1   **Stable carbon isotope biogeochemistry of lakes along a trophic gradient**

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3   Anna de Kluijver[1*], Petra L. Schoon[2], John A. Downing[3], Stefan Schouten[2,4], and Jack J.

4   Middelburg[1,4]

5

6   [1]Department of Ecosystems Studies, NIOZ Royal Netherlands Institute for Sea Research,

7   Yerseke, the Netherlands

8   [2]Department of Marine Organic Biogeochemistry, NIOZ Royal Netherlands Institute for Sea

9   Research, Den Burg, the Netherlands

10   [3]Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames,

11   Iowa, USA

12   [4]Utrecht University, Faculty of Geosciences, Utrecht, the Netherlands

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14   [*] present address corresponding author: Deltares, Delft, The Netherlands; e-mail:

15   Anna.deKluijver@deltares.nl

Abstract

16

17   The stable carbon (C) isotope variability of dissolved inorganic and organic C (DIC and

18   DOC), particulate organic carbon (POC), glucose and polar-lipid derived fatty acids (PLFA)

19   were studied in a survey of 22 North American oligotrophic to eutrophic lakes. The δ^{13}C of

20   different PLFA were used as proxy for phytoplankton producers and bacterial consumers. Lake

21   pCO_2 was primarily determined by autochthonous production (phytoplankton biomass),

22   especially in eutrophic lakes, and governed the δ^{13}C of DIC. All organic-carbon pools showed

23   larger isotopic variability in eutrophic lakes compared to oligo-mesotrophic lakes because of the

24   high variability in δ^{13}C at the base of the food web (both autochthonous and allochthonous

25   carbon). Phytoplankton δ^{13}C was negatively related to lake pCO_2 over all lakes and positively

26   related to phytoplankton biomass in eutrophic lakes, which was also reflected in a large range in

27   photosynthetic isotope fractionation ($\varepsilon_{CO_2-phyto}$, 8-25 ‰). The carbon isotope ratio of

28   allochthonous carbon in oligo-mesotrophic lakes was rather constant, while it varied in eutrophic

29   lakes because of maize cultivation in the watershed.

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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 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 studying inorganic and organic carbon pools and changes therein. It can provide information on the metabolic balance and the sources of organic matter fueling respiration. Respiration yields ^{13}C-depleted carbon dioxide from organic matter with the result that δ^{13}C of dissolved inorganic carbon 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 dissolved inorganic carbon will be enriched in ^{13}C (Herczeg, 1987; Parker et al., 2010). The δ^{13}C of the dissolved inorganic carbon pool thus integrates the relative importance of respiration and primary production (Parker et al., 2010). The δ^{13}C of organic carbon pools is primarily governed by the δ^{13}C of the dissolved inorganic carbon used by primary producers and the isotope fractionation during carbon fixation. Terrestrial plants use atmospheric carbon dioxide while aquatic primary producers utilize dissolved carbon dioxide or bicarbonate (Fry, 2006). The δ^{13}C of terrestrial-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).

61 Therefore, most studies use indirect methods to determine $\delta^{13}C$ of phytoplankton ($\delta^{13}C_{phyto}$),

62 bacteria ($\delta^{13}C_{bac}$) and allochthonous carbon ($\delta^{13}C_{allo}$). Common methods for determining $\delta^{13}C_{phyto}$

63 are the use of the $\delta^{13}C$ of particulate organic carbon (POC) with correction for non-

64 phytoplankton carbon and estimates based on $\delta^{13}C$ of dissolved inorganic carbon (DIC) with an

65 isotope fractionation factor (ε), obtained from experimental studies. Other methods are the use of

66 zooplankton consumers as a proxy for $\delta^{13}C_{phyto}$ or size fractionation of organic matter and

67 subsequent determination of $\delta^{13}C$ of different size classes (Marty and Planas, 2008).

68   Isotopic ratios of bacteria in field studies have been derived from re-growing bacteria in

69 bioassays (Coffin et al., 1989) or dialysis cultures (Kritzberg et al., 2004), with measurement of

70 ^{13}C in POC or respired CO_2 (McCallister et al., 2008) and from biomarkers like nucleic acids

71 (Coffin et al., 1990) and lipids (Bontes et al., 2006; Pace et al., 2007). Some studies used $\delta^{13}C$ of

72 DOC as proxy for $\delta^{13}C$ of bacteria, assuming that DOC was the primary carbon source for

73 bacteria (Taipale et al., 2008; Zigah et al., 2012).

74   A commonly used proxy for allochthonous $\delta^{13}C$ is the $\delta^{13}C$ of terrestrial C_3 plants, which

75 dominates most terrestrial vegetation and has a $\delta^{13}C$ of ~-28 ‰ (Fry, 2006; Kohn, 2010). When

76 vegetation is dominated by C_4 plants, however, common in tropical areas and agricultural areas

77 with maize production ($\delta^{13}C$ of ~-14‰; Fry, 2006), the isotopic composition of allochthonous

78 carbon can be significantly enriched. In lakes with large terrestrial input, $\delta^{13}C$ of DOC can be

79 used as a proxy for $\delta^{13}C_{allo}$, since terrestrial carbon forms the largest fraction of DOC (Kritzberg

80 et al., 2004; Wilkinson et al., 2013).

81   Compound specific isotope analysis (CSIA) of polar lipid-derived fatty acids (PLFA)

82 biomarkers has shown to be a valuable tool to determine the isotopic composition of plankton

83 producers and consumers (Boschker and Middelburg, 2002). Groups of phytoplankton and

84 bacteria have different fatty acid (FA) compositions, so by analyzing the $\delta^{13}C$ of specific FA, the

85 $\delta^{13}C$ of phytoplankton and bacteria can be inferred. The combined use of stable isotopes and FA

86 biomarkers has been successfully applied to study autochthonous and allochthonous carbon

87 contributions to zooplankton in a tidal river (Van den Meersche et al., 2009). Few studies have

88 applied CSIA to study carbon flows in plankton food webs in lakes. Examples are a

89 phytoplankton-zooplankton interaction study in a eutrophic lake (Pel et al., 2003), a

90 biomanipulation effect study (Bontes et al., 2006), a ^{13}C lake enrichment study (Pace et al.,

91 2007), and a cyanobacteria-zooplankton interaction study (de Kluijver et al., 2012).

92   In this study, we used compound-specific isotope analyses to examine carbon flows in

93 plankton food webs in temperate (North American) lakes. The lake survey encompassed a range

94 in trophic states from oligotrophic lakes, with an expected dominance of allochthonous input, to

95 eutrophic lakes, with an expected lower allochthonous input. In this trophic range, we explored

96 patterns of isotopic variability in dissolved inorganic and organic carbon, particulate organic

97 carbon and carbohydrates, phytoplankton, allochthonous carbon, heterotrophic bacteria and their

98 relationships.

