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Spatio-temporal variations of the bacterioplankton community composition in Chaohu Lake, China

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Abstract

Despite considerable attention paid to Chaohu Lake in China, the dynamics of bacterioplankton community composition (BCC) on spatial and seasonal scales are poorly understood. In this study, water samples were collected from autumn 2006 to summer 2007 at five positions in Chaohu Lake with different trophic status. BCC of these samples was determined by both the PCR amplification of the 16S rDNA gene and the denaturing gradient gel electrophoresis (DGGE). The abundance and diversity of bacterioplankton communities at different sampling positions showed similar seasonal patterns. The BCCs in the samples varied substantially, and the pattern of changes indicated that the seasonal difference might have a significant impact on the BCC's structure in the lake. Canonical correspondence analysis (CCA) on the DGGE patterns and physicochemical parameters indicated that the temperature and the levels of 5-d biochemical O_2 demand (BOD_5), NH_3–N, COD_{Mn}, total nitrogen, total phosphorous, and dissolved oxygen significantly influenced the BCC, and four of the seven variables were related to the level of eutrophication. Our results indicate that eutrophic status and season are the most influential factors in determining BCC in Chaohu Lake.
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Keywords: Chaohu Lake; Bacterioplankton community composition; Spatial and seasonal dynamics

1. Introduction

Bacteria play significant roles in carbon- and nutrient-cycling in aquatic ecosystems through many physiological and biochemical processes [1]. Therefore, bacterioplankton are not only abundant in freshwater lakes, but are also an important component in these ecosystems [2]. Bacterioplankton community composition (BCC) has been studied extensively, and research has shown that it is highly variable among different freshwater lakes [3–5]. Many studies have shown that BCC is greatly affected by certain environmental factors, such as pH, water temperature, water chemistry, nutrient conditions, and regional differences [6–12]. In addition, lake trophic status is thought to be a parameter potentially influencing BCC [2,13,14], but direct evidence for this is very scarce.

To obtain further insights into the dynamics of bacterial populations in lake ecosystems, it is important to explore temporal and spatial variations of BCC. Little is known about such variations, especially about the spatial and seasonal variations within freshwater lakes. To date, only a few studies have demonstrated vertical patterns in BCC within a lake [15–17] or temporal patterns among different lakes [13]. Horizontal heterogeneity of BCC within a single lake that was hundreds of meters wide was very limited [7]. One investigation showed horizontal differences in BCC in a lake hundreds of kilometers long [17], whereas another

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study showed seasonal dynamics of BCC in a large lake [18]. In general, the spatio-temporal within-lake dynamics of BCC has not been well studied.

Chaohu Lake, located in Anhui Province, eastern China (30°25′–31°43′ N and 117°16′–117°51′ E), is one of the five largest freshwater lakes in China. It is a typical, large, shallow, subtropical freshwater lake covering an area of 780 km^2 and with an average depth of approximately 3.0 m. In recent years, Chaohu Lake has become known as one of the three most polluted freshwater lakes in China [19]. The hydrology of the lake has resulted in a trophic gradient characterized by hypertrophic conditions in the western part of the lake, mesotrophic conditions in the eastern part of the lake, and light-trophic conditions in the center of the eastern lake area [20]. The climate at Chaohu Lake is influenced by East Asia monsoons, and has four distinct seasons. Thus, it is expected that there are horizontal and seasonal differences in the bacterial community structure in the lake. However, to date, there have been no reports on this topic [21]. Research on the BCC in this lake may help determine which environmental factors correlate with changes in BCC.

A recent study suggested that the microbial community in shallow lakes either sustains or further accelerates the process of lake eutrophication [21]. To predict ecosystem responses to environmental changes, it is necessary to investigate microbial dynamics and their interactions with environmental factors [22]. However, most studies on Chaohu Lake have focused on eutrophication [23], variations in physical or chemical parameters, eutrophication mechanisms [24], and strategies for eco-remediation [25]. No studies have been published on the potential relationships between changes in BCC and environmental factors in Chaohu Lake.

In this study, we investigated a full year cycle of BCC in Chaohu Lake at different locations to obtain insight into the spatio-temporal dynamics of BCCs. Furthermore, studying the spatio-temporal changes in the bacterioplankton community would allow us to explore the relationships between differences in BCC and the extent of eutrophication, and to identify which factors have significant impacts on BCC dynamics. These results could provide basic information for ecological restoration of Chaohu Lake.

