Effects of Stand Density on Soil Nutrients and Microbial Community in Robinia pseudoacacia Plantations (postprint)

Authors: Zhang Jianing, Zhang Jianjun, Lai Zongrui, Zhao Jiongchang, Hu Yawei, Li Yang, 卫朝阳

Date: 2025-02-27T00:00:00+00:00

Abstract

Soil nutrient levels and microbial community structural characteristics are important indicators for evaluating the ecosystem service functions of plantation forests. To investigate the effects of stand density on soil nutrients and microbial communities in plantations in arid and semi-arid regions, 30-year-old black locust (Robinia pseudoacacia) plantations on the eastern margin of the Loess Plateau were selected as the research object. Based on Reineke' s stand density effect law and regional management standards, the stands were divided into low-density (950-135 stems · ha^{-1}), medium-density (1600-2050 stems · ha^{-1}), and high-density (2400-3300 stems · ha^{-1}) groups. Through field investigation, soil nutrient determination, and 16S rRNA and ITS high-throughput sequencing, the effects of stand density on soil nutrients, microbial communities, and their ecological interactions were systematically analyzed, which is of great significance for optimizing plantation density and achieving sustainable management. The results showed that soil total nitrogen, nitrate nitrogen, total carbon, and organic carbon contents increased with stand density, with particularly significant differences in the high-density group ($P<0.05$); available phosphorus content peaked in the medium-density group. The bacterial community was mainly composed of Proteobacteria (38.70%), Actinobacteria (19.37%), Gemmatimonadetes (8.23%), and Chloroflexi (7.71%), with the relative abundance of Actinobacteria in the high-density group being significantly higher than that in the low-density group ($P<0.05$). In the fungal community, Ascomycota (51.79%), Mortierellomycota (30.70%), and Basidiomycota (10.07%) were the dominant phyla. The diversity of bacterial and fungal communities in the high-density group was significantly enhanced, with both Shannon index and Chao1 index significantly increased ($P<0.05$). PCoA analysis showed that the bacterial community structures of medium- and low-density groups exhibited aggregation and were significantly different from that of the high-density group ($P<0.05$);

fungal community structure showed no significant differences among different density groups. Mantel test indicated that both bacterial and fungal community structures were significantly correlated with soil total nitrogen ($P<0.05$). Co-occurrence network analysis revealed that moderately increasing stand density could enhance the interaction strength and complexity of microbial communities; however, when stand density exceeded 2400 stems $\cdot$ ha^{-1}, network stability decreased, which was not conducive to efficient resource utilization. In summary, when stand density is maintained at 1600-2050 stems $\cdot$ ha^{-1}, it can effectively improve soil nutrient levels and optimize microbial community structure, thereby providing a scientific basis for ecosystem management and sustainable operation of black locust plantations on the Loess Plateau.

Full Text

Effects of Stand Density on Soil Nutrients and Microbial Communities in *Robinia pseudoacacia* Plantations

ZHANG Jianing[1], ZHANG Jianjun[1,2], LAI Zongrui[1], ZHAO Jiongchang[1], HU Yawei[1], LI Yang[1], WEI Chaoyang[1]

[1]School of Soil and Water Conservation, Beijing Forestry University, Beijing 100083, China
[2]National Station for Forest Ecosystem Research in Jixian County, Jixian 042200, Shanxi, China

Abstract

Soil nutrient levels and microbial community structure are critical indicators for evaluating the ecosystem services of artificial forests. To investigate the effects of stand density on soil nutrients and microbial communities in plantations within arid and semi-arid regions, we examined a 30-year-old *Robinia pseudoacacia* plantation on the eastern Loess Plateau. Based on Reineke' s stand density effect law and regional management standards, the stands were divided into three density groups: low (950–1350 trees hm^{-2}), medium (1600–2050 trees hm^{-2}), and high (2400–3300 trees hm^{-2}). Through field surveys, soil nutrient measurements, and 16S rRNA sequencing, we systematically analyzed the effects of stand density on soil nutrients, microbial communities, and their ecological interactions. The results demonstrate that soil total nitrogen, nitrate nitrogen, total carbon, and organic carbon contents increased significantly with stand density, particularly in the high-density group ($P < 0.05$). Available phosphorus content, however, peaked in the medium-density group. The bacterial community was dominated by *Proteobacteria* (38.70%), *Actinobacteria* (19.37%), *Gemmatimonadetes* (8.23%), and *Chloroflexi* (7.71%), with the relative abundance of *Actinobacteria* in the high-density group significantly higher than in the low-density group ($P < 0.05$). In the fungal community, *Ascomycota* (51.79%), *Basidiomycota* (30.70%), and *Mortierellomycota* (10.07%) were the dominant phyla. Both bacterial and fungal community diversity increased significantly

in the high-density group, as reflected by higher Shannon and Chao1 indices ($P < 0.05$). Principal coordinates analysis revealed that bacterial community structures in the medium- and low-density groups showed significant clustering and differed markedly from the high-density group ($P < 0.05$), whereas fungal community structures showed no significant differences among density groups. Mantel analysis indicated that both bacterial and fungal community structures were significantly correlated with soil total nitrogen ($P < 0.05$). Co-occurrence network analysis showed that moderately increasing stand density enhanced microbial interaction strength and network complexity, but when stand density exceeded 2400 trees hm^{-2}, network stability declined, hindering efficient resource utilization. In conclusion, maintaining stand density between 1600–2050 trees hm^{-2} effectively improves soil nutrient levels and optimizes microbial community structure, providing a scientific basis for ecosystem management and sustainable operation of *R. pseudoacacia* plantations on the Loess Plateau.

