

# Sediment denitrification and nitrous oxide production in Chinese plateau lakes with varying watershed land uses

Wenzhi Liu · Zhixiu Wang · Quanfa Zhang · Xiaoli Cheng · Jing Lu · Guihua Liu

Received: 6 June 2014 / Accepted: 14 January 2015 / Published online: 4 February 2015  
© Springer International Publishing Switzerland 2015

**Abstract** Denitrification in lake sediments removes nitrogen and releases N<sub>2</sub>O to the atmosphere, contributing to global warming. However, the rates and controls of sediment denitrification and N<sub>2</sub>O production are still poorly understood in lakes. In this study, we measured potential denitrification, unamended denitrification and N<sub>2</sub>O production rate in sediments which were collected from 68 sites in 20 Chinese plateau lakes of varying watershed land uses. The result showed that potential denitrification rate of human-dominated lakes ( $37.94 \pm 8.91 \text{ ng N g}^{-1} \text{ h}^{-1}$ ) was significantly higher than that of reference lakes ( $18.50 \pm 3.22 \text{ ng N g}^{-1} \text{ h}^{-1}$ ). In addition, potential denitrification rate was positively related to

the proportion of human land uses in watersheds. At the lake level, unamended denitrification and N<sub>2</sub>O production rates were significantly related to water chemistry or sediment properties (e.g., conductivity and sediment total nitrogen). Water chemistry and sediment properties together accounted for 0–69 % of the variance in denitrification and N<sub>2</sub>O production rates. Variance partitioning showed that unamended denitrification and N<sub>2</sub>O production rates in human-dominated lakes were controlled primarily by sediment properties, while in reference lakes were generally controlled by water chemistry. Our findings suggest that Chinese plateau lakes can remove large quantities of nitrogen through sediment denitrification and produce small amounts of N<sub>2</sub>O. The relative contributions of water chemistry and sediment properties to the lake denitrification and N<sub>2</sub>O production vary widely.

Responsible Editor: Jennifer Leah Tank.

**Electronic supplementary material** The online version of this article (doi:10.1007/s10533-015-0072-9) contains supplementary material, which is available to authorized users.

W. Liu · Z. Wang · Q. Zhang · X. Cheng · G. Liu (✉)  
Key Laboratory of Aquatic Botany and Watershed Ecology Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan 430074, People's Republic of China  
e-mail: liugh@wbgcas.cn

Z. Wang  
College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

J. Lu  
Australian Rivers Institute, Griffith University, Brisbane, Queensland 4111, Australia

**Keywords** Eutrophication · Greenhouse gas · Nitrogen pollution · Potential denitrification · Unamended denitrification · Watershed landscape

## Introduction

Large increases in nitrogen (N) inputs from sewage, fertilizer and atmospheric deposition in terrestrial ecosystems have been reflected in increased N concentration in many lakes all over the world (Vitousek et al. 1997; Müller et al. 1998; Liu et al.

2010; McCrackin and Elser 2010). N has been recognized as an important and often the limiting or co-limiting nutrient for aquatic productivity in lake ecosystems (Elser et al. 2009; Abell et al. 2010). However, excess N in lakes may lead to eutrophication and many attendant environmental problems such as water quality decline, algal blooms, and loss of aquatic biodiversity (Carpenter et al. 1998; Abell et al. 2010; Liu et al. 2011). In China, the percentage of eutrophic lakes has rapidly increased from 41.2 % in 1980 to 84.5 % in 2005 (Liu et al. 2010).

Growing concern about the global N cycle and local N pollution, particularly in Europe and North America, has stimulated research on the ability and mechanism of lakes to remove excess N input from watersheds (Seitzinger 1988; van Luijn et al. 1996; Saunder and Kalff 2001; Piña-Ochoa and Álvarez-Cobelas 2006; Kreiling et al. 2011). The three main processes contribute to nitrogen retention are sedimentation, biological uptake and sediment denitrification (Saunder and Kalff 2001). In contrast to sedimentation and biological uptake, the microbial process of sediment denitrification results in the permanent removal of N through the conversion of nitrate ( $\text{NO}_3^-$ ) to nitrous oxide ( $\text{N}_2\text{O}$ ) and dinitrogen ( $\text{N}_2$ ) gases, which can be emitted into the atmosphere (Betz and Groffman 2012).

As an intermediate product of denitrification,  $\text{N}_2\text{O}$  can be emitted from sediment to the atmosphere in appropriate conditions (Hefting et al. 2006). Understanding the  $\text{N}_2\text{O}$  production of sediments is very important, as  $\text{N}_2\text{O}$  is both a potent greenhouse gas and a major source of nitric oxide (NO) and nitrogen dioxide ( $\text{NO}_2$ ), which play an important role in the destruction of stratospheric ozone (Beaulieu et al. 2011). Aquatic ecosystems, such as rivers and estuaries, are recognized as a globally significant source of  $\text{N}_2\text{O}$  (Ussiri and Lal 2013). River networks are estimated to account for approximately 10 % of the global anthropogenic  $\text{N}_2\text{O}$  emissions (Beaulieu et al. 2011). Although sediment denitrification is the predominant  $\text{N}_2\text{O}$  production process in lakes (Freymond et al. 2013), information on  $\text{N}_2\text{O}$  production of sediments is still limited (McCrackin and Elser 2010).

Factors that regulate denitrification and  $\text{N}_2\text{O}$  production can be categorized as proximal and distal controls (Wallenstein et al. 2006). Proximal controls, including available carbon,  $\text{NO}_3^-$  concentration and oxygen supply in overlying water and sediment, affect

the instantaneous rate of sediment denitrification (Saggar et al. 2013). In contrast, distal controls affect the denitrification indirectly by acting on the proximal controls (Roy et al. 1994). These include factors such as land use, plant communities, and soil texture. By generating point- and nonpoint-source pollutants (Liu et al. 2012), agricultural and urban land uses in watersheds can increase the N concentration in lakes, which may in turn regulate the sediment denitrification. Researchers have also found that agricultural landscapes can increase carbon inputs to lakes as a result of increased soil erosion or increased lake productivity resulting in higher rates of organic matter deposition in sediments (Bruesewitz et al. 2011). However, till now, very few studies have related watershed land use to sediment denitrification and  $\text{N}_2\text{O}$  production in lakes (but see Bruesewitz et al. 2011). Moreover, the relative roles of water chemistry and sediment properties in governing lake denitrification are still unclear.

