



## Soil microbial community structure along the ecological succession in Yellow River Delta

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**Abstract:** [Objective] The Yellow River Delta is one of the most important estuary wetland ecosystems in the world. *Suaeda glauca* (SG), *Glycine soja* and *Phragmites australis* (GP) are three typical plants that play important roles in the ecosystem of this region. In this work, we compared the microbial community composition and functional genes of the rhizosphere soils of these typical halophytes with those of bulk soil of the barren wetland (BW) along the saline gradient in the Yellow River Delta. [Methods] Metagenome sequencing was used to analyze the composition and abundance of the microbial communities. COG (Clusters of Orthologous Groups) and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analyses were used to determine the functional gene annotation of the predicted genes. [Results] The results demonstrated that *Proteobacteria* was the most abundant phylum in all samples. The relative abundance of *Proteobacteria* in the rhizosphere soils of SG and GP was 28.8% and 10.6% greater than that of the BW, respectively. In addition, *Bacteroidetes*, *Actinobacteria* and *Gemmatimonadetes* were detected as abundant species in the three samples. *Sinorhizobium* was an abundant species in the GP sample and may be responsible for nitrogen fixation in the rhizosphere soils. Sequence annotation indicated that the number of the predicted functional genes of BW was higher than that of SG and GP. Among them, amino acid metabolism, carbohydrate and energy metabolism, and inorganic ion transport and metabolism genes were abundant in the three samples. [Conclusion] This study demonstrated that different plants and soils have a coordinated effect on the soil microbial communities of the Yellow River Delta, and provided valuable insights on microbial resilience and their potential application on bioremediation of the coastal zone saline-alkali soils.

**Keywords:** metagenomic sequencing, microbial diversity, halophyte, ecological succession, Yellow River Delta

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The Yellow River Delta is the second largest estuary delta in China, which is one of the areas with the highest biodiversity in the world<sup>[1]</sup>. The succession process occurs from ocean to terrestrial land in this region, along with a decrease in the soil salt content and an increase in the organic matter content. At present, few studies of the microbial characteristic associated with different successional process in the wetlands have been reported. This estuarine delta is naturally characterized by extensive coverage of primary salinization, and different types of halophytes grow along the natural saline gradient. Saline-alkali stress is one of the main abiotic factors that greatly restricts the survival, growth, and productivity of plants by affecting their physiological and development processes<sup>[1-2]</sup>. However, soil microorganisms can promote the growth and yield of the plants by increasing their tolerance to salinity and nutrient availability<sup>[3-5]</sup>. In turn, halophytes could improve the microenvironments of the soil, which promotes the survival of rhizosphere microorganisms<sup>[6]</sup>.

Soil microorganism play critical roles in maintaining the ecological balance and have been regarded as sensitive indicators of the soil environments<sup>[7-8]</sup>. Microorganisms play important roles in biogeochemical cycling in the rhizosphere via nitrogen fixation, phosphorus uptake, and nutrient cycling<sup>[9-10]</sup>. The composition and abundance of soil bacteria are often related to soil chemistry and plant species<sup>[11-14]</sup>. Many studies have demonstrated that soil pH and salinity are the main factors that affect the composition of microbial communities<sup>[15-18]</sup>. In a global survey of bacterial communities from terrestrial environments, salinity has been reported to be the major environmental determinant of microbial community composition<sup>[19-20]</sup>. The structure of soil bacterial communities has also been shown to be associated with differences in soil pH<sup>[21-22]</sup>. In addition, plant species can influence the soil characteristics and

microbial communities through the excretion of root exudates and oxygen into the rhizosphere<sup>[23-25]</sup>. However, the combined effects of plant species and soil properties on soil microorganisms are poorly understood.

