

Responses of phytoplankton functional groups to environmental factors in the Maixi River, southwest China

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ABSTRACT

The functional groups approach is an efficient way to analyze seasonal changes in phytoplankton biomass as it is based on the physiological, morphological, and ecological attributes of the species. In this study, we identified the functional groups and driving factors behind short-term succession in phytoplankton communities. We analyzed physical, chemical, and biological factors of the Maixi River in Baihua Reservoir (BHR) from August to September, 2013 (summer, phase I) and March to May, 2014 (late spring and early summer, phase II). The 226 samples collected were divided into 23 functional groups. In phase I, phytoplankton biomass ranged from 4.88 to 30.59 mg·L⁻¹, and the group S1 (*Pseudanabaena limnetica*) had the greatest biomass. In phase II, phytoplankton biomass ranged from 2.22 to 50.61 mg·L⁻¹, and groups Y (*Cryptomonas* sp.) and S1 (*P. limnetica*) had the greatest biomass. Dominant functional groups in the Maixi River changed from S1 + D + Y + Lo in phase I to Y + S1 in summer. Changes in the phytoplankton community varied between 0 and 0.144 day⁻¹ in phase I and between 0.008 and 0.389 day⁻¹ in later spring and early summer. This showed a steady-state phytoplankton community during the two phases, in which the functional groups S1 (*P. limnetica*) and Y (*Cryptomonas* sp.) were dominant. *Pseudanabaena limnetica*, *Synedra* sp., *Peridinium* sp., and *Cryptomonas* sp. were dominant during summer, contributing to 70% of the total biomass in the steady-state community, and *P. limnetica*, *Synedra* sp., *Cryptomonas* sp., and *Chlamydomonas* sp. were dominant during later spring and early summer, contributing to 60% of the total biomass in the community. Groups S1, D, and Y formed easily in the Maixi River, but *P. limnetica* was the dominant species in the eutrophic conditions of the Maixi River. According to biotic and abiotic factors, we concluded that the Maixi River is hypertrophic, and water resource management should take blooms of *P. limnetica* occurring in May into account. Temperature and dissolved oxygen were the critical factors affecting the steady-state of the phytoplankton community in late spring and early summer and summer, respectively. Because the Maixi River is an important source in the BHR, its phytoplankton functional groups directly affect the ecological balance of the water environment.

Key words: Phytoplankton functional groups; short-term succession; steady-state; rate of change; Maixi River; Baihua Reservoir.

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INTRODUCTION

Phytoplankton is usually the primary producer in aquatic habitats, and its community composition and populations respond directly and rapidly to changes in the environment (Reynolds, 2006). Density and specific composition reorganization of the phytoplankton community are responses to the interactive processes of physical, chemical, and biological variables (Crossetti and Bicudo, 2008a). An approach to studying phytoplankton communities is by defining phytoplankton functional groups, which are based on phytoplankton ecological characteristics and growth strategies (Reynolds *et al.*, 2002). Functional groups include species that have similar physiology, morphology, and ecology, and frequently coexist. Moreover, functional groups share similar tolerances and sensitivities (Salmaso *et al.*, 2015) and, at present, 38 functional groups are described using alphanumerical

codes (Padisák *et al.*, 2009). Compared to phylogenetic groups, functional groups are more effective in evaluating the phytoplankton responses to changes in environmental conditions (Kruk *et al.*, 2002). The phytoplankton functional groups approach has also been applied to reservoirs (Nixdorf *et al.*, 2003; Naselli-Flores and Barone, 2003; Crossetti and Bicudo, 2005; Fonseca and Bicudo, 2008; Crossetti and Bicudo, 2008b; Becker *et al.*, 2009; Xiao *et al.*, 2011), estuaries (Smayda and Reynolds, 2003; Anderson and Rengefors, 2006; Costa *et al.*, 2009), lakes (Kruk *et al.*, 2002; Mieleitner *et al.*, 2008), and subtropical coastal system (Bonilla *et al.*, 2005).

Human activities have increased the nitrogen and phosphorus loads into water, accelerating eutrophication, and affecting the biological organization (Howarth, 1998). Phytoplankton growth and density can determine the potential productivity of the entire ecosystem (Wissel and Fry, 2005), by responding quickly to changes in the aquatic environment and indicating when physical, chem-

ical, or biological changes occur in the water column (Reynolds, 2006). Succession is the temporal change in the phytoplankton community and ecosystem, and a “steady-state phytoplankton community” is likely to appear in a period of relatively stable environmental factors. The definition of “steady-state” has been disputed: some researchers suggested that total biomass should allow a deviation of $\pm 15\%$ (Mischke and Nixdorf, 2003); others suggested that dominant species should represent at least 50–80% of the total abundance, and that the steady-state period should last at least two weeks (Dokulil and Teubner, 2003; Morabito *et al.*, 2003). The steady-state concept of succession emerged in phytoplankton ecology to explain the diversity-disturbance relationship (Naselli-Flores *et al.*, 2003). Establishing equilibrium requires consideration of many different factors, such as water depth, stratification, spatial heterogeneity, disturbance, stress, and the morphological and physiological plasticity of phytoplankton species (Naselli-Flores *et al.*, 2003). Padišák (2003) studied 80 Hungarian lakes and found equilibrium phases in only 17 lakes.

In this study, we analyzed phytoplankton functional groups, as well as phytoplankton composition, density, biomass, chlorophyll a (Chl a), and physical and chemical variables to assess the driving forces behind two short-term succession phases in the Maixi River, based on the adaptations of functional groups to environmental changes. An advantage of a short-term study is that the space-and-time substitution problem is avoided (Cardoso and Marques, 2003). Our primary objectives were to understand phytoplankton response mechanisms to environmental changes, by evaluating the phytoplankton functional groups in two succession phases and if there is a steady-state period of phytoplankton in this succession process. We expected variability in phytoplankton succession to be driven by the environment factors, which were steady-state phenomena during the short-term duration of the study and, therefore, functional groups would allow assessing the ecological status of the Maixi River.

METHODS

Study site

Maixi River is one of the four main tributaries of the Baihua Reservoir (BHR), flowing into its wharf area. It is a shallow (6–8 m depth), and the water quality is affected by the surrounding sewage from residents, agriculture, and industry. The BHR is located within the catchment of the Maotiao River, which is a branch of the Wujiang tributary of the Yangtze River in China, and acts as a second cascade of the hydropower station on the Maotiao River and provides drinking water for Guiyang City (Li *et al.*, 2013). As the linkage between rivers and

lakes, Maixi River ecological environment has a direct impact in phytoplankton composition structure and water quality in BHR. Previous studies showed that frequent activities, large amount of pollutants, and complex hydrological conditions affected phytoplankton composition structure of the Maixi River (Li *et al.* 2011, 2013).

