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Overall bacterial community composition and abundance of nitrifiers and denitrifiers in a typical macrotidal estuary

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ABSTRACT

Coupled nitrogen cycling processes can alleviate the negative effects of eutrophication caused by excessive nitrogen load in estuarine ecosystems. The abundance and diversity of nitrifiers and denitrifiers across different environmental gradients were examined in the sediment of Hangzhou Bay. Quantitative PCR and Pearson's correlation analyses suggested that the bacterial ammonia-oxidizers (AOB) were the dominant phylotypes capable of ammonia oxidation, while the *nirS*-encoding denitrifiers predominated in the denitrification process. Simultaneously, nitrite and pH were found to be the two major factors influencing *amoA* and *nir* gene abundances, and the distribution of bacterial communities. Moreover, the ratio of *nirS*/AOB *amoA* gene abundance showed negative correlation with nitrite concentration. Fluorescence *in situ* hybridization further demonstrated that AOB and acetate-denitrifying cells were closely connected and formed obvious aggregates in the sediment. Together, all these results provided us a preliminary insight for coupled nitrification-denitrification processes in the sediment of Hangzhou Bay.

1. Introduction

Over the last few decades, coastal eutrophication caused by excessive nitrogen (N) discharge has become a matter of global concern (Howarth et al., 2002). Estuarine eutrophication has resulted in a suite of environmental problems, such as hypoxic events (Rabalais, 2002) and harmful algal blooms (Paerl et al., 2002), and greatly threaten both the economy and human health. Accordingly, bioavailable N from landscapes to coastal waters can be removed by tightly-coupled microbial processes, such as coupled nitrification-denitrification (Seitzinger et al., 2006) or coupled nitrification-anammox (the anaerobic oxidation of ammonium) (Lam et al., 2007). Nitrification, the two-step conversion of ammonium (NH_4^+) to nitrate (NO_3^-) *via* nitrite (NO_2^-), is commonly thought to play a vital role in N cycle (Head et al., 1993). As is known to all, ammonia oxidation is the first and rate-limiting step of nitrification and is catalyzed by ammonia monooxygenase (AMO), which is encoded by the *amoA* gene from both archaea and bacteria. It is generally assumed that ammonia-oxidizing archaea (AOA) not ammonia-oxidizing bacteria (AOB) are the main contributors to ammonia oxidation process in marine environments (Francis et al., 2005; Könneke et al., 2005; Wuchter et al., 2006; Smith et al., 2014), however, the predominant ammonia oxidizers in estuarine ecosystems remain more uncertain. Being an intermediate zone between land and ocean, the estuarine area often experiences salinity and nutrient gradients, which may have important impacts on the temporal and spatial dynamics of ammonia oxidizers (Zheng et al., 2014b). As noted earlier, numerous studies suggest that AOA usually outnumber AOB in estuarine environments and play more important roles in nitrification process, such as the researches in Bahía del Tóbari, Mexico (Beman and Francis, 2006), Monterey Bay, USA (Mincer et al., 2007), the Yangtze Estuary, China (Dang et al., 2008; Zheng et al., 2014b), the Fitzroy Estuary, Australia (Abell et al., 2010), the Plum Island Sound Estuary, USA (Bernhard et al., 2010) and the Pearl River Estuary, China (Jin et al., 2011). Besides, a few studies found that nitrification might be driven by bacteria rather than archaea in estuarine environments, such as the researches in San Francisco Bay, USA (Mosier and Francis, 2008) and the Colne Estuary, UK (Li et al., 2014).

Denitrification, the sequential reduction of NO_3^- to dinitrogen gas (N_2) *via* oxidized intermediates, can remove more than half of the inorganic nitrogen (DIN) inputs from terrestrial ecosystems when coupled

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with nitrification (Seitzinger et al., 2006). Denitrification is considered to be the dominant loss pathway for fixed N in shallow coastal and estuarine systems (Bulow et al., 2008; Mosier and Francis, 2010), although anammox has been recently identified as an alternative microbial pathway of N_2 production (Ward et al., 2009). Two nitrite reductases are key enzymes in the denitrification pathway: copper-containing nitrite reductases and cytochrome cd_1 nitrite reductases (encoded by *nirK* gene and *nirS* gene respectively). Though these two forms of *nir* genes are supposed to be functionally equivalent (Zehr and Ward, 2002), *nirS* gene appears to be more abundant in estuaries according to previous studies, such as the researches in the Fitzroy Estuary, Australia (Abell et al., 2010), San Francisco Bay, USA (Mosier and Francis, 2010) and Laizhou Bay, China (Wang et al., 2014). Crucial factors known to influence the diversity and abundance of denitrifiers include substrate availability (Kemp et al., 1990), oxygen concentration (Smith et al., 2006), salinity, temperature and pH (Salehlakha et al., 2009).

Hangzhou Bay is located in the northern part of Zhejiang Province, China. It is the outer part of the Qiantang River Estuary and adjacent to the East China Sea. Covering an area of approximately 8500 km^2, it is one of the world's largest macrotidal embayments. The tidal amplitude at the mouth is 3–4 m, and it exceeds 4–6 m further upstream. Tidal currents are mainly rectilinear and the maximal flood velocity exceeds 4.0 m/s (Xie et al., 2009). The major rivers discharging directly into Hangzhou Bay are Qiantang River, Cao-e River and Yong River, with average water discharge of 44.4 km^3/a and DIN load of $> 3.8 \times 10^4$ t/a (Zhang et al., 2002). Over the past few decades, excessive anthropogenic N from agricultural production, domestic and industrial wastewater discharge and fish farming has resulted in severe eutrophication of Hangzhou Bay (Huo et al., 2010). Here, the water quality is far worse than Grade IV Sea Water Quality Standard of China (SOA, 2014). Influenced by tidal currents and waves, Hangzhou Bay has a high carrying capacity for suspended particulate matter, in which the net primary production tends to be light-limited (Xie et al., 2009). Thus, N biogeochemistry in such turbid environments, which is almost exclusively reliant on reduction-oxidation reactions, is facilitated primarily by non-phytoplankton microorganisms (Dang and Jiao, 2014). However, to the best of our knowledge, the microbial N cycling processes in this eutrophic macrotidal estuary as yet remain unclear.

This study aims to (I) evaluate the abundance and diversity of nitrifying (AOA *amoA* and AOB *amoA*) and denitrifying (*nirS* and *nirK*) phylotypes with quantitative PCR (qPCR), and examine the major environmental factors controlling the distribution of nitrifiers and denitrifiers in the estuary; (II) characterize the distribution of bacterial communities and its environmental regulation information using Illumina MiSeq sequencing; (III) demonstrate the ecological niche of nitrifiers and denitrifiers by fluorescence *in situ* hybridization (FISH).

2. Materials and methods

2.1. Sampling and environmental parameters

Sediment and overlying water samples were collected from Hangzhou Bay along a salinity gradient in May 2014, when the quantity of phytoplankton was quite low (Cai, 2006) (Fig. 1). Overlying water samples were collected with a 5-L Niskin bottle (Tianjin test center, Tianjin, China). Standard oceanographic properties, including water temperature, salinity, dissolved oxygen (DO) and pH, were measured on board immediately using a Horiba U-52 water quality checker (Horiba, Kyoto, Japan). Then the overlying water from each site was sampled triple from the Niskin bottle, and transferred to acid washed polyethylene bottles. The concentration of chlorophyll a (Chl a) was measured following the standard protocols described previously (Strickland and Parsons, 1972). Sediment samples were taken with a 250-cm^3 Van Veen Grab (Hydro-bios, Kiel, Germany). The upper layer (0–5 cm) sediment of each site was sliced, mixed and homogenized, and then put into a sterile plastic bag and quickly stored in the − 20 °C ice box for further analyses. Inorganic N (NH_4^+, NO_2^- and NO_3^-) were extracted from the sediment using 2 M KCl as previously described (Shen et al., 2013). The concentration of total phosphorus (TP) in sediment was measured colorimetrically by the ascorbic acid-molybdate blue method (Murphy and Riley, 1962).

