



Stable carbon isotope biogeochemistry of lakes along a trophic gradient

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Abstract. The stable carbon (C) isotope variability of dissolved inorganic and organic C (DIC and DOC), particulate organic carbon (POC), glucose and polar-lipid derived fatty acids (PLFAs) was studied in a survey of 22 North American oligotrophic to eutrophic lakes. The $\delta^{13}\text{C}$ of different PLFAs were used as proxy for phytoplankton producers and bacterial consumers. Lake $p\text{CO}_2$ was primarily determined by autochthonous production (phytoplankton biomass), especially in eutrophic lakes, and governed the $\delta^{13}\text{C}$ of DIC. All organic-carbon pools showed overall higher isotopic variability in eutrophic lakes ($n = 11$) compared to oligo-mesotrophic lakes ($n = 11$) because of the high variability in $\delta^{13}\text{C}$ at the base of the food web (both autochthonous and allochthonous carbon). Phytoplankton $\delta^{13}\text{C}$ was negatively related to lake $p\text{CO}_2$ over all lakes and positively related to phytoplankton biomass in eutrophic lakes, which was also reflected in a large range in photosynthetic isotope fractionation ($\varepsilon_{\text{CO}_2\text{-phyto}}$, 8–25 ‰). The carbon isotope ratio of allochthonous carbon in oligo-mesotrophic lakes was rather constant, while it varied in eutrophic lakes because of maize cultivation in the watershed.

on the one hand and respiration and burial of organic carbon on the other governs whether individual lakes are sources or sinks of CO_2 . This metabolic balance can be disturbed by changes in nutrient or organic matter inputs to the lake. Primary (autochthonous) production increases with increasing nutrient concentrations and lakes with high autochthonous carbon production, i.e. eutrophic lakes, may be sinks for CO_2 (Schindler et al., 1997). The loading of allochthonous (terrestrial) carbon is a key factor controlling community respiration of lakes. The metabolic balance of lakes is directly influenced by allochthonous organic carbon loading and trophic state (Del Giorgio and Peters, 1994; Hanson et al., 2003).

Stable carbon isotope analysis is a powerful tool for studying carbon cycling in lakes since it allows study of inorganic and organic carbon pools and changes therein. It can provide information on the metabolic balance and the sources of organic matter fuelling respiration. Respiration yields ^{13}C -depleted carbon dioxide from organic matter with the result that $\delta^{13}\text{C}$ of dissolved inorganic carbon (DIC) of the lakes becomes lower (Parker et al., 2010). Primary producers preferentially incorporate ^{12}C in their organic matter with the consequence that the remaining pool of DIC will be enriched in ^{13}C (Herczeg, 1987; Parker et al., 2010). The $\delta^{13}\text{C}$ of the DIC pool thus integrates the relative importance of respiration and primary production (Parker et al., 2010). The $\delta^{13}\text{C}$ of organic carbon pools is primarily governed by the $\delta^{13}\text{C}$ of the DIC used by primary producers and the isotope fractionation during carbon fixation. Terrestrial plants use atmospheric carbon dioxide while aquatic primary producers

1 Introduction

Studies suggest that lakes contribute significantly to the global carbon budget via organic matter burial and emission of CO_2 to the atmosphere (Cole et al., 2007). The balance between primary production and external organic carbon input

Table 1. Limnological characteristics of the sampled lakes. $p\text{CO}_2$ was determined from temperature, DIC, and pH. C_{phyto} is the average of chl *a* and fatty acid based phytoplankton biomass. C_{bac} is fatty acid derived bacteria carbon biomass. I, Iowa; M, Minnesota; TN, total nitrogen; TP, total phosphorus.

Name	US state	Trophic state	Temperature (°C)	pH	Alkalinity (mmol L ⁻¹)	DIC (mmol L ⁻¹)	$p\text{CO}_2$ (µatm)	TN (mg L ⁻¹)	TP (µg L ⁻¹)	Chl <i>a</i> (µg L ⁻¹)	DOC (mg L ⁻¹)	POC (mg L ⁻¹)	C_{phyto} (mg L ⁻¹)	C_{bac} (mg L ⁻¹)
Beaver	I	Eu	24.1	9.5	1.03	1.70	30	1.69	152.8	72.6	2.10	5.07	2.26	0.251
Beeds	I	Eu	23.8	8.5	2.28	4.15	835	9.32	36.1	9.1	0.97	0.93	0.38	0.023
Big Creek	I	Eu	25.0	8.5	1.97	3.89	795	6.12	21.3	8.0	1.56	1.30	0.32	0.047
Coralville	I	Eu	24.2	7.8	2.37	4.55	4545	6.50	207.1	10.9	1.16	1.91	0.49	0.074
Little Splithand	M	Eu	19.2	8.2	1.04	1.69	635	0.01	29.5	19.5	3.70	2.07	0.58	0.026
Lower Pine	I	Eu	24.1	8.6	1.44	2.51	400	4.15	128.3	60.2	1.46	4.00	2.32	0.213
McBride	I	Eu	22.0	8.8	1.18	1.98	195	1.27	67.9	42.6	1.71	2.84	1.80	0.103
Meyers	I	Eu	27.1	9.8	1.08	1.49	10	2.14	208.7	86.2	2.70	9.91	3.65	0.190
Rodgers Park	I	Eu	24.6	8.4	1.82	3.34	855	6.81	50.6	5.4	1.20	0.68	0.23	0.038
Saylorville	I	Eu	25.3	8.5	2.05	4.03	830	4.90	116.3	23.0	1.80	1.30	0.96	0.165
Three Mile	I	Eu	21.3	9.1	0.98	1.69	80	1.00	44.9	37.8	1.90	2.66	1.48	0.122
Beaver	M	O-M	19.7	6.9	0.09	0.09	520	0.12	16.2	3.6	4.34	1.50	0.13	0.017
Brush Shanty	M	O-M	20.3	7.4	0.28	0.29	655	0.34	10	1.4	4.75	0.56	0.06	0.014
Hatch	M	O-M	20.3	8.4	1.81	3.04	735	0.55	1.9	0.8	2.08	0.30	0.04	0.008
Horsehead	M	O-M	19.9	6.7	0.07	0.06	460	0.09	6.9	1.8	2.91	2.11	0.09	0.033
Kelly	M	O-M	20.2	6.9	0.08	0.05	310	0.01	8.9	2.6	2.50	1.22	0.09	0.017
Leighton	M	O-M	19.4	8.4	1.84	2.99	715	0.49	8.1	2.4	2.62	0.67	0.07	0.007
Little Sand	M	O-M	22.8	8.2	0.75	1.88	745	0.19	11.9	4.9	3.68	1.24	0.17	0.023
O'Leary	M	O-M	19.2	6.7	0.09	0.08	655	0.44	13.8	2.6	3.18	2.28	0.10	0.029
Sand Lake	M	O-M	19.9	7.7	1.18	0.61	710	0.66	20.4	3.1	3.81	0.85	0.12	0.016
South Sturgeon	M	O-M	18.6	6.1	0.22	0.20	3200	0.03	14.4	3.6	6.71	0.55	0.11	0.005
Thirty	M	O-M	22.0	7.1	0.13	0.12	525	0.10	15.7	6.3	3.65	3.02	0.23	0.066

