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Denitrification and Anaerobic Ammonium Oxidization Across the Sediment–Water Interface in the Hypereutrophic Ecosystem, Jinpu Bay, in the Northeastern Coast of China

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Abstract Denitrification and anaerobic ammonium oxidization (anammox) are considered the most important processes of removing reactive nitrogen from natural aquatic environments. We measured and compared in situ rates of the two processes across the sediment–water interface of Jinpu Bay using continuous-flow experiments combined with a $^{15}NO_3^-$ tracing technique to determine their relative importance in this hypereutrophic coastal ecosystem. Rates of denitrification and anammox ranged from 1.76 to 327.97 and 0.33 to 36.32 μmol N m^{-2} day^{-1}, respectively. Both the denitrification and anammox processes were observed to be associated closely with the bioavailability of organic matter and concentrations of sulfide and iron oxides in sediments. Denitrification was the dominant pathway of eliminating reactive nitrogen and on average accounted for about 90 % of the total removed nitrogen. Totally, both the denitrification and anammox processes removed about 20 % of the externally derived inorganic nitrogen within the system. Most of the external nitrogen was still retained in the ecosystem, which may cause the severe eutrophication and algae blooms occurring at the study area.

Keywords Nitrogen · Denitrification · Anammox · Sediment–water interface · Jinpu Bay

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Introduction

The annual input of anthropogenic reactive nitrogen into the global biosphere is presently estimated as much as 150 Tg N (Mosier and Kroeze 2000; Galloway and Cowling 2002). Much of the nitrogen, often in the form of nitrate, enters streams and is transported downriver to estuarine and coastal regions (Seitzinger 2008). Excessive nitrogen loading has exerted a serious threat to the environmental quality of these ecosystems (Burgin and Hamilton 2007; Diaz and Rosenberg 2008). Increased nitrogen concentration can fuel excessive growth and decay of phytoplankton, including harmful algae blooms, in the process of estuarine and coastal eutrophication. This scenario can lead to hypoxia and (or) anoxia in bottom waters of estuarine and coastal areas (Vitousek et al. 1997; Rabalais 2002; Burgin and Hamilton 2007; Deegan et al. 2012). An improved understanding of nitrogen transformations and fates on local and global scales in coastal regions, with different degrees of eutrophication, is required to develop strategies to protect the water quality and health of estuarine and coastal environments (Gardner and McCarthy 2009; Dong et al. 2011; Hou et al. 2012).

Denitrification and anaerobic ammonium oxidization (anammox) are important processes in the nitrogen cycle that remove reactive nitrogen from aquatic environments (Seitzinger 1988; Rysgaard et al. 2004; Kumar and Lin 2010; Fernandes et al. 2012; Hou et al. 2013). Both processes

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play a significant role in counteracting the effects of aquatic eutrophication induced by nitrogen overload. These nitrogen transformation processes are linked to temperature, dissolved oxygen, relative availability of labile carbon, sulfide, and (or) metal oxides (Postma et al. 1991; Straub et al. 1996; Strous et al. 1999; Thamdrup and Dalsgaard 2002; Weber et al. 2006; Jensen et al. 2009; Hou et al. 2012). However, exact factors controlling denitrification and anammox processes and their relative importance in the nitrogen removal remain unclear for specific hypereutrophic environments.

Jinpu Bay, which is located east of the Bohai Sea of China, is a shallow coastal region polluted heavily from increasing population, developing industry, and riverine inputs (Ma et al. 2001; Bu et al. 2011; Gao et al. 2012). Concentrations of inorganic nitrogen in overlying water of Jinpu Bay have on average increased up to 40-fold over the past several decades. Eutrophication caused by excessive enrichment of reactive nitrogen has been identified as the most serious environmental problem in this area (Liu and Yin 2010; Liu et al. 2011) and other comparable polluted regions. Information on the transformation rates and fates of nitrogen is needed to understand mechanisms and causes of eutrophication in hypereutrophic coastal ecosystems. However, little is known about exact nitrogen transformation processes in many severely polluted environments, represented by our study area. We conducted continuous-flow experiments to quantify the denitrification and anammox processes, in combination with a nitrogen isotope-pairing technique modified by Risgaard-Petersen et al. (2003) and Trimmer et al. (2006). In contrast with slurry incubations that often destroy sediment structure and redox gradients, the continuous-flow experiments with intact sediment cores can improve understanding of the in situ activities of denitrification and anammox in sediments. The main objectives of this study were (1) to investigate the actual denitrification and anammox rates across the sediment–water interface of Jinpu Bay, (2) to elucidate environmental factors controlling the denitrification and anammox processes, and (3) to evaluate the roles of both the denitrification and anammox processes in removing reactive nitrogen in these polluted coastal environments.

