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Contributed Paper

# The Structural Characteristics of Soil Microbial Composition in Different Wetland Plants in the Yellow River Delta

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## ABSTRACT

The soil microbial community composition of bare land and six kinds of plant vegetation were investigated in the Yellow River Delta Wetland, China. We analyzed the diversity and function of the soil microbial community composition using high-throughput sequencing technology in the different vegetation cover lands. *Tamarix chinensis* (*T. chinensis*) had the highest microbial richness and *Aeluropus sinensis* (*A. sinensis*) had the highest microbial diversity. *Phragmites australis* (*P. australis*) had the highest proportion of Pseudomonas (9.34%) showed the strongest denitrification and phosphorus-accumulation ability. *T. chinensis* had the highest read number of ammonia oxidizing bacteria (AOB) (41) indicated the highest ability of ammonia-oxidizing. The read number of Diazotrophs under *T. chinensis* (17) and *Suaeda salsa* (*S. salsa*) (88) was significantly higher than control ($p < 0.01$) mean the strongest nitrogen fixation effects. The relative abundance of anammox bacteria (273) was highest in *A. sinensis*. The read number of phosphorus-accumulating bacteria (PAOs) in *P. australis*, *T. chinensis*, *S. salsa* and *A. sinensis* were significantly higher than control ($p < 0.01$).

**Keywords:** yellow river delta wetland, microbial community, functional microbes, functional genes

## 1. INTRODUCTION

The Yellow River Delta is the youngest wetland ecosystem with a largest estuarine delta nature reserve in the warm-temperate zone of northern Shandong Province, China [1]. Wetland ecosystems represent the area bordering the interaction of land and water systems and can thus be considered a special type of ecological system. Given this characteristic of wetlands, the diversity of ecosystem functions can be occurred with the different kinds of wetlands [2], including nutrient cycling, sand deposition, pollutant treatment and soil erosion control. The Yellow River Delta wetland belongs to the typical coastal wetland type and is one of the world's richest biodiversity regions. A highly diverse microbial community inhabits this special land–sea interaction environment. These microorganisms take an important effect on

the cycle of mineral elements, the degradation of pollutants and maintaining the stability of the wetland ecosystem [2].

On the other side, the saline habitat bring about a fragile relationship between the vegetation and the environment [3]. In this ecosystem, Soil microorganisms build a nexus between the belowground and aboveground ecology by decomposing organic matter in the soil and benefiting plants via absorbance, fixing and release of nutrients. Furthermore, the composition of microbial communities is also affected by different vegetation [4]. Functional microbes such as diazotrophs, AOB [5], denitrifying bacteria (DNB), nitrite-oxidizing bacteria (NOB), anammox bacteria, denitrification and dissimilatory nitrate reduction to ammonium (DNRA) and phosphorus-accumulating bacteria (PAO) are present under different vegetation in wetlands [6]. The natural vegetation in the Yellow River Delta can be divided into the following six vegetation types: arbor, shrub, meadow, aquatic vegetation, swamp vegetation and psammophytic vegetation [7]. Given the various landforms, hydrology, and soil distribution of different vegetation types, the main vegetation types in this area are shrub and meadow [8]. Hygrophytes and halophytes are the main constructive and dominant vegetation types in the Yellow River Delta. Therefore, six species of halophyte and vigorously growing plants (*P. australis*, *T. chinensis*, *S. salsa*, *L. sinense*, *A. sinensis*, and *Beta vulgaris* (*B. vulgaris* )) have received in-depth research attention [9].

In this study, the soil microbial community in different kinds of wetland vegetation in the Yellow River Delta was analyzed by high-throughput sequencing technology [10]. The objectives of this study were to analyze the diversity and distribution of saline soil microbes and the functional composition of the soil microbial community. Different plants play different roles in nitrogen cycling, sulfur cycling and phosphorus cycling. The results of this study will be used to clarify the relationship of mutual influence between the microbes and plants will help us in protecting and restoring the wetland ecosystem.

