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ORIGINAL ARTICLE

Sources of organic matter affect depth-related microbial community composition in sediments of Lake Erhai, Southwest China

Wei XIONG,[1] Ping XIE,[2*] Shengrui WANG,[3*] Yuan NIU,[3] Xi YANG,[2] Wenjie CHEN[1]

[1]College of Fisheries, Huazhong Agricultural University, 430070 Wuhan; [2]Donghu Experimental Station of Lake Ecosystems, State Key Laboratory of Freshwater Ecology and Biotechnology of China, Institute of Hydrobiology, Chinese Academy of Sciences, 430072 Wuhan; [3]State Environmental Protection Key Laboratory for Lake Pollution Control, Research Center of Lake Eco-environment, Chinese Research Academy of Environmental Sciences, 100012 Beijing, China
*Corresponding author: xieping@ihb.ac.cn, shengruiwang@sohu.com

ABSTRACT

Sediment cores taken from different areas of the mesotrophic Lake Erhai were analysed to investigate the vertical distribution of bacterial community composition (BCC), as well as physicochemical parameters. PCR-denaturing gradient gel electrophoresis (DGGE), stable carbon isotope ($\delta^{13}C$), C/N atomic ratio and canonical correspondence analysis (CCA) were used to explore the relationships between the succession of bacterial communities and environmental variables, emphasising changes in the sources of organic matter (OM). The BCC in natural environments was characterised by DGGE of the 16S *rRNA gene with subsequent sequencing of bands of interest. The CCA revealed that the depth-related variation in sediment bacterial communities in different areas of the lake was significantly influenced by varying environmental factors. The OM source, however, played an important role in structuring BCC at all sites. The DGGE banding patterns revealed that the abundance of Deltaproteobacteria decreased with accompanying elevated levels of C4 plant-derived organic carbon. The sequencing of DGGE bands suggested that the majority of the sequences were affiliated with common phylogenetic groups in lake sediments:* Chloroflexi, Deltaproteobacteria *and* Firmicutes*.* Betaproteobacteria *detected in our study appeared as a prominent phylotype in the upper sediment. The Shannon-Wiener diversity index of bacterial communities was directly affected by the OM source. Constant OM sources resulted in a stable higher diversity of bacterial communities and broader enzymatic capabilities to access OM. We conclude that the differences in the diversity of bacterial communities in sediments differing in their sources of OM were related to environmental variables (*e.g. *water level, river runoff and terrestrial vegetation composition). Our study provided insights into the relationships between natural BCC and OM sources, facilitating a better understanding of microbial community structure in lake sediment.*

Key words: Bacterial community composition, organic matter, denaturing gradient gel electrophoresis, $\delta^{13}C$, canonical correspondence analysis, sediment.

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INTRODUCTION

Sediments are one of the most diverse microbial habitats with distinct redox gradients (Torsvik *et al.*, 2002) and represent an open system where physicochemical and biochemical reactions occur. Sediment microorganisms play a key role in the interchange of energy and matter between sediment and the water (Tong *et al.*, 2005), such as the ability to regulate the carbon cycle and to degrade a broad range of organic matter (OM) sources in aquatic systems (Kao and Prosser, 1999; Kao *et al.*, 2001). The vertical sequence of electron acceptors roughly follows the decreasing efficiency of energy metabolisms in undisturbed sediments, and the depth-related gradient of biogeochemical properties provides a niche for metabolically diverse microorganisms (Koizumi *et al.*, 2003). However, only few studies have investigated vertical changes of microbial communities in freshwater sediments and their OM sources. Primary production by phytoplankton, terrestrial plants, river runoff, municipal sewage and industrial discharge, *etc*., contribute to various sources of OM in sediments (Hedges and Keil, 1995; Hedges *et al.*, 1997). The $\delta^{13}C$ signatures of the various carbon sources are often different and serve as powerful tracers of carbon inputs in various environments, although some overlap occurs between different sources (Fry and Sherr, 1989; Meyers, 1994; Prahl *et al.*, 1994; Schelske and Hodell, 1995). The sources of OM, nutrient status and pollution may influence the microbial community structure (Bååth *et al.*, 1995; Pennanen, 2001). Traditional techniques of isolation and culturing have not been adequate for the characterisation of microorganisms in environmental samples, particularly in evaluating the natural microbial diversity (Fang *et al.*, 2000; Delong and Pace, 2001). In recent years, molecular methods based on 16S rRNA, such as PCR-denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), cloning and sequencing have been widely used to reveal intrinsic genetic diversity (Salles *et al*., 2002). PCR-DGGE fingerprinting has been widely used in environmental microbiology and

has been available to detect the similarities and differences of the dominant populations of microbial communities (Muyzer and Smalla, 1998; Lindstrom, 2000; Dorigo *et al.*, 2005). Although a few studies have focused on assessing microbial distribution in sediments and their function (Macalady *et al.*, 2000; Zhao *et al.*, 2008), data on the relationships between the vertical variation of bacterial community composition (BCC) and OM sources recorded in the sediments are still scarce.

This study represents the first attempt to investigate vertical gradients of natural microbial communities and environmental variables in Lake Erhai sediments. We especially focus on the relationship between BCC and OM sources represented by C/N ratios and $\delta^{13}C$ signatures. The two main objectives of our study are as follows: i) to describe the natural microbial community structure prole in sediment; and ii) to investigate the vertical gradients of environmental variables, particularly the content and sources of OM and their effects on natural microbial community structure. These may facilitate the understanding of relationships between natural microbial communities and environmental variables in plateau mesotrophic lake sediment.

METHODS

Study site and sampling

Sediment samples were collected at different locations and different depths from Lake Erhai, a mesotrophic plateau lake located in Dali, Yunnan province and the largest fault lake in southwest China (surface area: 249.8 km^2, mean depth: 10.5 m). Lake Erhai sits within an intermountain basin, there are high mountains stand up 2800~3500 m asl from the west and north of the lake, about 18 rivers and streams flow into the lake (Xu and Zheng, 2003). There is only one outlet (to the west in the southwest corner of the lake). Precipitation also influenced terrestrial runoff that supplied C from land to lake. The mean total phytoplankton biomass (in terms of Chl a) ranged from 5.5 μg/L (Feb) to 31.5 μg/L (Sep) in Lake Erhai (Li E. *et al.*, 2011). On the fluvial plain and lakeshore areas the vegetation is exclusively agricultural, with extensive irrigated areas supporting a double-cropping system of corn, rice, wheat, legumes and other vegetables (Dearing *et al.*, 2008). The lake plays an important role in the local socio-economic activities (Guo *et al.*, 2001; Wu *et al.*, 2001). In recent years, water quality deterioration associated with rapid socio-economic development in the Lake Erhai Basin has acquired increasing attention from the public and the government (Guo *et al.*, 2011).

Sediment cores (length: 50 cm, diameter: 9 cm) were collected from three sites (EH-1, EH-2 and EH-3) (Fig. 1) on 18 July 2010. The first sampling station (EH-1) was located in Hongshan Bay at a water depth of 11.2 m. Hongshan Bay, an area without plants, is located in the northern end of Lake Erhai. It is located at the lower edge of the alluvial fan of the Yongan River, an inflow river. This area has an influx of OM pollution through non-point source agricultural run-off (Guo *et al.*, 2001; Yang and Song, 2006). The second sampling station (EH-2), which was chosen because of its depth and lack of aquatic macrophytes, was located at the deepest point of the central lake body at a depth of 21 m. This zone is under the intersection interface of the northern part of the lake and south lake current. The third sampling station (EH-3) was located in a flat-bottom site that is 8.3 m deep in the southern part of the lake, which was characterised by the presence of submersed macrophyte communities prior to 2000. The submersed macrophyte communities in these zones gradually disappeared during the 2003-2006 time period due to rising water levels.

