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Diversity of endophytic and rhizoplane bacterial communities associated with exotic *Spartina alterniflora* and native mangrove using Illumina amplicon sequencing

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Abstract

Root-associated microbial communities are very important for biogeochemical cycles in wetland ecosystems and help to elaborate the mechanisms of plant invasions. In the estuary of Jiulong River (China), *Spartina alterniflora* has widely invaded *Kandelia obovata*-dominated habitats, which offers an opportunity to study the influence of root-associated bacteria. The community structures of endophytic and rhizophere bacteria associated with selected plant species were investigated using the barcoded Illumina paired-end sequencing technique. The diversity indices of root-associated bacteria in *S. alterniflora* were higher than the transition stands and *K.obovata* monoculture. Using principal coordinate analysis with UniFrac metrics, the comparison of β-diversity showed that all samples could be significantly clustered into three major groups, according to bacteria communities of origin. Four phyla, namely Proteobacteria, Bacteroidetes, Chloroflexi and Firmicutes, were enriched in the rhizoplane of both salt marsh plants, while they shared higher abundances of Cyanobacteria and Proteobacteria in endophytic bacteria. Phylum of Spirochaetes andChloroflexi were found in endophytic bacteria of *S. alterniflora*and *K. obovata*, respectively. One of the interesting findings was that endophytes were more sensitive response to plant invasion, compare to rhizophere bacteria.With linear discriminate analysis, we found some predominant rhizoplane and endophytic bacteria, including Methylococcales, Pseudoalteromonadacea,*Clostridium, Vibrio* and *Desulfovibrio*,which have the potential to affect the carbon, nitrogen and sulfur cycles. Thus, the results provide clues for the isolation of functional bacteria and the effects of root-associated microbial groups on *S. alterniflora* invasions.

Keywords: *Spartina alterniflora*; mangrove; endophytic bacteria; diversity; Illumina amplicon sequencing

Introduction

Coastal wetland research has broadly focused on the preservation and restoration aspects, due to a range of ecosystem services such as carbon (C) sequestration and climate regulation (Hensel and Silliman 2013; Mcleod et al. 2011). In subtropical and tropical coastlines, mangroves are considered C rich ecosystems, and are beneficial to biodiversity, hydrology, and global biogeochemistry (Alongi 2014). However, the functions of mangrove ecosystems are increasingly threatening by human activities including eutrophication, organic and inorganic pollution, and the introduction of invasive species.

The invasion of exotic plant species may alter ecosystem functions through a variety of mechanisms such as reducing plant and animal biodiversity, altering wetland hydrology, and changing C or nitrogen (N) cycling(Williams and Grosholz 2008). *Spartina alterniflora* invasion affected the community structures of methanogens and sulfate-reducing bacteria in *Phragmites australis*-vegetated sediments (Zeleke et al. 2013). On the other hand, plants may benefit from association with diverse root-associated microbial communities which respond rapidly to environmental changes (Bai et al. 2013; Lau and Lennon 2012). The microbiota colonizing the rhizosplane and endophytic environment, contribute to plant growth, productivity, C sequestration and phytoremediation (Badri et al. 2009; Bulgarelli et al. 2012; Weyens et al. 2009). Biological interactions, including resource availability and enemy release, have been considered as the two common hypotheses of plant invasion mechanisms (Blumenthal et al., 2009).

The perennial salt marsh grass *S. alterniflora*, originated from the northeastern United States, was introduced to China in 1979. Due to its extensive expansion, *S. alterniflora* is widely distributed in nine coastal provinces in East and South China, while their displacement of the

native species has caused a number of ecological impacts (Wan et al. 2009; Zhang et al. 2012). Some studies investigated the effects of *S. alterniflora* invasion on mangrove ecosystems, including biomass of mangrove seedlings, macro-invertebrate communities, microeukaryotic diversity, and the composition of ammonia oxidizers (Yu et al. 2014; Zhang et al. 2012 & 2013; Zhao et al. 2014). Rhizospheric bacterial diversity of *S. alterniflora* through denaturing gradient gel electrophoresis (DGGE) of PCR-amplified nifH gene or 16S rDNA fragments were also investigated (Lovell et al. 2000; Nie et al. 2010; Thomas et al. 2014). However, little attention has been paid to the bacterial diversity and structure of endophytic bacteria for both exotic and native plants (Debbab et al. 2013). Understanding community structures of root-associated bacteria from exotic plants would benefit to study their ecological functions and invasion mechanisms.

