Rhizosphere bacterial communities of Agriophyllum squarrosum (L.) Moq. during different developmental stages [postprint]

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Date: 2025-09-22T12:09:17+00:00

Abstract

Rhizosphere bacteria play crucial roles in plant health and growth as they are involved in nutrient assimilation and resisting adverse conditions such as nutrient stress, drought, and wind erosion. Agriophyllum squarrosum (L.) Moq. is a pioneer plant used for sand fixation due to its strong resistance to drought and wind erosion. However, the bacterial community characteristics and ecological functions in the rhizosphere of A. squarrosum are poorly understood. In this study, soil samples were collected from different developmental stages (seedling stage, vegetative stage, reproductive stage, and withering stage) of A. squarrosum. Illumina Miseq sequencing was used to detect differences in soil bacterial abundance. The Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) program was used to predict bacterial functions, and the relationships among bacteria, functional populations, and soil nutrients were examined using heatmap analysis. The results showed that the Shannon and Sobs indices of rhizosphere bacteria were significantly higher during the reproductive stage than during the other stages. Pantoea sp. (7.03%) was the dominant genus during the seedling stage; Arthrobacter sp. was the dominant genus during the vegetative (13.94%), reproductive (7.57%), and withering (12.30%) stages. The relative abundances of Chloroflexi, Acidobacteria, and Gemmatimonadetes were significantly high during the reproductive stage. According to the PICRUSt analysis, membrane transport, signal transduction, and environmental adaptation of the bacterial functional population occurred during the seedling stage. Carbohydrate metabolism increased during the vegetative stage, while energy metabolism, lipid metabolism, and biosynthesis of other secondary metabolites of the bacterial functional population significantly increased during the reproductive stage. The abundances of bacterial communities, functional genes, and soil nutrients were synergistically altered during various developmental stages. Our findings suggest that the developmental stages of A. squarrosum play a significant role in defining the composition and structure of bacterial communities in the rhizosphere. The results will provide a basis for better prediction and understanding of soil bacterial metabolic potential and functions of A. squarrosum rhizosphere in sandy areas.

Full Text

Preamble

Journal of Arid Land (2025) 17(9): 1282-1296
doi: 10.1007/s40333-025-0028-4; CSTR: 32276.14.JAL.02500284
Science Press Springer-Verlag

Rhizosphere bacterial communities of *Agriophyllum squarrosum* (L.) Moq. during different developmental stages

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Abstract: Rhizosphere bacteria play crucial roles in plant health and growth by assimilating nutrients and helping plants resist adverse conditions such as nutrient stress, drought, and wind erosion. *Agriophyllum squarrosum* (L.) Moq. is a pioneer plant widely used in sand fixation due to its strong resistance to drought and wind erosion. However, the characteristics and ecological functions of bacterial communities in the *A. squarrosum* rhizosphere remain poorly understood. In this study, we collected soil samples from different developmental stages (seedling, vegetative, reproductive, and withering) of *A. squarrosum* and used Illumina MiSeq sequencing to detect differences in bacterial abundance. We employed the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) program to predict bacterial functions and examined relationships among bacteria, functional populations, and soil nutrients through heatmap analysis. Our results showed that the Shannon and Sobs indices of rhizosphere bacteria were significantly higher during the reproductive stage than during other stages. *Pantoea* sp. (7.03%) dominated during the seedling stage, while *Arthrobacter* sp. was dominant during the vegetative (13.94%), reproductive (7.57%), and withering (12.30%) stages. The relative abundances of Chloroflexi, Acidobacteria, and Gemmatimonadetes were also significantly elevated during the reproductive stage. PICRUSt analysis revealed that membrane transport, signal transduction, and environmental adaptation functions were prominent during the seedling stage. Carbohydrate metabolism increased during the vegetative stage, while energy metabolism, lipid metabolism, and biosynthesis of other secondary metabolites became significantly enhanced during the reproductive stage. Bacterial community abundances, functional genes, and soil nutrients changed synergistically across developmental stages. Our findings suggest that *A. squarrosum* developmental stages significantly shape the composition and structure of rhizosphere bacterial communities, providing a foundation for better prediction and understanding of soil bacterial metabolic potential and functions in sandy ecosystems.

