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Carbohydrate metabolism genes dominant in a subtropical marine mangrove ecosystem revealed by metagenomics analysis[§]

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Mangrove sediment microorganisms play a vital role in the energy transformation and element cycling in marine wetland ecosystems. Using metagenomics analysis strategy, we compared the taxonomic structure and gene profile of the mangrove and non-mangrove sediment samples at the subtropical estuary in Beibu Gulf, South China Sea. Proteobacteria, Bacteroidetes, and Firmicutes were the most abundant bacterial phyla. Archaeal family Methanosarcinaceae and bacterial genera *Vibrio* and *Dehalococcoides* were significantly higher in the mangrove sediments than in the non-mangrove sediments. Functional analysis showed that "Carbohydrate metabolism" was the most abundant metabolic category. The feature of carbohydrate-active enzymes (CZs) was analyzed using the Carbohydrate-Active EnZymes Database. The significant differences of CZs between mangrove and non-mangrove sediments, were attributed to the amounts of polyphenol oxidase (EC 1.10.3.-), hexosyltransferase (EC 2.4.1.-), and β-*N*-acetylhexosaminidase (EC 3.2.1.52), which were higher in the mangrove sediment samples. Principal component analysis indicated that the microbial community and gene profile between mangrove and non-mangrove sediments were distinct. Redundancy analysis showed that total organic carbon is a significant factor that affects the microbial community and gene distribution. The results indicated that the mangrove ecosystem with massive amounts of organic carbon may promote the richness of carbohydrate metabolism genes and enhance the degradation and utilization of carbohydrates in the mangrove sediments.

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Introduction

Mangroves are unique ecosystems located at intertidal zones in the tropics and subtropics (Giri *et al*., 2011). They provide high ecological values, such as improvement of coastal water quality, maintenance of ecological diversity, and resistance to the wave destruction (Nagelkerken *et al*., 2008; Barbier *et al*., 2011; Bouma *et al*., 2014). Extensive nutrient exchanges occur between mangrove plants and various microorganisms. Microbial communities play unique roles in element and nutrient cycle processes, such as carbohydrate degradation, nitrogen fixation, sulfur metabolism, and phosphate solubilization, in mangrove ecosystems (Alongi *et al*., 1993; Bashan and Holguin, 2002; Andreote *et al*., 2012). Researchers found that the bacterial community is the main cause of carbon flux in tropical mangrove sediment (Alongi *et al*., 1989; Bano *et al*., 1997; Kristensen *et al*., 2008; Thatoi *et al*., 2013). Through classical culture methods, bacteria (e.g., Bacillales, Actinomycetales, Vibrionales), and fungi (e.g., *Pestalotiopsis foedans*, *Fusarium solani*) have been isolated from worldwide mangrove ecosystems with different carbon utilization characteristics, such as cellulose degradation, chitinolytic, lignocellulolytic, and amylase activities (Alias *et al*., 1995; Huang *et al*., 2008; Dias *et al*., 2009; Hong *et al*., 2009; Arfi *et al*., 2013).

Recently, comprehensive information about microbial diversity in mangrove sediment has been revealed through culture-independent strategies, such as denaturing gradient gel electrophoresis and 16S rDNA sequencing (Marcial Gomes *et al*., 2008; Jiang *et al*., 2013). However, the profile of mangrove microbial gene functions has not yet been studied well. A previous study on the features of microbial taxonomy and gene functions from Atlantic Ocean mangrove sediments (Brazilian) indicated that microbes involved in methane, nitrogen, and sulfur transformation and genes implicated in carbohydrate, energy, amino acid, cofactor, and vitamin metabolisms are highly abundant in the mangrove sediments (Andreote *et al*., 2012). Another study found that the genes involved in the "Metabolism of aromatic compounds," "Genetic mobile elements," and "Potassium metabolism" are enriched in the rhizosphere microbiomes of the mangrove ecosystem in Saudi Arabia (Alzubaidy *et al*., 2016). To better understand the characteristics of microbial genes and their metabolic functions in global mangrove ecosystems, analyzing more mangrove samples from different geographical locations is necessary.

Using metagenomics analysis strategy, high-throughput data provide convenience to gain more comprehensive genomic information on microbial structures and gene functions from various ecosystems (Kanokratana *et al*., 2011; Fang *et al*., 2014; Ganesh *et al*., 2014). In this work, mangrove sediment samples were collected from a subtropical estuary in Beibu Gulf, South China Sea, and the metagenomic DNA was sequenced to determine the following: (i) gene function profile and potential metabolic pathway and (ii) impacts of environmental factors on the distribution of microbial community and functional genes. This work provided detailed the information, particularly the carbohydrate metabolism profile, of the role of microorganisms in the subtropical mangrove ecosystem.

Materials and Methods

Sampling sites

An estuary in Beihai City in China (21°24′43.43″N, 109°9′50.98″E) was selected for sampling (Supplementary data Fig. S1). The south bank is a beach without plants, whereas the north bank is covered by mangroves, mostly *Avicennia marina*. The collected mangrove sediment samples in the north bank were labeled MS1, MS2, and MS3. The collected non-mangrove sediment samples in the south bank were labeled NMS4, NMS5, and NMS6. The 15 cm-deep sediment samples were collected using sterile polyvinyl chloride tubes from each sampling site (20 m × 20 m, five-point sampling method) and mixed sufficiently on August 7, 2017. All of the samples were stored at -80°C on the same day.

DNA extraction and high-throughput sequencing

Metagenomic DNA was extracted from the sediments using the FastDNA SPIN Kit for Soil (MP Biomedicals) according to the manufacturer's protocols. After quality verification by agarose gel electrophoresis and with NanoDrop 2000 Spectrophotometer (Thermo Scientific), at least 6 μg of DNA from each sample was submitted to the Shanghai Majorbio Bio-Pharm Technology Co., Ltd. for sequencing. The sequence libraries of approximately 300 bp DNA fragments were prepared and sequenced using Illumina HiSeq 4000. The data output from each DNA sample exceeded 5 Gb. The metagenomic sequencing data were deposited in the NCBI SRA database under BioProject PRJNA472209.

Analysis of environmental factors

Sediment properties, such as pH, total organic carbon (TOC), and metal elements (Cu, Zn, Hg, Cd, and metalloid As), were determined using Chinese standard methods (Ministry of Ecology and Environment of the People's Republic of China, 1997, 2016; Ministry of Agriculture of the People's Republic of China, 2006, 2007; Standardization Administration of the People's Republic of China, 2008a, 2008b). Briefly, pH was measured through potentiometry measurement; TOC was measured through $K_2Cr_2O_7$ oxidation method; Cu and Zn were determined through flame atomic absorption spectrophotometry; As and Hg were determined through atomic fluorescence spectrometry; Cd was determined using aqua regia extract - inductively coupled plasma mass spectrometry.

Data cleaning and assembly

Adapter sequences were removed using SeqPrep v1.33 (Yang *et al*., 2014). Sequences of less than 100 bp, sequences with a quality of less than 20, and reads containing N bases were removed using Sickle v1.2 (Yang *et al*., 2014). Clean reads were created.

Clean reads were assembled using MEGAHIT v1.1 (set as – presets meta-large), and the scaffolds were interrupted at the N joint, resulting in scaftigs (Mende *et al*., 2012; Nielsen *et al*., 2014). Unused reads were obtained using SoapAligner v2.21 (set as -u, -2, -m 200) for the global final assembly (with the same protocol as the assembly of single sample) (Karlsson *et al*., 2012).

Annotation of taxonomy

For rRNA-based taxonomic annotation, clean reads were aligned against SILVA database release 132 (Quast *et al*., 2012) using a local blastn program (E-value set as 10^{-6}) (Camacho *et al*., 2009). A blastn result was imported into MEGAN Community v6.12.6, and taxonomic classification was performed using the LCA algorithm (Huson *et al*., 2016). For taxonomic comparison, the number of SSU rRNA reads of each sample was normalized to the smallest sample in MEGAN Community v6.12.6.

Gene annotation and abundance calculation

Scaftigs longer than 500 bp were used for gene function analysis (Nielsen *et al*., 2014). Open reading frames (ORFs) were predicted with MetaGeneMark v2.10 (default setting), and ORFs with less than 100 nucleotides were filtered (Karlsson *et al*., 2013). A gene catalogue was constructed (identity 95%, coverage 90%) using CD-HIT v4.6.6 (set as -c 0.95, -G 0, -aS 0.9, -g 1, -d 0) (Fu *et al*., 2012; Li *et al*., 2014).

Clean reads were aligned against the gene catalogue using SoapAligner v2.21 (set as -m 200, -x 400, identity ≥ 95%) to obtain the gene reads number (Li *et al*., 2014). After the genes with a maximum read number of 2 were filtered, a unigenes catalogue was constructed for further analysis (Qin *et al*., 2010). The gene abundance of each sample could be calculated through the genes length and reads number (Karlsson *et al*., 2012). The gene reads number of each sample was normalized to the size of the smallest sample based on relative abundance (Qin *et al*., 2010).

