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ORIGINAL PAPER

Vertical distribution of bacterial community structure in the sediments of two eutrophic lakes revealed by denaturing gradient gel electrophoresis (DGGE) and multivariate analysis techniques

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Abstract Vertical distribution of bacterial community structure was investigated in the sediments of two eutrophic lakes of China, Lake Taihu and Lake Xuanwu. Profiles of bacterial communities were generated using a molecular fingerprinting technique, denaturing gradient gel electrophoresis (DGGE) followed by DNA sequence analysis, and the results were interpreted with multivariate statistical analysis. To assess changes in the genetic diversity of bacterial communities with changing depth, DGGE banding patterns were analysed by cluster analysis. Distinct clusters were recognized in different sampling stations of Lake Taihu. Canonical correspondence analysis (CCA) was carried out to infer the relationship between environmental variables and bacterial community structure. DGGE samples collected at the same sampling site clustered together in both lakes. Total phosphorus, organic matter and pH were considered to be the key factors driving the changes in bacterial community composition.

Keywords Bacterial community structure · Sediment · DGGE · Eutrophic lake · Multivariate statistical analysis

Introduction

Aquatic sediments are stratified habitats where the vertical sequence of electron acceptors roughly follows the decreasing efficiency of energy metabolisms. This vertical gradient of biogeochemical properties provides niches for metabolically diverse microorganisms. Fluorescence in situ hybridization (FISH) and phospholipid fatty acid analysis (PLFA) were effective approaches to characterizing the microbial community in aquatic sediments (Kieft et al. 1997; Tay et al. 2001). In recent years, molecular methods based on sequence variation of the 16S rRNA gene, such as denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (tRFLP), cloning and sequencing have been widely used to reveal intrinsic genetic diversity in ecosystems (Salles et al. 2002; van Elsas et al. 2002; Turpeinen et al. 2004; Lai et al. 2006; Hao et al. 2007; Sapp et al. 2007b). Using these technologies, depth-related changes of overall bacterial communities, sulfate-reducing and methanogen communities in freshwater and marine sediments have been reported (Sahm et al. 1999; Ravenschlag et al. 2000; Koizumi et al. 2003). However, only a few researches concerned the vertical distribution of bacterial community in relation to the physicochemical factors in the sediment. Multivariate techniques, such as principal component analysis (PCA), multidimensional scaling analysis (MDS) and canonical correspondence analysis (CCA) have been proved more effective in detecting the relationship between bacterial community composition and environmental factors than univariate methods (Iwamoto et al. 2000; Salles et al. 2006; Sapp et al. 2007a). Several studies have been conducted to investigate the relationship between bacterioplankton community composition and environmental factors in lake, costal marine and estuarine ecosystems (Rooney-Varga et al. 2005; Haukka et al. 2006). These studies showed that total phosphorus (TP), nitrogen-associated factors, organic matter (OM), pH and redox potential (E_h) were considered to be key factors driving the changes in community composition.

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Lake Taihu is a subtropical, eutrophic and polymictic lake in China. It was oligotrophic until the 1950s, however, increasing nutrient inputs have led to its eutrophication (Cai et al. 1997; Chen et al. 2003). Most pollutants were discharged into Meiliang Bay, located in the north part of Lake Taihu (Qin et al. 2007). Lake Xuanwu in Nanjing is a famous resort lake of China, as well as a typical urban, shallow and eutrophic lake. Eutrophication has greatly affected the recreational value of Lake Xuanwu and the outlook of Nanjing city is also influenced. So far, no investigation has been made into the vertical distribution of bacterial community in the sediment of Lake Xuanwu. The aim of this study was to investigate the vertical distribution of bacterial community present in the sediment of both lakes. Additionally, the effects of several environmental factors (which have been proven to be key factors for bacterioplankton community structure) on bacterial community distribution were assessed with the help of multivariate analysis techniques.

