Qinghai Lake in metagenomics Study on soil microbial diversity in Gangcha County

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Research Article

Keywords: Qinghai Lake, Poa alpigena Lindm, Rhizosphere microorganisms, Metagenomics

Posted Date: July 31st, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3194420/v1

Abstract

The Qinghai Lake Basin, situated in the northeastern part of the Qinghai-Tibet Plateau, is recognized as the "Third Pole" of the world. It serves as a pivotal aquatic ecosystem for upholding the ecological security of the northeastern Qinghai-Tibet Plateau, thereby carrying substantial significance for the conservation of this region's ecology. *Poa alpigena* Lindm, a prevalent and dominant grass species across the Qinghai-Tibet Plateau, plays a crucial role in soil and water conservation within the Qinghai Lake Basin. Soil microorganisms actively engage in root-soil interactions, exerting paramount influence on plant growth, health, and adaptability. In this study, we investigated the rhizosphere and non-rhizosphere soils of *Poa tableland* in the Gangcha region of Qinghai Lake. We examined the impact of *Poa tableland* on the composition and structure of soil microbial communities, while analyzing the diversity and disparities of microorganisms in these two soil types.The findings of this study indicate that the non-rhizosphere soil in the Gangcha region exhibits significantly higher microbial abundance and diversity compared to the rhizosphere soil. However, the proportions of dominant microorganisms show minimal variation between the two soil types. It is evident that the root system of *Poa* grass exerts a strong selective influence on the microbial assemblages in the soil environment. Analysis of KEGG metabolic pathways reveals notable enrichment of pathways related to photosynthesis and energy synthesis in the rhizosphere microbiota, whereas pathways associated with gene expression display significant enrichment in the non-rhizosphere soil microbiota. Moreover, the examination of dominant microorganisms across all soil samples reveals the presence of mutual inhibition or promotion relationships among different microbial taxa.

1. Introduction

Soil microorganisms play an indispensable role in terrestrial ecosystems, actively participating in the biogeochemical cycles of soil organic matter decomposition, nutrient mineralization, and energy transformation (Bakker et al. 2018; Ali & Xie 2020; Coban et al. 2022). Their activities are vital for maintaining soil ecological balance and have significant implications for soil development, nutrient cycling, and pollution remediation (Backer et al. 2018; Bhattacharyya et al. 2021). Rhizosphere microorganisms, as a subset of soil microbial communities, engage in intricate interactions with plants (Ali & Xie 2020). They contribute essential nutrients, facilitate plant growth, and play a pivotal role in plant development, as well as in the biological control of plant pests and diseases (Ding et al. 2019).

Qinghai Lake is situated within the environmentally sensitive arid/semi-arid region of northwestern China and represents the largest inland saline lake on the Qinghai-Tibet Plateau (Fu et al. 2021). Its significance extends to the ecological security of the northeastern region, as well as its role in mitigating the eastward expansion of desertification in western China, thereby holding profound implications for the ecological preservation of the Qinghai-Tibet Plateau (Jia et al. 2021). The dynamic changes in land use patterns within the Qinghai Lake basin have led to frequent fluctuations in vegetation coverage and continuous alterations in soil nutrient content, further exacerbating the susceptibility of the delicate ecosystem (Li et al. 2021). The growth and development of plants necessitate material exchanges and information

transmission with the soil, wherein the rhizosphere constitutes a distinct ecological milieu facilitating their intricate interactions (Wang et al. 2019). *Poa alpigena* L., as a predominant grass species widely distributed across the Qinghai-Tibet Plateau, finds frequent application in livestock production and the reseeding and rehabilitation of degraded grasslands (Fu et al. 2021). Therefore, investigating the diversity and characteristics of microbial communities in the rhizosphere and non-rhizosphere soils of *Poa alpigena* L. offers invaluable insights into the restoration and management of degraded grasslands on the Qinghai-Tibet Plateau.

This study utilized metagenomic sequencing in conjunction with bioinformatics tools to investigate the disparities in microbial communities between rhizosphere and non-rhizosphere soils associated with indigenous plant species. The objective was to discern the key factors influencing variations in microbial community structure and provide a theoretical foundation for examining the relationship between environmental changes and soil quality, thus contributing to the advancement of sustainable development in the Qinghai Lake region.

2. Materials and Methods

2.1 *Profile of the Study Area*

Gangcha County (GC) is situated on the northern shoreline of Qinghai Lake (longitude 99°20'-100°37', latitude 36°58'-38°04') at an elevation range of 3300–3800 meters. It falls within the domain of a high-altitude plateau continental climate, characterized by a meager annual average precipitation of less than 371 millimeters and an approximate average annual temperature of -0.6℃. The region is classified within the highland semi-arid and high-cold climate belt. The dominant vegetation types in this area encompass *Poa alpigena* L., *Stipa purpurea*, *Carex moorcroftii*, *Kobresia pygmaea* and *Kobresia humilis*. The soil samples collected for the present study were obtained from the specific location referred to as Gangcha County, the latitude and longitude of Poa rhizosphere soil and non-rhizosphere soil were 37°29 'N, 100°07' E (Fig. S1).

2.2 Sampling Treatment

In August 2020, the fenced and undisturbed *Poa alpigena* L. community in the National Positioning Observation and Research Station of the Qinghai Lake Wetland Ecosystem (Ganzi River Station) was selected as the research object. Five parallel plots of 2 m × 2 m were randomly set in this community, and each plot was more than 20 m away from the nearest neighboring plot. Within each quadrat, five individual plants of species *Poa alpigena* L., exhibiting similar developmental stages, were purposefully chosen using a systematic "S"-shaped sampling design.

For the collection of rhizosphere soil, the shaking method was employed. Soil adhering to the roots within a strong adhesion range of 0–1 mm was meticulously removed using a sterile soft-bristle brush, and this soil fraction was designated as the rhizosphere soil (GCG). Non-rhizosphere soil samples were collected from the vertical surface within the projected area of the plant roots, at a depth of 0–20 cm.

Subsequently, the four rhizosphere and four non-rhizosphere soil samples were aseptically transferred into sterile containers and accurately labeled as follows: rhizosphere soil samples were denoted as GCG1, GCG2, GCG3, and NG4, while non-rhizosphere soil samples were labeled as GCC1, GCC2, GCC3, and GCC4. Following the removal of roots, leaves, and stones, all collected soil samples were immediately stored at -80°C for subsequent DNA extraction and metagenomic sequencing.

