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ENVIRONMENTAL BIOTECHNOLOGY

Vertical profiles of water and sediment denitrifiers in two plateau freshwater lakes

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Abstract The present study investigated the abundance, richness, diversity, and community composition of denitrifiers (based on *nirS* and *nosZ* genes) in the stratified water columns and sediments in eutrophic Dianchi Lake and mesotrophic Erhai Lake using quantitative PCR assay and high-throughput sequencing analysis. Both *nirS*- and *nosZ* denitrifiers were detected in waters of these two lakes. Surface water showed higher *nosZ* gene density than bottom water, and Dianchi Lake waters had larger *nirS* gene abundance than Erhai Lake waters. The abundance of sediment *nirS*- and *nosZ* denitrifiers in Dianchi Lake was larger than that in Erhai Lake. *nirS* richness and diversity and *nosZ* richness tended to increase with increasing sediment layer depth in both lakes. The distinct structure difference of sediment *nirS*- and *nosZ* denitrifier communities was found between in Dianchi Lake and Erhai Lake. These two lakes also differed greatly in water denitrifier community structure. Moreover, phylogenetic analysis indicated the presence of several different groups of *nirS*- or *nosZ* denitrifiers in both lakes. The novel *nirS* denitrifiers were abundant in both Dianchi Lake and Erhai Lake, while most of the obtained *nosZ* sequences could be affiliated with known genera.

Keywords Denitrifier · *nirS* · *nosZ* · Freshwater lake · Vertical change

Introduction

Eutrophication of freshwater lake ecosystem is a major environmental concern all over the world (Smith and Schindler 2009). Large freshwater lakes may receive high nitrogenous loads from terrestrial sources, which leads to the deterioration of water quality and even eutrophication. Understanding nitrogen cycling in freshwater lake is of great importance for nitrogen removal. Denitrification process participates in nitrogen removal in freshwater lake and it can remove 30–50% of the total nitrogen inputs into aquatic systems (Annika and Christopher 2010; Chen et al. 2012; Gayle et al. 1989; Harrison et al. 2009). Denitrification is a facultative respiratory in which nitrate is reduced into molecular nitrogen, with some intermediate nitrogen oxide products containing nitrite, nitric oxide, and greenhouse gas nitrous oxide that are catalyzed by nitrate reductase (nar), nitrite reductase (nir), nitric oxide reductase (nor), and nitrous oxide reductase (nos), respectively (Beaulieu et al. 2011; Berk et al. 1995; Zumft and Körner 1997). The functional genes *nirS* and *nosZ* have been the commonly used biomarkers to elucidate the abundance, richness, and diversity of denitrifier communities (Bowen et al. 2015; Braker et al. 2015; Castro-González et al. 2005; Dang et al. 2009; Gao et al. 2016; Henry et al. 2006; Smith et al. 2015; Srinandan et al. 2011; Tatti et al. 2015; Zumft and Körner 1997).

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So far, the change of denitrifier community in a given freshwater lake with sampling site (horizontal change) has been well documented (Antti et al. 2011; Wang et al. 2013; Yang et al. 2013), while little is known about its change with sampling depth (vertical shift). Only Pina and Alvarez (2006) put forward that denitrification occurred readily in anoxic top sediment layer (2–5 cm) containing abundant nitrate nitrogen (NO_3^--N) and organic carbon. Moreover, few previous studies have paid attention to the difference of denitrifier community among various freshwater lakes (Guo et al. 2013; Yang et al. 2013). In addition, denitrification process can occur in both water column and sediment. Many previous studies have investigated the denitrifiers in either lake water or sediment, including their abundance, diversity, and community structure (Kim et al. 2011; Yang et al. 2013), yet the comparison of the difference of denitrifier community between in water and in sediment has not been addressed. Dianchi Lake and Erhai Lake, located on the Yunnan–Guizhou Plateau, are the two largest freshwater lakes in Yunnan Province (southwest China), characterized as eutrophic and mesotrophic, respectively (Wang et al. 2015; Yang et al. 2016a, 2016b). The main aim of the present study was to investigate the vertical profiles of *nirS*- and *nosZ*-type denitrifiers in water column and sediment of these two freshwater lakes.

Materials and methods

Study sites and sampling

In this study, water samples and sediment cores in triplicate were collected in December in 2015 from the profundal zone in Dianchi Lake (with water depth of 6.4 m) and Erhai Lake (with water depth of 11.6 m) using plexiglass water sampler and core sampler, respectively. Water samples from Dianchi Lake were collected from 50-cm depth below water surface (sample DW1) and 50 cm above sediment surface (sample DW2) (Fig. S1). In Erhai Lake, water samples in three different depths (sample EW1 0–0.5 m, sample EW2 5–5.5 m, sample EW3 9.5–10 m) were collected. In addition, in either lake, sediment core (upper 20 cm) was collected, and was then sliced into six layers (sample DS1 or ES10–5 cm, sample DS2 or ES2 5–8 cm, DS3 or ES3 8–11 cm, DS4 or ES4 11–14 cm, DS5 or ES5 14–17 cm, and DS6 or ES6 17–20 cm). Water and sediment samples were placed in sterile bottles and bags, respectively, and they were immediately transported to a laboratory on ice. The levels of lake water temperature, pH, dissolved oxygen, ammonium nitrogen, nitrate nitrogen, total nitrogen, total phosphorus, and dissolved organic carbon were 11–14.7 °C, 7.61–9.02, 4.76–8.33 mg/L, 0.062–0.278 mg/L, 0.02–0.17 mg/L, 0.39–0.9 mg/L, 0.011–0.15 mg/L, and 12.1–14.7 mg/L, respectively (Table S1). Moreover, the levels of lake sediment ammonium nitrogen, nitrate nitrogen, total nitrogen, total phosphorus, and total organic carbon were 1.14–75.49 mg/kg, 0.85–10.64 mg/kg, 781.48–8135.76 mg/kg, 259.33–2251 mg/kg, and 4.73–53.70 g/kg, respectively (Table S2). The methods of water and sediment physicochemical analyses were described in Supplementary Material.