The Yellow River Delta forms a natural saline-alkali gradient from the coastal to terrestrial land. *Suaeda glauca* (SG), *Glycine soja* and *Phragmites australis* (GP) are three typical plant communities along the succession region in the Yellow River Delta since 1990s. In this study, we compared the microbial community compositions and functional gene contents of rhizosphere microbial communities of *Suaeda glauca* (SG) and *Glycine soja-Phragmites australis* (GP). We compared halophyte rhizosphere microbial communities with the bulk soils of barren wetland in the coastal area of the Yellow River Delta. Therefore, the aim of this study was to investigate the combined effects of different vegetation covers and soil factors on both microbial communities and functional genes in Yellow River Delta rhizosphere soils.

## 1 Materials and Methods

### 1.1 Site description and sampling

Three sampling sites were located in the Yellow River Delta (119.1°E, 37.7°N) in Dongying City, Shandong Province, China. The rhizosphere soils were collected as described previously with a few modifications<sup>[26]</sup>. A nested five-point sampling method was used. At each sampling site, surface sediments (0–5 cm) were sampled at five points, and the distance between each point was 5 m. Three subsamples were collected in each sampling site. After that, all the subsamples of each site were manually mixed to homogeneity and immediately transported to the laboratory. The soil samples were divided into two parts. One was stored at 4 °C to analyze its soil properties, while the other was stored at –80 °C until DNA extraction.

## 1.2 Soil physiochemical properties

The soil pH was measured as previously described using a pH meter (Mettler Toledo Instruments, Shanghai, China)<sup>[27]</sup>. The electrical conductivity (EC) was measured using a conducting meter (DDS-11A, Shanghai, China). The total organic carbon (TC), total nitrogen (TN), and total phosphorus (TP) were measured as previously described<sup>[28]</sup>.

## 1.3 Metagenome sequencing and assembly

Illumina HiSeq-2000 sequencing of metagenomic DNA from the three samples generated 239489174 raw reads with a total of 24.19 billion bps. Sequences were cleaned and assembled using SeqPrep (<https://github.com/jstjohn/SeqPrep>), Sickle (<https://github.com/najoshi/sickle>) and SOAP<sup>[29]</sup>. Open reading frames (ORFs) were predicted using MetaGene software<sup>[30]</sup>. The ORFs with a length of  $\geq 100$  bp were selected and translated into amino acid sequences using the NCBI database. The predicted genes (with 95% sequence identity and 90% coverage) were clustered by CD-HIT software<sup>[31]</sup>, and the longest sequences from each cluster were selected as representative sequences to build a non-redundant gene catalog. The reads were then mapped to the representative sequences with 95% identity using SOAP aligner<sup>[32]</sup>, and the gene abundance in each sample was evaluated.

Representative sequences of the non-redundant gene catalog were aligned to the NR database using BLASTp for taxonomic annotations. The reads were

also used for the taxonomic assessment by screening for SSU rRNAs using Metaxa2<sup>[33]</sup>. The non-redundant gene catalog was aligned against the Non-supervised Orthologous Groups (eggNOG) database using BLASTp for Clusters of Orthologous Groups (COG) annotation with an E-value cut-off of  $1e^{-5}$ <sup>[34]</sup>. The non-redundant gene catalog was then aligned against the Kyoto Encyclopedia of Genes and Genomes (KEGG) using BLASTp against the KEGG database with an E-value cutoff of  $1e^{-5}$ <sup>[35]</sup>.

## 1.4 Accession number

The raw sequencing data have been deposited into the NCBI Sequence Read Archive (SRA) database. The SRP accession number is SRP253127.

# 2 Results and Discussion

## 2.1 Soil properties

Soil samples were taken from three sample sites, where the vegetation was *Suaeda glauca* (SG), *Glycine soja-Phragmites australis* (GP) and barren wetland (BW). The chemical composition of the soils is summarized in Table 1. All the soil samples were highly saline and had a high pH. The pH values are alkaline and decline along from the land to coast. The electrical conductivity (EC) values varied from 7.42 to 8.98. A previous study showed that the main component of soil salinity is sodium chloride, which exhibits a significant linear correlation with the EC in Yellow River Delta<sup>[36]</sup>. The total nitrogen and total carbon were highest in GP, which could have