Throughout the sampling procedure, the researchers strictly adhered to aseptic practices, wearing sterile masks and gloves. The entire plant was excavated using a sterile soil shovel (13 cm × 40 cm), and the rhizosphere soil was delicately brushed using a sterile soft-bristle brush. After each sample collection, masks, gloves, and brushes were replaced, and the soil shovel was disinfected using 75% ethanol to minimize any potential contamination.

Following the established protocol for soil DNA extraction and purification described by Vettraino et al., the sodium dodecyl sulfate (CTAB) method was employed for DNA extraction. The DNA concentration was determined using a fluorometric quantification assay with a Qubit fluorometer. Additionally, the integrity of the extracted DNA was assessed using 1% agarose gel electrophoresis. Subsequently, the DNA samples were submitted to BGI Genomics for high-throughput metagenomic sequencing using the BGISEQ-500 platform. Prior to sequencing library preparation, all DNA samples, encompassing bacterial, archaeal, fungal, viral, and protist DNA, were subjected to fragmentation into cDNA fragments (~ 300 bp) through controlled enzymatic shearing. The resulting cDNA fragments (~ 300 bp) were then subjected to paired-end sequencing on the BGISEQ-500 platform, generously provided by BGI Shenzhen, to generate raw sequencing data.

2.3 Data collection

In order to obtain high-quality and reliable sequencing data, the raw sequencing reads were subjected to data preprocessing using Trimmo-matic software (version 3.3). This preprocessing step involved the removal of adapter sequences, as well as the elimination of low-quality fragments, following the default parameters. The resulting cleaned reads, free from adapter contamination, are presented below.

PrefixPE/1: AAGTCGGAGGCCAAGCGGTCTTAGGAAG

PrefixPE/2:AAGTCGGATCGTAGCCATGTCGTTCTGTGA

The raw sequencing data from all samples were processed and organized, followed by metagenomic assembly using the Megahit software (https://github.com/voutcn/megahit). The assembled contigs were then compared to reference sequences using the Metaquast software (http://bioinf.spbau.ru/metaquast) to evaluate key metrics, including the number of high-quality contigs, the length of the longest contig, and the N50 value.To assess taxonomic composition, the obtained contigs were subjected to cross-mapping against the NCBI database, which encompasses genomes of bacteria, fungi, viruses, protozoa, and other organisms. The Kraken v2 software (https://anaconda.org/bioconda/kraken2) was employed for this purpose, enabling taxonomic annotation and analysis at various classification levels, including phylum, class, order, family, genus, and species. Alpha diversity analysis, incorporating observed species, Chao1,

Shannon, Simpson, and other diversity indices, was performed to evaluate the complexity of species diversity. Beta diversity analysis, employing Principal Coordinate Analysis (PCoA), was utilized to assess dissimilarities in species complexity among samples. Visualization of these analyses was accomplished using R software (version 4.2.1).

Identification of indicator microorganisms and characterization of metabolic pathways were achieved through LEfSe analysis of intergroup microbial data and cumulative metabolic pathway data (KEGG analysis), respectively. Finally, statistical analysis of experimental data was conducted using SPSS software (version 26.0).

The metagenomics sequencing data acquired in this study are available at the National Center for Biotechnology Information, USA, (https://www.ncbi.nlm.nih.gov/sra/PRJNA867494).

3. Results

3.1 Soil microbial composition of Poa alpigena L. *on Gangcha County*

Through comparative analysis using Kraken 2 and the NCBI database, the sequencing data obtained from soil samples of *Poa alpigena* L. in Gangcha County demonstrated sufficient sequencing depth, with an average number of sequences exceeding 36 million paired-end reads. This indicates a robust and reliable experimental outcome with minimal fluctuations. Subsequent sequence analysis revealed that more than 58% of the sequences were unassigned, of which approximately 36–39% could be attributed to the microbial domain. Microbial classification unveiled the presence of bacteria, fungi, viruses, and protozoa within the soil samples of the investigated area. Notably, bacteria emerged as the predominant microbial component, representing 28.55–31.32% of all microbial sequences. The bacterial fraction exceeded 50%, underscoring their crucial role in shaping the soil microbial community. Conversely, fungi, viruses, and protozoa exhibited relatively lower proportions in both rhizosphere and non-rhizosphere soils. Specifically, fungi accounted for 1.45% of the total sequences, viruses accounted for 0.15%, and protozoa accounted for 0.18% (See Table 1). However, these inter-group disparities did not attain statistical significance.

Table 1
Results of soil microbial sequencing.

Project	Non-rhizosphere soil (GCC)	Rhizosphere soil (GCG)
Number of raw reads	35127521.25 ± 1820525.18 a	35,588,777 ± 1508136.86 a
Classified reads (%)	43.32% ± 1.06% a	44.20% ± 1.08% a
Chordate reads (%)	6.02% ± 0.10% a	6.11% ± 0.04% a
Unclassified reads (%)	56.68% ± 1.06% a	55.80% ± 1.08% a
Microbial reads (%)	37.15% ± 1.15% a	37.95% ± 1.06% a
Bacterial reads (%)	29.72% ± 1.17% a	30.32% ± 1.00% a
Viral reads (%)	0.16% ± 0.00% a	0.15% ± 0.00% a
Fungal reads (%)	1.35% ± 0.03% a	1.43% ± 0.02% a
Protozoan reads (%)	0.17% ± 0.00% a	0.18% ± 0.00% a

3.2 Microbial β-Diversity

Principal Component Analysis (PCA) was employed to perform comparative analysis among the samples within the groups, demonstrating pronounced dissimilarities between the soil microbial communities. At the genus level, the cumulative interpretability of PC1, PC2, and PC3 reached 98.1%. The GCG group predominantly clustered towards the positive end of PC3, while the GCC group exhibited a positioning towards the negative end of PC3, indicating that the primary disparities between the two groups were predominantly discernible in the third principal component (Fig. 1).

