Article

Mineral-solubilizing microbial inoculums promote *Robinia pseudoacacia* L. growth by optimizing the rhizosphere soil microbial community structure

Zhaohui Jia [a], Chong Li [a], Shilin Ma [a], Xin Liu [a], Miaojing Meng [a], Xuefei Cheng [a], Hui Nie [a] and Jinchi Zhang [a,*]

[a] Co-Innovation Center for Sustainable Forestry in Southern China, Jiangsu Province Key Laboratory of Soil and Water Conservation and Ecological Restoration, Nanjing Forestry University, 159 Longpan Road, Nanjing 210037, China

* Correspondence: Jinchi Zhang; Email address: zhang8811@njfu.edu.cn

Abstract: The addition of mineral-solubilizing microbial inoculums is a biological measure used in vegetation restoration of rock mining areas. These inoculums accelerate soil weathering, improve soil fertility, and enhance plant growth. However, little is known about their effect on the rhizosphere soil microbial community. Therefore, the objective of this study was to investigate the pathways through which different mineral-solubilizing microbial inoculums positively affect the underground parts of *R. pseudoacacia*. A pot experiment was conducted, and 32 samples were taken from four different mineral-solubilizing microbial inoculum treatments to investigate the responses of rhizosphere soil bacterial and fungal communities in *R. pseudoacacia*. The results showed that the effect of mineral-solubilizing microbial inoculums on the structure of the fungal community was greater than that of the bacterial community. However, the relative abundance of *Proteobacteria* was increased, which had a strong positive correlation with root nodulation. Mineral-solubilizing microbial inoculums had a greater effect on the diversity and evenness of the bacterial community. Correlation analysis showed that *Proteobacteria* and *Verrucomicrobia* in bacteria, and *Ascomycota* and *Zoopagomycota* in fungi were positively correlated with soil enzyme activity and plant growth. RDA analysis showed that the relative abundance of these two phyla in bacteria had a positive effect on plant root nodulation. Our findings suggest that the addition of mineral-solubilizing microbial inoculums can optimize the rhizosphere soil microbial community structure, promote *R. pseudoacacia* root nodulation, and enhance the nitrogen fixation capacity of plants. This study provides a theoretical basis for the application of mineral-solubilizing microbial inoculums in slope ecological restoration.

Keywords: Bacterial communities; Fungal communities; Mineral-solubilizing microbial inoculums; *Robinia pseudoacacia* L.

1. Introduction

Limestone is a crucial industrial raw material used in metallurgy, construction, chemical, light, and agricultural industries, as well as other specialized sectors [1, 2]. As the steel and cement industries continue to develop, the importance of limestone is bound to increase [3, 4]. However, a number of environmental issues that have a significant negative influence on land degradation and ecological danger might come from the irrational development and use of mineral resources, which can destroy ecosystems. [5, 6]. To mitigate these issues, bioremediation measures such as mineral-solubilizing microbial inoculums have been developed to accelerate rock weathering [7-9]. Numerous studies have demonstrated that the inoculums can effectively promote plant growth, improve soil nutrient supply capacity, increase plant photosynthetic rate, enhance plant root tension, and improve plant nodulation [10-14]. As an environmentally friendly bioremediation measure, mineral-solubilizing microbial inoculums have wide-ranging applications and promising prospects.

 doi:10.20944/preprints202304.1136.v1

The complex biochemical processes between microorganisms and plants achieve the dynamic balance of nutrient supply [15, 16]. Although it is generally recognized that rhizosphere bacteria are crucial for plant health and nutrient uptake[17], our knowledge of the intricate plant-microbe interactions in the rhizosphere is still in its infancy. Plant rhizosphere soil microorganisms occupy the transition zone between the soil and root system, and they are the primary site of nutrient exchange. Therefore, the addition of mineral-solubilizing microbial inoculums will have a direct impact on the rhizosphere environment. *R. pseudoacacia*, which has a strong resistance to stress and a developed root system, is often used as a pioneer tree for soil consolidation and fertilizer conservation in slope ecological restoration [18]. Currently, research mainly focuses on the soil consolidation of *R. pseudoacacia* root systems and the growth of *R. pseudoacacia* as a building species on different slope sites [13]. However, exogenous microorganisms may augment, diminish, or have no effect on the native microbial activities. In order to comprehend rhizosphere nutrient dynamics, it is vital to understand how the structure of the soil's microbial community responds to the addition of mineral-solubilizing microbial inoculums.

The goals of this study were to: (i) evaluate the alterations in the bacterial and fungal community and structure in the rhizosphere soil of *R. pseudoacacia* under various mineral-solubilizing inoculum treatments; (ii) examine potential mechanisms by which mineral-solubilizing microbial inoculums may change microbial communities and functions to affect plant growth during *R. pseudoacacia* development; and (iii) carry out a niche analysis of mineral- Our investigation can act as a blueprint for additional study targeted at effectively extending the use of soil spray-sowing technology. In addition, it offers a fundamental theoretical framework for the global ecological rehabilitation of mining regions.

2. Materials and methods

2.1. Study area

At Nanjing Forestry University, the pot experiments were carried out in an intelligent greenhouse where relative humidity and maximum photosynthetic radiation were managed. The pot water content was measured every other day and watered quantitatively to ensure the consistency of soil water content in the pot experiments.

2.2. Seed test material, microbial strains, and soil

The experimental *Robinia pseudoacacia* L seeds are provided by Tianhe nursery garden company in Jiangsu, China. After germination is promoted, the seedlings are cultured together in the seedling substrate. *R. pseudoacacia* is a pioneer tree species often used for ecological restoration of slopes. These plants were chosen for this study so that we could examine the impact of growth substrates and mineral-solubilizing microorganisms on the soil microbial population.

Bacillus thuringiensis (NL-11), *Streptomyces thermocarboxydus* (NL-1), and *Gongronella butleri* (NL-15) were isolated from soil surrounding weathered dolostones [7-9]. The bacterial strain NL-11, actinomycetes NL-1, and fungal strain NL-15 were cultured in a liquid medium, subjected to oscillation for 24 hours, and then fermented in a fermentation tank. At regular intervals, the microorganisms' wet mass was calculated and recorded in order to create a curve graph. The bacteria were transferred to a sterilized plastic bottle and kept in a fridge before the curve initially peaked and started to drop. In order to evaluate the impacts on plant development and root nodulation, we picked these three inoculums and treatment approaches.

2.3. Pot experiment setup

Robinia pseudoacacia L. was used for each group, with eight replicates per group, totaling 32 pots. Each pot included 60 ml of mixed microorganisms in addition to 5 kg of nursery materials. The experiment consisted of four microbial groups: NL-11 (RPJ1), NL-11 + NL-15 (RPJ2), NL-1 + NL-11 + NL-15 (RPJ3), and a microbial liquid medium without microbes (CK).

The pot experiment began in December 2019, and sampling began in November 2020. Root and soil samples were removed, placed in low temperature heat insulation box and transferred to the laboratory for a short period of time to maintain freshness. Plant root nodules and rhizosphere soil were separated with sterilized tweezers for subsequent test.

2.4. Rhizosphere soil sampling

The *R. pseudoacacia* and soil samples were brought to the lab from the study location in a box with ice to maintain a consistent temperature. During the sampling period, most of the soil was removed by shaking and a small portion of the root soil was retained.

2.5. Rhizosphere soil physicochemical properties

The pH of the rhizosphere soil was measured using a glass electrode (PHS220-K, Mettler Shanghai, China) suspended in a 1 mol/L KCl solution (w: v, 1: 5). The carbon (TC) and nitrogen (TN) contents of the soil and biochar were measured using a various EL III elemental analyzer. 3,5-dinitro salicylic acid colorimetry was employed to measure the sucrase activity in soil, and the results were represented as mol glucose g^{-1} dry sample. Using urea as the substrate, soil urease activity was measured using indophenol colorimetry. The results were represented as μmol ammonium g^{-1} dry sample. Disodium phenyl phosphate colorimetry was used to measure the soil phosphatase activity, which was then reported as mol phenol per dry sample. Using 0.1 mol/L $KMnO_4$, soil catalase activity was titrated for 20 minutes, and the findings were reported as mol $KMnO_4$ g^{-1} dry sample.

2.6. Soil DNA extraction and amplification of sequencing

Total DNA was extracted from a total of 24 samples, with six samples obtained for each treatment. The extracted DNA samples underwent quality tests, and NanoDrop 2000 spectrophotometers were used to measure the quantities. Using the primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), bacterial 16S rRNA gene segments (V3-V4) were amplified from the isolated DNA. Using the primers CS1-ITS3 (5′-ACACTGACGACATGGTTCTACACAHCGATGAAGAACGYRG-3′) and CS2-ITS4 (5′-TACGGTAGCAGAGACTTGGTCTTTCCTSCGCTTATTGATATGC-3′), the partial ITS region (ITS3-ITS4) of fungi was amplified: 27 cycles at 55 ℃ for 30 s. The size of the amplicons was confirmed using agarose gel electrophoresis. The bacterial and fungal raw high-throughput sequencing data were deposited in the NCBI Genbank database with the accession number SRA accession: PRJNA638789, respectively.