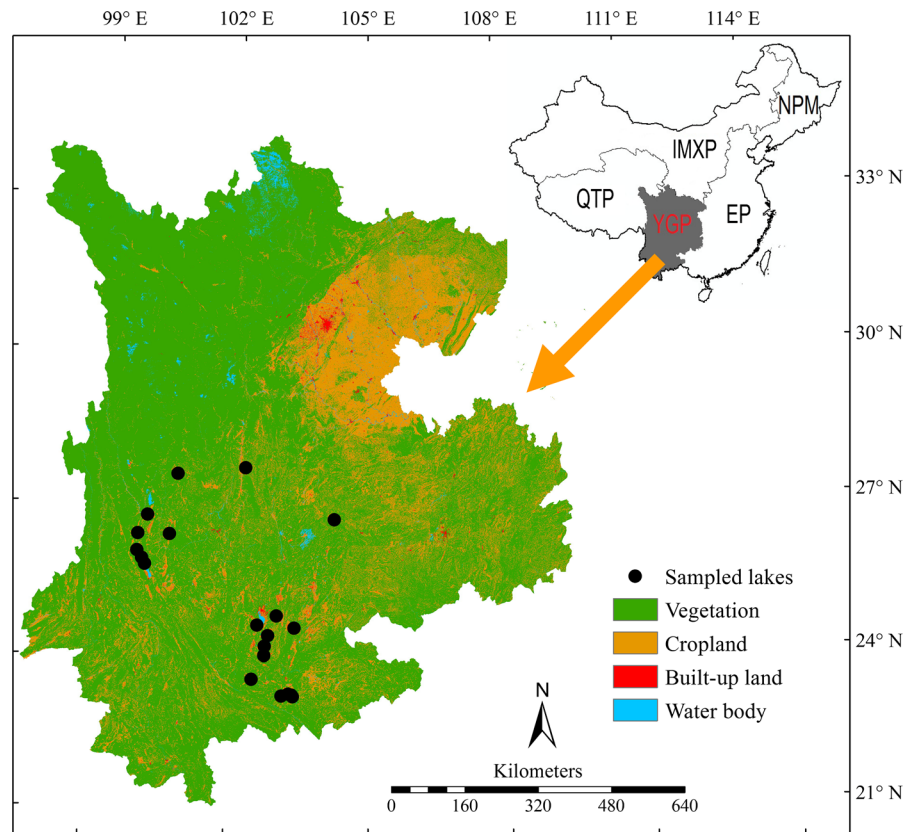
In this study, we measured the potential denitrification, unamended denitrification, and  $\text{N}_2\text{O}$  production rate in surface sediments which were collected from 68 sites in 20 Chinese plateau lakes of varying watershed land uses. We hypothesized that denitrification and  $\text{N}_2\text{O}$  production rates would be higher in human-dominated lakes than in reference lakes due to the greater input of N from watersheds. The objectives of this study therefore were (1) to examine the spatial variability in sediment denitrification and  $\text{N}_2\text{O}$  production rates in 20 plateau lakes in China and (2) to determine the relationships between denitrification and  $\text{N}_2\text{O}$  production rates and water chemistry and sediment properties at both the lake and site levels.

## Materials and methods

### Site description

China has 2,693 lakes with a surface area greater than  $1 \text{ km}^2$ , accounting for about 0.85 % of the total land area of China (Ma et al. 2011). On the basis of topography, climate and administrative division, China can be divided into five lake regions, as follows: the Qinghai–Tibetan Plateau, the Eastern Plains (e.g., the Yangtze floodplain), the Inner Mongolia–Xinjiang Plateau, the Northeast Plain and Mountain, and the Yunnan–Guizhou Plateau (Fig. 1). The Qinghai–Tibet

**Fig. 1** Locations of the 20 studied lakes in the Yunnan–Guizhou Plateau of China. *YGP* Yunnan–Guizhou Plateau, *QTP* Qinghai–Tibetan Plateau, *EP* Eastern Plains, *IMXP* Inner Mongolia–Xinjiang Plateau, *NPM* Northeast Plain and mountain



Plateau and Eastern Plains, which contain 1,055 and 634 lakes, respectively, are the two largest lake regions in China (Ma et al. 2011).

The Yunnan–Guizhou Plateau, located in the southwest of China (Fig. 1), is the smallest one among the five lake regions in China. It is the middle tier on the eastern slope of the Himalayas and has intensive neotectonic movement since late Pliocene time. The plateau receives 800–1,200 mm of precipitation annually, over 85 % of which falls between May and October. There are only 65 lakes (most are tectonic in origin) with a surface area greater than 1 km<sup>2</sup> (Ma et al. 2011). These plateau lakes play a very important role in providing freshwater resources and developing tourism and aquaculture, but their water quality has deteriorated rapidly in recent decades due to increasing point- and nonpoint-source pollution in watersheds. In a previous study (Liu et al. 2012), we reported that trophic status of these lakes ranges from extremely oligotrophic (e.g., Lake Luguhu and Lake Fuxianhu) to hyper-eutrophic (e.g., Lake Qiluhu and Lake Xingyunhu).

### Field sampling

In May 2012, we sampled 20 lakes located in the Yunnan–Guizhou Plateau of China (Fig. 1). Lakes with an area greater than 10 km<sup>2</sup> (N = 13) in the plateau were all included in this study. The other seven lakes with an area less than 10 km<sup>2</sup> were selected based on accessibility. The 20 selected lakes covered a wide range of morphometric, chemical and biological characteristics (Wang and Dou 1998). The name, geographic location, and morphology characteristics of these lakes are listed in Table S1 in supporting information. To obtain representative samples, we established one transect from the littoral to the pelagic zone for each lake (Yang et al. 2008). 3–6 sampling sites were chosen along each transect at regular intervals based on lake area (Table S1). Littoral sites were located at a water depth less than 2 m, approximately 100–300 m away from the shorelines. Pelagic sites were generally located at a water depth of about 10 m or at the approximate maximum depth if the lake was <10 m depth.

At each sampling site, surface sediments were randomly collected from a boat within an approximately 50 m<sup>2</sup> area using a Peterson dredge, and then mixed and homogenized to form a composite sample. Approximately 1 kg of sediment was placed in a sealed plastic bag for each site. Sediments were stored at about 5 °C in a refrigerator until return to the laboratory. At each sampling site, a 200-mL unfiltered water sample was collected at a depth of about 1 m above the sediments for use in the denitrification and N<sub>2</sub>O emission assays. At the same time, we collected an additional 200-mL water sample at the same depth for N analyses in laboratory. Water sampling was performed before collecting sediment to prevent sediments from being resuspended and thereby contaminating the water.

