CONTENTS

Editorial letter
We are integrating with the world – Journal of Environmental Sciences Journey of twenty five years
Qingcai Feng, Xiaoshan Tie ........................................................................................................ 1

Aquatic environment
Charaterization of the airborne bacteria community at different distances from the rotating brushes in a wastewater
treatment plant by 16S rRNA gene clone libraries
Yunping Han, Lin Li, Junxin Liu ............................................................................................. 5

Growth and nutrient accumulation of Phragmites australis in relation to water level variation
and nutrient loadings in a shallow lake
Ying Zhao, Xinghui Xia, Zhifeng Yang .................................................................................. 16

Cost-performance analysis of nutrient removal in a full-scale oxidation ditch process based on kinetic modeling
Zheng Li, Rong Qi, Bo Wang, Zhe Zou, Guohong Wei, Min Yang ............................................... 26

Sulfur-containing amino acid methionine as the precursor of volatile organic sulfur compounds in algae-induced black bloom
Xin Lu, Chengxin Fan, Wei He, Jiancai Deng, Hongbin Yin ...................................................... 33

Nitrous oxide reductase gene (nosZ) and N₂O reduction along the littoral gradient of a eutrophic freshwater lake
Chaoxu Wang, Guibing Zhu, Yu Wang, Shanyun Wang, Chengqing Yin .................................. 44

Influence of oxygen flow rate and compost addition on reduction of organic matter in aerated waste layer containing
mainly incineration residue
Hiroshi Asakura, Kei Nakagawa, Kazuto Endo, Masato Yamada, Yusaku Ono, Yoshiro Ono ........... 53

Removal and transformation of organic matters in domestic wastewater during lab-scale chemically enhanced primary
treatment and a trickling filter treatment
Qingliang Zhao, Huizhuang Zhang, Kun Wang, Liangliang Wei, Jinli Liu, Yu Liu .......................... 59

Occurrence and distribution of hexabromocyclododecane in sediments from seven major river drainage basins in China
Honghua Li, Hongtao Shang, Pu Wang, Yawei Wang, Haidong Zhang, Qinghua Zhang, Guibin Jiang .... 69

Influencing factors and degradation products of antipyrine chlorination in water with free chlorine
Meiquan Cai, Liqiu Zhang, Fei Qi, Li Feng ........................................................................... 77

Characterization of dissolved organic matter as N-nitrosamine precursors based on hydrophobicity,
molecular weight and fluorescence
Chengkun Wang, Xiaojian Zhang, Jun Wang, Chao Chen ...................................................... 85

Simultaneous removal of selected oxidized contaminants in groundwater using a continuously stirred
hydrogen-based membrane biofilm reactor
Siqing Xia, Jun Liang, Xiaoyin Xu, Shuang Shen .................................................................... 96

Effect of dissolved organic matter on nitrate-nitrogen removal by anion exchange resin and kinetics studies
Haiou Song, Zhijian Yao, Mengqiao Wang, Jinman Wang, Zhaolian Zhu, Aimin Li ................. 105

Natural organic matter quantification in the waters of a semiarid freshwater wetland (Tablas de Daimiel, Spain)
Montserrat Filella, Juan Carlos Rodríguez-Murillo, Franccís Quentel ..................................... 114

Atmospheric environment
Carbon dioxide capture using polyethyleneimine-loaded mesoporous carbons
Jitong Wang, Huichao Chen, Huanhuan Zhou, Xiaojun Liu, Wenming Qiao, Donghui Long, Licheng Ling .... 124

Simultaneous monitoring of PCB profiles in the urban air of Dalian, China with active and passive samplings
Qian Xu, Xiuhua Zhu, Bernhard Henkelmann, Karl-Werner Schramm, Jiping Chen, Yuwen Ni, Wei Wang, 
Gerd Pfister, Jun Mu, Songtao Qin, Yan Li ........................................................................... 133

Terrestrial environment
Profiling the ionome of rice and its use in discriminating geographical origins at the regional scale, China
Gang Li, Luis Nunes, Yijie Wang, Paul N. Williams, Maozhong Zheng, Qifang Zhang, Yongguan Zhu .... 144

Environmental biology
Effects of solution conditions on the physicochemical properties of stratification components of extracellular
polymeric substances in anaerobic digested sludge
Dongqin Yuan, Yili Wang ........................................................................................................ 155
Environmental health and toxicology

*In vitro* cytotoxicity of CdSe/ZnS quantum dots with different surface coatings to human keratinocytes HaCaT cells

Kavitha Pathakoti, Huey-Min Hwang, Hong Xu, Zoraida P. Aguilar, Andrew Wang .......................................................... 163

Effect of heavy metals and phenol on bacterial decolourisation and COD reduction of sucrose-aspartic acid Maillard product

Sangeeta Yadav, Ram Chandra ................................................................................................................................. 172

