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ENVIRONMENTAL BIOTECHNOLOGY

Spatial and seasonal variation of methanogenic community in a river-bay system in South China

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Abstract

River-bay system is a transitional zone connecting land and ocean and an important natural source for methane emission. Methanogens play important roles in the global greenhouse gas budget and carbon cycle since they produce methane. The abundance and community assemblage of methanogens in such a dynamic system are not well understood. Here, we used quantitative PCR and high-throughput sequencing of the *mcrA* gene to investigate the abundance and community composition of methanogens in the Shenzhen River-Bay system, a typical subtropical river-bay system in Southern of China, during the wet and dry seasons. Results showed that *mcrA* gene abundance was significantly higher in the sediments of river than those of estuary, and was higher in wet season than dry season. Sequences of *mcrA* gene were mostly assigned to three orders, including *Methanosarcinales*, *Methanomicrobiales*, and *Methanobacteriales*. Specifically, *Methanosarcina*, *Methanosaeta*, and *Methanobacterium* were the most abundant and ubiquitous genera. Methanogenic communities generally clustered according to habitat (river vs. estuary), and salinity was the major factor driving the methanogenic community assemblage. Furthermore, the indicator groups for two habitats were identified. For example, *Methanococcoides*, *Methanoculleus*, and *Methanogenium* preferentially existed in estuarine sediments, whereas *Methanomethylovorans*, *Methanolinea*, *Methanoregula*, and *Methanomassiliicoccales* were more abundant in riverine sediments, indicating distinct ecological niches. Overall, these findings reveal the distribution patterns of methanogens and expand our understanding of methanogenic community assemblage in the river-bay system.

Key Points

- *Abundance of methanogens was relatively higher in riverine sediments.*
- *Methanogenic community in estuarine habitat separated from that in riverine habitat.*
- *Salinity played a vital role in regulating methanogenic community assemblage.*

Keywords Methanogenic community · Abundance · *mcrA* · River-bay system · Seasonal variation · Salinity

Introduction

Methane, as a greenhouse gas, contributes 20% to global warming. Its emission from natural sources such as wetlands, hydrothermal vents, and oceans account for more than 40% of the global total methane emission, among which wetland is the largest natural source (Bridgham et al. 2013; Dalal and Allen 2008; Lyu et al. 2018). Coastal wetlands are important source of methane emission and the methane fluxes from coastal wetlands range from 100 to 231×10^2 g a^{-1} (Tong et al. 2010; Welti et al. 2016). The area of coastal wetlands in China are estimated at more than 1.2×10^4 km^2, making up a considerable proportion of the natural wetlands (Huang et al. 2006). Methane emission rates are higher from polluted wetlands than those from unpolluted ones (Purvaja and Ramesh 2000; Zheng et al. 2018).

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Methane is produced by methanogens through the process of methanogenesis under anaerobic conditions (Liu and Whitman 2008). Traditionally, methanogens from the phylum *Euryarchaeota* can be classified into seven orders (*Methanococcales*, *Methanopyrales*, *Methanobacteriales*, *Methanomicrobiales*, *Methanocellales*, *Methanosarcinales*, and *Methanomassiliicoccales*). Recent genome binning has revealed that the new class of *Methanofastidiosa*, the new phyla of *Verstraetearchaeota*, *Korarchaeota*, and *Nezhaarchaeota* also have potentials for methanogenesis (McKay et al. 2019; Nobu et al. 2016; Vanwonterghem et al. 2016; Wang et al. 2019b). Methanogens harbor three major pathways of methanogenesis, i.e., hydrogenotrophic, acetoclastic, and methylotrophic pathways (Conrad 2009). All the pathways have a common enzyme, methyl-coenzyme M reductase (MCR), for the final step of methane synthesis (Liu and Whitman 2008). The *mcrA* gene, which encodes alpha subunit of MCR, is a commonly used gene marker for surveying diversity of methanogens (Yang et al. 2014).

Previous studies based on *mcrA* gene have observed a vast diversity of methanogens in various environments including animal digestive tract, soil, sediments, and anaerobic digesters (Lu et al. 2015; Mihajlovski et al. 2008; Wilkins et al. 2015). The distribution and environmental preferences of methanogenic groups are also diverse. For example, *Methanofastidiosa* genomes are obtained from anaerobic sludge digester (Nobu et al. 2016); *Methanocellales* has been isolated from paddy field soils (Lyu and Lu 2012; Sakai et al. 2008); *Methanococcales* is preferentially identified in marine habitats (Liu and Whitman 2008); *Methanopyrales* is the only methanogens so far that could produce methane at temperature higher than 100 °C (Liu 2010); *Methanomassiliicoccales* has been isolated from human feces (Borrel et al. 2012). These studies indicate that methanogenic community composition could be influenced by environmental factors, such as salinity and temperature.

The river-bay system is located in coastal wetlands connecting land and ocean. Sediments in such environments are under tidal influences and are characterized by an absence of oxygen some or all of the time. In addition, coastal areas are normally densely populated and remarkably disturbed by anthropogenic activities. Due to lack of oxygen and relatively high carbon inputs, coastal sediments are optimal environments for methanogens (Taketani et al. 2010). Methanogenic communities are susceptible to environmental changes across the river-bay continuum where continental freshwater meets oceanic water. Understanding the spatial and seasonal variability of methanogens in the river-bay system is important for recognizing major players involved in methane production. However, few studies have reported the transition of methanogenic communities in the river-bay system.