The sampling site ($26^{\circ}65'78.02''$ N, $106^{\circ}54'28.06''$ E) was at the mouth of the Maixi River (Fig. 1). Sampling was conducted from August to September 2013 (summer, phase I) and from March to May 2014 (late spring and early summer, phase II). Daily water samples were taken at the surface (0.5 m layer) and weekly water samples were taken at depth (from the 0.5 m to the bottom, at 1 m intervals). Phase I and phase II sampling of surface water samples lasted 43 and 63 days, respectively. Stratified water samples were divided into seven layers (0.5–6 m) in phase I and into nine layers (0.5–8 m) in phase II; each phase was sampled eight times. This sampling strategy had two purposes: understanding the succession of phytoplankton from spring to summer and verifying the existence of a steady-state in the process of succession. Thus, a short-term continuous

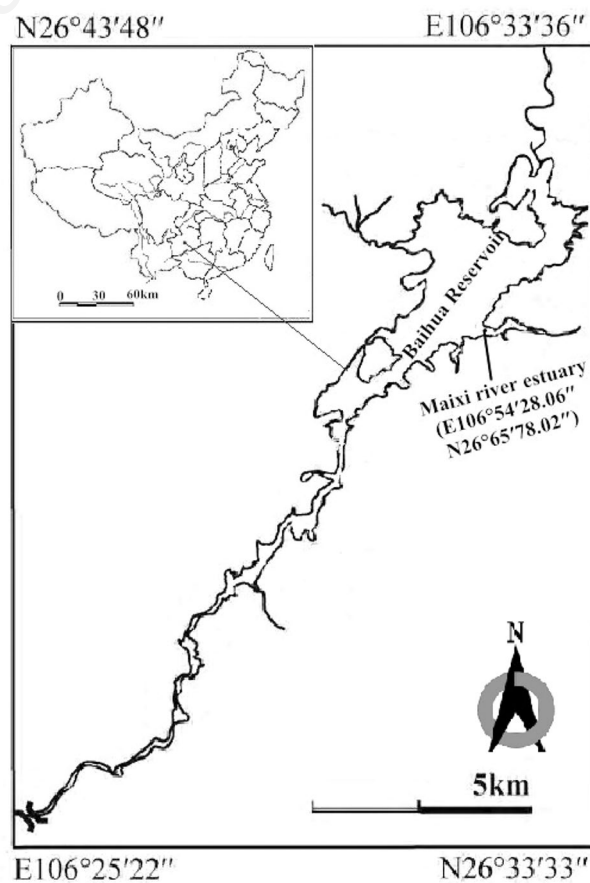


Fig. 1. Location of Baihua Reservoir and of the sampling site within Maixi River.

monitoring of phytoplankton was needed to monitor rate changes in phytoplankton communities.

Sample collection, processing, and evaluation

The 105 surface samples and the 128 vertically stratified samples collected in the two phases were fixed with formalin (3-5%) in the field. Firstly, each sample was concentrated by sedimentation (50 mL) after sedimentation for 48h in the laboratory, registering the volume before and after concentrate. After complete mixing, 0.1 mL of concentrated sample was counted in a 0.1 mL counting chamber under microscope to quantify phytoplankton. The phytoplankton were counted on a cell-by-cell basis. Taxa were identified, and the number of individuals of each taxon was counted under a direct microscope (Olympus BX41). Phytoplankton biomass was estimated using phytoplankton density and specific volumes, assuming a phytoplankton-specific density of 1 mg L⁻¹ (Sunjun *et al.*, 1999). Phytoplankton functional groups were used as described by Reynolds *et al.* (2002) and Padisák *et al.* (2009).

Physical and chemical parameters

Water temperature (WT), dissolved oxygen (DO), and pH were measured using an YSI meter (6600V2) on site. Transparent depth (SD) was measured using a Secchi disk. Physical and chemical characteristics of water, including total nitrogen (TN), ammonium (NH₄-N), nitrite (NO₂-N), nitrate (NO₃-N), total phosphorus (TP), dissolved inorganic phosphorus (PO₄-P) and chlorophyll a (Chl.a), were determined using Chinese standard methods for water quality analysis GB3838-2002.

Phytoplankton data and statistical analyses

The rate of succession of the community composition (σ) was calculated according to the methods of Cardoso and Marques (2003) and Pannard *et al.* (2008):

$$\sigma_s = \frac{\sum_{i=1}^n \left\{ \left[\frac{b_i(t_1)}{B(t_1)} \right] \right\} - \left\{ \left[\frac{b_i(t_2)}{B(t_2)} \right] \right\}}{(t_1 - t_2)}$$

where $b_i(t)$ is the density of the n species; $B(t)$ is the sum of individuals constituting the community sampled; and t_1 and t_2 are phase I and phase II, respectively. The rate value (σ) is low when the composition of the community does not fluctuate much and high values of σ generally represent abrupt changes (Cardoso and Marques, 2003).

The relationship between the vertical distribution of phytoplankton and environmental factors was drawn on an isoline map using Surfer 8.0. The relationships among environmental variables and phytoplankton functional groups were evaluated using Pearson's correlation in SPSS 19.0. Ordination analysis was performed in

CANOCO 4.5. A detrended correspondence analysis (DCA) was first run to test whether the explored gradient fitted a linear or unimodal model, which allowed deciding on using a redundancy analysis (RDA). This RDA, which is a direct gradient analysis technique, was conducted to analyze the relationship between environmental factors and phytoplankton functional groups in the Maixi River, to determine the main environmental factors affecting phytoplankton functional groups succession. Linear response models were used for the ordination analyses conducted within short-term gradients. More than 1% of total biomass was used for the statistical analysis of data. For the RDA, environmental variables (except pH) and biological data were log ($x + 1$)-transformed to reduce skewness. The statistical significance of environmental variables explaining the variance in phytoplankton species obtained in the RDA was tested using a Monte Carlo permutation test and considering a P-level of 0.05.

RESULTS

Changes in phytoplankton composition and community

Among the 101 species identified in the two phases, Chlorophyta were the most numerous, accounting for 56.43% of the total species count, followed by Bacillariophyta (20.79%) and Cyanobacteria (14.85%). Pyrrophyta, Cryptophyta, Chrysophyta, and Euglenophyta species were less abundant, accounting for approximately 8%.

Average biomass and density of vertical and surface were higher in phase I than in phase II (Fig. 2). In phase I, vertical biomass ranged from 4.88 to 30.59 mg L⁻¹ (mean 16.09 mg·L⁻¹), reaching the minimum at 6 m during the 3rd week and the maximum at 3 m during the 7th week. In phase II, vertical biomass ranged from 2.22 to 21.59 mg L⁻¹ (mean 8.60 mg·L⁻¹), reaching its minimum value at 8 m during the 4th week; the phytoplankton biomass was lower during the first three weeks and achieved its maximum value at 2 m during the 8th week.