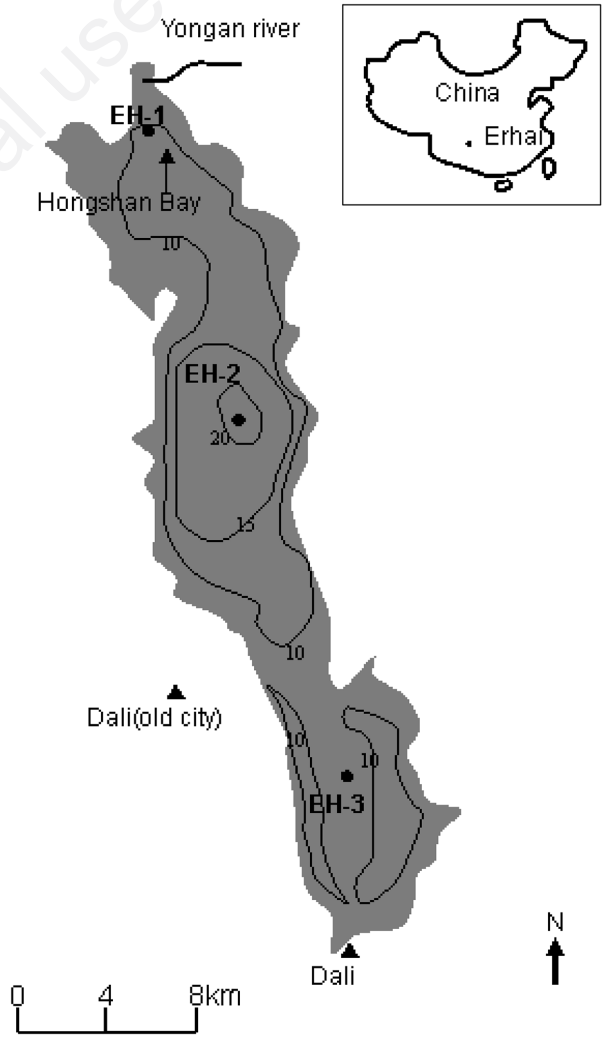

Fig. 1. Locations of the three sampling sites (EH-1, EH-2 and EH-3) in Lake Erhai.

Sediment cores were collected with a core sampler (HL-CN, Hengling Technology Ltd. Corp., China). The cores were sectioned in 2-cm intervals between depths of 2 cm and 12 cm, in 4-cm intervals between depths of 12 cm and 20 cm depth, and in 6-cm intervals between depths of 20 cm and 44 cm. Three replicate cores were taken by the same sampling procedure. pH and oxidation-reduction potential (Eh) were measured at each location. To minimize the risk of contamination, the samples were transferred to the laboratory in the dark at 4°C within 12 h in sterile plastic container by using alcohol-sterilized spatulas, the samples surfaces exposed to the air were discarded when processed. One portion of each sample was freeze dried, homogenized using agate mortar and pestle to a ne powder, then, used for physical and chemical analysis, and a second portion was frozen at -80°C and used for DNA extraction. DNA extractions were conducted under sterile condition.

Chemical analysis

The potential of hydrogen (pH) and the oxidation-reduction potential (Eh) were both measured with an ORP meter (pH/ion meter 225, Iwaki Glass, Tokyo, Japan). Total nitrogen concentrations (TN), total phosphorus concentrations (TP) and total organic carbon content (TOC) were measured according to Jin and Tu (1990) after the sediment samples were dried with a Freeze Dryer (labconco, Cole-Parmer Instrument Co., Vernon Hills, IL, USA). For TOC determination, about 0.5 g sediment was mixed into 10 mL potassium dichromate buffer solution (1/6×0.400 mol). The resulting slurry was then heated at 175°C for 10 min, followed by addition of 5 mL phosphoric acid buffer (acid:H_2O=1:1). Titration was performed using 0.2 mol ferrisulfas solution with sodium diphenylamine sulfonate as an indicator. The TOC:TN ratios (C/N) for sediments were determined using an elemental analyser (VarioEL, Elementar, Hanau, Germany). Subsamples of sediment for $\delta^{13}C$ contents were acidified with HCI (1N) to remove carbonates. Traces of HCI were removed by washing the sediments several times with distilled water. The sediments were then used for bulk $\delta^{13}C$ analysis. Values of $\delta^{13}C$ were determined using an isotope ratio mass spectrometer (Delta V Advantage, Finnigan MAT, Bremen, Germany). The overall analytical precision for replicate samples was within±0.5‰. The analysis of $^{13}C/^{12}C$ was cross-referenced with the international Pee Dee Belemnite (PDB) standards and international cellulose samples $IAEA_2C3$ ($\delta^{13}C$= -24.91‰) as a standard. The formula used for stable carbon isotope calculation was as follows:

$$\delta^{13}C(‰)=(R_{sample}/R_{standard}-1)\times 1000$$

where R is the value of $^{13}C/^{12}C$.

The age-depth relationship in this study has been estimated using ^{137}Cs and ^{210}Pb dating (Wan, 1999; Xu *et al.*, 1999). ^{210}Pb activity for $^{210}Pb_{ex}$ sedimentary dating was determined using α-spectroscopy. ^{137}Cs activity was determined using a Canberra (Meriden, CT, USA), S-100 Multi-channel Analyzer.

DNA extraction and PCR amplification

The subsamples of sediments belonging to the same depth from each replicate were pooled for DNA extraction. Genomic DNA was extracted from 500 mg of sediment from each depth using a Soil DNA kit (Omega Bio-Tek, Norcross, GA, USA) following the protocol of the manufacturer. The extracted DNA was used as template to amplify the *16S* rRNA gene fragment with the forward primer 341f (5'-CCTACGGGAGGCAGCAG-3') with a GC clamp (5'-CGCCCGCCGCGCGCG-GCGGGCGGGGCGGGGG CACGGGGGC-3'); 907r (5'-CCGTCAATTCCTTTGAGTTT-3') was used as the reverse primer (Teske *et al.*, 1996). These primers were capable of amplifying most bacteria from the sediment samples. PCR mixtures (50 μL) contained 1× PCR buffer, 1.5 mM $MgCl_2$, 200 mM each dNTP, 0.2 mM each primer, 2.5 U of Taq DNA polymerase (Takara, Shiga, Japan), and 20 ng of template DNA. A 5-min initial denaturation at 94°C was followed by a thermal cycling program as follows: 20 cycles of denaturation (1 min at 94°C), annealing (1 min at an initial temperature of 65°C, decreasing 0.5°C every cycle till a final temperature of 55°C), and extension (3 min at 72°C); 10 cycles of denaturation (1 min at 94°C), annealing (1 min at 55°C), and extension (3 min at 72°C), followed by a final 8-min extension at 72°C. A negative control, in which the template was replaced by an equivalent volume of sterile deionized water, was included. PCR products were confirmed by 1.5% agarose gel electrophoresis.

Denaturating gradient gel electrophoresis

A total of 600 ng of PCR product for each sample was loaded onto a 6% (w/v) polyacrylamide gel (37.5:1 acrylamide:bisacrylamide) with a denaturing gradient that ranged from 45% to 60%, where 100% denaturant is dened as 7 M urea and 40% deionized formamide. The denaturating gradient gel electrophoresis (DGGE) was performed with a Dcode system (Bio-Rad Laboratories, Philadelphia, PA, USA) using 1×TAE running buffer (20 mM Tris, 10 mM acetic acid, 0.5 mM EDTA, pH 8.0) at 60°C for 11 h at 100 V. The gel was stained with GelRed (Biotium, Hayward, CA, USA) nucleic acid staining solution (diluted 1:10,000) for 30 min and photographed using Gene Com. (Bio Image Systems, Inc., Jackson, MI, USA) under UV light. DGGE were performed separately for the three sites.