The Shenzhen River-Bay system is located in subtropical coastal areas in south China. It is an economically developed and densely populated area in China (Wang et al. 2019a). It extends the freshwater-saltwater interface from the Dasha River to Shenzhen Bay and is subjected to tidal influence (Fig. S1). The underlying mechanism of methanogenic community assemblage in such a dynamic and fluctuated system is still not well understood. Since riverine and estuarine sediments in the river-bay system are two types of habitats, some groups of methanogens might be found in distinct niches. In the current study, we analyzed samples collected during two seasons (wet and dry seasons) from the Shenzhen River-Bay system to explore the spatial and seasonal variation of methanogenic abundance and community composition. Our objectives are (i) to explore the transition of methanogen community structure in the Shenzhen River-Bay system, and (ii) to reveal the driving factors for the methanogenic community assemblage in the Shenzhen River-Bay system.

Materials and methods

Study sites, sediment sampling, and environmental parameter analysis

The Shenzhen River-Bay system includes Dasha River and Shenzhen Bay. The Dasha River originates in the north of Shenzhen and flows south towards Shenzhen Bay. Shenzhen Bay is a semi-enclosed bay located in the east coast of the Pearl River Estuary. We selected 6 sampling sites from Dasha River (R1, R3–R7) and 10 sampling sites from Shenzhen Bay (E1–E5, E7–E11) along salinity gradients (Fig. S1). The sampling sites were not named consecutively because the sediments of two sites (R2 and E6) were not enough for the following physical and molecular analysis and these two sites were removed. At each sampling site, sediments were collected using a stainless steel sampler in July 2016 and February 2017, representing the wet (W) and dry (D) seasons, respectively. Altogether, 32 sediment samples were collected. All samples were transferred to the laboratory on ice and stored at − 40 °C before analyses.

Water temperature, salinity, and pH were measured in situ by using a multiparameter water quality sonde (EXO2, USA). Sediments were air-dried in the laboratory for a few days until the weight kept unchanged. Sediment ammonium, nitrite, and nitrate were extracted from air-dried sediments with 1 M KCl and determined by a Continuous Segmented Flow Analyzer (SEAL AutoAnalyzer 3 HR, USA). Total organic carbon (TOC) and total nitrogen (TN) contents were measured using TOC Analyzer (Shimadzu, Japan), as previously described (Zhang et al. 2019).

DNA extraction and qPCR

Total DNA was extracted from 0.3 g of sediments using the DNeasy PowerSoil Kit (Qiagen, Germany) according to the manufacturer's instructions. The quantity and quality of the

extracted DNA were examined using NanoDrop® ND-2000c UV-Vis spectrophotometer (NanoDrop Technologies, USA). The DNA samples were stored at − 20 °C and used for later molecular analysis. Abundance of the *mcrA* genes was determined by quantitative PCR (qPCR) on an iCycler iQ 5 thermocycler (Bio-Rad, USA) using the primer pair mlas-mod-F (5′-GGY GGT GTM GGD TTC ACM CAR TA-3′) and mcrA-rev-R (5′-CGT TCA TBG CGT AGT TVG GRT AGT-3′) (Steinberg and Regan 2008). Each reaction with 25 μl contained 12.5 μl of 2 × SYBR® Premix Ex Taq™ (Takara Biotechnology, Japan), 0.5 μl of each primer (10 μM), and 2 μl of diluted DNA template (1–10 ng). The reaction protocol was as follows: 95 °C for 3 min, 30 cycles of 30 s at 95 °C, 45 s at 55 °C, and 30 s at 72 °C. Standard curves were generated using 10-fold serial dilutions of a plasmid containing the *mcrA* gene fragments. The PCR efficiency for different assays ranged between 90 and 100%, with R^2 value of 0.99.

DNA sequencing and data processing

For amplicon sequencing, *mcrA* gene fragments were amplified using the primer pair mlas-mod-F and mcrA-rev-R (Steinberg and Regan 2008). The purified amplification products were sequenced on the Illumina MiSeq platform at Novogene Bioinformatics Technology Co., Ltd. (Tianjin, China). Across the 32 samples, the high-throughput sequencing yielded 2,965,757 *mcrA* gene sequences in total, and the minimum sequence number for individual sample was 10,983.

The paired-end raw reads were merged using the join_paired_ends.py script of Quantitative Insights into Microbial Ecology (QIIME1) (Caporaso et al. 2010). The sequence data set was then analyzed using a modified QIIME2 pipeline (Bolyen et al. 2019). Briefly, chimeras were removed and sequences were trimmed by sequence length (478 bp) using DADA2. After denoising, feature table and feature sequences were generated. Each feature in the feature table will be represented by exactly one sequence. Feature sequences were BLAST against the NCBI-nr database to assign taxonomy. All the samples were randomly rarefied to the minimum number (10,983) of sequences per sample to ensure equal sampling depth.