Surface biomass ranged from 8.18 to 29.05 mg L⁻¹ (mean 15.40 mg L⁻¹) in phase I, registering the minimum value at the 7th day and increasing from the 28th to the 35th day, when it reached its maximum value, and then decreased. In phase II, surface biomass ranged between 2.46 and 50.61 mg L⁻¹ (mean 17.03 mg L⁻¹), reaching the highest value in the 67th day (May 27) and the lowest in the 50th day (March 27). From the 1st to the 51st day, biomass fluctuated slightly around 10 mg L⁻¹, but after the 52nd day (May 12) it increased gradually (Fig. 3).

Phytoplankton density ranged from 41.19×10⁶ to 184.40×10⁶ cells L⁻¹ during phase I; densities were similar before the 28th day, presented a slight increase from the 29th to the 39th day. In phase II, phytoplankton density

ranged from 8.26×10^6 to 473.09×10^6 cells L^{-1} density increased slowly until the 25th day, and rapidly from the 50th to 67th day (Fig. 3).

Phytoplankton biomass was positively correlated with DO ($r = 0.289$, $P < 0.05$, $n = 55$) and negatively correlated with TN ($r = -0.315$, $P < 0.05$, $n = 55$) and TP ($r = -0.307$, $P < 0.05$, $n = 55$) in phase I. In phase II, the biomass of phytoplankton was positively correlated with WT ($r = 0.480$, $P < 0.01$, $n = 72$) and negatively correlated with TN ($r = -0.367$, $P < 0.01$, $n = 72$), NO_3-N ($r = -0.478$, $P < 0.01$, $n = 72$), and NH_4-N ($r = -0.420$, $P < 0.01$, $n = 72$).

Short-term succession characteristics

Phytoplankton taxa were sorted into 22 functional groups in phase I and 21 functional groups in phase II (Tab. 1). We found similar changes in the relative biomass of vertical and surface phytoplankton functional groups determined weekly and daily, respectively (Figs. 2 and 3).

In phase I, functional groups S1 (*Pseudanabaena limnetica*), D (*Synedra* sp.), Y (*Cryptomonas* sp.), P (*Staurastrum* sp.), and Lo (*Peridinium* sp.) considerably contributed to total biomass. Dominant phytoplankton species of sur-

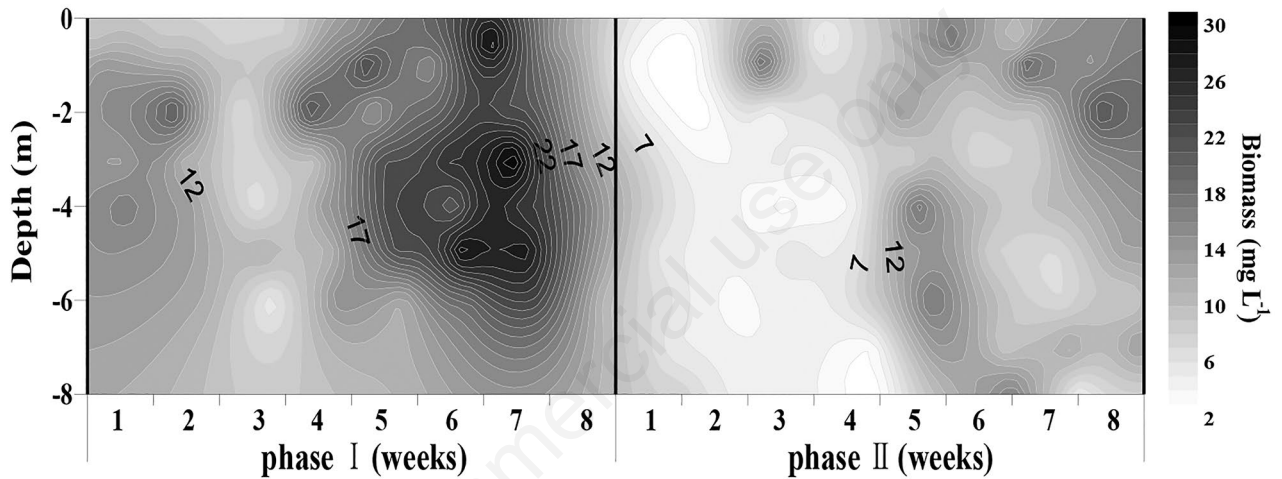


Fig. 2. Vertical distribution of phytoplankton biomass x-axis: vertical sampling once a week, eight times during each study phase. Phase I, summer, from August to September 2013; phase II, late spring and early summer, from March to May 2014.

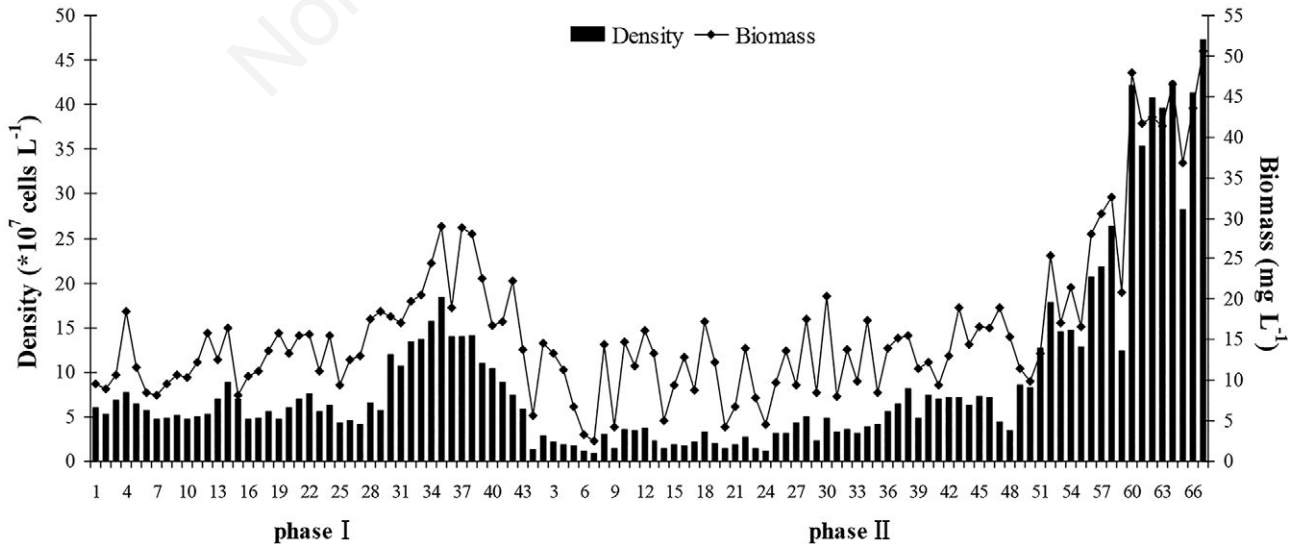


Fig. 3. Changes in surface phytoplankton biomass and density during each sampling period x-axis: phase I and phase II sampling of surface water samples lasted 42 and 63 days.

