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

kun li  
shandong agricultural university

xu han  
shandong agricultural university

ruiqiang ni  
shandong agricultural university

gi shi  
shandong agricultural university

Sergio de-Miguel  
Universitat de Lleida

chuanrong li (✉ chrlisd@126.com)  
shandong agricultural university  https://orcid.org/0000-0003-3933-6436

weixing shen  
mount tai scenic spot management committee

yikun zhang  
mount tai scenic spot management committee

xingzhong Zhang  
mount tai scenic spot management committee

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 plantations 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: Three 20×20 m plots were randomly selected in each seedling and coppice stand. The bulk soil and rhizosphere soil were sampled in the nine above-mentioned sample plots in the summer of 2017. Bulk soil was sampled at 10 cm from the soil surface using a soil auger. Rhizosphere soil samples were collected by 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 α-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 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 depends, 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 environment by effect of root exudation and litters (Sasse et al. 2018). So soil ecological transformation may provide a simple means of identifying stable state within the ecosystem (Macdonald 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 micro-ecology may be a key driver for predicting 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, unexpected problems have arisen in Mount Tai (China) forest ecosystems, including the decline of landscape quality, soil erosion and plant dwarving, 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). But there is a gap in knowledge concerning the effects of the transition 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 shift 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 by seedling direct planting. However, with increasing timber demand for use in construction, seedling plantations were gradually harvested leading to naturally-regenerated coppice plantations. Nowadays, most *R. pseudoacacia* stands are coppice plantations, mainly distributed along an elevational gradient from 500 to 1000 meters above sea level, and southern aspects.

The study was performed in first generation seedling plantation stands (First, F, 36°16′45″N, 117°3′26″E), first generation coppice plantations (Second, S, 36°16′40″N,117°3′ 21″E) and second generation coppice plantations (Third, T, 36°16′40″N,117°3′ 22″E) in Mount Tai, China (Figure S1). The understory vegetation is mainly composed of *Vitex negundo, Oplismenus undulatifolius, Digitaria sanguinali, Paspalum thunbergii, Rubia cordifolia* and *Oxalis corniculate*. 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 properties. The soil taxonomy is classified to the brown loamy soil. The soil moisture is about 10%. The microbial biomass of carbon in the three stages of stands are 247 mg/kg, 200 mg/kg, and 190 mg/kg, respectively, and those of nitrogen are 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 of 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 cm³). 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$_3^{-}$-N) and ammonium (NH$_4^{+}$-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$_3^{-}$-N and NH$_4^{+}$-N The available P in the soil was measured using the colorimetric method with 0.5M NaHCO$_3$ 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$^{2+}$, Mg$^{2+}$ and K$^{+}$) 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$^{-3}$) 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 $SQI$ could 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® 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 analyze alpha- (within samples) and beta- (among 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 \textit{Rhizobioum} 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 analysis 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 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 were 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 \textit{Proteobacteria} (30.54%), \textit{Actinobacteria} (25.30%), \textit{Acidobacteria} (13.94%), \textit{Firmicutes} (7.19%), \textit{Verrucomicrobia} (6.86%), \textit{Planctomycetes} (5.22%), \textit{Chloroflexi} (3.87%), \textit{Gemmatimonadetes} (2.37%), \textit{Bacteroidetes} (1.14%), and \textit{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 (≥1%) were \textit{Bacillus} (4.22%), \textit{Bradyrhizobium} (2.82%), \textit{Acidothermus} (1.88%), \textit{Bryobacter} (1.44%), \textit{Burkholderia-Paraburkholderia} (2.00%) and \textit{Streptomyces} (1.41%). The relative abundance of \textit{Bacillus} and \textit{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 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, NO$_3^-$-N and A.P were positively correlated with bacterial community composition by Mantel tests at both the 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, 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, 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, SOM and NO$_3^-$-N, the most relevant factors based on mantel tests 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 structure. The soil properties explained 75.46% of the observed variation, leaving 24.54% of the variation unexplained. Among them, C, N, SOM and NO$_3^-$-N explained 9.28%, 0.00%, 3.52% and 15.12%, respectively. Thus, NO$_3^-$-N, but not N, was the most important factor in shaping the bacterial community structure.
Discussion

