



Black locust coppice stands homogenize soil diazotrophic communities by reducing soil net nitrogen mineralization

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ABSTRACT

Background: Black locust (BL, *Robinia pseudoacacia*) is considered a promising tree species for reforestation due to its great ability to fix nitrogen. However, after two or three coppice-harvesting rotations, the productivity of BL declines. Whether soil microbial communities are affected and how these groups correlate with the nitrogen mineralization process across multi-generation stands remains unclear.

Methods: We investigated the composition and structure of free-living nitrogen-fixing microorganisms (diazotrophs) by sequencing the marker gene *nifH* and compared these results to levels of soil nitrogen mineralization in the bulk soil and rhizosphere in black locust plantations on Mount Tai, China.

Results: The results showed multi-generation BL coppice plantations decreased the total soil nitrogen (N), soil phosphorus (P), soil microbial biomass N (MBN), soil microbial biomass C (MBC), soil nitrification rate (Rn), soil ammonification rate (Ra), and net soil N mineralization rate (Rm), but significantly increased the concentration of soil $\text{NH}_4^+\text{-N}$ to maintain sufficient $\text{NO}_3^+\text{-N}$. The dominant species in bulk soil and rhizosphere changed from *Rhodopseudomonas* (22.62% and 15.76%), unclassified *c_Alphaproteobacteria* (22.37% and 29.28%), unclassified *o_Rhizobiales* (15.40% and 13.31%), *Bradyrhizobium* (12.00% and 11.74%) in seedling plantations to *Bradyrhizobium* (45.95% and 47.86%) and *Rhodopseudomonas* (43.56% and 41.84%) in coppice plantations, respectively. Mantel test and Redundancy analysis (RDA) revealed that Rn, Ra, and Rm were the most important factors shaping the diazotrophic communities.

Conclusions: Our results suggest that the multi-generation BL coppice plantation can homogenize soil diazotrophic communities, which is mainly regulated by the available N loss caused by nitrogen mineralization. Strengthening the management technology of coppice plantations will provide more beneficial external consumption.

1. Background

Black locust (BL, *Robinia pseudoacacia*) is considered a promising tree species for reforestation programs due to its rapid growth rate and ability to fix nitrogen (Medina-Villar et al., 2016). Reproduction of BL plantations is primarily vegetative through root suckering and stump sprouting (Carl et al., 2019), allowing vigorous regeneration after coppicing and disturbance. Unfortunately, BL coppice plantation productivity declines after two or three harvesting rotations (Cierjacks et al., 2013; Li et al.,

2021). Other adverse effects have also received widespread attention, such as soil fertility degradation, trunk bending, and limited tree height (Li et al., 2021).

BL roots are colonized with nitrogen-fixing microorganisms (diazotrophs) and thus enhance the nitrogen (N) content in soil organic fraction through N_2 -fixation (Cierjacks et al., 2013), which is an essential source of N to ecosystems (Reed et al., 2011). Nitrogen fixation reduces molecular nitrogen to ammonia and other nitrogen-containing compounds (Meng et al., 2019; Xu et al., 2019), mediated by bacteria and archaea that possess the enzyme nitrogenase (Reed et al., 2011; Ouyang et al.,

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Abbreviations

BL	Black locust	C/P	Ratio of soil carbon and phosphorus
F	First-generation seedling plantation stand	N/P	Ratio of soil nitrogen and phosphorus
S	First-regeneration coppice stand	NO ₃ ⁻ -N	Soil nitrate
T	Second-generation coppice stand	NH ₄ ⁺ -N	Soil ammonium
FR	Rhizosphere in first-generation seedling plantations	AN	Soil available nitrogen
SR	Rhizosphere in first-generation coppice stands	MBN	Microbial biomass N
TR	Rhizosphere in second-generation coppice stands	MBC	Microbial biomass C
FNR	Bulk soil in first-generation seedling plantations	MBC/MBN	Ratio of MBC and MBN
SNR	Bulk soil in first-generation coppice stands	Rn	Soil nitrification rate
TNR	Bulk soil in second-generation coppice stands	Ra	Soil ammonification rate
C	Total soil carbon	Rm	Net soil N mineralization rate
N	Total soil nitrogen	RDA	Redundancy analysis
P	Total soil phosphorus	PCoA	Principal coordinate analysis
C/N	Ratio of soil carbon and nitrogen	OTUs	Operational Taxonomic Units
		GLMMs	Generalized Linear Mixed Models
		BNF	biological nitrogen fixation

2018). The *nifH* gene regulates nitrogenize synthesis by producing an enzymatic compound that catalyzes the N₂-fixation to NH₄⁺-N in the plant (Gaby and Buckley, 2011; Zhou et al., 2021). Therefore, diazotrophic abundance, diversity, and community composition have been recognized as biotic factors crucial to determining soil nitrogen-fixing capacity (Stewart et al., 2011; Zhao et al., 2021). These factors have been extensively investigated, employing the *nifH* gene as a molecular marker (Fan et al., 2018; Chen et al., 2019a, 2019b). Generally, the rates of biological nitrogen fixation (BNF) are related to diazotrophic abundance and community composition (Hsu and Buckley, 2009; Stewart et al., 2011). The community composition, in turn, is influenced by land use, soil physical properties, nitrogen quantity, and availability (Calderoli et al., 2017; Chen et al., 2019a; Wang et al., 2021). Diazotrophs and nitrogen mineralization have a significant impact on soil nitrogen availability. Nevertheless, we still do not understand how they responded to converting BL seedling plantations to coppice stands.

Most researchers have focused on the bacteria and fungi communities, but little attention has been paid to functional genes, for instance, the *nifH* gene. For example, one study found that converting an Amazon rainforest to a cattle pasture altered the diazotrophic composition (Mirza et al., 2014). Another study found that forest conversion may directly or indirectly affect soil nitrogen mineralization rate by affecting soil biotic and abiotic factors such as available N (Rigby et al., 2016). Therefore, a study of the *nifH* gene and N mineralization can provide an insight into the influence of N dynamics on BL coppice plantations. Nitrogen is a crucial nutrient for plant and microbial growth that influences plant productivity and microbial decomposition in terrestrial ecosystems (LeBauer and Treseder, 2008). Soil N availability for plant growth is dependent on the rate of soil N mineralization, in which soil organic N is converted to inorganic N by soil microorganisms and invertebrates (Chapin III et al., 2011; Murphy et al., 2017). Such processes, including ammonification and nitrification, convert organic N (proteins, sugars, cellulose, lignin, etc.) to available inorganic forms (NH₄⁺ and NO₃⁻) (Fu et al., 2019). Soil N mineralization directly affects nitrification by providing substrates to nitrifying microorganisms (Petersen et al., 2012). The quantity of NH₄⁺-N is the rate-limiting step in soil nitrification. The soil abiotic properties, such as soil pH, soil moisture, and soil depth, could describe the N mineralization rate (Urakawa et al., 2016; Zhang et al., 2018; Thapa et al., 2021). At the same time, many recent studies have found that the vegetation type and microbial community also substantially affect N mineralization (Li et al., 2019). This process affects the quantity and quality of organic materials, soil biological activity, microbial community composition, and N plant-use efficiency (Rachid et al., 2013; Rahman et al., 2017; Kong et al., 2019; Yao et al., 2019). Therefore, studying vegetation types and soil properties is relevant to understanding nitrogen mineralization.

Reforestation decreases soil erosion and improves soil fertility, potentially affecting soil N fractions (Guillaume et al., 2018). Because of its effect on recovering N cycling processes, BL was used as a pioneer tree species for afforestation Mount Tai to preserve soil (Meng et al., 2020). Generally, different N requirements of specific plant species may be an underlying driver of the diazotrophic communities (Kong et al., 2019). However, the specific composition of diazotrophic communities and their associations with soil N availability under black locust coppice plantations are largely unknown, limiting our ability to evaluate the effectiveness of reforestation. Previous studies have confirmed that the conversion from seedling to coppice stands reduced soil quality and led to decreasing the relative abundance of *Rhizobium* in coppice plantations (Li et al., 2021). However, the impact of coppice plantation on nitrogen fixation and nitrogen mineralization is unknown. Accordingly, this study hypothesized that the coppice diazotrophic community negatively impacts soil fertility in the BL coppice plantations. Our objectives were to (1) determine the effects of conversion from seedling to coppice stands in BL plantations on the composition structure of diazotrophic community; (2) examine the effect of conversion on net N mineralization, and (3) explore the driving factors for the conversion in the diazotrophic communities.

2. Materials and methods

2.1. Study area

The study site is on Mount Tai, Shandong Province, China. The mean annual temperature is 12.8 °C, and the mean annual precipitation is 1,124.6 mm. The existing BL stands are mostly coppice plantations, mainly distributed on south-facing slopes at elevations of 500–1,000 m. The study was carried out in: i) a first-generation seedling plantation stand (from now on referred to as “First” or “F”, 36°16′45″ N, 117°3′26″ E), ii) a first-regeneration coppice stand generated after clear-cutting of a seedling stand (referred to as “Second” or “S”, 36°16′40″ N, 117°03′21″ E) and iii) a second-generation coppice stand generated after clear-cutting of a first-generation coppice stand (referred to as “Third” or “T”, 36°16′40″ N, 117°3′22″ E). The principal species in the understory vegetation community are *Vitex negundo*, *Oplismenus undulatifolius*, *Digitaria sanguinalis*, *Paspalum thunbergii*, *Rubia cordifolia*, and *Oxalis corniculata*. The soil type is classified as Alfisols. We sampled plants and collected rhizosphere and bulk soil samples for further analysis for each stand. Detailed information about the plots can be found in Li et al. (2021).

2.2. Sampling design

Three 20 m × 20 m plots were randomly selected in each seedling and coppice stand for a total of nine plots. The bulk soil and rhizosphere soil

were sampled in the nine plots in August 2018. Bulk soil was sampled at 10 cm from the soil surface 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 air-dried and stored at room temperature before chemical analysis, and the other stored at -80°C and -4°C for further analysis. In this article, 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.

2.3. Soil physicochemical property analysis

A subsample of 50 g of soil was stored at -4°C and later used for the determination of microbial biomass C (MBC) and N (MBN) by the method of chloroform fumigation extraction (Margeson and Schinner, 2005). During chloroform fumigation, soil incubation in gaseous CHCl_3 was extended from 24 to 48 h in our experiment to thoroughly kill all soil microorganisms (Tian et al., 2017). After chloroform fumigation, the soil samples were extracted using the solution of $0.5\text{ mol}\cdot\text{L}^{-1}\text{ K}_2\text{SO}_4$. The soil extraction solution was then analyzed by Multi N/C 3100 using the high-temperature combustion method (Analytik Jena AG, Germany). The factors in the transformations from the detected soil total organic carbon and nitrogen to microbial biomass carbon and nitrogen were 0.45 and 0.54, respectively (Margeson and Schinner, 2005).