3.3 Microbial α-Diversity

α-diversity serves as a measure of species richness within a given sample, thereby facilitating the assessment of potential differences in microbial species richness between rhizosphere and non-rhizosphere soil samples. Various diversity indices, including Chao1, richness index, Shannon index, and Simpson index, are employed to quantify the magnitude of species richness and diversity. Higher values of Chao1, richness index, and Shannon index are indicative of greater species richness, while the Simpson index reflects both species richness and evenness. In accordance with the data analysis, non-rhizosphere soil exhibited elevated levels of microbial species richness compared to the rhizosphere soil (Fig. 2).

3.4 Quantification of soil microbial populations in the rhizosphere and non-rhizosphere environments

Through statistical analysis and assessment of the dominant microbial taxa in the soil samples, a remarkable level of microbial diversity was observed across all analyzed samples (Table S1). At the

phylum level, the top 15 phyla present in both rhizosphere and non-rhizosphere soils accounted for a substantial portion of the microbial composition, with Proteobacteria exhibiting the highest representation, comprising approximately 56% of the total sequences, followed by Actinobacteria, which accounted for 37% (Fig. 3a). Notably, other significant microbial, including *Planctomycetes*, *Ascomycota*, *Firmicutes*, *Bacteroidetes*, *Euryarchaeota*, were detected in varying abundances in both types of soil. At the genus level, *Streptomyces* exhibited the highest relative abundance in both rhizosphere and non-rhizosphere soil microbial communities, representing approximately 31% of the total sequences, while *Pseudomonas* accounted for 12% (Fig. 3b).

3.5 Rhizosphere and non-rhizosphere dominant soil microorganisms

In order to gain further insights into the microbial community structure, we conducted differential species phylogenetic tree analysis and LEfSe (Linear discriminant analysis Effect Size) analysis for the root-associated (GCG) and non-root-associated (GCC) microorganisms, as depicted in Fig. 4. By employing the LRFSe differential analysis, we identified microbial taxa exhibiting differential abundances at the class and genus levels between the GCG and GCC groups. At the order level, taxa A and B demonstrated dominance within the GCG group. Similarly, at the family level, taxa C and D exhibited significant prevalence. Notably, at the class level, the abundance of taxa E and F was predominantly observed in the GCC group, while at the order level, taxa G and H exhibited higher abundance within the GCC group (Fig. 4a).

3.6 Differences in Metabolic Pathways between GCG and GCC

The KEGG enrichment analysis of the GCG and GCC groups revealed statistically significant enrichments in specific metabolic pathways. The GCG group exhibited significant enrichments in pathways related to carotenoid biosynthesis, monoterpenoid biosynthesis, phenylpropanoid biosynthesis, and the PI3K-Akt signaling pathway. Conversely, the GCC group displayed significant enrichments in pathways associated with pyrimidine metabolism, homologous recombination, ubiquinone and other terpenoid-quinone biosynthesis, RNA degradation, sulfur relay system, and non-homologous end joining.To elucidate the interrelationships among microorganisms, we conducted correlation analysis among the top 19 taxonomic groups in terms of their abundance within the GCG and GCC groups, as illustrated in Fig. 6. Each blue data point in the figure represents a distinct microbial taxon, with the size of the data point corresponding to its relative abundance. The dark red and light blue connecting lines denote positive and negative correlations, respectively, between soil microorganisms. The thickness of the connecting line indicates the magnitude of the correlation between the two bacterial taxa. The results revealed that *Sphingopyxis* exhibited the highest abundance, followed by *Sphingomonas*, while *Cupriavidus*, *Lysobacter*, *Mycobacterium* and *Baekduia* showed relatively elevated abundances (Fig. 5).

3.7 Correlation analysis of GCG and GCC

In order to investigate the interrelationships among microorganisms, we conducted a correlation analysis of the top 19 microorganisms in the GCC and GCG groups, as depicted in Fig. 6. Each data point in the figure represents a distinct microbial taxon, with the size of the point indicating its relative abundance. The connecting lines, shaded in dark red and light blue, represent positive and negative correlations, respectively, between soil microorganisms. The thickness of the lines represents the absolute magnitude of the correlation between the two bacterial taxa. The results unveiled that Sphingopyxis exhibited the highest abundance, followed by Sphingomonas, Cupriavidus, Lysobacter, Mycobacterium, and Baekduia (Fig. 6). Furthermore, notable positive and negative correlations were observed among the abundances of different microorganisms, suggesting that the composition of the plant rhizosphere microbial community is intricately influenced by both abiotic environmental factors and the complex interactions within the microbial community.

4. Discussion

4.1 The root system demonstrates a discerning selectivity towards microorganisms

The growth and development of plants are intricately influenced by the surrounding environment. Microorganisms such as bacteria and fungi in the soil are attracted to and accumulate in the rhizosphere soil due to the root exudates of plants (Brunel et al. 2020; Bhattacharyya et al. 2021). Within the context of plant growth and development, these microorganisms play a pivotal role. By decomposing plant and animal matter, soil microorganisms have the ability to directly impact soil fertility (Hemkemeyer et al. 2021; Coban et al. 2022; Gupta et al. 2022). The fluctuations in soil fertility directly correlate with the nutrient requirements for plant growth and development. In essence, the rhizosphere serves as a crucial interface for the intricate interactions among soil, microorganisms, and plants. It functions as a vital gateway and conduit for the transfer of nutrients or detrimental substances from the soil into the plant (Mendes et al. 2013; Kremer 2019; Ali & Xie 2020; Mesa 2022). Consequently, rhizosphere microorganisms hold significant importance in various aspects, including plant growth, development, and resilience to adverse conditions (Qu et al. 2020).

In our research, a comprehensive analysis of four indices, namely Chao 1, richness, Simpson, and Shannon, was conducted to investigate the microbial species richness in the rhizosphere and non-rhizosphere soil samples surrounding *Poa alpigena* L. in Gangcha County. The results indicated that the non-rhizosphere soil exhibited a higher microbial species richness compared to the rhizosphere soil. This finding supports the notion that plant roots are associated with complex physiological activities. Although different plant species exhibit distinct physiological and biochemical characteristics, a shared feature is the selective influence of root exudates on microbial communities (Niu et al. 2017; Huang et al. 2019). Consequently, the quantity of microorganisms in the rhizosphere soil is several to several tens of

times greater than that in the non-rhizosphere soil, while the transition from non-rhizosphere to rhizosphere soil is accompanied by a decline in bacterial community diversity (Li et al. 2016; Backer et al. 2018; Gupta et al. 2022; Han et al. 2022).