99

2. Material and Methods

2.1 Site description

102   The 22 lakes sampled in this study are located in Iowa and Itasca County in Minnesota,

103 USA. Iowa lakes are mostly man-made and situated in a highly agricultural region, with maize

104 and soya beans as main products. This type of row-crop agriculture has a large impact on the

105 nutrient load of the lake watershed (Arbuckle and Downing, 2001). However, Itasca lakes are

106 natural and situated in a highly forested area. The catchment areas have developed since the last

107 glaciation episode ca. 12,000 years ago and consist of carbonate-poor glacial deposits (till)

108 (Grimley, 2000).

2.2 Field sampling

110   The lakes were sampled in July -August 2009 as part of the ongoing lake monitoring

111 program of the limnology laboratories of Iowa State University and Itasca Community College.

112 Key parameters, such as temperature, pH, Secchi transparency, oxygen, inorganic nutrients and

113 carbon concentrations were measured as part of and according to the lake monitoring program.

114 All samples were taken from up to 2 m of the upper mixed zone at the deepest point of each lake.

115 Water samples were taken between 10h-16h, a period of the day that yields relatively stable

116 water chemistry readings in these lakes. More information on data collection, lake

117 characteristics, and methods can be found on http://limnoweb.eeob.iastate.edu/itascalakes and

118   http://limnology.eeob.iastate.edu/lakereport. All nutrients were analyzed using certified methods

119   and strict quality assurance procedures.

120   Triplicate water samples were taken for stable isotope analyses and concentrations of the

121   major carbon pools. Headspace vials (20 ml and 2 ml) were filled on board with sampled water

122   using the overflow method and sealed with gas-tight caps for DIC isotope analyses and

123   concentrations, respectively. Mercury chloride was added for preservation and the samples were

124   stored upside-down at room temperature. For DOC analyses, 20 ml of sampled water was filtered

125   over GF/F (0.7 µm pore size, 25 mm diameter) and stored frozen in clean (acid and milli-Q

126   rinsed) vials until further analysis.

127   Seston samples for particulate organic carbon (POC) and carbohydrates were collected by

128   filtering 0.4 to 1 L of sampled water on pre-weighed and pre-combusted GF/F filters (0.7 µm

129   pore size, 47 mm diameter), which were subsequently dried at 60° for POC analysis or freeze-

130   dried for carbohydrates; PLFA samples were collected by filtering ~2 L sampled water on pre-

131   combusted GF/F filters (0.7 µm, 47 mm) and filters were stored frozen. Pigment samples were

132   taken for concentrations only and collected by filtering ~600 ml sampled water on GF/F filters

133   (0.7 µm, 47 mm) in the dark and filters were stored frozen.

2.3 Laboratory analyses

135   POC samples were analyzed for carbon content and isotope ratios on a Thermo Electron

136   Flash EA 1112 analyzer (EA) coupled to a Delta V isotope ratio mass spectrometer (IRMS) (c.f.

137   Nieuwenhuize et al., 1994). For DIC isotope analyses, a helium headspace was created in the

138   headspace vials and samples were acidified with H_3PO_4 solution. After equilibration, the CO_2

139   concentration and isotope ratio in the headspace was measured using EA-IRMS (Gilikin and

140   Bouillon, 2007). DIC concentrations were measured using spectrophotometry according to Stoll

141   et al., (2001). For DOC analyses, the samples were acidified and flushed with helium to remove

142   DIC and subsequently oxidized with sodium persulfate ($Na_2S_2O_8$); the isotope ratio and

143   concentration of CO_2 resulting from this treatment was measured using high performance liquid

144   chromatography - isotope ratio mass spectrometry (HPLC-IRMS) (Boschker et al. 2008). PLFA

145   samples were extracted according to a modified Bligh and Dyer method (Bligh and Dyer, 1959,

146   Middelburg et al., 2000). The lipids were fractionated in different polarity classes by column

147 separation on a heat-activated silic acid column and subsequent elution with chloroform, acetone,

148 and methanol. The methanol fractions, containing most of the PLFA, were collected and

149 transformed to fatty acid methyl esters (FAME) using methanolic NaOH. The 12:0 and 19:0

150 FAME were added as internal standards. Concentrations and δ^{13}C of individual PLFA were

151 measured using gas chromatography-combustion isotope ratio mass spectrometry (GC-C-IRMS)

152 (Middelburg et al., 2000). The isotopic compositions were corrected for the carbon added during

153 derivatization. Pigment samples were extracted with 90% acetone in purified (miliQ) water with

154 intense shaking. Concentrations of individual pigments were measured on HPLC (Wright et al.

155 1991). Carbohydrate samples were hydrolyzed in H_2SO_4, neutralized with $SrCO_3$, and

156 precipitated with $BaSO_4$. The supernatant was collected and measured using HPLC-IRMS

157 according to Boschker et al., (2008).

158 **2.4 Data analyses**

159   The lakes were divided into eutrophic and oligo-mesotrophic lakes based on average

160 summer total phosphorus (TP) concentrations. Lakes with TP values >24 µg L^{-1} and a

161 corresponding trophic state index >50 were classified as eutrophic, and lakes with TP values <

162 24 µg L^{-1} as oligo-mesotrophic (Carlson 1977). All lakes in Iowa and one lake in Minnesota

163 were classified as eutrophic, while all oligo-mesotrophic lakes were located in Minnesota.

164 **2.4.1 CO_2 system and isotopic composition of CO_2**

165   The different components of the CO_2 system were calculated from temperature,

166 laboratory pH, and DIC concentrations using a salinity of 0 using the R package AquaEnv

167 (Hofmann et al., 2010). Stable isotope ratios were expressed in the delta notation (δ^{13}C), which is

168 the $^{13}C/^{12}C$ ratio relative to VPDB standard, in part per thousand (‰). The isotope ratio of CO_2

169 (aq) $(\delta^{13}C_{CO_2})$ was calculated from $\delta^{13}C_{DIC}$ according to Zhang et al., (1995):

170 $$\delta^{13}C_{CO_2} = \delta^{13}C_{DIC} - 0.0144 \times T(^{\circ}C) \times fCO_3^{2-} + 0.107 \times T(^{\circ}C) - 10.53 \qquad (1)$$

171 where fCO_3^{2-} is the fraction of CO_3^{2-} in total DIC, calculated from pH and DIC concentrations.

172 **2.4.2 δ^{13}C of phytoplankton and bacteria**

173   Poly-unsaturated fatty acids (PUFA) are abundant in most phytoplankton, and can

174   generally be used as chemotaxonomic markers for this group (Dijkman and Kromkamp, 2006).

