



## A Comparison of Soil Microbial Diversity of Marshes Covered by Plants *S. salsa* and *S. alterniflora* in Yancheng Wetland, China

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**Abstract:** The introduction of invasive species, *Spartina alterniflora*, in the tidal marshes of Yancheng Wetland has been viewed to be deleterious to habitat quality. Little is known, however, on the extent to which the replacement of *Suaeda salsa* by *S. alterniflora* affects soil environment of impacted marshes. In this study, the soil physiochemical characteristics and microbial community of rhizosphere and the non-rhizosphere soils in adjacent *Spartina* and *Suaeda* marshes have been examined. The microbial data indicate that dynamics of microbial community were similar in both types of plants and the roots of plants were conducive to microbial community. The total number of microorganisms and microbial activity showed seasonal fluctuation, increasing from spring through summer and then declining gradually to the lowest in spring. The data provided herein also indicated that values of organic carbon, biomass carbon, size of microbial population, and activity are higher in *Suaeda* marsh than *Spartina* marsh, which to suggest that difference in vegetation cover significantly affect soil environment and microbial community. Thus, we suggest that the introduction policy of *S. alterniflora* into this area should be reconsidered.

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**Keywords:** Microbial activity; Fluorescein diacetate; *Spartina alterniflora*; *Suaeda salsa*

### 1. Introduction

The importance of bacteria in marine and estuarine sediments as a decomposer of organic matter and major contributor to the cycling process of carbon and nitrogen in benthic ecosystem has been widely recognized. In sediment, a wide range of factors, such as aboveground plant type and soil formation, affect microbial diversity. Relationships are often observed between the abundance of soil microbial communities, soil and plant type, and ecosystem sustainability (Alongi et al. 1988). However, the details of these interactions are incompletely elucidated. Thus, the understanding of the link between environmental factors and microbial diversity in soil is becoming more and more important for us to predict the effect that the diversity of microbial communities has on ecological function and resilience to disturbances in soil ecosystems.

Coastal marshes are important ecosystems because of their unique ecological roles in nutrient cycling, sediment accretion, pollution filtration, and erosion control. Yancheng coastal marshes is located in province Jiangsu, eastern China, in which have two National Nature Reserve. In its intertidal region, the competing between *Suaeda salsa* and *Spartina alterniflora* has been staged since *S. alterniflora* was

introduced in 1979. In competition, the invading species, *S. alterniflora*, was prevail and its distribution area has been gradually expanded, while the area of *S. salsa* was flinching. A large area of native vegetation replaced by invasive *S. alterniflora* has attracted widespread attention of ecologists and managers. Nonetheless, the microbial differences of this two plants covered were remaining unknown. Therefore, it is highly relevant to investigate these questions across diverse ecosystem types and process, as it may contribute to our understanding of the interactions between soil microbial communities and plant type.

Total microbial activity is a general measure of organic matter turnover in natural habitats because of more than 90% of the energy flow generally passes through microbial decomposers (Green et al., 2006) and is also fundamental to our understanding of the abundance of microbial communities in soil. The fluorescein diacetate (FDA) has been widely used in determining overall soil microbial activity and has been widely accepted as an accurate and simple method for measuring total microbial activity in soils (Adam et al. 2001). FDA is hydrolyzed by a number of different enzymes, such as proteases, lipases and esterases (SchnÄurer et al. 1982). The product of this

enzymatic conversion is fluorescein, which can be quantified by spectrophotometry. In this paper, we was to examine the interactions between plant type and microbial activity in the intertidal zone of Yancheng coastal marshes. Specific objectives were (1) to qualify microbial activity and seasonal changes in two type of marshes characterized by different plant types: *S. alterniflora* and *S. salsa*; (2) to evaluate the effects of alien species, *S. alterniflora*, on microbial diversity in soil.

## 2. Material and Methods

### 2.1 Site description and soil samples collection

Soil sample were collected in the intertidal marshes in Dongtai, Yancheng City, Jiangsu Province, P. R. China. This Sampling site was located in a transit belt from the subtropics to a warm temperate zone, which to be characterized by a subtropical, humid monsoon ocean climate, with a mean annual temperature and precipitation of 14.6 °C and 960 mm. The average maximum and minimum temperatures are 29 to 31 °C (July) and 0 to 3 °C (January), respectively, with a mean of 230 frost free days. Dongtai bleach is a typical herbaceous temperate tidal salt marsh wetland, with a general community succession from bleach to a *S. alterniflora* or *S. salsa* community.