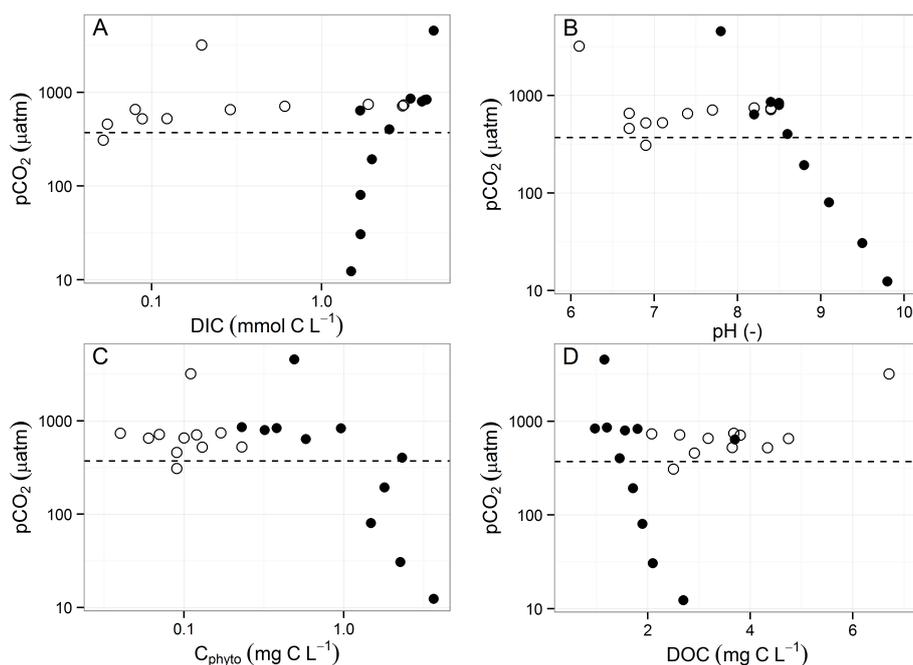


Figure 1. The relation of $p\text{CO}_2$ in eutrophic lakes (filled circles, $n = 11$) and oligo-mesotrophic lakes (open circles, $n = 11$) to (a) DIC; (b) pH; (c) C_{phyto} ; (d) DOC. The dashed line indicates atmospheric $p\text{CO}_2$ (385 µatm).

utilize dissolved carbon dioxide or bicarbonate (Fry, 2006). The $\delta^{13}\text{C}$ of terrestrially derived organic carbon is therefore often distinct from that of organic matter produced within the lakes and this difference can be used to trace carbon flows and origins in plankton food webs.

A major challenge in stable isotope studies is to elucidate the isotopic composition of microbial organisms (Middelburg, 2014), such as phytoplankton and bacteria, since it

is difficult to separate these potential carbon sources from bulk particulate organic carbon (POC). Therefore, most studies use indirect methods to determine $\delta^{13}\text{C}$ of phytoplankton ($\delta^{13}\text{C}_{\text{phyto}}$), bacteria ($\delta^{13}\text{C}_{\text{bac}}$) and allochthonous carbon ($\delta^{13}\text{C}_{\text{allo}}$). Common methods for determining $\delta^{13}\text{C}_{\text{phyto}}$ are the use of the $\delta^{13}\text{C}$ of POC with correction for non-phytoplankton carbon and estimates based on $\delta^{13}\text{C}$ of DIC with an isotope fractionation factor (ϵ), obtained from

experimental studies. Other methods are the use of zooplankton consumers as a proxy for $\delta^{13}\text{C}_{\text{phyto}}$ or size fractionation of organic matter and subsequent determination of $\delta^{13}\text{C}$ of different size classes (Marty and Planas, 2008).

Isotopic ratios of bacteria in field studies have been derived from re-growing bacteria in bioassays (Coffin et al., 1989) or dialysis cultures (Kritzberg et al., 2004), with measurement of ^{13}C in POC or respired CO_2 (McCallister and del Giorgio, 2008) and from biomarkers such as nucleic acids (Coffin et al., 1990) and lipids (Bontes et al., 2006; Pace et al., 2007). Some studies used $\delta^{13}\text{C}$ of dissolved organic carbon (DOC) as proxy for $\delta^{13}\text{C}$ of bacteria, assuming that DOC was the primary carbon source for bacteria (Taipale et al., 2008; Zigah et al., 2012).

A commonly used proxy for allochthonous $\delta^{13}\text{C}$ is the $\delta^{13}\text{C}$ of terrestrial C_3 plants, which dominates most terrestrial vegetation and has a $\delta^{13}\text{C}$ of $\sim -28\text{‰}$ (Fry, 2006; Kohn, 2010). When vegetation is dominated by C_4 plants, however, common in tropical areas and agricultural areas with maize production ($\delta^{13}\text{C}$ of $\sim -14\text{‰}$; Fry, 2006), the isotopic composition of allochthonous carbon can be significantly enriched in ^{13}C . In lakes with large terrestrial input, $\delta^{13}\text{C}$ of DOC can be used as a proxy for $\delta^{13}\text{C}_{\text{allo}}$, since terrestrial carbon forms the largest fraction of DOC (Kritzberg et al., 2004; Wilkinson et al., 2013).

Compound-specific isotope analysis (CSIA) of polar lipid-derived fatty acid (PLFA) biomarkers has shown to be a valuable tool to determine the isotopic composition of plankton producers and consumers (Boschker and Middelburg, 2002). Groups of phytoplankton and bacteria have different fatty acid (FA) compositions, so by analysing the $\delta^{13}\text{C}$ of specific FA, the $\delta^{13}\text{C}$ of phytoplankton and bacteria can be inferred. The combined use of stable isotopes and FA biomarkers has been successfully applied to study autochthonous and allochthonous carbon contributions to zooplankton in a tidal river (Van den Meersche et al., 2009). Few studies have applied CSIA to study carbon flows in plankton food webs in lakes. Examples are a phytoplankton–zooplankton interaction study in a eutrophic lake (Pel et al., 2004), a biomanipulation effect study (Bontes et al., 2006), a ^{13}C lake enrichment study (Pace et al., 2007) and a cyanobacteria–zooplankton interaction study (de Kluijver et al., 2012).

In this study, we used compound-specific isotope analyses to examine carbon flows in plankton food webs in temperate (North American) lakes. The lake survey encompassed a range in trophic states from oligotrophic lakes, with an expected dominance of allochthonous input, to eutrophic lakes, with an expected lower allochthonous input. In this trophic range, we explored patterns of isotopic variability in DIC, DOC, POC, carbohydrates, phytoplankton, allochthonous carbon and heterotrophic bacteria, and their relationships.

Table 2. Significant correlation coefficients (r) between tested variables in all lakes and in eutrophic and oligo-mesotrophic lakes separately. Significance levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Variables	Eutrophic lakes ($n = 11$) r	Oligo-mesotrophic lakes ($n = 11$) r	Overall ($n = 22$) r
log alkalinity and log $p\text{CO}_2$	0.79 **		
DIC and log $p\text{CO}_2$	0.82 **		
pH and log $p\text{CO}_2$	−0.98 ***		
log C_{phyto} and log $p\text{CO}_2$	−0.80 **		−0.59 ***
DOC and log $p\text{CO}_2$		0.75 **	
log TP and log C_{phyto}	0.61 *	0.77 **	0.89 ***
log TP and log C_{bac}	0.74 **		0.82 ***
log $p\text{CO}_2$ and $\delta^{13}\text{C}_{\text{DIC}}$	−0.63 *	−0.81 **	−0.48 *
POC and $\delta^{13}\text{C}_{\text{DIC}}$	0.61 *		
DOC and $\delta^{13}\text{C}_{\text{DIC}}$		−0.62 *	
log TP and $\Delta(\delta_{\text{gluc}}^{13}\text{C} - \delta_{\text{FA}}^{13}\text{C})$			0.52 *
log C_{phyto} and $\delta^{13}\text{C}_{\text{phyto}}$	0.90 ***		
log $p\text{CO}_2$ and $\delta^{13}\text{C}_{\text{phyto}}$	−0.79 **	−0.90 ***	−0.73 ***
$\delta^{13}\text{C}_{\text{CO}_2}$ and $\delta^{13}\text{C}_{\text{phyto}}$		0.82 **	0.54 **
log C_{phyto} and ε	−0.70 *		
$\delta^{13}\text{C}_{\text{DOC}}$ and $\delta^{13}\text{C}_{\text{detritus}}$		0.79 **	0.54 *

2 Material and methods

2.1 Site description

The 22 lakes sampled in this study are located in Iowa and Itasca County in Minnesota, USA. Iowa lakes are mostly man-made and situated in a highly agricultural region, with maize and soya beans as main products. This type of row-crop agriculture has a large impact on the nutrient load of the lake watershed (Arbuckle and Downing, 2001). Itasca lakes are natural and situated in a highly forested area.

