Wide distribution of anaerobic ammonium-oxidizing bacteria in the water column of the South China Sea: Implications for their survival strategies

Xiaohan Liu1,2 | Jiapeng Wu1,2 | Yiguo Hong1,2 | Yiben Li1,2 | Yaohao Hu3,4 | Limei Wang1,2 | Yu Wang1,2 | Aimin Long3,4

1Institute of Environmental Research at Greater Bay Area, Key Laboratory for Water Quality and Conservation of the Pearl River Delta, Ministry of Education, Guangzhou University, Guangzhou, China
2School of Environmental Science and Engineering, Guangzhou University, Guangzhou, China
3State Key Laboratory of Tropical Oceanography (LTO), South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, China
4University of Chinese Academy of Sciences, Beijing, China

Correspondence
Yiguo Hong, Institute of Environmental Research at Greater Bay Area, Key Laboratory for Water Quality and Conservation of the Pearl River Delta, Ministry of Education, Guangzhou University, Guangzhou 510006, China. Email: yghong@gzhu.edu.cn

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Abstract

Aim: Anammox bacteria are widely distributed in various environments, but the community composition and survival strategies of anammox bacteria in oligotrophic, deep-sea water rich in dissolved oxygen (DO) remain unknown. We aimed to understand the distribution of anammox bacteria in the water columns of the South China Sea (SCS).

Time period: Three cruises were in October 2014, October 2015 and September 2016, respectively.

Location: Six water columns were obtained in the SCS, China (E 111–120°, N 16–22°).

Methods: The diversity and community composition of anammox bacteria were investigated through a high-throughput sequencing technique targeting the anammox 16S rRNA gene.

Results: Unexpectedly, phylogenetic analysis showed that Ca. Brocadia (78.35%) was the dominant genus while Ca. Scalindua and unclassified species accounted for 12.62% and 9.03%, respectively. The highest diversity of anammox bacteria and concentrations of particulate organic matter (POM) were simultaneously observed in the 50–200 m layer, suggesting that particles may be a favourable niche for anammox bacteria. The wide distribution of anammox bacteria in the DO-rich sea waters suggests two possible survival strategies: one strategy is attaching to particles and using anaerobic anammox metabolism to produce energy, and the other strategy is free-living and using aerobic metabolism to produce energy.

Main conclusions: A high diversity of anammox bacteria was found in the aerobic water columns, and Ca. Brocadia was the clearly dominant genus. Furthermore, anammox microbes could improve their viability by switching their metabolic pathways between anaerobic and aerobic conditions. Overall, our study has improved the understanding of the distribution and survival strategies of anammox communities in an aerobic marine environment and could provide new ideas for engineering applications in wastewater treatment with anammox technology.
1 | INTRODUCTION

Anaerobic ammonium oxidation (Anammox) is a chemo lithotrophic process that oxidizes NH$_4^+$ under anoxic conditions, with NO$_2^−$ or NO$_3^−$ reduction, to produce N$_2$, thus contributing to the removal of nitrogen from different natural ecosystems (Hamasaki et al., 2017; Kuypers et al., 2003; Mulder, van de Graaf, Robertson, & Kuenen, 1995; Yang et al., 2015). At present, five anammox genera have been identified: Ca. Scalindua (Schmid et al., 2003), Ca. Brocadia (Strous et al., 1999), Ca. Kuuenenia (Schmid et al., 2000), Ca. Jettenia (Quan et al., 2008) and Ca. Anammoxoglobus (Kartal et al., 2007); these genera are affiliated with the phylum Planctomycetes. The existence of anammox processes has been proven in various ecosystems, including paddy soils (Yang et al., 2015), acidic red soils (Wu et al., 2018), marine sediments and water columns (Qian et al., 2018; Han & Gu, 2013; Hong, Yin, & Zheng, 2011; Kuypers et al., 2003; Song, Buckner, Hembury, Mills, & Palmer, 2014; Ward, 2003), lakes (Yang et al., 2016), land-freshwater interfaces (Zhu et al., 2015), mangroves (Wang, Li, & Gu, 2014; Xiao et al., 2018), wetlands and freshwater rivers (Sun et al., 2014).