This study is based on the analysis of microorganisms in samples using Stamp differences, revealing significant variations in the abundance of different types of microorganisms in different soil environments. *Mucor* was chosen as an example in this study because it is a typical endophytic bacterium in highland *Poa*, forming a symbiotic relationship with highland *Poa* and exhibiting strong degradation and survival capabilities. However, the abundance of *Mucor* is significantly lower in non-rhizosphere environments. In other words, the likelihood of microbial abundance variations in different soil environments is closely associated with the plants themselves (Moscatiello et al. 2010; Fu et al. 2021; Wang et al. 2022). Leaf analysis using differential species plane branch diagrams clearly indicates significant differences in the species composition of rhizosphere and non-rhizosphere soil microorganisms, with the activity of *γ-proteobacteria* being weakest in non-rhizosphere soil, while the abundance of monobacteria is highest in the rhizosphere soil. Studies exploring the impact of plant root exudates on microorganisms have found that amino acids, as carbon sources, are preferred by certain specific microorganisms, which can increase their species abundance. On the other hand, the lack of differences in certain microorganisms may be attributed to physical and chemical factors unrelated to the plant roots, affecting their abundance (Backer et al. 2018; Huang et al. 2019; Panchal et al. 2022).

4.2 The taxonomic composition of microbial communities

Studies have indicated that oligotrophic bacteria, such as *Acidobacteria*, prevail in non-rhizosphere soil in terms of abundance. In contrast, the microbial communities inhabiting the rhizosphere soil of plants are predominantly composed of copiotrophic bacteria characterized by fast growth rates, including *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Actinobacteria*. These copiotrophic bacteria possess the capability to release and facilitate the uptake of plant-available potassium, phosphorus, and other trace nutrients (Bakker et al. 2018; Bhattacharyya et al. 2021; Zhang et al. 2022a). In this study, metagenomic profiling was employed at six taxonomic levels (phylum, class, order, family, genus, and species) to investigate the microbial assemblages present in both rhizosphere and non-rhizosphere soil associated with *Poa*. Analysis of primary microbial content revealed *Actinobacteria* and *Actinobacteria* to exhibit the highest proportions, collectively constituting over 90% of the total microbial content. Specifically, the genus *Streptomyces* demonstrated the highest prevalence, followed by the genus *α-Proteobacteria*, which together accounted for over 60% of the total microbial abundance. These findings underscore the dominance of *Actinobacteria*, particularly the genus *Streptomyces*, within the soil microbial communities. However, notable variations were observed in terms of specific proportions, other prevalent bacterial groups, and higher taxonomic levels, consistent with previous research outcomes (Zhang et al. 2021; Sarkar et al. 2022). Comparative analysis with findings from other scholars suggests that these discrepancies may arise from substantial geographic disparities in sampling locations, including factors such as temperature, humidity, and altitude, contributing to divergent soil conditions (Ficano et al. 2021; Wang et al. 2021; Mesa 2022). Consequently, significant disparities exist in the composition of native

plant species across distinct soil environments, thereby influencing the physiological activities and metabolic outputs of plant roots. Consequently, different microbial groups are subject to varying degrees of inhibition or promotion. Notably, no significant distinctions were observed in the microbial content between the rhizosphere and non-rhizosphere soil of the same plant species (Zhang et al. 2021; Ma et al. 2022; Zhang et al. 2022b). This can be attributed to the fact that these plant populations exhibit characteristics of r-selected organisms, characterized by high birth rates, small individual sizes, strong dispersal capabilities, and adaptability to changing habitats. Consequently, when exposed to novel soil environments, they swiftly establish dominance within the soil microbial community, overriding extrinsic inhibitory factors (Li et al. 2015; Mikheenko et al. 2016; Qu et al. 2020; Jia et al. 2021; Hou et al. 2023).

4.3 Significant differences exist in the metabolic pathways of soil microorganisms

Previous research has demonstrated that under extreme environmental conditions, the synthesis of chlorophyll metabolism is reduced while the synthesis of carotenoid metabolism is enhanced. This is attributed to the protective role of carotenoids in mitigating phototoxic damage to plants (Panchal et al. 2022; Xu et al. 2022; Mathew et al. 2023). Microbial KEGG pathway analysis reveals that different soil environments exert diverse effects on microbial metabolism. Specifically, the rhizosphere soil exhibits significant enrichment of metabolic pathways related to plant photosynthesis and signal transduction, including carotenoid biosynthesis that is reliant on plant root systems. Furthermore, the PI3K-Akt signaling pathway is also prominently enriched. In addition to these representative metabolic pathways, the biosynthesis of monoterpenes and phenylpropanoids is significantly enriched as well (You et al. 2014; Bhattacharyya et al. 2021). These metabolic pathways serve as sources of energy for biological activities and contribute to the synthesis of precursor molecules for various biological processes. These findings highlight the robust metabolic activity and high energy demands of microorganisms in the vicinity of plant root systems (Panchal et al. 2022; Vettraino & Luchi 2022). To ensure their survival in the challenging environments surrounding Qinghai Lake, plants inevitably augment the synthesis of carotenoids. Likewise, rhizosphere soil microorganisms undergo comparable metabolic adaptations, suggesting a mutually beneficial symbiotic relationship between microorganisms and plants mediated through the rhizosphere.

Conversely, metabolic pathways associated with gene expression are notably enriched in non-rhizosphere soil microorganisms, such as pyrimidine metabolism and homologous recombination. The enrichment of these metabolic pathways correlates directly with the pronounced gene expression observed in non-rhizosphere soil microorganisms (Wang et al. 2019). This phenomenon can be attributed to the more rigorous environmental stressors encountered by microorganisms in non-rhizosphere environments, including drought and low temperature, without the assistance of plants. Consequently, microorganisms in these environments undergo autonomous adaptations to survive, leading to enhanced microbial respiration and metabolic efficiency (Coban et al. 2022; Panchal et al. 2022; Mathew et al. 2023). This observation aligns with the significant enrichment observed in the metabolism analysis of ubiquinone

and other terpenoid quinone biosynthesis processes. Ubiquinone, a well-known coenzyme Q in biochemical literature, plays a crucial role in microbial respiration (Jia et al. 2021; Han et al. 2022).