Table 1. Properties of rhizosphere soils of *Suaeda glauca* (SG), *Glycine soja-Phragmites australis* (GP) and Barren wetland bulk soil (BW)

| Sample ID | Description                              | TN/%        | TC/%        | TP/%        | C:N         | pH           | EC/( $\text{dsm}^{-1}$ ) |
|-----------|--|-------------|-------------|-------------|-------------|--------------|--------------------------|
| SG        | <i>Suaeda glauca</i>                     | 0.09±0.02 b | 1.79±0.07 b | 0.11±0.01 a | 20.43±2.4 a | 8.61±0.01 a  | 7.42±0.09 b              |
| GP        | <i>Glycine soja-Phragmites australis</i> | 0.22±0.14 a | 3.31±0.20 a | 0.11±0.02 a | 14.98±1.9 a | 8.16±0.101 b | 8.98±0.59 a              |
| BW        | Barren wetland                           | 0.10±0.13 b | 1.76±0.14 b | 0.09±0.04 b | 17.86±1.6 a | 7.93±0.02 c  | 7.52±0.01 b              |

Values are the means±standard deviation (SD; n=3). Values within a column followed by different lowercase letters indicate a statistically significant difference at  $P<0.05$ . TN: total nitrogen; TC: total organic carbon; TP: total phosphorus; EC: electrical conductivity.

resulted from the improvement of soil nutrients by the legume *Glycine soja*. It has been estimated that soil microorganisms require a C:N ratio of approximately 25:1 to reach 40% of their growth efficiency<sup>[37]</sup>. The C:N ratio varied from 14.98 to 20.43 in the three samples, indicating that the microbial communities facilitated a high degree of organic nitrogen mineralization of the soil organic matter.

## 2.2 Microbial taxonomic analysis at the phylum level

We first explored the microbial community composition by analyzing the reads using the ribosomal small subunit (SSU)<sup>[38]</sup>. The results demonstrated that the niche contains very high bacterial abundance (90%–93%), with a significantly lower proportion of Eukaryota (1.90%–2.88%) and Archaea (0.43%–3.56%) (Figure 1-A). The populations