Statistical analysis

The *mcrA* gene copies were log-transformed prior to statistical analysis to satisfy the normality assumptions. Shannon index, which was commonly used to represent the alpha diversity, was calculated by diversity function in "vegan" R package. Species abundance distribution (SAD) patterns of methanogenic genera were estimated according to their relative abundance and occurrence. According to the sampling habitats (R = river: Dasha River; E = estuary: Shenzhen Bay) and sampling seasons (W = wet season; D = dry season), the samples were separated into four groups: WR, DR, WE, and DE. The significant differences of the physicochemical properties, abundance, Shannon index, and relative abundances of major orders/families across groups were evaluated by single-factor analysis of variance (ANOVA) followed with Duncan's test ($P < 0.05$) by "agricolae" R package. Principal coordinate analysis (PCoA) and permutational multivariate analysis of variance (PERMANOVA) were performed to investigate shifts of the methanogenic community based on the genus level across groups using "vegan" R package. The indicator lineages, which were preferentially found in samples grouped by habitat (river vs. estuary), were calculated by IndVal index in "labdsv" R package (De Cáceres and Legendre 2009). The indicator lineages analysis combined relative abundance and frequency of occurrence of methanogenic genera.

Variation partitioning approach (VPA) was used to evaluate the relative importance of habitats, seasons, and environmental factors on methanogenic community using "vegan" R package. Furthermore, redundancy analysis (RDA) was used to explore relationships between methanogenic community and environmental factors using "vegan" R package. Here, environmental factors included water temperature, pH, salinity, and nutrients (ammonia, nitrite, nitrate, TOC, and TN) contents. Multivariate regression tree (MRT) was performed to predict relationships between methanogenic genera and environmental characteristics using "mvpart" R package (De'Ath 2002). The size of tree to be generated was set as 5. Pie chart under every leaf showed the community composition and how the relative abundances of the methanogenic genera contributed to the separation. Relationships between the relative abundances of methanogenic genera and environmental factors were calculated using Spearman's rank order correlations. All statistical analyses were performed using R software version 3.5.1 (Team 2018).

Results

Environmental parameters

Environmental properties in the Shenzhen River-Bay system varied according to habitat (river vs. estuary) and season (wet vs. dry) (Fig. S2). In general, the concentrations of nutrients (ammonia, nitrate, TOC, and TN) in riverine sediments were significantly higher than those in estuarine sediments ($P < 0.001$). Nitrite was higher in the wet season than those in the dry season ($P < 0.05$). Notably, pH and salinity were comparatively higher in estuarine sediments than those in riverine sediments ($P < 0.001$). In addition, salinity in the dry season was significantly higher than those in the wet season

($P < 0.001$). Water temperature in the wet season was significantly higher than in the dry season.

Abundance and alpha diversity of methanogens

Quantitative PCR analysis showed that the *mcrA* gene copies varied significantly according to habitat and season (Fig. 1a). It was significantly higher in the sediments of river than those of estuary ($P < 0.05$), and significantly higher in the wet season than in the dry season ($P < 0.05$). Alpha diversity, as measured by Shannon index, showed that there were no significant differences according to habitats and seasons (Fig. 1b).

Community composition of methanogens

The community composition of methanogens at each sampling site in the Shenzhen River-Bay system is presented in Fig. 2. The *mcrA* gene sequences were mostly assigned into one class and five orders from the phyla *Euryarchaeota*. *Methanosarcinales*, *Methanomicrobiales*, and *Methanobacteriales* were the top three abundant orders, contributing 44.4%, 30.6%, and 20.2% of the average relative abundance of methanogenic community, respectively. *Methanomassiliicoccales*, *Methanocellales*, and *Methanofastidiosa* were detected but at relatively low abundance, the average relative abundance of which were 2.4%, 1.9%, and 0.4%, respectively. Furthermore, the relative abundances of different genera were plotted against their occurrence to show the SAD patterns (Fig. S3). *Methanosarcina*, *Methanosaeta*, and *Methanobacterium* were the most abundant and ubiquitous genera in the Shenzhen River-Bay system.

There were significant differences of the relative abundance of methanogenic taxon according to habitat (river vs. estuary). The relative abundance of *Methanomicrobiaceae* was significantly higher in estuarine sediments than in the riverine zones, while those of *Methanoregulaceae* and *Methanomassiliicoccales* showed the opposite trends (Fig. S4). Specifically, *Methanomicrobiaceae* contributed 5.0% of the average relative abundance of the riverine community and 18.3% of the estuarine community. Conversely, *Methanoregulaceae* comprised 20.1% of the average relative abundance of the riverine community and 6.8% of the estuarine community. *Methanomassiliicoccales* contributed 3.5% of the average relative abundance of the riverine community and 1.7% of the estuarine community.

Beta diversity of methanogenic community and indicator groups for habitats

PCoA revealed that habitat (river and estuary) was a strong structuring factor (Fig. 3). Methanogenic community in estuarine habitat (WE and DE) clearly separated from that in riverine habitat (WR and DR), regardless of the season. PERMANOVA based on Bray-Curtis dissimilarity corroborated that habitat showed a significant influence on methanogenic community composition ($P = 0.001$, $R^2 = 0.137$).

