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Nitrous oxide reductase gene (*nosZ*) and N_2O reduction along the littoral gradient of a eutrophic freshwater lake

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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 N_2O 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 N_2O reduction, the *nosZ*-encoding denitrifier community composition/abundance, potential denitrification rate (DNR) and potential N_2O production rate (pN_2O) 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 pN_2O 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 $N_2O/(N_2O+N_2)$ ratio, indicating that *nosZ* gene abundance dominated N_2O 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 α-Proteobacteria. The community composition of *nosZ* gene-encoding denitrifiers appeared to be coupled with N_2O reduction along the littoral gradient.

Key words: littoral gradient; N_2O reduction; *nosZ* gene; abundance; community composition

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Introduction

Biological denitrification in the global nitrogen cycle is the main source of nitrous oxide (N_2O) released to the atmosphere (Fernandes et al., 2010). N_2O 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 N_2O 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 N_2O 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 N_2O reduction, because denitrifying bacteria can harbor a truncated denitrification pathway lacking the *nosZ* gene and thus be unable to reduce N_2O (Henry et al., 2006). Moreover, N_2O 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-

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lated to N_2O 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^2, including more than 140 small lakes. Reed (*Phragmites australis*) dominates the vegetative belts around Baiyangdian Lake. There are altogether 94.0 km^2 of reed fields with more than 3700 ditches (approximately 24.8 km^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 *nosZ*-encoding denitrifier community composition/abundance and N_2O 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^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 *in situ* using a DO-meter (Model 57, YSI, USA). Three replicas were carried out for every measurement.

1.2 Potential denitrification rate and potential N_2O production rate measurements

Potential denitrification rate (DNR) and potential N_2O production rate (pN_2O) were measured for each sampling site in triplicate sediment slurries or soils using the acetylene inhibition method as described by Magãlhaes et al. (2005) and Teixeira et al. (2010) with some modifications. The sediments or soils were prepared by adding 15 mL of *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_2 to remove O_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 *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_2. pN_2O was given by the N_2O accumulation in treatments without C_2H_2 and DNR (N_2O plus N_2 production) by the N_2O produced in acetylene incubations. The $N_2O/(N_2+N_2O)$ ratio, calculated from pN_2O/DNR, was used to indicate the proportion of N_2O 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 *nosZ* genes were amplified with the primer set nosZF/nosZR (699 bp) as described by Kloos et al. (2001). The PCR was performed in a C1000^{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 *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 *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® *Pre-*

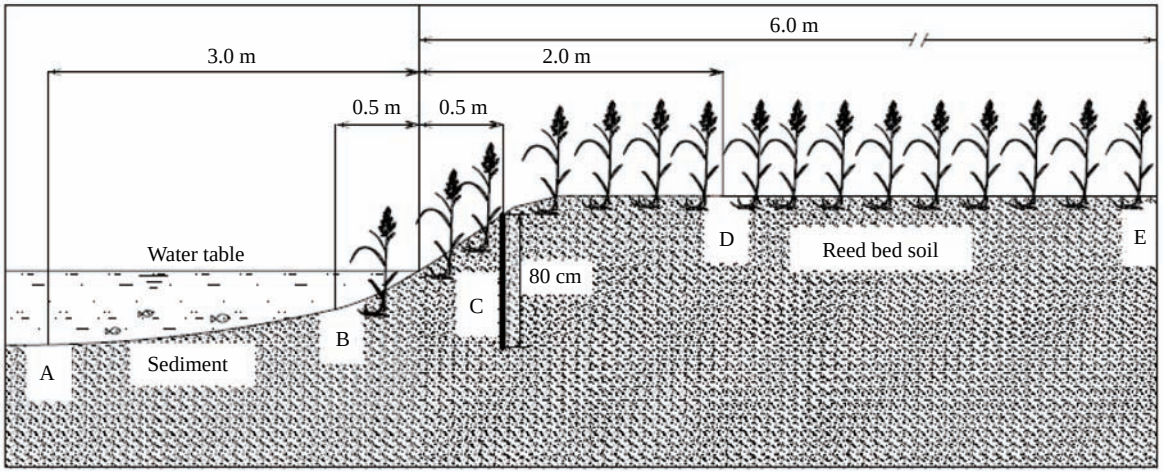

Fig. 1 Transect of the littoral zone and the sampling sites (A–E).

mix Ex Taq™ (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_4^+-N, NO_3^--N, NO_2^--N and organic matter (OM) showed great spatial differences along the littoral gradient. The average NH_4^+-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_3^--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_2-N content showed a trend similar to NO_3^--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

Site	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	NO_2^--N (mg/kg)	TN (g/kg)	TP (g/kg)	OM (g/kg)	pH (H_2O)
A	362.8 ± 12.1 a	7.18 ± 3.17 a	0.20 ± 0.03 a	3.67 ± 0.27 a	0.54 ± 0.01 a	50.3 ± 0.25 a	7.49 a
B	435.0 ± 4.02 b	5.89 ± 2.49 a	0.17 ± 0.03 a	3.87 ± 0.02 a	0.41 ± 0.01 b	71.5 ± 2.68 b	7.80 b
C	69.1 ± 0.93 c	1.73 ± 0.00 a	0.61 ± 0.28 b	1.97 ± 0.10 b	0.87 ± 0.01 c	29.9 ± 1.46 c	7.66 c
D	2.55 ± 1.03 d	57.2 ± 2.41 b	1.09 ± 0.00 c	1.41 ± 0.03 c	0.57 ± 0.00 d	21.8 ± 0.62 d	7.52 a
E	4.84 ± 1.31 d	13.4 ± 2.07 c	0.83 ± 0.19 bc	2.55 ± 0.00 d	0.89 ± 0.01 c	47.9 ± 0.03 a	7.33 d

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.

