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Phospholipid fatty acids analysis of the vertical distribution of microbial communities in eutrophic lake sediments

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ABSTRACT: Vertical distribution of microbial communities in a eutrophic lake sediments of Lake Xuanwu was quantified by phospholipid fatty acids analysis and multivariate statistical analysis was employed to interprete the data. Principle component analysis of sediment characteristics parameters, including total nitrogen, total phosphorus, organic matters and pH produced clustering of sampling sites for two distinct groups. These groups corresponded with the two sampling stations and the levels of nutrient enrichment. Total phospholipid fatty acids concentration, which is indicative of microbial biomass, reduced with depth, however, the relative percentage of anaerobic prokaryotes increased. To assess changes of microbial community along depth, phospholipid fatty acids compositions were analyzed by cluster analysis. Distinct clusters were observed in different sampling stations. Canonical correspondence analysis was carried out to infer the relationship between sediment characteristics and microbial communities. Phospholipid fatty acids samples collected at the same sampling site clustered together. Canonical correspondence analysis revealed that the environmental parameter with the greatest bearing on the phospholipid fatty acids profiles was pH. This study proved the successful application of phospholipid fatty acids and multivariate analysis to investigate the relationship between environment factors and microbial community composition.

Keywords: *Depth; Lake Xuanwu; Microbes; Multivariate analysis*

INTRODCTION

Lake sediments are vertically structured ecosystems in which microbial activity is predominantly influenced by the availability of nutrient elements and organic matters (OM). The close interchange of chemical, physical and biological processes along vertical gradient also provides niches for metabolically diverse microorganisms. Most previous studies in microbial ecology have focused exclusively on the surface sediment of the lake ecosystem (Amani *et al.*, 2011; Zeng *et al.*, 2008). However, large numbers of microorganisms residing in subsurface sediment, play an important role in nutrient cycling and contaminant degradation (Sahm *et al.*, 1999; Koizumi *et al.*, 2003a; Refaat, 2009). Meanwhile, the microbial communities in the deeper sediments may function differently from those at the surface and their metabolic properties could not be inferred by only studying the microbial communities found in the surface horizons (Ravenschlag *et al.*, 2000; Koizumi *et al.*, 2003b; Ajeagah *et al.*, 2007). There has been several studies concern the investigation of microbial community in the lake sediments based on cultivation, which could not avoid the biases of the true community composition (Jørgensen and Bak, 1991; Parkes *et al.*, 1994; Delille, 1995). Phospholipids are found exclusively in cell membranes and are rapidly metabolized after cell death (Macalady *et al.*, 2000; Mallet *et al.*, 2004). For these reasons, phospholipids could be used to characterize the community structure of viable microorganisms. Phospholipid

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fatty acid analysis (PLFA) is an established method for estimating microbial biomass and analyzing microbial community structure in complex environmental samples (Sundh *et al*., 2005; Dong *et al*., 2006; Zink *et al*., 2008). Additionally, most previous studies only focused on the distribution of pollutants in lake sediments (Nabulo *et al*., 2008; Nouri *et al*., 2008; 2009; Harikumar *et al*., 2009; Atafar *et al*., 2010; Mohiuddin *et al*., 2010), the effects of physicochemical factors of the lake sediment on the vertical distribution of microbial community structure should receive more concern (Babel *et al*., 2009).

Multivariate statistical analysis including principal components analysis (PCA), multidimensional scaling analysis (MDS) and canonical correspondence analysis (CCA) have been proven as promising approaches for investigating the profile of fatty acids.

In these approaches, samples could be clustered to several groups according to their overall similarity, and the effects of environmental parameters could also be considered.

Further analysis of the differentiation between fundamental taxonomic groups such as aerobic and anaerobic is also available. At last, the presence and abundance of discrete biomarkers could be used to assess the changes in the abundance of specific genera or groups (Ben-David *et al*., 2004; Nwuche and Ugoji, 2010). There have been studies investigating the impacts of acid rock drainage, petroleum hydrocarbon contamination and uranium on the microbial community compositions based on the PLFA and multivariable analysis (Ben-David *et al*., 2004; Syakti *et al*., 2006; Small *et al*., 2008).

