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Mechanisms controlling the carbon stable isotope composition of phytoplankton in karst reservoirs

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ABSTRACT

In order to systematically understand the mechanisms controlling the carbon stable isotope composition of phytoplankton ($\delta^{13}C_{PHY}$) in freshwater ecosystems, seasonal changes in $\delta^{13}C_{PHY}$ and related environmental factors were determined in karst reservoirs from the Wujiang river basin, China. Substantial and systematic differences within seasons and reservoirs were observed for $\delta^{13}C_{PHY}$, which ranged from -39.2‰ to -15.1‰. An increase in water temperature triggered fast growth of phytoplankton which assimilated more dissolved inorganic carbon (DIC), resulting in the increase of $\delta^{13}C_{PHY}$, $\delta^{13}C_{DIC}$ and pH. When the concentration of dissolved carbon dioxide (CO_2) was less than 10 μmol L^{-1}, phytoplankton shifted to using HCO_3^- as a carbon source. This resulted in the sharp increase of $\delta^{13}C_{PHY}$. The carbon stable isotope composition of phytoplankton tended to decrease with the increase of Bacillariophyta, which dominated in January and April, but tended to increase with the increase of Chlorophyta and Dinophyta, which dominated in July. Multiple regression equations suggested that the influence of biological factors such as taxonomic difference on $\delta^{13}C_{PHY}$ could be equal or more important than that of physical and chemical factors. Thus, the effect of taxonomic differences on $\delta^{13}C_{PHY}$ must be considered when explaining the $\delta^{13}C$ of organic matter in lacustrine ecosystem.

Key words: $\delta^{13}C$*, temperature, taxonomic difference, phytoplankton, karst reservoir.*

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INTRODUCTION

Carbon stable isotope analysis has been used to understand biologically driven carbon cycle (*e.g.* Lehmann *et al*., 2004) as natural carbon, which is derived from different sources and subject to different biogeochemical processes, has different carbon stable isotope compositions ($\delta^{13}C$). Furthermore, $\delta^{13}C$ values of aquatic biota contain important information about the contributions of different organic carbon sources to food web (Yoshii *et al*., 1999; Grey *et al*., 2000). Planktonic, benthic and terrestrial primary producers have distinct $\delta^{13}C$ values (Finlay *et al*., 1999; Doi *et al*., 2010; Milligan *et al*., 2010) that directly reflects their food sources as there is only a slight isotopic enrichment (<1‰) during the feeding process (Yoshii *et al*., 1999; Post, 2002). Therefore, carbon stable isotope analysis has been proved to be a powerful tool to study carbon sources in the aquatic ecosystem. To address these questions, however, it is necessary to understand the biogeochemical characteristics of the baseline $\delta^{13}C$ supporting the aquatic food web (Smyntek *et al*., 2012).

Phytoplankton plays an important role as it is the primary producer sustaining the lacustrine food web. The $\delta^{13}C$ of phytoplankton ($\delta^{13}C_{PHY}$) depends on the $\delta^{13}C$ of assimilated inorganic carbon and subsequent carbon isotope fractionation (ε_p) during assimilation. Thus, variability in $\delta^{13}C_{PHY}$ can either be induced by a succession of species with different carbon isotope fractionation levels (Zohary *et al*., 1994) or be a response to seasonal fluctuations in physical, chemical and biological factors influencing the $\delta^{13}C$ of inorganic carbon sources (Gu *et al*., 2006, 2011). Culture experiments have demonstrated that algal $\delta^{13}C$ is substantially influenced both by algal physiological characteristics such as growth rate, cell size, geometry (Laws *et al*., 1995; Popp *et al*., 1998) and by physical and chemical characteristics of the environment such as pH, temperature, daylength, light intensity (Descolas-Gros and Fontugne, 1990; Thompson and Calvert, 1994; Rost *et al*., 2002). These studies complicate the interpretation of carbon isotopic data in geochemical and palaeoceanographic applications (*e.g.* Rau *et al*., 1989; Freeman and Hayes, 1992) in the marine environment.

However, few field studies have been systematically conducted with respect to $\delta^{13}C_{PHY}$ in relation to controlling variables in freshwater (Bade *et al*., 2006; Vuorio *et al*., 2006; Marty and Planas, 2008). These studies have demonstrated that $\delta^{13}C_{PHY}$ show large spatial and temporal variation, but the mechanisms controlling these variations are mostly unknown. Many field studies have been carried out on $\delta^{13}C$ of particulate organic matter (POM) in lakes (Lehmann *et al*., 2004; Gu *et al*., 2006). Gu *et al.* (2011) integrated $\delta^{13}C_{POM}$ and related environmental data on a global scale and found that the seasonal average $\delta^{13}C_{POM}$ displayed weak relationships with total phosphorus (TP) and chlorophyll *a* (Chl *a*). However, the seasonal amplitude of $\delta^{13}C_{POM}$ significantly correlated with these two factors. This means that phytoplankton is not always the dominant source of POM, but the occurrence of phytoplankton influences the seasonal variation of $\delta^{13}C_{POM}$ in lakes.

In this study, we have seasonally analysed the $\delta^{13}C$ of different carbon species and related physical, chemical and biological factors in karst reservoirs from the Wujiang river basin. The main aim of our study was to discern which factors control variation in $\delta^{13}C_{PHY}$ and to better understand the mechanisms behind this control.

METHODS

The Wujiang river is a major hydropower source for China's massive West-to-East Power Transmission Project and a series of reservoirs have been constructed. The Wujiang river is a southern tributary of the Changjiang river, with a total length of 1037 km and a drainage area of 88,267 km^2. It has a runoff of 53.4×10^9 m^3 per year with a fall of 2124 m and it is the largest river in the Guizhou province. Investigations were carried out at eleven reservoirs (Tab. 1) and a total of 23 stations were selected (Fig. 1). Surface water samples (upper 0.5 m) were collected in July and October 2007 and in January and April 2008, which represent summer, autumn, winter and spring, respectively. Water temperature (T) in site M4 was only measured in October and April. Site M5 in January could not be sampled due to a blocked access road.

Water temperature, dissolved oxygen, pH and Chl *a* were measured *in situ* using a calibrated water quality probe (model: YSI 6600; YSI Inc., Yellow Springs, OH, USA). A small portion of each sample was stored for the analyses of total nitrogen (TN) and total phosphorus (TP). Both were determined spectrophotometrically (Unico UV-2000; Unico, Dayton, NJ, USA) after alkaline potassium persulfate digestion (EPA, 1988). Samples for major cations and anions were filtered through 0.45 μm filters. Samples for cation analysis were acidified to pH 2 with ultrapurified HNO_3. Major cations (Ca^{2+}, Mg^{2+}, K^+ and Na^+) were analysed by atomic absorption spectrometry (AAS, PE51002, America) and the anions (SO_4^{2-}, NO_3^-, and Cl^-) by high performance liquid chromatography (HP1100; Shimadzu Co., Kyoto, Japan). These major ions were used to calculate ionic strength. Alkalinity was titrated with HCl on the spot. HCO_3^- and dissolved CO_2 were calculated based on alkalinity, pH and T with corrections of dissociation constants by temperature and ionic strength (Stumm and Morgan, 1981; Maberly, 1996; Barth and Veizer, 1999).