The catchment areas have developed since the last glaciation episode ca. 12 000 years ago and consist of carbonate-poor glacial deposits (till) (Grimley, 2000).

2.2 Field sampling

The lakes were sampled in July–August 2009 as part of the ongoing lake monitoring programme of the limnology laboratories of Iowa State University and Itasca Community College. Key parameters, such as temperature, pH, Secchi transparency, oxygen, inorganic nutrients and carbon concentrations were measured as part of and according to the lake monitoring programme. All samples were taken from up to 2 m of the upper mixed zone at the deepest point of each lake. Water samples were taken between 10:00 and 16:00 LT, a period of the day that yields relatively stable water chemistry readings in these lakes. More information on data collection, lake characteristics and methods can be found on <http://limnoweb.eeob.iastate.edu/itascalakes> and <http://limnology.eeob.iastate.edu/lakereport>. All nutrients were analysed using certified methods and strict quality assurance procedures.

Triplicate water samples were taken for stable isotope analyses and concentrations of the major carbon pools. Headspace vials (20 and 2 mL) were filled on board with sampled water using the overflow method and sealed with

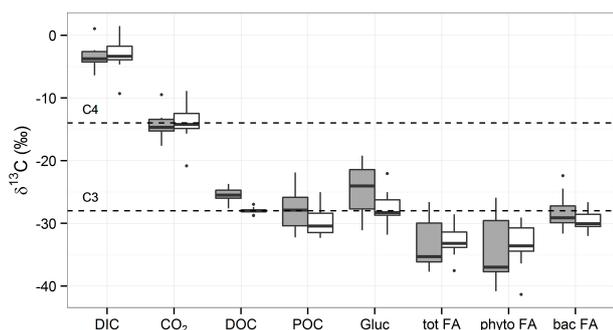


Figure 2. Box and whisker plot of the $\delta^{13}\text{C}$ of inorganic and organic carbon pools in eutrophic lakes (grey boxes, $n = 11$) and oligo-mesotrophic lakes (white boxes, $n = 11$). The dashed lines present typical values for C_3 and C_4 vegetation.

gas-tight caps for DIC isotope analyses and concentrations, respectively. Mercury chloride was added for preservation and the samples were stored upside-down at room temperature. For DOC analyses, 20 mL of sampled water was filtered over GF/F (0.7 μm pore size, 25 mm diameter) and stored frozen in clean (acid and Milli-Q rinsed) vials until further analysis.

Seston samples for POC and carbohydrates were collected by filtering 0.4 to 1 L of sampled water on pre-weighed and pre-combusted GF/F filters (0.7 μm pore size, 47 mm diameter), which were subsequently dried at 60 °C for POC analysis or freeze-dried for carbohydrates; PLFA samples were collected by filtering ~ 2 L sampled water on pre-combusted GF/F filters (0.7 μm , 47 mm) and filters were stored frozen. Pigment samples were taken for concentrations only and collected by filtering ~ 600 mL sampled water on GF/F filters (0.7 μm , 47 mm) in the dark and filters were stored frozen.

2.3 Laboratory analyses

POC samples were analysed for carbon content and isotope ratios on a Thermo Electron Flash EA 1112 analyser (EA) coupled to a Delta V isotope ratio mass spectrometer (IRMS) (cf. Nieuwenhuize et al., 1994). For DIC isotope analyses, a helium headspace was created in the headspace vials and samples were acidified with H_3PO_4 solution. After equilibration, the CO_2 concentration and isotope ratio in the headspace were measured using EA-IRMS (Gilikin and Bouillon, 2007). DIC concentrations were measured using spectrophotometry according to Stoll et al. (2001). For DOC analyses, the samples were acidified and flushed with helium to remove DIC and subsequently oxidized with sodium persulfate ($\text{Na}_2\text{S}_2\text{O}_8$); the isotope ratio and concentration of CO_2 resulting from this treatment was measured using high performance liquid chromatography – isotope ratio mass spectrometry (HPLC-IRMS) (Boschker et al., 2008). PLFA samples were extracted according to a modified Bligh and Dyer method (Bligh and Dyer, 1959, Mid-

delburg et al., 2000). The lipids were fractionated in different polarity classes by column separation on a heat-activated silicic acid column and subsequent elution with chloroform, acetone and methanol. The methanol fractions, containing most of the PLFAs, were collected and transformed to fatty acid methyl esters (FAME) using methanolic NaOH. The 12:0 and 19:0 FAME were added as internal standards. Concentrations and $\delta^{13}\text{C}$ of individual PLFAs were measured using gas chromatography–combustion isotope ratio mass spectrometry (GC-C-IRMS) (Middelburg et al., 2000). The isotopic compositions were corrected for the carbon added during derivatization. Pigment samples were extracted with 90 % acetone in purified (Milli-Q) water with intense shaking. Concentrations of individual pigments were measured on HPLC (Wright, 1991). Carbohydrate samples were hydrolysed in H_2SO_4 , neutralized with SrCO_3 , and precipitated with BaSO_4 . The supernatant was collected and measured using HPLC-IRMS according to Boschker et al. (2008).

2.4 Data analyses

The lakes were divided into eutrophic and oligo-mesotrophic lakes based on average summer total phosphorus (TP) concentrations. Lakes with TP values $> 24 \mu\text{g L}^{-1}$ and a corresponding trophic state index > 50 were classified as eutrophic, and lakes with TP values $< 24 \mu\text{g L}^{-1}$ as oligo-mesotrophic (Carlson, 1977). All lakes in Iowa and one lake in Minnesota were classified as eutrophic, while all oligo-mesotrophic lakes were located in Minnesota.

2.4.1 CO_2 system and isotopic composition of CO_2

The different components of the CO_2 system were calculated from temperature, laboratory pH and DIC concentrations using a salinity of 0 and the R package AquaEnv (Hofmann et al., 2010). Stable isotope ratios were expressed in the delta notation ($\delta^{13}\text{C}$), which is the $^{13}\text{C}/^{12}\text{C}$ ratio relative to VPDB standard, in parts per thousand (‰). The isotope ratio of CO_2 (aq) ($\delta^{13}\text{C}_{\text{CO}_2}$) was calculated from $\delta^{13}\text{C}_{\text{DIC}}$ according to Zhang et al. (1995):

$$\delta^{13}\text{C}_{\text{CO}_2} = \delta^{13}\text{C}_{\text{DIC}} - 0.0144 \cdot T(^{\circ}\text{C}) \cdot f\text{CO}_3^{2-} + 0.107 \cdot T(^{\circ}\text{C}) - 10.53, \quad (1)$$

where $f\text{CO}_3^{2-}$ is the fraction of CO_3^{2-} in total DIC, calculated from pH and DIC concentrations.

