% similarity level, there were 42 different phylotypes in all soils. The dominant groups (TOP 10) across all soil samples (Figure 1A) were *Proteobacteria* (30.54%), *Actinobacteria* (25.30%), *Acidobacteria* (13.94%), *Firmicutes* (7.19%), *Verrucomicrobia* (6.86%), *Planctomycetes* (5.22%), *Chloroflexi* (3.87%), *Gemmatimonadetes* (2.37%), *Bacteroidetes* (1.14%), and *Cyanobacteria* (0.40%), and these groups accounted for more than 96.43% of the bacterial sequences. Moreover, the Shannon and Simpson indices for alpha bacterial diversity declined from seedling to coppice plantations and from first-rotation to second-rotation coppice plantations by 2% and 0.2%, respectively (Table 2).

At the genus level (Figure 1B), the six most abundant bacteria ( $\geq 1\%$ ) were *Bacillus* (4.22%), *Bradyrhizobium* (2.82%), *Acidothermus* (1.88%), *Bryobacter* (1.44%), *Burkholderia-Paraburkholderia* (2.00%) and *Streptomyces* (1.41%). The relative abundances of *Bacillus* and *Burkholderia-Paraburkholderia* in the rhizosphere were lower than that of bulk soil in seedling plantations, but the opposite trend was found in coppice plantations. In addition, the relative abundance of other bacteria in the rhizosphere was higher than that of bulk soil in seedling and coppice plantations.

### Relative abundance of *Rhizobium* in seedling and coppice plantations

The relative abundance of *Rhizobium* in both bulk soil and rhizosphere in second generation coppice stands was significantly higher than in seedling and first generation coppice stands. The relative abundance of *Rhizobium* was the highest in the rhizosphere of T stands (0.32%), while the lowest was found in the bulk soil of seedling (F) stands (0.11%). Moreover, the difference in *Rhizobium* abundance between rhizosphere soil and bulk soil was significant in seedling plantations ( $p=0.002$ ), while there was no difference in coppice plantations (Figure 2).

### Bacterial community composition in seedling and coppice plantations

The results showed five replicates usually clustered closely (Figure 3). The first and second PCA axes revealed that the rhizosphere- and bulk soil-associated bacterial microbiota were inhomogeneous at phylum (12.77% and 8.23%, respectively, Figure 3A) and genus (17.21% and 13.16%, respectively, Figure 3B) levels. The soil layer and plantation type rendered a significant effect on bacterial community composition. The similarities in bacterial community composition within rhizosphere and bulk soil were lower in seedling plantations than in coppice plantations (Figure 3).

We found that C, N, SOM,  $\text{NO}_3^-$ -N and A.P were positively correlated with bacterial community composition by Mantel tests at both phylum and genus levels (Table 3). Spearman correlation analysis of the relationships between soil properties and bacterial community at the phylum (Figure 4A) and genus levels (Figure 4B) also confirmed the positive correlation between bacterial communities and nutrient concentrations. At the phylum level, SOM,  $\text{NO}_3^-$ -N, and A.P were significantly and negatively correlated with *Proteobacteria* ( $r=-0.66$ ,  $p=0.000$ ;  $r=-0.62$ ,  $p=0.000$  and  $r=-0.73$ ,  $p=0.000$ , respectively), and were significantly and positively correlated

with Actinobacteria ( $r=0.71$ ,  $p=0.000$ ;  $r=0.64$ ,  $p=0.000$  and  $r=0.59$ ,  $p=0.001$ , respectively), but there was no significant correlation with Acidobacteria. At the genus level, Acidothermus, Bryobacter and Mizugakiibacter were significantly and positively correlated with SOM,  $\text{NO}_3^-$ -N, and A.P ( $r=0.65$ ,  $0.62$  and  $0.68$ ;  $p=0.000$ ,  $p=0.000$  and  $p=0.000$ , respectively). Bacterial taxa were also more correlated with soil nutrient concentrations at the genus level than at the phylum level.

N, C, SOM and  $\text{NO}_3^-$ -N, the most significant factors for bacterial community composition according to the Mantel test results ( $p<0.001$ , at the phylum and genus levels), were selected for variance partitioning canonical correspondence analysis (VPA), to quantify the relative contributions of soil properties to bacterial community structure. The soil properties explained 75.46% of the observed variation, leaving 24.54% of the variation unexplained. Among them, C, N, SOM and  $\text{NO}_3^-$ -N explained 9.28%, 0.00%, 3.52% and 15.12%, respectively. Thus,  $\text{NO}_3^-$ -N, but not N, was the most important factor in shaping the bacterial community structure (Figure 5).