For analysis of microbial community structure, there are several previous culture-independent methods, such as 16S rRNA gene clone libraries, terminal restriction fragment length polymorphism (TRFLP), and DGGE (Franco Dias et al. 2011; Theron and Cloete 2000; Zhang and Xu 2008). However, these molecular approaches could not reveal the details of the highly diverse microbial communities (Vanwonterghem et al. 2014; Zhang and Xu 2008). In recent years, pyrosequencing and Illumina techniques were widely used to get a more detailed picture of the microbial communities in various environment matrices (Degnan and Ochman 2012; Hu et al. 2014). Therefore, this study investigated the endophytic and rhizosplane bacterial community structure and diversity from three sampling stands covered by monocultures of *S. alterniflora*, *Kandelia obovata* and both plants (transition stands). These results would ultimately help in better understanding the potential impacts of *S. alterniflora* invasion on ecological function of mangrove.

Materials and Methods

Study sites and sampling

Spartina alterniflora (n=6), *Kandelia obovata* (n=3) and sediment (n=9) samples were collected from Jiulong River Estuary Mangrove Nature Reserve (24°24′N, 117°55′E), China, in July 2012. In the sampled habitat, 3 mangrove plant species including *K. candel*, *Aegiceras corniculatum*, and *Avicennia marina* were present. In 2007, *S. alterniflora* invaded the mudflat zone and the *K. candel* habitat, and then formed monoculture and transition zone in the low tidal wetland .The details of different types of samples are given in Table 1. For rhizosphere sediment (RS), roots of *S.alterniflora* and *K.obovata* were dug up and shaken gently, and then rinsed with saline water to remove the root adhering sediments. RS were stored into pre-cleaned brown glass bottles. The top 5-cm layer of the mudflat sediment (MS) was carefully placed into a bucket using a stainless steel spoon. All types of samples were taken in triplicates, and stored at 4 °C in car refrigerator (CF-110DC, WAECO, Germany), and then transported to the lab at the Institute of Urban Environment, CAS for further analyses. Physiochemical properties such as pH, TC, TN and TS of the sediment have already been reported in a previous study (Zhang et al. 2011). Sediment pH ranged from 6.12 to 6.99 units. TC and TN concentrations in the mudflat were 12.4 and 1.33 g kg^{-1}, respectively, while in *S. alterniflora* zones were 12.4 and 1.49 g kg^{-1}, respectively. These values were obviously lower than those (18.2 and 1.81 g kg^{-1}) observed in native *K.obovata* zones.

Roots of *S.alterniflora* and *K.obovata* were thoroughly washed with deionized water, and rhizosplane bacteria were isolated by vigorously shaking of root segments (2 g) in 200 ml of PBS

buffer (140 mM NaCl, 2.5 mM KCl, 10 mM Na_2HPO_4, 1.5 mM KH_2PO_4[pH 7.4]) for 1 h (Mendes et al. 2007). Microbial cells were collected by centrifugation. Samples of endophytic bacteria in roots were collected using the method adopted in our previous studies (Hong et al., 2015). Briefly, washed root segments (5 g) were surface sterilized by sequential washing in 70% ethanol for 1 min, sodium hypochlorite (2%, vol/vol) for 3 min, and 70% ethanol for 30 s and five rinses with ample sterilized distilled water. Coated onto tryptic soy agar plates, the final rinse was used to verify surface sterilization. Plates were incubated at 28°C, and no colonies were found after 10 days.

DNA extraction, PCR and Illumina amplicon sequencing

Surface-sterilized root segments were ground in a mortar with liquid nitrogen. Total DNA from sediment and strains was extracted and purified according to the manufacturer's recommendation, using the Fast DNA spin kit for soil (MP Biomedicals, California, USA) and the DNA purification kit (Tiangen, China). The V3 region of bacterial 16S rRNA gene was amplified using 338F (5'-ACTCCTACGGGAGGCAGCAG-3' and 533R (5'-TTACCGCGGCTGCTGGCAC-3') with identified barcodes (Huse et al. 2008). PCR amplifications were performed using a thermocycler (Eppendorf, Hamburg, Germany) in 50 µL reaction volumes containing 25 µL DreamTaq Green PCR Master Mix (2×) (Thermo Scientific Co., USA), 0.5µL of 1% bovine serum albumin (BSA), 0.2 µM of each primer, 40-50 ngof template DNA and 20.5 µL of sterile water. PCR was carried out with the following temperature profiles: step one heated to 94 °C (3 min), 30 s of denaturation at 94 °C, 30 s at the primers annealing temperature (55 °C), 30 s of elongation at 72 °C. PCR products were purified using a UniversalDNA purification kit (Tiangen, China) following the instructions of the manufacturer.

The PCR productsfrom all samples were combined and stood in 0.1 vol 3mol/L sodium acetate and 3 vol 100% ethanol overnight. The pellet of combined DNAwas collected by centrifugation, washed with 75% ethanol, eluted in 50 μL water and submitted to BGI (Beijing Genomics Institute, Shenzhen,China) for sequencing (pair-end) on Illumina HiSeq 2000 platform. Duplicates in endophytic bacteria of *K. obovata* (EBM) were only included in the Figure 3 data set due to sample loss.

