Appl Microbiol Biotechnol (2013) 97:7035–7048
DOI 10.1007/s00253-012-4496-z

ENVIRONMENTAL BIOTECHNOLOGY

GeoChip-based analysis of the functional gene diversity and metabolic potential of soil microbial communities of mangroves

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Received: 24 August 2012 / Revised: 2 October 2012 / Accepted: 3 October 2012 / Published online: 8 November 2012

Abstract Mangroves are unique and highly productive ecosystems and harbor very special microbial communities. Although the phylogenetic diversity of sediment microbial communities of mangrove habitats has been examined extensively, little is known regarding their functional gene diversity and metabolic potential. In this study, a high-throughput functional gene array (GeoChip 4.0) was used to analyze the functional diversity, composition, structure, and metabolic potential of microbial communities in mangrove habitats from mangrove national nature reserves in China. GeoChip data indicated that these microbial communities were functionally diverse as measured by the number of genes detected, unique genes, and various diversity indices. Almost all key functional gene categories targeted by GeoChip 4.0 were detected in the mangrove microbial communities, including carbon (C) fixation, C degradation, methane generation, nitrogen (N) fixation, nitrification, denitrification, ammonification, N reduction, sulfur (S) metabolism, metal resistance, antibiotic resistance, and organic contaminant degradation. Detrended correspondence analysis (DCA) of all detected genes showed that *Spartina alterniflora* (HH), an invasive species, did not harbor significantly different microbial communities from *Aegiceras corniculatum* (THY), a native species, but did differ from other species, *Kenaelia candel* (QQ), *Aricennia marina* (BGR), and mangrove-free mud flat (GT). Canonical correspondence analysis (CCA) results indicated the microbial community structure was largely shaped by surrounding environmental variables, such as total nitrogen (TN), total carbon (TC), pH, C/N ratio, and especially salinity. This study presents a comprehensive survey of functional gene diversity of soil microbial communities from different mangrove habitats/species and provides new insights into our understanding of the functional potential of microbial communities in mangrove ecosystems.

Keywords Mangroves · GeoChip · Functional gene · Microbial communities · Invasive species · Native species

Electronic supplementary material The online version of this article (doi:10.1007/s00253-012-4496-z) contains supplementary material, which is available to authorized users.

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Introduction

Mangroves grow in saline coastal sediment habitats at transition zones between terrestrial, freshwater, and oceanic environments in tropic and subtropic regions, creating unique and important ecosystems with high productivity (Jennerjahn and Ittekkot 2002). The tidal action in these areas causes large changes in salinity, water temperature, and oxygen (O) level during the day, and these changes lead to unique flora, fauna, and microbial communities. Sediment microbial communities play important ecological and biogeochemical roles in

mangrove ecosystems, such as carbon (C), nitrogen (N), phosphorus (P), iron (Fe), O, and sulfur (S) cycling (Nealson 1997). Several factors have been identified that influence sediment microbial communities, including salinity (Silveira et al. 2011), organic matter quality (Dunaj et al. 2012), C content (He et al. 2012a), N content (Carreiro-Silva et al. 2012), and plant cover types (Yergeau et al. 2010). Therefore, it is necessary to examine the diversity, composition, and structure of sediment microbial communities and their linkages with environmental factors for improving our understanding of mangrove ecosystem functioning.

The phylogenetic diversity of mangrove microbial communities has been well studied by 16S rRNA-based approaches, such as PCR cloning (Li et al. 2011a), denaturing gradient gel electrophoresis (DGGE) (Tian et al. 2008), and pyrosequencing (dos Santos et al. 2011). However, little is known about the functional diversity, composition, and structure of sediment microbial communities from different mangrove habitats. GeoChip-based technologies have been widely used for functionally profiling microbial communities from different habitats (He et al. 2012a, b). GeoChip 4.0 contains 83,992 50-mer oligonucleotide probes targeting 152,440 genes in 410 categories for major microbial functional and biogeochemical functions, such as C, N, S, and P cycling; metal resistance; antibiotic resistance; and organic remediation. It has been used to analyze microbial communities in oil-contaminated marine ecosystems (Hazen et al. 2010; Lu et al. 2012).

In this study, GeoChip 4.0 was employed to address three key questions. (1) What are the functional gene diversities of sediment microbial communities from different mangrove habitats? (2) Are there any differences in the functional gene diversity, structure, and metabolic potential of sediment microbial communities among different mangrove habitats? (3) What is the relationship between the functional structure of sediment microbial communities and environmental variables? To answer these questions, surface sediment samples from four different mangrove fields and one mangrove-free field from Zhangjiang Estuary Mangrove National Nature Reserve in Fujian province, China were studied. Our results indicated that the functional diversity and structure of microbial communities in mangrove wetlands are largely shaped by environmental variables. In addition, the metabolic potential of soil microbial communities associated with mangroves is huge, and each habitat harbors unique microbial functional gene communities.

Materials and methods

Site description

A total of 15 mangrove sediment samples were collected in August 2010 from four different mangrove habitats: *Aegiceras corniculatum* (THY), *Kenaelia candel* (QQ), *Spartina alterniflora* (HH), and *Aricennia marina* (BGR), and one mangrove-free mud flat (GT) in Zhangjiang Estuary Mangrove National Natural Reserve (Fig. 1). This reserve, located north of the Tropic of Cancer is the largest and best-protected natural mangrove forest with the greatest diversity of mangrove species in China. Mangrove habitats were located at a Jiulongjiang River outlet (23°53′45″–23°56′00″N, 117°24′07″–117°30′00″E) approximately 2,360 hm^2. The tides are semidiurnal with an average tide amplitude of 2.32 m. The flood period lasts around 6–7 h and the ebb period lasts about 5 h. Annual rainfall is 1,714 mm and the average temperature is 21.2 °C (Wang et al. 2010).

Sampling collection

Five sampling points were chosen in the middle of the mangrove forest. In August 2010, three different surface sediment cores were collected randomly at each sampling point during low tide. Tubes, 10 cm in length and 5 cm in diameter, were inserted into the sediment to obtain cores and then packed on-site into sealed polythene bags. Samples were maintained on ice until transferred to the laboratory. The wet sediments of each core were thoroughly mixed and subsamples were used for nucleic acid extraction. Total carbon (TC), total nitrogen (TN), pH, and salinity of the sediments were measured using the methods of Zhang et al. (2012).