## Discussion

### Conversion from seedling to coppice stands reduced soil quality

Forest conversion has a great impact on plant and soil characteristics, altering soil bacterial community structure, soil nutrients and plant diversity and composition (Zhao et al. 2019). Previous studies have shown that *R. pseudoacacia* may induce significant changes on several physical and chemical properties of the soil (Khan et al. 2010; Du et al. 2019). In *R. pseudoacacia* coppice stands, intra-specific competition increases because of the high stem density, which may result in differences in microclimatic and ecological conditions as compared to seedling stands. In this regard, our results provide incremental knowledge to previous research by further showing that the conversion from seedling to coppice stands reduced soil quality (Table 1), consistently with the findings of Johnson (2001) and Luo (2006). Therefore, it supports our first hypothesis inasmuch as *R. pseudoacacia* is a  $\text{N}_2$ -fixing species with a strong nitrogen fixation ability. However, our results showed that soil N (N,  $\text{NO}_3^-$ -N and A.N) concentrations declined in coppice stands. It possibly indicates that the nitrogen fixation ability of *R. pseudoacacia* coppice decreased to a certain extent, and the N mineralization rate was significantly lower compared to seedling (F) stands (unpublished data). The main reason may be that the conversion decreased the net primary production and aboveground biomass and productivity (Liao et al. 2012). Specially, the coppice stands had lower stand productivity than the stand developed from seedlings (Figure S2), which could contribute to modifying soil structure and lead to less inputs and more losses of soil nutrients (Zheng et al. 2005), finally affecting the absorption of N by trees (Zhang et al. 2018b). Additionally, we found that the greater soil moisture content occurred in coppice plantations (13.95%), which might reduce root and microbial activity (Banerjee et al. 2016), then reduce the soil total N concentration, N storage, N cycling and availability (Wang et al. 2010).

Due to root exudations, microbiota activity, and plant absorption, which may lead to the accumulation of nutrients in the rhizosphere, the micro-environments between the rhizosphere and bulk soil may differ markedly (Philippot et al. 2013). Our results showed that N and C contents in bulk soil were higher than those in the rhizosphere, but the concentrations of other nutrients (e.g. SOM,  $\text{NO}_3^-$ -N and A.P) were lower in the bulk soil than in the rhizosphere (Table 1). These results are consistent with previous research (Chaudhary et al. 2015). One possible main reason is that plant roots directly uptake less available nutrients and reduce carbon loss in the rhizosphere (Jones et al. 2009), and they could also adapt to changes in soil nutrient availability through the elastic distribution of underground roots (Bardgett et al. 2014). The consumption of N for tree growth, the strong physiological metabolism function of root system and the activity of rhizosphere microorganisms drive the transformation of N to A.N, and this may be the reason why we found that rhizosphere soil had lower N content and higher A.N content (Table 1).

### Conversion from seedling to coppice stands altered the structure of bacterial communities

Changes in forest community types can affect soil microbial structure (Cardenas et al. 2015) and  $\alpha$ -diversity (Vitali et al. 2016). Our results showed that Shannon and Simpson indices declined from seedling to coppice stands (Table 2). These shifts can be accompanied by changes in bacterial functional activity (Kaiser et al. 2014), contributing to one of the reported changes of soil nutrients (Zhao et al. 2018). Previous research (Shi et al. 2016) found that rhizosphere microbes displayed higher levels of interactions than bulk soil microbes. However, we found that the bacterial community structures of bulk soil and rhizosphere were not significantly different in coppice plantations (Figure 3), which supports the hypothesis that the bacterial community structures of rhizosphere soil and bulk soil tend to be homogeneous. One possible explanation is the higher moisture content in coppice stands, which could better meet the needs of the microbial community (Cui et al. 2019). Another complementary possible reason may be that root activity was

weaker in coppice stands due to their lower productivity (Table S2). Thus, both soil environment and root activity may be responsible for the consistency of the bacterial community composition between the rhizosphere and bulk soil in coppice plantations.