Sequencing and phylogenetic analyses of DGGE bands

All visible DGGE bands were excised with a sterile razor blade and eluted overnight at 4°C in 40 µL MilliQ water. A volume of 3 µL of the supernatant was used as a template for reamplification with the same primer set as described above (without a GC clamp). The amplicons were electrophoresed again on a DGGE gel to check the position of the original band and then purified with a Gel Recovery Purification Kit (AxyPrepTM) and ligated into pMD18-T plasmid vector (Takara, Japan) following the manufacturer's instructions. The ligated DNA was transformed into *Escherichia coli* DH5a-competent cells. The recombinant clones were selected and then submitted for sequencing using M13 primers and an automated ABI sequencer at the Genomics Company (Wuhan, China). The *16S* rRNA gene sequences have been deposited to GenBank under the Accession No. KC788751 to KC788807.

CLUSTAL W program (Thompson *et al.*, 1994) and the phylogenetic analyses were performed with the MEGA4.0 (Tamura *et al.*, 2007) software package using neighbour-joining methods (Saitou and Nei, 1987). The sequences with similarities greater than 97% were grouped in one operational taxonomic unit (OTU).

Cluster analysis of DGGE proles and statistical analysis

Cluster analyses of DGGE proles were performed with the NTSYS program ver. 2.10e (Exeter software, Setauket, NY, USA). A binary matrix was constructed by scoring the presence or absence of DGGE bands. Pairwise similarities between gel banding patterns were quantified using the Dice coefficient as: $S_D=(2N_{AB})/(N_A+N_B)$, where N_{AB} is the number of bands common to the samples A and B, and N_A and N_B are the number of bands in samples A and B, respectively. The similarity coefficients were then used to construct a dendrogram using an unweight pair group method with the arithmetic average (UPGMA) through the sequential, hierarchical, agglomerative, and nested clustering (SHAN) routine of the NTSYS program.

To reveal the relationships between BCC and environmental variables (including the physical and chemical parameters), canonical correspondence analysis (CCA) was performed using CANOCO software (ver. 4.5, Microcomputer Power, Ithaca, NY, USA) as the length of the rst DCA axis run on species data was >4 (Ter Braak and Verdonschot, 1995). The binary matrix was constructed according to the cluster analyses. The environmental variables significantly related to the BCC were tested by forward selection and Monte Carlo permutation tests. The CCA was run separately for the datasets of each of the three sites.

Statistical analysis of the physical and chemical parameters was conducted using SPSS software, ver. 17.0 for Windows (Chicago, IL, USA). We tested the data normality using Shapiro-Wilk tests, and then the differences in environmental parameters between sites were evaluated with *t*-tests. Significance was determined at an alpha level of 0.05 ($P<0.05$).

Bacterial community diversity

Gel images were analysed using Gel-Pro Analyzer (version 4.5). A densitometric curve was calculated for each lane, and the relative intensities of all bands were obtained. The Shannon-Wiener Index (H', bits) was calculated to estimate changes in bacterial community diversity using the following formula:

$$H' = -\sum_{i=1}^{n} Pi \ln Pi$$

where:
Pi is the relative intensity of each band;
n is the total number of bands in each lane.

RESULTS

Vertical properties of bulk parameters

The vertical distributions of physicochemical and biological properties (pH, Eh, TN, TP, TOC, C/N, $\delta^{13}C$, H') of the sediments are shown in Fig. 2. The average and ranges of nutrient concentrations and ratios (TN, TP, TOC, C/N, $\delta^{13}C$), physical parameters (pH, Eh, water depth) and Shannon-Wiener diversity index (H') of each site are compiled in Tab. 1.

Site EH-1 in the northern end of Lake Erhai with an influx of OM pollution through non-point source agricultural run-off (Guo *et al.*, 2001; Yang and Song, 2006) had the highest average values of pH, C/N, and $\delta^{13}C$ and the lowest values of TN, TOC and H'; Site EH-2 in the deepest point of the central lake had the highest average value of Eh and TP; Site EH-3 in the southern part of the lake had the highest average concentrations of TN and TOC and the lowest average values of pH, TP, C/N, $\delta^{13}C$ and H'. The Eh, pH and C/N were significantly different ($P<0.05$) between Sites EH-1 and EH-2, as well as between Sites EH-1 and EH-3. The higher H' values coincided then with the highest nutrient and TOC concentration.

Vertical distributions of TOC and TN were highly variable with similar trends in both parameters at all sampling sites. There was a significant correlation between the concentration of TN and TOC (Sites EH-1: $r=0.95$, $P<0.01$; EH-2: $r=0.88$, $P<0.01$; EH-3: $r=0.92$, $P<0.01$). There were no significant differences ($P>0.05$) in the TOC concentrations among the three sites. An increase in the concentrations of TN and TOC were observed at 8-10 cm depth upward to the surface sediment sample at all sites, with the highest concentrations of TN and TOC found between the depths of 0 and 2 cm depths. Both the TN and

