1 **Cyanobacterial carbon concentrating mechanisms facilitate sustained CO_2 depletion in**

2 **eutrophic lakes**

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22 **Abstract**

23 Phytoplankton blooms are increasing in frequency, intensity, and duration in aquatic
24 ecosystems worldwide. In many eutrophic lakes, these high levels of primary productivity
25 correspond to periods of CO_2 depletion in surface waters. Cyanobacteria and other groups of
26 phytoplankton have the ability to actively transport bicarbonate (HCO_3^-) across their cell
27 membrane when CO_2 concentrations are limiting, possibly giving them a competitive advantage
28 over algae not using carbon concentrating mechanisms (CCMs). To investigate whether CCMs
29 can maintain phytoplankton bloom biomass under CO_2 depletion, we measured $\delta^{13}C$ signatures
30 of dissolved inorganic carbon ($\delta^{13}C_{DIC}$) and phytoplankton particulate organic carbon ($\delta^{13}C_{phyto}$)
31 in sixteen mesotrophic to hypereutrophic lakes during the ice-free season of 2012. We used mass
32 balance relationships to determine the dominant inorganic carbon species used by phytoplankton
33 under CO_2 stress. We found a significant positive relationship between phytoplankton biomass
34 and phytoplankton $\delta^{13}C$ signatures, as well as a significant non-linear negative relationship
35 between water column ρCO_2 and isotopic composition of phytoplankton, indicating a shift from
36 diffusive uptake to active uptake by phytoplankton of CO_2 or HCO_3^- during blooms. Calculated
37 photosynthetic fractionation factors indicated that this shift occurs specifically when surface
38 water CO_2 drops below atmospheric equilibrium. Our results indicate active HCO_3^- uptake via
39 CCMs may be an important mechanism maintaining phytoplankton blooms when CO_2 is
40 depleted. Further increases in anthropogenic pressure, eutrophication, and cyanobacteria blooms
41 are therefore expected to contribute to increased bicarbonate uptake to sustain primary
42 production.

43 **Key words:** Eutrophication, carbon cycling, Cyanobacteria, CCM, stable isotopes

44 **1. Introduction**

45 Cyanobacteria blooms resulting from anthropogenic eutrophication are among the greatest
46 current threats to inland water ecosystems, altering carbon cycling and ecosystem function,
47 impairing water quality, and endangering human health (Brooks et al., 2016; Paerl et al., 2011;
48 Visser et al., 2016). Forecasting models and macrosystem-scale analyses suggest the occurrence
49 of blooms is driven by the interactive effects of land use, nutrient inputs (nitrogen and
50 phosphorus), climate, weather, and in-lake processes (Anneville et al., 2015; Michalak et al.,
51 2013; Persaud et al., 2015; Rigosi et al., 2014). Mechanisms determining variability in timing
52 and duration of these events in lakes, however, remain poorly understood (Brooks et al., 2016),
53 and it is unclear what the large-scale feedbacks of sustained primary production are on lake
54 carbon cycling by phytoplankton. While temperate lakes have generally been considered net
55 sources of CO_2 to the atmosphere (Tranvik et al., 2009), eutrophic systems can maintain both
56 high levels of primary production and negligible concentrations of CO_2 in surface water (Balmer
57 and Downing, 2011; Gu et al., 2010; Laas et al., 2012), possibly increasing the flow of dissolved
58 inorganic C to organic C. Identifying drivers of the temporal variability of bloom formation and
59 maintenance will contribute to a better understanding of carbon dynamics in lakes with high
60 productivity.

61 Cyanobacteria have developed a suite of diverse strategies for obtaining and fixing carbon
62 and nutrients at growth-limiting concentrations. In addition to fixing atmospheric nitrogen , they
63 are able to maintain metabolic processes under severe CO_2 depletion by use of a carbon
64 concentrating mechanism (CCM; Badger and Price 2003; Raven et al. 2008). The cyanobacterial
65 CCM is not only the accumulation of inorganic carbon, but collectively active transport across
66 the cell membrane, partitioning of Rubisco into carboxysomes, and elevation of CO_2 around

67 enzyme complexes (Price et al., 2008b). When water column pH exceeds 8.5, CO_2 is negligible

68 and HCO_3^- is the dominant carbon species. HCO_3^- cannot passively diffuse across phytoplankton