At the phylum level, the three most abundant bacteria in both rhizosphere and bulk soil samples were *Proteobacteria*, *Actinobacteria* and *Acidobacteria*, in accordance with the findings of Fonseca et al. (2018). The relative abundance of *Actinobacteria* and *Verrucomicrobia* decreased from F to T stands, while *Proteobacteria* showed an opposite trend (Figure 1A). A possible explanation for this result is that the *Proteobacteria* are generally fast-growing *r*-strategists with the ability to use a wide range of root-derived carbon substrates (Philippot et al. 2013). Thus, the decline in soil quality would drive *Proteobacteria* to acquire more abundant carbon sources to sustain growth, but the underlying mechanisms need to be further explored. The main function of *Actinobacteria* is to absorb nutrients and excrete metabolic products, which results in the decline of soil quality (Wang et al. 2017a). At the genus level, the relative abundance of *Bacillus* and *Bradyrhizobium* increased from F to T stands, while *Acidothermus* and *Bryobacter* showed an opposite trend (Figure. 1B). Therefore, the proportion of dominant species changed, resulting in bacterial community composition homogeneity of bulk soil and rhizosphere in coppice stands. Such a homogenization in community composition is predicted to alter ecosystem function and reduce ecosystem resilience to disturbance (Olden et al. 2004) and result in a net loss of diversity (Rodrigues et al. 2013).

### Conversion from seedling to coppice stands increased the relative abundance of *Rhizobium*

*R. pseudoacacia* can increase the availability of soil inorganic N, presumably because of *Robinia's* ability to fix N<sub>2</sub> by association with *Rhizobium* (Zhang et al. 2019), which is the main source of nitrogen in *Robinia* stands (Papaioannou et al. 2016). Our results showed that the relative abundance of *Rhizobium* increased from seedling to coppice stands, which was against our third hypothesis. The reason may be that most of the *Rhizobium* bacteria are free-living individuals in the soil, resulting in the decrease of the symbiotic fixation of atmospheric N within the root nodules of legume hosts (Joëlle et al. 2010; Wang et al. 2018b). Another plausible reason may be that the biological nitrogen fixation requires an expenditure of more C and P (Tye and Drake, 2011; Liu and Deng, 1991). Deficit of C and P in *R. pseudoacacia* stands would decrease and, finally, inhibit symbiotic fixation of atmospheric nitrogen. In the meantime, some study has reported that soil nitrogen-fixing bacterial communities can increase the level of soil available N via biological N-fixation (Wang et al. 2018a), while our results showed an opposite trend. This may be related to the decline of soil C and N or to the reduced amount of litter biomass (Cao et al. 2018).

### Relationships between bacterial community and soil properties

Soil bacterial communities are strongly influenced by abiotic controls (Thoms and Gleixner, 2013), such as total organic carbon (TOC), total nitrogen (TN) (Zhou et al. 2012; Lazzaro et al. 2017). And, vice versa, shifts in microbial communities can affect multiple environmental factors (Fonseca et al. 2018), including potential negative impacts on soil health and plant nutrient acquisition. Therefore, environmental conditions mainly affect the diversity of bacterial communities by changing the physical and chemical properties of the soil (Zhang et al. 2018b). In this study, we found that bacterial communities in both the rhizosphere and bulk soil were strongly influenced by soil C, N, SOM, A.P and NO<sub>3</sub><sup>-</sup>-N (Table 2, Figure 4 and 5), which supports our second hypothesis, i.e., that nutrient availability plays an important role in shaping bacterial community. C and N contents exhibited a strong significantly positive correlation with *Bacteroidetes*, and a negative correlation with *Proteobacteria* and *Firmicutes*, whereas no correlation with *Actinobacteria* and *Acidobacteria* (Figure 4), which was consistent with the results reported by Fierer (2007) and Zhao (2018). *Proteobacteria* are considered to be rhizospheric-plant-promoting bacteria that can influence C accumulation (Ren et al. 2016), and have a significantly positive effect on C fractions. However, our results showed an opposite trend. The reason may be that *Bacteroidetes* can influence the rate of C mineralization and fix atmospheric nitrogen in symbiosis (Fierer et al. 2007).

Soil bacterial community can increase soil NO<sub>3</sub><sup>-</sup>-N content (Zhang et al. 2015; Lazzaro et al. 2017). The conversion from seedling to coppice stands altered the structure of the soil bacterial community and decreased soil resource availability (Zhang et al. 2017), which also partly supports the hypothesis that nutrient availability plays an important role in shaping the bacterial community. In this study, we found that bacterial communities in both the rhizosphere and bulk soil were strongly influenced by soil NO<sub>3</sub><sup>-</sup>-N (Figure 5), similarly to the results of Liu (2018). NO<sub>3</sub><sup>-</sup>-N may play an important role in shaping bacterial communities in *R. Pseudoacacia* plantations. Nitrogen in soil can be decomposed by bacteria to promote N absorption by trees. All N transformation and uptake processes are correlated with soil carbon resources and regulated by soil microbes (Geisseler et al. 2010). Our results showed that C and NO<sub>3</sub><sup>-</sup>-N contents in the coppice stands were lower than those in seedling plantations, leading to inhibition of microbial activity.