Environmental catalysis and materials

Mesoporous silicas synthesis and application for lignin peroxidase immobilization by covalent binding method

Zunfang Hu, Longqian Xu, Xianghua Wen ................................................................. 181

Adsorption of naphthalene onto a high-surface-area carbon from waste ion exchange resin

Qianqian Shi, Aimin Li, Zhaolian Zhu, Bing Liu .............................................................................. 188

Adsorption of lead on multi-walled carbon nanotubes with different outer diameters and oxygen contents:

Kinetics, isotherms and thermodynamics

Fei Yu, Yangqin Wu, Jie Ma, Chi Zhang ............................................................................................... 195

Environmental analytical methods

Application of comprehensive two-dimensional gas chromatography with mass spectrometric detection for the analysis of selected drug residues in wastewater and surface water

Petr Lacina, Ludmila Mravcová, Milada Vávrová ................................................................. 204

Determination of gaseous semi- and low-volatile organic halogen compounds by barrier-discharge atomic emission spectrometry

Yifei Sun, Nobuhisa Watanabe, Wei Wang, Tianle Zhu .......................................................... 213

Electrochemical treatment of olive mill wastewater: Treatment extent and effluent phenolic compounds monitoring using some uncommon analytical tools

Chokri Belaid, Moncef Khadraoui, Salma Mseddi, Monem Kallel, Boubaker Elleuch, Jean François Fauvarque .......................... 220

Municipal solid waste and green chemistry

Evaluation of PCDD/Fs and metals emission from a circulating fluidized bed incinerator co-combusting sewage sludge with coal

Gang Zhang, Jing Hai, Jiang Cheng, Zhiqi Cai, Mingzhong Ren, Sukun Zhang, Jieru Zhang ........................................ 231

Serial parameter: CN 11-2629/X*1989*m*235*en*P*26*2013-1
Nitrous oxide reductase gene (*nosZ*) and \( \text{N}_2\text{O} \) reduction along the littoral gradient of a eutrophic freshwater lake

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Received 04 February 2012; revised 17 April 2012; accepted 26 April 2012

**Abstract**

Lake littoral zones are characterized by heterogeneity in the biogeochemistry of nutrient elements. This study aimed to explore the relationship between the nitrous oxide reductase gene (*nosZ*)-encoding denitrifier community composition/abundance and \( \text{N}_2\text{O} \) reduction. Five samples (deep sediment, near-transition sediment, transition site, near-transition land and land soil) were collected along a littoral gradient of eutrophic Baiyangdian Lake, North China. To investigate the relationship between the *nosZ*-encoding denitrifier community structure and \( \text{N}_2\text{O} \) reduction, the *nosZ*-encoding denitrifier community composition/abundance, potential denitrification rate (DNR) and potential \( \text{N}_2\text{O} \) production rate (p\( \text{N}_2\text{O} \)) were investigated using molecular biological technologies and laboratory incubation experiments. The results showed that the average DNR of sediments was about 25 times higher than that of land soils, reaching 282.5 nmol N/(g dry weight (dw)/hr) and that the average p\( \text{N}_2\text{O} \) of sediments was about 3.5 times higher than that of land soils, reaching 15.7 nmol N/(g dw·hr). In the land area, the *nosZ* gene abundance showed a negative correlation with the \( \text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2) \) ratio, indicating that *nosZ* gene abundance dominated \( \text{N}_2\text{O} \) reduction both in the surface soils of the land area and in the soil core of the transition site. Phylogenetic analysis showed that all the *nosZ* sequences recovered from sediment clustered closely with the isolates *Azospirillum largimobile* and *Azospirillum irakense* affiliated to Rhodospirillaceae in alpha-Proteobacteria, while about 92.3% (12/13) of the *nosZ* sequences recovered from land soil affiliated to Rhizobiaceae and Bradyrhizobiaceae in \( \alpha \)-Proteobacteria. The community composition of *nosZ* gene-encoding denitrifiers appeared to be coupled with \( \text{N}_2\text{O} \) reduction along the littoral gradient.

**Key words**: littoral gradient; \( \text{N}_2\text{O} \) reduction; *nosZ* gene; abundance; community composition

**DOI**: 10.1016/S1001-0742(12)60005-9

**Introduction**

Biological denitrification in the global nitrogen cycle is the main source of nitrous oxide (\( \text{N}_2\text{O} \)) released to the atmosphere (Fernandes et al., 2010). \( \text{N}_2\text{O} \) emission and its relationship with corresponding environmental parameters, such as substrate availability, C/N ratio and soil water content in various ecosystems have been investigated extensively (Hunt et al., 2007; Chen et al., 2010; Liu et al., 2011). However, the study of connections between denitrifier community composition/abundance and function has been lacking and should be strengthened further (Wallenstein et al., 2006). In particular, recent studies have found that the littoral zones of eutrophic freshwater lakes were potential ‘hotspots’ of \( \text{N}_2\text{O} \) emissions in landscapes (Groffman et al., 2000; Wang et al., 2006). Therefore, it is necessary to understand the community composition and abundance of denitrifiers along the littoral gradient to determine if they may play an important role in \( \text{N}_2\text{O} \) transformation.