2.4.2 $\delta^{13}\text{C}$ of phytoplankton and bacteria

Poly-unsaturated fatty acids (PUFAs) are abundant in most phytoplankton, and can generally be used as chemotaxonomic markers for this group (Dijkman and Kromkamp, 2006). The most abundant PUFAs in all lakes were 18:3 ω 3 (α -linolenic acid), 18:4 ω 3 (stearidonic acid, SDA), 20:5 ω 3 (icosapentaenoic acid, EPA), 22:6 ω 3 (docosahexaenoic acid, DHA) and 20:4 ω 6 (arachidonic acid, ARA), common

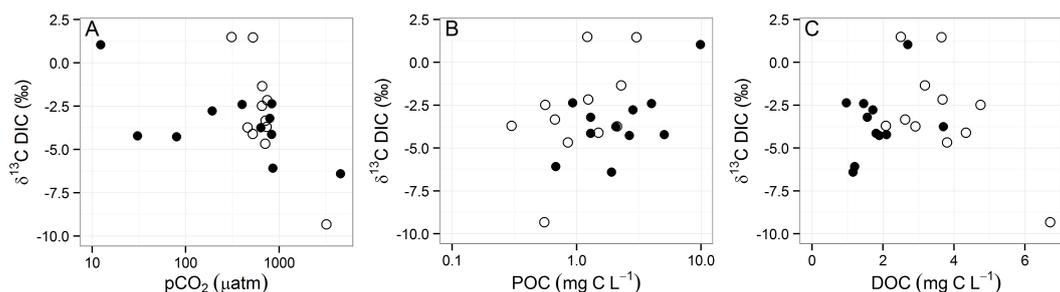


Figure 3. The relation of $\delta^{13}\text{C}_{\text{DIC}}$ in eutrophic lakes (filled circles, $n = 11$) and oligo-mesotrophic lakes (open circles, $n = 11$) to (a) $p\text{CO}_2$; (b) C_{phyto} ; (c) DOC.

PUFAs in freshwater phytoplankton (Taipale et al., 2013), and their concentration-weighted $\delta^{13}\text{C}$ were used to determine phytoplankton isotope ratios ($\delta^{13}\text{C}_{\text{phyto}}$). Note that phytoplankton is considered a mixture of eukaryotic algae and cyanobacteria. Branched fatty acids (BFAs) are abundant in heterotrophic bacteria (Kaneda, 1991) in contrast to phytoplankton. The most abundant BFAs were i15:0, ai15:0 and i17:0 and their weighted $\delta^{13}\text{C}$ were used as a proxy for heterotrophic bacteria isotope ratios ($\delta^{13}\text{C}_{\text{bac}}$), which we further consider bacteria. Isotope fractionation (ε) between CO_2 and phytoplankton was calculated as

$$\varepsilon_{\text{CO}_2\text{-phyto}}(\text{‰}) = \frac{\delta^{13}\text{C}_{\text{CO}_2} - \delta^{13}\text{C}_{\text{phyto,cor}}}{1 + \delta^{13}\text{C}_{\text{phyto,cor}}/1000} \quad (2)$$

$\delta^{13}\text{C}_{\text{phyto,cor}}$ was derived from $\delta^{13}\text{C}_{\text{phyto}}$ with a correction of +3 ‰ for the isotopic offset between PUFAs and total cells ($\Delta\delta^{13}\text{C}_{\text{FA-cell}}$) (Schouten et al., 1998; Hayes, 2001), although this isotopic offset can be highly variable (Schouten et al., 1998).

2.4.3 $\delta^{13}\text{C}$ of allochthonous carbon

Allochthonous organic carbon ($\delta^{13}\text{C}_{\text{allo}}$), i.e. organic matter delivered to lakes as DOC or POC, cannot be measured directly and we therefore used two proxies: the measured isotopic ratios of DOC ($\delta^{13}\text{C}_{\text{DOC}}$) and calculated isotopic composition of particulate detritus ($\delta^{13}\text{C}_{\text{det}}$). The latter was calculated from a mass balance and mixing model, similar to Marty and Planas (2008), amended with zooplankton and bacteria. We assumed that POC consists of phytoplankton, detritus, bacteria and zooplankton, and that the $\delta^{13}\text{C}$ of POC represents a mixture of the weighted $\delta^{13}\text{C}$ of the individual groups. Subsequently, $\delta^{13}\text{C}_{\text{det}}$ in each lake was derived from $\delta^{13}\text{C}_{\text{POC}}$:

$$\delta^{13}\text{C}_{\text{det}}(\text{‰}) = \left(\text{POC} \cdot \delta^{13}\text{C}_{\text{POC}} - C_{\text{phyto}} \cdot \delta^{13}\text{C}_{\text{phyto,cor}} - C_{\text{bac}} \cdot \delta^{13}\text{C}_{\text{bac}} - C_{\text{zoo}} \cdot \delta^{13}\text{C}_{\text{zoo}} \right) / C_{\text{det}} \quad (3)$$

$$C_{\text{det}}(\text{mg CL}^{-1}) = \text{POC} - C_{\text{phyto}} - C_{\text{bac}} - C_{\text{zoo}} \quad (4)$$

Equation (4) simply states that detrital organic matter is the non-living part of the total particulate organic matter pool.

Phytoplankton carbon (C_{phyto}) (mg CL^{-1}) was calculated as the average of biomass estimates based on chl *a* concentration ($C : \text{chl } a = 50$) as well as phytoplankton FA derived biomass, to minimize the error associated with each method. Phytoplankton FA biomass was calculated from the sum of phytoplankton PLFAs ($\sum 18:3\omega3, 18:4\omega3, 20:5\omega3, 22:6\omega3$, and $20:4\omega6$) and a C : specific FA ratio of 60 based on culture studies, summarized in Dijkman and Kromkamp (2006). The two approaches yielded similar results. Bacterial carbon (C_{bac}) (mg CL^{-1}) was calculated from the summed concentrations of bacteria-specific FA (i15:0, ai15:0, and i17:0) and a $C_{\text{bac}} : \text{FA}$ ratio of 50 (Middelburg et al., 2000). Zooplankton carbon (C_{zoo}) used in Eq. (3) was estimated to be $\sim 10\%$ of C_{phyto} (Del Giorgio and Gasol, 1995) and zooplankton $\delta^{13}\text{C}$ are based on de Kluijver (2012). Uncertainties in $\delta^{13}\text{C}$ and biomass of phytoplankton, bacteria and zooplankton were not considered in calculating $\delta^{13}\text{C}_{\text{det}}$.

2.4.4 Statistical analyses

Data that were part of the lake monitoring programme and $p\text{CO}_2$ values represent single samples of each lake. Data on carbon concentrations and isotopic compositions in each lake convey averages of triplicate samples. Statistical analyses were done with software package ‘‘R’’ (R development core team, 2013). Prior to correlation analyses, data were checked for normal distribution (Shapiro test) and log-transformed when necessary to achieve normal distribution. Correlation coefficients were calculated using Pearson product-moment correlation coefficient (normal distribution) or Spearman’s rank correlation coefficient (non-normal distribution). For completeness we present the average \pm sd values for eutrophic lakes ($n = 11$) and oligo-mesotrophic lakes ($n = 11$), but we do realize that any division based on a concentration is somewhat arbitrary. The correlations were tested for total lakes ($n = 22$) and for eutrophic lakes ($n = 11$) and oligo-mesotrophic lakes ($n = 11$). Differences between eutrophic and oligo-mesotrophic lakes were statistically tested using Student’s *t* tests.

3 Results

3.1 Lake chemistry

The sampled lakes covered a large range of nutrients and CO₂ system characteristics (Table 1). DIC values ranged from 0.05 to 4.55 mmol L⁻¹, alkalinity values ranged from 0.070 to 2.4 mmol L⁻¹ and pH ranged from 6.1 to 9.8 (Table 1). The calculated *p*CO₂ values were in the range 10–4500 μatm, covering a broad range from under- to super-saturation. The CO₂ system in eutrophic and oligo-mesotrophic lakes was clearly different. On average, the eutrophic lakes had higher DIC, alkalinity and pH than the oligo-mesotrophic lakes (Fig. 1, Table 1). In the eutrophic lakes, there were positive correlations between alkalinity and DIC and *p*CO₂ values (Fig. 1a, Table 2) and a negative correlation between pH and *p*CO₂ (Fig. 1b, Table 2). The *p*CO₂ values were not related to pH, alkalinity or DIC in the oligo-mesotrophic lakes. Both lake systems showed super-saturation (average *p*CO₂ 838 μatm in both), but the *p*CO₂ range was much larger in eutrophic lakes (10–4500 μatm) compared to oligo-mesotrophic lakes (310–3200 μatm) (Fig. 1, Table 1).