2.7. Data analysis and statistics

At a significance level of 5% ($p < 0.05$), the least significant difference (LSD) by Duncan's multiple range test was performed to examine the significance of differences in soil parameters among four treatments. Correlational analysis was performed using the "corrr" R (4.2.2) package [19], and certain figures were generated using the "ggplot2" R (4.2.2) package [20]. Additionally, redundancy analysis (RDA) was conducted to examine the relationship between the relative abundance of the top 10 microbial phyla and plant nodulation. After demultiplexing, the resulting sequences were processed by FLASH (v1.2.11) [21], fastq (0.19.6)[22], the DADA2 [23], the Qiime2 [24] (version 2020.2).

3. Result

3.1. Soil properties under different mineral-solubilizing microbial inoculums treatments

There were significant differences observed in soil enzyme activity and pH values across the treatments of mineral-solubilizing microbial inoculums, which were assessed based on 8 soil characteristics. The PRJ1 treatment showed a considerable reduction in soil pH, and an increase in soil phosphatase and urease activity, compared to the control (CK) ($P < 0.05$). The PRJ2 treatment resulted in a significant improvement in soil sucrase activity and total carbon content ($P < 0.05$).

However, there were no significant differences observed in other soil properties among the mineral-solubilizing microbial inoculum treatments (Table 1).

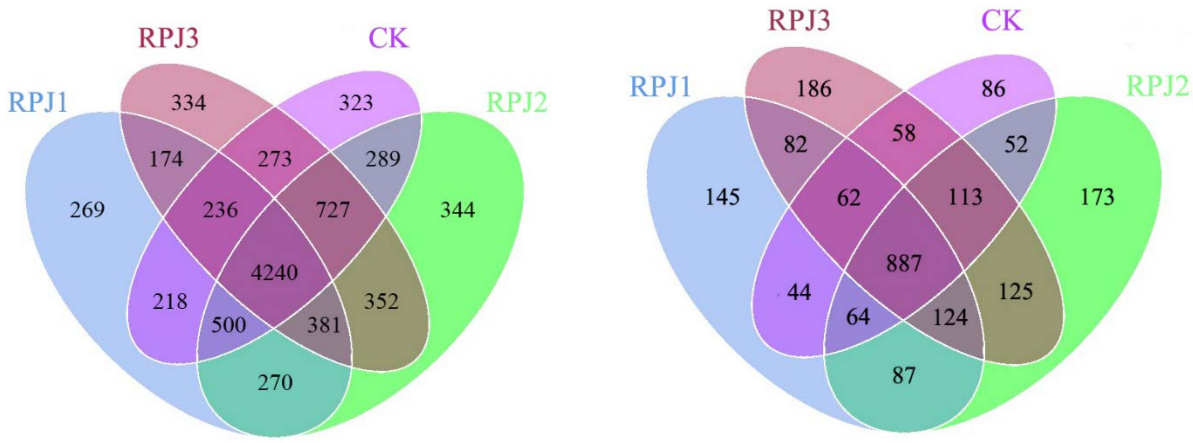
Table 1. Soil properties in different treatments.

Variables	CK		RPJ1		RPJ2		RPJ3	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CAT/（μmol/min）	0.78a	0.24	1.15a	0.19	1.16a	0.35	0.99a	0.18
SUC/（mg/g·24h）	12.40b	1.89	18.04ab	4.88	26.57a	15.21	17.71ab	2.50
HOS/（nmol/g·24h ）	338.31b	21.40	375.08a	20.64	365.38ab	56.66	358.94ab	50.54
URE/（μg/g·24h）	360.36b	40.06	407.22a	19.68	399.43ab	2.50	378.16ab	18.01
pH value	8.12a	0.03	8.04b	0.13	8.12a	0.11	8.11a	0.16
TC/%	7.37b	0.32	7.77ab	0.21	8.00a	0.30	7.93ab	0.35
TN/%	0.43a	0.06	0.50a	0.01	0.50a	0.10	0.43a	0.06
C/N/%	17.15a	1.80	15.53a	0.42	16.48a	3.58	18.52a	2.51

Note: Treatments: CK (control, add microbial liquid medium without microbes), RPJ1 (NL-11), RPJ2 (NL-11 + NL-15), and RPJ3 (NL-1 + NL-11 + NL-15).CAT: soil catalase activity; SUC: soil sucrose activity; HOS: soil alkaline phosphatase activity; URE: soil urease activity; pH value: soil pH; TC: total soil carbon; TN: total soil nitrogen; C/N: soil carbon to nitrogen ratio. Means are the average values of same treatment (n=3). Different letters indicate significance at a 0.05 probability level ($p < 0.05$) using the LSD test.

3.2. Soil microbial community composition under different mineral-solubilizing microbial inoculums treatments

After sequencing and quality filtering, a total of 2,753,009 bacterial and 2,537,567 fungal high-quality sequences were obtained from all 32 soil samples, with an average of 86,032 and 79,299 sequences per sample, respectively. These sequences were then clustered into 9,230 bacterial and 2,346 fungal OTUs. The Venn diagram analysis of bacterial OTUs indicated that 4,240 OTUs were shared across all four treatment groups, while RPJ1, RPJ2, RPJ3 and CK had 269, 344, 334, and 323 unique OTUs, respectively (Figure 1a). Similarly, the Venn diagram of fungal OTUs showed that 887 core OTUs were present in all four treatment groups, with 145, 173, 186, and 86 unique OTUs found in soils from RPJ1, RPJ2, RPJ3, and CK, respectively (Figure 1b).

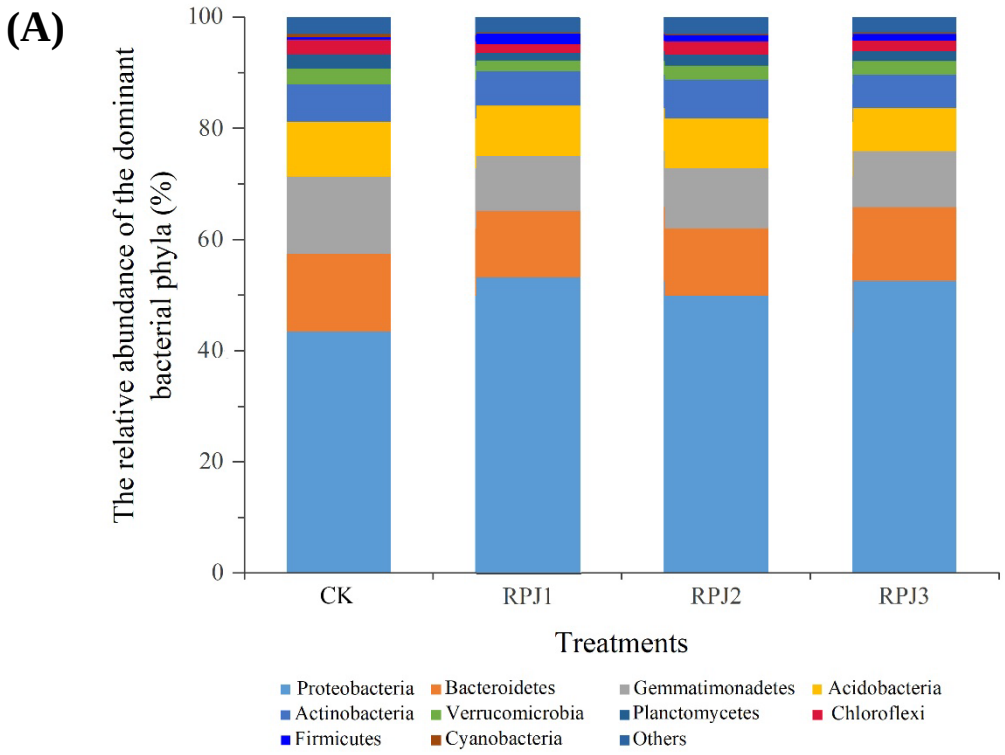


(A) **(B)**

Figure 1. Venn diagram of the number of unique and shared bacterial (A) and fungal (B) operational taxonomic units (OTUs) among the CK, RPJ1, RPJ2 and RPJ3 treatments based on a distance level of 97% similarity. Treatments: CK (control, add microbial liquid medium without microbes), RPJ1 (NL-11), RPJ2 (NL-11 + NL-15), and RPJ3 (NL-1 + NL-11 + NL-15).

The dilution curve is an essential tool for evaluating the quality of sequencing data in a sample library and its ability to cover all microbial groups. As the number of sequences increased in our investigation, the dilution curve for the bacteria and fungi (Figure S1) demonstrated that the curve for the sequencing samples tended to stabilize. This pattern suggests that the sequences have saturated and that further sequencing information has a smaller impact on the discovery of new OTUs. These findings collectively imply that the measured data amount was reasonable and that the sequencing data can accurately reflect the makeup of the soil microbial community structure.