175   The most abundant PUFA in all lakes were 18:3ω3 (α-linolenic acid), 18:4ω3 (stearidonic acid,

176   SDA), 20:5ω3 (icosapentaenoic acid, EPA), 22:6ω3 (docosahexaenoic acid, DHA) and 20:4ω6

177   (arachidonic acid, ARA), common PUFA's in freshwater phytoplankton (Taipale et al., 2013),

178   and their concentration-weighted $\delta^{13}C$ were used to determine phytoplankton isotope ratios

179   ($\delta^{13}C_{phyto}$). Note that phytoplankton is considered a mixture of eukaryotic algae and

180   cyanobacteria. Branched fatty acids (BFA) are abundant in heterotrophic bacteria (Kaneda,

181   1991) in contrast to phytoplankton. The most abundant BFA were i15:0, ai15:0 and i17:0 and

182   their weighted $\delta^{13}C$ were used as a proxy for heterotrophic bacteria isotope ratios ($\delta^{13}C_{bac}$),

183   which we further consider bacteria. Isotope fractionation (ε) between CO_2 and phytoplankton

184   was calculated as

185
$$\varepsilon_{CO_2-phyto}(‰) = \frac{\delta^{13}C_{CO_2} - \delta^{13}C_{phyto\_cor}}{1 + \delta^{13}C_{phyto\_cor}/1000} \qquad (2)$$

186        $\delta^{13}C_{phyto\_cor}$ was derived from $\delta^{13}C_{phyto}$ with a correction of +3‰ for the isotopic offset between

187   PUFA and total cells ($\Delta\delta^{13}C_{FA-cell}$) (Schouten et al., 1998; Hayes, 2001), although this isotopic

188   offset can be highly variable (Schouten et al. 1998).

189   **2.4.3 $\delta^{13}C$ of allochthonous carbon**

190   Allochthonous organic carbon ($\delta^{13}C_{allo}$), i.e. organic matter delivered to lakes as DOC or

191   POC, cannot be measured directly as such and we therefore used two proxies: the measured

192   isotopic ratios of DOC ($\delta^{13}C_{DOC}$) and calculated isotopic composition of particulate detritus

193   ($\delta^{13}C_{det}$). The latter was calculated from a mass balance and mixing model, similar to Marty and

194   Planas, (2008), amended with zooplankton and bacteria. We assumed that POC consists of

195   phytoplankton, detritus, bacteria, and zooplankton, and that the $\delta^{13}C$ of POC represents a mixture

196   of the weighted $\delta^{13}C$ of the individual groups. Subsequently, $\delta^{13}C_{det}$ in each lake was derived

197   from $\delta^{13}C_{POC}$:

198        $\delta^{13}C_{det}(‰) = (POC \times \delta^{13}C_{POC} - C_{phyto} \times \delta^{13}C_{phyto\_cor} - C_{bac} \times \delta^{13}C_{bac} - C_{zoo} \times \delta^{13}C_{zoo}) / C_{det}$ (3)

199        $C_{det}(mg\ C\ L^{-1}) = POC - C_{phyto} - C_{bac} - C_{zoo}$ (4)

200 The latter equation simply states that detrital organic matter is the non-living part of total

201 particulate organic matter pool.

202   Phytoplankton carbon (C_{phyto}) (mg C L^{-1}) was calculated as the average of biomass

203 estimates based on chl a concentration (C: Chl a = 50) as well as phytoplankton FA derived

204 biomass, to minimize the error associated with each method. Phytoplankton FA biomass was

205 calculated from the sum of phytoplankton PLFA ($\sum$ 18:3ω3, 18:4ω3, 20:5ω3, 22:6ω3, and

206 20:4ω6) and a C: specific FA ratio of 60 based on culture studies, summarized in Dijkman and

207 Kromkamp (2006). The two approaches yielded similar results. Bacterial carbon (C_{bac}) (mg C L^{-1})

208 was calculated from the summed concentrations of bacteria specific FA (i15:0, ai15:0, and

209 i17:0) and a C_{bac}: FA ratio of 50 (Middelburg et al., 2000). Zooplankton carbon (C_{zoo}) used in

210 equation 3 was estimated to be ~10 % of C_{phyto} (Del Giorgio and Gasol, 1995) and zooplankton

211 δ^{13}C are based on de Kluijver (2012). Uncertainties in δ^{13}C and biomass of phytoplankton,

212 bacteria and zooplankton were not considered in calculating $\delta^{13}C_{det}$.

213 **2.4.4 Statistical analyses**

214   Data that were part of the lake monitoring program and pCO_2 values represent single

215 samples of each lake. Data on carbon concentrations and isotopic compositionss in each lake

216 convey averages of triplicate samples. Statistical analyses were done with software package "R"

217 (R development core team, 2013). Prior to correlation analyses, data were checked for normal

218 distribution (Shapiro test) and log-transformed when necessary to achieve normal distribution.

219 Correlation coefficients were calculated using Pearson product-moment correlation coefficient

220 (normal distribution) or Spearman's rank correlation coefficient (non-normal distribution). For

221 reasons of instructiveness we present the average ± sd values for eutrophic lakes (n=11) and

222 oligo-mesotrophic lakes (n=11), but we do realize that any division based on a concentration is

223 somewhat arbitrary. The correlations were tested for total lakes (n=22) and for eutrophic lakes

224 (n=11) and oligo-mesotrophic lakes (n=11). Differences between eutrophic and oligo-

225 mesotrophic lakes were statistically tested using Student's t-tests.

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3. Results

3.1 Lake chemistry

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230 The sampled lakes covered a large range of nutrients and CO_2 system characteristics
231 (Table 1). DIC values ranged from 0.052 to 4.6 mmol L^{-1}, alkalinity values ranged from 0.07 to
232 2.4 mmol L^{-1} and pH ranged from 6.1 to 9.8 (Table 1). The calculated pCO_2 values were in the
233 range from 10-4500 µatm, covering a broad range from under- to super-saturation. The CO_2
234 system in eutrophic and oligo-mesotrophic lakes was clearly different. On average, the eutrophic
235 lakes had higher DIC, alkalinity, and pH than the oligo-mesotrophic lakes (Fig. 1, Table 1). In
236 the eutrophic lakes, there were positive correlations between alkalinity and DIC and pCO_2 values
237 (Fig. 1A, Table 2) and a negative correlation between pH and pCO_2 (Fig. 1B, Table 2). The
238 pCO_2 values were not related to pH, alkalinity, or DIC in the oligo-mesotrophic lakes. Both lake
239 systems showed super-saturation (average pCO_2 838 µatm in both), but the pCO_2 range was
240 much larger in eutrophic lakes (10-4500 µatm) compared to oligo-mesotrophic lakes (310-3200
241 µatm) (Fig. 1, Table 1).