To further identify the methanogenic lineages responsible for the observed community variations according to habitat (river vs. estuary), indicator taxa analysis was conducted. Overall, 7 methanogen genera showed a significant IndVal ($P < 0.01$) for habitat (Fig. 4). These genera were affiliated to *Methanomicrobiales*, *Methanosarcinales*, and *Methanomassiliicoccales*. Among them, *Methanolinea*, *Methanoregula* affiliated to *Methanomicrobiales*, and *Methanomethylovorans* affiliated to *Methanosarcinales* were more abundant in riverine sediments. By contrast, *Methanoculleus* and *Methanogenium* affiliated to *Methanomicrobiales* were more abundant in estuarine sediments. *Methanococcoides* affiliated to *Methanosarcinales* only occurred in estuarine sediments and were absent from riverine sediments.

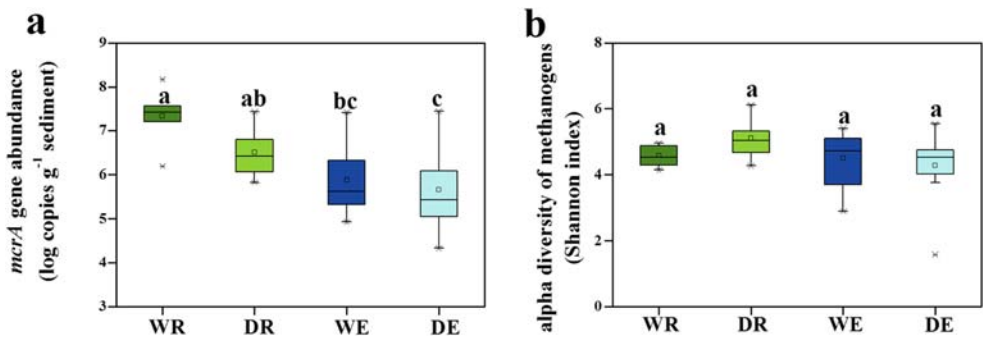

Fig. 1 Abundance and alpha diversity of methanogens. The samples were grouped by different habitats (river and estuary), and seasons (wet and dry seasons). **a** Log-transformed abundance of the *mcrA* genes. **b** Alpha diversity of methanogens, estimated with the Shannon index. Box plots were constructed to show median, interquartile range, and 1.5 × the interquartile range ($n = 6$ for riverine samples; $n = 10$ for estuarine samples). The lowercase letters indicate significant differences among groups (one-way ANOVA; Duncan test; $P < 0.05$). Sample names: W = wet season; D = dry season; R = river, Dasha River; E = estuary, Shenzhen Bay

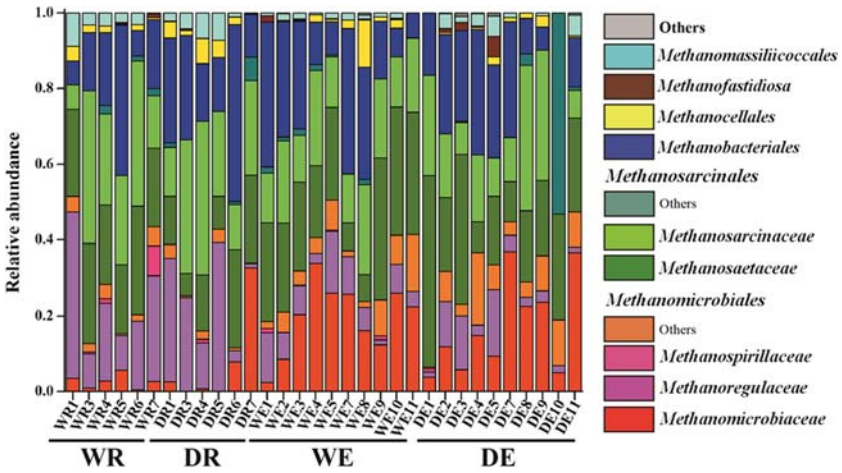

Fig. 2 Relative abundance of methanogenic taxa based on *mcrA* gene sequences. The top 6 methanogenic taxa (class or order) are represented. "Others" refers to the remained minor taxa. Sample names: W = wet season; D = dry season; R = river, Dasha River; E = estuary, Shenzhen Bay

Driving factors of methanogenic community assemblage

VPA revealed that habitat, season, and environmental factors contributed to the variations of methanogenic communities together. Environmental factors explained the largest proportion of variation (29.6%), followed by habitat (11.1%), and season (2.0%). RDA revealed that water temperature, pH, salinity, TN, TOC, ammonia, nitrite, and nitrate were significantly correlated with the variation of methanogenic communities. Among all the measured environmental factors, salinity and ammonia were more strongly associated with methanogenic community variation than other environmental factors (Fig. S5). MRT analysis was carried out to link the methanogenic community composition and environmental effects (Fig. 5). The analysis showed that salinity could divide the MRT into three major branches, one contained samples with freshwater condition (salinity < 1.11‰), one contained samples with saline condition (1.11‰ < salinity < 27.07‰), and the other one contained samples with hypersaline condition (salinity > 27.07‰). The most discriminated groups accounting for the separation were *Methanoregula*, *Methanoculleus*, and *Methanogenium*. *Methanoregula* was more abundant in freshwater environment, while *Methanoculleus* and *Methanogenium* were more abundant in saline environment. The branch with salinity less than 1.11‰ was then spilt by water temperature. The branch with salinity between 1.11‰ and 27.07‰ could be further divided by pH value.