Quantitative PCR analysis

Sediment DNA was extracted by Powersoil DNA extraction kit (Mobio Laboratories, USA), while Water DNA was extracted using E.Z.N.A. Water DNA kit (Omega, USA) after using 0.22-μm pore-size membrane (diameter 50 mm; Millipore) to retain microbial cells in water samples. Denitrifier community abundance was assessed using quantitative PCR (qPCR) assay with primer sets *nirS*cd3aF/*nirS*R3cd for *nirS* gene (Kandeler et al. 2006) and *nosZ*F/*nosZ*1622R for *nosZ* gene (Kloos et al. 2001; Throbäck et al. 2004). SYBR Green qPCR was performed using an ABI 7500 FAST (Applied Biosystems, Foster City, CA, USA) in 25-μL reaction mixture including10 μM primers (1 μL), 2× SYBR Green PCR master mix (12.5 μL), and template DNA (2 μL). The amplification conditions were as follows: an initial denaturation at 94 °C for 5 min; 35 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1.5 min; and a final extension of 10 min at 72 °C. The amplification specificity was confirmed via melting curve analysis. Standard curve was obtained by tenfold serial dilution of standard plasmids containing target functional gene. The amplification efficiency were 96 and 93% for *nirS* and *nosZ* genes, respectively, while the amplification coefficient (R^2) for these two genes were 0.997 and 0.995, respectively. Moreover, one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls test was used to determine the significant difference ($P < 0.05$) in the density of *nirS* or *nosZ* gene among samples.

High-throughput sequencing analysis

nirS and *nosZ* genes were also amplified with the abovementioned primer sets *nirS*cd3aF/*nirS*R3cd and *nosZ*F/*nosZ*1622R, respectively. The PCR products were purified using QIAquick PCR purification kit (Qiagen Inc.), and then were submitted for Illumina MiSeq high-throughput sequencing at Shanghai Majorbio Bio-pharm Technology Co., Ltd. (China). The raw Illumina reads obtained in the current study were deposited in the NCBI short-read archive under accession number SRP079048. The paired-end reads were assembled into composite reads using FLASH and further processed with the Quantitative Insights into Microbial Ecology (QIIME) software pipeline, as previously described (Caporaso et al. 2010). Putative chimeric composite reads were discarded using UCHIME (Edgar et al. 2011).

Chimeric-free sequences with ≥97% similarity were assigned to operational taxonomic units (OTUs) and α-diversity (Chao1 richness estimator and Shannon diversity index) were further generated using the UPARSE pipeline (Edgar 2013). Phylogenetic trees of representative *nirS* and *nosZ* gene sequences and their reference sequences from GenBank database were constructed with MEGA software version 6.0 (Tamura et al. 2013) using a maximum likelihood method. In addition, to identify the difference of denitrifier community composition among lake samples, unweighted pair group method with arithmetic mean (UPGMA) clustering was performed. Beta-diversity was calculated based on Bray–Curtis distance matrix of OTU composition using the QIIME program.

Results

Denitrifier community abundance

The density of *nirS* gene was 1.13×10^7 or 3.66×10^7 copies per liter water in two Dianchi Lake water samples, while the three Erhai Lake water samples showed the abundance of 3.21×10^5–7.22×10^5 *nirS* gene copies per liter water (Fig. 1a). The number of *nirS* gene varied from 2.04×10^5 to 1.04×10^9 and 5.05×10^5 to 1.32×10^8 copies per gram dry sediment in Dianchi Lake and Erhai Lake sediments, respectively (Fig. 1b). No significant difference in *nirS* gene abundance was found between two Dianchi Lake water samples, and among Erhai Lake water samples. In Dianchi Lake, sediment *nirS* gene abundance in samples DS1, DS2, and DS3 was significantly greater than that in other three samples (DS4, DS5, and DS6) ($P = 0$). In contrast, no significant difference in *nirS* gene abundance was found among Erhai lake sediment samples ($P > 0.05$). Moreover, *nirS* gene density in Dianchi Lake waters was significantly greater than that in Erhai lake waters ($P = 0.021$, 0.03, or 0.001). In the upper three sediment layers (0–5, 5–8, and 8–11 cm), Dianchi Lake had significantly greater *nirS* gene abundance than Erhai lake ($P = 0$), while no significant difference in *nirS* gene abundance was found among the lower three sediment layers (11–14, 14–17, and 17–20 cm) ($P = 1$).