2.2. DNA extraction

Total genomic DNA of each sediment sample (0.4–0.6 g) was extracted using a FastDNA spin kit for soil (Qbiogene, Carlsbad, CA, USA), following the manufacturer's instructions. Duplicate DNA extractions for each water sample were performed. DNA quality was detected through 1% agarose gel electrophoresis which was stained with SYBR Safe DNA Gel Stain (Invitrogen, Carlsbad, CA, USA). The duplicate DNA extractions were then merged together, and stored at − 80 °C for subsequent molecular analysis.

2.3. Quantitative PCR (qPCR)

The abundance of functional marker genes, including AOA *amoA*, AOB *amoA*, *nirK* and *nirS* gene, were quantified by qPCR analysis using a CFX 96C 1000™ Thermal Cycler (Bio-Rad, Hercules, CA, USA). Standard curves were generated using serial tenfold dilutions (10^{-1} to 10^{-5}) of linearized plasmids containing cloned AOA *amoA*, AOB *amoA*, *nirK* and *nirS* genes. The 20 μL reactions contained 0.4 μL of each primer (10 mM), 10 μL of SYBR Premix Ex Taq (Takara, Tokyo, Japan) and 2 μL of template DNA. Primers used in this study are listed in Table S1. The PCR cycle started with 3 min at 95 °C, followed by 40 cycles of 10 s at 95 °C, 30 s at the specific annealing temperature and 30 s at 72 °C. The specificity of amplification was checked by the observation of melt curves. The PCR amplification efficiencies were 83–100.7%, and correlation coefficients (R^2) for all assays were > 0.99. All samples and standard reactions were performed in triplicate, and average values were calculated.

2.4. Illumina MiSeq sequencing

Bacterial communities were investigated at the sediment samples of Hangzhou Bay, using high-throughput sequencing according to the protocols described by Caporaso et al. (2011). The V4 regions of the bacterial 16S rRNA gene were amplified from the DNA extracts using the primers 520F (5′-barcode-AYTGGGYDTAAAGNG-3′) and 802R (5′-TACNVGGGTATCTAATCC-3′) (Klindworth et al., 2015). The barcode is a seven-base sequence unique to each sample. The sequencing was then conducted on the Illumina MiSeq platform (Personalbio, Shanghai, China) and altogether generated 385,806 reads of 16S rRNA gene from nine sediment samples. Raw sequencing data were de-multiplexed and quality-filtered using the default parameters in Qiime version 1.7.0 (Caporaso et al., 2010). Criteria used for the filtering step were recommended by Bokulich et al. (2013). The remaining high quality 16S rRNA gene sequences were then clustered into operational taxonomic units (OTUs; 97% similarity) with uclust in Qiime (Edgar, 2010). A bootstrap cutoff of 50% suggested by the Ribosomal Database Project (RDP) was applied for taxonomic assignment (Wang et al., 2007). Based on OTU numbers, the alpha diversity measures (Chao 1 and Shannon index) were calculated in Mothur version 1.31.2 (Schloss et al., 2009).

All V4 sequence data are available in the NCBI Short Read Archive database (Accession Number: SRP091594).

2.5. FISH analysis

Three 16S rRNA-targeted oligonucleotide probes were used for *in situ* detection of nitrifying and denitrifying bacteria: (1) Cy3-labeled NSO190 probes, specific for ammonia-oxidizing β-subclass *Proteobacteria*; (2) Cy5-labeled DEN67 probes, specific for methanol-

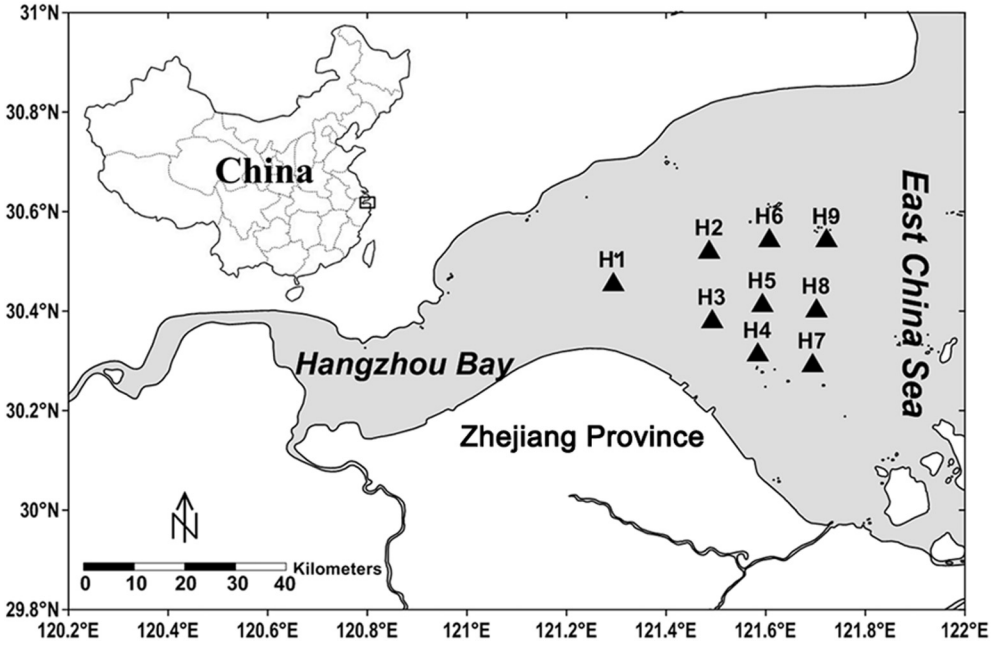

Fig. 1. Map of Hangzhou Bay showing sample sites on the west coast of the East China Sea.

denitrifying cluster; (3) FAM-labeled DEN124 probes, specific for acetate-denitrifying cluster. Sediment samples were fixed on ice for 3 h in freshly prepared 4% paraformaldehyde solution, rinsed with phosphate-buffered saline (PBS), and subsequently stored in PBS-ethanol (1:1) at − 20 °C. For *in situ* hybridization, 4 μL of each fixed sample was spotted onto adhesion microscope slides. Hybridization of each sediment sample was carried out as described previously (Amann, 1995). The sequences, specificities and hybridization temperatures of all probes are shown in Table S2. Then, fluorescent and phase-contrast images were recorded using a multiphoton confocal LSM 780 NLO microscope system (Carl Zeiss, AG, Germany).

2.6. Data analysis

Pearson's correlation analyses were used to test the correlations between environmental parameters (temperature, salinity, DO, pH, Chl a, TP, NH_4^+, NO_2^- and NO_3^-); between gene abundances (or gene copy ratios) and environmental parameters (temperature, salinity, DO, pH, Chl a, TP, NH_4^+, NO_2^- and NO_3^-). All calculations were performed using IBM SPSS statistics 20.0 software. To compare the phylogenetic similarities of bacterial community composition in different samples, hierarchical clustering was performed in the program PAST (Hammer et al., 2001) based on Bray-Curtis similarity index and unweighted pair group method average (UPGMA) algorithm. To test the effects of environmental parameters (temperature, salinity, DO, pH, Chl a, TP, NH_4^+, NO_2^- and NO_3^-) on the distribution of bacterial communities, redundancy analysis (RDA) was performed using the software Canoco version 5.0 (Lepš and Šmilauer, 2003).