For gene function annotation, unigenes were aligned against the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa *et al*., 2014), Non-supervised Orthologous Groups (eggNOG) (Powell *et al*., 2014), and Carbohydrate-Active EnZymes (CAZy) databases (Cantarel *et al*., 2009) using DIAMOND v0.9.14 (set as blastp, -e 1e-5) (Li *et al*., 2014). The sequences with the highest score (one HSP > 60 bits) were used for the subsequent analysis (Li *et al*., 2014).

Statistical analysis

Bar charts and pie charts were generated using Excel 2016. Differences between the two groups were analyzed using White's non-parametric *t*-test in STAMP v2.1.3 (Parks *et al*.,

2014). A heatmap was generated using MORPHEUS after the Z-score normalization (Fernandez *et al.*, 2017). Clustering of the samples was based on the weighted Unifrac distance using Fast UniFrac (Hamady *et al.*, 2010).

Principal component analysis (PCA), redundancy analysis (RDA), assessment of association between environmental factors and functional gene distribution in RDA, and Mantel test were performed using R3.4.3 with the vegan v2.4-6 package (Dixon, 2003). Confidence ellipse was generated using ggplot2 v3.1.0 package. The number of environmental factors exceeded the number of samples. As such, the first two axes of metal content PCA were chosen for RDA. These axes accounted for more than 80% of the variance in these factors.

Data accessibility

The metagenomic sequencing data analyzed in this work were deposited in the NCBI SRA database under BioProject PRJNA472209.

Results

Overview of the metagenomic data

Total DNA was extracted and sequenced using Illumina HiSeq 4000, thereby generating 31.75 Gbp data with an average sample size of approximately 5.29 Gbp. A total of 443,991 ORFs were predicted for functional annotation, and 73.88% of these ORFs were assigned to functional genes. The percentage of the identified SSU rRNA genes used for the taxonomic assignments in all of the samples was 0.07%.

Microbial community in mangrove and non-mangrove sediments

The taxonomic classification based on SSU rRNA annotation showed that bacteria, archaea, and fungi accounted for 93.83%, 1.91%, and 0.12%, respectively.

A total of 25 bacterial phyla, three archaeal phyla, and five phyla of fungi were found in the samples. The top three abundant bacterial phyla were Proteobacteria, Bacteroidetes, and Firmicutes (Fig. 1A). The major archaeal phyla were Crenarchaeota and Euryarchaeota (Fig. 1B). The main fungal phyla were Basidiomycota, Ascomycota, and Zoopagomycota (Fig. 1C).

To determine the taxonomic differences between the mangrove sediments and non-mangrove sediments, we assigned the mangrove-derived samples MS1, MS2, and MS3 as the MS (mangrove sediment) group, and the non-mangrove-derived samples NMS4, NMS5, and NMS6 as the NMS (non-mangrove sediment) group. The most abundant bacterial phyla in the MS group were Proteobacteria, Bacteroidetes, and Firmicutes (Supplementary data Fig. S2A). At the phylum level, the bacterial communities of the two groups were similar (Supplementary data Fig. S2A). Crenarchaeota and Euryarchaeota were the most abundant archaeal phyla in the two groups (Supplementary data Fig. S2B). The portion of Crenarchaeota was higher than that of Euryarchaeota in the MS group. By contrast, the NMS group showed different results (Supplementary data Fig. S2B). Basidiomycota was the most abundant fungal phylum in the two groups (Supplementary data Fig. S2C). The second-most abundant fungal phyla in the MS group and the NMS group were Zoopagomycota and Ascomycota, respectively (Supplementary data Fig. S2C).

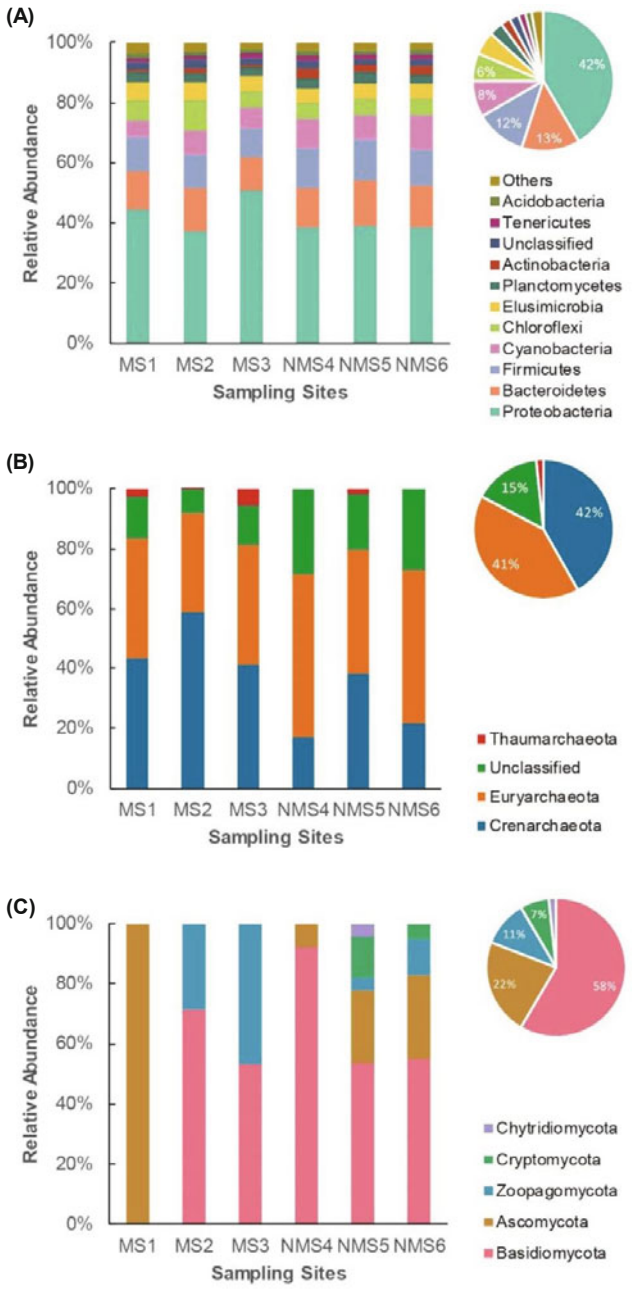

Fig. 1. Taxonomic structure of the samples. (A) The bar chart indicates the relative abundances of the 10 abundant bacterial phyla in each sample, and the pie chart indicates the relative abundances of the 10 abundant bacterial phyla in all the samples. (B) The bar chart indicates the relative abundances of the archaeal phyla in each sample, and the pie chart indicates the relative abundances of the archaeal phyla in all samples. (C) The bar chart indicates the relative abundances of the fungal phyla in each sample, and the pie chart indicates the relative abundances of the fungal phyla in all the samples.

Differences in taxonomic abundance were analyzed using white's non-parametric *t*-test in STAMP software. At the kingdom level, the abundance of archaea was significantly increased in the MS group, whereas that of fungi was significantly reduced (Fig. 2A). No significant difference in bacteria was found between the two groups. At different taxonomic levels, seven phyla (Supplementary data Fig. S3), 10 classes (Supplementary data Fig. S4), 19 orders (Supplementary data Fig. S5), 23 families (Supplementary data Fig. S6), 30 genera (Supplementary data Fig. S7), and 35 species (Supplementary data Fig. S8) significantly differed between the two groups. Of the 10 abundant bacterial phyla, three (Firmicutes, Cyanobacteria, and Actinobacteria) were significantly more abundant in the NMS group than in the MS group (Supplementary data Fig. S3). However, Proteobacteria and Bacteroidetes, the two most abundant phyla accounting for more than 50% of the bacteria (Fig. 1A and Supplementary data Fig. S2A), did not show a significant difference between the two groups (Supplementary data Fig. S3). Similarly, the classes of the two phyla did not significantly vary between the two groups (Supplementary data Fig. S4).

Six bacterial classes, namely, Actinobacteria, Bacilli, Planctomycetia, Mollicutes, Verrucomicrobiae, and Agaricomycetes, were enriched in the NMS group, whereas Dehalococcoidia and Phycisphaerae were enriched in the MS group (Supplementary data Fig. S4). Methanomicrobia and Thermoprotei, which belonged to archaea and showed significant difference between the two groups, were higher in the MS group than in the NMS group (Supplementary data Fig. S4). The most abundant fungal class Agaricomycetes, which belonged to Basidiomycota, was significantly abundant in the NMS group (Supplementary data Fig. S4).

Figure 2B shows the significantly different families between the MS and NMS groups (Supplementary data Fig. S6). Five bacterial families (Mycoplasmataceae, Streptococcaceae, Staphylococcaceae, Vibrionaceae, and Dehalococcoidaceae) were significantly different between the two groups. Mycoplasmataceae, Streptococcaceae, and Staphylococcaceae were higher in the NMS group, whereas Vibrionaceae and Dehalococcoidaceae were higher in the MS group (Fig. 2B and Supplementary data Fig. S6). Only one archaeal family, namely, Methanosarcinaceae, showed a significant difference between the MS and NMS groups (Fig. 2B and Supplementary data Fig. S6). At the genus level, *Vibrio* and *Dehalococcoides* were significantly higher in the MS group than in the NMS group, whereas *Desmonostoc*, *Mycoplasma*, and *Streptococcus* were significantly higher in the NMS group than in the MS group (Fig. 2C and Supplementary data Fig. S7).