The number of *nosZ* gene ranged between 6.65×10^6 and 1.22×10^7copies per liter water in Dianchi Lake water samples, and was 8.76×10^5–1.02×10^7 copies per liter water in Erhai Lake water samples. The *nosZ* gene abundance was 3.62×10^6–8.71×10^7 and 5.70×10^5–8.37×10^6 copies per gram dry sediment in Dianchi Lake and Erhai Lake sediment samples. In Dianchi Lake, sediment *nosZ* gene density in samples DS1, DS2, and DS3 was much greater than that in other three samples, and water *nosZ* gene density in sample DW1 was also greater than that in sample DW2. In Erhai Lake, no significant difference in *nosZ* gene abundance was found among sediment samples ($P = 1$), while water *nosZ* gene density in sample EW1 was significantly greater than in samples EW2 and EW3 ($P = 0.009$). Moreover, no significant difference in *nosZ* gene abundance was between surface water samples DW1 and EW1 ($P = 0.249$), while water sample DW2 had significantly greater *nosZ* gene density than water samples EW2 and EW3 ($P = 0.03$ or 0). In addition, in the upper three sediment layers (0–5, 5–8, and 8–11 cm), Dianchi Lake illustrated significantly greater *nosZ* gene density than Erhai lake ($P = 0$), while no significant difference in *nosZ* gene density was observed among the lower three sediment layers (11–14, 14–17, and 17–20 cm) ($P = 0.963$, 0.935, or 1).

Denitrifier community richness and diversity

In this study, after subsampling for the comparison of denitrifier community richness and diversity among different lake samples, a total of 16,547 *nirS* gene sequences were recovered from each water or sediment sample. High Good's coverage (≥99%) indicated that *nirS* OTUs of each lake sample were well captured. The number of *nirS* gene OTU in Dianchi Lake water samples ranged between 66 and 73, while Erhai Lake water *nirS* gene libraries included 49–267 OTUs (Table 1). Sediment samples from Dianchi Lake had 46–90 OTUs, while those from Erhai Lake had 78–100 OTUs. The Chao1 richness estimators of *nirS* denitrifier community were 69–85 and 39–90, and 49–266 and 76–106 in waters and sediments of Dianchi Lake and Erhai Lake, respectively. The Shannon diversity indices were 1.24–1.57 and 1.26–2.38, and 2.2–4.24 and 2–2.79 in waters and sediments of Dianchi Lake and Erhai Lake, respectively. The evident vertical variations of *nirS* OTU number, Chao1 richness, and Shannon diversity were observed in either lake, both in waters and sediments. In Dianchi Lake, surface water sample had lower *nirS* richness and diversity than bottom water, while in Erhai Lake, surface water sample had much higher *nirS* richness and diversity than middle-layer water and bottom water. In either Dianchi Lake or Erhai Lake, *nirS* richness and diversity tended to increase with increasing sediment layer depth. In addition, Erhai Lake waters displayed higher *nirS* diversity than Dianchi Lake waters. At a given sediment layer depth, Dianchi Lake generally had lower *nirS* richness and diversity than Erhai Lake.

In this study, the number of normalized *nosZ* gene sequences for each lake sample was 27,936. High Good's coverage (≥99%) indicated that most of *nosZ* OTUs in each lake sample were captured. Dianchi Lake waters contained 87 or 93 *nosZ* OTUs, while Erhai Lake waters comprised 69–108 *nosZ* OTUs. Dianchi Lake and Erhai Lake sediments included 39–146 and 17–281 *nosZ* OTUs, respectively. The Chao1 richness estimators of *nosZ* denitrifier community were 96–