In terms of the fungal community, *Ascomycota* was the most abundant phylum, followed by *Basidiomycota*, *Chytridiomycota*, and *Zygomycota*, covering 96.50, 95.92, 95.63, and 96.42% in CK, RPJ1, RPJ2, and RPJ3, respectively. Compared with CK, RPJ2 had a higher relative abundance of *Ascomycota* and *Basidiomycota*, while RPJ1 and RPJ3 had a higher relative abundance of *Chytridiomycota* and *Zygomycota* (Figure 2B). These results indicated that the rock-solubilizing microbial inoculums had different effects on the rhizosphere soil bacterial and fungal communities, which may be related to their different mineral solubilization capacities and the specific interactions between microbes and plants. *Ascomycota*, *Basidiomycota*, *Mortierellomycota,* and *Chytridiomycota*, which account for 31.23%, 34.67%, 48.41%, and 46.91% of the total fungal phyla, were found in CK, RPJ1, RPJ2, and RPJ3 at relative abundances larger than 1%. With a proportion of 44.15%, RPJ2 stood out as having the highest relative abundance of Ascomycota (Figure 2B).



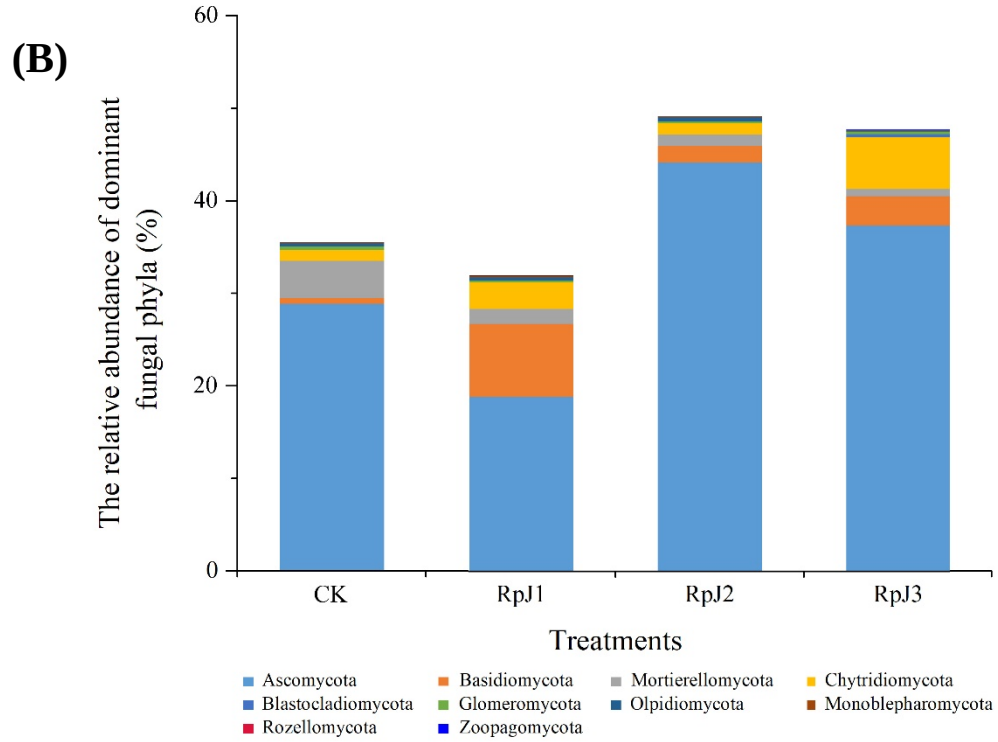
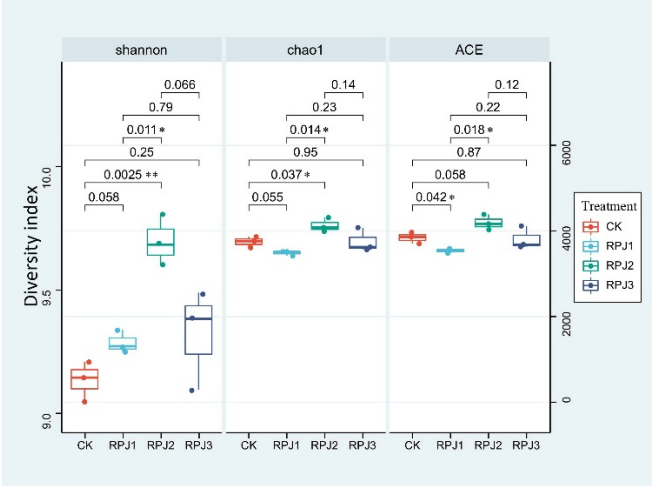


Figure 2. The relative abundance(%) of the dominant bacterial phyla (A) and fungal phyla (B). Treatments: CK (control, add microbial liquid medium without microbes), RPJ1 (NL-11), RPJ2 (NL-11 + NL-15), and RPJ3 (NL-1 + NL-11 + NL-15).

3.3. Soil microbial community diversity under different treatments

Alpha diversity differences between the various treatments were determined by the abundance-based Shannon index, richness estimator Chao1, and microbial community coverage ACE index. The results indicated that RPJ2 significantly increased the soil bacterial Shannon and chao1 indices compared with CK (Figure 3A). RPJ3 significantly improved the soil fungi Shannon index compared with CK (Figure 3B). In order to further illustrate the impacts of mineral-solubilizing microbial inoculums on soil bacteria (Figure 4A) and fungal (Figure 4B) communities, principal coordinate analysis (PCoA) based on the Weighted UniFrac metric was carried out. The first two axes (PCoA1 and PCoA2) explained 46% and 45% of the cumulative variance, respectively (Figure 4A and 4B). The PCoA plots showed that there was no overlap among the microbial communities of CK, RPJ1, RPJ2, and RPJ3, indicating significant differences in the soil microbial communities under different mineral-solubilizing microbial inoculum treatments (Figure 4).



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 April 2023 doi:10.20944/preprints202304.1136.v1

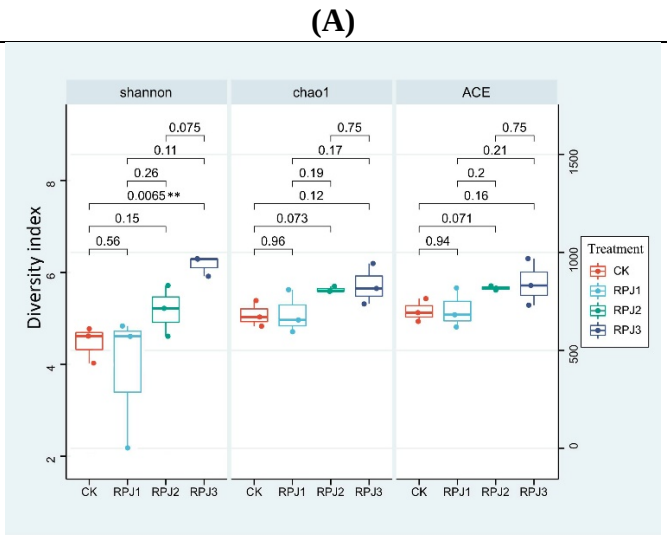


Figure 3. Bacterial (A) and fungal (B) alpha diversity under different treatments. The ordinate on the left corresponds to the Shannon index, and the ordinate on the right corresponds to the Chao1 and ACE indices. Differences in alpha diversity between different treatments were compared with the Kruskal-Wallis test. *, $p < 0.05$; **, $p < 0.01$. Treatments: CK (control, add microbial liquid medium without microbes), RPJ1 (NL-11), RPJ2 (NL-11 + NL-15), and RPJ3 (NL-1 + NL-11 + NL-15).

