

# Phytoplankton community reorganization driven by eutrophication and warming in Lake Biwa

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**Abstract** We compiled and analyzed long-term data, including chemical, physical and phytoplankton community data, for the Lake Biwa ecosystem from 1962 to 2003. Analyses on environmental data indicate that Lake Biwa had experienced intensified eutrophication (according to total phosphorus concentration) in the late 1960s and returned to a less eutrophic status around 1985, and then exhibited rapid warming and thus increased water column stability since 1990. Total phytoplankton cell volume

largely followed the trend of total phosphorus concentration, albeit short-term fluctuations existed. However, phytoplankton community shifted dramatically in response to those changes of environmental states. These shifts were caused by changes in trophic status driven by phosphorus loadings and physical properties in the water column driven by warming. Moreover, most phytoplankton species did not show a strong linear correlation with environmental variables, suggesting nonlinear transitions among different states.

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**Keywords** Ecological characteristics · Multiple stable states · Nutrient loading · Total phytoplankton volume · Water column stratification

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## Introduction

Anthropogenic impacts and climate effects on ecosystems are pressing concerns. For lake systems, one of the most serious anthropogenic impacts is eutrophication, and this issue has been studied for a 100 years and remains a serious concern today (Schindler 2006; Smith et al. 2006). Eutrophication of lakes caused by domestic sewage was well known in history and often was associated with an increase in phytoplankton abundance (Hasler 1947; Edmondson 1956; Davis 1964). In some lakes, phytoplankton abundance was found to track closely the eutrophic condition of the lakes (often measured by the total phosphorus, TP, in the water column). When TP increased, phytoplankton abundance increased, and to some extreme state, a phytoplankton bloom occurred; when TP decreased, phytoplankton reversed to its original abundance (Edmondson 1970; Ruggiu et al. 1998; Kohler and Hoeg 2000). However, in many other lakes, when TP

decreased, phytoplankton abundance remained high or delayed its decline (Anneville and Pelletier 2000; Horn 2003; Dokulil and Teubner 2005). Recent studies suggest that internal loading (release of phosphorus from the sediments mediated by microbial activities under an anoxic condition) is the main reason for the irreversible phenomenon or delayed response (Carpenter et al. 1998; Carpenter 2005). Theoretically, such phenomena are associated with nonlinear transition among alternative stable states (Scheffer et al. 2001; Hsieh et al. 2005).

Besides the bulk phytoplankton abundance, how phytoplankton communities respond to eutrophication and later oligotrophication is also a subject of intensive research. Some studies indicated that phytoplankton community changes responding to the trophic condition of lake was symmetrical; that is, phytoplankton community would return to its original structure when the trophic status of lake recovered (Sommer et al. 1993; Kohler and Hoeg 2000). Other studies indicated that the changes in the phytoplankton community were asymmetrical (Anneville et al. 2002b) or the reversal was delayed (Dokulil and Teubner 2005). Through trophic interactions, eutrophication effects also propagated to zooplankton (Molinero et al. 2006; Anneville et al. 2007).

In addition to eutrophication, warming in the past half century has also drastically influenced lake ecosystems. Increased water temperature resulted in intensified stratification of lakes that caused strong hypolimnetic oxygen depletion (Jankowski et al. 2006). Warming also changed the phenology of lake processes, such as earlier onset of stratification and consequent earlier spring phytoplankton blooms (Winder and Schindler 2004). Furthermore, warming altered phytoplankton communities, shifted the community structure to small-sized species (Winder and Hunter 2008), and favored the growth of cyanobacteria (Elliott et al. 2006). Moreover, trophic status and warming often had synergistic effects on the phytoplankton community as well as the whole ecosystem (Elliott et al. 2006; Huber et al. 2008; Wilhelm and Adrian 2008).

In this research, we investigated eutrophication and warming effects on the phytoplankton community of Lake Biwa. Lake Biwa is the largest lake in Japan; it contains a high biodiversity, including more than 60 endemic species. It also provides high economic values, including transportation, drinking water, and fisheries (Kumagai 2008). Along with urbanization, Lake Biwa had experienced increasingly nutrient loading since 1960s and subsequently suffered from blooms of *Uroglena americana* since 1977 and cyanobacteria since 1983 (Kumagai 2008). The initialization of eutrophication in the 1960s was reflected in the sediment records, showing pronounced changes in zooplankton composition (Tsugeki et al. 2003). The

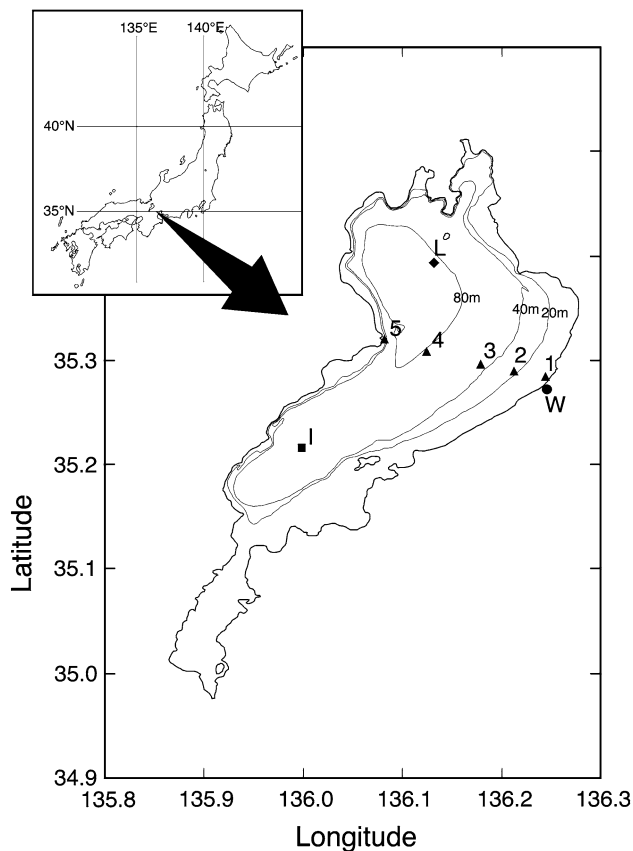
progress of eutrophication from the 1960s to early 1980s were revealed from stable nitrogen isotope analysis of sediment core samples and preserved gobiid fish, *Isaza*, *Chaenogobius isaza* (Ogawa et al. 2001). A water treatment regulation was enforced in 1982, and nutrient loading was progressively reduced and then stabilized after 1985 (Kumagai 2008). However since 1980, the air temperature rose quickly, putting another threat on the Lake Biwa ecosystem.

Due to its important ecological and economic value, Lake Biwa has been intensively monitored by the environmental agency, fisheries agency, and academic institutions. Despite of great amount of effort invested and various data (including physical, chemical, and biological) being collected, no attempt was ever made to compile those data from different agencies. Particularly, detailed phytoplankton community data have been collected; however, only total biovolume was reported in the Japanese literature (Ichise et al. 2001; Ichise et al. 2007). Detailed ecosystem-level time-series data from Lake Biwa are available, providing us a great opportunity to investigate eutrophication and climate effects on a large temperate lake. In this research, our purposes were to (1) compile and analyze historical (1962–2003) chemical, physical, and phytoplankton data collected by various institutes, and (2) investigate how eutrophication and climate warming affected the phytoplankton community in Lake Biwa. Although seasonal variation of lake environments and seasonal succession of phytoplankton communities were well known (Sommer et al. 1986; Anneville et al. 2002b; Lau and Lane 2002), here we focus on long-term variation and thus investigate only the annual average data in the current study.

## Materials and methods

### Environmental data

We compiled long-term time-series data from four research institutions. Chemical (concentration of phosphate, nitrate, and ammonium) and physical (water temperature) data from 1961 to 2005 (in Stations 1–5 in Fig. 1) were collected by the Shiga Prefecture Fisheries Experimental Station (SPFES). In addition, silicate data were collected after 1978. Only data from the deepest station (Station 4) are presented here to investigate the whole water column properties. Chemical data were collected monthly at depths of 0.5, 10, 20, 30 m and the bottom (~80 m). For each month, we calculated the averaged value for the surface layer (0–20 m); this surface layer approximates the living habitat of phytoplankton in Lake Biwa. Monthly chemical data for the surface layer are shown in Appendix 1 of



**Fig. 1** Map showing sampling stations in Lake Biwa. Stations 1–5 are the Shiga Prefecture Fisheries Experimental stations; station L is the long-term monitoring station of the Lake Biwa Environmental Research Institute; station I is the environmental monitoring station of the Kyoto University; station W is the Hikone Meteorological Station