69 cell membranes, and therefore requires an active transport system. CCMs are present in many

70 groups of aquatic photoautotrophs including green algae (Spalding, 2008) and diatoms

71 (Hopkinson et al., 2016), as well as some higher plants. These mechanisms are thought to have

72 evolved independently in eukaryotic algae and the cyanobacteria, corresponding to a large

73 decrease in atmospheric CO_2 and doubling of O_2 approximately 400 million years BP (Badger

74 and Price, 2003; Raven et al., 2008). There are, however, many similarities between eukaryotic

75 and cyanobacteria CCMs which are not fully resolved, so it is unclear whether or not

76 cyanobacteria CCMs represent a more efficient, competitive advantage over other phytoplankton

77 taxa (Moroney and Ynalvez, 2007).

78 The cyanobacterial CCM mechanism facilitates active transport of HCO_3^- across the

79 plasma membrane, where it is accumulated in the cytosol, transferred to Rubisco-containing

80 carboxysomes, and converted to CO_2 via carbonic anhydrases (Raven et al., 2008). Carboxysome

81 structures, unique to cyanobacteria CCMs, are thought to decrease CO_2 leakage rates via low

82 permeability for uncharged species (i.e., CO_2) across the carboxysome protein shell (Kaplan and

83 Reinhold, 1999; Price et al., 2008a). In an optimal CCM, diffusion of HCO3- across the

84 carboxysome shell is fast, and leakage of converted CO_2 is slow (Mangan and Brenner, 2014).

85 This results in reduced isotopic discrimination and an intracellular composition approaching that

86 of source material (Fielding et al., 1998).

87 In freshwaters, cyanobacteria use form 1B Rubisco, which facilitates acclimation to

88 inorganic carbon depletion via high cellular affinity for CO_2 and HCO_3^- (Raven and Beardall,

89 2016; Raven et al., 2008; Shih et al., 2015). While this process is energetically costly, it is

90 essential to both increase photosynthetic efficiency and local bioavailability of inorganic carbon
91 when CO_2 is depleted. In addition to inorganic carbon availability, cyanobacterial CCMs are
92 triggered by photosynthetically active radiation (PAR) and nitrogen availability. Because CCMs
93 are energetically costly (Raven and Beardall, 2016), decreased PAR lowers cellular affinity for
94 inorganic carbon (Giordano et al., 2005). Affinity increases with depletion of nitrate and iron, but
95 decreases with depletion of NH_4^+, and does not have a consistent response to phosphorus
96 limitation (Raven et al., 2008). CCM activation under carbon and nutrient stress thus may confer
97 a competitive advantage to cyanobacteria via efficient carbon fixation when CO_2 is low (Badger
98 and Price, 2003; Price et al., 2008b).

99 Shifts to alternative carbon assimilation strategies result in measureable changes in
100 isotopic fractionation. Stable isotopic signatures of phytoplankton are dependent both on the
101 isotopic composition of their DIC source and the physiological mechanism used to acquire it.
102 When phytoplankton use passive diffusion to take up ambient CO_2, photosynthetic fractionation
103 resembles that of C3 terrestrial plants (Yoshioka, 1997), resulting in typical mean $\delta^{13}C$ signatures
104 between -27‰ to -30‰ (Bade et al., 2004; Erez et al., 1998; O'Leary, 1988). In cyanobacteria
105 and other phytoplankton, carbon fixation can be equally limited by carboxylation and active
106 inorganic carbon transport into the cell. Cyanobacteria and eukaryotic algae that are actively
107 concentrating inorganic carbon via HCO_3^- uptake can have elevated $\delta^{13}C$ values as high as -8 to -
108 11‰ (Sharkey and Berry, 1985; Vuorio et al., 2006). This is largely attributable to the isotopic
109 signature of source material (Kaplan and Reinhold, 1999), as well as decreased carbon efflux
110 when CCMs are active, resulting in reduced photosynthetic fractionation (-1‰ to -3‰; Sharkey
111 and Berry 1985; Erez et al. 1998). Further, isotopic fractionation associated with active HCO_3^-
112 uptake is negligible (Sharkey and Berry, 1985; Yoshioka, 1997). In other words, discrimination

113 due to passive diffusion is reduced or negligible when active HCO_3^- uptake is occurring
114 (Giordano et al., 2005). Thus, if CCMs are activated during cyanobacteria blooms in eutrophic
115 lakes, we would expect the $\delta^{13}C$ signature of the phytoplankton to increase as ambient CO_2 is
116 depleted, and photosynthetic fractionation factors to decrease as the community approaches a
117 monoculture of phytoplankton using CCM.