## 2. MATERIALS AND METHODS

### 2.1 Study Site and Approach to Field Study

This study was conducted in the wetland of the Yellow River Delta, Shandong Province, China (37°10'~38°19'N and 118°15'~119°43'E). The region is located near the estuary of the Yellow River Delta and characterized by a temperate, semi-humid continental monsoon climate. The substrate is saline-alkali soil. Soil samples were collected from seven sites of different kinds of vegetation within this region in July 2015 by the diagonal five-point sampling method [11]. Collection of soil in the 0~20cm depths of different plant rhizosphere, because this depth of soil is rich in microbes. To focus on the correlation of rhizosphere on microbial populations, six dominant plant communities in this region were selected: a (*P. australis*), b (*T. chinensis*), c (*S. salsa*), d (*L. sinense*), e (*A. sinensis*), and f (*B. vulgaris*). In addition, a bare land (g) was selected as the control group. Three parallel samples were collected at each sampling point for high-throughput sequencing [12]. Plants were uprooted and Soil samples were taken back to lab after carefully removing fine roots and surface organic materials.

### 2.2 DNA Extraction and 16S rRNA Polymerase Chain Reaction (PCR) Amplification

Total DNA was extracted from Omega's magnetic use bead soil DNA Extraction Kit (Omega Bio-Tek, USA). Amplification the V4 region of the 16S rRNA gene in microorganism. By using the universal 16s primer pair forward primer: 520F (5'-AYTGGGYDTAAAGNG-3') and reverse primer: 802R (5'-TACNVGGGTATCTAATCC-3'). About 280 bp fragments were amplified in the V4 region. The PCR started with a denaturation

step of 98°C for 3 min. The second step was 25 cycles of denaturation (98°C for 3 min). The third step was primer annealing (30 s at 50°C). The forth step was extension (72°C for 30 s). There was a final extension step of 72°C for 5 min. This step was the key to ensuring full amplification. The PCR products were detected by 2% agarose gel electrophoretogram. In the Illumina MiSeq sequencing platform at Personal Biotechnology Co., Ltd. (Shanghai, China), the amplification products of V4 were sequenced by double terminal method.

### 2.3 High-throughput Sequencing

The project was sequenced by the Illumina MiSeq sequencing platform at Personal Biotechnology Co., Ltd. (Shanghai, China). The original data were stored in a paired-end sequencing FASTQ format. The original sequence processing, trimmed off sequences with average base quality values lower than 20, those containing ambiguous base ('N'), and those below 150 basis points. Base mismatches were not allowed. Finally, the effective sequence of each sample was extracted based on the index information (a sequence of bases in a sequence for distinguishing samples) [13].

### 2.4 Data Processing and Analysis

In Qiime [14], the uclust [15] and blast [16] methods were used to cluster the high quality sequences by sequence similarity of 97%, acquire the taxonomic information of every OTU and construct the phylogeny tree. Alpha diversity, which including Chao, ACE, Simpson and Shannon indices, were calculated by using the summary single command of the Mothur software. Statistical software (SPSS 20.0) was used to asses significant differences among the functional microbes at the $0.01<p<0.05$ and $p<0.01$ level. The microbial communities of 6 species of plants in phylum, class, family and genus were analyzed by Origin Pro 9.0. According to the abundance information of the OTU levels, LDA Effect Size (LEfSe) analysis was implemented using the R platform, the influence of functional genes with significant difference in the reduction and peacekeeping assessment of data by LDA [17].

## 3. Results and Discussion

### 3.1 Microbial Community Diversity under the Six Vegetation Species

#### 3.1.1 Overall taxonomic structure analysis

*A. sinensis*, *T. chinensis* and *S. salsa* had the maximum total OTU quantity, indicated that they had the highest community richness. The similarity was highest between *T. chinensis* and *S. salsa*. After quality control, we have obtained a total of 91,853 validated sequence reads which were classified into different OTUs at a 97% identity level [18]. Of all the OTUs in Figure 1, 1607 were from *P. australis*, 2459 were from *T. chinensis*, 2094 were from S. salsa, 1629 were from *L. sinense*, 2388 were from *A. sinensis* and 1476 were from *B. vulgaris*. *T. chinensis* had the maximum number of OTU (2459), this showed that it has the highest richness. As shown in Figure 1A, *T. chinensis* and *S. salsa* shared the most OTUs with 679 from both. This showed that they have the highest similarity.