## Conclusions

Our research revealed three important findings for assessing the impacts on soil habitat arising from the conversion of *R. pseudoacacia* seedling plantations to coppice stands. First, we found that this conversion can negatively affect soil properties. Second, the conversion from seedling to coppice stands could alter soil bacterial community composition, resulting in higher homogeneity of the bacterial community composition in bulk soil and rhizosphere in coppice stands. Furthermore, this can lead to the imbalance of soil microenvironment structure and the decline of soil functions. Additionally, stand conversion increased the relative abundance of *Rhizobium*, but the soil N and available N decreased, implying that the activity of *Rhizobium* was limited. Eventually, we found that  $\text{NO}_3^-$ -N is the most important factor in shaping soil bacterial structure in this ecosystem. Nevertheless, this does not necessarily mean that the contribution rate of N to bacterial community was equal to zero (Figure 5). Further research on N cycling and understory cover changes resulting from stand conversion from seedling to coppice stands can help to better assess this phenomenon, including mineralization, nitrification, anaerobic ammonium oxidation, denitrification and nitrogen fixation.

## Declarations

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### Authors Contributions statement

Conceived and designed the study: Kun Li, Xu Han, Chuanrong Li. Collected data and samples in the field: Kun Li, Ge Shi, Weixing Shen, Yikun Zhang, Xingzhong Zhang. Processed samples in the lab: Kun Li, Ge Shi. Analyzed the data: Kun Li and Xu Han. Wrote the paper: Kun Li, Ruiqiang Ni and Sergio de-Miguel. All authors read and approved the final manuscript.

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### Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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## Tables

**Table 1.** Bulk soil and rhizosphere soil properties (mean  $\pm$  standard error) of the three types of *Robinia pseudoacacia* stands following forest conversion from seedling to coppice stands.

	Rhizosphere			Bulk soil		
	FR	SR	TR	FNR	SNR	TNR
N/%	0.38±0.03a	0.32±0.01b	0.33±0.01b	0.43±0.02a	0.32±0.01c	0.36±0.02b
C/%	3.80±0.27a	3.31±0.14b	3.50±0.15b	4.56±0.16a	3.33±0.16c	3.77±0.30b
P/%	1.58±0.13b	1.88±0.05a	1.63±0.08b	1.66±0.05a	0.93±0.03b	0.85±0.02c
C/N	10.14±0.18b	10.22±0.31b	10.57±0.22a	10.52±0.26a	10.35±0.47a	10.51±0.17a
C/P	2.31±0.30a	1.76±0.11c	2.15±0.18b	2.75±0.10c	3.59±0.24b	4.43±0.28a
N/P	0.24±0.03a	0.17±0.01c	0.20±0.01b	0.26±0.01c	0.35±0.03b	0.42±0.02a
NO <sub>3</sub> <sup>-</sup> -N (mg/kg)	69.06±1.53a	63.29±1.63b	59.50±1.84c	67.20±2.99a	60.52±1.39b	60.71±2.14b
NH <sub>4</sub> <sup>+</sup> -N (mg/kg)	58.60±1.24a	58.81±1.62a	55.20±2.42b	45.99±4.02b	54.25±0.43a	39.41±1.48c
AN (mg/kg)	127.66±2.27a	122.10±2.95a	114.70±4.10b	113.19±4.19a	114.77±1.36b	100.12±2.87c
AP (mg/kg)	16.93±1.26a	11.18±0.9b	8.35±0.98c	14.41±0.91a	14.24±1.08a	9.60±1.27b
SOM (g/kg)	64.91±0.92a	62.84±1.29b	50.73±1.05c	61.93±1.78a	51.69±0.58b	46.70±1.47c
Exchangeable Ca (cmol/kg)	48.74±9.48b	44.63±11.21b	65.83±12.46a	61.78±13.15a	51.01±7.96a	65.26±11.27a
Exchangeable Mg (cmol/kg)	12.09±1.60b	12.01±1.17ab	13.17±0.75a	11.68±0.93a	12.66±0.78a	13.07±0.74a
Exchangeable K (cmol/kg)	0.71±0.05ab	0.70±0.03b	0.76±0.05a	0.72±0.05a	0.86±0.28a	0.72±0.07a
Soil moisture %				7.66±0.61b	13.28±0.52a	13.95±0.60a
Soil quality index	29.14±0.36a	27.42±0.33b	26.72±0.64b	27.59±0.29a	26.14±0.29b	24.33±0.97b