Keywords: bacterial diversity; developmental stage; drought; functional prediction; soil nutrient; wind erosion

Citation: ZHANG Shengnan, GAO Haiyan, YANG Shanshan, ZHANG Lei, YAN Deren, HUANG Haiguang, YANG Zhiguo, LI Junwen, TANG Yuekun, XU Hongbin. 2025. Rhizosphere bacterial communities of *Agriophyllum squarrosum* (L.) Moq. during different developmental stages. *Journal of Arid Land*, 17(9): 1282–1296.

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Received 2025-04-02; revised 2025-07-10; accepted 2025-07-23

Introduction

The rhizosphere microbiome plays vital roles in plant growth, nutrition, and stress tolerance [?, ?, ?]. Plants actively modify rhizosphere soil composition and physicochemical properties through root exudates, thereby influencing microbial activity [?, ?, ?]. In turn, recruitment of specific microbial taxa benefits the host by providing various ecological services [?, ?, ?, ?]. Recent studies indicate that plants experiencing iron and phosphorus deprivation increase secretion of oxalic, malic, and citric acids to enrich rhizosphere organic carbon and attract beneficial microorganisms [?, ?]. Yu et al. (2021) demonstrated that maize roots specifically recruit Oxalobacteraceae through flavonoid secretion (e.g., apigenin and luteolin) to enhance lateral root development and nitrogen uptake under nitrogen deprivation. Moreover, mounting evidence suggests that rhizosphere microbial community composition is closely linked to plant developmental stages. Chaparro et al. (2014) found significant differences in *Arabidopsis thaliana* (L.) Heynh. rhizosphere microbial communities across seedling, vegetative, bolting, and flowering stages. Similarly, wheat rhizosphere microbial species composition remained relatively stable during seedling, tillering, and flowering stages but changed significantly during maturation. Thus, rhizosphere microbial community assembly is not a stochastic process but rather a targeted process that meets plants' specific needs during different developmental stages [?, ?].

Desert ecosystems represent among the most challenging environments for plant survival, characterized by extreme abiotic stresses including drought, high temperatures, prevailing wind erosion, and nutrient deficiencies [?, ?]. Rhizosphere microorganisms become even more critical for plant establishment and growth under such adverse conditions [?, ?]. Previous studies have found that desert plants such as *Haloxylon ammodendron* (C. A. Mey.) Bunge and *Stipagrostis sabulicola* (Pilg.) De Winter harbor rhizosphere microbial communities enriched with drought-tolerant and plant growth-promoting bacteria that significantly enhance their survival in extremely arid and barren desert ecosystems [?, ?, ?]. However, research on rhizosphere microbiomes of pioneer desert plants, particularly those with exceptionally rapid life cycles adapted to ephemeral resource availability, remains scarce.

Agriophyllum squarrosum (L.) Moq. is a typical annual desert plant with numerous adaptive properties, including rapid seed germination and growth as well as a short growth period. *A. squarrosum* can survive in extremely hot and dry environments and tolerate sand burial. As a desert pioneer species, it creates conditions that enable other plant species to invade and establish themselves in fragile desert ecosystems [?, ?]. Desert conditions of wind erosion, sand burial, high temperature, and low precipitation affect plant population survival and reproduction. To adapt to these extreme conditions, *A. squarrosum* follows an r-selected reproductive strategy: following rainfall, seeds quickly germinate and plants grow, mature, and complete their life cycle within a short period. Previous research on *A. squarrosum* has focused on seed germination [?, ?, ?, ?, ?], environmental adaptation [?, ?, ?, ?], and sand fixation [?, ?]. Despite its important role in desert restoration, relatively little research has examined its rhizosphere microbiome.

Variation in rhizosphere bacterial communities represents a crucial link in *A. squarrosum*' s adaptation to harsh environments. Does a specific rhizosphere bacterial community assist *A. squarrosum*? What potential functions do these specialized bacteria perform during different developmental stages? To address these questions, our study aimed to: (1) analyze the composition and diversity of rhizosphere soil bacteria of *A. squarrosum* across different developmental stages; (2) examine changes in rhizosphere soil nutrients and determine relationships between bacterial community composition and environmental factors; and (3) predict the ecological functions of rhizosphere bacteria and explain relationships between these functions and bacterial groups.