Keywords: stand density; *Robinia pseudoacacia* plantation; soil nutrients; microbial community; arid and semi-arid regions

Introduction

Arid and semi-arid regions possess rich and unique biodiversity resources, yet their ecosystems face severe degradation risks. China' s arid and semi-arid regions cover 4.55$×10^{6}$ km^2, accounting for 47% of the country' s total land area, making China one of the world' s major arid nations. Since the reform and opening-up, China has implemented several large-scale ecological engineering projects, among which artificial afforestation has played a crucial role in ecological restoration. The Loess Plateau region, a key focus of ecological restoration efforts in China, has effectively controlled soil degradation and promoted vegetation recovery through artificial planting.

Robinia pseudoacacia, widely used in the Loess Plateau due to its drought tolerance, ability to thrive in poor soils, and nitrogen-fixing capacity, has become a primary species for ecological restoration projects in the region. However, as afforestation scale has expanded rapidly, some *R. pseudoacacia* plantations have exhibited slow growth and declining quality, with inappropriate stand density identified as a key factor limiting the ecological service functions of these artificial forests.

Stand density, as a core indicator of forest structure, regulates the distribution of environmental factors such as light, temperature, and water, playing a vital role in maintaining forest ecosystem balance. Research has shown that appropriate stand density enhances water conservation capacity in plantations by reducing surface evaporation and improving soil infiltration, thereby increasing soil moisture content and mitigating drought risk. Conversely, inappropriate stand density exacerbates soil drought and affects vegetation health. Additionally, optimal stand density improves light resource allocation, enhances photosynthetic efficiency, promotes biomass accumulation, and increases productivity. In the

resource-poor Loess Plateau region, stand density selection is particularly critical. Excessive stand density increases water demand, potentially exceeding local water resource carrying capacity, leading to soil moisture depletion and intensifying drought and soil degradation. Studies have found that when *R. pseudoacacia* plantation density ranges from 800–2200 trees hm^{-2}, soil infiltration capacity increases with stand density, peaking at 1950–2450 trees hm^{-2}. Furthermore, when stand density is maintained at 1600–2050 trees hm^{-2}, understory vegetation diversity and biomass accumulation reach optimal states, enabling plantations to better fulfill their ecological service functions.

Compared to traditional ecological evaluation indicators such as biomass or vegetation coverage, the soil microenvironment is more sensitive to stand density changes, particularly regarding soil nutrients and microbial communities, which can precisely reflect plantation ecosystem responses to stand density variation at finer scales. Research has demonstrated that stand density significantly affects soil physical properties, nutrient content, and stoichiometric ratios. In *R. pseudoacacia* plantations, soil organic matter and total nitrogen contents peak at stand densities of 1600–2050 trees hm^{-2}, with soil structure becoming more favorable for understory plant growth, while both excessively low and high densities hinder soil nutrient accumulation. Studies indicate that medium-density stands (1950–2450 trees hm^{-2}) effectively promote soil nutrient accumulation. However, most current research on *R. pseudoacacia* plantations in the Loess Plateau has focused on single-factor responses to stand density, with relatively few studies examining the interactive relationships between soil nutrients and microbial communities under different stand densities.

This study systematically analyzed changes in soil nutrient content and microbial community characteristics under different stand densities in a 30-year-old *R. pseudoacacia* plantation on the eastern Loess Plateau, and further explored the interaction mechanisms between these factors. The objective was to provide a scientific basis for regulating stand density, improving ecosystem service functions, and achieving sustainable management of *R. pseudoacacia* plantations in the Loess Plateau region.

1.1 Study Area Overview

The study area is located at the National Forest Ecosystem Research Station in Jixian County, Shanxi Province, in the southeastern Loess Plateau (110°39 45 - 110°47 45 E, 36°14 27 -36°18 23 N). The region has a temperate continental monsoon climate, with a mean annual temperature of 10 °C and mean annual precipitation of 579 mm, concentrated primarily during the May–October growing season. Soils are predominantly cinnamon soils, classified into three subtypes: hilly cinnamon soil, typical cinnamon soil, and leached cinnamon soil. These soils are relatively infertile, poorly resistant to erosion, and vulnerable to degradation. Current land cover types include forest, shrubland, grassland, farmland, orchard, and residential areas. The station' s artificial ecological forest consists mainly of *R. pseudoacacia*, *Platycladus orientalis*, and *Pinus tab-*

uliformis planted during the late 20th-century Grain-for-Green Program and the Sino-Japanese technical cooperation project.

1.2 Sample Plot Setup and Investigation

In July 2023, we selected nine *R. pseudoacacia* plantations with different stand densities in the Caijiachuan watershed within the Jixian Station area. Based on stand density, these were divided into three groups: low, medium, and high density, with three 20 m × 20 m standard plots established in each group, totaling nine sample plots. Each plot contained 15 sampling points, resulting in 135 sampling points across all plots. The study area and site distribution are shown in [Figure 1: see original paper]. Within each plot, we measured tree height, diameter at breast height (DBH), crown width, and canopy closure. Slope and aspect were measured using a compass, and altitude was recorded with a GPS data collector. Basic plot information is presented in .