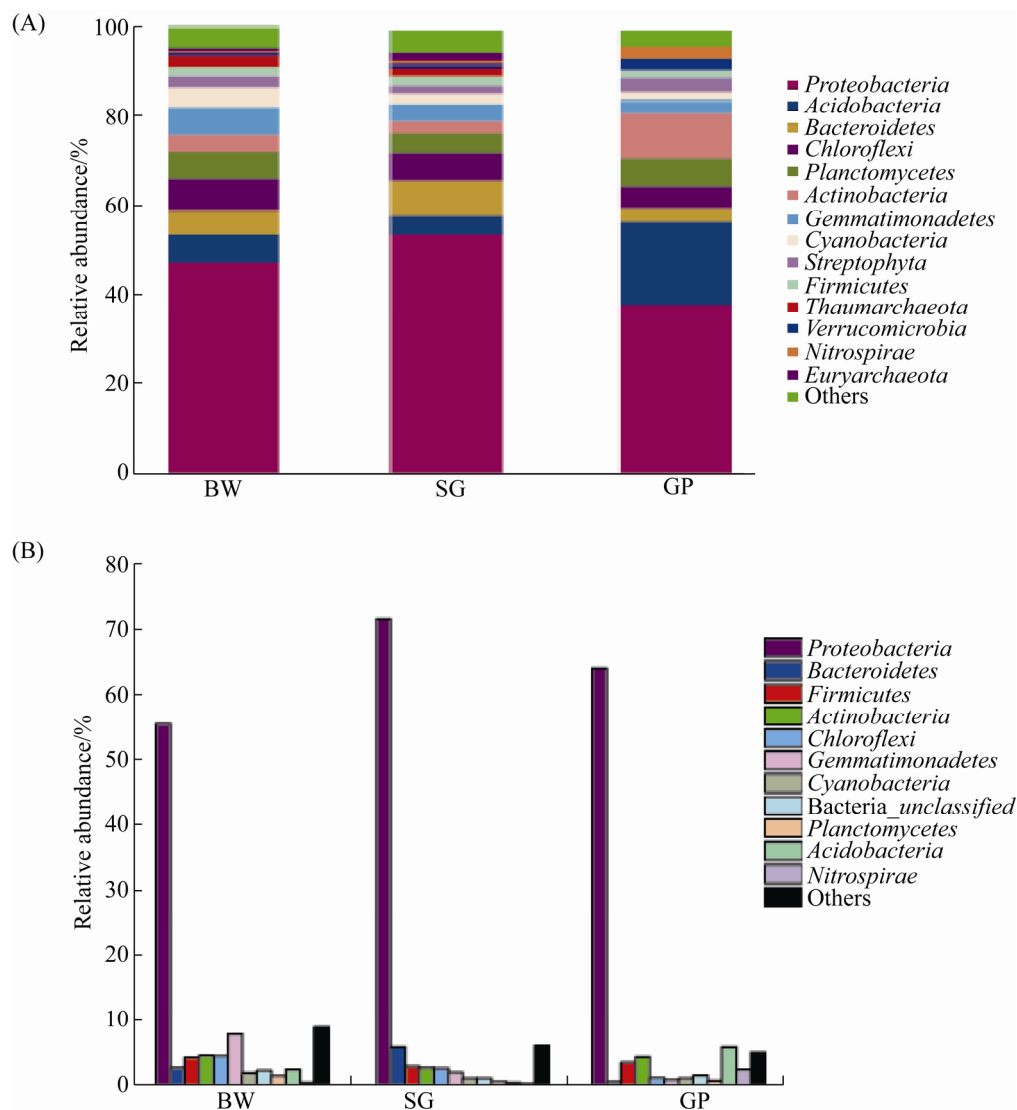


Figure 1. The relative abundance of different species at the phylum level by taxonomic classification using the ribosomal small subunit (SSU) genes (A) and the relative reads number (B). BW: barren wetland soil; SG: *Suaeda glauca* rhizosphere soil; GP: *Glycine soja-Phragmites australis* rhizosphere soil.

of Eukaryota and Archaea are found in low amounts in the three soil samples, which is consistent with the results of other environments<sup>[39–40]</sup>. The taxonomic analysis of SSUs of the bacterial fraction revealed that *Proteobacteria* was the most prevalent phylum represented in the metagenome, while other phyla, such as *Acidobacteria*, *Chloroflexi*, *Actinobacteria*, *Bacteroidetes*, *Planctomycetes*, and *Gemmatimonadetes*, were present as relatively minor contributors to the total bacterial abundance (Figure 1-A).

We further analyzed the microbial community structure of the three samples by comparing the relative reads number. The results showed that there were 10 phyla in common that comprised a total of 90.32%, 85.05% and 85.95% in SG, GP, and BW, respectively. As shown in Figure 1-B, the dominant phyla in the three sample soils were *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Chloroflexi*. *Proteobacteria* was the most abundant phylum in all samples, accounting for a total of 71.5%, 61.4% and 55.5% of SG, GP and BW, respectively. Many studies have shown that *Proteobacteria* predominate in different soil types, which could indicate an involvement in the biogeochemical process in ecosystems<sup>[41–44]</sup>. The differences in distribution are probably related to the organic matter content in the soil, since the relative abundance of *Proteobacteria* can be influenced by nutrients availability<sup>[45]</sup>.