Recently, examinations of denitrifier community composition and abundance were complemented by focusing on the amplification of functional genes involved in denitrification (Chroňáková et al., 2009). The quantification of nitrous oxide reductase gene (*nosZ*) can be crucial in terms of \( \text{N}_2\text{O} \) reduction, because denitrifying bacteria can harbor a truncated denitrification pathway lacking the *nosZ* gene and thus be unable to reduce \( \text{N}_2\text{O} \) (Henry et al., 2006). Moreover, \( \text{N}_2\text{O} \) reductase seems to be more sensitive to the primary environmental regulators, including oxygen, pH and carbon/nitrate ratio, than the other enzymes of the denitrification pathway (Tiedje, 1988). Overall, the combination of *nosZ*-encoding denitrifier community composition/abundance and the environmental factors re-
lated to N\textsubscript{2}O reduction along the heterogenous littoral zone is important and deserves attention.

Baiyangdian Lake is the largest natural freshwater lake in North China with a total area of approximately 366 km\textsuperscript{2}, including more than 140 small lakes. Reed (\textit{Phragmites australis}) dominates the vegetative belts around Baiyangdian Lake. There are altogether 94.0 km\textsuperscript{2} of reed fields with more than 3700 ditches (approximately 24.8 km\textsuperscript{2}) in Baiyangdian Lake, forming a characteristic reed-bed/ditch landscape (Wang and Yin, 2008). Baiyangdian Lake is a eutrophic lake now and much of the area has been converted to swamps. The eutrophication of Baiyangdian Lake is increasing as it receives municipal wastewater of Baoding City and non-point source pollutants. The hydrological and biogeochemical characteristics of Baiyangdian Lake were found to be heterogeneous according to our previous studies (Wang et al., 2002; Wang and Yin, 2008), and its littoral zone is an ideal field to explore the microbial mechanisms of denitrification.

Hence, the objectives of this study were: (1) to investigate the relationship between \textit{nosZ}-encoding denitrifier community composition/abundance and N\textsubscript{2}O reduction; (2) to examine the effects of environmental variables on the nature of denitrification end products under the spatially heterogeneous conditions of the littoral gradient of Baiyangdian Lake.

1 Materials and methods

1.1 Sample collection and analysis

The sampling campaign was carried out along a littoral gradient (38°54′16.8″N, 115°55′26.3″E) of the Fuhe River mouth area in Baiyangdian Lake in August, 2010 (Fig. S1). Five sampling sites were selected from open water area (sites A and B) to land area (sites C, D and E), named deep sediment, near-transition sediment, transition site, near-transition land and land soil, respectively (Fig. 1). Surface sediments (0–10 cm) from sites A and B were collected using a polymer glass tube (inner diameter, 6.0 cm; length, 2.0 m) with a rubber stopper at one end. Three sediment cores were randomly collected at each sampling site within the area of 1 m\textsuperscript{2}. The surface sediment was sliced with minimal disturbance and transferred quickly into glass jars which were completely filled to exclude oxygen, and the sediment in each glass jar was blended before use. Topsoils (0–10 cm) were collected from sites D and E with a spade. A soil core (80 cm) was collected at site C using a stainless-steel soil auger (inner diameter, 4.5 cm) and divided at intervals of 10 cm. Samples from each site were collected in triplicate and stored at 4°C before chemical analysis, and subsamples for molecular analysis were preserved at −20°C. Water temperature, pH, and dissolved oxygen (DO) were measured \textit{in situ} using a DO-meter (Model 57, YSI, USA). Three replicates were carried out for every measurement.

1.2 Potential denitrification rate and potential N\textsubscript{2}O production rate measurements

Potential denitrification rate (DNR) and potential N\textsubscript{2}O production rate (\textit{pN\textsubscript{2}O}) were measured for each sampling site in triplicate sediment slurries or soils using the acetylene inhibition method as described by Magalhães et al. (2005) and Teixeira et al. (2010) with some modifications. The sediments or soils were prepared by adding 15 mL of \textit{in situ} water sterilized by filtration through 0.22 μm polycarbonate filters (Table S1) to 250 mL serum bottles containing homogenized and weighed samples (5.0 g fresh weight). Serum bottles were hermetically sealed with butyl rubber stoppers and aluminum crimp seals and purged 15 min with N\textsubscript{2} to remove O\textsubscript{2}. Incubation sets with and without acetylene addition (20%, V/V) were run in parallel. The time zero sample was collected 0.5 hr after acetylene addition. All samples were incubated in the dark for 1.5 hr at \textit{in situ} temperature (30°C) with stirring (170 r/min). At the end of incubation, 20 mL of headspace sample was collected from each serum bottle (after shaking vigorously) by displacement with 20 mL high-purity N\textsubscript{2}. \textit{pN\textsubscript{2}O} was given by the N\textsubscript{2}O accumulation in treatments without C\textsubscript{2}H\textsubscript{2} and DNR (N\textsubscript{2}O plus N\textsubscript{2} production) by the N\textsubscript{2}O produced in acetylene incubations. The N\textsubscript{2}O/(N\textsubscript{2}+N\textsubscript{2}O) ratio, calculated from \textit{pN\textsubscript{2}O}/DNR, was used to indicate the proportion of N\textsubscript{2}O produced as the terminal product of denitrification.