Material and Methods

Study Area

Jinpu Bay consists of Jinzhou Bay and Pulandian Bay, a semi-enclosed bay with a total area of about 2,000 km^2 (Fig. 1). The average water depths of Jinzhou Bay and Pulandian Bay are 3.5 and 5.5 m, respectively. Salinity ranges from 29.3 to 31.8 ppt, with an average of about 31 ppt in the two bays. The coastal zone surrounding the Jinpu Bay is an important industrial base in China (Wang and Jia 2010). Annually, more than 3.1×10^7 t effluent enriched in organic matter, inorganic nitrogen, and phosphate is discharged from the coastal rivers, and industrial and municipal sewage outlets into the Bay, which makes the ecosystem one of the most nutrient-polluted areas along the coastal seas of China.

Sampling and Pretreatment

Sediments were collected during two cruises in late spring (June) of 2011 and early autumn (September) of 2012 with a box corer. Sub-cores of "intact" sediment (10–20 cm deep) were collected by sub-coring the box corers with six respective core-liner tubes (i.d. 7.0 cm) at each site (Fig. 1). Twenty liters of near-bottom water was sampled at each site for nutrient analysis and water column incubation experiments. After collection, all samples were transported on ice to the laboratory within 6 h, and three of six sediment sub-cores from each site were used for the continuous-flow experiments. Surface (0–5 cm) sediment was collected from the remaining three sub-cores inside a N_2-filled chamber for later analyses of sediment physiochemical characteristics. In detail, a portion of surface sediment was placed in a 60-ml centrifuge tube, centrifuged at a speed of 3,000 rpm and near in situ temperature for 40 min, and supernatant water was filtered through 0.2-μm pore-size nylon membrane syringe filters to provide porewater samples for dissolved inorganic nitrogen and sulfide analyses. The tubes were filled completely with N_2 before centrifugation to prevent oxidation of the sulfide. The remaining surface sediment of each site was freeze-dried for later analyses of grain size, amorphous Fe oxide, organic carbon, and nitrogen. In addition, about 300 ml overlying water at each site was filtered with glass microfiber filters (Whatman GF/F, No. 1845-047) for measurement of chlorophyll (Chl-a).

Continuous-Flow Experiments

Three intact sediment sub-cores from each site were installed respectively into a "gas-tight" continuous-flow system (Lavrentyev et al. 2000; Lin et al. 2011; Hou et al. 2012). The system consisted of a 20-L gas-tight bag (Tedlar) containing the site water, a multi-channel peristaltic pump, Peek transmission tubing, an acetol plunger with Viton o-ring, and an intact sediment core (McCarthy et al. 2008; Hou et al. 2012). The core was darkened with aluminum foil, and the plunger was positioned ~5 cm above the sediment surface to give ~230 ml of overlying water volume. The pump transfers water from the gas-tight bag and continuously displaces overlying water above the core at a flow rate of 1.5 ml min^{-1}. After the sediment core was incubated at near in situ temperature and dissolved oxygen for about 24 h to establish a steady-state exchange condition (Gardner and McCarthy 2009), the inflow water was enriched with $^{15}NO_3^-$ (final concentration ca. 100 μmol L^{-1}, final % ^{15}N ca. 90–99 %, depending on the

