Ann Microbiol (2012) 62:715–723
DOI 10.1007/s13213-011-0311-9

ORIGINAL ARTICLE

Bacterial diversity in the polluted water of the Dianchi Lakeshore in China

Donghui Wen · Yaohui Bai · Qing Shi · Zongxun Li · Qinghua Sun · Renhua Sun · Chuanping Feng · Xiaoyan Tang

Received: 28 January 2011 / Accepted: 27 June 2011 / Published online: 17 July 2011

Abstract Dianchi Lake is a typical Chinese eutrophic lake. The bacterial community in the polluted water of the Dianchi lakeshore was investigated by cultivation-independent approaches. The amplicon length heterogeneity polymerase-chain reaction (LH-PCR) was used to detect the major differences in bacterial structure among the nine different sampling sites. Cluster analysis shows that the bacterial communities in water blooming sites were similar. Three genes were employed to characterize bacteria in the two parts of Dianchi Lake. The 16S rRNA gene was used to analyze the total bacterial community, the ammonia monooxygenase (*amoA*) gene for detecting ammonia-oxidizing bacteria, and the nitrous oxide reductase (*nosZ*) gene for identifying denitrifying bacteria. The clone library results demonstrate that Proteobacteria, Bacteroidetes, and Cyanobacteria were the dominant bacteria in both parts of the lake. The communities of ammonia-oxidizing bacteria were very different in the two parts of the lake and belonged to *Nitrospira* in the north and *Nitrosomonas* in the south part, respectively. Denitrifying bacteria in the Dianchi lakeshore were related to several cultured denitrifiers such as *Pseudomonas*, *Paracoccus*, *Achromobacter*, and *Rubrivivax*.

Keywords Dianchi Lake · Bacterial diversity · Ammonia-oxidizing bacteria · Denitrifying bacteria

Electronic supplementary material The online version of this article (doi:10.1007/s13213-011-0311-9) contains supplementary material, which is available to authorized users.

D. Wen (✉) · Y. Bai (✉) · Q. Sun · R. Sun · X. Tang
College of Environmental Sciences and Engineering,
The Key Laboratory of Water and Sediment Sciences
(Ministry of Education), Peking University,
Beijing 100871, People's Republic of China
e-mail: dhwen@pku.edu.cn
e-mail: baiyh@pku.edu.cn

Q. Shi · C. Feng
School of Water Resource and Environmental Science,
China University of Geosciences (Beijing),
Beijing 100083, People's Republic of China

Z. Li
Kunming Institute of Environmental Science,
Kunming, Yunnan Province 650032, People's Republic of China

Introduction

Dianchi Lake (24°40′–25°02′N, 102°37′–102°48′E), located in Kunming, Yunnan Province of China, is a typical severely eutrophic lake. The watershed area is 2,920 km^2 and the average depth is 5.3 m. A dam divides the Dianchi Lake into two parts: Caohai, the northern part, with a water surface area of 10.8 km^2; and Waihai, the southern part, with a water surface area of 298.2 km^2. Eutrophication of the lake is caused mainly by human activities. Since the 1980s, a large amount of pollutants, mainly from industrial and domestic wastewater, runoff, and rural non-point source pollution, have been discharged into Dianchi Lake. The water quality has undergone a rapid decrease to far below the national standards for surface water. In recent years, the Chinese government has invested substantial financial and material resources in the remediation of Dianchi Lake with a number of measures, such as control of pollution sources, dredging of lake sediment, and ecological restoration. Since then, water quality has improved considerably. However, eutrophication is still a serious problem and represents a major challenge for the local government.

In order to understand the role of bacteria in lake ecosystems, a knowledge of the bacterial comminuty structure is essential (Baik et al. 2008). Studies on the variations in bacterial community at different times and locations may

provide an understanding of the effects of environmental variables or human activity on the bacterial diversity, composition, and species richness in water or soil (Lin et al. 2010). In recent decades, culture-independent molecular approaches based on 16S rRNA sequence have been used widely for studies of eutrophic lakes (Kolmonen et al. 2004; Muylaert et al. 2002; Tamaki et al. 2005; Tang et al. 2010). Previous studies have shown that cyanobacterial blooms are common in eutrophic freshwater and have caused deterioration of water quality (Bouvy et al. 1999; Dillon et al. 2009; Fabbro and Duivenvoorden 1996). Moreover, heterotrophic bacterial degradation of organic carbon synthesized during blooms of cyanobacteria may also deplete the dissolved oxygen to a level that is harmful to many organisms (Eiler and Bertilsson 2004). Therefore, the composition of the aquatic bacteria community may be used as an indicator for the level of eutrophication of the water body. Nitrogen is one of the primary resources for plant, alga, and microbe biomass production. Increased nutrient input can markedly alter aquatic ecosystems (Bernot et al. 2009). Studies on nitrification and denitrification are also important for exploring the mechanisms of eutrophication and greenhouse gas discharge (Altabet et al. 2002; Conley et al. 2009; Sakano et al. 2002).

To investigate whether sample location and associated water quality affect the composition and diversity of bacterial communities at the shores of eutrophic lakes, we collected water samples from nine different sites around the Dianchi Lake ecosystem. We employed amplicon length heterogeneity polymerase-chain reaction (LH-PCR) to analyze all samples, and 16S rRNA gene clone libraries to two typical samples to elucidate bacterial community diversity and composition. Furthermore, to investigate the composition of ammonia-oxidizing bacteria (AOB) and denitrifying bacteria in the Dianchi lakeshore, we constructed clone libraries of *amoA* (ammonia monooxygenase) and *nosZ* gene (nitrous oxide reductase)—two genes often used as functional markers for nitrification and denitrification (Purkhold et al. 2000; Rösch et al. 2002; Rich et al. 2003; Rotthauwe et al. 1997).

Materials and methods

Study sites and water sample collection

Nine sampling sites were selected around the Dianchi Lake (Fig. 1). All sites are located on the lakeshore and represent the most contaminated areas. The samples were collected on 12 and 13 January 2010. The temperature was 20°C. Surface water samples (approximately 2–10 cm from the surface) were taken in plastic bottles and transported immediately to the laboratory.