In marine environments, 30%-70% of total N$_2$ production could be contributed by anammox (Arrigo, 2005; Dalsgaard, Thamdrup, & Canfield, 2005; Hamasaki et al., 2017; Jetten et al., 2009), suggesting that it has an equally significant role as denitrification in the removal of fixed nitrogen (N). However, the contribution of anammox to N removal varies greatly in aquatic ecosystems, ranging from important to nearly negligible (Dang et al., 2010; Rysgaard, Glud, Risaard-Petersen, & Dalsgaard, 2004; Thamdrup & Dalsgaard, 2002). Many studies have shown the exclusive presence of the Ca. Scalindua genus of anammox bacteria in marine environments, presenting a low diversity of anammox microbes (Qian et al., 2018; Hamasaki et al., 2017; Han & Gu, 2015; Hong, Yin, et al., 2011; Schmid et al., 2007). The lower diversity of anammox bacteria may be due to the inadequate sequencing depths of clone libraries, which neglect some genera with low abundance in marine environments (Wu, Hong, Chang, et al., 2019; Wu, Hong, Ye, et al., 2019).

Thamdrup, Dalsgaard, Jensen, Ulloa, and Escribano (2006) and Hamersley et al. (2007) used an isotope incubation method to confirm the dominance of the anammox process in the eastern tropical South Pacific, one of the major oxygen minimum zones (OMZs) in the world that are characterized by very low dissolved oxygen (DO) and are generally suitable for the growth of anammox bacteria. Subsequently, an important role of anammox was also discovered in the South Pacific and Arabian Sea OMZs (Jayakumar, Naqvi, & Ward, 2009; Kalvelage et al., 2013; Pitcher et al., 2011). In contrast, the South China Sea (SCS) is considered to be an oligotrophic marine ecosystem with a comparatively high DO and minimal P and N contents; the SCS extends from the equator to 23°N and from 99°E to 121°E in the western Pacific Ocean (Chen, Chen, Karl, & Takahashi, 2004; Dong, Hong, Lu, & Xie, 2014; Hong, Li, Cao, & Gu, 2011). The presence of anammox bacteria was observed in deep-sea sediments from the SCS by amplifying the biomarkers of anammox microbes by targeting the hzo, 16S rRNA and Scalindua-nirS genes (Hong, Li, et al., 2011; Li, Hong, Cao, & Gu, 2013). Our previous research on the community composition and diversity of anammox bacteria in surface sediments of the SCS via a high-throughput sequencing technique found an unexpectedly higher diversity, and 87.29% OTUs were closely related to Ca. Scalindua (Wu, Hong, Chang, et al., 2019). Pajares and Ramos (2019) proposed that anammox activity was mainly regulated by the concentration of DO; however, the community composition and survival strategies of anammox bacteria in DO-rich deep-sea water remain poorly understood. Genomic analysis indicated that anammox bacteria could actually be generalists, not specialists, with a versatile lifestyle (Strous et al., 2006). Although the anammoxosome could have a possible endosymbiotic origin, anammox bacteria may possess a set of aerobic metabolic pathways (Hong, Cao, Li, & Gu, 2014). The ATP synthase that generally exists in aerobic bacteria was also identified in anammox bacteria (Deckers-Hebestreit & Altendorf, 1996; Glockner et al., 2003). Therefore, we assume that the metabolic pathways of anammox microbes could be able to switch between anaerobic and aerobic conditions and that anammox bacteria could survive in aerobic water by utilizing versatile metabolic pathways.

Here, six representative water column samples from the SCS were used to analyse the distribution characteristics of anammox bacteria by using a high-throughput sequencing technique. We found that there was a widespread and distinct distribution characteristic in the DO-rich water column of the SCS, indicating a different survival strategy compared to those observed in an anaerobic environment.