#### Measurements of denitrification and N<sub>2</sub>O production

We measured the potential denitrification, unamended denitrification and N<sub>2</sub>O production rate of 68 sediment samples in a laboratory located in Kunming City of the Yunnan–Guizhou Plateau. Potential and unamended denitrification rates were determined using the acetylene block technique, which inhibited the final conversion of N<sub>2</sub>O to N<sub>2</sub>. Although the technique has a number of limitations (Felber et al. 2012), it is still widely used and amenable to large-scale comparisons of denitrification, especially for systems with moderate or high NO<sub>3</sub><sup>-</sup> contents (Groffman et al. 2006). Potential denitrification rate was determined in the presence of added carbon and NO<sub>3</sub><sup>-</sup> and thus provided an upper-bound estimate of actual denitrification. Unamended denitrification rate was a conservative estimate of actual denitrification without carbon and NO<sub>3</sub><sup>-</sup> amendments because acetylene also inhibited the nitrification (Forshay and Dodson 2011). The N<sub>2</sub>O production rate was estimated by the N<sub>2</sub>O accumulation in treatments without acetylene (McCrackin and Elser 2010).

For potential denitrification assays, 50 g of homogenized sediment from each sampling site was slurried with 30 mL of incubation solution (final concentrations: 0.18 g/L glucose, 0.1 g/L KNO<sub>3</sub> and 1 g/L chloramphenicol) in a 250 mL serum bottle (Pell et al. 1996). Each bottle was then sealed and purged with N<sub>2</sub> for 2 min to induce anaerobic conditions. About 10 % of the bottle headspace was replaced with acetylene.

We measured unamended denitrification and N<sub>2</sub>O production rates using the 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<sub>2</sub>O production rates. All bottles were then incubated in the dark for 4 h at 25 °C (the approximate in situ water temperature). Short-term incubation (2–4 h) was recommended for measuring sediment denitrification of lakes (Bruesewitz et al. 2012), because the increase in N<sub>2</sub>O concentrations was always linear throughout the short assay duration. 5 mL of headspace gas was sampled from each bottle (after shaking vigorously) using a syringe at the beginning and end of incubation (McCrackin and Elser 2010).

The N<sub>2</sub>O 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<sub>2</sub>O production rates were calculated as the difference between the initial and final headspace N<sub>2</sub>O concentrations (corrected for N<sub>2</sub>O 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 (DM) of sediment ( ng N g<sup>-1</sup> h<sup>-1</sup>).

#### Measurements of water chemistry and sediment properties

At each sampling site, water depth and Secchi depth (SD) were recorded, and water temperature, pH, dissolved oxygen (DO), conductivity (Cond), nitrate (NO<sub>3</sub><sup>-</sup>) and ammonium (NH<sub>4</sub><sup>+</sup>) were measured on site at the sampling depth using a YSI Professional Plus multi-parameter water quality meter (YSI Inc., Yellow Springs, Ohio, USA). Total nitrogen (TN) and total dissolved nitrogen (TDN) were determined by alkaline persulfate digestion of the water samples and measuring NO<sub>3</sub><sup>-</sup> in the digests. Water samples were filtered through a 0.45-μm cellulose acetate membrane before TDN measurement.

Sediment moisture was measured gravimetrically (24 h at 105 °C) from 30 g sediment samples. Sediment pH was measured in a soil to water ratio of 1:5 (v/v) by a pH meter and bulk density was determined by weighing sediments of known volume after drying for 24 h at 105 °C. Sediment organic

matter (SOM) was determined by the loss on ignition for 4 h at 450 °C. Sediment total nitrogen (STN) was measured using a Kjeldahl method after digesting samples in a digester using sulfuric acid/mercuric oxide catalyst. Sediment total carbon (STC) content of air-dried samples was analyzed by a TOC analyzer (Vario TOC cube, Elementar, Germany).

#### Calculation of watershed land use

We obtained land use map of the Yunnan–Guizhou Plateau from the Data Sharing Infrastructure of Earth System Science in China (<http://www.geodata.cn/>). The map with a spatial resolution of 100 m was derived from Landsat TM images in 2005. We delineated the watershed boundaries of the 20 plateau lakes using a 1 km resolution digital elevation model. Land use data of each lake's watershed was extracted by using overlay functions on watershed boundaries and land cover data layers in ArcGIS version 10.0 (ESRI, Redlands, California, USA). The original land use classes were grouped into four main categories: (1) vegetation, including forest and grassland; (2) cropland, including dry land and paddy field; (3) built-up land, including urban area, rural settlements and others such as industrial areas, roads, and airports; (4) water body, including lakes, rivers, streams, reservoirs, ponds, and wetlands (Table S1). Finally, the 20 plateau lakes were roughly classified into human-dominated ( $N = 9$ ) and reference ( $N = 11$ ). Reference lakes were difficult to describe quantitatively because of the subjective judgment involved (Herlihy et al. 2013). Based on previous reports (e.g., Cheruvilil et al. 2008), reference lakes in the present study were defined as relatively undisturbed lakes with <30 % of human land uses (i.e., cropland and built-up land) in their watersheds (Table S1).

Lake morphology characteristics including mean depth, surface area, and water residence time of the 20 plateau lakes were obtained primarily from a monograph of Chinese lakes (Wang and Dou 1998). Water residence time was defined as the ratio of lake volume to the volume of water lost through outflow and evaporation during a given time period (Liu et al. 2011).

#### Statistical analyses

The data of water  $\text{NO}_3^-$  and  $\text{NH}_4^+$  concentrations had many zero values because the  $\text{NO}_3^-$  and  $\text{NH}_4^+$  probes

were not calibrated before measurements. Therefore, the two variables were not included in subsequent statistical analyses. Data were checked for normal distribution using the Shapiro–Wilk test before statistical analyses. When possible, non-normally distributed data were  $\ln(x)$  or  $\ln(x + 1)$  transformed to reach a normal distribution. We carried out a  $t$  test to examine the differences in sediment denitrification and  $\text{N}_2\text{O}$  production rates between human-dominated and reference lakes, and between littoral sites (0–2 m deep) and profundal sites (>2 m deep). Relationships between sediment denitrification and  $\text{N}_2\text{O}$  production rates and the watershed land use, water chemistry and sediment properties were examined using simple regression analyses. Finally, we conducted a series of partial multiple regression to partition the variance in sediment denitrification and  $\text{N}_2\text{O}$  production rates into the components uniquely explained by water chemistry, uniquely explained by sediment properties, explained jointly by water chemistry and sediment properties, and explained by none of these variables. Statistical analyses and graphs were performed using PASW Statistics 18.0 software (IBM SPSS Inc., Chicago, USA) and Origin 8.0 software (OriginLab, Northampton, US).

