Within-lake variability and environmental controls of sediment denitrification and associated N₂O production in a shallow eutrophic lake

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Abstract

Excess nitrogen (N) in lakes may lead to eutrophication and many attendant environmental problems such as water quality decline and loss of aquatic biodiversity. Denitrification in lake sediments can alleviate the effects of eutrophication through removal of N to the atmosphere as N_2O and N_2 . However, N_2O contributes to the greenhouse effect and global warming. In this study, we measured three denitrification parameters (i.e., potential denitrification rate, unamended denitrification rate and net N_2O production rate) in surface sediments which were collected from 7 sub-lakes of the Lake Donghu, one of the most eutrophic lakes in China. The results showed that a range of water quality and sediment characteristics (e.g., total N and total phosphorus) varied significantly among sub-lakes. The unamended denitrification rate varied between 0.51 and 26.0 $\text{ng N g}^{-1} \text{h}^{-1}$, while the N_2O production rate ranged from less than 0 to 1.68 $\text{ng N g}^{-1} \text{h}^{-1}$. However, there was no significant difference among the sub-lakes in these denitrification parameters. The unamended denitrification rate was positively related to the water NO_3^- concentration and sediment moisture and bulk density. The findings of the present study suggest that sediments in eutrophic lakes can remove large quantities of N through denitrification and may become a significant source of N_2O if the N input is maintained or to increase.

Keywords: Eutrophication; Denitrification potential; Donghu Lake; Greenhouse gas; Nitrogen pollution

1. Introduction

Nitrogen (N), one of the most abundant elements in our galaxy, constitutes approximately 78% of the Earth's atmosphere (Bebout et al., 2013). N is essential for the synthesis of nucleic acids and proteins and makes up about 14% of the weight of a microbial cell (Stolp 1988). However, human activities have dramatically altered the global N cycle in recent decades (Vitousek et al., 1997; Gruber and Galloway, 2008; Conley et al., 2009). Large increases in N inputs from industrial wastewater, domestic sewage, agricultural fertilizer and atmospheric deposition in terrestrial ecosystems have resulted in increased N concentration in lakes, rivers and other aquatic ecosystems in the world (Vitousek et al., 1997; McCrackin and Elser, 2010; Liu et al. 2012, Xiong et al., 2015).

Excess N in lakes may lead to eutrophication and numerous attendant ecological problems such as water quality deterioration, harmful algal blooms, and aquatic biodiversity loss (Carpenter et al., 1998; Abell et al., 2010; Liu et al., 2012). China has more than 2600 lakes with a surface area greater than 1 km², accounting for approximately 0.85% of the total land area of China (Ma et al., 2011). Unfortunately, as a result of increasing point- and nonpoint-source pollution in watersheds, the proportion of Chinese lakes classified as eutrophic has rapidly increased from about 41% in 1980 to nearly 85% in 2005 (Liu et al., 2010).

The lake ecosystem is one of the most important sink of N released by human activities (Jansson et al., 1994; Saunders and Kalff, 2001). By using a model called “NiRReLa”, Harrison et al. (2009) predicted that N removal by lakes larger than 1000 m² in the world was about 13 Tg year⁻¹, representing approximately 20% of estimated N inputs to surface freshwaters globally or 5% of N loading to continental surfaces.

Results from this NiRReLa model further indicated that small lakes (<50 km²) removed more than 2.5 times as much N from watersheds as large lakes (9.3 Tg N year⁻¹ for small lakes vs. 3.7 Tg N year⁻¹ for large lakes). This key role of small lakes acting as a biogeochemical sink in our earth was also detected in a study focusing on the fate of carbon in freshwater aquatic ecosystems (Cole et al., 2007). Approximately 91% of China's lakes have a surface area less than 50 km² (Ma et al., 2011). These small lakes can play a key role in regulating the local water quality and regional N cycle.

A number of studies have focused on the ability and mechanism of lake ecosystems to remove N and indicated that sediment denitrification, anaerobic ammonium oxidation, biological uptake and sedimentation are the main processes for N retention and removal (van Luijn et al., 1996; Saunder and Kalff, 2001; McCrackin and Elser 2010). It has been evidenced that sediment denitrification is the dominant process of N removal in freshwater lakes (e.g., Rissanen et al., 2013; Zhao et al., 2015). Sediment denitrification processes occur under anoxic or anaerobic conditions, resulting in the permanent removal of N through the conversion of nitrate (NO₃⁻) to nitrous oxide (N₂O) and dinitrogen (N₂) gases. Understanding the N₂O production during sediment denitrification is very important, as N₂O contributes notably to the greenhouse effect and global climate warming (Hefting et al., 2006). However, understanding of the mechanisms and factors regulating N₂O production from sediment denitrification is still incomplete in spite of extensive studies.