3.2 Organic carbon and fatty acid concentrations

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243 POC, C_{phyto} and C_{bac} concentrations were higher and DOC concentrations were lower in
244 the eutrophic lakes compared to the oligo-mesotrophic lakes (Table 1). C_{phyto} was on average 1.3
245 $\pm$ 1.1 mg C L^{-1} and 0.11 $\pm$ 0.03 mg C L^{-1}, corresponding to 44 % $\pm$ 17 % and 10 % $\pm$ 5 % of POC
246 in eutrophic and oligo-mesotrophic lakes, respectively. C_{phyto} and C_{bac} were significantly related
247 to TP (Table 2), but not to total nitrogen (TN) concentration. Average C_{bac} was 0.11 $\pm$ 0.08 mg C
248 L^{-1} and 0.021 $\pm$ 0.017 mg C L^{-1} in eutrophic and oligo-mesotrophic lakes, respectively.

249 Overall, lake pCO_2 decreased with increasing C_{phyto}, but the effect was strongest in
250 eutrophic lakes (Fig. 1C, Table 2). In the oligo-mesotrophic lakes, pCO_2 increased with
251 increasing DOC (Fig. 1D, Table 2), but this effect was caused by one point: the high pCO_2 at
252 high DOC in lake Sturgeon. In the eutrophic lakes, DOC concentrations were lower compared to
253 the oligo-mesotrophic lakes and did not act on lake pCO_2 (Fig. 1D, Table 1).

3.3 δ^{13}C of DIC and CO_2

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255   The isotope ratios of the major carbon pools in each lake are presented in Table 3 and in

256   boxplots (median and percentiles) in Figure 2. $\delta^{13}C_{DIC}$ ranged from -9.3 to +1.5 ‰ and $\delta^{13}C_{CO_2}$

257   (derived from $\delta^{13}C_{DIC}$) was on average 10.9 ± 0.3 ‰ depleted relative to DIC, with a range of -

258   20.8 to -8.9 ‰. $\delta^{13}C_{DIC}$ and $\delta^{13}C_{CO_2}$ showed no correlation with alkalinity, DIC, pH, temperature

259   and lake area. A weak negative relation between pCO_2 and $\delta^{13}C_{DIC}$ was observed, which was

260   stronger in oligo-mesotrophic lakes than in eutrophic lakes (Fig. 3A, Table 2). The highest pCO_2

261   lakes had the most depleted $\delta^{13}C_{DIC}$, suggesting that respiration of organic matter influenced

262    $\delta^{13}C_{DIC}$. Low CO_2 lakes had enriched $\delta^{13}C_{DIC}$, indicating influence of primary production. Weak,

263   but significant relations were observed for POC and DOC with $\delta^{13}C_{DIC}$ (Table 2). In eutrophic

264   lakes, $\delta^{13}C_{DIC}$ increased with increasing POC (Fig. 3B, Table 2), while in oligo-mesotrophic

265   lakes, $\delta^{13}C_{DIC}$ decreased with increasing DOC (Fig. 3C, Table 2).

266   **3.4 $\delta^{13}C$ of organic carbon pools**

267   The isotopic composition of DOC ($\delta^{13}C_{DOC}$) had the narrowest range of all carbon pools,

268   only -28.8 to -27.0 ‰ (mean -28.0 ‰) in the oligo-mesotrophic lakes and a slightly larger range

269   of -27.6 to -23.7 ‰ (mean -25.4 ‰) in the eutrophic lakes (Fig. 2, Table 3). The $\delta^{13}C$ isotopic

270   range of POC ($\delta^{13}C_{POC}$) was larger than of DOC in both lake types and on average 2.0 ‰

271   depleted compared to $\delta^{13}C_{DOC}$, with mean values of -27.8 ± 3.6 ‰ in eutrophic and -29.7 ± 2.8

272   ‰ in oligo-mesotrophic lakes (Fig. 2, Table 3). $\delta^{13}C$ of particulate glucose ($\delta^{13}C_{gluc}$), the most

273   abundant carbohydrate, was always enriched compared to $\delta^{13}C_{POC}$ and the enrichment was

274   similar in eutrophic (3.1 ± 1.7 ‰) and oligo-mesotrophic lakes (2.8 ± 1.5 ‰) (Fig. 2). On the

275   contrary, the concentration-weighted average $\delta^{13}C$ of all fatty acids ($\delta^{13}C_{FA_{tot}}$) was always

276   depleted compared to $\delta^{13}C_{POC}$ (Fig. 2). The depletion of $\delta^{13}C_{FA_{tot}}$ relative to POC was higher in

277   eutrophic lakes (5.2 ± 1.8 ‰) than in oligo-mesotrophic lakes (3.1 ± 1.1 ‰). The isotopic

278   difference between glucose and $\delta^{13}C_{FA_{tot}}$: $\Delta\delta^{13}C_{gluc-FA_{tot}}$ was highly variable with a range of 1.6 to

279   14.6 ‰. The isotopic differences between glucose and $\delta^{13}C_{FA_{tot}}$ did not correlate with CO_2, but

280   weakly with nutrient levels, i.e., $\Delta\delta^{13}C_{gluc-FA_{tot}}$ increased with increasing TP (Table 2).

281   There was a large variability among $\delta^{13}C$ of different FA with some consistent

282   differences over all lakes. Compared to the $\delta^{13}C$ of 16:0 (the most abundant FA), the bacterial

283   FA markers were always enriched by 1.4 - 5.0 ‰ (e.g., the *iso*-15:0 FA in Fig. 4), therefore the

284   overall $\delta^{13}C$ of bacterial FA ($\delta^{13}C_{bac}$) was more enriched than $\delta^{13}C_{FA_{tot}}$ in both lake systems (Fig.

285   2). The poly-unsaturated fatty acids (PUFA) used as markers for phytoplankton showed

286   consistent differences throughout the lakes. DHA (22:6ω3), common in dinoflagellates

287   (Dalsgaard et al. 2003), was found to be enriched with 4.6 ‰ compared to 16:0 while PLFA

288   18:3ω3 (α-linolenic acid), common in cyanobacteria (de Kluijver et al., 2012), was 4.7 ‰

289   depleted compared to 16:0 (Fig. 4). The other phytoplankton markers were not statistically

290   different from 16:0. The weighted $\delta^{13}C$ of phytoplankton FA ($\delta^{13}C_{phyto}$) was the most depleted of

291   all carbon pools (Fig. 2, Table 3) with an average of -33.8 ± 5.3 ‰ in eutrophic lakes and -33.4 ±

292   3.5 ‰ in oligo-mesotrophic lakes. $\delta^{13}C_{bac}$ were on average 4.7 ‰ enriched compared to $\delta^{13}C_{phyto}$

293   (Fig. 2).

