Stable carbon isotope biogeochemistry of lakes along a trophic gradient

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16 **Abstract**

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 $\delta^{13}C$ 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 $\delta^{13}C$ of DIC. All organic-carbon pools showed
23 overall higher isotopic variability in eutrophic lakes (n=11) compared to oligo-mesotrophic lakes
24 (n=11) because of the high variability in $\delta^{13}C$ at the base of the food web (both autochthonous
25 and allochthonous carbon). Phytoplankton $\delta^{13}C$ was negatively related to lake pCO_2 over all
26 lakes and positively related to phytoplankton biomass in eutrophic lakes, which was also
27 reflected in a large range in photosynthetic isotope fractionation ($\varepsilon_{CO_2-phyto}$, 8-25 ‰). The carbon
28 isotope ratio of allochthonous carbon in oligo-mesotrophic lakes was rather constant, while it
29 varied in eutrophic lakes because of maize cultivation in the watershed.

30 **1**. **Introduction**

31 Studies suggest that lakes contribute significantly to the global carbon budget via organic
32 matter burial and emission of CO_2 to the atmosphere (Cole et al., 2007). The balance between
33 primary production and external organic carbon input on the one hand and respiration and burial
34 of organic carbon on the other governs whether individual lakes are sources or sinks of CO_2.
35 This metabolic balance can be disturbed by changes in nutrient or organic matter inputs to the
36 lake. Primary (autochthonous) production increases with increasing nutrient concentrations and
37 lakes with high autochthonous carbon production, i.e. eutrophic lakes, may be sinks for CO_2
38 (Schindler et al., 1997). The loading of allochthonous (terrestrial) carbon is a key factor
39 controlling community respiration of lakes. The metabolic balance of lakes is directly influenced
40 by allochthonous organic carbon loading and trophic state (Del Giorgio and Peters, 1994;
41 Hanson et al., 2003).

42 Stable carbon isotope analysis is a powerful tool for studying carbon cycling in lakes
43 since it allows studying inorganic and organic carbon pools and changes therein. It can provide
44 information on the metabolic balance and the sources of organic matter fueling respiration.
45 Respiration yields ^{13}C-depleted carbon dioxide from organic matter with the result that $\delta^{13}C$ of
46 dissolved inorganic carbon of the lakes becomes lower (Parker et al., 2010). Primary producers
47 preferentially incorporate ^{12}C in their organic matter with the consequence that the remaining
48 pool of dissolved inorganic carbon will be enriched in ^{13}C (Herczeg, 1987; Parker et al., 2010).
49 The $\delta^{13}C$ of the dissolved inorganic carbon pool thus integrates the relative importance of
50 respiration and primary production (Parker et al., 2010). The $\delta^{13}C$ of organic carbon pools is
51 primarily governed by the $\delta^{13}C$ of the dissolved inorganic carbon used by primary producers and
52 the isotope fractionation during carbon fixation. Terrestrial plants use atmospheric carbon
53 dioxide while aquatic primary producers utilize dissolved carbon dioxide or bicarbonate (Fry,
54 2006). The $\delta^{13}C$ of terrestrial-derived organic carbon is therefore often distinct from that of
55 organic matter produced within the lakes and this difference can be used to trace carbon flows
56 and origins in plankton food webs.

57 A major challenge in stable isotope studies is to elucidate the isotopic composition of
58 microbial organisms (Middelburg, 2014), such as phytoplankton and bacteria, since it is difficult
59 to separate these potential carbon sources from bulk particulate organic carbon (POC).

60 Therefore, most studies use indirect methods to determine $\delta^{13}C$ of phytoplankton ($\delta^{13}C_{phyto}$),
61 bacteria ($\delta^{13}C_{bac}$) and allochthonous carbon ($\delta^{13}C_{allo}$). Common methods for determining $\delta^{13}C_{phyto}$
62 are the use of the $\delta^{13}C$ of particulate organic carbon (POC) with correction for non-
63 phytoplankton carbon and estimates based on $\delta^{13}C$ of dissolved inorganic carbon (DIC) with an
64 isotope fractionation factor (ε), obtained from experimental studies. Other methods are the use of
65 zooplankton consumers as a proxy for $\delta^{13}C_{phyto}$ or size fractionation of organic matter and
66 subsequent determination of $\delta^{13}C$ of different size classes (Marty and Planas, 2008).

67 Isotopic ratios of bacteria in field studies have been derived from re-growing bacteria in
68 bioassays (Coffin et al., 1989) or dialysis cultures (Kritzberg et al., 2004), with measurement of
69 ^{13}C in POC or respired CO_2 (McCallister et al., 2008) and from biomarkers like nucleic acids
70 (Coffin et al., 1990) and lipids (Bontes et al., 2006; Pace et al., 2007). Some studies used $\delta^{13}C$ of
71 DOC as proxy for $\delta^{13}C$ of bacteria, assuming that DOC was the primary carbon source for
72 bacteria (Taipale et al., 2008; Zigah et al., 2012).

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

80 Compound specific isotope analysis (CSIA) of polar lipid-derived fatty acids (PLFA)
81 biomarkers has shown to be a valuable tool to determine the isotopic composition of plankton
82 producers and consumers (Boschker and Middelburg, 2002). Groups of phytoplankton and
83 bacteria have different fatty acid (FA) compositions, so by analyzing the $\delta^{13}C$ of specific FA, the
84 $\delta^{13}C$ of phytoplankton and bacteria can be inferred. The combined use of stable isotopes and FA
85 biomarkers has been successfully applied to study autochthonous and allochthonous carbon
86 contributions to zooplankton in a tidal river (Van den Meersche et al., 2009). Few studies have
87 applied CSIA to study carbon flows in plankton food webs in lakes. Examples are a
88 phytoplankton-zooplankton interaction study in a eutrophic lake (Pel et al., 2003), a

89 biomanipulation effect study (Bontes et al., 2006), a ^{13}C lake enrichment study (Pace et al.,
90 2007), and a cyanobacteria-zooplankton interaction study (de Kluijver et al., 2012).

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

98

99 **2. Material and Methods**

100 **2.1 Site description**

101 The 22 lakes sampled in this study are located in Iowa and Itasca County in Minnesota,
102 USA. Iowa lakes are mostly man-made and situated in a highly agricultural region, with maize
103 and soya beans as main products. This type of row-crop agriculture has a large impact on the
104 nutrient load of the lake watershed (Arbuckle and Downing, 2001). However, Itasca lakes are
105 natural and situated in a highly forested area. The catchment areas have developed since the last
106 glaciation episode ca. 12,000 years ago and consist of carbonate-poor glacial deposits (till)
107 (Grimley, 2000).