In this work, we determined the sediment denitrification parameters (i.e., potential denitrification rate, unamended denitrification rate and net N₂O production rate) and their relationships to water quality and sediment characteristics in 7

sub-lakes of the Lake Donghu, the largest urban lake in China. We hypothesized that sediment denitrification parameters would be controlled by both water quality and sediment characteristics, such as N and phosphorus (P) concentrations. The objectives of this study were (1) to examine the spatial variation of sediment denitrification and N₂O production in the Lake Donghu, and (2) to determine the key environmental controls of sediment denitrification and N₂O production in the Lake Donghu.

2. Materials and methods

2.1. Study area

The Lake Donghu (30°31′-30°36′N, 114°21′-114°28′E) is a subtropical shallow lake close to the Yangtze River, located in the northeast of Wuhan, the largest city in central China (Fig. 1). It is the largest urban lake in China, covers a surface area of 28 km², and has a mean water depth of 2.2 m and maximum water depth of 4.8 m (Qiu et al., 2001). The lake area has a typically subtropical monsoon climate, with a mean annual temperature of 16.7 °C. The mean annual precipitation is approximately 1160 mm, and more than half of the total rainfall is concentrated in the summer monsoon season from June to August. In the Lake Donghu watershed, the dominant land use is urban land, which accounts for approximately 30% of the land (Li et al., 2006).

With the rapid urbanization and industrialization, a number of causeways have been constructed across the Lake Donghu, dividing it into several sub-lakes. The inter-basin water exchange occurs only via bridge under-passes and culverts beneath the causeways (Havens et al., 2001). Most of the sub-lakes are very important for the fishery production, aquatic sport and recreation. However, these sub-lakes have

experienced a drastic change in the ecosystem structure and function in the past five decades. During the 1970s and 1980s, nuisance cyanobacterial blooms frequently occurred and submerged vegetation declined considerably. The N and P concentrations in many of these sub-lakes have increased constantly and the trophic status has changed from mesotrophic to eutrophic or hypereutrophic conditions (Xie and Xie, 2002).

2.2 Field sampling

In 7 August 2012, seven main sub-lakes (i.e., Guozhenghu, HouHu, Lingjiaohu, Miaohu, Tanglinhu, Niuchaohu and Yujiahu) of the Lake Donghu were chosen for field sampling (Fig. 1). To obtain representative samples of each sub-lakes, one transect across the lake was randomly established and three sampling sites were selected along this transect at different water depth. At each sampling site, three replicate surface sediments were randomly collected using a grab sampler and then mixed and homogenised to form a composite sample. For each site, approximately 500 g of sediment was placed in a sealed plastic bag and stored at approximately 5°C in a refrigerator. At each sampling site, a 500-mL water sample was collected at a depth of approximately 1 m for use in the sediment denitrification and water quality analyses in the laboratory.

2.3 Measurements of denitrification and N₂O production

Sediment potential and unamended denitrification rates of Lake Donghu were measured using the acetylene (C₂H₂) inhibition technique (McCrackin and Elser, 2010). C₂H₂ was used to inhibit the reduction of N₂O to N₂ during denitrification

processes and to make N_2O as the end product of denitrification. The potential denitrification rate was measured under optimal conditions (by supplying an excess of NO_3^- and organic carbon and ensuring anoxia) and thus provided an upper-bound estimate of *in situ* denitrification (Liu et al., 2015a). The unamended denitrification rate was a conservative estimate of *in situ* denitrification without carbon and NO_3^- amendments.

For potential denitrification rate assays, 50 g of homogenised sediments from each sampling site were slurried with 30 mL of incubation solution (final concentrations: 0.18 g/L glucose, 0.1 g/L KNO_3 and 1 g/L chloramphenicol) in a 250 mL serum bottle. Each bottle was then sealed and purged with N_2 gas for 2 min to create anoxic conditions. Approximately 10 % of the bottle headspace was replaced with pure C_2H_2 . We measured unamended denitrification and N_2O production rates using a similar procedure, but with the addition of 30 mL of unfiltered lake water instead of the incubation solution. Parallel incubations with and without acetylene (10 % vol/vol in the bottle headspace) were used to differentiate between unamended denitrification and N_2O production (McCrackin and Elser, 2010). All bottles were then incubated in the dark for 4 h at 25°C (the approximate *in situ* water temperature). At the beginning and end of incubation, 5 mL of headspace gas samples were collected from each bottle (after shaking vigorously) using a syringe. The N_2O concentrations were measured using a gas chromatograph (Agilent 7890, Santa Clara, CA, USA) equipped with an electron capture detector.

Potential denitrification, unamended denitrification and N_2O production rates were calculated as the difference between the initial and final headspace N_2O concentrations (corrected for N_2O dissolved in water, Bunsen coefficient = 0.544)

divided by the incubation time (Hayakawa et al., 2012), and was expressed on the basis of dry matter of sediment ($\text{ng N g}^{-1} \text{h}^{-1}$).