The next most abundant phylum varied among the three samples, *Bacteroidetes* (5.87%) in SG, *Actinobacteria* (5.88%) in GP, and *Gemmatimonadetes* (7.89%) in BW, respectively. These results are consistent with those of previous studies, in which *Bacteroidetes*, *Actinobacteria* and *Gemmatimonadetes* were reported to be prevalent in the saline-alkaline sediment environment<sup>[46–48]</sup>. *Bacteroidetes* and *Actinobacteria* have been demonstrated to thrive in plant soils, while *Gemmatimonadetes* were enriched in barren soils in the Yellow River Delta<sup>[49]</sup>. The relative abundance of

*Bacteroidetes* was decreased from 5.87% (SG) to 0.6% (GP), whereas that of *Acidobacteria* was increased from 0.50% (SG) to 5.88% (GP) and that of *Nitrospirae* was increased from 0.32% (SG) to 2.49% (GP). Since *Acidobacteria* serve as decomposers, they participate in the cycling of organic matter derived from plants, fungi and insects<sup>[50]</sup>. In this study, the relative abundance of *Acidobacteria* was higher in the rhizospheres of GP, which could be related to the higher contents of root exudates in that region.

### 2.3 Microbial taxonomic analysis at the genus level

The richness of the bacterial community at the general level is illustrated in Figure 2. Twenty-four main genera (each with a relative abundance of >1%) were detected in the three soil samples, and the main genera represented 15.83%–22.88%. The composition of bacterial communities differed substantially across different samples. *Marinobacter* (3.94%), *Streptococcus* (3.92%), *Alcanivorax* (2.83%), *Gemmatimonas* (1.73%), *Pseudomonas* (1.25%), *Steroidobacter* (1.09%), *Thioalkalivibrio* (1.07%), and *Fluviicola* (1.03%) were the dominant genera in SG. *Sinorhizobium* (10.82%), *Nitrospira* (2.32%), *Pseudolabrys* (2.25%), *Pyrinomonas* (1.87%), *Bradyrhizobium* (1.68%), *Streptococcus* (1.49%), *Lysobacter* (1.40%), and *Rhodoplanes* (1.06%) were the dominant genera in GP. *Gemmatimonas* (6.81%), *Kiloniella* (1.51%), *Streptococcus* (1.38%), *Alcanivorax* (1.38%), *Fodincurvata* (1.37%), *Rhodovibrio* (1.25%), *Azospirillum* (1.14%), and *Thioalkalivibrio* (1.00%) were the dominant genera in BW. Among the top 24 genera, the dominant genera were composed of *Sinorhizobium*, *Gemmatimonas*, *Marinobacter* and *Streptococcus*, many species of which have been reported to have beneficial effects for C or N cycling in the soil<sup>[48]</sup>. In contrast with those in the BW and SG, the main microbes in the communities of GP at later succession stages included the genera of

*Sinorhizobium* (10.82%), *Nitrospira* (2.32%), and *Bradyrhizobium* (1.68%). These species have been demonstrated to play important roles in nitrogen cycle<sup>[51]</sup>. The legume *Glycine soja* appears to facilitate the expansion of the *Sinorhizobium* species in GP, since this microorganism is a nitrogen-fixing mutualistic symbiont of the legume. The relative lower population of *Bradyrhizobium* could be attributed to the influence of saline soil. Previous studies have identified that *Sinorhizobium* as the dominant species in alkaline-saline soil, whereas *Bradyrhizobium* was found to be dominant in neutral to acidic soil<sup>[52-54]</sup>. *Nitrospira* plays a role in nitrification, which is also exist in YRD agriculture soils in previous studies<sup>[55-56]</sup>. In concert, these results suggest that different vegetation covers and saline soils have an important impact on the bacterial communities.

## 2.4 Distribution of putative metabolic genes of microbial communities

To better understand the functional potential of the microorganisms represented in the three sampling sites, the metabolic functions of the microbial communities in SG, GP and BW were

investigated by aligning to the eggNOG databases. The results demonstrated that the number of total predicted functional genes of SG and GP was 25.2% and 71.2% lower than those of the BW, respectively (Figure 3-A). In addition to the function of unknown genes, the main functional gene classes in the three samples were amino acid metabolism, energy production and conversion, and inorganic ion transport and metabolism genes (Figure 3-A).