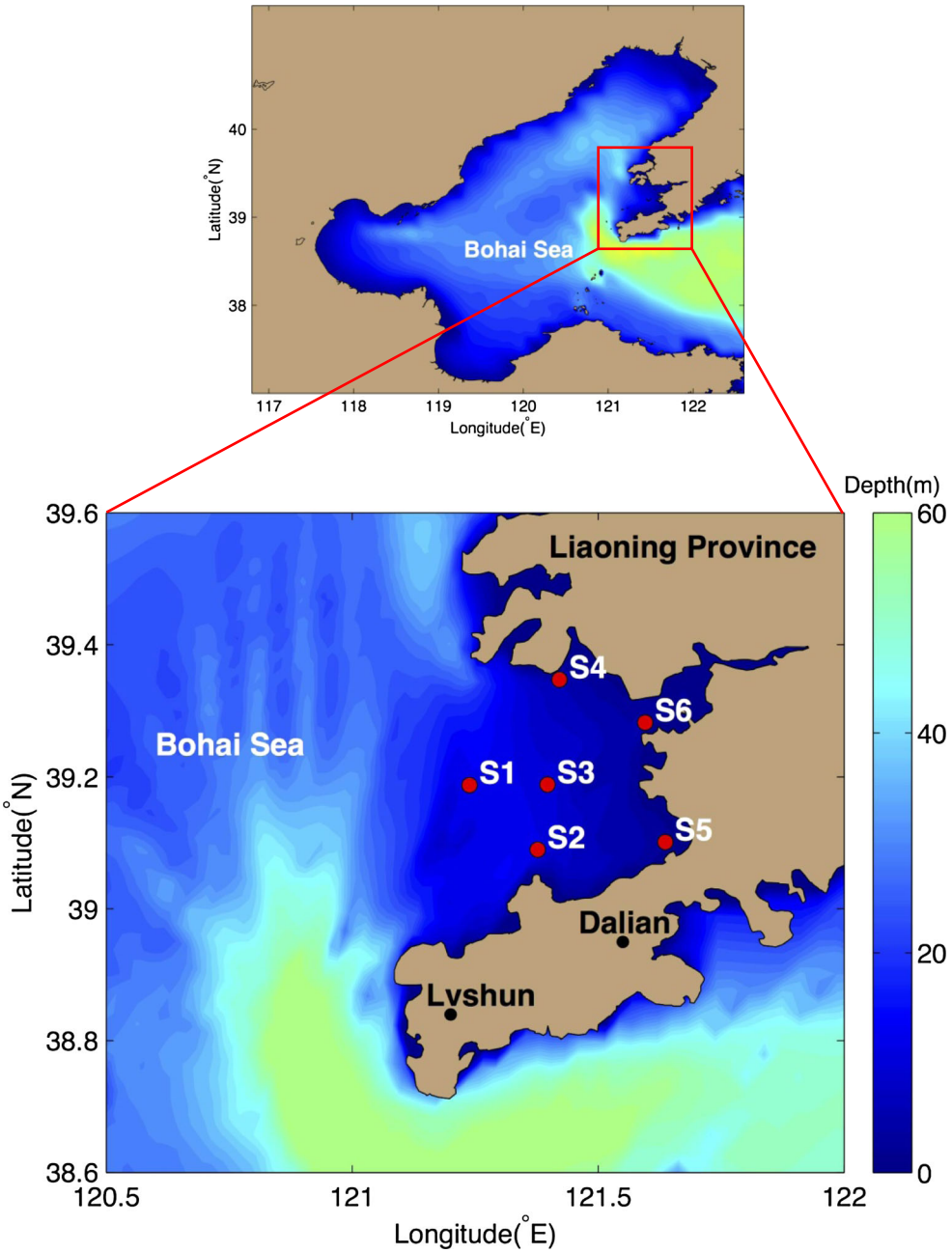

Fig. 1 Map of Jinpu Bay showing the sampling sites S1–6

background nitrate concentration). The incubation was continued overnight to allow the continuous-flow system to reach steady state (Gardner and McCarthy 2009). Within the next 48 h, inflow and outflow waters were sampled every 8 h for dissolved nitrogen gas analyses. In total, 36 water samples were collected from each core. After collection, water samples were preserved in gas-tight vials (12 ml Exetainer, Labco, High Wycombe, UK) with 250 μL 50 % $ZnCl_2$ solution and analyzed within 6 h. Dissolved nitrogen gases were measured by membrane inlet mass spectrometry with a detection limit of 0.06 μmol L^{-1} (An and Gardner 2002; Tortell 2005). In order to calculate the concentrations of dissolved $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$, 0- and 40-ppt artificial seawater were held at 25 °C as external standards (Kana et al. 1994; An et al. 2001). The rates of the denitrification and anammox processes at the sediment–water interface were calculated from the differences in concentrations of dissolved nitrogen gases between inflow and outflow samples, flow rates, and cross-sectional area of the cores (Lavrentyev et al. 2000; Gardner and McCarthy 2009), which were expressed as micromoles of N per square meter per day.