2.4 Measurements of water quality and sediment characteristics

At each sampling site, water depth and Secchi depth (SD) were measured. Water temperature, Conductivity (Cond), dissolved oxygen (DO), oxidation–reduction potential (ORP), nitrate (NO_3^-) and ammonium (NH_4^+) were measured on site at the sampling depth using a YSI 6920 multiparameter water quality probe (YSI Inc., Yellow Springs, Ohio, USA). The concentration of total organic carbon (TOC) in water was measured with a TOC analyser (Vario TOC cube, Elementar, Germany). Concentrations of total N (TN) and total P (TP) were analyzed using the Kjeldahl method after block digestion. Chlorophyll-*a* (Chl-*a*) was determined by filtration through $0.45 \mu\text{m}$ membrane filters followed by extraction with 90% acetone and fluorometry.

The trophic state index (TSI) of each sub-lake was calculated using the following equations (Carlson, 1977):

$$\text{TSI (SD)} = 10(6 - \frac{\ln \text{SD}}{\ln 2})$$

$$\text{TSI (TP)} = 10(6 - \frac{\ln(\frac{48}{\text{TP}})}{\ln 2})$$

$$\text{TSI (Chl-}a\text{)} = 10(6 - \frac{2.04 - 0.68 \ln \text{Chl-}a}{\ln 2})$$

Where SD was expressed in m, and concentrations of TP and Chl-*a* were in mg m^{-3} . TSI used in the present study was the average of TSI (SD), TSI (TP), and TSI (Chl-*a*). The trophic status of lakes could be classified as oligotrophic ($\text{TSI} < 40$), mesotrophic ($40 \leq \text{TSI} < 50$), eutrophic ($50 \leq \text{TSI} < 70$) and hypertrophic ($\text{TSI} \geq 70$) according to the TSI

values (Liu et al., 2010).

Sediment pH value was determined in a soil to water ratio of 1: 5 (v/v) using a pH meter and moisture was measured gravimetrically (24 h at 105 °C) from 30 g sediment samples. Sediment bulk density (BD) was measured by weighing soil cores of known volume after drying for 24 h at 105 °C. Sediment organic matter (SOM) was determined by mass loss on ignition at 500 °C for 2 h. Sediment total carbon (STC) was analyzed by using a TOC analyzer (Vario TOC cube, Elementar, Germany). Sediment total N (STN) and Sediment total P (STP) were measured using a Kjeldahl method after digesting samples in a digester using sulfuric acid/mercuric oxide catalyst.

2.5 Statistical analyses

Prior to statistical analyses, the raw data were tested with the Shapiro–Wilk test for normality. When possible, non-normally distributed data were natural log or square root transformed to reach a normal distribution. We carried out a one-way ANOVA followed by Tukey HSD post-hoc tests to evaluate the differences in water quality, sediment characteristics and denitrification parameters among seven sub-lakes. The relationships between denitrification parameters and water quality and sediment characteristics were assessed by Pearson correlation and regression analyses. All statistical analyses were conducted using PASW Statistics 18 software (IBM SPSS Inc., Chicago, USA).

3. Results

3.1 Water quality and sediment characteristics

The water TN concentration varied from a minimum value of 0.56 to a maximum value of 2.90 mg L⁻¹, while the TP concentration ranged from 0.02 to 0.12 mg L⁻¹ (Table 1). The averages of water TN and TP concentrations were 1.05 and 0.07 mg L⁻¹, respectively. The highest TN content (2.53 mg L⁻¹) and TP content (0.10 mg L⁻¹) in water were both found in the Lake Yujiahu (Table 2). Based on the TSI values, all sub-lakes could be classified as eutrophic or even hypertrophic (Fig. 2).

The sediment pH values varied from 7.26 to 8.25, and only two sediment samples had a value less than 7.5 (Table 1). The SOM varied between 2.24 and 16.3 %, with a mean value of 8.67%. The STN and STP varied significantly among the seven sub-lakes (Table 3). The highest STN (1.88 mg g⁻¹) and STP (1.65 mg g⁻¹) were both detected in the Lake Miaohu.

3.2 Spatial variation in sediment denitrification and N₂O production

The Lake Donghu showed a great spatial variation in sediment denitrification and N₂O production rates (Table 1). The potential denitrification rate varied from 31.4 to 154 ng N g⁻¹ h⁻¹ and averaged 77.0 ng N g⁻¹ h⁻¹. The unamended denitrification rates varied widely across the 21 sampling sites and ranged from 0.51 to 26.0 ng N g⁻¹ h⁻¹ (Table 1). N₂O production rates were lowest in Lake HouHu at 0.06 µg N g⁻¹ h⁻¹, and highest in Lake Lingjiaohu at 0.91 µg N g⁻¹ h⁻¹ (Fig. 3). However, the potential denitrification, unamended denitrification and net N₂O production rates were not significantly different among the seven sub-lakes (Fig. 3).

3.3 Relationships between water quality and sediment characteristics and sediment denitrification and N₂O production

Potential denitrification rate was positively related to the water TOC (Table 4). Among water quality and sediment characteristics, water NO_3^- , sediment moisture and BD were significantly correlated with the unamended denitrification rates. Stepwise regression analysis demonstrated that water NO_3^- could alone account for 39.9% of the variance in the unamended denitrification rate (date not shown). However, water quality and sediment characteristics had no significant relationships with net N_2O production rates (Table 4).