Furthermore, the predicted genes were aligned to the KO (Kyoto Encyclopedia of Genes and Genomes Orthology) database. The results demonstrated that the total number of predicted functional genes of SG and GP was 19.8% and 68.0% lower than that of the BW, respectively (Figure 3-B). Many genes were attributed to three categories, namely amino acid transport and metabolism, carbohydrate metabolism, and energy metabolism (Figure 3-B). Moreover, we compared the functional genes at KEGG level 3 using a heatmap analysis. As shown in Figure 3-C, a higher number of sequences in quorum sensing and ABC transporters was found in GP compared with those of SG and BW. The quorum sensing genes were

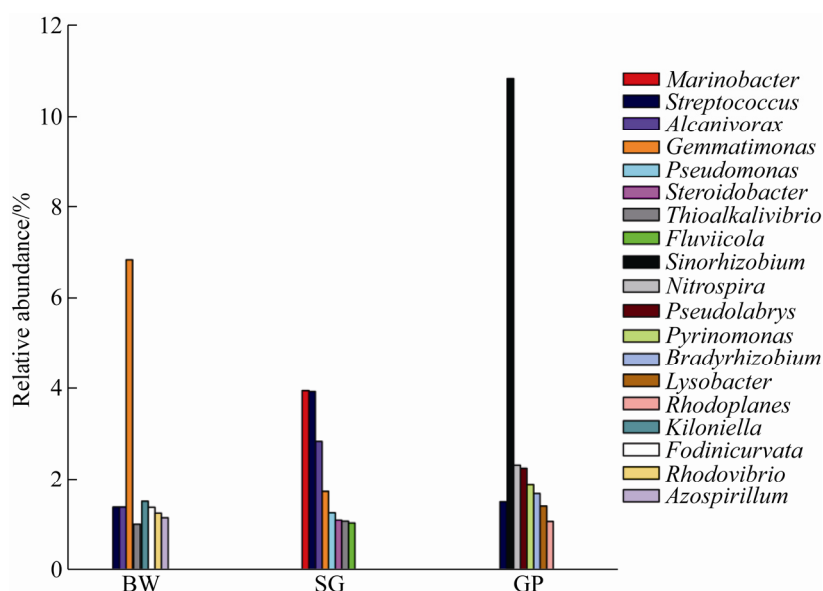


Figure 2. Bacterial community structure of each sampling point at the genus level.