Conversion from seedling to coppice plantations 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 plantations, 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 hypothesis 1 that *R. pseudoacacia* is a N₂-fixing species with a strong nitrogen fixation ability. However, our results showed that soil N (N, NO₃-N and A.N) concentrations declined in coppice plantations. 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 than seedling plantation (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 plantation had a lower stand productivity than seedling plantation (Figure S2), and which could modify soil structure and lead to less inputs and more losses of soil nutrients (Zheng et al. 2005), then finally affect the absorption of N by trees (Zhang et al. 2018b). Additionally, we found that the greater 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, NO₃-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 take up lower available nutrients and reduce carbon loss in the rhizosphere (Jones et al. 2009), and they could also adapt to the change of soil nutrient availability through the elastic distribution of underground roots (Bardgett et al. 2014). The consumption of N for 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 plantations altered the structure of bacterial communities

Changes in forest community types can affect soil microbial structure (Cardenas et al. 2015) and α-diversity (Vitali et al. 2016). Our results showed that Shannon and Simpson indices declined from seedling to coppice plantations (Table 2). These shifts can be accompanied by changes in bacterial functional activity (Kaiser et al. 2014), contributing to one of 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 consistent. The possible explanations were that one is the higher moisture content in coppice plantations, which could meet the needs of the microbes (Cui et al. 2019), the second was that the root activities were weaker due to the lower productivity (Table S2). Therefore, we concluded that the soil environment and root activities were responsible for this consistency of the bacterial communities 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*, consistent with the findings of Fonseca et al. (2018). The relative abundance of *Actinobacteria* and *Verrucomicrobia* decreased from F to T, while *Proteobacteria* showed an opposite trend (Figure 1A). A possible explanation for this result is that the *Proteobacteria* is generally a fast-growing r-strategist with the ability to use a wide range of root-derived carbon substrates (Philippot et al. 2013). Furthermore, the decline in soil quality will 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, while *Acidothermus* and *Bryobacter* showed opposite trend (Figure. 1B). Therefore, the proportion of dominant species changed, which resulted in bacterial community composition homogeneity of bulk soil and rhizosphere in coppice plantations. This homogenization 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 plantations 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\(_2\) by association with 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 plantations, which was against hypothesis 3. 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 little 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$_3^-$-N (Table 2, Figure 4 and 5), which supports hypothesis 2, 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* (Table 4), which was consistent with the results of 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. But 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$_3^-$-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 in part 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$_3^-$-N (Figure 5), which is similar to the results of Liu (2018). NO$_3^-$-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$_3^-$-N contents in the coppice plantations were lower than those in the seedling stands, leading to inhibition of microbial activity.

**Conclusions**

Our research revealed three important findings for assessing the impacts of converting seedling to coppice plantations on soil habitat. 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 plantations. 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 NO$_3^-$-N is the most important factor in shaping soil bacterial structure in this ecosystem. Nevertheless, we can not rule out that the contribution rate of N to bacterial community was equal to zero (Figure 5). Further research with N cycling and understory coverages conversion from seedling to coppice plantations can help to better assess this phenomenon, including mineralization, nitrification, anammox, 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 ± standard error) of the three types of Robinia pseudoacacia plantations 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$_3^-$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$_4^+$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 | 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 | 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 | 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 | 27.59±0.29a | 26.14±0.29b | 24.33±0.97b |
| 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 plantations and second generation coppice plantations, 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$_3^-$, nitrate nitrogen; NH$_4^+$, 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 α-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$_3^-$-N      | 0.5758 | 0.001 | 0.5383 | 0.001 |
| NH$_4^+$-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 plantations.

Figure 2

Differences in the relative abundance of Rhizobium between the rhizosphere and bulk soil seedling and coppice plantations. α=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 plantations (S); pink and yellow represent the bacterial community of bulk soil and rhizosphere in second generation coppice plantations (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, NO3–N: soil nitrate content, NH4+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$_3$-N and interaction among them. “Others” include other soil properties.

**Supplementary Files**

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

- FigS1.png
- FigS2.png