1.3 Sample Collection

In July 2023, after removing surface litter and loose topsoil, soil samples were collected using a root auger (60 cm length, 10 cm diameter) following a five-point sampling method [Figure 1: see original paper]. Samples were taken from 0–20 cm depth. The auger was sterilized with 75% ethanol after each sampling to prevent cross-contamination. Five soil samples from each plot were mixed to form a composite sample, which was then passed through a 2 mm sieve to remove large particles and debris. The sieved samples were placed in sterile sealed bags (1000 g per bag). One portion was transported in a dry ice container to the laboratory and stored at -20 °C before being shipped on dry ice to Guangdong Magigene Biotechnology Co., Ltd. (Guangzhou, China) for DNA extraction and high-throughput sequencing. The other portion was air-dried at room temperature, ground, and sieved through a 0.25 mm mesh for soil nutrient analysis according to standard soil physicochemical property measurement protocols.

1.4 Soil Nutrient Measurement

Soil total nitrogen (TN) content was determined using the Kjeldahl method after digestion with concentrated sulfuric acid. Ammonium nitrogen (NH_4^+-N) was extracted with potassium chloride solution and measured using the indophenol blue colorimetric method. Nitrate nitrogen (NO_3^--N) was extracted with potassium chloride solution and measured using UV spectrophotometry. Total carbon (TC) content was measured using an elemental analyzer. Soil organic carbon (SOC) content was determined using the potassium dichromate external heating method. Total phosphorus (TP) content was measured using the molybdenum-antimony anti-colorimetric method after digestion with sulfuric acid-perchloric acid. Available phosphorus (AP) content was extracted with sodium bicarbonate and measured using the molybdenum-antimony anti-colorimetric method.

1.5 Microbial Sequencing

DNA was extracted from soil samples using the Thermo Fisher Scientific DNA extraction kit (MA, USA). The V5–V7 region of the bacterial 16S rRNA gene was amplified using primers 5 -AACMGGATTAGATACCCKG-3 and 5 -ACGTCATCCCCACCTTCC-3 . The ITS region of the fungal gene was amplified using primers 5 -GCATCGATGAAGAACGCAG-3 and 5 -TCCTCCGCTTATTGATATGC-3 . PCR amplification was performed under the following conditions: initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s, with a final extension at 72 °C for 10 min. PCR products were detected using 1% agarose gel electrophoresis to verify length and concentration. Based on GeneTools Version 4.03.05.0 analysis, PCR products were mixed in equimolar ratios and purified using a gel extraction kit. Libraries were constructed using the NEBNext® Ultra™ DNA Library Prep Kit, and library quality was assessed using the Qubit®2.0 fluorometer and Agilent Bioanalyzer. Qualified libraries were sequenced on the Illumina HiSeq platform, and raw data were processed using DADA2 for splicing and filtering. Sequences were classified into amplicon sequence variants (ASVs) and annotated against the SILVA bacterial database and UNITE fungal database. Data were rarefied to the minimum sample sequence number for soil microbial community structure analysis. Sequencing was commissioned to Guangdong Magigene Biotechnology Co., Ltd.

1.6 Data Analysis

Statistical analysis and visualization were performed using R software (v.4.3.1). Data were first tested for normality and homogeneity of variance; non-normal data were log-transformed. One-way ANOVA with Tukey' s post hoc test was used to compare soil nutrient contents, microbial α-diversity, relative abundances at the phylum level, and network topological properties among different stand density groups (significance level $P < 0.05$). Microbial α-diversity was analyzed using the "vegan" package, with the Shannon, Chao1, Simpson, and Goods coverage indices selected as diversity metrics. Principal Coordinates Analysis (PCoA) based on Bray-Curtis distance was performed using the "vegan" ordinates function, with adonis2 used for PERMANOVA (999 permutations) to analyze differences in microbial community structure among density groups. Co-occurrence networks were constructed using the "igraph" package, with microbial community correlation matrices converted to data files and network topological parameters calculated, including node number, edge number, positive correlation count, average degree, average path length, and network diameter. Mantel analysis was performed using the "vegan" package to quantify the independent and interactive contributions of environmental factors (soil nutrients, stand structure) to microbial community variation.

2.1 Effects of Stand Density on Soil Nutrient Content

Stand density significantly affected soil nutrient content, with significant differences observed among density groups for total nitrogen, nitrate nitrogen, total carbon, organic carbon, and available phosphorus ($P < 0.05$), while ammonium nitrogen and total phosphorus showed no significant differences ($P > 0.05$). In the high-density group, total nitrogen ($F = 5.17$, $P = 0.02$), nitrate nitrogen ($F = 3.36$, $P = 0.04$), and total carbon ($F = 4.53$, $P = 0.02$) were significantly higher than in the low-density group. Organic carbon in the high-density group was significantly higher than in both the medium- and low-density groups ($P = 0.01$). Available phosphorus content was highest in the medium-density group, significantly exceeding that in the low-density group ($F = 5.17$, $P = 0.01$). Except for organic carbon, no significant differences were observed between the medium- and high-density groups for other soil nutrients ($P > 0.05$). Detailed soil nutrient contents are presented in .

2.2.1 Microbial Community Composition

After rarefaction, the dataset retained 45,000 bacterial sequences per sample, classified into 2,847 ASVs, and 45,000 fungal sequences per sample, classified into 1,234 ASVs. At the phylum level, the bacterial community was dominated by *Proteobacteria* (38.70%), *Actinobacteria* (19.37%), *Gemmatimonadetes* (8.23%), and *Chloroflexi* (7.71%). The relative abundance of *Actinobacteria* differed significantly among stand density groups, being significantly higher in the high-density group than in the low-density group ($F = 4.53$, $P = 0.02$). In the fungal community, *Ascomycota* (51.79%), *Basidiomycota* (30.70%), and *Mortierellomycota* (10.07%) were the dominant phyla, though no significant differences in relative abundance were observed among density groups ($P > 0.05$). The relative abundances of soil microbial communities at the phylum level are shown in [Figure 2: see original paper].