Materials and methods

Study sites

Lake Taihu is a large (2,338 km^2), shallow (1.9 m mean depth) and well-mixed lake in China. Annual water inflow is about 8×10^9 m^3 and the residence time of the lake is approximately 5 months (Qin et al. 2007). Additional information about Lake Taihu can be found elsewhere (Qin et al. 2007). Sampling station T1 (31°28′46.39″ N 120°12′05.38″ E) is located in Meiliang Bay, polluted by high concentrations of nitrogen and phosphorus. The eutrophication in Meiliang bay mainly results from domestic wastewater from the Lujiang and Liangxi rivers (Qin et al. 2007). The second sampling station T2 (31°16′44.79″ N 120°11′02.95″ E) is located in the open lake close to its centre.

Lake Xuanwu is an urban small (3.71 km^2) and shallow (1.14 m mean depth) lake located in the northern part of Nanjing city, Jiangsu province, China. About 5×10^4 m^3 wastewater is discharged into the lake everyday and the residence time of this lake is about 3 months. Hydrological information about Lake Xuanwu has been described by Gong et al. (2006); Zhang et al. (2007). The first sampling station X1 (32°05′13.83″ N 118°47′18.59″ E) is located near the Nanjing railway station, which is enriched with high concentrations of nitrogen and phosphorus. The eutrophication of this site was mainly caused by the wastewater discharged from a hotel. The second sampling station X2 (32°04′59.10″ N 118°47′25.86″ E) is located in the centre of the lake.

Sample collection and measurements of physicochemical parameters

Three replicate sediment samples from each sampling station were collected with a core sampler (DM60, Mingyu, China) and sectioned into seven depth strata with a sterile spatula (0–3, 3–6, 6–10, 10–13, 13–18, 18–23, 23–33 cm). pH and redox potential (E_h) were measured in situ with specific electrodes (PHB-5, REX, China). Three replicate sediment samples for bacterial community structure analysis from six depth strata (except 23–33 cm stratum) were homogenized and stored at −80°C. DNA extraction was performed within 24 h after sampling.

Sediment samples were dried with a Freeze Dryer (labconco, Cole-Parmer Instrument Co., USA). Total nitrogen (TN), TP and OM were measured according to Jin and Tu (1990) and the results were expressed as mg/g (dry weight).

DNA extraction

Sediment samples were prepared as follows: 500 mg (ww) of samples were suspended into 3 ml of the TENP buffer (50 mM Tris, 20 mM EDTA, 100 mM NaCl, 0.01 g/ml polyvinylpolypyrrolidone (PVPP), pH 10.0), vortexed for 10 min and centrifuged for 5 min at 12,000 *g*. Deposits were washed twice again with the TENP buffer and phosphate buffer solution (137 mM NaCl, 2.7 mM KCl, 4 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4) to remove PCR-inhibiting substances. DNA was extracted according to the in situ lysis method of Gillan (2004). The DNA concentration was measured with a spectrophotometer (752, Shanghai Analytical Instrument General Factory, China) and diluted to 10 ng/μl for PCR amplification.

PCR amplification and DGGE analysis

To amplify the V3 region of the bacterial 16S rRNA gene, the primers 357f-GC (5′-GC-clamp-CCTACGGGAGGC AGCAG-3′) and 518r (5′-ATTACCGCGGCTGCTGG-3′) were used with a 40-base-pair GC-clamp (CGCCCGCC GCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG) attached to the 5′ end of the forward primer (Muyzer et al. 1993).

PCR amplification was performed in a 25 μl reaction mixture containing 0.625 μl of each primer (10 μM), 1 μl of template DNA, 0.125 μl of Ex Taq (5 U/μl) (TaKaRa, Japan), 2.5 μl of 10× PCR buffer, 2 μl of $MgCl_2$ (25 mM), and 2 μl of deoxynucleotide triphosphates (2.5 mM each) (TaKaRa, Japan). PCR cycling was carried out in a thermocycler (My Cycler™ thermal cycler, Bio-Rad, USA) under the following conditions: an initial denaturing step at 94°C for 5 min and 30 cycles of denaturing at 94°C for

1 min, annealing at 55°C and extension at 72°C for 1 min. During the last cycling program, the extension step was held for an extra 7 min prior to cooling at 4°C.