Data processing and statistical analysis

The raw reads were assembled following the barcoded Illumina PE sequencing (BIPES) pipeline to reduce sequence and PCR errors (Zhou et al. 2011) and filtered by BGI in a pre-bioinformatics analysis. All the sequences were analyzed using QuantitativeInsights Into Microbial Ecology (QIIME, version 1.60) (Caporaso et al. 2010). As mentioned in previous studies (Xie et al., 2014), briefly, operational taxonomic units (OTUs) were picked at 97% sequence similarity, and their representative sequences were chosen for alignment and taxonomic assignment with RDP classifier. Chimeric sequences, mitochondrial, chloroplast and singleton OTUs were removed. We used phylogenetic diversity index (Phylogenetic Diversity (PD)), Shannon diversity index (H'), and an abundance-based coverage estimators Chao1 as measures of α-diversity according to our previous study (Hu et al. 2014). Rarefaction analysis, α-diversity and β-diversity were conducted according to the OTU table with minimal sequencing number (24,270) of the samples. For β-diversity analysis, dissimilarity of bacterial communities was determined using principal coordinate analysis (PCoA) on unweighted and weighted UniFrac distances among all samples. Venn diagrams were employed to characterize the shared bacterial communities among sample groups and to generate core microbiome in different samples. Linear discriminate

154 analysis (LDA) effect size (LEfSe) was employed to identify indicator taxa (from genus to phylum
155 level) associated with different sampling groups (Hu et al. 2014; Segata et al. 2011). All the
156 sequences were uploaded to the NCBI SRA database under the accession number SRX525654.

157 Mean and standard deviation for each set of data were calculated. One-way analysis of
158 variance (ANOVA) and least significant difference (LSD) were performed by SPSS (version 19.0)
159 for Windows (IBM Co., USA), and the diversity of endophytic, rhizoplane and rhizophere bacteria
160 from different wetland plants were compared.

161 ## Results and Discussion

162 **Diversity of endophytic and rhizoplane bacterial communities**

163 A total of 1,362,696 high-quality reads were obtained from 27 samples, and clustered into
164 44,094 OTUs at equal sequencing depth. RS samples presented the highest diversity of OTUs
165 followed by MS, rhizoplane and endophytic bacteria. (Table 2). Alpha diversity using
166 phylogeny-based metrics (PD) showed the significant difference between sediment and
167 root-associated samples (ANOVA test, $P < 0.01$). The root itself is generally considered as a more
168 stable niche, while RS represents more complex habitats providing microorganisms with a large
169 variety of C sources, including amino acids, organic acids and carbohydrates etc. (Berg and
170 Smalla 2009). In this study, diversity indices (Chao1 and Shannon) indicated higher diversity of
171 rhizoplane and endophytic bacteria in the *S. alterniflora* monoculture compared with the
172 *K.obovata*stands (ANOVA test, $P < 0.05$) (Table 2 and Fig.1).After *S. alterniflora* invasion, the
173 diversity indices of its root-associated bacterial community decreased by approximately 20%, due
174 to the interactions between exotic and native plants to a certain extent. Blumenthal et al (2009)

reported that plants classified as competitors hosted more than 4 times as many viruses and fungi as did stress tolerators. It also suggested that enemy release primarily contributes to invasion by fast-growing species adapted to resource-rich environments. In addition, the numbers of OTUs from the two rhizosphere sediments (RSSA and RSSAM) were higher than that in MS, indicating the "rhizosphere effect". Root exudates released from the exotic plants might cause changes in sediment parameters such as pH, C, N and S which significantly shaping rhizospheric bacterial community structure (Berg and Smalla 2009; Haichar et al. 2008; Zhang et al. 2011).

Overlapping of OTUs

According to the results of Venn analysis, consistent overlap patterns of OTU clusters among different samples were obtained (Fig.2). For endophytic bacteria, EBSA and EBSAM harbored 4,795 and 2,905 unique OTUs, respectively, while sharing250 and 612 OTUs with EBM. Moreover, EBM shared a large number of OTUs with EBSAM than EBSA. The results indicated the potential impact of *K.obovata* on the colonization of endophytic bacteria in roots of *S. alterniflora*. A similar variation pattern was observed for rhizoplane bacteria (Fig.2). These findings could be corroborated by the decreasing diversity indices of root-associated bacteria of *S. alterniflora* after its invasions. In comparison with MS, RSSA and RSSAM had higher OTUs overlap, which suggested the impact of plantation on the microbial community structure in the sediments. Sediment and rhizoplane bacterial communities had shown higher OTUs overlap as compared to those existing between endophytic and rhizoplane samples. Meanwhile, endophytic bacterial communities shared 4434 OTUs with rhizoplane and sediment samples, suggesting the colonization of endophyte in root partly originated from rhizopheric sediment environment.

