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ARTICLE

# Higher seasonal variation of actinobacterial communities than spatial heterogeneity in the surface sediments of Taihu Lake, China

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**Abstract:** Much more attention has been paid to the actinobacterial community in soils or water columns of aquatic habitats. However, there are few studies on their composition and diversity in lake sediments. Here, we used denaturing gradient gel electrophoresis and clone libraries of partial 16S rRNA gene to study the spatial variations of actinobacterial communities across 4 seasons in the surface sediments of the shallow, subtropical Taihu Lake. Cluster analysis based on fingerprints showed clear spatiotemporal variations of actinobacterial communities and higher seasonal variation than spatial heterogeneity. Based on clone libraries, this pattern was supported by the principal coordinates analysis in the phylogenetic context and by detrended correspondence analysis on the operational taxonomic unit table. Additionally, phylogenetic analysis showed that the putative freshwater-specific actinobacterial lineages (e.g., acI) were also detected in the lake sediments, which suggests that these subclusters may also adapt to the sediment environments. Summarily, our results suggested that actinobacterial communities of the surface sediments were more affected by seasonal variation than spatial heterogeneity in the intrahabitat of Taihu Lake.

*Key words: Actinobacteria*, surface sediments, spatiotemporal variations, lake intra-habitat, Taihu Lake.

**Résumé :** Beaucoup d'attention a été porté aux communautés actinobactériennes de sols ou de colonnes d'eau. Néanmoins, certains questions persistent à propos de leur composition et de leur diversité dans les sédiments de lacs. Nous avons utilisé dans la présente étude l'électrophorèse sur gel en gradient dénaturant et des banques géniques de clones partiels de l'ARNr 16S afin d'étudier les variations spatiales de communautés actinobactériennes au cours des quatre saisons, dans les sédiments de surface du lac Taihu, une étendue de faible profondeur située en région subtropicale. Une analyse de groupement fondée sur les empreintes a révélé des variations spatio-temporelles évidentes des communautés actinobactériennes et une variation saisonnière plus importante que l'hétérogénéité spatiale. À partir des banques de clones, une analyse en coordonnées principales dans le contexte phylogénétique et une analyse des correspondances redressée de la table des UTO ont permis d'appuyer cette configuration. En outre, une analyse phylogénétique a montré que les lignées actinobactériennes potentiellement spécifiques à l'eau douce (p. ex. acI) étaient détectables dans les sédiments du lac, ce qui laisse croire que ces sous-groupes pourraient également s'adapter aux environnements sédimentaires. En bref, nos résultats ont indiqué que les communautés actinobactériennes des sédiments de surface étaient davantage affectées par les saisons que par l'hétérogénéité spatiale dans l'habitat intérieur du lac Taihu. [Traduit par la Rédaction]

*Mots-clés : Actinobacteria*, sédiments de surface, variations spatio-temporelles, habitat intérieur de lac, lac Taihu.

## Introduction

The phylum *Actinobacteria* is widely distributed in soil, marine, and freshwater habitats (e.g., Warnecke et al. 2005; Allgaier et al. 2007; Maldonado et al. 2009; Jiang et al. 2010; Aizenberg-Gershtein et al. 2012). For instance, *Actinobacteria* can account for 70% of the total bacterial communities in lake water (Allgaier and Grossart 2006; Stevens et al. 2007) and approximately 6% in lake sediments (Wang et al. 2013). Evidence of these Gram-positive bacteria indicates their importance in biogeochemical cycles, such as the degradation of organic matter and xenobiotic compounds. Although the *Actinobacteria* assemblages have been recovered from various habitats, less is known about their spatiotemporal variations in different habitats and the ecological factors regulating these distribution patterns.