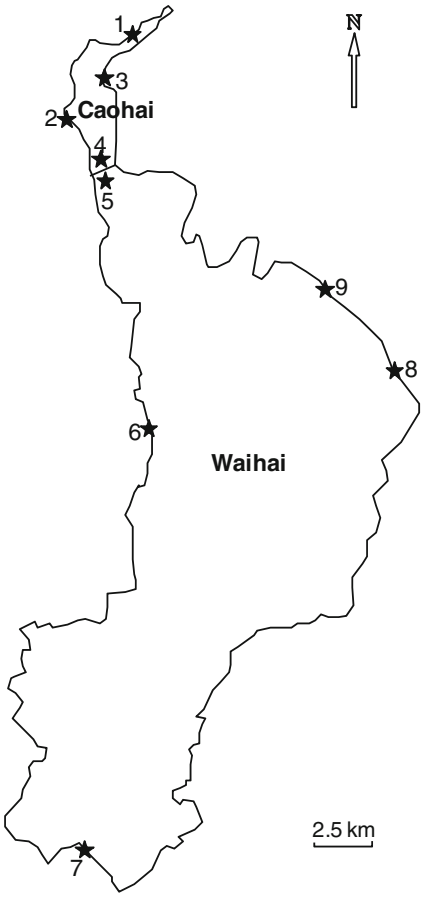


Fig. 1 Plan view of the Dianchi lakeshore sampling sites, Yunnan province, China. The *bold line* represents the dam that separates Caohai and Waihai. Site locations: *1* 25°00′53.3″N, 102°39′10.0″E; *2* 24°58′51.8″N, 102°37′51.2″E; *3* 24°59′43.8″N, 102°38′57.5″E; *4*, *5* 24°57′43.1″N, 102°38′42.0″E; *6* 24°50′29.6″N, 102°39′49.8″E; *7* 24°41′06.8″N, 102°38′05.6″E; *8* 24°52′33.0″N, 102°46′36.2″E; *9* 24°54′25.7″N, 102°45′01.1″E

Water quality analysis

Total organic carbon (TOC), pH and concentrations of NH_3-N, NO_2^--N, and NO_3^--N, were measured. The pH was measured by a pH meter (Model 868, Thermo Orion, Waltham,MA). TOC was measured by a TOC analyzer (TOC-vCPH, Shimadzu, Tokyo, Japan). The concentrations of NH_3-N, NO_2^--N, and NO_3^--N were measured using standard methods with a UV-VIS spectrometer (Shimadzu UV2401), i.e., salicylate-hypochlorous acid, N-1-naphthyl-ethylenediamine, and UV-spectrophotometric determination, respectively (State Environmental Protection Administration of China 1989).

Total DNA extraction from water samples

Water samples (300 ml) were filtered through Millipore membrane filters (pore size, 0.22 μm) using sterile syringes. Membranes were cut into pieces with sterile scissors and used

Table 1 Water properties of sampling sites on the Dianchi lakeshore. The data represent the mean of two independent replicate measurements. *TOC* Total organic carbon

Indicator	Site #								
	1	2	3	4	5	6	7	8	9
pH	7.64	7.67	7.64	7.79	7.97	7.97	8.04	8.06	8.05
TOC	8.93	12.92	11.72	14.26	18.74	18.42	20.40	10.37	16.76
NH_3-N	3.19	0.20	10.47	0.03	0.52	0.04	0.15	0.22	0.38
NO_2^--N	0.043	0.057	0.002	0.014	0.001	0.003	0.006	0.006	0.009
NO_3^--N	10.08	5.40	2.66	0.16	1.67	0.36	0.08	1.27	0.52

immediately for DNA extraction. DNA extraction was performed using a 3S spin DNA isolation kit for environmental samples K717 (Shanghai Biocolor Bioscience & Technology Company, Shanghai, China). The extracted DNA was stored in TE buffer in a −20°C freezer.

Length heterogeneity polymerase-chain reaction

LH-PCR was performed to detect the major differences in bacterial communities among samples from the nine sites. The 16S rRNA gene from the extracted total DNA was amplified with the universal primers 27 F (5′-AGAGTTTG ATCCTGGCTCAG-3′) and 338R (5′-GCTGCCTCC CGTAGGAGT-3′) (Ritchie et al. 2000). The 27 F primer was labeled with 6-carboxyfluorescein at the 5′ end. PCR amplification was performed in a thermocycler (Takara Bio, Otsu, Japan) with the program described by Ritchie et al. (2000). PCR products were purified using a QIAquick PCR purification kit (Qiagen, Hilden, Germany) and were sent to the SinoGenoMax (Beijing, China) for GeneScan analysis.

LH-PCR electropherograms were analyzed using the Genemapper 3.7 software. Signals with a peak height below 100 in relative fluorescence units were regarded as background noise and excluded from the analysis. LH-PCR for each sample was performed in triplicate.

LH-PCR data analysis

Relative area ratios for LH-PCR were calculated by dividing each individual peak area by the total peak area of each electropherogram. The peaks with a relative area ratio ≥1% were selected for further analysis (Lueders and Friedrich 2003). Three parameters were used to compare LH-PCR fingerprint patterns: the richness (S), (total number of peaks detected in a sample); the Shannon–Weaver's diversity index (H), (equal to $-\sum p_i \ln p_i$, where p_i is the relative area ratio detected in a sample), and the evenness (E), (equal to H/ln S) (Shannon and Weaver 1963). In addition, the profiles of LH-PCR were aligned and a binary matrix was created, where the presence or absence of peak was scored as 1 or 0. This binary matrix was used to compute the community similarity values among the nine sites based on the simple matching (SM) coefficient of similarity. The matrix of SM similarity was then used for unweighted pair group method with arithmetic mean (UPGMA) clustering, and the results were plotted with the software NTSYS-2.10e.

Clone library analysis

PCR amplification of the 16S rRNA gene (27 F and 1492R; Bai et al. 2008) from the total DNA of samples from sites #1 and #5 sites was performed for bacteria detection. In addition, PCR for the *amoA* gene of nitrifiers and the *nosZ* gene of denitrifiers from the total DNA of both these latter sites was performed as described in previous studies (Rösch et al. 2002; Rotthauwe et al. 1997).

