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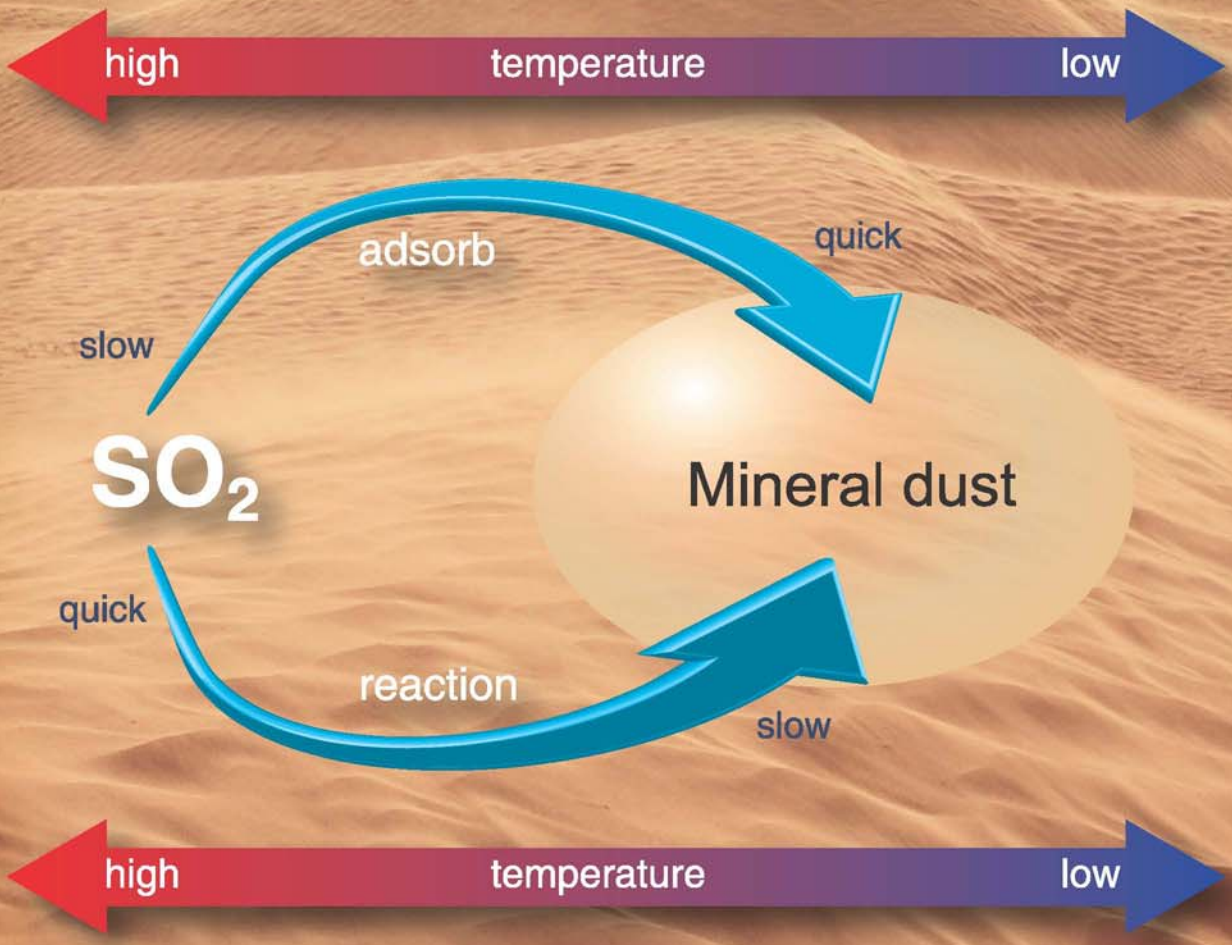

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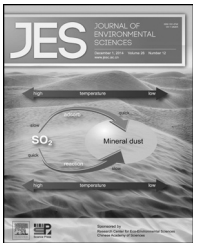

Salinity influence on soil microbial respiration rate of wetland in the Yangtze River estuary through changing microbial community