2.1 Study Area and Sampling

The study area was located in the eastern Hobq Desert, Inner Mongolia Autonomous Region, China (40°28 24 N, 109°82 19 E). This region has a midtemperate continental monsoon climate with average annual precipitation of 312 mm and evaporation of 2160 mm. Mean maximum and minimum temperatures are 40°C and -35°C, respectively. The average growing period for *A. squarrosum* is 130–140 days. Vegetation is dominated by *A. squarrosum*, with sparse *Corispermum* sp. and *Artemisia ordosica* Krasch.

Soil samples were collected during four developmental stages in 2022: late July (seedling stage), mid-August (vegetative stage), mid-September (reproductive stage), and early November (withering stage) [Fig. 1a [Figure 1: see original paper]-d]. For each stage, rhizosphere soil samples were collected from 20–30 randomly selected plants and combined into a single composite sample, yielding 60 soil samples total (15 per stage). Sample plots were approximately 0.2 hm^2. For each plant, we carefully excavated soil clods 5–15 cm from the plant, then removed soil 5 mm from the roots using a brush and tweezers. Soil shaken from root segments 1-5 mm from the root surface was defined as rhizosphere soil. Samples were divided into two portions: one placed in sterile ziplock bags and stored at 4°C for bacterial community analysis, and another air-dried, sieved (2 mm), and used for soil nutrient determination.

2.2 Soil Nutrient Analysis

Soil chemical characteristics were evaluated following established protocols [**?**, **?**]. Soil organic matter content was measured using the H_2SO_4-$K_2Cr_2O_7$ method. Available phosphorus was determined by sodium bicarbonate extraction, available potassium by ammonium acetate extraction, and available nitrogen by the diffuser method. Sucrase activity was measured according to Zhao et al. (2016), neutral phosphatase and catalase activities by Yang et al. (2008), urease activity by Kandeler and Gerber (1988), and neutral protease activity by Ladd et al. (1976).

2.3 DNA Extraction and 16S rRNA Gene Amplification

Approximately 5 g of soil was used for DNA extraction following Li et al. (2024). The V3–V4 regions of the 16S rRNA gene were amplified from total DNA using primers 338F (5 -ACTCCTACGGGAGGCAGCAG-3) and 806R (5 -GCACTACHVGGGTWTCTAAT-3) via the MiSeq system. Thermal cycling consisted of 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 30 s, with a final extension at 72°C for 7 min. The 20.0 L PCR mixture contained 4.0 L 5×TransStart FastPfu buffer, 2.0 L 2.5 mM dNTPs, 0.8 L each primer, 0.4 L TransStart FastPfu DNA Polymerase, 10.0 ng total DNA template, and ddH_2O to volume. Each sample was amplified in triplicate. DNA purity and concentration were assessed by 1.50% agarose gel electrophoresis, and the constructed library was sequenced on an Illumina MiSeq PE300 platform by Shanghai Majorbio Bio-pharm Technology Co., Ltd., China.

2.4 Sequence Processing and Analysis

Raw tag data were obtained by splicing offline sequencing data using Fast Length Adjustment of Short v.1.2.11 software. Quantitative Insights into Microbial Ecology was used for quality control to generate clean tags after filtering. Bioinformatics analysis was performed after normalization based on the minimum sample sequence number. Operational taxonomic units (OTUs) were clustered at 97.00% similarity using the Usearch Pipeline for Operational Taxonomic Unit Analysis software, followed by chimera sequence identification and removal. Sample composition statistics at various classification levels were obtained using the SILVA database. Sequences were submitted to the National Center for Biotechnology Information Sequence Read Archive under accession number PRJNA1031326.

After preparing the OTU table, we used the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) program to predict metagenomic functional gene composition and obtain Kyoto Encyclopedia of Genes and Genomes Orthology (KO) tables. Samples were compared for significant differences in level 1 and level 2 functions. Level 1 KEGG functions comprise six major categories, each further subdivided into level 2 functions for detailed analysis.

Bacterial alpha diversity indices (Good’ s coverage, Shannon, Shannon-Weiner, and Sobs) were computed using R v.3.3.2 software, which was also used for principal coordinates analysis (PCoA), principal component analysis (PCA), and heatmap analysis. Bar charts were generated using Origin v.2017 software, statistical analyses performed using SPSS v.21.0 software, and figures retouched using Adobe Illustrator v.2020 software.