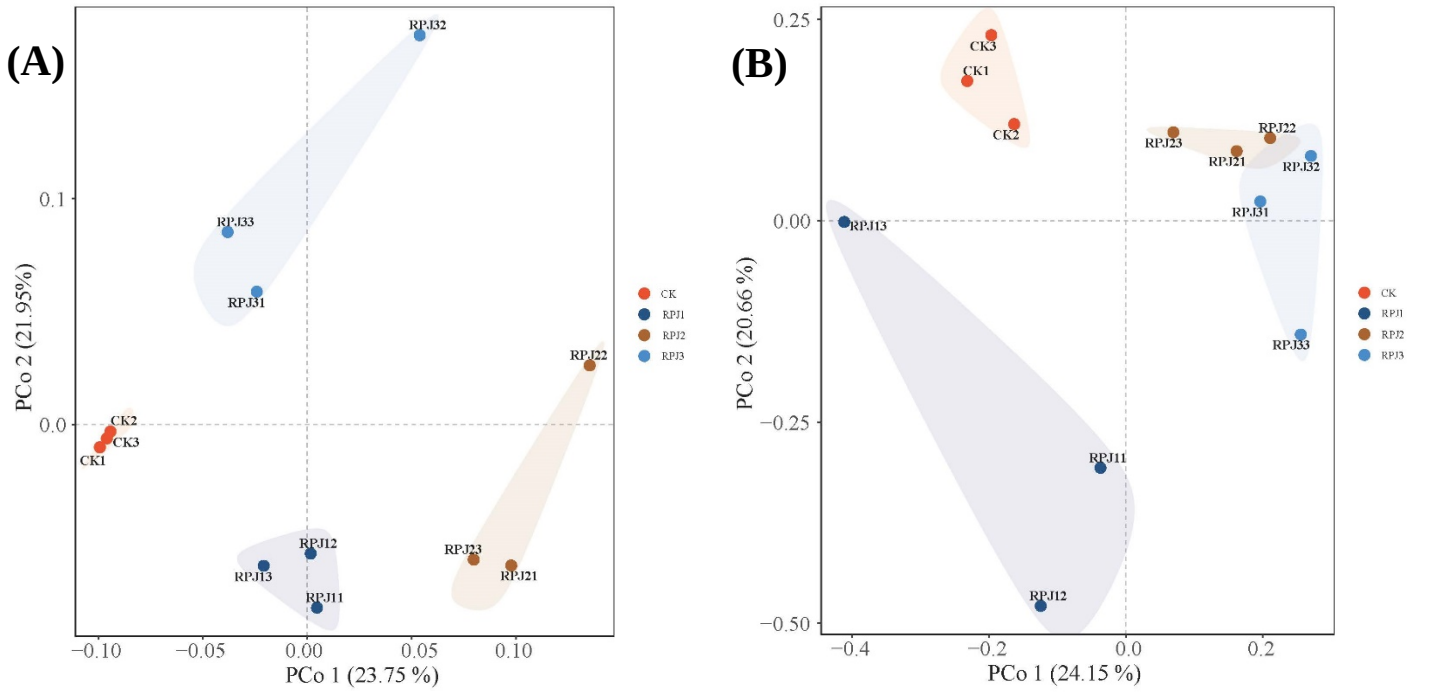


Figure 4. Principal coordinates analysis (PCoA) of changes in the operational taxonomic units of rhizosphere soil bacterial (A) and fungi (B) based on Weighted UniFrac metric among all treatments. Treatments: CK (control, add microbial liquid medium without microbes), RPJ1 (NL-11), RPJ2 (NL-11 + NL-15), and RPJ3 (NL-1 + NL-11 + NL-15).

The relative abundance of particular bacteria and fungus that significantly differed among the treatments were identified using LEfSe analysis (Figure 5). When compared to the other treatments, *Actinomarina*, *Promicromonspora*, and *Micromonospora* had a considerably higher concentration of CK (Figure 5A). Comparing RPJ1 to the other treatments, *Rubrobacter* and *Streptomycetes* were highly enriched (Figure 5A). In comparison to the other treatments, *Solirubrobacterales* and *Pseudonocardiaceae* were significantly enriched in RPJ2 (Figure 5A). Comparing RPJ3 to the other treatments, *acidimicrobiales* were substantially more abundant (Figure 5A). In comparison to the other treatments, *Macrophomina* and *Mycosphaerellaceae* were substantially CK enriched (Figure 5B). Comparing Pezizales to the other treatments, RPJ1 was considerably enriched in Pezizales (Figure 5B). In comparison to the other treatments, *Arthrographis*, *Eremomycetaceae*, and *Eurotiales* had considerably higher RPJ2 concentrations (Figure 5B). In comparison to the other treatments, *Pleosporales* and *Orbiliaceae* were significantly enriched in RPJ3 (Figure 5B).

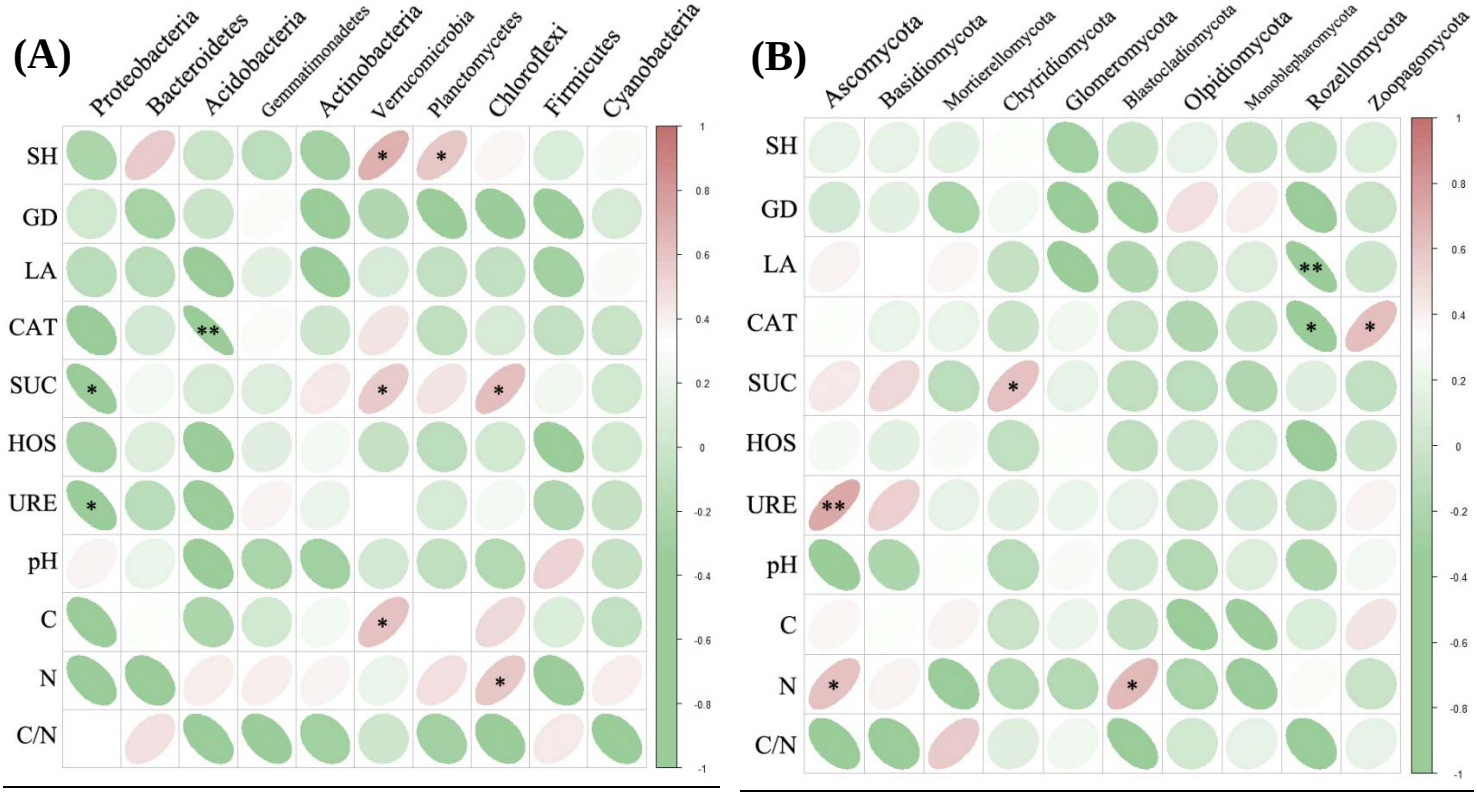


Figure 5 Relationships among Plant growth, soil chemical properties and relative abundance of bacterial community(A) and fungal community (B). Significant correlations are marked in red (positive) and green (negative). *** indicates significant correlation at p<0.001** indicates significant correlation at $p < 0.01$; * indicates significant correlation at $p < 0.05$. SH, height; GD, basal diameter; LA, leaf areas; CAT, soil catalase activity; SUC, soil sucrase activity; HOS, soil alkaline phosphatase activity; URE, soil urease activity; pH, soil pH; C, soil total carbon ; N, soil total nitrogen; C/N, soil carbon to nitrogen ratio.

3.4. The correlations between plant nodulation and soil microbial community composition

Spearman's correlation analyses revealed several significant associations. Specifically, the relative abundance of *Verrucomicrobia* and *Planctomycetes* showed a positive correlation with plant height ($P < 0.1$, Figure 6A), while *Acidobacteria* exhibited a negative correlation with soil catalase activity ($P < 0.001$, Figure 6A). In addition, *Verrucomicrobia* and *Chloroflexi* were positively correlated with soil sucrase activity ($P < 0.01$, Figure 6A) and soil total carbon ($P < 0.01$, Figure 6A), respectively. Moreover, the relative abundance of *Chloroflexi* showed a positive correlation with soil total nitrogen ($P < 0.01$, Figure 6A). On the other hand, the relative abundance of *Rozellomycota* was negatively correlated with plant leaf area ($P < 0.001$, Figure 6B) and soil catalase activity ($P < 0.01$, Figure 6B), while *Zoopagomycota* exhibited a positive correlation with soil catalase activity ($P < 0.001$, Figure 6B).