3.5 Carbon isotopic composition of phytoplankton

295          $\delta^{13}C_{phyto}$ depends on the isotopic composition of substrate ($\delta^{13}C_{CO_2}$), the isotope

296   fractionation ($\varepsilon_{CO_2-phyto}$) associated with primary production and the isotopic difference between

297   PUFA's and biomass. $\delta^{13}C_{phyto}$ in the eutrophic lakes became more enriched with increasing

298   C_{phyto} (Fig. 5A, Table 2) and decreasing pCO_2 (Fig. 5B, Table 2). No relation between $\delta^{13}C_{phyto}$

299   and C_{phyto} was observed in the oligo-mesotrophic lakes (Fig. 5A), but there was a strong negative

300   relation with pCO_2 (Fig. 5B, Table 2). The influence of C_{phyto} on $\delta^{13}C_{phyto}$ in the eutrophic lakes

301   was also reflected in fractionation; $\varepsilon_{CO_2-phyto}$ was highly variable in eutrophic lakes, while it was

302   less variable in oligotrophic lakes (Fig. 5C). The range of $\varepsilon_{CO_2-phyto}$ was 7.8 to 24.7 ‰ (mean 16.9

303   ‰) in eutrophic and 11.7 to 18.8 ‰ (mean 17.1 ‰) in oligo-mesotrophic lakes, when

304    $\delta^{13}C_{phyto\_cor}$ was used (Table 3). The less variable ε in oligo-mesotrophic lakes resulted in a

305   strong correlation between $\delta^{13}C_{CO_2}$ and $\delta^{13}C_{phyto}$ (Table 2), which was absent in the eutrophic

306   lakes. $\varepsilon_{CO_2-phyto}$ correlated negatively with C_{phyto} in eutrophic lakes, however (Table 2). The

307   variability in $\delta^{13}C_{phyto}$ in eutrophic lakes can be mainly attributed to the presence of two clusters:

308   a ^{13}C-enriched cluster at the highest C_{phyto} and a depleted cluster at lower C_{phyto} (Fig. 5A).

309   Interestingly, the eutrophic lakes within the enriched cluster also had high concentrations of

310   zeaxanthin, a marker pigment for cyanobacteria (data not shown here).

4. Discussion

4.1 Lake metabolism, pCO_2 and $\delta^{13}C_{DIC}$

In our study, about 3/4 of the lakes were supersaturated with pCO_2, consistent with the literature that lakes generally emit carbon dioxide to the atmosphere (Cole et al., 1994, Cole et al., 2007). This CO_2 excess can be due to in-lake respiration of terrestrially derived organic carbon outbalancing carbon dioxide 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 $\delta^{13}C_{DIC}$ dynamics. Previous studies have shown that $\delta^{13}C$ of DIC in lakes is driven by carbonate chemistry, hydrology (i.e., groundwater inflow), and metabolic activity (Bade et al., 2004). Primary production enriches $\delta^{13}C_{DIC}$ because of the preferential uptake of ^{12}C (isotope fractionation), while organic matter respiration depletes $\delta^{13}C_{DIC}$ (Fry, 2006).

If pCO_2 and $\delta^{13}C_{DIC}$ would have been only or primarily controlled by the balance between respiration and production of organic matter, one would expect a tight correlation between $\delta^{13}C_{DIC}$ and pCO_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 $\delta^{13}C_{DIC}$ (Bade et al., 2004). Moreover, carbon dioxide water-air exchange reactions may have modified $\delta^{13}C_{DIC}$ 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 $\delta^{13}C_{DIC}$ and pCO_2 in oligo-mesotrophic lakes was stronger (Table 2) and pCO_2 was highest and $\delta^{13}C_{DIC}$ was most depleted at the highest DOC in oligo-mesotrophic lakes (Fig. 1C, 3C). Such a depletion of $\delta^{13}C_{DIC}$ 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 $\delta^{13}C_{DIC}$ (Jones et al. 1999). However, anoxic hypolimnia are rare in these lakes, either due to low nutrients or polymixis, indicating that methanogenesis was not of major importance in the lakes investigated. Furthermore, we

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339   examined the $\delta^{13}C$ of fatty acids abundant in or specific to methanotrophs and these were not

340   more depleted in ^{13}C than other fatty acids.

341   Note that the lakes were only sampled at one point location and depth, representing

342   average conditions, so spatial variability per lake is not taken into account. Also diurnal variation

343   and variation over the year in each lake are not considered in this study. However, the aim of our

344   study is comparing snapshots of different lakes representing different metabolic states and not to

345   describe the biogeochemistry of each individual lake. So, although we miss some variation, this

346   shouldn't affect our main findings on carbon isotope biogeochemistry of these lakes.

347   **4.2 Allochthonous $\delta^{13}C$**

348   Dissolved and particulate organic carbon pools are mixtures of organic matter from

349   various sources with potentially different stable carbon isotopic compositions. The particulate

350   organic carbon pool comprises biomass from living organisms and remains from organisms

351   within the lake as well as allochthonous detritus. The relative importance of living biomass to

352   total POC pool, calculated based on equation 4, varies from 5.7 to 93 % (Table 1), with on

353   average about 53 ± 20 % in eutrophic lakes and only 13 ± 5 % in in oligo-mesotrophic lakes. In

354   our study we have explicit carbon isotope data for the most important living compartments

355   (algae, bacteria and zooplankton; De Kluijver, 2012), but we have no direct measurement of the

356    $\delta^{13}C$ of organic carbon delivered from the watershed via atmospheric, riverine and groundwater

357   inputs. We have therefore used two proxies for $\delta^{13}C_{allo}$; the carbon isotope ratio of dissolved

358   organic carbon and that of detrital particulate organic carbon calculated by difference (equations

359   3, 4). Both proxies for $\delta^{13}C_{allo}$ provide an estimate for the total detrital pool, i.e. the sum of

360   aquatic and terrestrial detritus.

361   The oligo-mesotrophic lakes are surrounded by forest (C_3 vegetation) and $\delta^{13}C_{DOC}$ was -

362   28.0 ± 0.5 ‰, corresponding to a C_3 vegetation signal, suggesting that the dissolved organic

363   carbon pool is dominantly terrestrially derived, consistent with a combined carbon and hydrogen

364   isotope study of Wisconsin and Michigan lakes by Wilkinson et al., (2013). The other proxy for

365   allochthonous carbon, $\delta^{13}C_{det,}$ was slightly more negative (-29.6 ± 2.1 ‰), but the two proxies

366   for allochthonous carbon were well correlated (Table 2). The 1.6 ‰ lighter isotopic composition

367   might reflect a relatively larger contribution of autochthonous detritus to the total detrital

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368   particulate organic matter pool than to the dissolved pool. Consistently, Wilkinson et al., (2013)

369   reported that a lower contribution of terrestrial organic matter to the particulate pool than to the

370   dissolved organic matter pools in North American lakes.