2.2.2 Microbial Community α-Diversity Analysis

In the high-density group, bacterial Shannon diversity and Chao1 richness indices were significantly higher than in the medium-density group ($P < 0.05$), while Simpson and Goods coverage indices showed no significant differences among groups ($P > 0.05$). In the fungal community, the Shannon diversity index in the high-density group was significantly higher than in the medium-density group ($P < 0.05$), but no significant differences were observed for Simpson, Chao1, or Goods coverage indices ($P > 0.05$). The α-diversity analysis of soil microbial communities is presented in [Figure 3: see original paper].

2.2.3 Microbial Community Structure Analysis

PCoA based on Bray-Curtis distance revealed that the first and second axes explained 19.44% and 10.07% of bacterial community variation, respectively, while the cumulative explanation for fungal communities was 27.50%. Bacterial

communities in the medium- and low-density groups exhibited significant aggregation, clearly distinct from the high-density group. Stand density explained 19.44% of bacterial community structure variation ($P = 0.03$), indicating a significant effect. In contrast, no significant differences in fungal community structure were observed among density groups ($P > 0.05$). The PCoA results are shown in [Figure 4: see original paper].

2.2.4 Correlation Between Microbial Community α-Diversity and Stand Density

Linear regression analysis revealed significant positive correlations between bacterial Chao1 and Shannon indices and stand density ($P < 0.05$), indicating that increased stand density enhanced bacterial diversity. No significant correlations were observed between fungal α-diversity indices and stand density ($P > 0.05$), reflecting the high stability of fungal communities in response to stand density variation. The linear regression models are presented in [Figure 5: see original paper].

2.3 Effects of Stand Density on Soil Microbial Co-Occurrence Network Structure

Co-occurrence networks for soil bacteria and fungi across different stand density groups are shown in [Figure 6: see original paper], with topological properties summarized in . The high-density group exhibited more complex networks with greater numbers of nodes and edges compared to the medium- and low-density groups. The bacterial network in the high-density group contained 1,218 nodes, substantially more than in the medium-density (1,134 nodes) and low-density (1,098 nodes) groups. Positive correlations far outnumbered negative correlations in both bacterial and fungal networks. The high-density bacterial network contained 51,790 positive correlations, exceeding those in the medium-density (19,370) and low-density (8,230) groups. Negative correlations were minimal across all groups (7,710, 3,870, and 1,640, respectively). In fungal networks, only positive correlations were observed, with 30,700 in the high-density group, compared to 13,770 in the medium-density and 11,630 in the low-density groups.

Average path length in bacterial networks remained relatively stable across density groups, indicating consistent node connectivity efficiency. In fungal networks, the medium-density group showed the lowest average path length (3.67) and smallest network diameter (4.04), demonstrating higher connectivity efficiency and stability. Network density and clustering coefficients were balanced in the medium-density group, reflecting moderate connectivity that facilitates resource sharing while avoiding excessive competition.

Natural connectivity analysis [Figure 7: see original paper] revealed significant differences among stand density groups. In the low-density group, fungal natural connectivity was significantly higher than bacterial ($P < 0.001$), a pattern

also observed in the medium-density group ($P < 0.001$). However, no significant difference was observed in the high-density group ($P > 0.05$). Low-density bacterial natural connectivity was significantly lower than in medium- and high-density groups, while fungal natural connectivity showed no significant differences among density groups ($P > 0.05$). Stability analysis through sequential node removal indicated that high-density bacterial networks were more vulnerable to disruption, while medium-density fungal networks exhibited the highest stability.

2.4 Interactive Effects of Soil Nutrients and Microbial Community Structure

Mantel test results revealed a significant positive correlation between total nitrogen and bacterial community structure ($R = 0.14$, $P = 0.02$), indicating that total nitrogen is a key factor influencing bacterial communities. No significant correlations were observed between bacterial community structure and other soil nutrients ($P > 0.05$). For fungal communities, significant positive correlations were found with total nitrogen ($R = 0.12$, $P = 0.04$), ammonium nitrogen ($R = 0.12$, $P = 0.03$), and total phosphorus ($R = 0.12$, $P = 0.04$). Variance partitioning analysis (VPA) showed that soil nutrients explained 32.33% of fungal community structure variation, higher than the 25.44% explained for bacterial communities. Stand characteristics explained approximately 17.00% of variation for both bacterial and fungal communities. The combined explanatory power of soil nutrients and stand characteristics for fungal community structure (10.17%) was higher than for bacterial communities (5.00%), indicating stronger synergistic effects on fungal communities [Figure 9: see original paper].

3.1 Effects of Stand Density on Soil Nutrient Content

Stand density significantly influenced soil total nitrogen, nitrate nitrogen, total carbon, organic carbon, and available phosphorus contents in *R. pseudoacacia* plantations on the Loess Plateau. Specifically, total nitrogen, nitrate nitrogen, total carbon, and organic carbon were significantly higher in high-density stands than in low-density stands, while available phosphorus peaked in medium-density stands. Increased stand density enhances litter accumulation, which decomposes through microbial activity, promoting nutrient cycling and increasing nutrient levels. High-density stands also experience more intense inter-tree competition, stimulating root activity and exudate release, which further accelerates organic matter decomposition and nutrient release. The higher available phosphorus in medium-density stands may be related to phosphorus mineralization processes, as available phosphorus is a key indicator of soil phosphorus supply capacity. Low-density stands have lower vegetation cover, making surface soils more susceptible to phosphorus loss, whereas medium- and high-density stands accumulate phosphorus from litter decomposition that exceeds tree uptake requirements.