2. Materials and methods

2.1. Sampling

Twenty water samples were collected in October 2006, and in January, April and July 2007 at five positions in Chaohu Lake (Fig. 1). The five sampling positions were selected based on a long-term environmental monitoring by the Chaohu Environmental Protection Monitoring Center. Since Chaohu Lake is a shallow lake with an average depth of approximately 3 m, samples were collected from approximately 30 cm below the surface using acid-rinsed and autoclaved polycarbonate bottles. They were sampled within a 3–4 h duration (9 am–1 pm) on the same day. Concurrent

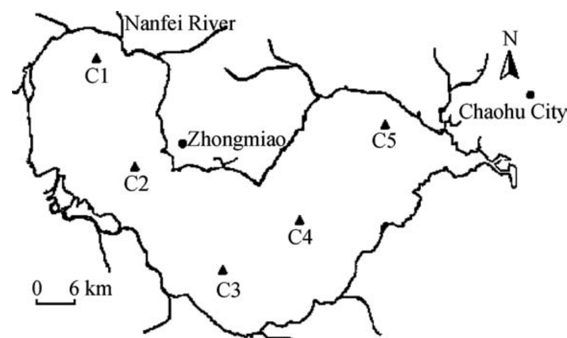

Fig. 1. Chaohu Lake and the five sampling positions (C1: 31°39′ 02″ N, 117°23′29″ E; C2: 31°33′55″ N, 117°27′19″ E, C3: 31°35′29″ N, 117°50′17″ E, C4: 31°31′02″ N, 117°35′23″ E, C5: 31°38′13″ N, 117°44′14″ E).

with bacterioplankton sampling, the water temperature and pH were determined *in situ* using electrodes [26]. Water samples were transported to the laboratory in thermo-boxes and stored in the dark at 4 °C until use. Samples (50 ml) were immediately filtered through 0.22 μm pore-size membranes (diameter 50 mm; Millipore), and the membranes with microorganisms were stored at −20 °C for further molecular analysis. Environmental variables were measured for water samples, including dissolved oxygen (DO), COD_{Mn}, 5-d biochemical oxygen demand (BOD_5), ammonia nitrogen (NH_3–N), total nitrogen (TN), and total phosphorous (TP), using standard methods [26].

2.2. Total bacterioplankton counts

The total bacterial abundance in each sample was determined by direct microscopic counts, using the DAPI-staining technique. Immediately after sampling, each water sample (4 ml) was fixed with buffered polyformaldehyde at a final concentration of 1%. Each water sample was measured in triplicate. The DAPI (4′,6′-diamidino-2-phenylindole, Sigma, USA) working solution (40 μg/ml) was added to water samples to a final concentration of 1 μg/ml. The samples were stained for 15–30 min in the dark, and then filtered through a black polycarbonate membrane (Nuclepore, 0.2 μm pore size; 25 mm diameter; shiny side up). Cells were counted at 1000× magnification with an Olympus epifluorescence microscope equipped with a 50 W/AC HBO lamp and a filter set No. 4 (BP 365 nm, FT 395 nm, LP 397 nm). The 4 ml samples, which sometimes required several-fold dilution, yielded 20–100 stained cells in a counting field (175 μm × 132 μm). A minimum of 400 cells (10–20 view fields) were counted.

2.3. DNA extraction and PCR amplification

For DNA extraction, the filters with microorganisms were cut into small pieces with a sterile scalpel, then DNA was extracted according to the protocol of Ausubel et al. by a combination of freezing-thawing and enzymolysis [27]. After rinsing with 70% ethyl alcohol, the nucleic

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acid was redissolved in 40 μl TE-buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0) and stored at −20 °C.

PCR amplification was performed with a Biometra Thermal Cycler (Biometra, Göttingen, Germany). The 16S rRNA gene fragment was amplified using the primer set GC357F (5′-CCTACGGGAGGCAGCAG-3′) and 519R (5′-GTATTACCGCGGCTGCTGG-3′) with GC-clamps (5′-CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGC CCCCGCCCC-3′) attached to the forward primer [28]. The PCR mixture contained 15 μl of diluted template DNA solution (approximately 30 ng DNA), each primer at 0.2 μM, each deoxynucleoside triphosphate at 200 μM, 5 μl of 10× PCR buffer, and 2.5 U of *Taq* DNA polymerase (Takara, Dalian, China). The final volume was adjusted to 50 μl with sterile water.