These studies showed that different environmental parameters could induce various effects on the changes of microbial community composition. However, the use of PLFA and multivariable analysis to characterize the vertical profile of microbial communities in eutrophic lake sediments was seldom previously described.

This study was carried out at Lake Xuanwu, which is a typical urban, shallow and eutrophic lake located in the north part of Nanjing city in May of 2010. The current status of Lake Xuanwu is eutrophication and the recreational value of this lake is also influenced. By far, there was only few studies concerned the concentrations of nitrogen and phosphorus in the sediment of Lake Xuanwu.

The aim of this study was to investigate the vertical distribution of microbial community present in the sediments of Lake Xuanwu. Additionally, the effects of several environmental factors, such as sampling site, depth, pH, nutrients and OM, on microbial community composition were assessed with multivariate analysis techniques.

MATERIALS AND METHODS

Study sites

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. Hydrological information about Lake Xuanwu has been described by Zhang *et al*. (2007). Two sampling stations were chosen for sediment sampling. The first sampling station S1 (32º052 14.523 N 118º472 17.713 E) is located near the Nanjing railway station, which is enriched with high concentrations of nitrogen and phosphorus nutrients. The eutrophication of this station was mainly caused by the domestic wastewater discharged from a hotel. The second sampling station S2 (32º042 58.573 N 118º472 22.923 E) is located in the centre of the lake.

Samples collection and measurements of physicochemical parameters

Three replicate sediment samples from each sampling station were collected with a columniform core sampler (DM60, Mingyu, China). The sediment sampler was made of plexiglasses.

The diameter and height of the sampler was 12 and 60 cm. The sampler was connected with a metal bar (length: 1.5 m) for sampling and the collected samples were sectioned into six strata with a sterile spatula (0-3, 3-6, 6-11, 11-16, 16-21, 21-26 cm). pH was measured in situ with specific electrodes (PHB-4, REX, China). Three replicate sediment samples for microbial community structure analysis from six depth strata were homogenized and stored at -80 °C. Sediment samples were dried with a Freeze Dryer (ALPHA 1-2, CHRIST, Germany). Total nitrogen (TN), total phosphorus (TP) and OM were measured according to Jin and Tu (1990) and the results were expressed as mg/g (dry weight). Each sample had three replicates.

Lipid extraction and gas chromatography mass spectrometry (GC-MS) analysis

Frozen sediment samples (5 g) were extracted overnight by the modified method described by Bligh and Dyer (1959). Phospholipids were converted to

fatty acid methyl esters (FAME) by heating with 3 mL of 0.5 % methanolic hydrochloric acid (HCl). Hexane/chloroform (4:1 v/v) were used to extract FAME and the solution was evaporated under a stream of nitrogen. Internal standards of C 20:0 ethyl ester was employed and the FAMEs were dissolved in hexane for chromatographic analysis. FAMEs were stored in GC vials at -20 °C until GC-MS analysis. Samples (1 µL) were injected by Triplus autosampler of a DSQ II Single Quadrupole GC/MS (ThermoQuest, San Jose, CA, USA) equipped with a fused-silica DB-5 MS capillary column (25 m × 0.32 mm i.d., 0.25 µm film thickness). Full scan mode was used for the quantification of FAMEs. Helium was the carrier gas at a constant flow of 1.0 mL/min. The ion source temperature was maintained at 200 °C. The column was maintained at 50 °C for 1 min and the oven was programmed to increase to 260 °C at 5 °C/min. Data collection was initiated after the hexane solvent was eluted (3.0 min) and continued until no further peaks were observed. All phospholipid ester-linked FAME were identified based on the relative percentage of the ions scanned and by comparison of retention times to the standard ethyl ester. Concentrations of individual FAME were determined by calibration with internal standard.

Multivariable statistics analysis

PCA was performed using MVSP 3.13i software package (Kovach Computing, Anglesey, Wales) to study the physicochemical parameters at different depths of the lake sediments. Clustering analysis of the phospholipid fatty acid composition was carried out to investigate the similarities of the microbial community structure in sediment samples.