Microbial community DNA isolation and purification

The community DNA was extracted using a freeze-grinding method as described previously (Zhou et al. 1996) and purified using a Promega Wizard DNA clean-up system (Madison, WI, USA) according to the manufacturer's directions. DNA quality was evaluated by the absorbance ratios at A_{260}/A_{280} and A_{260}/A_{230} using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA). Only DNA with A_{260}/A_{280} and A_{260}/A_{230} ratios of >1.7 and 1.8, respectively, were used for further analysis. DNA was quantified using PicoGreen (Ahn et al. 1996) and a FLUOstar OPTIMA plate reader (BMG LABTECH, Jena, Germany). Purified DNA was stored at −80 °C until used.

Microbial community DNA labeling, microarray hybridization, and scanning

The purified DNA (~1.5 μg) was labeled with the fluorescent dye Cy3 (GE Healthcare) by random priming (Van Nostrand et al. 2009; Wu et al. 2006a, b). Labeled DNA was purified with a QIAquick purification kit (Qiagen) and then dried in a SpeedVac (Thermo Savant) for 45 min at 45 °C.

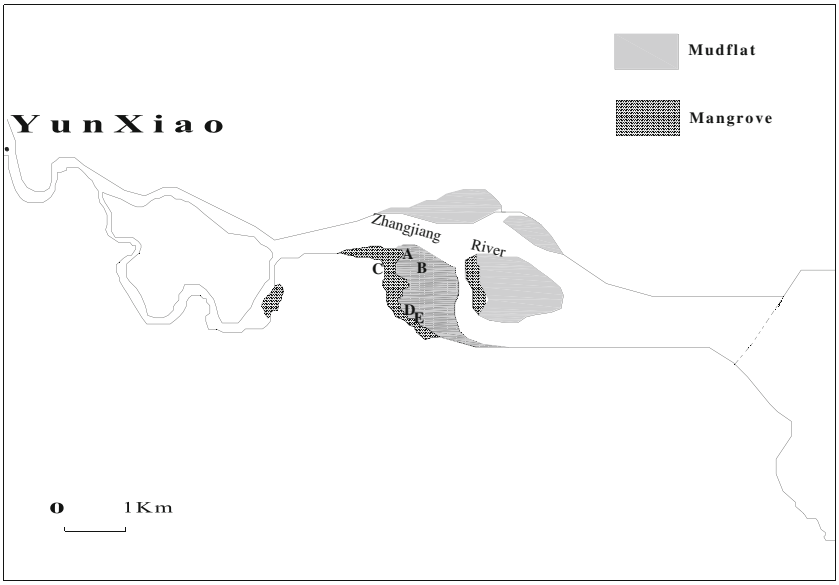

Fig. 1 Study sites in the Zhangjiang Estuary Mangrove National Natural Reserve. *A* HH, *Spartina alterniflora*; *B* GT, mangrove-free mud flat; *C* THY, *Aegiceras corniculatum*; *D* QQ, *Kenaelia candel*; *E*, BGR, *Aricennia marina*

Dried DNA was rehydrated with 2.68 μL sample tracking control (NimbleGen, Madison, WI, USA) to confirm sample identity. The samples were incubated at 50 °C for 5 min, vortexed for 30 s, and then centrifuged to collect all liquid at the bottom of the tube. Hybridization buffer (7.32 μL), containing 40 % formamide, 25 % SSC, 1 % SDS, 2.38 % Cy3-labeled alignment oligo (NimbleGen), and 2.8 % Cy5-labeled common oligonucleotide reference standard (CORS) target (Liang et al. 2009) for data normalization, was then added to the samples, vortexed, spun down, incubated at 95 °C for 5 min, and then maintained at 42 °C until ready for hybridization. CORS probes were placed randomly throughout the array and were used for signal normalization (Liang et al. 2010).

GeoChip 4.0 is a new generation of functional gene array (Hazen et al. 2010; Lu et al. 2012) containing 83,992 50-mer oligonucleotide probes targeting 152,414 genes in 410 categories for different microbial functional and biogeochemical processes including C, N, P, and S cycling, energy processing, metal resistance and reduction, organic contaminant degradation, stress responses, antibiotic resistance, and bacteriophages. GeoChip 4.0 was synthesized by NimbleGen in their 12-plex format (i.e., 12 arrays per slide). An HX12 mixer (NimbleGen) was placed onto the array using NimbleGen's Precision Mixer Alignment Tool (PMAT) and then the array was preheated to 42 °C on a Hybridization Station (MAUI, BioMicro Systems, Salt Lake City, UT, USA) for at least 5 min. Samples (6.8 μL) were then loaded onto the array surface and hybridized for approximately 16 h with mixing.

After hybridization, the arrays were scanned with a laser power of 100 % and 100 % photomultiplier tube (PMT) by an MS 200 Microarray Scanner (NimbleGen). Low quality spots were removed prior to statistical analysis as described previously (He et al. 2010). Spots were scored as positive if the signal-to-noise ratio (SNR) was ≥2.0 and the CV of the background was <0.8. Genes that were detected in only one sample were removed.

Statistical analysis

All GeoChip 4.0 hybridization data are available at the Institute for Environmental Genomics, University of Oklahoma (http://ieg.ou.edu/). Preprocessed data were then used for further analysis. Functional gene diversity was calculated using Simpson's 1/D, Shannon-Weiner's H′, and evenness. Statistical differences between the functional microbial communities from the different habitats were analyzed by a one-way analysis of variance (ANOVA) and Tukey's test. A significance level of $p<0.05$ was adopted for all comparisons (He and Wang 2011). Based on the standard error, the 95 % confident interval for each response variable was obtained and the statistical difference between different mangrove habitats and mangrove-free sediments was estimated. The total abundance of each gene category or family (the sum of the normalized intensity for the gene category or family) was used for ANOVA and the response ratio analysis.

Multivariate statistical analyses of GeoChip data including detrended correspondence analysis (DCA) for comparing the different functional gene communities and canonical correspondence analysis (CCA) for linking microbial communities to environmental variables (Zhou et al. 2008), and partial CCA for covariation analysis (variation partitioning analysis, VPA) were performed. Selection for CCA modeling was conducted by an iterative procedure of eliminating redundant environmental variables based on the variance inflation factor (VIF). All analyses were performed by the vegan package in R 2.9.1 (R Development Core Team 2006).