#### 3.1.2 Alpha diversity analysis

Alpha diversity index suggested that *T. chinensis* had the highest microbial richness and *A. sinensis* had the highest microbial diversity. The Alpha diversity can reflect the diversity and richness of soil microbial communities. The microbial community composition was correlated with the corresponding plant type [19]. The richness indices (Chao and ACE) of the six plant species also showed differences between species (Table 1), indicating that soil microbes could adapt to salinity to different degrees under different plants. Chao and ACE indices indicate the richness of microbes. Soil microbial community richness was highest under *T. chinensis* (CHAO 3039.963, ACE 3179.479),

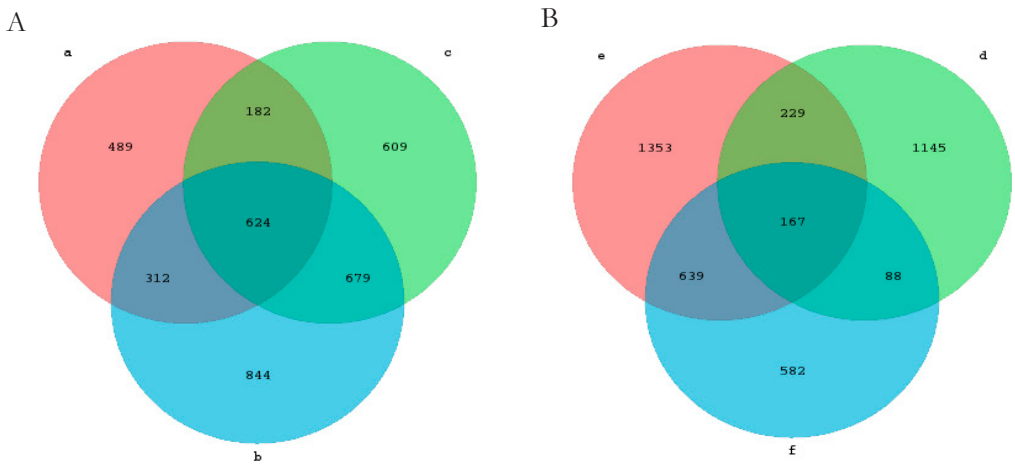


**Figure 1.** Venn diagram of OTUs of the microbial community under the six studied plant species. (a: *Phragmites australis* b: *Tamarix chinensis* c: *Suaeda salsa* d: *Limonium sinense* e: *Aeluropus sinensis* f: *Beta vulgaris*)

**Table 1.** Microbial community richness and diversity parameters.

| | CHAO | ACE | Shannon | Simpson |
|---|---|---|---|---|
| Control | 2390.346 | 2586.687 | 7.143 | 0.905 |
| *Phragmites australis* | 2226.688 | 2414.866 | 8.144 | 0.986 |
| *Tamarix chinensis* | 3039.963 | 3179.479 | 9.682 | 0.994 |
| *Suaeda salsa* | 2539.621 | 2667.592 | 9.360 | 0.995 |
| *Limonoum sinense* | 1704.845 | 1793.840 | 8.827 | 0.993 |
| *Aeluropus sinensis* | 2782.945 | 2954.744 | 9.834 | 0.996 |
| *Beta vulgaris* | 2008.648 | 2160.229 | 6.982 | 0.947 |

Notes: Chao and ACE indicate richness, Shannon indicate diversity.

followed by *A. sinensis* and *S. salsa*. The number of OTUs in Figure 1 also indicated that *A. sinensis*, *T. chinensis* and *S. salsa* had the highest community richness. The Shannon and Simpson indices results indicate that system biodiversity was different under different vegetation species, primarily because the selection pressure from the saline soil leads to elimination of microbial species that cannot adapt to salinity from the different vegetation [20]. The Shannon index ranked as follows: *A. sinensis* > *T. chinensis* > *S. salsa* > *L. sinense* > *P. australis* > control > *B. vulgaris*. The Simpson's index was also the highest in *A. sinensis* (0.996). The microbial biodiversity of soils under *A. sinensis* was highest. Overall, these results showed that *A. sinensis* and *T. chinensis* are the most suitable of the studied plant species for the high salinity environment in the Yellow River Delta.