3. Results and discussion

3.1. Environmental parameters of the sampling sites

Physical and chemical characteristics of the sampling sites are shown in Table 1. From the west side of Hangzhou Bay to the east, the overlying water temperature decreased from 21.20 to 18.80 °C, which was negatively correlated with salinity (17.17–27.46 psu) and DO (6.35–7.05 mg/L) (Table 1 and Table S3; $r = -0.840$, $P < 0.01$ and $r = -0.702$, $P < 0.05$). The concentration of Chl a also decreased from 1.03 to 0.03 μg/L (Table 1), showing a positive correlation with temperature (Table S3; $r = 0.902$, $P < 0.01$) and negative correlations with salinity and DO (Table S3; $r = -0.757$, $P < 0.05$ and $r = -0.736$, $P < 0.05$). Notably, the Chl a concentration in the overlying water was much lower than that measured in other estuaries, which is indicative of poor phytoplankton growth in the water column of Hangzhou Bay. For example, the Chl a concentration was peak (~25 μg/L) at the midsalinity sites (15–20 psu) of Delaware Bay, USA (Campbell and Kirchman, 2013), and the Chl a concentration was ~35 μg/L in summer of Tokyo Bay, Japan (Suzumura et al., 2004). As for the nutrient concentration, the results showed that the concentration of NH_4^+ ranged from 3.84 to 86.52 mg/kg, with a peak in sample H8. Ranging from 0.05 to 6.06 mg/kg, the concentration of NO_3^- was quite variable. As a whole, NH_4^+ was the main source of nitrogen in the sediment of Hangzhou Bay, which is accounted for 48.33–99.88% of DIN. It corresponds to the earlier study that NH_4^+-N is the primary anthropogenic nutrient entering Hangzhou Bay from the Qiantang River (Hu et al., 2012). Additionally, the concentration of TP, ranging from 0.50 to 0.64 mg/g, showed a gentle change.

3.2. Factors regulating nitrifying and denitrifying gene abundances

QPCR analysis was used to estimate the abundances of key nitrifying (AOA *amoA* and AOB *amoA*) and denitrifying (*nirK* and *nirS*) genes in the sediment samples. Among the nitrifying genes, the abundance of AOA *amoA* gene ranged from 5.91×10^5 to 2.62×10^7 copies/g dry sediment, with the abundance of AOB *amoA* gene ranging from 2.06×10^5 to 2.30×10^7 copies/g dry sediment (Fig. 2a). Specifically, the results indicated that AOB were more abundant in the west of the Bay (H1, H2, H3) with lower salinity (17.17–19.72 psu), while AOA were more abundant in the east of the Bay (H4, H5, H6, H7, H8, H9) with higher salinity (20.38–27.46 psu) (Fig. 2a). It's different from the previous studies that AOA phylotypes were more abundant than AOB in estuarine ecosystems (Beman and Francis, 2006; Mincer et al., 2007; Dang et al., 2008; Abell et al., 2010; Bernhard et al., 2010; Jin et al., 2011; Zheng et al., 2014b). Among the denitrifying genes, *nirS* gene appeared to be more numerous, with the abundance ranging from 1.90×10^6 to 4.69×10^7 copies/g dry sediment. Meanwhile, the abundance of *nirK* gene ranged from 1.21×10^4 to 3.27×10^6 copies/g dry sediment (Fig. 2b). The ratios of *nirS*/*nirK* gene copy number ranged from 6.15 to 863.23 in all samples, showing the clear predominance of *nirS* gene in the sediment of Hangzhou Bay. This finding is consistent with some earlier studies demonstrating that *nirS* gene was

Table 1
Physical and chemical properties of overlying water and sediment in Hangzhou Bay.

Environmental parament	Sampling site								
	H1	H2	H3	H4	H5	H6	H7	H8	H9
Latitude (°N)	30.45	30.52	30.38	30.31	30.41	30.54	30.29	30.40	30.54
Longitude (°E)	121.29	121.49	121.49	121.58	121.59	121.61	121.69	121.70	121.72
Depth (m)	8.00	8.50	10.00	10.00	11.00	9.00	12.00	9.00	11.00
Overlying water									
Temperature (°C)	21.20	20.30	19.80	18.80	19.60	20.10	20.10	18.90	19.20
Salinity (psu)	17.17	18.70	19.72	27.46	22.74	20.38	21.89	21.89	22.93
DO (mg/L)	6.35	6.50	6.54	6.99	6.82	6.69	6.76	7.05	6.43
pH	7.97	7.98	7.97	7.96	7.96	8.01	8.01	8.01	8.02
Chlorophyll a (μg/L)	1.03	0.78	0.80	0.45	0.66	0.78	0.56	0.30	0.48
Sediment									
Water content (%)	44.93	53.67	41.16	48.21	47.80	39.63	37.52	33.88	42.89
NH_4^+ (mg/kg)	7.31	4.74	5.88	9.94	11.21	8.06	5.76	86.52	3.84
NO_2^- (mg/kg)	0.16	0.24	0.18	0.15	0.22	0.11	0.10	0.06	0.16
NO_3^- (mg/kg)	2.20	3.82	2.42	3.29	2.73	0.62	6.06	0.05	0.09
TP (mg/g)	0.52	0.60	0.50	0.52	0.63	0.56	0.54	0.52	0.64

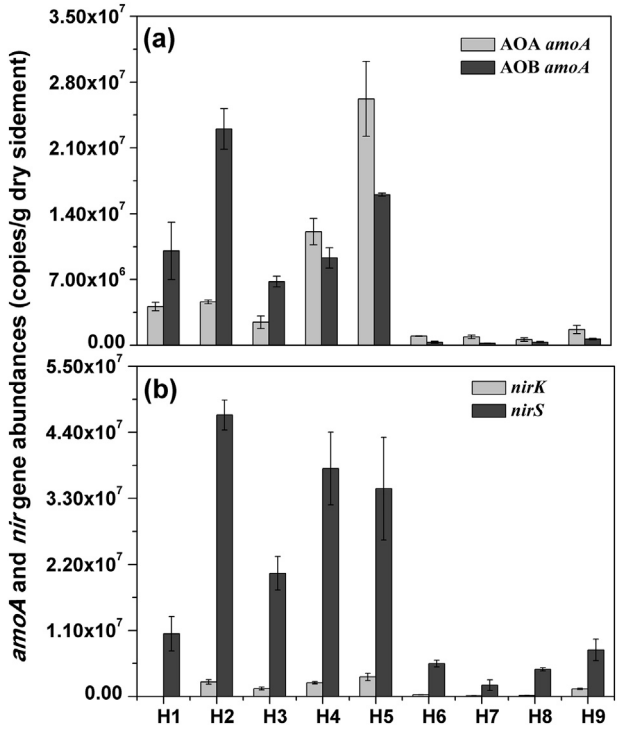

Fig. 2. Copy numbers (± 1SE) of (a) *amoA* and (b) *nir* genes detected in the sediment samples (per g dry sediment) of Hangzhou Bay.

more abundant than *nirK* gene in estuarine ecosystems (Abell et al., 2010; Mosier and Francis, 2010; Wang et al., 2014).