face phytoplankton in phase I accounted for 70.85% of the total biomass. Among this, S1 biomass ranged from 17.39 to 61.23% of the total biomass, followed by Lo (19%), P (17%), Y (10%), and D (15% before the 29th day and then gradually reduced to around 5%) (Fig. 4a). Early in phase I (1-32th day), relative biomass of S1 was low (<10%), but it gradually increased to 25% from the 33th to the 48th day, and then peaked to about 60% (Fig. 4a).

In phase II, the total biomass of the dominant phytoplankton functional groups S1 (*P. limnetica*), D (*Synedra* sp.), Y (*Cryptomonas* sp.), F (*Oocystis* sp.), and X2 (*Chlamydomonas* sp.) at the surface ranged from 29.95 to 89.74% (average 69.82%). The Y and S1 functional groups alternately accounted for 10% to 33% of the total biomass, and groups F and X2 were dominant from March 17 to March 24 with an average relative biomass as high as 30%; Y accounted for 32% of the total biomass, but this was gradually reduced from the 25th to 57th day. Early in phase II (1-32nd day), relative biomass of S1 was low (<10%) but it increased gradually to about 25% from the 33rd to the 48th day, and then peaked to about 60% (Fig. 4b).

Short-term succession rate (σ)

Rates of change in the phytoplankton community were broader in phase II than in phase I. In summer (phase I) rates ranged from 0 to 0.144 day⁻¹, attaining the highest value from 14th to 15th day (Fig. 5a). Rates of change were higher from 11th to 30th day, and relatively stable from the 1st to 10th day and from 31st to 41st day (Fig. 5a). In phase II, rates of change were between 0.009 and 0.370 day⁻¹ (Fig. 5b), being higher from the 1st to 53rd day and relatively stable from the 54th to 67th day (Fig. 5b). In phase I, the rate of change was the largest from the 8th to 15th day (Fig. 5a). In phase II, the rate of change was variable (Fig. 5b), and changes in density and relative biomass were also large (Fig. 3; Fig. 4). The relative density of S1, for example, averaged 31.87% from the 1st to 30th day (Fig. 5b).

Main environmental factors driving at the short-term succession

Average water temperature was higher in phase I than in phase II and there was no thermal stratification of water

Tab. 1. Phytoplankton functional groups and their typical habitat were used as described by Reynolds *et al.* (2002) and Padisák *et al.* (2009).

Functional groups	Most important taxa	Typical habitat	Phase	Phase
			I	II
B	<i>Cyclotella</i> sp.	Vertically mixed, mesotrophic small medium lakes	+	+
C	<i>Asterionella formosa</i> <i>Melosira ambigua</i>	Mixed, eutrophic small medium lakes	+	+
D	<i>Synedra acus</i>	Shallow, enriched turbid waters, including rivers	+	+
E	<i>Dinobryon divergens</i>	Usually small, oligotrophic, base poor lakes or heterotrophic ponds	+	+
F	<i>Oocystis</i> sp., <i>Kirchneriella</i> sp.	Clear epilimnia	+	+
G	<i>Eudorina elegans</i>	Short, nutrient-rich water columns	+	+
H1	<i>Aphanizomenon</i> sp.	Dinitrogen-fixing, nostocaleans	+	+
J	<i>Scenedesmus</i> sp., <i>Pediastrum</i> sp., <i>Coelastrum</i> sp.	Shallow, enriched lakes ponds and rivers	+	+
L _M	<i>Navicula</i> sp., <i>Achnanthes</i> sp.	Summer epilimnia in eutrophic lakes	+	+
LO	<i>Dactylocopsis</i> , <i>Peridinium</i>	Summer epilimnia in mesotrophic lakes	+	+
M	<i>Microcystis</i> sp.	Daily mixed layers of small eutrophic, low latitude lakes	+	+
MP	<i>Chlorella vulgaris</i>	Frequently stirred up, inorganically turbid shallow lakes	+	+
P	<i>Staurastrum</i> sp. <i>Fragilaria</i> sp.	Eutrophic epilimnia	+	+
S1	<i>Pseudanabaena limnetica</i>	Turbid mixed layers	+	+
S _N	<i>Raphidiopsis</i> , <i>Cylindrospermopsis</i> sp.	Warm mixed layers	+	+
T	<i>Quadrigula</i> sp.	Deep, well-mixed epilimnia	+	+
T _C	<i>Phormidium</i> sp.	Eutrophic standing waters, or slow-flowing rivers with emergent macrophytes	+	
W1	<i>Euglenophyta</i> sp.	Small organic ponds	+	+
W2	<i>Trachelomonas</i> sp.	Shallow mesotrophic lakes	+	+
X1	<i>Pyrrophyta</i> , <i>Chroococcus</i> sp.	Shallow mixed layers in enriched conditions	+	+
X2	<i>Chlamydomonas</i> sp.	Shallow, clear mixed layers in meso-eutrophic lakes	+	+
Y	<i>Cryptophyta</i>	Usually, small, enriched lakes	+	+

Phase I, August 16 - September 26, 2013; Phase II, March 21 - May 22, 2014.

column in any of the two seasons. In phase I (Fig. 6a), water temperature was usually above 21°C, despite a reduction in 5th and 8th week. In phase II, the temperature ranged between 9.2 and 21.3°C, with an average of 16.5°C (Tab. 2), gradually increasing from the 1st to 5th weeks, and slowly decreasing from the surface to the bottom after the second week; water temperature was always lower at the bottom (6–8 m) than at other depths (Fig. 6a).

In phase I, DO concentrations were lower than in phase II, ranging from 2.14 to 7.75 mg L⁻¹, with an average of 5.17 mg L⁻¹ (Tab. 2). The distributions of DO were similar to that of water temperature, with no obvious vertical stratification in the first two weeks (Fig. 6b). The

lowest concentration of DO was registered at the 3rd week, and the highest on the 3rd and 4th weeks. In phase II, DO ranged from 3.91 to 10.01 mg L⁻¹, with an average of 7.31 mg L⁻¹ (Tab. 2). In phase I, pH varied from 7.39 to 8.64, with an average of 8.16; in phase II, it ranged from 7.29 to 8.5, with a mean value of 8.08. There was a gradual decrease of pH with depth in both phases (Fig. 6c).