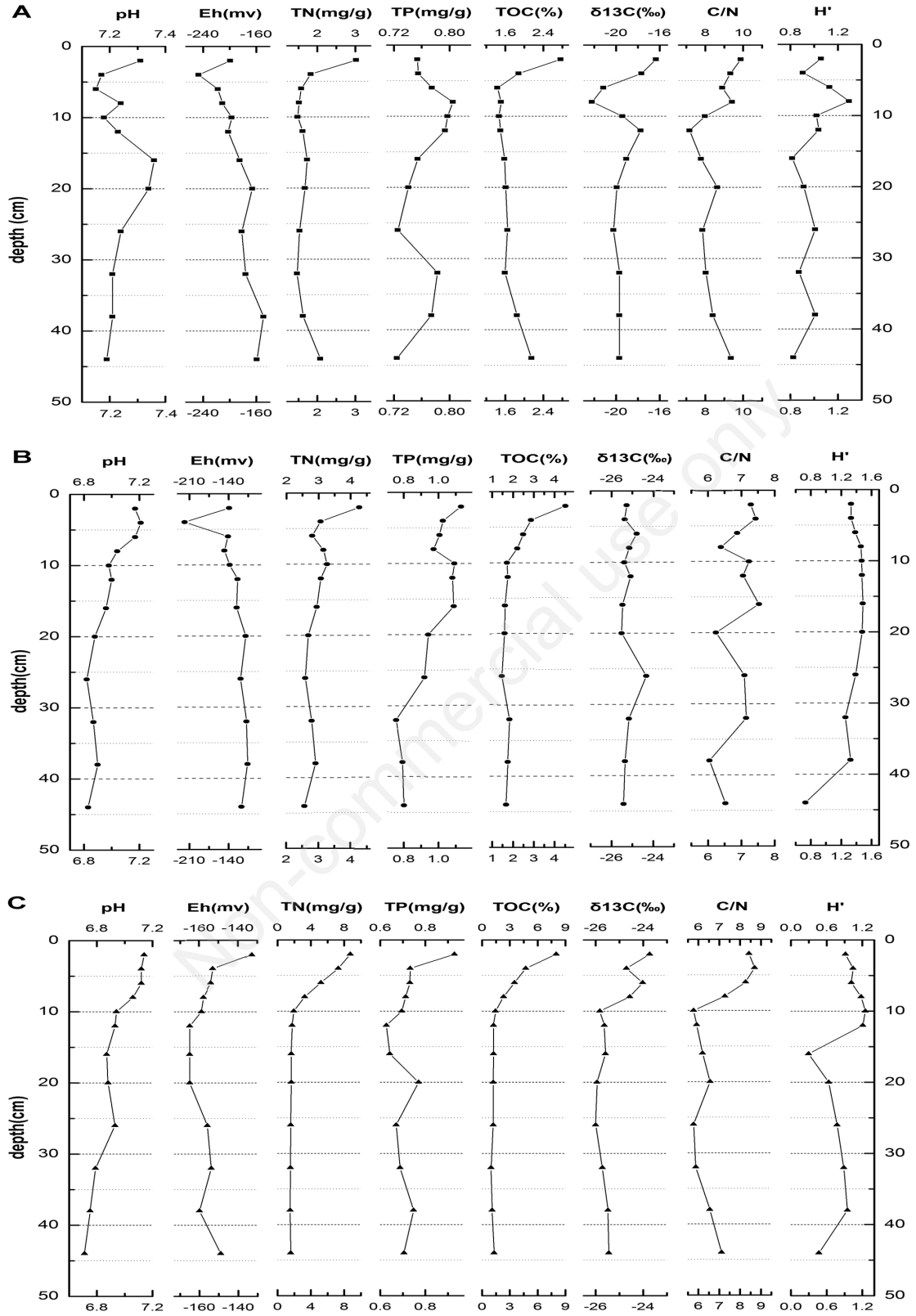

Fig. 2. Vertical properties of bulk parameters. Vertical changes in potential of hydrogen (pH), oxidation-reduction potential (Eh), total nitrogen concentrations (TN), total phosphorus concentrations (TP), total organic carbon content (TOC), values of $\delta^{13}C$(%) PDB, TOC:TN ratio (C/N), and Shannon-Wiener diversity index (H') of bacteria. The capital letters A, B and C were used to distinguish the data from different Sites EH-1, EH-2 and EH-3, respectively.

TOC showed high values in the H1 segment (from 10 to 0 cm sediment depths) and maintained a relatively stable value in the H2 segment (from 44 to 10 cm depths) at all sites (Fig. 2 A,B,C). The average concentrations of TOC (4.0%) and TN (5.3 mg/g) in the H1 segment were much higher than those in the H2 segment at Site EH-3 (TOC: 1.2% and TN: 1.6 mg/g) (Fig. 2).

Sediment dating

The sediments in Lake Erhai were dated using ^{137}Cs and ^{210}Pb (Wan, 1999; Xu *et al.*, 1999). The dating results of the sediments are shown in Tab. 2. The sedimentation rates in Lake Erhai were calculated based on these age constraints.

Vertical variations in diversity of bacterial communities

The spatial variations in the Shannon-Wiener diversity index (H') of bacteria community are shown in Fig. 2. Variations in the Shannon-Wiener diversity index revealed a similar pattern at Sites EH-1 and EH-3. The index was lower at the bottom of the core and increased from 16 cm to the surface sediment. It reached a peak at 8-10 cm and gradually decreased upward. The variation trend in the Shannon-Wiener diversity index of the cores from Site EH-2 was completely different from the other measured sites. The index was relatively stable and high at Site EH-2 ($P<0.05$).

Composition of the bacterial community

Visible changes were noted in the relative brightness and position of the DGGE banding patterns of *16S* rRNA gene fragments in various segments from the three sites (36 samples in total) (Supplementary Fig. 1). The cluster analysis (UPGMA) revealed remarkable spatial differences of BCC between different sites (Fig. 3). The bacterial communities in various layers from Site EH-1 (Fig. 3A) were grouped into 6 dened clusters. The samples from Site EH-2 formed four separate clusters (Fig. 3B). The 38-44 cm sample (lowest measured TN, TOC and $\delta^{13}C$) formed cluster 4. The samples from Site EH-3 distinctly formed three separate clusters along the depth gradient (Fig. 3C).

The bands from different layers at the same vertical position in a gel were assumed to have identical sequences (Riemann *et al.*, 1999). Eighteen, twenty-five and fourteen bands from different vertical positions in the DGGE proles from Sites EH-1, EH-2 and EH-3, respectively, were excised, reamplified, purified and sequenced. In the DGGE proles, the first band obtained from Site EH-1 was indicated by EH-1-1. The other bands were named in the same manner. The phylogenetic affiliation of clones obtained from the DGGE proles at the three sites are shown in Tab. 3. The majority of the sequences were affiliated with phylogenetic groups commonly found in sediment: *Deltaproteobacteria*, *Firmicutes* and *Chloroflexi*. A total of 55 clones were classified into 28 OTUs, where 8 OTUs belonged to *Deltaproteobacteria*, followed by *Firmicutes* (5 OTUs) and *Chloroflexi* (5 OTUs). The taxonomic descriptions of the 55 bands obtained from the DGGE profiles of the three sampling sites are shown in Supplementary Tab. 1.

It has been suggested that band intensity is related to the relative abundance of the corresponding phylotype in the template mixture (Murray *et al.* , 1996; Riemann *et al.* , 1999; Fromin *et al.*, 2002), and thus the bands with relatively high intensities in a lane were assumed to belong to a dominant target. Deltaproteobacteria (bands EH-1-11, EH-1-12 and EH-3-12) and *Chloroflexi* (bands EH-1-17, EH-3-3 and EH-3-8) were the most prominent when the values of TOC, $\delta^{13}C$ and C/N fluctuated (Site EH-1:0-12 cm; Site EH-3: 0-10 cm). When the values of TOC, $\delta^{13}C$ and C/N were relatively stable, *Firmicutes* (bands EH-1-3, EH-2-3 and EH-3-7) and *Acidobacteria* (bands EH-1-10, EH-2-6 and EH-2-12) were the most prominent. When the values of $\delta^{13}C$ and C/N were relatively stable but TOC fluctuated (Site EH-2: 0-8 cm), the

Tab. 1. Environmental parameters (mean and range) of the three sampling sites located in Lake Erhai.

Variable	Site EH-1		Site EH-2		Site EH-3	
	Mean	Range	Mean	Range	Mean	Range
Total nitrogen (mg/g)	1.76	1.47~3.02	2.21	2.56~4.27	3.01	1.51~8.71
Total phosphorus (mg/g)	0.76	0.725~0.805	0.97	0.757~1.131	0.72	0.627~0.929
Total organic carbon (%)	1.75	1.43~2.77	2.12	1.47~4.52	2.36	0.97~8.01
$\delta^{13}C$(%)	-19.3	-22.2~-16.3	-23.8	-25.4~-21.0	-25.2	-26.0~-23.7
C/N	8.5	7.2~9.9	7.0	6.2~7.5	6.9	5.8~8.7
Water depth (m)		11.2		21		8.3
pH	7.2	7.2~7.4	7.0	6.8~7.2	6.9	6.7~7.1
Eh (mv)	-191	-247~-149	-156	-218~-106	-133	-165~-133
Shannon-Wiener diversity index (H')	0.99	0.82~1.30	1.34	0.73~1.49	0.88	0.30~1.25

C/N, TOC:TN ratio (TOC, total organic carbon content; TN, total nitrogen concentration).

samples were more diverse and there was no obvious predominant band. *Firmicutes* (bands EH-3-5, EH-3-6, EH-3-7 and EH-3-11) were observed almost throughout the core at Site EH-3.