118 The purpose of this study was to evaluate the importance of CCMs in maintaining high
119 phytoplankton biomass during CO_2 depletion in eutrophic and hypereutrophic lakes. We
120 hypothesized that photosynthetic fractionation would be tightly coupled with inorganic carbon
121 limitation, resulting in decreased fractionation with shifts from atmospheric CO_2 to mineral
122 HCO_3^- in the water column. We further hypothesized that phytoplankton isotopic composition
123 and photosynthetic fractionation would correspond to CO_2 depletion in the water column,
124 reflecting CCM activation during blooms that are intense enough to lower water column CO_2.

125 **2. Methods**

126 16 lakes were chosen based on Iowa State Limnology Laboratory long-term survey data
127 (total phosphorus and phytoplankton community composition, 2000-2010, data publically
128 available via the Iowa Department of Natural Resources Lake Information System:
129 http://limnology.eeob.iastate.edu/lakereport/) along an orthogonal gradient of watershed
130 permeability (Fraterrigo and Downing, 2008) and interannual variability in Cyanobacteria
131 dominance. Long term survey data were used only for site selection. Duplicate stable isotope
132 samples for particulate organic and dissolved inorganic analyses were collected once following
133 ice off in 2012, weekly May-July, bi-weekly in August, and monthly September-November
134 (*n*=196). Standard physical, chemical, and biological parameters were measured at each
135 sampling event using US-EPA certified methods, including total nitrogen (TN), total phosphorus

136 (TP), chlorophyll a (Chl a), alkalinity and pH. Samples for phytoplankton community
137 characterization were collected three times during the summer in each lake using a vertical
138 column sampler from the upper mixed layer. Aqueous carbon dioxide concentration was
139 measured at 1 m using a Vaisala GMT2220 probe modified for water measurements (Johnson et
140 al., 2009). Partial pressure of carbon dioxide (pCO_2) was determined using temperature, depth,
141 and pressure corrections described in Johnson et al.(2009). Specifically, because pressure and
142 temperature respectively increase and decrease sensor output relative to their calibration,
143 measurements were reduced by 0.15% per unit increase hPa relative to calibration (1013 hPa),
144 and increased 0.15% per unit hPa decrease. An additional correction for depth was added to the
145 barometric pressure correction, because pressure is increased 9.81 hPa per 10 cm depth.
146 Measurements were taken at 1 m, equivalent to a 98.1 hPa increase. Similarly, measurements
147 were increased by 0.3% per degree Celsius increase in water temperature above instrument
148 calibration (25°C).

149 All water chemistry was performed in the Iowa State Limnology Laboratory using United
150 States Environmental Protection Agency (US EPA) certified methods. Total nitrogen was
151 determined using the second derivative method described in (Crumpton et al., 1989). Total
152 phosphorus was determined colorimetrically using the molybdate blue method (APHA, 2012).
153 Samples for Chl *a* analysis were filtered onto GF/C filters which were frozen then extracted and
154 sonicated in cold acetone under red light. Samples were then analyzed fluorometrically (Arar and
155 Collins, 1997; Jeffrey et al., 1997). Alkalinity was determined by acid titration and reported as
156 mg $CaCO_3$ L^{-1} (APHA, 2012). Field measurements of temperature, DO, pH, and conductivity
157 were taken with a YSI multi-parameter probe.

158 Phytoplankton community and biomass samples reported here were processed and
159 analyzed in the Iowa State Limnology Laboratory. These data can also be accessed via the Iowa
160 Department of Natural Resources Lake Information System. Samples were counted to 150
161 natural units of the most abundant genera, and biovolume determined following Hillebrand et al.
162 (1999). Biomass was determined from biovolume assuming cell density of 1.1 g cm^{-3} (Filstrup et
163 al., 2014; Holmes et al., 1969).