One and a half litres of surface water sample was preserved with Lugol's solution for quantitative analysis of phytoplankton. Phytoplankton for qualitative analysis was collected by a 64-μm nylon mesh and preserved with formaldehyde solution (2% final concentration). The method by Zhang and Huang (1991) was used for taxon identification, counting and cell dimensions using a standard light microscope. The wet weight (mg L^{-1}) of phytoplankton biomass was calculated according to its biovolume and cell density (Zhang and Huang, 1991).

Samples for $\delta^{13}C$ of dissolved inorganic carbon ($\delta^{13}C_{DIC}$) measurement were collected by filtering 100 mL of water through 0.45 μm filters with a syringe into polyethylene vials. Then, a saturated $HgCl_2$ solution was injected into the vials for sample preservation. The vials were immediately closed without headspace with caps and sealed with seal film (Parafilm). In the laboratory, the sample was injected into closed evacuated glass vessels con-

Tab. 1. Hydrographic parameters of the investigated reservoirs.

Reservoir	Drainage area (km^2)	Average flow (m^3/s)	Average annual precipitation (mm)	Total volume (10^8m^3)	Height of dam (m)	Residence time (d)	Impounded time
Hongjiadu	9900	155	1191	49.47	182	369.4	2001
Dongfeng	18,161	345	1118	8.64	162	28.9	1989
Suofengying	21,862	427	1061	2.012	113	5.5	2002
Wujiangdu	27,790	502	1124	23	165	53	1971
Hongfeng	1596	29	1585	7.53	54	300.5	1959
Baihua	1895	35	1362	2.21	50	73.1	1965
Xiuwen	2145	39	1230	0.114	49	3.4	1961
Honglin	2442	43	1169	0.0216	32	0.6	1967
Hongyan	2792	48	1108	0.304	60	7.3	1974
Puding	5871	123	1181	4.21	75	39.6	1989
Yizidu	6425	152	1267	5.43	130	41.3	2001

taining concentrated phosphoric acid and then heated at 50°C to extract CO_2 (Atekwana and Krishnamurthy, 1998).

Samples for $\delta^{13}C_{POM}$ measurement were stored in high-density polyethylene bottles with screw closure (1500 mL, leak-proof). Bottles were rinsed three times prior to storage of water samples. Water samples were filtered through pre-combusted (500°C, 5 h) 47 mm Whatman GF/F glass fibre filter (0.65 μm) (Whatman plc, Maidstone, UK) within 12 hours, stored at -20°C and freeze-dried before analysis. Samples for $\delta^{13}C_{PHY}$ measurement were collected using a 64-μm nylon mesh and immediately filtered with the same mesh and the phytoplankton obtained were transferred to vials and kept cool (0-4°C) in the field and dried at 45°C in the laboratory within 24 hours. Particulate organic matter collected by the 64-μm net tow comprised major phytoplankton and some zooplankton. Since phytoplankton sustains herbivorous zooplankton production (Brett *et al.*, 2009) and zooplankton shares δ^{13}C similar to its source (Yoshii *et al.*, 1999; Post, 2002), δ^{13}C of the mixture collected here was considered as a surrogate of $\delta^{13}C_{PHY}$, although the mixture did not include total phytoplankton. Samples for $\delta^{13}C_{POM}$ and $\delta^{13}C_{PHY}$ measurements were acidified with dilute hydrochloric acid and oven-dried overnight at 60°C just prior to carbon isotope determination. Particulate organic matter was converted into CO_2 using the high-temperature (850°C, 5 h) sealed-quartz tube combustion method with copper oxide as oxidant (Buchanan and Corcoran, 1959) as the low-temperature (550°C, 1 h) combustion method can lead to large analytical uncertainty (Tao *et al.*, 2001).

Carbon dioxide was cryogenically separated and its pressure and temperature were measured in a sensor (Edwards Barocel® 600; Edwards Ltd., Sanborn, NY, USA). The $^{13}C/^{12}C$ ratio of CO_2 was determined on a dual-inlet isotope ratio mass spectrometer (MAT 252; Thermo Fisher Scientific Inc., Waltham, MA, USA). Carbon isotope data were normalised and are reported following the *δ* denotation of Craig (1953) relative to the Vienna Pee Dee Belemnite (VPDB). The total precision for concentration and δ^{13}C analysis were better than 3% (1σ) and 0.1‰ (1σ), respectively.

The δ^{13}C of dissolved CO_2 ($\delta^{13}C_{CO2}$) was calculated

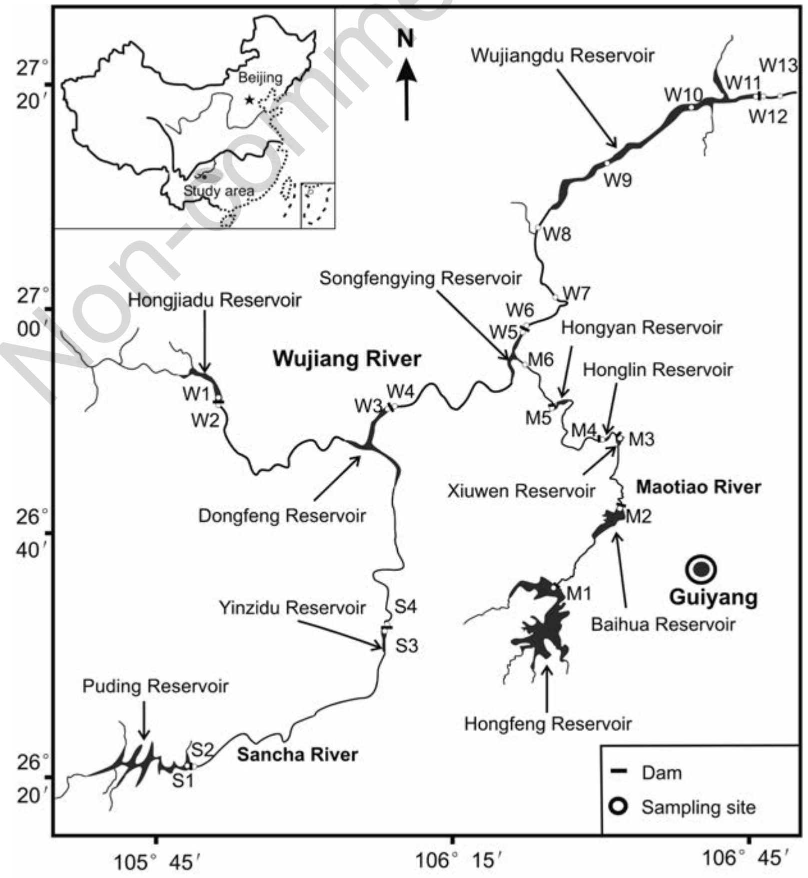

Fig. 1. Map showing sampling locations and numbers.

from $\delta^{13}C_{DIC}$ and absolute T (T_K, in Kelvin) according to Mook *et al.* (1974) by using the equation provided by Rau *et al.* (1996):

$$\delta^{13}C_{CO2}=\delta^{13}C_{DIC}+23.644-9701.5/T_K \quad \text{(eq. 1)}$$

Carbon isotope fractionation was calculated relative to CO_2 as a carbon source according to Freeman and Hayes (1992):

$$\varepsilon_p=(\delta^{13}C_{CO2}-\delta^{13}C_{PHY})/(1+\delta^{13}C_{PHY}/1000) \quad \text{(eq. 2)}$$

Pearson's correlation coefficient analysis and principal component analysis (PCA) were carried out using the software SPSS (version 11.5; SPSS Inc., Chicago, IL, USA). Multiple regression analysis was done with the Minitab 16 statistical software (Minitab Inc., Nerviano, Italy).