2 | METHODS

2.1 | Sampling

Seawater samples were obtained during three cruises in October 2014, October 2015 and September 2016 on the R/V Shiyan 3 in the SCS. Six water columns (W45, W11, M12, M18, K28 and K51) were selected for analysis, and the sampling details are shown in Figure 1 and Table S1. Seawater samples (2 L) were serially filtered by 0.20-μm pore-size polypropylene filters (Millipore, USA). Then, the membrane was immediately stored in liquid nitrogen until further processing. The salinity and temperature of the water samples were continuously recorded using a conductivity-temperature-depth (CTD) device (911 Plus V2, Sea-Bird Electronics, USA). The concentration of DO was determined by the Winkler titration
method (Chakraborty et al., 2016), as described by Bryan, Riley, and Williams (1976) and Broenkow and Cline (1969).

### 2.2 DNA extraction, PCR amplification and Illumina-based 16S rRNA sequencing

DNA extraction from the filter of each sample was performed using a Power Water DNA Isolation Kit (Mobio Laboratories, Inc./Qiagen) following the manufacturer’s instruction. The concentration of extracted DNA was determined using a NanoDrop Lite (Thermo Fisher Scientific) by spectrophotometric analysis, and then, electrophoresis was conducted with 1.0% agarose. The extracted DNA samples were stored at −80°C for further analysis. For amplification, a primer pair (A438f/A684r) was used to target the anammox 16S rRNA gene (Han & Gu, 2013). More details about the parameters of PCR amplification are presented in Table S2. Before high-throughput sequencing, a MiniBEST agarose gel DNA extraction kit (TaKaRa) was used to purify the PCR products. The PCR products were then subjected to Illumina MiSeq sequencing (Genewiz).

### 2.3 High-throughput sequencing analysis

High-throughput sequencing was performed using Mothur software V1.40.5 according to the standard protocol, as described previously by Schloss et al. (2009) and Wu et al. (2018). In short, the tags and primers were removed from quality-controlled sequences. The simplified sequences were aligned to the newly developed database of anammox 16S rRNA genes with the align.seqs command (Jiao et al., 2018; Wu, Hong, Chang, et al., 2019). Then, the badly aligned sequences were filtered and removed by the seqs.command. The pre.cluster, chimera.uchime and remove.seqs commands were used to reduce errors and remove chimeras. To conduct further diversity analyses, representative sequences were gathered into operational taxonomic units (OTUs) as defined by 97% sequence identity through the dist.seqs, cluster, make.shared, and classify.otu commands (Wu et al., 2018; Zhao et al., 2013). Rarefaction curves and Principal Coordinates analyses (PCoA) were conducted by the rarefaction.single and pcocao commands, respectively. Phylogenetic analysis of the representative sequences from the top 15 OTUs (covering 86% sequences) for the anammox 16S rRNA genes was accomplished by using MEGA7.0 software and Evolview (He et al., 2016). Microsoft Excel was used to construct a heat map according to the abundance of the dominant OTUs.

### 2.4 q-PCR analysis

q-PCR was performed on a LightCycler® R480 II Real-Time PCR system (Roche, Basel, Switzerland) in triplicate in 96-well plates with the same primer set of A438f/A684r (Table S2). A plasmid DNA (known copy number) was serially diluted to generate a standard curve with high-efficiency q-PCR (>0.9) and correlation coefficients of the standard curve (>0.95).

### 2.5 Mapping the diversity and distribution of anammox bacteria in the South China Sea

The Shannon index and the ratio of the Ca. Brocadia to the Ca. Scalindua genus were used to describe the diversity and distribution characteristics of the anammox bacteria, respectively, in the SCS. A total of 40 water samples from six water columns are presented using Ocean Data View (ODV) (https://odv.awi.de/en/software/download/) in this study.

### 2.6 Statistical analysis

The Shannon and Chao estimators were generated in Mothur as representatives of the alpha diversity index. PCoA was conducted with Prism 7 software (https://www.graphpad.com/scientific-software/prism/). Pearson correlation analysis was used to analyse the
correlation of environmental factors (depth, temperature, DO and salinity) of the water columns and anammox bacterial communities in the SCS via R software (version 3.6.3).