164 Samples collected for isotopic analysis of dissolved inorganic carbon ($\delta^{13}C_{DIC}$) were
165 filtered to 0.2 µm in the field using a syringe filter and cartridge containing a combusted GF/F
166 prefilter (Whatman) and 0.2 µm polycarbonate membrane filter (Millipore). Samples were then
167 injected into helium gas-flushed septa-capped vials with H_3PO_4 to cease biological activity and
168 to sparge CO_2 (Beirne et al., 2012; Raymond and Bauer, 2001). $\delta^{13}C_{DIC}$ samples were measured
169 via a Finnigan MAT Delta Plus XL mass spectrometer in continuous flow mode connected to a
170 Gas Bench with a CombiPAL autosampler. Reference standards (NBS-19, NBS-18, and LSVEC)
171 were used for isotopic corrections, and to assign the data to the appropriate isotopic scale (VPDB
172 for carbonates). Average analytical uncertainty (analytical uncertainty and average correction
173 factor) was ±0.06 ‰. Samples were analyzed by standard isotope ratio mass spectrometry
174 methods (IRMS), and reported relative to the Vienna Pee Dee Belemnite in ‰ (Equation 1).
175 $\delta^{13}C_{Sample} = [(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{VPDB} - 1] \times 1000$ Eq. 1
176 To determine the isotopic composition of phytoplankton organic carbon ($\delta^{13}C_{phyto}$),
177 samples were filtered onto pre-combusted GF/C filters. Zooplankton and detritus were removed
178 manually from filtered samples using a dissecting microscope. Samples were gently fumed in a
179 desiccator for 24 h with 1N HCl to remove inorganic carbon, dried in a low temperature oven,
180 then pulverized using a mortar and pestle and analyzed with standard methods (above IRMS

181 connected to a Costech Elemental Analyzer). Calcification is common in marine phytoplankton,

182 but not in eutrophic freshwater lakes and was not observed in our samples. For organic isotope

183 samples, three reference standards (Caffeine [IAEA-600], Cellulose [IAEA-CH-3], and

184 Acetanilide [laboratory standard]) were used for isotopic corrections, and to assign the data to

185 the appropriate isotopic scale (VPDB for carbonates). The average combined uncertainty for

186 $\delta^{13}C$ was ± 0.17‰ (1 sigma, VPDB). For all isotopic measurements, at least one reference

187 standard was used for every six samples.

188 Photosynthetic fractionation factors of biomass relative to ambient CO_2 (ε_p) were

189 calculated using published temperature dependent fractionation factors between carbon species

190 following methods described in Trimborn et al. 2009 (Mook, 1986; Trimborn et al., 2009),

191 reflecting cumulative fractionation occurring during phytoplankton growth. Inorganic carbon

192 fractions and total DIC concentration were calculated using discrete CO_2, alkalinity, and pH

193 measurements:

194

195 $$\delta^{13}C_{HCO_{3-}} = \frac{\delta^{13}C_{DIC}\,[DIC] - (\varepsilon_a[CO_2] + \varepsilon_b[CO_3^{2-}])}{(1+\varepsilon_a * 10^{-3})[CO_2] + [HCO_{3-}] + (1+\varepsilon_b * 10^{-3})[CO_3^{2-}]} \qquad \text{Eq. 2}$$

196 $$\delta^{13}C_{CO2} = \delta^{13}C_{HCO3-}\,(1 + \varepsilon_a \text{ x } 10^{-3}) + \varepsilon_a \qquad \text{Eq. 3}$$

197 $$\varepsilon_p = (\delta^{13}C_{CO2} - \delta^{13}C_{phyto}) / (1 + (\delta^{13}C_{phyto} / 1000)) \qquad \text{Eq. 4}$$

198 where ε_a and ε_b are temperature dependent fractionation factors between CO_2 and HCO_3^-, and

199 HCO_3^- and CO_2^{3-}, respectively (Trimborn et al. 2009, as referenced therein).

200 To test the hypothesized relationships between phytoplankton isotopic composition,

201 photosynthetic fractionation, and ambient pCO_2 (n=196), we used a nonlinear dynamic

202 regression and ran 199 model iterations (SigmaPlot 12, Systat Software) resulting in 100%

203 model convergence. The same approach was used to test the relationship between photosynthetic

204 fractionation (ε_p) and the isotopic composition of the DIC pool. The relationship between
205 phytoplankton biomass as chlorophyll *a* (Chl *a*) and phytoplankton isotopic composition using a
206 Pearson correlation. Prior to analyses, data were tested for normality using a Shapiro Wilk test.