RESULTS

Basic physical and chemical properties

Major physical and chemical variables are listed in Tab. 2. The study area has a subtropical monsoon humid climate and water T displayed clear seasonal variations (Fig. 2). Water T increased from January and reached a maximum value in July. Amplitude of water T within seasons was larger than within reservoirs (Fig. 3). pH had a similar fluctuation pattern to water T and therefore showed a significant positive correlation with it (Tab. 3). Total phosphorus showed a larger fluctuation within reservoirs than within seasons. Average value of TN was about 100 times larger than that of TP. However, TN showed less temporal and spatial variation than TP.

HCO_3^- and SO_4^{2-} were the dominant anions, while Ca^{2+} and Mg^{2+} were the dominant cations in the river water (Tab. 2). This is because the Wujiang river basin is mainly underlain by Permian and Triassic carbonate rocks and the river water chemistry is controlled by carbonate dissolution by both carbonic and sulfuric acid (Liu, 2007). Dissolved CO_2 displayed a larger fluctuation in concentration than HCO_3^-. Still, it showed a significant positive correlation with HCO_3^- and both displayed large variations within seasons and reservoirs (Figs. 2 and 3).

Phytoplankton

In these reservoirs there were four main algal groups: Chlorophyta, Dinophyta, Bacillariophyta, and Cyanophyta (Fig. 4). Phytoplankton showed a clear different variation in species composition within reservoirs and seasons. Chlorophyta and Dinophyta were dominant in July. Bacillariophyta increased from October and dominated in January and April. In the eutrophic reservoirs Hongfeng and Baihua, the dominant algae were Cyanophyta and Chloro-

Tab. 2. Averages and range of major biogeochemical variables in the studied reservoirs.

	Average	SD	Max	Min
T (°C)	18.10	4.79	30.00	9.68
pH	7.98*	9.37	7.39	
Ca (mmol L^{-1})	1.44	0.16	1.89	0.89
Mg (mmol L^{-1})	0.45	0.10	0.67	0.25
Na (mmol L^{-1})	0.27	0.14	0.71	0.12
K (mmol L^{-1})	0.05	0.02	0.16	0.03
HCO_3 (mmol L^{-1})	2.28	0.32	2.96	1.14
SO_4 (mmol L^{-1})	0.94	0.19	1.58	0.59
NO_3 (mmol L^{-1})	0.17	0.06	0.29	0.01
Cl (mmol L^{-1})	0.12	0.06	0.35	0.06
TN (mmol L^{-1})	0.22	0.06	0.39	0.05
TP (μmol L^{-1})	1.81	1.77	10	0.01
CO_2 (μmol L^{-1})	56.7	42.4	207.6	1.0
Chl *a* (μg L^{-1})	4.4	11.8	80.0	0.1
T-PHY (mg L^{-1})	7.02	10.2	53.5	1.1
CHLO (mg L^{-1})	3.38	7.03	49.70	0.00
BACI (mg L^{-1})	2.19	2.59	14.80	0.13
CYAN (mg L^{-1})	0.42	2.70	24.7	0.00
DINO (mg L^{-1})	1.03	3.95	27.5	0.00
$\delta^{13}C_{DIC}$ (‰)	-8.2	1.26	-3.27	-9.99
$\delta^{13}C_{POM}$ (‰)	-29.5	2.67	-19.6	-34.5
$\delta^{13}C_{PHY}$ (‰)	-30.8	4.34	-15.1	-39.2
ε_p (‰)	13.24	4.04	20.90	2.06

Geometric mean. T, temperature; Ca, calcium; Mg, magnesium; Na, sodium; K, potassium; HCO_3, hydrogen carbonate; SO_4, sulfate; NO_3, nitrate; Cl, chlorine; TN, total nitrogen; TP, total phosphorus; CO_2, dissolved CO_2; Chl a*, chlorophyll* a*; T-PHY, total phytoplankton biomass; CHLO, Chlorophyta; BACI, Bacillariophyta; CYAN, Cyanophyta; DINO, Dinophyta; $\delta^{13}C_{DIC}$, carbon stable isotope composition of dissolved inorganic carbon; $\delta^{13}C_{POM}$, particulate organic matter; $\delta^{13}C_{PHY}$, carbon stable isotope composition of phytoplankton; ε_p, carbon isotope fractionation.*