3.2 Organic carbon and fatty acid concentrations

POC, C_{phyto} and C_{bac} concentrations were higher and DOC concentrations were lower in the eutrophic lakes compared to the oligo-mesotrophic lakes (Table 1). C_{phyto} was on average 1.32 ± 1.10 and 0.11 ± 0.03 mg C L⁻¹, corresponding to 44 ± 17 and 10 ± 5 % of POC in eutrophic and oligo-mesotrophic lakes, respectively. C_{phyto} and C_{bac} were significantly related to TP (Table 2), but not to TN. Average C_{bac} was 0.114 ± 0.081 and 0.021 ± 0.017 mg C L⁻¹ in eutrophic and oligo-mesotrophic lakes, respectively.

Overall, lake *p*CO₂ decreased with increasing C_{phyto}, but the effect was strongest in eutrophic lakes (Fig. 1c, Table 2). In the oligo-mesotrophic lakes, *p*CO₂ increased with increasing DOC (Fig. 1d, Table 2), but this effect was caused by one point: the high *p*CO₂ at high DOC in lake Sturgeon. In the eutrophic lakes, DOC concentrations were lower compared to the oligo-mesotrophic lakes and did not act on lake *p*CO₂ (Fig. 1d, Table 1).

3.3 δ¹³C of DIC and CO₂

The isotope ratios of the major carbon pools in each lake are presented in Table 3 and in box plots (median and percentiles) in Fig. 2. δ¹³C_{DIC} ranged from -9.3 to +1.5 ‰ and δ¹³C_{CO₂} (derived from δ¹³C_{DIC}) was on average 10.9 ± 0.3 ‰ depleted in ¹³C relative to DIC, with a range of -20.8 to -8.9 ‰. δ¹³C_{DIC} and δ¹³C_{CO₂} showed no correlation with alkalinity, DIC, pH, temperature and lake area. A weak negative relation between *p*CO₂ and δ¹³C_{DIC} was observed, which was stronger in oligo-mesotrophic lakes than in eutrophic lakes (Fig. 3a, Table 2). The highest *p*CO₂

lakes had the lowest δ¹³C_{DIC}, suggesting that respiration of organic matter influenced δ¹³C_{DIC}. Low CO₂ lakes had enriched δ¹³C_{DIC}, indicating influence of primary production. Weak but significant relations were observed for POC and DOC with δ¹³C_{DIC} (Table 2). In eutrophic lakes, δ¹³C_{DIC} increased with increasing POC (Fig. 3b, Table 2), while in oligo-mesotrophic lakes, δ¹³C_{DIC} decreased with increasing DOC (Fig. 3c, Table 2).

3.4 δ¹³C of organic carbon pools

The isotopic composition of DOC (δ¹³C_{DOC}) had the narrowest range of all carbon pools, only -28.8 to -27.0 ‰ (mean -28.0 ‰) in the oligo-mesotrophic lakes and a slightly larger range of -27.6 to -23.7 ‰ (mean -25.4 ‰) in the eutrophic lakes (Fig. 2, Table 3). The δ¹³C range of POC (δ¹³C_{POC}) was larger than of DOC in both lake types and on average 2.0 ‰ lower compared to δ¹³C_{DOC}, with mean values of -27.8 ± 3.6 ‰ in eutrophic and -29.7 ± 2.8 ‰ in oligo-mesotrophic lakes (Fig. 2, Table 3). δ¹³C of particulate glucose (δ¹³C_{gluc}), the most abundant carbohydrate, was always enriched in ¹³C compared to δ¹³C_{POC} and the enrichment was similar in eutrophic (3.1 ± 1.7 ‰) and oligo-mesotrophic lakes (2.8 ± 1.5 ‰) (Fig. 2). On the contrary, the concentration-weighted average δ¹³C of all FA (δ¹³C_{FA_{tot}}) was always depleted in ¹³C compared to δ¹³C_{POC} (Fig. 2). The depletion in ¹³C of δ¹³C_{FA_{tot}} relative to POC was higher in eutrophic lakes (5.2 ± 1.8 ‰) than in oligo-mesotrophic lakes (3.1 ± 1.1 ‰). The isotopic difference between glucose and δ¹³C_{FA_{tot}}: Δδ¹³C_{gluc-FA_{tot}} was highly variable with a range of 1.6 to 14.6 ‰. The isotopic differences between glucose and δ¹³C_{FA_{tot}} did not correlate with CO₂, but correlated weakly with nutrient levels, i.e. Δδ¹³C_{gluc-FA_{tot}} increased with increasing TP (Table 2).

There was a large variability among δ¹³C of different FA, with some consistent differences over all lakes. Compared to the δ¹³C of 16:0 (the most abundant FA), the bacterial FA markers were always enriched in ¹³C by 1.4–5.0 ‰ (e.g. the iso-15:0 FA in Fig. 4), therefore the overall δ¹³C of bacterial FA (δ¹³C_{bac}) was more ¹³C-enriched than δ¹³C_{FA_{tot}} in both lake systems (Fig. 2). The PUFAs used as markers for phytoplankton showed consistent differences throughout the lakes. DHA (22:6ω3), common in dinoflagellates (Dalsgaard et al., 2003), was found to be ¹³C-enriched with 4.6 ‰ compared to 16:0 while PLFA 18:3ω3 (α-linolenic acid), common in cyanobacteria (de Kluijver et al., 2012), was 4.7 ‰ depleted in ¹³C compared to 16:0 (Fig. 4). The other phytoplankton markers were not statistically different from 16:0. The weighted δ¹³C of phytoplankton FA (δ¹³C_{phyto}) was the most ¹³C-depleted of all carbon pools (Fig. 2, Table 3) with an average of -33.8 ± 5.3 in eutrophic lakes and -33.4 ± 3.5 ‰ in oligo-mesotrophic lakes. δ¹³C_{bac} were on average 4.7 ‰ enriched in ¹³C compared to δ¹³C_{phyto} (Fig. 2).

Table 3. Carbon isotope values of sampled lakes. Isotope data are presented as average \pm SD ($n = 3$). $\delta^{13}\text{C}_{\text{CO}_2}$ was calculated from $\delta^{13}\text{C}_{\text{DIC}}$ (Eq. 1). $\delta^{13}\text{C}_{\text{phyto}}$ and $\delta^{13}\text{C}_{\text{bac}}$ are not corrected for the offset between fatty acids and total cells, but $\varepsilon_{\text{CO}_2\text{-phyto}}$ (Eq. 2) used the corrected $\delta^{13}\text{C}$ of phytoplankton.