Recently, growing attention has been paid to the distribution of actinobacterial communities in freshwater habitats (Glöckner et al. 2000; Warnecke et al. 2004; Lindstrom et al. 2005; Newton et al. 2007, 2011; Holmfeldt et al. 2009). Ecotypes of planktonic *Actinobacteria* with an identical 16S rRNA gene could adapt to different thermal niches in freshwater habitats (Hahn and Pöckl 2005). It has been reported that several specific subclusters of *Actinobacteria* were not isolated from other habitats but rather were indigenous to freshwater, and all these *Actinobacteria* from lake water columns could be clustered into acI, acII, acIII, acIV, and acTH, with some lineages (i.e., acI) particularly abundant (Warnecke et al. 2004; Newton et al. 2011). Generally, it is regarded that the freshwater lake *Actinobacteria* are free-living, open-water defense specialists (Newton et al. 2011).

Furthermore, potential factors underlying actinobacterial communities have been reported within seasonal and spatial scales in freshwater habitats (Glöckner et al. 2000; Allgaier and Grossart 2006; Allgaier et al. 2007; Holmfeldt et al. 2009). For instance, there was strong seasonal succession of actinobacterial communities in the water columns of 4 distinct tropic lakes, while there was less correlation between tropic status and community composition (Allgaier and Grossart 2006). At the spatial scale, it was revealed that the variation of actinobacterial communities in the lake water columns was related to several limnological features, such as conductivity, salinity, total phosphorous, and alkalinity

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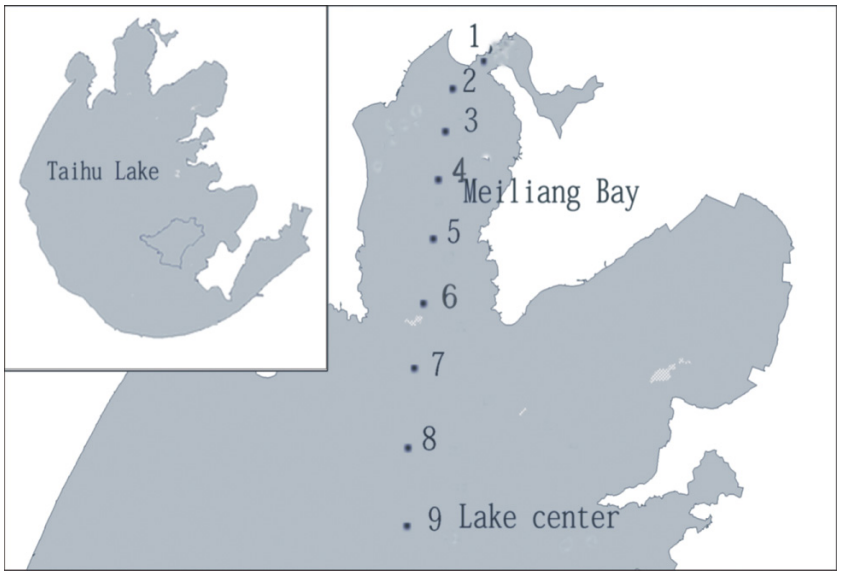
**Fig. 1.** The sampling locations in Taihu Lake (Sites 1–9). Three sites (1, 5, and 9) were sampled in February, May, and August 2007. In autumn 2007, all 9 sites were sampled.




(Allgaier et al. 2007; Holmfeldt et al. 2009). Although biogeographic signals have not been observed for actinobacterial taxa defined by any 16S rRNA gene groupings, pH differences among lakes and particle attachment and carbon substrate preferences within lakes have been shown to differentiate the clades (Newton et al. 2011).

Yet, still fewer studies investigate the variations of actinobacterial communities in lake surface sediments, especially within the seasonal and spatial scales, although there is a high relative abundance of *Actinobacteria*, e.g., approximately 6% in Taihu Lake sediments (Wang et al. 2013). Here, we studied surface sediment actinobacterial communities of Taihu Lake across 4 seasons using denaturing gradient gel electrophoresis (DGGE) and clone libraries. As the third largest freshwater lake in China, Taihu Lake covers large spatial scales with notorious algae blooms especially at the northern part (Duan et al. 2009). The aims of this study are (*i*) to examine the spatiotemporal variations of actinobacterial communities and (*ii*) to reveal whether the typical freshwater actinobacterial clusters (e.g., acI) can be observed in surface sediments.