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ABSTRACT

Estuarine wetland, where freshwater mixes with salt water, comprises different regions (rivers and marine ecosystems) with significantly varying tidal salinities. Two sampling areas, ZXS and JS, were selected to investigate the effect of tidal salinity on soil respiration (SR). ZXS and JS were located in Zhongxia Shoal and Jiangyanan Shoal of Jiuduansha Wetland respectively, with similar elevation and plant species, but significantly different in salinity. The results showed that with almost identical plant biomass, the SR and soil microbial respiration (SMR) of the tidal wetland with lower salinity (JS) were significantly higher than those of the tidal wetland with higher salinity (ZXS) ($p < 0.05$). However, unlike SMR and SR, the difference in the soil microbial biomass (SMB) was not significant ($p > 0.05$) with the SMB of ZXS a little higher than that of JS. The higher SMR and SR of JS may be closely connected to the soil microbial community structures and amount of dominant bacteria. Abundant β- and γ-Proteobacteria and Actinobacteria in JS soil, which have strong heterotrophic metabolic capabilities, could be the main reason for higher SMR and SR, whereas a high number of ε-Proteobacteria in ZXS, some of which have carbon fixation ability, could be responsible for relatively lower carbon output. Path analysis indicated that soil salinity had the maximum negative total influencing coefficient with SMR among the various soil physical and chemical factors, suggesting that higher soil salinity, restricting highly heterotrophic bacteria, is the principle reason for lower SMR and SR in the ZXS.

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Introduction

Soil is the largest terrestrial carbon pool in earth. The soil pool can be as high as 1.5×10^3–2×10^3 Pg, which is greater than the atmospheric and vegetation carbon pool (Post and Kwon, 2000). Soil carbon pool consists of soil inorganic carbon (SIC) pool and soil organic carbon (SOC) pool (Lal, 2004a). Furthermore, depletion of SOC pool has contributed to (78 ± 12) Pg C (carbon) in the atmosphere (Lal, 2004b). Hence, the storage of SOC is of great importance for the circulation of C.

Input and output are two aspects that can affect SOC pool. The input is mainly from plant biomass materials, while the output is mainly affected by soil respiration (SR), which comprises three biological components (soil microbial respiration (SMR), root respiration, and soil animal respiration), and one chemical component. Among them, SMR accounts for half of the carbon flow from terrestrial ecosystems to the atmosphere, and is closely associated with microbial activity, microbial community, as well as soil environmental conditions (Zou et al., 2006; Xi et al., 2012).

Wetlands are water–land complex ecosystems. Undisturbed natural wetlands are considered to be very important for carbon sequestration because of their low rate of organic decomposition and SR, which might be related to long flooded periods (Bernal and Mitsch, 2012).

Many previous studies had primarily focused on interior freshwater wetlands, especially the boreal wetland, and demonstrated their ability of high SOC accumulation (Hirota et al., 2006). Estuarine salt marsh has high primary productivity and its carbon sequestration ability has drawn a lot of attention with the increase of global greenhouse effect (Li et al., 2011; Tang et al., 2011; Hu et al., 2012, 2014). Estuarine wetland is usually formed from the upstream sediments. It is an ecotone between river and marine ecosystems, and is located where the fresh water meets the salt water and the land connects to the sea. This property often leads to its combination of riverine and coastal mud flat. Tiding is an important factor that separates estuarine wetland from the interior wetland, and the duration of being submerged and nutrient input from the tide vary with the succession of different elevations. As a result, tiding can have an effect on soil property and SR, and thus, on the carbon sequestration ability of estuarine wetland. The soil salinity of different regions (river and marine ecosystem) of the estuarine wetland may vary significantly owing to the difference in tidal salinity. Wichern et al. (2006) indicated that SR and SMB decreased with the increasing levels of salinity in the pasture sites in Heringen (Germany). Furthermore, the study by Wong et al. (2008) with soil samples collected from a vegetated soil under controlled conditions demonstrated that low- and mid-salinity treatments produced the highest and lowest SR, but lowest and highest SMB, respectively. In general, soil salinity restrains SR, and an increase in soil salinity leads to a decrease in SMB and soil enzyme activities. Studies on the effects of salinity on SMR and SMB in the estuarine wetland are relatively rare and have not focused on the microbial community structures of the soil.

Jiuduansha, which is the only original wetland situated in the Yangtze River estuary, Shanghai, China, has a significant ecological environment function (Qiao et al., 2012). This wetland spans about 40 km from west to east. Similar to other estuarine wetlands, Jiuduansha is also an ecotone between a river and a marine ecosystem and from its west to east, river water reaches the marine water, resulting in obvious difference in the tidal water salinity. Some studies have been conducted on the SR and carbon turnover of the Jiuduansha Wetland. Tang et al. (2011) investigated the response of soil microbial community in Jiuduansha Wetland to different successional stages and its effects on SMR and carbon turnover. Qiao et al. (2012) studied the distribution and characteristics of the total petroleum hydrocarbons in the Jiuduansha Wetland, and indicated their potential impact on SMR. Nevertheless, few studies had been conducted to clarify the effect of tidal salinity on SR and carbon turnover of the Jiuduansha Wetland.

In the present study, two sampling areas, ZXS and JS, located in Zhongxia and Jiangyanan Shoals of the Jiuduansha Wetland, respectively, in the Yangtze River estuary were selected. These two sampling areas were found to have similar elevation and plant species, but significantly different tidal salinities. The effect of tidal salinity on SR and its underlying mechanism were investigated by analyzing the SMR, soil microbial characteristics, and physicochemical properties.