Canonical correspondence analysis

Canonical correspondence analysis (CCA) based on DGGE data and environmental variables was carried out separately for the three sites. The results of CCA (Fig. 4) illustrate that the differences in the BCC were related to the three environmental variables (TN, TOC, and C/N; $P<0.05$) at Site EH-1 (Fig. 4A). The three variables and the two axes explained 35% and 27% of the observed variation in BCC, respectively. The first axis was positively related with the TN, TOC and C/N (r=0.91, 0.86 and 0.75, respectively). Alternatively, the results of CCA (Fig. 4B) illustrate that the differences in the BCC were related to the three environmental variables (pH, TP, and depth; $P<0.05$) at Site EH-2 (depth as a catagorical variable). The three variables and the two axes explained 48% and 41% of the observed variation in BCC, respectively. The first axis was positively related to depth (r=0.98) and negatively related to TP (r=-0.93), and the second axis was positively related to pH (r=0.63). Similarly, the results of CCA (Fig. 4C) indicated that the differences in BCC were related to the five environmental variables (pH,

Tab. 2. Dating results for sediments in Lake Erhai.

	Year		
Depth (cm)	Site EH-1	Site EH-2	Site EH-3
2	2001	1997	2000
4	1993	1984	1992
6	1985	1971	1984
8	1978	1959	1976
10	1970	1947	1968
12	1962	1934	1960
16	1943	1910	1936
20	1926	1884	1908
26	1906	1846	1870
32	1880	1808	1834
Sedimentation rate (cm/y)	0.25	0.16	0.18

Tab. 3. Phyogenetic affiliation of clones obtained from the DGGE profiles at the three sampling sites.

	No. of clones		
Taxon	Site EH-1	Site EH-2	Site EH-3
Betaproteobacteria	3	1	0
Gammaproteobacteria	0	1	0
Deltaproteobacteria	6	6	1
Firmicutes	3	3	4
Acidobacteria	2	3	0
Chloroflexi	2	7	6
Bacteroidetes	1	0	1
Thermodesulfobacteria	0	1	0
Spirochaetes	0	0	1
Bacillariophyta	1	1	0
Actinobacteria	0	1	0
Total	18	24	13

DGGE, denaturing gradient gel electrophoresis.

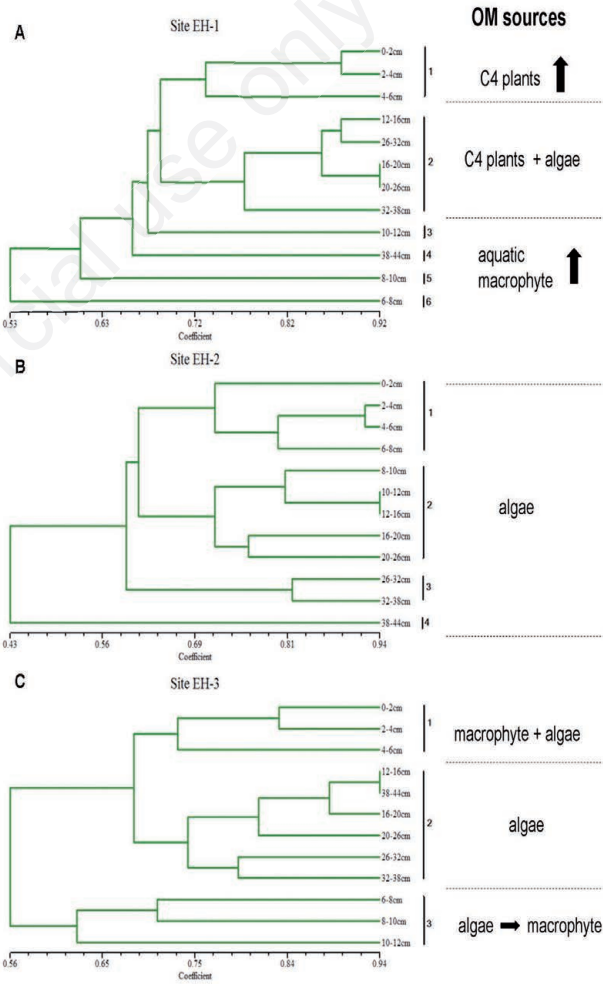

Fig. 3. Cluster analysis of bacterial community composition based on denaturing gradient gel electrophoresis (DGGE) profiles. The cluster results of bacterial community composition (BCC) with depths are shown. The capital letters A, B and C were used to distinguish the data of different Sites EH-1, EH-2 and EH-3, respectively. Organic matter (OM) sources in different segment of the cores are showed at the right side, the up arrow represent the increase of the OM source, the right arrow represent the succession of the OM source.

C/N, $\delta^{13}C$, TN, and depth; $P<0.05$) at Site EH-3. The five variables and the two axes explained 51% and 28% of the observed variation in BCC, respectively. The first axis was positively related to depth ($r=0.86$), and the second axis was negatively related to the pH, C/N, $\delta^{13}C$ and TN ($r=-0.93$, -0.79, -0.77 and -0.69, respectively).

The CCA biplot revealed that the samples from Sites EH-1 and EH-2 formed three clusters, whereas Site EH-3 formed two clusters (Fig. 4). The samples collected at Site EH-1 from 2 to 10 cm and from 12 to 20 cm formed one cluster, whereas the remaining samples (except the 0~2 cm sample) formed the second cluster. The 0~2 cm sample was quite different from the others due to its high concentrations of TN and TOC. The samples collected at Site EH-2 were grouped into 3 clusters. Cluster 1 consisted of samples from 0 to 8 cm, cluster 2 contained samples from 8 to 26 cm, and cluster 3 was composed of samples from 26 to 44 cm. These results are similar to those obtained via the cluster analysis of BCC based on the DGGE proles (Fig. 3B). The samples collected at Site EH-3 grouped into two clusters. Cluster 1 consisted of samples from 0 to 8 cm, and cluster 2 contained samples from 8 to 44 cm (Fig. 4C).

DISCUSSION

In recent years, Lake Erhai has been considered as a transitional body of water, shifting between a mesotrophic and eutrophic state (Pan *et al.*, 1999). Lake Erhai is a closed or semi-closed plateau lake, whose sediment records provide a sensitive indicator of environmental changes, as well as fine- and high-resolution information (Chen and Wan, 1999). Several studies have investigated the climate and environmental changes recorded in Lake Erhai sediment by determining OM source and characterisation (Fu *et al.*, 2006; Kitagawa *et al.*, 2007; Guo *et al.*, 2011). Our study not only detected the variation in OM sources with depth but also revealed the major driving factors for bacterial community composition in the sediment of Lake Erhai.

C/N and $\delta^{13}C$ identifiers of organic matter source

In general, the C/N value can be used to distinguish between aquatic and terrestrial sources of OM in lacustrine environments (Meyers and Lallier-Vergès, 1999; Filippi and Talbot, 2005). Protein-rich lake phytoplankton have C/N values between 4 and 10, whereas the C/N values of higher plants on land, which are cellulose-rich and protein-poor, have C/N atomic ratios of >20 (Meyers and Ishiwatari, 1993). In contrast, the C/N values in aquatic macrophytes are higher than phytoplankton (Hedges *et al.*, 1988; Meyers, 1994). This distinction arises from the absence of cellulose in algae and its abundance in vascular plants. All the C/N ratios of the three sites are less than 10, with average values of 8.5 (Site EH-1), 7.0 (Site EH-2) and 6.9 (Site EH-3) (n=12) (Tab. 1). The values throughout the cores suggested that the contributors of sedimentary OM were predominantly aquatic sources.