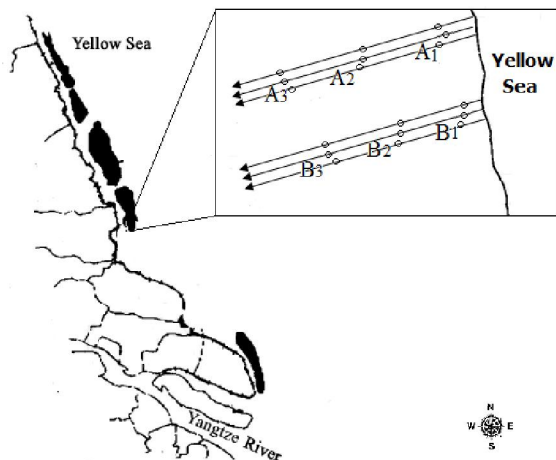


Figure 1. Location and sampling sites (A, B) on the Yancheng wetland, Jiangsu Province, China

Sampling was conducted from October to July, 2009 to 2010. The sampling sites were selected based on deposition conditions and plant distribution (Site A and B; Figure 1). Site A is dominate by *S. alterniflora*, and site B consists of *S. salsa*. Three parallel transects with an interval of approximately 10 m were set at each study site. Three sampling sites

were selected along each transect perpendicular to the coastline, extending from the sea to the inland, with an interval of approximately 500 m. Core soil samples, to be composed of roots and soils, were collected at three depths: -2 to -10 cm, -10 to -20 cm, and -20 to -30 cm. The fresh core samples were stored in valve plastic bags at 4 °C during the sampling process. All sample has been taken to the lab within 12 hrs after sampling and further treatment was conducted. Picked out plant roots from each samples and the remaining soils were considered as non-rhizosphere soil, while soils separated from roots by shaking roots vigorously were considered as rhizosphere soil.

Samples were homogenized and sieved through a 2-mm mesh. Microbial analyses were carried out with the field moist sub-samples, and physicochemical analyses were performed on air dried sub-samples (Xing et al. 2005). All results reported are the means of triplicate analyses and expressed on an oven-dry basis. All sample analyses were conducted within 2 weeks.

### 2.2 Determination of Microbial activity

The FDA hydrolytic activity was determined as a general indicator of total microbial activity (SchnÄurer et al., 1982). Each 5 g (dry weight) soil samples was added into 50 ml of 60 mM sodium phosphate buffer, pH 7.6, and then was simultaneously added with FDA to a final concentration 10µg ml<sup>-1</sup>. The mixture was incubated at 24 °C on a rotary shaker for 3 h and then the hydrolysis of FDA was terminated by the addition of acetone (final concentration, 50% [vol/ vol]). Soil was removed from the incubation solution by centrifugation for 5 min at 6000 rpm followed by filtration through Munktell no. 3 filter paper. The amount of FDA hydrolyzed was measured as absorbance at 490 nm (A<sub>490</sub>), and samples giving absorption values of greater than 0.8 were always diluted before final absorbance determinations.

### 2.3 Bacterial counting

Prior to dispersion, the soil samples were incubated for 20 min with Tween 80 (final concentration, 1 mg L<sup>-1</sup>). An ultrasonicator (100-W output) equipped with a 3-mm tapered micro-tip and the amplitude set at 40% of the maximum was used for the bacterial dispersion from the soil and the dispersion time for samples in the tubes was 1 min. To prevent denaturation of nucleic acids caused by overheating, the tubes were placed in ice water during the dispersion treatments.

After dispersion, the samples were diluted 50 to 250 times with particle-free seawater. Diluted samples were stained with DAPI to a final

concentration of 5  $\mu\text{g mL}^{-1}$  for staining. After more than 30 min of staining, 0.5 to 2 ml of the samples was filtered through polycarbonate black filters (0.2-mm pore size) and then rinsed with particle-free seawater. The filters were immersed in nonfluorescent oil on microscope slides and covered with cover slips. Bacteria retained on the filters were examined within 24 h after dispersion under an Olympus BX-FLA-3 epifluorescence microscope (UV excitation) equipped with a 100 $\times$  oil immersion objective. On each filter, no fewer than 200 clear-edged cells in 20 microscopic fields were counted (Porter et al. 1980).