PCR products were loaded onto 8% (w/v) polyacrylamide gel cast in 1× TAE (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH 8.0). The polyacrylamide gels (acrylamide: bisacrylamide, 37.5:1) were made with denaturing gradients ranging from 35 to 55%. 100% denaturing contained 7 M urea and 40% formamide. DGGE was carried out with a D-code DGGE system (Bio-Rad laboratories, USA). Electrophoresis was conducted in 1× TAE buffer at 130 V and 60°C for 6 h. Gels were stained with ethidium bromide (50 μg/l) for 30 min, rinsed with Milli-Q water and visualized with u.v. in the Fluor-S MultiImager (Bio-Rad, USA) to acquire DGGE band image.

Analysis of DGGE banding pattern

To evaluate the bacterial community in different strata of the sediments, the positions and signal intensities of detected bands in each gel track were determined with a gel documentation system, Gel Doc 2000, Quantity-One 4.5.2 (Bio-Rad, USA). Lane background was subtracted by using the rolling disk size at 8 (Salles et al. 2004). Band-matching function with tolerance and optimization setting at 0.75% was used to identify and compare the bands present in each lane (Salles et al. 2004). We calculated the relative intensity of band to eliminate the bias caused by different PCR product amounts loaded in the gel (Salles et al. 2004).

Multivariable statistics analysis

Clustering analysis of the DGGE banding pattern was performed with the Quantity-One 4.5.2 software package (Bio-Rad, USA) using the unweighted pair-group method. The relationship between DGGE profiles and the sediment physicochemical properties was also investigated. The initial detrended correspondence analysis (DCA) results demonstrated that the data exhibited unimodal rather than linear responses to the environmental variables (Lepš and Šmilauer 2003; Sapp et al. 2007b), so we chose CCA performed by CANOCO 4.5 (Biometris, Wageningen, Netherlands) to explain our data. The analysis was performed without transformation of data and focus scaling on intersample distances. Manual selection of environmental variables, applying a partial Monte Carlo permutation test (499 permutations) with unrestricted permutation, was performed to investigate the statistical significance. The marginal effects of environmental variables were selected according to their significance level ($P < 0.05$) prior to permutation (Salles et al. 2004; Sapp et al. 2007b). Ordination biplots including the environmental variables and DGGE samples (lanes) were used to explain our data. The nominal variable "sampling site" was represented as centroids. The detailed interpretation of the ordination plots has been described by Ter Braak (1987); Salles et al. (2004).

Sequencing of excised bands and phylogenetic analysis

DGGE bands were excised from the gel, re-amplified and individual bands verified by a subsequent DGGE. Isolated bands from second DGGE gels were excised and 16S rRNA gene fragments were re-amplified, except that no GC clamp was attached to the forward primer. The PCR products were purified using Qiaquick PCR preps (Qiagen, Valencia, CA) and sequenced with the ABI PRISM 3730 automated sequencer (Applied Biosystems, Foster City, CA) by Shanghai Sangon Biological Technology Co. Ltd. Sequencing primer was 357f (5′-CCTACGGGAGGCAG-CAG-3′). For some bands, the above strategy was not able to obtain quality sequences. After re-amplified with primers 357f (without the GC clamp) and 518r, the resulting amplification products were cloned using a TA cloning kit (Invitrogen, USA) according to the manufacturer's instructions. Afterwards, sequencing analysis was carried out to the inserts as described above. The sequences obtained were checked for the presence of PCR-amplified chimeric sequences with the check_chimera program available at http://rdp8.cme.msu.edu/cgis/chimera.cgi (Cole et al. 2003). Sequences containing no chimera were compared to the GenBank database using the Basic Local Alignment Search Tool (BLAST) algorithm (Altschul et al. 1997).

Nucleotide sequence accession numbers

Sequences obtained in this study are available in the GenBank database under accession numbers EU181171 and EU186387 to EU186401.