All PCR products were purified with the Qiaquick gel extraction kit and cloned into pGEM-T Easy vectors (Promega, Madison, WI). The recombinant plasmids were transformed into competent *E. coli* JM109 cells. Fifty-five clones for 16S rRNA gene and 30–50 clones for *amoA* and *nosZ* genes from sites #1 and #5 were randomly selected and sequenced with vector-specific primers M13F and M13R using an ABI 3730*xl* DNA Analyzer (Applied Biosystems, Foster City, CA). Chimeric sequences in the 16S rRNA clone library were detected using the Mallard (Ashelford et al. 2006) programs, and excluded from further analysis.

Using the Mothur software, diversity statistics, including library coverage (Good 1953), nonparametric richness Chao 1 (Chao 1987), and Shanon-Weaver index, were calculated (Schloss et al. 2009). Operational taxonomic units (OTUs) were defined as groups where the sequence similarities were greater than 97%.

Representative 16S rRNA gene sequences from the OTUs combined from sites #1 and #5 were blasted against the published gene sequences from the Ribosomal Database Project (RDP) (Cole et al. 2009), and the *amoA* and *nosZ* sequences were blasted against the National Center for Biotechnology Information (NCBI) database. Sequence analysis was performed by the BioEdit software and phylogenetic trees were constructed using the neighbor-joining method with the software MEGA 4.0 (Tamura et al. 2007).

The representative sequences (OTUs) of 16S rRNA, *amoA*, and *nosZ* in this study have been deposited in the

Table 2 Diversity indices including richness (S), Shannon–Weaver's diversity index (H) and evenness (E) from the length heterogeneity polymerase-chain reaction (LH-PCR) analysis of water samples from the nine sites on the Dianchi lakeshore. Samples 1–4 are from Caohai and 5–9 samples are from Waihai

	Site #								
Index	1	2	3	4	5	6	7	8	9
S	13	13	14	14	13	8	16	12	18
H	2.03	1.89	2.17	1.98	2.16	1.30	2.43	1.68	2.39
E	0.80	0.74	0.82	0.75	0.84	0.63	0.88	0.68	0.83

NCBI database under accession numbers HQ324827–HQ324887.

Results

Water quality

The water characteristics of the nine different sites in the Dianchi lakeshore are listed in Table 1. The concentrations of NH_3^--N, NO_2^--N and NO_3^--N, show that the level of N pollutants in Caohai was higher than that in Waihai. This is consistent with field observations, with blooms of algae being more abundant in Caohai than in Waihai.

LH-PCR analysis

The LH-PCR technique was used to determine the major differences in bacterial communities in the nine sampling sites. Statistical analysis of the LH-PCR profiles showed that the number of LH-PCR fragments varied from 8 to 18. The 341 bp (#1 and #2), 319 bp (#3) and 318 bp (#4) fragments were the dominant groups in Caohai according to the statistical results of relative abundances of the LH-PCR fragments (Supporting information, Fig. S1), The 341 bp and 342 bp fragments were both present in the four Caohai samples. In Waihai, the 319 bp (#5), 315 bp (#6 and #7), 322 bp (#8), and 316 bp (#9) fragments were the dominant groups. The 319 bp, 341 bp, and 346 bp fragments were detected in the five Waihai samples.

As shown in Table 2, the diversity of bacterial communities in the Dianchi lakeshore as indicated by the Shannon-Weaver's index (H), ranged from 1.30 to 2.43. In comparison with Caohai, the H values in different Waihai sites were more distinct. The same pattern was also found when the evenness index was compared.

The similarity of bacterial LH-PCR profiles within the dataset was expressed in terms of an SM similarity coefficient. These coefficients were then used to generate distances in order to construct a dendrogram that depicts the similarities between different profiles of the Dianchi lakeshore samples (Fig. 2). The dendrogram showed a distinct grouping among samples obtained from Caohai and Waihai sites, except that sample #3 from Caohai was grouped with samples #5 and #8 from Waihai. The LH-PCR profile from Caohai site #4 was distinctively different from Waihai site #5, although these two sites are located very close to each other.

Clone libraries

We characterized the total, nitrifying, and denitrifying bacterial communities in two water samples (#1 and #5) of the Dianchi lakeshore using the three target genes coding for 16S rRNA, ammonia monooxygenase (*amoA*), and nitrous oxide reductase (*nosZ*).

Nearly complete bacterial 16S rRNA gene (1.5 kb) of 110 random clones from Caohai site #1 and Waihai site #5 were sequenced. All sequences were checked for chimeras, yielding 64 sequences (37 from site #1 and 27 from site #5) for further analysis.

The number of OTUs for each site as well as estimates of species coverage, richness, and diversity were calculated for the 16S rRNA, *amoA*, and *nosZ* libraries retrieved from sites #1 and #5 (Table 3). Coverages of *amoA* and *nosZ*

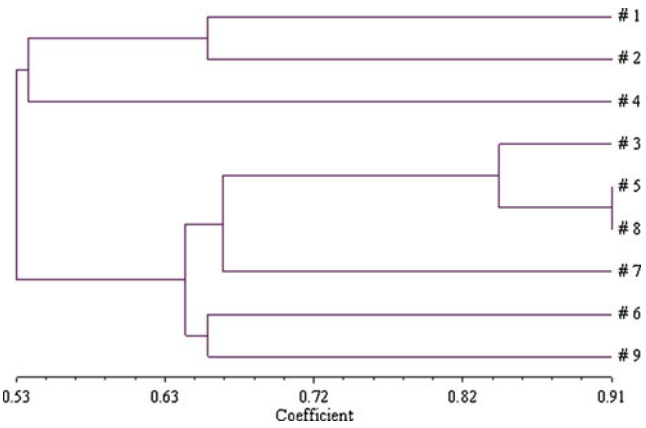


Fig. 2 Dendrogram of unweighted pair group method with arithmetic mean (UPGMA) cluster analysis based on the length heterogeneity polymerase-chain reaction (LH-PCR) profiles in the nine sites of Dianchi lakeshore