Following incubation for 5 min at 94 °C, a touchdown PCR was performed using 15 cycles as follows: denaturation at 94 °C for 1 min, annealing at 65 °C (with temperature decreasing by 1 °C every cycle until the touchdown temperature of 56 °C was reached) for 1 min, and primer extension at 72 °C for 2 min. Fifteen additional cycles were carried out at an annealing temperature of 55 °C, and followed by incubation for 10 min at 72 °C. The presence of PCR products and their concentrations was determined by electrophoresis of 4 μL of the product on 1.2% agarose gels.

2.4. DGGE

Twenty samples from Chaohu Lake were analyzed on two parallel DGGE gels. DGGE was performed with the DCode™ system from Bio-Rad Laboratories (Hercules, CA, USA). PCR samples were loaded onto 8% polyacrylamide (acrylamide/bis, 37.5:1) gels in 0.5× TAE (20 mM Tris acetate, pH 7.4, 10 mM sodium acetate, 0.5 mM Na_2–EDTA). The denaturing gradient contained 45%–60% denaturant (100% denaturant corresponded to 7 M urea and 40% formamide). Electrophoresis was carried out for 16 h at a constant voltage of 60 V at 60 °C. After electrophoresis, the gels were stained for 50 min in Milli-Q water containing SYBR Green I (1:10000 dilution), and destained for 15 min with Milli-Q water. Finally, the gels were placed on a UV transilluminator and photographed with Bio-Rad Gel Doc 2000 equipment.

2.5. Statistical analyses

The DGGE profiles were analyzed using Quantity One software (Bio-Rad). First, the DGGE banding patterns were converted to a binary matrix using presence-absence data, i.e., 1 represents presence and 0 represents absence. Then, a pairwise similarity of the banding patterns of the different samples was calculated with the Dice coefficient $Sd = 2j/(a + b)$, where j is the number of bands common to both samples, a is the number of bands in sample A, and b is the number of bands in sample B. Using these pairwise similarity values, an unweighted pair group with mathematical averages (UPGMA) cluster analysis was performed to determine whether the samples revealed a non-random pattern and whether the samples clustered according to seasons or to positions. The binary matrix was used as data for the multidimensional scaling map (MDS) method, in which the data were presented in a Euclidean plane. Every band pattern was shown as one plot, and highly similar band patterns were plotted close together. MDS analysis was performed using SPSS 13.0.

We used H, representing the Shannon index, as a parameter for the bacterial community diversity to obtain some information about the differences among these 20 samples. H was calculated using the function $H = -\sum P_i \ln P_i$ [29], where P_i is the importance probability of the bands in a gel lane for DGGE bands analysis. P_i was calculated using the equation $P_i = n_i/N$, where n_i is the height of a peak for DGGE band, and N is the sum of all peak heights of bands in the whole lane.

To investigate relationships between BCC and measured environmental variables mentioned above a direct gradient analysis was carried out using the software CANOCO ver. 4.5 [30]. The obtained ordination axes (based on community composition data) were linear combinations of environmental variables, assuming a unimodal species–environment relationship. Environmental variables significantly related to BCC were determined by the forward selection and 99 unrestricted Monte Carlo permutations test. Since the data could not be assumed to be normally distributed, $\log(x + 1)$-transformed environmental data were used in the analysis.

3. Results

3.1. Changes in environmental parameters

Spatial and seasonal fluctuations of the selected environmental variables (temperature, pH, BOD_5, COD_{Mn}, DO, TN, NH_3–N, and TP) are shown in Table 1. We found that there was little variation in temperature at the five sampling positions in the same season, since all the samples were collected within a 3–4 h duration on the same day. The seasonal temperature variation at the five sampling positions showed a typical trend, with the highest values in summer and the lowest values in winter. The pH ranged from 6.91 to 8.84, and the lake was almost alkaline, except at sampling positions C1 and C3 in spring. In addition, the pH at C4 was always higher than that at C1. The values for BOD_5, COD_{Mn}, TN, NH_3–N, and TP concentrations at C1 were almost always significantly higher than those at the other four positions, except for TN concentration in summer at C1. DO concentration at C1 was lower than that at the other four sampling positions.