1.3 DNA extraction, PCR, cloning, sequencing and phylogenetic analysis

The homogenized soil/sediment of each site was used for DNA extraction after freeze-drying. DNA was extracted from 0.3 g dry soil/sediment with three replicates using a FastDNA SPIN Kit for soil (Bio 101, Vista, CA) following the manufacturer’s instructions. Fragments of \textit{nosZ} genes were amplified with the primer set \textit{nosZF}/\textit{nosZR} (699 bp) as described by Kloos et al. (2001). The PCR was performed in a C1000\textsuperscript{TM} thermal cycler (BioRad, USA) and included initial denaturation at 94°C for 5 min and 35 cycles consisting of denaturation at 94°C for 30 sec, annealing at 52°C for 45 sec, and extension at 72°C for 1 min. The detailed information on cloning and sequencing were described by Wang et al. (2011) and Zhu et al. (2011). The partial \textit{nosZ} gene sequences were aligned using CLUSTAL X software version 1.0.1. Phylogenetic analyses were conducted using MEGA version 3.0 (Kumar et al., 2004) and the neighbor-joining method was used to calculate the distances and to construct phylogenetic trees. All the \textit{nosZ} gene sequences obtained in this study are available in the GenBank nucleotide sequence database under the Accession Numbers shown in Table S2.

1.4 Quantitative PCR assay

SybrGreenI-based real-time PCR assays were carried out in a volume of 20 μL, containing 10 μL SYBR\textsuperscript{®} Pre-
mix Ex Taq\textsuperscript{TM} (TAKARA, Dalian, China), 10 pmol of each primer and 1 μL of 10-fold diluted DNA template, and the reaction mixture was supplemented to 20 μL with sterilized water. The primer pairs used in our study were nosZ1F/nosZ1R (259 bp) (Henry et al., 2006) and 341F/534R (193 bp) (Muyzer et al., 1993) for nosZ and bacterial 16S rRNA gene (as a measure of total community numbers), respectively. The thermal cycling conditions for nosZ were 95°C for 2 min, followed by 40 cycles of 95°C for 15 sec, 63°C for 20 sec and 72°C for 26 sec (acquisition data step). The conditions for total bacterial 16S rRNA gene were 95°C for 30 sec, followed by 38 cycles of 95°C for 10 sec, 62°C for 10 sec and 72°C for 27 sec (acquisition data step). Thermal cycling, fluorescent data collection and data analysis were carried out with an ABI Prism 7300 Real-Time PCR System. Quantitative PCR assays were performed in triplicate for each sample. Three no-template controls were run for each quantitative PCR assay. Positive clones were selected to isolate plasmid DNA using a GeneJet Plasmid Miniprep Kit (Fermentas MBI, Lithuania). The concentration of plasmid DNA was determined on a Nanodrops ND-1000 UV-Vis Spectrophotometer (NanoDrop Technologies, USA). A standard curve was calculated on serially 10 fold diluted plasmid DNA containing the respective target DNA fragment. The amplification efficiencies were in the range of 95%–98% (Figs. S2 and S3).

1.5 Data analyses

Variance analysis (one-way ANOVA) was examined by the protected LSD multiple range test \((p<0.05)\) using the software Statistica 6.0. Canonical Correspondence Analysis (CCA) was performed using the software Canoco for Windows 4.5 (Biometris, Wageningen, The Netherlands). The indices of microbe diversity were calculated by the software program DOTUR.

2 Results

2.1 Physico-chemical parameters

The contents of NH\textsubscript{4}\textsuperscript{+}-N, NO\textsubscript{3}\textsuperscript{-}-N, NO\textsubscript{2}\textsuperscript{-}-N and organic matter (OM) showed great spatial differences along the littoral gradient. The average NH\textsubscript{4}\textsuperscript{+}-N content of sediments (sites A and B) (398.9 mg/kg) was about 150 times higher than that of land soils (sites D and E). The average NO\textsubscript{3}\textsuperscript{-}-N content of sediments (6.54 mg/kg) was significantly lower than that of land soils (average 35.3 mg/kg) and the average NO\textsubscript{2}\textsuperscript{-}-N content showed a trend similar to NO\textsubscript{3}\textsuperscript{-}-N along the littoral gradient (0.19 and 0.96 mg/kg for sediment and land soil, respectively). As an electron donor in denitrification chains, the OM content of the near-transition sediment (site B) (71.5 g/kg) was significantly higher than the other sites, while there was no significant difference between the OM contents of deep sediment (site A) and the land soil (site E) (Table 1).