Next, correlation analyses were performed to examine the relationships between the environmental factors and relative abundances of methanogenic genera (Fig. 6). Spearman's rank analysis found that pH and salinity were significantly positively correlated with relative abundances of *Methanogenium*, *Methanoculleus*, and *Methanococcoides* ($P < 0.05$). Moreover, significant positive correlations were found between sediment nutrients concentration (TOC, TN, ammonia, and nitrate) and relative abundances of *Methanoregula*, *Methanomethylovorans*, and *Candidatus Methanomethylophilus* ($P < 0.05$).

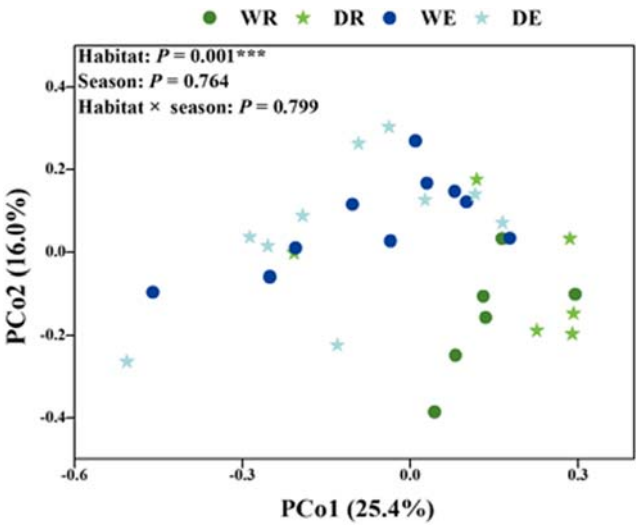

Fig. 3 Principal Coordinates Analysis (PCoA) using Bray-Curtis indices of methanogenic communities at genus level. Sample names: W = wet season; D = dry season; R = river, Dasha River; E = estuary, Shenzhen Bay

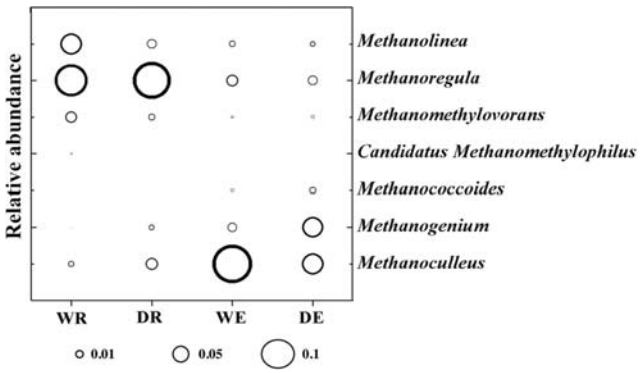

Fig. 4 Bubble plot showing the average relative abundance of methanogenic genera in the samples grouped following Fig. 1. The genera represent significant indicator lineages according to the IndVal index ($P < 0.05$). Sample names: W = wet season; D = dry season; R = river, Dasha River; E = estuary, Shenzhen Bay