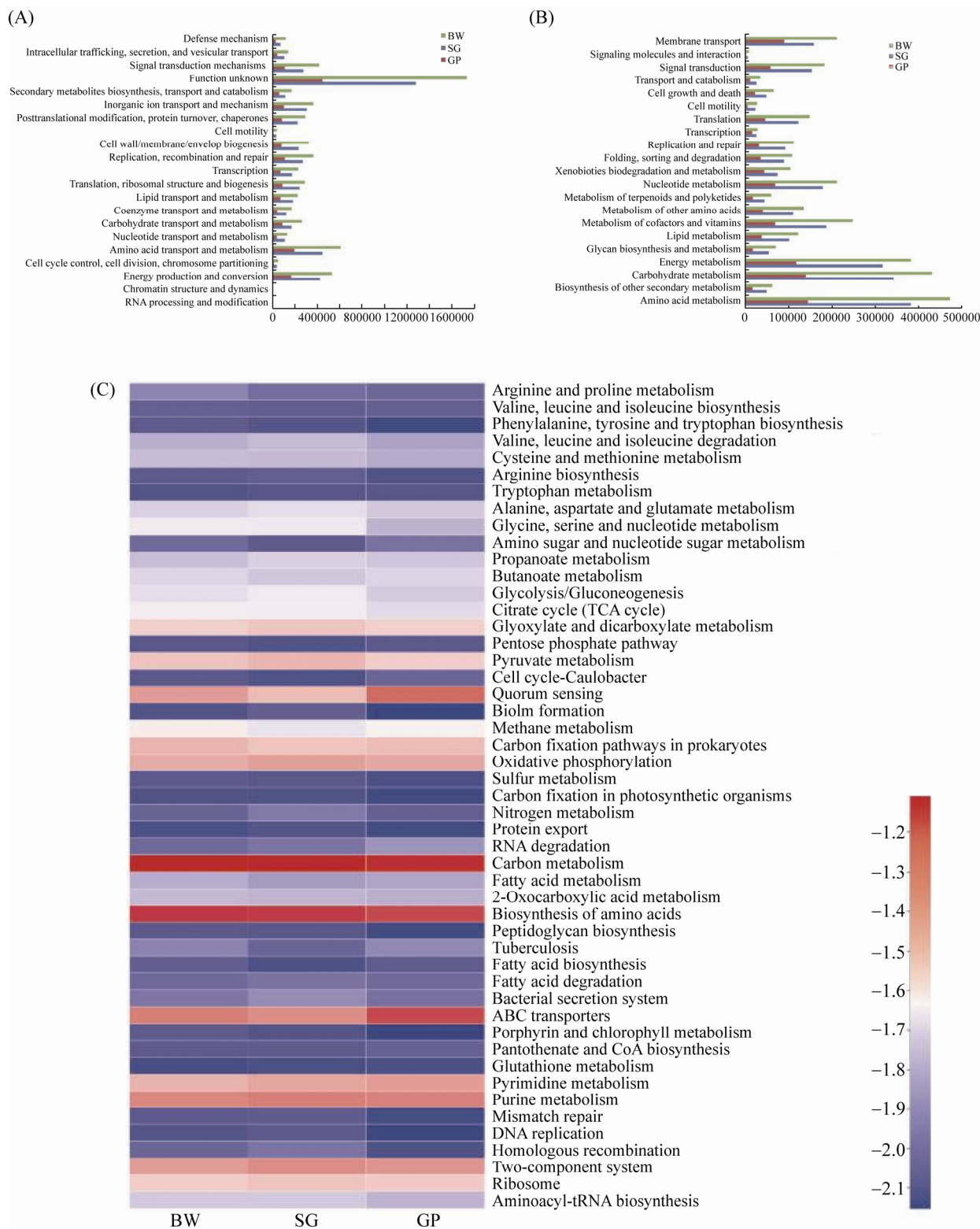


Figure 3. COG (A) and KEGG analysis (B) of the predicted functional genes in the three samples. Heatmap showing the differences of the three investigated communities based on the KEGG level 3 (C).

potentially implicated in regulating the legume-rhizobia nodulation symbiosis process<sup>[57]</sup>. The ABC transporters genes could be related to the high EC values of the soil sample in GP, since the ABC transporter proteins have been demonstrated to play a role in osmotolerance<sup>[46,58]</sup>. In summary, our data show that the vegetation types and soil characteristics play important roles in the structure of bacterial communities.

We further compared functional genes that participate in the nitrogen cycling in the three samples. As shown in Figure 4, the ATP-dependent glutamine synthetase-encoding gene *glnA* had the highest abundance in each sample. The next abundant genes were *gltB* and *gltD*, which encode

glutamate-oxoglutarate amidotransferase and can cooperate with *glnA* in indirect ammonia fixation. The abundance of N fixation gene (*nifH*) in SG and GP was higher than that of BW. And more dissimilatory N reduction gene (*nasA*, *nrfA*, and *nirADK*) was detected in SG than that of GP and BW. For the ammonification process, the abundance of *gdh* of SG was lower than those of GP and BW. This observation could be related to C:N ratio in the soil, as the ammonification process can be influenced by nitrogen availability<sup>[59]</sup>. The mere presence of gene does not necessarily mean the function, and metatranscriptomic analysis and cultivation of targeted functional guilds are warranted for further study.