## Results

### Denitrification and $\text{N}_2\text{O}$ production rates

Potential denitrification rate ranged from  $3.97 \pm 2.90 \text{ ng N g}^{-1} \text{ h}^{-1}$  in Lake Changhu to  $99.71 \pm 11.93 \text{ ng N g}^{-1} \text{ h}^{-1}$  in Lake Qiluhu (Table S1). Unamended denitrification rate was considerably lower than potential denitrification rate, with the lowest ( $0.02 \pm 0.03 \text{ ng N g}^{-1} \text{ h}^{-1}$ ) in Lake Luguahu and the highest ( $8.13 \pm 3.93 \text{ ng N g}^{-1} \text{ h}^{-1}$ ) in Lake Qiluhu (Table S1). Five lakes showed negative values for  $\text{N}_2\text{O}$  production rate. The lowest value of  $\text{N}_2\text{O}$  production rate ( $-0.05 \pm 0.03 \text{ ng N g}^{-1} \text{ h}^{-1}$ ) was recorded in Lake Luguahu (Table S1), an oligotrophic and deep lake (mean depth 40 m) in the Yunnan–Guizhou Plateau.

### Denitrification and $\text{N}_2\text{O}$ production rates related to within-lake variables

At the lake level, potential denitrification rate was not significantly related to water chemistry and sediment

properties. Unamended denitrification rate increased with TN concentrations in overlying water ( $R^2 = 0.26$ ,  $P = 0.02$ , Fig. 2).  $N_2O$  production rate was positively related to Cond in water ( $R^2 = 0.27$ ,  $P = 0.02$ ) and STN concentrations in sediment ( $R^2 = 0.20$ ,  $P = 0.05$ , Fig. 2). Unamended denitrification rate in littoral sites ( $1.28 \pm 0.39 \text{ ng N g}^{-1} \text{ h}^{-1}$ ) was significantly higher than that in profundal sites ( $0.28 \pm 0.10 \text{ ng N g}^{-1} \text{ h}^{-1}$ ,  $P = 0.02$ , Fig. S1). At the site level, both potential and unamended denitrification rates were significantly correlated with N concentrations in water and sediment (Fig. S2).  $N_2O$  production rate was significantly related to Cond and the concentrations of SOM and STN (Fig. S2).

Denitrification and  $N_2O$  production rates related to watershed land use

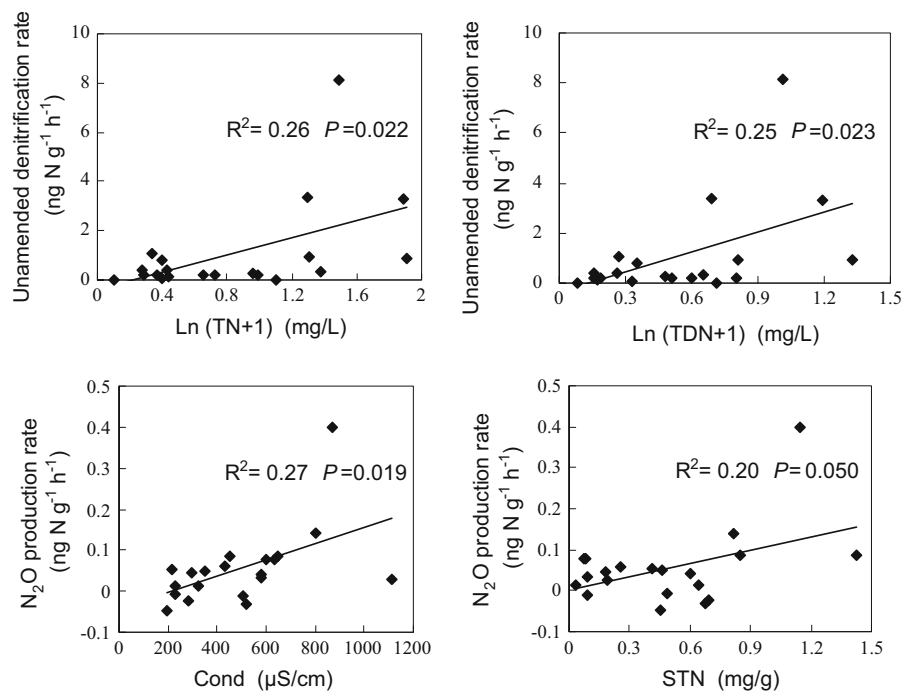
Although the potential denitrification, unamended denitrification and  $N_2O$  production rates were all higher in human-dominated lakes than in reference lakes (Table 1), only the potential denitrification rate had a significant difference ( $P = 0.04$ ). The proportion of human land uses in watersheds was positively related to potential denitrification rate ( $R^2 = 0.38$ ,  $P < 0.01$ ), but not to unamended denitrification rate and  $N_2O$  production rate (Fig. 3).

## Controls on denitrification and $N_2O$ production rates

Multiple regression analyses demonstrated that, at the lake level, water chemistry and sediment properties together accounted for 21.7–69.0 % of the variance in unamended denitrification and  $N_2O$  production rates (Table 2). However, water chemistry and sediment properties did not explain any variance in potential denitrification for all lakes group and human-dominated lakes group (Table 2). Variance partitioning analyses indicated that unamended denitrification and  $N_2O$  production rates in human-dominated lakes were controlled primarily by sediment properties, while in reference lakes were generally controlled by water chemistry (Fig. 4a).

At the site level, 11.0–47.9 % of the variance in potential denitrification, unamended denitrification and  $N_2O$  production rate were explained by TN, TDN, Cond, SOM, STN and sediment moisture (Table 2). Variance partitioning analyses showed that for the two groups (all sites and sites from reference lakes), potential denitrification rate was primarily influenced by sediment properties, while unamended denitrification and  $N_2O$  production rates were largely controlled by overlying water chemistry (Fig. 4b).