4. Discussion

4.1 Characteristics of sediment denitrification and N_2O production

The unamended denitrification rate was generally recognized as a conservative estimate of actual *in situ* denitrification. In the present study, the unamended denitrification rate varied widely across the sampling sites in the Lake Donghu, ranging from 0.51 to 26.0 $\text{ng N g}^{-1} \text{h}^{-1}$ with a mean of 3.97 $\text{ng N g}^{-1} \text{h}^{-1}$ (Table 1). In China, previous studies have reported that sediment unamended denitrification rates in lakes from the Yangtze River basin and the Yunnan-Guizhou Plateau were approximately 3.0 and 1.06 $\text{ng N g}^{-1} \text{h}^{-1}$, respectively (Liu et al. 2015a, b). It has been reported that the potential for N removal by sediment denitrification is generally high in eutrophic lakes (Seitzinger, 1990; Jansson et al., 1994). Therefore, the relatively higher unamended denitrification rate in the Lake Donghu may be due to the fact that all sub-lakes are eutrophic to hypertrophic. Although the sediment denitrification can alleviate the negative effects of eutrophication, the question remains whether the sediment denitrification capacity is large enough to remove current N pollution in the Lake Donghu.

Scaled to an areal basis using the density of the top 7 cm of sediments (McCrackin and Elser, 2010), unamended denitrification rate of the 21 sampling sites in this study varied between 274 to 14,651 kg N km⁻² year⁻¹, with an average of 2133 kg N km⁻² year⁻¹. This value was much lower than the mean N retained capacity of global small lakes (3577 kg N km⁻² year⁻¹) estimated by the NiRReLa model (Harrison et al., 2009). This result may be partly due to the fact that the NiRReLa model does not distinguish between N removal via sediment denitrification and N removal via other processes such as sediment burial. It has been estimated that sedimentation can account for anywhere from 25% to 250% of the total NiRReLa-based estimate of N retention in small lakes (Harrison et al., 2009). In the Lake Donghu, the mean sediment accumulation rate was approximately 0.33 cm yr⁻¹ (Yang et al., 1994). According to the density and N concentration of the sediments, we roughly estimated annual mean N burial in the Lake Donghu to be 1608 kg N km⁻² year⁻¹, or about 75% of the N removal by sediment denitrification process. McCrackin and Elser (2010) have estimated that sediment denitrification can remove 24–39% of annual N inputs to Norwegian lakes. In addition, Bruesewitz et al. (2011) have predicted that 30–60% of N loading to Rotorua lakes in New Zealand may be removed by denitrification process. These studies and the present investigation suggest that both denitrification and sedimentation are key processes controlling the N retention in lakes.

Sediment denitrification process is a large source of N₂O emitted into the atmosphere from lakes and other aquatic ecosystems (Mengis et al., 1997; Beaulieu et al., 2011). Determining the net N₂O production (i.e., N₂O production in the absence of acetylene) is important, because N₂O is a powerful greenhouse gas which play a key role in the destruction of stratospheric ozone (Hefting et al., 2006). The

mean net N₂O production rate of the sampling sites in the Lake Donghu was 0.32 ng N g⁻¹ h⁻¹ or 157 kg N km⁻² year⁻¹. This value is much greater than the average of 32 Norwegian lakes (68.60 kg N km⁻² year⁻¹) calculated by McCrackin and Elser (2010), but similar to the value of 20 Yangtze lakes (143 kg N km⁻² year⁻¹) reported by Liu et al (2015b). It should be noted that N₂O may also be produced by nitrification under incomplete anaerobic conditions (García-Ruiz et al., 1998), although denitrification is generally recognized as the dominant process for N₂O formation. In comparison with the N₂O emission from Chinese paddy fields (354 kg N km⁻² year⁻¹, Xing 1998), the Lake Donghu also seems to be a significant source of N₂O.

4.2 Environmental drivers of sediment denitrification and N₂O production

The effects of water quality on sediment denitrification in lakes were frequently described in previous studies (Hasegawa and Okino, 2004; Bruesewitz et al., 2011; Liu et al. 2015a). Consistent with studies conducted in rivers and streams (e.g., García-Ruiz et al., 1998), the present study found that water NO₃⁻ concentration had significant a relationship with the unamended denitrification rate in the Lake Donghu (Table 4). Because sediment denitrification is an anaerobic process that converts NO₃⁻ into N₂O or N₂, a high concentration of NO₃⁻ in overlying water may directly accelerate the denitrification rates. Therefore, N availability in water is generally recognized as one of the primary factors limiting the sediment denitrification in aquatic ecosystems (García-Ruiz et al., 1998; Inwood et al., 2005). Seitzinger et al. (2006) combined data from a range of aquatic ecosystems (e.g., lakes, estuaries, coastal seas, and continental shelves) and found that N loading could explain 77% of the variability in the total amount of annual denitrification. Meanwhile, using a

meta-analysis, Piña-Ochoa and Álvarez-Cobelas (2006) reported that water NO_3^- concentration could account for approximately 70% of the variation in sediment denitrification rate.