108 **2.2 Field sampling**

109 The lakes were sampled in July -August 2009 as part of the ongoing lake monitoring
110 program of the limnology laboratories of Iowa State University and Itasca Community College.
111 Key parameters, such as temperature, pH, Secchi transparency, oxygen, inorganic nutrients and
112 carbon concentrations were measured as part of and according to the lake monitoring program.
113 All samples were taken from up to 2 m of the upper mixed zone at the deepest point of each lake.
114 Water samples were taken between 10h-16h, a period of the day that yields relatively stable
115 water chemistry readings in these lakes. More information on data collection, lake
116 characteristics, and methods can be found on http://limnoweb.eeob.iastate.edu/itascalakes and

117 http://limnology.eeob.iastate.edu/lakereport. All nutrients were analyzed using certified methods
118 and strict quality assurance procedures.

119 Triplicate water samples were taken for stable isotope analyses and concentrations of the
120 major carbon pools. Headspace vials (20 ml and 2 ml) were filled on board with sampled water
121 using the overflow method and sealed with gas-tight caps for DIC isotope analyses and
122 concentrations, respectively. Mercury chloride was added for preservation and the samples were
123 stored upside-down at room temperature. For DOC analyses, 20 ml of sampled water was filtered
124 over GF/F (0.7 µm pore size, 25 mm diameter) and stored frozen in clean (acid and milli-Q
125 rinsed) vials until further analysis.

126 Seston samples for particulate organic carbon (POC) and carbohydrates were collected by
127 filtering 0.4 to 1 L of sampled water on pre-weighed and pre-combusted GF/F filters (0.7 µm
128 pore size, 47 mm diameter), which were subsequently dried at 60° for POC analysis or freeze-
129 dried for carbohydrates; PLFA samples were collected by filtering ~2 L sampled water on pre-
130 combusted GF/F filters (0.7 µm, 47 mm) and filters were stored frozen. Pigment samples were
131 taken for concentrations only and collected by filtering ~600 ml sampled water on GF/F filters
132 (0.7 µm, 47 mm) in the dark and filters were stored frozen.

133 **2.3 Laboratory analyses**

134 POC samples were analyzed for carbon content and isotope ratios on a Thermo Electron
135 Flash EA 1112 analyzer (EA) coupled to a Delta V isotope ratio mass spectrometer (IRMS) (c.f.
136 Nieuwenhuize et al., 1994). For DIC isotope analyses, a helium headspace was created in the
137 headspace vials and samples were acidified with H_3PO_4 solution. After equilibration, the CO_2
138 concentration and isotope ratio in the headspace was measured using EA-IRMS (Gilikin and
139 Bouillon, 2007). DIC concentrations were measured using spectrophotometry according to Stoll
140 et al., (2001). For DOC analyses, the samples were acidified and flushed with helium to remove
141 DIC and subsequently oxidized with sodium persulfate ($Na_2S_2O_8$); the isotope ratio and
142 concentration of CO_2 resulting from this treatment was measured using high performance liquid
143 chromatography - isotope ratio mass spectrometry (HPLC-IRMS) (Boschker et al. 2008). PLFA
144 samples were extracted according to a modified Bligh and Dyer method (Bligh and Dyer, 1959,
145 Middelburg et al., 2000). The lipids were fractionated in different polarity classes by column

146 separation on a heat-activated silic acid column and subsequent elution with chloroform, acetone,
147 and methanol. The methanol fractions, containing most of the PLFA, were collected and
148 transformed to fatty acid methyl esters (FAME) using methanolic NaOH. The 12:0 and 19:0
149 FAME were added as internal standards. Concentrations and $\delta^{13}C$ of individual PLFA were
150 measured using gas chromatography-combustion isotope ratio mass spectrometry (GC-C-IRMS)
151 (Middelburg et al., 2000). The isotopic compositions were corrected for the carbon added during
152 derivatization. Pigment samples were extracted with 90% acetone in purified (miliQ) water with
153 intense shaking. Concentrations of individual pigments were measured on HPLC (Wright et al.
154 1991). Carbohydrate samples were hydrolyzed in H_2SO_4, neutralized with $SrCO_3$, and
155 precipitated with $BaSO_4$. The supernatant was collected and measured using HPLC-IRMS
156 according to Boschker et al., (2008).

157 **2.4 Data analyses**

158 The lakes were divided into eutrophic and oligo-mesotrophic lakes based on average
159 summer total phosphorus (TP) concentrations. Lakes with TP values >24 µg L^{-1} and a
160 corresponding trophic state index >50 were classified as eutrophic, and lakes with TP values <
161 24 µg L^{-1} as oligo-mesotrophic (Carlson 1977). All lakes in Iowa and one lake in Minnesota
162 were classified as eutrophic, while all oligo-mesotrophic lakes were located in Minnesota.

163 **2.4.1 CO_2 system and isotopic composition of CO_2**

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

169 $$\delta^{13}C_{CO_2} = \delta^{13}C_{DIC} - 0.0144 \times T(^{\circ}C) \times fCO_3^{2-} + 0.107 \times T(^{\circ}C) - 10.53 \qquad (1)$$

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

171 **2.4.2 $\delta^{13}C$ of phytoplankton and bacteria**

172 Poly-unsaturated fatty acids (PUFA) are abundant in most phytoplankton, and can
173 generally be used as chemotaxonomic markers for this group (Dijkman and Kromkamp, 2006).
174 The most abundant PUFA in all lakes were 18:3ω3 (α-linolenic acid), 18:4ω3 (stearidonic acid,
175 SDA), 20:5ω3 (icosapentaenoic acid, EPA), 22:6ω3 (docosahexaenoic acid, DHA) and 20:4ω6
176 (arachidonic acid, ARA), common PUFA's in freshwater phytoplankton (Taipale et al., 2013),
177 and their concentration-weighted $\delta^{13}C$ were used to determine phytoplankton isotope ratios
178 ($\delta^{13}C_{phyto}$). Note that phytoplankton is considered a mixture of eukaryotic algae and
179 cyanobacteria. Branched fatty acids (BFA) are abundant in heterotrophic bacteria (Kaneda,
180 1991) in contrast to phytoplankton. The most abundant BFA were i15:0, ai15:0 and i17:0 and
181 their weighted $\delta^{13}C$ were used as a proxy for heterotrophic bacteria isotope ratios ($\delta^{13}C_{bac}$),
182 which we further consider bacteria. Isotope fractionation (ε) between CO_2 and phytoplankton
183 was calculated as

184 $$\varepsilon_{CO_2-phyto}(‰) = \frac{\delta^{13}C_{CO_2} - \delta^{13}C_{phyto_cor}}{1+\delta^{13}C_{phyto_cor}/1000} \quad (2)$$

185 $\delta^{13}C_{phyto_cor}$ was derived from $\delta^{13}C_{phyto}$ with a correction of +3‰ for the isotopic offset between
186 PUFA and total cells ($\Delta\delta^{13}C_{FA-cell}$) (Schouten et al., 1998; Hayes, 2001), although this isotopic
187 offset can be highly variable (Schouten et al. 1998).