The results of Pearson correlation analyses showed that the abundance of AOB *amoA* gene was significantly correlated with NO_2^- concentration (Table 2; $r = 0.869$, $P < 0.01$), while the abundance of AOA *amoA* gene did not. It does seem likely that the ammonia oxidation process may be driven by bacteria rather than archaea in the sediment of Hangzhou Bay (Mosier and Francis, 2008; Li et al., 2014). Furthermore, Pearson's correlation analysis showed that the abundance of *nirS* gene was strongly correlated with NO_2^- concentration (Table 2; $r = 0.798$, $P < 0.01$), with the abundance of *nirK* gene weakly correlating with NO_2^- concentration (Table 2; $r = 0.783$, $P < 0.05$). This is likely then, that *nirS*-type denitrifiers may play a major role in the denitrification process in the sediment of Hangzhou Bay. Furthermore, pH may also play an important role in the N cycling processes, with the abundances of AOA *amoA*, AOB *amoA* and *nirS* genes negatively correlating with pH (Table 2; all $P < 0.05$). As previous research has pointed out, pH exerts a strong selection pressure on microbes and thus, has a pervasive effect on bacterial abundance and diversity (An and Joye, 2001; Hartman et al., 2008). Nevertheless, unlike some earlier studies that salinity is an important factor affecting the abundance and distribution of both AOA and AOB (Mosier and Francis, 2008; Sahan and Muyzer, 2008; Abell et al., 2010; Bernhard and Bollmann, 2010), there was no correlation between salinity and AOA *amoA* or AOB *amoA* gene abundance in this study (Table 2). We think the reason could probably be attributed to the small variation of salinity gradient across the sampling sites. The relationships between environmental parameters and gene copy number ratios were also detected by Pearson's correlation analysis. The ratio of *nirS*/AOB *amoA* gene copy number was negatively correlated with NO_2^- concentration (Table 2; $r = -0.749$, $P < 0.05$), potentially linking coupled nitrification-denitrification processes to N metabolism in the sediment of Hangzhou Bay. Otherwise, no significant correlation was found between NO_2^- or NO_3^- and AOA *amoA*/*nirK*, AOA *amoA*/*nirS*, or *nirK*/AOB *amoA* values (Table 2).

3.3. Factors regulating the bacterial community compositions

To investigate the distribution of bacterial communities in the sediment of Hangzhou Bay, Illumina MiSeq sequencing was conducted. The Illumina MiSeq platform produced > 385,000 raw reads of the V4 amplicons. After removing short- and low-quality reads, 34,786–49,886 bacterial reads of each sample were available for further analyses. OTU Numbers, Chao 1 and Shannon's indices at cutoff levels of 3% are summarized in Table S4. On the basis of OTU numbers, sample H1 (4164 OTUs) had the richest abundance, gradually reducing from the west of the Bay to the east, and only 2236 OTUs were found at sample H7. Very similar trend in the Chao 1 index was observed in comparison with OTU richness (Table S4). As to the Shannon diversity index, it also indicated a higher bacterial diversity in the west of the Bay (H1, H2, H3, H4, H5) relative to the west of the Bay (H6, H7, H8, H9) (Table S4). Overall, these results suggest that the abundance and diversity of bacterial communities in the nearshore area are much higher than those in the offshore area.

Cluster analysis at the phylum level revealed similarities of bacterial community structure in each sample of Hangzhou Bay (Fig. 3). The nine sediment samples could be clustered into two groups: (1) Group I contains five sediment samples from the western Bay (H1, H2, H3, H4, H5); (2) Group II contains four sediment samples from the eastern Bay (H6, H7, H8, H9) (Fig. 3). Specifically, *Proteobacteria* was the most abundant phylum across all sediment samples, accounting for 27.25–31.10% of the total effective bacterial sequences. Within the

Table 2
Pearson's correlation coefficients (*r*) between environmental parameters and gene abundances and the ratios of target genes across the sampling sites.

	Temperature	Salinity	DO	pH	Chl a	TP	NH_4^+	NO_2^-	NO_3^-
	(°C)	(psu)	(mg/L)		(μg/L)	(mg/g)	(mg/kg)	(mg/kg)	(mg/kg)
AOA *amoA*	− 0.178	0.377	0.285	**− 0.677***	− 0.001	0.394	− 0.170	0.538	0.179
AOB *amoA*	0.285	− 0.204	− 0.212	**− 0.721***	0.413	0.287	− 0.308	**0.869****	0.353
nirK	− 0.313	0.386	0.125	− 0.616	− 0.067	0.517	− 0.299	**0.783***	0.189
nirS	− 0.093	0.188	0.048	**− 0.736***	0.124	0.246	− 0.292	**0.798****	0.323
AOA *amoA*/*nirK*	**0.704***	− 0.531	− 0.495	− 0.278	0.636	− 0.280	− 0.123	0.041	− 0.017
AOA *amoA*/*nirS*	0.104	0.230	0.167	− 0.372	0.067	0.302	− 0.234	0.208	0.381
nirK/AOB *amoA*	− 0.366	0.249	− 0.163	**0.754***	− 0.404	0.506	− 0.042	− 0.268	− 0.518
nirS/AOB *amoA*	− 0.344	0.166	0.287	**0.876****	− 0.482	0.060	0.394	**− 0.749***	− 0.503

Data in bold indicate significant correlations, $^*P < 0.05$, $^{**}P < 0.01$.

Proteobacteria group, *Deltaproteobacteria* (12.10%) was the most dominant class (average abundance), followed by *Gammaproteobacteria* (10.38%), *Alphaproteobacteria* (4.74%) and *Betaproteobacteria* (1.59%). Being the most dominant community in estuarine environments, the abundance of *Proteobacteria* described in this study was much lower than that measured in other estuaries, such as Liaodong Bay, China (Zheng et al., 2014a) and Laizhou Bay, China (Wang et al., 2014). The other dominant phyla of sediment samples were *Planctomycetes* (13.29%), *Bacteroidetes* (7.31%), *Acidobacteria* (4.86%), *Firmicutes* (4.67%) and *Chloroflexi* (4.55%), accounted for 32.57–35.72% of total effective bacterial sequences. Followed by a few other major phyla (abundance > 1% in each sample), including: *Chlamydiae*, *Verrucomicrobia* and *Actinobacteria*; a few phyla (*e.g. Spirochaetes*, *Gemmatimonadetes*, *Lentisphaerae*, *Cyanobacteria*, *Nitrospirae*, *Armatimonadetes* and *Fibrobacteres*) were only major contributors (abundance > 1%) to community compositions in at least one sample.

To investigate the potential effects of environmental parameters on the distribution of bacterial communities, redundancy analysis (RDA) was performed (Fig. 4). The results showed that the first two RDA dimensions explained 77.8% (62.0% and 15.8% by RDA1 and RDA2, respectively) of the cumulative variance of the relationship between environmental parameter and bacterial community (999 times Monte-Carlo permutation tests). RDA1 clearly distinguished the bacteria communities in Group I samples (H1, H2, H3, H4, H5) from that in Group II samples (H6, H7, H8, H9). In particular, the phyla *Planctomycetes*, *Bacteroidetes*, *Acidobacteria*, *Chlamydiae*, and the class *Beta-* and *Gammaproteobacteria* were found to predominate in the Group I samples (characterized by high NO_2^-). The phyla *Firmicutes*, *Chloroflexi*, *Actinobacteria*, *Gemmatimonadetes*, *Lentisphaerae*, the classes *Alpha-* and *Deltaproteobacteria* predominated in the Group II samples (characterized by high pH) (Fig. 4). Among the environmental parameters measured, pH and NO_2^- were the two major factors influencing the distributions of bacterial communities (Fig. 4; $F = 7.876$, $P = 0.002$ and $F = 3.064$, $P = 0.044$, respectively). A previous investigation of Laizhou Bay also