Some studies also reported significant relationships between denitrification and sediment characteristics (e.g., Bruesewitz et al., 2011; Liu et al., 2015a). Sediment organic carbon can act as an electron supplier in the denitrification process. Through the decomposition of sediment organic matter, the supply of carbon becomes available for denitrifying microbes; hence, positive relationships are often found between denitrification rates and organic matter or carbon contents of sediment (Bruesewitz et al., 2011). However, such significant relationship was not observed in the Yangtze lakes, probably because the average of sediment TC concentration was over 25 mg g^{-1} and was not limiting to the denitrification process (Liu et al. 2015b). Result from the present study was consistent with some previous work (e.g., Wall et al. 2005; McCrackin and Elser, 2010) and indicated that sediment unamended denitrification rate of the Lake Donghu was not limited by the availability of sediment organic matter.

The effect of P concentration on the sediment denitrification in aquatic ecosystems has gained growing attention in recent years (Piña-Ochoa and Álvarez-Cobelas, 2006; Finlay et al., 2013). Piña-Ochoa and Álvarez-Cobelas (2006) reported that low concentration of total P in water column could enhance denitrification rates at the yearly scale. They explained that lakes with high P level might lead to an imbalance of N and P supply to bacterial denitrification metabolism. In other words, a low N: P ratio could result in a higher competition for N from other organisms, such as benthic algae. In contrast, Finlay et al. (2013) indicated that

increased P inputs had stimulated N removal process in many lakes. This result may be due to the fact that increased P level in water stimulates algal production and inorganic N uptake and therefore increases the transfer of N and organic matter to sediments. In the present study, however, there was no significant correlation between sediment denitrification and phosphorus content in overlying water. Therefore, the effects of increased P on N removal in lakes are not uniform, and the mechanism of action of P on sediment denitrification has remained unclear.

5. Conclusion

In summary, the Lake Donghu showed a great spatial variation in sediment denitrification and N₂O production rates. Unamended denitrification rates varied widely across the sampling sites and averaged 3.97 ng N g⁻¹ h⁻¹, indicating that eutrophic lakes could remove large quantities of N through sediment denitrification. As hypothesized, unamended denitrification rate in sediments was found to be controlled primarily by water NO₃⁻ concentration and sediment moisture and bulk density. Our results, however, also showed that eutrophic lakes might become a significant source of N₂O. Further studies are needed to explore the seasonal dynamic of sediment denitrification to estimate the N removal capacity of the Lake Donghu more accurately. Also further direct measurements of N₂O emission are warranted to better understand the contribution of such eutrophic lakes to regional N₂O fluxes in China.

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REFERENCES

- Abell, J.M., Özkundakci, D., Hamilton, D.P., 2010. Nitrogen and phosphorus limitation of phytoplankton growth in New Zealand lakes: implications for eutrophication control. *Ecosystems* 13, 966-977.
- Beaulieu, J.J., Tank, J.L., Hamilton, S.K., Wollheim, W.M., Hall Jr, R.O., Mulholland, P.J., Peterson, B.J., Ashkenas, L.R., Cooper, L.W., Dahm, C.N., Dodds, W.K., Grimm, N.B., Johnson, S.L., McDowell, W.H., Poole, G.C., Valett, H.M., Arango, C.P., Bernot, M.J., Burgin, A.J., Crenshaw, C.L., Helton, A.M., Johnson, L.T., O'Brien, J.M., Potter, J.D., Sheibley, R.W., Sobota, D.J., Thomas, S.M., 2011. Nitrous oxide emission from denitrification in stream and river networks. *Proc. Natl. Acad. Sci. U. S. A.* 108, 214-219.
- Bebout, G.E., Fogel, M.L., Cartigny, P., 2013. Nitrogen: Highly volatile yet surprisingly compatible. *Elements* 9, 333-338.
- Bruesewitz, D.A., Hamilton, D.P., Schipper, L.A., 2011. Denitrification potential in lake sediment increases across a gradient of catchment agriculture. *Ecosystems* 14, 341-352.
- Carlson, R.E., 1977. A trophic state index for lakes. *Limnol. Oceanogr.* 22, 361-369.
- Carpenter, S.R., Caraco, N.F., Correll, D.L., Howarth, R.W., Sharpley, A.N., Smith, V.H., 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecol. Appl.* 8, 559-568.
- Cole, J.J., Prairie, Y.T., Caraco, N.F., McDowell, W.H., Tranvik, L.J., Striegl, R.G., Duarte,