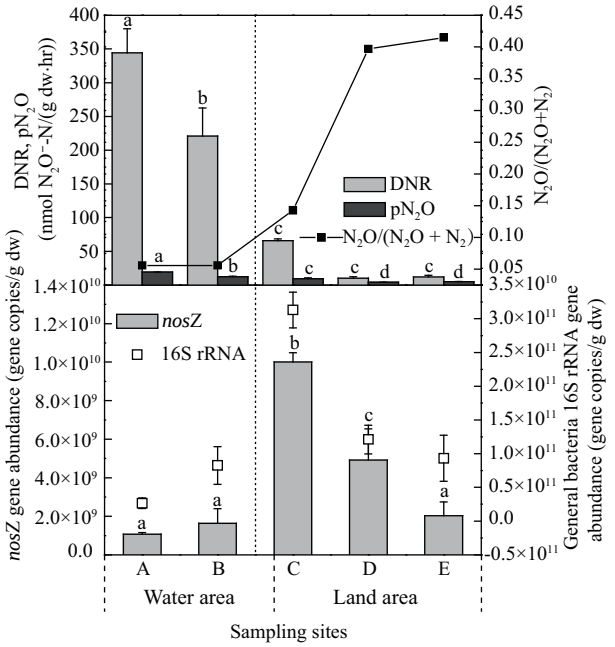

Fig. 3 Potential denitrification rates (DNR), potential N_2O production rates (pN_2O) and $N_2O/(N_2O+N_2)$ ratios and *nosZ*/bacteria 16S rRNA gene abundance along the littoral zone. Values are means ± SD (n=3). Different letters above the columns of DNR, pN_2O and *nosZ* gene abundance denote a significant difference at $p< 0.05$.

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 NH_4^+-N, NO_3^--N and NO_2^--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 N_2O reduction

2.2.1 Along the littoral gradient

To show the nature of denitrification end products and distinguish the abilities of denitrifiers to reduce N_2O along the littoral gradient, DNR and pN_2O 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 pN_2O of sediments was about 3.5 times higher than land soils, reaching 15.7 nmol N/(g dw·hr). The average $N_2O/(N_2O+N_2)$ ratio (N_2O 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 N_2O was transformed into N_2 in sediments than in land soils (**Fig. 3**).

The abundance of *nosZ*, the key enzyme for N_2O reduction, was investigated with quantitative PCR to explore its relationship with the N_2O 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, 1.00×10^{10} gene copies/g dw) accompanied by the highest 16S rRNA gene abundance (3.13×10^{11} gene copies/g dw). The average *nosZ* gene abundance of sediments (1.35×10^9 gene copies/g dw) was 2.6 times lower than that of land soils; for 16S rRNA, the average value of sediments was 5.46×10^{10} gene copies/g dw, 2.0 times lower than that of land soils (**Fig. 3**). The results showed that DNR, pN_2O and *nosZ*/16S rRNA gene abundance all had high spatial heterogeneity along the littoral gradient.

In the land area, it was observed that the $N_2O/(N_2O+N_2)$ ratio increased with declining *nosZ* gene abundance, indicating that *nosZ* gene abundance dominated N_2O reduction. However, in the water area, a relatively lower $N_2O/(N_2O+N_2)$ 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 $N_2O/(N_2O+N_2)$ ratio was also explored in the vertical profile of site C. In the soil core of site C, the DNR and pN_2O 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 pN_2O) higher than the other layers, indicating that the topsoil accounted for the largest part of N_2O emission. In the reed root channel distribution zone, the increase of pN_2O and decrease of DNR resulted in $N_2O/(N_2O+N_2)$ ratios increasing from 0.14 to 0.76 (**Fig. 4**).

The highest *nosZ* gene abundance (1.00×10^{10} gene copies/g dw) and 16S rRNA gene abundance (3.13×10^{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 9.23×10^9 to 1.22×10^9 gene copies/g dw; for 16S rRNA, the abundance decreased from 5.69×10^{10} to 1.76×10^{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 $N_2O/(N_2O+N_2)$ 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 N_2O 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 3.7×10^8 to 1.0×10^{10} 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

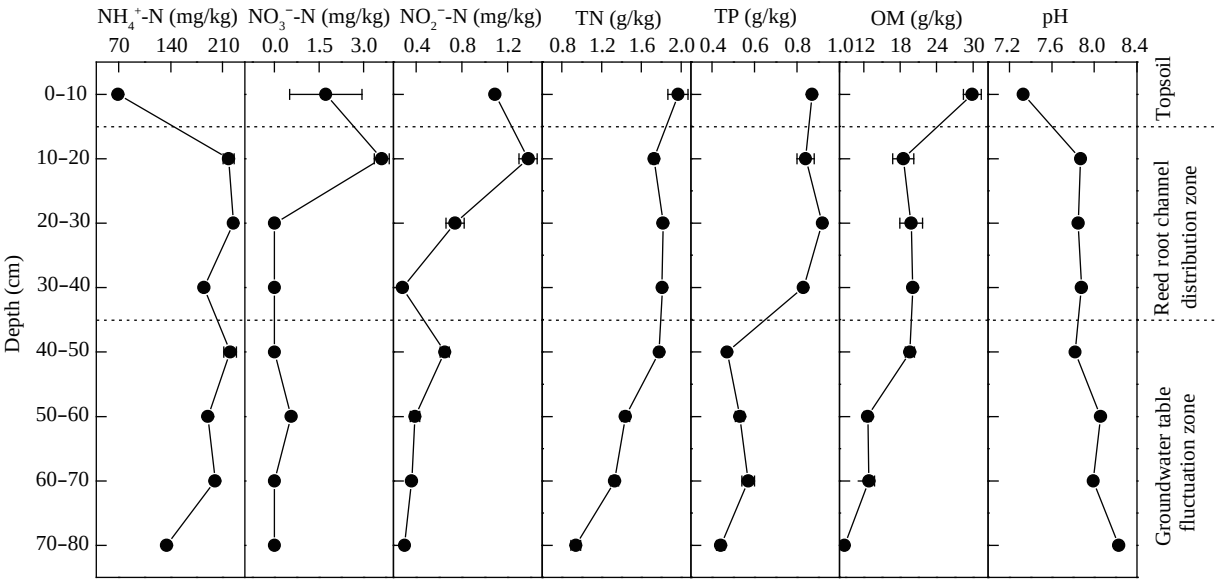

Fig. 2 Profile of the physico-chemical characteristics of the soil core at site C.