196 **Structure of bacterial communities**

197 Ordination plots based on PCoA analyses were constructed using unweighted and weighted
198 UniFrac distances to identify the pattern of community structure of endophyte, rhizoplane and
199 sediment bacteria (Fig. 3). RS and MS samples grouped tightly, and endophytic bacteria could be
200 separated into two lineages; one included *K. obovata* samples, while the other group consisted on
201 monocultures of *S. alterniflora* and transition stands. Rhizoplane bacteria from monocultures of *S.*
202 *alterniflora*, *K. obovata* and transition stands were also grouped. Rhizoplane samples of *S.*
203 *alterniflora* were closer to that of *K.obovata*, indicating the major influence of sediment properties.
204 However, there were obvious difference in endophytic bacteria between*S. alterniflora* and
205 *K.obovata*. This may be related to the difference between salt marsh plants. The result also showed
206 the difference of endophyte and rhizoplane bacteria in the monoculture and transition stands of *S.*
207 *alterniflora*, due to the influence of *K.obovata* (Fig.3). Overall, the clustered patterns of all
208 samples were consistent with the OTUs and alpha analysis results.

209

210 **Taxonomic characteristics**

211 All representative sequences clustered into different groups (phyla or classes) according to
212 the taxonomic classification of the EzTaxon-e database (Fig.4). In this study, 12 known phyla with
213 a predominance of five major phyla (Proteobacteria, Cyanobacteria, Bacteroidetes, Firmicutes and
214 Chloroflexi) were observed. It is obvious that Proteobacteria was the predominant phylum and
215 contributed to 41-88 % of the total tags. These results suggested high bacterial diversity across the
216 rhizosphere, rhizoplane, endophyte of *S. alterniflora* and *K. obovata*. The number of phylum

observed here was much greater than those reported inprevious studies using the 16S rRNA clone library (Li et al. 2014).

In *S. alterniflora* monoculture, major phyla of rhizoplane bacteria were Proteobacteria (78.0±4.6%), Bacteroidetes (5.6±1.0%), Chloroflexi (3.3±0.4%) and Firmicutes (1.6±0.5%), while Proteobacteria (45.0±1.9%), Cyanobacteria (23.7±3.2%), Bacteroidetes (11.4±1.8%), Firmicutes (4.3±1.5%) and Spirochaetes (4.1±0.6%) were predominant in their endophytic bacteria of roots (Fig.4). In contrast, dominant phyla in rhizoplane bacterial community of *K.obovata*included Proteobacteria (88.0±7.7%), Bacteroidetes (2.1±0.5%), Chloroflexi (2.0±0.3%) and Firmicutes (1.1±0.2%), while most of endophytic bacteria belong to Cyanobacteria (46.9±1.2%), Proteobacteria (41.7±6.3%), Firmicutes (6.7±4.5%) and Chloroflexi (1.1±0.8%). The results indicated that Cyanobacteria phylum was largely enriched in endophytic bacterial communities of both plants, while Spirochaetes only existed in *S. alterniflora*. The invasion and growth of *S. alterniflora* within *K.obovata* forest may increased the percentage of Cyanobacteria and decreased the abundance of Bacteroidetes in rhizoplane and endophytic bacteria. For the abundance of Cyanobacteria,a significant difference ($p < 0.001$) was observed between EBSA and EBSAM, suggesting the influence of *S. alterniflora* invasion on the abundance of endophytic bacterial communities. This is possibly attributed to the impact of *K. obovata* stands, including root exudates, rhizophere bacteria, and the properties of sediments. Previous studies suggested the composition and temporal variation of soil microbial communities were associated with habitat characteristics and vegetation types, such as the influence of root exudates (Jiang et al. 2013; Lau and Lennon 2012). Meanwhile, soil type and host genotype also determined the composition of root-inhabiting bacterial communities, especially for soil-derived root endophytes (Badri et al.

2009; Bulgarelli et al. 2012; Reinhold-Hurek and Hurek 2011).In this study, compared with MS, the abundances of Proteobacteria and Bacteroidetes in RSwere enhanced while numbers of Chloroflexi were inhibited. However, RS showed no difference of bacterial communities between *S. alterniflora* monoculture and its ecotone with *K.obovata*, mainly including Proteobacteria (55.7±4.0%), Chloroflexi (10.3±1.4%), Bacteroidetes (4.7±0.3%) and Firmicutes (2.0±0.3%). It suggested more sensitive response of endophytes to environment change, compare to rhizophere bacteria (Bulgarelli et al. 2012). These limited samples reflect the variation of typical bacterial species in roots of the two plants, but we recommend that more samples be collected to identify the interactions of root-associated bacteria between different plants.