## Materials and methods

### Sampling sites and methods

Taihu Lake is located in the southeast of China (31.181445°N, 120.137462°E) (Fig. 1). It is one of the main drinking water sources for local residents but also one of the most severely polluted freshwater lakes in China. The sampling sites were located from the Liangxi River mouth to Meiliang Bay to the lake center (Fig. 1). In February (winter), May (spring), and August (summer) 2007, the samples were collected in 3 locations. Site 1 was located approximately 1 km away from the Liangxi River discharge into Meiliang Bay, Site 5 at the inner Meiliang Bay, and Site 9 at the lake center (Fig. 1). In November (autumn) 2007, 6 extra sites were sampled along the transect (sites 2, 3, 4, 6, 7, and 8) (Fig. 1).

Three replicates of surface sediments (0–1 cm) for each site were sampled aseptically. The samples of each site were then homogenized, stored in one sterilized tube, and shipped to the laboratory in a 4 °C cooler within 12 h. In the laboratory, all the samples were freeze-dried and stored at –20 °C until molecular biological and geochemical analyses.

### Geochemical analysis

The temperature of the water and surface sediments was measured in the field. Total nitrogen (TN) and total phosphorus (TP) of the sediment were analyzed by molybdate reagent colorimetry after $HClO_4$–$H_2SO_4$ digestion (Wang et al. 2008).

### Genomic DNA extraction

Genomic DNA of the surface sediments was extracted using a modified method from Zhou et al. (1996). Briefly, about 0.3 g of each freeze-dried sediment sample was mixed with 1.65 mL of extraction buffer (100 mmol/L Tris–HCl, 100 mmol/L sodium EDTA, 100 mmol/L sodium phosphate, 1.5 mol/L NaCl, 1% CTAB, pH 8.0). The samples were mixed for 2 min and incubated for 30 min at 37 °C. Then 10 μL of proteinase K (20 mg/L) was added and incubated for 30 min at 37 °C. After this, 50 μL of 10% SDS was added to the tubes and incubated for 2 h at 65 °C, with periodic (every 10 min) mixing. The extractions were centrifuged (15 min, 10 000*g*, room temperature), and the supernatants were mixed with an equal volume of chloroform – isoamyl alcohol (24:1, *v*/*v*) and centrifuged as before. The supernatant was transferred into a new sterile tube by the addition of phenol – chloroform – isoamyl alcohol (25:24:1, by volume) (the equal volume with the supernatant). The above steps were repeated and after the final step, a 0.6 volume of cold isopropanol was added to the supernatant solutions. The samples were mixed at 37 °C, and the genomic DNA was precipitated by centrifugation (20 min, 13 000*g*, room temperature). The precipitate was washed with ice-cold 70% ethanol and dissolved in 100 μL of TE buffer. Furthermore, the total genomic DNA was purified with a UNIQ-10 Column DNA Gel Extraction kit (Shanghai Sangon, China) according to the manufacturer's instructions.

### PCR–DGGE and 16S rRNA gene clone library construction

For the partial 16S rRNA gene of *Actinobacteria* amplification, nested polymerase chain reactions (PCR) were performed with the first primers: Bac27F (5′-AGAGTTTGGATCMTGGCTCAG-3′) and Univ1492R (5′-CGGTTACCTTGTTACGACTT-3′). PCR reagents were mixed as follows: total 50 μL of volume containing 0.2 mmol/L dNTP, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L (each) primer, 5 μL of buffer (10× Pyrobest Buffer II, Takara, China), 1.5 U of rTaq (Takara, China), and 3 μL of template DNA. The mixture was amplified with the following conditions: 1 cycle at 95 °C for 1 min; 30 cycles