### 2.4 Soil characteristics

The soil organic C, biomass C, total N, pH and soluble salt were analyzed. Total salt content was

measured by using the distillation residue method as described by Lu et al. (1999). Soil pH was measured with a glass electrode in 0.01 mol L<sup>-1</sup> CaCl<sub>2</sub> with a soil-to-solution ratio of 1: 2.5. Biomass carbon was determined as described by Vance et al. (1987). Soil organic carbon was determined by the Walkley and Black dichromate oxidation method. Total nitrogen was estimated by Kjeldahl method (Bremner et al. 1982).

### 2.5 Statistical analysis

Statistical analyses were conducted using Excel 2003. The significance of the various parameters was tested by one-way analysis of variance using the Duncan's new multiple range method.

Table 1. Physiochemical characteristics of upper (-5 to -10 cm) soil layer in sampling seasons (mean  $\pm$  SE)

Item	<i>Suaeda</i> marsh				<i>Spartina</i> marsh			
	Oct.	Jan.	Apr.	Jul.	Oct.	Jan.	Apr.	Jul.
Soil salinity (%)	1.12 $\pm$ 0.11a	0.97 $\pm$ 0.13a	0.38 $\pm$ 0.07b	0.45 $\pm$ 0.09b	1.08 $\pm$ 0.15a	1.05 $\pm$ 0.15a	0.31 $\pm$ 0.08b	0.34 $\pm$ 0.06b
Soil pH	8.35	8.35	8.42	8.35	8.43	8.50	8.42	8.37
Total N (g/kg)	0.29 $\pm$ 0.06a	0.32 $\pm$ 0.05a	0.20 $\pm$ 0.05b	0.07 $\pm$ 0.04c	0.32 $\pm$ 0.06a	0.34 $\pm$ 0.04a	0.21 $\pm$ 0.04b	0.14 $\pm$ 0.04bc

## 3. Results

### 3.1 Characteristics of the upper soil layer

The mean values for all physical and chemical properties of the upper soil layer are shown in Table 1. Soluble salt content varied significantly from 0.31 to 1.12 %, and the highest salt content was recorded in autumn at *Suaeda* marsh. Salt concentrations in the marshes were higher in autumn and winter than spring and summer, and showed no differences between *Suaeda* and *Spartina* marshes. Soil pH ranged between 8.35 and 8.50. There were no significant differences among seasons and marsh types in pH. Total nitrogen in the marshes ranged significantly between 0.07 and 0.34 g kg<sup>-1</sup> and there were no significant differences between the marshes in total nitrogen.

### 3.2 Soil carbon content in the upper soil layer

Soil organic carbon content at site A was significantly higher than that at site B throughout the year except in summer (Figure 2. a). In site B, soil

organic carbon content varied seasonally between 2.26 and 3.75 g kg<sup>-1</sup> dry soil; while in site A, soil organic carbon varied seasonally between 2.17 and 5.23 g kg<sup>-1</sup> dry soil. The organic carbon content of the two types of wetland soils showed similar trend, being highest from autumn through winter, and then declining gradually to the lowest in summer (Figure 2. a). In addition, soil organic carbon content showed an increasing trend from the lower tideland to the inland. Soil organic carbon content were determined by the difference between organic inputs (from the death of plant and soil animals) and organic carbon outputs. A possible for this may be that upper tidal mudflat soils had more plant and a shorter water-logging time; therefore, organic carbon inputs of the upper tidal soils were much higher. Organic carbon inputs into the low tide flat soils were, however, much low.