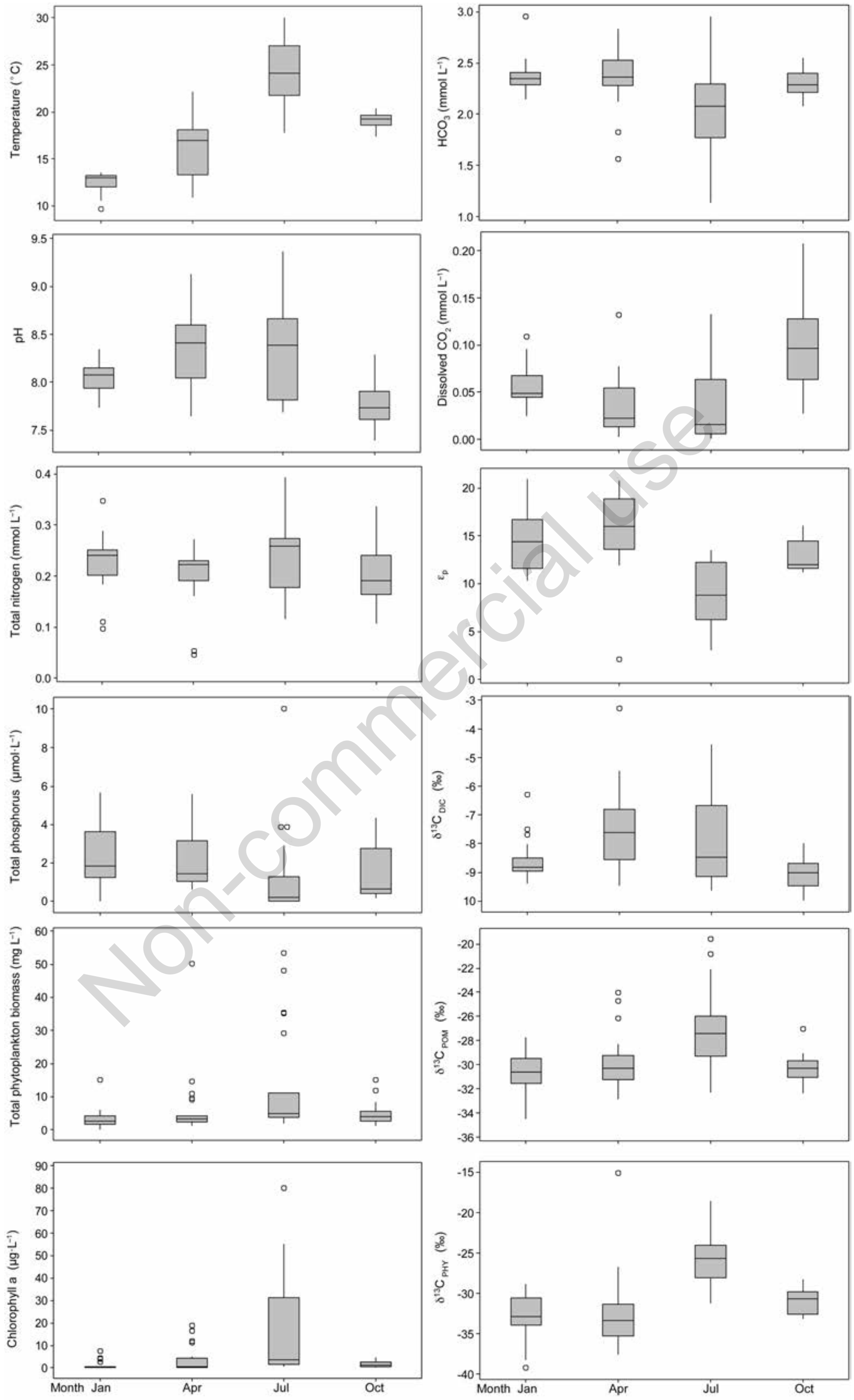

Fig. 2. Box plots of physical, chemical, and biological variables in different seasons. Box boundaries indicate the 25th and 75th percentiles; whiskers extend to a maximum of 1.5 times the inter-quartile range. The inner horizontal line is the median, while circles indicate outliers.

phyta, and in the others, the dominant algae were Bacillariophyta and Chlorophyta. Dinophyta was only abundant in July 2007. Different reservoir showed different phytoplankton community structure: for example, in April 2008 the dominant algae in Hongfeng reservoir were Chlorophyta and those in Wujiangdu reservoir were Bacillariophyta.

Phytoplankton biomass was significantly correlated with Chl *a* concentration (R=0.802, P<0.001). Phytoplankton biomass also showed a large variation within seasons and reservoirs. Chl *a* was significantly correlated with TP (Tab. 3), suggesting that P was a limiting factor for phytoplankton growth in these reservoirs. Temperature was also highly significantly correlated with Chl *a*, indicating that increasing water T could stimulate algal growth.

Carbon stable isotope composition of different carbon species

The $\delta^{13}C_{DIC}$, $\delta^{13}C_{POM}$ and $\delta^{13}C_{PHY}$ values for all samples are listed in Tab. 2. The seasonal fluctuation of $\delta^{13}C_{PHY}$ was comparable to that of $\delta^{13}C_{POM}$ and larger than that of $\delta^{13}C_{DIC}$ (Fig. 2). The average $\delta^{13}C_{PHY}$ value was -25.7‰ in July 2007 and -33.1‰ in January 2008; however, the average $\delta^{13}C_{DIC}$ value was -7.8‰ in July 2007 and -8.6‰ in January 2008. The $\delta^{13}C_{PHY}$ value differed significantly among reservoirs (Fig. 3), being -15.1‰ in the Hongfeng reservoir and -31.7‰ in the Wujiangdu reservoir in April 2008. The carbon stable isotope composition of phytoplankton showed a significant relationship with $\delta^{13}C_{POM}$ (Tab. 3), suggesting that POM was mainly derived from phytoplankton in these reservoirs. Soil organic matter and phytoplankton are the possible contributors of riverine POM; however, after damming a river to produce a reservoir, a riverine heterotrophic ecosystem may be transformed into an autotrophic one (Wetzel, 2001), and phytoplankton becomes the dominant contributor of POM.

Principal component analysis was conducted for the whole dataset and each season, respectively (Fig. 5). The first three principal components with an eigenvalue more than 1 were extracted. For the whole dataset, the principal components 1, 2, and 3 represented 55.7, 17.1 and 9.3% of the total variance, respectively (Fig. 5a). The first component (PC1) showed a positive correlation with T, pH, $\delta^{13}C_{DIC}$, $\delta^{13}C_{POM}$, $\delta^{13}C_{PHY}$, and algal biomass (total phytoplankton, Chlorophyta, Dinophyta, and Chl *a*), respectively (R>0.803; Fig. 5a), suggesting that they are the coupling factors. The results of PCA in relation to $\delta^{13}C_{PHY}$ from each season differed from one another (Figs. 5b-e), indicating that the factors controlling $\delta^{13}C_{PHY}$ were different in different seasons.

Multiple regression analysis was conducted for some variables (Tab. 4). According to this analysis, T, HCO_3^-, contribution of Bacillariophyta to total phytoplankton (CBTP) (%), and total phytoplankton biomass (T-PHY) were the main four factors controlling $\delta^{13}C_{PHY}$.