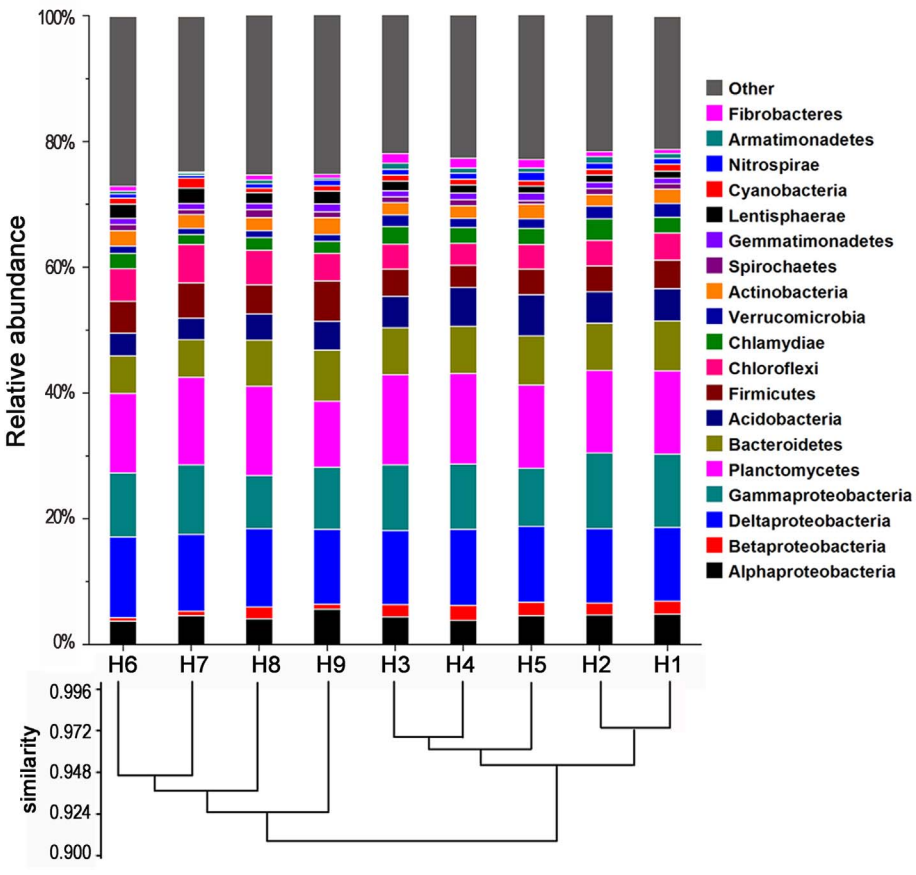

Fig. 3. Dendrogram of hierarchical clustering of bacterial communities based on Bray-Curtis similarity. Bacterial taxonomic information is shown at the phylum level (and subdivision level for *Proteobacteria*). Taxa represented occurred at > 1% abundance in at least one sample.

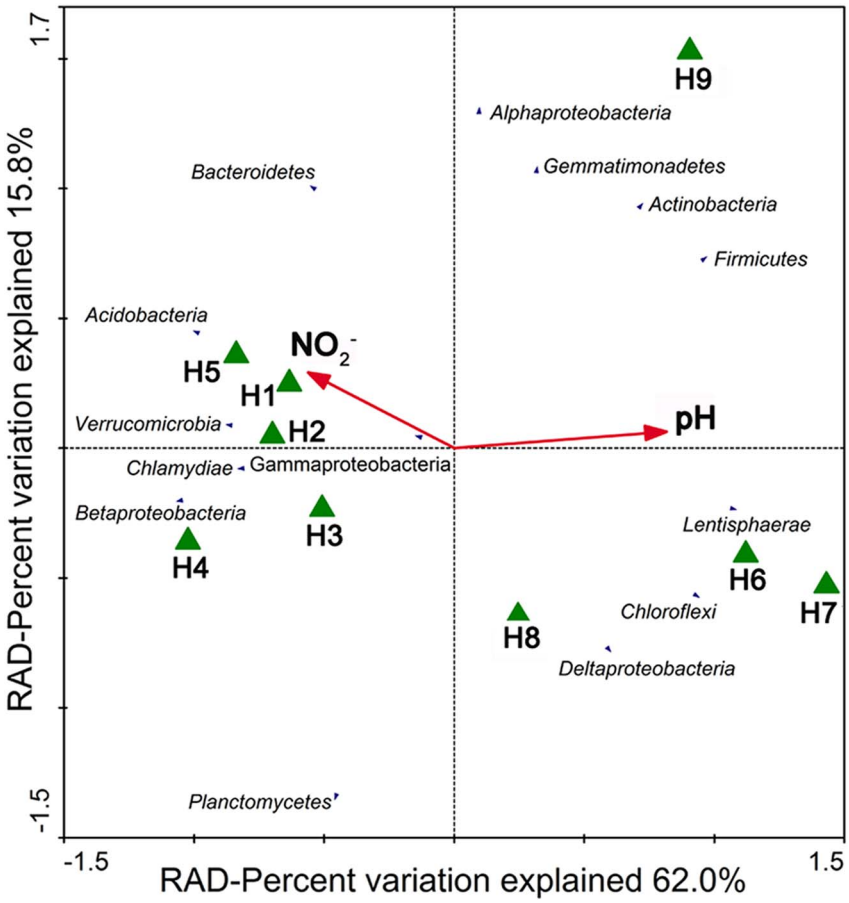

Fig. 4. Redundancy analysis of the relationship between environmental parameters (red arrows) and bacterial communities of sediment samples (green triangles) in Hangzhou Bay. Taxonomic information is shown at the phylum level (and subdivision level for *Proteobacteria*). Only *P* value of environmental parameter < 0.05 (999 times Monte-Carlo permutation test) and average abundance of taxa > 1% are shown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

suggests that pH and NO_2^- are important factors shaping the bacterial community patterns (Wang et al., 2014). Other parameters (*e.g.* temperature, DO, salinity, Chl a, TP, NH_4^+ and NO_3^-) detected in this study showed weaker effects on bacterial assemblages (Fig. 4). Except for pH and NO_2^-, the crucial environmental parameters known to influence the bacterial communities in estuarine environments also include salinity, NO_3^-, DO, TOC and sediment texture (Campbell and Kirchman, 2013; Wang et al., 2014; Zheng et al., 2014a).

3.4. Abundances of nitrifying and potential denitrifying genera

Taxonomically, AOB are phylogenetically restricted to two lineages within the *Proteobacteria*: the *Betaproteobacteria*, including the genera *Nitrosomonas*, *Nitrosospira*, *Nitrosovibrio* and *Nitrosolobus* (Purkhold et al., 2000); and the *Gammaproteobacteria*, including the genus *Nitrosococcus* (Ward and O'Mullan, 2002). Three AOB lineages were observed in this study: the Betaproteobacterial *Nitrosomonas* and *Nitrosospira*, and the Gammaproteobacterial *Nitrosococcus*. The *Nitrosomonas* and *Nitrosococcus* groups were the two dominant genera of AOB, accounting for 0.02–0.16% and 0.09–0.19% of the total sample sequences respectively, while the genus *Nitrosospira* was nearly absent in this study (Fig. 5a). The abundance of *Nitrosococcus* was higher than that of *Nitrosomonas* in most sediment samples, which is different from earlier studies in coastal and marine environments (Ward et al., 2007; Bernhard et al., 2010).

Previous studies provide a list of ~100 denitrifying bacterial genera (Heylen et al., 2006; Shapleigh, 2006; Yu et al., 2014; Zhong et al., 2015), among which only 43 genera were detected in the sediment of Hangzhou Bay (Fig. 5b). In the present study, majority of the potential denitrifying genera belonged to *Proteobacteria* (31 genera; 0.66–1.58% of the total 16S rRNA sequences). In particular, the potential denitrifying sequences were mainly (*i.e.* average abundance > 0.05%) assigned to the genus *Bradyrhizobium* of *Alphaproteobacteria*, the genus *Thauera* of *Betaproteobacteria*, the genera *Pseudomonas*, *Pseudoalteromonas*, *Stenotrophomonas*, *Halomonas* and *Psychrobacter* of *Gammaproteobacteria*, the genus *Anaeromyxobacter* of *Deltaproteobacteria*, and the genus *Arcobacter* of *Epsilonproteobacteria*. The second largest group of denitrifiers was categorized as *Bacteroidetes* (4 genera; 0.33–0.76%), followed by the group of *Firmicutes* (5 genera; 0.02–0.25%) and *Actinobacteria* (3 genera; 0–0.14%) (Fig. 5b). However, more research is required to clarify the mechanisms driving the denitrification process in the sediment of Hangzhou Bay and resolve the roles played by denitrifying bacteria in this process.