No significant differences in ammonium nitrogen and total phosphorus were ob-

served among density groups, likely because these nutrients are more strongly influenced by moisture and temperature than by stand density. Except for organic carbon, no significant differences existed between medium- and high-density groups for other nutrients, with medium-density stands showing slightly higher total and available phosphorus. This may be attributed to more suitable soil aeration and moisture conditions in medium-density stands, which promote phosphorus mineralization while maintaining relative balance among other nutrients. Our findings support that the relationship between soil nutrient content and stand density is not simply linear; both excessively low and high densities reduce nutrient use efficiency. When stand density is maintained at 1600–2050 trees hm^{-2}, soil nutrient levels remain high and stable, promoting soil fertility improvement and providing a suitable habitat for microorganisms.

3.2 Effects of Stand Density on Soil Microbial Community Structure and Diversity

Stand density significantly affected microbial community structure, particularly the relative abundance of specific bacterial taxa. The bacterial community was dominated by *Proteobacteria*, *Actinobacteria*, *Gemmatimonadetes*, and *Chloroflexi*, which play crucial roles in soil nutrient cycling. While most bacterial phyla showed no significant differences among density groups, *Actinobacteria* abundance increased significantly in high-density stands, likely due to their strong capacity to decompose complex organic compounds, enabling them to thrive in the organic-rich environment of high-density stands. Other bacterial groups, such as *Acidobacteria* and *Proteobacteria*, exhibit strong adaptability to varying soil pH and nutrient conditions, while *Chloroflexi* can survive in diverse environments, including anaerobic and low-light conditions, maintaining relatively stable abundances across density groups.

In the fungal community, *Ascomycota*, *Basidiomycota*, and *Mortierellomycota* were dominant, with no significant differences in relative abundance among stand density groups. This indicates that fungal communities are highly adaptable to stand density changes, primarily due to their ability to decompose complex organic matter and maintain nutrient cycling functions under various environmental conditions. PCoA revealed significant differences in bacterial community structure among stand density groups, with low-density group samples showing high aggregation, suggesting similar environmental conditions, while high-density group bacterial communities exhibited differentiation due to more complex environmental conditions. Fungal community structure remained relatively stable across density groups, likely because fungi can acquire nutrients through mycelial networks even in relatively nutrient-poor environments.

Linear regression analysis showed significant positive correlations between bacterial Chao1 and Shannon indices and stand density, consistent with previous research showing that complex environmental conditions promote microbial diversity. In contrast, fungal α-diversity indices showed no significant correlation with stand density, further demonstrating their strong ecological adaptability

and stable diversity across different stand densities.

3.3 Effects of Stand Density on Soil Microbial Co-Occurrence Network Structure

Microbial network analysis revealed that the high-density group had significantly more nodes and edges, indicating more complex network structures. The complex environment in high-density stands enhanced microbial interactions and improved ecosystem robustness. However, excessive nodes and edges may intensify resource competition, negatively affecting community stability. In contrast, medium- and low-density groups had fewer but more balanced nodes and edges, facilitating microbial cooperation and reflecting more efficient resource utilization strategies. Positive correlations dominated these networks, indicating that microbial interactions in *R. pseudoacacia* plantation soils rely more on cooperation than competition, revealing flexible adaptation to environmental conditions.

The medium-density group exhibited balanced network density and clustering coefficients, reflecting moderate connectivity that facilitates resource sharing while avoiding excessive competition in high-density stands. This balance benefits microbial community stability and sustained ecological function, suggesting that medium-density stands are more suitable for maintaining soil microbial community health.

Natural connectivity analysis showed that while high stand density increased bacterial network complexity, it also enhanced sensitivity to environmental disturbance, reducing network stability. In contrast, low- and medium-density bacterial networks showed greater robustness. In fungal networks, medium-density stands provided more suitable conditions for microbial survival, increasing network robustness, while excessively low or high densities adversely affected stability. Fungal networks had significantly higher natural connectivity than bacterial networks, indicating greater ecological adaptability. Optimal natural connectivity promotes efficient resource allocation and stress resistance; excessively low connectivity reduces these capabilities, while excessively high connectivity intensifies competition in resource-limited environments. Medium-density groups exhibited moderate natural connectivity, promoting efficient resource allocation without excessive competition, thereby maintaining network stability and ecosystem health.

3.4 Interactive Effects of Soil Nutrients and Microbial Community Structure

Mantel analysis revealed significant correlations between microbial community structure and soil total nitrogen, emphasizing nitrogen' s importance for microbial communities. Total nitrogen is a key factor influencing soil microbial community structure and an effective predictor of bacterial community structure. Fungal community structure also correlated significantly with ammonium

nitrogen and total phosphorus, indicating differential nutrient requirements and responses between bacterial and fungal communities. However, some studies suggest bacterial communities are more sensitive to nitrogen and phosphorus than fungal communities, a result that differs from our findings. This discrepancy may be related to ecosystem type, as microbial community responses to nutrients vary across forest, grassland, and farmland ecosystems. Temperature, moisture, and soil texture may also indirectly regulate microbial nutrient responses by affecting soil physicochemical properties. In the nutrient-limited environment of the Loess Plateau, fungi' s high sensitivity to nitrogen and phosphorus may reflect unique regional characteristics.