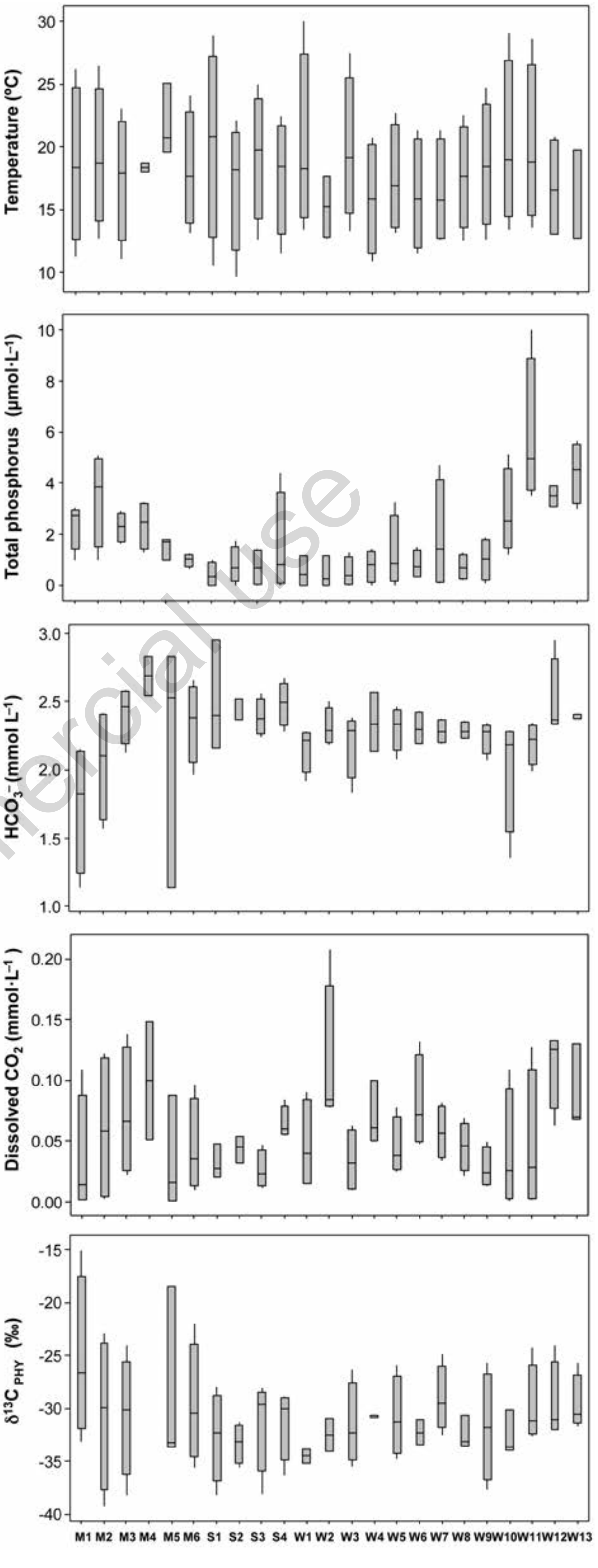

Fig. 3. Box plots of physical, chemical, and biological variables in different reservoirs. Box boundaries indicate the 25th and 75th percentiles; whiskers extend to a maximum of 1.5 times the inter-quartile range. The inner horizontal line is the median, while circles indicate outliers (see Fig. 1 for site names).

Tab. 3. Results of Pearson's correlation coefficient analysis.

	T	pH	CO$_2$	HCO$_3^-$	TN	TP	Chl *a*	T-PHY	CHLO	BACI	CYAN	DINO	δ^{13}C$_{DIC}$	δ^{13}C$_{POM}$	δ^{13}C$_{PHY}$
pH	0.336**														
CO$_2$	-0.212	-0.874**													
HCO$_3^-$	-0.501**	-0.537**	0.351**												
TN	0.078	-0.137	0.059	0.107											
TP	-0.220*	0.142	0.048	0.025	-0.205										
Chl *a*	0.461**	0.593**	-0.324**	-0.478**	0.120	0.454**									
T-PHY	0.421**	0.661**	-0.384**	-0.656**	-0.245**	0.206	0.802**								
CHLO	0.331**	0.552**	-0.310**	-0.555**	-0.237*	0.259*	0.636**	0.883**							
BACI	-0.100	0.151	-0.135	0.172	-0.093	0.004	0.044	0.108	-0.141						
CYAN	0.189	0.243	-0.136	-0.396**	-0.234	-0.017	-0.021	0.305**	0.076	-0.055					
DINO	0.495**	0.629**	-0.406**	-0.732**	0.048	0.145	0.874**	0.910**	0.798**	-0.171	-0.050				
δ^{13}C$_{DIC}$	0.370**	0.810**	-0.704**	-0.515**	-0.149	-0.019	0.358**	0.583**	0.543**	0.207*	-0.242	0.435**			
δ^{13}C$_{POM}$	0.463**	0.499**	-0.301**	-0.614**	-0.038	0.209	0.608**	0.634**	0.573**	-0.261*	0.293*	0.733**	0.333**		
δ^{13}C$_{PHY}$	0.572**	0.349**	-0.190	-0.630**	-0.075	0.045	0.392**	0.592**	0.625**	-0.318**	0.126	0.645**	0.337**	0.769**	
εp	0.419**	-0.087	-0.040	0.499**	0.003	-0.102	-0.276*	-0.413**	-0.479**	0.423**	-0.022	-0.568**	-0.015	-0.696**	-0.938**

***Correlation is significant at the0.01 level (2-tailed);* **correlation is significant at the 0.05 level (2-tailed). CO$_{2,}$ dissolved CO$_2$; HCO$_3$, hydrogen carbonate; TN, total nitrogen; TP, total phosphorus; Chl* a*, chlorophyll* a*; T-PHY, total phytoplankton biomass; CHLO, Chlorophyta; BACI, Bacillariophyta; CYAN, Cyanophyta; DINO, Dinophyta; δ^{13}C$_{DIC}$, carbon stable isotope composition of dissolved inorganic carbon; δ^{13}C$_{POM}$, particulate organic matter; δ^{13}C$_{PHY}$, carbon stable isotope composition of phytoplankton; ε$_p$, carbon isotope fractionation.*

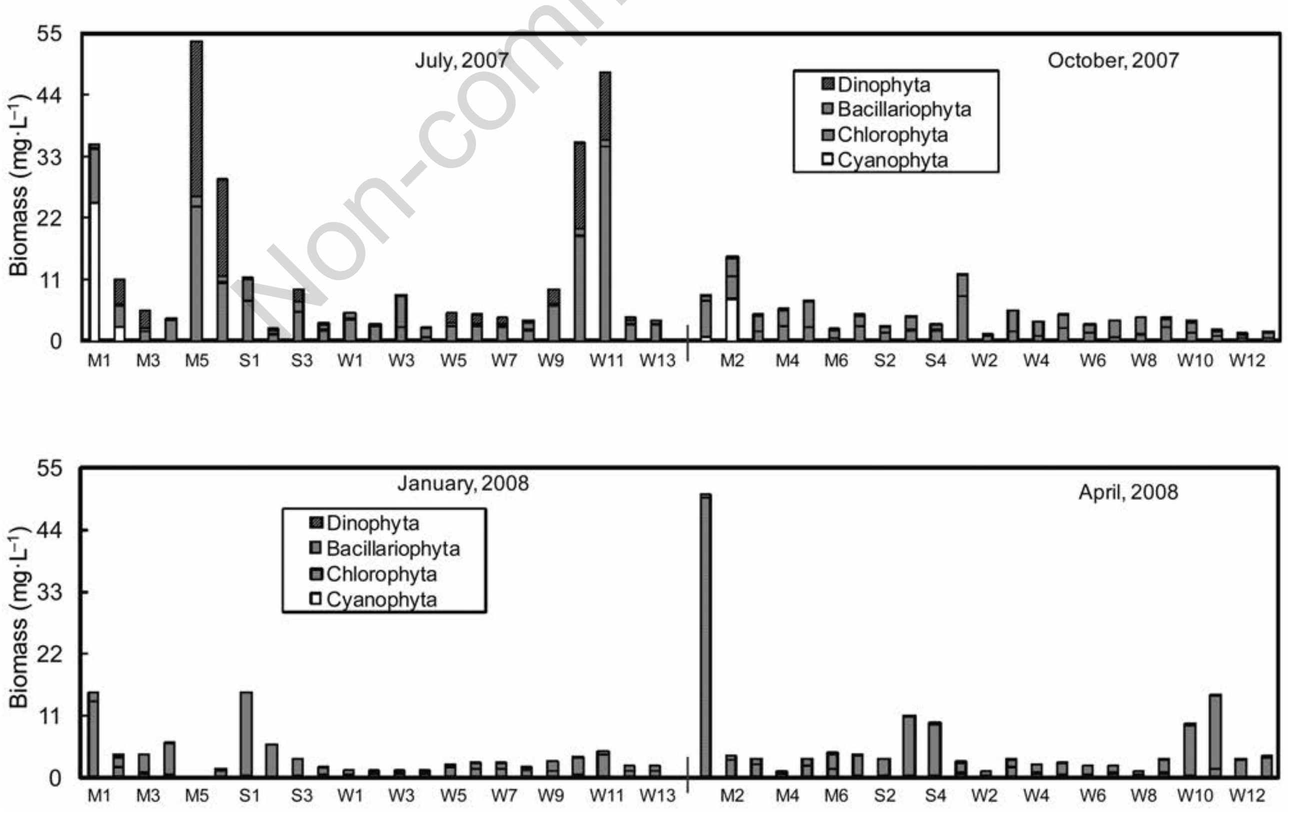

Fig. 4. Seasonal variation of phytoplankton species composition and biomass (see Fig. 1 for site names).