### Table 1  Physico-chemical characteristics of soil samples along the littoral zone

<table>
<thead>
<tr>
<th>Site</th>
<th>(\text{NH}_4^+\text{-N} \ (\text{mg/kg}))</th>
<th>(\text{NO}_3^-\text{-N} \ (\text{mg/kg}))</th>
<th>(\text{NO}_2^-\text{-N} \ (\text{mg/kg}))</th>
<th>TN (g/kg)</th>
<th>TP (g/kg)</th>
<th>OM (g/kg)</th>
<th>pH (H\textsubscript{2}O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>362.8 ± 12.1 a</td>
<td>7.18 ± 3.17 a</td>
<td>0.20 ± 0.03 a</td>
<td>3.67 ± 0.27 a</td>
<td>0.54 ± 0.01 a</td>
<td>50.3 ± 0.25 a</td>
<td>7.49 a</td>
</tr>
<tr>
<td>B</td>
<td>435.0 ± 4.02 b</td>
<td>5.89 ± 2.49 a</td>
<td>0.17 ± 0.03 a</td>
<td>3.87 ± 0.02 a</td>
<td>0.41 ± 0.01 b</td>
<td>71.5 ± 2.68 b</td>
<td>7.80 b</td>
</tr>
<tr>
<td>C</td>
<td>69.1 ± 0.93 c</td>
<td>1.73 ± 0.00 a</td>
<td>0.61 ± 0.28 b</td>
<td>1.97 ± 0.10 b</td>
<td>0.87 ± 0.01 c</td>
<td>29.9 ± 1.46 c</td>
<td>7.66 c</td>
</tr>
<tr>
<td>D</td>
<td>2.55 ± 1.03 d</td>
<td>57.2 ± 2.41 b</td>
<td>1.09 ± 0.00 c</td>
<td>1.41 ± 0.03 c</td>
<td>0.57 ± 0.00 d</td>
<td>21.8 ± 0.62 d</td>
<td>7.52 a</td>
</tr>
<tr>
<td>E</td>
<td>4.84 ± 1.31 d</td>
<td>13.4 ± 2.07 c</td>
<td>0.83 ± 0.19 b</td>
<td>2.55 ± 0.00 d</td>
<td>0.89 ± 0.01 c</td>
<td>47.9 ± 0.03 a</td>
<td>7.33 d</td>
</tr>
</tbody>
</table>

Values represent means ± SD \((n = 3)\). Data in the same column followed by the same letter are not significantly different at \(p < 0.05\). TN: total nitrogen; TP: total phosphorus; OM: organic matter.
As a representative of the interaction zone of water and soil, the physico-chemical parameter profile of the soil core (80 cm) at site C was also studied (Fig. 2). The concentrations of NH4+–N, NO3−–N and NO2−–N in topsoil were relatively lower than the next layer, but the concentrations of TN and OM in topsoil were the highest, reaching 1.97 and 29.9 g/kg, respectively. The nutrient concentrations in the reed root channel distribution zone were higher than that in the groundwater table fluctuation zone, and both decreased with depth. pH values increased slightly with depth in the core.

2.2 Relationship between nosZ gene abundance and N2O reduction

2.2.1 Along the littoral gradient

To show the abilities of denitrifiers to reduce N2O along the littoral gradient, DNR and pN2O were determined with incubation experiments. The average DNR of sediments (sites A and B) was about 25 times higher than that of land soils (sites D and E), reaching 282.5 nmol N/(g dry weight (dw)-hr), while the average pN2O of sediments was about 3.5 times higher than land soils, reaching 15.7 nmol N/(g dw-hr). The average N2O/(N2O+N2) ratio (N2O proportion produced as terminal denitrification product) of sediments (0.056) was seven times lower than that of land soils, indicating that a much higher proportion of N2O was transformed into N2 in sediments than in land soils (Fig. 3).

The abundance of nosZ, the key enzyme for N2O reduction, was investigated with quantitative PCR to explore its relationship with the N2O proportion produced as terminal denitrification product along the littoral gradient. At the same time, general bacteria 16S rRNA gene abundance was also determined with q-PCR. A significantly higher nosZ gene abundance was observed at the transition site (site C, 1.00 x 10^11 gene copies/g dw) accompanied by the highest 16S rRNA gene abundance (3.13 x 10^11 gene copies/g dw). The average nosZ gene abundance of sediments (1.35 x 10^9 gene copies/g dw) was 2.6 times lower than that of land soils; for 16S rRNA, the average value of sediments was 5.46 x 10^10 gene copies/g dw, 2.0 times lower than that of land soils (Fig. 3). The results showed that DNR, pN2O and nosZ/16S rRNA gene abundance all had high spatial heterogeneity along the littoral gradient.