VPA results showed that soil nutrients explained more variation in fungal community structure than in bacterial community structure, indicating that fungal community structure is more strongly driven by soil nutrient content. The combined explanatory power of soil nutrients and stand characteristics for fungal community structure was significantly higher than for bacterial communities, further demonstrating that fungal community structure is co-regulated by soil nutrients and stand characteristics. Based on these results, integrated management of *R. pseudoacacia* plantations in the Loess Plateau should consider the synergistic effects of stand characteristics and soil nutrients to optimize ecosystem service functions and promote healthy, sustainable development.

4 Conclusion

Based on systematic analysis of soil nutrients and microbial communities in a 30-year-old *R. pseudoacacia* plantation under different stand densities on the Loess Plateau, we draw the following conclusions:

1) High-density stands significantly increased soil total nitrogen, nitrate nitrogen, total carbon, and organic carbon contents, while available phosphorus peaked in medium-density stands, with other nutrients showing no significant differences from high-density stands. This indicates that moderately increasing stand density effectively improves soil nutrient levels, but excessively high density may reduce the utilization efficiency of key nutrients.

2) Stand density significantly affected microbial community structure, particularly increasing the relative abundance of *Actinobacteria* in medium- and high-density stands, which exhibited stronger niche advantages and enhanced decomposition of complex organic matter, promoting nutrient cycling and optimizing soil ecological function.

3) Moderately increasing stand density enhanced microbial community diversity and ecosystem function. However, when stand density exceeded 2400 trees hm^{-2}, microbial network stability declined, compromising ecosystem health and resource use efficiency.

4) Integrating soil nutrient and microbial community characteristics, we rec-

ommend maintaining *R. pseudoacacia* plantation stand density at 1600–2050 trees hm^{-2} in the Loess Plateau. This range improves soil fertility while optimizing microbial community structure, thereby enhancing plantation ecosystem stability and sustainability.

This study highlights the importance of appropriate stand density in improving soil nutrient levels, optimizing microbial community structure, and maintaining ecosystem stability, providing a scientific basis for sustainable management of *R. pseudoacacia* plantations in the Loess Plateau.

Acknowledgments

This field experiment relied on the support of the National Forest Ecosystem Research Station in Jixian County, Shanxi Province, which provided essential equipment, technical guidance, and site access. The station's assistance enabled our team to obtain high-quality data, laying a solid foundation for subsequent scientific analysis. We sincerely thank all team members for their diligent work and collaborative efforts.

References

[1] Zhang L, Lü N, Cheng L H. Regime shifts and early warning signals in dryland ecosystems—an identification method based on landscape pattern characteristics[J]. Acta Ecologica Sinica, 2023, 43(15): 6486-6498.

[2] Yu G R, Hao T X, Yang M. Ecosystem principles and main issues in regional ecological restoration and environmental governance in China[J]. Chinese Journal of Applied Ecology, 2023, 34(2): 289-304.

[3] Kou M, Jiao J Y. Changes in vegetation and soil properties across 12 years after afforestation in the hilly gully region of the Loess Plateau[J]. Global Ecology and Conservation, 2021, 33(6): e01989.

[4] Wang Y X, Dong G, Qu L P, et al. Ecosystem functioning of the Loess Plateau in China from vegetation restoration relied largely on climate[J]. Forests, 2023, 14(1): 27.

[5] Jiang J, Chen C Q, Chen B B, et al. Effects of stand density on carbon, nitrogen, and phosphorus stoichiometry and nutrient resorption in *Platycladus orientalis* plantations in rocky mountainous areas of Beijing[J]. Journal of Beijing Forestry University, 2024, 46(10): 33-41.

[6] Wang S Q, Zhang J J, Zhang Y Q, et al. Understory plant community diversity of *Robinia pseudoacacia* plantation with different densities in the loess plateau of western Shanxi Province[J]. Arid Zone Research, 2023, 40(7): 1141-1151.

[7] Jiang S X. Effects of density on growth and understory light environment of 6-year old Chinese fir plantation[J]. Subtropical Agriculture Research, 2022, 18(4): 223-228.

[8] Jijijiamen, Cheng Y B, Chen L L, et al. Dynamic changes in soil moisture and its response to rainfall in *Pinus sylvestris* var. *mongolica* plantation in Horqin Sandy Land[J]. Arid Zone Research, 2023, 40(5): 756-766.

[9] Cui Y H, Bi H X, Hou G R, et al. Soil infiltration characteristics and influencing factors of *Robinia pseudoacacia* plantation in the loess gully region of western Shanxi Province, northern China[J]. Journal of Beijing Forestry University, 2021, 43(1): 77-87.

[10] Wei J S, Li Z S, Feng X Y, et al. Ecological and physiological mechanisms of growth decline of *Robinia pseudoacacia* plantations in the Loess Plateau of China: A review[J]. Chinese Journal of Applied Ecology, 2018, 29(7): 2433-2444.

[11] Cong W. Study on the Soil Microbial Community and Coexistence with Plants of Typical Forest[D]. Beijing: Chinese Academy of Forestry, 2020.

[12] Wang Y S, Ma B M, Gao H P, et al. Response of soil nutrients and their stoichiometric ratios to stand density in *Pinus tabuliformis* and *Robinia pseudoacacia* plantations in the loess region of western Shanxi Province, northern China[J]. Journal of Beijing Forestry University, 2020, 42(8): 81-93.