Calculation of Denitrification and Anammox Rates

The denitrification rates, together with anammox rates in the continuous-flow experiments, were calculated via the revised isotope pairing technique (r-IPT) proposed by

Risgaard-Petersen et al. (2003) and Trimmer et al. (2006). Briefly, the total production of N_2 (p_{14}) from both the denitrification and anammox processes during the incubations was quantified by the following equation:

$$p_{14} = 2 \times r_{14} \times \left(p^{29}N_2 + p^{30}N_2 \times (1 - r_{14})\right) \quad (1)$$

where $p^{29}N_2$ and $p^{30}N_2$ are the total measured $^{29}N_2$ and $^{30}N_2$ production rates and r_{14} is the ratio between $^{14}NO_3^-$ and $^{15}NO_3^-$ in the NO_3^- reduction zone. The r_{14} was estimated from the contribution of anammox to the total production of N_2 (r_a) measured within anaerobic slurry experiments and $p^{29}N_2$ and $p^{30}N_2$ in the light of:

$$r_{14} = \left((1 - r_a) \times p^{29}N_2 / p^{30}N_2 - r_a\right) / (2 - r_a) \quad (2)$$

The detailed information on the sediment slurry experiments was described in Engström et al. (2005) and Trimmer et al. (2006). Meanwhile, the anammox rate ($p_{14\text{-AAO}}$) during the incubations was determined according to:

$$p_{14\text{–AAO}} = 2 \times r_{14} \times \left(p^{29}N_2 - 2 \times r_{14} \times p^{30}N_2\right) \quad (3)$$

Then, the rate of denitrification was estimated as the difference between Eqs. 1 and 3.

Geochemical Analysis

Salinity was measured in situ with an YSI-30 portable salinity meter. Water temperature and dissolved oxygen (DO) were determined using an YSI-550A portable DO Meter immediately after the water samples were collected. The concentrations of nutrients were measured by a SKALAR Segmented Flow Analyzer (SAN plus System) with detection limits of 0.5 μmol L^{-1} for ammonium and 0.1 μmol L^{-1} for nitrate (plus nitrite). Chl-a was determined using a spectrophotometric assay and standard equations, after extraction with a 45:45:10 solvent mixture (acetone/methanol/water) for 24 h at 0 °C (Liu et al. 2006). The contents of organic carbon and nitrogen were determined by CHNOS Elementary Analyzer (Vario EL III) with detection limits of 0.4 μg for carbon and 0.1 μg for nitrogen, after removing carbonate by leaching with 0.1 mol L^{-1} HCl (Hou et al. 2012). Grain size of sediment was measured using a LS 13 320 Laser grain sizer. Concentration of amorphous Fe oxides was quantified by extracting 0.5 g of sediment with 30 ml of 0.5 mol l^{-1} HCl, followed by colorimetric (Ferrozine) determination (Roden and Lovley 1993). Sulfide was measured using an Orion Sure-flow® combination silver–sulfide electrode (Thermo Scientific Orion) with a detection limit of 0.09 μmol l^{-1}. All physiochemical parameters of sediments were analyzed in triplicate.

Data Analysis

In this work, one-way ANOVA was performed to examine whether temporal and spatial differences in obtained data were statistically significant at the 95 % confidence level, and correlation analyses were also done for comparisons. All statistical analyses were conducted using Statistical Package of Social Sciences (SPSS, version-11.5).