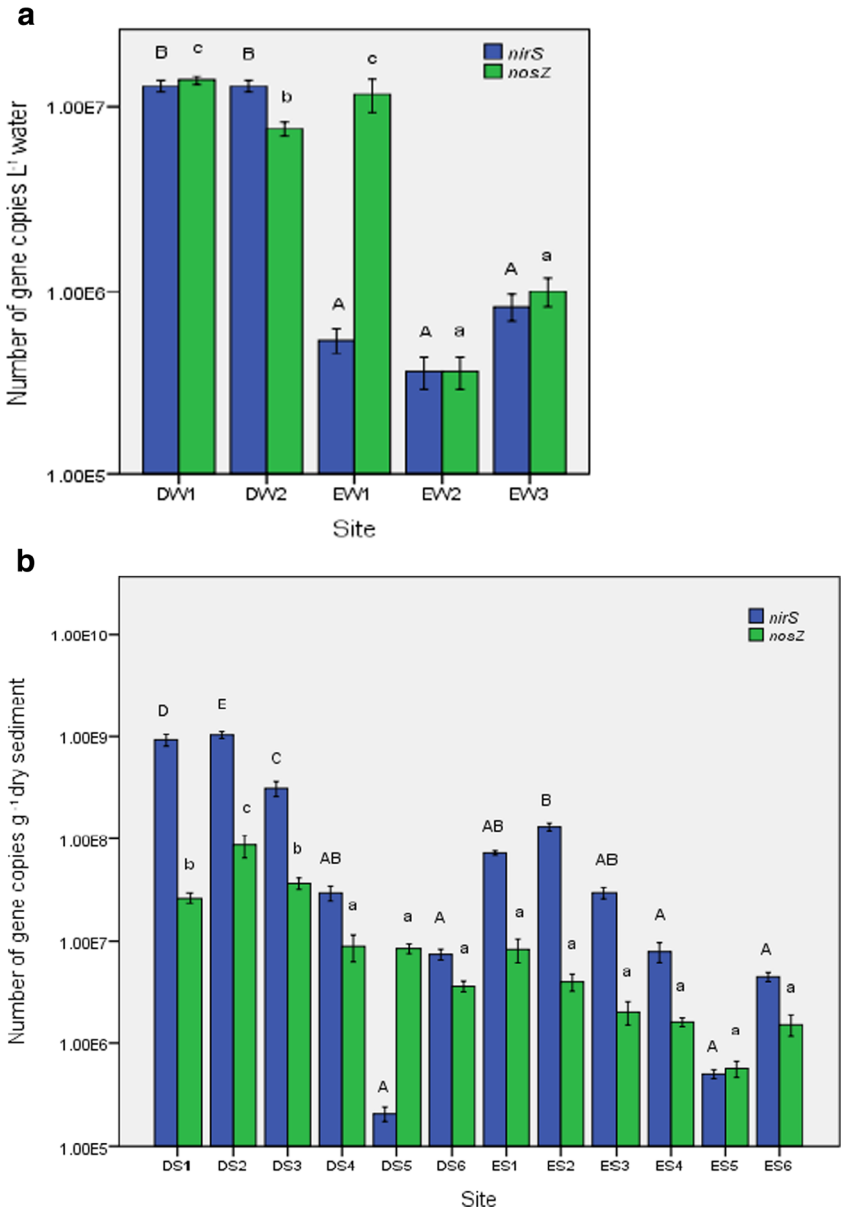

Fig. 1 Abundance of *nirS* gene and *nosZ* gene in water samples (**a**) and sediment samples (**b**) from different depths in Dianchi Lake and Erhai Lake. Different *letters above* the columns indicate significant differences in gene abundance ($P < 0.05$)

102 and 37–153, and 76–105 and 35–277 in waters and sediments of Dianchi Lake and Erhai Lake, respectively. The Shannon diversity indices were 0.98–1.56 and 0.37–2.1, and 1.95–2.49 and 0.1–2.71 in waters and sediments of Dianchi Lake and Erhai Lake, respectively. The evident vertical variations of *nosZ* OTU number, Chao1 richness, and Shannon diversity also occurred in either lake, both in waters and sediments. In Dianchi Lake, surface water sample had lower *nosZ* richness but higher diversity than bottom water, while in Erhai Lake, surface water sample had both lower richness and diversity than middle-layer water and bottom water. In either Dianchi Lake or Erhai Lake, *nosZ* denitrifier community richness tended to increase with increasing sediment layer depth; however, the trend for the vertical change of *nosZ* denitrifier community diversity was not clear.

UPGMA clustering analysis of denitrifier communities

The result of UPGMA clustering illustrated that, based on *nirS* gene, lake sediment samples were distantly separated from water samples (Fig. 2a), suggesting the distinct *nirS* denitrifier community structure difference between in water columns and sediments. Erhai Lake water sample EW1 alone formed a clade, while other water samples were grouped together. Moreover, Dianchi Lake sediments tended to be separated from Erhai Lake ones, suggesting these two lakes differed in sediment *nirS* denitrifier community structure.

The result of UPGMA clustering indicated that, based on *nosZ* gene, lake sediment samples were also clearly separated from water samples (Fig. 2b), suggesting the distinct *nosZ* denitrifier community structure difference between in water

Table 1 Denitrifier community OTUs, Chao1 estimator and Shannon index

Sample	*nirS*			*nosZ*		
	OTU	Chao1	Shannon	OTU	Chao1	Shannon
DW1	66	69	1.24	93	96	1.56
DW2	73	85	1.57	87	102	0.98
DS1	54	50	1.26	50	37	0.37
DS2	46	39	1.33	46	43	0.85
DS3	58	64	1.43	39	78	0.6
DS4	82	87	2.27	104	113	2.1
DS5	72	68	1.88	61	54	0.97
DS6	90	90	2.38	146	153	2.01
EW1	267	266	4.24	69	76	1.95
EW2	49	49	2.34	108	105	2.49
EW3	53	53	2.2	83	84	2.26
ES1	81	83	2	17	35	0.1
ES2	78	76	2.03	84	96	1.69
ES3	93	89	2.34	146	131	1.69
ES4	89	94	2.25	160	171	1.1
ES5	92	93	2.53	281	277	2.71
ES6	100	106	2.79	88	80	1.72

columns and sediments. For lake water samples, Dianchi Lake water samples DW1 and DW2 were clustered together, while Erhai Lake water samples EW1, EW2, and EW3 formed another group. Moreover, Dianchi Lake sediments also tended to be separated from Erhai Lake ones. Sample ES1 alone formed a clade and it was distantly separated from other Erhai Lake sediment samples. In addition, samples DS4 and DS6 were clearly separated from other Dianchi Lake sediment samples.

Phylogenetic analysis of denitrifier communities

In this study, the major OTUs (with the relative abundance of ≥5% in at least one lake sample) were used for further phylogenetic analysis. Lake *nirS* denitrifier communities could be divided into five distinct clusters (Fig. 3). Cluster 1 was the largest *nirS* group in both waters and sediments, and they accounted for a larger proportion in water samples (EW1 50%, EW2 53%, EW3 82%, DW1 78%, and DW2 55%) than sediment ones (ES1–ES6 9–25% and DS4–DS6 10–42%). The *nirS* sequences in this cluster were related (with ≥90% similarity) to those from epithilitic biofilm, tributary and lake sediments, rice paddy soil, river, and wastewater bioreactor, and could also be related to the sequence from *Ideonella* sp. NC3L-43b. Cluster 4 was the second largest *nirS* group. The *nirS* sequences affiliated with this cluster displayed a greater proportion in sediments DS1, DS4, DS5, DS6, ES1, ES3, ES4, ES5, and ES6 (30–71%) than in other sediments (22–24%). In addition, they comprised a greater proportion in