Diversity analysis of microbial community in mangrove and non-mangrove sediments

The α-diversities of bacteria, archaea, and fungi were indicated by the Shannon index. At the class level, the average

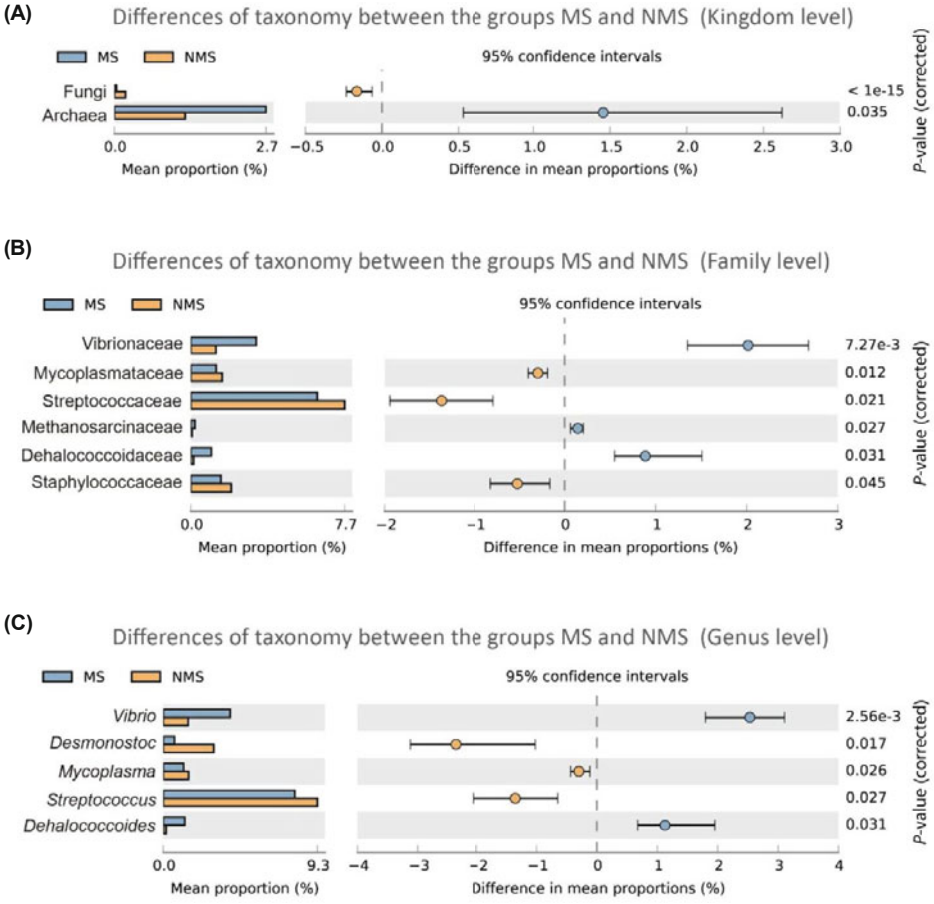

Fig. 2. Comparison of taxonomic abundances between the mangrove and non-mangrove sediments. (A) Significant differences of the taxonomic abundance between the MS and NMS groups (kingdom level). (B) Significant differences of the taxonomic abundance between the MS and NMS groups (family level). (C) Significant differences of the taxonomic abundance between the MS and NMS groups (genus level).

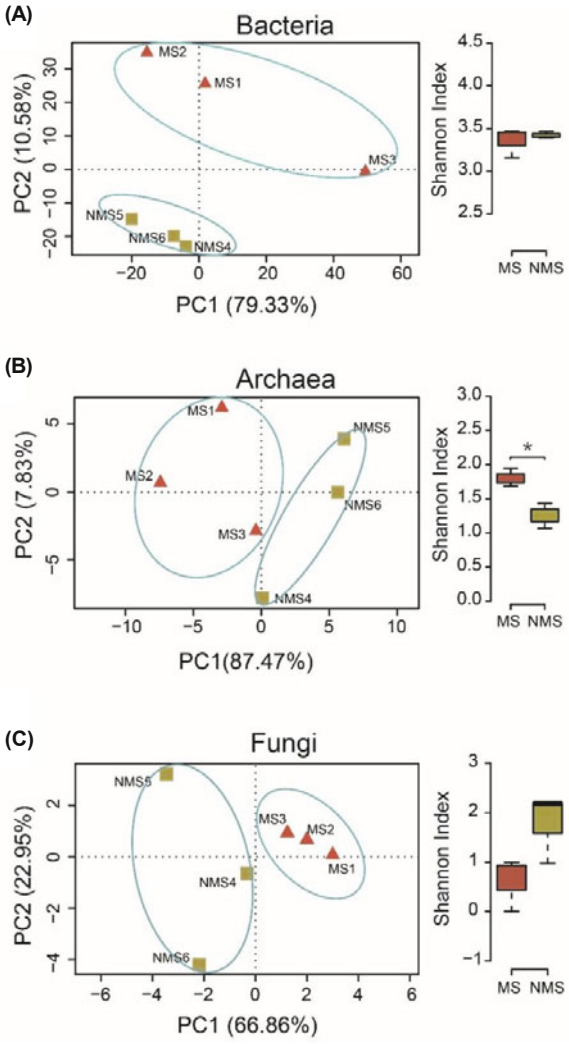

Fig. 3. Diversity analysis of the bacteria, archaea, and fungi in the MS and NMS groups (class level). (A) PCA plot resulting from bacterial abundance of each sample; boxplot shows the bacterial Shannon indexes of the MS and NMS groups. (B) PCA plot resulting from archaeal abundance of each sample; boxplot shows the archaeal Shannon indexes of the MS and NMS groups. (C) PCA plot resulting from the fungal abundance of each sample; boxplot shows the fungal Shannon indexes of the MS and NMS groups. Ellipses represented 95% confidence level.

Shannon indexes of bacteria, archaea, and fungi were 3.36, 1.81, and 0.62 in the MS group and 3.42, 1.25, and 1.79 in the NMS group, respectively (Fig. 3). The Shannon index of archaea was significantly higher in the MS group than in the NMS group (Fig. 3B). Meanwhile, the Shannon index of fungi was higher in the NMS group than in the MS group. The PCA of the three kingdoms showed that the samples of the two groups could be divided (Fig. 3). For bacteria, the samples in the MS group were more dispersed than those in the NMS group on the PCA plot (Fig. 3A). Conversely, the samples in the NMS group were more dispersed than in the MS group in the case of fungi (Fig. 3C). Similar to the PCA results, clustering (weighted Unifrac) among the samples showed that bacterial, archaeal, and fungal communities were different between the MS and NMS groups (Fig. 4).

Gene function profile in mangrove and non-mangrove sediments

KEGG, eggNOG, and CAZy databases were used for functional gene annotation to explore the functional gene features of the microbial community in the mangrove sediments (Cantarel *et al*., 2009; Kanehisa *et al*., 2014; Powell *et al*., 2014). Functional analysis results showed that approximately 53.27% of the predicted protein coding sequences were matched to 4,637 KEGG Orthologies and 20 KEGG categories (level 2) (Fig. 5 and Supplementary data Fig. S9). The results suggested that the genes associated with core-housekeeping functions, such as "Carbohydrate metabolism," "Energy metabolism," and "Amino acid metabolism" were the most abundant metabolic categories (Fig. 5 and Supplementary data Fig. S9), accounting for 14.36%, 13.44%, and 12.96% of the total functional genes in the MS group, respectively.

The structures of the functional genes in the two groups were similar (Fig. 5). The gene abundances of "Membrane transport," "Translation," and "Signal transduction" were higher in the MS group, whereas genes associated with "Amino acid metabolism" and "Nucleotide metabolism" were higher in the NMS group (Fig. 5).

In addition to the functional gene structures of the two groups shown in Fig. 5, the gene distribution pattern of the samples is illustrated in Fig. 6. The genes involved in "Membrane transport," "Xenobiotics biodegradation and metabolism," and "Energy metabolism" were abundant in sam-

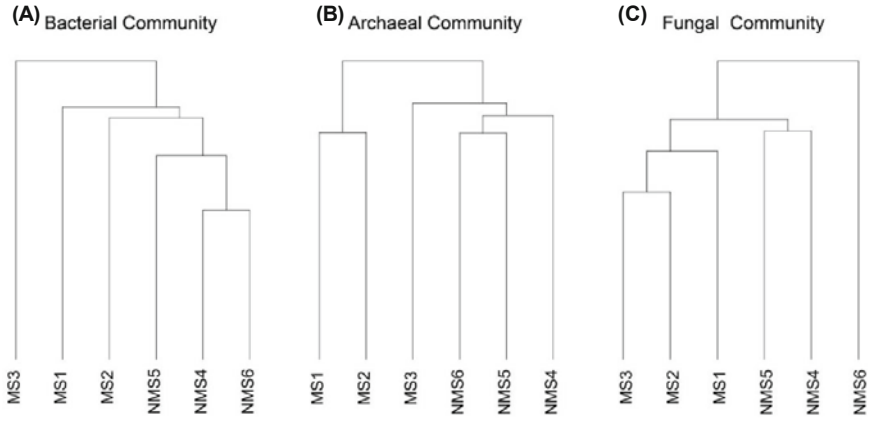

Fig. 4. Clustering of microbial communities associated with the Beibu gulf samples. (A) Bacterial community, (B) Archaeal community, (C) Fungal community.