There were large seasonal variations in BOD_5, COD_{Mn}, TN, NH_3–N and TP concentrations at position C1. Only weak fluctuations in BOD_5 and COD_{Mn} concentrations were observed at C2, while TN, NH_3–N and TP concentrations fluctuated at C3. With respect to DO concentration, the greatest seasonal variation was observed at C5 and

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Table 1
Spatial and seasonal variations in environmental parameters

		C1	C2	C3	C4	C5
Temperature (°C)	Autumn	24.4	25.0	24.0	23.0	24.0
	Winter	4.5	5.0	5.0	5.0	5.0
	Spring	11.2	11.0	11.0	11.0	11.0
	Summer	29.5	29.0	29.0	29.0	29.0
pH	Autumn	7.32	8.50	8.51	8.84	8.24
	Winter	7.60	7.42	7.65	7.88	7.39
	Spring	6.92	7.50	6.91	7.63	7.96
	Summer	7.37	7.91	7.62	7.80	7.75
BOD_5 (mg/l)	Autumn	3.60	2.10	2.04	1.82	2.25
	Winter	9.62	2.04	1.93	1.50	2.47
	Spring	3.70	2.18	2.08	1.72	2.04
	Summer	5.96	1.93	1.82	1.61	2.26
COD_{Mn} (mg/l)	Autumn	8.11	4.66	4.88	4.59	4.44
	Winter	9.58	4.53	4.03	3.11	3.96
	Spring	11.38	4.58	3.35	3.35	3.97
	Summer	6.55	4.68	4.01	3.84	4.70
DO (mg/l)	Autumn	5.09	8.26	8.05	9.44	8.57
	Winter	5.71	10.62	12.12	12.33	12.01
	Spring	4.20	8.90	8.69	9.22	8.90
	Summer	4.98	5.21	6.71	6.92	6.49
TN (mg/l)	Autumn	1.89	1.77	1.54	1.43	1.41
	Winter	2.49	1.81	1.24	1.07	1.51
	Spring	1.74	2.09	1.31	1.31	0.94
	Summer	1.49	1.63	1.49	1.57	1.84
NH_3–N (mg/l)	Autumn	1.44	0.50	0.47	0.55	0.44
	Winter	2.12	0.36	0.41	0.39	0.32
	Spring	1.46	0.40	0.44	0.34	0.30
	Summer	0.50	0.36	0.47	0.52	0.52
TP (mg/l)	Autumn	0.38	0.10	0.11	0.12	0.09
	Winter	0.44	0.11	0.10	0.08	0.10
	Spring	0.31	0.11	0.09	0.06	0.10
	Summer	0.29	0.18	0.11	0.10	0.13

the least at C1 (Table 1). Compared horizontally, the greatest variations in the concentrations of the chemical parameters at the different sampling positions were observed in winter, with two exceptions: COD_{Mn} was highest in spring and lowest in summer, and BOD_5 showed greatest variations in autumn (Table 1).

3.2. Total bacterioplankton count

Total number of bacterioplanktons in Chaohu Lake was obtained by counting DAPI-stained cells. Cell counts ranged from 0.075×10^6 to 6.75×10^6 cells/ml at the five positions during four seasons. The highest and the lowest values at position C4 were in summer and winter, respectively (Fig. 2). Spatially, C4 showed the highest average abundance of bacterioplankton over the four seasons, and C5 the lowest. Seasonally, the bacterioplankton was the most abundant at C1 in winter, C2 in spring, C3 in autumn and C4 in summer.

3.3. Bacterioplankton community composition

DGGE fingerprint patterns were obtained from 20 samples (Fig. 3). Generally, the bands were dispersed across the entire gel gradient. Three obvious bands were found in all

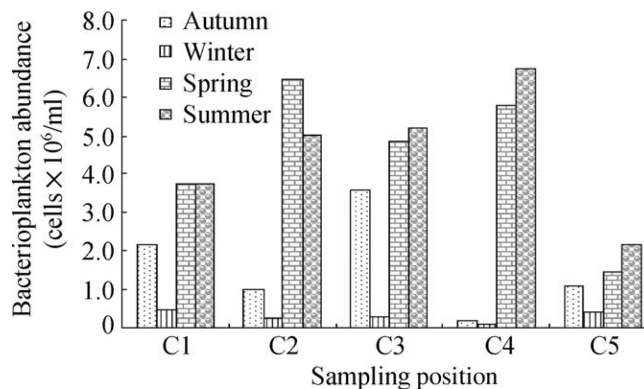

Fig. 2. Bacterioplankton abundance at different sampling positions.