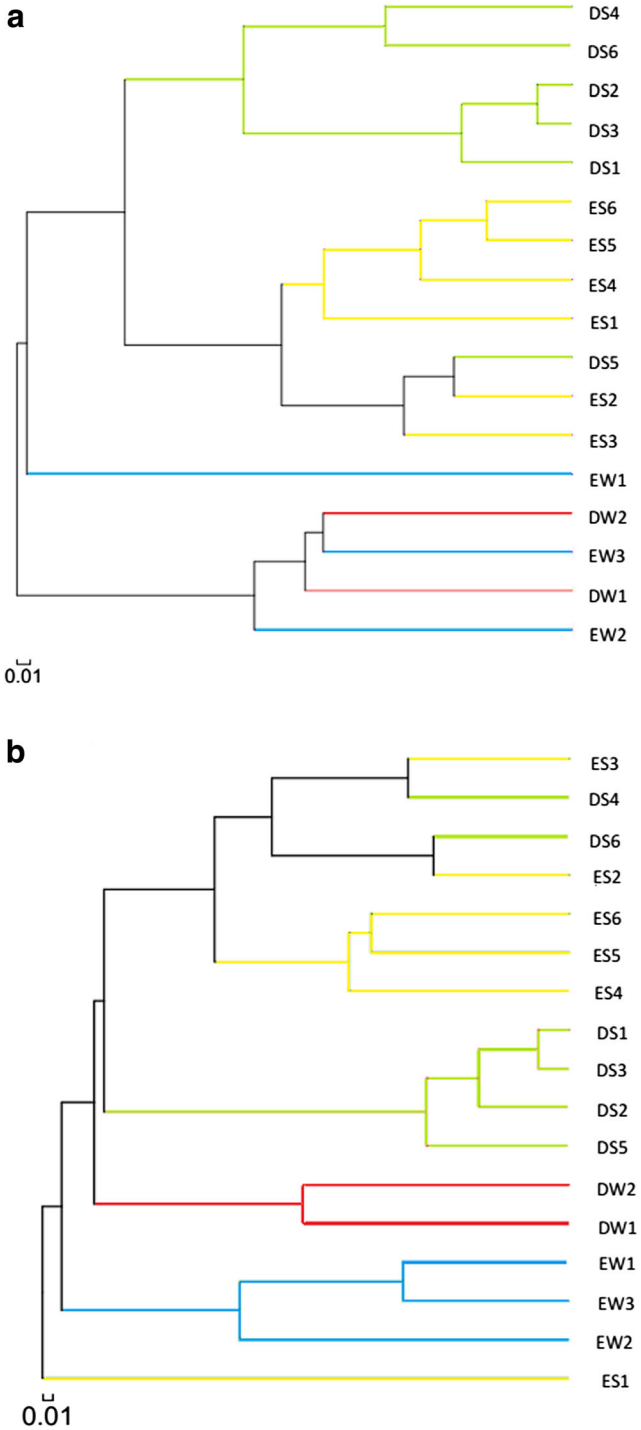

Fig. 2 UPGMA clustering of water and sediment samples from Dianchi Lake and Erhai Lake based on *nirS* (**a**) and *nosZ* genes (**b**)

Dianchi Lake water sample DW2 (39%) than Erhai Lake water samples EW2 (20%) and EW3 (9%). The sequences in cluster 4 were related with ≥90% similarity to the *nirS* gene sequences from a variety of ecosystems, such as Zoige plateau soil, wetland, intertidal marsh, tributary sediment, freshwater lake sediment, and wastewater treatment plant. Cluster 3 was the third largest *nirS* group. The sequences in this group could