In the land area, it was observed that the N2O/(N2O+N2) ratio increased with declining nosZ gene abundance, indicating that nosZ gene abundance dominated N2O reduction. However, in the water area, a relatively lower N2O/(N2O+N2) ratio and lower nosZ gene abundance both occurred, which may result from a different community composition of nosZ gene-encoding denitrifiers harbored in the sediment.

2.2.2 Profile of the transition site

Given that the wet/dry shift caused by water table fluctuation usually occurred at the transition site (site C), as well as the heterogeneous conditions in the soil core of site C, the relationship between nosZ gene abundance and the N2O/(N2O+N2) ratio was also explored in the vertical profile of site C. In the soil core of site C, the DNR and pN2O of topsoil (65.8 and 9.41 nmol N/(g dw-hr), respectively) were significantly (ANOVA, p < 0.05 for DNR; p < 0.001 for pN2O) higher than the other layers, indicating that the topsoil accounted for the largest part of N2O emission. In the reed root channel distribution zone, the increase of pN2O and decrease of DNR resulted in N2O/(N2O+N2) ratios increasing from 0.14 to 0.76 (Fig. 4).

The highest nosZ gene abundance (1.00 x 10^10 gene copies/g dw) and 16S rRNA gene abundance (3.13 x 10^11 gene copies/g dw) were both observed in topsoil (0–10 cm). In the reed root channel distribution zone, the nosZ gene abundance decreased from 9.23 x 10^9 to 1.22 x 10^9 gene copies/g dw; for 16S rRNA, the abundance decreased from 5.69 x 10^10 to 1.76 x 10^10 gene copies/g dw. Moreover, the nosZ gene abundance showed no significant difference in the groundwater table fluctuation zone (Fig. 4). Overall, in the soil core of the transition site, the N2O/(N2O+N2) ratio also showed negative correlation (r = -0.766, p < 0.05) with the nosZ gene abundance as observed in the surface soils of the land area, suggesting that the nosZ gene abundance controlled N2O reduction both in the surface soils of the land area and in the soil core of the transition site.

The nosZ gene abundances of Baiyangdian Lake (ranging from 3.7 x 10^8 to 1.0 x 10^9 gene copies/g dw) were
one or two magnitudes higher than those reported in previous studies (1.0 × 10^7 to 1.0 × 10^8 copies/g dw) (Ma et
al., 2008; Chon et al., 2011). The great discrepancy may be attributed to the different NH$_4^+$-N contents in Baiyangdian Lake (sediment: 187.2 mg N/kg dw) and other wetlands (e.g., North American uncultivated wetland sediment: 2.6–7.0 mg N/kg dw; Suncheon estuarine wetland overlying water: 2–3 mg/L) (Ma et al., 2008; Chon et al., 2011).

2.3 Diversity and phylogenetic analysis of the nosZ sequences

The phenomenon that a relatively larger amount of N$_2$O reduction but lower nosZ gene abundance was observed in sediments along the littoral gradient drove us to analyze the biodiversity of nosZ-encoding denitrifiers. In total, 153 clones were screened from two nosZ libraries, including sediment (mixed sample of sites A and B) and land soils (mixed sample of sites C, D and E), and 25 final nosZ OTUs (97% cut-off) were identified after sequencing and translation. The coverage (C) values indicated that more than 70% of the nosZ sequence types were captured in all the libraries (Table 2). The dominant and common nosZ encoding denitrifiers in the littoral zone of Baiyangdian Lake were probably detected. Sediments showed relatively higher diversity than land soils based on the values of Shannon-Weiner (H), Simpson (D), and the richness estimators $\text{S}_{\text{Chao1}}$ and $\text{S}_{\text{ACE}}$ (Table 2).

The phylogenetic analysis of nosZ gene sequences showed that nosZ-encoding denitrifiers had high biodiversity along the littoral zone, including the families Rhodospirillaceae, Rhizobiaceae and Bradyrhizobiaceae in $\alpha$-Proteobacteria. All the OTUs of sediment were clustered in Group 1 and all the recovered nosZ sequences clustered closely with the isolates Azospirillum lari-mobile (AY072228) and Azospirillum irakense (AY072230) affiliated to Rhodospirillaceae. For land soil, 90% (9/10) of the OTUs were clustered in Group 2 and about 92.3% (12/13) of the recovered nosZ sequences affiliated to Rhizobiaceae and Bradyrhizobiaceae (Fig. 5). In all, the community composition of nosZ-encoding denitrifiers was site-specific and had high spatial heterogeneity along the littoral gradient.