Results

Station Characteristics

Site water depths during the two cruises ranged from 5 to 15 m (Table 1). Salinity in June was slightly higher than that in September, with the respective ranges of 31.1–31.6 and 30.5–30.9 ppt. Temperature ranged from 13.4 to 15.7 °C in June and 21.1 to 21.4 °C in September. Dissolved oxygen was near saturation in June and ranged from 6.21 to 7.40 mg L^{-1}. However, it was lower in September, ranging from 3.49 to 4.27 mg L^{-1}. The concentrations of bottom water ammonium were relatively low at the sampling sites, ranging from 3.73 to

Table 1 Physiochemical characteristics of near-bottom water at the sampling sites

Station	S1		S2		S3		S4		S5		S6	
	Jun	Sep	Jun	Sep	Jun	Sep	Jun	Sep	Jun	Sep	Jun	Sep
Water depth (m)	15	15	12	14	11	11	5	9	9	5	5	6
S (ppt)	31.6	30.9	31.4	30.7	31.4	30.8	31.4	30.7	31.1	30.7	31.3	30.5
Temp (°C)	13.4	21.1	14.6	21.3	15.3	21.4	15.7	21.1	16.4	21.3	15.1	21.3
DO (mg L^{-1})	7.40	3.87	7.30	3.75	6.71	4.27	6.83	3.76	6.21	3.49	6.62	3.82
NH_4^+ (μmol L^{-1})	5.45	3.77	4.94	3.36	3.73	3.75	5.56	3.74	10.4	7.63	5.71	4.29
NO_x^- (μmol L^{-1})	7.21	8.38	11.0	6.26	10.1	3.44	11.3	8.46	38.97	9.17	11.01	10.5
Chl a (μg L^{-1})	9.51	10.3	12.5	13.4	13.2	11.6	17.9	14.4	21.7	23.5	22.7	21.3

S salinity, *Temp* temperature, *DO* dissolved oxygen, NO_x^- nitrate plus nitrite

10.40 μmol L^{-1} in June and 3.36 to 7.63 μmol L^{-1} in September. Concentrations of bottom water nitrate were higher with the ranges of 7.21–38.97 and 3.44–10.46 μmol L^{-1} in June and September, respectively. During the two sampling periods, concentrations of chlorophyll a were relatively high, with values of 10.33–23.47 μg L^{-1} in June and 9.51–22.72 μg L^{-1} in September.

Study area sediments were characterized as sand-silt, with respective clay (<4 μm), silt (4–63 μm), and sand (>63 μm) contents accounting for about 9.55–24.70, 19.75–66.90, and 13.96–70.70 % of the total sediments (Table 2). The contents of organic carbon and nitrogen in June sediments ranged from 400 to 600 μmol g^{-1} and from 19 to 33 μmol g^{-1}, respectively. In contrast, the contents of organic carbon and nitrogen in most September sediments were lower, with ranges of 55–545 and 2.3–42 μmol g^{-1}, respectively. The concentrations of reactive Fe oxides in June sediments were lower than those in September sediments, with the ranges of 38–50 and 34–68 μmol g^{-1}, respectively. The concentrations of nitrate (plus nitrite) in porewater varied between 25 and 48 μmol L^{-1} in June and were lower in September with the values of 6.2–14.6 μmol L^{-1}. Similarly, the concentrations of ammonium in sediment porewater were higher in June than in September, with values of 34–80 and 9.7–22 μmol L^{-1}, respectively. The concentrations of sulfide in sediment porewater ranged from 1.5 to 6.5 μmol L^{-1} in June and from 1.6 to 5.0 μmol L^{-1} in September.

Denitrification Rates

The denitrification rates ranged from10 to 328 and 1.8 to 289 μmol N m^{-2} day^{-1} in June and September, respectively (Fig. 2). A significant temporal variation of the denitrification rates was observed at the study area (one-way ANOVA, F=2.82, n=36, df=30, p=0.041). Meanwhile, there was a remarkable spatial difference in denitrification rates among sampling sites (one-way ANOVA, F=5.53, n=18, df=12, p=0.007 for June; F=6.25, n=18, df=12, p=0.004 for September). Generally, the lowest denitrification rate in June was detected at site S1, whereas the highest denitrification rate was measured at site S5. However, in September, higher rates of denitrification appeared at sites S1 and S2, and the lowest rate occurred at S5. The denitrification rates were correlated positively with concentrations of organic carbon and nitrogen and reactive Fe oxides in sediments (Table 3; Fig. S1). In contrast, the denitrification rates related negatively to C/N molar ratios and sulfide concentrations in sediment porewater.