188 **2.4.3 $\delta^{13}C$ of allochthonous carbon**

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

197 $$\delta^{13}C_{det}\ (‰) = (POC \times \delta^{13}C_{POC} - C_{phyto} \times \delta^{13}C_{phyto_cor} - C_{bac} \times \delta^{13}C_{bac} - C_{zoo} \times \delta^{13}C_{zoo}) / C_{det} \quad (3)$$

198 $$C_{det}\ (mg\ C\ L^{-1}) = POC - C_{phyto} - C_{bac} - C_{zoo} \quad (4)$$

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

201 Phytoplankton carbon (C_{phyto}) (mg C L^{-1}) was calculated as the average of biomass
202 estimates based on chl *a* concentration (C: Chl *a* = 50) as well as phytoplankton FA derived
203 biomass, to minimize the error associated with each method. Phytoplankton FA biomass was
204 calculated from the sum of phytoplankton PLFA (∑ 18:3ω3, 18:4ω3, 20:5ω3, 22:6ω3, and
205 20:4ω6) and a C: specific FA ratio of 60 based on culture studies, summarized in Dijkman and
206 Kromkamp (2006). The two approaches yielded similar results. Bacterial carbon (C_{bac}) (mg C L^{-}
207 ^{1}) was calculated from the summed concentrations of bacteria specific FA (i15:0, ai15:0, and
208 i17:0) and a C_{bac}: FA ratio of 50 (Middelburg et al., 2000). Zooplankton carbon (C_{zoo}) used in
209 equation 3 was estimated to be ~10 % of C_{phyto} (Del Giorgio and Gasol, 1995) and zooplankton
210 $\delta^{13}C$ are based on de Kluijver (2012). Uncertainties in $\delta^{13}C$ and biomass of phytoplankton,
211 bacteria and zooplankton were not considered in calculating $\delta^{13}C_{det}$.

212 **2.4.4 Statistical analyses**

213 Data that were part of the lake monitoring program and pCO_2 values represent single
214 samples of each lake. Data on carbon concentrations and isotopic compositions in each lake
215 convey averages of triplicate samples. Statistical analyses were done with software package "R"
216 (R development core team, 2013). Prior to correlation analyses, data were checked for normal
217 distribution (Shapiro test) and log-transformed when necessary to achieve normal distribution.
218 Correlation coefficients were calculated using Pearson product-moment correlation coefficient
219 (normal distribution) or Spearman's rank correlation coefficient (non-normal distribution). For
220 reasons of instructiveness we present the average ± sd values for eutrophic lakes (n=11) and
221 oligo-mesotrophic lakes (n=11), but we do realize that any division based on a concentration is
222 somewhat arbitrary. The correlations were tested for total lakes (n=22) and for eutrophic lakes
223 (n=11) and oligo-mesotrophic lakes (n=11). Differences between eutrophic and oligo-
224 mesotrophic lakes were statistically tested using Student's t-tests.

225

226

227 **3. Results**

228 **3.1 Lake chemistry**

229 The sampled lakes covered a large range of nutrients and CO_2 system characteristics
230 (Table 1). DIC values ranged from 0.05 to 4.55 mmol L^{-1}, alkalinity values ranged from 0.070 to
231 2.4 mmol L^{-1} and pH ranged from 6.1 to 9.8 (Table 1). The calculated pCO_2 values were in the
232 range from 10-4500 µatm, covering a broad range from under- to super-saturation. The CO_2
233 system in eutrophic and oligo-mesotrophic lakes was clearly different. On average, the eutrophic
234 lakes had higher DIC, alkalinity, and pH than the oligo-mesotrophic lakes (Fig. 1, Table 1). In
235 the eutrophic lakes, there were positive correlations between alkalinity and DIC and pCO_2 values
236 (Fig. 1A, Table 2) and a negative correlation between pH and pCO_2 (Fig. 1B, Table 2). The
237 pCO_2 values were not related to pH, alkalinity, or DIC in the oligo-mesotrophic lakes. Both lake
238 systems showed super-saturation (average pCO_2 838 µatm in both), but the pCO_2 range was
239 much larger in eutrophic lakes (10-4500 µatm) compared to oligo-mesotrophic lakes (310-3200
240 µatm) (Fig. 1, Table 1).

241 **3.2 Organic carbon and fatty acid concentrations**

242 POC, C_{phyto} and C_{bac} concentrations were higher and DOC concentrations were lower in
243 the eutrophic lakes compared to the oligo-mesotrophic lakes (Table 1). C_{phyto} was on average
244 1.32 ± 1.10 mg C L^{-1} and 0.11 ± 0.03 mg C L^{-1}, corresponding to 44 % ± 17 % and 10 % ± 5 %
245 of POC in eutrophic and oligo-mesotrophic lakes, respectively. C_{phyto} and C_{bac} were significantly
246 related to TP (Table 2), but not to total nitrogen (TN) concentration. Average C_{bac} was 0.114 ±
247 0.081 mg C L^{-1} and 0.021 ± 0.017 mg C L^{-1} in eutrophic and oligo-mesotrophic lakes,
248 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).

254 **3.3 δ^{13}C of DIC and CO_2**

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 in ^{13}C relative to DIC, with a range
258 of -20.8 to -8.9 ‰. $\delta^{13}C_{DIC}$ and $\delta^{13}C_{CO_2}$ showed no correlation with alkalinity, DIC, pH,
259 temperature and lake area. A weak negative relation between pCO_2 and $\delta^{13}C_{DIC}$ was observed,
260 which was stronger in oligo-mesotrophic lakes than in eutrophic lakes (Fig. 3A, Table 2). The
261 highest pCO_2 lakes had the lowest $\delta^{13}C_{DIC}$, suggesting that respiration of organic matter
262 influenced $\delta^{13}C_{DIC}$. Low CO_2 lakes had enriched $\delta^{13}C_{DIC}$, indicating influence of primary
263 production. Weak, but significant relations were observed for POC and DOC with $\delta^{13}C_{DIC}$ (Table
264 2). In eutrophic lakes, $\delta^{13}C_{DIC}$ increased with increasing POC (Fig. 3B, Table 2), while in oligo-
265 mesotrophic 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 $\delta^{13}C$ range of
270 POC ($\delta^{13}C_{POC}$) was larger than of DOC in both lake types and on average 2.0 ‰ lower compared
271 to $\delta^{13}C_{DOC}$, with mean values of -27.8 ± 3.6 ‰ in eutrophic and -29.7 ± 2.8 ‰ in oligo-
272 mesotrophic lakes (Fig. 2, Table 3). $\delta^{13}C$ of particulate glucose ($\delta^{13}C_{gluc}$), the most abundant
273 carbohydrate, was always enriched in ^{13}C compared to $\delta^{13}C_{POC}$ and the enrichment was similar in
274 eutrophic (3.1 ± 1.7 ‰) and oligo-mesotrophic lakes (2.8 ± 1.5 ‰) (Fig. 2). On the contrary, the
275 concentration-weighted average $\delta^{13}C$ of all fatty acids ($\delta^{13}C_{FA_{tot}}$) was always depleted in ^{13}C
276 compared to $\delta^{13}C_{POC}$ (Fig. 2). The depletion in ^{13}C 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 $\delta^{13}C_{FA_{tot}}$: $\Delta\delta^{13}C_{gluc\text{-}FA_{tot}}$ 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., $\Delta\delta^{13}C_{gluc\text{-}FA_{tot}}$ 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 in ^{13}C by 1.4 - 5.0 ‰ (e.g., the *iso*-15:0 FA in Fig. 4),