The second subsample of 10 g was air-dried and used to determine soil nitrogen mineralization rates. N mineral fractions ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) were determined using the aerobic incubation method (Hart et al., 1994). For this purpose, 10 g soil through a 2-mm sieve was adjusted to 40% of the maximum water holding capacity. Samples were incubated in a constant temperature incubator at 25°C , and N extractions were performed at 0 and 14 d after incubation. The $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ contents were extracted using 50 mL of $2\text{ mol}\cdot\text{L}^{-1}\text{ KCl}$ solution. The soil mineralization ratio was obtained by subtracting the initial (zero-day) and final (14 days) ammonium ($\text{NH}_4^+\text{-N}$) and nitrate ($\text{NO}_3^-\text{-N}$) concentrations (Hart et al., 1994). Net N mineralization rates were calculated as the changes in mineral N contents and reported in mg N dry soil per 14 d.

The third subsample of 30 g was air-dried and used to determine soil nutrient content, including total soil carbon (C), total soil nitrogen (N), soil nitrate ($\text{NO}_3^-\text{-N}$), soil ammonium ($\text{NH}_4^+\text{-N}$), and soil phosphorus (P). The C and N contents were measured by combustion in an elemental analyzer (Costech ECS4010, Italy). $\text{NO}_3^-\text{-N}$ and $\text{NH}_4^+\text{-N}$ were extracted by shaking 20 g of fresh soil in 100 mL of $2\text{ mol}\cdot\text{L}^{-1}\text{ KCl}$ solution for 1 h and were analyzed with a continuous flow analytical system (AA3, German). Available N (AN) was a sum of $\text{NO}_3^-\text{-N}$ and $\text{NH}_4^+\text{-N}$. P content was extracted using the Olsen method and measured by a continuous flow analytical system (AA3, German).

2.4. DNA extraction, *nifH* gene amplification

Microbial DNA was extracted from soil samples (frozen at -80°C) using the E.Z.N.A.® soil DNA Kit (Omega Bio-Tek, Norcross, GA, US) according to the manufacturer's protocols. The final DNA concentration and purification were determined by NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was checked by 1% agarose gel electrophoresis. The primer pair PolF (5'-TGCGAYCCSAARGCBGACTC-3') and PolR (5'-ATSGCCATCA-TYTCRCGGA-3') was used for amplifying the *nifH* gene. Each reaction was carried out in a 25- μL volume which included 0.5 μL of each primer ($10\text{ }\mu\text{mol}\cdot\text{L}^{-1}$), 2 μL DNA template (1–10 ng), 12.5 μL of Premix Taq™ ($2\times$) (Takara) and 9.5 μL ddH₂O. The PCR conditions were as follows: 5 min at 95°C , and then 35 cycles of 30 s at 95°C , 30 s at 58°C , and 45 s at 72°C , and a final extension at 72°C for 6 min (Wang et al., 2018b). With three replicates, each sample was amplified. Then, three PCR replicates of the same sample were mixed with the same quality based on concentration and purified with AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA).

2.5. Illumina MiSeq sequencing

Purified amplicons were pooled in equimolar and paired-end sequenced on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1, <http://drive5.com/uparse/>), and chimeric sequences were identified and removed using UCHIME. The taxonomy of each *nifH* gene sequence was analyzed by the RDP Classifier algorithm (<http://rdp.cme.msu.edu/>) against the Silva (SSU123) *nifH* database using a confidence threshold of 70% (Gaby and Buckley, 2014).

2.6. Statistical analysis

The rates of ammonification (Ra), nitrification (Rn), and net N mineralization (Rm) during incubation were defined as the difference between initial and final $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and net mineral N (sum of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) concentrations, respectively (Kong et al., 2019):

$$\text{Ra} = (\text{NH}_4^+\text{-N}_{i+1} - \text{NH}_4^+\text{-N}_i)/t$$

$$\text{Rn} = (\text{NO}_3^-\text{-N}_{i+1} - \text{NO}_3^-\text{-N}_i)/t$$

$$\text{Rm} = \text{Ra} + \text{Rn}$$

where $\text{NH}_4^+\text{-N}_i$ and $\text{NO}_3^-\text{-N}_i$ are the concentrations of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ before incubation, $\text{NH}_4^+\text{-N}_{i+1}$ and $\text{NO}_3^-\text{-N}_{i+1}$ are the concentrations of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ after incubation, respectively; and t is the number of days of the incubation period.

All responses were modeled as two-way factorial designs using Generalized Linear Mixed Models (GLMMs) that included the main effects of soil, generation, and soil $\times$ generation interaction. A random heteroscedasticity component for each soil $\times$ generation combination was also included for all models. Each model utilized the appropriate conditional distribution of the response based on the response range. Specifically, C/N, C/P, MBC, MBC/BN, MBN, N/P, $\text{NH}_4^+\text{-N}$, and $\text{NO}_3^-\text{-N}$ were modeled as Gamma with a log link, Ra, Rm, and Rn were modeled as Gaussian with an identity link, and C, N, P were modeled as Beta with a logit link. In addition, we computed Hill Numbers analogous to Shannon's Diversity Index ($q = 1$) for each treatment combination (Hill, 1973; Chao et al., 2014). The responses are thus defined as effective species counts and modeled as a Poisson distribution with a log link.

All model prediction confidence bounds were controlled for family-wise experiment rates using the Sidak adjustment method with $\alpha = 0.05$ (Westfall and Young, 1993). In addition, multiple comparisons were adjusted for family-wise experiment rates using Tukey's HSD method, with $\alpha = 0.05$ (Kramer, 1956). Data were analyzed using the R statistical environment version 4.0.5 (R Core Team, 2021) using R packages glmmTMB (Brooks et al., 2017) and emmeans (Lenth, 2021).

To illustrate the clustering of different samples and to further determine the microbial community structure, Principal coordinate analysis (PCoA), Permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis distance were used to analyze the diazotrophic communities at the genus level. Redundancy analysis (RDA), Random Forest, and Mantel tests were used to link the variation within the microbial community, functional structure, and soil properties to environmental variables. Spearman correlation analysis examined the relationships between the abundance of the *nifH* gene, soil nutrients, and net N mineralization. PCoA, RDA, PERMANOVA, Mantel tests were conducted using the *vegan* package with R software. Random Forest analysis was conducted using the *randomForest* package with R software. Circos plot was created with the Circos-0.67-7 software (<http://circos.c.a/>). Data analysis and graphing were performed with SPSS version 26.0 statistical software (SPSS, Chicago, USA) and R software (version 3.6.1).

3. Results

3.1. Effects of conversion on soil properties

Bulk soil and rhizosphere soil properties of seedlings and coppice plantations were significantly different (Fig. 1). NO_3^- -N is the main form of available soil N in BL plantations, ranging from 59.28% to 76.01%. We found that TN, MBN, and MBC in both bulk soil and rhizosphere soil were significantly higher in seedlings plantations than coppice plantations (23.32%–80.24% and 18.71%–58.30%, respectively).

3.2. Effects of conversion on soil nitrogen mineralization rate

The bulk soil and rhizosphere nitrification (Rn), ammonification (Ra), and net N mineralization (Rm) were significantly higher in seedling plantations than in coppice plantations (Fig. 2). In addition, we found that the Rn and Rm were positive, while the Ra was negative. For example, in bulk soil of F, Rn, Ra, and Rm of seedling plantations were 30.89, -5.45 and $25.44 \text{ mg N}\cdot\text{kg}^{-1}\cdot 14 \text{ d}^{-1}$. Rn, Ra, and Rm in the rhizosphere were 30.16, -8.10 and $22.05 \text{ mg N}\cdot\text{kg}^{-1}\cdot 14 \text{ d}^{-1}$, respectively. Conversion from seedling to coppice plantations resulted in

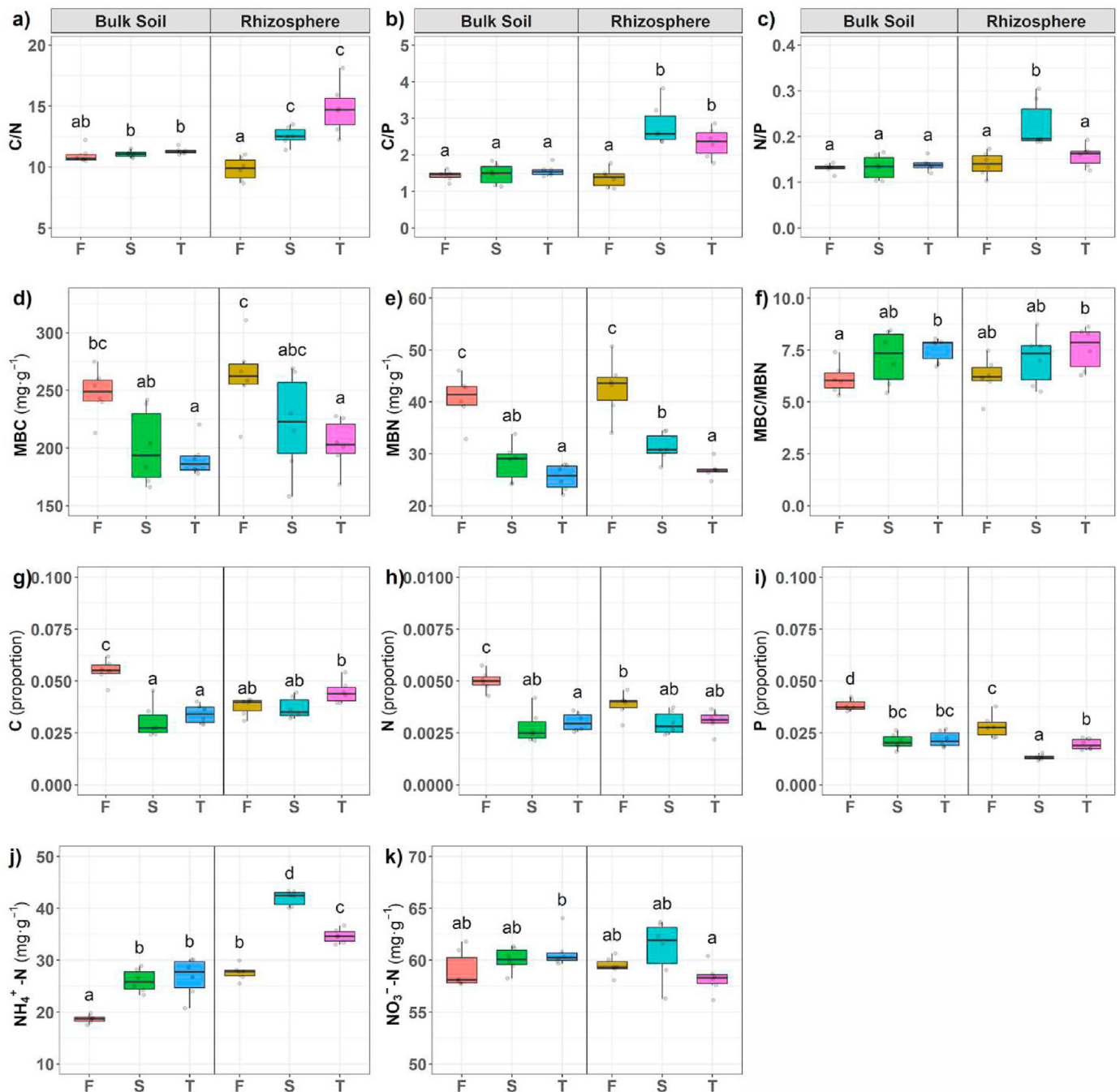


Fig. 1. Bulk soil and rhizosphere physicochemical properties in seedlings plantations and coppice plantations. Appropriate 95% confidence intervals were constructed from estimates derived from the GLMM. F, S, and T represent seedling plantations, first-generation coppice plantations, and second-generation coppice plantations, respectively. Different letters in columns show significant differences as determined by Tukey's HSD method ($p < 0.05$).

decreases of 31.98%, 40.18%, 47.44% in bulk soil, and 25.00%, 130.12%, 81.95% in the rhizosphere, respectively.