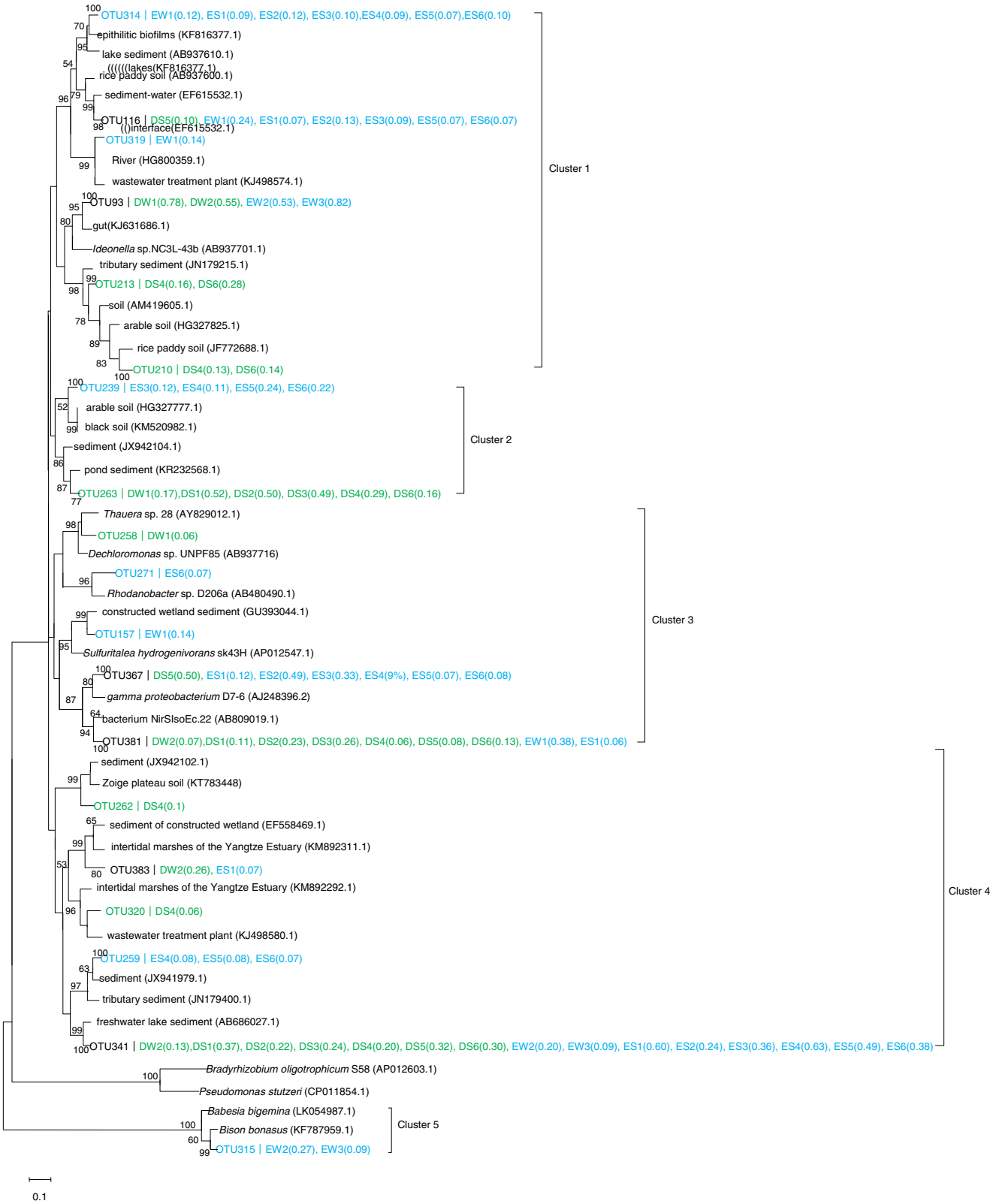

Fig. 3 Phylogenetic tree of the representative *nirS* sequences and their reference sequences from GenBank. The *number* in parentheses represents the ratio of the sequences in a given OTU to the total sequence number in a given lake sample. *Numbers* at the nodes indicate the levels of bootstrap support based on a maximum likelihood analysis of 1000 resampled datasets. The values less than 50 are not listed. The *bar* represents 10% sequence divergence

be related to ≥90% similarity to those from *Thauera* sp. 28, *Rhodanobacter* sp. D206a, *Dechloromonas* sp. UNPF85, and *Sulfuritalea hydrogenivorans* sk43H. They were observed in water samples DW1 (6%), DW2 (7%), and EW1 (52%), and sediment samples DS1 (11%), DS2 (23%), DS3 (26%), DS4 (6%), DS5 (58%), DS6 (13%), ES1 (18%), ES2 (49%), ES3 (33%), ES4 (9%), ES5 (7%), and ES6 (15%). Moreover, clusters 2 and 5 were the minor *nirS* groups. Cluster 2 was mainly composed of the sequences from sediment samples, and they showed ≥90% similarity to the *nirS* sequences from a variety of soil and sediment ecosystems. In Dianchi Lake, they illustrated a larger proportion in sediments DS1 (52%), DS2 (50%), and DS3 (49%) than in sediments DS4 (29%) and DS6 (16%). In Erhai Lake, *nirS* sequences from sediments ES3 (12%), ES4 (11%), ES5 (24%), and ES6 (22%) were also found in this cluster. In addition, cluster 5 only included the *nirS* sequences from Erhai Lake water samples EW2 (27%) and EW3 (9%) that could be related to *Babesia bigemina* and *Bison bonasus*.

Lake *nosZ* denitrifier communities could be grouped into four clusters (Fig. 4). The *nosZ* sequences from Dianchi Lake were distributed in all clusters, while those from Erhai Lake were mainly distributed in clusters a, b, and d. Cluster a was the largest *nosZ* group in both waters and sediments. Cluster a-related *nosZ* sequences displayed a greater proportion in Dianchi Lake waters (DW1 and DW2 90–100%) than Erhai Lake ones (EW1–EW3 22–60%). A high proportion of cluster a-related *nosZ* sequences was found in Dianchi Lake sediments (DS1–DS6 56–100%) and Erhai Lake sediments (ES1100%, ES2 61%, and ES3 80%). The *nosZ* sequences in cluster a were related with ≥90% similarity to those from a number of known species, such as *Niveispirillum irakense*, *Herbaspirillum* sp. TSO26–2, *Massilia* sp. TSO8, *Azospirillum largimobile*, *Thiobacillus denitrificans* ATCC 25259, *Burkholderia pseudomallei*, *Pseudogulbenkiania* sp. NH33B, *Bradyrhizobium oligotrophicum* S58, and *Alicycliphilus denitrificans* BC. Cluster d was the second largest *nosZ* group. It contained a greater proportion of *nosZ* sequences in water samples EW1 (53%), EW2 (59%), and EW3 (41%) than sediment samples (DS1–DS4 5–17%; ES3, ES5, and ES6 7–25%). These *nosZ* sequences were related with ≥95% similarity to those from *Aeromonas hydrophila* NJ-35, *A. hydrophila* NJ-35, *Haliangium ochraceum* DSM 14365, and *Stenotrophomonas maltophilia* K279a. Moreover, clusters b and c were the minor *nosZ* groups. Cluster b was only consisted of the *nosZ* sequences from lake sediment samples. They were related with ≥95% similarity to that from *Alicycliphilus denitrificans* K601. Cluster b-related *nosZ* sequences showed a greater proportion in sediment samples ES4 (100%), ES5 (77%), and ES6 (77%) than in other sediment samples (0–39%). Cluster c only included the *nosZ* sequences from a Dianchi Lake sediment sample (DS4 7%), and they had ≥90% similarity to the sequences from many known species, such as *Desulfarculus baarsii* DSM 2075, *Bordetella hinzii*, *Myxococcus fulvus* HW-1, A. *hydrophila* NJ-35, and *Bordetella parapertussis*.