284 therefore the overall $\delta^{13}C$ of bacterial FA ($\delta^{13}C_{bac}$) was more ^{13}C-enriched than $\delta^{13}C_{FA_{tot}}$ in both

285 lake systems (Fig. 2). The poly-unsaturated fatty acids (PUFA) used as markers for

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

287 dinoflagellates (Dalsgaard et al. 2003), was found to be ^{13}C-enriched with 4.6 ‰ compared to

288 16:0 while PLFA 18:3ω3 (α-linolenic acid), common in cyanobacteria (de Kluijver et al., 2012),

289 was 4.7 ‰ depleted in ^{13}C compared to 16:0 (Fig. 4). The other phytoplankton markers were not

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

291 ^{13}C-depleted of all carbon pools (Fig. 2, Table 3) with an average of -33.8 ± 5.3 ‰ in eutrophic

292 lakes and -33.4 ± 3.5 ‰ in oligo-mesotrophic lakes. $\delta^{13}C_{bac}$ were on average 4.7 ‰ enriched in

293 ^{13}C compared to $\delta^{13}C_{phyto}$ (Fig. 2).

294 **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 in ^{13}C with

298 increasing C_{phyto} (Fig. 5A, Table 2) and decreasing pCO_2 (Fig. 5B, Table 2). No relation between

299 $\delta^{13}C_{phyto}$ and C_{phyto} was observed in the oligo-mesotrophic lakes (Fig. 5A), but there was a strong

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

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

302 lakes, while it was less variable in oligotrophic lakes (Fig. 5C). The range of $\varepsilon_{CO_2-phyto}$ was 7.8 to

303 24.7 ‰ (mean 16.9 ‰) in eutrophic and 11.7 to 18.8 ‰ (mean 17.1 ‰) in oligo-mesotrophic

304 lakes, when $\delta^{13}C_{phyto_cor}$ was used (Table 3). The less variable ε in oligo-mesotrophic lakes

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

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

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

308 clusters: a ^{13}C-enriched cluster at the highest C_{phyto} and a ^{13}C-depleted cluster at lower C_{phyto}

309 (Fig. 5A). Interestingly, the eutrophic lakes within the ^{13}C-enriched cluster also had high

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

311 **4. Discussion**

312 **4.1 Lake metabolism, pCO_2 and $\delta^{13}C_{DIC}$**

313 In our study, about 3/4 of the lakes were supersaturated with pCO_2, consistent with the
314 literature that lakes generally emit carbon dioxide to the atmosphere (Cole et al., 1994, Cole et
315 al., 2007). This CO_2 excess can be due to in-lake respiration of terrestrially derived organic
316 carbon outbalancing carbon dioxide fixation by primary producers (negative metabolic balance)
317 or due to river and groundwater input of CO_2 -rich waters (McDonald et al., 2013). Lake
318 metabolism also impacts $\delta^{13}C_{DIC}$ dynamics. Previous studies have shown that $\delta^{13}C$ of DIC in
319 lakes is driven by carbonate chemistry, hydrology (i.e., groundwater inflow), and metabolic
320 activity (Bade et al., 2004). Primary production increases $\delta^{13}C_{DIC}$ because of the preferential
321 uptake of ^{12}C (isotope fractionation), while organic matter respiration decreases $\delta^{13}C_{DIC}$ (Fry,
322 2006).

323 If pCO_2 and $\delta^{13}C_{DIC}$ would have been only or primarily controlled by the balance between
324 respiration and production of organic matter, one would expect a tight correlation between
325 $\delta^{13}C_{DIC}$ and pCO_2, which was not observed overall (Fig. 3A, Table 2), indicating that other
326 factors are involved. High inorganic carbon loadings of inflowing rivers and groundwater inputs
327 may sustain the CO_2 excess (McDonald et al., 2013) and govern the $\delta^{13}C_{DIC}$ (Bade et al., 2004).
328 Moreover, carbon dioxide water-air exchange reactions may have modified $\delta^{13}C_{DIC}$ values
329 because of isotope fractionation during water-air exchange, in particular at high pH/low CO_2
330 values (Herczeg and Fairbanks, 1987; Bade et al., 2004; Bontes et al., 2006).

331 The correlation between $\delta^{13}C_{DIC}$ and pCO_2 in oligo-mesotrophic lakes was stronger (Table 2) and
332 pCO_2 was highest and $\delta^{13}C_{DIC}$ was most depleted in ^{13}C at the highest DOC in oligo-mesotrophic
333 lakes (Fig. 1C, 3C). Such a depletion of $\delta^{13}C_{DIC}$ with increasing DOC, as an indicator of the
334 importance of respiration in oligo-mesotrophic lakes, has been shown previously in North-
335 American lakes (Lennon et al., 2006). In addition to community respiration, methanotrophic
336 bacteria in high DOC lakes could decrease $\delta^{13}C_{DIC}$ (Jones et al. 1999). However, anoxic
337 hypolimnia are rare in these lakes, either due to low nutrients or polymixis, indicating that
338 methanogenesis was not of major importance in the lakes investigated. Furthermore, we

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

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 ^{13}C-enriched and variable: -
372 25.4 ± 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 in ^{13}C of $\delta^{13}C_{allo}$ in eutrophic lakes can be partly
374 explained by land use in the water shed; almost all eutrophic lakes were located in the state of
375 Iowa, where an average of 92% of the land is under periodic cultivation for maize (C_4 plants, -14
376 ‰). There was more uncertainty in $\delta^{13}C_{allo}$ in the eutrophic lakes for two main reasons. First, we
377 expect a substantial autochthonous contribution to DOC and detritus in productive lakes (Bade et
378 al., 2007), which contributes to the larger range in $\delta^{13}C_{DOC}$ and $\delta^{13}C_{det}$ (Fig. 2). Second, the
379 presence of C_3 and C_4 vegetation with their distinct isotopic compositions can create a variable
380 $\delta^{13}C_{allo}$. Variability in $\delta^{13}C_{allo}$ has received far less attention than that of aquatic primary
381 producers. Our results show distinct differences in the isotopic composition of external subsidies
382 and argue against a fixed value for allochthonous carbon, especially in areas with abundant C_4
383 vegetation, such as maize.