Results

Geochemical properties in sampling sites

Fifteen samples were obtained from five different sites, with THY, HH, and GT being close to each other, and QQ and BGR being close to each other (Fig. 1). In general, the pH of GT was significantly higher than that of the other habitats ($P<0.05$); however, total C and total N of GT were significantly lower than those of others ($P<0.05$). The salinity of QQ and BGR was significantly higher than that of THY, HH ($P<0.01$), and GT ($P<0.05$) (Table 1). Therefore, THY, HH, and GT appeared to be similar, and QQ and BGR were similar based on the results of environmental parameters.

Overview of mangrove habitat microbial community functional gene diversity and structure

To understand the functional diversity and structure of microbial communities, the number of detected genes, Shannon index, Simpson index, and Simpson evenness were measured. The number of detected genes ranged from 30,000 to 35,000 in each of the sites. One-way ANOVA showed that the number of genes detected in THY, HH, and GT was significantly higher than in QQ and BGR. No significant difference was observed between QQ and BGR or among THY, HH, and GT. Similar results were observed with the Shannon-Weaver index (H′), Simpson index (1/D), and Simpson evenness (see Online Resource Table S1–S5).

DCA of all detected genes showed that QQ was well separated from BGR by DCA I and II and from the other samples by DCA II. BRG was separated from QQ by DCA I and II and from the other samples by DCA I. THY, HH, and GT were well separated from BGR by DCA I, and from QQ by DCA II. Generally, the functional structure of microbial communities was similar between THY, HH, and GT and differed from QQ and BGR (Fig. 2).

Patterns of detected functional gene categories

To understand the effects of different mangroves habitats on sediment microbial communities, microbial functional gene categories for major biogeochemical/metabolic processes were examined (Fig. 3). Functional genes related to organic remediation were most abundant in all samples followed by stress-related functional genes. There were no significant differences in energy process, P cycling, and S cycling genes among the five groups of samples. However, the other gene categories generally showed significant differences between BGR and HH. First, many antibiotic resistance genes were detected in all mangrove habitats like those encoding beta-lactamases, which could cause antimicrobial resistance. Our results indicated the signal intensity of antibiotic resistance genes in HH was highest, while that of antibiotic resistance genes in the BGR habitat was lowest. Based on ecological theory, bacteria and their phages should coexist in the environment. Our results indicated that the abundance of bacterial phage genes in BGR was lowest. Second, due to human activities, the mangrove environments are rich in various metals and we detected metal resistance/reduction genes for Al, As, Cd, Zn, Co, Cr, Ni, Cu, Pb, Hg, Ag, and Te in all mangrove habitats, and QQ and BGR had lower signal intensities for these gene categories than the other three habitats. Third, the organic remediation-related functional genes were the most abundant genes of all categories. This included genes for aromatic carboxylic acid, BTEX and related aromatics, chlorinated aromatics, chlorinated solvents, herbicide-related compound, heterocyclic aromatics, nitroaromatics, pesticide-related compound, and polycyclic aromatics. Our results indicated the THY, HH, and GT habitats have higher organic remediation abilities than QQ and BGR. Fourth, the stress category had many functional genes related to cold shock, heat shock, glucose limitation, N limitation, osmotic stress, O limitation, O stress, phosphate limitation, protein stress, sigma factors, and radiation stress. Our results showed the QQ and BGR had lower signal intensities than the other three habitats. In addition, key functional genes were detected for virulence factors, such as adhesion,

Table 1 The environmental parameters of different mangrove habitats

	THY	HH	QQ	BGR	GT
TC	1.584±0.152	1.266±0.035	1.904±0.048	1.442±0.075	0.984±0.020
TN	0.154±0.009	0.147±0.009	0.156±0.001	0.131±0.004	0.096±0.002
C/N	10.256±0.369	8.648±0.403	12.240±0.270	11.041±0.292	10.254±0.124
pH	6.567±0.145	6.667±0.061	6.567±0.033	6.633±0.033	7.213±0.035
Salinity	21.000±0.000	21.000±0.000	30.000±0.000	30.000±0.000	22.333±0.333

All data are presented as mean±SE

THY Aegiceras corniculatum, *HH Spartina alterniflora*, *QQ Kenaelia candel*, *BGR Aricennia marina*, *GT* mangrove-free mud flat

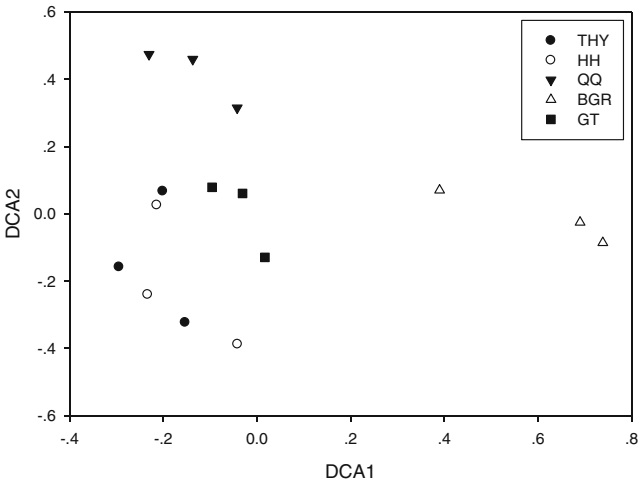

Fig. 2 Detrended correspondence analysis (DCA) of GeoChip 4.0 data showing the sediment microbial community composition and functional structure of different mangrove habitats. THY, *Aegiceras corniculatum*. HH, *Spartina alterniflora*. QQ, *Kenaelia candel*. BGR, *Aricennia marina*. GT, mangrove-free mud flat

aerobactin, capsule, colonization factor, fimbriae, hemolysin, invasion, pilin, secretion, surface protein, toxin, and virulence proteins. Our results indicated that the signal intensity of virulence genes in HH was highest and that in BGR was lowest.