3 | RESULTS

3.1 | Environmental parameters

The environmental characteristics of the water samples from the SCS are presented in Table S1. The depths of the water columns ranged from 0 to 1,500 m, and the temperature and salinity varied from 2.82 to 29.45°C and 33.29 to 34.64 psu, respectively. The content of DO in the water samples of the SCS varied from 3.22 to 7.22 mg/L and tended to decrease as the water depth increased ($p < .01, R^2 = -0.596$) (Figure S1). The temperature also varied greatly with the increase in water depth ($p < .01, R^2 = -0.878$), while the salinity was positively correlated with the depth of the water columns in the SCS ($p < .01, R^2 = 0.430$).

3.2 | Diversity and abundance of the anammox bacteria

We ran a total of 0.2 million of anammox raw 16S rRNA gene sequences from 40 water samples (5,000 sequences per sample). After denoising and trimming, approximately 84,920 gene sequence reads were used for further analysis (Table S3).

Based on the 97% sequences similarity threshold, 1,070 OTUs (25 ± 15 OTUs per sample) were obtained with the dominant OTUs (>1,723 sequences per OTU) accounting for 86% of all sequences. Shannon-Wiener (Figure S2) indicated that the sequencing results were sufficient to express the bioinformation about the diversity and structure of the anammox bacterial community in this study. In addition, the Good’s Coverage values of anammox 16S rRNA genes for each sample were higher than 0.99, suggesting that the OTUs of every anammox bacterial library had been reliably captured (Table S3 and Figure S3).

The Chao1 and ACE estimators ranged from 24.33 to 64.97 and 38.15 to 155.91, respectively, and the highest richness was found in the K28-1000 m. The Shannon index and Simpson diversity ranged from 0.23 to 1.71 and 0.59 to 0.94, respectively (Table S3). The profile map of the Shannon index was used to describe the diversity characteristics of the anammox bacteria in the SCS in detail (Figure 2a). The highest diversity of anammox bacteria was present in W45. Vertically, the highest diversity of anammox bacteria was found in the 50–200 m layer of all water columns rather than in the surface water (0–50 m) (Figure 2b).

However, anammox bacterial abundances in the water columns of the SCS were under the limit of detection ($1 \times 10^3$ copies L$^{-1}$).
3.3 | Phylogenetic and community composition of anammox bacteria

We found that 15 dominant OTUs in all samples were affiliated with Ca. Brocadia (78.35%), Ca. Scalindua (12.62%) and unclassified (9.03%) with 97% similarity, suggesting a high relative abundance of Ca. Brocadia in the water columns of the SCS (Figure 3a).

The heat map of the dominant OTUs and the phylogenetic analysis of representative sequences of the anammox 16S rRNA gene are presented in Figure S4 and Figure 3b (Figure S5), respectively. OTU 01, affiliated with Ca. Brocadia, was the dominant genus in most of the water samples. The proportion of Ca. Scalindua increased with the increasing water depth and decreasing temperature (Figures 3a and 4). Almost all of the samples from K51 were closely related to Ca. Brocadia, while Ca. Scalindua was not found in this column (Figure 3a). A similar trend also occurred in K28 except for K28-1400 m.

3.4 | Spatial distribution of the anammox bacteria

The spatial distribution of the anammox bacteria was demonstrated by PCoA, and the first two (P1 and P2) principal coordinates explained 41.71% of the community variation of anammox bacteria in all the samples (Figure S6). The samples from K28 and K51 were grouped together, suggesting a similar community composition of anammox bacteria at similar depths. The anammox bacterial compositions in W45 and M12 were different from the others, indicating they had distinct horizontal and vertical distribution patterns. High diversity was also
detected in W45 and M12 compared with the other water columns (Figure 2a and Table S3). In the basin of the SCS, significant stratification may be the main factor affecting the vertical distribution of the anammox bacterial community, especially in M12 (Han & Gu, 2013). In contrast, no obvious vertical distribution pattern of anammox bacterial communities was detected in K28 (Qian et al., 2018).