384 **4.3 Phytoplankton δ^{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 δ^{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 higher $\delta^{13}C_{phyto}$ in high zeaxanthin lakes was not a
413 direct consequence of ^{13}C-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 ^{13}C-depleted of all FA (Fig 4). Cyanobacteria grown in laboratory cultures also showed
416 higher fractionation (up to 9 ‰) in lipids relative to total biomass than eukaryotic phytoplankton
417 (summarized in Hayes, 2001).

418 The most ^{13}C-enriched phytoplankton FA was 22:6ω3, which is abundant in
419 dinoflagellates (Fig. 4) (Dalsgaard et al., 2003). Dinoflagellates were also more enriched in ^{13}C
420 compared to other phytoplankton in a subtropical lake (Zohary et al., 1994). A possible
421 explanation for ^{13}C-enriched dinoflagellates in field studies, can be their mixotrophic character,
422 so that part of their isotopic composition reflects consumer $\delta^{13}C$. However, PUFAs of
423 autotrophic dinoflagellates grown in continuous cultures were also more ^{13}C-enriched to C16:0
424 than PUFA of other phytoplankton (Schouten et al., 1998).

425 Finally, variability in $\Delta\delta^{13}C_{FA-cell}$ 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 in ^{13}C of carbohydrates and depletion in ^{13}C of lipids relative to total
433 cells (mainly amino acids) has been shown in culture studies of phytoplankton (Van Dongen et
434 al., 2002) and in culture studies of several primary producers and consumers (Teece and Fogel,
435 2007). Results of this study show that the enrichment in ^{13}C in glucose as well as the ^{13}C-
436 depletion in in fatty acids relative to bulk material can also be detected in field samples (Fig. 2,
437 Table 3). We observed that $\Delta\delta^{13}C_{gluc\text{-}FA_{tot}}$ increased with TP (Table 2), but whether this represents
438 a general phenomenon for lakes needs further exploration.

439 Bacterial FA were more enriched in ^{13}C than phytoplankton FA in all lakes (Fig. 2, Fig.
440 4). This observation can be explained by differences in carbon source or differences in $\Delta\delta^{13}C_{FA\text{-}}$
441 $_{cell}$ between phytoplankton and bacteria. Carbohydrates, present in high concentrations in DOC,
442 form an important carbon source for bacteria. Since carbohydrates were the most ^{13}C-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 in ^{13}C than its
451 potential carbon sources in half of the studied lakes, which is rather unlikely.

452

453

454

455 **5. Conclusions**

456 Our results show that trophic state has a large influence on lake metabolism and carbon
457 cycling in plankton food webs. Overall, eutrophic lakes had larger variability in $\delta^{13}C$ in all
458 organic carbon pools than oligo-mesotrophic lakes, caused by larger isotopic variability in the
459 base of the food web in eutrophic lakes (both allochthonous and autochthonous carbon). In
460 eutrophic lakes, $\delta^{13}C_{phyto}$ showed that two clusters of phytoplankton were present, with the most
461 ^{13}C-enriched phytoplankton at high CO_2 and high chl *a*. Dominance of cyanobacteria played a
462 role, but enrichment in ^{13}C was present in all phytoplankton, as seen in specific PLFA.

463

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474

475 **References**

476 Arbuckle, K. E., and Downing, J. A.: The influence of watershed land use on lake N: P in a
477 predominantly agricultural landscape. Limnol. Oceanogr., 46, 970-975, 2001
478 Bade D. L. , Carpenter , S. R., Cole, J. J., Hanson, P. C., and Hesslein, R. H.: Controls of $\delta^{13}C$-
479 DIC in lakes: Geochemistry, lake metabolism, and morphometry, Limnology and
480 Oceanography, 49, 1160-72, 2004.

481 Bade D. L., Carpenter S. R., Cole J. J., Pace M. L., Kritzberg E., Van de Bogert M. C. et al.:
482 Sources and fates of dissolved organic carbon in lakes as determined by whole-lake carbon
483 isotope additions, Biogeochemistry, 84, 115–129, 2007.
484 Bade, D. L., Pace, M. L. Cole, J. J., and Carpenter, S. R.: Can algal photosynthetic inorganic
485 carbon isotope fractionation be predicted in lakes using existing models?, Aquat. Sci., 68,
486 142-153, 2006.
487 Bidigare, R. R., Fluegge, A., Freeman, K. H., Hanson, K. L., Hayes, J. M., Hollander, D., Jasper,
488 J. P., King, L. L., Laws, E. A., Milder, J., Millero, F. J., Pancost, R., Popp, B. N., Steinberg,
489 P. A., and Wakeham, S. G.: Consistent fractionation of ^{13}C in nature and in the laboratory:
490 Growth-rate effects in some haptophyte algae, Glob. Biogeochem. Cycle, 11, 279-292, 1997.
491 Bligh, E. G., and Dyer, W. J.: A rapid method of total lipid extraction and purification, Canadian
492 Journal of Biochemistry and Physiology, 37, 911-917, 1959.
493 Bontes, B. M., Pel, R., Ibelings, B. W., Boschker, H. T. S., Middelburg, J. J., and Van Donk, E.:
494 The effects of biomanipulation on the biogeochemistry, carbon isotopic composition and
495 pelagic food web relations of a shallow lake, Biogeosciences, 3, 69-83, 2006.
496 Boschker, H. T. S., and Middelburg, J. J.: Stable isotopes and biomarkers in microbial ecology,
497 FEMS Microbiol. Ecol., 40, 85-95, 2002.
498 Boschker, H. T. S., Moerdijk-Poortviiet, T. C. W., van Breugel, P., Houtekamer, M., and
499 Middelburg, J. J.: A versatile method for stable carbon isotope analysis of carbohydrates by
500 high-performance liquid chromatography/isotope ratio mass spectrometry, Rapid Commun.
501 Mass Spectrom., 22, 3902-3908, 10.1002/rcm.3804, 2008.
502 Bouillon, S., and Boschker, H. T. S.: Bacterial carbon sources in coastal sediments: a cross-
503 system analysis based on stable isotope data of biomarkers, Biogeosciences, 3, 175-185,
504 2006.
505 Burke, R. A., Molina, M., Cox, J. E., Osher, L. J., and Piccolo, M. C.: Stable carbon isotope ratio
506 and composition of microbial fatty acids in tropical soils, J. Environ. Qual., 32, 198-206,
507 10.2134/jeq2003.1980, 2003.
508 Carlson, R. E.: A trophic state index for lakes, Limnology and Oceanography, 22, 361-369,
509 1977.
510 Coffin, R. B., Fry, B., Peterson, B. J., and Wright, R. T.: Carbon isotopic compositions of
511 estuarine bacteria, Limnology and Oceanography, 34, 1305-1310, 1989.