Functional genes involved in the carbon cycle

Mangrove habitats have complex plant and animal communities, which may provide nutrient resources to mangrove ecosystems and affect microbial C cycling, which plays an important role in this ecosystem. Cellulose, lignin, starch, hemicellulose, and chitin are the most abundant C sources derived from plant and animal sources in soil ecosystems. Key functional genes for C degradation, C fixation, methane generation, and methane oxidation were detected in all groups. There were no significant differences for starch (e.g., *cda*, *pulA*, and glucoamylase genes) hemicellulose (e.g., *xylA*), cellulose (e.g., exoglucanase and cellobiase genes), chitin (e.g., exochitinase genes), and lignin (e.g., *lip*, *mnp*, and phenol oxidase genes) degradation genes among these five groups. Other genes involved in C degradation did show significant differences between groups (Fig. 4). The gene *pcc* involved in C fixation showed a significantly lower abundance in QQ and BGR compared to the other groups, while there was no significant difference observed for *AclB*, CO-dehydrogenase (*CODH*), and ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) genes (Fig. 5). The signal intensity of functional genes involved in C degradation and C fixation showed that THY, HH, and GT had a greater potential for C degradation and fixation than QQ and BGR. The genes *mcrA* involved in methane production, and *mmoX* and *pmoA* involved in methane oxidation showed no significant differences among the five groups (Fig. 6), indicating that all of these mangrove habitats shared similar potential for methane metabolism.

Functional genes involved in the nitrogen cycle

Microbial activity is responsible for the major N transformations within a mangrove ecosystem. The microbial N removal pathway in mangrove sediments is interesting not only because it mitigates N loading but also because it

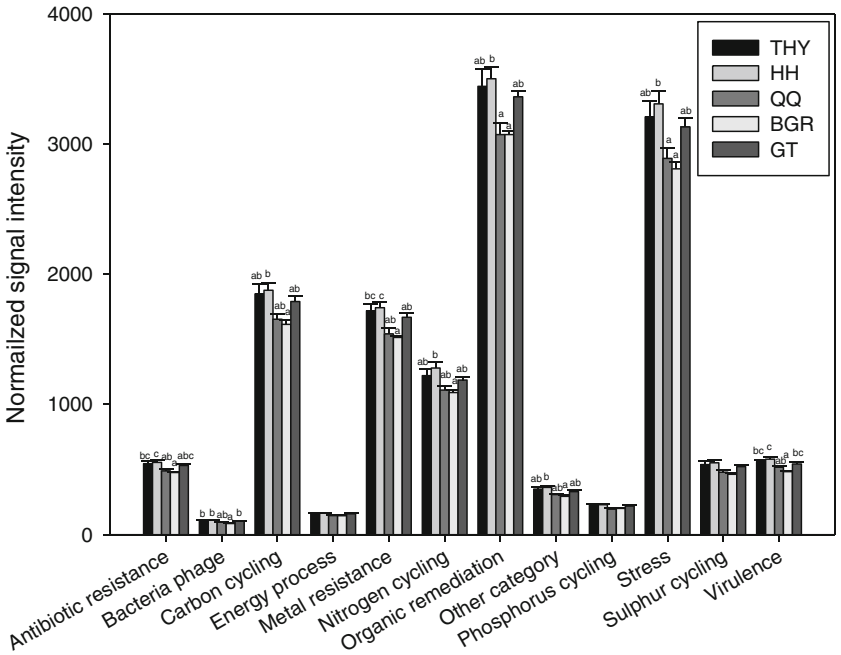

Fig. 3 The normalized signal intensity of detected genes from different gene categories. The signal intensity for each functional gene category is the average of the total signal intensity from all the replicates. All data are presented as mean± SE

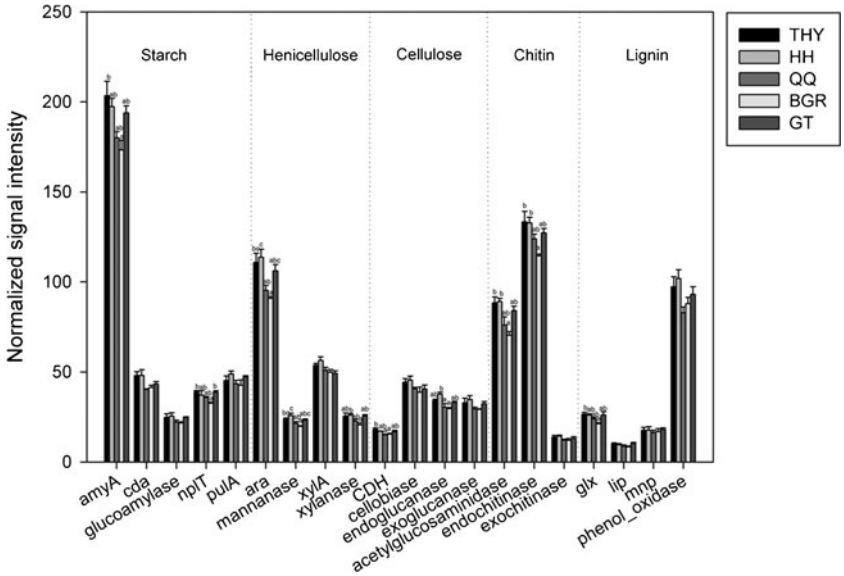

Fig. 4 The normalized signal intensity of detected key genes involved in carbon degradation. The signal intensity for each functional gene category is the average of the total signal intensity from all replicates. All data are presented as mean±SE

means a loss of fixed N from an ecosystem where N is frequently a limiting nutrient (Holguin et al. 2001). Key functional genes for ammonification, anammox (Anx), denitrification, assimilatory N reduction, dissimilatory N reduction, nitrification, and nitrification fixation were detected in all groups. The gene *ureC* involved in ammonification showed a significant difference between THY and HH, and between QQ and BGR. The gene *hzo* involved in Anx showed a significant difference between HH and BGR. THY had the highest *nirA* (involved in assimilatory N reduction) signal intensity. For denitrification, only *nosZ* had a significant difference between HH and BGR. The gene *nrfA* involved in dissimilatory N reduction showed a significant difference between HH and BGR. There was a significant difference in *amoA* (nitrification) between THY and HH and QQ and BGR. For N fixation, the gene *nifH* showed a significant difference between HH and BGR. The other N cycling genes did not show significant differences among the five groups (Fig. 7). Generally speaking, the THY, HH, and GT habitats had higher metabolic potential for N cycling than the QQ and BGR habitats.