4.4 Interactions among soil microorganisms

The rhizosphere soil harbors a substantial population of microorganisms that possess specific adaptations to the root environment, exerting influences on the structure of rhizosphere microbial communities (Trivedi et al. 2020; Bhattacharyya et al. 2021; Sarkar et al. 2022; Mathew et al. 2023). To gain a comprehensive understanding of these potential relationships, we investigated the correlations among dominant microbial abundances. Previous studies have revealed that the bacterial community structure in the rhizosphere of sand dropseed grass is predominantly shaped by the bacterial community composition in the non-rhizosphere soil, suggesting that sand dropseed grass does not exhibit preferential selection of specific detrimental or beneficial microbial communities (Bulgarelli et al. 2015; Niu et al. 2017; Gao et al. 2020). Thus, the composition of microbial communities in the non-rhizosphere soil plays a pivotal role in determining the assembly of rhizosphere microbial communities. By conducting correlation analysis on rhizosphere and non-rhizosphere soil microorganisms, it was observed that *Pseudomonas* and *Burkholderia* mutually promote their growth, while *Streptomyces* and *Rhizobium*, as well as *Actinomycetes* and *Bradyrhizobium*, exhibit mutualistic interactions in terms of growth promotion and reproduction. Conversely, *Pseudomonas* growth is inhibited by copper, and it exerts inhibitory effects on *Nocardia* growth (Trivedi et al. 2020; Jia et al. 2021). Moreover, in comparison to the non-rhizosphere soil, the rhizosphere microenvironment exhibits a higher microbial cell density and more intricate interactions among rhizosphere microorganisms, predominantly characterized by mutualistic associations. This implies that rhizosphere microorganisms possess greater potential for mutualistic symbiosis. For instance, under nutrient-restricted conditions, the interactions between rhizobia and arbuscular mycorrhizal fungi (AMF) with leguminous plants involve the provision of diverse nutrients that facilitate plant growth, consequently influencing the composition of rhizosphere microbial communities associated with leguminous plants (Luo et al. 2020; Ficano et al. 2021; Thanni et al. 2022). Nonetheless, the mechanisms underlying plant-microorganism interactions are complex, and several aspects remain elusive. In the realm of agricultural production, elucidating effective agricultural practices for sustaining a healthy rhizosphere microbial community in plants, thereby enhancing disease resistance, stress tolerance, and nutrient utilization efficiency, represents a crucial avenue for future research endeavors (Raklami et al. 2019; Thanni et al. 2022).

Declarations

Acknowledgements

This work was supported by The Natural Science Research Project of Anhui Provincial Colleges and Universities (Grant No. KJ2020A0118). Natural Science Foundation of Anhui Province (grant No. 2208085QC68); Qinghai Provincial science and technology Plan (Grant No. 2023-ZJ-905T).

Funding

Not applicable.

Competing interest

There are no conflicts of interest.

Data availability

The metagenomics sequencing data have been uploaded to NCBI.

Author contributions

Zhiqiang Dong performed the experiments, prepared figures and/or tables, wrote original draft, authored or reviewed drafts of the paper, and approved the final draft. Xuewei Xu, Xia Wang, Nannan Dong, Kelong Chen, carried out the plot investigation, performed the experiments, Lingling Li assisted with data curation and formal analysis, and reviewed and edited the paper. Cheng Cheng and Yahui Mao carried out the experimental conceptualization, conceived and designed the experiments, and supervised the final draf.

Declaration of competing interest

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

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Figures

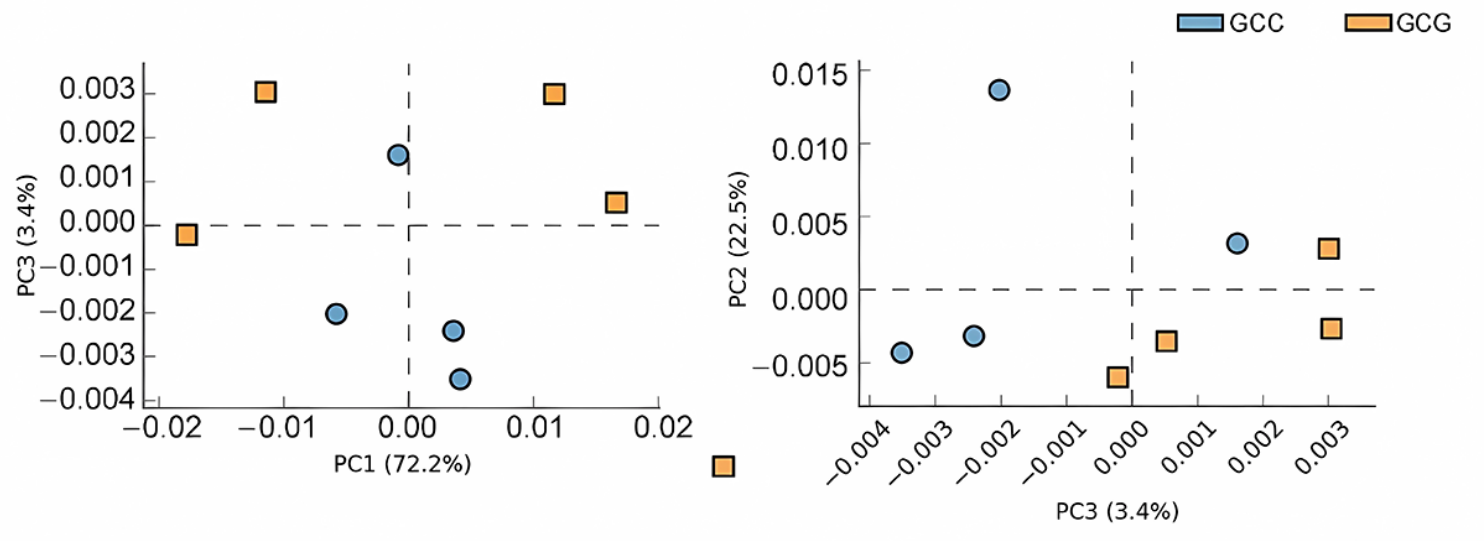

Figure 1

PCA score/load diagram analysis of rhizosphere and non-rhizosphere microorganisms. GCC, plant rhizosphere; GCG, rhizosphere soil.