Mean values ±SE (n=5) are shown. FR, SR and TR represent the rhizosphere of seedling plantations, first generation coppice plantations and second generation coppice plantations, respectively; FNR, SNR and TNR represent bulk soil of seedling plantations, first generation coppice stands and second generation coppice stands, respectively. N, nitrogen; C, carbon; P, phosphorus; C/N, carbon and nitrogen ratio; C/P, carbon and phosphorus ratios; N/P, nitrogen and phosphorus ratios; NO<sub>3</sub><sup>-</sup>-N, nitrate nitrogen; NH<sub>4</sub><sup>+</sup>-N, ammonium nitrogen; AN, available nitrogen; AP, available phosphorus; SOM, soil organic carbon. Different lowercase letters indicate significant differences in soil properties among the bulk soil or rhizosphere in different *R. pseudoacacia* plantations (p<0.05).

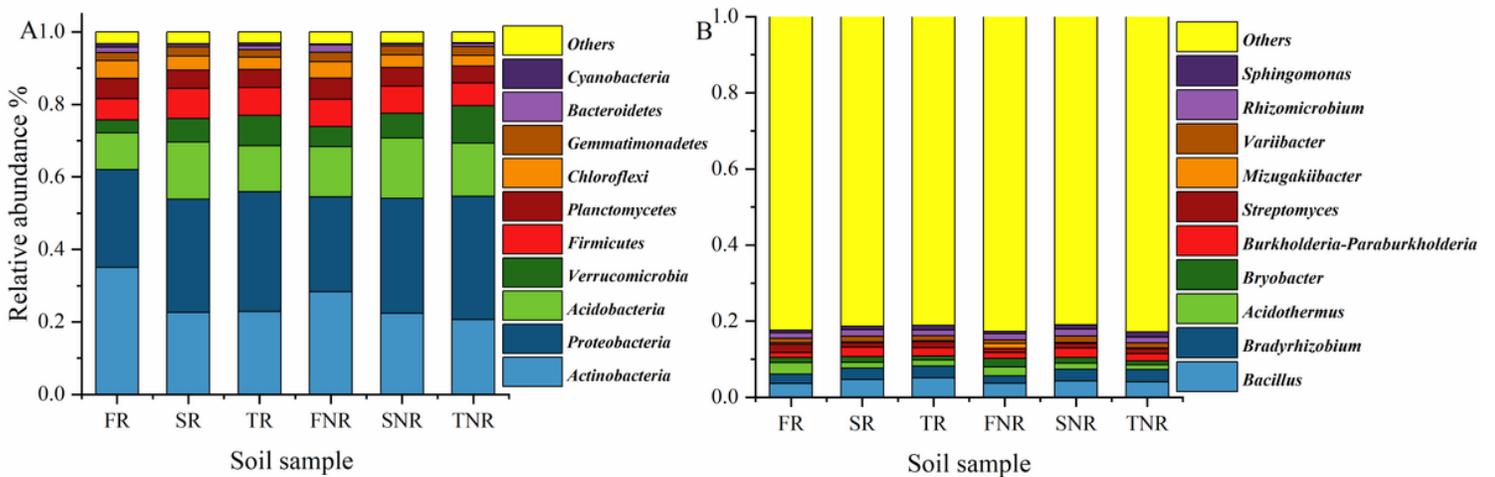
**Table 2.** Differences in bacterial  $\alpha$ -diversity in the rhizosphere and bulk soil between seedling and coppice *R. pseudoacacia* plantations.

	FR	SR	TR	FNR	SNR	TNR
Shannon	9.2205±0.0473a	9.1055±0.0119bc	9.1803±0.0155ab	9.2186±0.0482a	9.0159±0.0329c	9.0336±0.0339c
Simpson	0.9954±0.0001a	0.9949±0.0001ab	0.9945±0.0002b	0.9954±0.0003a	0.9945±0.0002b	0.9934±0.0004c

**Table 3** Mantel-test between bacteria phylum and genus and soil properties.

Soil properties	Phylum		Genus	
	r	p	r	p
N	0.495	0.001	0.505	0.001
C	0.4433	0.001	0.4419	0.001
P	0.08502	0.07	0.04408	0.236
C/N	0.008762	0.499	0.04093	0.665
C/P	0.1329	0.046	0.09815	0.106
N/P	0.1254	0.044	0.09049	0.13
SOM	0.4002	0.001	0.3239	0.001
NO <sub>3</sub> <sup>-</sup> -N	0.5758	0.001	0.5383	0.001
NH <sub>4</sub> <sup>+</sup> -N	0.2009	0.016	0.1892	0.017
A.N	0.2144	0.007	0.2028	0.015
A.P	0.4528	0.001	0.4239	0.001
Exchangeable Ca	0.03213	0.308	0.04071	0.285
Exchangeable Mg	0.1988	0.017	0.187	0.033
Exchangeable K	0.06525	0.767	0.07211	0.764

## Figures



**Figure 1**

Relative abundance of the dominant bacteria phylum and genus among the soil bacterial phyla via sequencing of 16S rRNA gene amplicons in bulk soil and rhizosphere of different stands.