2.4 Canonical correspondence analysis

The influences of geochemical factors on DNR, pN$_2$O, N$_2$O/(N$_2$O+N$_2$) ratio and nosZ gene abundance were analyzed by CCA (Fig. 6). CCA resulted in the first two canonical axes explaining 96.1% of the total cumulative variance in the response variable data and 97.7% of the total cumulative variance of the response variable-environment relationship. The first canonical axis explained 45.0% of the total variation and was dominated by the environmental variables pH (0.86), OM (−0.75) and TN (−0.83). The second canonical axis explained an additional 8.8% of the total variation and was dominated by NO$_3^-$-N (0.52) and NH$_4^+$-N (−0.65). NO$_3^-$-N showed a stronger effect on all the response variables than NO$_3^-$-N.

NO$_3^-$-N and NO$_3^-$-N both showed positive effects on the N$_2$O/(N$_2$O+N$_2$) ratio ($r = 0.73$ for NO$_3^-$-N, $r = 0.43$ for NO$_3^-$-N), while the effects of OM and pH were negative ($r = −0.78$ for OM, $r = −0.73$ for pH).

3 Discussion

An incubation experiment and nosZ-encoding denitrifier community structure analysis were carried out along the littoral gradient of Baiyangdian Lake. The results showed that the land/water ecotone had high spatial heterogeneity in DNR, pN$_2$O and nosZ-encoding denitrifier community structure. Based on the above measurements, the relationship between the nosZ-encoding denitrifier community composition/abundance and N$_2$O reduction was addressed along the littoral gradient of Baiyangdian Lake.

In the land area of the littoral gradient, the nosZ gene abundance showed a negative correlation ($r = −0.757$, $p < 0.05$) with the N$_2$O/(N$_2$O+N$_2$) ratio, indicating that the nosZ gene abundance dominated N$_2$O reduction. Ma et al. (2011) indicated that the abundance of denitrifier nosZ is related to potential N$_2$O emission because nosZ abundance directly affects nitrous oxide reductase activity, and also found that the N$_2$O/(N$_2$O+N$_2$) ratio increased as nosZ abundance declined in North American ephemeral wetland soils. Similar results were also confirmed in grassland soils (Philippot et al., 2009). However, compared with the land area, a relatively lower N$_2$O/(N$_2$O+N$_2$) ratio and nosZ gene abundance were both observed in the water area. The phenomenon suggested that besides denitrifier nosZ abundance, the different community composition of nosZ

![CCA ordination plot for the first two dimensions of a CCA of the relationship between the potential denitrification rate (DNR), potential N$_2$O production rate (pN$_2$O), N$_2$O/(N$_2$O+N$_2$) ratio, nosZ gene abundance and environmental variables. Correlations between environmental variables and CCA axes are represented by the length and angle of arrows.](jesc.ac.cn)
another important factor influencing the reduction of N gene-encoding denitrifiers harbored in sediments may be Rhizobiaceae and Bradyrhizobiaceae in previous studies (Stres et al., 2004; Enwall et al., 2005), grouped closely with the representatives of -Proteobacteria. Homologous sequences were also recovered from Australian Lyrebird Creek sediments (Perryma et al., 2008) and aquatic invertebrates in Aarhus Bay. The aquatic invertebrates ‘foraminifer’ could carry out different evolutionary distance from A. largimobile and Azospirillum irakense affiliated to Rhodospirillaceae in -Proteobacteria. Most of the sequences recovered from the land soil of the littoral gradient possessed the same homology as previous studies (Stres et al., 2004; Enwall et al., 2005), grouped closely with the representatives of Rhizobiaceae and Bradyrhizobiaceae in -Proteobacteria. However, all the sequences recovered from the sediment clustered closely with Azospirillum largimobile and Azospirillum irakense and Bradyrhizobiaceae bacterium D210a in surface and subsurface upland soils (AB480512, Group 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of clones</th>
<th>No. of OTUs</th>
<th>C (%)</th>
<th>H</th>
<th>I/D</th>
<th>S_{Chao1}</th>
<th>S_{ACE}</th>
<th>Group 1</th>
<th>Group 2</th>
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</thead>
<tbody>
<tr>
<td>Sediment</td>
<td>81</td>
<td>15</td>
<td>76.5</td>
<td>2.67</td>
<td>68.0</td>
<td>41.0</td>
<td>63.8</td>
<td>100%</td>
<td>90%</td>
</tr>
<tr>
<td>Land soil</td>
<td>72</td>
<td>10</td>
<td>80.6</td>
<td>2.06</td>
<td>13.0</td>
<td>30.0</td>
<td>27.1</td>
<td>10% (1/10)</td>
<td>90% (9/10)</td>
</tr>
</tbody>
</table>

C: coverage; H: Shannon-Winer; D: Simpson; S_{Chao1}, S_{ACE}: richness estimators.