- C.M., Kortelainen, P., Downing, J.A., Middelburg, J.J., Melack, J., 2007. Plumbing the global carbon cycle: integrating inland waters into the terrestrial carbon budget. *Ecosystems* 10, 171-184.
- Conley, D.J., Paerl, H.W., Howarth, R.W., Boesch, D.F., Seitzinger, S.P., Havens, K.E., Lancelot, C., Likens, G.E., 2009. Controlling eutrophication: nitrogen and phosphorus. *Science* 323, 1014-1015.
- Finlay, J.C., Small, G.E., Sterner, R.W., 2013. Human influences on nitrogen removal in lakes. *Science* 342, 247-250.
- García-Ruiz, R., Pattinson, S.N., Whitton, B.A., 1998. Denitrification in river sediments: relationship between process rate and properties of water and sediment. *Freshw. Biol.* 39, 467-476.
- Gruber, N., Galloway, J.N., 2008. An earth-system perspective of the global nitrogen cycle. *Nature* 451, 293-296.
- Harrison JA, Maranger RJ, Alexander RB, Giblin AE, Jacinthe PA, Mayorga E, Seitzinger SP, Sobota DJ, Wollheim WM (2009) The regional and global significance of nitrogen removal in lakes and reservoirs. *Biogeochemistry* 93: 143-157.
- Hasegawa, T., Okino, T., 2004. Seasonal variation of denitrification rate in Lake Suwa sediment. *Limnology* 5, 33-39.
- Havens, K.E., Fukushima, T., Xie, P., Iwakuma, T., James, R.T., Takamura, N., Hanazato, T., Yamamoto, T., 2001. Nutrient dynamics and the eutrophication of shallow lakes Kasumigaura (Japan), Donghu (PR China), and Okeechobee (USA). *Environ. Pollut.* 111, 263-272.
- Hayakawa, A., Nakata, M., Jiang, R., Kuramochi, K., and Hatano, R., 2012. Spatial variation of denitrification potential of grassland, windbreak forest, and

- riparian forest soils in an agricultural catchment in eastern Hokkaido, Japan, *Ecol. Eng.* 47, 92-100.
- Hefting, M.M., Bobbink, R., Janssens, M.P., 2006. Spatial variation in denitrification and N₂O emission in relation to nitrate removal efficiency in a N-stressed riparian buffer zone. *Ecosystems* 9, 550-563.
- Inwood, S.E., Tank, J.L., Bernot, M.J., 2005. Patterns of denitrification associated with land use in 9 midwestern headwater streams, *J. N. Am. Benthol. Soc.* 24, 227-245.
- Jansson, M., Andersson, R., Berggren, H., Leonardson, L., 1994. Wetlands and lakes as nitrogen traps. *Ambio* 23, 320-325.
- Li, Y., Zhao, S.Q., Zhao, K., Xie, P., Fang, J.Y., 2006. Land-cover changes in an urban lake watershed in a mega-city, Central China. *Environ. Monit. Assess.* 115, 349-359.
- Liu, W.Z., Li, S.Y., Bu, H.M., Zhang, Q.F., Liu, G.H., 2012. Eutrophication in the Yunnan Plateau lakes: the influence of lake morphology, watershed land use, and socio-economic factors. *Environ. Sci. Pollut. Res.* 19, 858-870.
- Liu, W.Z., Wang, Z.X., Zhang, Q.F., Cheng, X.L., Lu, J., Liu, G.H., 2015a. Sediment denitrification and nitrous oxide production in Chinese plateau lakes with varying watershed land uses. *Biogeochemistry* 123, 379-390.
- Liu W.Z., Yao, L., Wang, Z.X., Xiong, Z.Q., Liu, G.H., 2015b. Human land uses enhance sediment denitrification and N₂O production in Yangtze lakes primarily by influencing lake water quality. *Biogeosciences* 12, 6059-6070.
- Liu, W.Z., Zhang, Q.F., Liu, G.H., 2010. Lake eutrophication associated with geographic location, lake morphology and climate in China. *Hydrobiologia* 644, 289-299.
- Ma, R.H., Yang, G.H., Duan, H.T., Jiang, J.H., Wang, S.M., Feng, X.Z., Li, AN., Kong, F.X.,

- Xue, B., Wu, J.L., Li, S.J., 2011. China's lakes at present: number, area and spatial distribution. *Sci. China-Earth Sci.* 54, 283-289.
- McCrackin, M.L., Elser, J.J., 2010. Atmospheric nitrogen deposition influences denitrification and nitrous oxide production in lakes. *Ecology* 91, 529-539.
- Mengis, M., Gächter, R., Wehrli, B., 1997. Sources and sinks of nitrous oxide (N₂O) in deep lakes. *Biogeochemistry* 38, 281-301.
- Piña-Ochoa, E. Álvarez-Cobelas, M., 2006. Denitrification in aquatic environments: a cross-system analysis. *Biogeochemistry* 81, 111-130.
- Qiu, D.R., Wu, Z.B., Liu, B.Y., Deng, J.Q., Fu, G.P., He, F., 2001. The restoration of aquatic macrophytes for improving water quality in a hypertrophic shallow lake in Hubei Province, China. *Ecol. Eng.* 18, 147-156.
- Rissanen, A.J., Tirola, M., Hietanen, S., Ojala, A., 2013. Interlake variation and environmental controls of denitrification across different geographical scales. *Aquat. Microb. Ecol.* 69, 1-16.
- Saunders, D.L., Kalff, J., 2001. Nitrogen retention in wetlands, lakes and rivers. *Hydrobiologia* 443, 205-212.
- Seitzinger, S.P., 1990. Denitrification in aquatic sediments. In: Revsbech, N.P., Sørensen, J. (Eds.). *Denitrification in Soil and Sediment*. Plenum Press, New York, pp. 301-322.
- Seitzinger, S.P., Harrison, J.A., Böhlke, J.K., Bouwman, A.F., Lowrance, R., Peterson, B., Tobias, C., Van Drecht, G., 2006. Denitrification across landscapes and waterscapes: a synthesis, *Ecol. Appl.* 16, 2064-2090.
- Stolp, H., 1988. *Microbial ecology: organisms, habitats, activities*. Cambridge University Press, Cambridge, pp. 16-17.