[13] Jia Y J, Yang J Y, Zhang J J, et al. Effects of stand density on biomass and soil physicochemical properties of *Pinus tabuliformis* forest in the loess area of western Shanxi[J]. Journal of Zhejiang A & F University, 2024, 41(6): 1211-1221.

[14] Cui G L, Li Q F, Gao Y, et al. Characteristics of soil microbial communities structure and influencing factors in typical vegetation in the Beichuan River source area of Datong, Qinghai[J]. Arid Zone Research, 2024, 41(7): 1195-1206.

[15] Zhao M, Sun Y R, Liu S H, et al. Effects of stand density on the structure of soil microbial functional groups in *Robinia pseudoacacia* plantations in the hilly and gully region of the Loess Plateau, China[J]. Science of the Total Environment, 2024, 912: 169337.

[16] Chang Y F. Soil Moisture Characteristics and Simulation in Typical Forestlands in Loess Region of Western Shanxi Province[D]. Beijing: Beijing Forestry University, 2018.

[17] Bao S D. Soil Agrochemical Analysis[M]. Beijing: China Agricultural Press, 2000.

[18] Callahan B, McMurdie P, Holmes S. Exact sequence variants should replace operational taxonomic units in marker gene data analysis[J]. The ISME Journal, 2017, 11(12): 2639-2643.

[19] Tedersoo L, Tooming-Klunderud A, Anslan S. PacBio metabarcoding of fungi and other eukaryotes: Embracing full-length amplicons and completeness[J]. New Phytologist, 2018, 217(3): 1370-1385.

[20] Fu Z G, Xiao Y H, Xu H, et al. Interannual dynamics of soil microbial biomass carbon and nitrogen in subtropical evergreen broad-leaved forest and

its environmental impact factors[J]. Acta Ecologica Sinica, 2024, 44(3): 1092-1103.

[21] Zeng X H, Zhang W J, Cao J S, et al. Changes in soil organic carbon, nitrogen, phosphorus, and bulk density after afforestation of Beijing-Tianjin Sandstorm Source Control program in China[J]. Catena, 2014, 118: 186-194.

[22] Shi S W, Peng C H, Wang M, et al. A global meta-analysis of changes in soil carbon, nitrogen, phosphorus, and sulfur, and stoichiometric shifts after forestation[J]. Plant and Soil, 2016, 407: 343-355.

[23] Huang H B, Bi H X, Zhao D Y, et al. Ecological stoichiometry of soil, microbial biomass, and extracellular enzyme in *Robinia pseudoacacia* with different stand densities in the Loess Plateau[J/OL]. Acta Ecologica Sinica, 2025, (03): 1-11.

[24] Zhang N N, Huang S H, Lei H, et al. Assessment of soil erosion and nutrient loss characteristics based on ^{137}Cs tracer technique[J]. Acta Ecologica Sinica, 2022, 42(22): 9274-9283.

[25] Wang Z M, Zhao M M, Ren Y M, et al. Response of growth and soil properties of Chinese pine building timber forest at felling age to stand density[J]. Journal of Beijing Forestry University, 2022, 44(12): 88-101.

[26] Zhong Y M, Chen Z N, Wang H H, et al. Responses of stand characteristics of *Pinus tabuliformis* plantation to density regulation[J]. Journal of Forest and Environment, 2023, 43(6): 606-613.

[27] Hu Y W, Shi Z L, Liu C, et al. Effects of stand densities on understory vegetation diversity and soil physicochemical properties of *Robinia pseudoacacia* forest in loess region of western Shanxi Province[J]. Chinese Journal of Ecology, 2023, 42(9): 2072-2080.

[28] Fang X M, Zhang X L, Zong Y Y, et al. Soil phosphorus functional fractions and tree tissue nutrient concentrations influenced by stand density in subtropical Chinese fir plantation forests[J]. Plos One, 2017, 12(10): e0186905.

[29] Wang X, Bi Y L, Wang Y, et al. Effects of planting density of *Hippophae rhamnoides* and inoculation of AMF on understory vegetation growth and soil improvement[J]. Scientia Silvae Sinicae, 2023, 59(10): 138-149.

[30] Liu S H, Zhao M, Wang Y J, et al. Effects of stand density on soil physicochemical properties and enzyme activities in *Robinia pseudoacacia* plantations in the Loess hilly gully region[J]. Research of Soil and Water Conservation, 2024, 31(5): 123-129.

[31] Guo J, Wu Y Q, Wu X H, et al. Soil bacterial community composition and diversity response to land conversion is depth-dependent[J]. Global Ecology and Conservation, 2021, 32: e01923.

[32] Zhang S G, Liu X, Zhao P S, et al. Soil bacterial networks in *Pinus sylvestris* var. *mongolica* plantations of the Hulunbuir Desert[J]. Arid Zone Research, 2023,

40(6): 905-915.

[33] Zhao F Z, Fan X D, Ren C J, et al. Changes of the organic carbon content and stability of soil aggregates affected by soil bacterial community after afforestation[J]. Catena, 2018, 171: 622-631.

[34] Deng J J, Zhang Y, Yin Y, et al. Comparison of soil bacterial community and functional characteristics following afforestation in arid areas[J]. PeerJ, 2019, 7: e7141.

[35] Zuo Y W, Li W Q, Zeng Y L, et al. Changes in the soil microenvironment during ecological restoration of forest parks in megacities[J]. Ecological Indicators, 2024, 66: 112261.