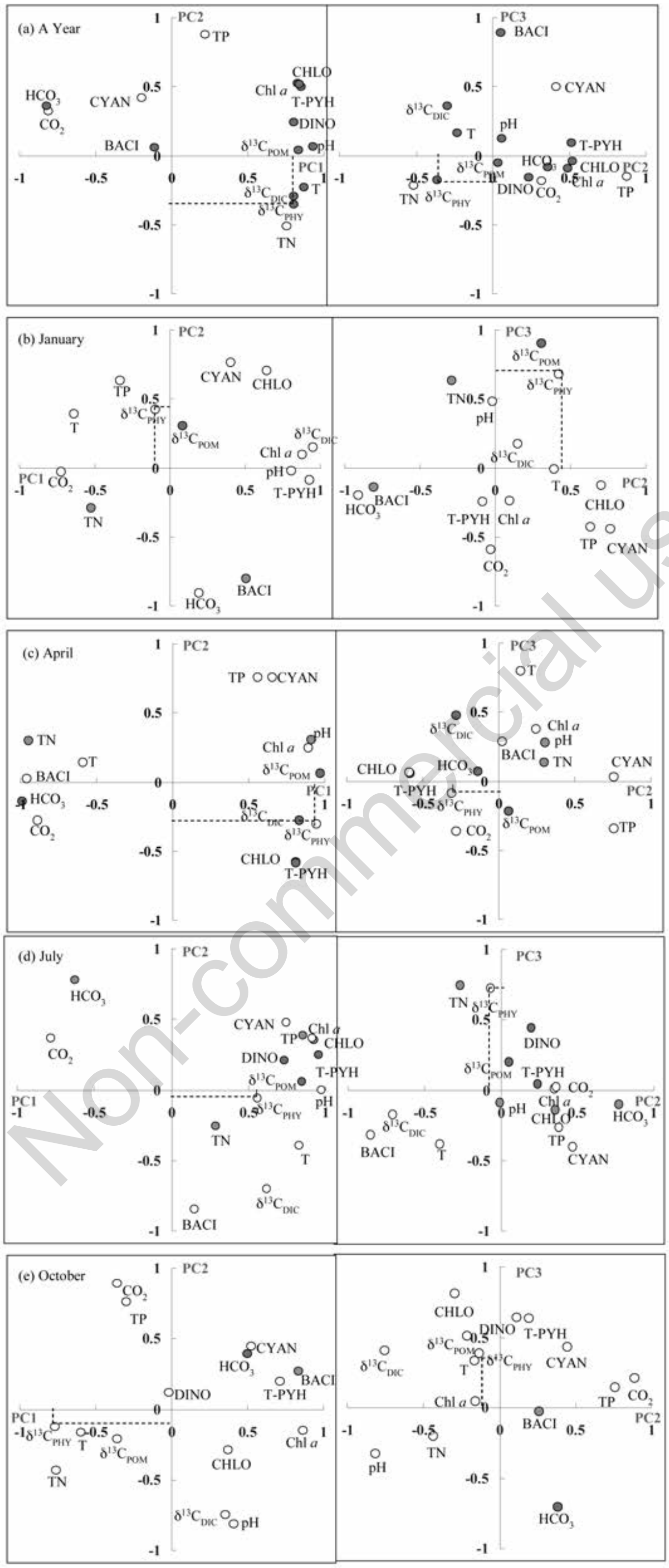

Fig. 5. Scatter plots of principal component 1 (PC1) *versus* principal component 2 (PC2) and PC2 *vs* 3 (PC3) in all the datasets and each season, respectively. Black circles mean that correlation with carbon stable isotope composition of phytoplankton ($\delta^{13}C_{PHY}$) is significant at the 0.01 level (2-tailed); grey circles mean that correlation with $\delta^{13}C_{PHY}$ is significant at the 0.05 level (2-tailed); blank circles mean that correlation with $\delta^{13}C_{PHY}$ is not significant.

DISCUSSION

Effect of temperature on the carbon stable isotope composition of phytoplankton

Sackett *et al.* (1965) first reported the relationship between phytoplankton $\delta^{13}C$ and sea surface T and this has been extended in subsequent work (Wong and Sackett, 1978; Hinga *et al.*, 1994). Variation of T induces a change of algal physiology and biochemistry, such as growth rate and carboxylase activity (Li *et al.*, 1984; Wang *et al.*, 2008) and therefore results in a change in algal $\delta^{13}C$. In this study, we found that $\delta^{13}C_{PHY}$ increased with T, and this result is consistent with another experimental study (Johnston, 1996). On the basis of the correlation analyses and PCA, we concluded that, as T increased, phytoplankton assimilated more DIC and biomass also increased. In turn, this led to an increase of $\delta^{13}C_{PHY}$ and $\delta^{13}C_{DIC}$, accompanied by the increase of pH. Undoubtedly, T is an important controlling factor on $\delta^{13}C_{PHY}$, though the variation of $\delta^{13}C_{PHY}$ cannot be explained by the thermal effect alone. Compared to T, pH was passively changed by seasonal changes in biological activity (*i.e.* photosynthesis and respiration), which was triggered by seasonal change of T as mentioned above. Furthermore, increasing of T reduced the solubility of CO_2, which may also have affected pH, as the temperature dependence of the carbonate dissociation constants and the high concentrations of dissolved CO_2 due to respiration in the bottom water of the reservoir may have led to the low pH value of the release water (Wang *et al.*, 2008, 2011). Therefore, pH was not considered as an important direct controlling factor on $\delta^{13}C_{PHY}$ in these reservoirs, though it also showed a significant correlation with $\delta^{13}C_{PHY}$ (Tab. 3).

Effect of different carbon sources on the carbon stable isotope composition of phytoplankton

Both HCO_3^- and CO_2 can be the inorganic carbon source for phytoplankton growth (Burkhardt *et al.*, 1999; Cassar *et al.*, 2004). An equilibrium isotope effect in the hydration/dehydration reactions between HCO_3^- and CO_2 concentrates isotopically light carbon in the CO_2 in a temperature-sensitive manner, with $\delta^{13}C$ of CO_2 lower than those of HCO_3^- by 12‰ at 0°C and 8.4‰ at 30°C (Mook *et al.*, 1974). HCO_3^- dominated DIC due to pH value greater than 8 in these reservoirs and $\delta^{13}C$ of HCO_3^- was therefore considered as $\delta^{13}C_{DIC}$ with an average value of about -8‰ (Tab. 2). The average $\delta^{13}C$ of CO_2 was -17.9‰ according to equation 1 (eq. 1) which is 9.7‰ lower than average $\delta^{13}C$ of HCO_3^-. Therefore, the shift from CO_2 to HCO_3^- as inorganic carbon source can strongly affect the $\delta^{13}C_{PHY}$ value.