1. Materials and methods

1.1. Site description

Jiuduansha Wetland (31°03′N–31°17′N, 121°46′E–122°15′E) is located between the southern and northern watercourses of the Yangtze Estuary, Shanghai, China. This wetland initially emerged above the water surface in the 1920s and rapidly became an independent wetland in the 1960s. It is affected by the East Asian subtropical monsoon climate, with an average annual temperature of 17.3°C, average summer temperature of 28.9°C, and average winter temperature of 5.6°C (Gaoqiao Monitoring Station, Shanghai, China). Furthermore, the mean salinity of the wetland water from west to east varies significantly.

The wetland covers 423.2 km^2 and consists of the following three shoals from west to east: Jiangyanan Shoal, Shangsha Shoal, and Zhongxia Shoal. The vegetation mainly includes *Zizania aquatica* L., *Phragmites australis*, *Spartina alterniflora*, and *Scirpus triqueter* L.

1.2. Experimental design

The two sampling areas, JS and ZXS, with significantly different tidal salinities, were selected for investigation. JS is the nearest to the Yangtze River, while ZXS is located at the outermost part of the Yangtze Estuary area, near East China Sea.

To eliminate the effect of different kinds of plants and elevation on SR, sampling areas with similar elevation and identical plant species (*S. triqueter*) at the low tidal flat were selected, where carbon flux was more sensitive to tides than at the high-elevation (Guo et al., 2009). *S. triqueter* is the typical and pioneer plant of the Jiuduansha Wetland, and is widely spread in areas with an elevation of about 2.6 m. The sampling areas are shown in Fig. 1 and the characteristics of the sampling areas are listed in Table 1.

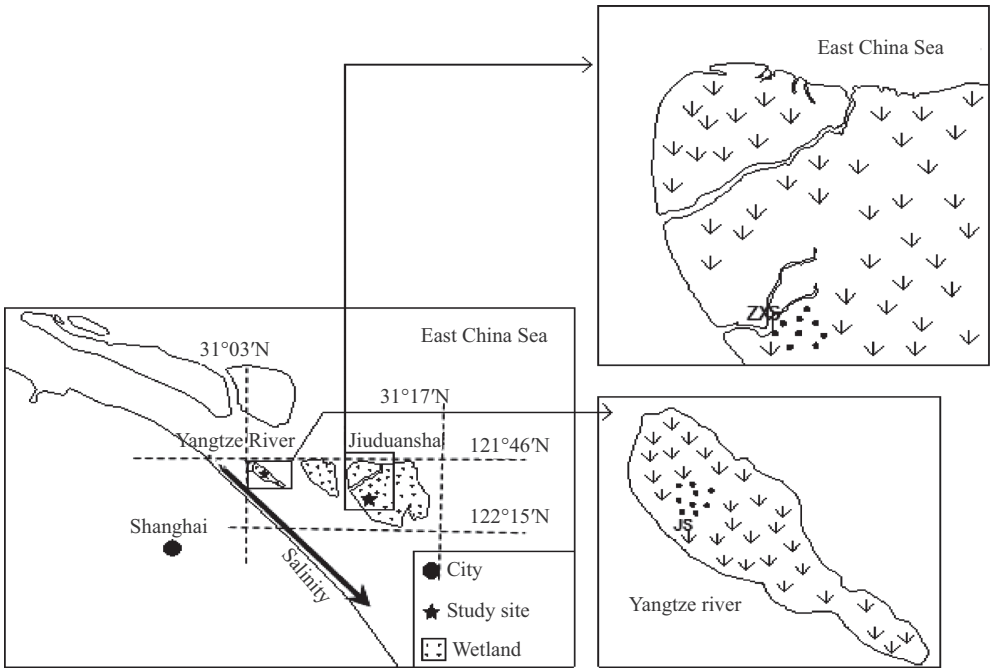

Fig. 1 – Map of Jiuduansha Wetland, Shanghai, China. From west to east, JS and ZXS are sampling areas. ZXS: sampling area in Zhongxia Shoal; JS: sampling area in Jiangyanan Shoal.

1.3. Soil sampling and pretreatment

Nine random points (1 m × 1 m each) were selected in each sampling area and the distance between the successive points was about 10 m. At each point, soil samples were collected from the sub-surface layer (−5 to −20 cm) according to the measure of Pennock and Yates (2007). Thus, a total of nine samples for each area were collected in January, April, July and October 2011, respectively. At the same time, SR at every sampling point was assayed in the field. Each point was monitored three times per day and night, respectively. Totally, each point was monitored for six times in one sampling quarter and 24 times in one sampling year, *i.e.*, each area (9 points) was monitored for 216 times in one sampling year.