**Fig. 2** The relationships between denitrification and  $N_2O$  production rates and water chemistry and sediment properties at the lake level ( $N = 20$ ). Each point corresponds to a lake

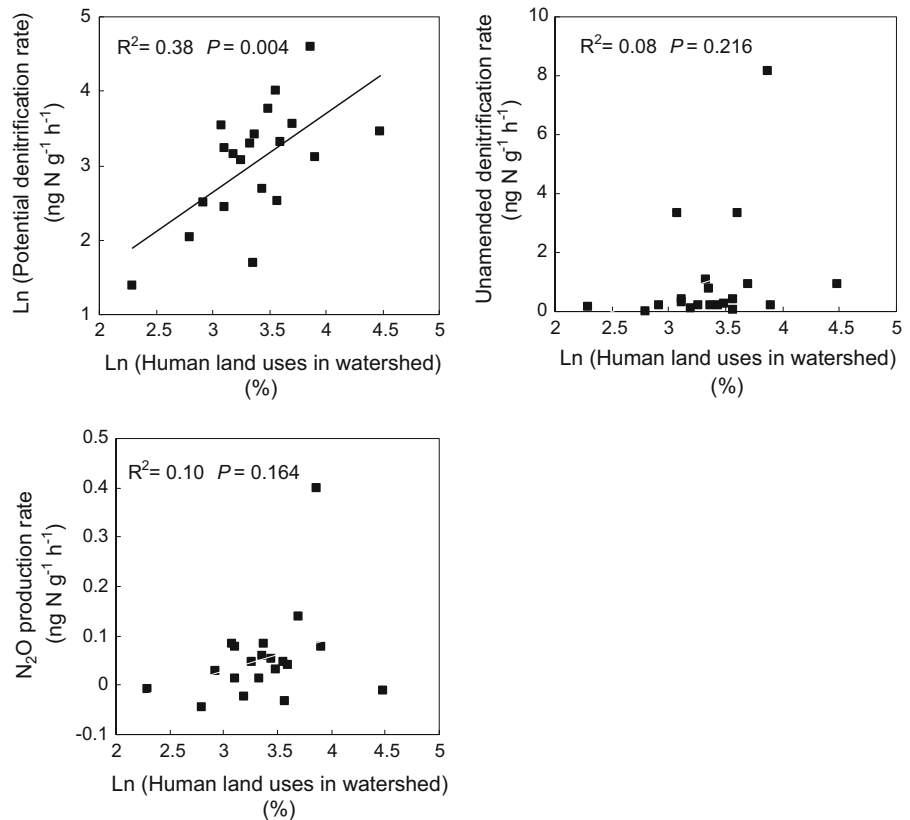


**Table 1** The mean values and standard error of sediment denitrification and N<sub>2</sub>O production rates in 20 plateau lakes

	Units	Human-dominated lakes (N = 9)	Reference lakes (N = 11)	All lakes (N = 20)
Potential denitrification rate	ng N g <sup>-1</sup> h <sup>-1</sup>	37.94 ± 8.91*	18.50 ± 3.22*	27.24 ± 4.79
Unamended denitrification rate	ng N g <sup>-1</sup> h <sup>-1</sup>	1.61 ± 0.88	0.62 ± 0.29	1.06 ± 0.43
N <sub>2</sub> O production rate	ng N g <sup>-1</sup> h <sup>-1</sup>	0.08 ± 0.04	0.03 ± 0.01	0.05 ± 0.02

\* Significant difference between the human-dominated and reference lakes at  $P < 0.05$

**Fig. 3** The relationships between denitrification and N<sub>2</sub>O production rates and the proportion of human land uses in the watershed at the lake level (N = 20). Each point corresponds to a lake



Interestingly, at both lake and site levels, water chemistry and sediment properties together explained a larger fraction of the variance of reference lakes than of human-dominated lakes (Table 2; Fig. 4).

## Discussion

Controls on sediment denitrification and N<sub>2</sub>O production in lakes

Consistent with previous studies conducted in streams and rivers (García-Ruiz et al. 1998; Martin et al. 2001;

Forshay and Stanley 2005; Inwood et al. 2005), our study finds that both overlying water chemistry and sediment properties have significant relationships with sediment denitrification and N<sub>2</sub>O production in freshwater lakes (Fig. 2; Fig S2). N concentration, SOM, sediment moisture, and Cond are found to be the major factors determining sediment denitrification in the 20 plateau lakes (Table 2). In previous studies (García-Ruiz et al. 1998; Martin et al. 2001), water N concentrations generally explained the greatest amount of variance in sediment denitrification. (García-Ruiz et al. 1998) have found that water NO<sub>3</sub><sup>-</sup> concentration can alone explain about 53 % of

**Table 2** Adjusted  $R^2$  for the multiple regression models which investigated the effect of water chemistry and sediment properties on denitrification and  $N_2O$  production rates at the lake and site levels

	Water chemistry (W)	Sediment properties (S)	W + S	Significant variables
All lakes (N = 20)				
Potential denitrification rate	0	0	0	
Unamended denitrification rate	0.217	0	0.217	TN*
$N_2O$ production rate	0.228	0.152	0.360	Cond*; STN*
Human-dominated lakes (N = 9)				
Potential denitrification rate	0	0	0	
Unamended denitrification rate	0	0.447	0.447	STN*
$N_2O$ production rate	0	0.410	0.410	STN*
Reference lakes (N = 11)				
Potential denitrification rate	0	0.331	0.331	STC*
Unamended denitrification rate	0.690	0	0.690	TDN**
$N_2O$ production rate	0.366	0	0.366	TDN*
All sites (N = 68)				
Potential denitrification rate	0.067	0.182	0.206	TDN*; Moisture**
Unamended denitrification rate	0.139	0.076	0.173	TDN**; Moisture*
$N_2O$ production rate	0.092	0.052	0.110	Cond**; SOM*
Sites from man-dominated lakes (N = 30)				
Potential denitrification rate	0.120	0.125	0.176	Cond*; Moisture*
Unamended denitrification rate	0.198	0.140	0.252	Cond**; Moisture*
$N_2O$ production rate	0.116	0.164	0.185	Cond*; STN*
Site from reference lakes (N = 38)				
Potential denitrification rate	0	0.203	0.203	Moisture**
Unamended denitrification rate	0.419	0.080	0.479	TDN**; Moisture*
$N_2O$ production rate	0.261	0.103	0.268	TN**; SOM*

TN total nitrogen, Cond conductivity, TDN total dissolved nitrogen, DO dissolved oxygen, STN sediment total nitrogen, STC sediment total carbon, Moisture sediment moisture, SOM sediment organic matter