Lake name	Trophic state	$\delta^{13}\text{C}_{\text{DIC}}$ (‰)	$\delta^{13}\text{C}_{\text{CO}_2}$ (‰)	$\delta^{13}\text{C}_{\text{POC}}$ (‰)	$\delta^{13}\text{C}_{\text{DOC}}$ (‰)	$\delta^{13}\text{C}_{\text{phyto}}$ (‰)	$\varepsilon_{\text{CO}_2\text{-phyto}}$	$\delta^{13}\text{C}_{\text{bac}}$ (‰)	$\delta^{13}\text{C}_{\text{gluc}}$ (‰)
Beaver (I)	Eu	-4.2 ± 0.6	-15.2	-21.9 ± 0.2	-24.2 ± 0.0	-25.9 ± 1.9	7.8	-22.4 ± 0.3	-19.2
Beeds (I)	Eu	-2.4 ± 0.1	-13.1	-32.2	-23.7 ± 0.1	-38.0 ± 2.5	22.7	-29.1 ± 0.0	-31.1
Big Creek (I)	Eu	-3.2 ± 0.1	-14.1	-32.2 ± 0.1	-26.4 ± 0.9	-40.8 ± 2.2	24.7	-31.5 ± 0.7	-30.4
Coralville (I)	Eu	-6.4 ± 0.0	-17.6	-27.9 ± 0.5	-24.6 ± 0.3	-37.4 ± 1.5	17.4	-29.4 ± 0.9	-21.4
Little Split-hand (M)	Eu	-3.8 ± 0.1	-14.7	-32.3 ± 0.4	-27.6 ± 0.4	-37.0 ± 0.5	20.0	-31.7 ± 0.7	-27.3
Lower Pine (I)	Eu	-2.4 ± 0.1	-13.2	-26.2 ± 0.2	-25.3 ± 0.5	-30.6 ± 1.1	14.8	-27.2 ± 0.8	-24.0
McBride (I)	Eu	-2.8 ± 0.1	-13.6	-25.6 ± 0.4	-25.6 ± 0.3	-27.3 ± 0.6	11.0	-27.2 ± 1.0	-21.5
Meyers (I)	Eu	1.0 ± 0.3	-9.5	-22.1 ± 0.1	-26.2 ± 0.6	-29.3 ± 1.7	17.3	-24.5 ± 0.6	-19.7
Rodgers Park (I)	Eu	-6.1 ± 0.0	-17.3	-29.0	-24.8 ± 0.3	-38.6	19.0	-30.3	-26.3
Saylorville (I)	Eu	-4.1 ± 0.0	-15.1	-29.2 ± 0.1	-25.8 ± 1.2	-37.3 ± 1.4	19.8	-29.5 ± 0.4	-28.1
Three Mile (I)	Eu	-4.3 ± 0.3	-15.3	-27.8 ± 0.4	-25.5 ± 1.0	-29.8 ± 2.3	11.9	-28.5 ± 0.6	-23.7
Beaver (M)	O-M	-4.1 ± 0.5	-15.1	-30.5 ± 0.2	-28.7 ± 0.2	-32.4 ± 0.2	14.8	-30.4 ± 2.0	-28.3
Brush Shanty (M)	O-M	-2.5 ± 0.2	-13.3	-29.6 ± 0.2	-28.1 ± 0.4	-33.6 ± 2.1	17.9	-30.0 ± 3.0	-25.0
Hatch (M)	O-M	-3.7 ± 0.1	-14.6	-31.4 ± 0.4	-28.8 ± 0.2	-34.5 ± 1.3	17.4	-30.6 ± 2.4	-28.6
Horsehead (M)	O-M	-3.7 ± 0.9	-14.7	-25.0 ± 0.1	-27.0 ± 0.1	-29.1 ± 0.7	11.7	-27.8 ± 2.0	
Kelly (M)	O-M	1.5 ± 0.5	-8.9	-27.8 ± 0.7	-27.9 ± 0.4	-30.1 ± 1.0	18.7	-26.6 ± 2.6	-22.1
Leighton (M)	O-M	-3.3 ± 0.0	-14.2	-32.3 ± 0.3	-28.2 ± 0.7	-34.4 ± 1.3	17.7	-30.9 ± 1.3	-31.8
Little Sand (M)	O-M	-2.2 ± 0.1	-12.9	-30.4 ± 0.2	-28.1 ± 0.0	-33.7 ± 1.7	18.4	-29.4 ± 1.8	
O'Leary (M)	O-M	-1.3 ± 0.7	-12.0	-28.1 ± 0.3	-27.4 ± 0.7	-31.3 ± 0.4	16.7	-26.8 ± 0.8	-26.3
Sand Lake (M)	O-M	-4.7 ± 0.1	-15.7	-31.5 ± 0.4	-28.0 ± 0.1	-36.4 ± 0.1	18.3	-29.6 ± 1.8	-28.7
South Sturgeon (M)	O-M	-9.3 ± 0.1	-20.8	-31.8 ± 0.9	-28.0 ± 0.0	-41.4 ± 1.4	18.2	-30.5 ± 1.2	-29.4
Thirty (M)	O-M	1.5 ± 0.6	-8.9	-28.7 ± 0.1	-27.8 ± 0.1	-30.2 ± 1.4	18.8	-32.0 ± 1.2	-26.4

3.5 Carbon isotopic composition of phytoplankton

$\delta^{13}\text{C}_{\text{phyto}}$ depends on the isotopic composition of substrate ($\delta^{13}\text{C}_{\text{CO}_2}$), the isotope fractionation ($\varepsilon_{\text{CO}_2\text{-phyto}}$) associated with primary production and the isotopic difference between PUFAs and biomass. $\delta^{13}\text{C}_{\text{phyto}}$ in the eutrophic lakes became more enriched in ^{13}C with increasing C_{phyto} (Fig. 5a, Table 2) and decreasing $p\text{CO}_2$ (Fig. 5b, Table 2). No relation between $\delta^{13}\text{C}_{\text{phyto}}$ and C_{phyto} was observed in the oligomesotrophic lakes (Fig. 5a), but there was a strong negative relation with $p\text{CO}_2$ (Fig. 5b, Table 2). The influence of C_{phyto} on $\delta^{13}\text{C}_{\text{phyto}}$ in the eutrophic lakes was also reflected in fractionation; $\varepsilon_{\text{CO}_2\text{-phyto}}$ was highly variable in eutrophic lakes, while it was less variable in oligotrophic lakes (Fig. 5c). The range of $\varepsilon_{\text{CO}_2\text{-phyto}}$ was 7.8 to 24.7 ‰ (mean 16.9 ‰) in eutrophic and 11.7 to 18.8 ‰ (mean 17.1 ‰) in oligomesotrophic lakes, when $\delta^{13}\text{C}_{\text{phyto,cor}}$ was used (Table 3). The less variable ε in oligo-mesotrophic lakes resulted in a strong correlation between $\delta^{13}\text{C}_{\text{CO}_2}$ and $\delta^{13}\text{C}_{\text{phyto}}$ (Table 2), which was absent in the eutrophic lakes. $\varepsilon_{\text{CO}_2\text{-phyto}}$ correlated negatively with C_{phyto} in eutrophic lakes, however (Table 2). The variability in $\delta^{13}\text{C}_{\text{phyto}}$ in eutrophic lakes can be mainly attributed to the presence of two clusters: a ^{13}C -enriched cluster at the highest C_{phyto} and a ^{13}C -depleted cluster at lower C_{phyto} (Fig. 5a). Interestingly, the eutrophic lakes within the ^{13}C -enriched cluster also had high concentrations of zeaxanthin, a marker pigment for cyanobacteria (data not shown here).

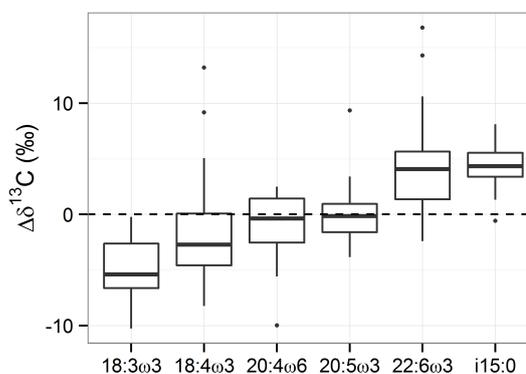


Figure 4. Box and whisker plots of the $\Delta\delta^{13}\text{C}$ of individual biomarker fatty acids relative to 16:0 FA ($\delta^{13}\text{C}_{\text{FA}} - \delta^{13}\text{C}_{16:0}$) in all lakes.