The relationship between phospholipid fatty acid composition 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*., 2007) so CCA was performed to explain the data by CANOCO 4.5 (Biometris, Wageningen, Netherlands) (Salles *et al*., 2004; Sapp *et al*., 2007). Ordination biplots including the phospholipid fatty acid composition and environmental variables were used to explain the data. The detailed interpretation of the ordination plots could be referred to Ter Braak (1987).

RESULTS AND DISCUSSION

Physicochemical properties of the sediments

The vertical variations of the physicochemical properties of the sediments at two sampling stations are shown in Fig. 1. pH values at station S2 (7.53 to 8.27) are higher than S1 and it decreased along depth gradually. The concentrations of OM, TN and TP at sampling station S1 ranged from 78.6 to 104.6 mg/g, 3.84 to 7.24 mg/g and 1.02 to 1.25 mg/g, respectively, which were clearly higher than those of station S2 (OM: 17.4 to 45.9 mg/g; TN: 1.57 to 2.91 mg/g; TP: 0.49 to 0.69 mg/g).

The OM concentrations decreased gradually with depth at station S2. However, significant increases of OM were observed at the depths of 13.5 and 18.5 cm at station S1. TN contents decreased with depths at station S2 (2.91 to 1.57 mg/g). However, the highest content of TN was registered at the deeper strata (18.5 cm, 7.24 mg/g) at station S1. Data on TP variations along depths are also presented in Fig. 1. Station S1 presented the higher values of TP, while at station S2 it kept nearly constant with the increased depths (0.49 to 0.69 mg/g). Additionally, the total microbial biomass could be calculated by summation of all detectable PLFAs (Findlay, 1996). In this study, the total concentrations of PLFA varied dramatically at station S1 (1.66 to 8.32 µg/g) while it decreased with depths gradually at station S2 (12.24 to 2.05 µg/g). The PCA ordination of the twelve sediment samples collected from two sampling sites of Lake Xuanwu was shown in Fig. 2a. The first axis and the second axis explained 86.59 % and 8.77 % of the total variance, respectively. Variables such as TN and OM weighed most heavily on the first axis, while the second axis was better correlated with pH and TP. Six samples from station S2 formed individual cluster, indicating the relative stable sediment properties in the lake center. Samples collected from S1 station show discrete distribution, which suggests remarkable difference in physicochemical parameters along depth. The influence of domestic wastewater discharged from a hotel near station S1 may explain the elevated nutrient loading and fluctuating physicochemical characteristics at this station.

PLFA composition

PLFA extracts of sediments samples from Lake Xuanwu were analyzed by gas chromatogram mass analysis and individual PLFA was quantified