All of the soil samples, were packed in individual sterile plastic bags and immediately stored in an ice-box. A part of the fresh soil stored at 4°C was used immediately for SMB and soil enzyme activity analysis (sieved <2 mm) in 1 week; another part was stored at −70°C for subsequent DNA extraction; and the rest of the soil sample was air-dried, sieved to <1 mm, and stored at room temperature for routine assay. All the results were expressed on an oven-dry basis.

1.4. Analysis methods

1.4.1. Soil respiration

In each sample point, 10-cm-diameter PVC soil collars were installed for SR test. In accordance with the study by Han et al. (2014), living plants inside the collars were carefully clipped from the soil surface. There were one to two plants inside the collar, with a plant density similar to that of the sampling area. The SR values obtained from the gradient method were calibrated with SR values acquired using a soil chamber connected to a soil respiration system (LI-8100A Automated Soil CO_2 Flux System, LI-COR Biotechnology, Lincoln, Nebraska, USA).

1.4.2. Soil microbial respiration (SMR)

The CO_2 released by the microorganisms within 24 hr from 40 g of original fresh soil samples incubated in 250-mL serum bottles at 28°C was measured by gas chromatography (GC-14B,

Table 1 – The geographical parameters, vegetation and water quality of the sampling areas in Jiuduansha Wetland.

	Location	Height (m)	Time of being submerged (hr/day)	Vegetation type	Plant biomass (kg/m^2)	Water		
						pH	Salinity (g/kg)	Total organic carbon (mg/L)
ZXS	31 10.236°′N 121 57.707°′E	2.5–2.8	6–8	*S. triqueter* L.	1.61	7.74 ± 1.01	3.4 ± 0.31	13.7 ± 2.98
JS	31 14.046°′N 121 48.902°′E	2.5–2.8	6–8	*S. triqueter* L.	1.06	7.50 ± 1.24	0.22 ± 0.10	15.8 ± 2.74

ZXS: sampling area in Zhongxia Shoal; JS: sampling area in Jiangyanan Shoal.

Shimadzu, Kyoto, Japan) using a stainless steel column (10 m × 2 mm) and a TCD detector (Tang et al., 2010). The column, inlet, and detector temperatures were 40°C, 40°C, and 90°C, respectively. Nitrogen gas was applied as the carrier at a flow rate of 30 mL/min. The CO_2 injection volume was 0.2 mL, and the CO_2 released per unit of time from the microorganisms was assayed and reported as the SMR.

1.4.3. Soil microbial biomass

The SMB was represented by ATP concentration, which was measured by using Profile-1 Bioluminometer (Model3560, 1×, New Horizons Diagnostics Corp., Washington D.C., USA), for determining the microbial biomass in the soil samples. A total of 5 g of fresh soil was added to 50 mL of sterile water with several glass beads (diameter: 3–4 mm) and then placed on an oscillator for 60 min (120 r/min) to suspend the soil. Subsequently, 2 mL of the soil suspension was mixed with 8 mL of 0.05% benzalkonium bromide to break up the cells, and the mixture was filtered. Then, 100 μL of luciferase (FL-AA, Sigma, ST. LOUIS, USA) was added to 100 μL of the filtrate, and 200 μL of this mixture was added to the Profile-1 Bioluminometer to obtain the fluorescence value for determining the microbial biomass in the soil samples (Hu et al., 2014).

1.4.4. Enzyme activities

Soil dehydrogenase and β-glucosidase activities were analyzed based on the standard method described by the Soil Science Society of America (Tabatabai, 1994).

1.4.5. Clone library

Samples for clone analysis were obtained from the mixture of four seasons' samples. Total DNA extraction was conducted using a FastDNA® spin kit for soil (Qbiogene Inc., Carlsbad, California, USA). The extracted DNA was visualized on 1% agarose gels, and then stored at −20°C for the construction of clone library.

The clone library was constructed as follows: PCR amplification of bacterial 16S rRNA gene fragments was performed using the universal bacterial primers 8f and 1492r (Lane et al., 1985). The PCR products were purified with a PCR purification kit (Biomiga Inc., San Diego, California, USA). Then, 4.95 μL of the PCR product of about 1.5 kb was linked to pMD® 18-T vector (TaKaRa, Dalian, Liaoning Province, China), and 6 μL of the ligation products was subsequently transformed into *Escherichia coli* TOP10, which allowed screening of positive clones on Lysogeny broth (LB) agar plate containing 100 μg/mL of ampicillin. About one-fifth of the clones were randomly selected for amplification by PCR with vector-specific primers M13, and the clone with target PCT PCR products were sequenced using bacteria-specific 8f primer. A total of 100 effective sequences were obtained. The sequences and their closely related 16S rDNA sequences obtained were then manually proof-read and corrected. The sequences were compared with the 16S rRNA gene databases using a BLAST search of the GenBank database. All consensus sequences were checked for chimeras using the CHIMERA CHECK program from the Ribosomal Database Project II, and no chimeras were detected.