3.5. In situ characterization of nitrifying and denitrifying bacteria

To investigate the spatial distribution of nitrifying and denitrifying bacteria in the sediment of Hangzhou Bay, simultaneous *in situ* hybridization with specific probes was performed. *In situ* hybridization clearly indicated that AOB were in greater abundance than acetate-denitrifying cells (Fig. 6a–b and Fig. S1a, b), while nearly no methanol-denitrifying cell was detected. Specifically, NSO190 probe-stained AOB (red signals) and DEN124 probe-stained acetate-denitrifying cells (green signals) formed irregularly shaped (~20 μm), dense aggregates (yellow signals) (Fig. 6c-d and Fig. S1c–d). It is common knowledge that nitrification is an aerobic process and denitrification an anaerobic process, therefore, the two processes are usually carried out in separate ecological niches. The reasons for spatial niche overlap between nitrifiers and denitrifiers in the sediment could be attributed as follows: (1) nitrification and denitrification are closely coupled in time because of the small scale spatial separation of nitrification and denitrification (Seitzinger et al., 2006); (2) nitrification produces nitrite or nitrate,

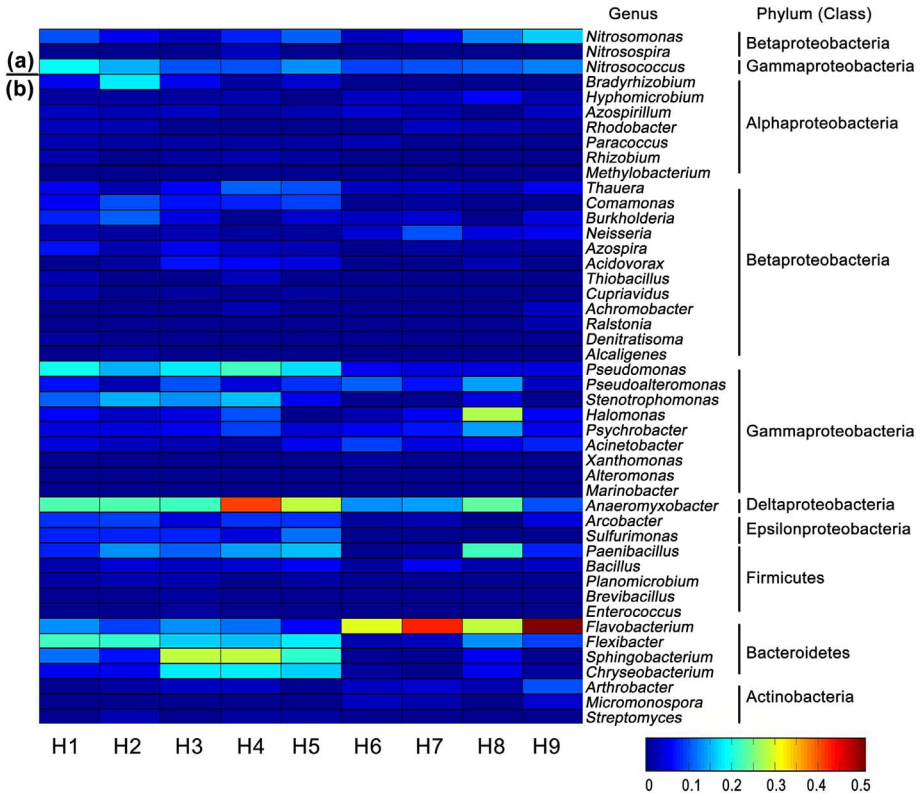

Fig. 5. Heatmap of (a) nitrifying and (b) potential denitrifying genera in each sediment sample of Hangzhou Bay. Nitrifying (3 genera) and potential denitrifying genera (43 genera) were compared with their relative abundances among different samples. The color intensity in each panel shows the percentage of a genus in a sample, referring to color key at the right bottom.

which is a reactant in denitrification; (3) nitrification reduces the pH and oxygen around, and denitrification generates the alkalinity that is required in nitrification (Menoud et al., 1999). Aggregates consisting of nitrifiers and denitrifiers detected by FISH analysis provide us an evidence for the co-existence of nitrifiers and denitrifiers in the sediment of Hangzhou Bay.

4. Conclusion

We have examined the abundance and diversity of nitrifying and denitrifying genes in the sediment of Hangzhou Bay. Though AOA *amoA* gene predominated AOB *amoA* gene in most of the samples, correlation analysis suggesting that AOB was probably the major

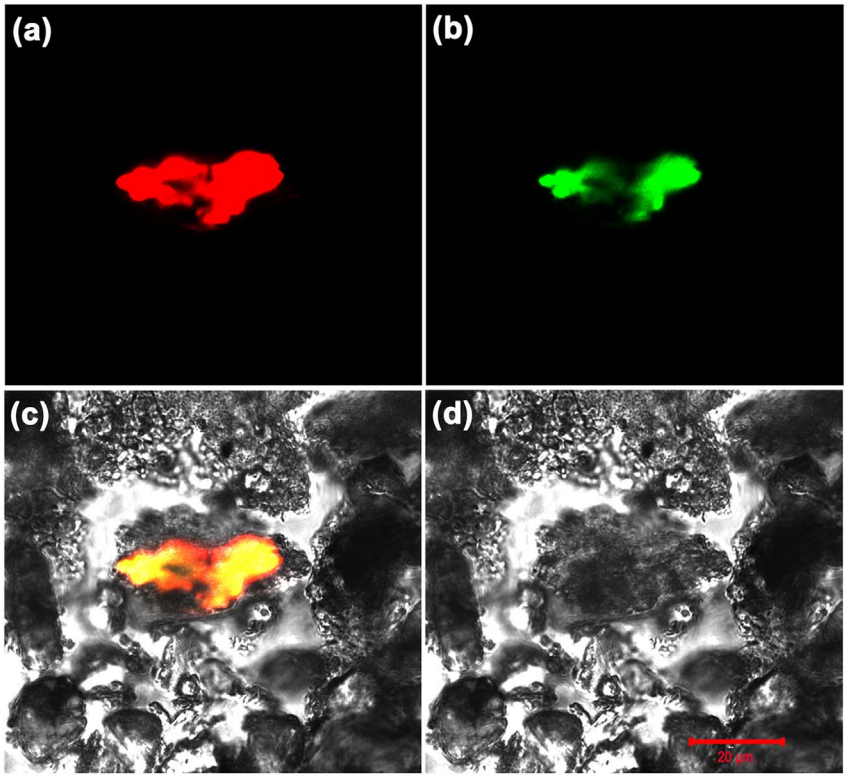

Fig. 6. Simultaneous *in situ* hybridization of nitrifying and denitrifying bacteria in the sediment of Hangzhou Bay. Fluorescence micrograph of (a) ammonia-oxidizing bacteria hybridization with Cy3-labeled probe NSO190 (red); (b) acetate-denitrifying cluster hybridization with FAM-labeled probe DEN124 (green); (c) combined image of the two fluorescence micrographs, where the yellow cell aggregates are double labeled with NSO190 and DEN124; A phase contrast-micrograph of the floc section, where the red bar = 20 μm, is depicted in (d). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

phylotype responsible for ammonia oxidation in the sediment of the Hangzhou Bay. Likewise, *nirS*-encoding denitrifiers were supposed to play an important role in the denitrification process in the estuary. Then, nitrite and pH were found to be the two important factors influencing the abundance and diversity of the nitrifiers and denitrifiers, as well as the distribution of bacterial communities. Furthermore, negative correlation between the ratio of *nirS*/AOB *amoA* gene abundance and nitrite, with aggregates consisting of nitrifiers and denitrifiers, together provided us a preliminary insight for coupled nitrification-denitrification processes in the sediment of Hangzhou Bay. To our knowledge, this study is the first investigation on N cycling functional microorganisms in the sediment of Hangzhou Bay. Further research with ^{15}N isotopic tracer technology, will provide us deeper insights into the exact extents of N flux mediated by coupled N cycling processes.