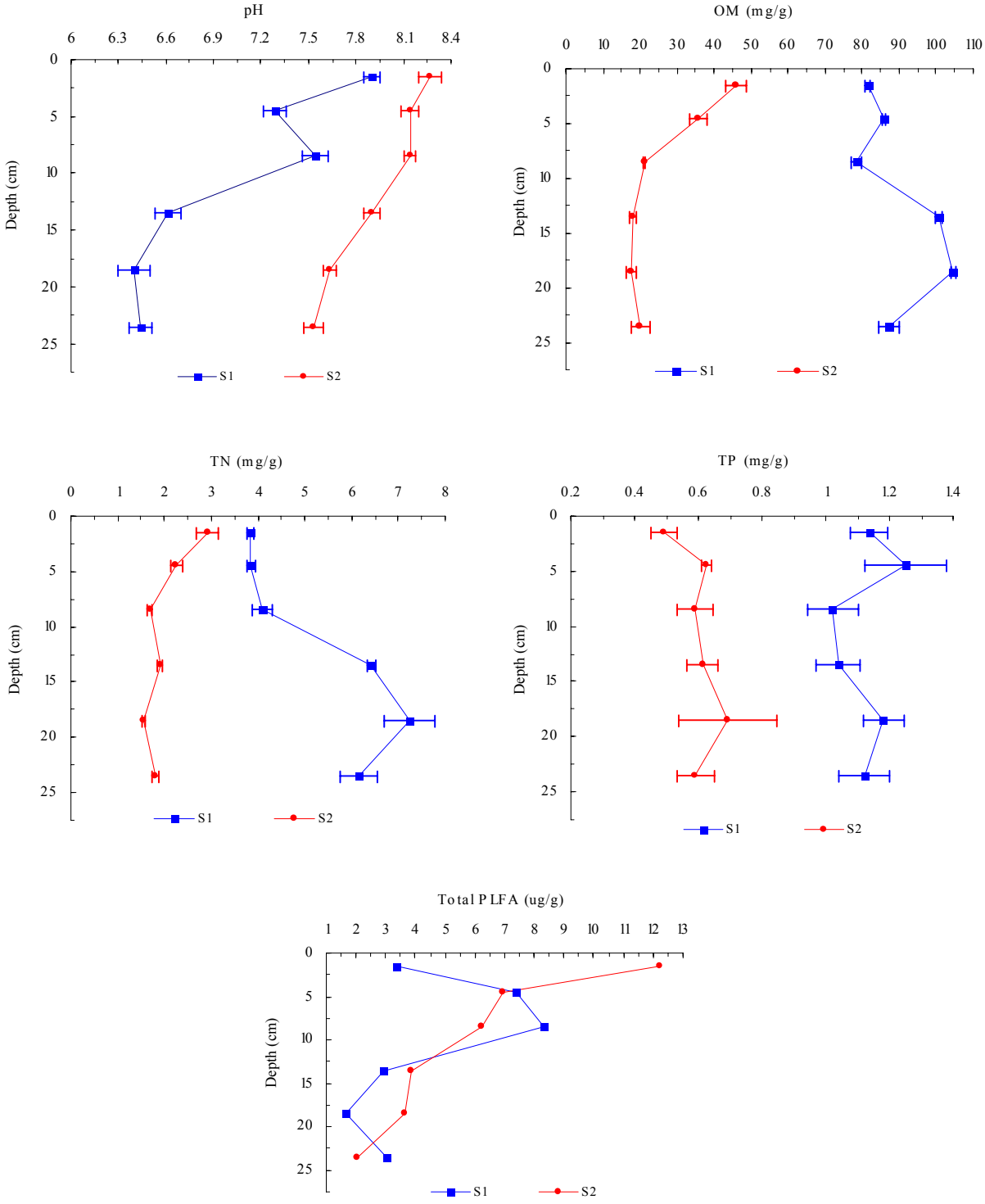

Fig. 1: The vertical variation of the physicochemical parameters in the sediments of Lake Xuanwu. Error bars represent the standard deviation of three replicates

(Table 1). Twelve kinds of fatty acids containing 15~19 carbon atoms, including saturated (odd and even), chain branched (iso and anteiso) and monounsaturated fatty acids were detected. The PLFA 16:0, 17:0 and 17:1w7 were the fatty acids with the highest relative abundance in the samples. Major PLFA components also included a18:0 and 19:1w8. The relative abundances of saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) in each sample were also calculated and the results are shown in Table 1.

Dendrograms were constructed based on the relative percentages of fatty acids present in different depths of the lake sediments (Fig. 2b). Generally, sampling stations of S1 and S2 demonstrated distinct clusters, indicating the composition of phospholipid fatty acid differed significantly at the two sites. S1-5 and S2-6 grouped far from the other clusters due to the sharp increase of fatty acids i19:0 and 17:0 at the two strata. These groupings suggest that the sampling stations that had similar sediment characteristics also had similar microbial community composition. Mucha *et al*. (2004) also reported that similar environmental parameters in the sediment resulted in similar macrobenthic community structures. Fig. 3 shows the vertical changes in the relative abundance of microbial functional groups in sediments of Lake Xuanwu. At station S1 (Fig. 3a), the relative percentage of microbial group I (aerobic prokaryotes) was 33.99 % at the first strata (0-3 cm) and it decreased gradually with depth, the minimize percentage (10.42 %) appears at the 16-21 cm strata. Generally, the relative percentages of gram-positive and other anaerobic bacteria group increased with depth at station S1. The vertical distribution of sulfate reducing bacteria (SRB) increased a little with depth at station S2 (Fig. 3b). However, the relative percentages of gram-positive and other anaerobic bacteria did not show remarkable variations at this station. Aerobic prokaryotes decreased from 37.06 % in the top stratum to 26.43 % in the deepest stratum. According to Komagata and Suzuki (1987), most of the identified 12 PLFAs in the sediments are affiliated to be prokaryotic. The presence of high proportional MUFAs in all the samples is a further indication of dominated prokaryotes (Ratledge and Wilkinson, 1988). Interpretation of the PLFA data based on the functional group approach revealed aerobic prokaryotes followed by SRB and other