[36] Liu W X, Li Y M, Jiang H X, et al. Comparative analysis of soil microbial composition of four typical plant communities in Momoge National Nature Reserve, Jilin Province[J]. Chinese Journal of Ecology, 2024, 43(10): 2988-2998.

[37] Zhai K T, Hua Y C, Liang J W, et al. Soil microbial diversity under different types of interference in birch secondary forest in the Greater Khingan Mountains in China[J]. Frontiers in Microbiology, 2023, 14: 1267746.

[38] Zhong C, Ren Y Y, Lu A H, et al. Survival strategies of microorganisms in low-energy environments[J]. Acta Microbiologica Sinica, 2024, 64(12): 4480-4503.

[39] Xu M P, Wang J Y, Zhu Y F, et al. Plant biomass and soil nutrients mainly explain the variation of soil microbial communities during secondary succession on the Loess Plateau[J]. Microbial Ecology, 2022, 83(3): 114-126.

[40] Song Y H, Zhai J Y, Zhang J Y, et al. Forest management practices alter soil organic carbon stability in *Pinus tabulaeformis* plantations by adjusting microbial characteristics on the Loess Plateau of China[J]. Science of The Total Environment, 2021, 766: 144209.

[41] Luo J Y, Song R Q, Deng X, et al. PGPR interacts with ectomycorrhizal fungi to promote growth of *Pinus sylvestris* var. *mongolica* and to effect rhizosphere micro-ecological environment[J]. Journal of Central South University of Forestry & Technology, 2021, 41(9): 22-34.

[42] Li D D, Li J W, Gao G L, et al. Soil fungal community structure and functional characteristics associated with *Pinus sylvestris* var. *mongolica* plantations in the Horqin Sandy Land[J]. Journal of Desert Research, 2023, 43(4): 241-251.

[43] Xue Y Y, Chen H H, Yang J R, et al. Distinct patterns and processes of abundant and rare eukaryotic plankton communities following a reservoir cyanobacterial bloom[J]. ISME Journal, 2018, 12(9): 2263-2277.

[44] Li W T, Liu Q H, Xie L L, et al. Interspecific plant-plant interactions increase the soil microbial network stability, shift keystone microbial taxa, and

enhance their functions in mixed stands[J]. Forest Ecology and Management, 2023, 533: 120851.

[45] Creamer R, Hannula S, van Leeuwen J, et al. Ecological network analysis reveals the interconnection between soil biodiversity and ecosystem function as affected by land use across Europe[J]. Applied Soil Ecology, 2016, 97: 112-124.

[46] Zarafshar M, Vincent G, Korboulewsky N, et al. The impact of stand composition and tree density on topsoil characteristics and soil microbial activities[J]. Catena, 2024, 234: 107541.

[47] Chen J, Feng K, Hannula S E, et al. Interkingdom plant-microbial ecological networks under selective and clear cutting of tropical rainforest[J]. Forest Ecology and Management, 2021, 491: 119182.

[48] Wang C, Shi Z Y, Li A G, et al. Long-term nitrogen input reduces soil bacterial network complexity by shifts in life history strategy in temperate grassland[J]. iMeta, 2024, 3(3): e194.

[49] Yang T, Tedersoo L, Liu X, et al. Fungi stabilize multi-kingdom community in a high-elevation timberline ecosystem[J]. iMeta, 2022, 1(4): e49.

[50] Li C C, Jin L, Zhang C, et al. Destabilized microbial networks with distinct performances of abundant and rare biospheres in maintaining networks under increasing salinity stress[J]. iMeta, 2023, 2(1): e87.

[51] Gong H Y, Wang H X, Wang Y, et al. Topological change of soil microbiota networks for forest resilience under global warming[J]. Physics of Life Reviews, 2024, 50: 228-251.

[52] Wu W C, Yue P, Cui X Q, et al. Response of soil microbial biomass carbon and nitrogen deposition to precipitation and temperature in the Gurbantunggut Desert[J]. Arid Zone Research, 2018, 35(3): 515-523.

[53] Wang J Q, Shi X Z, Zheng C Y, et al. Different responses of soil bacterial and fungal communities to nitrogen deposition in a subtropical forest[J]. Science of the Total Environment, 2021, 755(1): 142471.

[54] Zhang L, Wang Q, Yang X Y, et al. Effect of the process of returning farmland to forest in the Loess Hilly area on soil fungal communities[J]. Environmental Science, 2023, 44(3): 1758-1767.

[55] Chen Y C, Yin S W, Shao Y, et al. Soil bacteria are more sensitive than fungi in response to nitrogen and phosphorus enrichment[J]. Frontiers in Microbiology, 2022, 13: 999385.

[56] Chen X, Hao B H, Jing X, et al. Minor responses of soil microbial biomass, community structure and enzyme activities to nitrogen and phosphorus addition in three grassland ecosystems[J]. Plant and Soil, 2019, 444: 21-37.

[57] Zhang S Y, Fan D D, Kong W D. Effects of warming on soil microbial diversity and carbon sequestration function in drylands[J]. Chinese Journal of

Ecology, 2024, 43(6): 1817-1823.

[58] Zhang T, Liu J, Han X, et al. Effects of grazing on soil nutrients and microbial community of *Pinus sylvestris* var. *mongolica* forest in sandy land[J]. Arid Zone Research, 2023, 40(2): 194-202.

[59] Haas J C, Street N R, Sjödin A, et al. Microbial community response to growing season and plant nutrient optimisation in a boreal Norway spruce forest[J]. Soil Biology and Biochemistry, 2018, 125: 197-209.

Note: Figure translations are in progress. See original paper for figures.