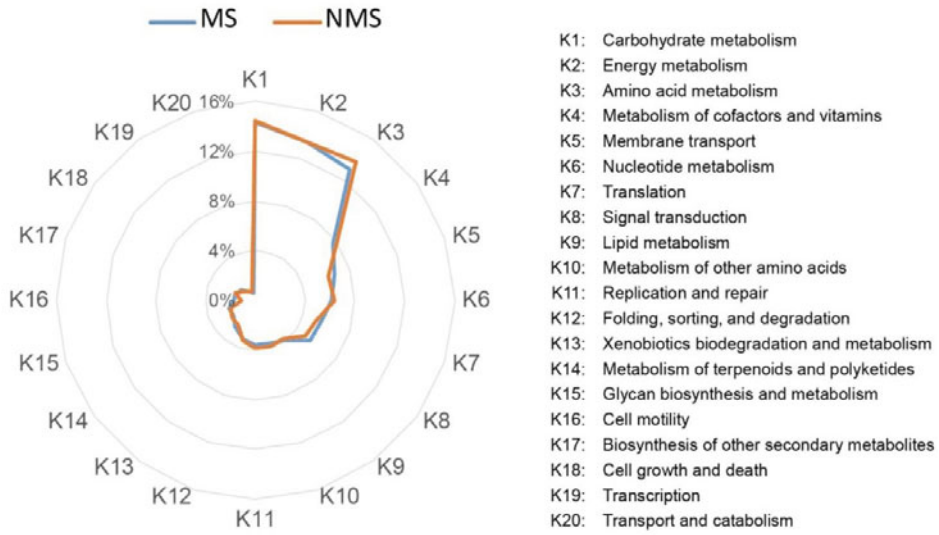

Fig. 5. Abundances of the functional genes in the MS and NMS groups.

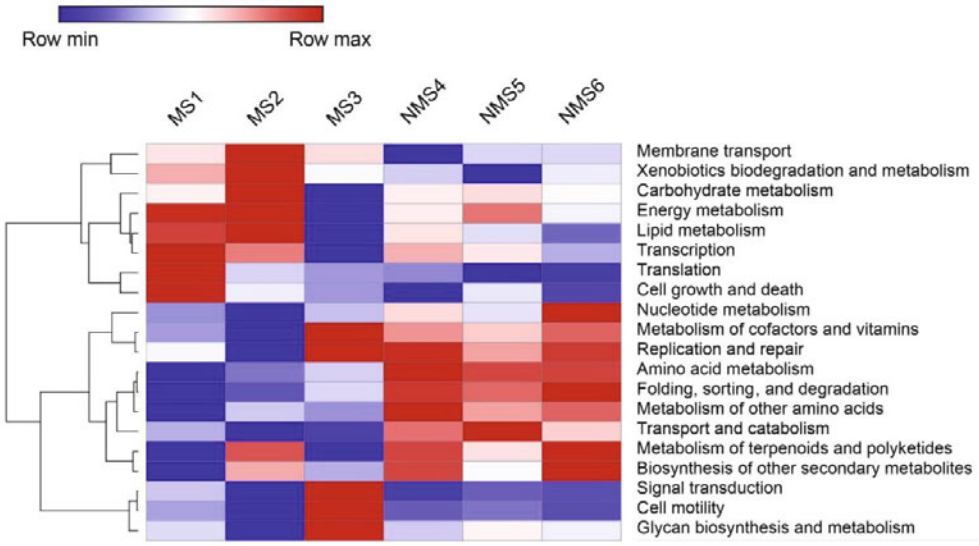

Fig. 6. Distribution pattern of the functional genes among samples. The abundances were normalized to Z-scores.

ples of the MS group. And genes involved in "Amino acid metabolism," "Folding, sorting and degradation," "Metabolism of other amino acids," and "Transport and catabolism" were abundant in the NMS group (Fig. 6).

For β-diversity analysis, PCA showed that the functional gene compositions were different between the MS and NMS groups (Fig. 7). As shown in the PCA plot, samples from the two groups could be obviously divided, and the samples in the MS group were more dispersed than those in the NMS group (Fig. 7).

White's non-parametric *t*-test was performed to analyze the significant differences between the two groups, and four significantly different functional gene categories, namely, "Transport and catabolism," "Metabolism of other amino acids," "Folding, sorting and degradation," and "Amino acid metabolism," were found. These categories were more abun-

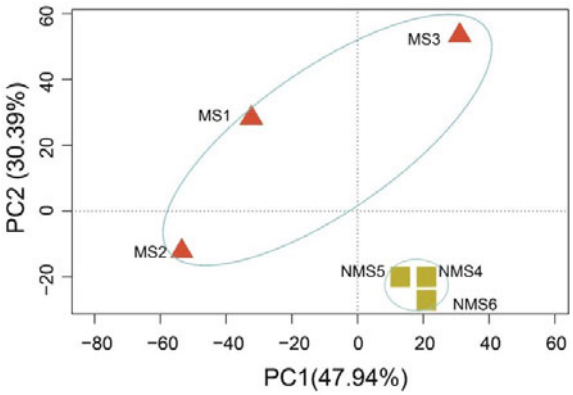

Fig. 7. PCA plot using the KEGG Orthology abundances of each sample.

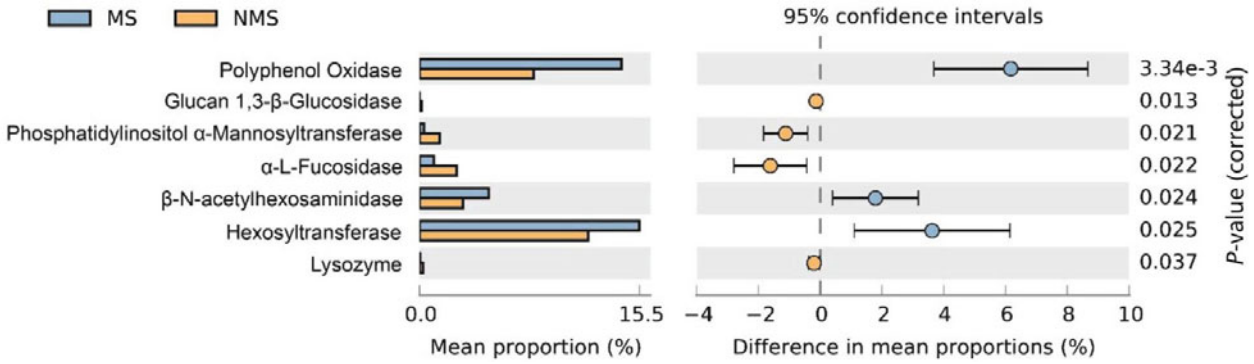

Fig. 8. **Significant differences of CZs between the MS and NMS groups.**

dant in the NMS group (Supplementary data Fig. S10). "Carbohydrate metabolism" and "Energy metabolism" were the most abundant functional genes in the samples and did not show a significant difference between the two groups. CZs were analyzed using the CAZy database to further determine the difference in the genes involved in carbohydrate metabolism between the MS and NMS groups. Seven types of CZs were significantly different between the MS and NMS groups (Fig. 8). Polyphenol oxidase (EC 1.10.3.-), hexosyltransferase (EC 2.4.1.-), and β-*N*-acetylhexosaminidase (EC 3.2.1.52) were enriched in the MS group. According to CAZy annotation, 19 CZ families were significantly different between the MS and NMS groups ($P < 0.05$) (Fig. 9). The GT51, GH23, and CE11 families were significantly higher in the MS group than in the NMS group (Fig. 9).