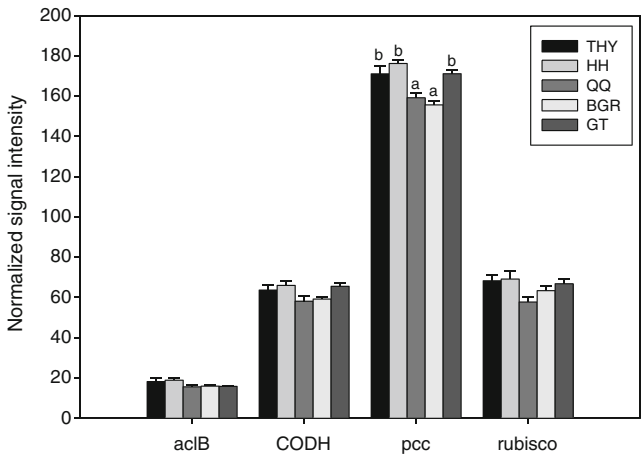

Fig. 5 The normalized signal intensity of the detected key genes involved in carbon fixation. The signal intensity for each functional gene category is the average of the total signal intensity from all the replicates. All data are presented as mean±SE

Phosphorus metabolism

P metabolism is considered an important process in mangrove microbial communities. GeoChip 4.0 contains probes for the key enzymes phytase, *ppk*, and *ppx* to analyze P utilization and associated microbial populations. One-way ANOVA showed no significant differences in the abundances of *ppk* and phytase genes among the five groups; however, *ppx* abundance showed a significant difference between THY and HH and QQ and BGR (Fig. 8). These results indicate that the THY, HH, and GT habitats have a higher potential for P metabolism than QQ and BGR habitats.

Sulfur metabolism

S metabolism is also considered an important process in mangrove microbial communities. GeoChip 4.0 contains *sox* probes for S oxidation, *dsrA* and *dsrB* probes for S reduction, and *aprA* and *aprB* probes for sulfate-reducing bacterial populations. One-way ANOVA showed no significant differences in the abundance of *sox*, *dsrA*, and *aprB* among the five groups. THY and HH had higher signal intensities for *dsrB* than the other habitats and significantly higher than BGR. The abundance of *aprA* showed significantly higher signal intensity in HH than BGR, but no significant differences were observed with the other groups (Fig. 9). In general, these results indicate that the THY, HH,

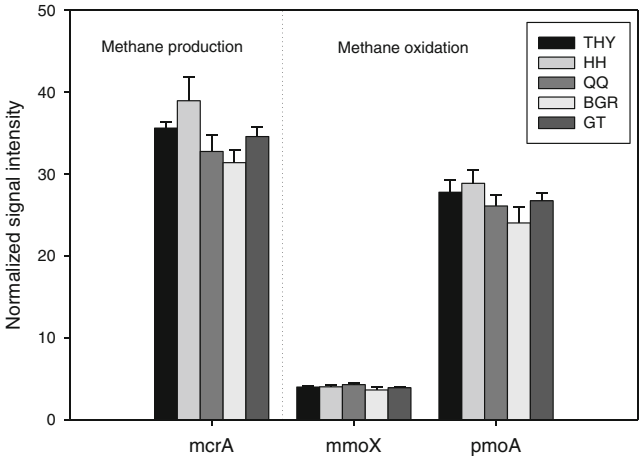

Fig. 6 The normalized signal intensity of the detected key genes involved in methane cycle. The signal intensity for each functional gene category is the average of the total signal intensity from all the replicates. All data are presented as mean±SE

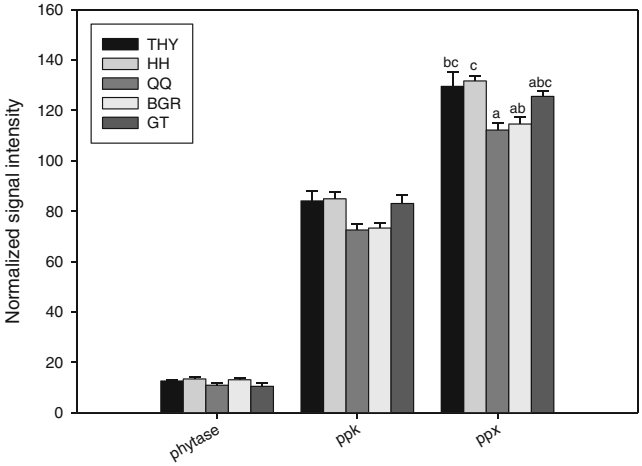

Fig. 8 The normalized signal intensity of the detected key genes involved in phosphorus metabolism. The signal intensity for each functional gene category is the average of the total signal intensity from all the replicates. All data are presented as mean±SE

and GT habitats had higher potential for S metabolism than the QQ and BGR habitats.

Relationship between microbial functional diversity and environmental variables

CCA was performed to discern possible linkages between the microbial functional structure and sediment geochemical parameters (Fig. 10). The first axis was positively correlated with C/N and salinity, and the second axis was positively correlated with TC and TN, but negatively correlated with pH. On the basis of the relationship between environmental variables and the microbial functional structure, pH seemed to be a major factor influencing the microbial functional structure in GT. TC seemed to be a major factor influencing the microbial functional structure in HH and THY. In addition, TN, salinity and C/N could have key roles in shaping the microbial functional structure in QQ and BGR.

Discussion

Analyzing microbial functional genes encoding key enzymes involved in major biogeochemical processes is important to link microbial community structure to their potential ecological functions (Torsvik and Øvreås 2002). However, characterization and quantification of microbial communities is challenging. It is extremely difficult to survey key microbial functional genes involved in important geochemical cycles in a rapid and comprehensive way using conventional molecular ecology approaches. Microarray-based genomic technology provides a powerful tool for monitoring various microbial functional genes (He et al. 2012b). In this study, microbial

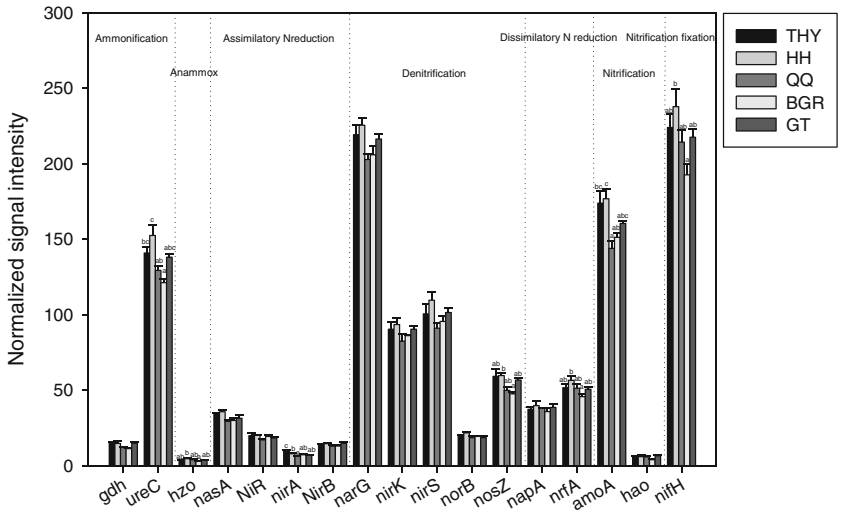

Fig. 7 The normalized signal intensity of the detected key genes involved in nitrogen cycle. The signal intensity for each functional gene category is the average of the total signal intensity from all the replicates. All data are presented as mean± SE