3.3. Diversity and community structure of soil diazotrophs

The Hill diversity indices of soil diazotrophs ranged from 22.35 to 39.84 and were significantly different between the seedling and coppice plantations (Fig. 3a). The diazotrophs Hill diversity indices in the seedling plantations were 29.02 and 35.34, significantly higher than those in the coppice plantations ($p < 0.05$, S (22.35 and 24.30), T (29.56 and 39.84), respectively) for bulk soil and rhizosphere.

The relative abundance of the soil diazotrophs at the genus level based on the *nifH* gene is displayed in Figs. 3b and 4. There were significant differences in soil diazotrophic communities between seedling and coppice plantations for bulk soil and rhizosphere soil (PERMANOVA, bulk soil, $p = 0.001$, Rhizosphere, $p = 0.001$). Overall, the BL soil diazotrophic communities in the seedling plantations were dominated by Proteobacteria at the phylum level (Fig. S1), unclassified-c-*Alphaproteobacteria* (22.37% and 29.28%), unclassified-o-*Rhizobiales* (15.40% and 13.31%), and *Bradyrhizobium* (12.00% and 11.74%) for rhizosphere and bulk soil at the genus level (Fig. 3b), respectively. However, in the first-generation coppice plantations (S), the dominant genus was *Rhodopseudomonas* (43.56% and 41.84%), followed by *Bradyrhizobium* (17.47% and 22.72%). In the second-generation coppice plantations (T), the dominant genus was *Bradyrhizobium* (45.95% and 47.86%), followed by unclassified-k-norank-d-*Bacteria* (16.40% and 14.92%) for rhizosphere and bulk soil at the genus level, respectively. The relative abundances of *Rhodopseudomonas* and *Bradyrhizobium* were close to half of the total in

coppice plantations (Fig. 3, Fig. S2), suggesting an increasingly homogenized composition of diazotrophic communities.

We used Principal coordinate analysis (PCoA) to evaluate the similarity of diazotrophic communities in bulk soil and rhizosphere. The PCoA plot (Fig. 4) exhibited the Bray-Curtis distance for nitrogen-fixing microorganism communities found in seedling and coppice plantations for bulk soil and rhizosphere. In bulk soil, the distribution of the nitrogen-fixing microorganism communities in the seedling plantations was clearly separated from coppice plantations; the same results appeared in rhizosphere soil. Based on Adonis tests, a significant difference was observed between the BL seedling and coppice plantations, bulk soil ($R^2 = 0.586$, $p < 0.001$), and rhizosphere ($R^2 = 0.625$, $p < 0.001$).

Based on the composition and abundance of OTUs, the dominant diazotrophic communities are clustered into four categories (Fig. 5). *Spe01* includes *Rhodopseudomonas* and *Bradyrhizobiaceae*; *Spe02* includes *Alphaproteobacteria*, *Rhizobiales*, and *Mesorhizobium*; *Spe03* includes *Bradyrhizobium*; and *Spe04* include *Beijerinckiacae*, *Rhodospirillaceae*, *Azospirillum*, *Skermanella*, unclassified-k-norank-d-*Bacteria*, and unclassified-p-*Proteobacteria*. *Spe02* and *Spe03* are significantly correlated with soil properties (Fig. 6). Notably, *Spe02* consists of only the *Bradyrhizobium* community, a special group in the nitrogen-fixing bacteria community of *Robinia pseudoacacia*.

3.4. Relationships among soil properties, soil N mineralization rate, and diazotrophic communities

The Spearman rank-order correlation was used to evaluate the relationships between soil properties and the diazotrophic communities

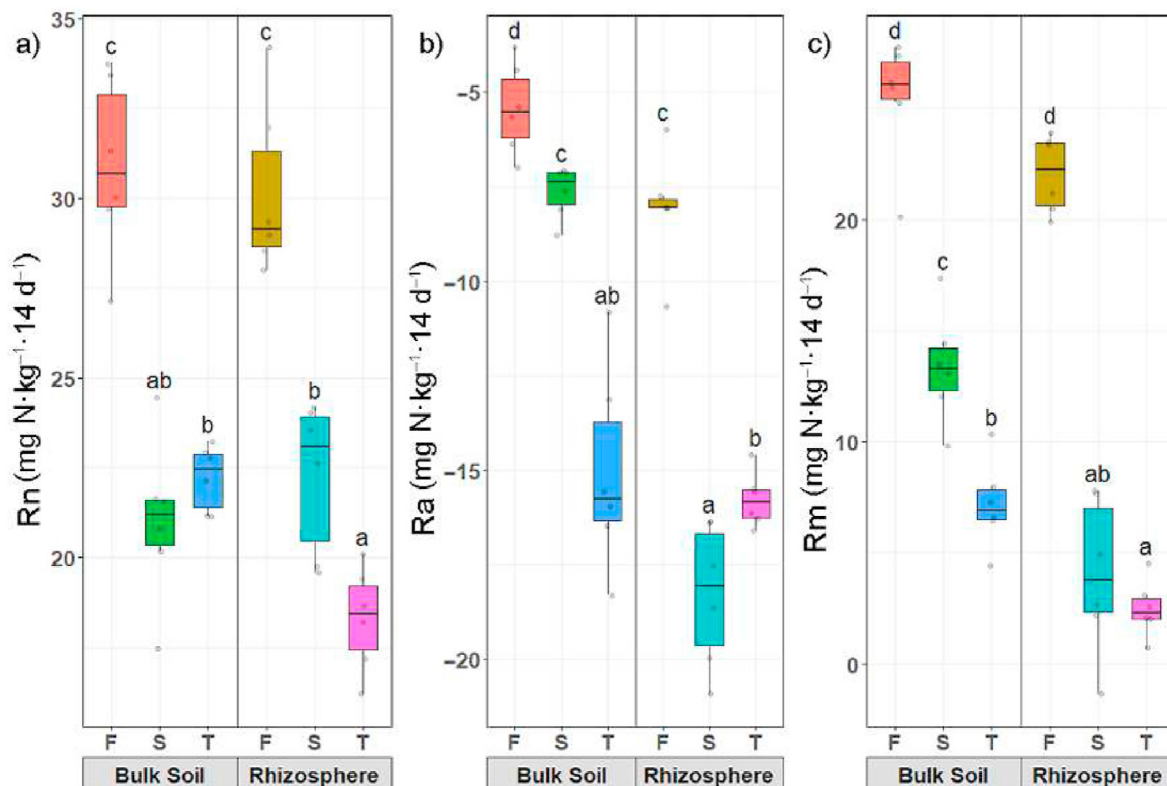


Fig. 2. Bulk soil and rhizosphere mineralization rate in seedlings plantations and coppice plantations. F, S, and T represent seedling plantations, first-generation coppice plantations, and second-generation coppice plantations, respectively. Subfigures a, b, and c represent nitrification (Rn), ammonification (Ra), and net N mineralization (Rm). All data are presented as boxplots calculated from six biological replicates. Multiple comparisons were adjusted for family-wise experiment rates using Tukey's HSD method, also with $\alpha = 0.05$. The lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles), and the centerline corresponds to the median. The upper whisker extends from the upper hinge to the largest value no further than $1.5 \times \text{IQR}$ from the hinge (where IQR is the inter-quartile range or distance between the first and third quartiles). Likewise, the lower whisker extends from the lower hinge to the smallest value no further than $1.5 \times \text{IQR}$ from the hinge. Different letters indicate significant differences among the treatments by Tukey's HSD method at ($p < 0.05$). The same is below.

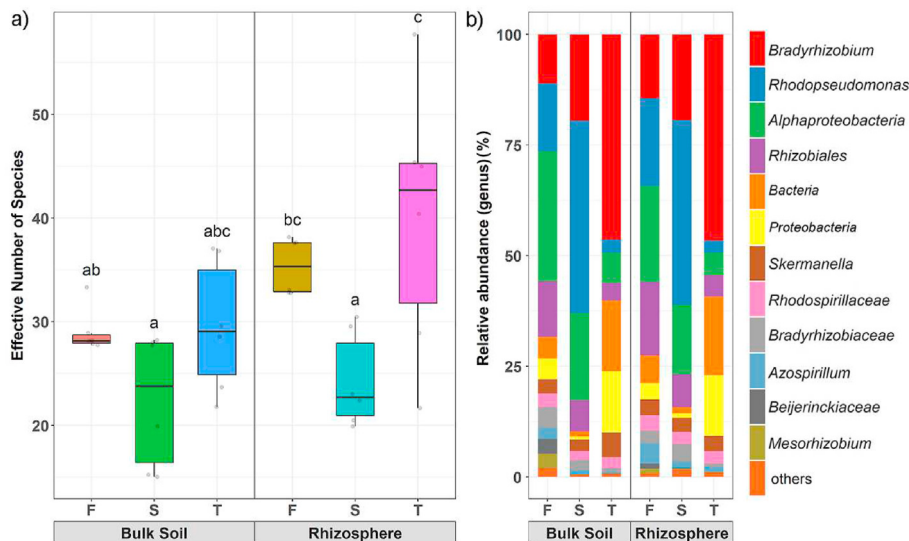


Fig. 3. Hill diversity indices (a) and Taxonomic classification of *nifH* gene reads (b) of bulk soil and rhizosphere in seedling plantations and coppice plantations at the genus level, using RDP classifier. Rhodocyclaceae: unclassified_f_Rhodocyclaceae; Alphaproteobacteria: unclassified_c_Alphaproteobacteria; Rhizobiales: unclassified_o_Rhizobiales; Bacteria: unclassified_k_norank_d_Bacteria; Proteobacteria: unclassified_p_Proteobacteria; Beijerinckiaceae: unclassified_f_Bejerinckiaceae; Rhodospirillaceae: unclassified_f_Rhodospirillaceae.