Furthermore, *Chytridiomycota* was positively correlated with soil sucrase activity ($P < 0.001$, Figure 6B), while *Ascomycota* showed a positive correlation with soil urease activity ($P < 0.001$, Figure 6B) and soil total nitrogen ($P < 0.01$, Figure 6B). Additionally, the relative abundance of *Blastocladiomycota* was positively correlated with soil total nitrogen ($P < 0.001$, Figure 6B).

RDA analysis was employed to investigate the relationship between soil microbial community composition and plant nodulation (Figure 7). For soil bacterial communities, RDA1 and RDA2 accounted for 85.85% and 9.71% of the total variation, respectively (Figure 7A). These ten soil bacterial communities explained 95.56% of the variation in plant nodulation. Notably, the relative abundance of *Proteobacteria*, *Verrucomicrobia*, and *Gemmatimonadetes* exhibited significant positive correlations with plant nodulation (Figure 7A). Regarding soil fungal communities, 92.37% of the variation in plant nodulation was significantly explained by these communities. Specifically, the relative abundance of *Ascomycota*, *Basidiomycota*, and *Zoopagomycota* showed significant positive correlations with plant nodulation (Figure 7B).

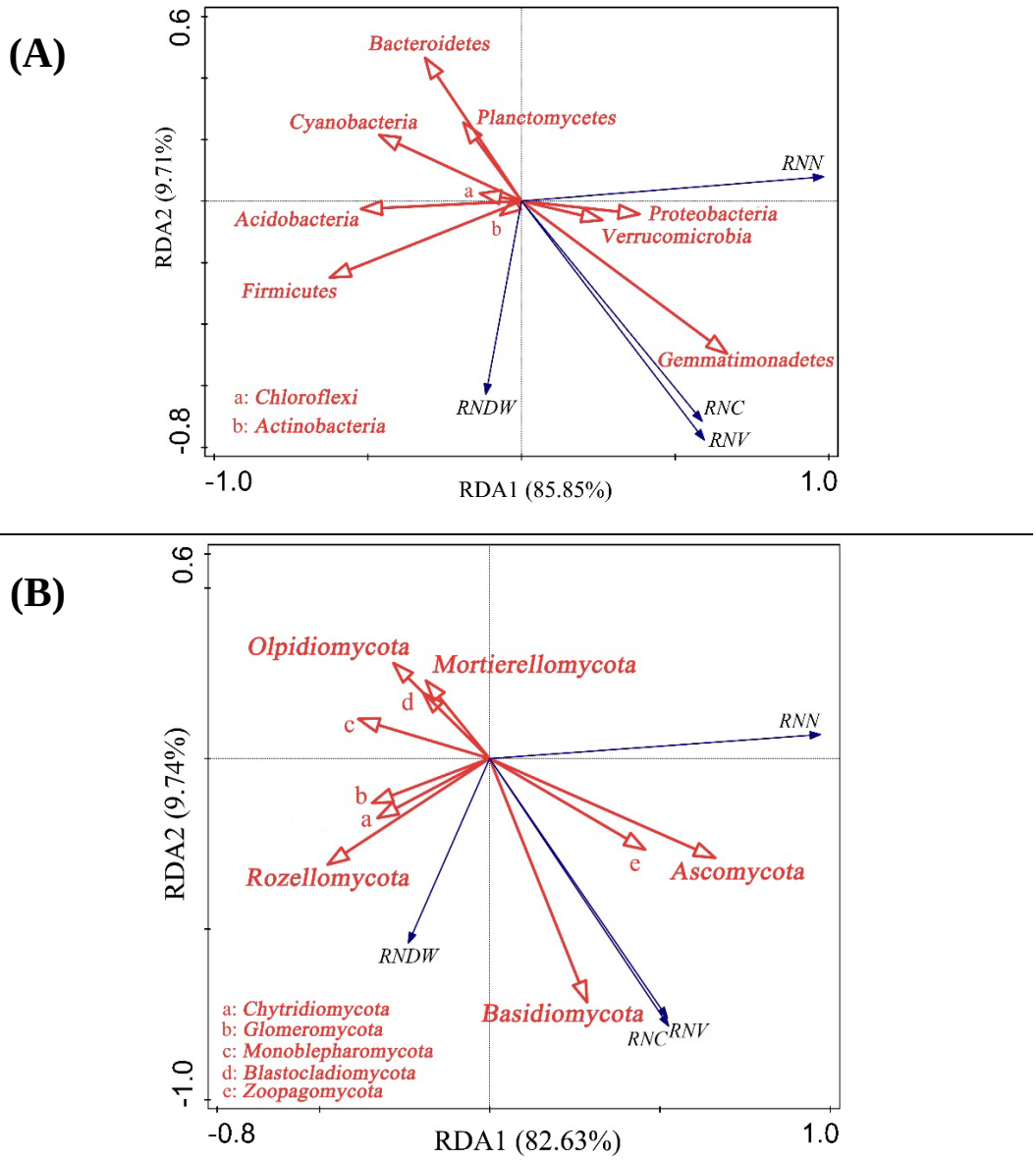


Figure 6. Redundancy analysis (RDA) of plant nodulation and microbial community for bacterial (A) and fungal (B) in *R. pseudoacacia* rhizosphere. RNN, root nodules number; RNV, root nodules volume; RNDW, root nodules dry weight; RV, root volume; RNC, root nodules contribution.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 April 2023 doi:10.20944/preprints202304.1136.v1

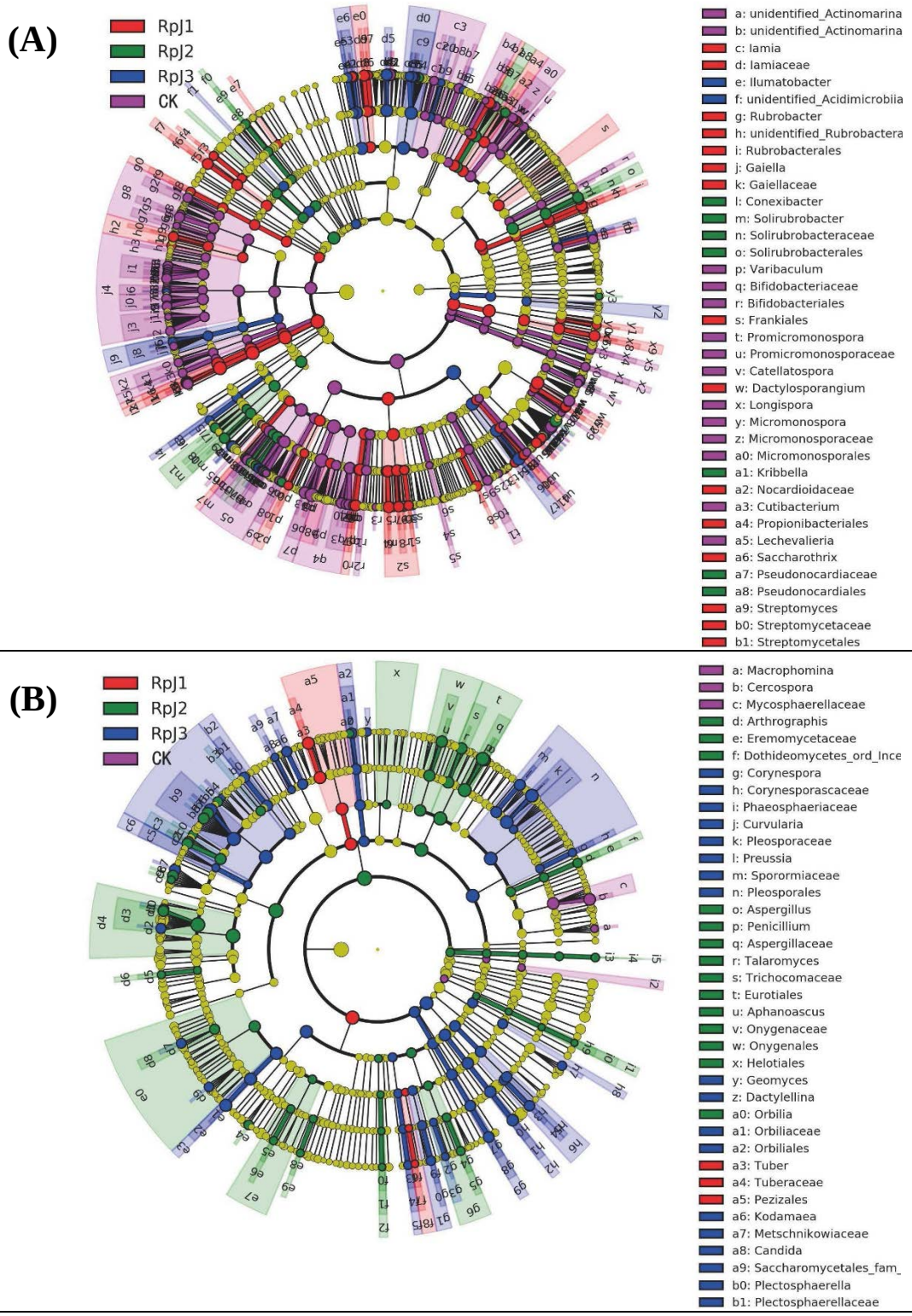


Figure 7 Cladogram of rhizosphere soil bacteria (A) and fungi (B) in different treatments via LEfSe method identifies the significantly different abundant taxa. CK (control, add microbial liquid medium without microbes), RPJ1 (NL-11), RPJ2 (NL-11 + NL-15), and RPJ3 (NL-1 + NL-11 + NL-15).