3.1 Soil Nutrients of the Rhizosphere

Soil chemical properties and enzyme activities are presented in Table 1 . Sucrase activity was significantly higher during the seedling stage than during vegetative, reproductive, and withering stages ($P<0.050$). Available nitrogen and neutral protease values were significantly lower during seedling, reproductive, and withering stages compared to the vegetative stage. Available nitrogen and potassium contents and sucrase, urease, and catalase activities were significantly lower during the reproductive stage than during other stages. Available phosphorus and potassium contents were significantly higher during the withering stage, while neutral protease activity decreased significantly. Soil organic matter and neutral phosphatase showed similar variation patterns across developmental stages. These results indicate that *A. squarrosum* primarily absorbed carbon sources during the seedling stage, causing significant sucrase activity increases. During the vegetative stage, nitrogen source absorption dominated, resulting in significantly higher available nitrogen and neutral protease activities. During the reproductive stage, large nitrogen and potassium requirements caused significant decreases in available nitrogen and potassium in rhizosphere soil.

3.2 Bacterial Alpha Diversity

Alpha diversity of rhizosphere bacterial communities varied significantly among developmental stages [Fig. 2a [Figure 2: see original paper]1-a4]. Good' s coverage indices ranged from 0.97 to 0.99, indicating that sequencing results accurately represented bacterial community composition. Shannon and Sobs indices were consistent: values were highest during the reproductive stage and significantly higher during vegetative and withering stages than during the seedling stage ($P<0.050$). The Shannon-Weiner index was significantly higher during the reproductive stage than during seedling and vegetative stages, with the lowest value occurring during the seedling stage. These results demonstrate greater bacterial diversity during the reproductive stage, while bacterial abundance and variety were significantly lower during the seedling stage, indicating that rhizosphere bacteria became more abundant as *A. squarrosum* grew and developed.

Different developmental stages of *A. squarrosum* significantly affected rhizosphere bacterial communities, explaining 38.53% of total variance ($R^2=0.6969$, $P=0.001$) [Fig. 2b]. PCoA revealed clear separation of samples based on developmental stage, with seedling, vegetative, reproductive, and withering stage samples clustering tightly, indicating good repeatability.

3.3 Bacterial Community Composition of Rhizosphere Soil

We identified 38 phyla, 117 classes, 292 orders, 501 families, and 953 genera. Bacterial community structure varied significantly among developmental stages ($P<0.050$; Fig. 3a [Figure 3: see original paper]). Proteobacteria and Actinobacteria were dominant phyla, with relative abundances of 31.69%-54.86% and 26.20%-39.16%, respectively. Less abundant phyla (<10.00%) included Bacteroidetes, Chloroflexi, Patescibacteria, Acidobacteria, Myxococcota, Firmicutes, Gemmatimonadetes, and Cyanobacteria. We also detected 28 trace phyla with low relative abundance, including Verrucomicrobia and Nitrospirae, plus some unclassified bacteria (1.31%-2.74%). Proteobacteria abundance decreased significantly from seedling to vegetative and reproductive stages, while Actinobacteria abundance increased significantly from seedling to vegetative and withering stages. Bacteroidetes, Chloroflexi, Acidobacteria, and Gemmatimonadetes abundances changed in concert, with significantly higher values during the reproductive stage than during other stages. Bacterial abundance distribution was more uniform at the phylum level during the reproductive stage, likely due to varied nutrient requirements during *A. squarrosum* reproduction that increased root secretions and enriched diverse bacterial groups.

Approximately 15 genera had relative abundances >1.00% [Fig. 3b]. Unclassified bacteria and genera <1.00% were categorized as "others," comprising 55.14%-66.28% of the total. During the seedling stage, dominant bacteria were *Arthrobacter* (6.71%) and *Pantoea* (7.03%). During vegetative, reproductive, and withering stages, *Arthrobacter* (7.57%-13.94%) dominated. About 14 genera, including *Microbacterium* and *Nocardioides*, had relative abundances of 1.00%-10.00%. *Pantoea* and *Pseudomonas* abundances increased significantly during the seedling stage ($P<0.050$). *Arthrobacter*, *Paenarthrobacter*, and *Delftia* abundances were significantly higher during the vegetative stage than during seedling and reproductive stages, while *Streptomyces* decreased significantly during the vegetative stage. *Nocardioides*, *Streptomyces*, *Agromyces*, and *Microvirga* abundances increased significantly during the reproductive stage. The withering stage showed no significant variation across the four phases. Generally, bacterial abundance distribution was more uniform at the genus level during seedling and vegetative stages. The appearance of different bacterial groups during different *A. squarrosum* developmental stages likely reflects plant recruitment to meet stage-specific needs.