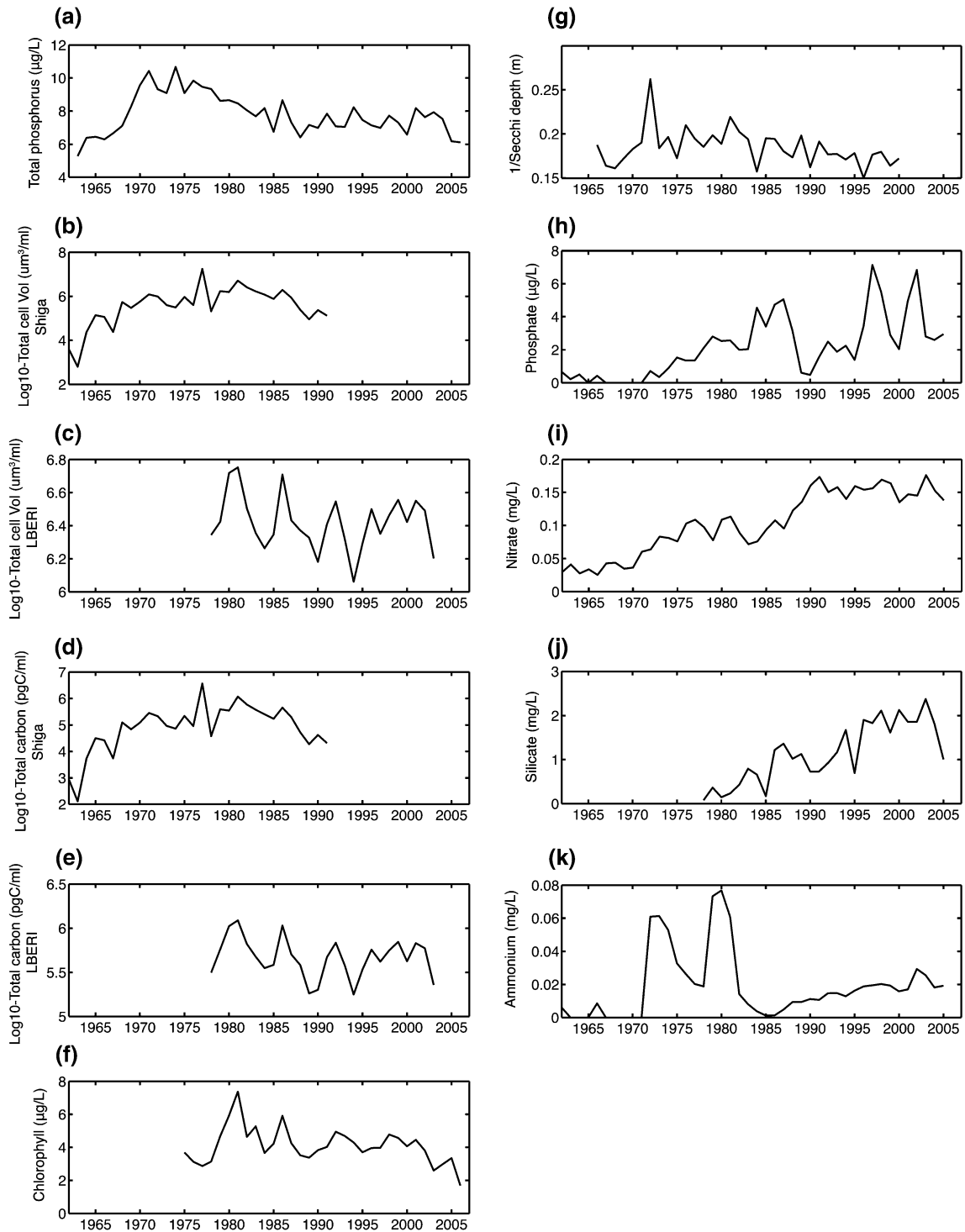
Supplementary material. Annual averaged values are shown in Fig. 2. We further calculated monthly anomalies by subtracting the long-term monthly mean and averaged them into annual anomalies (Appendix 1 of Supplementary material). In addition to the nutrient concentrations, we also investigated the phosphate/total phosphorus ratio and ammonium/nitrate ratio as indicators to nutrient regimes. Water temperature data from the surface (0.5 m) to the bottom were measured at 5-m intervals. Following Hsieh et al. (2009b), we interpolated the temperature data into 1-m intervals using cubic spline. With the interpolated data, we calculated the thermocline depth defined as the depth where the greatest temperature gradient occurs and computed the maximal buoyancy frequency representing the buoyancy frequency at the thermocline depth, which is indicative of water column stability (Hsieh et al. 2009b). Monthly values, annual averages, and annual anomalies of thermocline depth, maximal buoyancy frequency, and surface (averaged 0–10 m) and bottom (80 m) temperature were provided in Fig. 3 and Appendix 1 of Supplementary material.

We further compiled chlorophyll *a* (chl<sub>a</sub>) concentration data from 1975 to 2005 collected by the Lake Biwa Environmental Research Institute (LBERI, formerly Shiga Prefecture Institute of Public Health and Environmental Science) in Station L (Fig. 1). The chl<sub>a</sub> data were treated in the same manner and presented in Fig. 2 and Appendix 1 of Supplementary material. In fact, the LBERI have also collected similar physical and chemical data as the SPFES at Station L since 1975. Patterns of these LBERI physical and chemical data are consistent with those of SPFES (correlation analyses for each of the aforementioned physical and chemical variables,  $p < 0.001$ ). Because the SPFES contain longer time series, only SPFES data were used in further analyses.

We used total phosphorus (TP) in the surface layer (<20 m) as a proxy to the trophic status of Lake Biwa. TP data were collected by LBERI (Station L in Fig. 1) only since 1978. To extend the time series backward, we compiled TP data from 1963 to 1980 collected by the Kyoto University (Station I in Fig. 1). We combined these two datasets to form a TP time series (Fig. 2, Appendix 1 of Supplementary material). For the overlapped period (1978–1980), the TP of these two datasets were at a similar concentration, and thus we took the average of the series for that period. We acknowledge that these two stations are far apart, the two datasets overlap by only 3 years, and it is difficult to test the consistency of these two datasets. However, the combined TP series exhibits a history of changes in trophic condition of Lake Biwa that is consistent with that uncovered by Ogawa et al. (2001) based on sediment records and isaza fish. Therefore, we consider that the TP time series we reconstructed here is representative of the trophic status of Lake Biwa for the past half century.

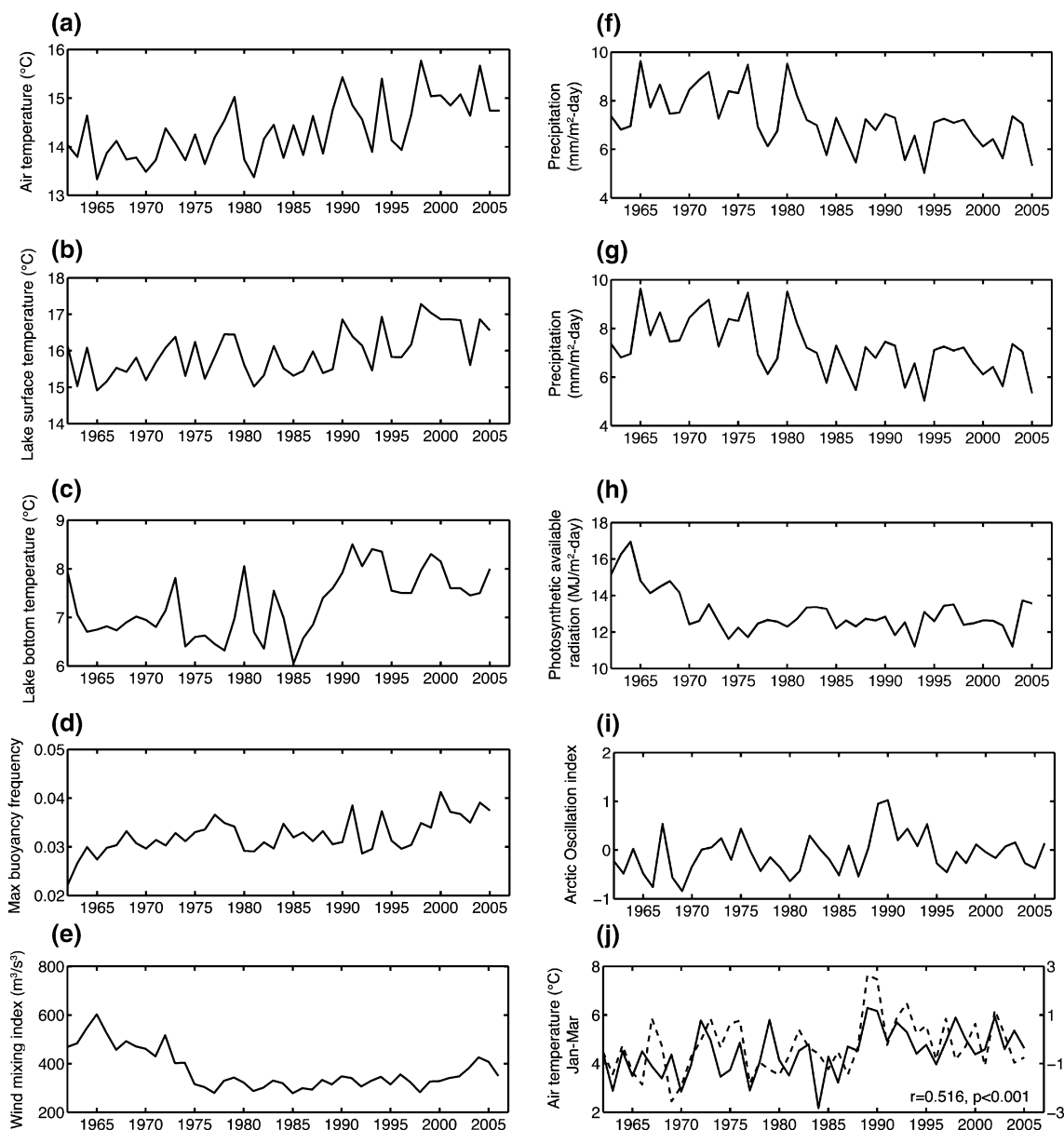
Since the chl<sub>a</sub> data were only available after 1975, we used 1/Secchi depth as a surrogate of phytoplankton biomass. Monthly Secchi depth data from 1966 to 2000 were collected by the Kyoto University at Station I (Fig. 1). The inverse Secchi depth and chl<sub>a</sub> data are significantly correlated (correlation analysis,  $p < 0.001$ ). Inverse Secchi depth data are shown in Fig. 2 and Appendix 1 of Supplementary material.