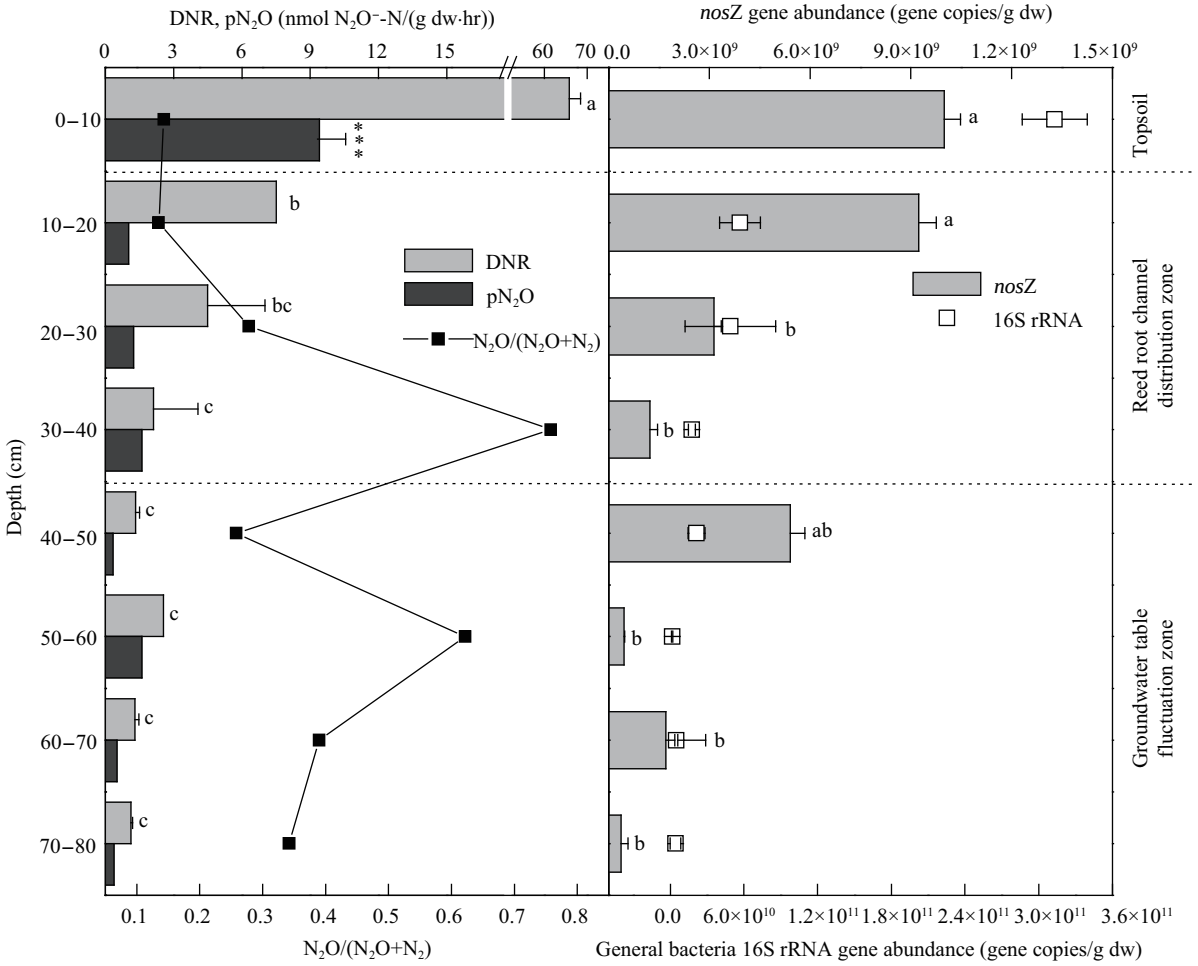

Fig. 4 Vertical distribution of DNR, pN_2O and $N_2O/(N_2O+N_2)$ ratios and *nosZ*/bacteria 16S rRNA gene abundance of the soil core at the transition site (site C). Values are means ± SD (n = 3). Different letters beside the column of DNR denote a significant difference at $p< 0.05$. ***Beside the columns of pN_2O denotes a significant difference at $p<0.001$.

