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Nitrogen removal by denitrification and anammox processes in freshwater rivers of high nitrogen loading region of China

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Abstract

The importance of anaerobic ammonium oxidation (anammox) – a metabolic pathway that can generate dinitrogen – remains poorly understood in freshwater river system. Using the ^{15}N-isotope pairing technique (^{15}N IPT) combined with membrane inlet mass spectrometry (MIMS), the potential rates of denitrification, anammox and total N removal, and their respective contributions to total N_2 production were evaluated in 11 rivers in the Taihu Lake region of China. The measured potential rates of denitrification, total N removal and anammox varied from 18.9±0.3 to 70.0±11.4, 26.3±0.4 to 71.3±11.1 and 1.3±0.3 to 11.0±2.5 μmol N m^{-2} h^{-1}, respectively. The relative contribution of anammox to total N_2 production (ra%) ranged from 2.0±0.8% to 29.9±0.7%. The mean potential denitrification and the total N removal rates varied spatially in these 11 rivers, with the highest rates occurring in the western rivers of the region, while the mean potential anammox rates and ra% displayed the opposite trend with the highest values occurring in the southern rivers of the region. The contents of nitrate and dissolved organic carbon in sediments appeared to be the primary controlling factors for denitrification and anammox in these studied rivers. Our results indicated that the potential rates of N removal varied spatially, and denitrification is the dominant activity for removing fixed N but the role of anammox is not negligible in freshwater rivers.

Keywords: Taihu Lake region; membrane inlet mass spectrometry; ^{15}N isotope pairing technique; sediment; denitrification; anammox

1. Introduction

Anthropogenic addition of bioavailable nitrogen (N) to aquatic systems have increased in recent decades, and surplus N affects the ecology, quality, and value of aquatic ecosystems (Mulholland *et al.,* 2008; Sun *et al.,* 2012; Psarropoulou and karatzas, 2014). Understanding the mechanisms of N removal in aquatic systems is especially important. Denitrification was assumed to be the dominant mechanism of N loss in aquatic systems (Seitzinger, 1988). However, the discovery of anaerobic oxidation of ammonium (anammox) revolutionized the N cycle by demonstrating that ammonium was also oxidized under anoxic conditions in 1995 (Mulder *et al.,* 1995). The anammox reaction uses nitrite as an electron acceptor to convert ammonium into dinitrogen gas under strictly anoxic conditions (Dalsgaard *et al.,* 2005). Since their initial discovery in engineered environments (Jetten *et al.,* 2009), anammox bacteria have been implicated in substantial losses of fixed N from many naturally occurring anoxic environments, including marine and estuarine sediments (Thamdrup and Dalsgaard, 2002; Risgaard-Petersen *et al.,* 2004; Wang *et al.,* 2012a), anoxic freshwater lakes (Schubert *et al.,* 2006; Yoshinaga *et al.,* 2011; Wu *et al.,* 2012), paddy soils (Zhu *et al.,* 2011), wetlands (Erler *et al.,* 2008) and contaminated groundwater (Moore *et al.,* 2011). Anammox appears to be widespread, and a high N-loading freshwater river system has the potential to provide a suitable environment to support anammox processes. However, studies on the occurrence and role of anammox in freshwater river systems are limited, although anammox bacterial communities have been detected by molecular technologies in some freshwater river systems (Zhang *et al.,* 2007; Hu *et al.,* 2012; Han and Li 2016; Sun *et al.,* 2016).

In a single habitat, competition between denitrification and anammox is expected. The differences in the relative importance of denitrification and anammox may be controlled by the environmental variables that favor one process over the other. The controlling factors of denitrification have been identified in many studies in freshwater river system, including temperature, pH, nitrate concentrations, organic carbon, and oxygen concentrations at the bottom of water bodies (Seitzinger, 1988; Inwood *et al.,* 2007; Herrman *et al.*, 2008; Zhao *et al.*, 2014, 2015a). The important regulating factors for anammox activity in marine and estuarine systems have also been examined, including temperature (Thamdrup and Dalsgaard, 2002; Teixia *et al.,* 2012), NO_3^- availability in water column (Rich *et al.,* 2008; Nicholls and Trimmer, 2009), the content of

organic carbon (Thamdrup and Dalsgaard, 2002), NO_3^- and NO_2^- concentrations in sediments (Meyer *et al.,* 2005; Zhao *et al.,* 2013). However, data on the factors affecting the anammox process and its relative contributions to total nitrate reduction in freshwater river systems is yet very limited. This restricts our ability to fully understand N removal and formulate effective management plans in freshwater river systems. Potential factors influencing denitrification and anammox can be incorporated into ecosystem modeling of N removal and are commonly used in supporting management and policy decisions to mitigate the severe N pollution of water.