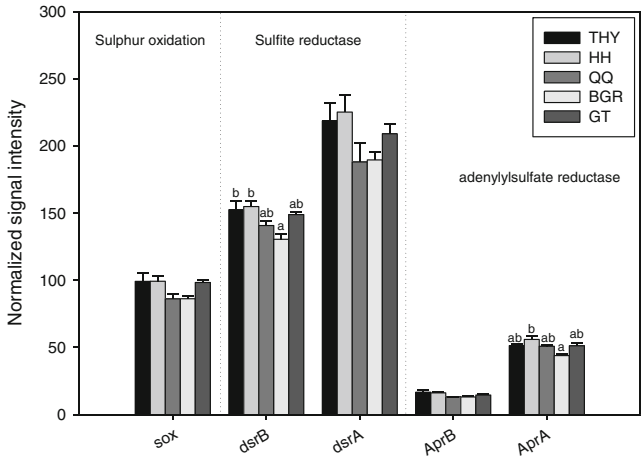

Fig. 9 The normalized signal intensity of the detected key genes involved in sulfur metabolism. The signal intensity for each functional gene category is the average of the total signal intensity from all the replicates. All data are presented as mean±SE

functional gene diversity in different mangrove habitats and a mangrove-free field in south China was analyzed using GeoChip. Our results showed that most of the functional processes targeted by GeoChip 4.0, including C and N fixation, C degradation, methane metabolism, ammonification, nitrification, denitrification, N reduction, S reduction, organic contaminant degradation, and antibiotic and metal resistance were detected in all mangrove habitats samples. As such, results from this study should provide a more comprehensive view of the functional structure and metabolic potential of mangrove microbial communities in general.

Mangrove ecosystems consist of a series of vegetation communities depending on the elevation gradient with

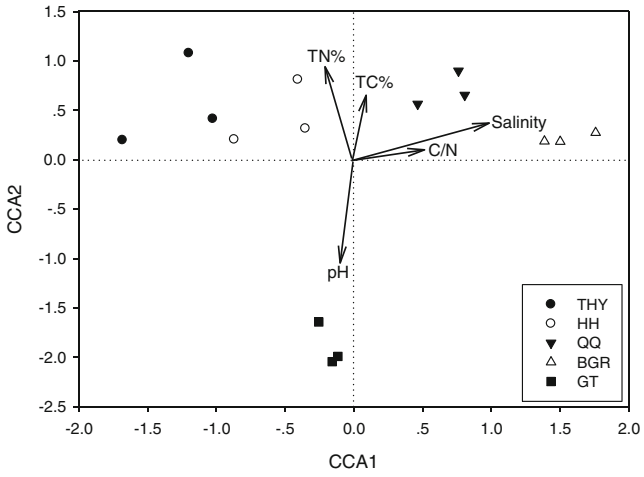

Fig. 10 Canonical correspondence analysis (CCA) of all genes detected by GeoChip 4.0 and sediment geochemistry variables of different mangrove habitats. THY, *Aegiceras corniculatum*; HH, *Spartina alterniflora*; QQ, *Kenaelia candel*; BGR, *Aricennia marina*; GT, mangrove-free mud flat

mangroves, saltmarsh, and cyanobacterial mats organized in parallel zones along the coast (Ewel et al. 1998; Adame et al. 2010). Because the location of these zones is principally controlled by tidal elevation, any disturbance linked to changes in tidal height or amplitude will result in an adaptation and ecological response (Rogers and Saintilan 2008). Disturbances can arise from either large external perturbations such as tsunami and storm surge events or from smaller changes such as variations in salinity due to fluctuating tidal levels (Feagin et al. 2010). Our DCA results indicated that the microbial community functional structure are similar between THY (*A. corniculatum*), HH (*S. alterniflora*), and GT (mangrove-free) and differ from QQ (*K. candel*) and BGR (*A. marina*). The sample sites THY, HH, and GT are very close together, while the QQ and BGR sites are close together. In addition, the salinity of QQ and BGR were similar and higher than the other groups. So, the differences observed in the microbial communities may be caused by the sampling location and environmental variables.

Based on the global pattern of bacterial diversity determined using annotated sequences from 202 globally distributed natural environments, Lozupone and Knight (2007) demonstrated that salinity is the major environmental determinant of microbial communities rather than temperature, pH, or other physical and chemical factors. Another study used three different methods, fluorescent in situ hybridization (FISH), DGGE with subsequent band sequencing, and reverse line blot hybridization (RLB), to investigate the bacterioplankton community composition spanning a salinity gradient from 0.02 % (freshwater) to 22.3 % (hypersaline), and results showed salinity was the major environmental factor controlling bacterioplankton community composition (Wu et al. 2006a, b). Similar results were observed by Jiang et al., and systematic changes in microbial community composition were correlated with the salinity gradient (Jiang et al. 2007). Environmental factors, such as salinity, can make it difficult for some specific microorganisms to survive. Across a gradient of salinity values from zero to extremely high, different microbial community compositions and diversity would be found which could result in differing functional abilities of the communities (Wang et al. 2011). Community composition variations across salinity gradients probably leads to changes in gene expression patterns that can modify the way in which microorganisms interact with each other and the environment (Vieira et al. 2008; Silveira et al. 2011). Increased salinity has also been shown to cause decreases in the O concentration and in the microdiversity of bacteria and Archaea (Rodriguez-Valera et al. 1985; Benlloch et al. 2002). The salinity differences could explain why the functional structure of sediment microbial communities in the Everglades freshwater marsh, fringing mangrove forest, and Florida Bay seagrass meadows were different (Ikenaga et al. 2010).