To link the physical environment of the lake to atmospheric forcing, we also compiled air temperature, photosynthetic available radiation (PAR), wind velocity, and precipitation from the Hikone Meteorological Station (Station W in Fig. 1). Note that the PAR data were from the Meteorological Station but not from the water column and may not represent light availability in deeper layers. The turbulent mixing condition of the upper water column was estimated by a wind-mixing index, which is proportional to the cube of the wind velocity (Bakun and Parrish 1991; Hsieh et al. 2009a). The wind mixing index was



**Fig. 2** Annual time series of chemical and biological variables: **a** total phosphorus, **b** total phytoplankton cell volume for the SPFES data, **c** total phytoplankton cell volume for the LBERI data, **d** total phytoplankton carbon for the SPFES data, **e** total phytoplankton carbon for the LBERI data, **f** chlorophyll concentration, **g** inverse

Secchi depth, **h** phosphate concentration, **i** nitrate concentration, **j** silicate concentration (only available since 1978), **k** ammonium concentration. For **a**, **f**, and **h–k**, shown are averaged value for the 0–20 m, while the whole water column average shows a similar pattern



**Fig. 3** Annual time series of physical variables: **a** air temperature, **b** lake surface temperature (average 0–10 m), **c** lake bottom temperature (80 m), **d** buoyancy frequency at the thermocline depth, **e** wind mixing index, **f** thermocline depth, **g** precipitation,

**h** photosynthetic available radiation, **i** Arctic Oscillation index, and **j** winter averaged air temperature (*solid line*) and Arctic Oscillation index (*dashed line*)

calculated from daily maximum wind velocity (extracted from hourly measurements) and then averaged into monthly means. In addition, we investigated the Arctic Oscillation index (AO), a climate pattern defined by winds circulating counterclockwise around the Arctic at about 55° north latitude. The AO has been known to influence weather condition of Japan (Thompson and Wallace 1998). Time series of these atmospheric data are shown in Fig. 3 and Appendix 1 of Supplementary material. We investigated also the Pacific Decadal Oscillation (PDO) (Mantua

et al. 1997) and Southern Oscillation index (SOI) (Trenberth 1984) but found no significant correlation and do not present these data.

#### Phytoplankton data

Phytoplankton community data include the time series from 1978 to 2003 collected by LBERI (Stations L in Fig. 1) and those from 1962 to 1991 collected by SPFES (Stations 1–5 in Fig. 1). (In this paper, both eukaryotic

algae and prokaryotic cyanobacteria are referred to as phytoplankton for simplicity.) Phytoplankton from the LBERI were collected using a Van Dorn water sampler at 0.5 m depth. We acknowledge that this sampling depth may not cover the habitats for all species in Lake Biwa; however, this depth likely includes the representative samples of phytoplankton cells. Samples were taken fortnightly (at the beginning and middle of each month during day time). For each sampling date, all phytoplankton in a 1-ml water sample were identified and counted using a microscope by a single expert (S. Ichise). Some colony-forming cyanobacteria were counted as per colony unit and the others were counted as per cell unit (detailed in Appendix 2 of Supplementary material) to facilitate counting. Average number of cells per colony was also recorded (Appendix 2 of Supplementary material). Only species with occurrence of more than 5 years during the span were analyzed in this study (Appendix 3 of Supplementary material). The taxonomy of phytoplankton changed over time, and we have carefully adjusted the database to ensure the consistency.

Phytoplankton from SPFES (Stations 1–5 in Fig. 1) were collected monthly during daytime using a plankton net of 95- $\mu\text{m}$  mesh size towed at four depth intervals: 0–10, 10–20, 20–40, and 40–75 m. Experts carrying out species identification changed in different periods. As in the LBERI dataset, we have carefully adjusted the change of taxonomy of phytoplankton in the SPFES database. To be consistent with the LBERI data, only data from the 0–10 m interval were analyzed here. Indeed, this surface layer contained higher abundance of phytoplankton compared with the other depths. The cell density in the surface layer (0–10 m) is significantly higher than the average density of other deep layers (with a grand average of all species ranging 2.5 fold at Station 1 to 172 fold at Station 5). Only species with occurrence of more than 5 years during the span were analyzed (Appendix 4 of Supplementary material). We averaged the data from the five stations to form a single value. Data from the deepest station (Station 4, with a water depth closest to Station L in LBERI) exhibited a similar pattern with the averaged values for most species we analyzed here (correlation analysis,  $p < 0.00001$ ) but showed a higher variability. Only 4 species, *Eudorina elegans*, *Aphanothece nidulans*, *Fragilaria* sp., and *Synedra* sp. did not show a high correlation ( $p > 0.05$ ), and we noticed that these species occurred in <10 years in the database. We chose to show the results based on the average data because these data exhibited less variance. The results based on analyzing only Station 4 are qualitatively the same as the results based on the average data. Missing data were imputed using the best fitting state-space model based on the Kalman filter (Durbin and Koopman 2001; Hsieh et al. 2009a). We noticed that much fewer

phytoplankton taxa were identified in the SPFES data; this is due to the large mesh size of the sampling gear that misses most small phytoplankton species. This problem makes it difficult to integrate the LBERI and SPFES datasets, and thus these two datasets were analyzed independently.

To compute total biomass of phytoplankton, we first converted species abundance to biovolume and then to carbon biomass. Biovolume of phytoplankton species (Appendix 2 of Supplementary material) was measured by LBERI and reported in Ichise et al. (2007). Biovolume of colony-forming species was measured as the colony volume. Single cell volume was estimated as the colony volume divided by averaged cell number per colony (Ichise et al. 2007). Taxa-specific empirical equations (Rocha and Duncan 1985; Verity et al. 1992; Menden-Deuer and Lessard 2000) were then used to convert biovolume to carbon biomass (Appendix 5 of Supplementary material). We summed these values to construct the time series of total biovolume and total carbon biomass for the SPFES and LBERI datasets, respectively (Fig. 2).

#### Data analyses

Two main environmental issues are associated with the Lake Biwa ecosystem: eutrophication and warming. To understand these effects on the Lake Biwa ecosystem, first, correlation analyses were used to investigate long-term relationships among physical, chemical, and bulk biological properties (shown in Figs. 2, 3) at an interannual scale. Stationary bootstrap approach (Politis and Romano 1994) with accelerated bias correction was used to compute 95% confidence limits and to perform a hypothesis test in order to account for serial dependence in the time-series data (Hsieh et al. 2009a). For correlation analyses, only data in the overlapped time period between pair-wise series were considered. Consequently, sample sizes were not equal for different comparisons, and thus paired series with a short overlap tended to show no statistically significant correlation when there was no sufficient variation within the short overlap period.

Second, warming in water temperature in Lake Biwa around 1990 (Fig. 3a–c) has caught particular attention, because this warming event has resulted in significant reduction of dissolved oxygen in bottom waters, which might have negative effects on benthic organisms (Kumagai 2008). We tested whether significant changes in magnitude and variance of physical variables had happened since 1990, using a permutation test. Specifically, we calculated the difference in mean and variance for the two periods (before and after 1990) for each variable, and then generated a null distribution by randomly shuffling the data to perform hypothesis tests (Hsieh et al. 2009b). Prior to



calculating the variance, we removed a long-term trend in time-series data to avoid inflation in variance caused by the trend (Hsieh et al. 2006, 2009a). To do this, we extracted the trend by using the locally weighted scatter plot smoother (LOWESS) and calculated the magnitudes of residuals deviated from the trend (Hsieh et al. 2006). We used a window of 20 years to estimate the LOWESS, but sensitivity analysis indicated that our results were not sensitive to the choice of window size, given that the window is sufficiently large (>15 years).