Table 3 Sequence diversity and library coverage estimations in clone libraries. *OTU* Operational taxonomic unit

Type	No. of sequences	No. of OTU (>97% identity)	Coverage %[a]	Chao 1 value	Shannon's index
#1 16S rRNA	37	24	51%	62 (36, 150)	2.95
#5 16S rRNA	27	21	37%	66 (34, 178)	2.95
#1 *amoA*	46	9	93%	10 (9, 17)	1.69
#5 *amoA*	50	4	96%	4 (4, 12)	0.36
#1 *nosZ*	16	5	69%	16 (8, 58)	1.23
#5 *nosZ*	28	6	89%	8 (6, 21)	1.03

[a] Library coverage was calculated using C=1-*n*/*N*, where *n* is the number of OTUs without a replicate and *N* is the total number of sequences. Numbers in parentheses are lower and upper 95% confidence intervals for the Chao 1 estimators. The Shannon–Weaver's index $H = -\sum p_i \ln p_i$, where $p_i = n_i/N$, n_i is the number of OTUs with *i* individuals and *N* is the total number of individuals

clone libraries in site #1 and #5 were greater than 60%, indicating that these gene sequences represented the majority of genes in all libraries. The Chao 1 values and Shannon's indices of 16S rRNA clone libraries in #1 and #5 sites were similar, but the values and indices of *amoA* and *nosZ* in site #1 were higher than those in site #5.

A phylogenetic tree was constructed based on the analysis of 16S rRNA sequences (Fig. 3). Three groups including Proteobacteria, Bacteroidetes and Cyanobacteria were detected in Caohai, and five groups including Proteobacteria, Bacteroidetes, Cyanobacteria, Verrucomicrobia, and Planctomycetes were found in Waihai. The Proteobacteria were dominant in the Caohai clone library (45.9% of the total OTUs) but were less represented in the Waihai clone library (22.2%). In both Caohai and Waihai, the Proteobacteria consisted of three classes, α-proteobacteria, β-proteobacteria and γ-proteobacteria, with the latter two classes being the major groups. One-third of the total OTUs belonged to two classes of Bacteroidete in both Caohai and Waihai, i.e., Flavobacteria and Sphingobacteria. Cyanobacteria were the predominant bacteria in Waihai clone library (37.0%), but accounted for only 18.9% of Caohai clone library. Verrucomicrobia and Planctomycetes were two minor groups in the Waihai clone library. Most of the clones were highly related to sequences previously described in the GenBank database.

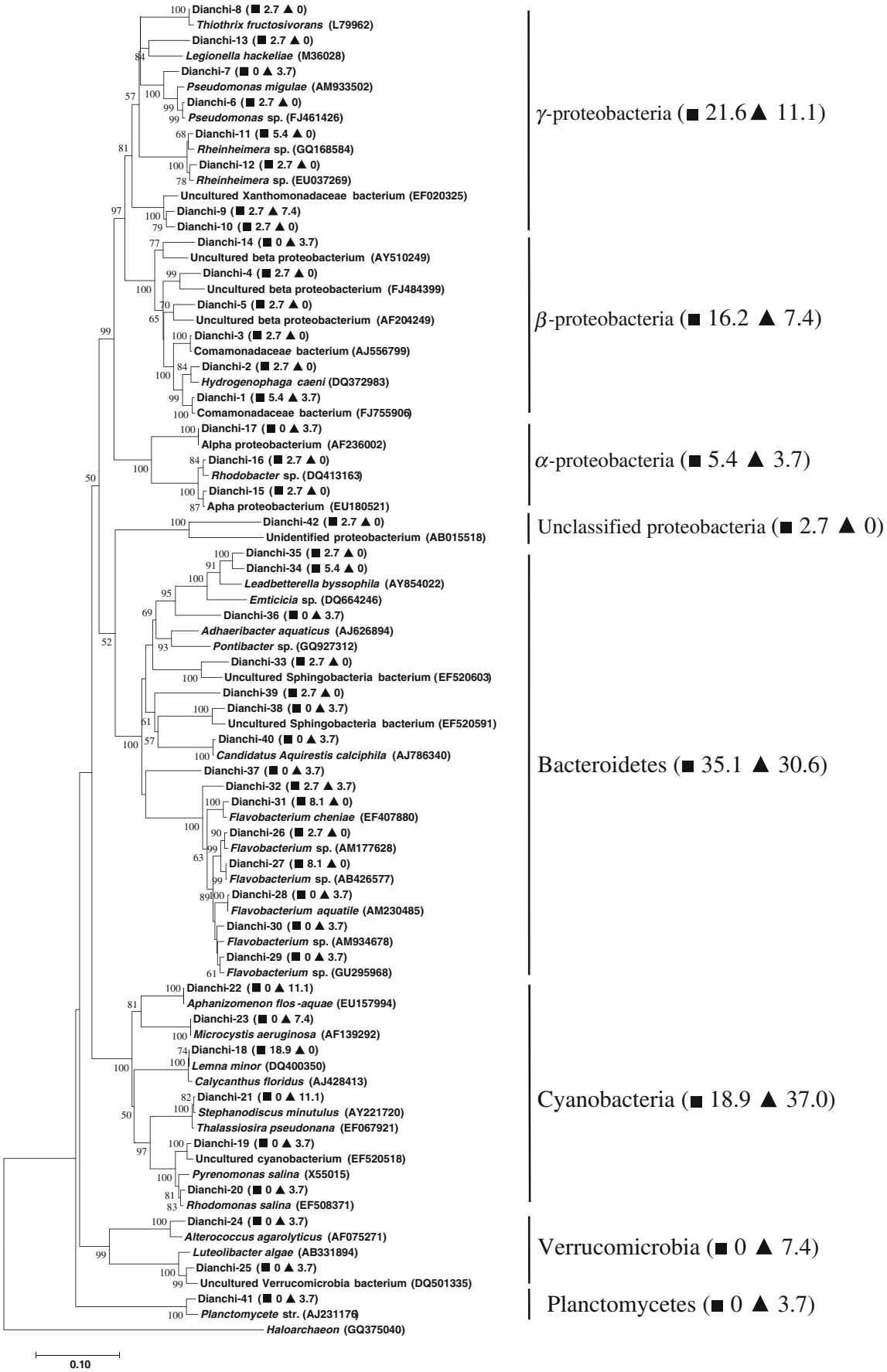
The oxidation of ammonia to nitrite by autotrophic nitrifiers is a key process in nitrogen transformation and its removal from the lake. The first crucial step, the oxidation of ammonia to hydroxylamine, is catalyzed by ammonia monooxygenase (AMO). A combined phylogenetic tree based on the partial *amoA* genes in site #1 and #5 was constructed as shown in Fig. 4. Most of the *amoA* genes shared high levels of sequence similarity (>80%) to known sequences registered in the NCBI database. The results indicated that the ammonia-oxidizing bacteria in sites #1 and #5 were different. In Caohai site #1, 69.5% of AOB were grouped in the genus of *Nitrosospira* based on *amoA* gene. Other *amoA* genes had a great similarity with the *amoA* gene of unidentified bacteria in the database. However, in Waihai site #5, all AOB were grouped in the *Nitrosomonas* lineage based on *amoA* gene.