- Van Luijn, F., Boers, P., Lijklema, L., 1996. Comparison of denitrification rates in lake sediments obtained by the N₂ flux method, the ¹⁵N isotope pairing technique and the mass balance approach. *Water Res.* 30, 893-900.
- Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D.G., 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecol. Appl.* 7, 737-750.
- Wall, L.G., Tank, J.L., Royer, T.V., Bernot, M.J., 2005. Spatial and temporal variability in sediment denitrification within an agriculturally influenced reservoir. *Biogeochemistry*, 76, 85-111.
- Xie, L.Q., Xie, P., 2002. Long-term (1956–1999) dynamics of phosphorus in a shallow, subtropical Chinese lake with the possible effects of cyanobacterial blooms. *Water Res.* 36, 343-349.
- Xing, G.X., 1998. N₂O emission from cropland in China. *Nutr. Cycl. Agroecosyst.* 52, 249-254.
- Xiong, Z.Q., Li, S.C., Yao, L., Liu, G.H., Zhang, Q.F., Liu, W.Z., 2015. Topography and land use effects on spatial variability of soil denitrification and related soil properties in riparian wetlands. *Ecol. Eng.* 83, 437-443.
- Yang, H.D., Nong, S.W., Cai, S.M., Cai, Q.H., 1994. Environmental geochemistry of sediments in Lake Donghu, Wuhan. *Acta Hydrobiol. Sin.* 18, 208-214.
- Zhao, J.W., Zhu, D.W., Fan, J.N., Hua, Y.M., Zhou, W.B., 2015. Seasonal variation of anammox and denitrification in sediments of two eutrophic urban lakes. *Pol. J. Environ. Stud.* 24, 2779-2783.

Table 1 Summary statistics for sediment denitrification variables, water quality and sediment characteristics of the Lake Donghu (N=21).

	Abbr	Min.	Max.	Mean	Standard deviation
Sediment denitrification variables					
Potential denitrification rate (ng N g ⁻¹ h ⁻¹)		31.4	154	77.0	31.8
Unamended denitrification rate (ng N g ⁻¹ h ⁻¹)		0.51	26.0	3.97	5.51
N ₂ O production rate (ng N g ⁻¹ h ⁻¹)		-0.01	1.68	0.32	0.39
Water quality					
Depth (m)		1.70	3.50	2.40	0.41
Secchi depth (m)	SD	0.20	0.60	0.35	0.12
Temperature (°C)	T	26.1	27.6	26.8	0.40
Conductivity (µS cm ⁻¹)	Cond	152	362	273	80.5
Dissolved oxygen (mg L ⁻¹)	DO	4.62	8.17	6.90	1.04
Oxidation–reduction potential (mv)	ORP	63.0	138	108	19.3
Total organic carbon (mg L ⁻¹)	TOC	3.59	18.4	6.03	3.90
Nitrate (mg L ⁻¹)	NO ₃ ⁻	0.27	0.83	0.41	0.15
Total organic carbon: nitrate	TOC: NO ₃ ⁻	4.86	31.7	14.9	6.76
Ammonium (mg L ⁻¹)	NH ₄ ⁺	0.16	0.38	0.25	0.05
Total nitrogen (mg L ⁻¹)	TN	0.56	2.90	1.05	0.71
Total phosphorus (mg L ⁻¹)	TP	0.02	0.12	0.07	0.03
Chlorophyll- <i>a</i> (mg m ⁻³)	Chl- <i>a</i>	5.60	44.3	15.4	10.3
Sediment characteristics					
pH		7.26	8.25	7.86	0.25
Moisture (%)		36.5	83.2	58.8	14.0
Bulk density (g cm ⁻³)	BD	0.71	0.98	0.84	0.09
Sediment organic matter (%)	SOM	2.24	16.3	8.67	3.88
Sediment total carbon (mg g ⁻¹)	STC	13.2	66.9	27.0	13.6
Sediment total nitrogen (mg g ⁻¹)	STN	0.04	2.85	0.58	0.73
Sediment total phosphorus (mg g ⁻¹)	STP	0.00	2.63	0.57	0.61

Table 2 The mean values of water quality parameters in different sub-lakes of the Lake Donghu.