Third, long-term variation of phytoplankton communities were examined using principal component analysis (PCA) for the SPEFS and LBERI data separately. Prior to analysis, abundance data were transformed as  $\log_2(X + 1)$  to account for the aggregation effects of phytoplankton. We linked the temporal pattern of phytoplankton community to environmental variables using redundancy analysis (RDA) (Legendre and Legendre 1998). Stepwise procedure was used to select the significant variables (using  $\alpha = 0.05$ ) to exclude irrelevant variables, and such a procedure will select only the variable that explains the highest variance when multiple variables in the model are linearly dependent (Peres-Neto et al. 2006). In addition, correlation analyses were carried out to relate environmental variables to individual species.

Fourth, we categorized phytoplankton species into groups according to their time-series pattern (based on scores extracted from the PCA), using K-means clustering to obtain the optimal classification (Legendre and Legendre 1998). The optimal number of groups was determined by the Davies-Bouldin index (Davies and Bouldin 1979). The categorized phytoplankton species were plotted on the RDA biplot to exhibit the loadings of phytoplankton species on the community pattern and to link phytoplankton species to environmental variables (Legendre and Legendre 1998).

## Results

### Long-term environmental variation

The time series of total phosphorus (TP) in Lake Biwa exhibited a history of eutrophication and a later return to a less eutrophic state in the past half century. TP increased quickly after 1967, reached a maximum in 1974 and then declined until 1985, and fluctuated around a stable value thereafter (Fig. 2a). This variation of trophic status also was reflected in bulk phytoplankton biovolume (Fig. 2b) and biomass (Fig. 2d) for the SPFES data (1962–1991) as well as the water column transparency (Secchi depth) for the whole period (Fig. 2g). Significant correlations were found among these variables (Table 1). Correlations

between bulk phytoplankton variables of LBERI data (1978–2003) and TP were not significant (Fig. 2c, e, f; Table 1), presumably because the variation of trophic status was much less pronounced during this later period. Nevertheless, correlations among those bulk phytoplankton variables (total biovolume and carbon) of SPFES and LBERI data for the overlap period (14 years) were all significant (Fig. 2b, c, d, e, f; Table 1), suggesting that the bulk phytoplankton biomass from the two institutes showed a similar response to environmental change, although these two sets of data were collected with different methods and in different locations. This supports our use of these two datasets in parallel to investigate environmental effects on phytoplankton in Lake Biwa.

Changes in the trophic status of Lake Biwa were also seen in other chemical variables. Phosphate concentration was low in the early period when TP started to increase (Fig. 2h). Phosphate accumulated over time from 1972 to 1987, dropped rapidly since 1987, and then fluctuated dramatically within a period of roughly 5 years. Nitrate concentration was also low in the early period when TP started to increase (Fig. 2i). It then accumulated over time and leveled off after 1991. Silicate concentration was only available after 1978, and it showed an increasing trend (Fig. 2j). Ammonium concentration was almost undetectable in the early period when TP started to increase (Fig. 2k). It increased suddenly in 1972, exhibited two large peaks between 1972 and 1985, dropped to very low value in 1985, and has increased since then.

Several chemical variables as well as their ratios were correlated (Table 1). Phosphate/TP ratio correlated positively with nutrient concentrations and bulk phytoplankton of SPFES series, and negatively correlated with mixing condition and precipitation (Table 1). Ammonium/nitrate ratio positively correlated with TP and ammonium concentration and bulk phytoplankton of SPFES series (Table 1).

Physical data exhibited significant interannual variability and a warming trend. Air temperature showed interannual fluctuations (Fig. 3a), superimposed by a significant warming trend (stationary bootstrapped correlation;  $r = 0.641$ ,  $p < 0.05$ ). Before 1990, air temperature surpassing 15°C was only observed in 1979, but this high value was observed frequently after 1990 (Fig. 3a). Albeit strong short-term fluctuations, the average air temperature after 1990 was significantly higher than that before 1990 (permutation test of the mean;  $p < 0.001$ ). One can also notice a higher variability after 1990 ( $\text{var} = 0.311$ ) than that before 1990 ( $\text{var} = 0.166$ ), although this difference was only marginally significant ( $p = 0.082$ ). A similar situation was found in the surface water temperature (Fig. 2b). A significant warming trend was found ( $r = 0.563$ ,  $p < 0.05$ ), and the average surface water

Table 1 Result of correlation analysis among environmental variables

	TP	Phosphate	Nitrate	Ammonium	Silicate	Air T	Lake surface T	Lake bottom T	Wind mixing	Thermocline depth	Buoyancy frequency	AO	Shiga cell volume	LBBERI cell volume	Shiga total carbon	LBBERI total carbon	I/Secchi depth	Chla	Precipitation	PAR	Phosphate/TP	Ammonium/nitrate
TP	NA	0	0	1	-1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	-1	0	1
Phosphate	-0.102	NA	1	0	1	0	0	0	-1	-1	1	0	1	0	1	0	0	0	-1	0	1	0
Nitrate	-0.101	0.562	NA	0	1	1	1	1	-1	0	1	0	0	0	0	0	0	0	-1	-1	1	0
Ammonium	0.446	0.101	0.159	NA	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	-1	0	1
Silicate	-0.467	0.462	0.662	-0.286	NA	0	0	0	0	-1	0	0	0	0	0	0	0	0	0	0	1	0
Air T	-0.212	0.399	0.583	0.025	0.315	NA	1	1	0	0	1	1	0	0	0	0	0	0	-1	0	1	0
Lake surface T	-0.020	0.371	0.507	0.134	0.260	0.868	NA	1	0	0	1	1	0	0	0	0	0	0	-1	0	0	0
Lake bottom T	-0.344	0.194	0.601	0.135	0.343	0.627	0.613	NA	0	0	0	1	0	0	-1	0	0	0	-1	0	0	0
Wind mixing	-0.194	-0.585	-0.663	-0.202	0.264	-0.292	-0.245	-0.184	NA	1	-1	0	-1	-1	-1	0	0	-1	0	1	-1	0
Thermocline depth	-0.066	-0.473	-0.298	0.109	-0.350	-0.203	-0.265	0.188	0.358	NA	-1	0	0	0	0	0	0	1	1	0	-1	0
Buoyancy frequency	0.121	0.349	0.508	0.070	0.236	0.561	0.557	0.089	-0.348	-0.448	NA	0	1	0	1	0	0	-1	-1	-1	1	0
AO	0.022	-0.061	0.349	-0.049	-0.017	0.388	0.291	0.401	-0.247	0.163	0.107	NA	0	0	0	0	0	0	0	-1	0	0
Shiga cell volume	0.596	0.441	0.356	0.368	-0.256	-0.038	0.048	-0.271	-0.552	-0.177	0.516	-0.087	NA	1	1	1	1	0	-1	-1	1	1
LBBERI cell volume	0.260	0.253	-0.155	0.430	-0.104	-0.406	-0.192	-0.240	-0.418	0.067	-0.231	-0.434	0.629	NA	1	1	0	1	0	0	0	0
Shiga total carbon	0.594	0.437	0.328	0.371	-0.232	-0.059	0.028	-0.284	-0.538	-0.169	0.496	-0.100	0.999	0.628	NA	1	1	1	0	-1	1	1
LBBERI total carbon	0.290	0.354	-0.219	0.436	-0.086	-0.408	-0.149	-0.267	-0.404	-0.007	-0.203	-0.553	0.797	0.947	0.788	NA	0	1	0	0	0	0
I/Secchi depth	0.448	-0.137	-0.196	0.479	-0.454	-0.139	-0.242	-0.128	0.075	0.170	-0.150	-0.049	0.326	0.420	0.327	0.366	NA	0	0	0	0	1
Chla	0.053	0.148	-0.182	0.377	-0.385	-0.292	-0.180	-0.034	-0.409	0.345	-0.458	-0.171	0.425	0.724	0.434	0.769	0.343	NA	0	0	0	0
Precipitation	0.341	-0.491	-0.401	0.253	-0.171	-0.526	-0.486	-0.310	0.378	0.324	-0.407	-0.139	0.028	0.356	0.039	0.297	0.408	0.148	NA	0	-1	0
PAR	-0.509	-0.332	-0.604	-0.370	-0.242	-0.135	-0.134	-0.159	0.701	0.150	-0.394	-0.212	-0.652	0.021	-0.639	0.060	-0.191	0.040	0.039	NA	0	0
Phosphate/TP	-0.143	0.989	0.564	0.050	0.527	0.408	0.373	0.242	-0.552	-0.464	0.317	-0.068	0.368	0.202	0.364	0.300	-0.177	0.100	-0.509	-0.289	NA	0
Ammonium/nitrate	0.424	-0.057	-0.094	0.936	-0.412	-0.059	0.051	0.046	-0.013	0.221	-0.049	-0.080	0.291	0.375	0.297	0.403	0.572	0.346	0.315	-0.232	-0.104	NA

Correlation coefficients are shown in the lower triangle, and results of stationary bootstrap tests ( $\alpha = 0.05$ ) are shown in the upper triangle with 1 indicating a significant positive correlation, -1 indicating a significant negative correlation, and 0 indicating no significant correlation