Results

Physicochemical properties of the sediments

Properties of sediment samples are shown in Fig. 1. Total nitrogen (TN) and total phosphorus (TP) contents were higher in the sediments of Lake Xuanwu, especially in station X1. The vertical variation of TN concentration was relatively small in the sediments of Lake Taihu. However, it changed drastically within the upper 10 cm then

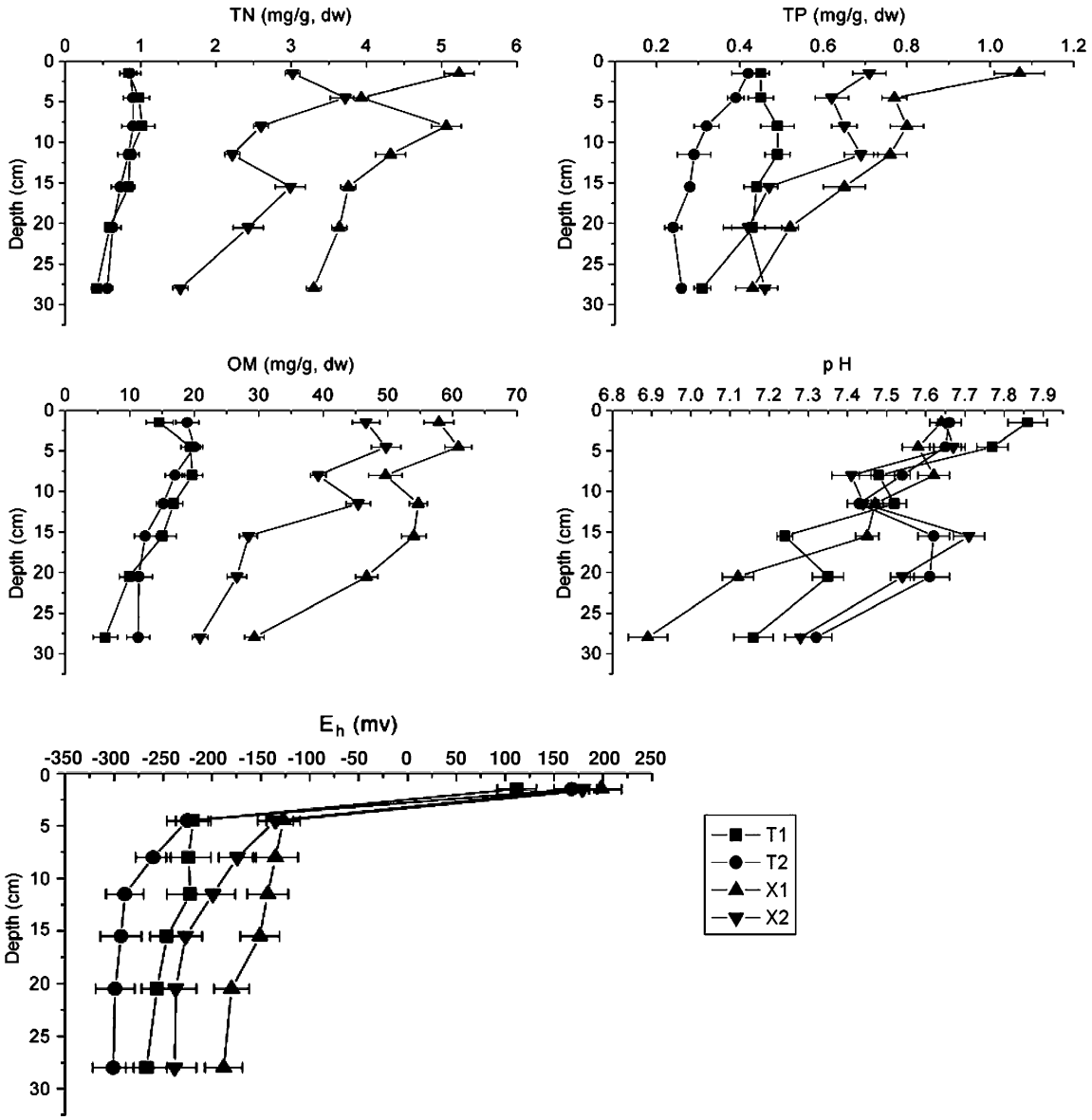

Fig. 1 Total nitrogen (TN), total phosphorus (TP), organic matter (OM), pH and redox potential (E_h) variations with depth at the four sampling stations. The data of TN, TP and OM were expressed as mg/g sediment (dry weight). *Error bars* represent the standard deviation of three replicates

decreased gradually in the sediments of Lake Xuanwu. The TP contents decreased with depths at all stations. Data on organic matter (OM) variations along depths are also presented in Fig. 1. Station X1 presented the highest values of OM, while station X2 presented intermediate and stations T1 and T2 the lowest values. In stations T1, X1 and X2, the OM contents decreased with depths, while in station T2 it kept nearly constant with the increased depths. Relatively high pH values were registered in the upper strata and then it decreased with depths gradually. E_h decreased drastically from 200 mV to −165 mV within the upper 5 cm at all stations then decreased gradually.