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Author contributions

Z.W.J. and W.W.X. designed and conducted the experiments. Z.W.J. and W.C. wrote the main manuscript text. Z.W.J. and Z.L.C performed the experiments. W.W.X., M.Z.H., S.F.Q. and D.W.J. contributed to the data analysis and manuscript revision and language editing. All authors reviewed the manuscript and have given approval to the final version of the manuscript.

Compliance with ethical standards

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

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2017.09.062.

References

Abell, G.C., Revill, A.T., Smith, C., Bissett, A.P., Volkman, J.K., Robert, S.S., 2010. Archaeal ammonia oxidizers and *nirS*-type denitrifiers dominate sediment nitrifying and denitrifying populations in a subtropical macrotidal estuary. ISME J. 4, 286–300. http://dx.doi.org/10.1038/ismej.2009.105.

Amann, R.I., 1995. *In situ* identification of micro-organisms by whole cell hybridization with rRNA-targeted nucleic acid probes. In: Akkerman, A.D.L., van Elsas, J.D., de Bruijn, F.J. (Eds.), Molecular Microbial Ecology Manual. Kluwer Academic Publishers, Dordrecht, pp. 1–15.

An, S., Joye, S.B., 2001. Enhancement of coupled nitrification-denitrification by benthic photosynthesis in shallow estuarine sediments. Limnol. Oceanogr. 46, 62–74.

Beman, J.M., Francis, C.A., 2006. Diversity of ammonia-oxidizing archaea and bacteria in the sediments of a hypernutrified subtropical estuary: Bahía del Tóbari, Mexico. Appl. Environ. Microbiol. 72, 7767–7777.

Bernhard, A.E., Bollmann, A., 2010. Estuarine nitrifiers: New players, patterns and processes. Estuar. Coast. Shelf Sci. 88, 1–11. http://dx.doi.org/10.1016/j.ecss.2010.01.023.

Bernhard, A.E., Landry, Z.C., Blevins, A., de la Torre, J.R., Giblin, A.E., Stahl, D.A., 2010. Abundance of ammonia-oxidizing archaea and bacteria along an estuarine salinity gradient in relation to potential nitrification rates. Appl. Environ. Microbiol. 76, 1285–1289. http://dx.doi.org/10.1128/AEM.02018-09.

Hu, B.L., Shen, L.D., Zheng, P., Hu, A.H., Chen, T.T., Cai, C., Liu, S., Lou, L.P., 2012. Distribution and diversity of anaerobic ammonium-oxidizing bacteria in the sediments of the Qiantang River. Environ. Microbiol. Rep. 4, 540–547. http://dx.doi.org/10.1111/j.1758-2229.2012.00360.x.

Bokulich, N.A., Subramanian, S., Faith, J.J., Gevers, D., Gordon, J.I., Knight, R., Mills, D.A., Caporaso, J.G., 2013. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nat. Methods 10, 57–59.

Bulow, S.E., Francis, C.A., Jackson, G.A., Ward, B.B., 2008. Sediment denitrifier community composition and *nirS* gene expression investigated with functional gene microarrays. Environ. Microbiol. 10, 3057–3069.

Cai, Y.H., 2006. The Diversity of Marine Phytoplankton in Hangzhou Bay. Ocean University of China, Dissertation.

Campbell, B.J., Kirchman, D.L., 2013. Bacterial diversity, community structure and potential growth rates along an estuarine salinity gradient. ISME J. 7, 210–220. http://dx.doi.org/10.1038/ismej.2012.93.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. U. S. A. 108, 4516–4522.

Dang, H., Jiao, N., 2014. Perspectives on the microbial carbon pump with special reference to microbial respiration and ecosystem efficiency in large estuarine systems. Biogeosciences 11, 3887–3898.

Dang, H., Zhang, X., Sun, J., Li, T., Zhang, Z., Yang, G., 2008. Diversity and spatial distribution of sediment ammonia-oxidizing crenarchaeota in response to estuarine and environmental gradients in the Changjiang Estuary and East China Sea. Microbiology 154, 2084–2095. http://dx.doi.org/10.1099/mic.0.2007/013581-0.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461.

Francis, C.A., Roberts, K.J., Beman, J.M., Santoro, A.E., Oakley, B.B., 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proc. Natl. Acad. Sci. U. S. A. 102, 14683–14688. http://dx.doi.org/10.1073/pnas.0506625102.

Hammer, Ø., Harper, D., Ryan, P., 2001. Past: paleontological statistics software package for education and data analysis. Palaeontol. Electron. 4, 9.

Hartman, W.H., Richardson, C.J., Vilgalys, R., Bruland, G.L., 2008. Environmental and anthropogenic controls over bacterial communities in wetland soils. Proc. Natl. Acad. Sci. U. S. A. 105, 17842–17847.

Head, I.M., Hiorns, W.D., Embley, T.M., McCarthy, A.J., Saunders, J.R., 1993. The phylogeny of autotrophic ammonia-oxidizing bacteria as determined by analysis of 16S ribosomal RNA gene sequences. J. Gen. Microbiol. 139, 1147–1153.

Heylen, K., Vanparys, B., Wittebolle, L., Verstraete, W., Boon, N., De Vos, P., 2006. Cultivation of denitrifying bacteria: optimization of isolation conditions and diversity study. Appl. Environ. Microbiol. 72, 2637–2643.

Howarth, R.W., Sharpley, A., Dan, W., 2002. Sources of nutrient pollution to coastal waters in the United States: Implications for achieving coastal water quality goals. Estuaries 25, 656–676.

Huo, Y.Z., SN, Xu, Wang, Y.Y., Zhang, J.H., Zhang, Y.J., WN, Wu, Chen, Y.Q., He, P.M., 2010. Bioremediation efficiencies of *Gracilaria verrucosa* cultivated in an enclosed sea area of Hangzhou Bay, China. J. Appl. Phycol. 23, 173–182. http://dx.doi.org/10.1007/s10811-010-9584-9.

Jin, T., Zhang, T., Ye, L., Lee, O.O., Yue, H.W., Qian, P.Y., 2011. Diversity and quantity of ammonia-oxidizing Archaea and Bacteria in sediment of the Pearl River Estuary, China. Appl. Microbiol. Biotechnol. 90, 1137–1145.

Kemp, W., Sampou, P., Caffrey, J., Mayer, M., Henriksen, K., Boynton, W.R., 1990. Ammonium recycling versus denitrification in Chesapeake Bay sediments. Limnol. Oceanogr. 35, 1545–1563.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O., 2015. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 41, e1. http://dx.doi.org/10.1093/nar/gks808.

Könneke, M., Bernhard, A.E., Jr, D.L.T., Walker, C.B., Waterbury, J.B., Stahl, D.A., 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437, 543–546.

Lam, P., Jensen, M.M., Lavik, G., McGinnis, D.F., Muller, B., Schubert, C.J., Amann, R., Thamdrup, B., Kuypers, M.M., 2007. Linking crenarchaeal and bacterial nitrification to anammox in the Black Sea. Proc. Natl. Acad. Sci. U. S. A. 104, 7104–7109.

Lepš, J., Šmilauer, P., 2003. Multivariate Analysis of Ecological Data Using CANOCO. Cambridge university press, Cambridge, UK.

Li, J., Nedwell, D.B., Beddow, J., Dumbrell, A.J., McKew, B.A., Thorpe, E.L., Whitby, C., 2014. *amoA* gene abundances and nitrification potential rates suggest that benthic ammonia-oxidizing bacteria (AOB) not archaea (AOA) dominate N cycling in the Colne estuary, UK. Appl. Environ. Microbiol. 81, 159–165. http://dx.doi.org/10.1128/AEM.02654-14.