371   Allochthonous carbon proxies in eutrophic lakes were more enriched and variable: -25.4

372   ± 1.1 ‰ for $\delta^{13}C_{DOC}$ and -26.6 ± 4.2 ‰ for $\delta^{13}C_{det}$. Moreover, $\delta^{13}C_{DOC}$ and $\delta^{13}C_{det}$ were not

373   significantly correlated. The enrichment of $\delta^{13}C_{allo}$ in eutrophic lakes can be partly explained by

374   land use in the water shed; almost all eutrophic lakes were located in the state of Iowa, where an

375   average of 92% of the land is under periodic cultivation for maize (C_4 plants, -14 ‰). There was

376   more uncertainty in $\delta^{13}C_{allo}$ in the eutrophic lakes for two main reasons. First, we expect a

377   substantial autochthonous contribution to DOC and detritus in productive lakes (Bade et al.,

378   2007), which contributes to the larger range in $\delta^{13}C_{DOC}$ and $\delta^{13}C_{det}$ (Fig. 2). Second, the presence

379   of C_3 and C_4 vegetation with their distinct isotopic compositions can create a variable $\delta^{13}C_{allo}$.

380   Variability in $\delta^{13}C_{allo}$ has received far less attention than that of aquatic primary producers. Our

381   results show distinct differences in the isotopic composition of external subsidies and argue

382   against a fixed value for allochthonous carbon, especially in areas with abundant C_4 vegetation,

383   such as maize.

384   **4.3 Phytoplankton $\delta^{13}C$**

385   The determination of $\delta^{13}C_{phyto}$ is one of the major challenges in aquatic ecology. Fatty

386   acid biomarkers as proxies for $\delta^{13}C_{phyto}$ have the advantage that there is a larger certainty that

387   measured $\delta^{13}C$ values represent parts of phytoplankton carbon. The main uncertainty using

388   $\delta^{13}C_{FA}$ as marker for $\delta^{13}C_{phyto}$ comes from the isotopic offset between lipids and total cells

389   $(\Delta\delta^{13}C_{FA-cell})$ which depends on species composition (summarized in, e.g., Hayes, 2001), growth

390   conditions (e.g., Riebesell et al., 2000) and the FA considered (Fig. 5).

391   Isotope fractionation between CO_2 and phytoplankton was variable (8 – 25 ‰) in our

392   study (Table 3). This implies that calculations of $\delta^{13}C_{phyto}$ from $\delta^{13}C_{CO_2}$ with a constant

393   fractionation factor provides inaccurate results, consistent with methodological comparisons by

394   Marty and Planas, (2008) and McCallister et al., (2008). The usual value for photosynthetic

395   fractionation in phytoplankton is ~20 ‰, based on C_3 photosynthesis (Fry, 2006), but several

396   studies that determined ε in lakes showed that actual fractionation is usually lower than this value

397 (Cole et al., 2002; Bade et al., 2006). Also, in our study, fractionation was lower (~17 ‰) on

398 average, and highly variable, especially in eutrophic lakes. There are several explanations for this

399 variability. 1) Actual fractionation has been shown to be dependent on several variables,

400 including growth rate (Bidigare et al., 1997) and CO_2 availability (Laws et al., 1995).

401 Fractionation is highest under high CO_2 availability and low growth rates. In the less productive

402 oligo-mesotrophic lakes, the conditions favor optimal fractionation, and therefore, fractionation

403 was rather constant (Fig. 5C). In the productive, eutrophic lakes, actual fractionation was

404 influenced by pCO_2 and C_{phyto}, with lowest fractionation and therefore most enriched ^{13}C

405 phytoplankton in the most productive (low CO_2 and high C_{phyto}) lakes (Fig. 5A, 5B).

406   Two clusters in $\delta^{13}C_{phyto}$ were present in the eutrophic lakes (Fig. 5A, 5B) and the shift

407 occurred when lakes were below 20 µmol L^{-1} CO_2 in the eutrophic lakes. When CO_2 becomes

408 limiting, some phytoplankton can also shift to bicarbonate utilisation, which is isotopically

409 enriched by ~8 ‰ compared to CO_2. Direct uptake of carbonate and conversion in the

410 carboxysomes is very common in the Cyanobacteria that dominate eutrophic lakes (Bontes et al.,

411 2006). The lakes with ^{13}C-enriched phytoplankton had high concentrations of zeaxanthin, a

412 biomarker for cyanobacteria. However, the enrichment in $\delta^{13}C_{phyto}$ in high zeaxanthin lakes was

413 not a direct consequence of enrichment in Cyanobacteria. FA that are abundant in Cyanobacteria

414 (18:nωn) were not more enriched than FA that are absent in Cyanobacteria; in fact, they were the

415 most depleted of all FA (Fig 4). Cyanobacteria grown in laboratory cultures also showed higher

416 fractionation (up to 9 ‰) in lipids relative to total biomass than eukaryotic phytoplankton

417 (summarized in Hayes, 2001).

418   The most enriched phytoplankton FA was 22:6ω3, which is abundant in dinoflagellates

419 (Fig. 4) (Dalsgaard et al., 2003). Dinoflagellates were also more enriched in ^{13}C compared to

420 other phytoplankton in a subtropical lake (Zohary et al., 1994). A possible explanation for ^{13}C-

421 enriched dinoflagellates in field studies, can be their mixotrophic character, so that part of their

422 isotopic composition reflects consumer $\delta^{13}C$. However, PUFAs of autotrophic dinoflagellates

423 grown in continuous cultures were also more enriched to C16:0 than PUFA of other

424 phytoplankton (Schouten et al., 1998).

425   Finally, variability in $\Delta\delta^{13}C_{FA\text{-}cell}$ can contribute to the observed variability. In laboratory

426 studies, the offset between lipids and bulk material has shown to be variable (van Dongen et al.,

427 2002, Fiorini et al., 2010). One can expect that in field studies, with multiple species, however,

428 these cellular variations would probably disappear within broader trends. If we assume an overall

429 mean $\Delta\delta^{13}C_{FA\text{-}cell}$, then the uncertainty in the actual value would affect the absolute fractionation

430 values, but not the observed variability in fractionation.

431 **4.4 Carbohydrates and lipid $\delta^{13}C$**

432   The enrichment of carbohydrates and depletion of lipids relative to total cells (mainly

433 amino acids) has been shown in culture studies of phytoplankton (Van Dongen et al., 2002) and

434 in culture studies of several primary producers and consumers (Teece and Fogel, 2007). Results

435 of this study show that the enrichment in glucose as well as the depletion in fatty acids relative to

436 bulk material can also be detected in field samples (Fig. 2, Table 3). We observed that

437 $\Delta\delta^{13}C_{gluc\text{-}FA_{tot}}$ increased with TP (Table 2), but whether this represents a general phenomenon for

438 lakes needs further exploration.