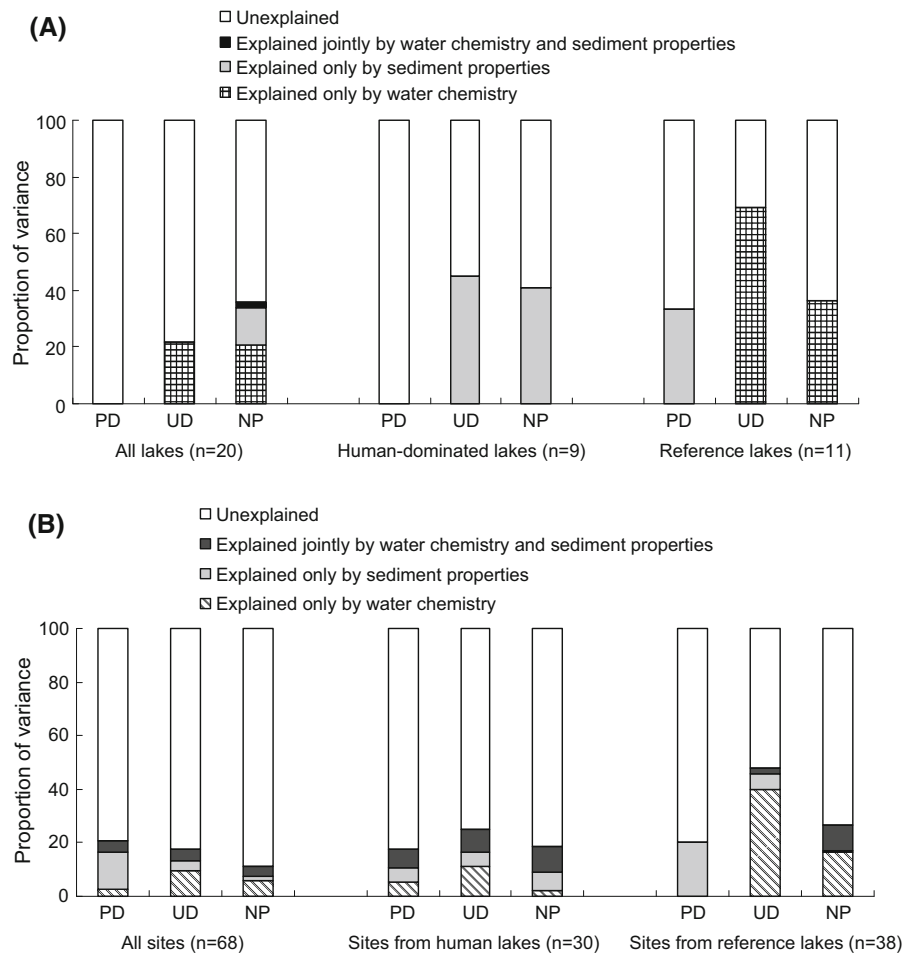
\* Significant at the 0.05 level, \*\* significant at the 0.01 level

denitrification variance for sediment samples collected from rivers in north-east England. In the present study, at both the lake and site levels, water TN and TDN contents are the most important determinants of unamended denitrification and  $N_2O$  production rates among reference lakes (Table 2). However, these relationships are not significant among human-dominated lakes, probably because water N concentrations in human-dominated lakes were high (mean TN concentration = 2.31 mg/L) and not limiting to sediment denitrification (Inwood et al. 2005). These results imply that the importance of water N concentration in modeling sediment denitrification at low N concentration and the need to select alternative parameters at high N concentration.

One key to understanding the spatial and temporal patterns of sediment denitrification is the role of soil moisture (Boyer et al. 2006; Saggari et al. 2013). The oxygen availability and sediment denitrification rate change rapidly depending on sediment moisture and the consequent rate of oxygen diffusion through sediments (Seitzinger et al. 2006). Consistent with earlier work (García-Ruiz et al. 1998), we found significant positive relationships between sediment moisture and potential and unamended denitrification rates at the site level. The higher moisture in sediments may inhibit oxygen diffusion to soil microorganisms, thus creating an anaerobic environment favorable for denitrification (García-Ruiz et al. 1998). However, a study indicates that the condition of complete anoxia is



**Fig. 4** Proportion of variance in denitrification and  $N_2O$  production rates explained by water chemistry and sediment properties at the lake level (a) and the site level (b). Variance was partitioned among water chemistry-only, sediment properties-only, water chemistry correlated to sediment properties, and unexplained variance. *PD* potential denitrification, *UD* unamended denitrification, *NP*  $N_2O$  production



not preferred for sediment denitrification, because  $NO_3^-$  is preferentially reduced to  $NH_4^+$  when no oxygen is present (Boyer et al. 2006). It should be noted that the selected water and soil variables explain small amounts of variance in sediment denitrification of the 20 Chinese plateau lakes. A portion of unexplained variance remained that would require the consideration of more predictors, such as water  $NO_3^-$  and  $NH_4^+$  concentrations. Some studies have reported that  $NO_3^-$  concentration in water is the most important explanatory variable for sediment denitrification rates (García-Ruiz et al. 1998; McCrackin and Elser 2010).

Only one study has examined the effects of watershed land uses on sediment denitrification in lakes (Bruesewitz et al. 2011). Our research found that only potential denitrification rate of sediments significantly increases with the proportion of human land

uses in watersheds (Fig. 3), consistent with the result of Bruesewitz et al. (2011). However, the relationship between unamended denitrification rate and human land uses in watershed was not significant (Fig. 3). Inwood et al. (2007) indicated that the indirect effects of watershed land use on stream sediment denitrification and  $N_2O$  emission are mainly mediated through stream water chemistry or sediment properties. Lakes with a high percentage of agriculture and urban land uses in watersheds may receive higher nitrogen loads (Bruesewitz et al. 2011; Powers et al. 2014). Our study also revealed a positive correlation between percentage of human land use in watersheds and water N contents in lakes (Table S2). This result may imply that human landscapes in watersheds indirectly affect the sediment denitrification and  $N_2O$  production rates of the plateau lakes in China primarily via effects on lake water chemistry.