1.4.6. Routine analysis

The soil pH was measured using a sure-flow combined glass-calomel electrode in H_2O and 1 mol/L KCl solution (solid: liquid mass ratio of 1:2.5) (Du and Gao, 2006). The soil moisture content was determined after drying at 105°C for 8 hr (Du and Gao, 2006). Salinity was determined in a 1:5 (solid: liquid mass ratio) slurry using a conductivity meter (HQ400d, multi, HACH, Loveland, Colorado, USA). Oxidation-reduction potential (ORP) was measured using a Model IQ150 hand-held pH/mV/temperature meter (Spectrum technologies, Inc., Aurora, Illinois, USA). The SOC was measured using a total organic carbon analyzer (TOC-VCPN-SSM, Shimadzu, Kyoto, Japan), maintaining accuracy within 5%. Available phosphorous (AP) was assayed based on the Olsen method (Olsen et al., 1954), and inorganic nitrogen (IN) was extracted from freshly thawed soil samples with 1 mol/L of KCl (soil: solution ratio = 25 g dry wt: 80 g) (Recous et al., 1995).

1.5. Data processing and statistical analysis

Besides clone library, the data of each point represented the average of three measurements and those of each area were the average of the nine points. Furthermore, all the data were presented as the average of 1 year. Statistical analyses included one-way analysis of variance (ANOVA) and path analysis. The one-way ANOVA was conducted to determine the significance of the difference in the soil parameters between the two areas. Path analysis was performed to determine the relationship among the variables, as well as to show the emphasis of reason on the results. Furthermore, the correlation coefficients can be divided into those with direct and indirect effects, which suggest the relative importance of the factors on the results. The path coefficient (R) was calculated using Eq. (1):

$$R_{23} = \beta_{31.2} R_{12} + \beta_{32.1} \quad \text{(if there were only three parameters)} \quad (1)$$

where, R_{23} denotes the correlation between 2 and 3 and $\beta_{31.2}$ indicates the regression of 3 on 1 (Alwin and Hauser, 1975). All analyses were conducted using the SPSS software (version 19.0, SPSS Inc., International Business Machines Corporation, Armonk, New York, USA).

The clones were considered to belong to the same operational taxonomic unit (OTU) if they were ≥97% identical to the region of the 16S rRNA gene sequenced (Stackebrandt et al., 1993). The Shannon index was described as being the most sensitive to changes in the importance of rare species in the sample and was calculated by Eq. (2):

$$H = -\sum Pi \ln Pi \quad (2)$$

where, H is The Shannon index and P_i is the proportion of clones in the OTU (Hill et al., 2003). The analyses of OTUs and the Shannon index were conducted by using mothur software (version 1.32.0, initiated by Dr. Patrick Schloss and his software development team, Department of Microbiology and Immunology at The University of Michigan, Michigan, USA).

2. Results

2.1. Variability of SR, SMR, and soil microbial activity of tidal wetland with different tidal salinity

The results (Fig. 2) showed that the SR varied significantly between the two areas differing in tidal salinity. The SR of ZXS

was greatly lower than that of JS ($p < 0.05$). The two areas exhibited identical plant biomass (Table 1). JS showed higher SR, demonstrating that the tidal wetland with lower salinity had weaker SOC sequestration ability. Besides, the SMR of ZXS was also lower than that of the JS ($p < 0.05$).

The results, shown in Fig. 3, indicated that, similar to SMR and SR, the dehydrogenase and β-glucosidase activities of JS were both higher than those of ZXS. The difference in β-glucosidase activity between JS and ZXS was insignificant ($p > 0.05$), whereas the difference in the dehydrogenase activity was significant ($p < 0.05$). Therefore, it can be speculated that higher soil enzyme activity may be one of the main factors responsible for higher SMR and SR of the tidal wetland with lower salinity.

The above-mentioned findings indicated that the difference in the tidal characteristics may have resulted in the variance in the soil microbial activity and thus the SMR and SR of the two areas examined.

2.2. SMB and microbial community structures responsible for different microbial activities and SR

To identify the reasons leading to the difference in the SMR and microbial activity between JS and ZXS, the SMB and microbial community structures were analyzed. The difference in the SMB between JS and ZXS was not significant ($p > 0.05$). And unlike SMR and SR, the SMB of ZXS was a little higher than that of JS ($1.20 \times 10^{-10} \pm 6.17 \times 10^{-11}$ mol/g for ZXS, and $9.92 \times 10^{-11} \pm 3.12 \times 10^{-11}$ mol/g for JS).

The complete results of the soil bacterial community structures of the two types of tidal wetland are provided in the Supplementary information (Table S1). The sequences obtained in this study are available in the GenBank database under the accession numbers KM188064–KM188253.