Isotopic signatures of photosynthetically fixed organic

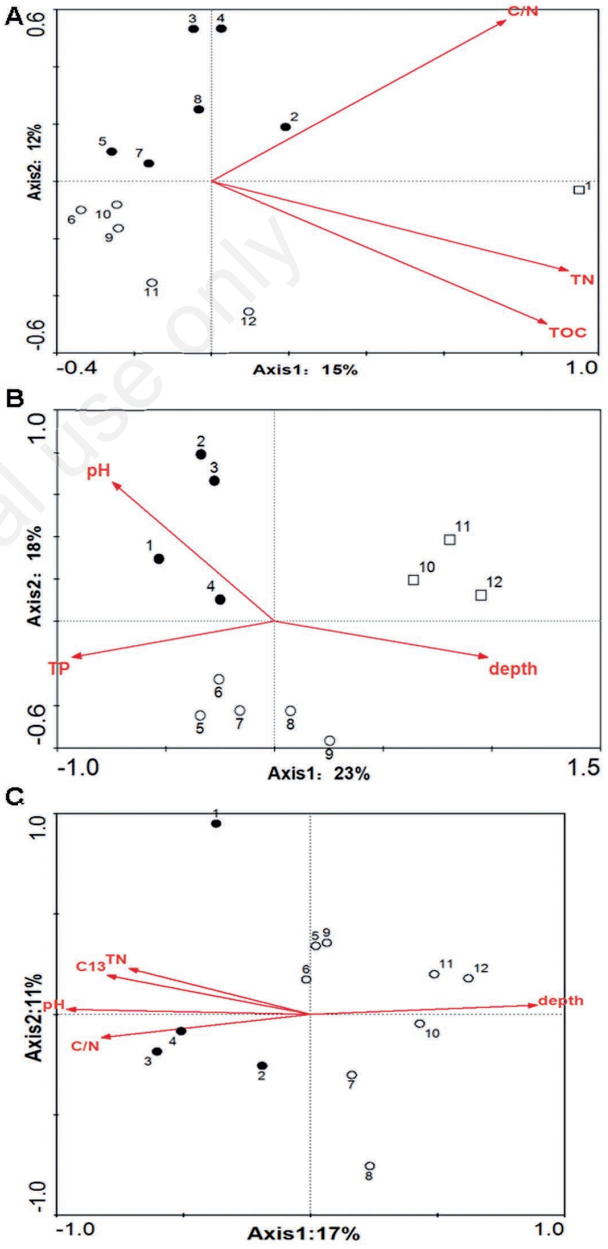

Fig. 4. Canonical correspondence analysis (CCA) biplot based on data from the denaturing gradient gel electrophoresis and environmental variables. The capital letters A, B and C were used to distinguish the data of different Sites EH-1, EH-2 and EH-3, respectively. The spots represent sediment samples in different layers. 1, 0~2 cm; 2, 2~4 cm; 3, 4~6 cm; 4, 6~8 cm; 5, 8~10 cm; 6, 10~12 cm; 7, 12~16 cm; 8, 16~20 cm; 9, 20~26 cm; 10, 26~32 cm; 11, 32~38 cm; 12, 38~44 cm.

carbon offer opportunities to trace sources of OM in sediment. Carbon isotopic ratios are useful for distinguishing between lacustrine and continental plant sources of sedimentary organic matter (Meyers, 1994). Algal organic matter typically has a distinctly different carbon isotopic composition than material produced by C4 plants growing either on land or the lake bottom (Meyers and Lallier-Vergès, 1999). The isotope carbon ratios for the C4 pathway is -10 to -15‰, whereas that of the CAM pathway can vary from -10‰~-20‰; the C3 pathway has a variability of -24~-30‰ (Fontugne and Jouanneau, 1987). Lake sediments with multiple sources of OM have variable $\delta^{13}C$ values (Talbot and Johannessen, 1992). OM produced by land plants using the C3 pathway consequently has an average $\delta^{13}C$ (PDB) value of ca -28‰, and the OM of plants using the C4 pathway has an average $\delta^{13}C$ (PDB) value of ca -14‰ (O'leary, 1988). Because submerged plants use carbon dioxide and carbonate in lake water to produce carbohydrate through photosynthesis, the $\delta^{13}C$ value is relatively positive; however, phytoplankton use atmospheric CO_2 for photosynthesis, thus resulting in more negative values of $\delta^{13}C$ (Stuiver, 1975). The $\delta^{13}C$ values for algae, which are similar to C3 plants, are below -25‰ (Lücke *et al.*, 2003).

Origin of sedimentary organic matter from aquatic as opposed to land sources can be distinguished by the characteristic C/N ratio compositions and carbon isotopic ratios (Fig. 5). The C/N and $\delta^{13}C$ values both increased from 6 cm to 0 cm of the sediment at Site EH-1, indicating a rise in the relative proportion of OM derived from C4 plants ($\delta^{13}C$ ranged from -10 to -15‰). An increase in the C/N ratios coupled with a decrease in the $\delta^{13}C$ values from 12 cm to 6 cm of the sediment suggested growth in the proportion of OM derived from aquatic macrophytes. The $\delta^{13}C$ values (-22.2 ~ -16.3‰) and C/N ratios recorded in the lower layers indicate that the source of OM is a mixture of C4 plant-derived and plankton-derived organic carbon (Fig. 2A). All C4 plants have a significant advantage over C3 plants under low atmospheric CO_2 condition and have the ability to evade drought. Within the monocots, C4 photosynthesis is quite common among grasses (*Poaceae*) and sedges (*Cyperaceae*) (Hattersley and Watson, 1992), for examples, corn (*Zea mays*), *Cyperis rothundus* and *Heleocharis dulcis*. Site EH-1 was located at the alluvial fan of the Yongan River and has an influx of OM pollution through non-point source agricultural run-off. It was also reported that the sources of sedimentary organic matter of Lake Erhai changed due to changes in terrestrial vegetation composition (Tareq *et al.*, 2011). We speculated that the C4 plant-derived OM observed at Site EH-1 were mainly from the water-carried plant debris by fluvial action of the inflowing rivers and terrestrial runoff. According to the dating data, the segment (6-12 cm depths) of the core covered the period from 1972-