At the class level, *α-proteobacteria*, *δ-proteobacteria* and *γ-proteobacteria* were the major groups for all samples (Fig. 4). In the *S. alterniflora* monoculture,*γ-proteobacteria*(52.2±4.6%) was significantly more prominent in rhizoplane bacteria, followed by *δ-proteobacteria* (15.9±0.2%) and *α-proteobacteria* (6.9±1.3%). In contrast, there were different pattern of endophytic bacteria, showing the distribution of *δ-proteobacteria* (16.4±1.9%), *γ-proteobacteria*(13.5±1.0%) and *α-proteobacteria* (12.1±1.3%). In the transition stands, *α-proteobacteria*,*γ-proteobacteria*and *δ-proteobacteria* in endophytic bacteria of *S. alterniflora* accounted for 23.0±5.4, 8.9±0.6 and 8.2±1.4%, respectively. In addition, *δ-proteobacteria* and *γ-proteobacteria* were greatly enriched in sediment samples with plantations, although there were no obvious difference for *α-proteobacteria* between RS and MS.

Indicator bacterial taxa and their ecological functional implication

In addition to α- and β-diversity, LEfSe was used to demonstrate potentially discriminating

taxa among three major habitats, including *S. alterniflora* and *K.obovata* stands (Fig.5). There were only three replicates for rhizoplane bacteria, therefore, we combined LDA score of exotic and native plants to determine their difference with other bacterial groups. The results showed that there were 33 taxa (4 phyla, 9 classes, 12 orders, 11 families, and 7 genera) distinguishing the two groups based on LDA scores > 3.0. Phylum Proteobacteria and several *γ-proteobacteria*-associated taxa were significantly predominant in root-associated bacteria of *S. alterniflora* and *K.obovata* (RB group). For endophytic bacterial community in the roots of *S. alterniflora*, Bacteroideteswere enriched from different classified levels, such asBacteroidia, Flavobacteria and Sphingobacteriia. In addition, EBSA contained greater abundance of α-*proteobacteria* (Proteobacteria), *Clostridia* (Firmicutes), *Spirochaetes* (*Spirochaetes*) and several taxa of δ-*proteobacteria* than those in other groups. In comparison, Phylum Firmicutes were significantly more prominent the endophytic bacterial community in the roots of *K.obovata*, including Clostridiaceae, Pseudoalteromonadaceae, Oceanospirillaceae and Planococcaceae. However, Proteobacteria was enriched in the rhizoplane bacterial community in root of both plants.

Ecological functional implications of root-associated bacteria of *S. alterniflora* and *K.obovata*were discussed in this study. In past, some studies reported that invasion of *S. alterniflora* would alter the community structure of related functional microorganisms, and affect the C, N and S cycles in the salt marshes ecosystem (Liao et al. 2007; Thomas et al., 2014). In this study, the community structure of rhizoplane and endophyte were investigated using samples from different stands covered by *S. alterniflora* and *K.obovata* (Fig.5), so as to further understand their impacts on natural ecology of salt marsh.

Nitrogen-fixation cyanobacteria are able to form symbiotic associations with various plants,

such as wheat and potato (Gantar et al. 1995; Ringelberg et al. 2012). Fuernkranz et al.（2008) also reported that leaf-associated diazotrophic bacterial communities primarily belongs to Cyanobacteria, which may provide significant N input into this rainforest ecosystem. In this study, Cyanobacteria presence in root endophytes is valuable for the two plants to incorporate N into plant biomass. These results indicated that endophytic bacteria from these salt marsh plants may provide an advantage as N-fixer agents. We also found the genus Clostridium, which is considered a ubiquitous endophytic bacterium in gramineous plants and has exhibited N_2-fixing capability in association with nondiazotrophic endophytes (Miyamoto et al. 2004). Nitrogen-fixing bacteria have been isolated from mangrove rhizosphere and were identified as members of the genera *Azospirillum, Azotobacter, Rhizobium, Klebsiella, Vibrio, Phyllobacterium, Arthrobacter, Corynebacterium*, and *Oceanomonas* (Flores-Mireles et al. 2007; Holguin et al. 1992). Thus, they have a significant impact on the N cycles in coastal wetland ecosystem. In Fig.5, some rhizophere bacteria of salt marsh plants belonged to the families of *Alteromonadacea*, *Pseudoalteromonadacea*, *Vibrionacea* and *Methylophilacea*, which play key roles in the decomposition processes of dissolved organic matter. Pseudomonads are often found in contaminated aquifers, due to use a large number of substances as C sources (Moore et al., 2006).