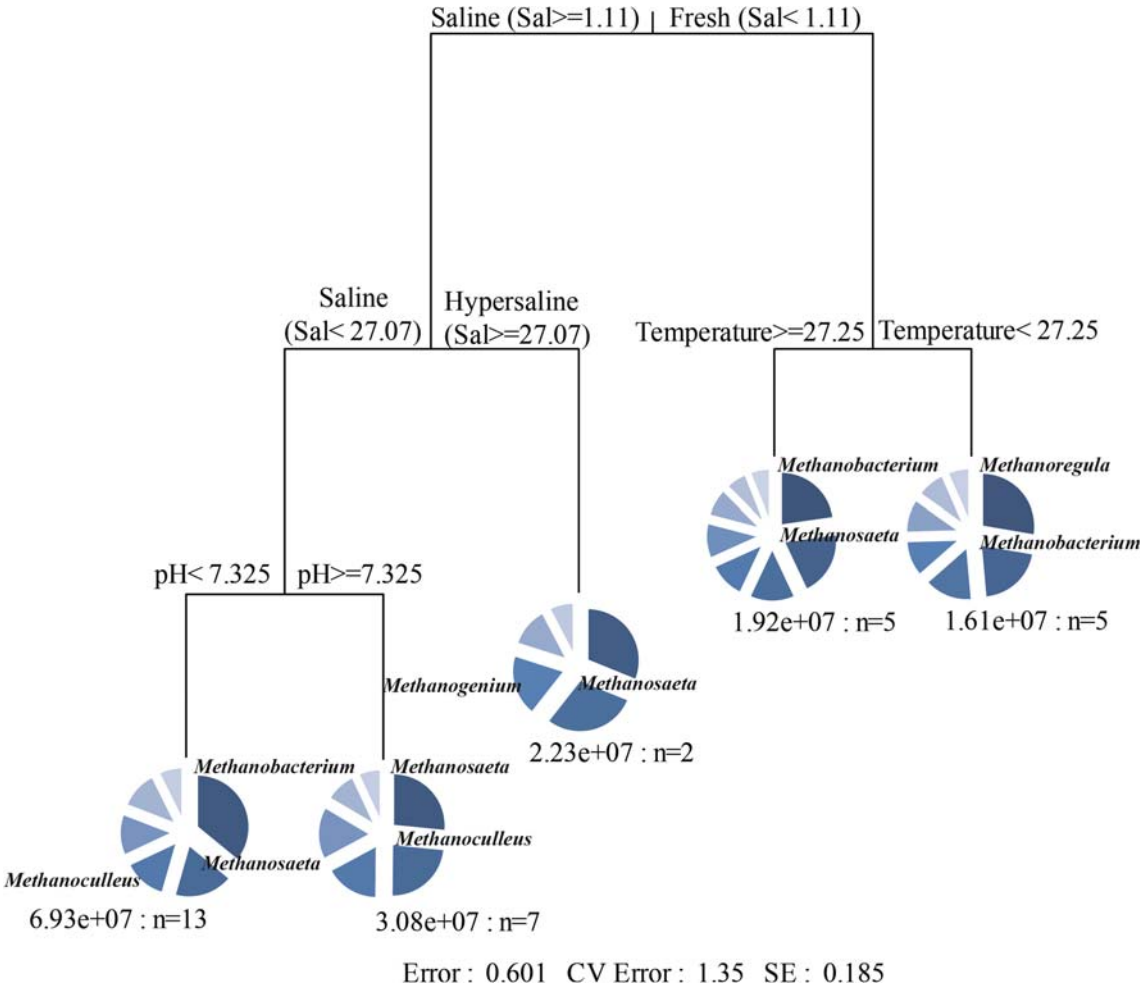

Fig. 5 Multivariate regression tree (MRT) showing the relationships between the methanogenic community and environmental factors. Five-split tree is visualized. Statistics information is listed under tree, including the residual error, cross-validated error, and standard error. Pie charts under each leaf represent the methanogenic community composition at genus level. The corresponding residual error and sample numbers under each leaf are also listed

Discussion

In the current study, we systematically investigated the transition of methanogenic abundance and community composition in the Shenzhen River-Bay system. The presented findings provided novel evidence that salinity was a major factor driving the methanogen community assemblage in the river-bay system. Salinity and nutrients' contents regulated the relative abundance of a few particular methanogenic groups. For example, *Methanoregula* were positively influenced by nutrients' contents, whereas *Methanogenium* and *Methanoculleus* were positively influenced by salinity. These results implied that particular methanogenic groups had environmental preference.

Quantitative PCR analysis revealed that *mcrA* gene copies ranged from 10^4 to 10^8 per g sediment and differed according to habitats and seasons. The abundance of methanogens was significantly higher in the riverine sediments than that in the estuarine sediments. One of the possible reasons is that methanogenic abundance is associated with nutrients availability (Zhou et al. 2014). Human activities, such as industrial and domestic wastewater, discharge relatively high contents of nutrients in riverine sediments, which might provide substrates for methanogenesis (Liu et al. 2018). Higher concentration of nutrients in the riverine sediments we measured in the current study supported the above opinion. We also found that the methanogenic abundance was higher in the wet season than in the dry season. It might be because of a higher temperature in the wet season than in the dry season, which promoted the growth of methanogens (Chafee et al. 2017; Lu et al. 2015).

We identified diverse methanogens, including one class and five orders from the phylum *Euryarchaeota*, in the Shenzhen River-Bay system. *Methanosarcinales*, *Methanomicrobiales*, and *Methanobacteriales* were the three dominant methanogen orders identified in the current study. This was consistent with previous reports that *Methanomicrobiales* and *Methanosarcinales* were abundant in coastal sediments (Chen and Yin 2013). *Methanomassiliicoccales*, *Methanocellales*, and