No stratification was found for TP and TN (Fig. 6 d,e). The TP concentrations ranged from 10 to 120 µg L⁻¹, with an average of 16 µg L⁻¹ in phase I and 39 µg L⁻¹ in phase II (Tab. 2). The lowest TP concentration occurred on the 3rd week of phase I (Fig. 6d). Because PO₄-P concentration was extremely low, it was not a main component of TP. TN was

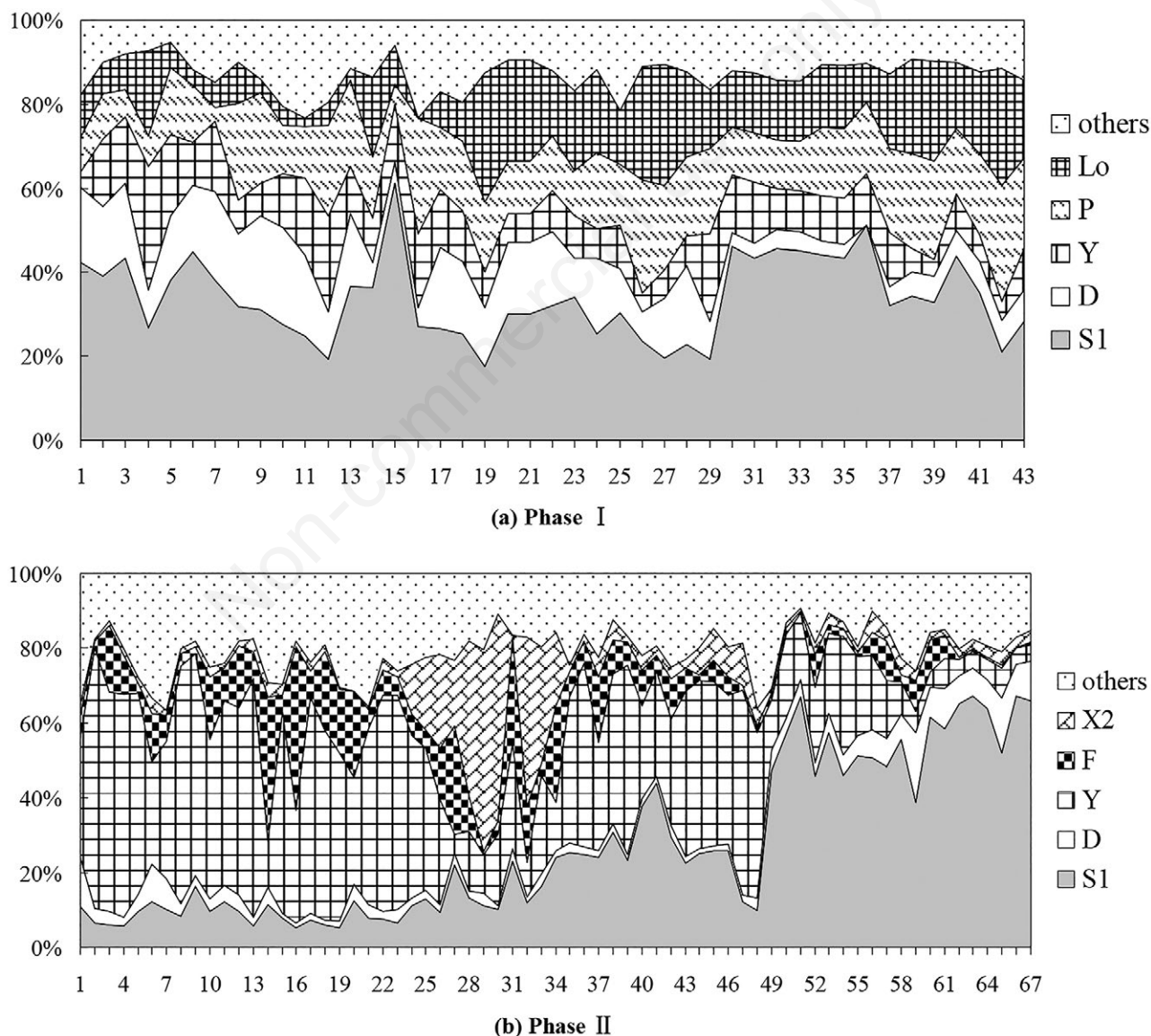
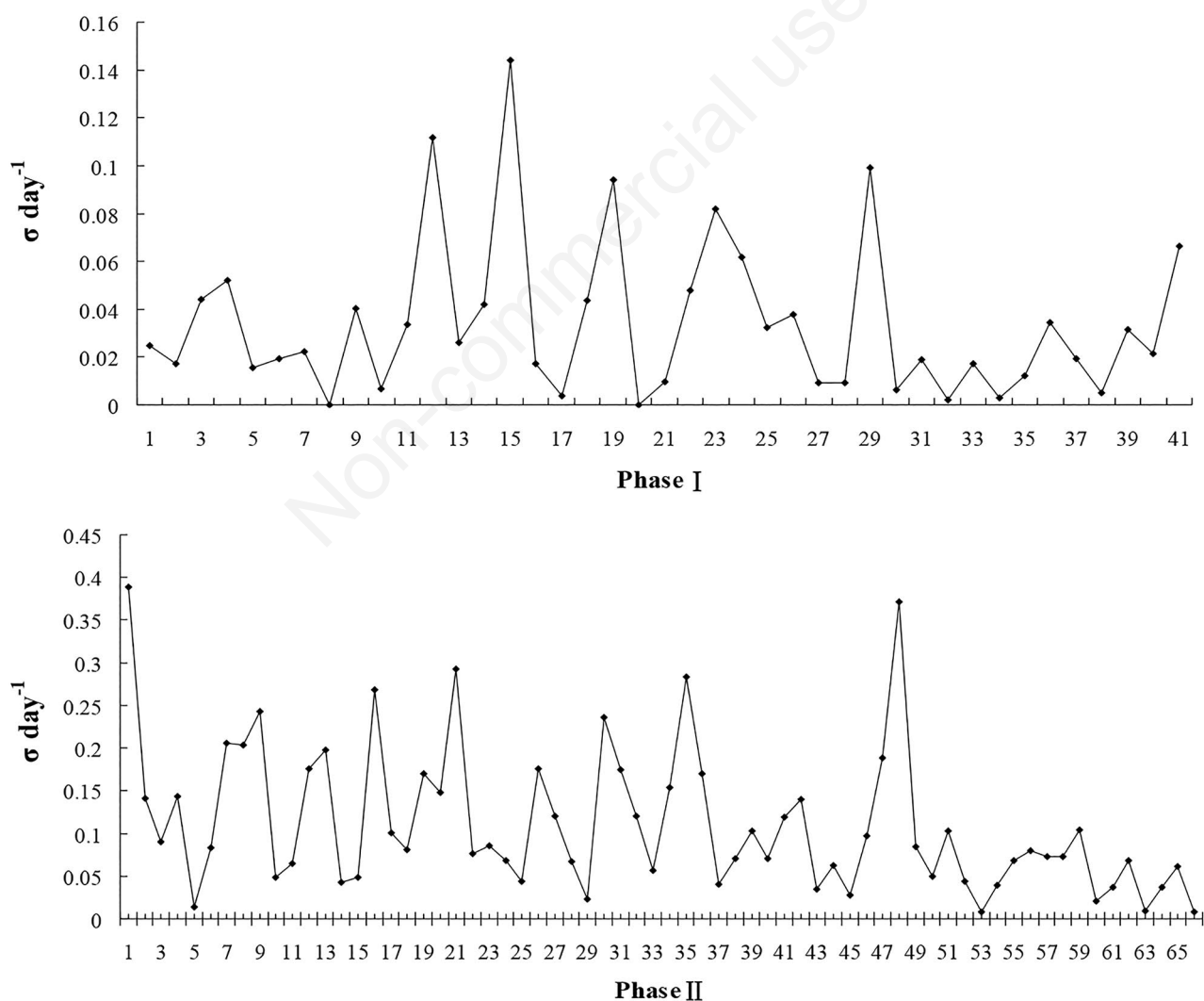