20 samples, though the intensity of bands differed among samples. Other bands showed different distribution patterns; some were present in one or several sampling positions in one or several seasons. Dominant bands in the spring and winter samples were distributed near the top of the DGGE gel, while the bands in autumn and summer samples distributed near the bottom of the gel (Fig. 3b). There was a greater seasonal variation of band patterns in C1 samples than that in other samples (Fig. 3a).

Fig. 4 shows the bacterioplankton richness defined as the number of DGGE bands [31]. A total of 65 different bands were detected in the 20 samples, indicating that there were 65 taxa of bacterioplanktons [2]. For the individual samples, the number of bands varied from 7 to 22. At each sampling position, bacterioplankton richness in autumn samples was higher than those in samples from the other three seasons. The five sampling positions showed different seasonal trends (Fig. 4). In contrast to other positions, richness in C4 samples varied little according to season.

The Shannon index among samples ranged from 2.56 to 3.10, with the lowest value in autumn at position C1 and the highest in spring at position C5 (Fig. 5). The variation in the Shannon index indicated that the diversity of bacterioplankton varied greatly among the 20 samples. Among samples collected from the same position in different seasons, the Shannon index ranged from 0.11 to 0.44. The range in samples collected in the same season at different positions was 0.27–0.38.

Fig. 5 shows the same seasonal patterns of bacterioplankton diversity at positions C4 and C5, but the diversities in the four seasons in the C4 samples were always lower than those in the C5 samples. The other three positions (C1, C2, and C3) showed different seasonal change trends. With respect to season, the lowest diversities for each position occurred in autumn, except at C2. The highest diversities for C1 occurred in summer, C2 in winter, and C3, C4 and C5 in spring. Interestingly, the bacterial species richness and diversity in the C2 winter sample were clearly higher than those in the other three C2 samples.

Cluster analysis by UPGMA showed that there was low similarity among BCCs in the 20 samples (Fig. 6). Furthermore, the C1 samples were the most divergent over the four

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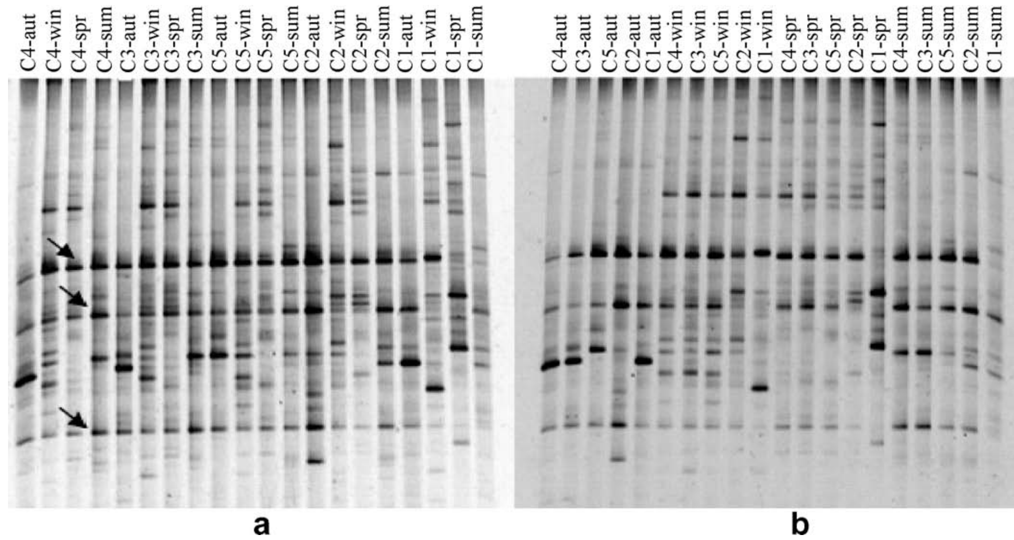

Fig. 3. DGGE patterns. The two DGGE gels contained the same samples loaded in different order. (a) The four samples taken from each position were loaded according to sampling time. (b) Samples were loaded according to sampling position. Arrows show the position of the three bands that were detected in every sample collected at Chaohu Lake. aut, autumn; win, winter; spr, spring; sum, summer.