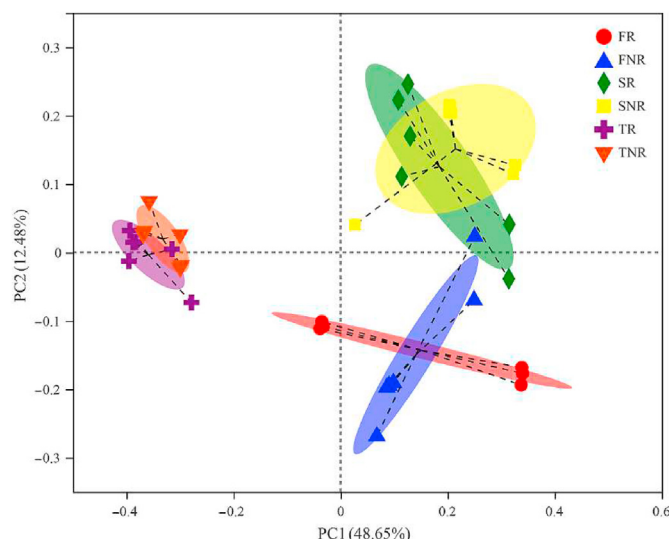


Fig. 4. Principal coordinate analysis (PCoA) of the relative abundances of diazotrophs at the genus level based on Bray-Curtis distance. Red Circle and blue triangle represent the bacterial community of rhizosphere and bulk soil in seedling plantations (FR and FNR); Green diamond and yellow square represent the bacterial community of rhizosphere and bulk soil in first generation coppice stands (SR and SNR); Purple cross and orange triangle represent the bacterial community of rhizosphere and bulk soil in second generation coppice stands (TR and TNR). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(Fig. 6). N mineralization was closely correlated with soil properties. Nitrogen had a positive correlation with Rn ($r = 0.78, p < 0.01$) and Rm ($r = 0.77, p < 0.01$), but negative correlation with Ra ($r = -0.59, p < 0.01$). Ra was positively correlated with C/N ($r = 0.48, p < 0.01$) and N/P ($r = 0.50, p < 0.01$). The soil properties exhibited a significant correlation with *Mesorhizonium*, *f_Bejerinckiaceae*, *o_Rhizobiales*, *c_Alphaproteobacteria*, and *Bradyrhizobium*. *c_Alphaproteobacteria* was significantly and positively correlated with MBN, Rn, Ra and Rm ($r = 0.713, p < 0.001$; $r = 0.636, p < 0.001$; $r = 0.70, p < 0.001$; $r = 0.77, p < 0.001$, respectively). *Bradyrhizobium* was significantly and negatively correlated with MBN, Rn, Ra and Rm ($r = -0.592, p < 0.001$; $r = -0.524, p < 0.01$; $r = -0.455, p < 0.01$; $r = -0.566, p < 0.001$, respectively).

Thirteen variables were selected to perform RDA on diazotrophic community composition. The RDA analysis for the soil attributes

explained 64.33% of the data variability in the first two axes (Fig. 7). There were obvious differences in type and quantity of factors affecting community compositions among samples. For FR and FNR samples, diazotrophic communities were closely related to C, P, N, MBN, MBC, Rn, Ra, and Rm. In terms of SR and SNR samples, four factors affected the diazotrophic structures including NO_3^- -N, N/P, NH_4^+ -N, C/P and MBC/MBN. However, only C/N factor has an influence on the communities of TR and TNR samples (Fig. 7). The Mantel test analysis showed that Rn, Ra, Rm, and MBN were the important factors in shaping the diazotrophic communities (Table 1). In addition, the results of random forest analysis also showed that Rn, Ra, Rm, and MBN were the most important environmental variables linked to changes in diazotrophic community composition (Fig. S3).

4. Discussion

4.1. Multi-generation coppice plantations affect the soil nutrients

Black locust is an ideal tree species for short-rotation biomass production with many advantages such as rapid growth, high drought tolerance, and nitrogen-fixation, as well as the ability to reproduce via stump shoots in response to harvest (Carl et al., 2019). Nevertheless, the multi-generation coppice plantations on Mount Tai negatively impacted soil quality (Li et al., 2021). Our study supports previous observations of the adverse effects of forest conversion on soil properties, soil microbial communities, and enzyme activities (Wang et al., 2017; Cai et al., 2018; Yang et al., 2018). Additionally, our results revealed that the plantation conversion from seedling to coppice stands significantly decreased the concentration of soil N, P, MBC, and MBN, while increased soil NH_4^+ -N and C/N concentrations (Fig. 1). There were no significant differences in other soil variables, indicating that the impact of forest conversion on soil nutrients is inconsistent, partly in line with the results of Li et al. (2021). These outcomes may be associated with complex nutrient pools in forest ecosystems (Yang et al., 2018) and negative ammonification (Fig. 2).

Forest conversion has a specific effect on soil C and N transformations (Yang et al., 2018). Our results showed that plantation conversion increased the rhizosphere carbon and decreased bulk soil carbon by including N-fixers that improved the soil C, but the response may be site- and species-specific (Hoogmoed et al., 2014). As a light-demanding tree species, black locust growth is limited if the light availability is weak (Carl et al., 2019). In BL coppice plantations, the density of black locust regeneration is higher. Due to the already-established root systems, BL grows faster and obtains energy from light. Subsequently, the microbial community may require higher amounts of rhizosphere C for their energy

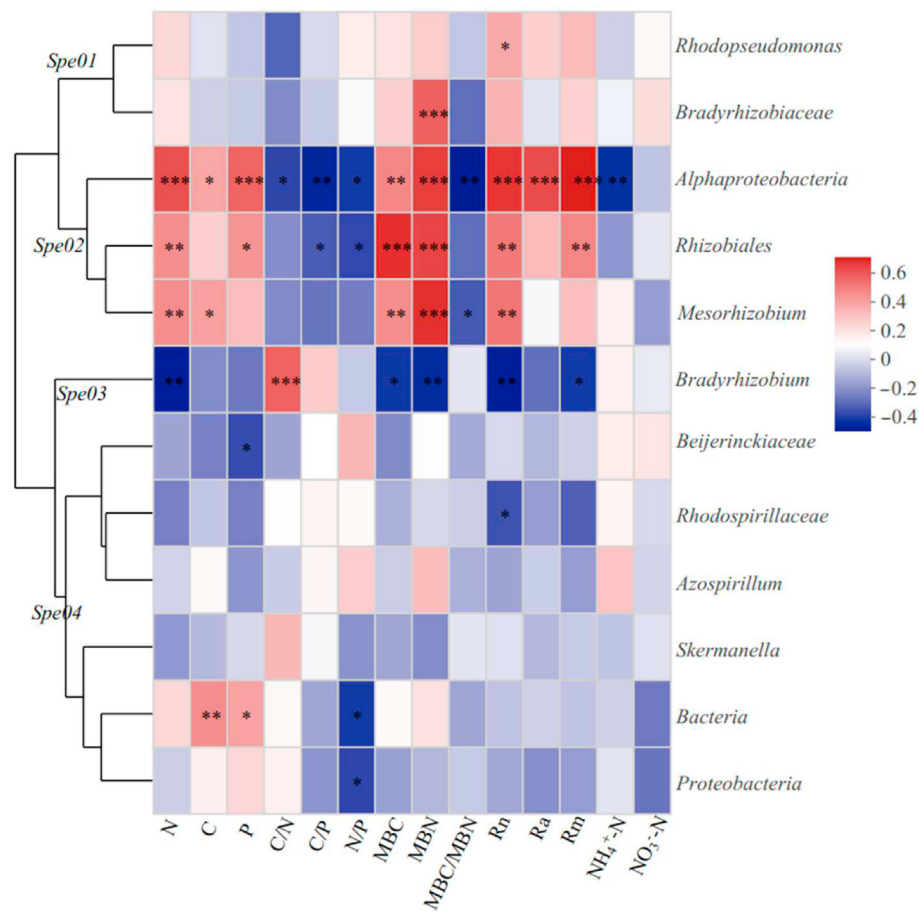


Fig. 5. Hierarchical clustering tree on OTU level using Weighted-UniFrac distance. *Spe01* include *Rhodopseudomonas*, *Bradyrhizobiaceae*; *Spe02* include *Alphaproteobacteria*, *Rhizobiales*, *Mesorhizobium*; *Spe03* include *Bradyrhizobium*; *Spe04* include *Beijerinckiaceae*, *Rhodospirillaceae*, *Azospirillum*, *Skermanella*, *Bacteria*, *Proteobacteria*. Spearman correlation coefficients between soil properties and dominant diazotrophic communities were calculated for all soil samples. Colors indicate the direction of the correlation. Blue cells correspond to significant negative correlations and red cells correspond to significant positive correlations. Only the relative abundance of diazotrophic communities >1% was shown in the plot. C: total carbon, N: total nitrogen, P: total phosphorus, C/N: C:N ratio, C/P: C:P ratio, N/P: N:P ratio, $\text{NH}_4^+\text{-N}$: soil ammonium, $\text{NO}_3^-\text{-N}$: soil nitrate, Ra: ammonification rate, Rn: nitrification rate, Rm: net N mineralization rate, MBC: soil microbial biomass carbon, MBN: soil microbial biomass nitrogen, MBC/MBN: MBC:MBN ratio. * $0.01 < p \leq 0.05$, ** $0.001 < p \leq 0.01$, *** $p \leq 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

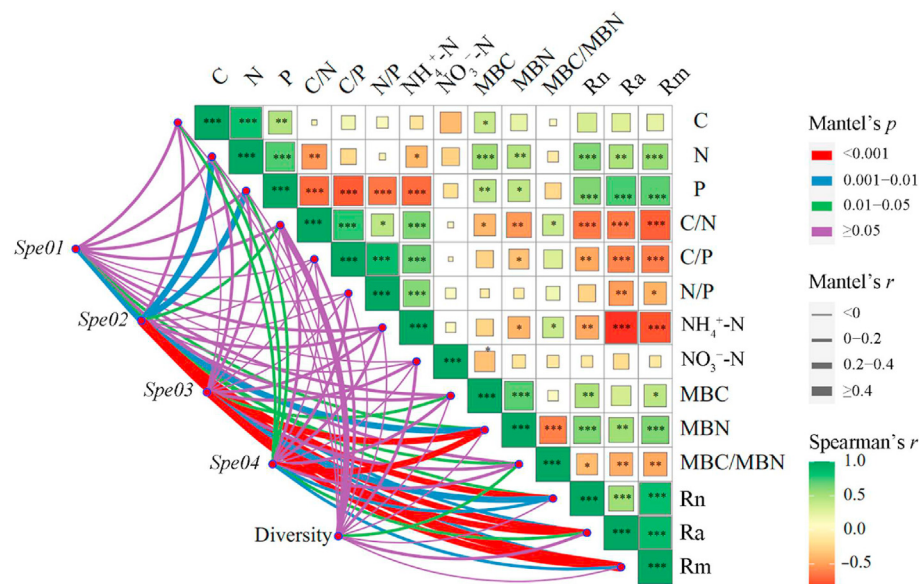


Fig. 6. Spearman correlation coefficients (r) between soil properties and soil net nitrogen mineralization rates. Mantel test of the relationships between soil environmental factors and the diazotrophic community structure were based on hierarchical clustering tree. * Significant at $p < 0.05$, ** Significant at $p < 0.01$, *** Significant at $p < 0.001$.