Menoud, P., Wong, C.H., Robinson, H.A., Farquhar, A., Barford, J.P., Barton, G.W., 1999. Simultaneous nitrification and denitrification using Siporax™ packing. Water Sci. Technol. 40, 153–160.

Mincer, T.J., Church, M.J., Taylor, L.T., Preston, C., Karl, D.M., Delong, E.F., 2007. Quantitative distribution of presumptive archaeal and bacterial nitrifiers in Monterey Bay and the North Pacific Subtropical Gyre. Environ. Microbiol. 9, 1162–1175.

Mosier, A.C., Francis, C.A., 2008. Relative abundance and diversity of ammonia-oxidizing archaea and bacteria in the San Francisco Bay estuary. Environ. Microbiol. 10, 3002–3016. http://dx.doi.org/10.1111/j.1462-2920.2008.01764.x.

Mosier, A.C., Francis, C.A., 2010. Denitrifier abundance and activity across the San Francisco Bay estuary. Environ. Microbiol. Rep. 2, 667–676. http://dx.doi.org/10.

1111/j.1758-2229.2010.00156.x.

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural water. Anal. Chim. Acta 27, 31–36. http://dx.doi.org/10.1016/S 0003-2670(00)88444-5.

Paerl, H.W., Dennis, R.L., Whitall, D.R., 2002. Atmospheric deposition of nitrogen: implications for nutrient over-enrichment of coastal waters. Estuaries 25, 677–693.

Purkhold, U., Juretschko, S., Schmid, M.C., 2000. Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and *amoA* sequence analysis: implications for molecular diversity surveys. Appl. Environ. Microbiol. 66, 5368–5382.

Rabalais, N.N., 2002. Nitrogen in aquatic ecosystems. Ambio 31, 102–112.

Sahan, E., Muyzer, G., 2008. Diversity and spatio-temporal distribution of ammonia-oxidizing Archaea and Bacteria in sediments of the Westerschelde estuary. FEMS Microbiol. Ecol. 64, 175–186.

Salehlakha, S., Shannon, K.E., Henderson, S.L., Goyer, C., Trevors, J.T., Zebarth, B.J., Burton, D.L., 2009. Effect of pH and temperature on denitrification gene expression and activity in Pseudomonas mandelii. Appl. Environ. Microbiol. 75, 3903–3911.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541.

Seitzinger, S., Harrison, J.A., Böhlke, J., Bouwman, A., Lowrance, R., Peterson, B., Tobias, C., Drecht, G.V., 2006. Denitrification across landscapes and waterscapes: a synthesis. Ecol. Appl. 16, 2064–2090.

Shapleigh, J.P., 2006. The Denitrifying Prokaryotes. In: Dworkin, M. (Ed.), The Prokaryotes. Springer, New York, pp. 769–792.

Shen, L.D., Liu, S., Lou, L.P., Liu, W.P., XY, Xu, Zheng, P., BL, Hu, 2013. Broad distribution of diverse anaerobic ammonium-oxidizing bacteria in Chinese agricultural soils. Appl. Environ. Microbiol. 79, 6167–6172.

Smith, L.K., Voytek, M.A., Böhlke, J.K., Harvey, J.W., 2006. Denitrification in nitrate-rich streams: application of N_2: Ar and ^{15}N-tracer methods in intact cores. Ecol. Appl. 16, 2191–2207.

Smith, J.M., Casciotti, K.L., Chavez, F.P., Francis, C.A., 2014. Differential contributions of archaeal ammonia oxidizer ecotypes to nitrification in coastal surface waters. ISME J. 8, 1704–1714.

SOA, 2014. Bulletin of China's Marine Environmental Status. People's Republic of China, State Oceanic Administration.

Strickland, J.D., Parsons, T.R., 1972. A practical handbook of seawater analysis, 2nd edn. Bull. Fish. Res. Bd. Canada 167, 310.

Suzumura, M., Kokubun, H., Arata, N., 2004. Distribution and characteristics of suspended particulate matter in a heavily eutrophic estuary, Tokyo Bay, Japan. Mar. Pollut. Bull. 49, 496–503.

Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73, 5261–5267.

Wang, L., Zheng, B., Nan, B., Hu, P., 2014. Diversity of bacterial community and detection of *nirS*- and *nirK*-encoding denitrifying bacteria in sandy intertidal sediments along Laizhou Bay of Bohai Sea, China. Mar. Pollut. Bull. 88, 215–223. http://dx.doi.org/10.1016/j.marpolbul.2014.09.002.

Ward, B.B., O'Mullan, G.D., 2002. Worldwide distribution of *Nitrosococcuss* oceani, a marine ammonia-oxidizing γ-proteobacterium, detected by PCR and sequencing of 16S rRNA and *amoA* genes. Appl. Environ. Microbiol. 68, 4153–4157.

Ward, B.B., Eveillard, D., Kirshtein, J.D., Nelson, J.D., Voytek, M.A., Jackson, G.A., 2007. Ammonia-oxidizing bacterial community composition in estuarine and oceanic environments assessed using a functional gene microarray. Environ. Microbiol. 9, 2522–2538.

Ward, B.B., Devol, A.H., Rich, J.J., Chang, B.X., Bulow, S.E., Naik, H., Pratihary, A., Jayakumar, A., 2009. Denitrification as the dominant nitrogen loss process in the Arabian Sea. Nature 461, 78–81.

Wuchter, C., Abbas, B., Coolen, M.J., Herfort, L., van Bleijswijk, J., Timmers, P., Strous, M., Teira, E., Herndl, G.J., Middelburg, J.J., 2006. Archaeal nitrification in the ocean. Proc. Natl. Acad. Sci. U. S. A. 103, 12317–12322.

Xie, D., Wang, Z., Gao, S., De Vriend, H.J., 2009. Modeling the tidal channel morphodynamics in a macro-tidal embayment, Hangzhou Bay, China. Cont. Shelf Res. 29, 1757–1767. http://dx.doi.org/10.1016/j.csr.2009.03.009.

Yu, Z., Yang, J., Liu, L., 2014. Denitrifier community in the oxygen minimum zone of a subtropical deep reservoir. PLoS One 9, e92055.

Zehr, J.P., Ward, B.B., 2002. Nitrogen cycling in the ocean: new perspectives on processes and paradigms. Appl. Environ. Microbiol. 68, 1015–1024. http://dx.doi.org/10.1128/aem.68.3.1015-1024.2002.

Zhang, J., Shi, Q., Wu, A., Ye, X., Zhu, G., 2002. Distribution characteristic analysis of main pollution factor in rainy season in the Hangzhou Bay. Donghai Mar. Sci. 20, 35–41.

Zheng, B., Wang, L., Liu, L., 2014a. Bacterial community structure and its regulating factors in the intertidal sediment along the Liaodong Bay of Bohai Sea, China. Microbiol. Res. 169, 585–592. http://dx.doi.org/10.1016/j.micres.2013.09.019.

Zheng, Y., Hou, L., Newell, S., Liu, M., Zhou, J., Zhao, H., You, L., Cheng, X., 2014b. Community dynamics and activity of ammonia-oxidizing prokaryotes in intertidal sediments of the Yangtze estuary. Appl. Environ. Microbiol. 80, 408–419. http://dx.doi.org/10.1128/AEM.03035-13.

Zhong, F., Wu, J., Dai, Y., Yang, L., Zhang, Z., Cheng, S., Zhang, Q., 2015. Bacterial community analysis by PCR-DGGE and 454-pyrosequencing of horizontal subsurface flow constructed wetlands with front aeration. Appl. Microbiol. Biotechnol. 99, 1499–1512.