Fig. 5 Neighbor-joining phylogenetic tree of partial nosZ gene fragments and bootstrap values (100 iterations) greater than 50% are shown. Clones with > 97% sequence similarity were considered to be the same OTU. Clone numbers are shown in parentheses and corresponding accession numbers are shown in Table S2.

Table 2 Biodiversity and predicted richness of the bacterial nosZ sequences recovered from sediment and land soil
species belonging to Bradyrhizobiaceae were found to carry the nosZ gene, it has not yet been fully proved that they could definitely mediate the evolution of $N_2$ from $N_2O$, e.g. *Bradyrhizobium japonicum* USDA110 (Velasco et al., 2004). Zhong et al. (2009) also referred to the fact that the end-product from many of the most active *Rhizobium* denitrifiers is $N_2O$, rather than $N_2$. The community composition appeared to be coupled with $N_2O$ reduction along the littoral gradient. In agreement, Magalhães et al. (2008) demonstrated that the denitrifying community composition may contribute to the variability of denitrification rates in the sediment of the Douro river estuary, and a similar result was also observed in meadow and forest soils (Rich et al., 2003).

The high disparities in the nature of the denitrification end products along the littoral gradient may result from the heterogeneous geochemical conditions. Along the littoral gradient, the $NO_3^-\cdot N$ ($NO_3^-\cdot N$ and $NO_2^-\cdot N$) contents of sediments were significantly lower than those of land soils (Table 1). The low $NO_2^-\cdot N$ supply in sediments did not appear to limit the denitrification process, but rather increased the reduction of $N_2O$ to $N_2$, resulting in $N_2$ being the dominant end product. This is consistent with the results of previous work that led to the conclusion that $N_2$ is the dominant end product of denitrification where there is a short supply of $NO_2^-$ as the electron acceptor for the denitrification process (Gillam et al., 2008). The higher content of OM in sediment also resulted in the $N_2O$ reduction along the littoral gradient of a eutrophic freshwater lake.

**4 Conclusions**

Taken together, the littoral zone had high spatial heterogeneity in potential denitrification rate, potential $N_2O$ production rate, and nosZ-encoding denitrifier community structure. In the land area, the nosZ gene abundance dominated $N_2O$ reduction both in the surface soils and in the soil core of the transition site. The community composition of nosZ gene-encoding denitrifiers appeared to be coupled with $N_2O$ reduction along the littoral gradient. Putatively, denitrifiers having short evolutionary distance from *Azospirillum largimobile* and *Azospirillum irakense* may be the key players in $N_2O$ reduction.

**Acknowledgments**

The authors would like to thank Professor Yujing Mu and associate Professor Weidong Wang for their kind help in gas sampling and measuring. This work was supported by the National Natural Science Foundation of China (No. 21077119), the National Basic Research Program of China (No. 2009CB421103), the Key Project of Water Pollution Control and Management of China (No. 2008ZX07209-006, 2009ZX07209-005 and 2008ZX07421-001) and the Special Fund of Tianjin Science and Technology Innovation Project (No. 08FDZDSF03200). Moreover, the author Guibing Zhu gratefully acknowledges the support of the Beijing Nova Program (No. 2011095) and the K. C. Wong Education Foundation, Hong Kong, China.

**Supporting materials**

Supplementary data associate with this article can be found in the online version.

**References**


Nitrous oxide reductase gene (nosZ) and N₂O reduction along the littoral gradient of a eutrophic freshwater lake

Supporting materials

Fig. S1 Location of the sampling site in the Baiyangdian Lake.

Fig. S2 The dissociation curve of nosZ gene quantitative PCR assay.
Nitrous oxide reductase gene (nosZ) and \text{N}_2\text{O} \text{ reduction along the littoral gradient of a eutrophic freshwater lake}

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**Fig. S3** The dissociation curve of total bacteria 16S rRNA gene quantitative PCR assay.

---

**Table S1** Physico-chemical characteristics of the overlying water used for incubation experiment (means ± SD, n=3)

<table>
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<tr>
<th>Item</th>
<th>Value</th>
<th>Item</th>
<th>Value</th>
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</thead>
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<td>TN (mg/L)</td>
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<td>Water depth (m)</td>
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<tr>
<td>NH\textsubscript{4}\textsuperscript{+}-N (mg/L)</td>
<td>9.69 ± 0.22</td>
<td>Temperature (°C)</td>
<td>29.1 ± 0.07</td>
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<td>NO\textsubscript{2}\textsuperscript{-}-N (mg/L)</td>
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<td>7.89 ± 0.03</td>
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TN: total nitrogen; TP: total phosphorus; SRP: soluble reactive phosphorus; DO: dissolved oxygen; Chl-\textalpha: chlorophyll-\textalpha; COD: chemical oxygen demand.
Nitrous oxide reductase gene (nosZ) and N\textsubscript{2}O reduction along the littoral gradient of a eutrophic freshwater lake

Table S2  Distribution of bacterial nosZ gene clones of each OTU and accession numbers in Genebank

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