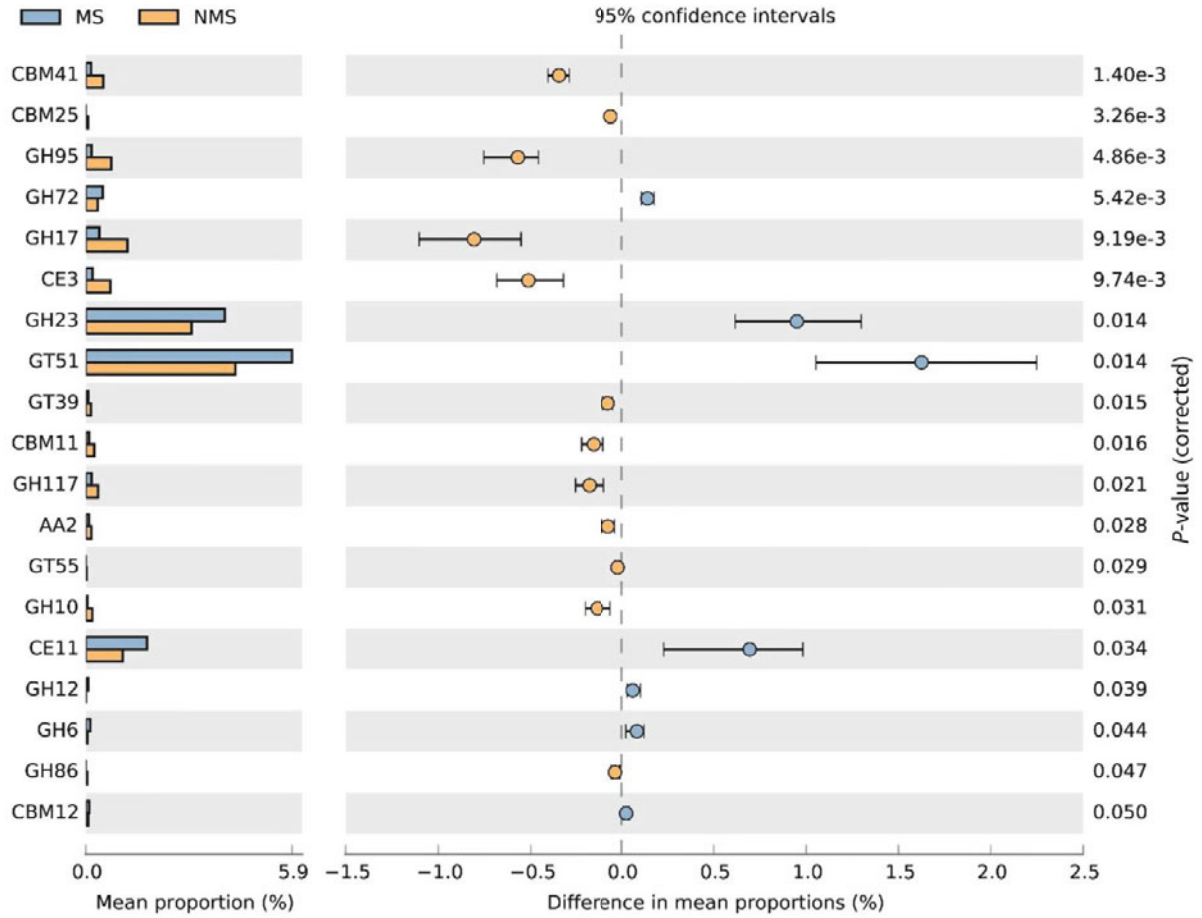

Fig. 9. **Significant differences of CZ families between the MS and NMS groups.**

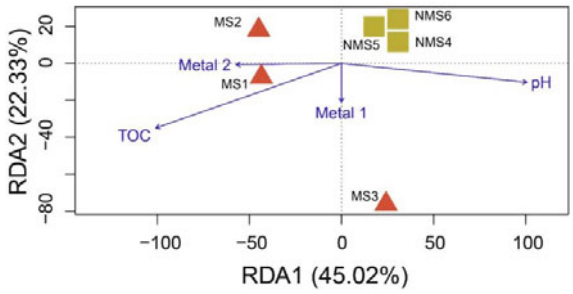

Fig. 10. RDA plot according to the KEGG Orthology abundances and environmental factors information of each sample.

Impact of environmental factors on the functional gene profile

The Mantel test showed a significant correlation between functional genes and environmental factors (Pearson's r = 0.545, P = 0.019, Euclidean distance, permutations: 9999). RDA results also showed that pH and TOC were the main factors affecting the functional gene constructure (Fig. 10). TOC significantly affected the gene distribution ($P < 0.05$) (Supplementary data Table S1).

Discussion

Mangroves are coastal ecosystems around the world (Giri *et al.*, 2011) and are deeply involved in biogeochemical processes, mainly in carbon cycling (Bouillon *et al.*, 2002). Given the essential roles of microorganisms in the ecosystem, investigating the microbial community and gene functions in the mangrove sediment environments is necessary. Researchers have investigated the taxonomy and gene functions based on high-throughput sequencing of the mangrove sediment samples from the Arabian Sea (India), Red Sea (Saudi Arabia), and Atlantic (Brazil) (Andreote *et al.*, 2012; Thompson *et al.*, 2013; Alzubaidy *et al.*, 2016; Imchen *et al.*, 2018). In this study, we analyzed the gene function profile of the microbial community in the mangrove sediments at the subtropical estuary of Beibu Gulf, South China Sea.

Bacteria was the predominant kingdom rather than archaea and fungi in our mangrove sediment samples. At the phylum level, Proteobacteria, Bacteroidetes, and Firmicutes were the dominant bacteria in the mangrove sediment samples MS1, MS2, and MS3. The most abundant phylum Proteobacteria accounted for 44.31% of the bacteria in the MS group (Supplementary data Fig. S2A). A remarkable portion of Proteobacteria was also found in the mangrove sediment samples from India, Brazil, Saudi Arabia, and Hong Kong, accounting for more than 54.0% (Andreote *et al.*, 2012; Jiang *et al.*, 2013; Mangrola *et al.*, 2015; Alzubaidy *et al.*, 2016). This result indicated that Proteobacteria is the dominant phylum in mangrove sediment. Proteobacteria is widely involved in material cycling in the natural environment (Kersters *et al.*, 2006). In Proteobacteria, three genera with high abundance (*Klebsiella*, *Vibrio*, and *Pseudomonas*) were found in the Beibu Gulf samples, and they are considered as bacteria that promote the growth of mangroves by participating in nitrogen fixation and phosphate solubilization (Bashan and Holguin, 2002). Chloroflexi, Cyanobacteria, Planctomycetes, and Actinobacteria constitute the other abundant bacterial phyla in the mangrove sediment samples, and this observation is consistent with other mangrove studies (Andreote *et al.*, 2012; Jiang *et al.*, 2013; Mangrola *et al.*, 2015; Alzubaidy *et al.*, 2016). Interestingly, Elusimicrobia was the highly abundant phylum in our work, accounting for 5.60% of the total bacteria (Fig. 1A). Most of its species were detected only by the 16S rRNA-based method from anoxic environments, such as marine sediments (Brune, 2018) and the gut microbiome of animals or insects with a rich cellulose content (Huang *et al.*, 2013; Wong *et al.*, 2016). The anoxic and cellulose-rich sediment in the mangrove ecosystem may facilitate their growth (Holguin *et al.*, 2001). Euryarchaeota and Crenarchaeota, which were the most abundant archaeal phyla in this study, are also the major archaeal phyla in India, Brazil, and Saudi Arabia mangrove sediment samples (Imchen *et al.*, 2018). Basidiomycota and Ascomycota were the major fungal phyla in the Beibu Gulf and Saudi Arabia mangrove sediment samples (Alzubaidy *et al.*, 2016). These results suggested the similar phylum structure of bacteria, archaea, and fungi in the global mangrove ecosystems.

We also found that the genera *Vibrio* and *Dehalococcoides* were significantly higher in the MS group than in the NMS group (Fig. 2C). *Vibrio* is widely found in marine and estuarine environments (Thompson *et al.*, 2004). Members of *Vibrio* are involved in nitrogen fixation and organic matter degradation, which are important metabolic processes in mangrove ecosystems (Bashan and Holguin, 2002; Ray *et al.*, 2014; Badur *et al.*, 2015). In addition, plant, fish, shrimp, and shellfish often serve as *Vibrio* carriers, which are abundant in mangrove ecosystems (Rönnbäck, 1999; Walters *et al.*, 2008). *Dehalococcoides* is known as the only bacterial genus that degrades chlorinated organic compounds, which are human-derived contaminants that easily accumulate in sediments (Olutona *et al.*, 2016). The special properties of the mangrove ecosystem at the urban estuary may contribute to the richness of these bacteria.

The result also demonstrated that archaea were significantly higher in the MS group than in the NMS group, whereas fungi were significantly higher in the NMS group (Fig. 2A). For archaea, the family Methanosarcinaceae was significantly higher in the MS group than in the NMS group (Fig. 2B). Members of Methanosarcinaceae, such as *Methanosarcina* and *Methanococcoides*, have been found in various anaerobic environments where methane is produced (Hedderich and Whitman, 2013; Oren, 2014). A previous study indicated that the methanogenic bacteria are important components of mangrove sediment microorganisms (Holguin *et al.*, 2001). Human activity and contamination, such as sewage of aquaculture, may increase the methanodynamic activity in mangrove ecosystems (Holguin *et al.*, 2001). We found that "Methane metabolism" was the sixth-most abundant metabolic function (KEGG level 3) in the MS group and was higher than that in the NMS group (Supplementary data Table S2). The emission of municipal and aquaculture sewage upstream of the sampling estuary might promote the enrichment of Methanosarcinaceae and enhance methane metabolism in the mangrove ecosystem.