consumption that in turn fixes more gaseous N_2 . An earlier study found that the N content in plantations' soils decreased remarkably following the conversion from natural forests (Xu et al., 2010), and our results support this finding (Fig. 1). In BL plantations, soil nitrogen input

primarily comes from the decomposition of plant detrital input and roots and biological nitrogen fixation (Yang et al., 2011). The outputs are the harvesting and removal of the plant (a loss from the ecosystem) and nitrogen leaching to groundwater or emission to the atmosphere (Li et al.,

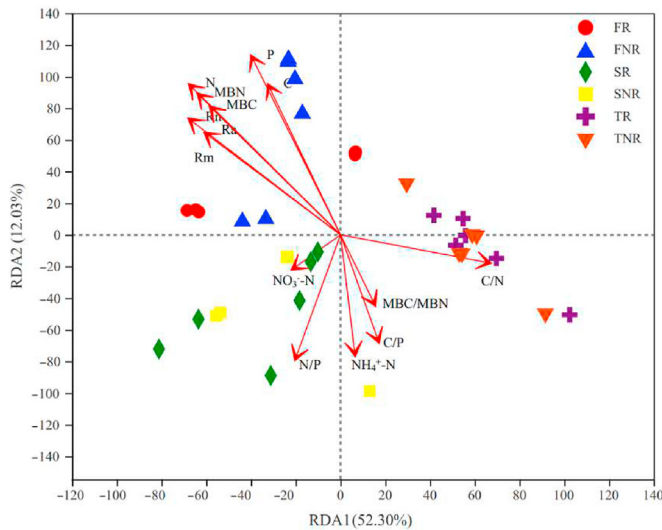


Fig. 7. Redundancy analysis (RDA) of the relationships of soil environmental variables with the diazotrophic communities' structure at the genus level. The environmental variables are indicated with arrows and include C (total carbon), N (total nitrogen), P (total phosphorus), C/N (C:N ratio), C/P (C:P ratio), N/P (N:P ratio), $\text{NH}_4^+\text{-N}$ (soil ammonium), $\text{NO}_3^-\text{-N}$ (soil nitrate), Ra (ammonification rate), Rn (nitrification rate), Rm (net N mineralization rate), MBC (soil microbial biomass carbon), MBN (soil microbial biomass nitrogen), MBC/MBN (MBC:MBN ratio). Red Circle and blue triangle represent the bacterial community of rhizosphere and bulk soil in seedling plantations (FR and FNR); Green diamond and yellow square represent the bacterial community of rhizosphere and bulk soil in first generation coppice stands (SR and SNR); Purple cross and orange triangle represent the bacterial community of rhizosphere and bulk soil in second generation coppice stands (TR and TNR). The negative data (Ra) uses a log format technique by $y' = \log(y + 1)$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1

Mantel test of the relationships of soil environmental variables with the diazotrophic community structure at the genus level.

Variables	R	p
Rm	0.359	0.001
Ra	0.307	0.001
Rn	0.281	0.001
MBN	0.274	0.001
N	0.194	0.002
P	0.188	0.003
MBC	0.193	0.004
C/N	0.100	0.062
$\text{NH}_4^+\text{-N}$	0.068	0.102
C	0.068	0.108
MBC/MBN	0.031	0.230
$\text{NO}_3^-\text{-N}$	-0.025	0.645
C/P	-0.051	0.803
N/P	-0.069	0.858

2012). BL coppice plantations have lower net mineralization and decreased nitrogen inputs, further decreasing soil nitrogen.

Phosphorus is an essential nutrient for BL trees (Carl et al., 2018), which could affect the nitrogen fixation of plants (Augusto et al., 2013). Our results suggest that the decrease in soil P was caused by plantation conversion, which was in accord with carbon and nitrogen reduction (Li et al., 2021). The same trend also appeared in MBN and MBC, which are considered indicators of soil quality and as a measure of N availability for plants due to their sensitivity to soil disturbance by land-use changes and tillage activities (Yang et al., 2010). MBC and MBN provide the basis for the survival of soil microbes (Vitali et al., 2016; Moghimian et al., 2017). Additionally, our results showed that MBN and MBC were negatively

correlated with soil C/N ratio and positively with total N, results which were in line with the findings of Moghimian et al. (2017) and Hoogmoed et al. (2014). The C/N ratio can be a critical indicator for determining biological processes. Earlier work has shown that soil C/N ratio decreased after planting black locust (Cao et al., 2017). By contrast, our results revealed soil C/N ratio increased in coppice plantations due to lower soil nitrogen content. Lower soil C/N will reduce the turnover and retention of soil nutrients (Ng et al., 2014).

4.2. Multi-generation coppice plantations reduce the soil N mineralization

Net N mineralization rate and mineral N content in seedling plantations were significantly higher in coppice plantations (Fig. 2), and Rn and Rm were positive, while Ra was negative. The results suggest that soil net nitrogen mineralization decreased in coppice plantations. Our results demonstrated that nitrification exceeded the net mineralization, consistent with earlier observations (Wei et al., 2011; Kong et al., 2019). Typically, soil microorganisms are C limited, and it has been shown that increased N can depress total microbial biomass. Generally, net soil N mineralization rate decreases with a decrease in root biomass (Wang et al., 2018a) due to the immobilization of inorganic N to satisfy the microbial N demand. Furthermore, a lower soil C/N ratio accelerates the activities of microorganisms (Usman et al., 2000; Wei et al., 2011), which would explain our experimental results. In seedlings plantations, the C/N ratio was lower than that of coppice plantations. We found that $\text{NH}_4^+\text{-N}$ content in coppice plantations was higher than that in seedling plantations, and the higher $\text{NH}_4^+\text{-N}$ level in the soil might inhibit soil N mineralization rate (Geisseler et al., 2010).

As microbial communities drive soil net nitrogen mineralization, the reductions in soil microbial biomass carbon (MBC) were positively related to N mineralization rates (Geisseler et al., 2010; Wu et al., 2021). Our results showed that the MBC was lower in coppice plantations (Table 1), which can increase the efficiency of soil N mineralization (Li et al., 2020). Still, we found lower N mineralization in coppice plantations, suggesting that the N cycle was limited. N mineralization of soil depends strongly on the substrate contents, including total N, soil organic C, water-soluble N, and dissolved organic C (Zhang et al., 2016). Our results also showed that the Rn and Rm had positive correlations with soil C, N, and P, consistent with Booth et al. (2005). Therefore, it might be that the BL coppice plantation grows faster, requiring a large amount of nutrients, and the uptake of mineral N by roots may result in decreased mineral N (Kong et al., 2019).

4.3. Multi-generation coppice plantations normalize soil diazotrophic communities

The diazotrophic microorganisms are primary drivers of soil BNF that may be significantly correlated with the communities' attributes, including abundance, composition, and activity (Reed et al., 2011; Pogoreutz et al., 2017). Our results show that diazotrophic community composition was significantly altered by the coppice plantations. In our study, the Proteobacteria phylum was the most dominant diazotrophic group (Fig. S1), generally similar to results from other studies (Wang et al., 2017; Chen et al., 2019a). However, the diazotrophic communities' structures at the genus level were significantly different in seedling and coppice plantations (Figs. 3–5 and Fig. S2). Our results suggest that forest conversion-induced variation in diazotrophic β -diversity and reduced α -diversity (Hill index) is consistent with Peerawat et al. (2018). At the genus level, the diazotrophic communities in coppice plantations commonly transition to *Rhodopseudomonas* or *Bradyrhizobium*-dominated communities (Fig. 3 and Fig. S2), but in seedling plantations, there were higher relative abundances of several diazotrophic genera, including *Rhodopseudomonas*, *Bradyrhizobium*, *o_Rhizobiales*, and *c_Alphaproteobacteria*, which could favor distinct subsets of diazotrophic communities, suggesting that there is a tendency of simplification of diazotrophs in coppice plantations. The change in beta diversity in response to forest

conversion is an indication of biotic homogenization, and the process by which the similarity of communities increases over time and/or space (Rodrigues et al., 2013). In our results, the increasing similarity in the beta diversity of coppice plantations (Fig. S4) shows the soil diazotrophic homogenization in coppice plantations. The homogeneity of this characteristic will change the function of the ecosystem and reduce the ecosystem's ability to recover from disturbances.

Generally, BNF is carried out by the *Rhizobia* in the anaerobic environment of the nodule. The *Rhizobiales* are well known for forming root nodules with legumes and conducting symbiotic nitrogen-fixation (Schmidt et al., 2017). In our results, the relative abundance of *Rhizobiales* in seedling plantations was higher than that in coppice plantations, which indicated it had more root nodules and, to some extent, it had a stronger BNF ability. Therefore, it would produce more ammonium. Because of the lower nitrification in coppice plantations, the rate of ammonium transferring to nitrate decreased, which would lead to the accumulation of ammonium (Fig. 1). It has also shown that increased available soil N inhibits colonization by *Rhizobia*. If the plant demands for N are being met by available soil N, then the need for the symbiotic relationship with *Rhizobia* is no longer advantageous to the plant.

4.4. Nitrogen mineralization and soil properties affect the diazotrophic community composition

Soil nutrient content has been recognized as a key determinant for soil microorganism survival and species compositions (Kerfahi et al., 2016; Meng et al., 2017; Wood et al., 2017). The composition and abundance of the diazotrophic communities in soil may be correlated with several environmental factors and vegetation types (Rodríguez-Blanco et al., 2015; Saleem et al., 2018; Chen et al., 2019a). In our study, the soil nutrient (N and P) was significantly correlated with the diazotrophs. Mantel test results showed that the diazotrophic community presence was explained mainly by nitrogen mineralization, MBN, MBC, N, and P (Table 1). Nitrogen mineralization was the most significant contributor to diazotrophs in measured variables. Previous studies have shown that soil N and MBC drive diazotrophic community structure dominance (Chen et al., 2019a; Zhao et al., 2021). Our results showed that the soil nitrogen had a significantly positive correlation with diazotrophic community structures, consistent with the results of Che et al. (2018). In our study, coppice plantations had higher levels of inorganic nitrogen ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$), which may reduce the activity of diazotrophs (DeLuca et al., 1996; Xu et al., 2019) and reduce *nifH* gene expression (Mirza et al., 2014), and finally lower the BNF (Chen et al., 2019b). Along with the growth of BL, more rRNA is needed to increase the protein synthesis, which leads to an increase in the N content of leaves (Xu et al., 2019). Our results showed that coppice plantations had a lower P and higher N/P ratio, further inhibiting the BNF. The reason was that phosphorus is one of the critical factors limiting nitrogen fixation due to the high adenosine triphosphate (ATP) demand of BNF (Bell et al., 2014) and P deficiency-induced more restrictive effects on diazotrophic communities (Tang et al., 2017).