DGGE profiles and cluster analysis

DGGE profiles of bacterial community structures in the sediments of Lake Taihu (a) and Lake Xuanwu (b) are shown in Fig. 2. The average number of recognized DGGE bands derived from sites X1 and X2 throughout all layers were 29.7 and 32.7, respectively, compared to 26.7 and 28.3 of sites T1 and T2. Sequenced fragments clustered into eight major phylogenetic groups, including the Nitrospirae group (Band 1, 2, 4, 9, 10, 12, 15 and 16), α-(Band 14), β-(Band 7 and 8), γ-Proteobacteria group (Band 13), Verrucomicrobia group (Band 5), Chloroflexi

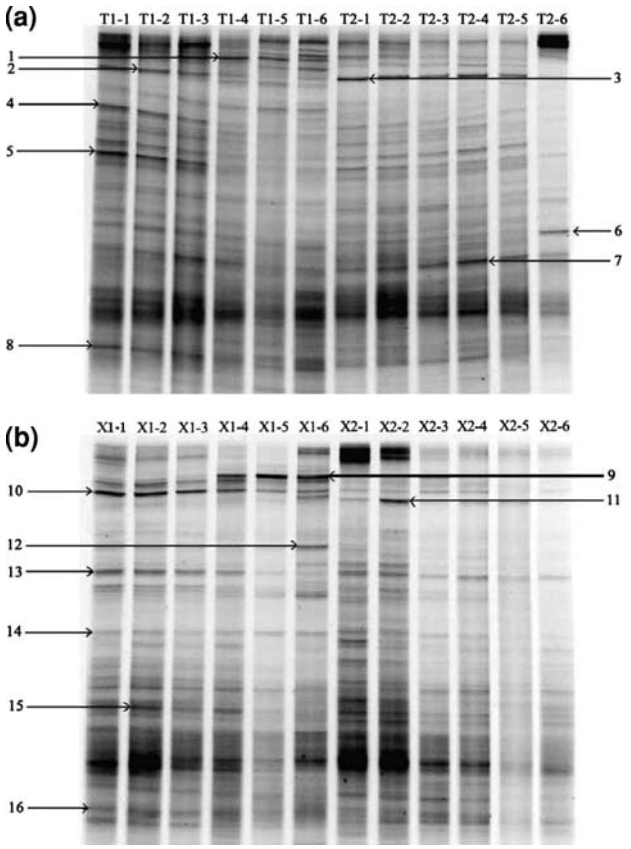

Fig. 2 DGGE analysis of sediments in Lake Taihu **a** and Lake Xuanwu **b**. Sequenced bands are numbered

group (Band 11), Actinobacteria group (Band 3) and Deferribacter group (Band 6) (Table 1).

Dendrograms were constructed from the matrix of similarity values of the DGGE banding patterns based on the presence of bands. Different dendrograms were derived from the cluster analysis of DGGE banding patterns of Lake Taihu (Fig. 3a) and Lake Xuanwu (Fig. 3b). In Fig. 3a, sampling stations of T1 and T2 demonstrated distinct clusters, indicating that the bacterial community structure differed significantly at the two sites. T2-5 and T2-6 grouped far from the other clusters due to the loss of bands in the lower layers of sediment at this station. The third and fourth slices at X1 and X2 were similar (though not clustering together obviously) (Fig. 3b).

Bacterial community composition in relation to sediment physicochemical properties

Biplots consisting of environmental variables and DGGE samples (lanes) were chosen to analyse the effects of sediment physicochemical properties (Fig. 4). In order to eliminate the bias arising from different DGGE gels, we studied the effect of environmental factors separately with each lake. Environmental variables with inflation factor (>20) were not observed during the CCA analysis, indicating that each variable was not collinear with other variables.