All clones were grouped into different OTUs on the basis of >97% sequence similarity. There were 48 and 84 OTUs in the ZXS and JS respectively. The community diversity, as reflected by the Shannon index, was higher in the JS (4.40) and lower in the ZXS (3.82) at a level of 0.03. Thus, it can be concluded that the JS comprised abundant microorganisms than the ZXS.

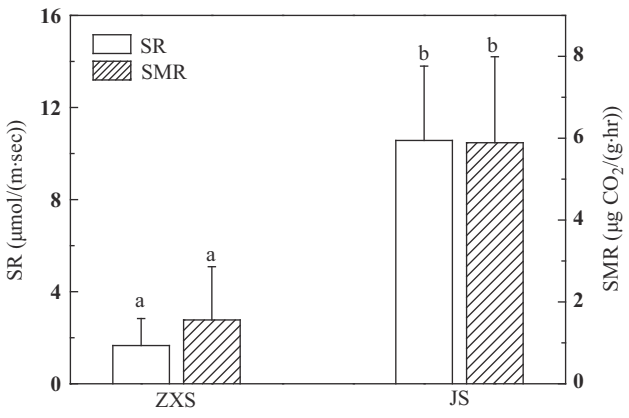

Fig. 2 – The annual average SR and SMR of the two the sampling areas of Jiuduansha Wetland. Parameters designated by identical letter represent insignificant ($p > 0.05$). SR: soil respiration; SMR: soil microbial respiration.

Furthermore, there was a significant difference in the soil microbial community between the two areas. To investigate this difference in detail, the number of dominant bacteria in the two areas was analyzed.

A summary of the bacterial sequence identification carried out according to the closest matched sequences in the two sampling areas is presented in Fig. 4. The clone library included α-, β-, γ-, δ-, and ε-Proteobacteria, Cyanobacteria, Bacteroidetes, Firmicutes, Nitrospirae, Actinobacteria, *etc*. The bacterial sequences that were affiliated to the different types of Proteobacteria accounted for 55% and 52% in ZXS and JS, respectively. α-, β-, γ-, δ-, and ε-Proteobacteria were all observed in the soil of ZXS and JS. However, ε-Proteobacteria was the most dominant Proteobacteria in ZXS (19%), whereas γ- and β-Proteobacteria played the most important role in JS (29% in total). Actinobacteria were found only in JS. Thus, there was a significant difference in the number of dominant bacteria between the two areas. The difference in the soil microbial community structures and the number of dominant bacteria in the two sampling areas in the tidal wetlands may be the reason for the difference in their soil enzyme activities and SMR, and thus the SR.

2.3. Difference in soil physical and chemical characters of the two kinds of tidal wetlands and its effects on microbial properties and SR

Soil physical and chemical characters, such as ammonia nitrogen salinity, organic carbon, water content, and ORP are important factors that influence soil microbial growth, community diversity, soil enzyme activity, and hence SMR and SR (Kirschbaum, 1995).

To study the effect of environmental features on microbial activity and SMR, the soil physical and chemical characteristics of the two kinds of tidal wetlands were analyzed, and the results are listed in Table 2. The results of the path analysis of those physical–chemical characters and SMR are shown in Table 3. Path analysis indicated that the sequence of the total effect of soil physical and chemical characters on SMR is salinity > SOC > AP (absolute value). However, stepwise regression analysis revealed that the water content, pH, ORP (Eh), and IN might not be significantly correlated with SMR. The results indicated that salinity had the greatest negative total influencing coefficient with respect to SMR, while SOC had the greatest direct negative correlation with SMR. In general, SOC is considered to be proportional to the microbial biomass (Xi et al., 2012); however, the results of the present study are in contrast to this observation. The reason for this may be the higher SMR and soil microbial activities when the organic matter input was almost identical, suggesting that more organic carbon in the soil was used, leading to the decrease in the remaining organic carbon in the soil (Li et al., 2010). Although AP had a positive direct effect on SMR, on the whole, it had a negative influence, implying that, in general, the negative effect of salinity on SMR was more evident than the positive effect of AP.