The Lake Taihu region is located in the center of the Yangtze River Delta in southeastern China, in one of China's most developed economic zones. This region has both dense river networks and dense human populations, with people tending to reside along rivers, leading to developed industries and intensive agriculture (Qian *et al.,* 2007; Zhao *et al.*, 2015a). More than 40% of urban sewage and 80% of rural sewage is left untreated, as a result, large amounts of N-enriched sewage were discharged into the rivers and have caused serious environmental problem in recent years (Sun *et al.,* 2012; Xia *et al.,* 2013). The total N (TN) input of the region was up to 14.2×10^4 tons in 2005, which included industrial waste, urban and rural human excreta and domestic sewage, leaching and runoff from farmland, aquaculture and animal excreta (Environmental Protection Department of Jiangsu Province 2008). With the deterioration of the water environment, eutrophication and seasonal incidents of algal blooms (Microcystis spp.) have occurred in Lake Taihu, causing a drinking water crisis in Wuxi, Jiangsu Province, in 2007 (Qin *et al.,* 2007). There are a large number of inflow rivers in the western Lake Taihu region, which have different pollution levels and ecological types (Chen *et al.*, 2012; Zhao *et al.,* 2015a). These characteristics may directly affect the capacity of N removal and the N-loading flowing into Lake Taihu from these inflow rivers.

Accordingly, in this study, we choose 11 rivers in the Lake Taihu region to measure their potential activities of denitrification and anammox using membrane inlet mass spectrometry (MIMS) and ^{15}N-isotope pairing technique (^{15}N IPT), and determine the key factors that regulate denitrification and anammox activity in these rivers. This study will help to identify the capacity of N removal and the limiting factors of the main inflow rivers in the Lake Taihu region, and provide the theoretical basis for understanding the migration and transportation of N and its mechanism.

2. Materials and methods

2.1. Study area

Study site and sample collection. Eleven inflow rivers in the west of Lake Taihu region (Figure 1) were selected to study the spatial variation of denitrification and anammox activities.

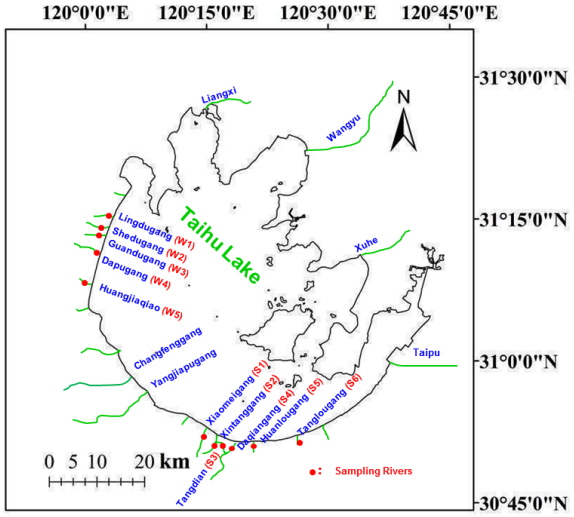

Figure 1. Sampling locations for the measured $^{15}N_2$-N fluxes across the sediment–water interface in the Lake Taihu region, China. We sampled Huangjiaqiao River (W5) in July 2013, Xintanggang River (S2) in August 2013; Xiaomeigang (S1), Tangdian (S3), Daqiangang (S4), Huanlougang (S5) and Tanglougang Rivers (S6) in October 2013; and Shedugang (W2), Lingdugang (W1), Guandugang (W3) and Dapugang (W4) in December 2013.

Table 1. Surface water and sediment characteristics in the sampled 11 rivers in the Lake Taihu region; WT, water temperature; DOC, dissolved organic carbon; TDN, total dissolved nitrogen. Values are means (SE), n=3.

