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 (δ13C PHY) in freshwater ecosystems, seasonal changes in δ13C 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 δ13C 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 δ13C PHY, δ13C DIC and pH. When the concentration of dissolved carbon dioxide (CO2) was less than 10 μmol L–1, phytoplankton shifted to using HCO3⁻ as a carbon source. This resulted in the sharp increase of δ13C 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 δ13C PHY could be equal or more important than that of physical and chemical factors. Thus, the effect of taxonomic differences on δ13C PHY must be considered when explaining the δ13C of organic matter in lacustrine ecosystem.

Key words: δ13C, 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 (δ13C). Furthermore, δ13C 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 δ13C 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 δ13C 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 δ13C of phytoplankton (δ13C PHY) depends on the δ13C of assimilated inorganic carbon and subsequent carbon isotope fractionation (εp) during assimilation. Thus, variability in δ13C 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 δ13C of inorganic carbon sources (Gu et al., 2006, 2011). Culture experiments have demonstrated that algal δ13C 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 (Descals-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 δ13C 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 δ13C PHY show large spatial and temporal variation, but the mechanisms controlling these variations are mostly unknown. Many field studies have been carried out
on δ13C of particulate organic matter (POM) in lakes (Lehmann et al., 2004; Gu et al., 2006). Gu et al. (2011) integrated δ13C_POM and related environmental data on a global scale and found that the seasonal average δ13C_POM displayed weak relationships with total phosphorus (TP) and chlorophyll a (Chl a). However, the seasonal amplitude of δ13C_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 δ13C_POM in lakes.

In this study, we have seasonally analysed the δ13C 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 δ13C_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 km². It has a runoff of 53.4×10⁹ m³ 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 HNO₃. Major cations (Ca²⁺, Mg²⁺, K⁺ and Na⁺) were analysed by atomic absorption spectrometry (AAS, PE51002, America) and the anions (SO₄²⁻, NO₃⁻, 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. HCO₃⁻ and dissolved CO₂ 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 δ13C of dissolved inorganic carbon (δ13C_DIC) measurement were collected by filtering 100 mL of water through 0.45 μm filters with a syringe into polyethylene vials. Then, a saturated HgCl₂ 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 (km²) | Average flow (m³/s) | Average annual precipitation (mm) | Total volume (10⁸ m³) | Height of dam (m) | Residence time (d) | Impounded time (d) |
|-----------|---------------------|---------------------|----------------------------------|----------------------|------------------|-------------------|-------------------|
| 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               | 75.1              | 1965              |
| Xiuwen    | 2145                | 39                  | 1230                             | 0.114                | 49               | 3.4               | 1961              |
| Hongsun   | 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              |
Mechanisms controlling phytoplankton δ13C
taining concentrated phosphoric acid and then heated at
50°C to extract CO2 (Atekwana and Krishnamurthy, 1998).

Samples for δ13C_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 fil-
tered through pre-combusted (500°C, 5 h) 47 mm What-
man 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 δ13C PHY meas-
urement were collected using a 64-μm nylon mesh and
immediately filtered with the same mesh and the phyto-
plankton 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 herbiv-
orous zooplankton production (Brett et al., 2009) and zoo-
plankton shares δ13C similar to its source (Yoshii et al.,
1999; Post, 2002), δ13C of the mixture collected here was
considered as a surrogate of δ13C PHY, although the mixture
did not include total phytoplankton. Samples for δ13C_POM
and δ13C PHY measurements were acidified with dilute hy-
drochloric acid and oven-dried overnight at 60°C just
prior to carbon isotope determination. Particulate organic
matter was converted into CO2 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 (Ed-
wards Barocel® 600; Edwards Ltd., Sanborn, NY, USA).
The 13C/12C ratio of CO2 was determined on a dual-inlet isotope ratio mass spectrometer (MAT 252; Thermo
Fisher Scientific Inc., Waltham, MA, USA). Carbon iso-
tope 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 concen-
tration and δ13C analysis were better than 3% (1σ) and
0.1‰ (1σ), respectively.