512 Coffin, R. B., Velinsky, D., Devereux, R., Price, W., and Cifuentes, L.: Stable carbon isotope
513 analysis of nucleic acids to trace sources of dissolved substrates used by estuarine bacteria,
514 Appl. Environ. Microbiol., 56, 2012-2020, 1990.
515 Cole, J. J., Caraco, N. F., Kling, G. W., and Kratz, T. K.: Carbon dioxide supersaturation in the
516 surface waters of lakes, Science, 265, 1568-1570, 1994.
517 Cole, J. J., Carpenter, S. R., Kitchell, J. F., and Pace, M. L.: Pathways of organic carbon
518 utilization in small lakes: Results from a whole-lake ^{13}C addition and coupled model,
519 Limnology and Oceanography, 47, 1664-1675, 2002.
520 Cole, J. J., Prairie, Y. T., Caraco, N. F., McDowell, W. H., Tranvik, L. J., Striegl, R. G., Duarte,
521 C. M., Kortelainen, P., Downing, J. A., Middelburg, J. J., and Melack, J.: Plumbing the
522 global carbon cycle: Integrating inland waters into the terrestrial carbon budget, Ecosystems,
523 10, 171-184, 2007.
524 Dalsgaard, J., St John, M., Kattner, G., Muller-Navarra, D., and Hagen, W.: Fatty acid trophic
525 markers in the pelagic marine environment, in: Advances in Marine Biology, Vol 46,
526 Advances in Marine Biology, Academic Press Ltd, London, 225-340, 2003.
527 de Kluijver, A., Yu, J., Houtekamer, M., Middelburg, J. J., and Liu, Z.: Cyanobacteria as a
528 carbon source for zooplankton in eutrophic Lake Taihu, China, measured by ^{13}C labeling
529 and fatty acid biomarkers, Limnol. Oceanogr, 57, 1245-1254, 2012.
530 De Kluijver, A.: Carbon flows in natural plankton communities in the Anthropocene. Thesis
531 Utrecht University, 2012.
532 Del Giorgio, P. A., and Peters, R. H.: Patterns in planktonic P:R ratios in lakes: Influence of lake
533 trophy and dissolved organic carbon, Limnology and Oceanography, 39, 772-787, 1994.
534 Del Giorgio, P. A., and Gasol, J. M.: Biomass distribution in freshwater plankton communities,
535 The American Naturalist, 146, 135-152, 1995.
536 Dijkman, N. A., and Kromkamp, J. C.: Phospholipid-derived fatty acids as chemotaxonomic
537 markers for phytoplankton: application for inferring phytoplankton composition, Mar. Ecol.-
538 Prog. Ser., 324, 113-125, 2006.
539 Fiorini, S., Gattuso, J.-P., van Rijswijk, P., and Middelburg, J.: Coccolithophores lipid and
540 carbon isotope composition and their variability related to changes in seawater carbonate
541 chemistry, J. Exp. Mar. Biol. Ecol., 394, 74-85, 2010.
542 Fry, B.: Stable isotope ecology, Springer, 2006.

543 Gillikin, D. P., and Bouillon, S.: Determination of $\delta^{18}O$ of water and $\delta^{13}C$ of dissolved inorganic
544 carbon using a simple modification of an elemental analyser-isotope ratio mass
545 spectrometer: an evaluation, Rapid Commun. Mass Spectrom., 21, 1475-1478, 2007.
546 Grimley, D. A.: Glacial and nonglacial sediment contributions to Wisconsin Episode loess in the
547 central United States. Geological Society of America Bulletin, 112, 1475-1495, 2010.
548 Hanson, P. C., Bade, D. L., Carpenter, S. R., and Kratz, T. K.: Lake metabolism: Relationships
549 with dissolved organic carbon and phosphorus, Limnology and Oceanography, 48, 1112-
550 1119, 2003.
551 Hayes, J. M.: Fractionation of carbon and hydrogen isotopes in biosynthetic processes, in: Stable
552 Isotope Geochemistry, Reviews in Mineralogy & Geochemistry, Mineralogical Soc
553 America, Washington, 225-277, 2001.
554 Herczeg, A.: A stable carbon isotope study of dissolved inorganic carbon cycling in a softwater
555 lake, Biogeochemistry, 4, 231-263, 10.1007/BF02187369, 1987.
556 Herczeg, A. L., and Fairbanks, R. G.: Anomalous carbon isotope fractionation between
557 atmospheric CO_2 and dissolved inorganic carbon induced by intense photosynthesis,
558 Geochim. Cosmochim. Acta, 51, 895-899, 1987.
559 Hofmann, A. F., Soetaert, K., Middelburg, J. J., Meysman, F. J. R.: AquaEnv - an Aquatic acid-
560 base modeling Environment in R, R-package version 1, 2010
561 Jones, R. I., Grey, J., Sleep, D., and Arvola, L.: Stable isotope analysis of zooplankton carbon
562 nutrition in humic lakes, Oikos, 97-104, 1999.
563 Kaneda, T.: Iso-fatty and anteiso-fatty acids in bacteria – biosynthesis, function, and taxonomic
564 significance. Microbiol. Rev. 55, 288-302, 1991.
565 Kohn, M. J.: Carbon isotope compositions of terrestrial C_3 plants as indicators of (paleo)ecology
566 and (paleo)climate, Proceedings of the National Academy of Sciences, 107, 19691-19695,
567 10.1073/pnas.1004933107, 2010.
568 Kritzberg, E. S., Cole, J. J., Pace, M. L., Granéli, W., and Bade, D. L.: Autochthonous versus
569 allochthonous carbon sources of bacteria: Results from whole-lake ^{13}C addition experiments,
570 Limnology and Oceanography, 49, 588-596, 2004.
571 Laws, E. A., Popp, B. N., Bidigare, R. R., Kennicutt, M. C., and Macko, S. A.: Dependence of
572 phytoplankton carbon isotopic composition on growth-rate and $[CO_2]$ (aq) – theoretical
573 considerations and experimental results, Geochim. Cosmochim. Acta, 59, 1131-1138, 1995.