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_2O 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 S_{Chao1} and S_{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 α-Proteobacteria. All the OTUs of sediment were clustered in Group 1 and all the recovered *nosZ* sequences clustered closely with the isolates *Azospirillum largimobile* (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_2O, $N_2O/(N_2O+N_2)$ 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_2^--N (0.52) and NH_4^+-N (–0.65). NO_2^--N showed a stronger effect on all the response variables than NO_3^--N. NO_2^--N and NO_3^--N both showed positive effects on the $N_2O/(N_2O+N_2)$ ratio (r = 0.73 for NO_2^--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_2O and *nosZ*-encoding denitrifier community structure. Based on the above measurements, the relationship between the *nosZ*-encoding denitrifier community composition/abundance and N_2O 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 $N_2O/(N_2O+N_2)$ ratio, indicating that the *nosZ* gene abundance dominated N_2O reduction. Ma et al. (2011) indicated that the abundance of denitrifier *nosZ* is related to potential N_2O emission because *nosZ* abundance directly affects nitrous oxide reductase activity, and also found that the $N_2O/(N_2O+N_2)$ 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 $N_2O/(N_2O+N_2)$ 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*

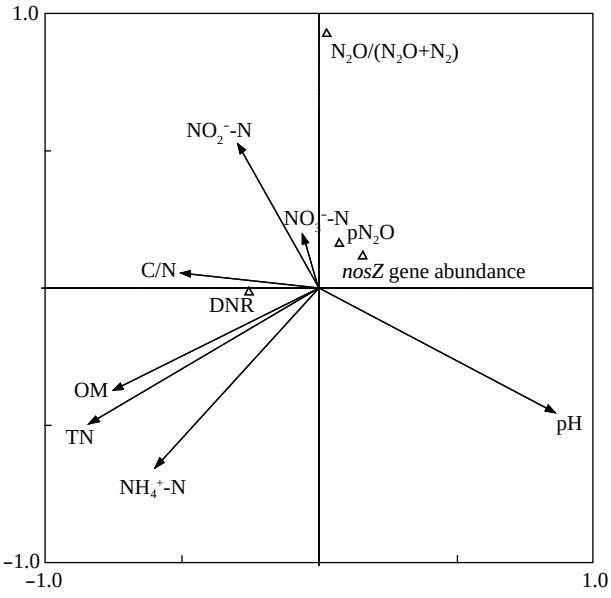

Fig. 6 CCA ordination plot for the first two dimensions of a CCA of the relationship between the potential denitrification rate (DNR), potential N_2O production rate (pN_2O), $N_2O/(N_2O + N_2)$ ratio, *nosZ* gene abundance and environmental variables. Correlations between environmental variables and CCA axes are represented by the length and angle of arrows.

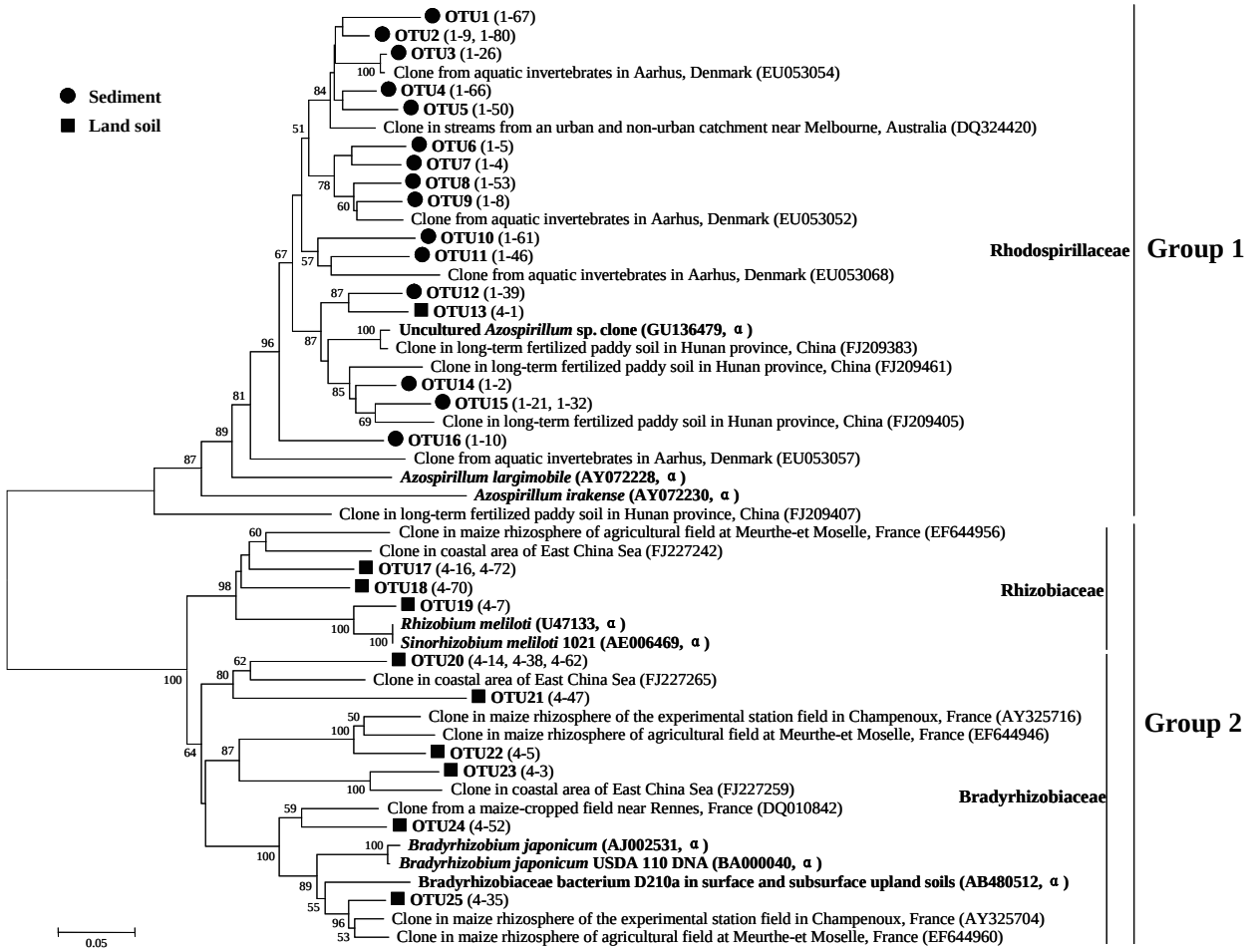

Fig. 5 Neighbor-joining phylogenetic tree of partial *nosZ* gene fragments and bootstrap values (100 iterations) greater than 50% are shown. Clones with > 97% sequence similarity were considered to be the same OTU. Clone numbers are shown in parentheses and corresponding accession numbers are shown in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