at 95 °C for 1 min, 52 °C for 1.5 min, and 72 °C for 2 min; followed by a final elongation step at 72 °C for 10 min. The PCR products were purified with a QIAprep PCR purification kit (Shanghai Sangon, China). For DGGE, an approximate 648 bp fragment of the 16S rRNA gene of bacteria was amplified with the forward primer GC-517f (5′-GTGCCAGCAGCCGCGG-3′) and the reverse primer 1165R (5′-ACCTTCCTCCGAGTTRAC-3′) (Gich et al. 2005) from the 20-fold dilution of purified PCR products with the primers Bac27F and Univ1492R. Each 50 μL PCR mixture contained 5 μL of 10× PCR buffer, 200 μmol/L nucleotide mixtures, 1.5 mmol/L $MgCl_2$, 0.5 μmol/L (each) primers, 1 U of rTaq (Takara, China), and 3 μL of templates. The PCR procedure was performed as before. The DGGE was performed on a DCode Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, California, USA). The PCR products were analyzed in 6% acrylamide gel with a denaturing gradient from 55% to 70% (100% denaturant corresponds to 7 mol/L urea and 40% formamide). Then the gel was run in 1× TAE buffer at 60 °C and 100 V for 16 h, stained for 30 min in 1× TAE containing SYBR Green I (Roche, Mannheim, Germany), and documented by the Gel Doc EQ gel documentation system (Bio-Rad).

For the clone libraries construction, we chose 9 sites: 8 from the river mouth (Site 1) and lake center (Site 9) for the 4 seasons, and 1 at Site 5 in the summer. A second PCR was performed with the special actinobacterial primers 517F and 1165R (Gich et al. 2005), and PCR amplified conditions were the same as before. Then the PCR products were ligated into the pGEM-T vector (Promega, Madison, Wisconsin) and transformed into TOP10 competent cells. Over 50 randomly chosen colonies per sample were checked for inserted 16S rRNA gene sequences, then sequenced commercially (Invitrogen, China).

### Statistical analysis

The DGGE bands were identified with software Bionumerics version 5.0 (Applied Maths, Sint-Martens-Latem, Belgium) and then the unweighted pair group method with arithmetic averages cluster analysis with Pearson's coefficient was used to find the main clustering groups following the previous study (Wang et al. 2008). Obtained clone sequences were manually checked for chimeras using the Ribosomal Database Project II, and identified chimera sequences were removed. A total of 283 actinobacterial sequences were subjected to phylogenetic analysis using the QIIME pipeline (version 1.2) (Caporaso et al. 2010; Wang et al. 2012). In brief, sequences were clustered into OTUs at 97% pairwise identity with the seed-based UCLUST algorithm (Edgar 2010). Chimeras were further removed via Chimera Slayer (Haas et al. 2011), and then representative sequences from each OTU were aligned to the Greengenes imputed core reference alignment (DeSantis et al. 2006). After removing gaps and hypervariable regions using a Lane mask, the alignments were then used to construct an approximate maximum-likelihood tree using RAxML version 7.0.3 (Stamatakis 2006) for phylogenetic analyses.

The principal coordinates analysis (PCoA) of weighted UniFrac metric (Lozupone and Knight 2005) with even-sampling was used to investigate the changes of actinobacterial communities within the spatiotemporal scales. Detrended correspondence analysis (DCA) based on OTUs table was also performed to reveal the main factors or gradients in the spatiotemporal distribution patterns. Chao1 richness (Chao 1984) and Faith's phylogenetic diversity (PD) (Faith 1992) were used to estimate the community diversity (Wang et al. 2012). Chao1 richness is a nonparametric estimator of richness (Chao 1984) that is computed as Chao1 richness = $S_{obs} + (a^2/[2b])$, where $S_{obs}$ is the number of species observed, and $a$ or $b$ are the number of species that are observed just once or twice, respectively. Faith's PD measures the total phylogenetic branch length that joins the basal node to the tips of all the species in the sample (Faith 1992). Multivariate analyses were conducted in R environment with Vegan package.

### Nucleotide sequences accession numbers

The partial 16S rRNA gene sequences were deposited in GenBank under the accession Nos. HQ214682–HQ214964.