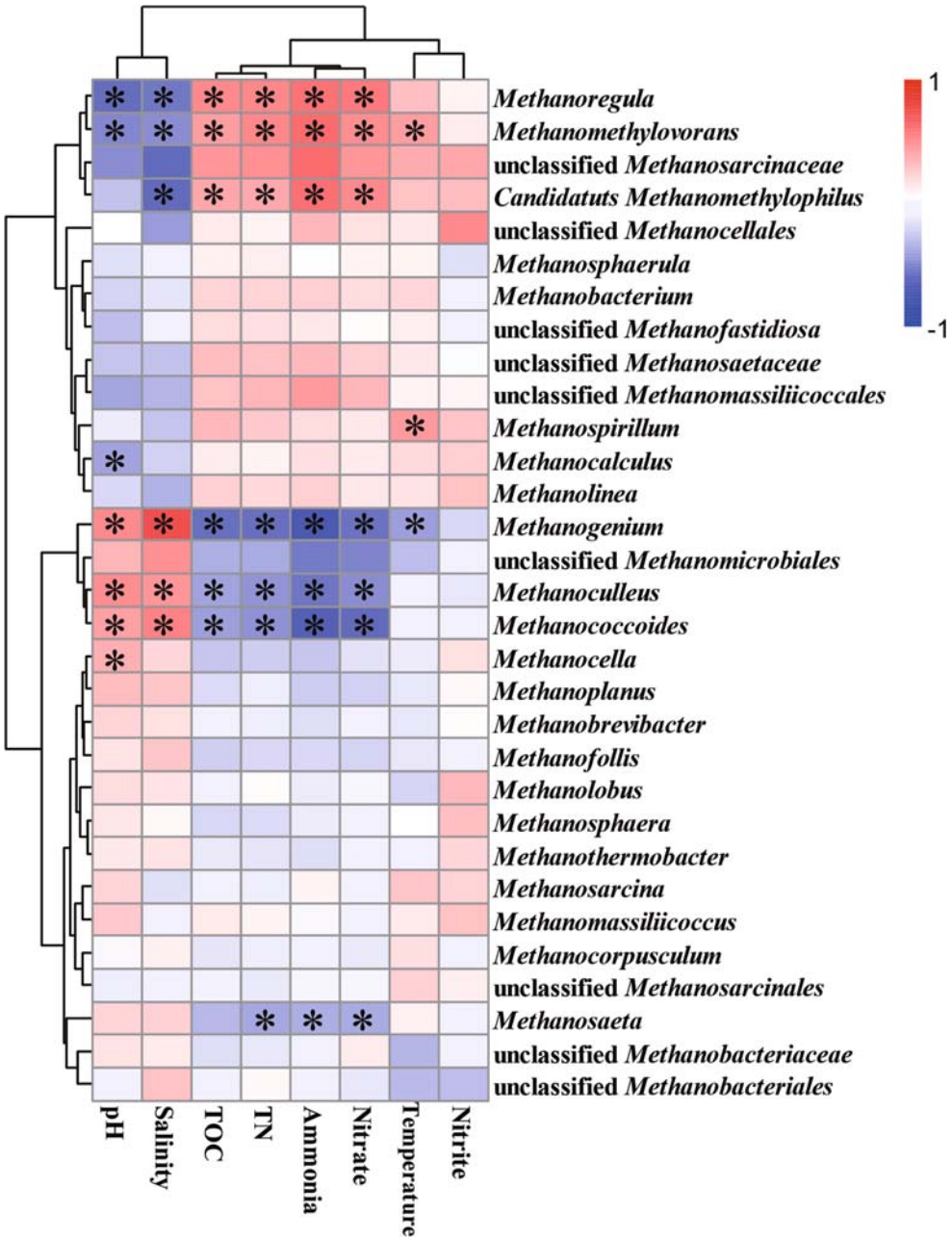

Fig. 6 Heat map representing Spearman's rank order correlations between the methanogenic genera and environmental properties. Significant correlations are labeled with asterisk ($P < 0.05$)

Methanofastidiosa were also detected, but their relative abundance were low, indicating that these methanogens contribute less to methane production in the river-bay system. *Methanomassiliicoccales* has been isolated from human feces and considered to be the seventh order of methanogens according to phylogenetic analysis (Borrel et al. 2013; Dridi et al. 2012). It has been reported that *Methanomassiliicoccales* contain two main clades: environmental clade and gastro-intestinal tract (GIT) clade (Söllinger and Urich 2019). *Methanomassiliicoccales* in river-bay system has to face higher variations in environmental parameters such as salinity, oxygen exposure, and nutrient availability than *Methanomassiliicoccales* in the GIT clade, which might explain the low abundance in the river-bay system. *Methanocellales* has been isolated from paddy field soils (Lyu and Lu 2012; Sakai et al. 2008). *Methanocellales* are key methanogen groups in rice soils, and they are at a small fraction in other environments including riverine sediments (Angel et al. 2012; Li et al. 2012). *Methanofastidiosa* is a newly described class of methanogens. There is no pure culture of this class for now. The first draft genomes retained from the anaerobic digester were reported in 2016 (Nobu et al. 2016). *Methanofastidiosa* is suspected to produce methane using methylated thiol as substrates strictly. In addition, genome annotations revealed that *Methanofastidiosa* lack abilities to fix carbon/nitrogen and synthesize many amino acids (Nobu et al. 2016). Since they require exogenous organic carbon as a carbon source

for growth, there is no competitive advantage over other methanogens in the river-bay system.

In the current study, methanogenic communities were clustered according to habitat (river vs. estuary). Salinity was the major environmental factor regulating the methanogenic community assemblage, as indicated by RDA and MRT analysis. It is in agreement with previous studies disclosing that salinity is a primary factor shaping microbial communities (Wang et al. 2019a; Wen et al. 2017). Effects of salinity on growth and metabolisms of methanogens depend on the level of salinity and the pathways of methanogenesis. Low salinity (0.1–0.2‰) is essential to methanogens, probably because of its role in the oxidation of NADH and in the formation of ATP (Lu et al. 2019). In contrast, high salinity (larger than 8‰) inhibits hydrogenotrophic and acetotrophic methanogens but not methylotrophic methanogens. It is because high levels of salinity favor growth of sulfate reducing bacteria (SRB). Due to higher affinity for H_2 and acetate, SRB have a thermodynamic advantage over hydrogenotrophic and acetotrophic methanogens. Methylotrophic methanogens could coexist with SRB because they use methyl compounds as substrates (Xiao et al. 2017).