Discussion

Denitrifier abundance in freshwater lake

Denitrification usually occurs in anaerobic condition. However, microorganisms from genera *Thiosphaera*, *Pesudomonas*, and *Alcaligenes* have been characterized as aerobic denitrifiers (Ozeki et al. 2001; Su et al. 2001). Aerobic denitrification process can exist in many aquatic systems (Huang et al. 2012; Kim et al. 2011; Patureau et al. 2000; Robertson and Kuenen 1984; Zheng et al. 2011). Moreover, the abundance of *nirS* denitrifiers can change with water depth (Kim et al. 2011). In this study, qPCR was applied to estimate the denitrifier abundance in waters and sediments of freshwater Dianchi Lake and Erhai Lake. The abundance of both *nirS* and *nosZ* genes was found in aerobic waters in Dianchi Lake and Erai Lake. The density of water *nirS* denitrifiers did not illustrate an evident vertical variation in either Dianchi Lake or Erhai Lake. However, in Dianchi Lake, water *nosZ* gene density in surface sample DW1 was greater than that in bottom sample DW2, and in Erhai Lake, *nosZ* denitrifiers in surface water (sample EW1) outnumbered those in middle-layer and bottom waters (samples EW2 and EW3). To the authors' knowledge, this was the first study reporting the vertical change of *nosZ* denitrifier abundance in lake water. Moreover, Dianchi Lake waters had relatively greater *nirS* gene abundance than Erhai Lake waters, which suggested that water *nirS* gene abundance might be influenced by lake tropic status.

So far, information on the sediment layer depth-related of denitrifier abundance in freshwater lake ecosystems is still lacking. In Dianchi Lake, *nirS* and *nosZ* gene abundance in sediments DS1, DS2, and DS3 were much greater than those in lower-layer sediments, while the vertical change of sediment denitrifier abundance in Erhai Lake was not evident. Therefore, the vertical change of sediment denitrifier abundance might be lake-specific. Moreover, in the upper three sediment layers (0–5 cm, 5–8 cm, and 8–11 cm), Dianchi Lake displayed much greater *nirS* and *nosZ* gene density than Erhai lake, suggesting that sediment *nirS* and *nosZ* gene abundance might be influenced by lake tropic status.

Denitrifier richness and diversity in freshwater lake

So far, several previous studies have investigated the spatial change of denitrifier community richness and diversity in aquatic systems (Christopher et al. 2013; Zhang and Li 2012), while little is known about the denitrifier richness and diversity in water column of freshwater lake. Junier et al.