4 | DISCUSSION

4.1 | Diversity and community distributions of anammox bacteria

The ubiquitous distribution of anammox bacteria in anoxic marine environments exhibits low diversity, and only the Ca. Scalindua genus has been found (Hamasaki et al., 2017; Schmid et al., 2007). Unexpectedly, our results provided sufficient bioinformation about the diversity of anammox bacteria through the high-throughput sequencing of deep-sea water samples, and Ca. Brocadi and unclassified species were found in addition to Ca. Scalindua.

Compared with the typical OMZs around the world, the abundance and activity of anammox microbes in the water columns of the oligotrophic SCS were lower, but their diversity was relatively high (Jayakumar et al., 2009; Kalvelage et al., 2013). The richness estimators and diversity indices of the anammox bacteria in this study were also higher than those of other deep-sea water samples (Bandekar, Nagappa, & Meena, 2018; Qian et al., 2018; Sonthiphand, Hall, & Neufeld, 2014; Wu, Hong, Chang, et al., 2019). The profile map of the Shannon index (Figure 2a) indicated the highest diversity of anammox bacteria in W45, presumably due to the shallow water...
and human activities on and around Hainan island (Han & Gu, 2015). Domestic emissions may lead to an increase in inorganic nitrogen concentrations in small areas of the marine environment, further enriching the diversity of anammox bacteria in station W45 (Li, Cao, Hong, & Gu, 2013). The vertical distribution of anammox bacterial diversity (Figure 2b) showed a peak diversity in the 50–200 m layer water samples, which was possibly due to the content of particulate organic matter (POM). As an example, consider KS1, which is closest to the Luzon strait. The diversity of anammox bacteria was positively correlated with the concentration of POM and reached its maximum value at 75 m (Figure 2c) (Hung, Wang, & Chen, 2007). Previous studies suggested that in the microscale hypoxic environment of marine snow, an organic particle was thought to be the place most conducive to the growth of anaerobic microorganisms in incompletely anoxic or oxic conditions (Hamasaki et al., 2017; Wright, Konwar, & Hallam, 2012). Anammox bacteria may use a similar strategy to survive in aerobic marine environments. Moreover, the intrusion of Kuroshio tributaries in the form of a loop current and the obvious influence on the SCS ranged from 115 to 119°E and 20 to 21°N, modifying the nutrient inventory and subsequently the ecological cycles in the northeastern SCS (Chen, Hu, Hou, & Chu, 2011; Li et al., 2017, 2018; Lu et al., 2019; Xu et al., 2018). The maximum velocity of the Kuroshio was at a depth of 100 m, transferring nutrients to nearby oligotrophic oceans, which makes the high diversity of anammox bacteria in the SCS (50–200 m layer) more significant (Chen, Jan, Kuo, & Li, 2017).

To the best of our knowledge, this study is the first to identify the dominance of the Ca. Brocadia genus of anammox bacteria in a marine ecosystem (Hamasaki et al., 2017; Li, Hong, Cao, & Gu, 2013; Qian et al., 2018). Generally, Ca. Brocadia is associated with freshwater, terrestrial environments and wastewater treatment systems, and it is thought to be a freshwater genus of anammox bacteria (Hu et al., 2012; Jetten et al., 2003; Oshiki, Satoh, & Okabe, 2016). These findings are inconsistent with the findings in this study that Ca. Brocadia is widespread in the high-salinity waters of the SCS. However, Ca. Brocadia possesses metabolic diversity and could make better use of organic compounds for energy than most other anammox bacteria (Gori, Tringe, Kartal, Marchiori, & Jetten, 2011; Yang et al., 2017). This metabolic diversity may explain why Ca. Brocadia is mostly distributed above the 200-m layer (Figure 4), where there is a relatively high organic matter concentration due to decomposed plankton in the surface waters. In addition, the dominance of Ca. Scalindua in marine environments is commonly attributed to the low temperature of deep-sea water (Hong, Yin, et al., 2011). Influenced by the Kuroshio tributaries, a stronger water exchange process occurs in the SCS. The Kuroshio has fewer impurities and a higher temperature (Li et al., 2017; Lu et al., 2019), which may provide favourable growth conditions for the Ca. Brocadia genus.