It is well documented that δ-proteobacteria is a major group of sulfur-reducing bacteria in anaerobic environments (Castro et al. 2000; Zeleke et al. 2013), which may explain higher sulfur concentrations in *S. alterniflora*-invaded zones as compared to with native plant and unvegetated zones (Zhou et al. 2009). In this study, after the invasion of *S. alterniflora* within *K.obovata* forest, percentage of *γ-proteobacteria*in rhizoplane bacteria increased, while the abundance of *α-proteobacteria* in endophytic bacteria decreased. For *K.obovata*, *γ-proteobacteria* (75.0±7.7%)

was observed as more significant and prominent rhizoplane bacteria, followed by δ-proteobacteria (7.4±3.9%) and *α-proteobacteria* (3.0±0.4%) (Fig.4). This was consistent with the fact that *γ-Proteobacteria* and *δ-Proteobacteria* were the two major classes of phylum Proteobacteria in the rhizophere of mangrove (Jiang et al. 2013). These abundant classes were clearly habitat-specific, due to the different nutrition patterns. As shown in Fig.5, some endophytic bactera in root of *S. alterniflora* were members of the order *Desulfovibrionales*. The genus *Desulfovibrio*, belonging to sulphate-reducing bacteria, is known to oxidize acetate and other organic compounds (Muyzer and Stams 2008). These species are considered as numerically important members on macrophyte root surfaces, such as *Phragmites australis* (Vladar et al. 2008). *Desulfovibrio* spp. also had strong affinity for sulphate under sulphate-limited conditions (Basso et al. 2005; Laanbroek et al. 1984) . Thus, root-associated bacteria of *S. alterniflora* might make a great contribution to S accumulation in *S. alterniflora*-invaded stands.

Based on analysis of microbial community, root-associated bacteria in *S. alterniflora* roots have the potential to affect nutrient metabolism in wetland ecosystems, especially with regard to the N and S cycles, as well as the removal of some organic matter. Variations in community structures of root-associated bacteria also provide some clues for *S. alterniflora* invasions. However, the culture-independent method cannot provide direct information on the function of the individual community members, therefore, further work is necessary to improve our understanding about the mechanisms through the isolation of endophytic bacteria in salt marsh plants.

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Fig. 1 Shannon and equitability index ofbacterial communities of sediment, rhizoplane and endophyte associated with *Spartina alterniflora* and *Kandelia obovata*. Error bars denote the standard deviation. Lowercase letters show significantly different at $P<0.05$ for different samples, ANOVA with LSD test. RB: rhizoplane bacteria; EB: endophytic bacteria; SA: *S.alterniflora* monoculture; SAM: *S. alterniflora* from the transition stands; RBM (EBM): Rhizoplane (endophytic) bacteria in root of *K. obovata*monoculture; RS: Rhizopheric sediment; MS: Mudflat sediment.

Fig. 2 Venn diagrams showing the number of OTUs shared among different groups of samples.RB: rhizoplane bacteria; EB: endophytic bacteria; SA:*S. alterniflora* monoculture; SAM: *S. alterniflora* from the transition stands; RBM (EBM): Rhizoplane (endophytic) bacteria in root of *K. obovata*monoculture; RS: Rhizopheric sediment; MS: Mudflat sediment.

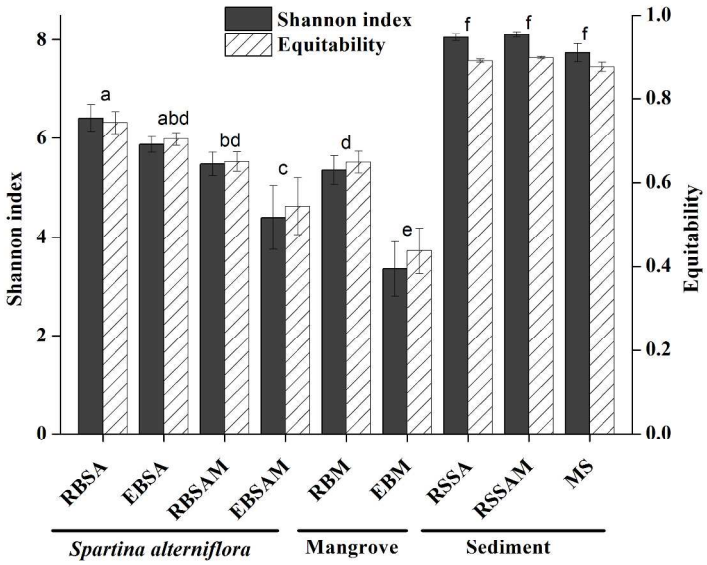
Fig. 3 PCoA analysis of bacterial community structures using the unweighted (a) and weighted (b) UniFrac distances. RB: rhizoplane bacteria; EB: endophytic bacteria; SA: *S. alterniflora* monoculture; SAM: *S. alterniflora* from the transition stands; RBM (EBM): Rhizoplane (endophytic) bacteria in root of *K. obovata* monoculture; RS: Rhizopheric sediment; MS: Mudflat sediment.