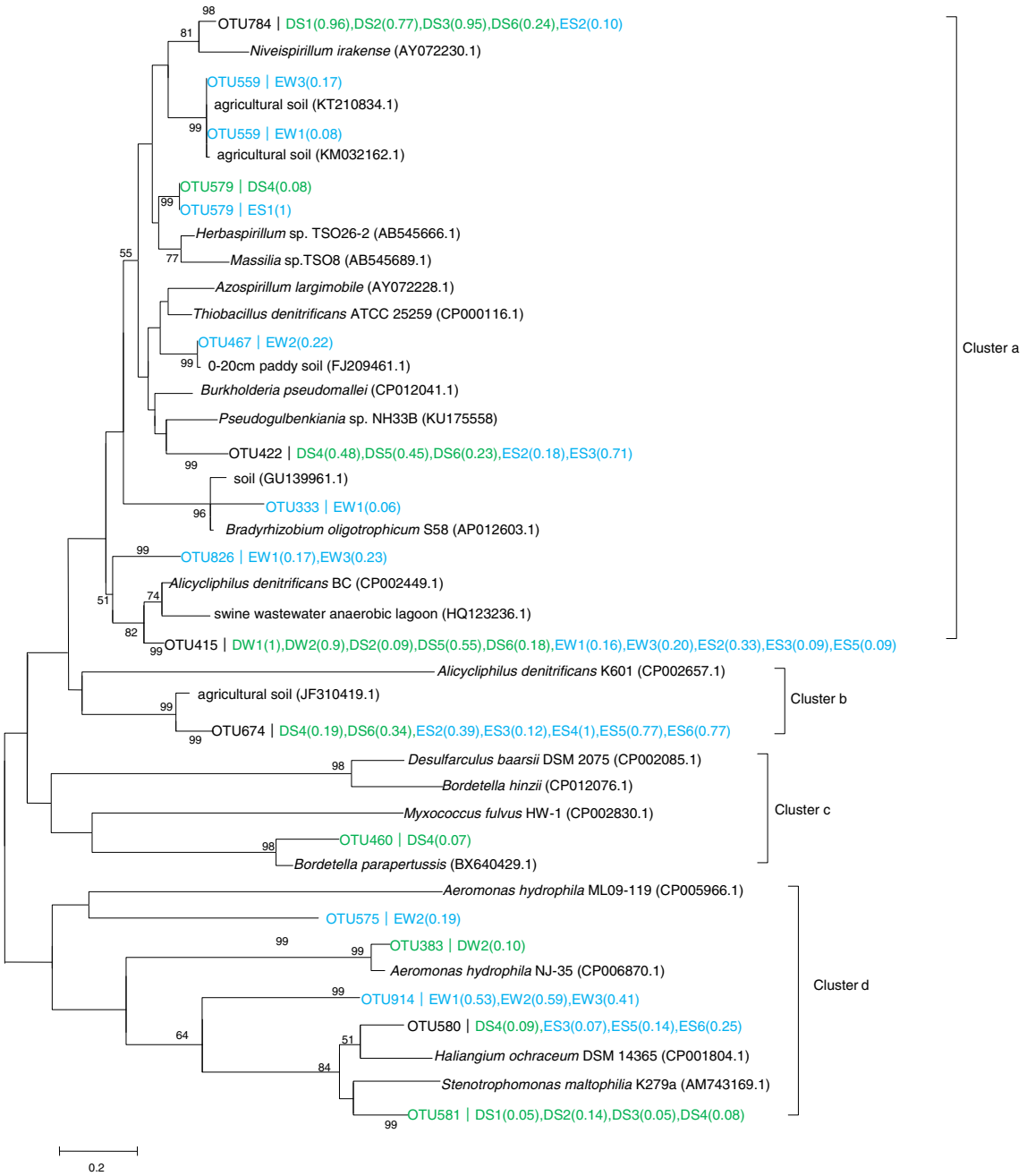

Fig. 4 Phylogenetic tree of the representative *nosZ* sequences and their reference sequences from GenBank. The *number* in parentheses represents the ratio of the sequences in a given OTU to the total sequence number in a given lake sample. *Numbers* at the nodes indicate the levels of bootstrap support based on a maximum likelihood analysis of 1000 resampled datasets. The values less than 50 are not listed. The *bar* represents 20% sequence divergence

(2008) found that *nirS* denitrifier diversity shifted in stratified water columns of Lake Kinneret. In Dianchi Lake, surface water had lower *nirS and nosZ* gene richness than bottom water, while in Erhai Lake, surface water had much higher *nirS* but lower *nosZ* richness and diversity than middle-layer and bottom waters. Hence, water denitrifier community richness and diversity in Dianchi Lake and Erhai Lake changed with water depth, but the vertical change of *nirS* denitrifier richness and diversity might be lake-specific. In addition, the present study provided the first evidence that the diversity of *nirS*- and *nosZ* denitrifiers differed in freshwater lake.

The evident spatial heterogeneity of sediment denitrifier community richness and diversity has been documented (Antti et al. 2011; Catarina et al. 2011). These previous studies focused on the horizontal variation, yet the change of denitrifier richness and diversity with sediment layer depth in freshwater ecosystems has not been addressed. In the present study, the result of Illumina MiSeq high-throughput sequencing analysis

indicated that *nirS* and *nosZ* richness tended to increase with increasing sediment layer depth in both Dianchi Lake and Erhai Lake. *nirS* diversity also tended to rise with increasing sediment layer depth; however, the trend for the vertical change of sediment *nosZ* diversity was not clear. These results suggested that the vertical change of lake sediment denitrifier diversity could depend on denitrifying functional gene.

Information on the comparison of denitrifier richness and diversity in water columns and sediments is still very limited. A previous study showed that *nirS* denitrifiers in sediments were more diverse than in waters in freshwater lake (Kim et al. 2011). In contrast, in both Dianchi Lake and Erhai Lake, denitrifier richness and diversity illustrated no evident difference between in sediments and in water columns.

Denitrifier community structure in freshwater lake

Limited information existed on the community structures of denitrifiers in either freshwater lake sediment or water column (Antti et al. 2013; Wang et al. 2013). In this study, in either Dianchi Lake or Erhai Lake, water columns and sediments differed sharply in both *nirS*- and *nosZ* denitrifier community structure. Our previous studies also indicated the distinct structure difference of both bacterial and archaeal communities between lake water columns and sediments (Dai et al. 2016; Yang et al. 2016a). Therefore, habitats may select microbial community structure. Moreover, Antti et al. (2013) reported that denitrifier community structure varied among different freshwater lakes. In the present study, the studied two freshwater lakes also differed in both sediment *nirS*- and *nosZ* denitrifier community structure. The difference of water *nosZ* denitrifier community structure could be also observed between in Dianchi Lake and Erhai Lake. Hence, lake trophic status might shape denitrifier community structure. Our previous studies also illustrated the structure difference of both bacterial and archaeal communities between these two lakes (Dai et al. 2016; Yang et al. 2016a).

In conclusion, the vertical changes of denitrifier community abundance, richness, diversity, and structure occurred in waters and sediments of either Dianchi Lake or Erhai Lake. Dianchi Lake waters had larger *nirS* and sediment *nirS* and *nosZ* abundance than Erhai Lake waters. *nirS* richness and diversity tended to increase with increasing sediment layer depth. Dianchi Lake and Erhai Lake showed distinctly different denitrifier community structure in both water column and sediment. Moreover, water column and sediment also differed sharply in denitrifier community structure.

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Conflict of interest The authors declare that they have no competing interests.

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