Although no significant difference in the total bacterial abundance was observed between the two groups of the Beibu Gulf samples at the kingdom level, the bacterial structure was more diverse in the MS group than in the NMS group (Figs. 3A and 4A). Plants and animals, whose community structures are much different between the mangrove ecosystem and the non-mangrove area, have extensive nutritional exchanges with bacteria (Holguin *et al*., 2001; Sahoo and Dhal, 2009), which may cause more complex bacterial community in the Beibu Gulf mangrove sediments.

In addition to taxonomic investigation, the gene function profile of the Beibu Gulf mangrove sediment samples was determined using the KEGG, eggNOG, and CAZy databases. We found that "Carbohydrate metabolism," "Energy metabolism," and "Amino acid metabolism" were the major functional types of the Beibu Gulf mangrove sediment samples (Fig. 5). The three functional types are also predominant in the samples from Brazilian mangrove sediments (Andreote *et al*., 2012; Thompson *et al*., 2013). In the two mangrove sediment studies, "Carbohydrate metabolism" is also the most abundant type (Andreote *et al*., 2012; Thompson *et al*., 2013). The three functional types are involved in basic metabolism in various environmental samples (Costa *et al*., 2015; Badhai *et al*., 2016; Meneghine *et al*., 2017). However, previous studies on the mucus of the coral *Fungia echinata* and zoo agricultural soil demonstrated that "Amino acid metabolism" is the only abundant functional type (Badhai *et al*., 2016; Meneghine *et al*., 2017).

Microbes in mangrove sediment dominate organic matter degradation, and carbon and energy can enter the food web by the uptake of animal consumers (Holguin *et al*., 2001; Rajendran *et al*., 2016). In our study, "Carbon metabolism" was the highest (43.80%) functional type of metabolic genes (KEGG: Global and overview maps of metabolism) in the MS group, and this type was higher than that in the NMS group (Supplementary data Table S3). In addition, the genes associated with "Carbon fixation pathways in prokaryotes" were the third-most abundant (4.30%) metabolic genes in the MS group (KEGG level 3) and were higher than those in the NMS group (Supplementary data Table S2). Mangrove plants contain highly abundant carbohydrates, such as glucose (cellulose form), xylose, arabinose, and galactose as organic carbon (Opsahl and Benner, 1999; Rajendran *et al*., 2016). This condition may contribute to the particularly high ratio of carbohydrate metabolism genes in the mangrove sediments. In this work, the difference in "Carbohydrate metabolism" genes was not significant ($P = 0.48$) between the MS and NMS groups as indicated by White's non-parametric *t*-test (Supplementary data Fig. S10). This result is understandable because of tidal brush and estuary water flow, and detritus from mangrove ecosystems can be carried to the surrounding area, thus influencing the distribution of functional genes in the area (Holguin *et al*., 2001). These results implied that carbohydrate metabolism genes are dominant in the mangrove ecosystem.

The differences in CZs between the two groups were further analyzed using the CAZy database, and our results revealed that polyphenol oxidase (EC 1.10.3.-), hexosyltransferase (EC 2.4.1.-), and β-*N*-acetylhexosaminidase (EC 3.2.1.52) were significantly higher in the MS group than in the NMS group (Fig. 8). These enzymes collectively accounted for 90.84% of the significantly different CZs. Polyphenol compounds, such as tannins, are widely found as secondary metabolites of plant tissues, which are also rich in mangrove (Hättenschwiler and Vitousek, 2000; Rajendran *et al*., 2016). Polyphenol oxidases are found in bovine rumen microflora, and they improve the digestion of lignin (Beloqui *et al*., 2006; Cantarel *et al*., 2009). We speculated that this enzyme promotes lignin degradation in mangrove sediments, which is similar to that in bovine rumen (Lee *et al*., 2014), because of the richness of polyphenol and lignin in the mangrove ecosystem (Rajendran *et al*., 2016). Hexoses such as cellulosic glucose, galactose, and hemicellulosic glucose are rich in the wood and leaves of mangrove plants (Rajendran *et al*., 2016). The higher abundance of hexosyltransferase in mangrove sediment microflora conforms to the degradation requirement of these hexoses (Rajendran *et al*., 2016). β-*N*-acetylhexosaminidase, which hydrolyzes the terminal non-reducing *N*-acetyl-D-hexosamine residues in *N*-acetyl-beta-D-hexosaminides and belongs to glycoside hydrolases, was previously detected in the mangrove sediment samples (Soares *et al*., 2017). This enzyme contributes to the degradation of chitin, a polysaccharide that widely exists in marine animals, such as crabs and shrimps, which inhabit mangrove ecosystems (Patil *et al*., 2000; Holguin *et al*., 2001). This finding indicated that a strong chitinolytic process occurred in mangrove sediments.

The significantly different CZ families between MS and NMS groups were GT51, GH23, and CE11 (Fig. 9). The families belonged to glycosyltransferase, glycoside hydrolase, and carbohydrate esterase classes in CAZy, respectively (Cantarel *et al*., 2009). These families correspond to α-l-arabinofuranosidase, glycosidic bonds cleavage, and UDP-3-*O*-acyl-*N*-acetylglucosamine deacetylase activities, respectively (Cantarel *et al*., 2009; Artimo *et al*., 2012). Thus, the results of CAZy family analysis also indicated the higher activities of carbohydrate metabolism in the Beibu Gulf mangrove sediments.

Large amounts of organic matter are retained in sediments by falling wood and leaves of mangroves (Holguin *et al*., 2001). In this study, the TOC value of the mangrove sediments was significantly higher than that of non-mangrove sediments ($P < 0.05$) (Supplementary data Table S4). The RDA results showed that TOC was the significant factor that affects the microbial community and functional gene distribution in the samples ($P < 0.05$) (Supplementary data Table S1). We found that microbial communities and functional genes were distinct in the MS and NMS samples (Figs. 3, 4, 6, and 7), which were similar to previous findings on mangroves (Jiang *et al*., 2013; Simões *et al*., 2015; Alzubaidy *et al*., 2016).

In summary, this work provided the detailed profiles of microbial communities and functional genes in the subtropical mangrove sediments in the South China Sea through metagenomics analysis. Functional gene analysis showed the richness of carbohydrate metabolism genes in the mangrove sediment microorganisms. Significantly abundant CZs were found in the mangrove samples. High TOC, a characteristic of mangroves, might contribute to the enrichment of carbohydrate metabolism genes, thereby promoting the utiliza-

tion and transformation of large amounts of carbon in mangrove sediments. The results of this study would help elucidate the key microorganisms in the matter flow of subtropical marine mangrove ecosystems.

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Ethics Statement

The study was carried out under the stipulation of ethics approved by the State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, College of Life Science and Technology, Guangxi University.

Conflict of Interest

All authors declare no competing interests.

References

Alias, S.A., Kuthubutheen, A.J., and Jones, E.B.G. 1995. Frequency of occurrence of fungi on wood in Malaysian mangroves. *Hydrobiologia* **295**, 97–106.

Alongi, D.M., Boto, K.G., and Tirendi, F. 1989. Effect of exported mangrove litter on bacterial productivity and dissolved organic carbon fluxes in adjacent tropical nearshore sediments. *Mar. Ecol. Prog. Ser.* **56**, 133–144.

Alongi, D.M., Christoffersen, P., and Tirendi, F. 1993. The influence of forest type on microbial-nutrient relationships in tropical mangrove sediments. *J. Exp. Mar. Biol. Ecol.* **171**, 201–223.

Alzubaidy, H., Essack, M., Malas, T.B., Bokhari, A., Motwalli, O., Kamanu, F.K., Jamhor, S.A., Mokhtar, N.A., Antunes, A., Simões, M.F., *et al.* 2016. Rhizosphere microbiome metagenomics of gray mangroves (*Avicennia marina*) in the Red Sea. *Gene* **576**, 626–636.

Andreote, F.D., Jiménez, D.J., Chaves, D., Dias, A.C.F., Luvizotto, D.M., Dini-Andreote, F., Fasanella, C.C., Lopez, M.V., Baena, S., Taketani, R.G., *et al.* 2012. The microbiome of Brazilian mangrove sediments as revealed by metagenomics. *PLoS One* **7**, e38600.

Arfi, Y., Chevret, D., Henrissat, B., Berrin, J.G., Levasseur, A., and Record, E. 2013. Characterization of salt-adapted secreted lignocellulolytic enzymes from the mangrove fungus *Pestalotiopsis* sp. *Nat. Commun.* **4**, 1810.

Artimo, P., Jonnalagedda, M., Arnold, K., Baratin, D., Csardi, G., de Castro, E., Duvaud, S., Flegel, V., Fortier, A., Gasteiger, E., *et al.* 2012. ExPASy: SIB bioinformatics resource portal. *Nucleic Acids Res.* **40**, W597–W603.