The δ13C of dissolved CO2 (δ13C CO2) was calculated

Fig. 1. Map showing sampling locations and numbers.
from $\delta^{13}\text{C}_{\text{DIC}}$ and absolute T ($T_K$, in Kelvin) according to Mook et al. (1974) by using the equation provided by Rau et al. (1996):

$$\delta^{13}\text{C}_{\text{CO}_2} = \delta^{13}\text{C}_{\text{DIC}} + 23.644 - 9701.5/T_K \quad \text{(eq. 1)}$$

Carbon isotope fractionation was calculated relative to CO$_2$ as a carbon source according to Freeman and Hayes (1992):

$$\epsilon_p = (\delta^{13}\text{C}_{\text{CO}_2} - \delta^{13}\text{C}_{\text{PHY}})/(1+\delta^{13}\text{C}_{\text{PHY}}/1000) \quad \text{(eq. 2)}$$

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 carboxic and sulfcuric 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.

| Variable | Average | SD | Max | Min |
|----------|---------|----|-----|-----|
| T (°C)   | 18.10   | 4.79 | 30.00 | 9.68 |
| pH       | 7.98*   | 9.37 | 7.39 | 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 | 0.11 | 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$_4^{2-}$ (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 (mmol L$^{-1}$) | 1.81 | 1.77 | 10 | 0.01 |
| CO$_2$ (mmol 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 (µg L$^{-1}$) | 7.02 | 10.2 | 53.5 | 1.1 |
| CHLO (µg L$^{-1}$) | 3.38 | 7.03 | 49.70 | 0.00 |
| BACI (µg L$^{-1}$) | 2.19 | 2.59 | 14.80 | 0.13 |
| CYAN (µg L$^{-1}$) | 0.42 | 2.70 | 24.7 | 0.00 |
| DINO (µg L$^{-1}$) | 1.03 | 3.95 | 27.5 | 0.00 |
| $\delta^{13}$C$_{\text{DIC}}$ (%) | -8.2 | 1.26 | -3.27 | -9.99 |
| $\delta^{13}$C$_{\text{PO4}}$ (%) | -29.5 | 2.67 | -19.6 | -34.5 |
| $\delta^{13}$C$_{\text{PHY}}$ (%) | -30.8 | 4.34 | -15.1 | -39.2 |
| $\epsilon_p$ (%) | 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$_4^{2-}$, 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$_{\text{DIC}}$, carbon stable isotope composition of dissolved inorganic carbon; $\delta^{13}$C$_{\text{PO4}}$ particulate organic matter; $\delta^{13}$C$_{\text{PHY}}$, carbon stable isotope composition of phytoplankton; $\epsilon_p$, 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 δ¹³CDIC, δ¹³CPOM and δ¹³CPHY values for all samples are listed in Tab. 2. The seasonal fluctuation of δ¹³CPHY was comparable to that of δ¹³CPOM and larger than that of δ¹³CDIC (Fig. 2). The average δ¹³CPHY value was -25.7‰ in July 2007 and -33.1‰ in January 2008; however, the average δ¹³CDIC value was -7.8‰ in July 2007 and -8.6‰ in January 2008. The δ¹³CPHY 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 δ¹³CPOM (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, δ¹³CDIC, δ¹³CPOM, δ¹³CPHY 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 δ¹³CPHY from each season differed from one another (Figs. 5b-e), indicating that the factors controlling δ¹³CPHY were different in different seasons.

Multiple regression analysis was conducted for some variables (Tab. 4). According to this analysis, T, HCO₃⁻, contribution of Bacillariophyta to total phytoplankton (CBTP) (%), and total phytoplankton biomass (TPHY) were the main four factors controlling δ¹³CPHY.