	Guozhenghu L.	HouHu L.	Lingjiaohu L.	Miaohu L.	Tanglinhu L.	Niuchaohu L.	Yujiahu L.
Depth	2.13	2.50	2.65	2.83	2.43	2.27	1.97
SD	0.33 ^{ab}	0.53 ^a	0.33 ^{ab}	0.23 ^b	0.37 ^{ab}	0.43 ^a	0.23 ^b
T	26.4 ^a	27.4 ^b	26.4 ^a	26.8 ^{ab}	26.7 ^{ab}	26.8 ^{ab}	26.8 ^{ab}
Cond	348	213	275	304	344	222	206
DO	6.60	7.48	6.92	6.91	7.03	7.93	5.46
ORP	99.0 ^{bc}	107 ^b	101 ^b	113 ^{ab}	135 ^a	121 ^{ab}	75.7 ^c
TOC	5.54	5.82	4.96	4.43	4.38	8.24	8.86
NO ₃ [−]	0.34 ^b	0.33 ^b	0.31 ^b	0.40 ^b	0.39 ^b	0.39 ^b	0.74 ^a
TOC: NO ₃ [−]	16.5	17.7	15.9	11.3	11.2	19.5	12.7
NH ₄ ⁺	0.21 ^b	0.24 ^{ab}	0.23 ^{ab}	0.32 ^a	0.29 ^{ab}	0.24 ^{ab}	0.23 ^{ab}
TN	0.79 ^b	0.63 ^b	0.72 ^b	1.35 ^b	0.64 ^b	0.68 ^b	2.53 ^a
TP	0.09 ^{ab}	0.05 ^{ab}	0.03 ^b	0.08 ^{ab}	0.07 ^{ab}	0.06 ^{ab}	0.10 ^a
Chl- <i>a</i>	9.90 ^b	10.2 ^b	15.7 ^b	13.6 ^b	10.0 ^b	10.1 ^b	38.6 ^a

Within a row, mean values followed by different letter are significantly different ($p < 0.05$). See Table 1 for explanation of the abbreviations.

Table 3 The mean values of sediment characteristics in different sub-lakes of the Lake Donghu.

	Guozhenghu L.	HouHu L.	Lingjiaohu L.	Miaohu L.	Tanglinhu L.	Niuchaohu L.	Yujiahu L.
pH	8.01	7.98	8.01	7.57	8.03	7.59	7.84
Moisture	62.1	44.2	54.1	66.4	58.9	57.7	68.2
BD	0.84	0.76	0.80	0.88	0.83	0.84	0.90
SOM	9.76	4.76	8.09	10.8	8.03	7.77	11.5
STC	30.4	13.5	29.4	37.3	23.7	23.8	31.1
STN	0.31 ^{ab}	0.39 ^{ab}	0.19 ^b	1.88 ^a	0.32 ^{ab}	0.62 ^{ab}	0.33 ^{ab}
STP	0.51 ^{ab}	0.36 ^b	0.18 ^b	1.65 ^a	0.30 ^b	0.15 ^b	0.81 ^{ab}

Within a row, mean values followed by different letter are significantly different ($p < 0.05$). See Table 1 for explanation of the abbreviations.

Table 4 Pearson correlation coefficients between sediment denitrification variables and water quality and sediment characteristics (N = 21).

	Potential denitrification rate	Unamended denitrification rate	N ₂ O production rate
Water quality			
Depth	-0.23	-0.14	-0.14
SD	-0.14	-0.15	0.00
T	-0.16	0.00	-0.28
Cond	0.02	-0.36	0.05
DO	-0.11	-0.07	0.20
ORP	-0.16	-0.10	-0.24
TOC	0.62**	0.36	0.23
NO ₃ ⁻	0.43	0.63**	0.01
TOC: NO ₃ ⁻	0.37	-0.07	0.21
NH ₄ ⁺	-0.03	0.23	-0.30
TN	0.13	0.30	-0.12
TP	0.07	0.16	-0.06
Chl- <i>a</i>	0.24	0.18	0.07
Sediment characteristics			
pH	-0.40	-0.36	0.04
Moisture	0.20	0.51*	-0.07
BD	0.20	0.49*	-0.21
SOM	0.15	0.34	-0.05
STC	0.12	0.29	-0.05
STN	-0.27	0.16	-0.21
STP	-0.16	0.20	-0.36

*Significant at the 0.05 level; ** Significant at the 0.01 level. See Table 1 for explanation of the abbreviations.

Figure captions

Fig. 1 Locations of the sampling sites in the Lake Donghu of China

Fig. 2 Trophic state index of seven sub-lakes within the Lake Donghu of China

Fig. 3 Sediment denitrification and N_2O production rate (mean $\pm$ SD) for seven sub-lakes within the Lake Donghu of China

Fig. 1

Fig. 2

Fig. 3