The *nosZ* gene encoding nitrous oxide reductase is unique to denitrifying bacteria, and it has been used recently to detect denitrifier-specific DNA in environmental samples (Scala and Kerkhof 1999; Stres et al. 2004). A phylogenetic tree based on the partial *nosZ* gene in samples from the #1 and #5 sites was constructed as shown in Fig. 5. The *nosZ* sequences detected in this study were similar to those from bacterial isolates affiliated with the α, β, and γ subclass of the class Proteobacteria. In Caohai site #1, the dominant sequence was the Dianchi-N1, and most of sequences (64.3%) in the library were assigned to the β-Proteobacteria lineage. In Waihai #5, the sequence of Dianchi-N6 (71.4%) was dominant, and it was assigned to the α-Proteobacteria lineage.

Discussion

The bacterial composition of the water samples in the Dianchi lakeshore was related to water quality and blooms. Based on clustering analysis (Fig. 2) and the observation of sampling sites, #1, #2 and #4 sites were allocated to the same group of water-blooming sites. The Shannon's indices (Table 3) showed that the AOB based on *amoA* gene and denitrifying bacteria based on *nosZ* gene in site #1 encompassed more diverse phylotypes than that in site #5 because inorganic N compounds at site #1 were signifi-

Fig. 3 Neighbor-joining trees showing the phylogenetic affiliation of ▶ the operational taxonomic units (OTUs) retrieved from the samples of Caohai (#1) and Waihai (#5). The numbers on the branch nodes represent percentage of bootstrap resamplings based on 1,000 replicates (only values greater than 50% are shown). The scale bar indicates the average numbers of nucleotide substitutions per site. *Haloarchaeon* was used as the outgroup in the tree. The relative abundances of OTUs in the libraries of #1 (■) and #5 (▲) are indicated in parenthesis


Dianchi-8 (■ 2.7 ▲ 0)
Thiothrix fructosivorans (L79962)
Dianchi-13 (■ 2.7 ▲ 0)
Legionella hackeliae (M36028)
Dianchi-7 (■ 0 ▲ 3.7)
Pseudomonas migulae (AM933502)
Dianchi-6 (■ 2.7 ▲ 0)
Pseudomonas sp. (FJ461426)
Dianchi-11 (■ 5.4 ▲ 0)
Rheinheimera sp. (GQ168584)
Dianchi-12 (■ 2.7 ▲ 0)
Rheinheimera sp. (EU037269)
Uncultured Xanthomonadaceae bacterium (EF020325)
Dianchi-9 (■ 2.7 ▲ 7.4)
Dianchi-10 (■ 2.7 ▲ 0)
Dianchi-14 (■ 0 ▲ 3.7)
Uncultured beta proteobacterium (AY510249)
Dianchi-4 (■ 2.7 ▲ 0)
Uncultured beta proteobacterium (FJ484399)
Dianchi-5 (■ 2.7 ▲ 0)
Uncultured beta proteobacterium (AF204249)
Dianchi-3 (■ 2.7 ▲ 0)
Comamonadaceae bacterium (AJ556799)
Dianchi-2 (■ 2.7 ▲ 0)
Hydrogenophaga caeni (DQ372983)
Dianchi-1 (■ 5.4 ▲ 3.7)
Comamonadaceae bacterium (FJ755906)
Dianchi-17 (■ 0 ▲ 3.7)
Alpha proteobacterium (AF236002)
Dianchi-16 (■ 2.7 ▲ 0)
Rhodobacter sp. (DQ413163)
Dianchi-15 (■ 2.7 ▲ 0)
Apha proteobacterium (EU180521)
Dianchi-42 (■ 2.7 ▲ 0)
Unidentified proteobacterium (AB015518)
Dianchi-35 (■ 2.7 ▲ 0)
Dianchi-34 (■ 5.4 ▲ 0)
Leadbetterella byssophila (AY854022)
Emticicia sp. (DQ664246)
Dianchi-36 (■ 0 ▲ 3.7)
Adhaeribacter aquaticus (AJ626894)
Pontibacter sp. (GQ927312)
Dianchi-33 (■ 2.7 ▲ 0)
Uncultured Sphingobacteria bacterium (EF520603)
Dianchi-39 (■ 2.7 ▲ 0)
Dianchi-38 (■ 0 ▲ 3.7)
Uncultured Sphingobacteria bacterium (EF520591)
Dianchi-40 (■ 0 ▲ 3.7)
Candidatus Aquirestis calciphila (AJ786340)
Dianchi-37 (■ 0 ▲ 3.7)
Dianchi-32 (■ 2.7 ▲ 3.7)
Dianchi-31 (■ 8.1 ▲ 0)
Flavobacterium cheniae (EF407880)
Dianchi-26 (■ 2.7 ▲ 0)
Flavobacterium sp. (AM177628)
Dianchi-27 (■ 8.1 ▲ 0)
Flavobacterium sp. (AB426577)
Dianchi-28 (■ 0 ▲ 3.7)
Flavobacterium aquatile (AM230485)
Dianchi-30 (■ 0 ▲ 3.7)
Flavobacterium sp. (AM934678)
Dianchi-29 (■ 0 ▲ 3.7)
Flavobacterium sp. (GU295968)
Dianchi-22 (■ 0 ▲ 11.1)
Aphanizomenon flos-aquae (EU157994)
Dianchi-23 (■ 0 ▲ 7.4)
Microcystis aeruginosa (AF139292)
Dianchi-18 (■ 18.9 ▲ 0)
Lemna minor (DQ400350)
Calycanthus floridus (AJ428413)
Dianchi-21 (■ 0 ▲ 11.1)
Stephanodiscus minutulus (AY221720)
Thalassiosira pseudonana (EF067921)
Dianchi-19 (■ 0 ▲ 3.7)
Uncultured cyanobacterium (EF520518)
Pyrenomonas salina (X55015)
Dianchi-20 (■ 0 ▲ 3.7)
Rhodomonas salina (EF508371)
Dianchi-24 (■ 0 ▲ 3.7)
Alterococcus agarolyticus (AF075271)
Luteolibacter algae (AB331894)
Dianchi-25 (■ 0 ▲ 3.7)
Uncultured Verrucomicrobia bacterium (DQ501335)
Dianchi-41 (■ 0 ▲ 3.7)
Planctomycete str. (AJ231176)
Haloarchaeon (GQ375040)
0.10
γ-proteobacteria (■ 21.6 ▲ 11.1)
β-proteobacteria (■ 16.2 ▲ 7.4)
α-proteobacteria (■ 5.4 ▲ 3.7)
Unclassified proteobacteria (■ 2.7 ▲ 0)
Bacteroidetes (■ 35.1 ▲ 30.6)
Cyanobacteria (■ 18.9 ▲ 37.0)
Verrucomicrobia (■ 0 ▲ 7.4)
Planctomycetes (■ 0 ▲ 3.7)