The above-mentioned finding indicated that higher salinity of ZXS might be the main reason for the relatively weaker SMR, and consequently, low SR. According to the results presented in Section 3.2, tidal salinity might influence the SR

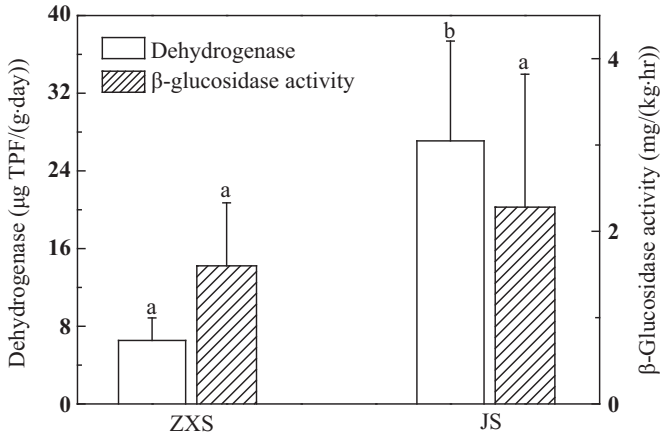

Fig. 3 – Soil enzyme activity of the two the sampling areas of Jiuduansha Wetland. The different letters above ($p > 0.05$).

intensity by changing the soil microbial community structure and dominant bacteria. As soil salinity mainly results from tide, tidal salinity could be considered to be the major abiotic reason for the difference in the microbial activity, SMR, and thus SR.

3. Discussion

3.1. Effect of soil microbial community structures on microbial activity, SMR, and SR

As indicated earlier, the soil enzyme activity of ZXS was lower than that of JS, which was similar to SR and SMR, whereas unlike SMR and SR, the SMB of ZXS was higher than that of JS. Although higher SMB might enhance soil microbial activity and SR, some studies have indicated that a positive correlation between SMB and SMR or SR was not absolute. Bardgett and Leemans (1996) examined four kinds of mountain soils and reported that, in general, the SMB could promote soil microbial activity; however, they found that one sample with lower SMB exhibited higher respiration and soil enzyme activities. Li et al. (2011) analyzed two different sites in Chongming Dongtan Wetland and found that the microbial biomass did not differ significantly, but the SMR showed obvious variation. In addition to SMB, microbial community and the amount of dominant bacteria were noted to be more closely connected to soil enzyme activities, and, hence, SR and carbon retention capability (Li et al., 2011).

Janssen (2006) reported that Proteobacteria constituted 40% of the prokaryotes. In the present study, Proteobacteria accounted for 55% and 52% in the ZXS and JS, respectively, and α-, β-, γ-, δ-, and ε-Proteobacteria were found in both types of wetlands. Different kinds of Proteobacteria have

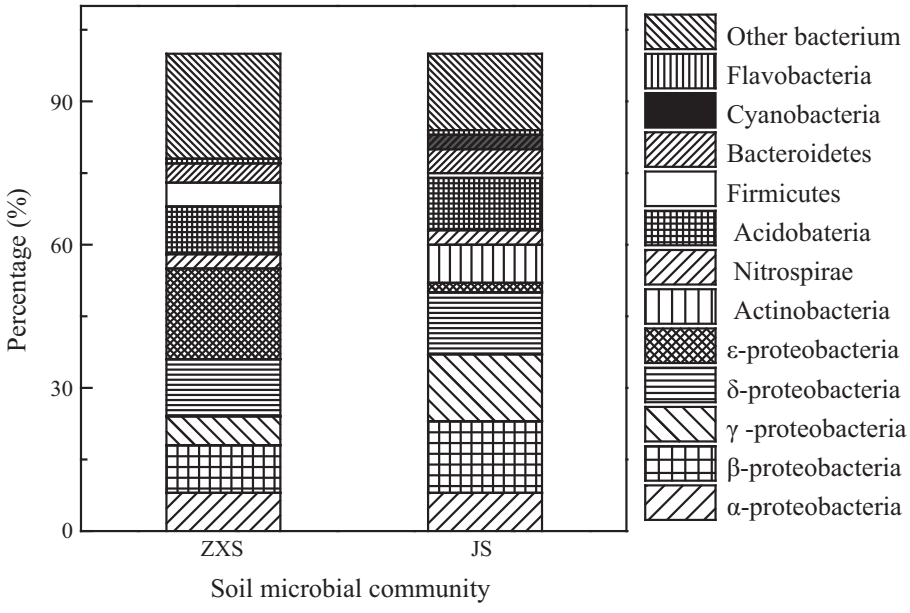

Fig. 4 – Soil microbial community of the two the sampling areas of Jiuduansha Wetland.

Table 2 – Soil physical and chemical factors of the two the sampling areas of Jiuduansha Wetland.