### 3.2 Microbial Community Dynamics

The taxonomic classification of validated sequences from soil under the six species were classified at four different levels (i.e. phylum, class, family and genus) shown in Figure 2. The results present a significant distinction in the microbial community compositions between species. Moreover, a total of 48 bacterial

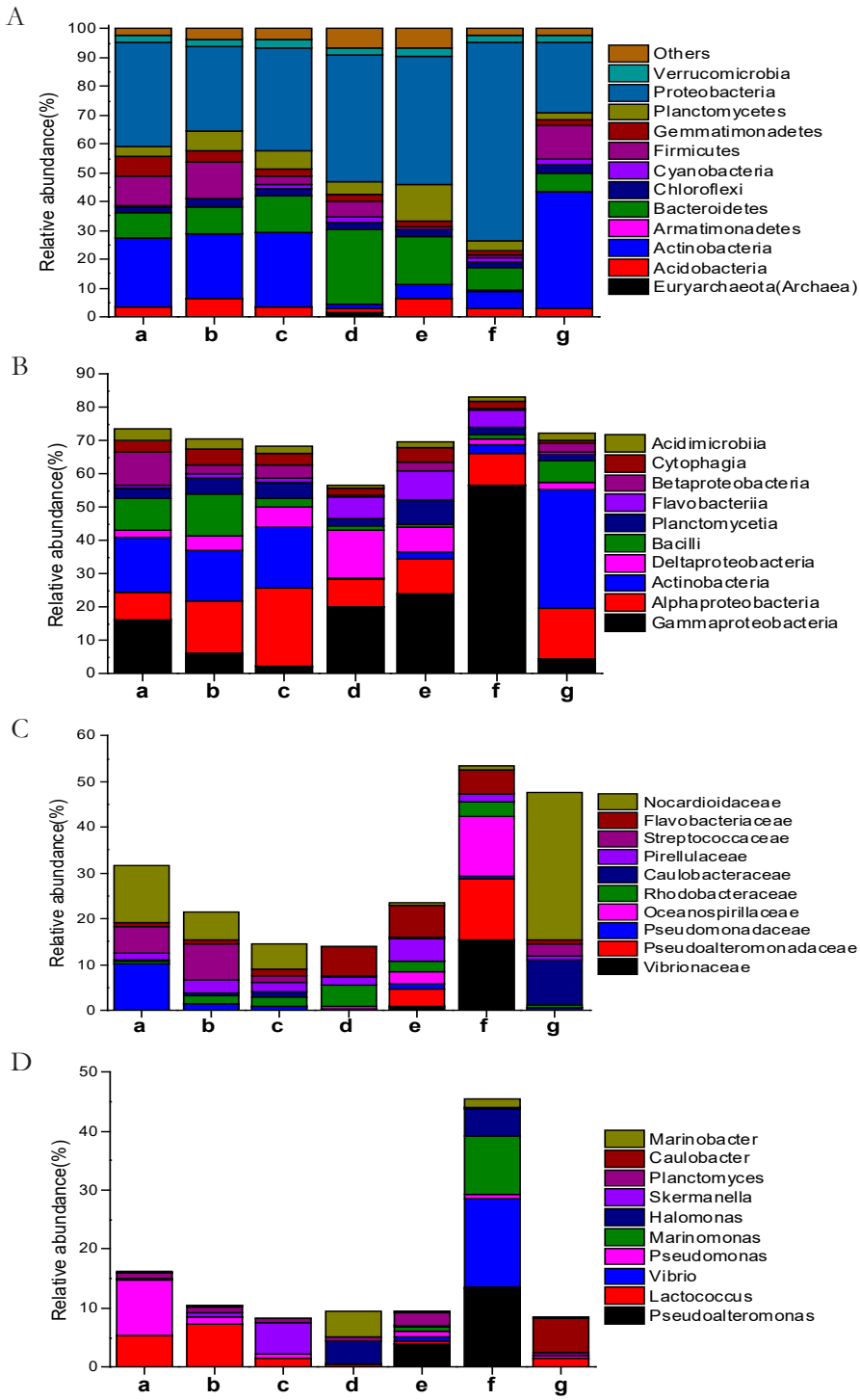


**Figure 2.** Taxonomic classification of the microbial communities under the six studied plant species at (A) phylum, (B) class, (C) family and (D) genus levels.
(a: *Phragmites australis* b: *Tamarix chinensis* c: *Suaeda salsa* d: *Limonium sinense* e: *Aeluropus sinensis* f: *Beta vulgaris* g: Control)

phyla and 2 archaeal phyla were identified across all samples [21]. Figure 2A lists the dominant bacterial phyla (>0.1% sequence abundance in at least one site) and an archaeal phylum. Euryarchaeota were only observed in *L. sinense*. Euryarchaeota contains most of the archaea, including those living in very high salt concentrations (Halobacterium). Proteobacteria showed the highest abundance in all samples [22], accounting for 24.7–68.9%. In soils under *B. vulgaris* in particular, the relative abundance of Proteobacteria was 68.9%. Other dominant phyla were Actinobacteria (1.1–40.3%) and Bacteroidetes (6.5–26.3%). Bacteroidetes was the dominant phylum in *L. sinense* (26.3%).

At the class level (Figure 2B), Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria and Deltaproteobacteria all belong to Proteobacteria. Proteobacteria include the bacteria with the function of nitrogen fixation [22] and important AOB such as Gammaproteobacteria [23]. Gammaproteobacteria was the most abundant of the Proteobacteria for all samples, accounting for 2.2–56.5% and the relative abundance of Gammaproteobacteria was