The ratio of Ca. Brocadia to Ca. Scalindua in the total anammox bacterial sequences in the six water columns of the SCS (Figure 4) demonstrated that a high relative abundance of Ca. Scalindua was detected in the deep-sea samples that were near marine sediments, such as W18-1,000 m, W18-1,500 m, W28-1,400 m, W28-1,500 m and W12-1,500 m. This trend corresponded to the results that Ca. Scalindua, with its excellent adaptability to high-salinity conditions, is the dominant genus in marine sediments (Fu et al., 2015; Hong, Li, et al., 2011; Hong, Yin, et al., 2011; Takenori, Tomonori, Noriatsu, & Akiyoshi, 2015; Wu, Hong, Chang, et al., 2019). Furthermore, the depth, temperature and salinity of seawater are thought to be important factors that affect the distribution of anammox bacteria (Li, Hong, Cao, Klotz, & Gu, 2013; Trimmer & Nichols, 2009; Wu, Hong, Chang, et al., 2019). Nonetheless, no significant correlation between the patterns of anammox bacterial distribution and environmental parameters (depth, temperature, DO and salinity) (p > 0.05) (Figure S1) in the SCS was found in the current study. Future studies need to combine multiple environmental factors to confirm the key factors that influence the microbial characteristics of anammox bacteria.

### 4.2 | Survival characteristics of anammox bacteria in water columns

In this study, anammox bacteria were widespread in the water columns of the SCS, where DO was relatively abundant (the minimum value was 3.22 mg/L). Similarly, anammox bacteria were found to exhibit higher oxygen tolerance than denitrifiers in anoxic zones, possibly due to the evolution of specific enzyme mechanisms that are used to sustain the microbial community functions of anammox (Dalsgaard et al., 2014; Zakem, Mahadevan, Lauderdale, & Follows, 2019). Furthermore, anammox bacteria are also considered to be rather unique microorganisms with a complicated cell plan (Kartal et al., 2013). Therefore, we speculated that the tolerance of anammox bacteria to oxygen was related to the origin of the cell structure. The anammoxosome, the unique organelle of anammox bacteria with ATP-generation capability, has been unambiguously identified as the compartment where metabolism occurs (Kartal et al., 2013). Hong et al. (2014) proposed the endosymbiotic origin of anammoxosomes, derived from the anammox archaea and an ancestral planctomycete (the host cell). It was also deduced that the host planctomycete cell would be an independent microorganism with a set of metabolic pathways and the capability of generating energy (Hong et al., 2014). Thus, conceivably, the unique anammox bacterium might be able to perform aerobic respiration and utilize the energy generated by the host bacterial cell to sustain its growth in aerobic marine environments. According to an analysis of the genome of the anammox bacterium K. stuttgartiensis, four ATPases were identified, and one of the ATP synthases was a homolog of those generally found in all bacteria (Deckers-Hebestreit & Altendorf, 1996; Glockner et al., 2003; Hong et al., 2014), further proving the ATP-generation ability of anammox bacteria. Although there are no direct experimental or genomic data to suggest that anammox bacteria are capable of aerobic metabolism, deeper genome sequencing may provide evidence for speculation in the future.