Rivers	Surface water							Sediments			
	WT (°C)	pH	DO (mg L^{-1})	NO_3^- (mg L^{-1})	NH_4^+ (mg L^{-1})	TDN (mg L^{-1})	DOC (mg L^{-1})	NO_3^- (mg Kg^{-1})	NH_4^+ (mg Kg^{-1})	TDN (mg Kg^{-1})	DOC (mg Kg^{-1})
S1	21.5	6.7±0.1	5.5±0.3	2.3±0.5	0.2±0.1	3.3±0.8	4.1±0.4	2.5±1.0	88.7±9.6	129.5±15.6	21.1±4.3
S2	29.3	7.4±0.3	5.2±1.6	0.1±0.0	0.8±0.3	1.1±0.2	7.5±1.7	4.9±3.5	203.9±11.3	253.7±17.1	40.3±1.2
S3	21.3	6.8±0.0	3.3±0.2	0.9±0.2	0.3±0.1	2.4±0.3	6.2±0.4	0.7±0.4	156.6±29.0	167.1±33.7	26.1±4.7
S4	21.4	6.7±0.0	2.8±0.3	0.8±0.1	0.3±0.0	2.9±1.1	5.4±0.8	1.6±0.1	136.8±23.8	143.3±25.6	44.1±2.1
S5	21.8	6.7±0.2	2.7±0.6	0.4±0.1	0.5±0.1	2.1±0.2	4.5±0.9	2.5±0.1	87.7±34.5	146.1±39.4	61.5±18.5
S6	21.5	6.7±0.1	1.6±0.6	0.7±0.0	0.5±0.2	2.5±0.3	5.0±0.7	0.2±0.1	91.9±38.4	114.6±38.2	43.6±1.4
W1	13.6	7.1±0.3	3.0±0.7	0.8±0.1	2.4±0.3	4.5±0.3	8.1±1.3	0.4±0.4	108.0±10.4	201.3±134.5	101.4±39.8
W2	13.3	6.7±0.2	4.8±1.3	0.6±0.1	1.9±0.8	3.9±0.9	9.7±3.3	0.4±0.1	117.4±30.3	127.1±38.2	94.6±2.1
W3	13.2	6.9±0.0	3.7±0.4	1.2±0.2	1.6±0.6	4.1±1.6	9.6±3.4	0.2±0.1	61.8±54.2	84.3±54.9	76.9±34.7
W4	13.6	7.2±0.1	7.3±0.3	1.3±0.1	1.0±0.8	3.6±1.2	8.5±1.5	0.1±0.0	71.9±48.9	77.1±50.4	65.3±13.4
W5	25.1	6.7±0.1	1.9±1.0	0.3±0.1	2.4±0.3	4.6±1.4	7.9±0.3	0.8±0.1	138.1±28.8	82.3±25.8	80.5±20.2

We sampled the Huangjiaqiao River (W5) in July 2013, the Xintanggang River (S2) in August 2013; the Xiaomeigang (S1), Tangdian (S3), Daqiangang (S4), Huanlougang (S5) and Tanglougang Rivers (S6) in October 2013; and the

Shedugang (W2), Lingdugang (W1), Guandugang (W3) and Dapugang (W4) Rivers in December 2013. Each of these 11 rivers was sampled at three sites to assess the potential denitrification and anammox rates, and associated sediment and water properties. The three sites were located in the upper, middle and lower reaches of each river, respectively. Selected sediment and overlying water characteristics of the studied sites are shown in Table 1. These sampled rivers were affected by different levels of N pollution that originate from crops and livestock production as well as untreated sewage effluents (Zhang *et al.,* 2014; Zhao *et al.*, 2015a).

2.2. Sampling and preparation of sediments

For each sampling event at each site, we collected triplicate, intact sediment cores (10 cm depth; 8 cm inner diameter) using a core sampler (length, 30 cm; Uwitec, Austria). Upon collection at the field sites, cores were sealed at the bottom with rubber stoppers, immediately submerged in ambient water using ice packs for transport (Zhao *et al.,* 2013). After collection, the cores were sealed and returned to the laboratory. The cores were then combined, homogenized, transferred into tubes to form a slurry layer of 10 cm, and then stored in a container with NO_3^--free freshwater for 96 h (Zhao *et al.,* 2013). NO_3^--free freshwater was used to ensure that the sediment nitrification was the only possible source of $^{14}NO_3^-$ (Nielsen, 1992). Storage and incubation were carried out at the same temperature as that of in situ water.