Global warming is believed to be associated with increases in the concentration of atmospheric CO_2, due primarily to the increased use of fossil fuels since the industrial revolution (Pachauri and Reisinger 2007). Terrestrial ecosystems have been recognized as major sinks for global CO_2 emissions and pose significant potential for mitigating atmospheric CO_2 (Lal 2008). The soil C pool has been estimated as twice that of the atmospheric pool (Falkowski et al. 2000). The Calvin–Benson–Bassham cycle is the major and most widely distributed pathway for CO_2 fixation. In terrestrial ecosystems, the Calvin cycle is found in diverse organisms, from bacteria and algae to green plants (Tabita 1999). RuBisCO catalyses the first, rate-limiting step in the Calvin cycle, which enables 10^2 Pg of inorganic atmospheric CO_2 to be converted into organic cellular constituents every year (Siegenthaler and Sarmiento 1993). Another important pathway for autotrophic C fixation present in some anaerobic and microaerobic bacteria is based on tricarboxylic acid operating in reverse (rTCA) (Buchanan and Arnon 1990). The key enzymes in this cycle are Adenosine Triphosphate (ATP) citrate lyase (encoded by the *acl* gene), 2-oxoglutarate:ferridoxin oxidoreductase, and fumarate reductase. Some evidence for the occurrence of the rTCA cycle in marine microbial communities has been obtained recently (Campbell et al. 2003; Campbell and Cary 2004; Voordeckers et al. 2008). *CODH*, a key enzyme of the CO-oxidizing system in aerobic carboxydobacteria, has been detected in various habitats by two degenerate primer pairs that specifically target the *coxL* gene, encoding the large catalytic subunit of *CODH* (Weber and King 2010; King and Weber 2007). This has substantially broadened the diversity of bacteria capable of utilizing CO as an electron donor compared with those previously recognized as capable of utilizing CO based on cultivation at high CO concentrations. In our study, we didn't detect significant differences in the abundance of RuBisCO, *CODH*, or *aclB* between the mangrove habitats. However, abundance of *pcc*, encoding the CO_2 fixation enzyme propionyl-CoA for the 3-hydroxypropionate cycle, showed that the THY, HH, and GT had a higher capacity for C fixation than the other samples.

Generally, THY, HH, and GT had higher signal intensities for C degradation genes than QQ and BGR, with some differences being statistically significant. Substrates for this group of genes ranged from labile C to more recalcitrant C (e.g., starch, hemicelluloses, cellulose, chitin, and lignin). These results suggest that THY, HH, and GT have a greater capacity for C degradation than the other two communities. Soil microbial communities have commonly been viewed as black boxes into which organic C flows and is converted into CO_2 or biomass. Flow rates through this black box are affected by environment variables, but in some cases, microbial community, size, and physiology also may affect the degradation rates of C substrates primarily through shifts in enzymatic capacity (Gulledge and Schimel 1998; Wall and Moore 1999; Zogg et al. 1997). Process rates have, however, been measured with no accompanying detectable change in community composition (Balser et al. 2002; Houston et al. 1998). Alternatively, differences in microbial communities may lead to no change in function (Finlay et al. 1997), and different plant communities may not alter the profiles of microbial communities responsible for microbial functions (Waldrop and Firestone 2004).

With the rapidly increasing concentrations of atmospheric CO_2, our planet has been warming (Forster et al. 2007). Terrestrial ecosystems could help to reduce this increase in atmospheric CO_2 and thereby slow climate change (Gifford 1994). However, the capacity of land ecosystems to slow climate warming has been overestimated (Van Groenigen et al. 2011). This may be because increased CO_2 can also stimulate soil emissions of nitrous oxide (N_2O) and CH_4 (Pendall et al. 2004), and the global warming potentials of these gases are much higher than for CO_2: 298 times higher for N_2O and 25 times higher for CH_4 (Forster et al. 2007). Wetlands, including mangroves, contribute 32–53 % to global emissions of CH_4 (Denman et al. 2007). Our results showed no significant difference between the capacity of methane production of each mangrove habitat and methane oxidation; however, C oxidation and C degradation did show some difference. These results indicate that the mangrove ecosystem may provide little contribution to the slowing down of global warming.

Microbes are major drivers of the soil N cycle, including N fixation, nitrification, denitrification, ammonification, anaerobic ammonium oxidizing (Anx), and dissimilatory nitrate reduction to ammonium (Lindsay et al. 2010). Here, we detected all N cycle-related functional genes involved in ammonification, Anx, assimilatory N reduction, denitrification, dissimilatory N reduction, nitrification, and nitrification fixation covered by GeoChip. Based on our results, we found that the potential metabolic ability of THY, HH, and GT was higher than QQ and BGR. N fixation is considered a major source of combined N input in mangrove forest habitats (Hicks and Silvester 1985; Kyaruzi et al. 2003). High rates of N fixation have been found associated with dead and decomposing leaves, pneumatophores, rhizosphere soil, tree bark, cyanobacterial mats covering the surface of sediment, and the sediments themselves (Zuberer and Silver 1978; Hicks and Silvester 1985; Holguin et al. 2001; Lugomela and Bergman 2002). Microbes containing *nifH* have been detected in mangrove sediments, revealing the high fundamental diazotrophic biodiversity in mangrove ecosystems (Zhang et al. 2008). The *amoA* gene, which encodes ammonia monooxygenase subunit A, from ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB), and *hzo*, which encodes the key enzyme for the transformation of hydrazine to N_2 in

anaerobic ammonium oxidizing (Anx) bacteria, were detected in mangrove habitats (Li et al. 2011b). In marine ecosystems, a variety of taxonomically unrelated bacterial groups are capable of denitrification. Of these, 96 % of cultured denitrifiers belong to the gammaproteobacteria (Braker et al. 2001). Culture-independent approaches have been employed to probe the diversity of denitrifying genes like *narG*, *nirK*, *nirS*, *norB*, *norC*, and *nosZ* (Braker et al. 2000; Priemé et al. 2002; Liu et al. 2003). The *nosZ* gene, encoding N_2O reductase, an enzyme catalyzing the final step of denitrification, is largely unique to denitrifying bacteria (Scala and Kerkhof 1999). It represents the process leading to the loss of biologically available N from the sediment (Mills et al. 2008) and has been used for determining the diversity of denitrifiers (Horn et al. 2006). Anx is a process in which NH_4^+ is oxidized to N_2 at the expense of NO_2^- (Meyer et al. 2005). The NO_2^- could be produced by either heterotrophic NO_3^- reduction (Dalsgaard et al. 2003) or nitrification by aerobic ammonium oxidation (Francis et al. 2005). In mangrove systems, ~55 % of the N loss (as NO, N_2O, or N_2) is known to occur through denitrification (Chiu et al. 2004). However, Anx can account for up to 67 % of N removal (Thamdrup and Dalsgaard 2002) in marine sediments by shunting N directly from NH_4^+ and NO_2^- under anaerobic conditions. In organically rich estuarine sediments, Anx has been shown to be significant under low concentrations of NO_2^- (Trimmer et al. 2003). Mangrove sediments could potentially present such characteristics since they are largely anaerobic and nitrate/nitrite availability is the factor controlling denitrification rates (Seitzinger 1990).