## Results

### Physiochemical properties

TN and TP levels in the surface sediments decreased from Liangxi River mouth (mean = 1.48 and 16.76 mg/g, respectively) to Meiliang Bay (mean = 0.61 and 9.66 mg/g, respectively) to the lake center (mean = 0.56 and 7.62 mg/g, respectively) within the 4 seasons. TN and TP at Site 1 (2.69 and 2.01 mg/g, respectively) were highest in summer compared with the other seasons. However, less variation in TN or TP across seasons was observed for the other 2 sites.

### DGGE analysis

A total of 63 bands in different sampling sites were detected from the DGGE fingerprints. The highest band numbers were in summer (20, 20, and 21 for Sum-01, Sum-05, and Sum-09, respectively) and the lowest were in winter (9 bands, Win-09). Cluster analysis on DGGE fingerprints revealed that the actinobacterial communities were mainly clustered by seasons rather than by spatial differences (Fig. 2A). Actinobacterial communities in summer were clearly differentiated from other seasons (Fig. 2A).

### Clone library analysis

A total of 297 partial 16S rRNA gene sequence was obtained. After removing putative chimera, 283 *Actinobacteria* sequences were used for subsequent analyses. Unifrac PCoA plot (Fig. 2B) and DCA (Fig. 2C) both indicated a strong spatiotemporal variation in actinobacterial communities, but with a clearer change in seasons than spatial sites. This is supported by permutational multivariate analysis of variance (ANOVA), which showed that seasons and sites explained 31.2% and 18.7% of the variation in community composition with the weighted Unifrac metric ($P < 0.001$, 999 permutations).

Chao1 richness showed a high variation from 13 to 29 (Fig. 3). The samples in winter and autumn showed the low Chao1 richness (mean value of 18 for both seasons), while the highest Chao1 richness was in summer with a mean value of 26. Generally, higher Chao1 richness was observed in the Liangxi River mouth than in the lake center. Consistent variation across seasons was observed for PD, with an increasing trend from winter to autumn (Fig. 3).

Based on phylogenetic analyses, we found putative typical freshwater cluster acI and acTH2 (Fig. 4), which account for 12% and 38% of the total obtained sequences, respectively. When the seasons were assigned to each detected species, there was no clear pattern in the species presence or absence across the seasons. But the clear changes in species abundance were observed across seasons (Fig. 4), which is consistent with the previous PCoA and DCA analyses.

## Discussion

This is the first report on the actinobacterial communities in the surface sediments within a lake intrahabitat. In previous aquatic studies, most studies are related to *Actinobacteria* in water columns, and *Actinobacteria* is usually described as free-living (Newton et al. 2011). In the surface sediments of Taihu Lake, however, we recovered abundant actinobacterial sequences and observed clear temporal–spatial variations of actinobacterial communities with culture-independent methods.

In Taihu Lake, it is revealed that there is a clear horizontal heterogeneity in both bacterioplankton and aggregates-associated bacterial communities (Wu et al. 2007; Xing and Kong 2007; Tang et al. 2009), and the dominance of submersed macrophytes is the most influential factor for bacterioplankton commu-

**Fig. 2.** (A) Unweighted pair group method with arithmetic averages cluster analysis using Pearson's coefficient of denaturing gradient gel electrophoresis band patterns for *Actinobacteria*. (B) Principal coordinates analysis of weighted UniFrac metric and (C) detrended correspondence analysis based on operational taxonomic units table for the 9 clone libraries. Samples are labeled by season (Sum, summer; Aut, autumn; Spr, spring; Win, winter) and site number.