Bradyrhizobium is a common slow-growing bacterium (Koponen et al., 2003), which plays an essential role in the nitrogen cycle in forest ecosystems (Meng et al., 2017). However, *Bradyrhizobium* can only fix N and increase N input after inoculating legume plants (Liu et al., 2019). We found that the relative abundance of *Bradyrhizobium* in coppice plantations was higher than that in seedling plantations (Figs. 3 and 4, and Fig. S2), which suggests that coppice could increase its abundance. The explanation may be that the low nitrogen content and higher C/N in the soil of the coppice plantations stimulated the increase in the activity of nitrogen-fixing microorganisms (Nie et al., 2021). Studies have found that *Bradyrhizobium* has a pronounced capacity for N_2 fixation (Straub et al., 2004; Sánchez et al., 2011). However, its nitrogen fixation function is not sufficient to meet the growth needs of coppice plantations. In addition, we found that the relative abundance of *Bradyrhizobium* was negatively correlated with N, MBN, MBC, Rn and Rm (Fig. 5), which was

consistent with the results of Hu et al. (2018). Therefore, it may be that N inhibits the activity of *Bradyrhizobium* (Xu et al., 2019), and soil nutrient availability was the most dominant factor in shaping diazotrophic community structure (Wang et al., 2017).

5. Conclusions

Our results suggest the conversion of black locust plantations impacts *nifH* microorganism communities, N mineralization, microbial biomass N, and soil properties. We found that the dominant species changed from *Rhodopseudomonas*, *Bradyrhizobium*, o_ *Rhizobiales*, and c_ *Alphaproteobacteria* in seedling plantations to *Bradyrhizobium* and *Rhodopseudomonas* in coppice plantations. This outcome suggests that the coppice stand can normalize diazotrophic soil communities, which in turn affects the function of diazotrophic communities.

As the forest converted to different plantation types, the tendency of dominant species varied. Rm, Rn, and MBN were the most sensitive factors to diazotrophic microorganisms. Converting seedling plantation to a coppice plantation decreased the soil MBN, MBC, Rn, Rm, N, and P, but significantly increased the concentration of $\text{NH}_4^+\text{-N}$, demonstrating that plantation conversion had a positive impact on inorganic N, while the effects on soil mineralization and *nifH* were negative. $\text{NH}_4^+\text{-N}$ produced by biological nitrogen fixation is more synthesized into organic nitrogen. Therefore, to increase the nitrogen fixation and nitrogen use efficiency of black locust, reducing the proportion of the multi-generation coppice plantations in this region is advisable.

Authors' contributions

KL and HMT conceived and designed the experiment; RQN and KL analyzed the data; HMT and WXS obtained the data in the field; KL and CRL wrote the first draft of the paper and WKM, STO and LSB contributed to the writing and data analysis. All the authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed 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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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fecs.2022.100025>.

References

- Augusto, L., Delerue, F., Gallet-Budynek, A., Achat, D.L., 2013. Global assessment of limitation to symbiotic nitrogen fixation by phosphorus availability in terrestrial ecosystems using a meta-analysis approach. *Global Biogeochem. Cycles* 27, 804–815. <https://doi.org/10.1002/gbc.20069>.
- Bell, C., Carrillo, Y., Boot, C.M., Rocca, J.D., Pendall, E., Wallenstein, M.D., 2014. Rhizosphere stoichiometry: are C:N:P ratios of plants, soils, and enzymes conserved at the plant species level? *New Phytol.* 201, 505–517. <https://doi.org/10.1111/nph.12531>.
- Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Maechler, M., Bolker, B.M., 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *Rev. Javer.* 9 (2), 378–400. <https://doi.org/10.32614/rj-2017-066>.
- Booth, M.S., Stark, J.M., Rastetter, E., 2005. Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. *Ecol. Monogr.* 75, 139–157. <https://doi.org/10.1890/04-0988>.
- Cai, X., Lin, Z., Penttinen, P., Li, Y., Li, Y., Luo, Y., Yue, T., Jiang, P., Fu, W., 2018. Effects of conversion from a natural evergreen broadleaf forest to a Moso bamboo plantation on the soil nutrient pools, microbial biomass and enzyme activities in a subtropical area. *For. Ecol. Manag.* 422, 161–171. <https://doi.org/10.1016/j.foreco.2018.04.022>.
- Calderoli, P.A., Collavino, M.M., Behrends Kraemer, F., Morras, H.J.M., Aguilar, O.M., 2017. Analysis of *nifH*-RNA reveals phylotypes related to *Geobacter* and *Cyanobacteria* as important functional components of the N₂-fixing community depending on depth and agricultural use of soil. *MicrobiologyOpen* 6. <https://doi.org/10.1002/mbo3.502>.
- Cao, Y., Zhang, P., Chen, Y., 2017. Soil C:N:P stoichiometry in plantations of N-fixing black locust and indigenous pine, and secondary oak forests in Northwest China. *J. Soils Sediments* 18, 1478–1489. <https://doi.org/10.1007/s11368-017-1884-0>.
- Carl, C., Biber, P., Veste, M., Landgraf, D., Pretzsch, H., 2018. Key drivers of competition and growth partitioning among *Robinia pseudoacacia* L. trees. *Forest Ecol. Manag.* 430, 86–93. <https://doi.org/10.1016/j.foreco.2018.08.002>.
- Carl, C., Lehmann, J., Landgraf, D., Pretzsch, H., 2019. *Robinia pseudoacacia* L. in short Rotation Coppice: seed and stump shoot reproduction as well as UAS-based spreading analysis. *Forests* 10 (3), 235. <https://doi.org/10.3390/f10030235>.
- Chao, A., Gotelli, N.J., Hsieh, T.C., Sander, E.L., Ma, K.H., Colwell, R.K., Ellison, A.M., 2014. Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. *Ecol. Monogr.* 84, 45–67. <https://doi.org/10.1890/13-0133.1>.
- Chapin III, F.S., Matson, P.A., Vitousek, P., 2011. *Principles of Terrestrial Ecosystem Ecology*. Springer Science & Business Media, New York.
- Che, R.X., Deng, Y.C., Wang, F., Wang, W.J., Xu, Z.H., Hao, Y.B., Xue, K., Zhang, B., Tang, L., Zhou, H.K., Cui, X.Y., 2018. Autotrophic and symbiotic diazotrophs dominate nitrogen-fixing communities in Tibetan grassland soils. *Sci. Total Environ.* 639, 997–1006. <https://doi.org/10.1016/j.scitotenv.2018.05.238>.
- Chen, J., Shen, W.J., Xu, H., Li, Y.D., Luo, T.S., 2019a. The composition of nitrogen-fixing microorganisms correlates with soil nitrogen content during reforestation: a comparison between legume and non-legume plantations. *Front. Microbiol.* 10, 508. <https://doi.org/10.3389/fmicb.2019.00508>.
- Chen, J., Wang, P., Wang, C., Wang, X., Miao, L., Liu, S., Yuan, Q., 2019b. Dam construction alters function and community composition of diazotrophs in riparian soils across an environmental gradient. *Soil Biol. Biochem.* 132, 14–23. <https://doi.org/10.1016/j.soilbio.2019.01.020>.
- Cierjacks, A., Kowarik, I., Joshi, J., Hempel, S., Ristow, M., von der Lippe, M., Weber, E., 2013. Biological flora of the British isles: *Robinia pseudoacacia*. *J. Ecol.* 101, 1623–1640. <https://doi.org/10.1111/1365-2745.12162>.
- DeLuca, T.H., Drinkwater, L.E., Wiefeling, B.A., DeNicola, D.M., 1996. Free-living nitrogen-fixing bacteria in temperate cropping systems: influence of nitrogen source. *Biol. Fertil. Soils* 23, 140–144. <https://doi.org/10.1007/BF00336054>.
- Fan, K.K., Weisenhorn, P., Gilbert, J.A., Shi, Y., Bai, Y., Chu, H.Y., 2018. Soil pH correlates with the co-occurrence and assemblage process of diazotrophic communities in rhizosphere and bulk soils of wheat fields. *Soil Biol. Biochem.* 121, 185–192. <https://doi.org/10.1016/j.soilbio.2018.03.017>.
- Fu, W., Wang, X., Wei, X.R., 2019. No response of soil N mineralization to experimental warming in a northern middle-high latitude agro-ecosystem. *Sci. Total Environ.* 659, 240–248. <https://doi.org/10.1016/j.scitotenv.2018.12.315>.
- Gaby, J.C., Buckley, D.H., 2011. A global census of nitrogenase diversity. *Environ. Microbiol.* 13 (7), 1790–1799. <https://doi.org/10.1111/j.1462-2920.2011.02488.x>.
- Gaby, J.C., Buckley, D.H., 2014. A Comprehensive Aligned *nifH* Gene Database: a Multipurpose Tool for Studies of Nitrogen-Fixing Bacteria. *Database*. <https://doi.org/10.1093/database/bau001>, 2014.
- Geisseler, D., Horwath, W.R., Joergensen, R.G., Ludwig, B., 2010. Pathways of nitrogen utilization by soil microorganisms – a review. *Soil Biol. Biochem.* 42, 2058–2067. <https://doi.org/10.1016/j.soilbio.2010.08.021>.
- Guillaume, T., Kotowska, M.M., Hertel, D., Knoch, A., Krashevskaya, V., Murtlaksono, K., Scheu, S., Zuyakov, Y., 2018. Carbon costs and benefits of Indonesian rainforest conversion to plantations. *Nat. Commun.* 9, 2388. <https://doi.org/10.1038/s41467-018-04755-y>.
- Hart, S.C., Stark, J.M., Davidson, E.A., Firestone, M.K., 1994. Nitrogen mineralization, immobilization, and nitrification. *Methods of Soil Analysis: Part 2 Microbiological and Biochemical Properties* 5, 985–1018. <https://doi.org/10.2136/sssabookser5.2.c42>.
- Hill, M., 1973. Diversity and evenness: a unifying notation and its consequences. *Ecology* 54, 427–432. <https://doi.org/10.2307/1934352>.
- Hoogmoed, M., Cunningham, S.C., Baker, P.J., Beringer, J., Cavagnaro, T.R., 2014. Is there more soil carbon under nitrogen-fixing trees than under non-nitrogen-fixing trees in mixed-species restoration plantings? *Agric. Ecosyst. Environ.* 188, 80–84. <https://doi.org/10.1016/j.agee.2014.02.013>.
- Hsu, S.F., Buckley, D.H., 2009. Evidence for the functional significance of diazotroph community structure in soil. *ISME J.* 3, 124–136. <https://doi.org/10.1038/ismej.2008.82>.
- Hu, X.J., Liu, J.J., Zhu, P., Wei, D., Jin, J., Liu, X.B., Wang, G.H., 2018. Long-term manure addition reduces diversity and changes the community structure of diazotrophs in a neutral black soil of northeast China. *J. Soils Sediments* 18, 2053–2062. <https://doi.org/10.1007/s11368-018-1975-6>.
- Kerfahi, D., Tripathi, B.M., Dong, K., Go, R., Adams, J.M., 2016. Rainforest conversion to rubber plantation may not result in lower soil diversity of bacteria, fungi, and nematodes. *Microb. Ecol.* 72, 359–371. <https://doi.org/10.1007/s00248-016-0790-0>.
- Kong, W.B., Yao, Y.F., Zhao, Z.N., Qin, X., Zhu, H.S., Wei, X.R., Shao, M.A., Wang, Z., Bao, K.Q., Su, M., 2019. Effects of vegetation and slope aspect on soil nitrogen mineralization during the growing season in sloping lands of the Loess Plateau. *Catena* 172, 753–763. <https://doi.org/10.1016/j.catena.2018.09.037>.
- Koponen, P., Nygren, P., Domenach, A.M., Le Roux, C., Saur, E., Roggy, J.C., 2003. Nodulation and dinitrogen fixation of legume trees in a tropical freshwater swamp forest in French Guiana. *J. Trop. Ecol.* 19, 655–666. <https://doi.org/10.1017/S0266467403006059>.
- Kramer, C.Y., 1956. Extension of multiple range tests to group means with unequal numbers of replications. *Int. Biomet. Soc.* 12, 307–310. <https://doi.org/10.2307/3001469>.
- LeBauer, D.S., Treseder, K.K., 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* 89, 371–379. <https://doi.org/10.1890/06-2057.1>.
- Lenth, R.V., 2021. Emmeans: Estimated Marginal Means, Aka Least-Squares Means. R Package Version 1.6.0. <https://CRAN.R-project.org/package=emmeans>.
- Li, D.J., Niu, S.L., Luo, Y.Q., 2012. Global patterns of the dynamics of soil carbon and nitrogen stocks following afforestation: a meta-analysis. *New Phytol.* 195, 172–181. <https://doi.org/10.1111/j.1469-8137.2012.04150.x>.
- Li, K., Han, X., Ni, R.Q., Shi, G., de-Miguel, S., Li, C.R., Shen, W.X., Zhang, Y.K., Zhang, X.Z., 2021. Impact of *Robinia pseudoacacia* stand conversion on soil properties and bacterial community composition in Mount Tai, China. *Forest Ecosyst.* 8, 19. <https://doi.org/10.1186/s40663-021-00296-x>.
- Li, Z.L., Tian, D.S., Wang, B.X., Wang, J.S., Wang, S., Chen, H.Y.H., Xu, X.F., Wang, C.H., He, N.P., Niu, S.L., 2019. Microbes drive global soil nitrogen mineralization and availability. *Global Change Biol.* 25, 1078–1088. <https://doi.org/10.1111/gcb.14557>.
- Li, Z.L., Zeng, Z.Q., Tian, D.S., Wang, J.S., Fu, Z., Wang, B.X., Tang, Z., Chen, W.N., Chen, H.Y.H., Wang, C.H., Yi, C.X., Niu, S.L., 2020. The stoichiometry of soil microbial biomass determines metabolic quotient of nitrogen mineralization. *Environ. Res. Lett.* 15 (3), 034005. <https://doi.org/10.1088/1748-9326/ab6a26>.
- Liu, X.Y., Liu, C., Gao, W.H., Xue, C., Guo, Z.H., Jiang, L., Li, F., Liu, Y., 2019. Impact of biochar amendment on the abundance and structure of diazotrophic community in an alkaline soil. *Sci. Total Environ.* 688, 944–951. <https://doi.org/10.1016/j.scitotenv.2019.06.293>.
- Margeson, R., Schinner, F., 2005. *Manual for Soil Analysis-Monitoring and Assessing Soil Bioremediation*. Springer Science & Business Media, New York.
- Medina-Villar, S., Rodríguez-Echeverría, S., Lorenzo, P., Alonso, A., Pérez-Corona, E., Castro-Díez, P., 2016. Impacts of the alien trees *Ailanthus altissima* (Mill.) Swingle and