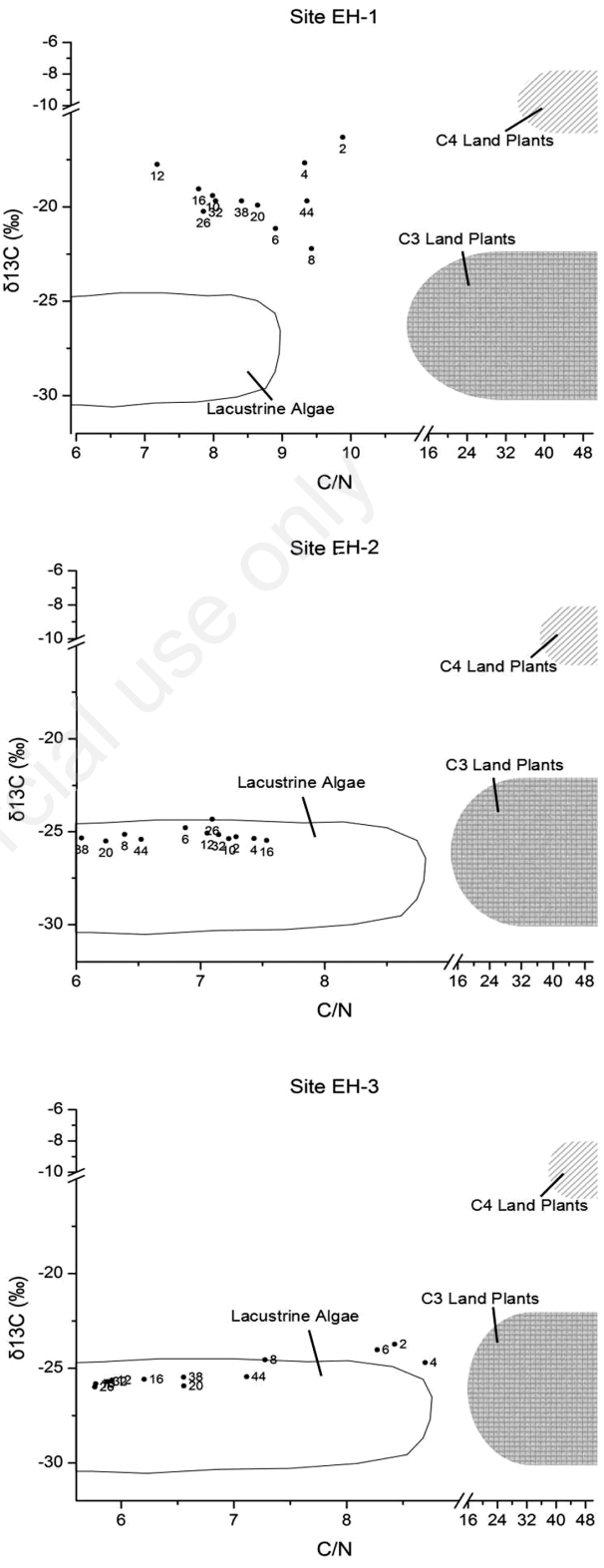

Fig. 5. C/N ratio and carbon isotopic compositions of organic matter from lacustrine algae, C3 land plants, C4 land plants that use CO_2 as their source of carbon during photosynthesis, and sediment samples of different depths. The spots represent sediment samples in different depths.

1982. The water level dropped during this period (Li Y. *et al.*, 2011), which could explain the growth in the proportion of OM derived from aquatic macrophytes in the lower (6-12 cm depths) part of the core.

The C/N ratios were smaller than 7, and the $\delta^{13}C$-values were below -25‰ across the core from Site EH-2, clearly indicating that the OM consistently had an algae origin (Fig. 2B). The identical changes in the C/N and $\delta^{13}C$ values revealed that the OM was also mostly derived from algae in the lower (below 10 cm) parts of the core from Site EH-3. An increase in the C/N ratio and $\delta^{13}C$ values was observed from samples taken from 10 cm below the interface up to surface sediment sample, indicating a change from algae to macrophytes as the major source of OM (Fig. 2C). The segment (6-10 cm depths) of the core covered the period from 1972-1982. As we mentioned above, the water level dropped during this period. Our result was consistent with the observation that the submersed macrophyte communities appeared before the year 2000 at Site EH-3. Water level rose from 1983 to 2006, during the fall of 1996, 1998, 2003, and 2006, there were four lake- wide Anabaena spp. bloom breakouts, especially in 1998 when the bloom lasted for more than 50 days (Wang *et al*., 2011). An decrease in the C/N ratio from 4 cm up to surface sediment indicated that the growth in the proportion of OM derived from algae during the 1994-2006 time period. These results maybe the main reason why the submersed macrophyte communities in these zones gradually disappeared during the 2003-2006 time period.

Environmental factors regulating the bacterial community composition

Canonical correspondence analysis is a powerful tool to illustrate how bacterial community structure varies along gradients of environmental variables (Salles *et al.*, 2004). The CCA results in this study revealed that the TN, TOC, C/N, $\delta^{13}C$, pH and TP, as well as depth significantly influenced BCC ($P<0.05$) in the lake sediments. We also observed that the depth-related BCC in different locations of Lake Erhai were significantly influenced by different environmental factors (Fig. 4 A,B,C).

The vertical profile of BCC ($P<0.05$) in the sediment at Site EH-1 was significantly influenced by the content of TOC, TN and the C/N ratio (Fig. 4A), indicating that both the content and source of OM have important influence on the microbial community structure. As mentioned above, the source of OM at Site EH-1 was a mixture of autochthonous and terrestrial organic carbon, both of which had an indirect effect on the bacterial community composition (Rooney-Varga *et al.*, 2005). Thus, changes in the content of organic carbon derived from different sources result in variations in the composition of the bacterial communities utilising the OM at Site EH-1. Similarly, the concentration of TN, C/N ratio and the $\delta^{13}C$ values at Site EH-3 were significantly related to the BCC ($P<0.05$); however, the concentration of TOC at Site EH-3 was not significantly related to BCC ($P>0.05$) (Fig. 4C), which demonstrated that the sediment bacterial community at Site EH-3 was much more sensitive to the content of TN than the content of TOC. These results suggested that the concentration of TN and OM sources, rather than the content of OM, had a pronounced impact on the vertical changes in BCC in the underwater platform of south Lake Erhai.

Our study indicated that TP significantly influenced ($P<0.05$) the sediment BCC at Site EH-2 (Fig. 4B). The concentration of TP explained over 20% of the observed variation in BCC at this site, indicating that the concentration of TP may be the major shaping force in the deepest point in middle Lake Erhai. The effect of TP on the diversity and composition of bacterial communities has been reported (Lindström and Bergström, 2005), they noted that TP concentration statistically explained the differences between the microbial community compositions in two different drainage areas. The TP values were significantly higher ($P<0.05$) at Site EH-2 (Tab. 1) compared to the other sites, which suggests that the substrate phosphorus content is rich in middle lake sediment. Moreover, the concentrations of TP in the 0-26 cm sediment layers were greater than those in the 26-44 cm layers (Fig. 2B). The measurement of BCC in samples with a higher phosphorus content (0-20 cm) were positively related with TP, whereas the BCC in layers with a lower phosphorus content (20-44 cm) were negatively related with TP (Fig. 4B). Our results indicate the sediment bacterial community composition was directly driven by the total phosphorus concentration rather than the content and source of OM in middle lake sediment. We speculated that at the deepest point of the lake, the P contained in organic matter that reaches the bottom is of a lower concentration because they remain in the water column longer; thus, there is more time to be degraded by planktonic bacteria. Therefore, the bottom bacteria are limited by this nutrient.

The CCA results revealed that pH has a pronounced impact on the bacterial community composition ($P<0.05$) at Site EH-2 and EH-3 (Figs. 4 B,C). pH may not only reflect changes in other environmental factors (Koski-Vähälä *et al.*, 2001), which can have both inhibitory and stimulative effects on bacterial communities (Zeng *et al.*, 2009), but may also influence bacterial communities through direct biological mechanisms (Yannarell and Triplett, 2005).

We found that depth was one of the most influential factors ($P<0.05$) on sediment BCC at sites EH-2 and EH-3 (Fig. 4 B,C). Our results agree with the observation of several studies that BCC in sediment is related to a depth gradient (Hewson and Fuhrman, 2006; Zhao *et al.*, 2008; Liu *et al.*, 2010). The depth gradients in redox potential

and oxygen concentration should consistently affect the vertical distribution of bacterial communities (Tšertova *et al.*, 2013). Nevertheless, the CCA results suggested that depth did not have a marked effect on BCC in the sediments at Site EH-1. Site EH-1 is located in Hongshan Bay, which is the lower edge of the alluvial fan of the Yongan River. This area has an influx of OM pollution through non-point source agricultural run-off. We assume that ambient interference is efficient at vertically shaping bacterial communities, and the intensive resuspension of the soft sediment is a major factor that causes the homogeneous community distribution.