2.3. ^{15}N sediment slurry incubation and analysis

After pre-incubation for 96 h, the tubes were filled with N solutions, sealed with an airtight Plexiglas plate (without any headspace), and then incubated in a water bath at in situ temperature. The overlaying water was gently shaken at approximately 14 rpm by a suspended magnet driven by an external rotating magnet. Three treatments with different N species in the overlaying water were prepared: (1) 100μM $^{15}NH_4^+$ (99.29%^{15}N), (2) 100μM $^{15}NH_4^+$ (99.29% ^{15}N)+100μM $^{14}NO_3^-$, and (3) 100μM $^{15}NO_3^-$ (99.19% ^{15}N).

Three replicate water samples (15 of 1005 mL of overlying water) for N_2 isotope composition analysis were withdrawn from the outlet port by allowing water to overflow (Ferguson and Eyre, 2007), directly into 5 mL Exetainer vials (Labco Limited, Buckinghamshire, UK) with glass stoppers filled to overflowing, and treated with 20 μL of 5% $HgCl_2$ solution at five points (2 h interval) from each core during the 8 h incubation. So, treated, the Exetainers were then stored underwater at the incubation temperature without cover until analysis. During the experiment, the overall sample water removed did not exceed 8% of the total water volume in each core. A gravity-feed system compensated each core with corresponding concentrations of N species in three treatments through the inlet port during sample removal. The physicochemical properties of the sediments were determined immediately after the incubation.

2.4. Measurement and calculation of N_2 isotope composition

Dissolved ^{15}N-N_2 gas ($^{29}N_2$ and $^{30}N_2$) was converted to gaseous form through a membrane device and then directly measured using a membrane inlet mass spectrometer (MIMS, Bay Instruments, Easton, MD, USA) (Kana *et al.,* 1994). The ion currents were standardized and drift-corrected by measuring thermally equilibrated water at intervals and applying gas solubility equations of Weiss (1970). Calibration of masses 28, 29, and 30 was performed using the rationale of Zhao *et al.,* (2013). Potential rates of anammox and denitrification and their contribution to N_2 production were calculated from the production of $^{29}N_2$ and $^{30}N_2$ in the incubation with $^{15}NO_3^-$ using the equations of Thamdrup and Dalsgaard, (2002).

2.5. Environment factors and statistical analysis

At each field sample site, a portable parameter detector (Hach, USA) was used to measure water temperature (WT, °C), pH and dissolved oxygen (DO, % saturation). Additionally, 100 mL water samples for chemical analyses were also collected and placed on ice for transport to the laboratory, then filtered (0.7 μm, Whatman GF/F filters) and frozen until analysis. The filtered water was analyzed for nitrate (NO_3^--N), ammonium (NH_4^+-N), total nitrogen (TN), and dissolved organic carbon (DOC).

Sediment (approximately 50 g) from each assay core was dried (60 °C), pulverized (0.15 mm), and analyzed for total carbon (TC) and total nitrogen (TN) content. Well-mixed fresh sediment samples (approximately 15 g each) were mixed with 50 mL of 2 M KCl, and the resulting suspension shaken, filtered, and analyzed for NO_3^--N, NH_4^+-N and total dissolved nitrogen (TDN). DOC was extracted from the sediment samples (approximately 10 g each) with 50 mL of deionized water, and measured on a Multi N/C 3000 (with a detection limit of 0.05 mg L^{-1}). Concentrations of NO_3^--N, NH_4^+-N, and TDN were determined using a flow injection analyzer (Skalar Analytical, Breda, The Netherlands).

Temporal and spatial differences were examined using one-way analysis of variance (ANOVA), followed by testing for the least significant difference (LSD). The relationship among potential anammox rates, the relative contribution of anammox to total N_2 production (ra%) and the measured independent variables (water column: temperature, pH, NO_3^-–N, NH_4^+–N, TN and DOC; sediment: NO_3^-–N, NH_4^+–N, TDN, DOC) were examined by a simple linear regression. All statistical analyses were performed in SPSS (version 19.0, USA).