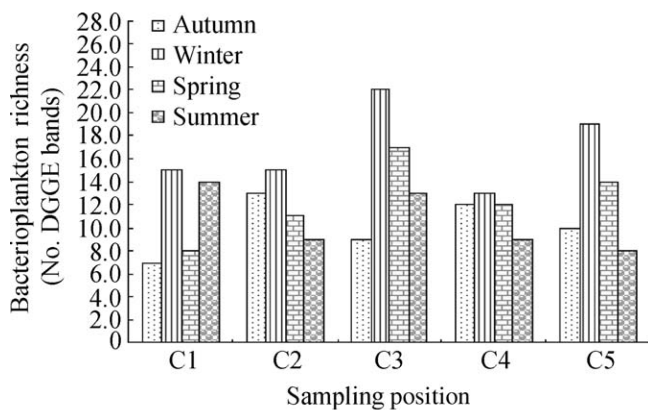

Fig. 4. Bacterioplankton richness at different sampling positions. Richness is indicated by the number of bands on the DGGE gel.

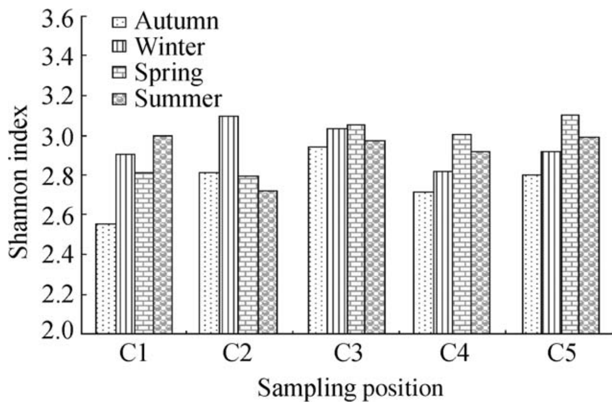

Fig. 5. Shannon index at different sampling positions.

seasons. Cluster analysis indicated that the BCCs in spring at positions C2, C3, and C5 formed one cluster. BCCs in winter at positions C3, C4, and C5 were grouped into another cluster, those in summer at positions C3, C4, and

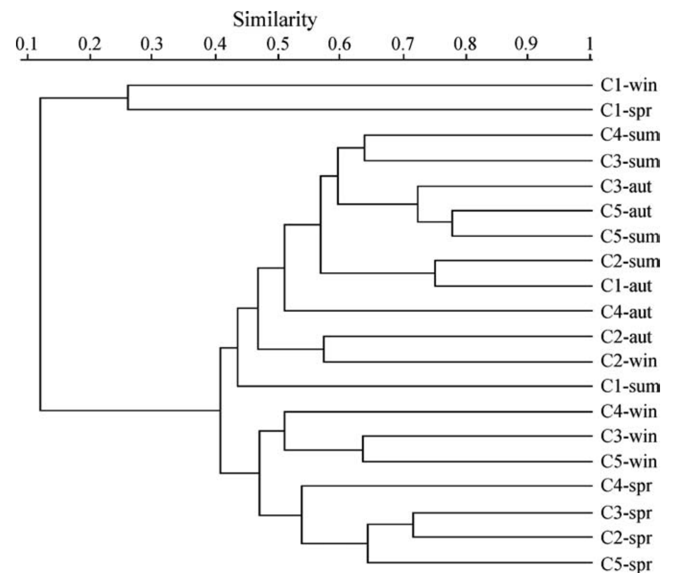

Fig. 6. Dendrogram obtained by UPGMA clustering of DGGE patterns from Chaohu Lake samples. Similarity is expressed as the Dice correlation coefficient. aut, autumn; win, winter; spr, spring; sum, summer.

C5 and autumn at positions C3 and C5 tended to cluster, while the BCCs of samples obtained from different positions were separated (Fig. 6).