4 Discussion

4.1 Lake metabolism, $p\text{CO}_2$ and $\delta^{13}\text{C}_{\text{DIC}}$

In our study, about three-quarters of the lakes were supersaturated with $p\text{CO}_2$, consistent with the finding in the literature that lakes generally emit carbon dioxide to the atmosphere (Cole et al., 1994, 2007). This CO_2 excess can be due to in-lake respiration of terrestrially derived organic carbon outbalancing CO_2 fixation by primary producers (negative metabolic balance) or due to river and groundwater input of CO_2 -rich waters (McDonald et al., 2013). Lake metabolism also impacts $\delta^{13}\text{C}_{\text{DIC}}$ dynamics. Previous studies have shown that $\delta^{13}\text{C}$ of DIC in lakes is driven by carbonate chemistry,

hydrology (i.e. groundwater inflow), and metabolic activity (Bade et al., 2004). Primary production increases $\delta^{13}\text{C}_{\text{DIC}}$ because of the preferential uptake of ^{12}C (isotope fractionation), while organic matter respiration decreases $\delta^{13}\text{C}_{\text{DIC}}$ (Fry, 2006).

If $p\text{CO}_2$ and $\delta^{13}\text{C}_{\text{DIC}}$ had been only or primarily controlled by the balance between respiration and production of organic matter, one would expect a tight correlation between $\delta^{13}\text{C}_{\text{DIC}}$ and $p\text{CO}_2$, which was not observed overall (Fig. 3a, Table 2), indicating that other factors are involved. High inorganic carbon loadings of inflowing rivers and groundwater inputs may sustain the CO_2 excess (McDonald et al., 2013) and govern the $\delta^{13}\text{C}_{\text{DIC}}$ (Bade et al., 2004). Moreover, CO_2 water–air exchange reactions may have modified $\delta^{13}\text{C}_{\text{DIC}}$ values because of isotope fractionation during water–air exchange, in particular at high pH/low CO_2 values (Herczeg and Fairbanks, 1987; Bade et al., 2004; Bontes et al., 2006).

The correlation between $\delta^{13}\text{C}_{\text{DIC}}$ and $p\text{CO}_2$ in oligo-mesotrophic lakes was stronger (Table 2) and $p\text{CO}_2$ was highest and $\delta^{13}\text{C}_{\text{DIC}}$ was most depleted in ^{13}C at the highest DOC in oligo-mesotrophic lakes (Figs. 1c, 3c). Such a depletion of $\delta^{13}\text{C}_{\text{DIC}}$ with increasing DOC, as an indicator of the importance of respiration in oligo-mesotrophic lakes, has been shown previously in North American lakes (Lennon et al., 2006). In addition to community respiration, methanotrophic bacteria in high DOC lakes could decrease $\delta^{13}\text{C}_{\text{DIC}}$ (Jones et al., 1999). However, anoxic hypolimnia are rare in these lakes, due to either low nutrients or polymixis, indicating that methanogenesis was not of major importance in the lakes investigated. Furthermore, we examined the $\delta^{13}\text{C}$ of fatty acids abundant in or specific to methanotrophs and these were not more depleted in ^{13}C than other fatty acids.

Note that the lakes were sampled at only one point location and depth, representing average conditions, so spatial variability per lake is not taken into account. Also diurnal variation and variation over the year in each lake are not considered in this study. However, the aim of our study is to compare snapshots of different lakes representing different metabolic states and not to describe the biogeochemistry of each individual lake. So, although we miss some variation, this should not affect our main findings on carbon isotope biogeochemistry of these lakes.

4.2 Allochthonous $\delta^{13}\text{C}$

DOC and POC pools are mixtures of organic matter from various sources with potentially different stable carbon isotopic compositions. The POC pool comprises biomass from living organisms and remains from organisms within the lake as well as allochthonous detritus. The relative importance of living biomass to total POC pool, calculated based on Eq. (4), varies from 5.7 to 93% (Table 1), with on average about $53 \pm 20\%$ in eutrophic lakes and only $13 \pm 5\%$ in oligo-mesotrophic lakes. In our study we have explicit carbon iso-

tope data for the most important living compartments (algae, bacteria and zooplankton; De Kluijver, 2012), but we have no direct measurement of the $\delta^{13}\text{C}$ of organic carbon delivered from the watershed via atmospheric, riverine and groundwater inputs. We have therefore used two proxies for $\delta^{13}\text{C}_{\text{allo}}$: the carbon isotope ratio of DOC and that of detrital POC calculated by difference (Eqs. 3, 4). Both proxies for $\delta^{13}\text{C}_{\text{allo}}$ provide an estimate for the total detrital pool, i.e. the sum of aquatic and terrestrial detritus.

The oligo-mesotrophic lakes are surrounded by forest (C_3 vegetation) and $\delta^{13}\text{C}_{\text{DOC}}$ was $-28.0 \pm 0.5\%$, corresponding to a C_3 vegetation signal, suggesting that the DOC pool is dominantly terrestrially derived, consistent with a combined carbon and hydrogen isotope study of Wisconsin and Michigan lakes by Wilkinson et al. (2013). The other proxy for allochthonous carbon, $\delta^{13}\text{C}_{\text{det}}$, was slightly more negative ($-29.6 \pm 2.1\%$), but the two proxies for allochthonous carbon were well correlated (Table 2). The 1.6% lighter isotopic composition might reflect a relatively larger contribution of autochthonous detritus to the total detrital particulate organic matter pool than to the dissolved pool. Consistently with this, Wilkinson et al. (2013) reported that a lower contribution of terrestrial organic matter to the particulate pool than to the dissolved organic matter pools in North American lakes.

Allochthonous carbon proxies in eutrophic lakes were more ^{13}C -enriched and variable: $-25.4 \pm 1.1\%$ for $\delta^{13}\text{C}_{\text{DOC}}$ and $-26.6 \pm 4.2\%$ for $\delta^{13}\text{C}_{\text{det}}$. Moreover, $\delta^{13}\text{C}_{\text{DOC}}$ and $\delta^{13}\text{C}_{\text{det}}$ were not significantly correlated. The enrichment in ^{13}C of $\delta^{13}\text{C}_{\text{allo}}$ in eutrophic lakes can be partly explained by land use in the water shed; almost all eutrophic lakes were located in the state of Iowa, where an average of 92% of the land is under periodic cultivation for maize (C_4 plants, -14%). There was more uncertainty in $\delta^{13}\text{C}_{\text{allo}}$ in the eutrophic lakes for two main reasons. First, we expect a substantial autochthonous contribution to DOC and detritus in productive lakes (Bade et al., 2007), which contributes to the larger range in $\delta^{13}\text{C}_{\text{DOC}}$ and $\delta^{13}\text{C}_{\text{det}}$ (Fig. 2). Second, the presence of C_3 and C_4 vegetation with their distinct isotopic compositions can create a variable $\delta^{13}\text{C}_{\text{allo}}$. Variability in $\delta^{13}\text{C}_{\text{allo}}$ has received far less attention than that of aquatic primary producers. Our results show distinct differences in the isotopic composition of external subsidies and argue against a fixed value for allochthonous carbon, especially in areas with abundant C_4 vegetation, such as maize.

4.3 Phytoplankton $\delta^{13}\text{C}$

The determination of $\delta^{13}\text{C}_{\text{phyto}}$ is one of the major challenges in aquatic ecology. Fatty acid biomarkers as proxies for $\delta^{13}\text{C}_{\text{phyto}}$ have the advantage that there is a larger certainty that measured $\delta^{13}\text{C}$ values represent parts of phytoplankton carbon. The main uncertainty using $\delta^{13}\text{C}_{\text{FA}}$ as marker for $\delta^{13}\text{C}_{\text{phyto}}$ comes from the isotopic offset between lipids and total cells ($\Delta\delta^{13}\text{C}_{\text{FA-cell}}$) which depends

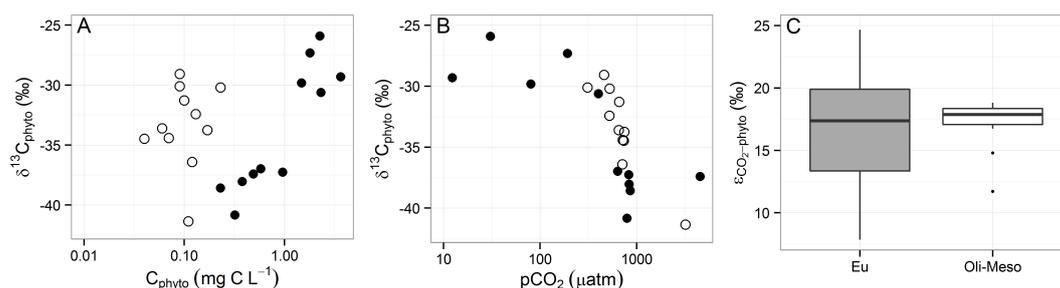


Figure 5. The relation of $\delta^{13}\text{C}_{\text{phyto}}$ in eutrophic lakes (filled circles, $n = 11$) and oligo-mesotrophic lakes (open circles, $n = 11$) to (a) C_{phyto} and (b) $p\text{CO}_2$. Panel (c) presents a box whisker plot of calculated $\varepsilon_{\text{CO}_2\text{-algae}}$ in eutrophic (Eu) and oligo-mesotrophic (Oli-Meso) lakes.

on species composition (summarized in, e.g., Hayes, 2001), growth conditions (e.g. Riebesell et al., 2000) and the FA considered (Fig. 5).