3. Results and discussion

3.1. ^{15}N experiments

Slurry $^{29}N_2$ and $^{30}N_2$ production rates were measured with homogenized sediments under in situ temperature using a ^{15}N isotope pairing technique. The results showed that in the case of the incubation with $^{15}NH_4^+$ alone, no significant accumulation of $^{15}N_2$-labeled gas could be observed in the slurries, indicating that the sediment background nitrate

had been consumed during preincubation (Figure 2a). For slurries amended with both $^{15}NH_4^+$ and $^{14}NO_3^-$, $^{29}N_2$ significantly accumulated in every sample with no $^{30}N_2$ accumulation, indicating that anammox occurred in the sediment samples (Figure 2b). In the case of the slurries amended with $^{15}NO_3^-$ only, significant accumulation of both $^{29}N_2$ and $^{30}N_2$ was observed (Figure 2c), indicating the presence of anammox and denitrification in these river sediments. The potential rates of denitrification and anammox were estimated in the incubations with $^{15}NO_3^-$.

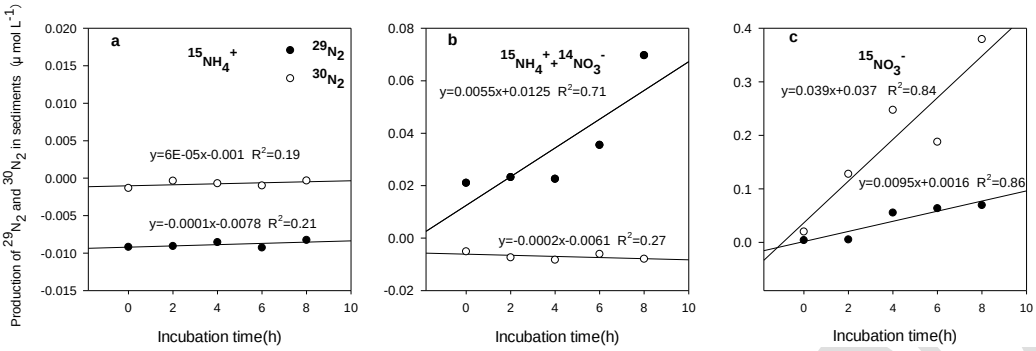

Figure 2. Examples of the accumulation of $^{29}N_2$ and $^{30}N_2$ (μmol L^{-1}) in sampled sediment slurries treated with either $^{15}NH_4^+$ (a), $^{15}NH_4^+ + ^{14}NO_3^-$ (b), or $^{15}NO_3^-$ (c) in the case of Huangjiaqiao River

3.2. Spatial variability in potential denitrification and the total N removal rates

The measured potential rates of denitrification and the total N removal at these 11 rivers ranged from 18.9±0.3 to 70.0±11.4μmol N m^{-2} h^{-1}, and 26.3±0.4 to 71.3±11.1μmol N m^{-2} h^{-1}, respectively (Figure 3). Using the ^{15}N IPT and MIMS, Zhang *et al.* (2012) measured that the total N removal rates in the rivers of Jiangsu and shanghai in the Taihu Lake region, and reported rates ranged from 10.8 to 32.9 μmol N m^{-2} h^{-1}, which are similar to our measured rates (ranged from 26.3±0.4 to 71.3±11.1μmol N m^{-2} h^{-1}). Similarly, using the ^{15}N IPT, Pind *et al.,* (1997) reported that the N removal rates ranged from 0 to 750 μmol N m^{-2} h^{-1} at the Gelbæk River in the eastern of Denmark, higher than our measured rates in these 11 rivers, likely due to the higher water NO_3^--N concentrations in the Gelbæk river (range, 0.6 to 7.3 mg L^{-1}) than those in our studied rivers (range, 0.1 to 2.3 mg L^{-1}) (Table 1). The potential rates of denitrification and the total N removal were all significantly different among these sampled sediments (ANOVA, $p<0.05$, Figure 3). The highest rates were all determined in the Huangjiaqiao River (W5) which was sampled in July 2013 (ANOVA, $p<0.05$, Figure 3), and the lowest rates were all observed in the Xiaomeigang River (S1) which was sampled in October, 2013. The mean potential denitrification and the total N removal rates of river sediments in the western rivers (W1, W2, W3, W4 and W5; 54.7±13.6 μmol N m^{-2} h^{-1}, 57.6±12.2 μmol N m^{-2} h^{-1}, respectively) were all significantly higher than the southern rivers of the Taihu Lake region (S1, S2, S3, S4, S5 and S6; 28.9±6.6 μmol N m^{-2} h^{-1}, 36.0±5.2 μmol N m^{-2} h^{-1}, respectively) (ANOVA, $p<0.05$, Figure 3). The potential total N removal rates measured within these rivers in the Lake Taihu region showed high spatial heterogeneity (Figure 3), as has also been reported in other aquatic systems (Schubert *et al.,* 2006; Moore *et al.,* 2011; Zhu *et al.,* 2013). Thus, large variation in space may be a characteristic feature of the total N removal rates in aquatic systems.