Fig. 4. Relative biomass (%) of surface functional groups. a) Others: X2, L_M, F, B, C, E, G, H1, J, M, MP, S2, S_N, T, T_C, W1, W2, X1. b) Others: B, P, Lo, C, E, G, H1, J, L_M, M, MP, S2, S_N, T, T_C, W1, W2, X1.

Tab. 2. Environmental variables in the Maixi River during the two periods.

	Unit	Phase I		Phase II	
		Mean	Range	Mean	Range
Water temperature	°C	23.65	21.1~25.9	16.5	9.2~21.3
DO	mg L ⁻¹	5.17	4.09~7.75	7.31	3.91~10.01
Conductivity	μs m ⁻¹	470.11	420~545	532.04	498~689
pH		8.16	7.39~8.64	8.08	7.29~8.5
TP	μg L ⁻¹	16	10~40	39	10~120
TN	mg L ⁻¹	1.83	1.55~2.48	2.43	1.14~5.87
TN/TP		126.4	50.9~200.3	72.7	17.0~233.1
NO ₃ -N	mg L ⁻¹	1.28	1.007~2.17	1.86	0~4.81
NO ₂ -N	mg L ⁻¹	0.05	0.031~0.08	0.035	0.022~0.056
NH ₃ -N	mg L ⁻¹	0.011	0~0.05	0.057	0~0.37
PO ₄ -P	μg L ⁻¹	2	0~4	3	0~7
Chlorophyll a	μg L ⁻¹	24.7	16.09~32.3	17.1	0.37~33.45
SD	m	1.1	0.8~1.4	1.36	0.9~1.75

**Fig. 5.** Rate of change in the phytoplankton community ($\sigma \text{ day}^{-1}$) during the two sampling phases in the Maixi River.

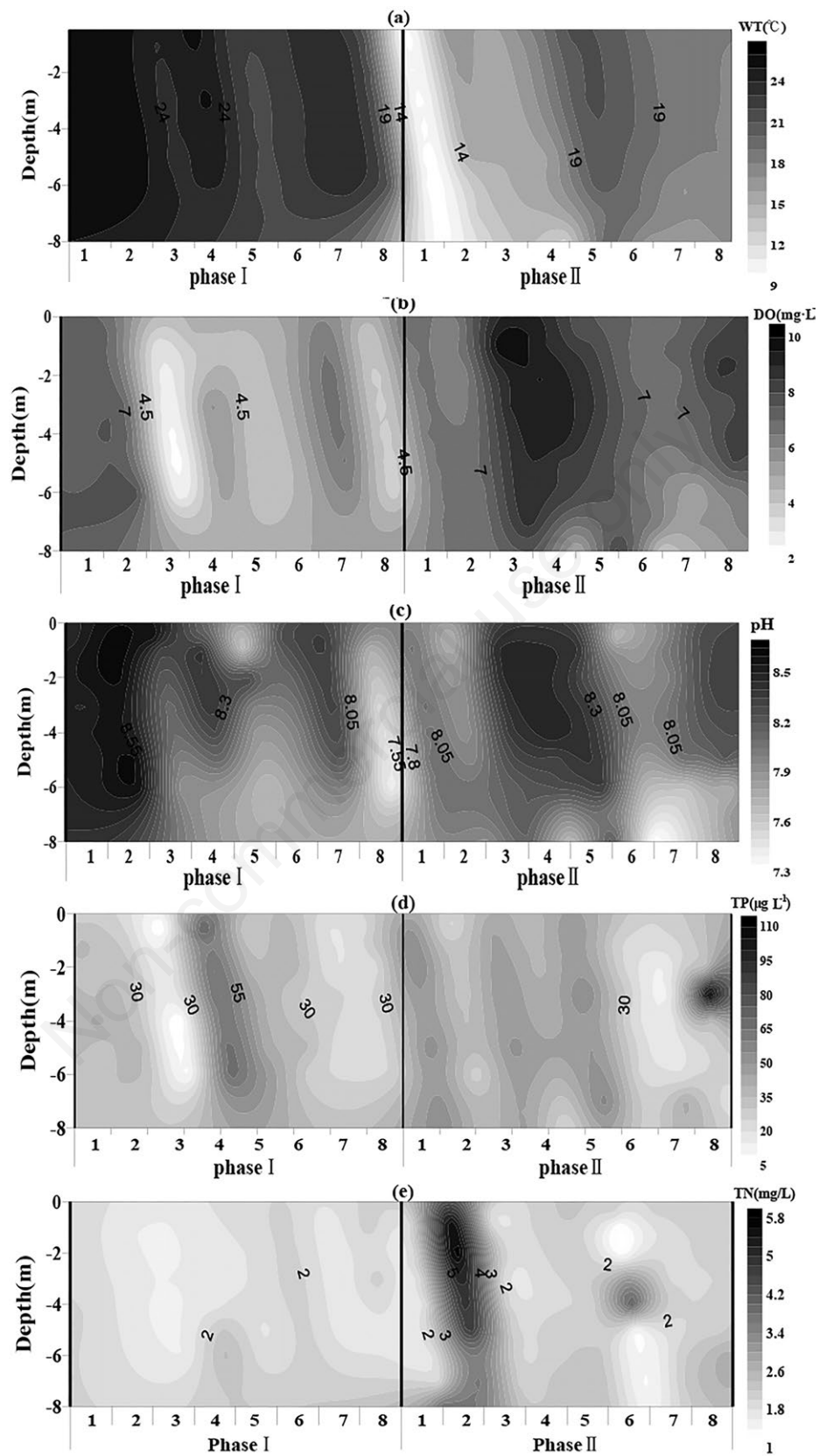


Fig. 6. Spatiotemporal distribution of the values of the main environmental variables. a) Water temperature (°C); b) dissolved oxygen (mg L⁻¹); c) pH; d) total phosphorus (mg L⁻¹); e) total nitrogen (mg L⁻¹).