As shown in Fig. 4, the DGGE samples clearly clustered according to sampling site rather than sampling depth. Based on the 5% level in a partial Monte Carlo permutation

Table 1 Sequence analysis of bands excised from DGGE gels

Band no.	Taxonomic description	Accession no.	Most closely related bacterial sequence	Identity (%)	Accession no. of related sequence
1	Nitrospirae	EU181171	Uncultured Nitrospiraceae bacterium, D15_30	99	EU266868
2	Nitrospirae	EU186387	Uncultured bacterium, Ev219h1bfT3b4	99	EF446805
3	Actinobacteria	EU186397	Uncultured *Actinobacterium*, DOK_NOFERT_680	98	DQ829589
4	Nitrospirae	EU186389	Uncultured *Nitrospira* bacterium, 356	98	AB252945
5	Verrucomicrobia	EU186392	Uncultured bacterium, ORS25C_d09	97	EF392991
6	Deferribacteres	EU186401	Uncultured *Deferribacter* bacterium, MSB-4E9	99	DQ811935
7	β-Proteobacteria	EU186391	*Comamonas testosteroni* strain TacK2	98	AM937260
8	β-Proteobacteria	EU186393	*Comamonas testosteroni* strain TacK2	99	AM937260
9	Nitrospirae	EU186394	Uncultured Nitrospiraceae bacterium, D15_30	98	EU266868
10	Nitrospirae	EU186395	Uncultured *Nitrospira* bacterium, Ev219h1bfT3b4	98	EF446805
11	Chloroflexi	EU186396	Uncultured *Chloroflexus* bacterium, MSB-5B10	99	DQ811888
12	Nitrospirae	EU186400	Uncultured *Nitrospira* bacterium, Ev219h1bfT3b4	99	EF446805
13	γ-Proteobacteria	EU186390	*Methylocaldum* sp. E10a	99	AJ868426
14	α-Proteobacteria	EU186399	*Sphingomonas* sp. B28161	97	AJ001052
15	Nitrospirae	EU186398	Uncultured bacterium, Ev219h1bfT3b4	98	EF446805
16	Nitrospirae	EU186388	Uncultured *Nitrospira* sp. GASP-0KB-579-C01	99	EU043639

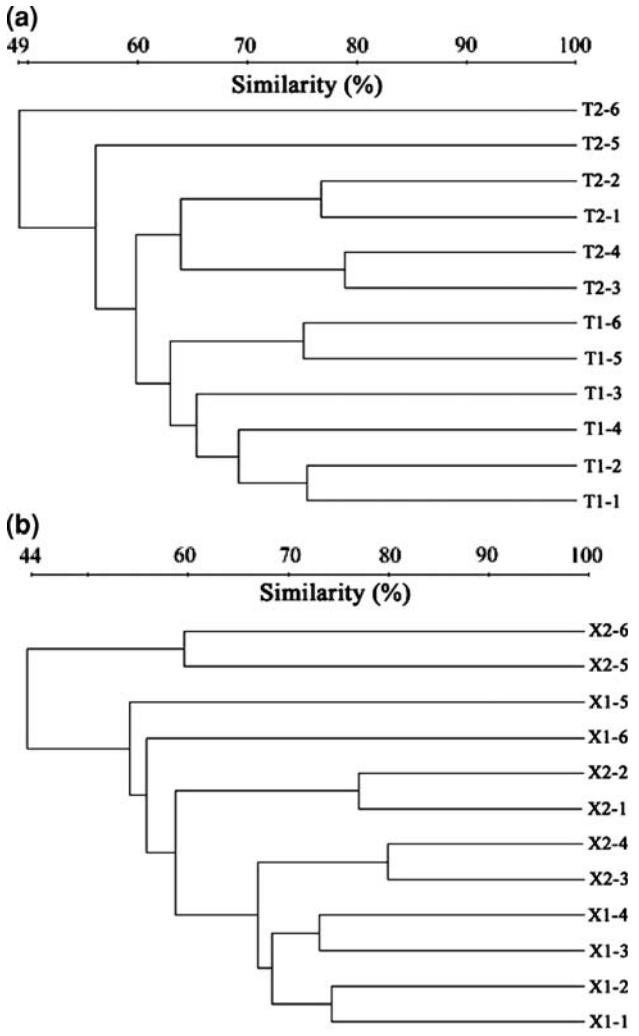

Fig. 3 Dendrogram for samples from the four stations over different depths. Similarities were calculated using the unweighted pair group method. **a** Lake Taihu, **b** Lake Xuanwu