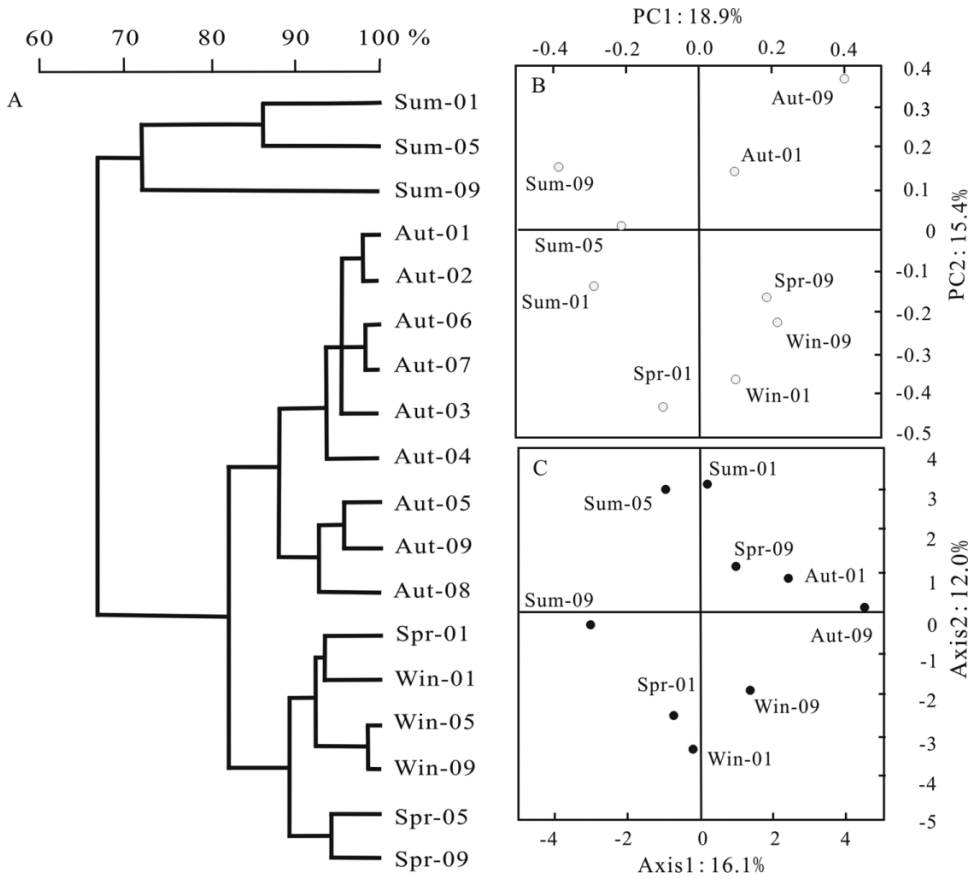
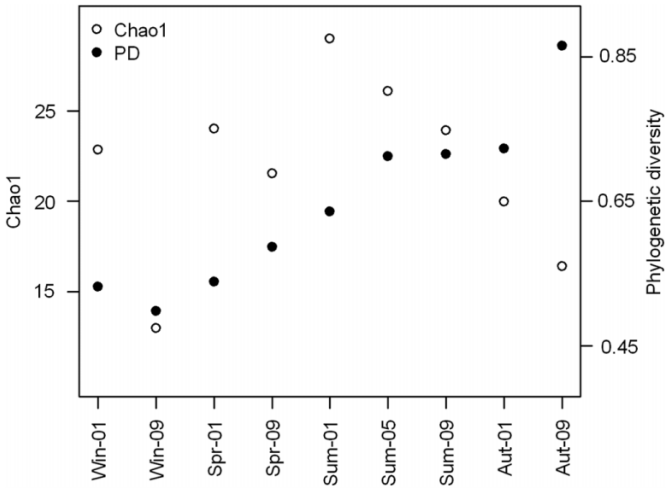