Table 2 Biodiversity and predicted richness of the bacterial *nosZ* sequences recovered from sediment and land soil

Sample	No. of clones	No. of OTUs	*C* (%)	*H*	1/*D*	S_{Chao1}	S_{ACE}	Group 1	Group 2
Sediment	81	15	76.5	2.67	68.0	41.0	63.8	100% (15/15)	0% (0/15)
Land soil	72	10	80.6	2.06	13.0	30.0	27.1	10% (1/10)	90% (9/10)

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

gene-encoding denitrifiers harbored in sediments may be another important factor influencing the reduction of N_2O. Cavigelli and Robertson (2000) showed that it was the denitrifier community composition that led to the different denitrification rates and $N_2O/(N_2O+N_2)$ ratios in two kinds of soil under controlled incubation conditions.

Phylogenetic analysis showed that all the OTUs were found to be restricted to sediment or land soil alternatively, suggesting that the micro-environmental conditions of the sampling sites constrain the denitrifying bacteria. 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 α-Proteobacteria. However, all the sequences recovered from the sediment clustered closely with *Azospirillum largimobile* and *Azospirillum irakense* affiliated to Rhodospirillaceae in α-Proteobacteria. Homologous sequences were also recovered from Australian Lyrebird Creek sediments (Perryman et al., 2008) and aquatic invertebrates in Aarhus Bay. The aquatic invertebrates 'foraminifer' could carry out complete denitrification (Risgaard-Petersen et al., 2006), resulting in N_2 being the main denitrification product in sediments. Moreover, considering that a relatively lower $N_2O/(N_2O+N_2)$ ratio and lower *nosZ* gene abundance both occurred in the water area compared to that of the land area, it could be inferred that the denitrifiers having short evolutionary distance from *A. largimobile* and *A. irakense* in Rhodospirillaceae may be the key players in N_2O reduction compared with the members of Rhizobiaceae and Bradyrhizobiaceae. In previous work, although some

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_x^--N (NO_3^--N and NO_2^--N) contents of sediments were significantly lower than those of land soils (**Table 1**). The low NO_x^--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_x^- as the electron acceptor for the denitrification process (Gillam et al., 2008). The higher content of OM in sediment also resulted in the tremendous reduction of N_2O to N_2, and subsequently a lower $N_2O/(N_2O+N_2)$ ratio. Moreover, the stronger effect of NO_2^--N on DNR and pN_2O compared to NO_3^--N was consistent with the result of previous works (Dong et al., 2002, 2004). Perhaps, this was because the nitrite denitrifiers in the sediment only use nitrite as their terminal electron acceptor, or that the nitrate denitrifiers use nitrite more efficiently than nitrate (Dong et al., 2002). Although instantaneous denitrification or N_2O reduction is affected by environmental factors, these drivers act through the biotic community. Moreover, different denitrifying communities respond to the same environmental regulator in different manners. Thus, it can be assumed that a network of environmental conditions shapes the *nosZ*-encoding denitrifier community structure, and consequently results in different N_2O reduction patterns along the littoral gradient.

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.

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Supporting materials

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

References

Cavigelli M A, Robertson G P, 2000. The functional significance of denitrifier community composition in a terrestrial ecosystem. *Ecology*, 81(5): 1402–1414.

Chen H, Yuan X Z, Gao Y H, Wu N, Zhu D, Wang J X, 2010. Nitrous oxide emissions from newly created littoral marshes in the drawdown area of the Three Gorges Reservoir, China. *Water, Air, and Soil Pollution*, 211(1-4): 25–33.

Chon K, Chang J S, Lee E, Lee J, Ryu J, Cho J, 2011. Abundance of denitrifying genes coding for nitrate (*narG*), nitrite (*nirS*), and nitrous oxide (*nosZ*) reductases in estuarine versus wastewater effluent-fed constructed wetlands. *Ecological Engineering*, 37(1): 64–69.

Chroňáková A, Radl V, Čuhel J, Šimek M, Elhottová D, Engel M et al., 2009. Overwintering management on upland pasture causes shifts in an abundance of denitrifying microbial communities, their activity and N_2O-reducing ability. *Soil Biology and Biochemistry*, 41(6): 1132–1138.

Dong L F, Nedwell D B, Colbeck I, Finch J, 2004. Nitrous oxide emission from some English and Welsh rivers and estuaries. *Water, Air, and Soil Pollution*, 4(6): 127–134.

Dong L F, Nedwell D B, Underwood G J C, Thornton D C O, Rusmana I, 2002. Nitrous oxide formation in the Colne Estuary, England: The central role of nitrite. *Applied and Environmental Microbiology*, 68(3): 1240–1249.

Enwall K, Philippot L, Hallin S, 2005. Activity and composition of denitrifying bacterial community respond differently to long-term fertilization. *Applied and Environmental Microbiology*, 71(12): 8335–8343.

Fernandes S O, Bharathi P A L, Bonin P C, Michotey V D, 2010. Denitrification: An important pathway for nitrous oxide production in tropical mangrove sediments (Goa, India).

Journal of Environment Quality, 39(4): 1507–1516.

Gillam K M, Zebarth B J, Burton D L, 2008. Nitrous oxide emissions from denitrification and the partitioning of gaseous losses as affected by nitrate and carbon addition and soil aeration. *Canadian Journal of Soil Science*, 88(2): 133–143.

Groffman P M, Gold A J, Addy K, 2000. Nitrous oxide production in riparian zones and its importance to national emission inventories. *Chemosphere*, 2(3-4): 291–299.