Taxonomic groups and their distribution associated with organic matter sources

In the present study, the majority of the bacteria sequences obtained from the three sampling sites were affiliated with the divisions *Proteobacteria*, *Firmicutes*, and *Chloroflexi* (Tab. 3). Clones affiliated with *Acidobacteria, Bacteroidetes*, *Thermodesulfobacteria*, *Spirochaetes* and *Actinobacteria* were also found in low numbers (<6 sequences per division).

A total of 18 clones were affiliated with *Proteobacteria*, and most of these clones were from the subdivision *δ-Proteobacteria* (Supplementary Fig. 2). The majority of the *δ-Proteobacteria* sequences fell into two distinct families, *Pelobacteraceae* and *Desulfobacteraceae*, both of which are sulphate-reducing bacteria. Sulphur-reducing bacterial communities are important in organic carbon oxidation in sediments. This observation is supported by strong evidence that sulphates are one of the main electron acceptors present in these environments (Ghosh *et al.*, 2010). The oxidation of reduced S species and/or sulphur-reduction is thought to be essential for energetic metabolism in these areas (López-García *et al.*, 2003). In the present study, we found that 4 of the 5 *δ-Proteobacteria* clones (bands EH-1-11, EH-1-12, EH-1-16 and EH-1-18) at Site EH-1 seemed to be predominant in the middle part (6-12 cm depth) of the core and then decreased upward to the water-sediment interface. One clone (band EH-1-16) was absent in shallower sediment levels (Supplementary Fig. 1A). In total, 6 *δ-Proteobacteria* clones (bands EH-2-13, EH-2-14, EH-2-15, EH-2-19, EH-2-20 and EH-2-22) at Site EH-2 appeared in all layers throughout the core. As mentioned above, the OM in the middle (6-12 cm depth) part of the core from Site EH-1 mainly originated from aquatic macrophytes, and C4 plants contributed to the increased sediment OM in the upper (0-6 cm depths) portion of the core. By contrast, the OM at Site EH-2 originated from algae across all sediment layers investigated in this study (Fig. 2 A,B). It was reported that compared to autochthonous OM, terrestrial carbon is relatively more resistant to biodegradation due to its complex structure (Meyers, 1994; Mccallister *et al.*, 2006). The cultured members of sulphur-reducing bacterial families predominantly oxidise low-molecular-weight organic acids, and their substrate range does not include high molecular weight carbohydrates in the sediment (Widdel and Bak, 1992), which may explain the decreased abundance of δ-*Proteobacteria* subdivisions coinciding with the elevated proportions of C4 plant-derived organic carbon (Fig. 2A).

A total of four clones grouped in the β-*Proteobacteria* and one clone in the *γ- Proteobacteria* subdivisions were detected at the three sites (Tab. 3). The members of the β-*Proteobacteria* constituted an important fraction in the sediments of the more eutrophic reservoirs, whereas γ-subgroup *Proteobacteria* were most frequently detected in sediment samples from the dystrophic reservoir (Wobus *et al.*, 2003). In our study, 3 of the 4 clones grouped in the β-*Proteobacteria* subdivisions and no clone belonging to *γ-Proteobacteria* were observed in the sediment at Site EH-1 (Tab. 3), indicating that the water body at Site EH-1 was eutrophic. Our results agree with the observation of several studies that the water quality in the northern area of Lake Erhai has deteriorated (Guo *et al*. , 2001, Yang and Song, 2006). In Addition, we found that all β-*Proteobacteria* clones (bands EH-1-6, EH-1-7, EH-1-8, EH-1-9, EH-2-4) detected in our study appeared as prominent phylotypes in the upper sediments (Supplementary Fig. 1 A,B). The results agree with previous reports that β-*Proteobacteria* was the predominant group in upper sediments (Liu *et al.*, 2010); the prevalence of β-*Proteobacteria* might be a general feature of microbial communities in habitats with high loads of organic carbon. DGGE is not sufficient to catch the whole community composition and reveals only the most abundant taxons. Thus, there could be other results when using another method.

It was reported that C*hloroflexi* are likely to contribute to the diversified repertoire of polysaccharide hydrolases in sediments (Teske *et al.*, 2011). *Chloroflexi* are a dominant microbial community component of deep marine sediments and include obligate anaerobic dehalogenators and diverse sugar and amino acid fermenters within the recently defined class *Anaerolineae* (Teske, 2006). *Anaerolineae* are often isolated from anaerobic digesters in wastewater treatment plants or from industrial fermenting bioreactors; some recently isolated strains within Anaerolineae are able to grow slowly on xylan and starch (Yamada *et al.*, 2006). Clones affiliated with the class *Anaerolineae* of the *Chloroflexi* phylum were also detected in our study (Supplementary Fig. 2). Several uncultured *Chloroflexi* populations were shown to express chitinase, esterase, galactosidase, and glucuronidase activity (Kragelund *et al.*, 2007). Therefore, in the present study, *Chloroflexi* was the most prominent when both the source and content of OM changed. In total, 30% and 46% of the clones grouped in the C*hloroflexi* were detected at Site EH-2 and EH-3, respectively, indicating that the sedimentary microbial communities at the two sites had broad enzymatic capabilities

on diversified OM. This result may explain why the BCC in the sediments from Site EH-2 and Site EH-3 were not sensitive to the content of TOC.

Firmicutes are seldom discussed in previous studies of microbial ecology due to their low frequencies in the libraries (Eiler and Bertilsson, 2004; Wu *et al.*, 2007). Nevertheless, three, three and four clones were detected in our study at Sites EH-1 EH-2 and EH-3, respectively (Tab. 3). Most of the clones fell into the class Bacilli, may be that Bacilli sequences found in the sediments belonged to spores from soil-derived Bacilli. It was reported that members of the *Firmicutes* assimilate algal extracts quickly (Teske *et al.*, 2011). In the present study, the appearance of *Firmicutes* in all layers of the core from all sites suggested that their distributions were not significantly influenced by the OM source.

The relationship between the organic matter sources and the diversity of bacterial community

In the present study, no significant correlation ($P<0.05$) could be observed between the physicochemical parameters and the diversity of the bacterial community (H') at all the observed sites, which was consistent with a previous study in sediment of Lake Taihu by Zhao *et al.* (2008). They speculated that the bacterial community structure in sediment was primarily regulated by integrative conditions rather than a single factor. Nevertheless, we also found that the bacterial community diversity was directly influenced by the OM source. The constant OM sources at Site EH-2 resulted in a stable higher diversity of the bacterial community as compared to other two sites, whereas the variable OM sources at Sites EH-1 and EH-3 contributed to the changes in H'. We can conclude that differences in the diversity of bacterial communities in samples differing in their source of OM were related to environmental variables (*e.g.*, water level, river runoff and terrestrial vegetation composition). The greater number of phylum- and subphylum-level lineages and operational taxonomic units in Site EH-2 compared to the other sites may reflect a wider range of enzymatic capabilities and strategies to access OM.

CONCLUSIONS

Our detailed field survey on the relationships between depth-related bacterial community composition and physicochemical parameters showed that the depth-related variation in sediment bacterial communities in different areas of the lake was significantly influenced by varying environmental factors, however, the OM sources played important roles in structuring the BCC at all the sites. The sequencing of DGGE bands suggested that the majority of the sequences were affiliated with the following common phylogenetic groups in lake sediment: *Chloroflexi, Deltaproteobacteria*, and *Firmicutes*. The DGGE banding patterns revealed that the abundance of *Deltaproteobacteria* decreased with accompanying elevated levels of C4 plant-derived organic carbon. The Shannon-Wiener diversity index of bacterial communities was directly affected by the OM source, and constant OM sources resulted in a stable higher diversity of bacterial community. These may lead to broader understanding of microbial community structure and ecosystem functioning in mesotrophic lakes, although the mechanisms need further investigations.

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