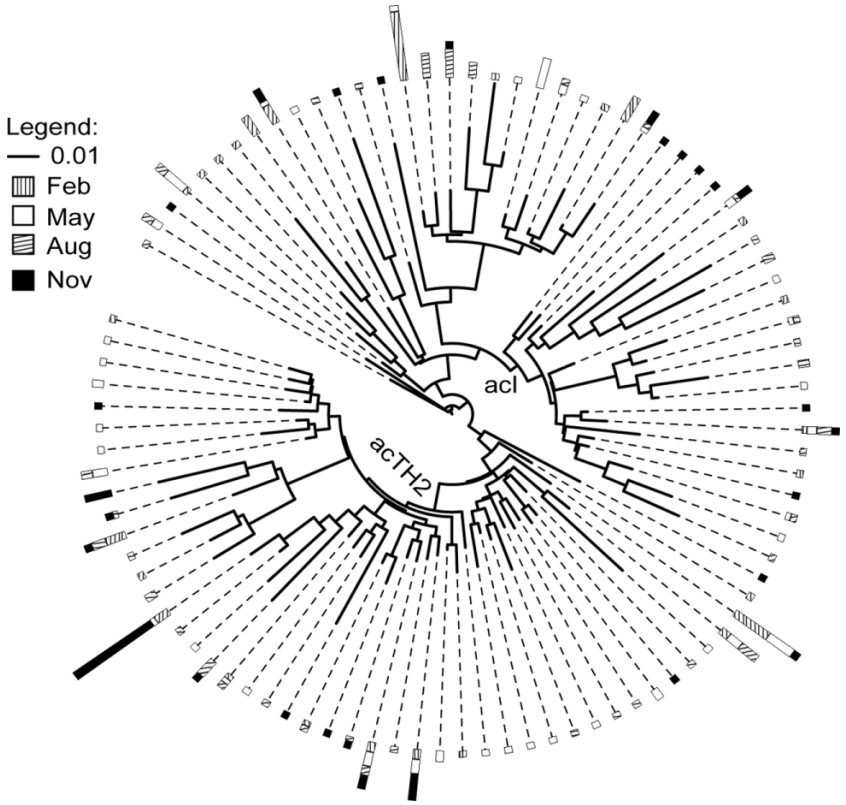

**Fig. 3.** Community richness as indicated by Chao1 richness and phylogenetic diversity as indicated by Faith's phylogenetic diversity (PD) for the 9 clone libraries. Samples are labeled by season (Sum, summer; Aut, autumn; Spr, spring; Win, winter) and site number.




nities (Wu et al. 2007). Here we did not include the samples from regions with macrophytes. But we still observed great differences in actinobacterial communities and diversity (e.g., Chao1 richness) between the northern part of Meiliang Bay and the lake center. This may be attributed to the nutrient conditions in the surface sediments. For instance, the sediment nutrients consistently declined from the Liangxi River mouth to Meiliang Bay to the lake center across the 4 seasons. Also, the nutrient differences in the lake water also generally decreased from the northern part of Meiliang Bay to the lake center (e.g., Wu et al. 2007), and there is a clear difference in algal blooms in the studied region (Duan et al. 2009). For actinobacterial communities in lake water columns, numerous environmental or biological variables were proposed to affect the spatial variations (Newton et al. 2011), such as nutrients (i.e., total phosphorus) (Holmfeldt et al. 2009), Protistan grazing, solar UV radiation (Warnecke et al. 2005), pH, and temperature (Lindstrom et al. 2005). However, in lake sediments, the effects of protistan grazing and solar UV radiation are unlikely because of the attachment of *Actinobacteria* to sediments and low UV transparency of Northern Taihu Lake. Our results suggest that the nutrient conditions, along with the algal blooms, may affect the spatial differences in sediment *Actinobacteria*.

Furthermore, the spatial horizontal heterogeneity had less influence on the actinobacterial communities than do the seasons. For instance, when we increased the sampling sites in autumn up to 9 samples, the DGGE lanes from the same season were clustered together. This seasonal variation in communities is also consistent with PCoA results with clone libraries. These clear seasonal successions suggest that the temperature changes mainly influence the actinobacterial communities. For instance, it has been observed that aquatic actinobacterial communities exhibit clear seasonal successions, which seem to be independent of basic limnological features, with maxima in spring and autumn (Glöckner et al. 2000; Allgaier and Grossart 2006).

**Fig. 4.** Maximum-likelihood phylogenetic trees with seasons as bar charts, drawn with iTOL (Letunic and Bork 2006). The length of the bar indicates the sequence number for each season, with the lowest value of 1 sequence/operational taxonomic unit.