Culture experiment demonstrated that marine diatom *Phaeodactylum tricomutum* may obtain inorganic carbon via active transport of HCO_3^- and/or CO_2 when the concentration of aqueous CO_2 is 10 μmol kg^{-1} or less (Laws *et al.*, 1995). Our results also found that, when the concentration of dissolved CO_2 was less than 10 μmol L^{-1}, the availability of aqueous CO_2 was not enough to support phytoplankton growth, so phytoplankton began to use HCO_3^- as inorganic carbon source and this finally resulted in a substantial increase of $\delta^{13}C_{PHY}$ (Fig. 6). In the southern ocean, approximately half of the DIC uptake observed was attributable to direct HCO_3^- uptake by phytoplankton (Cassar *et al.*, 2004). Aqueous CO_2 concentrations in the ocean typically fall in the range 10-20 μmol kg^{-1} (Rau *et al.*, 1992), while dissolved CO_2 concentrations in this study were usually more than 10 μmol L^{-1} with the average value of 56.7μmol L^{-1} and the concentrations of HCO_3^- were only found to decrease sharply when the concentrations of dissolved CO_2 were less than 10 μmol L^{-1}.

The average partial pressure of CO_2 (1588 μatm, equal to the average dissolved CO_2 concentration of 56.7 μmol L^{-1}) was higher in these reservoirs than in air (380 μatm), suggesting that the dissolved CO_2 was mainly from HCO_3^- instead of atmosphere. Dissolved CO_2 did not show a significant correlation with $\delta^{13}C_{PHY}$, but strongly correlated with HCO_3^- and pH (Tab. 3). This implies that the depletion of CO_2 by algal uptake was quickly compensated by HCO_3^- in the growing seasons. The concentration of HCO_3^- decreased during periods of high algal biomass and the residual inorganic carbon pool became increasingly enriched in ^{13}C, leading to further enrichment in algal ^{13}C. This explains why $\delta^{13}C_{PHY}$ showed a signifi-

Tab. 4. Results of multiple regression analysis of variables concerned. Coefficient and standard error of the coefficient are listed in parenthesis.

Variable	Constant	Temp	pH	TP	SO_4	HCO_3	CBTP	T-PHY	Adj R^2	P
$\delta^{13}C_{PHY}$	-24.4 (3.9)	0.20 (0.08)				-3.52 (1.39)	-0.053 (0.012)	0.086 (0.039)	62.2%	0.000
HCO_3	3.43 (0.56)	-0.013 (0.006)	-0.15 (0.07)		0.44 (0.13)			-0.013 (0.003)	54.6%	0.000
CO_2	0.01 (0.04)						0.024 (0.018)	-0.001 (0.0005)	14.4%	0.001
T-PHY	-110.3 (14.84)	0.60 (0.18)	12.85 (1.95)	1.13 (0.47)					50.5%	0.000

TP, total phosphorus; SO_4, sulfate; HCO_3, hydrogen carbonate; CBTP, contribution of Bacillariophyta to total phytoplankton; T-PHY, total phytoplankton biomass; Adj R^2, adjusted R-squared; $\delta^{13}C_{PHY}$, carbon stable isotope composition of phytoplankton; CO_2, dissolved CO_2.

cant positive correlation with $\delta^{13}C_{DIC}$ and a negative correlation with HCO_3^- concentration.

Effect of taxonomic difference on the carbon stable isotope composition of phytoplankton

Taxonomic differences in the carbon concentrating mechanism (CCM) and cell morphology may account for the observed differences in ε_p responses (Laws *et al.*, 1995; Popp *et al.*, 1998; Burkhardt *et al.*, 1999). Therefore, as for phytoplankton from the same sample, different algal groups have different $\delta^{13}C$. In this study, $\delta^{13}C_{PHY}$ tended to decrease with the increase of Bacillariophyta (Fig. 7), suggesting that Bacillariophyta had lower $\delta^{13}C$ values than other algal groups. Low $\delta^{13}C$ value of Bacillariophyta has been reported at about -31‰ in lakes (Zohary *et al.*, 1994; Jones *et al.*, 1998; Vuorio *et al.*, 2006). In this study $\delta^{13}C_{PHY}$ had an average value of about -34‰ when the phytoplankotn community was dominated by Bacillariophyta (>90%, Fig. 7). Bacillariophyta were abundant in January and April, and the low water T in these seasons may have decreased the growth rate of Bacillariophyta. This low growth rate can lead to low $\delta^{13}C$ in Bacillariophyta (Laws *et al.*, 1995; Fry, 1996). However, there is a lack of convincing evidence to explain why Bacillariophyta have low $\delta^{13}C$ values in freshwater ecosystem.

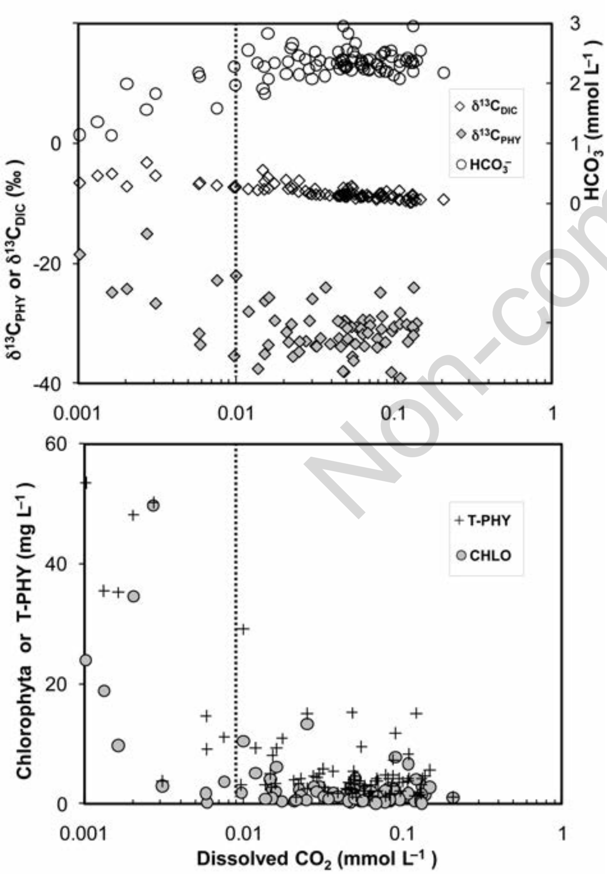

Fig. 6. Scatter plots of dissolved CO_2 *vs* each related factor. Labels used are: $\delta^{13}C_{DIC}$, carbon stable isotope composition of dissolved inorganic carbon; $\delta^{13}C_{PHY}$, carbon stable isotope composition of phytoplankton; T-PYH, total phytoplankton biomass; CHLO, Chlorophyta.