574 Lennon, J. T., Faiia, A. M., Feng, X. H., and Cottingham, K. L.: Relative importance of CO_2
575 recycling and CH_4 pathways in lake food webs along a dissolved organic carbon gradient,
576 Limnology and Oceanography, 51, 1602-1613, 2006.
577 Marty, J., and Planas, D.: Comparison of methods to determine algal $\delta^{13}C$ in freshwater, Limnol.
578 Oceanogr. Meth., 6, 51-63, 2008.
579 McCallister, S. L., and del Giorgio, P. A.: Direct measurement of the $\delta^{13}C$ signature of carbon
580 respired by bacteria in lakes: Linkages to potential carbon sources, ecosystem baseline
581 metabolism, and CO2 fluxes, Limnology and Oceanography, 53, 1204-1216, 2008.
582 McDonald, C. P., Stets, E. G., Striegl, R. G., and Butman, D.: Inorganic carbon loading as a
583 primary driver of dissolved carbon dioxide concentrations in the lakes and reservoirs of the
584 contiguous United States, Glob. Biogeochem. Cycle, 27, 1-11, 10.1002/gbc.20032, 2013.
585 Middelburg, J.J. Stable isotope dissect aquatic foodwebs from the top to the bottom.
586 Biogeosciences, 11, 2357-2371, 10.5194/bg-11-2357-2014, 2014.
587 Middelburg, J. J., Barranguet, C., Boschker, H. T. S., Herman, P. M. J., Moens, T., and Heip, C.
588 H. R.: The fate of intertidal microphytobenthos carbon: An in situ ^{13}C-labeling study,
589 Limnology and Oceanography, 45, 1224-1234, 2000.
590 Nieuwenhuize, J., Maas, Y. E. M., and Middelburg, J. J.: Rapid analysis of organic carbon and
591 nitrogen in particulate materials, Mar. Chem., 45, 217-224, 1994.
592 Pace, M. L., Carpenter, S. R., Cole, J. J., Coloso, J. J., Kitchell, J. F., Hodgson, J. R.,
593 Middelburg, J. J., Preston, N. D., Solomon, C. T., and Weidel, B. C.: Does terrestrial organic
594 carbon subsidize the planktonic food web in a clear-water lake?, Limnology and
595 Oceanography, 52, 2177-2189, 2007.
596 Parker, S., Poulson, S., Smith, M. G., Weyer, C., and Bates, K.: Temporal variability in the
597 concentration and stable carbon isotope composition of dissolved inorganic and organic
598 carbon in two Montana, USA rivers, Aquatic Geochemistry, 16, 61-84, 10.1007/s10498-
599 009-9068-1, 2010.
600 Pel, R., Floris, V., and Hoogveld, H.: Analysis of planktonic community structure and trophic
601 interactions using refined isotopic signatures determined by combining fluorescence-
602 activated cell sorting and isotope-ratio mass spectrometry, Freshw. Biol., 49, 546-562, 2004.

603 Riebesell, U., Burkhardt, S., Dauelsberg, A., and Kroon, B.: Carbon isotope fractionation by a
604 marine diatom: dependence on the growth-rate-limiting resource, Mar. Ecol.-Prog. Ser., 193,
605 295-303, 2000.
606 Schindler, D. E., Carpenter, S. R., Cole, J. J., Kitchell, J. F., and Pace, M. L.: Influence of food
607 web structure on carbon exchange between lakes and the atmosphere, Science, 277, 248-
608 251, 1997.
609 Stoll, M., Bakker, K., Nobbe, G., and Haese, R.: Continuous-flow analysis of dissolved inorganic
610 carbon content in seawater, Anal. Chem, 73, 4111-4116, 2001.
611 Taipale, S., Kankaala, P., Tiirola, M., and Jones, R. I.: Whole-lake dissolved inorganic C-13
612 additions reveal seasonal shifts in zooplankton diet, Ecology, 89, 463-474, 2008.
613 Taipale, S., Strandberg, U., Peltomaa, E., Galloway, A. W., Ojala, A., and Brett, M. T.: Fatty
614 acid composition as biomarkers of freshwater microalgae: analysis of 37 strains of
615 microalgae in 22 genera and in seven classes, Aquat Microb Ecol, 71, 165-178, 2013.
616 Teece, M. A., and Fogel, M. L.: Stable carbon isotope biogeochemistry of monosaccharides in
617 aquatic organisms and terrestrial plants, Org. Geochem., 38, 458-473,
618 10.1016/j.orggeochem.2006.06.008, 2007.
619 Van den Meersche, K., Van Rijswijk, P., Soetaert, K., and Middelburg, J. J.: Autochthonous and
620 allochthonous contributions to mesozooplankton diet in a tidal river and estuary: Integrating
621 carbon isotope and fatty acid constraints, Limnol. Oceanogr, 54, 62-74, 2009.
622 van Dongen, B. E., Schouten, S., and Damste, J. S. S.: Carbon isotope variability in
623 monosaccharides and lipids of aquatic algae and terrestrial plants, Mar. Ecol.-Prog. Ser.,
624 232, 83-92, 2002.
625 Wilkinson, G. M., Pace, M. L., and Cole, J. J.: Terrestrial dominance of organic matter in north
626 temperate lakes, Glob. Biogeochem. Cycle, 27, 43–51, 10.1029/2012GB004453, 2013.
627 Wright, S. W.: Improved HPLC method for the analysis of chlorophylls and carotenoids from
628 marine phytoplankton, Marine Ecology Progress Series, 77, 183-196, 1991.
629 Zhang, J., Quay, P. D., and Wilbur, D. O.: Carbon-isotope fractionation during gas-water
630 exchange and dissolution of CO_2, Geochim. Cosmochim. Acta, 59, 107-114, 1995.
631 Zigah, P., Minor, E., Werne, J., and Leigh McCallister, S.: An isotopic ($\Delta^{14}C$, $\delta^{13}C$, and $\delta^{15}N$)
632 investigation of particulate organic matter and zooplankton biomass in Lake Superior and

633 across a size-gradient of aquatic systems, Biogeosciences, 9, 3663-3678, 10.5194/bg-9-
634 3663-2012, 2012.
635 Zohary, T., Erez, J., Gophen, M., Bermanfrank, I., and Stiller, M.: Seasonality of stable carbon
636 isotopes within the pegalic food-web of lake Kinneret, Limnology and Oceanography, 39,
637 1030-1043, 1994.

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 $\Delta(\delta_{gluc}\text{-}\delta_{FA})$			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