- Robinia pseudoacacia* L. on soil nutrients and microbial communities. *Soil Biol. Biochem.* 96, 65–73. <https://doi.org/10.1016/j.soilbio.2016.01.015>.
- Meng, H., Wu, R.N., Wang, Y.F., Gu, J.D., 2017. A comparison of denitrifying bacterial community structures and abundance in acidic soils between natural forest and re-vegetated forest of Nanling Nature Reserve in southern China. *J. Environ. Manag.* 198, 41–49. <https://doi.org/10.1016/j.jenvman.2017.04.066>.
- Meng, H., Zhou, Z.C., Wu, R.N., Wang, Y.F., Gu, J.D., 2019. Diazotrophic microbial community and abundance in acidic subtropical natural and re-vegetated forest soils revealed by high-throughput sequencing of *nifH* gene. *Appl. Microbiol. Biotechnol.* 103, 995–1005. <https://doi.org/10.1007/s00253-018-9466-7>.
- Meng, Y., Cao, B.H., Mao, P.L., Dong, C., Cao, X.D., Qi, L., Wang, M.X., Wu, Y., 2020. Tree species distribution change study in Mount Tai based on Landsat remote sensing image data. *Forests* 11 (2), 130. <https://doi.org/10.3390/f11020130>.
- Mirza, B.S., Potisap, C., Nusslein, K., Bohannan, B.J., Rodrigues, J.L., 2014. Response of free-living nitrogen-fixing microorganisms to land-use change in the Amazon rainforest. *Appl. Environ. Microbiol.* 80, 281–288. <https://doi.org/10.1128/AEM.02362-13>.
- Moghimi, N., Hosseini, S.M., Kooch, Y., Darki, B.Z., 2017. Impacts of changes in land use/cover on soil microbial and enzyme activities. *Catena* 157, 407–414. <https://doi.org/10.1016/j.catena.2017.06.003>.
- Murphy, C.J., Baggs, E.M., Morley, N., Wall, D.P., Paterson, E., 2017. Nitrogen availability alters rhizosphere processes mediating soil organic matter mineralisation. *Plant Soil* 417, 499–510. <https://doi.org/10.1007/s11040-017-3275-0>.
- Ng, E.L., Patti, A.F., Rose, M.T., Scheffe, C.R., Wilkinson, K., Cavagnaro, T.R., 2014. Functional stoichiometry of soil microbial communities after amendment with stabilised organic matter. *Soil Biol. Biochem.* 76, 170–178. <https://doi.org/10.1016/j.soilbio.2014.05.016>.
- Nie, H.J., Qin, T.L., Yan, D.H., Lv, X.Z., Wang, J.W., Huang, Y.H., Lv, Z.Y., Liu, S.S., Liu, F., 2021. How do tree species characteristics affect the bacterial community structure of subtropical natural mixed forests? *Sci. Total Environ.* 764, 144633. <https://doi.org/10.1016/j.scitotenv.2020.144633>.
- Ouyang, Y., Evans, S.E., Friesen, M.L., Tiemann, L.K., 2018. Effect of nitrogen fertilization on the abundance of nitrogen cycling genes in agricultural soils: a meta-analysis of field studies. *Soil Biol. Biochem.* 127, 71–78. <https://doi.org/10.1016/j.soilbio.2018.08.024>.
- Peerawat, M., Blaud, A., Trap, J., Chevallier, T., Alonso, P., Gay, F., Thaler, P., Spor, A., Sebag, D., Choosai, C., Suvannang, N., Sajjaphan, K., Brauman, A., 2018. Rubber plantation ageing controls soil biodiversity after land conversion from cassava. *Agric. Ecosyst. Environ.* 257, 92–102. <https://doi.org/10.1016/j.agee.2018.01.034>.
- Petersen, D.G., Blazewicz, S.J., Firestone, M., Herman, D.J., Turetsky, M., Waldrop, M., 2012. Abundance of microbial genes associated with nitrogen cycling as indices of biogeochemical process rates across a vegetation gradient in Alaska. *Environ. Microbiol.* 14, 993–1008. <https://doi.org/10.1111/j.1462-2920.2011.02679.x>.
- Pogoreutz, C., Radecker, N., Cardenas, A., Gardes, A., Wild, C., Voolstra, C.R., 2017. Nitrogen Fixation Aligns with *nifH* abundance and expression in two coral trophic functional groups. *Front. Microbiol.* 8, 1187. <https://doi.org/10.3389/fmicb.2017.01187>.
- R Core Team, 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>. (Accessed 10 April 2021).
- Rachid, C.T.C.C., Balieiro, F.C., Peixoto, R.S., Pinheiro, Y.A.S., Piccolo, M.C., Chaer, G.M., Rosado, A.S., 2013. Mixed plantations can promote microbial integration and soil nitrate increases with changes in the N cycling genes. *Soil Biol. Biochem.* 66, 146–153. <https://doi.org/10.1016/j.soilbio.2013.07.005>.
- Rahman, M.M., Bárcena, T.G., Vesterdal, L., 2017. Tree species and time since afforestation drive soil C and N mineralization on former cropland. *Geoderma* 305, 153–161. <https://doi.org/10.1016/j.geoderma.2017.06.002>.
- Reed, S.C., Cleveland, C.C., Townsend, A.R., 2011. Functional ecology of free-living nitrogen fixation: a contemporary perspective. *Annu. Rev. Ecol. Syst.* 42, 489–512. <https://doi.org/10.1146/annurev-ecolsys-102710-145034>.
- Rigby, H., Clarke, B.O., Pritchard, D.L., Meehan, B., Beshah, F., Smith, S.R., Porter, N.A., 2016. A critical review of nitrogen mineralization in biosolids-amended soil, the associated fertilizer value for crop production and potential for emissions to the environment. *Sci. Total Environ.* 541, 1310–1338. <https://doi.org/10.1016/j.scitotenv.2015.08.089>.
- Rodrigues, J.L.M., Pellizari, V.H., Mueller, R., Baek, K., Jesus, E.d.C., Paula, F.S., Mirza, B., Hamaoui, G.S., Tsai, S.M., Feigl, B., Tiedje, J.M., Bohannan, B.J.M., Nusslein, K., 2013. Conversion of the Amazon rainforest to agriculture results in biotic homogenization of soil bacterial communities. *Proc. Natl. Acad. Sci. U. S. A.* 110 (3), 988–993. <https://doi.org/10.1073/pnas.1220608110>.
- Rodríguez-Blanco, A., Sicardi, M., Frioni, L., 2015. Plant genotype and nitrogen fertilization effects on abundance and diversity of diazotrophic bacteria associated with maize (*Zea mays* L.). *Biol. Fertil. Soils* 51, 391–402. <https://doi.org/10.1007/s00374-014-0986-8>.
- Saleem, M., Law, A.D., Sahib, M.R., Pervaiz, Z.H., Zhang, Q.M., 2018. Impact of root system architecture on rhizosphere and root microbiome. *Rhizosphere* 6, 47–51. <https://doi.org/10.1016/j.rhisph.2018.02.003>.
- Sánchez, C., Tortosa, G., Granados, A., Delgado, A., Bedmar, E.J., Delgado, M.J., 2011. Involvement of *Bradyrhizobium japonicum* denitrification in symbiotic nitrogen fixation by soybean plants subjected to flooding. *Soil Biol. Biochem.* 43, 212–217. <https://doi.org/10.1016/j.soilbio.2010.09.020>.
- Schmidt, J., Fester, T., Schulz, E., Michalzik, B., Buscot, F., Gutknecht, J., 2017. Effects of plant-symbiotic relationships on the living soil microbial community and microbial necromass in a long-term agro-ecosystem. *Sci. Total Environ.* 581–582, 756–765. <https://doi.org/10.1016/j.scitotenv.2017.01.005>.
- Stewart, K.J., Coxson, D., Siciliano, S.D., 2011. Small-scale spatial patterns in N₂-fixation and nutrient availability in an arctic hummock-hollow ecosystem. *Soil Biol. Biochem.* 43, 133–140. <https://doi.org/10.1016/j.soilbio.2010.09.023>.
- Straub, K.L., Schönhuber, W.A., Buchholz-Cleven, B.E.E., Schink, B., 2004. Diversity of ferrous iron-oxidizing, nitrate-reducing bacteria and their involvement in oxygen-independent iron cycling. *Geomicrobiol. J.* 21, 371–378. <https://doi.org/10.1080/01490450490485854>.
- Tang, Y.F., Zhang, M.M., Chen, A.L., Zhang, W.Z., Wei, W.X., Sheng, R., 2017. Impact of fertilization regimes on diazotroph community compositions and N₂-fixation activity in paddy soil. *Agric. Ecosyst. Environ.* 247, 1–8. <https://doi.org/10.1016/j.agee.2017.06.009>.
- Thapa, R., Tully, K.L., Cabrera, M.L., Dann, C., Schomberg, H.H., Timlin, D., Reberg-Horton, C., Gaskin, J., Davis, B.W., Mirsky, S.B., 2021. Effects of moisture and temperature on C and N mineralization from surface-applied cover crop residues. *Biol. Fertil. Soils* 57, 485–498. <https://doi.org/10.1007/s00374-021-01543-7>.
- Tian, D., Jiang, L., Ma, S.H., Fang, W.J., Schmid, B., Xu, L.C., Zhu, J.X., Li, P., Losapio, G., Jing, X., Zheng, C.Y., Shen, H.H., Xu, X.N., Zhu, B., Fang, J.Y., 2017. Effects of nitrogen deposition on soil microbial communities in temperate and subtropical forests in China. *Sci. Total Environ.* 607–608, 1367–1375. <https://doi.org/10.1016/j.scitotenv.2017.06.057>.
- Urakawa, R., Ohte, N., Shibata, H., Isobe, K., Tateno, R., Oda, T., Hishi, T., Fukushima, K., Inagaki, Y., Hirai, K., Oyanagi, N., Nakata, M., Toda, H., Kenta, T., Kuroiwa, M., Watanabe, T., Fukuzawa, K., Tokuchi, N., Ugawa, S., Enoki, T., Nakanishi, A., Saigusa, N., Yamao, Y., Kotani, A., 2016. Factors contributing to soil nitrogen mineralization and nitrification rates of forest soils in the Japanese archipelago. *For. Ecol. Manag.* 361, 382–396. <https://doi.org/10.1016/j.foreco.2015.11.033>.
- Usman, S., Singh, S.P., Rawat, Y.S., 2000. Nitrification mineralisation and inorganic-N uptake in evergreen forests of the central Himalayas. *Eur. J. Soil Biol.* 36 (2), 65–71. [https://doi.org/10.1016/S1164-5563\(00\)01053-0](https://doi.org/10.1016/S1164-5563(00)01053-0).
- Vitali, F., Mastromei, G., Senatore, G., Caroppo, C., Casalone, E., 2016. Long-lasting effects of the conversion from natural forest to poplar plantation on soil microbial communities. *Microbiol. Res.* 182, 89–98. <https://doi.org/10.1016/j.micres.2015.10.002>.
- Wang, C., Liu, D.W., Bai, E., 2018a. Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen addition. *Soil Biol. Biochem.* 120, 126–133. <https://doi.org/10.1016/j.soilbio.2018.02.003>.
- Wang, C., Zheng, M.M., Song, W.F., Wen, S.L., Wang, B.R., Zhu, C.Q., Shen, R.F., 2017. Impact of 25 years of inorganic fertilization on diazotrophic abundance and community structure in an acidic soil in southern China. *Soil Biol. Biochem.* 113, 240–249. <https://doi.org/10.1016/j.soilbio.2017.06.019>.
- Wang, C., Zheng, M.M., Chen, J., Shen, R.F., 2021. Land-use change has a greater effect on soil diazotrophic community structure than the plant rhizosphere in acidic ferralsols in southern China. *Plant Soil* 462, 445–458. <https://doi.org/10.1007/s11040-021-04883-3>.
- Wang, Q., Wang, J.L., Li, Y.Z., Chen, D.W., Ao, J.H., Zhou, W.L., Shen, D.C., Li, Q.W., Huang, Z.R., Jiang, Y., 2018b. Influence of nitrogen and phosphorus additions on N₂-fixation activity, abundance, and composition of diazotrophic communities in a Chinese fir plantation. *Sci. Total Environ.* 619–620, 1530–1537. <https://doi.org/10.1016/j.scitotenv.2017.10.064>.
- Wei, X.R., Shao, M.A., Fu, X.L., Ågren, G.I., Yin, X.Q., 2011. The effects of land use on soil N mineralization during the growing season on the northern Loess Plateau of China. *Geoderma* 160, 590–598. <https://doi.org/10.1016/j.geoderma.2010.11.007>.
- Westfall, P.H., Young, S.S., 1993. Resampling-based Multiple Testing: Examples and Methods for P-Value Adjustment. John Wiley & Sons, New York.
- Wood, S.A., Gilbert, J.A., Leff, J.W., Fierer, N., D'Angelo, H., Bateman, C., Gedallovich, S.M., Gillikin, C.M., Gradoville, M.R., Mansor, P., Massmann, A., Yang, N., Turner, B.L., Brearley, F.Q., McGuire, K.L., 2017. Consequences of tropical forest conversion to oil palm on soil bacterial community and network structure. *Soil Biol. Biochem.* 112, 258–268. <https://doi.org/10.1016/j.soilbio.2017.05.019>.
- Wu, H., Cai, A., Xing, T., Huai, S., Zhu, P., Xu, M., Lu, C., 2021. Fertilization enhances mineralization of soil carbon and nitrogen pools by regulating the bacterial community and biomass. *J. Soils Sediments* 21, 1633–1643. <https://doi.org/10.1007/s11368-020-02865-z>.
- Xu, Y.D., Wang, T., Li, H., Ren, C.J., Chen, J.W., Yang, G.H., Han, X.H., Feng, Y.Z., Ren, G.X., Wang, X.J., 2019. Variations of soil nitrogen-fixing microorganism communities and nitrogen fractions in a *Robinia pseudoacacia* chronosequence on the Loess Plateau of China. *Catena* 174, 316–323. <https://doi.org/10.1016/j.catena.2018.11.009>.
- Xu, Z.F., Hu, R., Xiong, P., Wan, C., Cao, G., Liu, Q., 2010. Initial soil responses to experimental warming in two contrasting forest ecosystems, Eastern Tibetan Plateau, China: nutrient availabilities, microbial properties and enzyme activities. *Appl. Soil Ecol.* 46, 291–299. <https://doi.org/10.1016/j.apsoil.2010.07.005>.
- Yang, K., Zhu, J.J., Xu, S., Zheng, X., 2018. Conversion from temperate secondary forests into plantations (*Larix* spp.): impact on belowground carbon and nutrient pools in northeastern China. *Land Degrad. Dev.* 29, 4129–4139. <https://doi.org/10.1002/ldr.3169>.
- Yang, K., Zhu, J.J., Zhang, M., Yan, Q., Sun, O.J., 2010. Soil microbial biomass carbon and nitrogen in forest ecosystems of Northeast China: a comparison between natural secondary forest and larch plantation. *J. Plant Ecol.* 3, 175–182. <https://doi.org/10.1093/jpe/rtq022>.
- Yang, Y.H., Luo, Y.Q., Finzi, A.C., 2011. Carbon and nitrogen dynamics during forest stand development: a global synthesis. *New Phytol.* 190, 977–989. <https://doi.org/10.1111/j.1469-8137.2011.03645.x>.
- Yao, Y.F., Zhao, Z.N., Wei, X.R., Shao, M.A., 2019. Effects of shrub species on soil nitrogen mineralization in the desert-loess transition zone. *Catena* 173, 330–338. <https://doi.org/10.1016/j.catena.2018.10.016>.