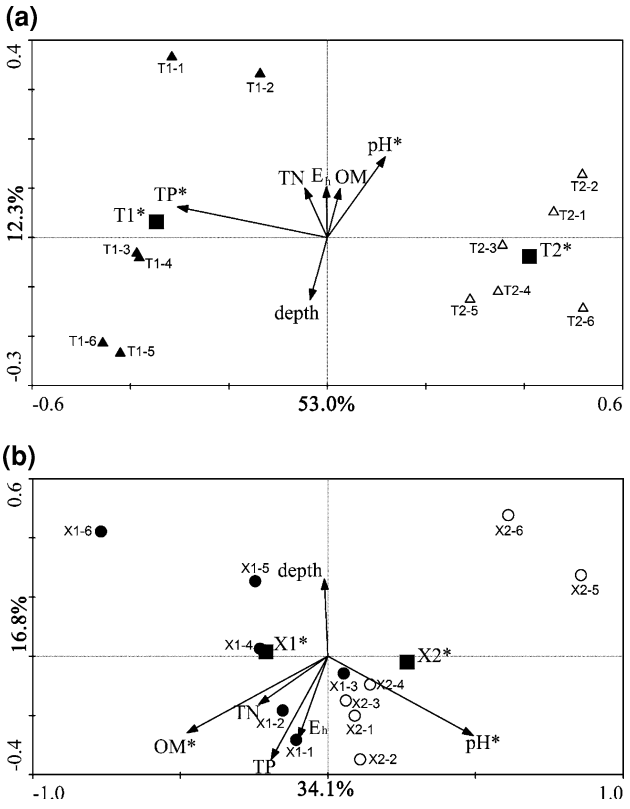

Fig. 4 Canonical correspondence analysis (CCA) ordination diagram of bacterial communities associated with environmental variables of total nitrogen (TN), total phosphorus (TP), organic matter (OM), pH, redox potential (E_h) and sampling depth. **a** Lake Taihu; **b** Lake Xuanwu. Environmental variables were indicated as *arrows*. The nominal variable "sampling site" was represented as centroids. DGGE samples were indicated as (▲) T1 site, (△) T2 site, (●) X1 site, (○) X2 site. Environmental variables marked with *asterisks* were significant ($P < 0.05$)

test, sampling site emerged as a highly significant explanatory variable in both lakes (Fig. 4a, b). Sampling depth was not a significant environmental variable in both lakes ($P > 0.05$). In Lake Taihu, TP and pH were the significant environmental factors, correlated with the first and second ordination axes, respectively (Fig. 4a). In the ordination plot of Lake Xuanwu, sampling site was also the significant explanatory variable, separating the DGGE samples along the first axis. OM and pH emerged as the significant environmental variables explained by the first axis in Fig. 4b.

Discussion

Microbial community plays a crucial role in decomposition of organic materials and nutrient element transformation in freshwater sediment. Vertical related gradient in sediment physicochemical properties provides niches for metabolically diverse microorganisms. The aim of this study was to investigate the vertical variations of physicochemical factors and bacterial communities in two eutrophic lakes and examine which environmental factor has the largest influence on bacterial community structure.

Analysis of sediment physicochemical properties revealed that the variations of TN, TP and OM in the sediments of Lake Taihu were smaller than those of Lake Xuanwu, indicating the relative stable sediment properties in Lake Taihu. E_h decreased with the sampling depth, although the decreases were not obvious when the depth increased to 15 cm. The reduction of E_h along with depth means the decreased concentrations of oxygen, and the increased CO_2 and methane indicated an anaerobic environment, which may influence the community composition (Sheppard and Lloyd 2002).

CCA is powerful in illustrating how bacterial community structure varies along gradients of environmental variables (Salles et al. 2004). Bacterioplankton community dynamics in freshwater and marine ecosystems have been well-documented (Rooney-Varga et al. 2005; Haukka et al. 2006; Sapp et al. 2007b). However, a correlation between the bacterial community in sediments and environmental factors has seldom been reported. The CCA results in this study suggested that TP, pH and OM significantly influenced the bacterial community composition in the sediments.