3.4 Correlations Between Soil Nutrients and Bacterial Community Structure

Spearman correlation analysis between soil nutrient factors and bacterial taxa with >1.00% relative abundance revealed significant negative correlations between *Streptomyces*, *Agromyces*, *Nocardioides*, *Microviga*, and several soil indices [Fig. 4 [Figure 4: see original paper]]. *Paenarthrobacter*, *Arthrobacter*, and *Microbacterium* showed highly significant positive correlations with soil organic matter, available nitrogen, available phosphorus, urease, neutral phosphatase, and catalase ($P<0.010$). *Acinetobacter*, *Noviherbaspirillum*, *Pantoea*, and *Pseudomonas* demonstrated extremely significant positive correlations with soil organic matter, available nitrogen, sucrase, urease, and neutral phosphatase. *Acinetobacter* showed an extremely significant positive correlation with neutral protease. Bacterial taxa >1.00% relative abundance responded more strongly to soil organic matter, available nitrogen, sucrase, urease, and neutral phosphatase than to available phosphorus, available potassium, neutral protease, and catalase, indicating that *A. squarrosum* rhizosphere bacteria had higher demands for carbon and nitrogen than for phosphorus and potassium.

3.5 Prediction of Rhizosphere Bacterial Functions During Different Developmental Stages

PCA based on PICRUSt functional predictions showed clear separation of the four developmental stages along principal component 1 (PC1) and PC2 axes [Fig. 5 [Figure 5: see original paper]]. Seedling stage points were distant from all other stages, suggesting that rhizosphere bacterial functions during the seedling stage differed significantly from other stages and that some functions changed with plant development.

Table 2 illustrates predicted level 1 KEGG pathway functions, covering metabolism (50.96%-52.60% of total), environmental information processing, genetic information processing, cellular processes, human diseases, and organismal systems. Comparison between seedling and vegetative-reproductive-withering stages revealed four significantly different functions: metabolism and genetic information processing increased significantly in the vegetative-reproductive-withering group, while cellular processes and human diseases decreased significantly. These results indicate that cellular process functions were relatively strong during the *A. squarrosum* seedling stage, while metabolism and genetic information processing functions were enhanced as *A. squarrosum* entered its fast-growing stage.

Table 3 shows predicted level 2 KEGG functions (relative abundance >1.00%), comprising 18 functions. Global and overview maps, carbohydrate metabolism, and amino acid metabolism were main components, accounting for 38.96%-39.77%, 9.07%-9.39%, and 7.94%-8.30% of the total, respectively. During the seedling stage, carbohydrate metabolism, amino acid metabolism, lipid metabolism, and metabolism of terpenoids and polyketides were significantly decreased ($P<0.050$). During the vegetative stage, carbohydrate and nucleotide metabolism were relatively enhanced. During the reproductive stage, energy metabolism, lipid metabolism, biosynthesis of other secondary metabolites, and metabolism of terpenoids and polyketides increased significantly. During the seedling stage, membrane transport, signal transduction, and cellular community-prokaryotes increased significantly, while translation, replication, repair, folding, sorting, and degradation decreased significantly. No significant changes occurred during the withering stage.

3.6 Correlations of Functional Genes and Bacterial Community Structure

Correlation analysis between bacterial taxa >1.00% relative abundance and functional genes >1.00% relative abundance [Fig. 6 [Figure 6: see original paper]] revealed that *Arthrobacter*, *Paenarthrobacter*, and *Delftia* were significantly positively correlated with carbohydrate metabolism ($P<0.010$). *Arthrobacter*, *Nocardioides*, *Streptomyces*, and *Delftia* showed significantly positive correlations with amino acid metabolism. *Nocardioides*, *Streptomyces*, and *Agromyces* were significantly positively correlated with energy metabolism, lipid metabolism, biosynthesis of other secondary metabolites, and metabolism of terpenoids and polyketides. *Pantoea* and *Pseudomonas* showed significantly positive correlations with membrane transport, signal transduction, cellular community-prokaryotes, and metabolism of other amino acids. Additionally, *Pantoea* was significantly positively correlated with nucleotide metabolism and glycan biosynthesis and metabolism. These results suggest that *Arthrobacter*, *Paenarthrobacter*, *Nocardioides*, *Streptomyces*, and *Delftia* play major roles in regulating carbon and nitrogen metabolism and represent important bacterial groups during *A. squarrosum* vegetative and reproductive stages. *Nocardioides*, *Streptomyces*, and *Agromyces* regulate metabolism and biosynthesis of various compounds and are important during the reproductive stage. *Pantoea* and *Pseudomonas* are major regulators of cellular processes and important contributors to *A. squarrosum* stress resistance.