Henry S, Bru D, Stres B, Hallet S, Philippot L, 2006. Quantitative detection of the *nosZ* gene, encoding nitrous oxide reductase, and comparison of the abundances of 16S rRNA, *narG*, *nirK*, and *nosZ* genes in soils. *Applied and Environmental Microbiology*, 72(8): 5181–5189.

Hunt P G, Matheny T A, Ro K S, 2007. Nitrous oxide accumulation in soils from riparian buffers of a coastal plain watershed-carbon/nitrogen ratio control. *Journal of Environment Quality*, 36(5): 1368–1376.

Kloos K, Mergel A, Rösch C, Bothe H, 2001. Denitrification within the genus *Azospirillum* and other associative bacteria. *Australian Journal of Plant Physiology*, 28(9): 991–998.

Kumar S, Tamura K, Nei M, 2004. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics*, 5(2): 150–163.

Liu Y S, Zhu R B, Ma D W, Xu H, Luo Y H, Huang T et al., 2011. Temporal and spatial variations of nitrous oxide fluxes from the littoral zones of three alga-rich lakes in coastal Antarctica. *Atmospheric Environment*, 45(7): 1464–1475.

Ma W K, Bedard-Haughn A, Siciliano S D, Farrell R E, 2008. Relationship between nitrifier and denitrifier community composition and abundance in predicting nitrous oxide emissions from ephemeral wetland soils. *Soil Biology and Biochemistry*, 40(5): 1114–1123.

Ma W K, Farrell R E, Siciliano S D, 2011. Nitrous oxide emissions from ephemeral wetland soils are correlated with microbial community composition. *Frontiers in Microbiology*, 2: 110–117.

Magalhães C, Bano N, Wiebe W J, Bordalo A A, Hollibaugh J T, 2008. Dynamics of nitrous oxide reductase genes (*nosZ*) in intertidal rocky biofilms and sediments of the Douro river estuary (Portugal), and their relation to N-biogeochemistry. *Microbial Ecology*, 55(2): 259–269.

Magalhães C M, Joye S B, Moreira R M, Wiebe W J, Bordalo A A, 2005. Effect of salinity and inorganic nitrogen concentrations on nitrification and denitrification rates in intertidal sediments and rocky biofilms of the Douro River estuary, Portugal. *Water Research*, 39(9): 1783–1794.

Muyzer G, De Waal E C, Uitterlinden A G, 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology*, 59(3): 695–700.

Perryman S E, Rees G N, Walsh C J, 2008. Analysis of denitrifying communities in streams from an urban and non-urban catchment. *Aquatic Ecology*, 42(1): 95–101.

Philippot L, Čuhel J, Saby N P A, Chèneby D, Chroňáková A, Bru D et al., 2009. Mapping field-scale spatial patterns of size and activity of the denitrifier community. *Environmental Microbiology*, 11(6): 1518–1526.

Rich J J, Heichen R S, Bottomley P J, Cromack K Jr, Myrold D D, 2003. Community composition and functioning of denitrifying bacteria from adjacent meadow and forest soils. *Applied and Environmental Microbiology*, 69(10): 5974–5982.

Risgaard-Petersen N, Langezaal A M, Ingvardsen S, Schmid M C, Jetten M S M, Op den Camp H J M et al., 2006. Evidence for complete denitrification in a benthic foraminifer. *Nature*, 443(7107): 93–96.

Stres B, Mahne I, Augustin C, Tiedje J M, 2004. Nitrous oxide reductase (*nosZ*) gene fragments differ between native and cultivated Michigan soils. *Applied and Environmental Microbiology*, 70(1): 301–309.

Teixeira C, Magalhães C, Boaventura R A R, Bordalo A A, 2010. Potential rates and environmental controls of denitrification and nitrous oxide production in a temperate urbanized estuary. *Marine Environmental Research*, 70(5): 336–342.

Tiedje J M, 1988. Ecology of denitrification and dissimilatory nitrate reduction to ammonium. In: Biology of Anaerobic Microorganisms (Zehnder A J B, ed.). Wiley, Chichester, UK. 179–244.

Velasco L, Mesa S, Xu C A, Delgado M J, Bedmar E J, 2004. Molecular characterization of *nosRZDFYLX* genes coding for denitrifying nitrous oxide reductase of *Bradyrhizobium japonicum*. *Antonie van Leeuwenhoek*, 85(3): 229–235.

Wallenstein M D, Myrold D D, Firestone M, Voytek M, 2006. Environmental controls on denitrifying communities and denitrification rates: insights from molecular methods. *Ecological Applications*, 16(6): 2143–2152.

Wang H J, Wang W D, Yin C Q, Wang Y C, Lu J W, 2006. Littoral zones as the "hotspots" of nitrous oxide (N_2O) emission in a hyper-eutrophic lake in China. *Atmospheric Environment*, 40(28): 5522–5527.

Wang S Y, Wang Y, Feng X J, Zhai L M, Zhu G B, 2011. Quantitative analyses of ammonia-oxidizing archaea and bacteria in the sediments of four nitrogen-rich wetlands in China. *Applied Microbiology and Biotechnology*, 90(2): 779–787.

Wang W D, Yin C Q, 2008. The boundary filtration effect of reed-dominated ecotones under water level fluctuations. *Wetlands Ecology and Management*, 16(1): 65–76.

Wang W D, Wang D L, Yin C Q, 2002. A field study on the hydrochemistry of land/inland water ecotones with reed domination. *Acta Hydrochimica et Hydrobiologica*, 30(2-3): 117–127.

Zhong Z Z, Lemke R L, Nelson L M, 2009. Nitrous oxide emissions associated with nitrogen fixation by grain legumes. *Soil Biology and Biochemistry*, 41(11): 2283–2291.