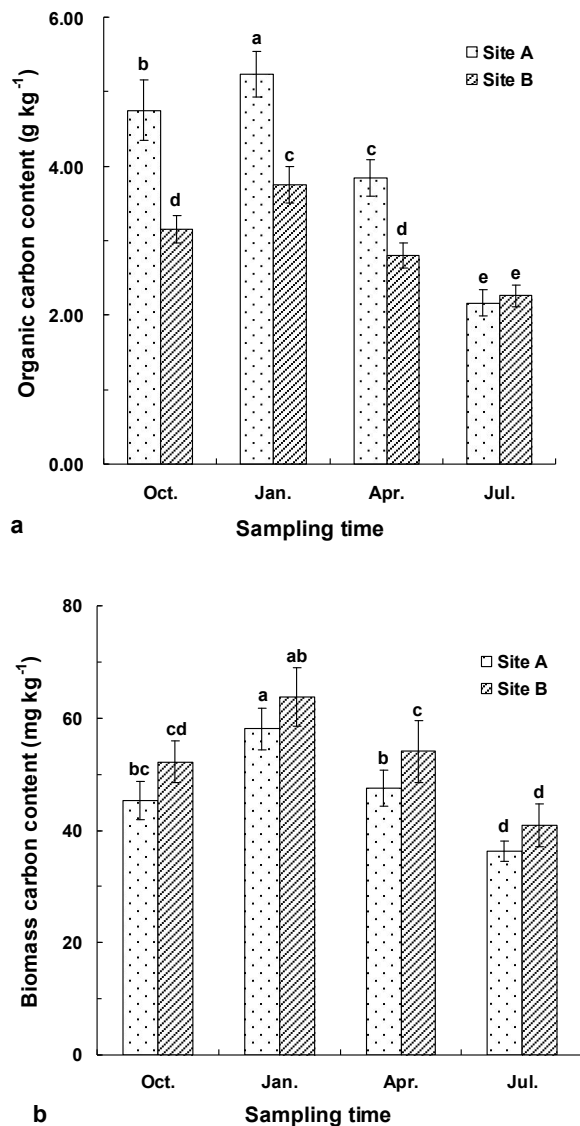


Figure 2. a: Seasonal variation in soil organic carbon content; b: Seasonal variation in soil microbial biomass carbon content. The different lower case letters indicated significant differences among the columns ( $p < 0.05$ ).

Seasonal changing trends of soil microbial biomass carbon content were similar to that of soil organic carbon content (Figure 2. b). In both sampling sites (A, B), the highest content of biomass carbon appeared in winter, and while the lowest content was in summer. Our results showed that biomass carbon content at site B with *S. salsa* was higher than the site A with *S. alterniflora* through the year. The microbial quantity and activity in site B was higher than the site A, which may result in

higher biomass carbon in the soil of the site B. Soil organic matter is usually used as an indicator to indicate soil fertility, and biomass carbon may be also used as indicator of soil fertility in beach areas with different plant type. Parffit et al. (2005) pointed out that microbial biomass was more effectively reflect the status of soil fertility, because of the level of soil organic carbon content can not directly explain the availability of nutrients, and soil microbial biomass activity can directly reflect condition of soil nutrient pool.

### 3.3 Changes of soil microbial community in the marshes

Total microbial activity provides a general measure of organic matter turnover in natural habitats. In other words, the FDA hydrolysis rate is positively related to the concentration and activity of soil enzymes. In this study, microbial activity was positively correlated with the microbial population size ( $R^2 = 0.8362$ ) (Figure 3). For the non-rhizosphere soil samples, the soil enzymes were primarily secreted by soil microorganisms. Thus, microbial activity determined by measuring the amount of FDA hydrolyzed can reflect the microbial population size.

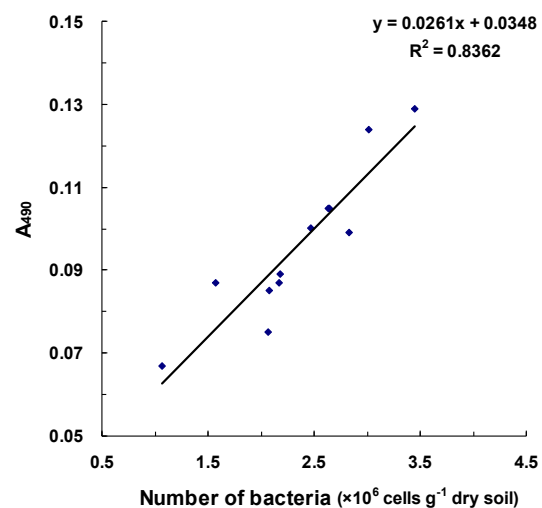


Figure 3. Relationships of total number of bacteria with microbial activity ( $A_{490}$ ). The equation describes the linear fit line through sampling sites.

The seasonal dynamics of microbial community characteristics of soil samples in the upper (-2 to -10 cm) soil layer collected in October 2009 (Autumn), January 2010 (Winter), April 2010 (Spring), and July 2010 (Summer) are shown in Figure 4. For *Suaeda* marsh, the total number of microorganisms and microbial activity varied

seasonally between  $2.17 \times 10^6$  and  $3.45 \times 10^6$ , and between 0.087 and 0.129, respectively, being high from spring through summer, and then declining gradually to the lowest in spring. For *Spartina* marsh, the numbers and microbial activity varied between  $2.07 \times 10^6$  and  $2.64 \times 10^6$ , and between 0.075 and 0.105, respectively. The seasonal fluctuation of microbial number and activity were similar compared to *Suaeda* marsh. In general, the *Suaeda* marsh has bigger microbial population and higher activity than the *Spartina* marsh.