The effect of TP on the diversity and composition of bacterial communities has been reported. Lindström and Bergström (2005) pointed out that TP concentration statistically explained the differences between the microbial community compositions in two different drainage areas. Li et al. (2005) also reported that the number of phosphate-dissolving/decomposing bacteria was directly correlated to the total phosphorus concentration in the sediments of Guanting reservoir. OM played an important role in driving the changes in bacterial community in the sediments of Lake Xuanwu. Macalady et al. (2000) reported that bacterial community structure was strongly related to sediment organic carbon content in a mercury-polluted lake. The phytoplankton and its exudates are the major sources of autochthonous organic matter in lake; both of them had an indirect effect on the bacterial community (Rooney-Varga et al. 2005). A correlation between bacterial community structure and pH has also been reported previously (Lindström and Bergström 2005). pH may merely reflect changes in other environmental factors, such as the availability of ions and trace metals (Koski-Vähälä et al. 2001), which can have both inhibitory and stimulative effects on bacterial community. However, a fluctuant pH may also influence bacterial community through direct biological mechanisms (Yannarell and Triplett 2005).

DGGE samples collected in the same sampling site clustered together in both lakes and sampling depth was not a significant environmental factor (Fig. 4). These results were consistent with the previous reports that depth-related change of the 5′-terminal restriction fragments of 16S rRNA was small in marine sediment (Urakawa et al. 2000); Novitsky (1990) reported that the microbial community structure on the sediment surface was similar to that of sedimenting particles, suggesting that the sediment microbial community originated from sedimenting particles. One possible explanation for the similar community structure between surface and deeper layers of the sediment may be the microorganisms bound on sedimenting particles deposited on the surface and buried. Further research is needed to investigate the origin and formative process of microbial communities in aquatic sediments.

The bacterial groups detected by sequence analysis in this study are commonly found in freshwater environments (Haukka et al. 2006). According to previous reports, Nitrospirae present in various environments, i.e., sewage disposal plants (Daims et al. 2000), soils (Bartosch et al. 2002), freshwater aquaria (Hovanec and DeLong 1996) and sediments (Stein et al. 2001). Another group of bacteria present in our samples was Proteobacteria. Two of our sequences belonged to β-Proteobacteria subgroup (Bands 7 and 8), one (Band 14) to α-subgroup and one (Band 13) to γ-Proteobacteria. β-Proteobacteria are often the dominant group in freshwater ecosystem, whereas α- and γ-Proteobacteria appear to be predominant indigenous community in marine (Sekiguchi et al. 2002; Eiler and Bertilsson 2004); Kolmonen et al. (2004) pointed out that Verrucomicrobia are relatively more prevalent in eutrophic lakes than in oligo- or mesotrophic lakes. Many Verrucomicrobia are prosthecate, offering them an advantage in nutrient uptake or making them more resistant to gazing. Actinobacteria are prevalent in freshwaters and estuaries with varying hydrological and limnological characteristics (Kolmonen et al. 2004).

Although PCR-DGGE has recently become a useful molecular technology in analysis of microbial ecology and dynamics, several associated biases related to DNA extraction efficiency and PCR primer annealing efficiency should be taken into consideration (Clement et al. 1998; Ranjard et al. 2000). In our study, all samples were subjected to the same procedure of DNA extraction and PCR amplification, using low-stringency annealing temperature and small number of amplification cycles, in order to minimize biases and make possible the comparison of samples. Several studies have shown that DGGE patterns, including relative intensity of DGGE bands, are reproducible (Rooney-Varga et al. 1999; Schauer et al. 2000; Diez et al. 2001).

Conclusions

In conclusion, the results obtained in this study demonstrated that the variational characteristics of sediments may lead to different bacterial communities. Multivariate statistical analysis indicated that TP, OM and pH had remarkable effects on bacterial community structure in lake sediments. The bacterial divisions detected in this study remained the same as previous research. These results are important, because microbial community in sediment plays a crucial role in nutrients cycling, which is necessary to maintain aquatic ecosystem health.

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