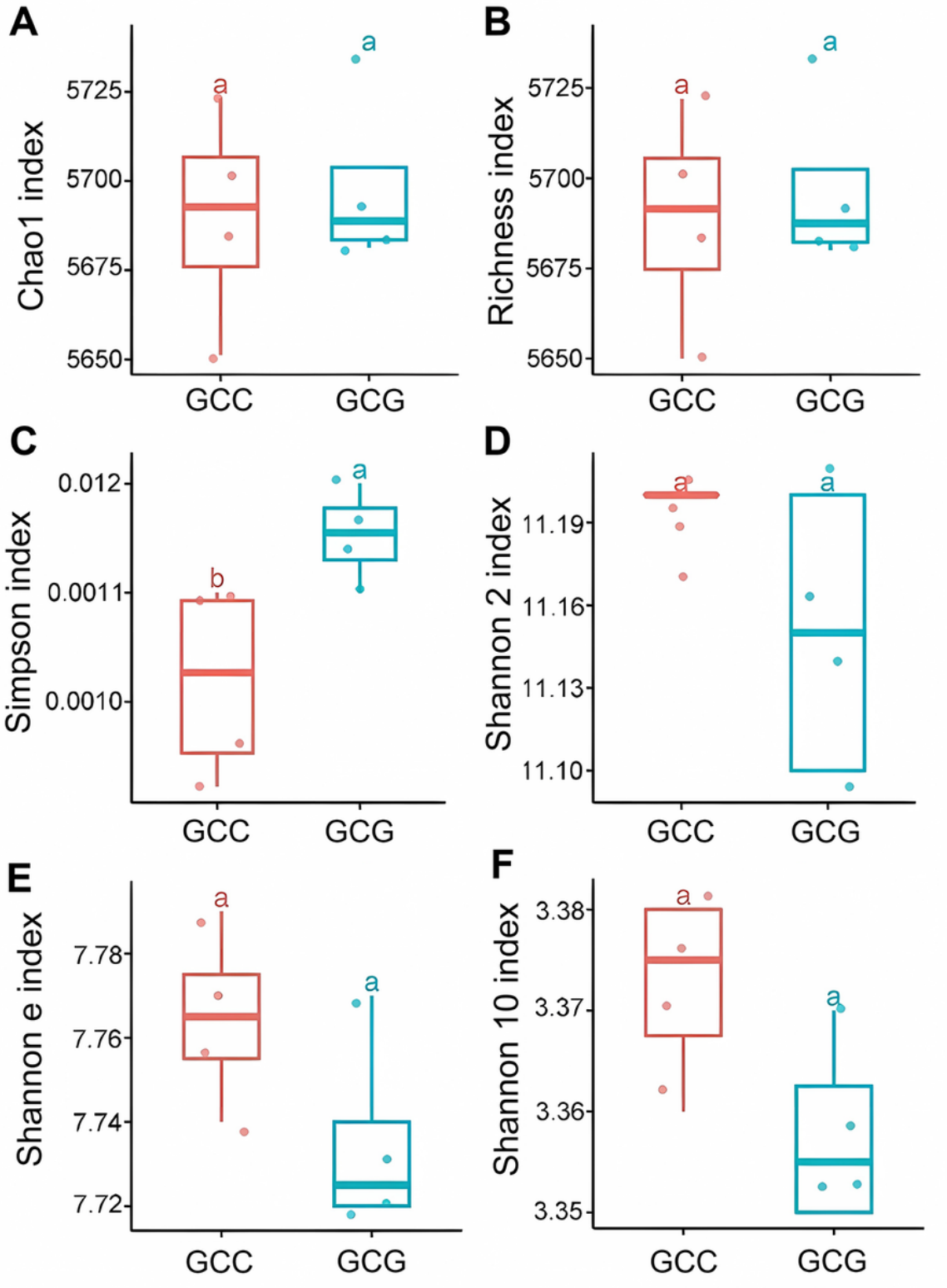

Figure 2

Box-plot of α-diversity index. (A) Chao1 index. (B) Richness index. (C) Simpson index. (D) Shannon 2 index. (E) Shannon e index. (F) Shannon 10 index. GCC: plant rhizosphere; GCG: rhizosphere soil.

Note: The same colored letters in the figure indicate no significant difference, and the different colored letters indicate significant differences.