highest under *B. vulgaris* (56.5%). Alphaproteobacteria was the secondary dominant species of the Proteobacteria with abundance of 8.1–23.7%. The following bacterial classes were Actinomycetes and Bacilli. The proportion of Actinomycetes was highest in control (35.7%), followed by *P. australis* (16.8%), *T. chinensis* (15.3%) and *S. salsa* (18.1%). Actinomycetes can break down many organic compounds, play a positive role in the biological treatment of sewage and organic solid wastes, and also promote the formation of soil aggregates and improve soil. Pseudoalteromonadaceae, Oceanospirillaceae, Vibrionaceae and Pseudomonadaceae are members of the Deltaproteobacteria class [24]. They were more abundant than other families, especially in soils under *B. vulgaris*. *P. australis* had the most Pseudomonadaceae (10.0%, include Pseudomonas), *T. chinensis* had the most Streptococcaceae (7.7%, including Lactococcus). Flavobacteriaceae was present in *L. sinense*, *A. sinensis*, and *B. vulgaris* were more abundant than other families. From the genus level assignment result (Figure 2D), the proportion of Pseudomonas in soils under *P. australis* (9.34%) was about three or four times that of other plants at the genus level. Pseudomonas are denitrifying bacteria, the abundance of it was the highest under *B. vulgaris*. These three genera belong to Gammaproteobacteria. The relative abundance of Lactococcus was enriched highly in *T. chinensis* (7.4%).

As explained above, *B. vulgaris* had the most Proteobacteria (Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria and Deltaproteobacteria), which primary functions of the bacteria were concentrated on nitrogen fixation and AOB. *L. sinense* had the most Bacteroidetes, suggesting that the selective preference was inclined to bacteria for sulfur cycling [2].