Fig. 4 Bacterial community composition in sediment, rhizoplane and endophyte of *Spartina alterniflora* and *Kandelia obovata*. Minor phyla which accounting for < 0.5% of total sequences are summarized in the group 'other and unclassified bacteria'. Error bars denote the standard deviation.RB: rhizoplane bacteria; EB: endophytic bacteria; SA:*S. alterniflora* monoculture; SAM: *S. alterniflora* from the transition stands; RBM (EBM): Rhizoplane (endophytic) bacteria in root of *K. obovata*monoculture; RS: Rhizopheric sediment; MS: Mudflat sediment.

Fig. 5 Indicator bacterial groups associated with different groups of samples identifying using LEfSe algorithm (LDA > 3). EBM: endophytic bacteria in root of *Kandelia obovata*; EBS: endophytic bacteria in root of *Spartina alterniflora*; RB: Rhizoplane bacteria of both plants.

Table 1 Details of different types of samples used in this study

Table 2 Estimated OTU richness and diversity indices of different type of samples collected from coastal wetland



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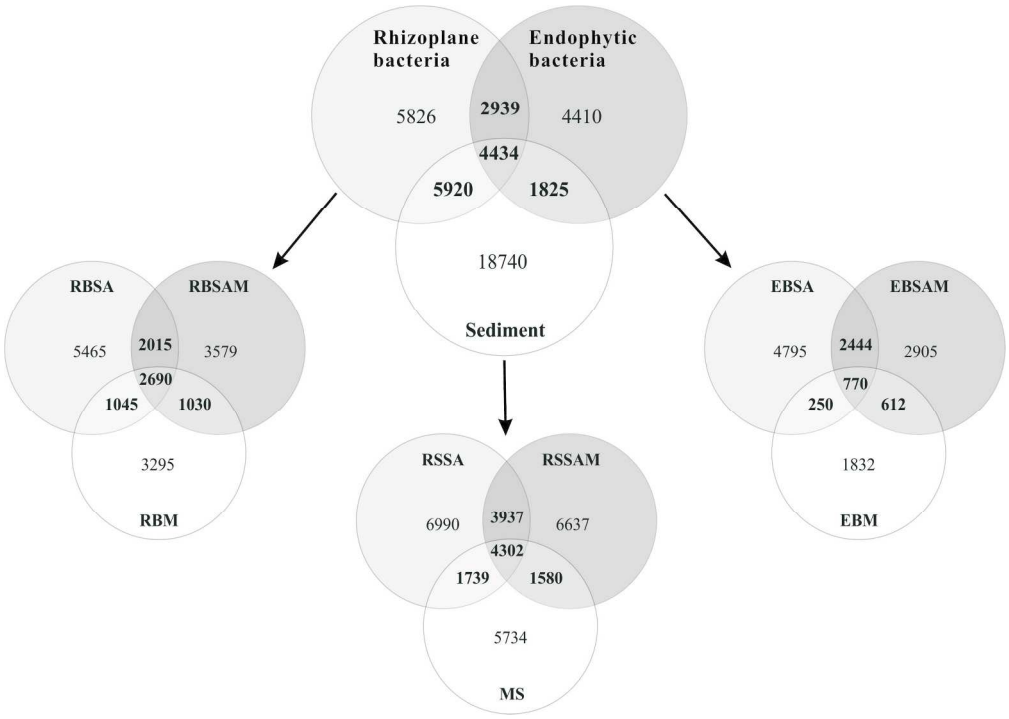

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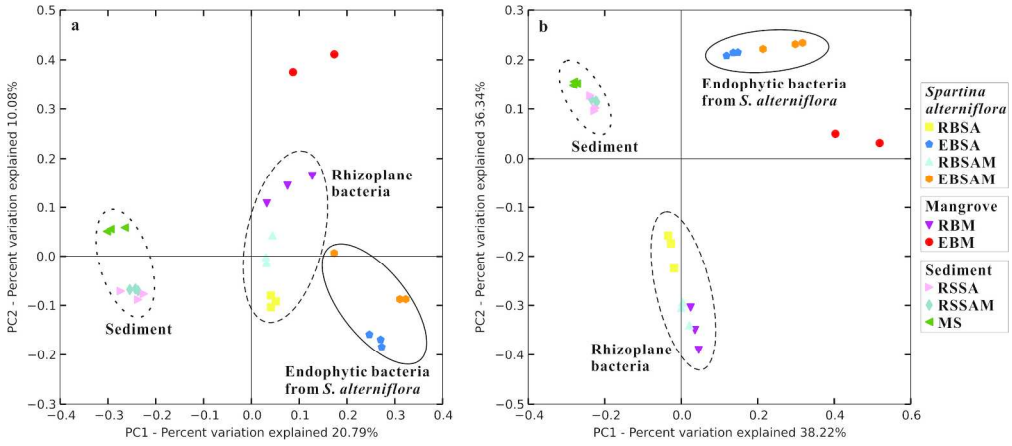