439   Bacterial FA were more enriched than phytoplankton FA in all lakes (Fig. 2, Fig. 4). This

440 observation can be explained by differences in carbon source or differences in $\Delta\delta^{13}C_{FA\text{-}cell}$

441 between phytoplankton and bacteria. Carbohydrates, present in high concentrations in DOC,

442 form an important carbon source for bacteria. Since carbohydrates were the most enriched

443 carbon source, a preferential use of carbohydrates, would result in ^{13}C enriched bacteria (Fig. 2).

444 Another explanation is that isotope fractionation during FA synthesis was smaller in bacteria

445 compared to phytoplankton. There are no field studies on $\Delta\delta^{13}C_{FA\text{-}cell}$ in freshwater bacteria, but

446 field studies on sediment and marine bacteria report a range of $0-5$ ‰ in $\Delta\delta^{13}C_{FA\text{-}cell}$ (Hayes,

447 2001, Burke et al., 2003; Bouillon and Boschker, 2006). Burke et al., (2003) suggested that in

448 field samples with complex communities and substrates, $\Delta\delta^{13}C_{FA\text{-}cell}$ would be ~0 ‰. The results

449 of our study support this idea, since bacterial FA had a similar $\delta^{13}C$ as POC. If a similar $\Delta\delta^{13}C_{FA\text{-}}$

450 $_{cell}$ for phytoplankton and bacteria would be used, bacteria would be more enriched than its

451 potential carbon sources in half of the studied lakes, which is rather unlikely.

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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 $\delta^{13}C$ 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, $\delta^{13}C_{phyto}$ showed that two clusters of phytoplankton were present, with the most enriched phytoplankton at high CO_2 and high chl *a*. Dominance of cyanobacteria played a role, but enrichment was present in all phytoplankton, as seen in specific PLFA.

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Table 1: Limnological characteristics of the sampled lakes. pCO_2 was determined from temperature, DIC, and pH. C_{phyto} presents the average of chl *a* and fatty acid based phytoplankton biomass. C_{bac} presents fatty acid derived bacteria carbon biomass.

name	U.S. state	trophic state	temperature (°)	pH	Alkalinity (mmol L⁻¹)	DIC (mmol L⁻¹)	pCO2 (µatm)	TN (mg L⁻¹)	TP (µg L⁻¹)	Chl *a* (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

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 pCO_2	0.79 **		
DIC and log pCO_2	0.82 **		
pH and log pCO_2	-0.98 ***		
log C_{phyto} and log pCO_2	-0.80 **		-0.59 ***
DOC and log pCO_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 pCO_2 and $\delta^{13}C_{DIC}$	-0.63 *	-0.81 **	-0.48 *
POC and $\delta^{13}C_{DIC}$	0.61 *		
DOC and $\delta^{13}C_{DIC}$		-0.62 *	
log TP and $\Delta(\delta_{gluc}-\delta_{FA})$			0.52 *
log C_{phyto} and $\delta^{13}C_{phyto}$	0.90 ***		
log pCO_2 and $\delta^{13}C_{phyto}$	-0.79 **	-0.90 ***	-0.73 ***
$\delta^{13}C_{CO2}$ and $\delta^{13}C_{phyto}$		0.82 **	0.54 **
log C_{phyto} and ε	-0.70 *		
$\delta^{13}C_{DOC}$ and $\delta^{13}C_{detritus}$		0.79 **	0.54 *

Table 3: Carbon isotope values of sampled lakes. Isotope data are presented as average ± sd (n=3). $\delta^{13}C_{CO_2}$ was calculated from $\delta^{13}C_{DIC}$ (equation 1). $\delta^{13}C_{phyto}$ and $\delta^{13}C_{bac}$ are not corrected for the offset between fatty acids and total cells, but $\varepsilon_{CO_2-algae}$ (equation 2) used the corrected $\delta^{13}C$ of phytoplankton.

lake name	trophic state	$\delta^{13}C_{DIC}$ (‰)	$\delta^{13}C_{CO_2}$ (‰)	$\delta^{13}C_{POC}$ (‰)	$\delta^{13}C_{DOC}$ (‰)	$\delta^{13}C_{Phyto}$ (‰)	$\varepsilon_{CO_2-algae}$	$\delta^{13}C_{bac}$ (‰)	$\delta^{13}C_{gluc}$ (‰)
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

Figure legends

Figure 1: The relation of pCO_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 pCO_2 (385 µatm).

Figure 2: Box and whisker plot of the $\delta^{13}C$ 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.

Figure 3: The relation of $\delta^{13}C_{DIC}$ in eutrophic lakes (filled circles, n=11)) and oligo-mesotrophic lakes (open circles, n=11) to A) pCO_2; B) C_{phyto}; C) DOC.

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

Figure 5: The relation of $\delta^{13}C_{phyto}$ in eutrophic lakes (filled circles, n=11) and oligo-mesotrophic lakes (open circles, n=11) to A) C_{phyto} and B) pCO_2. Panel C presents a box whisker plot of calculated $\varepsilon_{CO_2-algae}$ in eutrophic (Eu) and oligo-mesotrophic (Oli-Meso) lakes.
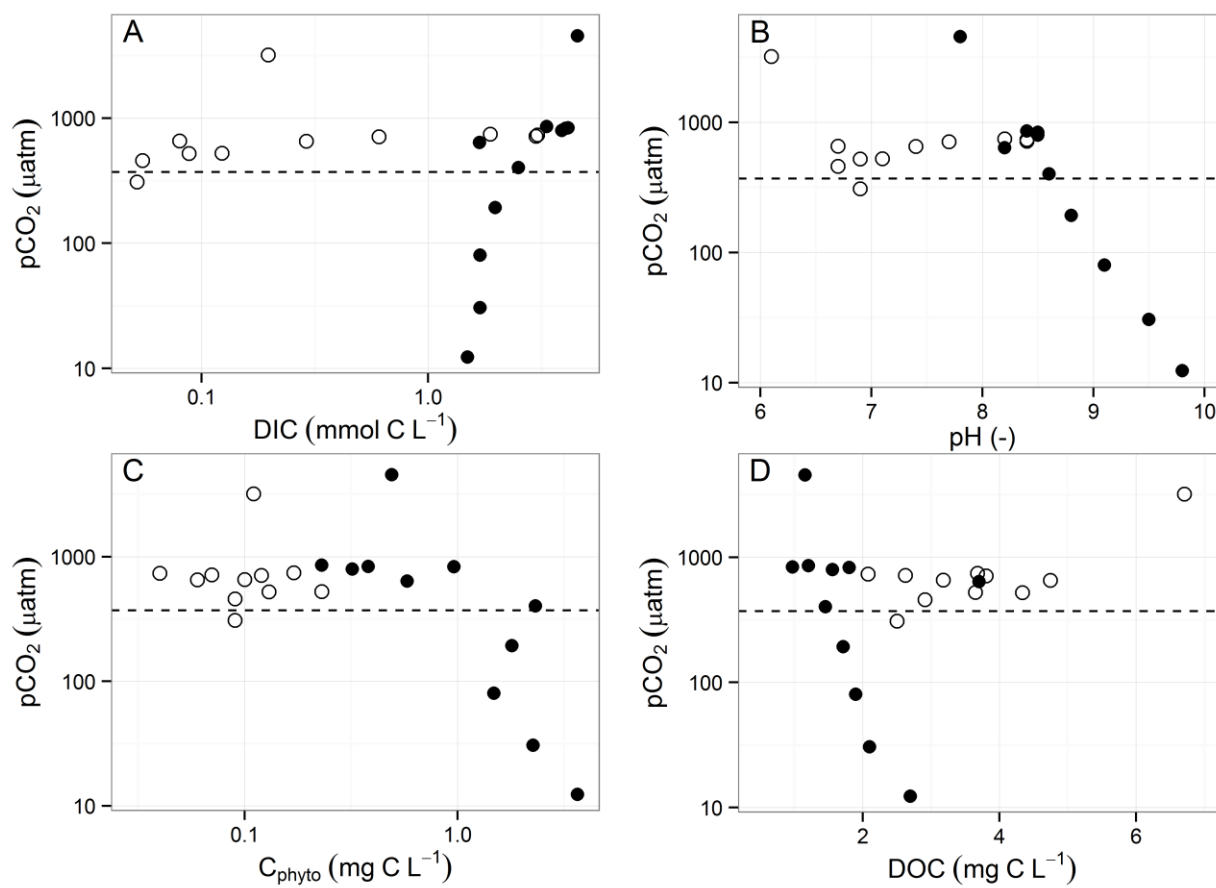
Fig. 1