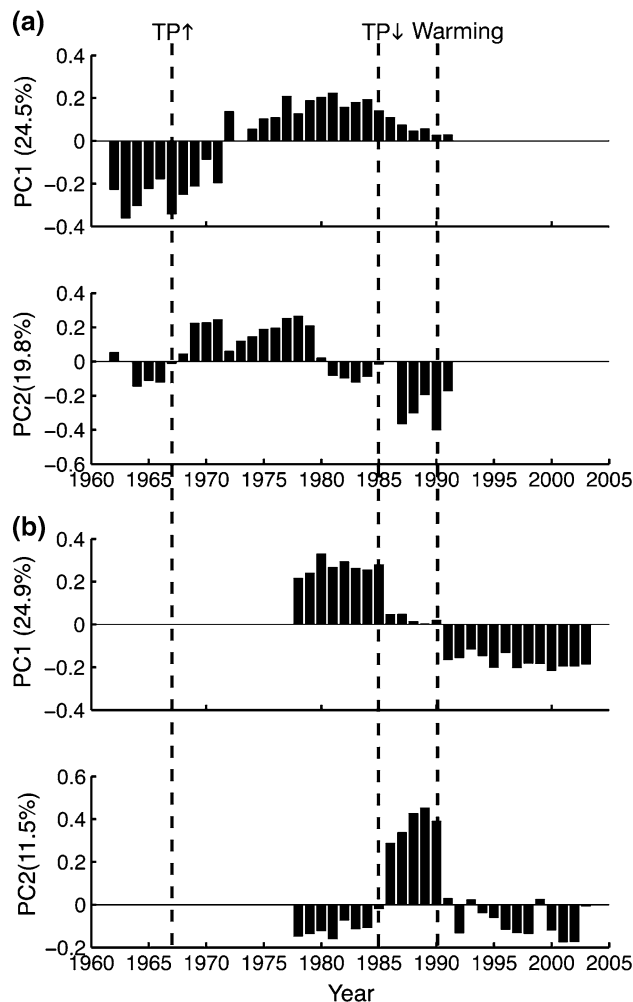
temperature after 1990 was significantly higher than that before 1990 ( $p < 0.001$ ). A higher variability of surface water temperature was seen in the later period ( $\text{var} = 0.326$ ) compared with the earlier period ( $\text{var} = 0.201$ ), but there was only a marginally significant difference ( $p = 0.110$ ). The warming signal was especially strong in the bottom water temperature (Fig. 3c). The average bottom temperature after 1990 was significantly higher ( $p < 0.001$ ), but the variability was significantly lower ( $p = 0.048$ ).

Along with warming, the buoyancy frequency across the thermocline also increased over time ( $r = 0.606$ ,  $p < 0.05$ ; Fig. 3d). The mean value and variance in the later period is significantly higher than that in the earlier period ( $p = 0.002$  and  $0.021$ , respectively). Wind mixing strength declined initially from 1961 to 1975 and then leveled off (Fig. 3e), and the long-term declining trend was significant ( $r = -0.597$ ,  $p < 0.05$ ). The mean mixing index was slightly higher in the earlier period ( $p = 0.078$ ), and no significant difference in variance was found ( $p = 0.226$ ). The mean and variance in thermocline depth in the earlier period is slightly higher than the later period ( $p = 0.107$  and  $p = 0.051$ , respectively), but no significant long-term declining trend existed ( $r = -0.333$ ,  $p > 0.05$ ; Fig. 3f). Precipitation showed a long-term declining trend ( $r = -0.546$ ,  $p < 0.05$ ; Fig. 3g), and its magnitude in the earlier period was significantly higher than the latter ( $p = 0.003$ ); however, the variance was not different for the two periods ( $p > 0.05$ ). The photosynthetic available radiation (Fig. 3h) was high before 1970 and remained steady after 1970 (no significant trend and no difference in mean magnitude and variance between the two periods,  $p > 0.05$ ).

Most physical variables were correlated. Both air and water temperatures were significantly correlated with the Arctic Oscillation index (Table 1). Air and surface temperatures had a positive effect on maximal buoyancy frequency (Table 1), as one would expect. However, temperature did not significantly affect thermocline depth (Table 1); instead, it was the wind mixing strength that determined thermocline depth (Table 1). In addition, the wind mixing strength showed a negative effect on maximal buoyancy frequency (Table 1). Temperature and temperature related variables were negatively correlated with precipitation (Table 1). PAR was negatively correlated with several nutrients, AO, and SPFES (1962–1991) phytoplankton abundance (Table 1).

#### Responses of phytoplankton community

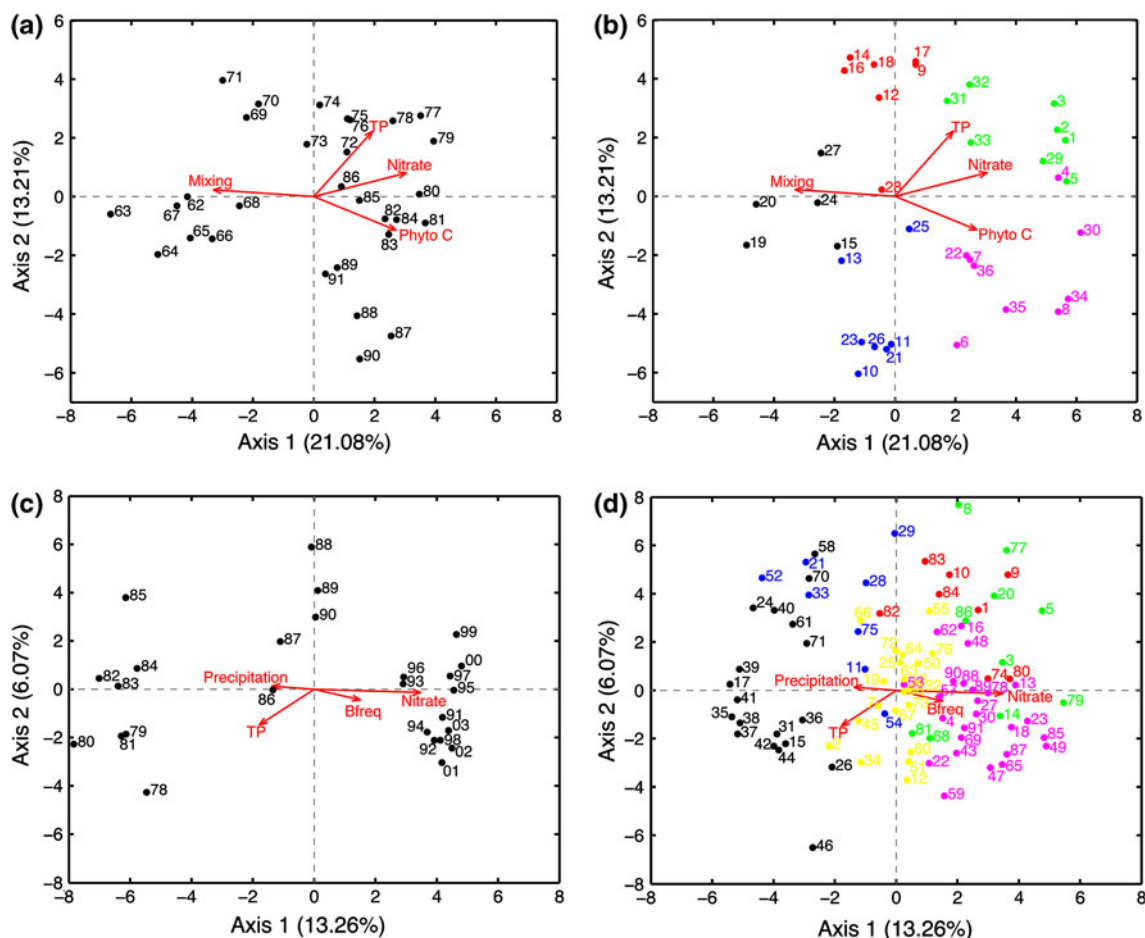
Variation in trophic status and warming considerably affected the phytoplankton community in Lake Biwa. The increase in TP around 1967 was detected by the PC2 of SPFES phytoplankton data, and the decrease in TP around



**Fig. 4** Principal components based on phytoplankton data from **a** the SPFES and **b** the LBERI. Vertical dashed lines indicate roughly the years when the lake environment changed

1985 was reflected in the PC2 of SPFES data and the PC1 and PC2 of LBERI data (Fig. 4). The PC1 of SPFES data seemed to follow the variation of TP but with a time delay (Fig. 4). The dramatic warming around 1990 also significantly altered the phytoplankton community (with 1 year delay), as shown by the PC1 and PC2 of LBERI data (Fig. 4). The phytoplankton community clearly signaled environmental variations.