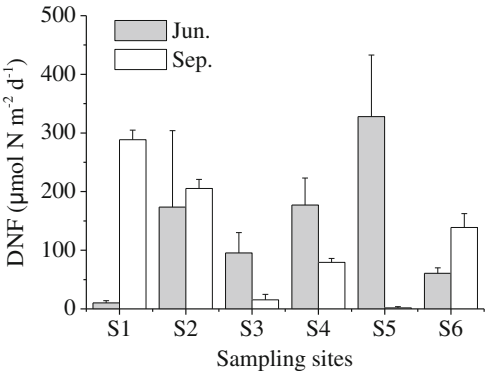

Fig. 2 Denitrification (*DNF*) rates in the sediments of Jinpu Bay in June and September, respectively. *Bars* denote standard error of triplicate samples

Anammox Rates

Compared to denitrification rates, the anammox process accounted for only a small proportion of the nitrate reduction at the study area, with the rates of 0.3–36 μmol N m^{-2} day^{-1} (Fig. 3). A significant seasonal variation in the anammox rates was observed (one-way ANOVA, F=3.02, n=36, df=30, p=0.032). In general, the anammox rates were higher in June than in September at the sampling sites, except for site S2. At the study area, the spatial difference in the anammox rates was

Table 2 Physiochemical characteristics of sediments at the sampling sites

Station	S1		S2		S3		S4		S5		S6	
	Jun	Sep	Jun	Sep	Jun	Sep	Jun	Sep	Jun	Sep	Jun	Sep
Clay (%)	17.3	16.9	19.3	19.1	20.1	16.8	16.7	24.7	17.0	9.6	19.1	13.1
Silt (%)	42.2	37.1	66.7	45.6	50.5	43.3	51.3	60.9	56.7	19.8	66.9	35.4
Sand (%)	40.5	46.0	14.0	35.3	29.5	39.9	32.0	14.4	26.3	70.7	14.0	51.5
OC (μmol g^{-1})	510	545	403	420	442	187	600	471	575	55	567	322
ON (μmol g^{-1})	26	42	19	24	29	11	25	27	33	2	32	14
Fe oxides (μmol g^{-1})	40.2	67.7	45.2	59.8	38.4	53.2	43.1	50.7	41.6	33.9	49.5	56.3
NO_x^- (μmol L^{-1})	24.8	14.6	26.0	11.5	32.9	6.2	22.2	13.0	29.8	12.7	48.4	14.3
NH_4^+ (μmol L^{-1})	34.4	15.9	73.8	20.4	62.1	9.7	41.9	22.2	56.9	21.4	80.2	16.0
Sulfide (μmol L^{-1})	6.5	2.4	2.7	1.6	1.9	2.4	1.5	3.7	3.2	5.0	3.1	3.6

OC organic carbon, *ON* organic nitrogen, NO_x^- nitrate plus nitrite

Table 3 Correlation analyses between nitrogen transformation (denitrification and anammox) rates and physiochemical characteristics of sediments from the Jinpu Bay (n=36)

Transformations (μmol N m^{-2} day^{-1})	Clay (%)	Silt (%)	Sand (%)	OC (μmol g^{-1})	ON (μmol g^{-1})	C/N	NO_x^- (μmol L^{-1})	NH_4^+ (μmol L^{-1})	Fe oxides (μmol g^{-1})	Sulfides (μmol L^{-1})
Correlation										
DNF	0.138	0.165	−0.159	0.539[a]	0.607[a]	−0.313[b]	0.047	0.061	0.438[a]	−0.289[b]
Anammox	0.125	0.259	−0.222	0.430[a]	0.410[a]	−0.189	0.237	0.193	−0.107	0.397[a]
Sig. (1-tailed)										
DNF	0.212	0.168	0.177	0.000	0.000	0.032	0.392	0.362	0.004	0.044
Anammox	0.233	0.064	0.093	0.004	0.007	0.135	0.082	0.129	0.268	0.008

DNF denitrification, *anammox* anaerobic ammonium oxidization, *OC* organic carbon, *ON* organic nitrogen, *C/N* the molar ratio of organic carbon to nitrogen, NO_x^- nitrate plus nitrite

[a] Means that correlation is significant at the 0.01 level

[b] Means that correlation is significant at the 0.05 level

also remarkable (one-way ANOVA, F=16.39, n=18, df=12, p<0.0001 for June; F=3.11, n=18, df=12, p=0.05 for September). In June, the highest anammox rate occurred at site S5 and the lowest at site S2. In September, the highest and lowest anammox rates, however, were detected at site S1 and S5, respectively. Additionally, anammox rates correlated positively with organic carbon and sulfide (Table 3; Fig. S1).