## Benefits and risks of sediment denitrification in lakes

N removal by lakes larger than 0.001 km<sup>2</sup> predicted by a global model is about 13 Tg year<sup>-1</sup>, representing 20 % of estimated inputs to aquatic ecosystems or 5 % of N loading to continental surfaces (Harrison et al. 2009). Scaled to an areal basis using the bulk density of the top 7 cm of sediment, unamended denitrification rate of the 20 plateau lakes ranged from 5 to 4,552 kg N km<sup>-2</sup> year<sup>-1</sup>, with a mean of 521 kg N km<sup>-2</sup> year<sup>-1</sup>. According to the areal unamended denitrification rate and surface area of the 20 lakes (Table S1), we find that the Lake Qiluhu and Lake Fuxianhu can at least remove 167,777 and 119,728 kg of N every year, respectively. The Lake Changhu, a small and oligotrophic water body, can only remove 40 kg of N every year. It is difficult to quantify the N inputs to the plateau lakes and calculate the proportion of N load removed via denitrification. However, Seitzinger et al. (2006) propose a relationship between percentage N removed by denitrification and water residence time (WRT) based on a synthesis of data from lakes, rivers, estuaries, and the continental shelf:

$$\% \text{ N removed by denitrification} = 23.4 (\text{WRT})^{0.204} \quad (1)$$

This simple model can account for 56 % of the total variance (Seitzinger et al. 2006). Applying WRT of the 20 plateau lakes (see Table S1) to this model, we estimated that 36.43–81.40 % of annual N inputs can be removed via denitrification. McCrackin and Elser (2010) have used this model and predicted that denitrification may remove 24–39 % of N loading to Norwegian lakes. Bruesewitz et al. (2011) also estimate that 30–60 % of annual N inputs to Rotorua lakes in New Zealand can be removed through denitrification.

Denitrification is a large source of N<sub>2</sub>O emitted into the atmosphere from sediments in aquatic ecosystems (Mengis et al. 1997; Beaulieu et al. 2011). Determining the N<sub>2</sub>O production rate in lake systems is important not only for understanding sediment biogeochemical processes, but also for evaluating the risk of N<sub>2</sub>O emissions at broad spatial scales (McCrackin and Elser 2010). The average of N<sub>2</sub>O production rate of the 20 Chinese plateau lakes was 0.05 ng N g<sup>-1</sup> h<sup>-1</sup> or 28.50 kg N km<sup>-2</sup> year<sup>-1</sup>. This value is slightly

less than half of the average of 32 Norwegian lakes (68.60 kg N km<sup>-2</sup> year<sup>-1</sup>) predicted by McCrackin and Elser (2010). Compared to N<sub>2</sub>O emission from paddy fields in China (354 kg N km<sup>-2</sup> year<sup>-1</sup>, Xing 1998), these plateau lakes seem not to contribute significantly to atmospheric N<sub>2</sub>O emissions. Five plateau lakes show negative values for N<sub>2</sub>O production rate, which suggests a net consumption of the N<sub>2</sub>O produced. N<sub>2</sub>O production in lakes depends not only on its synthesis but also on the extent of reduction to N<sub>2</sub> by reductase enzyme (García-Ruiz et al. 1998). It is generally admitted that under anaerobic conditions, denitrification is the dominant process for N<sub>2</sub>O formation, while nitrification generally occurs under aerobic conditions (Mathieu et al. 2006). The available information on N<sub>2</sub>O production and consumption processes in freshwater lakes are still rather limited (Ussiri and Lal 2013). Further direct measurements of N<sub>2</sub>O emission are needed to better understand the contribution of lakes to global N<sub>2</sub>O fluxes.

In summary, the results of the present study indicate that plateau lakes in southwestern China can remove large quantities of N through sediment denitrification and produce small amounts of N<sub>2</sub>O. Littoral zones had an unamended denitrification rate considerably higher than profundal zones, which suggested that the littoral zones in plateau lakes were hotspots of sediment denitrification and N removal. Therefore, the littoral zones must be considered when characterizing N dynamics and restoring eutrophic lakes (Bruesewitz et al. 2012). The quantity of N exported from agricultural and urban land uses in watersheds should be controlled to improve the water quality of these plateau lakes, although our findings suggest that lake sediments have the capacity to remove much of the anthropogenic N.

**Acknowledgments** The authors would like to thank Lu Yao, Haoping Wu, and Beibei Hao for their assistance with field sampling and laboratory analyses. Funding was provided by the Special S&T Project on Treatment and Control of Water Pollution (2013ZX07102–005) and the National Science Foundation of China (31270583 and 31370550).

**Conflict of interest** The authors declare that there is no conflict of interest.

## References

Abell JM, Özkundakci D, Hamilton DP (2010) Nitrogen and phosphorus limitation of phytoplankton growth in New