The calculated potential denitrification rates ranged from 18.9±0.3 to 70.0±11.4μmol N m^{-2} h^{-1}, which accounted for 70.1±0.7% to 98.0±0.8% of the total N_2 production in these selected rivers. The results indicated that the role of denitrification was the dominant pathway for N removal in these studied rivers. The spatial variation of the potential denitrification rates showed similar trend with the total N removal rates, increased from the southern rivers to western rivers in the region. The contents of DOC in sediments were greater in the western than the southern rivers in the studied region (Table 1).

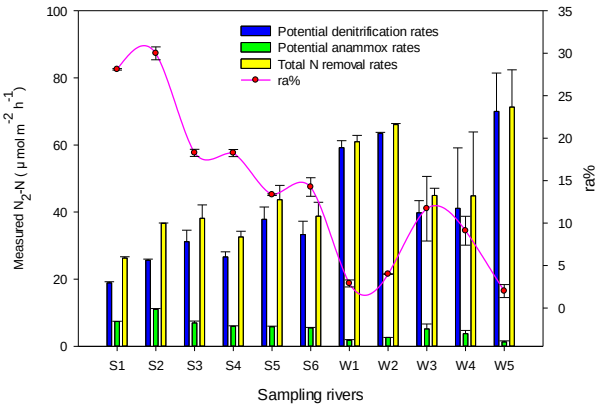

Figure 3. Measured potential denitrification, anammox, total N removal rates and ra% for the 11 sampling rivers in the Taihu Lake region.
Values are means ±1 standard deviation

Compared with southern rivers, the higher concentrations of sediment DOC in the western rivers may provide a more favorable habitat for the activities of denitrification bacteria. Previous studies have reported that the level of sediment DOC content was a significant limiting factor for the activities of denitrification bacteria, since organic carbon can serve as an electron donor for the denitrifying bacteria (Knowles, 1982; Arango *et al.,* 2007; Moore *et al.,*

2011). We also observed a significantly positive relationship between potential denitrification rates and sediment DOC concentrations (R=0.89, p<0.01) (Figure 4). However, no statistically significant relationship with sediment nitrate (R=-0.51, p<0.05), and ammonium concentrations (R=-0.09, p<0.05) was observed. The results indicated that the content of DOC in sediments was the limiting factor that controls the activities of denitrification bacteria in these studied rivers.