Table 1: Relative percentages of PLFA in different depths of sediments from Lake Xuanwu

PLFA	S1-1	S1-2	S1-3	S1-4	S1-5	S1-6	S2-1	S2-2	S2-3	S2-4	S2-5	S2-6
i15:0	4.07	1.72	1.18	4.25	1.83	3.17	1.93	2.25	1.3	3.3	2.33	1.8
15:1w3	2.01	2.18	4.84	N.D.	1.05	0.47	0.2	0.1	0.1	0.1	0.1	0.1
15:0	0.2	0.1	0.2	0.1	0.1	0.1	3.55	1.81	0.82	2.15	4.08	1.91
16:0	12.47	15.01	10.03	15.84	8.83	14.81	18.79	15.87	11.02	13.16	16.73	10.32
i16:0	3.64	4.88	4.63	6.99	8.96	7.35	0.1	0.1	0.2	0.2	0.1	0.3
17:0	23.18	26.5	25.65	31.21	26.43	34.82	22.62	26.15	27.21	28.71	26.32	35.25
17:1w7	17.51	16.49	13.43	6.03	N.D.	5.81	15.65	17.53	15.77	12.25	13.95	N.D.
a17:0	1.83	1.91	3.5	3.91	2.82	2.42	3.71	1.75	5.39	1.41	2.21	2.13
18:0	0.2	0.1	0.1	0.1	0.1	0.1	1.2	4.04	N.D.	1.41	N.D.	1.6
a18:0	13.79	13.18	21.79	12.92	7.96	10.53	4.63	4.71	5.43	5.21	3.44	6.72
19:1w8	14.47	13.05	9.25	8.49	9.33	11.11	21.21	19.63	24.83	22.25	19.51	26.33
i19:0	6.64	4.88	5.4	10.16	32.59	9.31	6.41	6.06	7.93	9.85	11.23	13.54
ΣSFA*	66.02	68.28	72.48	85.48	89.62	82.61	62.94	62.74	59.3	65.4	66.44	73.57
ΣMUFA**	33.99	31.72	27.52	14.52	10.38	17.39	37.06	37.26	40.7	34.6	33.56	26.43