- Zealand lakes: implications for eutrophication control. *Ecosystems* 13:966–977
- Beaulieu JJ, Tank JL, Hamilton SK, Wollheim WM, Hall RO Jr, Mulholland PJ, Peterson BJ, Ashkenas LR, Cooper LW, Dahm CN, Dodds WK, Grimm NB, Johnson SL, McDowell WH, Poole GC, Valett HM, Arango CP, Bernot MJ, Burgin AJ, Crenshaw CL, Helton AM, Johnson LT, O'Brien JM, Potter JD, Sheibley RW, Sobota DJ, Thomas SM (2011) Nitrous oxide emission from denitrification in stream and river networks. *Proc Natl Acad Sci USA* 108:214–219
- Bettez ND, Groffman PM (2012) Denitrification potential in stormwater control structures and natural riparian zones in an urban landscape. *Environ Sci Technol* 46:10909–10917
- Boyer EW, Alexander RB, Parton WJ, Li C, Terbach-Bahl K, Donner SD, Skaggs RW, Del Gross SJ (2006) Modeling denitrification in terrestrial and aquatic ecosystems at regional scales. *Ecol Appl* 16:2123–2142
- Bruesewitz DA, Hamilton DP, Schipper LA (2011) Denitrification potential in lake sediment increases across a gradient of catchment agriculture. *Ecosystems* 14:341–352
- Bruesewitz DA, Tank JL, Hamilton SK (2012) Incorporating spatial variation of nitrification and denitrification rates into whole-lake nitrogen dynamics. *J Geophys Res* 117, G00N07. doi:10.1029/2012JG002006
- Carpenter SR, Caraco NF, Correll DL, Howarth RW, Sharpley AN, Smith VH (1998) Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecol Appl* 8:559–568
- Cheruvilil KS, Soranno PA, Bremigan MT, Wagner T, Martin SL (2008) Grouping lakes for water quality assessment and monitoring: the roles of regionalization and spatial scale. *Environ Manag* 41:425–440
- Elser JJ, Kyle M, Steger L, Nydick KR, Baron JS (2009) Nutrient availability and phytoplankton nutrient limitation across a gradient of atmospheric nitrogen deposition. *Ecology* 90:3062–3073
- Felber R, Conen F, Flechard CR, Neftel A (2012) Theoretical and practical limitations of the acetylene inhibition technique to determine total denitrification losses. *Biogeochemistry* 9:4125–4138
- Forshay KJ, Dodson SI (2011) Macrophyte presence is an indicator of enhanced denitrification and nitrification in sediments of a temperate restored agricultural stream. *Hydrobiologia* 668:21–34
- Forshay KJ, Stanley EH (2005) Rapid nitrate loss and denitrification in a temperate river floodplain. *Biogeochemistry* 75:43–64
- Freymond CV, Wenk CB, Frame CH, Lehmann MF (2013) Year-round N<sub>2</sub>O production by benthic NO<sub>x</sub> reduction in a monomictic south-alpine lake. *Biogeochemistry* 10:8373–8383
- García-Ruiz R, Pattinson SN, Whitton BA (1998) Denitrification in river sediments: relationship between process rate and properties of water and sediment. *Freshw Biol* 39:467–476
- Groffman PM, Altabet MA, Böhlke JK, Butterbach-Bahl K, David MB, Firestone MK, Giblin AE, Kana TM, Nielsen LP, Voytek MA (2006) Methods for measuring denitrification: diverse approaches to a difficult problem. *Ecol Appl* 16:2091–2122
- 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
- Hayakawa A, Nakata M, Jiang R, Kuramochi K, 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 MM, Bobbink R, Janssens MP (2006) Spatial variation in denitrification and N<sub>2</sub>O emission in relation to nitrate removal efficiency in a N-stressed riparian buffer zone. *Ecosystems* 9:550–563
- Herlihy AT, Sobota JB, McDonnell TC, Sullivan TJ, Lehmann S, Tarquinio E (2013) An a priori process for selecting candidate reference lakes for a national survey. *Freshw Sci* 32:385–396
- Inwood SE, Tank JL, Bernot MJ (2005) Patterns of denitrification associated with land use in 9 midwestern headwater streams. *J N Am Benthol Soc* 24:227–245
- Inwood SE, Tank JL, Bernot MJ (2007) Factors controlling sediment denitrification in midwestern streams of varying land use. *Microb Ecol* 53:247–258
- Kreiling RM, Richardson WB, Cavanaugh JC, Bartsch LA (2011) Summer nitrate uptake and denitrification in an upper Mississippi River backwater lake: the role of rooted aquatic vegetation. *Biogeochemistry* 104:309–324
- Liu W, Zhang Q, Liu G (2010) Lake eutrophication associated with geographic location, lake morphology and climate in China. *Hydrobiologia* 644:289–299
- Liu W, Zhang Q, Liu G (2011) Effects of watershed land use and lake morphometry on trophic state of Chinese lakes: implications for eutrophication control. *Clean-Soil Air Water* 39:35–42
- Liu W, Li S, Bu H, Zhang Q, Liu G (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
- Ma RH, Yang GH, Duan HT, Jiang JH, Wang SM, Feng XZ, Li AN, Kong FX, Xue B, Wu JL, Li SJ (2011) China's lakes at present: number, area and spatial distribution. *Sci China Earth Sci* 54:283–289
- Martin LA, Mulholland PJ, Webster JR, Valett HM (2001) Denitrification potential in sediments of headwater streams in the southern Appalachian Mountains, USA. *J N Am Benthol Soc* 20:505–519
- Mathieu O, Hénault C, Lévêque J, Baujard E, Milloux MJ, Andreux F (2006) Quantifying the contribution of nitrification and denitrification to the nitrous oxide flux using <sup>15</sup>N tracers. *Environ Pollut* 144:933–940
- McCrackin ML, Elser JJ (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 (N<sub>2</sub>O) in deep lakes. *Biogeochemistry* 38:281–301
- Müller B, Lotter AF, Sturm M, Ammann A (1998) Influence of catchment quality and altitude on the water and sediment composition of 68 small lakes in central Europe. *Aquat Sci* 60:316–337
- Pell M, Stenberg B, Stenström J, Torstensson L (1996) Potential denitrification activity assay in soil—With or without chloramphenicol? *Soil Biol Biochem* 28:393–398
- Piña-Ochoa E, Álvarez-Cobelas M (2006) Denitrification in aquatic environments: a cross-system analysis. *Biogeochemistry* 81:111–130

- Powers SM, Robertson DM, Stanley EH (2014) Effects of lakes and reservoirs on annual river nitrogen, phosphorus, and sediment export in agricultural and forested landscapes. *Hydrobiologia* 28:5919–5937
- Roy R, Legendre P, Knowles R, Charlton MN (1994) Denitrification and methane production in sediment of Hamilton Harbour (Canada). *Microb Ecol* 27:123–141
- Saggar S, Jha N, Deslippe J, Bolan NS, Luo J, Giltrap DL, Kim DG, Zaman M, Tilman RW (2013) Denitrification and  $N_2O:N_2$  production in temperate grasslands: processes, measurements, modelling and mitigating negative impacts. *Sci Total Environ* 465:173–195
- Saunders DL, Kalff J (2001) Nitrogen retention in wetlands, lakes and rivers. *Hydrobiologia* 443:205–212
- Seitzinger SP (1988) Denitrification in freshwater and coastal marine ecosystems: ecological and geochemical significance. *Limnol Oceanogr* 33:702–724
- Seitzinger SP, Harrison JA, Böhlke JK, Bouwman AF, Lowrance R, Peterson B, Tobias C, Van Drecht G (2006) Denitrification across landscapes and waterscapes: a synthesis. *Ecol Appl* 16:2064–2090
- Ussiri D, Lal R (2013) Soil emission of nitrous oxide and its mitigation. Springer, Dordrecht
- Van Luijn F, Boers P, Lijklema L (1996) Comparison of denitrification rates in lake sediments obtained by the  $N_2$  flux method, the  $^{15}N$  isotope pairing technique and the mass balance approach. *Water Res* 30:893–900
- Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman DG (1997) Human alteration of the global nitrogen cycle: sources and consequences. *Ecol Appl* 7:737–750
- Wallenstein MD, Myrold D, Voytek M, Firestone M (2006) Environmental controls on denitrifying communities and denitrification rates: insights from molecular methods. *Ecol Appl* 16:2143–2152
- Wang SM, Dou HS (1998) Chinese lake catalogue. Science Press, Beijing
- Xing G (1998)  $N_2O$  emission from cropland in China. *Nutr Cycl Agroecosys* 52:249–254
- Yang X, Anderson NJ, Dong X, Shen J (2008) Surface sediment diatom assemblages and epilimnetic total phosphorus in large, shallow lakes of the Yangtze floodplain: their relationships and implications for assessing long-term eutrophication. *Freshw Biol* 53:1273–1290