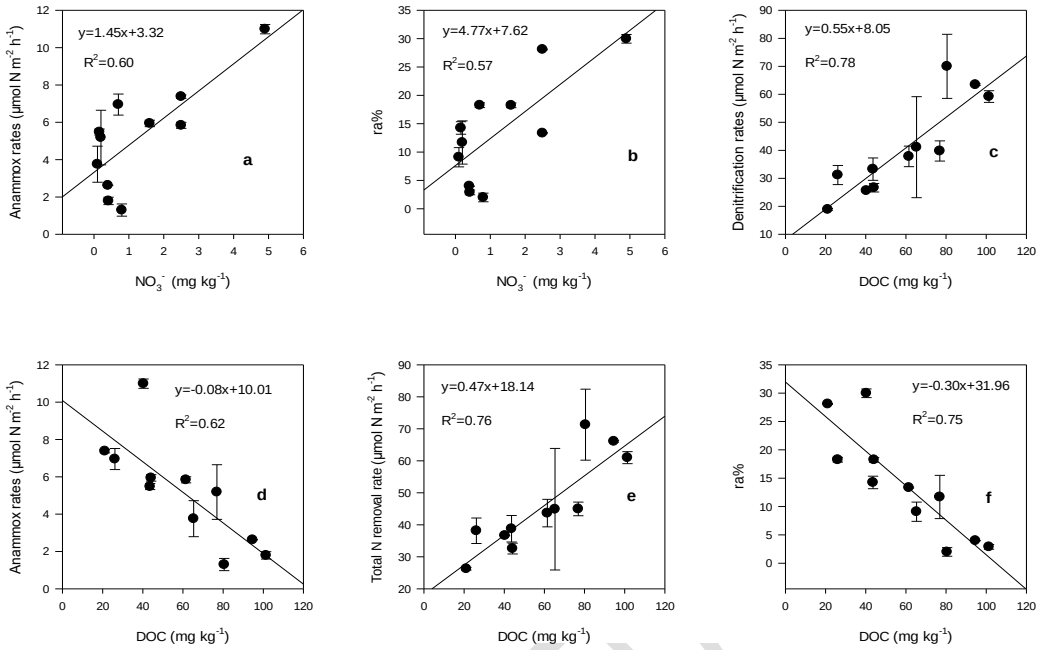

Figure 4. Relationships between the content of NO_3^-–N in sediments and (a) potential anammox rates and (b) the relative contribution of anammox to total N_2 production (ra%); relationships between the content of DOC in sediments and (c) potential denitrification rates, and (d) potential anammox rates and (e) potential total nitrogen removal rates and (f) the relative contribution of anammox to total N_2 production

3.3. Spatial variability in potential anammox rates

The presence of anammox bacteria in rivers of the Taihu Lake region using barcode pyrosequencing has been confirmed by Zhao *et al.,* (2013). The 16S rRNA anammox gene sequences in the river sediments were affiliated with *Candidatus Kuenenia, Candidatus Jettenia,* and *Candidatus Scalindua*, among which *C. Kuenenia* dominated the anammox bacterial communities. The results confirmed the presence of anammox bacteria in the rivers of Taihu Lake region.

The potential anammox rates and the relative contribution of anammox to total N_2 production (ra%) can be calculated from the incubation with $^{15}NO_3^-$. The calculated potential anammox rates and ra% ranged from 1.3±0.3 to 11.0±2.5 μmol N m^{-2} h^{-1}, and 2.0±0.8% to 29.9±0.7% in these rivers between July 2013 and December 2013, respectively (Figure 3). Our measured rates are comparable with the activities of anammox bacteria in the Yincungang and Henangeng river sediments of the Taihu Lake region (0.1 to 6.8μmol N $m^{-2}h^{-1}$) (Zhao *et al.*, 2013), and are within the range of reported rates in other freshwater environments, ranged from 0 to 137±77 μmol N m^{-2} h^{-1} (e.g., Hamersley *et al.,* 2009; Xu *et al.,* 2009; Zhao *et al.,* 2015b). In the present study, the relative contribution of anammox activity to the total N_2 production ranged from 2.0±0.8% to 29.9±0.7%, whereby the remainder would be due to denitrification activity. Our measured values are lower than those reported for shelf and deep marine sediments, which contributed to up to 79% of total N_2 production (Thamdrup and Dalsgaard, 2002; Engström *et al.*, 2005; Trimmer and Nicholls, 2009), and are comparable with those of values reported for N-polluted groundwater and freshwater environments. For example, based on ^{15}N isotope pairing technique, Moore et al (2011) found that the relative contribution of anammox to total N_2 production ranged from 18-36% in an N-polluted groundwater system in Canadian, while Zhu *et al.*, (2013) reported that the anammox activities contributed to 11-35% of the total N_2 production at land–freshwater interfaces of lake riparian zones in North China. Obviously, the anammox process was not the dominant pathway for removing fixed N in these studied freshwater rivers, but its role cannot be ignored. In rivers such as these with highly anthropogenic reactive N-loading (Table 1), the activity of anammox bacteria may not be able to keep up with denitrification bacteria when electron donor availability (DOC) for denitrification is high in sediments (Dalsgaard *et al.,* 2005; Trimmer and Nicholls, 2009; Zhao *et al.,* 2013). In the present study, the hypothesis has been demonstrated by the significantly negative correlate relationship between potential anammox rates and the sediment DOC concentrations (R=-0.79, p<0.01) (Figure 4). In summary, the results indicated that these sampled rivers utilize the same N removal mechanism by anammox process that has been found in marine, freshwater lake, and containment

groundwater systems (Thamdrup and Dalsgaard, 2002; Schburt *et al.,* 2006; Moore *et al.,* 2011; Zhu *et al.,* 2013).