Isotope fractionation between CO_2 and phytoplankton was variable (8–25‰) in our study (Table 3). This implies that calculations of $\delta^{13}\text{C}_{\text{phyto}}$ from $\delta^{13}\text{C}_{\text{CO}_2}$ with a constant fractionation factor provide inaccurate results, consistent with methodological comparisons by Marty and Planas (2008) and McCallister and del Giorgio (2008). The usual value for photosynthetic fractionation in phytoplankton is ~ 20 ‰, based on C_3 photosynthesis (Fry, 2006), but several studies that determined ε in lakes showed that actual fractionation is usually lower than this value (Cole et al., 2002; Bade et al., 2006). Also, in our study, fractionation was lower (~ 17 ‰) on average, and highly variable, especially in eutrophic lakes. There are several explanations for this variability. Actual fractionation has been shown to be dependent on several variables, including growth rate (Bidigare et al., 1997) and CO_2 availability (Laws et al., 1995). Fractionation is highest under high CO_2 availability and low growth rates. In the less productive oligo-mesotrophic lakes, the conditions favour optimal fractionation, and therefore, fractionation was rather constant (Fig. 5c). In the productive, eutrophic lakes, actual fractionation was influenced by $p\text{CO}_2$ and C_{phyto} , with lowest fractionation and therefore most enriched ^{13}C phytoplankton in the most productive (low CO_2 and high C_{phyto}) lakes (Fig. 5a, b).

Two clusters in $\delta^{13}\text{C}_{\text{phyto}}$ were present in the eutrophic lakes (Fig. 5a, b) and the shift occurred when lakes were below $20 \mu\text{mol L}^{-1} \text{CO}_2$ in the eutrophic lakes. When CO_2 becomes limiting, some phytoplankton can also shift to bicarbonate utilization, which is isotopically enriched by ~ 8 ‰ compared to CO_2 . Direct uptake of carbonate and conversion in the carboxysomes is very common in the cyanobacteria that dominate eutrophic lakes (Bontes et al., 2006). The lakes with ^{13}C -enriched phytoplankton had high concentrations of zeaxanthin, a biomarker for cyanobacteria. However, the higher $\delta^{13}\text{C}_{\text{phyto}}$ in high zeaxanthin lakes was not a direct consequence of ^{13}C -enrichment in cyanobacteria. FA that are abundant in cyanobacteria (18:1n-7) were not more enriched than FA that are absent in cyanobacteria; in fact, they

were the most ^{13}C -depleted of all FA (Fig. 4). Cyanobacteria grown in laboratory cultures also showed higher fractionation (up to 9‰) in lipids relative to total biomass than eukaryotic phytoplankton (summarized in Hayes, 2001).

The most ^{13}C -enriched phytoplankton FA was 22:6 ω 3, which is abundant in dinoflagellates (Fig. 4) (Dalsgaard et al., 2003). Dinoflagellates were also more enriched in ^{13}C compared to other phytoplankton in a subtropical lake (Zohary et al., 1994). A possible explanation for ^{13}C -enriched dinoflagellates in field studies, can be their mixotrophic character, so that part of their isotopic composition reflects consumer $\delta^{13}\text{C}$. However, PUFAs of autotrophic dinoflagellates grown in continuous cultures were also more ^{13}C -enriched to C16:0 than PUFAs of other phytoplankton (Schouten et al., 1998).

Finally, variability in $\Delta\delta^{13}\text{C}_{\text{FA-cell}}$ can contribute to the observed variability. In laboratory studies, the offset between lipids and bulk material has been shown to be variable (van Dongen et al., 2002; Fiorini et al., 2010). One can expect that in field studies, with multiple species, however, these cellular variations would probably disappear within broader trends. If we assume an overall mean $\Delta\delta^{13}\text{C}_{\text{FA-cell}}$, then the uncertainty in the actual value would affect the absolute fractionation values, but not the observed variability in fractionation.

4.4 Carbohydrates and lipid $\delta^{13}\text{C}$

The enrichment in ^{13}C of carbohydrates and depletion in ^{13}C of lipids relative to total cells (mainly amino acids) has been shown in culture studies of phytoplankton (Van Dongen et al., 2002) and of several primary producers and consumers (Tece and Fogel, 2007). Results of this study show that the enrichment in ^{13}C in glucose as well as the ^{13}C -depletion in fatty acids relative to bulk material can also be detected in field samples (Fig. 2, Table 3). We observed that $\Delta\delta^{13}\text{C}_{\text{gluc-FA}_{\text{tot}}}$ increased with TP (Table 2), but whether this represents a general phenomenon for lakes needs further exploration.

Bacterial FA were more enriched in ^{13}C than phytoplankton FA in all lakes (Figs. 2, 4). This observation can be explained by differences in carbon source or differences in $\Delta\delta^{13}\text{C}_{\text{FA-cell}}$ between phytoplankton and bacteria.

Carbohydrates, present in high concentrations in DOC, form an important carbon source for bacteria. Since carbohydrates were the most ^{13}C -enriched carbon source, a preferential use of carbohydrates would result in ^{13}C -enriched bacteria (Fig. 2). Another explanation is that isotope fractionation during FA synthesis was smaller in bacteria compared to phytoplankton. There are no field studies on $\Delta\delta^{13}\text{C}_{\text{FA-cell}}$ in freshwater bacteria, but field studies on sediment and marine bacteria report a range of 0–5‰ in $\Delta\delta^{13}\text{C}_{\text{FA-cell}}$ (Hayes, 2001; Burke et al., 2003; Bouillon and Boschker, 2006). Burke et al. (2003) suggested that in field samples with complex communities and substrates, $\Delta\delta^{13}\text{C}_{\text{FA-cell}}$ would be $\sim 0\%$. The results of our study support this idea, since bacterial FA had a similar $\delta^{13}\text{C}$ as POC. If a similar $\Delta\delta^{13}\text{C}_{\text{FA-cell}}$ for phytoplankton and bacteria were used, bacteria would be more enriched in ^{13}C than its potential carbon sources in half of the studied lakes, which is rather unlikely.

5 Conclusions

Our results show that trophic state has a large influence on lake metabolism and carbon cycling in plankton food webs. Overall, eutrophic lakes had larger variability in $\delta^{13}\text{C}$ in all organic carbon pools than oligo-mesotrophic lakes, caused by larger isotopic variability in the base of the food web in eutrophic lakes (both allochthonous and autochthonous carbon). In eutrophic lakes, $\delta^{13}\text{C}_{\text{phyto}}$ showed that two clusters of phytoplankton were present, with the most ^{13}C -enriched phytoplankton at high CO_2 and high chl *a*. Dominance of cyanobacteria played a role, but enrichment in ^{13}C was present in all phytoplankton, as seen in specific PLFAs.

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