between one and two orders of magnitude higher than TP (Tab. 2). Nitrate (NO₃-N) was the main component of TN, its strongest correlations with TN obtained by Pearson's correlation analysis were $r = 0.741$ ($P < 0.01$, $n = 55$) and $r = 0.891$ ($P < 0.01$, $n = 55$). The SD was generally low, with an average of 1.1 m in phase I and an average of 1.36 m in phase II (Tab. 2).

RDA of phytoplankton functional groups and environmental factors

The results of the DCA ordination with phytoplankton functional groups accounted for gradient lengths of 1.267 and 1.542 in phase I and II, respectively. Thus, linear ordination methods such as RDA should be used. In the diagram, the cosine of the angle of environmental factors and species variables represents the correlation between them; an acute angle is a positive correlation and an obtuse angle is a negative correlation. There were 9 environmental factors in both phases, (TP, TN, NO₃-N, NO₂-N, NH₄-N, WT, DO, pH and SD), and 12 functional groups in phase I and 16 functional groups in phase II. In phase I, the Monte Carlo test ($P < 0.05$) demonstrated that the ordination along axis 1 was statistically significant with an eigenvalue of 0.284; 75.8% of the cumulative variance in species distribution was explained by the first two axes (Tab. 3). The most effective explanatory factors were SD ($P < 0.01$, $F = 8.51$, $n = 499$) and TN ($P < 0.01$, $F = 5.224$, $n = 499$), both playing a significant role in the short-term succession. Correlations also showed that WT was the most important variable ($P < 0.01$, $F = 5.134$, $n = 499$). Regarding phytoplankton functional groups, S1 was located towards higher DO and SD values, whereas X2, P, T and L₀ towards higher SD. Most phytoplankton functional groups (T, L₀, P, X2, L_M, Y, F and J) were closely related to four environmental factors, appearing at the bottom left of the diagram. In addition, D and S2 were placed towards higher values of NO₂-N, G and B were towards higher NH₄-N concentrations, MP was placed towards of water temperature (Fig. 7a).

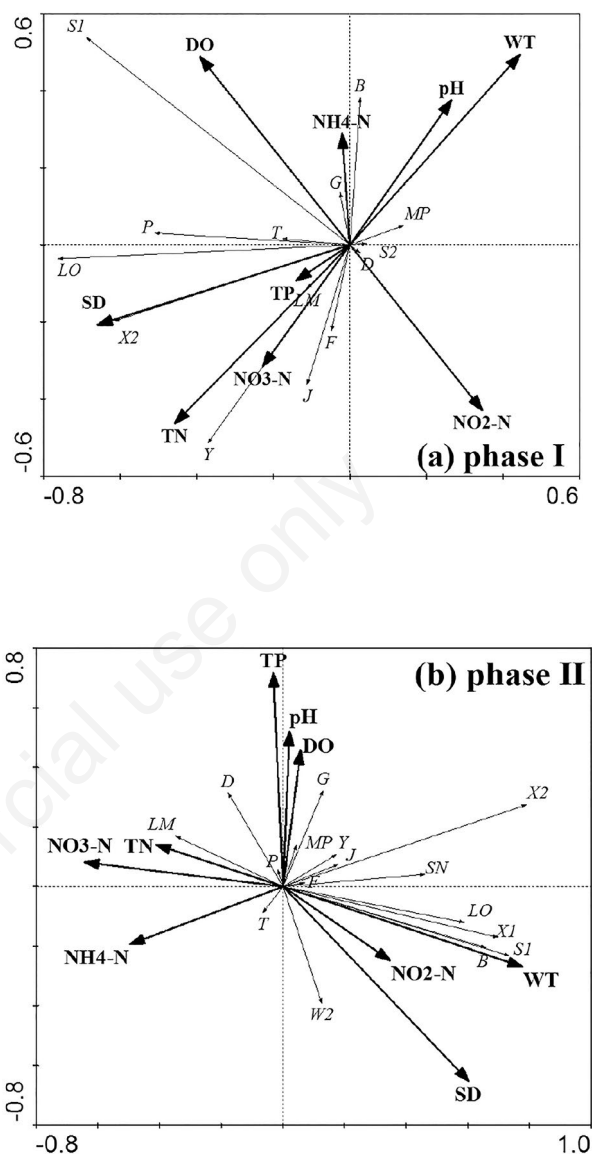


Fig. 7. Redundancy analysis of phytoplankton functional groups related to environmental factors in the Maixi River.

Tab. 3. Main results of redundancy analysis and Monte Carlo permutation test for the relationship between biovolume of phytoplankton species and environment variables.

Axes	Phase I				Total variance	Phase II				Total variance
	λ_1	λ_2	λ_3	λ_4		λ_1	λ_2	λ_3	λ_4	
Eigenvalues	0.284	0.087	0.038	0.022	1	0.325	0.040	0.028	0.022	1
Species-environment correlations	0.793	0.758	0.580	0.741		0.881	0.611	0.492	0.467	
Cumulative percentage variance of species data	28.4	37.1	40.9	43.1		32.5	36.6	39.3	41.5	
of species-environment relation	62.7	81.9	90.2	95.0		74.0	83.1	89.4	94.3	
Sum of all eigenvalues					1					1
Sum of all canonical eigenvalues					0.453					0.440

In phase II, the Monte Carlo test ($P < 0.05$) demonstrated that axis 1 was statistically significant ($P < 0.01$) with an eigenvalue of 0.325; 61.1% of the cumulative variance in species distribution was explained by the first two axes (Tab. 3). The most effective explanatory factors were WT ($P < 0.01$, $F = 18.27$, $n = 499$), $\text{NO}_3\text{-N}$ ($P < 0.01$, $F = 11.88$, $n = 499$), SD ($P < 0.01$, $F = 11.24$, $n = 499$), $\text{NH}_4\text{-N}$ ($P < 0.01$, $F = 6.66$, $n = 499$), and TN ($P < 0.01$, $F = 5.12$, $n = 499$), and played significant roles in the short-term succession of phytoplankton functional groups. While WT (0.684), $\text{NO}_3\text{-N}$ (-0.566) and SD (0.531) were highly correlated with axis 1, TP (0.438), pH (0.317), and SD (-0.398) were highly correlated with axis 2. Most phytoplankton functional groups (S1, B, X1, Lo, J, S_N , F, X2, J and Y) were located towards higher WT, $\text{NO}_2\text{-N}$ and SD. In addition, G, MP, P and D were located towards higher DO, pH, and TP, whereas D, L_M , and P towards higher TN and TP concentrations (Fig. 7b).