Discussion

The rhizosphere microbiome is crucial for plant growth and health, often termed the plant' s secondary genome [?, ?, ?]. Our 16S rRNA data provide snapshots of the microbiome at each developmental stage. Different plant developmental stages showed significant variations in bacterial abundances and functions, suggesting that soil microbial communities may selectively adopt particular functions as plants develop. During the *A. squarrosum* seedling stage, *Pantoea* contributed most significantly, with notable increases in membrane transport and signal transduction functions that correlated extremely positively with *Pantoea*. *Pantoea* is widely distributed in various plant seeds and typically dominates during seedling stages [?, ?, ?]. It not only promotes seedling growth and development but also secretes exopolysaccharides (EPSs) to retain water, thereby enhancing seedling drought tolerance [?, ?, ?]. Zhang et al. (2022) showed that rice core bacterial endophytes included *Pantoea* and *Xanthomonas*, with all *Pantoea* strains producing indole-3-acetic acid, a plant growth-promoting compound. Walitang et al. (2018) found that endophytic community profiles in salt-sensitive and salt-tolerant rice seeds changed significantly under salt stress, with core microbiota gradually shifting to include *Pantoea*. We therefore speculate that *Pantoea* may reach *A. squarrosum* seedling rhizospheres through vertical transmission. Since *A. squarrosum* seedlings face wind erosion and drought threats during desert colonization, the rhizosphere bacterial community responds positively to environmental stress by expressing genes involved in membrane transport and signal transduction. This expression helps maintain stable intracellular osmotic pressure and improves seedling adaptability. We also observed significantly increased sucrase activity during the seedling stage, with *Pantoea* showing significant positive correlations with sucrase and glycan biosynthesis and metabolism. Carbohydrates are essential cell membrane components that regulate shoot development, hypocotyl elongation, and cotyledon greening [?, ?, ?]. *A. squarrosum* seedlings grow rapidly by absorbing large amounts of nitrogen for amino acid and protein conversion, requiring carbohydrates [?, ?]. Enhanced sucrase activity promotes glycan biosynthesis and metabolism, thereby supporting rapid seedling growth.

During the *A. squarrosum* vegetative stage, *Arthrobacter* contributed most significantly, showing positive correlations with carbohydrate and amino acid metabolism, which were dominant level 2 functions. Among the 15 genera with >1.00% relative abundance, 13 showed significantly positive correlations with soil carbon and nitrogen cycle-related environmental factors. Nitrogen is a key factor regulating soil carbon supply and microbial activity [?, ?, ?]. *A. squarrosum* requires both nitrogen and carbon sources during its fast-growing stage, promoting soil nitrogen and carbon cycles and leading to significant increases in functional gene and enzyme expression involved in these cycles. Ye et al. (2014) found that *Arthrobacter ps-5* was a high EPS-producing strain;

EPS has high water retention capacity, with each gram retaining more than 70 g of water [?, ?]. Thus, PICRUSt functional predictions indicated significantly increased carbohydrate metabolism gene abundances during the vegetative stage, which may promote carbon-rich substrate availability. These carbon substrates could stimulate dominant *Arthrobacter* to produce large amounts of EPS, improving *A. squarrosum* drought tolerance.