	Water content (%)	Salty (g/kg)	pH	Eh (mV)	SOC (g/kg)	IN (mg N/kg)	AP (mg/kg)
ZXS	35.2 ± 7.4 a	3.75 ± 0.34 a	6.97 ± 0.30 a	−36.19 ± 13.23 a	4.84 ± 0.74 a	18.73 ± 9.93 a	23.34 ± 9.70 a
JS	32.7 ± 8.1 a	1.27 ± 0.60 b	6.86 ± 0.11 a	−27.60 ± 15.00 b	2.60 ± 0.65 b	12.00 ± 3.90 a	19.07 ± 9.44 a

In the table parameters designated by identical letters represent insignificant difference between these two sampling areas ($p > 0.05$).
Eh: oxidation–reduction potential; SOC: soil organic carbon; IN: inorganic nitrogen; AP: available phosphorus.

their own distinct characteristics: some β-Proteobacteria have greater ability to degrade cellulose and various carbon sources (Sato et al., 2009); some γ-Proteobacteria have the ability to decompose different forms of carbon compounds (Rocker et al., 2012); some ε-Proteobacteria are considered to have the ability to fix CO_2 (Hugler and Sievert, 2011). Thus, a relatively small amount of β- and γ-Proteobacteria and a larger amount of ε-Proteobacteria in the ZXS may be responsible for weaker SR than that observed in the JS.

In addition to Proteobacteria, Actinobacteria and Acidobacteria also are important compositions in the soil bacterial community. The percentages of Acidobacteria in the JS and ZXS were found to be similar, and hence, it was conjectured that they were not the main reason for the difference in SR of JS and ZXS. Actinobacteria was only found in the JS. Lee et al. (2008) reported that Actinobacteria might probably be the most metabolically active organism in the rhizosphere soils. In other words, the existence of Actinobacteria might also contribute to the higher SR of the JS.

3.2. *Effect of salinity on bacterial community and microbial activity*

In the present study, path analysis showed that salinity had the maximum negative total influencing coefficient with respect to SMR. In other words, because of the different water qualities of the two shoals in the conjunction of fresh and sea water, the SMR and SR were found to vary. This might be because salinity could have changed the microbial community structure or the number of dominant bacteria or suppressed the heterotrophic metabolic capabilities.

The number of ε-Proteobacteria in the high salinity tidal wetland (ZXS) was observed to be high. Furthermore, a large number of one species of ε-Proteobacteria, more prevalent in the deep sea (Hugler and Sievert, 2011) with the ability to fix CO_2, could have led to lower OTUs and low Shannon index (Kennedy and Smith, 1995) as well as low SMR and SR in the ZXS. On the contrary, a relatively high number of various species of heterotrophic bacteria, such as Actinobacteria, γ- and β-Proteobacteria was noted in the JS. Many studies have illustrated that β-Proteobacteria with higher heterotrophic metabolic capacity are always dominant in estuarine freshwater areas (Garneau et al., 2006). Zwart et al. (2002) noted that β-Proteobacteria were the most numerically dominant cell and clone-type organism in the world's freshwater areas. Furthermore, Tang et al. (2012) observed that the abundance of β-Proteobacteria in an oligosaline lake was dominated by soil salinity. Besides, γ-Proteobacteria were noted to show sporadic peaks along the transect of the salinity gradient, which may be related to the local environment (Bouvier and Del Giorgio, 2002). Actinobacteria, the typical soil microorganism, generally exist in interior areas with low salinity, such as grassland, agricultural land, or forest soil (Singh et al., 2007).

In other words, low salinity condition in the JS was observed to be suitable for the survival and inhabitation of β- and γ-Proteobacteria and Actinobacteria, which have the ability to utilize the organic carbon source to some extent. Furthermore, when compared with terrestrial soil, ε-Proteobacteria were more prevalent in the submarine sediment, such as the ZXS. In addition, salinity not only affected the soil micro flora structure, but also had an inhibitory effect on the growth and metabolism of most of the microorganisms. Some studies have indicated that salinity has a negative influence on the microbial biomass and metabolic activity (Garcia and Hernandez, 1996).

4. Conclusions

Based on the results of the present study, the following conclusions could be drawn:

(1) When the plant biomass was almost identical, the SR of estuarine tidal wetland with lower salinity (JS) was significantly higher than that of tidal wetland with higher salinity (ZXS). Higher SMR and soil enzyme activities in the JS may be the major biotic reason for higher SR.
(2) The presence of large numbers of β- and γ-Proteobacteria and Actinobacteria, which have strong heterotrophic metabolic capabilities, might be the main reason for higher microbial activity and SMR, and thus, higher SR of

Table 3 – Path analysis of SMR.

Soil physical and chemical factors	Direct path coefficient	Indirect path coefficient			Total influencing coefficient
		Salinity	SOC	AP	
Salinity	−0.463		−0.531	0.146	−0.848
SOC	−0.633	−0.388		0.195	−0.826
AP	0.430	−0.158	−0.287		−0.015

SOC: soil organic carbon; AP: available phosphorous.

the JS, whereas abundant ε-Proteobacteria, some of which have carbon fixation ability, and a small number of β- and γ-Proteobacteria in the ZXS could be responsible for the reduced carbon output of the ZXS.

(3) The difference in soil physical and chemical characters, especially, soil salinity, resulting from tides was observed to be the principle reason for the variance in the microbial community structures, microbial activity, and, hence SR between the JS and ZXS.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2014.07.016.

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