DISCUSSION

The short-term succession of phytoplankton response mechanisms to environmental changes

Phytoplankton functional group composition in the Maixi River had particular spatial and temporal distribution characteristics. Our study site is connected to the river and lake ecosystems; thus, its hydrological environment is complex, which influences phytoplankton community dynamics. There was no thermal stratification of water during the study period, phytoplankton community changes were similar between layers, as was observed in shallow water by Nixdorf and Deneke (1997). The dominant functional groups in late spring and early summer were Y and S1, but in summer S1 absolutely dominated. Based on the phytoplankton steady-state conditions, we defined a steady-state in summer and two steady-state periods in late spring and early summer. The steady-state of dominant phytoplankton was controlled by many factors, including nutrients, light, and water stagnation, which changed frequently from deficiency to superfluity (Mischke and Nixdorf, 2003), and by coexistence mechanisms in the relative equilibrium state. There were competition and coexistence mechanism of phytoplankton; both S1 and Y tolerate highly light-deficient conditions, but the first one is sensitive to flushing, contrarily to D that is tolerant to it. In Maixi river S1 is well adapted to the less transparency conditions. Functional group S1 is usually characterized by cyanobacteria, are adapted to high temperature, forming groups, floating, and multiplying fast, which dominate during the summer period (Çelekli and Öztürk, 2014). Some studies have shown that populations of Cyanobacteria, which comprise a few species within the functional group of S1, regularly occur in summer in different water bodies around the world

(Rücker *et al.*, 1997; Padišák *et al.*, 2003; Gemelgo *et al.*, 2009; Borics *et al.*, 2012; Li *et al.*, 2013; Stević *et al.*, 2013; Hu *et al.*, 2016). Both Lo and Y have a flagellum that allows their survival in multiple water layers when adverse conditions such as weather changes and water body disturbance are encountered (Padišák *et al.*, 2009; Naselli-Flores and Barone, 2003).

Seasonal variation in phytoplankton is closely related to changes in the water environment, such as sudden changes in temperature, nutritional status, disturbance patterns, zooplankton grazing pressure, and hydrodynamics (Padišák *et al.*, 2003; Wilk-Woźniak and Żurek, 2006; Marija *et al.*, 2007). In fact, changes in water temperature was the main environmental factor affecting the growth of S1 during late spring and early summer (*i.e.*, phase II). Many studies also pointed out that water temperature plays a major role in seasonal changes, growth, and community structure of phytoplankton (Padišák *et al.*, 2003; Salmaso and Zignin, 2010). In summer, with continuously high temperature, this variable did not have a significant effect and DO was the main factor influencing the rate of succession.

Besides, ratios of TN/TP have also been used to examine nutrient limitations on phytoplankton growth (Becker *et al.*, 2010). Given the high TN concentrations observed during the two study periods, our results suggest that TP was probably a limiting factor for phytoplankton growth; the N present in the Maixi River was mainly in the form of $\text{NO}_3\text{-N}$, which reflected the amount of exogenous pollutants in the water. In addition, this showed that the water self-purification process was ongoing, with the high ammonia utilization ratio leading to increasing nitrate contents. Therefore, these stable and suitable environmental conditions are able to the establishment of a steady state for a longer time, and a less marked rate of phytoplankton change. Our hypothesis was confirmed, the variability in phytoplankton succession to be driven by the physical and chemical factors, which were steady-state phenomena during the short-term duration of the study.

Reaction of phytoplankton functional groups to ecological status

Because the phytoplankton functional groups approach can be used without geographic limitations (Padišák *et al.*, 2009; Crossetti and Bicudo, 2008b; Becker *et al.*, 2009; Pasztaleniec and Poniewozik, 2010), and habitat requirements and phytoplankton are sensitive to changes in water quality (Çelekli and Öztürk, 2014), it is a potential monitoring tool for the assessment of ecological status in the context of the Water Framework Directive (Cellamare *et al.*, 2012). For instance, phytoplankton functional groups estimated reliable water quality states between hypertrophic and oligotrophic conditions in a reservoir of Turkey (Çelik and Sevindik, 2015), indicated eutrophication due to human impacts

along the river Loire in France (Abonyi *et al.*, 2012), and allowed an ecological assessment of French Atlantic lakes (Cellamare *et al.*, 2012).

This system is hypereutrophic based on chlorophyll, SD, TN and sometimes due to TP, and also the presence of the dominant functional groups S1, Lo, and P indicated this trophic condition. In addition, we found high values of biomass and density from spring to summer, which is the most likely period for the occurrence of phytoplankton blooms of *P. limnetica* (in May particularly). Many studies have confirmed that the worse the ecological condition of the water, the higher the thin filamentous cyanobacteria biomass (Hajnal and Padisák, 2008). The water management should take preventive measures against this phenomenon during this month in future. Baihua reservoir is one of the suppliers of drinking water for Guiyang City, and Maixi River has a direct impact on the water quality of this reservoir, threatening the safety of human life. On the other hand, the human activities in this area varied frequently, and a large amount of pollutants and complex hydrological conditions affected phytoplankton composition (Li *et al.*, 2011, 2013). For that situation, we need to pay attention to control pollution in key river valleys and regions, and we should take appropriate measures to reduce nutrient input, especially during the period of the temperature rise and continuous rainfall.

CONCLUSIONS

- This study confirmed the potential utility of the phytoplankton functional groups approach in the Maixi River. Two consecutive short-term sampling phases yielded 23 functional groups; phase I was dominated by the functional group S1, and phase II was dominated by the functional groups Y and S1.
- Water temperature was a key factor in the selection of phytoplankton species and were likely to be the critical factors affecting phytoplankton communities in the steady-states occurring in later spring to early summer, whereas SD and DO were key factors during summer. Functional groups S1, D, and Y were in a steady-state dynamics in the Maixi River.
- Based on phytoplankton functional groups and the environmental factors Maixi River can be classified as hypertrophic. Functional group S1 may begin to grow in May, and this period may coincide with phytoplankton blooms. Therefore, water resources management should focus on this period.

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