Badhai, J., Ghosh, T.S., and Das, S.K. 2016. Composition and functional characterization of microbiome associated with mucus of the coral *Fungia echinata* collected from Andaman Sea. *Front. Microbiol.* **7**, 936.

Badur, A.H., Jagtap, S.S., Yalamanchili, G., Lee, J.K., Zhao, H., and Rao, C.V. 2015. Alginate lyases from alginate-degrading *Vibrio splendidus* 12B01 are endolytic. *Appl. Environ. Microbiol.* **81**, 1865–1873.

Bano, N., Nisa, M.U., Khan, N., Saleem, M., Harrison, P.J., Ahmed, S.I., and Azam, F. 1997. Significance of bacteria in the flux of organic matter in the tidal creeks of the mangrove ecosystem of the Indus River delta, Pakistan. *Mar. Ecol. Prog. Ser.* **157**, 1–12.

Barbier, E.B., Hacker, S.D., Kennedy, C., Koch, E.W., Stier, A.C., and Silliman, B.R. 2011. The value of estuarine and coastal ecosystem services. *Ecol. Monogr.* **81**, 169–193.

Bashan, Y. and Holguin, G. 2002. Plant growth-promoting bacteria: a potential tool for arid mangrove reforestation. *Trees* **16**, 159–166.

Beloqui, A., Pita, M., Polaina, J., Martínez-Arias, A., Golyshina, O. V., Zumárraga, M., Yakimov, M.M., García-Arellano, H., Alcalde, M., Fernández, V.M., *et al.* 2006. Novel polyphenol oxidase mined from a metagenome expression library of bovine rumen. *J. Biol. Chem.* **281**, 22933–22942.

Bouillon, S., Koedam, N., Raman, A., and Dehairs, F. 2002. Primary producers sustaining macro-invertebrate communities in intertidal mangrove forests. *Oecologia* **130**, 441–448.

Bouma, T.J., van Belzen, J., Balke, T., Zhu, Z., Airoldi, L., Blight, A.J., Davies, A.J., Galvan, C., Hawkins, S.J., Hoggart, S.P.G., *et al.* 2014. Identifying knowledge gaps hampering application of intertidal habitats in coastal protection: Opportunities & steps to take. *Coast. Eng.* **87**, 147–157.

Brune, A. 2018. *Elusimicrobia*, pp. 1–3. *In* Whitman, W.B., Rainey, F., Kämpfer, P., Trujillo, M., Chun, J., DeVos, P., Hedlund, B., and Dedysh, S. (eds.), Bergey's manual of systematics of archaea and bacteria-2015. John Wiley & Sons Ltd., Chichester, UK.

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., and Madden, T.L. 2009. BLAST+: architecture and applications. *BMC Bioinformatics* **10**, 421.

Cantarel, B.L., Coutinho, P.M., Rancurel, C., Bernard, T., Lombard, V., and Henrissat, B. 2009. The carbohydrate-active enzymes database (CAZy): an expert resource for Glycogenomics. *Nucleic Acids Res.* **37**, D233–D238.

Costa, P.S., Reis, M.P., Ávila, M.P., Leite, L.R., de Araújo, F.M.G., Salim, A.C.M., Oliveira, G., Barbosa, F., Chartone-Souza, E., and Nascimento, A.M.A. 2015. Metagenome of a microbial community inhabiting a metal-rich tropical stream sediment. *PLoS One* **10**, e0119465.

Dias, A.C.F., Andreote, F.D., Dini-Andreote, F., Lacava, P.T., Sá, A.L.B., Melo, I.S., Azevedo, J.L., and Araújo, W.L. 2009. Diversity and biotechnological potential of culturable bacteria from Brazilian mangrove sediment. *World J. Microbiol. Biotechnol.* **25**, 1305–1311.

Dixon, P. 2003. VEGAN, a package of R functions for community ecology. *J. Veg. Sci.* **14**, 927–930.

Fang, H., Cai, L., Yang, Y., Ju, F., Li, X., Yu, Y., and Zhang, T. 2014. Metagenomic analysis reveals potential biodegradation pathways of persistent pesticides in freshwater and marine sediments. *Sci. Total Environ.* **470–471**, 983–992.

Fernandez, N.F., Gundersen, G.W., Rahman, A., Grimes, M.L., Rikova, K., Hornbeck, P., and Ma'ayan, A. 2017. Clustergrammer, a web-based heatmap visualization and analysis tool for high-dimensional biological data. *Sci. Data* **4**, 170151.

Fu, L., Niu, B., Zhu, Z., Wu, S., and Li, W. 2012. CD-HIT: accelerated for clustering the next-generation sequencing data. *Bioinformatics* **28**, 3150–3152.

Ganesh, S., Parris, D.J., DeLong, E.F., and Stewart, F.J. 2014. Metagenomic analysis of size-fractionated picoplankton in a marine oxygen minimum zone. *ISME J.* **8**, 187–211.

Giri, C., Ochieng, E., Tieszen, L.L., Zhu, Z., Singh, A., Loveland, T.,

Masek, J., and Duke, N. 2011. Status and distribution of mangrove forests of the world using earth observation satellite data. *Glob. Ecol. Biogeogr*. **20**, 154–159.

Hamady, M., Lozupone, C., and Knight, R. 2010. Fast UniFrac: facilitating high-throughput phylogenetic analyses of microbial communities including analysis of pyrosequencing and PhyloChip data. *ISME J*. **4**, 17–27.

Hättenschwiler, S. and Vitousek, P.M. 2000. The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends Ecol. Evol.* **15**, 238–243.

Hedderich, R. and Whitman, W.B. 2013. Physiology and biochemistry of the methane-producing archaea, pp. 635–662. *In* Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., and Thompson, F. (eds.), The prokaryotes-2013. Springer Berlin Heidelberg, Berlin, Heidelberg, Germany.

Holguin, G., Vazquez, P., and Bashan, Y. 2001. The role of sediment microorganisms in the productivity, conservation, and rehabilitation of mangrove ecosystems: an overview. *Biol. Fertil. Soils* **33**, 265–278.

Hong, K., Gao, A.H., Xie, Q.Y., Gao, H.G., Zhuang, L., Lin, H.P., Yu, H.P., Li, J., Yao, X.S., Goodfellow, M., *et al.* 2009. Actinomycetes for marine drug discovery isolated from mangrove soils and plants in China. *Mar. Drugs* **7**, 24–44.

Huang, X.F., Bakker, M.G., Judd, T.M., Reardon, K.F., and Vivanco, J.M. 2013. Variations in diversity and richness of gut bacterial communities of termites (*Reticulitermes flavipes*) fed with grassy and woody plant substrates. *Microb. Ecol.* **65**, 531–536.

Huang, H., Lv, J., Hu, Y., Fang, Z., Zhang, K., and Bao, S. 2008. *Micromonospora rifamycinica* sp. nov., a novel actinomycete from mangrove sediment. *Int. J. Syst. Evol. Microbiol.* **58**, 17–20.

Huson, D.H., Beier, S., Flade, I., Górska, A., El-Hadidi, M., Mitra, S., Ruscheweyh, H.J., and Tappu, R. 2016. MEGAN community edition - Interactive exploration and analysis of large-scale microbiome sequencing data. *PLoS Comput. Biol.* **12**, e1004957.

Imchen, M., Kumavath, R., Barh, D., Vaz, A., Góes-Neto, A., Tiwari, S., Ghosh, P., Wattam, A.R., and Azevedo, V. 2018. Comparative mangrove metagenome reveals global prevalence of heavy metals and antibiotic resistome across different ecosystems. *Sci. Rep.* **8**, 11187.

Jiang, X.T., Peng, X., Deng, G.H., Sheng, H.F., Wang, Y., Zhou, H.W., and Tam, N.F.Y. 2013. Illumina sequencing of 16S rRNA tag revealed spatial variations of bacterial communities in a mangrove wetland. *Microb. Ecol.* **66**, 96–104.

Kanehisa, M., Goto, S., Sato, Y., Kawashima, M., Furumichi, M., and Tanabe, M. 2014. Data, information, knowledge and principle: back to metabolism in KEGG. *Nucleic Acids Res.* **42**, D199–D205.

Kanokratana, P., Uengwetwanit, T., Rattanachomsri, U., Bunterngsook, B., Nimchua, T., Tangphatsornruang, S., Plengvidhya, V., Champreda, V., and Eurwilaichitr, L. 2011. Insights into the phylogeny and metabolic potential of a primary tropical peat swamp forest microbial community by metagenomic analysis. *Microb. Ecol.* **61**, 518–528.

Karlsson, F.H., Fåk, F., Nookaew, I., Tremaroli, V., Fagerberg, B., Petranovic, D., Bäckhed, F., and Nielsen, J. 2012. Symptomatic atherosclerosis is associated with an altered gut metagenome. *Nat. Commun.* **3**, 1245.

Karlsson, F.H., Tremaroli, V., Nookaew, I., Bergström, G., Behre, C.J., Fagerberg, B., Nielsen, J., and Bäckhed, F. 2013. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* **498**, 99–103.