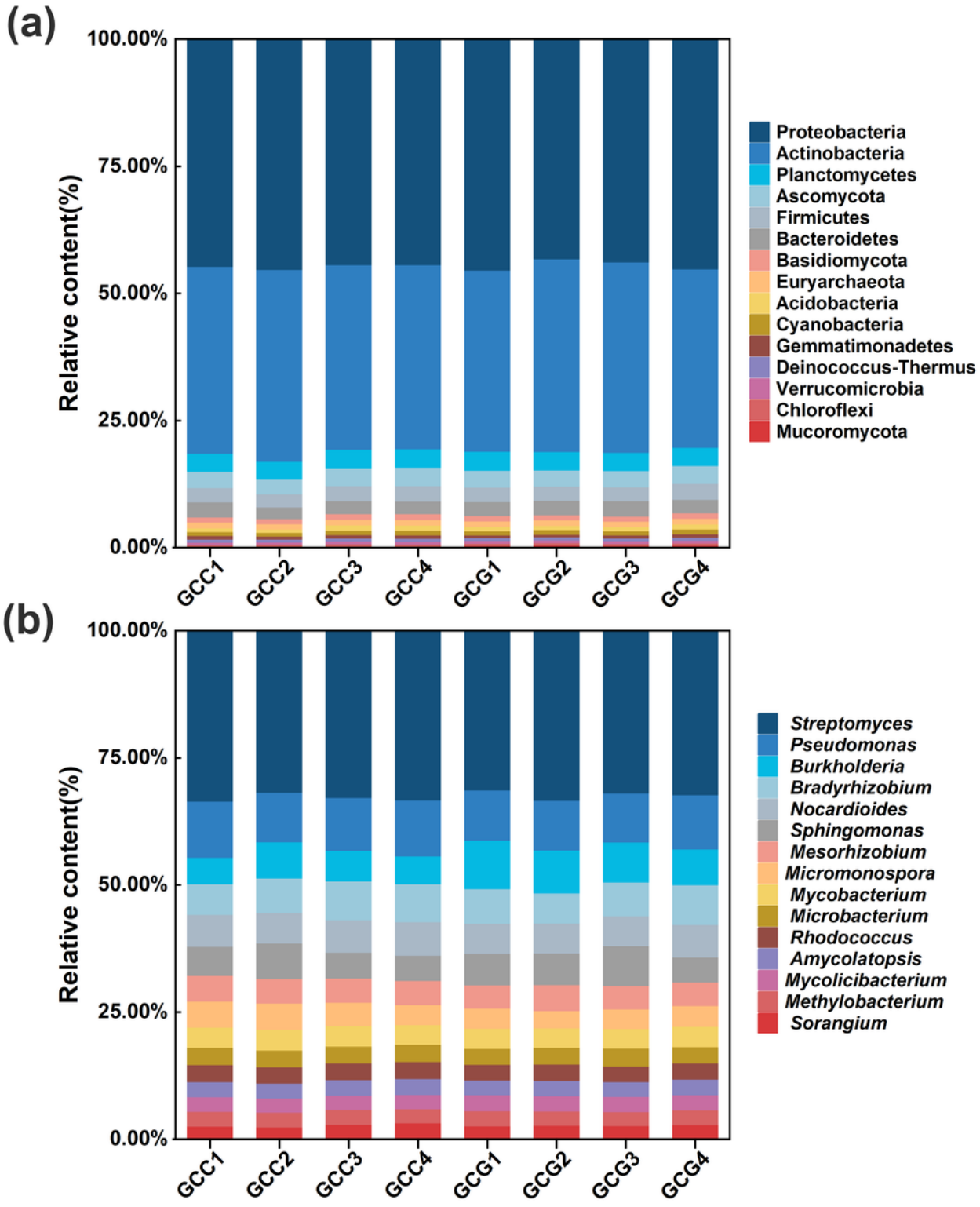

Figure 3

The relative abundances of microorganisms at phylum (a) and genus (b) in rhizosphere (NG) and non-rhizosphere (NC) soil

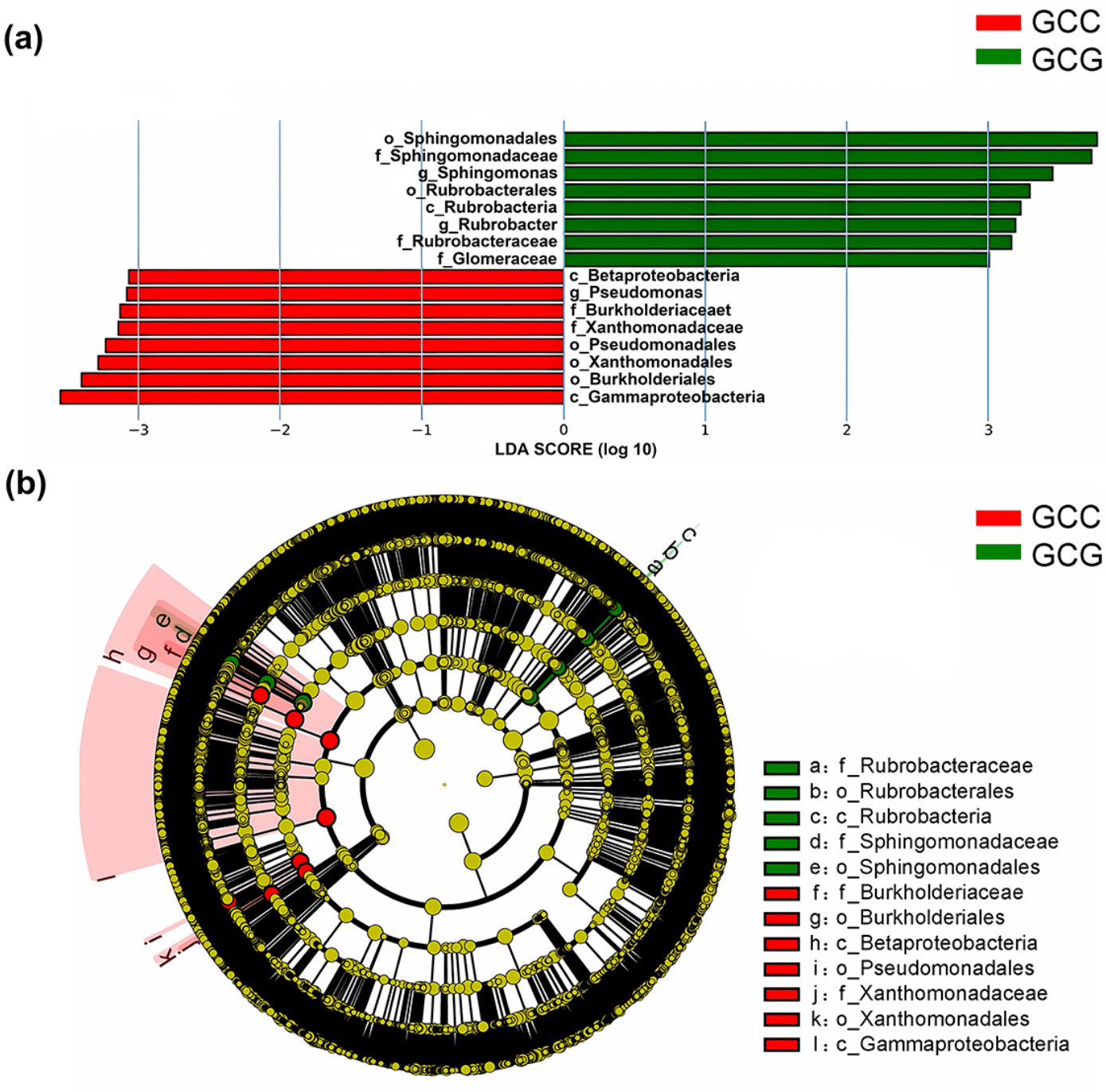

Figure 4

Classification level discriminant analysis (LDA) (a) and Cladogram (b) of key microorganism by LEfSe analysisFigure 5 LEfSe analysis of rhizospheric microorganisms and natural state