* ΣSFA: Saturated fatty acid; ** ΣMUFA: Monounsaturated fatty acid

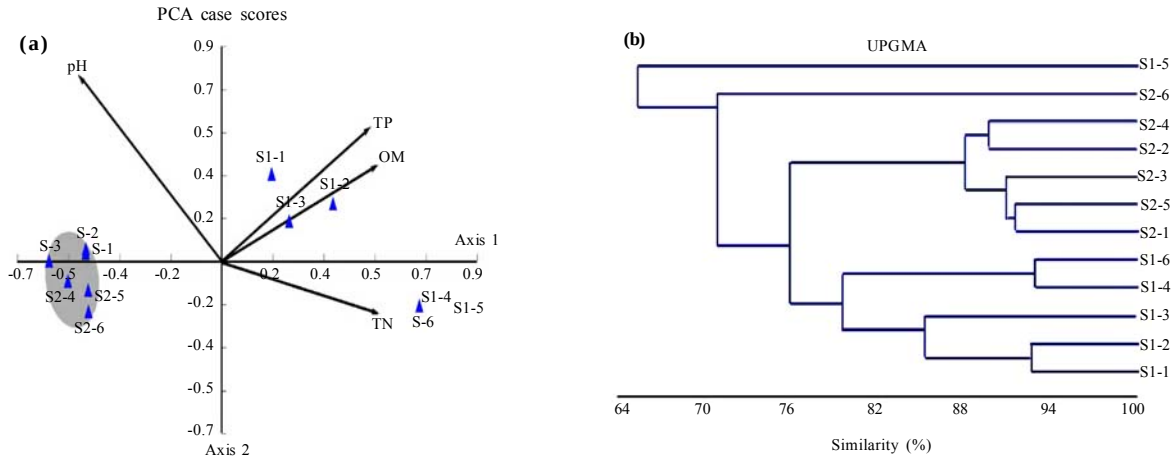

Fig. 2: PCA of the chemical parameters from sediments of Lake Xuanwu (a). Group was indicated with a gray background. Dendrogram for the PLFA from sediments of Lake Xuanwu over different depths (b). Similarities were calculated according to the unweighted pair group method analysis (UPGMA)

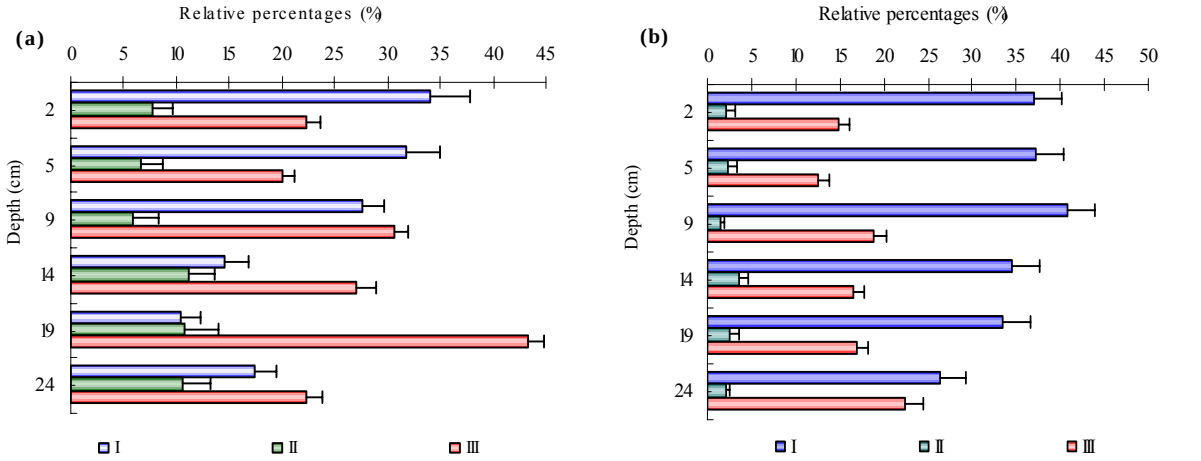

Fig. 3: Vertical changes in the relative abundance of microbial functional groups in sediments of Lake Xuanwu. (a) Station S1; (b) Station S2. I: Aerobic prokaryotes; II: Gram-positive and other anaerobic bacteria; III: SRB and other anaerobic prokaryotes

anaerobic prokaryotes were the predominant components of the prokaryotic community in the sediments (Findlay, 1996). However, SRB may be overestimated due to several SFAs may have a broader phylogenetic distribution. At the same time, a shift in the microbial community structure along depth was observed at both sampling stations. This alteration was manifested by the reduction in relative abundance of aerobic prokaryotes and the increase of SRB and other anaerobic prokaryotes.

Microbial community composition in relation to sediment physicochemical properties

In the freshwater sediment ecosystem, microorganisms play an important role in the process of nutrients transformation and decomposition. Vertical variation of sediment property provides niches for metabolically diverse microorganisms. In this study, the vertical variations of physicochemical factors and phospholipid fatty acid composition in the sediments of Lake Xuanwu were investigated to determine which

environmental parameter has the strongest effect on microbial community structure (Zink and Mangelsdorf, 2004; Deines *et al*., 2007; Shrestha *et al*., 2008; Bodelier *et al*., 2009; Gómez-Brandón *et al*., 2010).