Zhu G, Wang S, Wang Y, Wang C, Risgaard-Petersen N, Jetten M S M et al., 2011. Anaerobic ammonia oxidation in a fertilized paddy soil. *The ISME Journal*, 5(12): 1905–1912.

Supporting materials

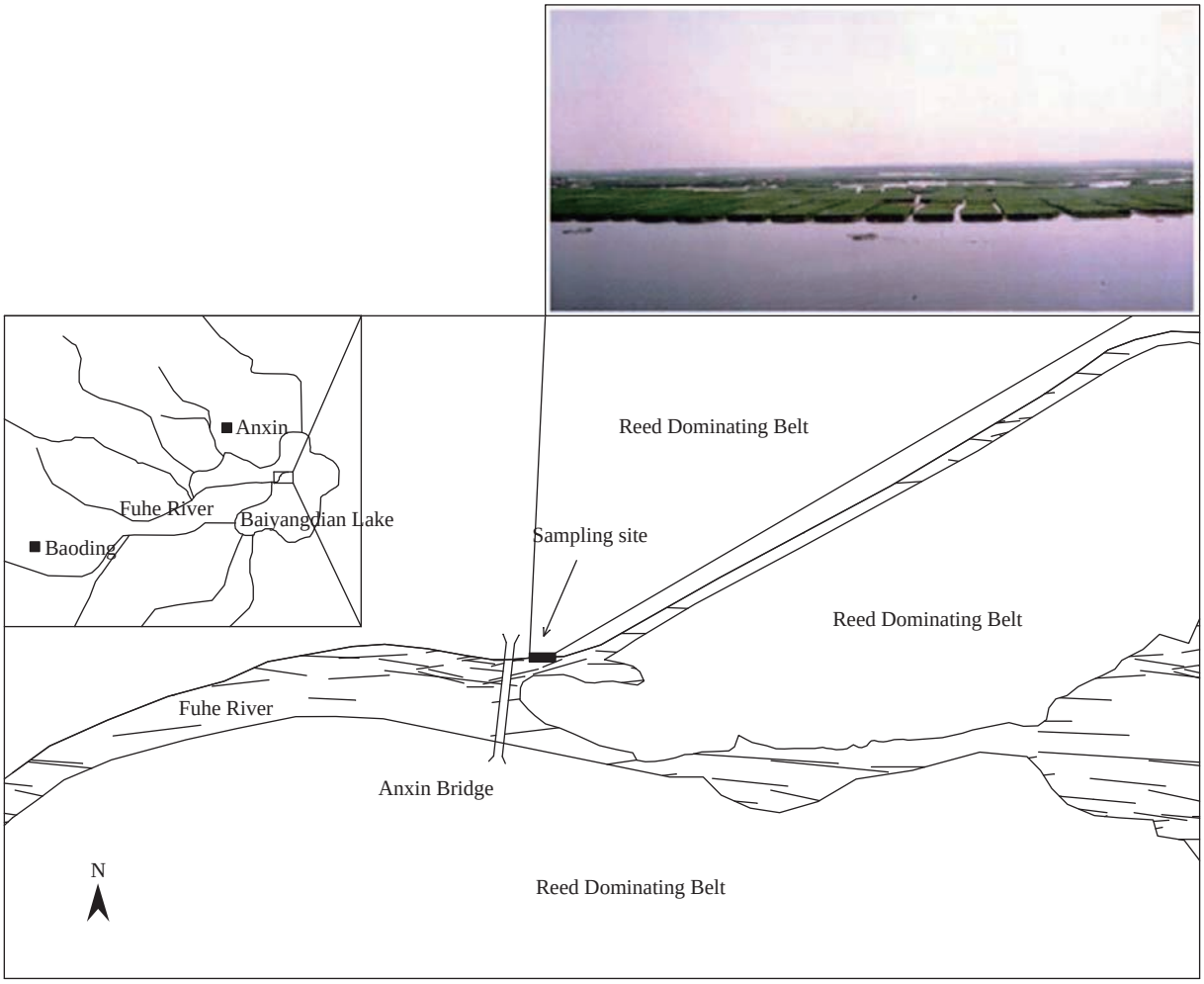

Fig. S1 Location of the sampling site in the Baiyangdian Lake.

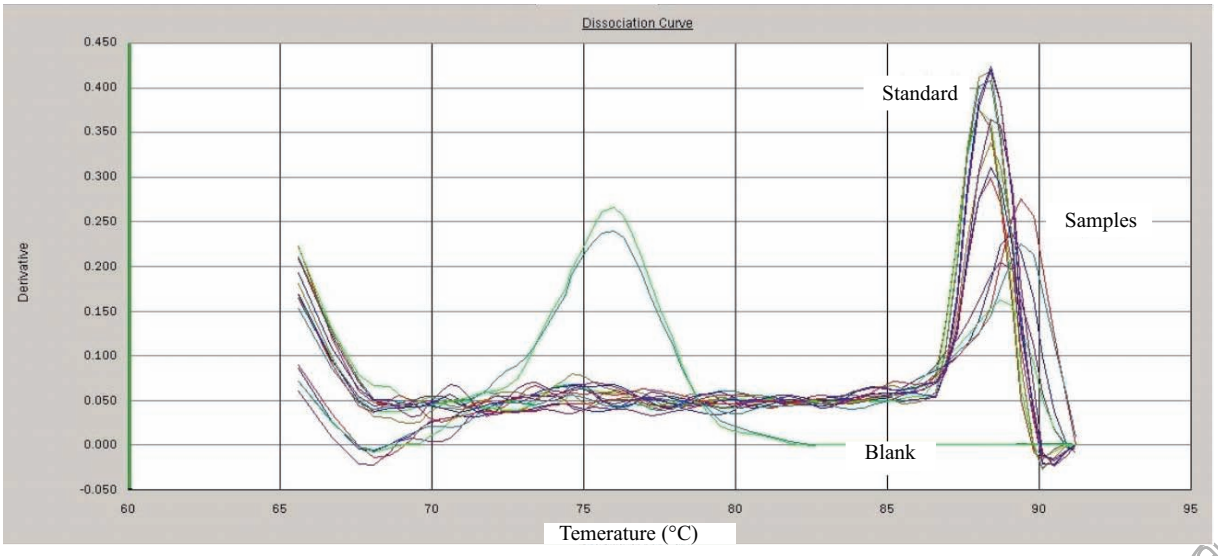

Fig. S2 The dissociation curve of *nosZ* gene quantitative PCR assay.