The linkage between temporal variation of the phytoplankton community and environmental variables are illustrated in RDA plots (Fig. 5). We separated the ordination of years and species into two plots for better visualization. When overlapped, one can find the relationships among sampling years, phytoplankton taxa, and environmental factors. For the SPFES data, the change in the phytoplankton community followed mainly the trophic status of the lake, with a strong correlation with TP, nitrate and total phytoplankton carbon (Fig. 5a). In addition, the



**Fig. 5** Ordination biplots exhibiting the relationships among years and phytoplankton species and their linkage to environmental variables for (a, b) SPES and (c, d) LBERI data. a and c represents ordination of years, and b and d represents ordination of species. Only significant ( $p < 0.05$ ) environmental variables (red arrows) were used

early period was affected by mixing condition (Fig. 5a). The associations between phytoplankton taxa and the environmental factors were also revealed (Table 2, Fig. 5b). The phytoplankton taxa can be statistically categorized into five groups, and these groups largely depicted the evolution of the environmental state of the lake from 1962 to 1991 (Fig. 5b). For the LBERI data, the phytoplankton community was related to TP and precipitation in the early period (1978–1985) and then to maximum buoyancy frequency and nitrate concentration in a later period (Fig. 5c). The associations between phytoplankton taxa and the environmental factors are shown in Fig. 5d. The phytoplankton taxa can be categorized into six groups; however, the separation of these groups was less distinct (Table 2, Fig. 5d). The group L6 showed no significant variation throughout the period, while group L1 to L5 revealed a transition (Fig. 5d).

based on stepwise selection. Color coding indicates phytoplankton groups classified from K-means classification: black, group 1; blue, group 2; red, group 3; green, group 4; magenta, group 5; cyan, group 6. See Table 2 for species names

The signals of phytoplankton in response to environmental events remained strong even when only presence/absence data of species were considered (Fig. 6). As can be seen in Fig. 6a (SPES data), some species mainly occurred during the eutrophic period, while others only appeared when the eutrophic condition diminished. Similarly in Fig. 6b (LBERI data), some species mainly occurred between 1978 and 1985, while others only appeared when the eutrophication faded away. Interestingly, some species emerged after the temperature increased.

Species-specific responses to each environmental factor corroborated with the results of RDA. To visualize this, we plotted correlation structures between each phytoplankton species and environmental variables categorized by group (Fig. 7). For groups S1, S2, and S3 of SPES data, many species showed no significant correlation with

**Table 2** List of frequent species (occurring >5 years in samples) used in the analyses. Shown are species ID along with group classifications. The ID corresponds to the order in Fig. 6

Species	ID	Group
<b>SPFES</b>		
<i>Staurastrum dorsidentiferum</i>	1	S4
<i>Pediastrum biwae</i>	2	S4
<i>Closterium aciculare</i>	3	S4
<i>Asterionella formosa</i>	4	S5
<i>Melosira solida</i>	5	S4
<i>Stephanodiscus carconensis</i>	6	S5
<i>Melosira varians</i>	7	S5
<i>Melosira granulata</i>	8	S5
<i>Sphaerocystis Schroeteri</i>	9	S3
<i>Ceratium hirundinella</i>	10	S2
<i>Eudorina elegans</i>	11	S2
<i>Xanthidium antilopaeum</i>	12	S3
<i>Staurastrum limneticum</i>	13	S2
<i>Aphanocapsa elachista</i>	14	S3
<i>Cosmocladium constrictum</i>	15	S1
<i>Synedra ulna</i>	16	S3
<i>Fragilaria capucina</i>	17	S3
<i>Aphanothece nidulans</i>	18	S3
<i>Aphanocapsa</i> sp.	19	S1
<i>Staurastrum</i> sp.	20	S1
<i>Dinobryon</i> sp.	21	S2
<i>Spirogyra</i> sp.	22	S5
<i>Fragilaria</i> sp.	23	S2
<i>Synedra</i> sp.	24	S1
<i>Oscillatoria</i> sp.	25	S2
<i>Aphanothece</i> sp.	26	S2
<i>Diatoma</i> sp.	27	S1
<i>Microcystis</i> sp.	28	S3
<i>Staurastrum arctiscon</i>	29	S4
<i>Melosira italica</i>	30	S5
<i>Spondylosium moniliforme</i>	31	S4
<i>Oocystis</i> sp.	32	S4
<i>Chroococcus dispersus</i>	33	S4
<i>Fragilaria crotonensis</i>	34	S5
<i>Xanthidium hastiferum</i>	35	S5
<i>Mougeotia</i> sp.	36	S5
<b>LBERI</b>		
<i>Stephanodiscus carconensis</i>	1	L3
<i>Staurastrum dorsidentiferum</i>	2	L6
<i>Melosira granulata</i>	3	L4
<i>Fragilaria crotonensis</i>	4	L5
<i>Cryptomonas</i> sp.	5	L4
<i>Coelastrum cambricum</i>	6	L6
<i>Closterium aciculare</i>	7	L6
<i>Chroococcus dispersus</i>	8	L4
<i>Chlamydomonas</i> sp.	9	L3

**Table 2** continued

Species	ID	Group
<i>Aphanothece clathrata</i>	10	L3
<i>Ankistrodesmus falcatus</i>	11	L2
<i>Uroglena americana</i>	12	L6
<i>Nitzschia</i> sp.	13	L5
<i>Asterionella formosa</i>	14	L4
<i>Pediastrum biwae</i>	15	L1
<i>Nitzschia acicularis</i>	16	L5
<i>Melosira solida</i>	17	L1
<i>Gymnodinium helveticum</i>	18	L5
<i>Cyclotella glomerata</i>	19	L6
<i>Cosmocladium constrictum</i>	20	L4
<i>Elakatothrix gelatinosa</i>	21	L2
<i>Closterium</i> sp.	22	L5
<i>Ceratium hirundinella</i>	23	L5
<i>Oocystis lacustris</i>	24	L1
<i>Staurastrum pingue</i>	25	L6
<i>Mougeotia</i> sp.	26	L1
<i>Gloeocystis</i> sp.	27	L5
<i>Kirchneriella contorta</i>	28	L2
<i>Cyclotella stelligera</i>	29	L2
<i>Scenedesmus</i> sp.	30	L5
<i>Melosira italica</i>	31	L1
<i>Mallomonas fastigata</i>	32	L6
<i>Oocystis parva</i>	33	L2
<i>Spondylosium moniliforme</i>	34	L6
<i>Oocystis</i> sp. (a)	35	L1
<i>Actinastrum hantzschii</i>	36	L1
<i>Planktosphaeria gelatinosa</i>	37	L1
<i>Gloeocystis vesiculosa</i>	38	L1
<i>Gloeocystis gigas</i>	39	L1
<i>Glenodinium</i> sp.	40	L1
<i>Cryptomonas erosa</i>	41	L1
<i>Quadrigula lacustris</i>	42	L1
<i>Coelastrum microporum</i>	43	L5
<i>Chodatella citriformis</i>	44	L1
<i>Staurastrum longiradiatum</i>	45	L6
<i>Scenedesmus denticulatus</i>	46	L1
<i>Synedra acus</i>	47	L5
<i>Gymnodinium</i> sp.	48	L5
<i>Cyclotella</i> sp.	49	L5
<i>Eudorina elegans</i>	50	L6
<i>Tetraspora lacustris</i>	51	L6
<i>Oocystis submarina</i>	52	L2
<i>Staurastrum arctiscon</i>	53	L5
<i>Aphanocapsa elachista</i>	54	L2
<i>Navicula</i> sp.	55	L6
<i>Ankistrodesmus</i> sp.	56	L6
<i>Dictyosphaerium pulchellum</i>	57	L5

**Table 2** continued

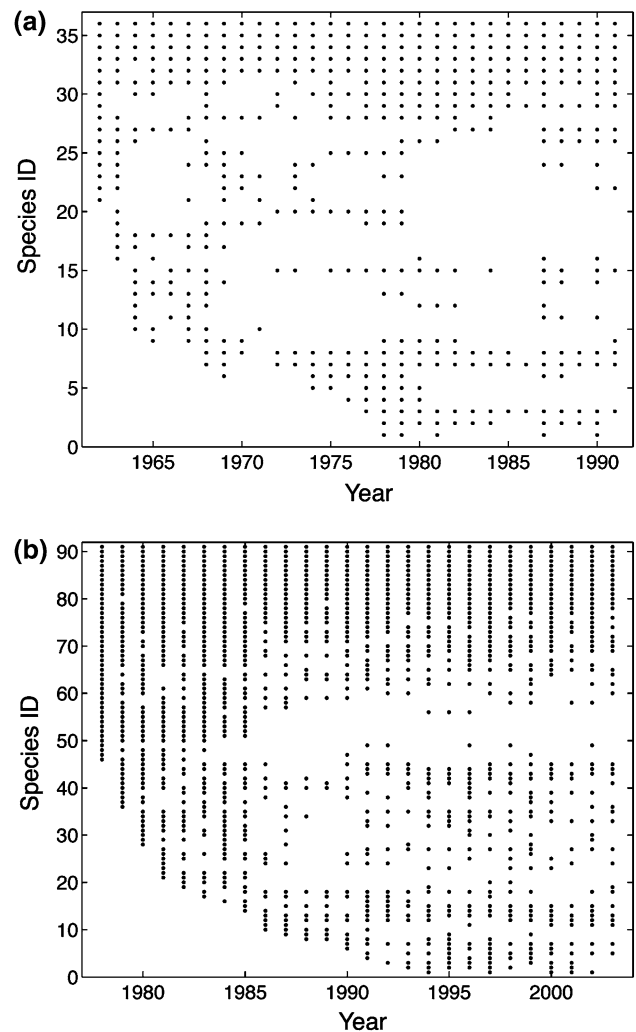
Species	ID	Group
<i>Schroederia judayi</i>	58	L1
<i>Staurastrum</i> sp.	59	L5
<i>Micractinium pusillum</i>	60	L6
<i>Crucigenia irregularis</i>	61	L1
<i>Cocconeis placentula</i>	62	L5
<i>Synedra</i> sp.	63	L6
<i>Chroococcus</i> sp.	64	L6
<i>Peridinium</i> sp.	65	L5
<i>Melosira distans</i>	66	L6
<i>Mallomonas tonsurata</i>	67	L6
<i>Dinobryon bavaricum</i>	68	L4
<i>Microcystis</i> sp.	69	L5
<i>Gomphonema</i> sp.	70	L1
<i>Chodatella subsalsa</i>	71	L1
<i>Xanthidium hastiferum</i>	72	L6
<i>Mallomonas akrokomos</i>	73	L6
<i>Chrysamoeba radians</i>	74	L3
<i>Chromulina</i> sp.	75	L2
<i>Trachelomonas</i> sp.	76	L6
<i>Gomphosphaeria lacustris</i>	77	L4
<i>Mallomonas</i> sp.	78	L5
<i>Rhodomonas</i> sp.	79	L4
<i>Planktosphaeria</i> sp.	80	L3
<i>Peridinium berolinense</i>	81	L4
<i>Pseudokephyron gallicum</i>	82	L3
<i>Monoraphidium tortile</i>	83	L3
<i>Chlorocloster</i> sp.	84	L3
<i>Oocystis</i> sp.	85	L5
<i>Oocystis solitaria</i>	86	L4
<i>Microcystis wesenbergii</i>	87	L5
<i>Peridinium elpatiewskyi</i>	88	L5
<i>Microcystis incerta</i>	89	L5
<i>Microcystis aeruginosa</i>	90	L5
<i>Quadrigula chodatii</i>	91	L5