Kersters, K., De Vos, P., Gillis, M., Swings, J., Vandamme, P., and Stackebrandt, E. 2006. Introduction to the Proteobacteria, pp. 3–37. *In* Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.H., and Stackebrandt, E. (eds.), The Prokaryotes-2006. Springer New York, New York, USA.

Kristensen, E., Bouillon, S., Dittmar, T., and Marchand, C. 2008. Organic carbon dynamics in mangrove ecosystems: A review. *Aquat. Bot*. **89**, 201–219.

Lee, S., Cantarel, B., Henrissat, B., Gevers, D., Birren, B.W., Huttenhower, C., and Ko, G. 2014. Gene-targeted metagenomic analysis of glucan-branching enzyme gene profiles among human and animal fecal microbiota. *ISME J*. **8**, 493–503.

Li, J., Jia, H., Cai, X., Zhong, H., Feng, Q., Sunagawa, S., Arumugam, M., Kultima, J.R., Prifti, E., Nielsen, T., *et al.* 2014. An integrated catalog of reference genes in the human gut microbiome. *Nat. Biotechnol.* **32**, 834–841.

Mangrola, A.V., Dudhagara, P., Koringa, P., Joshi, C.G., and Patel, R.K. 2015. Shotgun metagenomic sequencing based microbial diversity assessment of Lasundra hot spring, India. *Genomics Data* **4**, 73–75.

Marcial Gomes, N.C., Borges, L.R., Paranhos, R., Pinto, F.N., Mendonça-Hagler, L.C.S., and Smalla, K. 2008. Exploring the diversity of bacterial communities in sediments of urban mangrove forests. *FEMS Microbiol. Ecol.* **66**, 96–109.

Mende, D.R., Waller, A.S., Sunagawa, S., Järvelin, A.I., Chan, M.M., Arumugam, M., Raes, J., and Bork, P. 2012. Assessment of metagenomic assembly using simulated next generation sequencing data. *PLoS One* **7**, e31386.

Meneghine, A.K., Nielsen, S., Varani, A.M., Thomas, T., and Carareto Alves, L.M. 2017. Metagenomic analysis of soil and freshwater from zoo agricultural area with organic fertilization. *PLoS One* **12**, e0190178.

Ministry of Agriculture of the People's Republic of China. 2006. Method for determination of soil organic matter. NY/T 1121.6-2006. (in Chinese).

Ministry of Agriculture of the People's Republic of China. 2007. Determination of pH in soil. NY/T 1377-2007. (in Chinese).

Ministry of Ecology and Environment of the People's Republic of China. 1997. Soil quality-determination of copper, zinc-flame atomic absorption spectrophotometry. GBT 17138-1997. (in Chinese).

Ministry of Ecology and Environment of the People's Republic of China. 2016. Soil and sediment-determination of aqua regia extracts of 12 metal elements-inductively coupled plasma mass spectrometry. HJ 803-2016. (in Chinese).

Nagelkerken, I., Blaber, S.J.M., Bouillon, S., Green, P., Haywood, M., Kirton, L.G., Meynecke, J.O., Pawlik, J., Penrose, H.M., Sasekumar, A., *et al.* 2008. The habitat function of mangroves for terrestrial and marine fauna: A review. *Aquat. Bot*. **89**, 155–185.

Nielsen, H.B., Almeida, M., Juncker, A.S., Rasmussen, S., Li, J., Sunagawa, S., Plichta, D.R., Gautier, L., Pedersen, A.G., Le Chatelier, E., *et al.* 2014. Identification and assembly of genomes and genetic elements in complex metagenomic samples without using reference genomes. *Nat. Biotechnol.* **32**, 822–828.

Olutona, G.O., Olatunji, S.O., and Obisanya, J.F. 2016. Downstream assessment of chlorinated organic compounds in the bed-sediment of Aiba Stream, Iwo, South-Western, Nigeria. *Springerplus* **5**, 67.

Opsahl, S. and Benner, R. 1999. Characterization of carbohydrates during early diagenesis of five vascular plant tissues. *Org. Geochem*. **30**, 83–94.

Oren, A. 2014. The family methanosarcinaceae, pp. 259–281. *In* Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., and Thompson, F. (eds.), The prokaryotes-2014. Springer Berlin Heidelberg, Berlin, Heidelberg, Germany.

Parks, D.H., Tyson, G.W., Hugenholtz, P., and Beiko, R.G. 2014. STAMP: statistical analysis of taxonomic and functional profiles. *Bioinformatics* **30**, 3123–3124.

Patil, R.S., Ghormade, V., and Deshpande, M.V. 2000. Chitinolytic enzymes: an exploration. *Enzyme Microb. Technol.* **26**, 473–483.

Powell, S., Forslund, K., Szklarczyk, D., Trachana, K., Roth, A., Huerta-Cepas, J., Gabaldón, T., Rattei, T., Creevey, C., Kuhn, M., *et al.* 2014. eggNOG v4.0: nested orthology inference across 3686 organisms. *Nucleic Acids Res*. **42**, D231–D239.

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K.S., Manichanh,

C., Nielsen, T., Pons, N., Levenez, F., Yamada, T., *et al.* 2010. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**, 59–65.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glöckner, F.O. 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res*. **41**, D590–D596.

Rajendran, N., Saravanakumar, K., and Kathiresan, K. 2016. Mangroves: A potent source of polysaccharides, pp. 393–399. *In* Kim, S.K. (ed.), Marine glycobiology: principles and applications. CRC Press, Taylor & Francis Group, Boca Raton, USA.

Ray, R., Majumder, N., Das, S., Chowdhury, C., and Jana, T.K. 2014. Biogeochemical cycle of nitrogen in a tropical mangrove ecosystem, east coast of India. *Mar. Chem*. **167**, 33–43.

Rönnbäck, P. 1999. The ecological basis for economic value of seafood production supported by mangrove ecosystems. *Ecol. Econ.* **29**, 235–252.

Sahoo, K. and Dhal, N.K. 2009. Potential microbial diversity in mangrove ecosystems:A review. *Indian J. Mar. Sci*. **38**, 249–256.

Simões, M.F., Antunes, A., Ottoni, C.A., Amini, M.S., Alam, I., Alzubaidy, H., Mokhtar, N.A., Archer, J.A.C., and Bajic, V.B. 2015. Soil and rhizosphere associated fungi in gray mangroves (*Avicennia marina*) from the Red Sea – A metagenomic approach. *Genomics Proteomics Bioinformatics* **13**, 310–320.

Soares, F.L., Marcon, J., Pereira e Silva, M.D.C, Khakhum, N., Cerdeira, L.T., Ottoni, J.R., Domingos, D.F., Taketani, R.G., de Oliveira, V.M., Lima, A.O.S., *et al.* 2017. A novel multifunctional β-*N*-acetylhexosaminidase revealed through metagenomics of an oil-spilled mangrove. *Bioengineering* **4**, 62.

Standardization Administration of the People's Republic of China. 2008a. Soil quality - Analysis of total mercury, arsenic and lead contents in soils - Atomic fluorescence spectrometry - Part 1: analysis of total mercury contents in soils. GB/T 22105.1-2008. (in Chinese).

Standardization Administration of the People's Republic of China. 2008b. Soil quality - Analysis of total mercury, arsenic and lead contents in soils - Atomic fluorescence spectrometry - Part 2: Analysis of total arsenic contents in soils. GB/T 22105.2-2008. (in Chinese).

Thatoi, H., Behera, B.C., Mishra, R.R., and Dutta, S.K. 2013. Biodiversity and biotechnological potential of microorganisms from mangrove ecosystems: a review. *Ann. Microbiol*. **63**, 1–19.

Thompson, C., Beys-da-Silva, W., Santi, L., Berger, M., Vainstein, M., Guima rães, J., and Vasconcelos, A.T. 2013. A potential source for cellulolytic enzyme discovery and environmental aspects revealed through metagenomics of Brazilian mangroves. *AMB Express* **3**, 65.

Thompson, F.L., Iida, T., and Swings, J. 2004. Biodiversity of vibrios. *Microbiol. Mol. Biol. Rev*. **68**, 403–431.

Walters, B.B., Rönnbäck, P., Kovacs, J.M., Crona, B., Hussain, S.A., Badola, R., Primavera, J.H., Barbier, E., and Dahdouh-Guebas, F. 2008. Ethnobiology, socio-economics and management of mangrove forests: A review. *Aquat. Bot*. **89**, 220–236.

Wong, M.T., Wang, W., Lacourt, M., Couturier, M., Edwards, E.A., and Master, E.R. 2016. Substrate-driven convergence of the microbial community in lignocellulose-amended enrichments of gut microflora from the Canadian beaver (*Castor canadensis*) and north American moose (*Alces americanus*). *Front. Microbiol*. **7**, 961.

Yang, Q., Sun, F., Yang, Z., and Li, H. 2014. Comprehensive transcriptome study to develop molecular resources of the copepod *Calanus sinicus* for their potential ecological applications. *Biomed Res. Int.* **2014**, 1–12.