4. Discussion

An increasing body of research has demonstrated the potential of external microorganisms to enhance crop resilience against abiotic stresses, such as drought, heat, and salinity, as well as biotic stresses, such as soil-borne pathogen [25-29]. These microorganisms can serve as mineral-solubilizing microbial inoculums to improve soil texture, with strong ecological adaptability that makes them ideal for use in revegetation initiatives.

4.1. Rhizosphere soil activity

In the process of ecosystem reconstruction, the plant rhizosphere soil, being an active nutrient exchange area, is often the focus of research to understand the mechanisms underlying plant growth changes [30-35]. Soil enzyme activity is a crucial indicator of soil quality [36]. Our study has shown that mineral-solubilizing microbial inoculums can enhance soil enzyme activity, thus improving the soil's nutrient supply capacity and having a positive effect on promoting plant growth. Previous studies have demonstrated that the use of exogenous microorganisms can directly or indirectly increase the number of microorganisms and root exudates in the rhizosphere soil, contributing to the enhancement of soil enzyme activities [37-39]. These results concur with our findings..

The soil environment typically hosts a stable microbial community, and mining activities can disrupt the ecological balance and induce adaptations in the original microorganisms, leading to changes in microbial community structure and diversity [40-42]. Our study found that the addition of mineral-solubilizing microbial inoculums altered the structure of the rhizosphere soil microbial community, with the fungal community showing particularly significant changes. These findings are in line with previous research that has investigated the impact of exogenous microbial additives on microbial community structure [43, 44].

The addition of mineral-solubilizing microbial inoculums had a significant effect on bacterial abundance and diversity during the potting experiment, indicating changes in bacterial α-diversity under the test conditions. However, the effect of microbial inoculums on soil microbial abundance and diversity was more controversial. Our study showed that there was no significant effect of bacterial agent addition on bacterial diversity. However, some studies on the effects of microbial agent addition have shown a reduction in the diversity of microbial communities in soil [45]. Therefore, it is suggested that microbial diversity may depend on specific site conditions and may vary with the input of microbial inoculums.

4.2. Effect of addition of mineral-solubilizing microbial inoculums on root nodulation of plant

In our study, we observed that mineral-solubilizing microbial inoculums had a positive impact on nodule number and total volume. Previous research has demonstrated that exogenous microorganisms can enhance plant root growth and improve their ability to withstand drought and infertile soil conditions [46]. In addition, the addition of exogenous substances like synthetic nodulation factors can increase the number of root nodules [47]. Our study achieved a similar effect by altering the rhizosphere soil microbial community through the action of mineral-solubilizing inoculums. We observed an increase in the relative abundance of *Proteobacteria* with the addition of mineral-solubilizing microbial inoculums. This is consistent with previous studies that found a positive correlation between the relative abundance of *Proteobacteria* and total carbon, total nitrogen, available potassium, and available phosphorus [48]. *Proteobacteria* also contain rhizobia, which are required for symbiotic nodulation in leguminous plants. Our findings suggest that an increase in the abundance of rhizobia in soil may lead to an increase in bacteria activated by nodulation factors in the root system, thereby promoting plant nodulation [49]. While plants play a leading role in regulating root nodulation, mineral-solubilizing microbial inoculums also play an important role in promoting nitrogen fixation and absorption during plant growth.

5. Conclusions

This study provides valuable information on the effects of adding mineral-solubilizing microbial inoculums to the rhizosphere soil of *R. pseudoacacia*, including changes in the taxonomic and functional characteristics of the microbial community. The addition of different mineral-solubilizing microbial inoculums had a significant impact on the relative abundance of fungal communities and bacterial diversity. The RPJ1 treatment was found to be the most effective in altering the rhizosphere soil microbial community of *R. pseudoacacia*. Additionally, our study revealed a mechanism for the effects of mineral-solubilizing microbial inoculums on promoting plant growth, specifically through the regulation of soil microbial community to increase the number and volume of R. *pseudoacacia* root nodules. Our findings demonstrate a linkage between enhanced symbiotic nitrogen fixation capacity of plants and the associated soil microbial community. The results of this study provide valuable insights into the impact of mineral-solubilizing microbial inoculums addition on plant growth for ecological restoration.

Author Contributions: Conceptualization, Zhaohui Jia; Methodology, Zhaohui Jia, Shilin Ma and Xuefei Cheng; Software, Zhaohui Jia and Chong Li; Validation, Zhaohui Jia; Formal analysis, Zhaohui Jia and Chong Li; Investigation, Xuefei Cheng and Jingchi Zhang; Resources, Shilin Ma; Data curation, Miaojing Meng, Xuefei Cheng, Hui Nie and Jingchi Zhang; Writing – original draft, Zhaohui Jia; Writing – review & editing, Chong Li, Shilin Ma, Xin Liu, Miaojing Meng and Jingchi Zhang. All authors have read and agreedto the published version of the manuscript.

Funding: This research was funded by the Jiangsu Science and Technology Plan Project [BE2022420]; the Innovation and Promotion of Forestry Science and Technology Program of Jiangsu Province [LYKJ (2021) 30]; the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD); and the Scientific Research Project of Baishanzu National Park (2021KFLY05).

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: We are grateful to Mr. Frank and Mrs. Leng for the editing of this manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Mymrin, V., E. K. Aibuldinov, K. Alekseev, M. A. Avanci, P. H. B. Rolim, R. E. Catai and K. Q. Carvalho. "Sustainable material manufacturing from hazardous bauxite red mud in composites with clay slate waste and lime production waste." *The International Journal of Advanced Manufacturing Technology* 111 (2020): 1375-85. 10.1007/s00170-020-06187-9. https://doi.org/10.1007/s00170-020-06187-9.
2. Shehata, N., E. T. Sayed and M. A. Abdelkareem. "Recent progress in environmentally friendly geopolymers: A review." *Science of The Total Environment* 762 (2021): 143166. https://doi.org/10.1016/j.scitotenv.2020.143166. https://www.sciencedirect.com/science/article/pii/S0048969720366961.
3. Chang, Z., G. Long, J. L. Zhou and C. Ma. "Valorization of sewage sludge in the fabrication of construction and building materials: A review." *Resources, Conservation and Recycling* 154 (2020): 104606. https://doi.org/10.1016/j.resconrec.2019.104606. https://www.sciencedirect.com/science/article/pii/S0921344919305129.
4. Li, T., A. Wang, W. Xing, Y. Li and Y. Zhou. "Assessing mineral extraction and trade in china from 1992 to 2015: A comparison of material flow analysis and exergoecological approach." *Resources Policy* 63 (2019): 101460. https://doi.org/10.1016/j.resourpol.2019.101460. https://www.sciencedirect.com/science/article/pii/S0301420719300911.