Fig. 2

Fig. 3

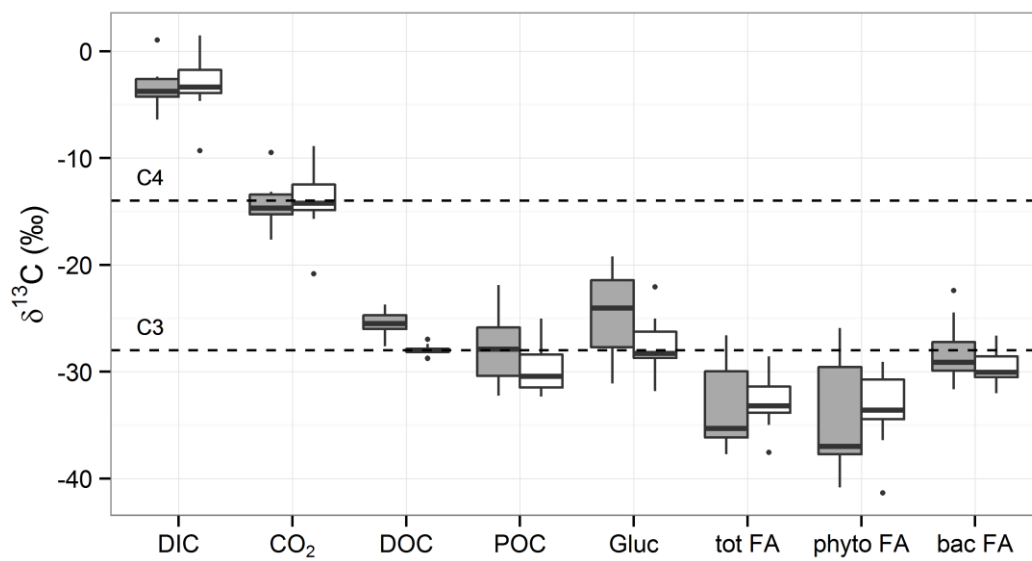
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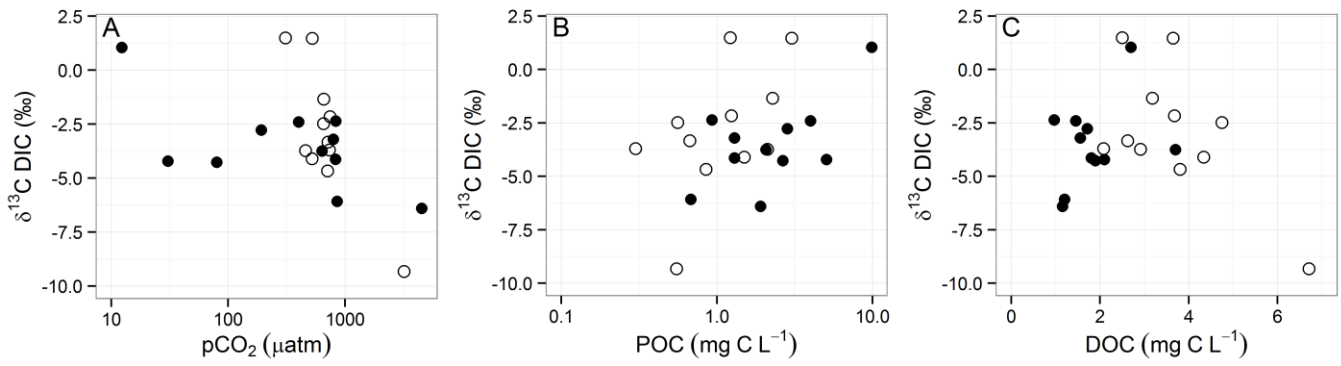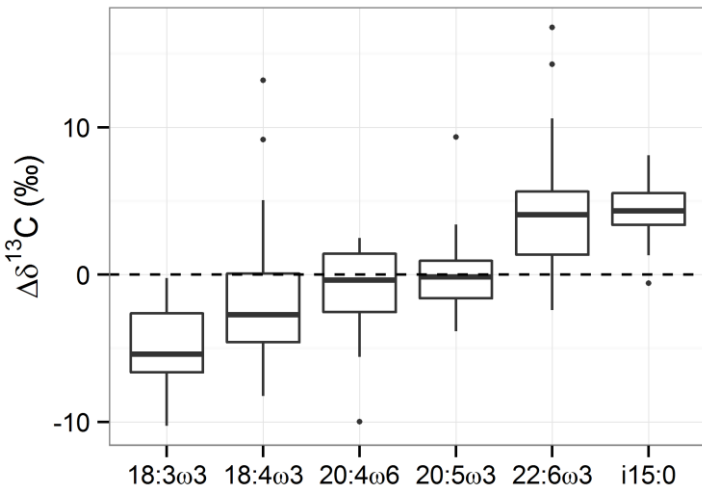

Fig. 5