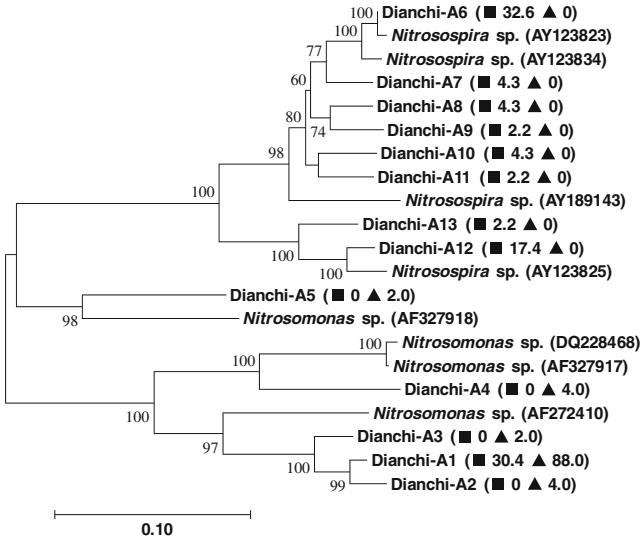


Fig. 4 Phylogenetic tree constructed for *amoA* OTU sequences (490 bp) retrieved from samples from Caohai (#1) and Waihai (#5). The numbers (only those >50% are shown) on the branch nodes indicate the percentages of bootstrap support for the clades based on 1,000 bootstrap resamplings. Numbers in the brackets are each strain's GenBank accession in the NCBI. The relative abundances of OTUs in the libraries of #1 (■) and #5 (▲) are indicated in parenthesis

cantly higher with regard to NH_3-N and NO_3^--N concentration. Based on 16S rRNA sequences in the #1 and #5 samples, most of them corresponded to aquatic bacteria, which, according to the blast results from the NCBI database, are able to degrade different types of organic substances. A number of bacterial species were also found to be plant symbionts, such as algal-bacteria. These results suggest that there was a clear relationship between the bacterial community and the eutrophication process, as was shown in a study of another Chinese lake, Taihu Lake (Liu et al. 2009). In addition, the bacterial diversity and composition between sites of #4 and #5 differed significantly (Fig. 2, Table 2), although they are separated only by a dam. This indicates that differences in the level of pollution contribute to the variation in the structure of the microbial community (Table 1).

The 16S rRNA clone libraries of sites #1 and #5 indicated that Proteobacteria, Bacteroidetes and Cyanobacteria were the most dominant phyla at these two sites. Proteobacteria and Bacteroidetes are known to be the most prominent heterotrophic microorganisms in marine (Dillon et al. 2009; Stevens et al. 2005) and lake (Martinez-Alonso et al. 2008; Ye et al. 2009) waters. Cyanobacteria are ubiquitous, ecologically important, and phylogenetically diverse components of the phytoplankton of marine and freshwater environments (Talbot et al. 2008). According to the clone library results, they are the dominant bacteria in sites #1 and #5. This is a clear indication of the inferior water quality in Dianchi lake. The massive growth of cyanobacteria, in particular, can lead to toxic outcomes and endanger human health and safety (de Figueiredo et al. 2010). Plantomycetes and Verrucomicrobia, which are also common members of aquatic or soil environments (Hugenholtz et al. 1998; Wagner and Horn 2006), were present only in the sample from site #5. These results indicates the presence of more polluted water and a less diverse bacterial population (Table 1, Fig. 3).

In order to identify the peaks from the LH-PCR profiles of the samples from nine sites, we also performed LH-PCR analysis for each OTU in the clone libraries from sites #1 and #5. The results show that several OTUs shared the same peak length (data not shown). Therefore, it was difficult to identify the genus information of each peak and community distribution from the LH-PCR profiles because one LH-PCR peak may represent more than one genus. However, the results demonstrated that the dominant peaks in the LH-PCR profiles were identical to the peaks of OTUs belonging to Proteobacteria, Bacteroidetes, or Cyanobacteria. This indicated that these three phyla might be dominant in the nine sampling sites.