environmental variables, as did many species in the group L6 of LBERI data. Further, the patterns were clear in the LBERI data but not very strong in the SPFES data.

## Discussion

### Ecosystem changes

Dramatic changes of trophic status and water column physical properties have occurred during the past half century in Lake Biwa. The start of eutrophication in the late 1960s and return to a less eutrophic state in the mid

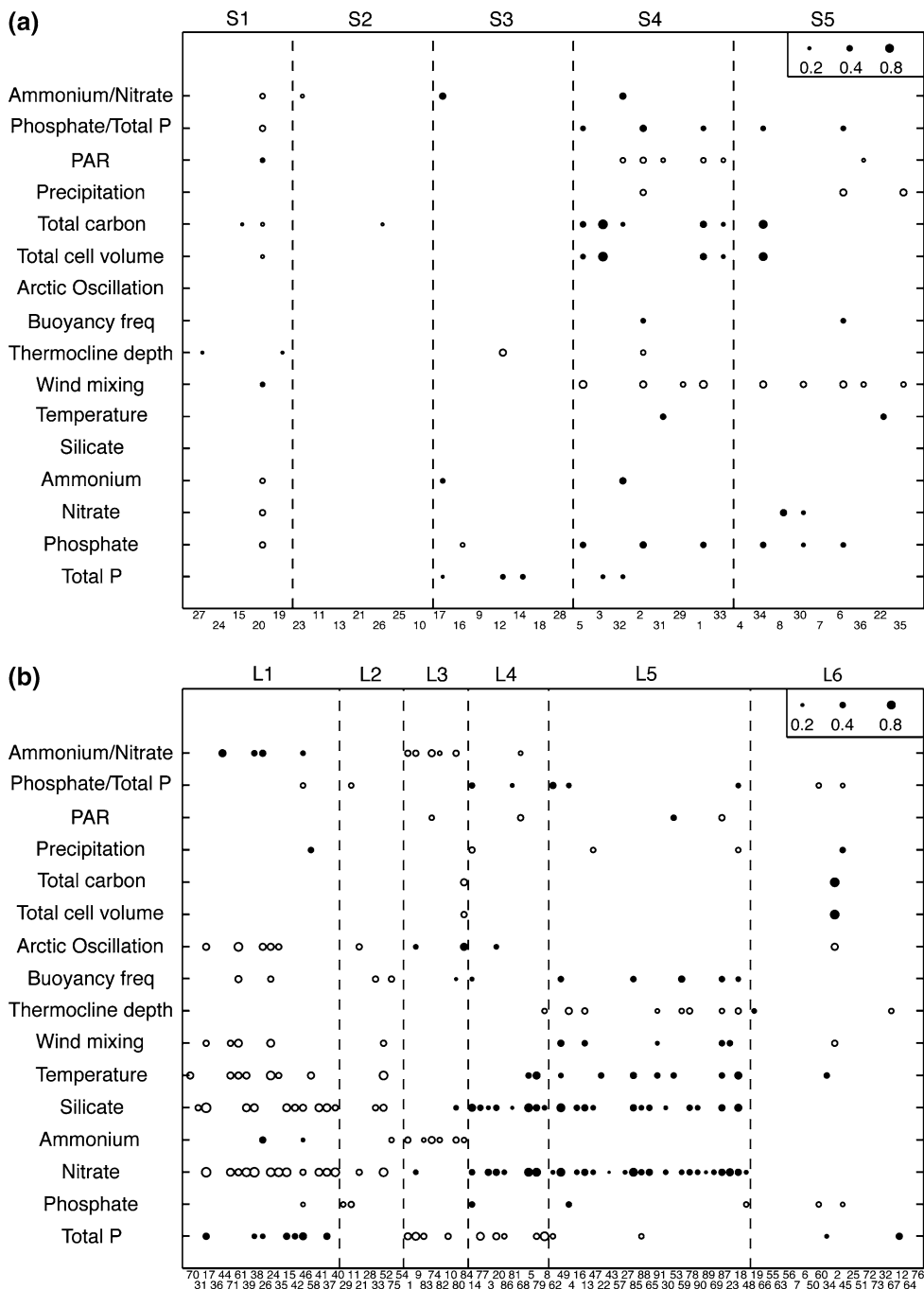


**Fig. 6** Species occurrence for phytoplankton species collected from (a) the SPFES and (b) the LBERI. The order (top-down) follows the first occurrence of species in the data series. Only species occurred for more than five sampling years are included. See Table 2 for species names

1980s had been suggested according to anecdotal evidence, sediment records, and museum fish samples (Ogawa et al. 2001; Nakazawa et al. 2010). However, here for the first time, the history of the trophic status of Lake Biwa is documented based on direct measurements of water column chemical data. These chemical data overcome the issues of coarse temporal resolution and high uncertainty associated with sediment records and museum fish samples.

The total phytoplankton biomass was mainly determined by trophic status. The TP data clearly showed a rapid increase after 1967, reached maximum in 1974, then declined until 1985, and fluctuated around a stable value thereafter (Fig. 2a). The return to a less eutrophic condition in the mid 1980s was due to a successful governmental

**Fig. 7** Correlation structures between phytoplankton species and environmental variables categorized by group for **a** SPFES and **b** LBERI data. *Filled* and *open circles* indicate significant positive and negative correlations, respectively. See Table 2 for species ID



mitigation enforced since 1982 (Kumagai 2008). The total phytoplankton biomass (or its surrogate) largely followed the trend of TP (Table 1, Fig. 2b, d, g), at least for the period from 1962 to 1991 (SPFES data). Further, cross-correlation analyses between TP and phytoplankton biomass indicators showed that the best correlation occurred when phytoplankton biomass lagged 1 year behind TP, suggesting a quick response of phytoplankton total biomass to the control of nutrient loading. This is in contrast to many studies (e.g. Anneville and Pelletier 2000; Horn

2003; Dokulil and Teubner 2005) that discovered none or delayed response of phytoplankton biomass to nutrient mitigation. The negative correlation between total phytoplankton biomass and light (photosynthetic available radiation measured from the meteorological station) may be a spurious result; however, we do not have measurements of light intensity in the water column to clarify this issue. The negative correlation between mixing strength and SPFES phytoplankton biomass implies negative effects of strong mixing on phytoplankton, perhaps by destructing



vertical structure of phytoplankton that is critical for their light acclimation (Tirok and Gaedke 2007; Winder and Hunter 2008).

The correlations between TP and LBERI phytoplankton bulk measurements (Fig. 2c, e, f) were not significant (Table 1). This is maybe because the variation of TP has been at a low level during this period (1978–2003) and factors in addition to nutrients had also contributed to affecting phytoplankton, such as mixing regime (Table 1). The negative correlation between mixing strength and LBERI phytoplankton biomass also existed, as in the SPFES data series. The differential responses of the SPFES and LBERI data series to environmental variables may be because they covered different periods. The critical environmental forcing on phytoplankton may have changed through time, and the phytoplankton species compositions were different for these two periods (Table 2, Figs. 4, 6).