5. Zhang, J., H. Li, D. Huang and X. Wang. "Evaluation study of ecological resilience in southern red soil mining areas considering rare earth mining process." *Sustainability* 15 (2023): 2258. 10.3390/su15032258.
6. Liu, W., D.-R. Lee, S.-Y. Wang and H.-W. Yu. "Assessing the ecological loss of mining areas in taiwan." *Environmental Monitoring and Assessment* 195 (2023): 10.1007/s10661-022-10780-8.
7. Wu, Y., J. Zhang, L. Wang and Y. Wang. "A rock-weathering bacterium isolated from rock surface and its role in ecological restoration on exposed carbonate rocks." *Ecological Engineering* 101 (2017): 162-69. https://doi.org/10.1016/j.ecoleng.2017.01.023. https://www.sciencedirect.com/science/article/pii/S0925857417300575.
8. Wu, Y., J. Zhang, X. Guo, Y. Wang and Q. Wang. "Isolation and characterisation of a rock solubilising fungus for application in mine-spoil reclamation." *European Journal of Soil Biology* 81 (2017): 76-82. https://doi.org/10.1016/j.ejsobi.2017.06.011. https://www.sciencedirect.com/science/article/pii/S1164556317301371.
9. Wu, Y., J. Zhang and X. Guo. "An indigenous soil bacterium facilitates the mitigation of rocky desertification in carbonate mining areas." *Land Degradation & Development* 28 (2017): 2222-33. https://doi.org/10.1002/ldr.2749. https://onlinelibrary.wiley.com/doi/abs/10.1002/ldr.2749.
10. Jia, Z., M. Meng, C. Li, B. Zhang, L. Zhai, X. Liu, S. Ma, X. Cheng and J. Zhang. "Rock-solubilizing microbial inoculums have enormous potential as ecological remediation agents to promote plant growth." *Forests* 12 (2021): 357. https://www.mdpi.com/1999-4907/12/3/357.
11. Li, C., Z. Jia, X. Peng, L. Zhai, B. Zhang, X. Liu and J. Zhang. "Functions of mineral-solubilizing microbes and a water retaining agent for the remediation of abandoned mine sites." *Science of The Total Environment* 761 (2021): 143215. https://doi.org/10.1016/j.scitotenv.2020.143215. https://www.sciencedirect.com/science/article/pii/S0048969720367462.
12. Li, C., Z. Jia, L. Zhai, B. Zhang, X. Peng, X. Liu and J. Zhang. "Effects of mineral-solubilizing microorganisms on root growth, soil nutrient content, and enzyme activities in the rhizosphere soil of robinia pseudoacacia." *Forests* 12 (2021): 60. https://www.mdpi.com/1999-4907/12/1/60.
13. Li, C., J. Zhaohui, Y. Yingdan, C. Xuefei, S. Jichang, T. Xinggang, W. Yuhao, P. Xiaonan, D. Yiqiao, M. Shilin, *et al.* "Effects of mineral-solubilizing microbial strains on the mechanical responses of roots and root-reinforced soil in external-soil spray seeding substrate." *Science of The Total Environment* (2020):
14. Wang, Y., J. Zhang, W. U. Yanwen, L. Wang and Z. Jia. "Effects of soil bacteria inoculation in spray seeding matrix on photosynthesis characteristics and chlorophyll fluorescence parameters of amorpha fruticose." *Research of Environmental Sciences* 30 (2017): 902-10.
15. Srivastava, S., J. Randhawa, V. Anand, V. Bist, P. Singh, S. Rastogi, S. Yadav and S. Srivastava. "Phosphate starvation responses in plants and microbe mediated phosphorus recycling in soil: A review." *INTERNATIONAL JOURNAL OF PLANT AND ENVIRONMENT* 8 (2022): 25-37. 10.18811/ijpen.v8i01.03.
16. Girme, M. and D. Sarnobat. "Effect of nutrient supply system on biometry and rhizosphere microflora of soybean, pigeon pea and sorghum." *Agricultural Science Digest - A Research Journal* (2018): 10.18805/ag.D-4516.
17. Sun, S., R. Xue, M. Liu, L. Wang and W. Zhang. "Research progress and hotspot analysis of rhizosphere microorganisms based on bibliometrics from 2012 to 2021." *FRONTIERS IN MICROBIOLOGY* 14 (2023): 10.3389/fmicb.2023.1085387.
18. Zhao, M., Y. Li, Y. Wang, Y. Sun and Y. Chen. "High stand density promotes soil organic carbon sequestration in robinia pseudoacacia plantations in the hilly and gully region of the loess plateau in

china." *Agriculture, Ecosystems & Environment* 343 (2023): 108256. 10.1016/j.agee.2022.108256.

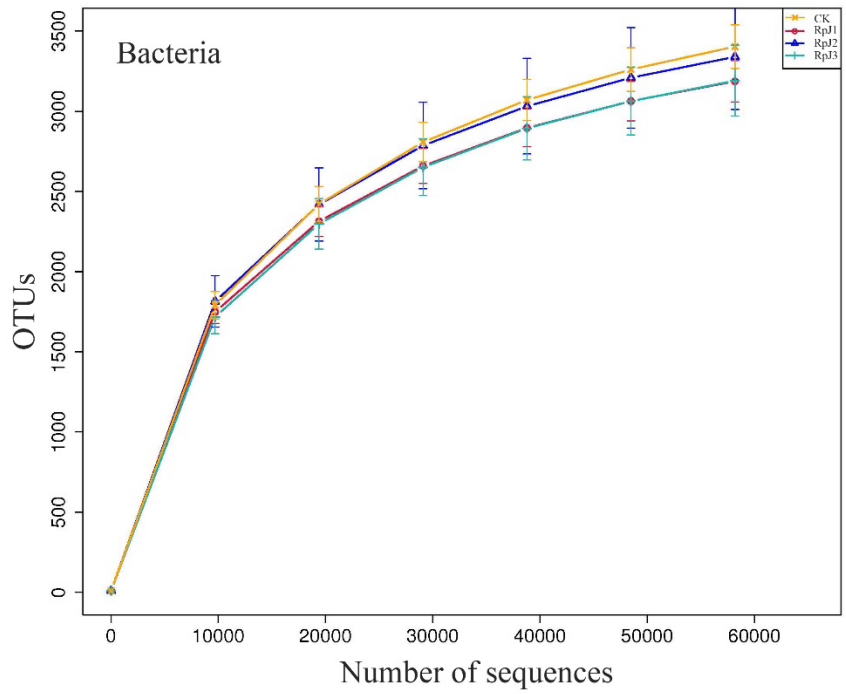
19. Jackson, S. "Correlations in r [r package corrr version 0.2.1]." (2016):
20. Wickham, H. "Ggplot2." *Wiley Interdisciplinary Reviews: Computational Statistics* 3 (2011): 180-85. 10.1002/wics.147.
21. Magoc, T. and S. L. Salzberg. "Flash: Fast length adjustment of short reads to improve genome assemblies." *BIOINFORMATICS* 27 (2011): 2957-63. 10.1093/bioinformatics/btr507.
22. O'Halloran, D. M. "Fastq_brew: Module for analysis, preprocessing, and reformatting of fastq sequence data." *BMC research notes* 10 (2017): 275-75. 10.1186/s13104-017-2616-7.
23. Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson and S. P. Holmes. "Dada2: High-resolution sample inference from illumina amplicon data." *NATURE METHODS* 13 (2016): 581-+. 10.1038/NMETH.3869.
24. Bolyen, E., J. R. Rideout, M. R. Dillon, N. A. Bokulich, C. C. Abnet, G. A. Al-Ghalith, H. Alexander, E. J. Alm, M. Arumugam, F. Asnicar, *et al.* "Reproducible, interactive, scalable and extensible microbiome data science using qiime 2 (vol 37, pg 852, 2019)." *NATURE BIOTECHNOLOGY* 37 (2019): 1091-91. 10.1038/s41587-019-0252-6.
25. Buntić, A., S. Tošić, M. Knežević, M. Jovković, B. Sikiric, N. Koković and E. Saljnikov. "Synergistic effect of bacillus isolates and biomass ash on soil and plant quality: A preliminary pot- experiment with the analysis of potentially toxic elements." *Zemljiste i Biljka* 70 (2021): 42-55. 10.5937/ZemBilj2102042B.
26. Chatterjee, S., K. Mondal and S. Chatterjee. "Role of soil microbes in soil health and stability improvement." In 2022, 579-92.
27. Gong, D., S. ll, L. Xinghao, W. Zhang, D. Zhang and J. Cai. "Micro/nanofabrication, assembly, and actuation based on microorganisms: Recent advances and perspectives." *Small Structures* (2023): 10.1002/sstr.202200356.
28. Kaboosi, E., A. Rahimi, M. Abdoli and M. Ghabooli. "Comparison of serendipita indica inoculums and a commercial biofertilizer effects on physiological characteristics and antioxidant capacity of maize under drought stress." *Journal of Soil Science and Plant Nutrition* 23 (2022): 10.1007/s42729-022-01091-5.
29. Treesubsuntorn, C., P. Dhurakit, G. Khaksar and P. Thiravetyan. "Effect of microorganisms on reducing cadmium uptake and toxicity in rice (oryza sativa l.)." *ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH* 25 (2018): 1-12. 10.1007/s11356-017-9058-6.
30. Gang, L., L. Nan, N. Wang and W. Yang. "Effects of soil organic construction on regional surface et and gpp of coal mine goaf." *Journal of Physics: Conference Series* 1549 (2020): 022091. 10.1088/1742-6596/1549/2/022091.
31. Herrington, R. and M. Tibbett. *Cradle-to-cradle mining: A future concept for inherently reconstructive mine systems?* 2022,
32. Hu, J., B. ye, Z. Bai and Y. Feng. "Remote sensing monitoring of vegetation reclamation in the antaibao open-pit mine." *Remote Sensing* 14 (2022): 5634. 10.3390/rs14225634.
33. Metsaranta, J., S. Beauchemin, S. Langley, B. Tisch and P. Dale. "Assessing the long-term ecosystem productivity benefits and potential impacts of forests re-established on a mine tailings site." *Forests* 9 (2018): 707. 10.3390/f9110707.
34. Pietrzykowski, M. "Tree species selection and reaction to mine soil reconstructed at reforested post-mine sites: Central and eastern european experiences." *Ecological Engineering: X* 3 (2019): 100012. 10.1016/j.ecoena.2019.100012.
35. Zhao, J., J. ma, Y. Yang, H. Yu, S. Zhang and F. Chen. "Response of soil microbial community to

vegetation reconstruction modes in mining areas of the loess plateau, china." *FRONTIERS IN MICROBIOLOGY* 12 (2021): 10.3389/fmicb.2021.714967.