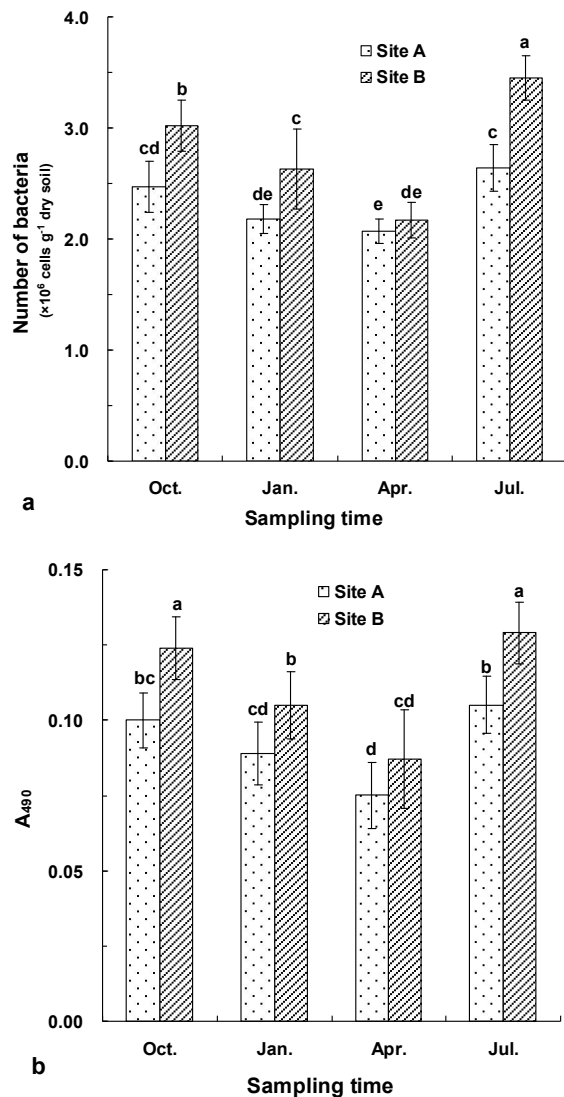


Figure 4. a: Seasonal dynamics of the total number of bacteria in upper (-2 to -10 cm) soil layer; b: Seasonal dynamics of microbial activity in upper (-2 to -10 cm) soil layer.

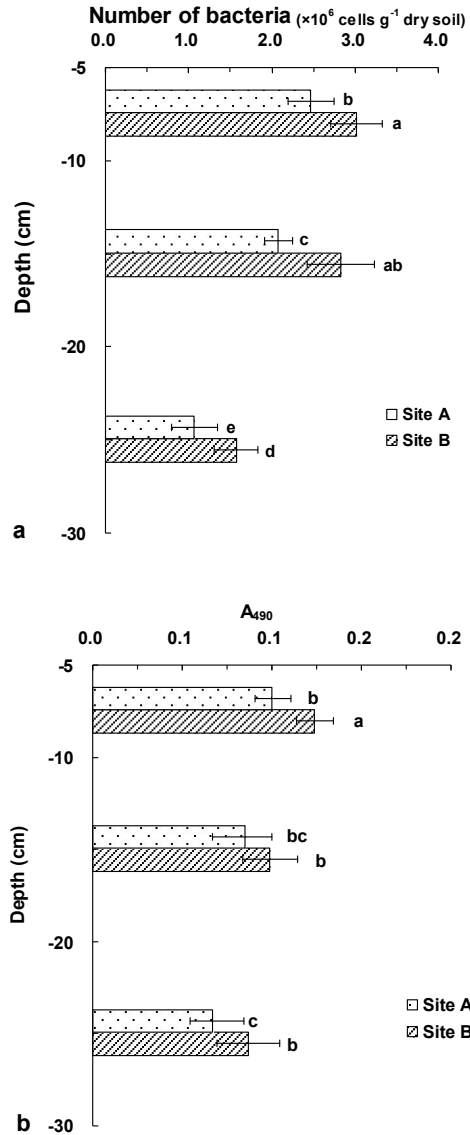


Figure 5. a: Dynamics of the total number of soil bacteria following depth in July; b: Dynamics of microbial activity following depth in July.