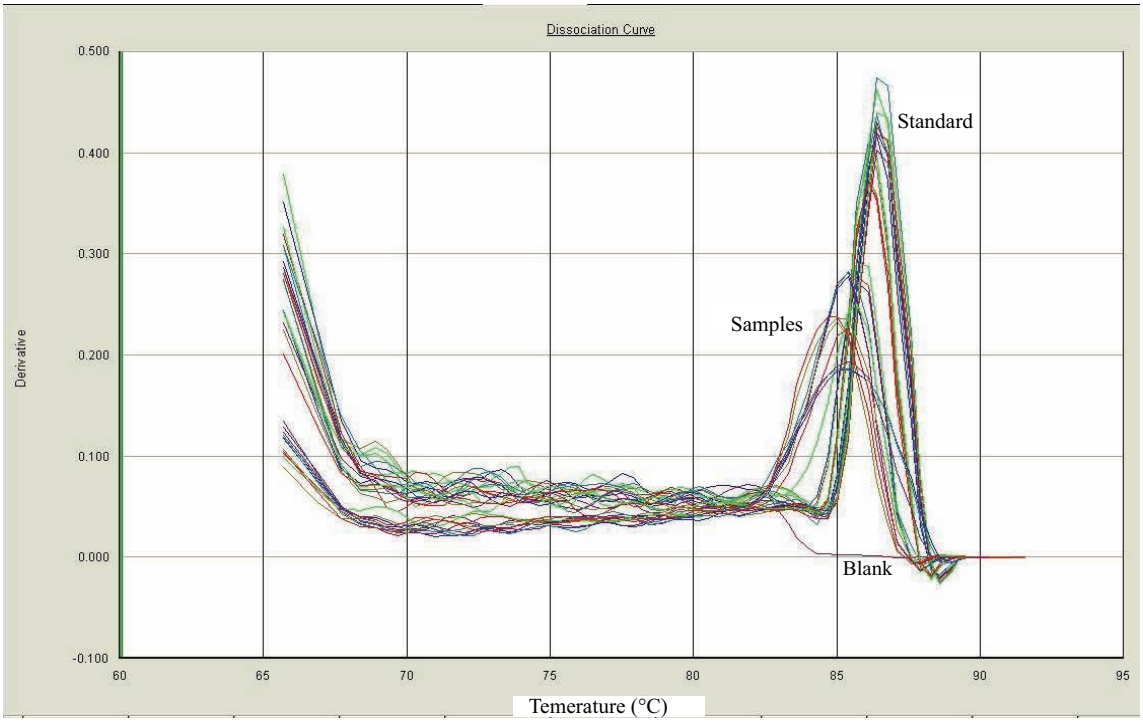

Fig. S3 The dissociation curve of total bacteria 16S rRNA gene quantitative PCR assay.

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

Item	Value	Item	Value
TN (mg/L)	15.7 ± 0.21	Water depth (m)	1.60
NH_4^+-N (mg/L)	9.69 ± 0.22	Temperature (°C)	29.1 ± 0.07
NO_3^--N (mg/L)	0.48 ± 0.02	pH	7.89± 0.03
NO_2^--N (mg/L)	0.02 ± 0.00	DO (mg/L)	1.47 ± 0.11
TP (mg/L)	1.00 ± 0.01	Chl-*a* (μg/L)	7.02 ± 3.96
SRP (mg/L)	0.90 ± 0.01	COD (mg/L)	45.0

TN: total nitrogen; TP: total phosphorus; SRP: soluble reactive phosphorus; DO: dissolved oxygen; Chl-*a*: chlorophyll-a; COD: chemical oxygen demand.

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

OTU (number of sequence)	Sequences		Genebank Accession Number
	Sediment	Land soil	
OTU 1# (1)	1-67		JF509058
OTU 2# (2)	1-9		JF509063
	1-80		JF509062
OTU 3# (1)	1-26		JF509051
OTU 4# (1)	1-66		JF509057
OTU 5# (1)	1-50		JF509054
OTU 6# (1)	1-5		JF509046
OTU 7# (1)	1-4		JF509045
OTU 8# (1)	1-53		JF509055
OTU 9# (1)	1-8		JF509047
OTU 10# (1)	1-61		JF509056
OTU 11# (1)	1-46		JF509053
OTU 12# (1)	1-39		JF509052
OTU 13# (1)		4-1	JF509084
OTU 14# (1)	1-2		JF509043
OTU 15# (2)	1-21		JF509049
	1-32		JF509061
OTU 16# (1)	1-10		JF509048
OTU 17# (2)		4-16	JF509089
		4-72	JF509097
OTU 18# (1)		4-70	JF509096
OTU 19# (1)		4-7	JF509087
OTU 20# (3)		4-14	JF509088
		4-38	JF509099
		4-62	JF509098
OTU 21# (1)		4-47	JF509093
OTU 22# (1)		4-5	JF509086
OTU 23# (1)		4-3	JF509085
OTU 24# (1)		4-52	JF509094
OTU 25# (1)		4-35	JF509091

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