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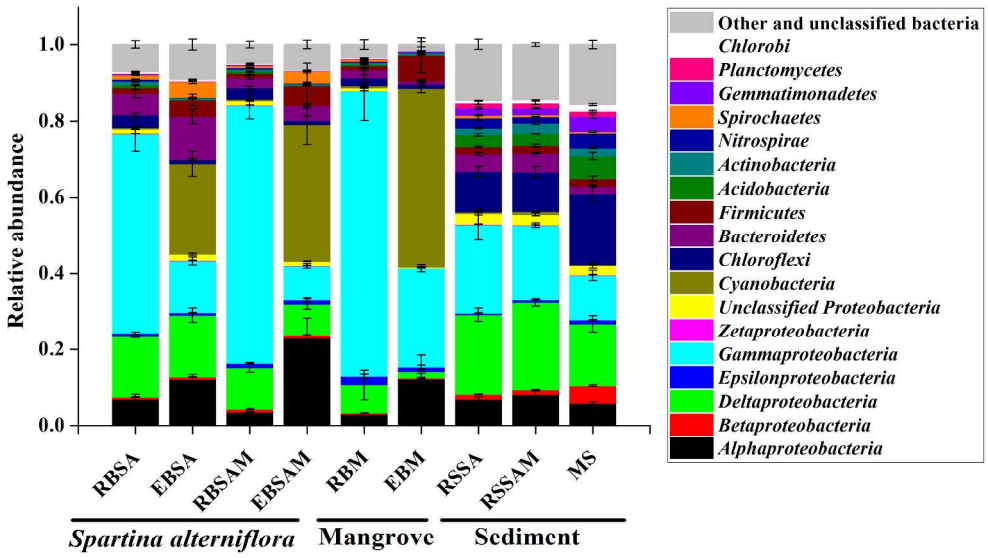

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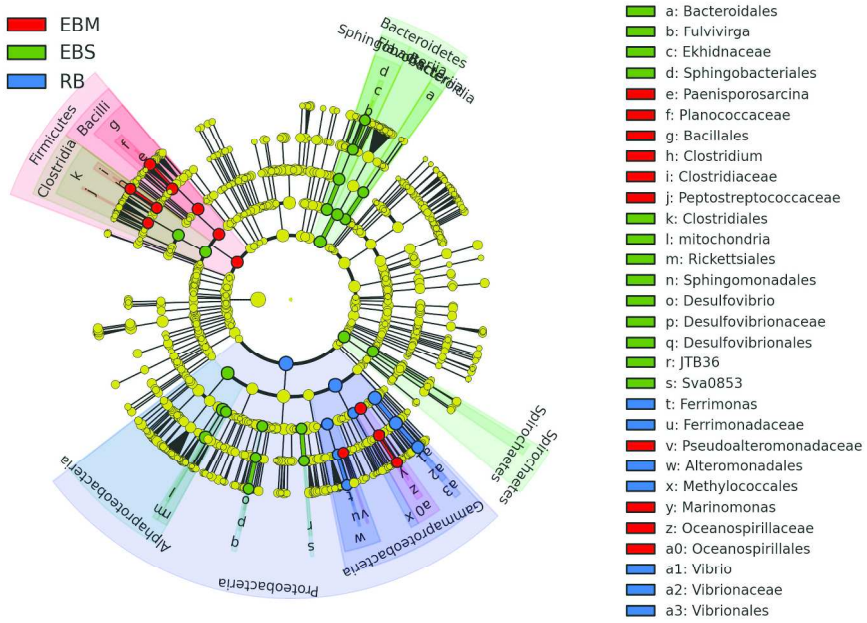

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Table 1 Details of different types of samples used in this study

Types	Samples	Descriptions
Spartina alterniflora	RBSA (EBSA)	Rhizoplane (endophytic) bacteria in root of *S. alterniflora* monoculture
	RBSAM (EBSAM)	Rhizoplane (endophytic) bacteria in root of *S. alterniflora* from the transition stands
Kandelia obovata	RBM (EBM)	Rhizoplane (endophytic) bacteria in root of *K. obovata* monoculture
Sediment	RSSA	Rhizopheric sediment in the *S. alterniflora* monoculture
	RSSAM	Rhizopheric sediment in the *S. alterniflora* from the transition stands
	MS	Mudflat sediment

Table 2 Estimated OTU richness and diversity indices of different type of samples collected from coastal wetland

Types	Samples	PD	Chao1	OTUs
Spartina altoriflora	RBSA	333	14168	5577
	EBSA	279	9858	4213
	RBSAM	285	11901	4524
	EBSAM	232	7980	3220
Kandelia obovata	RBM	241	9837	3865
	EBM	162	5791	2142
Sediment	RSSA	453	20753	8382
	RSSAM	448	20280	8371
	MS	388	15337	6837

All data (PD, Chao1 and OTUs) with the mean value are shown.