36. Liu, X., L. Yaying, Y. Yu and H. Yao. "Effect of nonbiodegradable microplastics on soil respiration and enzyme activity: A meta-analysis." *Applied Soil Ecology* 184 (2023): 104770. 10.1016/j.apsoil.2022.104770.
37. Huang, K., J. Zhang, G. Tang, D. Bao, T. Wang and D. Kong. "Impacts and mechanisms of biochar on soil microorganisms." *Plant, Soil and Environment* 69 (2023): 10.17221/348/2022-PSE.
38. Li, Q., D. Zhang, H. Cheng, L. Ren, X. Jin, W. Fang, D. Yan, Y. Li, Q. Wang and A. Cao. "Organic fertilizers activate soil enzyme activities and promote the recovery of soil beneficial microorganisms after dazomet fumigation." *Journal of Environmental Management* 309 (2022): 114666. 10.1016/j.jenvman.2022.114666.
39. Umar, A., A. Turaki, A. Bagudo and R. Yeldu. "Production of fibrinolytic enzyme by soil actinobacteria." *Proceedings of the Pakistan Academy of Sciences: B. Life and Environmental Sciences* 60 (2023): 49-56. 10.53560/PPASB(60-1)733.
40. Krause, S., A. Näther, V. Ortiz Cortes, E. Mullins, G. Kessel, B. Lotz and C. Tebbe. "No tangible effects of field-grown cisgenic potatoes on soil microbial communities." *Frontiers in Bioengineering and Biotechnology* 8 (2020): 10.3389/fbioe.2020.603145.
41. Saghaï, A., L. Wittorf, L. Philippot and S. Hallin. "Loss in soil microbial diversity constrains microbiome selection and alters the abundance of n-cycling guilds in barley rhizosphere." *Applied Soil Ecology* 169 (2021): 104224. 10.1016/j.apsoil.2021.104224.
42. Zhou, Z., G. Ding, M.-H. Yu, G. Gao and G. Wang. *Diversity and structural variability of bacterial microbial communities in rhizocompartments of desert leguminous plants*. 2020,
43. Huang, L., W. Bao, F. Li and H. Hu. "Effects of soil structure and vegetation on microbial communities." *Chinese Journal of Applied and Environmental Biology* 27 (2021): 1725-31. 10.19675/j.cnki.1006-687x.2020.08001.
44. Wang, Q.-Y., W. Na, Y. Liu, G. Chen, H. He, J. Gao, X.-L. Zhuang and G.-Q. Zhuang. "Microbial community structure of soil methanogens and methanotrophs during degradation and restoration of reed wetlands in the songnen plain." *Huan jing ke xue= Huanjing kexue / [bian ji, Zhongguo ke xue yuan huan jing ke xue wei yuan hui "Huan jing ke xue" bian ji wei yuan hui.]* 42 (2021): 4968-76. 10.13227/j.hjkx.202102172.
45. Chen, J., L. Mo, Z. Zhang, J. Nan, D. Xu, L. Chao, X. Zhang and Y. Bao. "Evaluation of the ecological restoration of a coal mine dump by exploring the characteristics of microbial communities." *Applied Soil Ecology* 147 (2020): 103430. https://doi.org/10.1016/j.apsoil.2019.103430. http://www.sciencedirect.com/science/article/pii/S0929139319310078.
46. Liu, X.-Q., M.-M. Xie, A. Hashem, E. F. Abd Allah and Q.-S. Wu. "Arbuscular mycorrhizal fungi and rhizobia synergistically promote root colonization, plant growth, and nitrogen acquisition." *Plant Growth Regulation* (2023): 10.1007/s10725-023-00966-6.
47. Ayala-Garcia, P., I. Jiménez-Guerrero, C. Jacott, F. López-Baena, F. Ollero, P. del Cerro and F. Pérez-Montaño. "The rhizobium tropici ciat 899 nodd2 protein promotes symbiosis and extends rhizobial nodulation range by constitutive nodulation factor synthesis." *Journal of Experimental Botany* 73 (2022): 10.1093/jxb/erac325.
48. Zhang, H., F. Ullah, R. Ahmad, S. Shah, A. Khan and M. Adnan. "Response of soil proteobacteria to biochar amendment in sustainable agriculture-a mini review." 01 (2022): 16-30. 10.56946/jspae.v1i2.56.
49. Mondal, S., K. Pramanik, P. Pal, S. Mitra, S. Ghosh, T. Mondal, T. Soren and T. Maiti. "Multifaceted roles

 doi:10.20944/preprints202304.1136.v1

of root exudates in light of plant-microbe interaction." In 2022, 49-76.



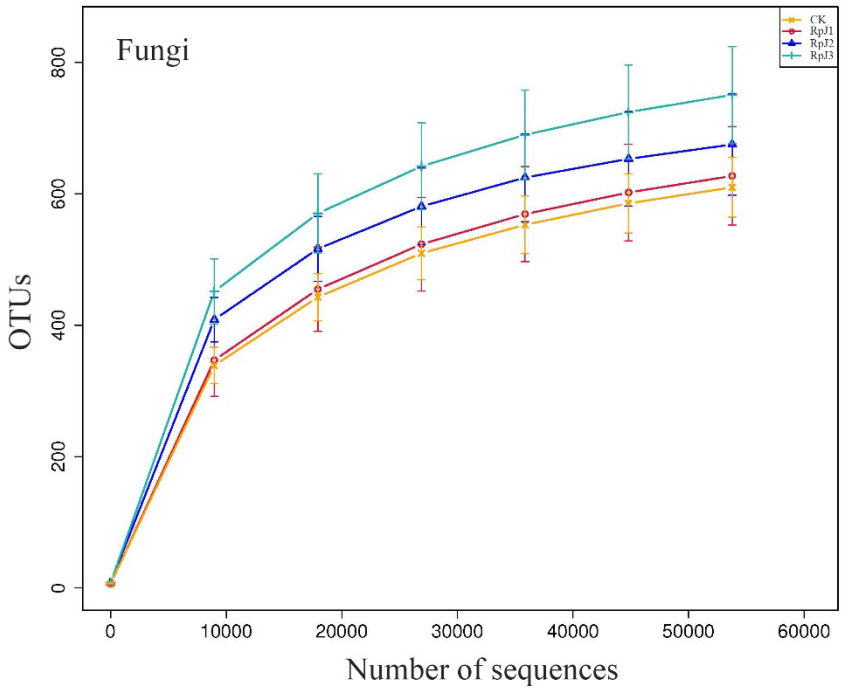


Figure S1 Rarefaction curve of soil bacteria (A) and fungi (B). AH: *Robinia pseudoacacia;*

Table S1 Soil properties in different treatments

Variables	CK		RPJ1		RPJ2		RPJ3	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
SH/ (cm)	74.00 c	12.30	88.17 bc	10.02	94.53 ab	21.64	107.80 a	10.33
GD/ (mm)	7.75 a	1.37	8.34 a	0.35	6.80 a	0.78	7.50 a	0.84
LA/ (cm^2)	541.42 a	278.82	857.34 a	167.28	606.24 a	90.41	728.83 a	192.89
RNN	31.33 b	7.64	161.67 a	67.17	55.33 b	25.74	76.00 b	38.97
RNV/ (cm^3)	0.45 b	0.15	1.14 a	0.22	0.67 ab	0.42	0.45 b	0.42
RNDW/ (g)	0.18 a	0.13	0.17 a	0.20	0.19 a	0.12	0.13 a	0.12
RNC/%	0.53 b	0.16	1.07 a	0.26	0.55 b	0.16	0.45 b	0.25

Note: Treatments: CK (control, add microbial liquid medium without microbes), RPJ1 (NL-11), RPJ2 (NL-11 + NL-15), and RPJ3 (NL-1 + NL-11 + NL-15). SH, height; GD, basal diameter; LA, leaf areas; RNN, root nodules number; RNV, root nodules volume; RNDW, root nodules dry weight; RV, root volume; RNC, root nodules contribution. Means are the average values of same treatment (n=3). Different letters indicate significance at a 0.05 probability level ($p < 0.05$) using the LSD test.