- Zhang, M.Y., Wang, W.J., Wang, D.J., Heenan, M., Xu, Z.H., 2018. Short-term responses of soil nitrogen mineralization, nitrification, and denitrification to prescribed burning in a suburban forest ecosystem of subtropical Australia. *Sci. Total Environ.* 642, 879–886. <https://doi.org/10.1016/j.scitotenv.2018.06.144>.
- Zhang, Y.H., Xu, X.J., Li, Y., Huang, L.D., Xie, X.J., Dong, J.M., Yang, S.Q., 2016. Effects of *Spartina alterniflora* invasion and exogenous nitrogen on soil nitrogen mineralization in the coastal salt marshes. *Ecol. Eng.* 87, 281–287. <https://doi.org/10.1016/j.ecoleng.2015.12.003>.
- Zhao, J.W., Tao, Q., Li, B., Luo, J.P., Zhang, H.Y., Lu, C.L., Li, Q.Q., Xu, Q., Huang, R., Li, H.X., Li, B., Chen, Y.L., Wang, C.Q., 2021. Low-pyrolysis-temperature biochar promoted free-living N₂-fixation in calcareous purple soil by affecting diazotrophic composition. *Geoderma* 388, 114969. <https://doi.org/10.1016/j.geoderma.2021.114969>.
- Zhou, L.T., Li, J.J., Pokhrel, G.R., Chen, J., Zhao, Y.L., Bai, Y., Zhang, C., Lin, W.X., Wu, Z.Y., Wu, C.Z., 2021. *nifH* Gene sequencing reveals the effects of successive monoculture on the soil diazotrophic microbial community in *Casuarina equisetifolia* plantations. *Front. Plant Sci.* 11, 578812. <https://doi.org/10.3389/fpls.2020.578812>.