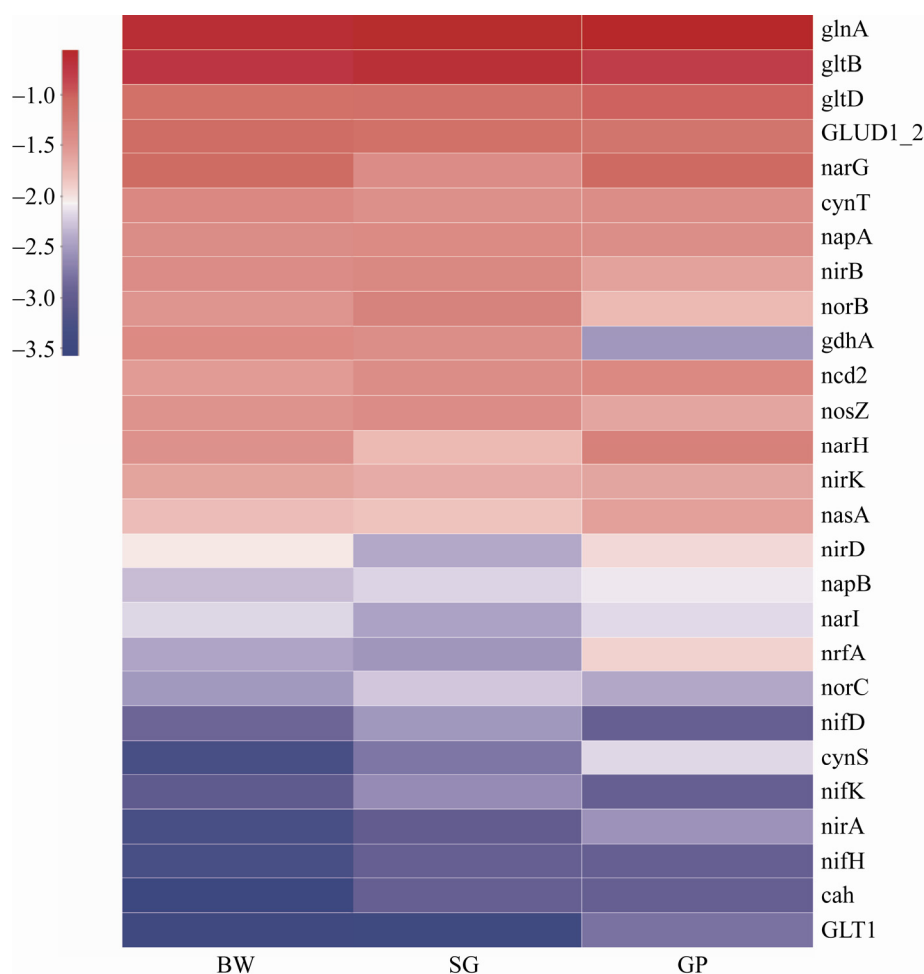


Figure 4. Heatmap analysis of genes potentially involved in nitrogen cycle in the three samples.



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# 黄河三角洲原生演替中土壤微生物群落结构分析

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**摘要:**【目的】黄河三角洲区域具有重要的湿地生态系统。碱蓬、野大豆和芦苇是该地区典型的盐生植物。本研究针对碱蓬、野大豆和芦苇混生植物的根际土壤微生物群落组成和功能基因进行了分析比较。

【方法】对碱蓬、野大豆-芦苇混生植物的根际微生物菌群和光滩土壤菌群进行了宏基因组测序, 使用 COG 和 KEGG 数据库对微生物菌群的功能进行了注释和比较。【结果】本研究结果表明, 变形菌门是 3 个取样点的主要菌门, 其在碱蓬、野大豆-芦苇根际土壤中的相对含量比光滩土壤分别多 28.8% 和 10.6%。此外, 拟杆菌门、放线菌门和芽单胞菌门是 3 个取样点中的优势物种。中华根瘤菌属是野大豆-芦苇混生植物根际土壤的最主要的属。对功能基因进行分析表明, 光滩土壤中的功能基因的数量多于碱蓬根际土壤和野大豆-芦苇混生植物根际土壤的功能基因数。在这 3 个位点中, 氨基酸代谢、碳水化合物代谢和能量代谢, 以及无机离子转运和代谢的基因最多。【结论】本研究为发掘有价值的微生物资源和海岸带盐碱土壤修复提供了一定的理论基础。

**关键词:** 宏基因组测序, 微生物多样性, 盐生植物, 生态演替, 黄河三角洲

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