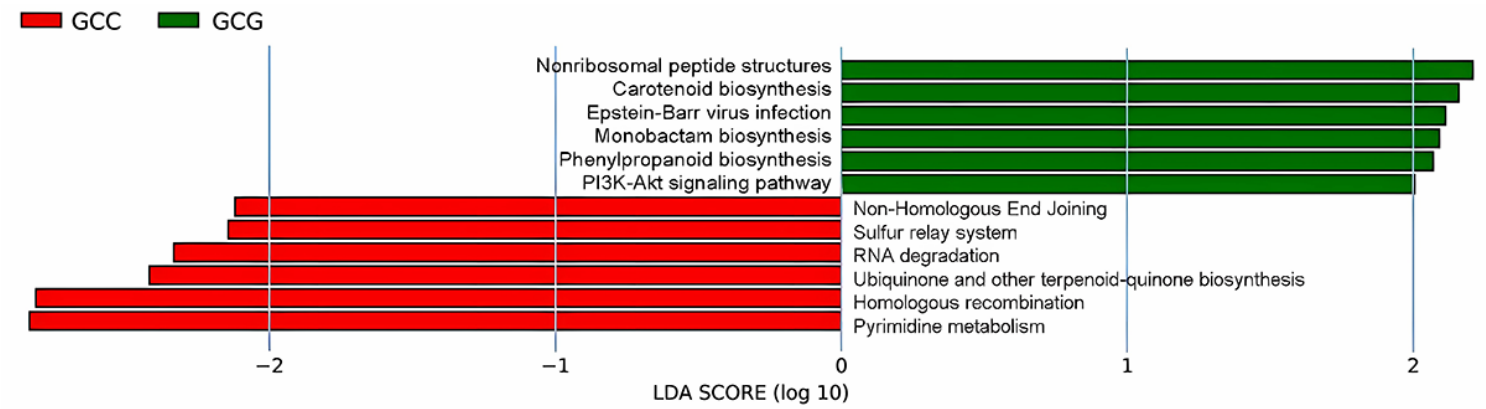

Figure 5

Results of analysis of KEGG pathway

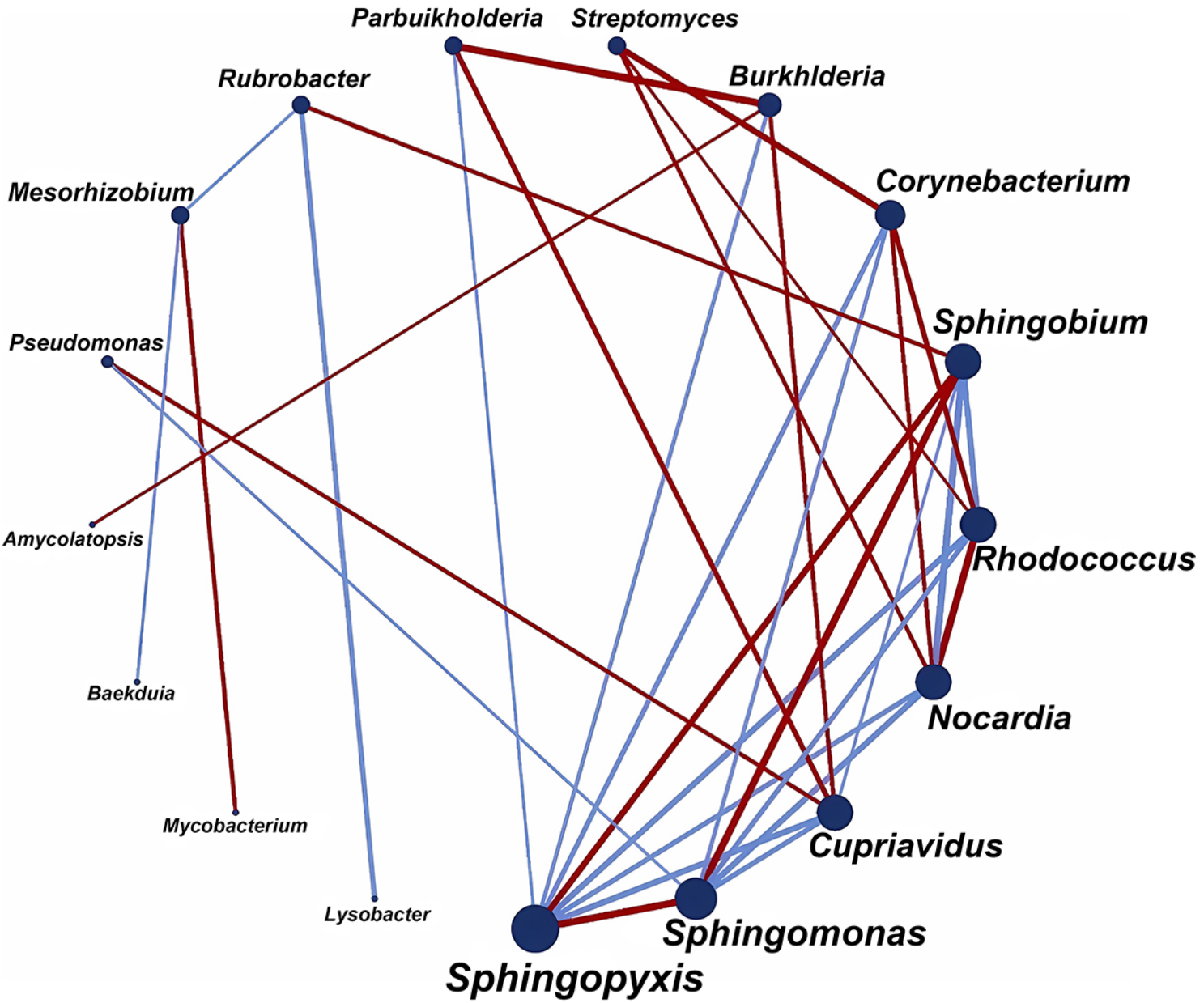

Figure 6

Correlation analysis between rhizosphere and non-rhizosphere soil microorganisms

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