The results of the quantitative analysis of soil microbial populations in different depths are presented in Figure 5. The total number decreased from the uppermost layer to the lowest layer. For *Suaeda* marsh, the total number varied from  $1.57 \times 10^6$  to  $3.02 \times 10^6$  cells  $g^{-1}$  dry soil; for *Spartina* marsh, the numbers changed between  $1.07 \times 10^6$  and  $2.08 \times 10^6$  cells  $g^{-1}$  dry soil. Each layer of the *Suaeda* marsh had more microorganisms than that of the *Spartina* marsh. Microbial activity ( $A_{490}$ ) also decreased with depth. In *Suaeda* marsh microbial activity varied seasonally between 0.087 and 0.124, while it varied between 0.067 and 0.100 in *Spartina* marsh. On the whole, microbial communities in upper (-2 to -10 cm)



layer had the biggest size and highest activity among the three soil layers studied.

Significantly higher total microbial numbers and activity were observed in the rhizosphere soils as compared to the non-rhizosphere soils (Figure 6). For the two rhizosphere soil samples, total microbial number were  $10.83 \times 10^6$  (*Suaeda* marsh) and  $7.08 \times 10^6$  (*Spartina* marsh) cells  $g^{-1}$  dry soil, being significantly higher than the corresponding non-rhizosphere samples. The *Suaeda* marsh had the larger difference between the rhizosphere and non-rhizosphere microbial number compared to the *Spartina* marsh. Rhizosphere soil microbial activity ( $A_{490}$ ) significantly increased up to 0.202 in the *Suaeda* marsh, while it increased up to 0.141 in the *Spartina* marsh. In summary, roots of *S. Salsa* have more influence on the microbial community compared to *S. alterniflora* roots.

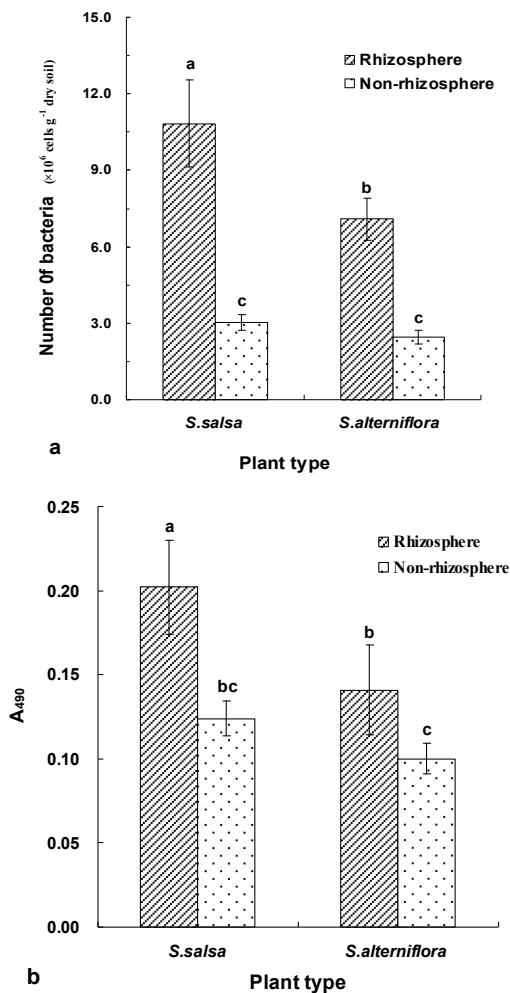


Figure 6. a: Difference of the total number of soil bacteria Values between rhizosphere soils and non-rhizosphere soils; b: Difference of microbial activity between rhizosphere soils and non-rhizosphere soils.

## 4. Discussions

### 4.1 Seasonal dynamics of microbial community in the upper soil layer

The total numbers and microbial activity were found to have a pronounced seasonal fluctuation. In this study, the highest values always occurred in summer. These results suggested that summer might be the most proper season for microbial reproduction (Wang et al. 2010). The lowest values occurred in spring, not as expected in winter. The region is located in a transit belt from the subtropics to a warm temperate zone, which to be characterized by a subtropical, humid monsoon climate, with a relatively high winter temperature. Though winter temperature reduced the microbial activity and reproductive ability, the soil microorganisms would keep proliferation with adequate energy supply from decomposing litter. On the other hand, microorganisms can decrease metabolism with lower organic matters to surviving lower winter temperatures (Zhou et al. 2005). These explain why the lowest microbial size and activity did not occur in winter.