In addition, a positive correlation between anammox bacterial diversity and POM was presented in the current research (Figure 2c). This correlation suggests that POM is the habitat of anammox in...
aerobic water columns. Consistent with other studies, Jayakumar et al. (2009) also found that the abundance and activity of anammox bacteria seemed to increase with increased organic degradation in the Arabian Sea OMZ. Additionally, it has been proven that the rates of nitrification and denitrification are increased in response to an increase in the concentration of suspended sediments (SPS) (Xia et al., 2017). Xia et al. (2017) proposed the existence of aerobic–anaerobic gradient within POM, exhibitingoxic and anoxic conditions at the surface layer and the inner layer of POM, respectively (Xia et al., 2004; Xia, Yang, & Zhang, 2009). Based on these findings, we thought that the inner layer of POM was a favorable microenvironment for the survival of anammox bacteria. Furthermore, anammox bacteria presented a high affinity for their substrates and derived energy from the reaction of ammonium and nitrite for growth (Jetten et al., 2009). The substrates of anammox could be obtained by other N-cycle processes in the POM. When POM is surrounded by oxic waters, nitrite could be provided by nitrifiers in the oxic layer of POM and denitrifying microbes in the anoxic zone near the particle centre (Figure 5) (Song, Liu, Marchant, Kuypers, & Lavik, 2013; Xia et al., 2017). Ammonium could be supplied by dissimilatory reduction of nitrate to ammonium (DNRA) and the mineralization of organic matter (Dalsgaard, Canfield, Petersen, Thamdrup, & Acuña-González, 2003; Kalvelage et al., 2013; Pajares & Ramos, 2019). Anammox bacteria reduce nitrate to ammonium via DNRA, and subsequently convert nitrite and ammonium into N₂ by the usual anammox metabolism (Kartal et al., 2013). In addition, the concerted action between the ammonia-oxidizing bacteria (AOB) and anammox microbes inside the POM is another pathway by which anammox bacteria obtain substrates (Hannig, Lavik, Kuypers, & Jürgens, 2007; Jetten et al., 2009). Under anoxic conditions, AOB oxidized ammonium to nitrate or nitrite for the anammox bacteria, and this synergy has also been identified in other studies carried out in the Arabian Sea OMZ and laboratory experiments (Jayakumar et al., 2009; Third, Slikkers, Kuenen, & Jetten, 2001; Vlaeminck, Geets, Vervaeren, Boon, & Verstraete, 2007). In addition to the AOB, there may be other microorganisms involved in the nitrification process to supply nitrate for anammox under oxygen-limited conditions, such as Crenarchaea (Francis, Beman, & Kuypers, 2007; Jetten et al., 2009; Köneke et al., 2005). Indeed, the discovery and practical application of a new wastewater treatment technology were biologically based on the synergy between nitrification and anammox, further promoting sustainable sewage treatment (Kartal, Kuenen, & van Loosdrecht, 2010). According to the widespread distribution of anammox bacteria found in the current study, we speculated that an aerobic environment may be more conducive to the growth of anammox bacteria, which provides a new perspective on nitrogen removal from nitrogenous wastewater. However, a further confirmation is needed in future studies.

The anammox bacteria found in the water columns of the oligotrophic SCS in the current study may not only exist locally but also be acquired through exogenous factors (strong hydrological movement circulation of subsea floor fluids or seawater). Furthermore, there could also be a residual community of inactive anammox bacteria (DNA fragments) with no ecological significance. Further researches from the perspective of RNA could help identify the truly active anammox bacteria in deep-sea water environments.

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PEER REVIEW

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DATA AVAILABILITY STATEMENT

The sequences of the anammox bacteria in this study were deposited in the NCBI under the accession No. PRJNA607617.

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ORCID

Yiguo Hong https://orcid.org/0000-0002-6255-4100

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BIOSKETCH
The research team (http://www.labmeg.icoc.bz/) is interested in the microbiological and geochemical processes that control nitrogen cycling in the estuary and marine environment. The team employed geochemical, microbiological and molecular techniques to study the environmental regulation of these processes, and their effects on the global biogeochemical cycles. Our goal is to provide fundamental insights into microbial-mediated nitrogen cycling in the estuary and coast that ultimately affect coastal chemistry, biology and climate.

Author contribution: Y. H., X. L. and J.W. conceptualized the study and led the writing, Y. W. and A. L. oversaw the research, and all authors contributed to the writing. The research team (http://www.labmeg.icoc.bz/) is interested in microbial-mediated nitrogen cycling in the estuary and marine environment. The team employed geochemical, microbiological and molecular techniques to study the environmental regulation of these processes, and their effects on the global biogeochemical cycles. Our goal is to provide fundamental insights into microbial-mediated nitrogen cycling in the estuary and coast that ultimately affect coastal chemistry, biology and climate.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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