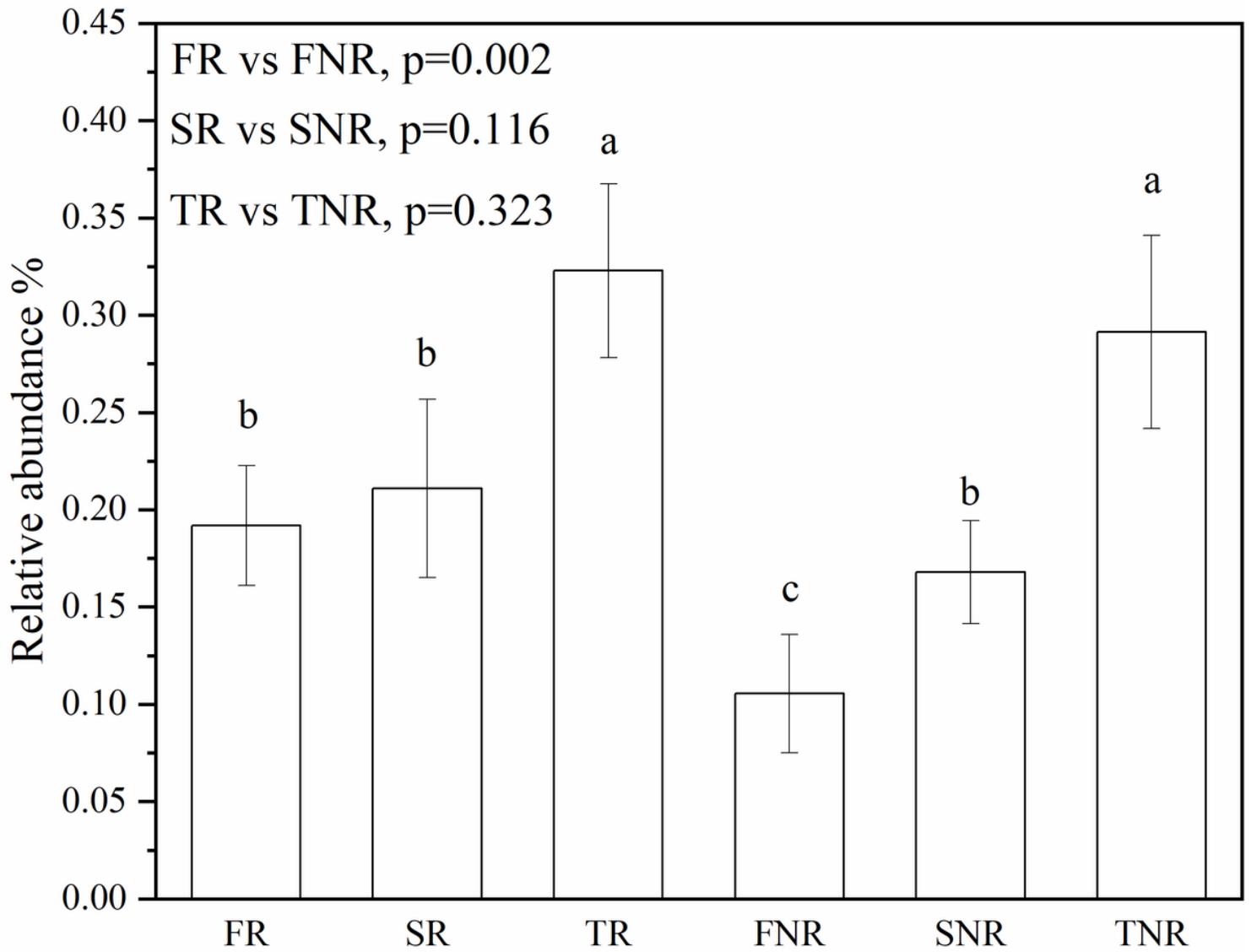