The AOB form a tight cluster within the β subclass of Proteobacteria and include members of the genera *Nitrosomonas* and *Nitrosospira* (Chen et al. 2009; Teske et al. 1994). Our study found that the dominant AOB in Caohai and Waihai regions of the Dianchi lakeshore are significantly different (Fig. 4). This characteristic of the AOB

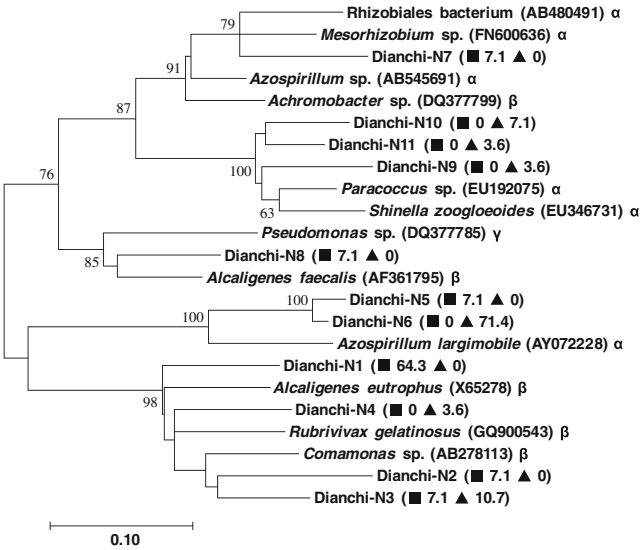


Fig. 5 Phylogenetic tree constructed for partial *nosZ* OTU sequences (700 bp) retrieved from samples from Caohai (#1) and Waihai (#5). The numbers (only those >50% are shown) on the branch nodes indicate the percentages of bootstrap support for the clades based on 1,000 bootstrap resamplings. Numbers in the brackets are each strain's GenBank accession in the NCBI. The relative abundances of OTUs in the libraries of #1 (■) and #5 (▲) are indicated in parenthesis. The phylogenetic positions of known cultures based on 16S rDNA gene are indicated by α, β, and γ for the α, β, and γ subclasses of the Proteobacteria

community is possibly caused by the substantial differences in nitrogen forms and their concentration levels between the two parts of the lake (Table 1). In addition, the heterogeneity in substrate affinity of the ammonia-oxidizing enzyme system among the dominant AOB species may also help account for differences found (Chen et al. 2009). The cloning library results of *nosZ* gene suggest that the denitrifying bacteria in Dianchi Lake are related to some cultured denitrifiers such as the *Pseudomonas*, *Paracoccus*, *Achromobacter*, and *Rubrivivax* group (Fig. 5). It is inconsistent with another study of natural environment where the *nosZ* gene was not found to be associated with any cultivable denitrifying bacteria (Scala and Kerkhof 1999). However, our result was supported by a study of denitrifying bacteria in a wastewater treatment reactor (Sakano et al. 2002).

The bacterial community structure of the polluted water in the Dianchi Lake can be used as an indicator to reveal ecological change under the developing pressure in the watershed. This microbial information about the lake environment will be beneficial in evaluating the remediation efforts made by local government in this region.

Acknowledgments This study was supported by a General Project (No. 51008003) grant from the National Natural Science Foundation of China and a National Science & Technology Mega-program on Water Pollution Control for the 11th Five-Year Plan (2009ZX07102-002-01). The authors thank Dr. Zbigniew Cichacz in the Biodesign Institute at Arizona State University for carefully checking of this paper.

References

Altabet MA, Higginson MJ, Murray DW (2002) The effect of millennial-scale changes in Arabian Sea denitrification on atmospheric CO_2. Nature 415:159–162

Ashelford KE, Chuzhanova NA, Fry JC, Jones AJ, Weightman AJ (2006) New screening software shows that most recent large 16S rRNA gene clone libraries contain chimeras. Appl Environ Microbiol 72:5734–5741

Bai YH, Sun QH, Zhao C, Wen DH, Tang XY (2008) Microbial degradation and metabolic pathway of pyridine by a *Paracoccus* sp. strain BW001. Biodegradation 19:915–926

Baik K, Park S, Kim E, Bae K, Ann JH, Ka JO, Chun J, Seong C (2008) Diversity of bacterial community in freshwater of Woopo wetland. J Microbiol 46:647–655

Bernot MJ, Bernot RJ, Morris JT (2009) Nutrient cycling relative to delta N-15 and delta C-13 natural abundance in a coastal wetland with long-term nutrient additions. Aquat Ecol 43:803–813

Bouvy M, Molica R, De Oliveira S, Marinho M, Beker B (1999) Dynamics of a toxic cyanobacterial bloom (Cylindrospermopsis raciborskii) in a shallow reservoir in the semi-arid region of northeast Brazil. Aquat Microb Ecol 20:285–297

Chao A (1987) Estimating the population size for capture recapture data with unequal catchability. Biometrics 43:783–791

Chen GY, Qiu SL, Zhou YY (2009) Diversity and abundance of ammonia-oxidizing bacteria in eutrophic and oligotrophic basins of a shallow Chinese lake (Lake Donghu). Res Microbiol 160:173–178

Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, Kulam-Syed-Mohideen AS, McGarrell DM, Marsh T, Garrity GM, Tiedje JM (2009) The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res 37:D141–D145

Conley DJ, Paerl HW, Howarth RW, Boesch DF, Seitzinger SP, Havens KE, Lancelot C, Likens GE (2009) Controlling eutrophication: nitrogen and phosphorus. Science 323:1014–1015

de Figueiredo DR, Pereira MJ, Correia A (2010) Seasonal modulation of bacterioplankton community at a temperate eutrophic shallow lake. World J Microbiol Biotechnol 26:1067–1077

Dillon JG, McMath LM, Trout AL (2009) Seasonal changes in bacterial diversity in the Salton Sea. Hydrobiologia 632:49–64

Eiler A, Bertilsson S (2004) Composition of freshwater bacterial communities associated with cyanobacterial blooms in four Swedish lakes. Environ Microbiol 6:1228–1243