Discussion

Among nitrogen transformation processes, denitrification, anammox, and dissimilatory nitrate reduction to ammonium (DNRA) are the most important processes of dissimilatory nitrate reduction in aquatic environments (Ward 1996; Rysgaard et al. 2004; Dalsgaard et al. 2005; Piña-Ochoa and Álvarez-Cobelas 2006; Burgin and Hamilton 2007). However, these processes play different roles in controlling the fates of nitrogen. DNRA retains transformed inorganic nitrogen in ecosystems as a bioavailable form, whereas denitrification and anammox processes can remove bioavailable nitrogen from aquatic ecosystems (Seitzinger 1988; Hietanen and Kuparinen 2008). Denitrification and anammox, the dominant processes eliminating reactive nitrogen from aquatic environments (Dong et al. 2009; Crowe et al. 2012), are closely associated with the environmental characteristics of sediments (Burgin and Hamilton 2007; Hou et al. 2012). In this study, the rates of denitrification and anammox resemble those in intact sediment cores from other estuarine and coastal ecosystems (Rysgaard et al. 2004; Gardner et al. 2006; Trimmer and Nicholls 2009; Neubacher et al. 2011; Crowe et al. 2012).

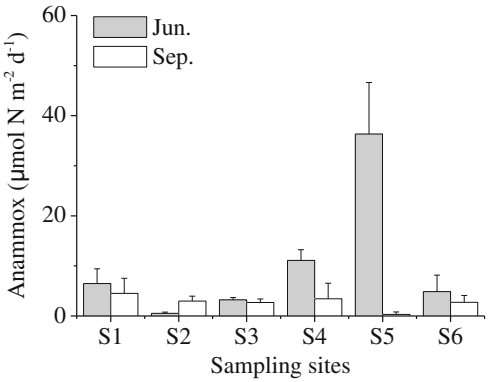

Fig. 3 Anaerobic ammonium oxidation (anammox) rates in Jinpu Bay sediments during June and September, respectively. *Bars* denote standard error of triplicate samples

Denitrification rates have been shown to increase exponentially with temperature over the range of ca. 10–30 °C (Nowicki 1994; Bachand and Horne 2000). However, no positive response of denitrification rates to temperature changes was observed in our study, although temperature showed high seasonality. Thus temperature was not a significant factor affecting the denitrification process. In contrast, the temporal and spatial fluctuations of denitrification rates may be attributed to geochemical characteristics of the site sediments (Table 3). Significant relationships of denitrification rates with organic carbon and nitrogen contents suggest that denitrification at the study area was driven mainly by microbial decomposition of organic matter (Burgin and Hamilton 2007), consequently affecting the temporal and spatial variations of denitrification activities. The changes in the activities of denitrifying bacteria were influenced by the bioavailability of organic matter in other environments (Thamdrup and Dalsgaard 2002; Engström et al. 2005; Dodla et al. 2008), as evidenced by the negative correlation of denitrification rates with C/N molar ratios. Both quantity and quality of organic matter were important in controlling denitrification rates (Dodla et al. 2008 and references therein). In addition, at this bay, the

denitrification may be coupled to ferrous Fe oxidation according to the following expected stoichiometric equation (Straub et al. 1996):

$$10Fe^{2-} + 2NO_3^- + 12\,H^+ \rightarrow 10Fe(OH)_3 + N_2 + 6H_2O \quad (4)$$

The positive correlation of denitrification rates with Fe oxides contents may, to some extent, support the facilitation of that pathway (Table 3). Nitrate may be reduced to nitrite by ferrous Fe oxidation, followed by rapid reaction of nitrite to dinitrogen (Postma et al. 1991; Weber et al. 2001, 2006). The observed negative correlation of denitrification rates with sulfide concentrations in porewater implies that the denitrification activity at the study area may be inhibited by sulfide in the sediments (Table 3), even though denitrification may be coupled to sulfide oxidation as an electron donor for nitrate reduction (Jensen et al. 2009). This inhibition may be associated with the adverse effect of sulfide on nitrogen monoxide and nitrous oxide reductases (Sørensen et al. 1980; Brunet and Garcia-Gil 1996; and references therein), thereby decreasing the reduction of N_2O to N_2 in denitrification. Therefore, denitrification rates were influenced concurrently by the geochemical compositions of site sediments.