Changes in BCC among these 20 samples were further investigated using MDS analysis of DGGE banding patterns. The resulting MDS graph shows similar cluster patterns to those generated by UPGMA analysis, that is, many samples were dispersed. Only a few samples clustered, mainly according to season. For example, the spring samples plotted together on the lower right side of the graph, and the summer and autumn samples clustered on the upper center side of the graph (Fig. 7). This result, taken together with the UPGMA analysis, suggested that

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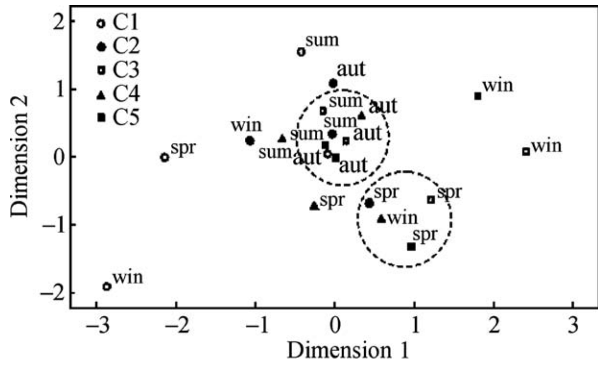

Fig. 7. MDS analysis of BCC data in different seasons and at different positions. aut, autumn; win, winter; spr, spring; sum, summer.

the similarity of BCCs within the lake horizon was not higher than the similarity among seasons.

3.4. Bacterioplankton community composition in relation to environmental parameters

Fig. 8 shows the CCA ordination of the bacterioplankton community composition data with respect to environmental variables. Fig. 8(b) shows the positions of the different samples in the ordination. The C1 samples in winter and spring are both outliers, and are positioned on the right of the plot, whereas the other samples are positioned on the left of the plot. Fig. 8(a), which displays the positions of the different DGGE bands in the ordination, shows that a very large part of the variation in the ordination is due to the appearance of a few taxa in the winter and spring C1 samples. Except for temperature, the vectors indicate increasing eutrophic status from the left to the right. This result indicates that the trophic status had the most pronounced effect on the ordination of the bacterioplankton community compositions.

The results from the CCA also showed that the taxa–environment correlation was high (Fig. 8a), and there was a strong relationship between the environmental variables and the BCC (Table 2). The eigenvalues were higher for the first two ordination axes compared with the third and the fourth, indicating that the variations along the first two axes were most important (Table 2). Axis 1 was mainly composed of BOD_5 ($r = 0.8778$), NH_3–N ($r = 0.8958$), COD_{Mn} ($r = 0.7732$), TP ($r = 0.7686$), and TN ($r = 0.6797$), while axis 2 consisted mainly of DO and temperature ($r = -0.5712$ and $r = 0.4837$, respectively). Moreover, the separation of BCC among samples was strongly related to the seven chosen environmental variables ($p < 0.05$), especially the concentrations of the former five variables (Table 2). In total, the CCA plot of samples, taxa, and environmental variables based on the first four axes explained 36.7% of the taxonomic variation in the bacterioplankton communities.

4. Discussion

The DGGE technique used in this study does not allow a complete characterization of the bacterioplankton community [32]. For instance, bands found at the same

Table 2
Summary of CCA results on bacterioplankton community composition data and significant environmental variables ($p < 0.05$)

Total inertia:				3.970
Sum of all canonical eigenvalues:				1.981
	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.613	0.329	0.295	0.222
Species-environment correlations	0.985	0.972	0.971	0.969
Cumulative percentage variance species data	15.5	23.7	31.2	36.7
Species-environment relation	31.0	47.6	62.5	73.7