Fabbro LD, Duivenvoorden LJ (1996) Profile of a bloom of the cyanobacterium Cylindrospermopsis raciborskii (Woloszynska) Seenaya and Subba Raju in the Fitzroy River in tropical central Queensland. Mar Freshwater Res 47:685–694

Good IJ (1953) The population frequencies of species and the estimation of population parameters. Biometrika 40:237–264

Hugenholtz P, Goebel BM, Pace NR (1998) Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. J Bacteriol 180:4765–4774

Kolmonen E, Sivonen K, Rapala J, Haukka K (2004) Diversity of cyanobacteria and heterotrophic bacteria in cyanobacterial blooms in Lake Joutikas, Finland. Aquat Microb Ecol 36:201–211

Lin YT, Huang YJ, Tang SL, Whitman WB, Coleman DC, Chiu CY (2010) Bacterial Community Diversity in Undisturbed Perhumid Montane Forest Soils in Taiwan. Microb Ecol 59:369–378

Liu FH, Lin GH, Gao G, Qin BQ, Zhang JS, Zhao GP, Zhou ZH, Shen JH (2009) Bacterial and archaeal assemblages in sediments of a large shallow freshwater lake, Lake Taihu, as revealed by denaturing gradient gel electrophoresis. J Appl Microbiol 106:1022–1032

Lueders T, Friedrich MW (2003) Evaluation of PCR amplification bias by terminal restriction fragment length polymorphism analysis of small-subunit rRNA and *mcrA* genes by using defined template mixtures of methanogenic pure cultures and soil DNA extracts. Appl Environ Microbiol 69:320–326

Martinez-Alonso M, Mendez-Alvarez S, Ramirez-Moreno S, Gonzalez-Toril E, Amils R, Gaju N (2008) Spatial heterogeneity of bacterial populations in monomictic Lake Estanya (Huesca, Spain). Microb Ecol 55:737–750

Muylaert K, Van der Gucht K, Vloemans N, De Meester L, Gillis M, Vyverman W (2002) Relationship between bacterial community composition and bottom-up versus top-down variables in four eutrophic shallow lakes. Appl Environ Microbiol 68:4740–4750

Purkhold U, Pommerening-Roser A, Juretschko S, Schmid MC, Koops HP, Wagner M (2000) Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and *amoA* sequence analysis: Implications for molecular diversity surveys. Appl Environ Microbiol 66:5368–5382

Rösch C, Mergel A, Bothe H (2002) Biodiversity of denitrifying and dinitrogen-fixing bacteria in an acid forest soil. Appl Environ Microbiol 68:3818–3829

Rich JJ, Heichen RS, Bottomley PJ, Cromack K, Myrold DD (2003) Community composition and functioning of denitrifying bacteria from adjacent meadow and forest soils. Appl Environ Microbiol 69:5974–5982

Ritchie NJ, Schutter ME, Dick RP, Myrold DD (2000) Use of length heterogeneity PCR and fatty acid methyl ester profiles to characterize microbial communities in soil. Appl Environ Microbiol 66:1668–1675

Rotthauwe JH, Witzel KP, Liesack W (1997) The ammonia monooxygenase structural gene amoA as a functional marker: Molecular fine-scale analysis of natural ammonia-oxidizing populations. Appl Environ Microbiol 63:4704–4712

Sakano Y, Pickering KD, Strom PF, Kerkhof LJ (2002) Spatial distribution of total, ammonia-oxidizing, and denitrifying bacteria in biological wastewater treatment reactors for bioregenerative life support. Appl Environ Microbiol 68:2285–2293

Scala DJ, Kerkhof LJ (1999) Diversity of nitrous oxide reductase (*nosZ*) genes in continental shelf sediments. Appl Environ Microbiol 65:1681–1687

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF (2009) Introducing mothur: Open-source, platform-Independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541

Shannon CE, Weaver W (1963) The mathematical theory of communication. Universiy of Illinois Press, Urbana

State Environmental Protection Administration of China (1989) Monitoring and analysis method of water and wastewater, 3rd edn (in Chinese). China Environmental Science Press, Beijing

Stevens H, Stubner M, Simon M, Brinkhoff T (2005) Phylogeny of *Proteobacteria* and *Bacteroidetes* from oxic habitats of a tidal flat ecosystem. FEMS Microbiol Ecol 54:351–365

Stres B, Mahne I, Avgustin G, Tiedje JM (2004) Nitrous oxide reductase (*nosZ*) gene fragments differ between native and cultivated Michigan soils. Appl Environ Microbiol 70:301–309

Talbot HM, Summons RE, Jahnke LL, Cockell CS, Rohmer M, Farrimond P (2008) Cyanobacterial bacteriohopanepolyol signatures from cultures and natural environmental settings. Org Geochem 39:232–263

Tamaki H, Sekiguchi Y, Hanada S, Nakamura K, Nomura N, Matsumura M, Kamagata Y (2005) Comparative analysis of bacterial diversity in freshwater sediment of a shallow eutrophic lake by molecular and improved cultivation-based techniques. Appl Environ Microbiol 71:2162–2169

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. Mol Biol Evol 24:1596–1599

Tang XM, Gao G, Chao JY, Wang XD, Zhu GW, Qin BQ (2010) Dynamics of organic-aggregate-associated bacterial communities and related environmental factors in Lake Taihu, a large eutrophic shallow lake in China. Limnol Oceanogr 55:469–480

Teske A, Alm E, Regan JM, Toze S, Rittmann BE, Stahl DA (1994) Evolutionary relationships among ammonia-oxidizing and nitrite-oxidizing bacteria. J Bacteriol 176:6623–6630

Wagner M, Horn M (2006) The *Planctomycetes*, *Verrucomicrobia*, *Chlamydiae* and sister phyla comprise a superphylum with biotechnological and medical relevance. Curr Opin Biotechnol 17:241–249

Ye WJ, Liu XL, Lin SQ, Tan J, Pan JL, Li DT, Yang H (2009) The vertical distribution of bacterial and archaeal communities in the water and sediment of Lake Taihu. FEMS Microbiol Ecol 70:263–276