The value of $\delta^{13}C_{PHY}$ increased with the increase of abundance of Chlorophyta and Dinophyta, which were the dominant algal groups in July (Fig. 4). Chlorophyta and Cyanophyta can use HCO_3^- directly or indirectly by extracellular conversion to CO_2 catalysed by carbonic anhydrase (CA) (Moroney and Ynalvez, 2007). This process would eliminate the isotopic difference between HCO_3^- and CO_2 and make the two carbon sources isotopically indistinguishable (Riebesell and Wolf-Gladrow, 1995). Our previous study found that the activity of external CA was positively correlated with the density of Chlorophyta and Cyanophyta in the karst reservoirs (Wu *et al.*, 2008). So, it is expected that with their rapid growth, Chlorophyta and Cyanophyta begin to convert HCO_3^- into CO_2 by external CA due to the lack of CO_2 availability. This CCM may result in high $\delta^{13}C$ in these algae. Furthermore, Cyanophyta possess Rubisco II, for which the maximum carbon isotopic fractionation is 22‰, lower by 8‰ than ε_p via Rubisco I that belongs to green algae and diatoms (Hayes, 2001). On the basis of our dataset, Dinophyta, like Chlorophyta, seemed to have a CCM, however, further physiological evidence is needed to support this hypothesis.

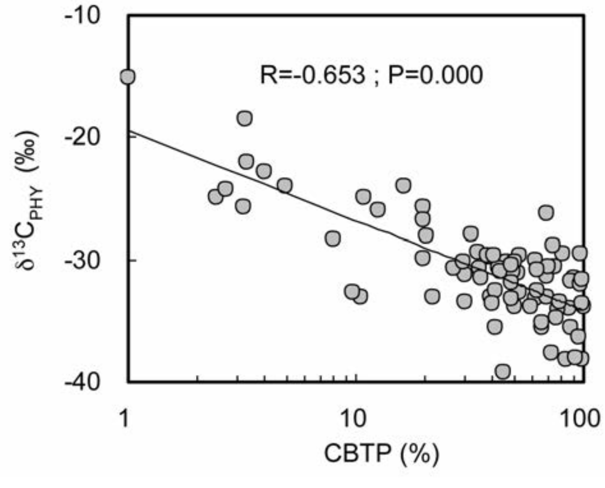

Fig. 7. Scatter plot of carbon stable isotope composition of phytoplankton ($\delta^{13}C_{PHY}$) *vs* contribution of Bacillariophyta (CBTP) to total phytoplankton.

Mechanisms controlling the carbon stable isotope composition of phytoplankton

There were several factors controlling $\delta^{13}C_{PHY}$ in freshwater ecosystem. In summer, with high T, Chlorophyta, Dinophyta, and Cyanophyta bloomed and assimilated dissolved CO_2 actively. When the concentration of dissolved CO_2 was less than 10 μmol L^{-1}, these algal groups shifted to use HCO_3^- as their carbon source. This shift from CO_2 to HCO_3^- and rapid algal growth resulted in high $\delta^{13}C_{PHY}$ in summer. In autumn, T decreased and total phytoplankton biomass also began to decrease. Nonetheless, the CBTP increased and this resulted in a decrease of $\delta^{13}C_{PHY}$. Temeperature decreased in winter and Bacillariophyta became the dominant algal group and consequently the phytoplankton had low $\delta^{13}C$ values. Water T increased in spring and Chlorophyta became the dominant phytoplankton in some eutrophic reservoirs, while Bacillariophyta still dominated in other reservoirs. Therefore, $\delta^{13}C_{PHY}$ showed a large variation within reservoirs in spring. Multiple regression equations of $\delta^{13}C_{PHY}$ quantitatively expressed the relationship between $\delta^{13}C_{PHY}$ and its controlling factors. For instance, $\delta^{13}C_{PHY}$ theoretically decreased by 5.3‰ with the increase of CBTP from 1% to 100%, as show in Tab. 4. Therefore, the influence of biological factors such as CBTP and T-PHY on $\delta^{13}C_{PHY}$ could be equal to, or more than, that of physical and chemical factors such as T and HCO_3^-.

Carbon isotope fractionation showed a significant negative correlation with $\delta^{13}C_{PHY}$, but a weak correlation with $\delta^{13}C_{DIC}$ (Fig. 8), indicating the main influence of $\delta^{13}C_{PHY}$ on ε_p. This was attributed to larger variation of $\delta^{13}C_{PHY}$ than that of $\delta^{13}C_{DIC}$. Therefore, the main factors influencing ε_p were evaluated by their influences on $\delta^{13}C_{PHY}$.

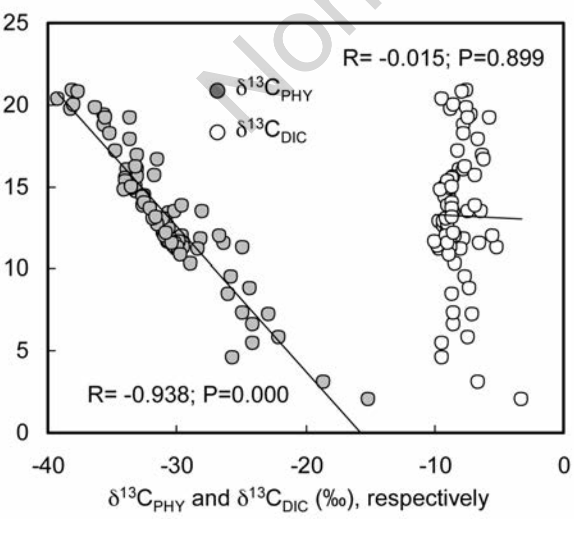

Fig. 8. Scatter plots of carbon isotope fractionation (ε_p) *vs* carbon stable isotope composition of phytoplankton ($\delta^{13}C_{PHY}$), and carbon isotope fractionation *vs* carbon stable isotope composition of dissolved inorganic carbon ($\delta^{13}C_{DIC}$).

There were weak relationships between $\delta^{13}C_{PHY}$ and TP and between $\delta^{13}C_{PHY}$ and TN (Tab. 3). However, TN was significantly correlated with $\delta^{13}C_{PHY}$ in April and July at the 0.05 level (Fig. 5). This could be attributed to passive reduction of TN by algal uptake. Therefore, chemical trophic state indices (*i.e.* TP and TN) were not important direct controlling factors of $\delta^{13}C_{PHY}$ on a regional scale.

CONCLUSIONS

The carbon stable isotope composition of phytoplankton showed substantial and systematic differences within seasons and reservoirs. An increase of T and a shift from CO_2 to HCO_3^- as a carbon source increased $\delta^{13}C_{PHY}$. The carbon stable isotope composition of phytoplankton tended to be lower when Bacillariophyta were dominant, as in January, and April, but tended to be higher when Chlorophyta and Dinophyta were dominant, as in July. This study indicated that the effect of taxonomic differences on $\delta^{13}C_{PHY}$ must be considered to explain the $\delta^{13}C$ of organic matter in lacustrine ecosystems.

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