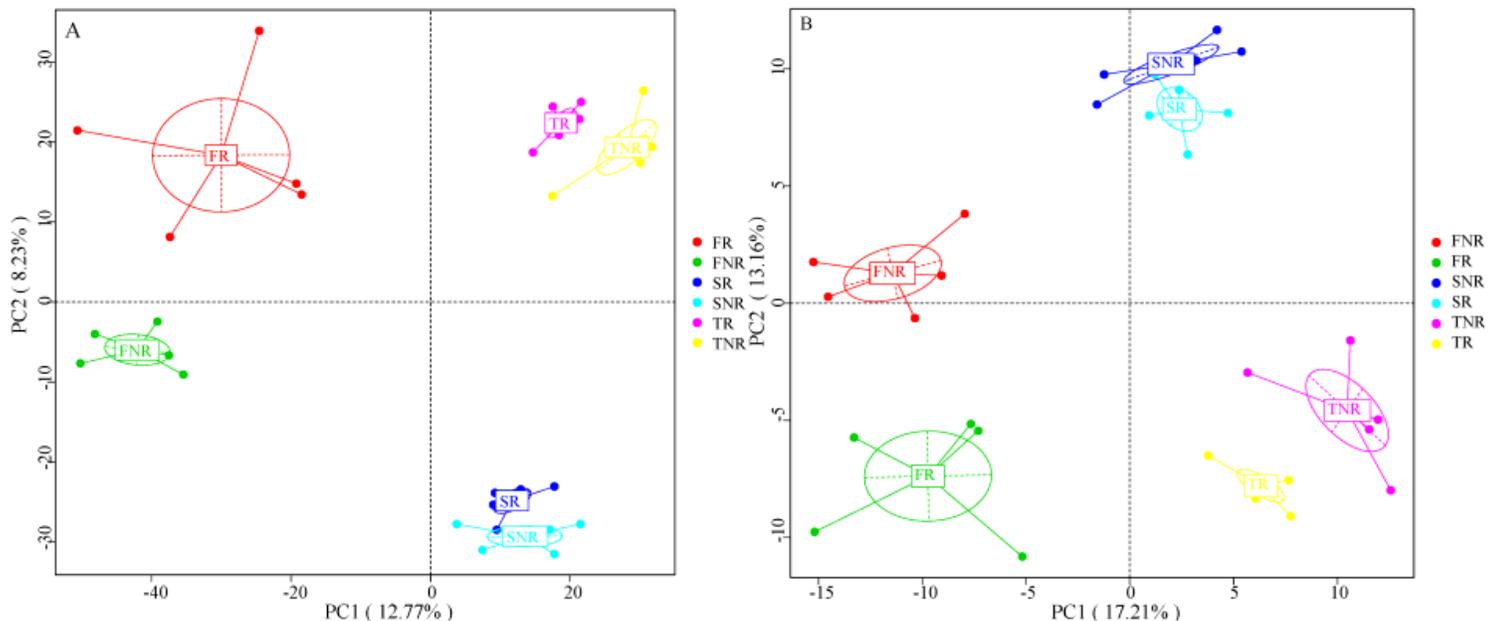