A significant spatial variation of the potential anammox rates and ra% were also observed in these sampled rivers (ANOVA, $p<0.05$, Figure 3). The highest anammox rate and ra% were all observed in the Xintanggang River (S2) which was sampled in August 2013 (ANOVA, $p<0.05$), and the lowest anammox rate and ra% were all observed in the Huangjiaqiao River (W5) which was sampled in Nov, 2013 (Figure 3). The mean potential anammox rates and ra% of river sediments in the southern rivers (S1, S2, S3, S4, S5 and S6; 7.1±2.0μmol N m^{-2} h^{-1}, 20.4±7.0%, respectively) were significantly higher than the western rivers of the Taihu Lake region (W1, W2, W3, W4 and W5; 2.9±1.6μmol N m^{-2} h^{-1}, 5.9±4.2 %, respectively) (ANOVA, $p<0.05$, Figure 3). Compared with the potential denitrification rates, the sediment potential anammox rates and ra% all displayed an opposite trend of spatial variation, all increased from the western rivers to southern rives in the region. The relatively high sediment DOC contents in the western rivers may have enhanced the activities of denitrifying bacteria, then contributed to increases the concentrations of substrates for the anammox bacteria through the denitrification process since most denitrifying bacteria are heterotrophic and capable of utilizing organic matter to generate nitrite and ammonium (Dalsgaard *et al.,* 2005), and further stimulated the anammox activities. However, the strength of stimulation for denitrification activities was greater than the anammox, resulting in the anammox rates and ra% displaying an opposite trend to the denitrification activity, namely, decreased from the southern rivers to the western rivers in the studied region.

Not surprisingly, the potential anammox rates were significantly limited by sediment nitrate concentrations in these rivers based on correlation analysis, as observed in other aquatic systems (Figure 4) (Risgaard-Petersen *et al.*, 2004; Trimmer and Nicholls, 2009; Zhao *et al.,* 2013). The contents of ammonium were much higher than nitrate in selected river sediments (Table 1), however, the contents of sediment ammonium were not significantly correlated with potential anammox rates and ra% (R=-0.09 and 0.44, respectively, $p>0.05$) based on the correlation analysis in the present study. Since the relationship between ammonium and nitrate is 1: 1 molar ratio in the reaction system of anammox process (Thamdrup and Dalsgaard, 2002; Wang *et al.,* 2012b), we therefore concluded that the content of sediment ammonium was not the limiting factor for the anammox activities in these 11 rivers.

In addition, there were no statistically significant relationships among sediment ammonium concentrations, potential denitrification and anammox rates, indicated that the nitrification process was not a major source of nitrate in sediments in these studied rivers. Namely, coupled nitrification-denitrification was not a major source of N_2 production in these studied rivers.

4. Conclusions

In the present study, the potential rates of N removal in the 11 rivers of Taihu Lake region (China) were evaluated using the ^{15}N IPT combined with MIMS. The measured potential rates of denitrification and total N removal ranged from 18.9±0.3 to 70.0±11.4 and 26.3±0.4 to 71.3±11.1μmol N m^{-2} h^{-1}, respectively. The potential rates of denitrification and the total N removal varied spatially in these 11 rivers, with the highest rates occurring in the western rivers of the region. The measured anammox rates ranged from 1.3±0.3 to 11.0±2.5 μmol N m^{-2} h^{-1}, and contributed 2.0±0.8% to 29.9±0.7% of the total N_2 production. The anammox rates and ra% displayed the opposite trend to denitrification with the highest values occurring in the southern rivers of the region. The contents of nitrate and dissolved organic carbon in sediments were identified as important factors controlling denitrification and anammox activities in these studied rivers. Our results indicated that the activities of N removal varied spatially, and denitrification is the dominant process for removing fixed N but the role of anammox is not negligible in freshwater rivers.

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