Anammox had a remarkable seasonal variation at our respective sites. In general, there were relatively higher anammox rates in June than in September. However, we did not observe a temperature effect on the anammox rates even though temperature can be important in modulating seasonal fluctuations in anammox rates (Strous et al. 1999; Dosta et al. 2008; Hou et al. 2012). Factors other than temperature were considered to be more important regulators of anammox activity during our investigation. Anammox activity and its relative contribution to total N_2 production were linked closely to the availability of organic carbon in other studies (Thamdrup and Dalsgaard 2002; Engström et al. 2005; Trimmer and Nicholls 2009). In agreement with those studies, anammox rates related positively with the content of sedimentary organic carbon at our sites. This association may be attributed to a coupling among anammox, denitrification, and organic carbon decomposition. During heterotrophic denitrification driven by organic matter, nitrite may be generated as intermediate product and provided for metabolic activities of anammox bacteria (Thamdrup and Dalsgaard 2002; Trimmer et al. 2003; Hou et al. 2013). This hypothesis is supported by a close positive correlation between the anammox and denitrification rates ($n=36$, $r=0.43$, $p=0.009$). Interestingly, the anammox rates were also observed to positively relate to sulfide concentrations in the sediments. This relationship may imply that sulfide can enhance nitrite accumulation in the incomplete denitrification process via inhibiting nitrogen monoxide and nitrous oxide reductases (Sørensen et al. 1980; Brunet and Garcia-Gil 1996), thus stimulating the anammox reaction indirectly. Alternatively, the observed correlation between anammox and sulfide is likely attributed to the association that denitrification locally reduces sulfide concentrations so that immediately surrounding denitrification hot spots, anammox can also occur (Prokopenko et al. 2013). However, further work is still needed to verify these hypotheses.

In agreement with the idea that denitrification is the dominant process removing reactive nitrogen from aquatic ecosystems, denitrification contributed 60–100 and 85–98 % to the total N_2 production in June and September, respectively, at Jinpu Bay. The contributions of anammox to the total dinitrogen production ranged from 0.3 to 38.8 % in June and 1.5 to 14.7 % in September, with an average of 8.8 %. Thus, anammox also removed significant reactive nitrogen at the study area. If the measured denitrification and anammox rates were extrapolated to the entire bay, about 1.4×10^3 t N was removed annually from the aquatic ecosystem by the sum of these two processes. This removal accounts for about 20 % of the total inorganic nitrogen (about 7.0×10^3 t N) transported annually by the coastal rivers, sewage outlets, and atmospheric deposition into the bay (Cui 2001; Yang et al. 2012; Zong et al., unpublished data). The removal amount is lower than the 30–60 % removed from other estuarine and coastal ecosystems (e.g., Seitzinger 1988; Seitzinger and Kroeze 1998; and references therein), although there are comparable denitrification and anammox rates (Rysgaard et al. 2004; Gardner et al. 2006; Trimmer and Nicholls 2009; Neubacher et al. 2011; Crowe et al. 2012; Hou et al. 2012). Interestingly, this comparison may imply that the efficiency of nitrogen removal decreases with increasing eutrophication because the nitrogen removal capacity in those aquatic ecosystems becomes relatively saturated once the nitrate concentrations are increased to a certain level. In addition, most external inorganic nitrogen was still retained in this hypereutrophic ecosystem, which may be the main reason for high levels of chlorophyll and frequent occurrence of harmful algal blooms at the study area (Wei et al. 2008). These results should help guide future efforts to control reactive nitrogen pollution within the Jinpu Bay catchment and other hypereutrophic coastal environments.

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