Furthermore, some indicator genera of methanogens were observed to show preferences for freshwater or saline environments, explaining the observed community structure variation. The relative abundances of *Methanomethylovorans*, *Methanolinea*, and *Methanoregula* were significantly higher in freshwater riverine sediments (Fig. 4). *Methanomethylovorans*, a methanogen belonging to *Methanosarcinales*, have been isolated from freshwater sediment (Lomans et al. 1999). *Methanolinea* and *Methanoregula*, affiliated to the order *Methanomicrobiales*, are detected from diverse habitats including digester sludge, wastewater treatment systems, rice field soils, and riverine sediments (Chen and Yin 2013; Kuroda et al. 2015; Sakai et al. 2012; Yashiro et al. 2011). The relative abundance of *Methanoregula* was positively and significantly correlated to nutrient contents, which might explain its preference for riverine sediments. The current results also showed that the relative abundance of *Methanomassiliicoccales* was significantly higher in riverine sediments during wet season. *Methanomassiliicoccales* could utilize methyl-compounds such as methanol, methylamine, and methyl thiol for methane production (Borrel et al. 2014). The upstream riverine zone (sites R3) had a larger input of nutrients due to discharges of wastewater from the nearby Xili wastewater treatment plants, thus *Methanomassiliicoccales* had relatively higher abundance in the riverine sediments.

In contrast, the relative abundances of *Methanococcoides*, *Methanoculleus*, and *Methanogenium* were significantly higher in saline estuarine sediments (Fig. 4). *Methanococcoides*, a genus in *Methanosarcinales*, was absent from riverine sediments, suggesting that *Methanococcoides* is indicator group of saline environments. It has been reported that *Methanococcoides* is a predominant lineage in marine sediments and barely exist in riverine sediments (Wen et al. 2017; Xiao et al. 2017). It might be explained by that *Methanococcoides* are methylotrophic methanogens and could compete with sulfate reducer in saline environments (L'Haridon et al. 2014). *Methanoculleus* and *Methanogenium*, belonging to the order *Methanomicrobiales*, were significantly more abundant in estuary than in riverine sediments. It is in agreement with the previous analyses that *Methanoculleus* and *Methanogenium* are commonly detected in marine and estuarine environments, but rarely found in river habitats (Romesser et al. 1979; Wen et al. 2017; Weng et al. 2015). They are hydrogenotrophic methanogens and well adapt to low H_2 concentration. Therefore, the above genera might have an advantage over other methanogens in saline environments.

It is observed that some methanogenic genera such as *Methanosarcina*, *Methanosaeta*, and *Methanobacterium* were abundant and ubiquitous in all the samples, indicating they had high adaptation to various salinity conditions including freshwater to saline environments. *Methanosarcina* could utilize multiple substrates including $H_2 + CO_2$, acetate, and methyl compounds for methanogenesis, which gave them an advantage to exist in different environments (Thauer et al. 2008). *Methanosaeta* was acetoclastic methanogens and was frequently detected in wetlands (Narrowe et al. 2017; Welte and Deppenmeier 2014). It has been reported that *Methanobacterium* were detected in diverse environments, such as freshwater sediments and anaerobic digester (Kern et al. 2015; Schirmack et al. 2014) *Methanobacterium* was proposed to be involved in syntrophic methane production with syntrophs such as *Geobacter*, *Desulfovibrio*, *Pelobacter*, *Pelotomaculum*, and *Syntrophomonas* (Losey et al. 2017; Yin et al. 2019), which might favor the widespread of *Methanobacterium*. In addition, we used traditional *mcrA* gene primer pair that targeted *Euryarchaeotal* methanogens in this study. It has low specificity to ANaerobic MEthanotrophic (ANME) archaea and the novel methanogens in other phyla such as *Verstraetearchaeota*, *Korarchaeota*, and *Nezhaarchaeota*, potentially not reaching the highest diversity of methanotrophs and methanogens (McKay et al. 2017). Therefore, the response obtained could slightly vary if the groups not considered here were taken into account. Further studies should be conducted to design new primers and investigate the novel methanogens in future.

In conclusion, we found that abundance of methanogens was significantly higher in the riverine than estuarine sediments. Community of methanogens was separated by habitat (river vs. estuary). According to RDA and MRT analysis, salinity was the major factor regulating methanogenic community assemblage. Interestingly, the indicator groups for riverine and estuarine environments were identified, suggesting some methanogenic groups were environment-specific. *Methanomethylovorans*, *Methanolinea*, *Methanoregula*, and *Methanomassiliicoccales* preferentially dwelled in riverine sediments, while *Methanococcoides*, *Methanoculleus*, and

Methanogenium preferred existing in estuarine sediments. Collectively, the current study contributes to the understanding of the spatial and seasonal patterns of methanogens and addresses the underlying mechanisms explaining methanogenic community assemblage in the Shenzhen River-Bay system.

Author contributions ML conceived the study. YC and YW sampled sediments in the field and conducted experiments. CZ, YC, and JP analyzed data. CZ wrote the manuscript with help from all coauthors. All authors have given approval to the final version of the manuscript.

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Data availability All the raw reads in the current study have been deposited in the sequencing read archive (SRA) of NCBI under BioProject accession number "PRJNA601619".

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement This article does not contain any studies with human participants performed by any of the authors.

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