Phytate is the most abundant organic P compound in soil and dominates biotic P input from terrestrial runoffs into aquatic systems. Microbial mineralization of phytate by phytases is a key process for recycling P in the biosphere (Lim et al. 2007). Phytate can be detected in marine waters (Matsuda et al. 1985) and marine sediments (Suzumura and Kamatani 1995). In addition, it constitutes approximately 20 % of total organic P in soil leachates (Toor et al. 2003). Phytases play a crucial role in the recycling of organic P in the aquatic biosphere. Up to 50 % of the filterable, nonreactive P harvested from preconcentrated lake water is exclusively hydrolyzed by phytase (Herbes et al. 1975). For sediment P, up to 34 % of residual organic P can be hydrolyzed by phytase (De Groot and Golterman 1993). Four classes of phytases have been characterized in terrestrial organisms: histidine acid phosphatase (HAP), cysteine phytase (CPhy), purple acid phosphatase (PAP), and β-propeller phytase (BPP) (Mullaney and Ullah 2003; Chu et al. 2004). BPPs play a major role in phytate-P cycling in both soil and aquatic microbial communities (Lim et al. 2007). The enzyme polyphosphate (poly-p) kinase, which is encoded by *ppk*, promotes the transfer of the terminal phosphate of ATP to a growing chain of inorganic poly-p and is reversible in vitro (Kornberg 1957). The presence of poly-p kinase could be used to reflect the ability of biological P removal (McMahon et al. 2002). In our results, we didn't observe significant differences in the abundance of phytase and poly-p genes. However, the abundance of *ppx*, encoding exopolyphosphatase which can catalyze the poly-p transfer to phosphate, did show a significant difference. These results showed that HH had the highest potential enzymatic activity of exopolyphosphatase and that QQ had the lowest.

The S-oxidizing multienzyme complex is comprised of the thiosulfate-induced periplasmic proteins, *SoxXA*, *SoxYZ*, *SoxB*, and *SoxCD* (Friedrich 1998; Friedrich et al. 2000). The same S-oxidizing gene cluster (soxSRTsoxVW-soxXYZABCD) also produces proteins such as *SoxV* and *SoxW*, which are believed to be involved in the biosynthesis or maintenance of the multienzyme complex system (Bardischewsky et al. 2006; Friedrich 1998). It is widely accepted that the reduction of sulfate by sulfate-reducing bacteria (SRB) is one of the most significant mechanisms of H_2S production (Ligthelm et al. 1991; Sunde et al. 1993). The best-studied enzyme system responsible for sulfite reduction is encoded by the dissimilatory sulfite reductase (*dsr*) genes, dsrABEFHCMKLJOPNRS (Grimm et al. 2010). The cytoplasmic dissimilatory sulfite reductase, encoded by *dsrAB*, is homologous to the sulfite reductase of sulfate-reducing prokaryotes (SRP) (Loy et al. 2009). The latter enzyme is indispensable for reduction of sulfite to sulfide in sulfate reducers. SRP are ubiquitous and quantitatively important members in many ecosystems, especially in marine sediments (Blazejak and Schippers 2011). The functional gene of SRP is the adenosine 5′-phosphosulfate reductase gene *aprA*. In sulfate reducers, APS reductase catalyzes the two-electron reduction of APS to sulfite and AMP. APS reductase consists of an alpha and beta subunit, encoded by the genes *aprA* and *aprB* (Meyer and Kuever 2007; Friedrich 2002). Our results indicate that the different mangrove habitats have comparative S oxidation abilities; nevertheless, HH has a higher capacity of S reduction than the other habitats, and BGR had the lowest ability of S reduction.

In conclusion, our results indicate that a variety of functional genes related to C, N, P, and S biogeochemical cycling are present in different mangrove habitats. Our results also imply that there is a huge bioremediation potential of mangrove wetland ecosystems. We also detected many antibiotic and metal resistance genes, reflecting that the antibiotic abuse and heavy metal pollution problems have become more and more serious. However, this also suggests that we could take advantage of mangrove habitats to isolate functional microorganisms capable of bioremediation and other purposes. The physical and chemical sediment properties, microbial metabolic diversity, functional gene diversity, and structure were affected by environmental variables, especially salinity. *S. alterniflora* (HH) was introduced into

China in 1979 from the United States and has invaded mangrove habitats continuously and vigorously. In China, *S. alterniflora* is an invasive species that could gradually replace these mangroves in the regions of Chinese estuaries (Zhang et al. 2012). Wang et al. suggests that genotypic diversity enhances the invasive ability of *S. alterniflora* (Wang et al. 2012), which may be why *S. alterniflora* can adapt to different environments. In addition, invasive alien plants could increase the plant N and soil inorganic N pool in some terrestrial ecosystems, and *S. alterniflora* invasion increases soil inorganic N pools (Peng et al. 2011). In this study, we found that the potential microbial N metabolic capacity from the sediments of *S. alterniflora* were higher than for the other habitats. In general, *S. alterniflora* didn't show much difference based on the potential microbial community functions detected. GeoChip is considered a powerful tool for analyzing microbial communities; however, it only detects known sequences already present in a database at the time of probe design. In order to gain a comprehensive understanding of microbial community functions, high-quality, full-length sequencing should be done as a complementary approach.

Acknowledgments This work was supported by the National Nature Science Foundation (40930847, 30930017, 31070442), public science and technology research funds projects of ocean (201305016, 201305041, 201305022) and the Program for Changjiang Scholars and Innovative Research Team in University (41121091).

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