### 3.3 Functional Bacteria Under the Six Plant Species

Nitrification and denitrification of microorganisms, together with nitrogen fixation, constitute the global nitrogen cycle system [25]. Six important functional microbes were extracted diazotrophs, AOB, NOB, DNB, anammox bacterium, denitrification and DNRA and PAO [6] (Table 2). The relative abundance of functional microorganisms is expressed by their read numbers. *T. chinensis* and *S. salsa* had the highest nitrogen fixation ability of the studied plants, whereas *P. australis*, *T. chinensis* and *S. salsa* had the higher ammonia-oxidizing ability. *L. sinense* had the lowest nitrification capacity.

Beijerinckia (Diazotrophs) can fix nitrogen in the atmosphere and their abundance under *T. chinensis* (17) and *S. salsa* (88) was significantly higher than control ($p < 0.01$), indicating that

**Table 2.** Functional microbes under the six studied plant species.

| Name of bacteria | | Level | Control | *Phragmites australis* | *Tamarix chinensis* | *Suaeda salsa* | *Limonium sinense* | *Aeluropus sinensis* | *Beta vulgaris* |
|---|---|---|---|---|---|---|---|---|---|
| Total | All | All | 16503 | 12600 | 14779 | 14979 | 13919 | 12556 | 12397 |
| Diazotrophs | *Beijerinckia* | Family | 0 | 3 | 17** | 88** | 0-- | 4 | 0-- |
| AOB | *Nitrosomonadaceae* | Family | 4 | 24** | 41** | 15** | 2 | 7 | 1 |
| NOB | *Nitrospirales* | Order | 101 | 33** | 43** | 21** | 37** | 19** | 67 |
| DNB | *Pseudomonas* | Genus | 71 | 1177** | 155** | 109** | 3** | 135** | 71 |
| | *Bacillus* | Genus | 32 | 274** | 325** | 20 | 14* | 14 | 20 |
| Anammox | *Planctomyces* | Genus | 58 | 142** | 148** | 111** | 91** | 273** | 45 |
| DNRA | *Enterobacteriaceae* | Family | 40 | 40 | 4** | 2** | 0** | 8** | 10** |
| | *Desulfovibrio* | Genus | 2 | 0 | 0 | 1 | 29** | 0 | 0 |
| PAOs | *Pseudomonas* | Genus | 71 | 1177** | 155** | 109** | 3** | 135** | 71 |

Notes: -- can't calculated, **p<0.01,*0.05>p>0.01.

these plants have stronger nitrogen fixation effects than the other studied plants. Microbial nitrogen fixation is an important way to increase soil nitrogen content and bioavailability [22]. Nitrification includes two processes: ammonia oxidation and nitrite oxidation [26]. The abundance of NOB decreased significantly in all samples relative to control ($p < 0.01$). The AOB was detected in Nitrosomonadaceae and was largest in *T. chinensis* (41). The abundance of AOB under *P. australis* (24) and *T. chinensis* (41) were significantly higher than in control ($p < 0.01$). This showed that these plants had the higher ammonia-oxidizing ability. Nitrospirales (NOB) in the six sample plants were significantly less than control ($p < 0.01$), which result in low nitrate and therefore enhanced denitrifying dephosphatation [7].

*P. australis* had the strongest denitrification and phosphorus-accumulation abilities. *L. sinense* had the lowest capacity of denitrification [27], but the highest sulfur reduction potential. The relative abundance of anammox bacteria was highest in *A. sinensis*. The relative abundance of DNRA decreased significantly in all samples ($p < 0.01$). DNB including Pseudomonas, and Bacillus (archaea) were existed in soils of all six species. The read numbers of Pseudomonas in *P. australis* (1177) was significantly higher than control ($p < 0.01$). The read numbers of Pseudomonas in *L. sinense* (3) was significantly decreased than control ($p < 0.01$). The relative abundance of Bacillus was the highest in *T. chinensis* (325), followed by *P. australis* (274), both significantly higher than control ($p < 0.01$). Anammox bacteria indicating that the six plants can also improve the soil nitrogen content [28] and they were significantly higher than control ($p < 0.01$). Planctomyces is an anammox bacterial phylum with the highest relative abundance under *A. sinensis* (273). The ammonia fixation is converted to nitrogen removal, which is important to the global nitrogen cycle [29]. The relative abundance of DNRA decreased significantly relative to control, only in *L. sinense* (29) the Desulfovibrio increased significantly ($p < 0.01$). Desulfovibrio is also a sulfur-reducing bacterium. Previous studies have shown that more alkaline conditions are benefit to DNRA. Microbial nitrate-reduction processes can be inhibited by the trade off between denitrification and dissimilatory nitrate reduction to ammonium [30]. The relatively low abundance of DNRA can also enhance denitrification. For phosphorus-accumulating bacteria (PAOs), the read number of Pseudomonas in *P. australis*, *T. chinensis*, *S. salsa* and *A. sinensis* were significantly higher than control ($p < 0.01$).