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.

|          | pH     | CO₂    | HCO₃⁻ | TN     | TP     | Chl a   | T-PHY  | CHLO   | BACI   | CYAN   | DINO   | δ¹³C_DIC | δ¹³C_POM | δ¹³C_PHY |
|----------|--------|--------|-------|--------|--------|---------|--------|--------|--------|--------|--------|---------|---------|---------|
| pH       | 0.336  | **     |       |        |        |         |        |        |        |        |        |         |         |         |
| CO₂      | -0.212 | -0.874 | **    |        |        |         |        |        |        |        |        |         |         |         |
| HCO₃⁻    | -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 | **     | 0.206   | 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  | **     | -0.171 | -0.050  |         |         |
| δ¹³C_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  |
| δ¹³C_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  |
| δ¹³C_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  |
| 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 |

**Correlation is significant at the 0.01 level (2-tailed); *correlation is significant at the 0.05 level (2-tailed). CO₂, dissolved CO₂; HCO₃⁻, 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; δ¹³C_DIC, carbon stable isotope composition of dissolved inorganic carbon; δ¹³C_POM, particulate organic matter; δ¹³C_PHY, carbon stable isotope composition of phytoplankton; εp, 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 δ¹³C 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 δ¹³C. In this study, we found that δ¹³CPHY 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 δ¹³CDIC and δ¹³CPHY, accompanied by the increase of pH. Undoubtedly, T is an important controlling factor on δ¹³CPHY, though the variation of δ¹³CPHY 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 CO₂, which may also have affected pH, as the temperature dependence of the carbonate dissociation constants and the high concentrations of dissolved CO₂ 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 δ¹³CPHY in these reservoirs, though it also showed a significant correlation with δ¹³CPHY (Tab. 3).

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

Both HCO₃⁻ and CO₂ 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 HCO₃⁻ and CO₂ concentrates isotopically light carbon in the CO₂ in a temperature-sensitive manner, with δ¹³C of CO₂ lower than those of HCO₃⁻ by 12%o at 0°C and 8.4%o at 30°C (Mook et al., 1974). HCO₃⁻ dominated DIC due to pH value greater than 8 in these reservoirs and δ¹³C of HCO₃⁻ was therefore considered as δ¹³CDIC with an average value of about -8%o (Tab. 2). The average δ¹³C of CO₂ was -17.9%o according to equation (1) (eq. 1) which is 9.7%o lower than average δ¹³C of HCO₃⁻. Therefore, the shift from CO₂ to HCO₃⁻ as inorganic carbon source can strongly affect the δ¹³CPHY value.

Culture experiment demonstrated that marine diatom Phaeodactylum tricornutum may obtain inorganic carbon via active transport of HCO₃⁻ and/or CO₂ when the concentration of aqueous CO₂ is 10 μmol kg⁻¹ or less (Laws et al., 1995). Our results also found that, when the concentration of dissolved CO₂ was less than 10 μmol L⁻¹, the availability of aqueous CO₂ was not enough to support phytoplankton growth, so phytoplankton began to use HCO₃⁻ as inorganic carbon source and this finally resulted in a substantial increase of δ¹³CPHY (Fig. 6). In the southern ocean, approximately half of the DIC uptake observed was attributable to direct HCO₃⁻ uptake by phytoplankton (Cassar et al., 2004). Aqueous CO₂ concentrations in the ocean typically fall in the range 10-20 μmol kg⁻¹ (Rau et al., 1992), while dissolved CO₂ concentrations in this study were usually more than 10 μmol L⁻¹ with the average value of 56.7 μmol L⁻¹ and the concentrations of HCO₃⁻ were only found to decrease sharply when the concentrations of dissolved CO₂ were less than 10 μmol L⁻¹.