Bacterial diversity and richness in the *A. squarrosum* rhizosphere increased significantly during the reproductive stage, with *Bacteroidetes*, *Chloroflexi*, *Acidobacteria*, *Gemmatimonadetes*, and the genera *Nocardioides*, *Streptomyces*, *Agromyces*, and *Microvirga* showing significant increases. Higher bacterial diversity during the reproductive stage suggests that diverse bacterial co-operation may be driven by increasing metabolic exchange. The lack of a complex bacterial community during other stages may indicate that many root-related functions are inactive or dormant [?, ?]. Studies have shown that root exudates' main components are soluble sugars that serve as primary energy and carbon sources for rhizosphere microorganisms near growing roots [?, ?, ?]. Yuan et al. (2016) found that root exudate carbon was rapidly assimilated by rhizosphere microorganisms, with secretion rates increasing with rice maturity. Correlation analysis also showed significant associations between microbial biomass and photosynthetic products. We therefore expect that increased root secretions during *A. squarrosum*' s reproductive stage elevated Shannon, Shannon-evenness, and Sobs indices of rhizosphere bacteria. During the withering stage, root secretion cessation leads to significant rhizosphere bacterial diversity reduction. Highly diverse taxa likely play distinct roles in bacterial communities during the reproductive stage. *Streptomyces* showed positive correlations with amino acid metabolism, energy metabolism, lipid metabolism, biosynthesis of other secondary metabolites, and metabolism of terpenoids and polyketides. Host plants induce rhizosphere microbial reassembly during development in natural environments [?, ?, ?]. According to Rajguru et al. (2024), rhizosphere microbial community structure varies with developmental stages as plants secrete substances that benefit specific bacteria while suppressing others, ultimately increasing dominant bacterial taxa. This phenomenon was observed from *A. squarrosum*' s vegetative to reproductive stages as nutrient allocation shifted from vegetative to reproductive organs. Root exudates may recruit specialized bacterial groups to perform specialized functions, inducing significant increases in functional genes involved in complex compound metabolism [?, ?].

During *A. squarrosum*' s withering stage, neutral protease activity decreased significantly. Neutral proteases are serine proteases secreted by various microorganisms during growth that hydrolyze proteins into small peptides or amino acids usable by plants and microorganisms, thereby improving viability and environmental adaptability under stress [?, ?, ?]. Plant metabolite secretion relates to rhizosphere bacterial recruitment and induction. During *A. squarrosum*' s withering stage, weakened recruitment and induction reduce plant rhizosphere community richness [?, ?], consequently affecting neutral protease activity in rhizosphere soil.

Conclusions

We analyzed variations in *A. squarrosum* rhizosphere bacterial communities across developmental stages, revealing significant changes in both diversity and functions that highlight their important roles in *A. squarrosum* growth. *Pantoea* dominated during the seedling stage, with functional genes associated with environmental stress response showing synergistic alterations in the *Pantoea* group. *Arthrobacter* dominated during the vegetative stage, with functional genes related to carbon and nitrogen metabolism showing synergistic alterations in the *Arthrobacter* group. Bacterial diversity increased significantly during the reproductive stage, with functional genes involved in energy metabolism, complex compound synthesis, and metabolism showing significantly higher abundances compared to other stages. We speculate that greater bacterial abundance and more functional interactions occurred during the reproductive stage than during other developmental stages. These results guide screening of plant growth-promoting bacteria, with special attention warranted for *Pantoea* and *Arthrobacter*. *Pantoea* may serve as a seed coating agent for psammophytes due to its ability to help seedlings resist adverse conditions, while *Arthrobacter* may function as a topdressing microbial fertilizer (root fertilization) for psammophytes by enhancing carbon and nitrogen metabolism in the rhizosphere during vegetative growth. These findings provide a basis for "microbial-driven" vegetation construction in desertified lands. It is important to note that PICRUSt prediction has limitations, and future work should combine this approach with methods such as metagenomic sequencing to predict functional genes.

Conflict of Interest

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

Acknowledgements

This research was supported by the Natural Science Foundation Project of Inner Mongolia Autonomous Region (2024LHMS03017) and the Research Capacity Enhancement "Unveiling and Leading" Project of Inner Mongolia Forestry Science Research Institute (2024NLTS03).

Author Contributions

Conceptualization: ZHANG Shengnan, GAO Haiyan, YAN Deren; Methodology: ZHANG Shengnan, YANG Shanshan; Formal analysis: ZHANG Shengnan, ZHANG Lei; Writing –original draft preparation: ZHANG Shengnan, GAO Haiyan, YANG Shanshan; Writing –review and editing: ZHANG Shengnan, GAO Haiyan; Funding acquisition: ZHANG Shengnan, ZHANG Lei; Resources: HUANG Haiguang, YANG Zhiguo, LI Junwen, TANG Yuekun, XU Hongbin; Supervision: ZHANG Lei. All authors approved the manuscript.

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