### 3.4 Functional Gene Prediction of the Six Samples

Abundance of functional genes was forecasted based on the COG (Clusters of Orthologous Groups) database. In order to determine the functional genes that differ significantly in each sample, supervised comparisons by LEfSe ($p<0.05$) were performed. LDA score represent the extent to which functional genes with significant differences between different groups affect plants [31]. In Figure 3, the functional genes were related to *Transcriptional regulator* (LDA score = 4.98) and *Signal transduction histidine kinase* (LDA score = 4.98) in control were significant higher than that others. The functional genes with significant differences in the control group were those related to gene and environmental information processing, and their LDA values were the largest. With the succession, the root system of different salt-tolerant plants has an effect on the function of microorganisms in soil. As for the functional gene related to *Transposase and inactivated derivatives* (LDA score = 4.81) and *Serinethreonine protein kinase* (LDA score = 4.63), they have the highest influence on *S. salsa*. In addition, it can be seen from Figure 3 that functional genes associated with environmental information processing, metabolism and cell processing differ significantly in other samples, with a downward trend in the influence of *L. sinense*, *P. australis*, *B. vulgaris* and *T. chinensis*.

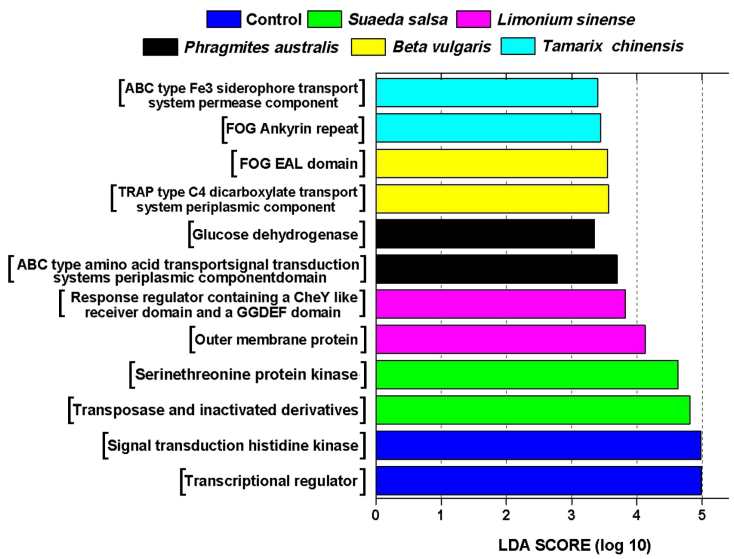


**Figure 3.** Supervised comparison identified differential abundance of functional gene prediction using LEfSe ($p<0.05$).

## 4. Conclusion

The microbial communities under different vegetation in the Yellow River Delta showed a significant spatial heterogeneity of microbial community diversities and functions. *A. sinensis* and *T. chinensis* were more adapted to the saline alkaline environment of the Yellow River Delta. The similarity in microbe community structures was highest between *T. chinensis* and *S. salsa*, which are both tolerant to severe salinity. *P. australis* had the strongest ability of denitrification and phosphorus-accumulation. Moreover, *T. chinensis* and *S. salsa* had significantly stronger nitrogen fixation effects than control ($p < 0.01$). The relative abundance of anammox bacteria was highest in *A. sinensis* (273).

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