Significantly higher soluble salt content was observed in autumn and spring than in summer and winter. To define factors leading salt content variations, we analyzed the rainfall data of Jiangsu Province, China. This region is characterized by rainy summer and arid winter, during rainy season more than 50% of the total annual precipitation recorded. The reason might be that there was strong evaporation and little rainfall which led to the salt accumulation in the upper layers in autumn and winter. Although rainfall helped to washing away soluble salt in summer, the rapid evaporation of the saline shallow groundwater in the coastal region during the summer months brings salts to the surface through capillary action. These explained that soluble salt content was similar between spring and summer. There was no obvious correlation between soluble salt content and microbial activity.

### 4.2 Effects of depth on microbial community

The aeration gradient may be the predominant determinant of active microbial communities (Bossio et al. 2006) and there are usually more organic matter and nutrients in the upper layer (Niemi et al., 2007), which make it a better environment for the aerobic microorganisms' survival. In this study, the zonation may indicate that the microbial communities in 5 to 30 cm layers are largely dominated by aerobic species that are unable to survive in deeper, more anoxic conditions. With further increases in depth, the aforementioned soil properties might decrease and would result in smaller and less active microbial communities with depth.

The obvious zonation of microbial community was consistent with results of other researches. In costal wetland of the Yellow River Delta, for example, the species richness of microorganisms decreased with depth (Liu et al. 2007; Wang et al. 2010).

#### 4.3 Effects of plants on microbial community

Significantly higher total microbial numbers and activity were observed in the rhizosphere soils as compared to the non-rhizosphere soils. The existence of plants improved the soil quality and increased the nutrition for microbial communities, which in turn created environmental conditions suitable for the establishment of a more diverse and active microbial community.

Zhou et al. (2005, 2006) found that the structure of microbial community has improved when plants were present. Plant roots release a wide variety of compounds into the surrounding soil, including amino acids, sugars, ethylene, polysaccharides, and enzymes. These materials create unique environments for microorganisms living in association with plant roots and thus different compositions of root exudates are expected to select different rhizosphere communities. In other words, plant type is the major factor influencing the structure of microbial communities.

This conclusion makes sense for two reasons. Firstly, plants are the main providers of specific carbon and energy sources (Garbeva et al. 2004). Different species of plants have different kinds and quantities of root exudates, and thus the rhizosphere effects on microbial communities are not the same among different vegetative communities (Xiang et al. 2004). Secondly, the influence of soil is negligible due to the carefully selected sampling sites.

There were higher microbial numbers and activity in both rhizosphere and non-rhizosphere soils in *Suaeda* marsh compared to *Spartina* marsh. The *Suaeda* had the larger difference between the rhizosphere and non-rhizosphere microbial size and activity. The smaller difference was found in *Spartina*. In general, *S. Salsa*, one of the most popular native plants in the wetland of Yellow Sea, had the more obvious rhizosphere effect. Lin et al. (2005) also reported that *Suaeda glauca* decreases the EC of soil, and increases organic matter and total nitrogen. The common seepweed had the most obviously rhizosphere effect among the wetland plants (Wang et al. 2010). Thus it is self-evident that the microbial levels were higher in native plants' rhizosphere compared to alien *S. alterniflora*'s.

#### 5. Conclusions

In the wetland of Yellow Sea, China, *S. Salsa* is noted for acting as an environmental engineer. It grows at the seaward edge of a salt marsh,

and enables other habitat-engineering species, such as hermit crab, to settle. Since the invasion of *S. alterniflora*, all the situations have changed. *S. alterniflora* has a variety of traits that allow it to outcompete the native *S. Salsa*, including a high saline tolerance and the ability to perform photosynthesis productively at lower temperatures. Meadows of *S. alterniflora* crowds out *S. Salsa*, altering the soil environment, as a result of *S. alterniflora*'s growth, microorganisms that live in soil decreased as their habitat quality deteriorated. The invasion of *S. alterniflora* brings deleterious consequences to microorganisms, and may alter the developing trend of wetland ecosystem. Thus, the policy, introducing *S. alterniflora* into this area, should be reconsidered.

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