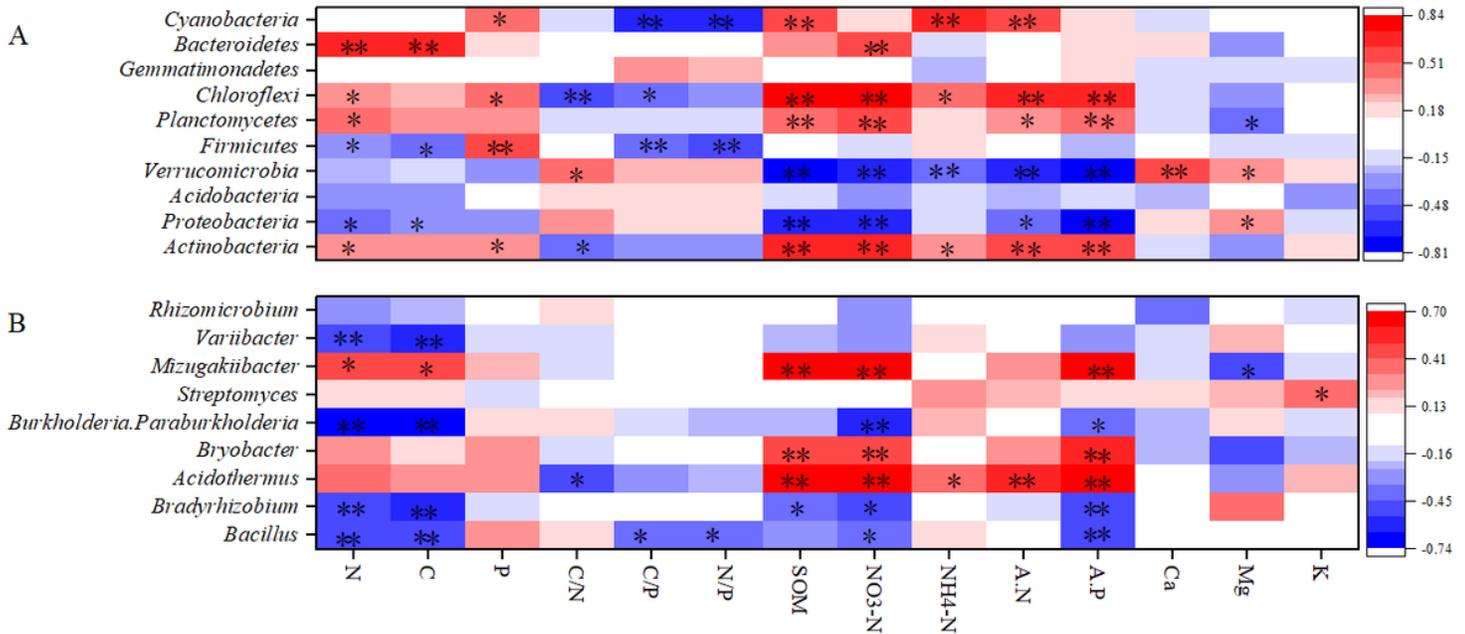
Figure 2

Differences in the relative abundance of Rhizobium between the rhizosphere and bulk soil seedling and coppice stands.  $\alpha=0.05$ .



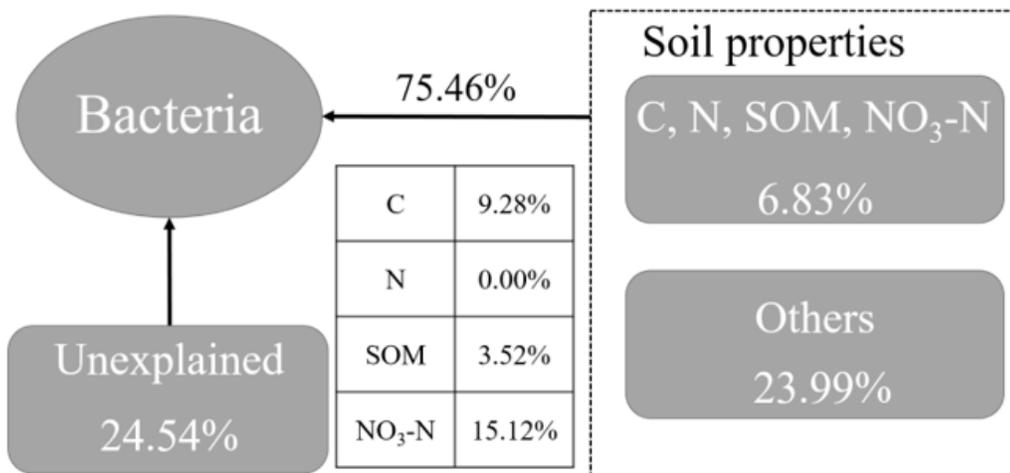
**Figure 3**

Principal Component Analysis (PCA) (Bray-Curtis distance) among bulk soil and rhizosphere bacterial communities at phylum (A) and genus (B) level. Red and green represent the bacterial community of bulk soil and rhizosphere in seedling plantations (F); blue and cyan represent the bacterial community of bulk soil and rhizosphere in first generation coppice stands (S); pink and yellow represent the bacterial community of bulk soil and rhizosphere in second generation coppice stands (T).



**Figure 4**

Spearman correlation matrix between soil physicochemical characteristics and bacterial communities at phylum level (A) and genus level (B). Note: N: soil nitrogen content, C: soil carbon content, P: soil phosphorus content, NO<sub>3</sub>-N: soil nitrate content, NH<sub>4</sub>+N: soil ammonium content, A.N: available nitrogen content, A.P: available phosphorus content, Ca: soil exchangeable calcium content, Mg: soil exchangeable magnesium content, K: soil exchangeable potassium, SOM: soil organic matter. \*P<0.05, \*\* P<0.01.



**Figure 5**

Variance partition analysis (VPA) of the effects of soil properties on the bacterial community structure. Soil properties include C, N, SOM and NO<sub>3</sub>-N and interaction among them. "Others" include other soil properties, such as P, NH<sub>4</sub>+N, Ca and so on.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [FigS1.png](#)
- [FigS2.png](#)