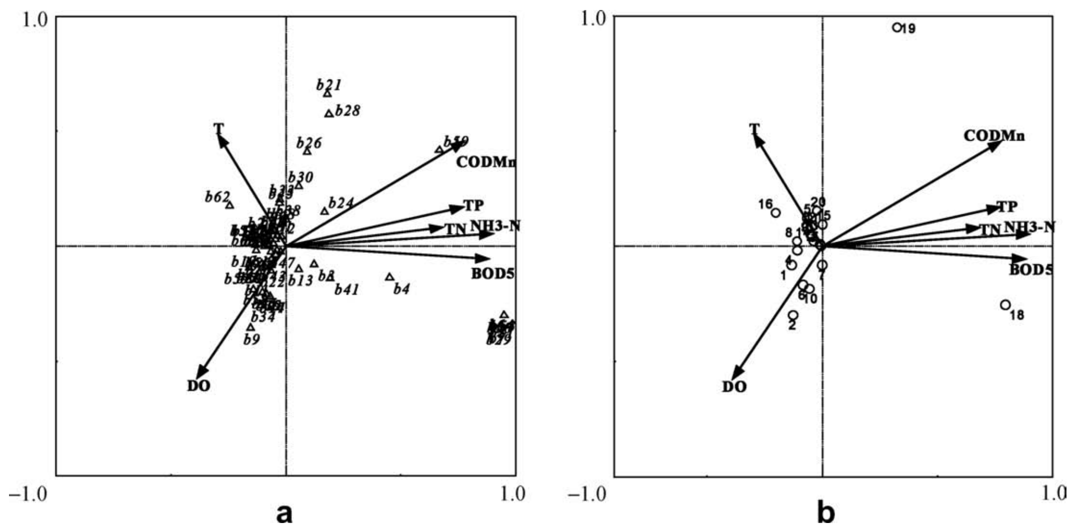

Fig. 8. Canonical correspondence analysis ordination plots of the samples, taxa (DGGE bands), and environmental variables. (a) Triangles indicate different taxa; numbers following 'b' in band codes refer to the positions on the DGGE gel according to electrophoresis distance; (b) open circles indicate different samples.

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positions on the gels do not always represent the same taxon. Therefore, differences and similarities in gel patterns may not completely correspond to differences and similarities in nature. However, the technique is capable of detecting differences in taxa composition among different samples, and can be used to assess the changes in bacterioplankton community composition [33]. Twenty samples were collected from five locations over four seasons, and 65 different bands were obtained from their DGGE patterns. Three bands were found in all the samples (Fig. 2), suggesting that only a few bacterioplankton taxa might survive at all the different positions in their different physicochemical environments, in all four seasons. Most bacterioplankton taxa had their unique habitat, and required different environmental conditions. This result agrees with those from previous studies in freshwater lakes [3].

Both spatial and temporal variations in the richness and diversity in bacterioplankton communities were observed in this study. With the exception of position C2, the horizontal differences among the sites were similar, as were the seasonal differences (Figs. 4 and 5). At the same time, our results show that the bacterioplankton community compositions in 20 samples differed substantially in horizon and season, but the seasonal difference accounted for most of the variation (Fig. 7). This is in contrast to a previous study on three temperate lakes with different trophic status [13], while it is consistent with the observations of Lindström in five eutrophic lakes [3].

In freshwater ecosystems, shifts of BCC are, to a great extent, the consequence of fluctuations in environmental factors [8,11]. In this study, the physicochemical parameters varied with sampling position and seasons in Chaohu Lake (Table 1). The CCA analysis indicated that BCC was mainly influenced by BOD_5, COD_{Mn}, TP, TN, NH_3–N, and DO concentrations as well as temperature. BOD_5, COD_{Mn}, TP, and TN are all related to the level of eutrophication. Thus, it appeared that eutrophic status and season were the major factors affecting BCC in the lake. For example, the C1 samples showed the highest BOD_5, COD_{Mn}, TN, TP, and NH_3–N concentrations, and the lowest DO concentration. There were marked seasonal variations in these variables (Table 1); except for the autumn sample, these samples plotted separately (Fig. 7). The result of CCA analysis also implied that the changes of BCC on spatial and seasonal scales are, to an extent, determined by the changes of physicochemical parameters on the same spatio-temporal scales in Chaohu Lake.

We attempted to sequence some of the major bands present. However, the small universal bacterial PCR product size used for DGGE (<200 bp) meant that we were unable to obtain enough phylogenetic information to construct a tree, so no further bands were sequenced. We chose a primer set that would yield a small PCR product, because previous studies have shown that the upper size limit for adequate DGGE band separation is smaller than 500 bp. This study could contribute to an identification of important taxa, which could eventually lead to an understanding of the mechanisms behind the statistical relationships. We will further construct a 16S rDNA library to analyze the characteristics of bacterioplankton species at different sampling positions and seasons. This work, together with previous experimental findings, should help determine the most important factors affecting bacterioplankton community structures.

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