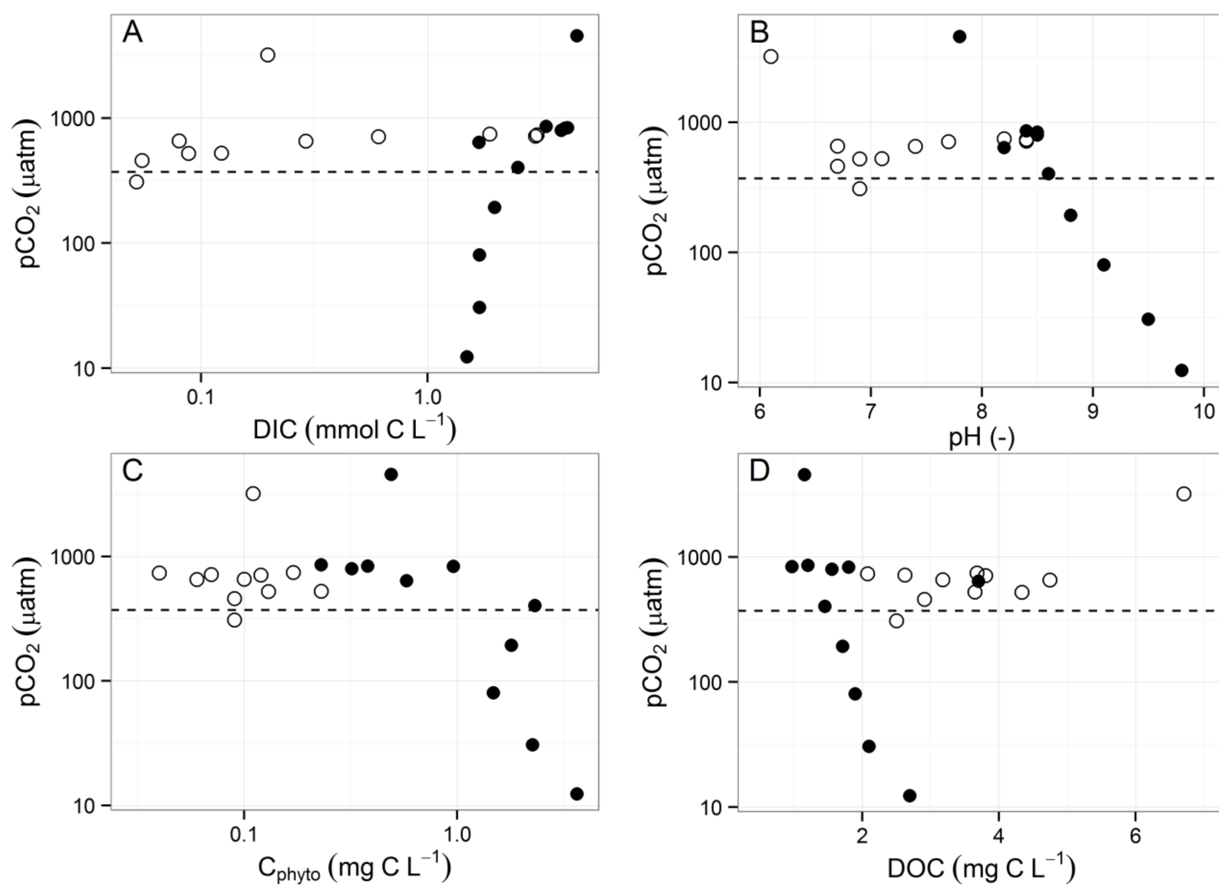

Fig. 2

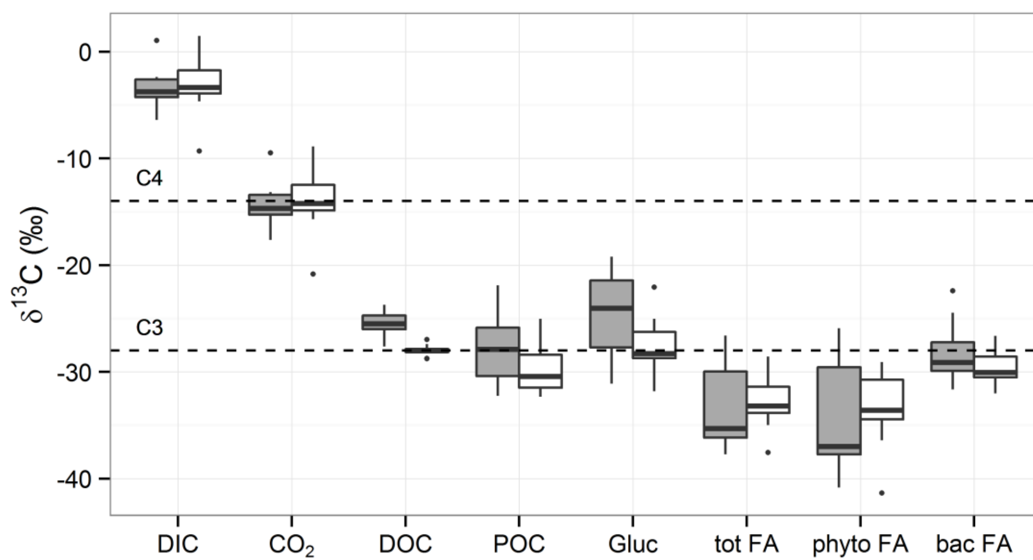

Fig. 3

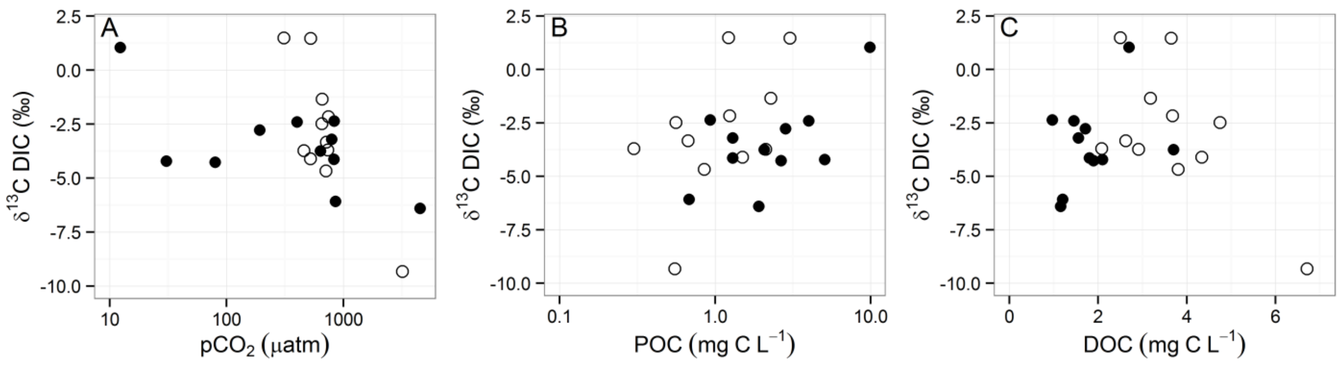

Fig. 4

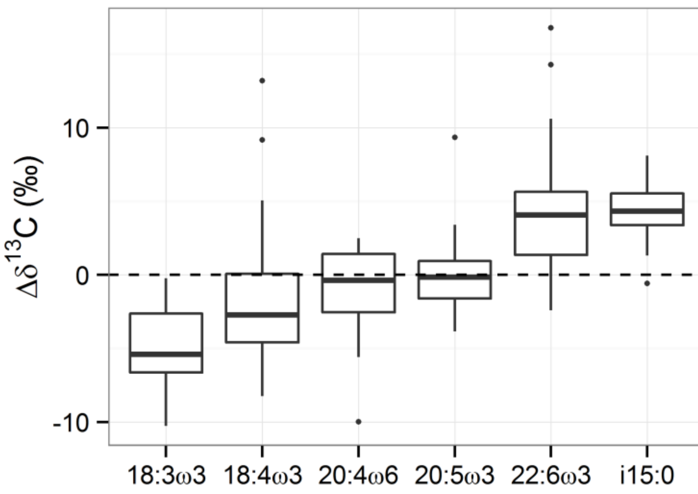

Fig. 5