Inorganic nutrients exhibited complex variation irrespective of TP. Phosphate concentration was low during the early period of eutrophication presumably due to rapid uptake of phytoplankton, and then accumulated gradually up to 1987 (Fig. 2h). Such kinds of progression were commonly observed during eutrophication in lakes (Anneville et al. 2002a). However after 1987, phosphate fluctuated with a high amplitude despite TP remaining stable during that period (Fig. 2a, h); that is, even though nutrient loading had been controlled, phosphate still showed episodic high pulses. Preliminary analyses suggested this is related to internal loading from the sediments (Murphy et al. 2001; Kumagai 2008). The nitrate (Fig. 2i) and silicate (Fig. 2i) accumulating over time was likely due to both external and internal loading (Kumagai 2008). Ammonium (Fig. 2k) was low during the early period of eutrophication presumably due to rapid uptake of phytoplankton, and showed two strong peaks between 1972 and 1985, which was likely associated with eutrophication. The fluctuation of ammonium is not a result of measurement error, as the ammonium data from LBERI also showed a similar pattern. We found a significant correlation between the phosphate/TP ratio and air temperature, mixing index, and precipitation (Table 1), suggesting climate variations might have effects on nutrient dynamics through regional physical processes.

Global climate changes have affected Lake Biwa through atmospheric forcing. This can be seen from significant correlations between the Arctic Oscillation index versus air and water temperature (Table 1, Fig. 3). This association is especially strong in winter (Fig. 3j). It is worth noting that the temperature of Lake Biwa has warmed significantly in the past half century, with a warming rate of 0.031°C/year for air temperature (Fig. 3a) and 0.028°C/year for surface water temperature (Fig. 3b). Increased temperature enhanced water column stability as

signified by the max buoyancy frequency (Table 1, Fig. 3d), which potentially reduced nutrient mixing and upwelling (Hsieh et al. 2009b). Temperature did not directly affect the thermocline depth (Table 1, Fig. 3f). Rather, thermocline depth was influenced by the strength of wind mixing (Table 1, Fig. 3e), as also shown in other lakes (Coats et al. 2006). The strength of wind mixing has weakened along with warming (Fig. 3e), and further reinforced water column stability (Table 1). The enhanced water column stability due to the combination of increased water temperature and reduced wind mixing is attributed to the reduced bottom water renewal and consequently anoxic benthic condition, which might lead to catastrophic mass mortality of benthic fishes (Kumagai 2008).

Air and water temperature and max buoyancy frequency after 1990 was significantly higher than that before 1990 (Fig. 3a, b, c). In addition, the variance was also higher during the later period, suggesting that the lake physical environment in this later period was unstable. The cause of this high variability is not known at this time. However, such increased variance has been suggested to be indicative of ecosystem transition, often to undesired states (Brock and Carpenter 2006; Carpenter and Brock 2006). Thus, the effects of high variability of the living environment on organisms warrant further investigation.

#### Reorganizations of phytoplankton community

Variation in the phytoplankton community was clearly affected by changes in trophic status and the physical environment of lake Biwa (Fig. 5). For the SPFES data, the changes in community were mainly caused by nutrient-related variables through time (roughly clock-wise evolution in Fig. 5a, b). Some species developed after eutrophication started, and others occurred before eutrophication and after recovery. The phytoplankton community showed a clear change as revealed by the statistically defined five groups (Figs. 5b, 6a, Appendix 3 of Supplementary material). For the LBERI data, the changes in community were caused by nutrient-related and temperature-related variables (roughly clock-wise evolution in Fig. 5c, d). One can replace the max buoyancy frequency with lake water temperature or AO to obtain qualitatively similar RDA results, indicating that climate driven changes in physical properties of the water column have had significant impacts on the phytoplankton community in Lake Biwa. One can replace nitrate concentration with ammonium/nitrate ratio and obtain qualitatively similar RDA results, indicating that nutrient dynamics also contributed to variations in the phytoplankton community. In addition, we found precipitation was a significant variable in RDA. The precipitation might affect phytoplankton community by changing nutrient input or turbidity of the water column.



Some species only appeared during eutrophication (1978–1985 for the LBERI series), and others only occurred after warming (1990) (Figs. 5d, 6b, Appendix 2 of Supplementary material). Interestingly, several cyanobacteria taxa (such as *Microcystis* spp.) established large concentrations during the warming period (Appendix 2 of Supplementary material). This may be because they prefer stable water conditions (Elliott et al. 2006), and at the same time, the nutrient supply was sufficient (Fig. 2h, i) for them to prosper. In fact, nutrients could come from internal loading due to anoxic bottom conditions caused by warming and thus stronger water column stability. These results suggest synergistic effects of eutrophication and warming in Lake Biwa in driving phytoplankton community dynamics.

The changes in trophic status and warming have driven reorganization of the phytoplankton community in Lake Biwa. On the one hand, while the total phytoplankton biomass has largely followed TP (Fig. 2), the community structure has changed in the SPFES data (Figs. 4, 5, 6). On the other hand, while the total phytoplankton biomass remained at a stable level, the phytoplankton community almost completely reorganized in response to eutrophication and warming in the LBERI data (Figs. 4, 5, 6). Such a phenomenon was also observed in other lakes (Zohary 2004; Winder and Hunter 2008), and we believe this is the norm rather than the exception. One might consider these reorganizations as nonlinear transitions among multiple stable states (Scheffer et al. 2001; Hsieh et al. 2008). Conceptually, total phytoplankton live at the limit of the carrying capacity of the lake, determined by resource availability (e.g. nutrients). Phytoplankton species compete for resources; some species were sensitive to different nutrients, whereas others were sensitive to temperature or the mixing condition of the water column, as exemplified in the current study (Figs. 5, 6, 7). The winners are decided by a certain threshold of resource factors, or more likely by multiplication of several factors; that is, several conditions (both environmental and biological) need to be fulfilled simultaneously for a new state to emerge (Dixon et al. 1999; Hsieh et al. 2005). Once the tipping point is reached, the winners are determined. Often through some kind of positive feedback, the winner can stay at the stage for a prolonged period until a different threshold is reached, owing to external forcing such as a nutrient pulse or change in temperature (Scheffer and Carpenter 2003).

Nonlinear transitions among stable states might have happened in the Lake Biwa phytoplankton community. Significant changes in relative abundance (Fig. 4) and even species members (Fig. 6) in different trophic regimes or physical conditions were observed. Although community patterns showed correlations with environmental factors (Fig. 5), most of the correlations were weak. When individual species was considered, the correlations were even

weaker or non-existent (Fig. 7). Nonetheless, the changes of community as an ensemble (Fig. 4) were consistent with the eutrophication and warming in timing. The weak linear correlations and threshold responding of the community implies regime shifts of phytoplankton in Lake Biwa (Hsieh and Ohman 2006). However, we cannot rule out the possibility that such results may arise due to the change in vertical distribution of phytoplankton species (Anneville et al. 2002a).

It is likely that the trophic status and phytoplankton community in the large north basin was influenced by the small, shallow and eutrophic south basin, because flows between two basins have been observed (Kumagai 2008). It was speculated that cyanobacteria (*Microcystis*) observed in the north basin might be seeded from the south basin (Kumagai 2008). However, to what extent the north basin was influenced by the south basin needs further study.

#### Limitations of the data

We had hoped to completely integrate the historical data collected in Lake Biwa when we started to compile those time series. However, our data contain several caveats. First, the discrepancy between the sampling methods for phytoplankton by the two institutes makes data integration difficult. Second, the phytoplankton sampling in the LBERI was limited to the surface layer, which prevented us from investigating the vertical distribution of species. Third, the large mesh size used in sampling phytoplankton in the SPFES data likely missed important small species. Fourth, the TP data are compiled from Station I and L (Fig. 1), which are far apart, and these two series have limited overlap to facilitate rigorous calibration. Here, we faithfully present the data (Appendices 1–4 of Supplementary material). Readers can judge our analyses with those caveats in mind.

#### Conclusion

We analyzed long-term time-series data for the Lake Biwa ecosystem from 1962 to 2003. Analyses on environmental data indicate that Lake Biwa had experienced intensified eutrophication in the late 1960s and return to a less eutrophic status around 1985, and then exhibited rapid warming since 1990. The phytoplankton clearly responded to the change in trophic status and to more recent warming in Lake Biwa. Generally, the total phytoplankton biomass followed the rise and fall of total phosphorus loadings in the Lake (Fig. 2), exhibiting a symmetrical behavior. This suggests a quick response of phytoplankton total biomass to the control of nutrient loading. However, the phytoplankton community changed dramatically (Figs. 4, 5, 6),

exhibiting an asymmetrical behavior, with the community in the later period (since 1990) likely being modified by water warming. In addition, the correlations between individual species and environmental variables are weak (Fig. 7). These results imply possible nonlinear transitions of phytoplankton communities among multiple stable states. Phytoplankton species can be categorized into groups depending on their time series behavior (Fig. 5). We still have limited understanding of the mechanisms underlying these patterns and cannot discuss every species here. Further investigations on their life history and ecological characteristics as well as their inter-specific interactions may shed lights on this issue (Reynolds et al. 2002). While acknowledging the limitation of our data, we hope our analyses will stimulate more research on this large temperate ancient lake. We hope the information presented here may be useful for lake management.

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