CCA is a suitable statistic technique to investigate how microbial community structure varies along gradients of environmental variables (Salles *et al*., 2004). Bacterioplankton community compositions in relation to water chemistry in marine and freshwater ecosystems have been documented (Rooney-Varga *et al*., 2005; Haukka *et al*., 2006; Sapp *et al*., 2007). However, relationships between the microbial community in sediments and environmental factors were rarely reported.

Biplots consisting of environmental variables and phospholipid fatty acid samples were chosen to analyze the relationship between sediment physicochemical properties and the microbial community composition (Fig. 4). In the ordination plot, the first two CCA axes accounted for 68.0 % of the total variance and the first axis alone explained 40.0 %. The microbial community composition clearly clustered according to sampling site rather than sampling depth. Based on the 5 % level in a partial Monte Carlo permutation test, sampling site emerged as a highly significant explanatory variable ($p < 0.05$). However, sampling depth was not a significant environmental variable ($p > 0.05$).

Additionally, environmental parameter that has the

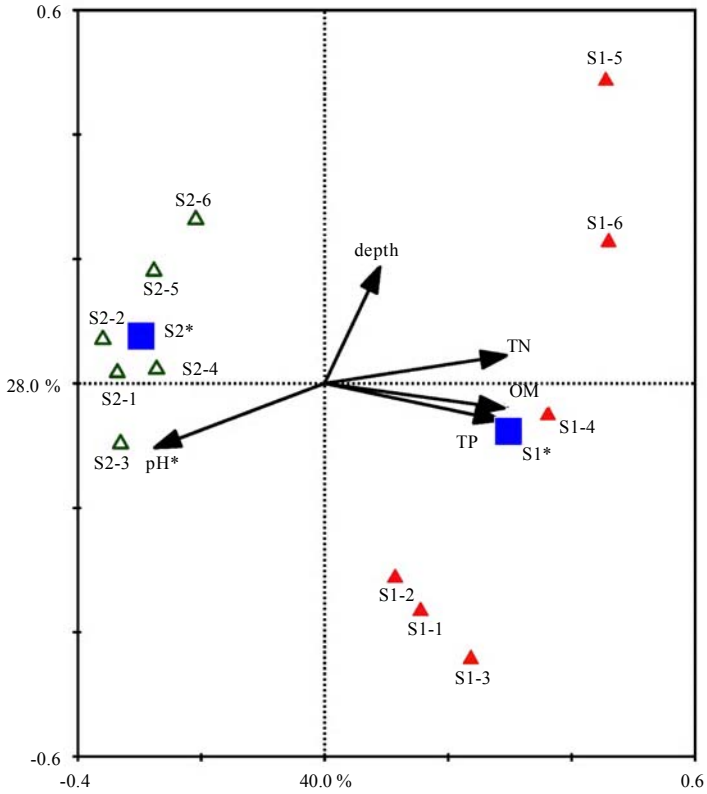

Fig. 4: Ordination diagram of phospholipid fatty acid composition associated with environmental variables of sampling depth, pH, TN, TP and OM. Environmental variables were indicated as arrows. The nominal variable ''sampling station'' was represented as centroids. Phospholipid fatty acid samples were indicated as (▲) Station S1, (△) Station S2. Environmental variables marked with asterisks were significant ($p < 0.05$)

greatest bearing on the PLFA profiles was pH. Variation of pH would influence other environmental factors, such as the availability of ions and trace metals (Koski-Vähälä *et al*., 2001), which could have both stimulative and inhibitory effects on microbial community. However, pH could also affect microbial community directly by biological mechanisms (Yannarell and Triplett, 2005). In this study, phospholipid fatty acid samples collected at the same sampling site clustered together and sampling depth did not present as 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) also 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 studies are needed to investigate the origin and formative process of microbial communities in lake sediments.

CONCLUSION

In conclusion, the results obtained in this study demonstrated that the PLFA for measuring microbial abundance and community structure could be productively applied to lake sediment. Multivariate statistical methods are effective in explaining the relationship between sediment quality and microbial PLFA. pH showed remarkable effects on microbial community structure in lake sediments. Investigating the relationship between sediment characteristics and microbial community composition would be useful, because sediment microorganisms play a crucial role in nutrients cycling, which is necessary to maintain aquatic ecosystem health.

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