However, we speculated that other factors, such as algal blooms or nutrients, will also significantly affect the seasonal variations. This is evidenced from 2 facts. (*i*) There is a high PD in autumn, rather than maximum diversity in summer. In soil environments, for instance, *Actinobacteria* are important organic matter decomposers, and rich actinobacterial communities can be expected in ecosystems that vary in quantity and quality of organic matter (Kopecky et al. 2011). The increase of PD in sediment *Actinobacteria*, but not of species richness (i.e., Chao1), may be directly related to the decomposition of algal sedimentations in autumn when phytoplankton (dominated by *Microcystis* spp.) die and sink to the surface sediments. (*ii*) The actinobacterial communities in summer were significantly different from the other 3 seasons, which is especially clear from the results of DGGE and the PCoA results. This is consistent with the peak of cyanobacterial blooms in summers, which greatly differentiate the summer season from the others (Duan et al. 2009).

The second aim of this study was to examine whether we can find typical freshwater column lineages (i.e., acI, acII, and acIII) in the lake sediments using phylogenetic analyses. The acI lineage is suggested to be particularly abundant among the freshwater lake *Actinobacteria* and can compose >90% of the identified actinobacterial taxa in the free-living fraction (Newton et al. 2007). In previous studies, sequences related to the proposed clusters acI and acIV (Warnecke et al. 2004) have been recovered from the water column of Taihu Lake using universal bacterial primers, and the acTH were proposed as new clusters from Taihu Lake (Wu et al. 2007). Here, however, in the sediment, we also obtained the abundant sequences putatively affiliated with acI (12%), with specific *Actinobacteria* primers across 4 seasons. There are also some sequences (38%) that are putatively grouped with acTH2 following Wu et al (2007). The classification of the other sequences is not very clear based on the typical lineages from lake epilimnia (i.e., named as acII, etc.). The co-occurrence of acI lineages in both sediments and lake water can be explained in 2 aspects. First, it may be possible that some *Actinobacteria* species came from the freshwater columns *Actinobacteria* assemblages. The occurrence of particular groups of *Actinobacteria* in the surface lake sediments does not necessarily indicate that these groups are active in these habitats (Warnecke et al. 2005). It is known to us that many cultivated *Actinobacteria* have a different life cycle that may involve a vegetative and a resting stage (i.e., spores). The spores can freely survive in unfavorable conditions for a long time. This life strategy can promote survival of some particular *Actinobacteria* groups in distinct habitats with different life stages (Warnecke et al. 2005). Second, it is likely that particular subgroups *Actinobacteria* can adapt in either freshwater columns or surface sediments of the freshwater habitats. The present knowledge about the particular *Actinobacteria* assemblages adapted to lake sediments is rather rare (Maldonado et al. 2009). Likewise, it is true that sediment resuspension is one of the important features of the shallow lake, and wind may cause strong mixing of the water columns and sediment over two thirds of the year at the center of Taihu Lake (Qin et al. 2007). In this case, the actinobacterial species can be passively exchanged between the water columns and surface sediments. Meanwhile, it has been suggested that *Actinobacteria* have various ecotypes and pronounced ecological plasticity in distinct habitats (Allgaier et al. 2007; Babalola et al. 2009). Therefore, it is mostly likely that particular *Actinobacteria* subclusters (i.e., acI) could adapt to both the lake water column and sediments.

In summary, our results clearly indicate that there are significant spatiotemporal variations in actinobacterial communities in the surface sediments of the Taihu Lake, and the seasonal successions of actinobacterial communities were obviously stronger than the spatial changes. The algal blooms, along with nutrients and temperature, are suggested to affect the spatial variations of actinobacterial communities. Meanwhile, the phylogenetic analysis indicated that the proposed typical freshwater columns acti-

nobacterial lineages (e.g., acI) also existed in the surface sediments across 4 seasons. This indicates that there is no strict differentiation in the specific lineages between lake water columns and sediments. This observation can be tested by more strict experiments. For instance, one can deeply sequence the actinobacterial assemblages and phylogenetically compare them between the sediments and water columns from the same time and location. Novel or unique lineages specific to aquatic sediments like to be differentiated from those of lake water columns.

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