The average partial pressure of CO₂ (1588 μatm, equal to the average dissolved CO₂ concentration of 56.7 μmol L⁻¹) was higher in these reservoirs than in air (380 μatm), suggesting that the dissolved CO₂ was mainly from HCO₃⁻ instead of atmosphere. Dissolved CO₂ did not show a significant correlation with δ¹³CPHY, but strongly correlated with HCO₃⁻ and pH (Tab. 3). This implies that the depletion of CO₂ by algal uptake was quickly compensated by HCO₃⁻ in the growing seasons. The concentration of HCO₃⁻ decreased during periods of high algal biomass and the residual inorganic carbon pool became increasingly enriched in ¹³C, leading to further enrichment in algal ¹³C. This explains why δ¹³CPHY 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   | SO₄  | HCO₃  | CBTP  | T-PHY | Adj R² | P       |
|--------------|----------|--------|------|------|------|-------|-------|-------|--------|---------|
| δ¹³CPHY      | -24.4 (3.9) | 0.20 (0.08) |      | -3.52 (1.39) | -0.053 (0.012) | 0.086 (0.039) | 62.2% | 0.000 |
| HCO₃         | 3.43 (0.56) | -0.013 (0.006) | -0.15 (0.07) | 0.44 (0.13) |       |       |       |       | 54.6% | 0.000   |
| CO₂          | 0.01 (0.04) |       |      |       |       | 0.024 (0.018) | -0.013 (0.003) | 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; SO₄: sulfate; HCO₃⁻: hydrogen carbonate; CBTP: contribution of Bacillariophyta to total phytoplankton; T-PHY: total phytoplankton biomass; Adj R²: adjusted R-squared; δ¹³Cphy: carbon stable isotope composition of phytoplankton; CO₂: dissolved CO₂.
cant positive correlation with $\delta^{13}C_{\text{DIC}}$ 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 $\varepsilon_p$ 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, $\delta^{13}C_{\text{PHY}}$ 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 $\delta^{13}C_{\text{PHY}}$ had an average value of about -34‰ when the phytoplankton 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.

The value of $\delta^{13}C_{\text{PHY}}$ 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 $\varepsilon_p$ 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. 6. Scatter plots of dissolved CO$_2$ vs each related factor. Labels used are: $\delta^{13}C_{\text{DIC}}$, carbon stable isotope composition of dissolved inorganic carbon; $\delta^{13}C_{\text{PHY}}$, carbon stable isotope composition of phytoplankton; T-PHY, total phytoplankton biomass; CHLO, Chlorophyta.](image)

![Fig. 7. Scatter plot of carbon stable isotope composition of phytoplankton ($\delta^{13}C_{\text{PHY}}$) vs contribution of Bacillariophyta (CBTP) to total phytoplankton.](image)
Mechanisms controlling the carbon stable isotope composition of phytoplankton

There were several factors controlling $\delta^{13}C_{\text{PHY}}$ 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 $\mu$mol 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 $\delta^{13}C_{\text{PHY}}$ 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 $\delta^{13}C_{\text{PHY}}$. Temperature 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, $\delta^{13}C_{\text{PHY}}$ showed a large variation within reservoirs in spring. Multiple regression equations of $\delta^{13}C_{\text{PHY}}$ quantitatively expressed the relationship between $\delta^{13}C_{\text{PHY}}$ and its controlling factors. For instance, $\delta^{13}C_{\text{PHY}}$ 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 $\delta^{13}C_{\text{PHY}}$ 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 $\delta^{13}C_{\text{PHY}}$, but a weak correlation with $\delta^{13}C_{\text{DIC}}$ (Fig. 8), indicating the main influence of $\delta^{13}C_{\text{PHY}}$ on $\varepsilon_p$. This was attributed to larger variation of $\delta^{13}C_{\text{PHY}}$ than that of $\delta^{13}C_{\text{DIC}}$. Therefore, the main factors influencing $\varepsilon_p$ were evaluated by their influences on $\delta^{13}C_{\text{PHY}}$.

There were weak relationships between $\delta^{13}C_{\text{PHY}}$ and TP and between $\delta^{13}C_{\text{PHY}}$ and TN (Tab. 3). However, TN was significantly correlated with $\delta^{13}C_{\text{PHY}}$ 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 $\delta^{13}C_{\text{PHY}}$ 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 $\delta^{13}C_{\text{PHY}}$. 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 $\delta^{13}C_{\text{PHY}}$ must be considered to explain the $\delta^{13}C$ of organic matter in lacustrine ecosystems.

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![Fig. 8. Scatter plots of carbon isotope fractionation ($\varepsilon_p$) vs carbon stable isotope composition of phytoplankton ($\delta^{13}C_{\text{PHY}}$), and carbon isotope fractionation vs carbon stable isotope composition of dissolved inorganic carbon ($\delta^{13}C_{\text{DIC}}$).](image-url)
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