207 **3. Results**

208 Phytoplankton biomass during productive summer months (May-August) ranged from 4.3
209 mg L^{-1} in Springbrook Lake in August to 4120.35 mg L^{-1} in Lake Orient in June. Phytoplankton
210 communities were consistently dominated by cyanobacteria with the exceptions of East Lake
211 Osceola in June and August and Springbrook Lake in August, which were both dominated by
212 diatoms (Figures 1 and 2). Maximum cyanobacteria biomass was measured in Lake Orient in
213 June (4119.34 mg L^{-1}) and the minimum occurred in Silver Lake-D in August (3.70 mg L^{-1}).

214 Phytoplankton $\delta^{13}C$ signatures in this study ranged from -29.86 ‰ to -13.48 ‰ with an
215 average -25.26 ± 2.8 ‰. The highest values were measured when algal biomass peaked (i.e.,
216 during summer months, Table 2). Overall, pH increased slightly and CO_2 decreased during
217 blooms relative to non-bloom conditions (Tables 1 and 2). All lakes except Arrowhead and
218 George Wyth experienced cyanobacteria blooms. Phytoplankton $\delta^{13}C$ and phytoplankton
219 biomass inferred from Chl a concentration were positively correlated (Pearson correlation, µg
220 Chl *a* L^{-1}, $R = 0.60$, $P < 0.001$, Figure 3), suggesting a shift from diffusive to active uptake of
221 inorganic carbon during blooms. Over the course of this study, bloom conditions, defined as > 40
222 µg Chl *a* L^{-1} (Table 1; Bachmann et al. 2003), were observed in 46% of our observations with
223 varying degrees of intensity. TN and TP measured across the study were on average in the
224 eutrophic to hypereutrophic range (Table 1).

225 To evaluate the predicted shift in algal carbon assimilation strategies below atmospheric
226 equilibrium, we used a nonlinear dynamic model to analyze the relationships between ambient
227 pCO_2 and $\delta^{13}C_{phyto}$ across lakes and sampling events. We found that while no relationship existed
228 between these variables above atmospheric equilibrium, there was a rapid, significant increase in
229 $\delta^{13}C_{phyto}$ (Figure 4, top; R^2=0.58, P<0.001) and decrease in fractionation (Figure 4, bottom;
230 R^2=0.66, P<0.001) as CO_2 was depleted below atmospheric equilibrium (393 ppm, NOAA Earth
231 System Research Laboratory, http://www.esrl.noaa.gov/). Relationships between pCO_2 and
232 $\delta^{13}C_{phyto}$ for individual lakes can be found in supplemental information (Figures S1 and S2).

233 We found a significant, positive, non-linear relationship between the stable isotopic
234 composition of the DIC pool and photosynthetic fractionation (ε_p, R^2=0.72, P<0.001, Figure 5).
235 Specifically, the lowest ε_p was observed when the $\delta^{13}C_{DIC}$ values were less than -8 ‰, or
236 atmospheric levels. Below this level, ε_p decreased exponentially toward zero.

237

238 **4.Discussion**

239 Our results indicate that alternative carbon assimilation strategies may be an important
240 mechanism sustaining cyanobacteria blooms in anthropogenically eutrophic and hypereutrophic
241 lakes. While previous studies found no predictive relationship between ambient pCO_2 and
242 photosynthetic fractionation (Bade et al., 2006), others have shown long term relationships
243 between pCO_2 and the isotopic composition of phytoplankton (Smyntek et al., 2012). Here we
244 demonstrate that the relationship between pCO_2 and photosynthetic fractionation exists only
245 when pCO_2 drops below atmospheric equilibrium during blooms. We found a similar clear
246 breakpoint below atmospheric equilibrium between pCO_2 and phytoplankton isotopic

247 composition, together suggesting that CCM mechanisms are switched on in phytoplankton
248 communities when ambient water column CO_2 is depleted below atmospheric levels.
249 The range of values for both $\delta^{13}C_{phyto}$ and ε_p associated with these trends is consistent with
250 previous laboratory and marine field studies demonstrating shifts from diffusive to active
251 inorganic carbon assimilation via CCM activation (Boller et al., 2011; Cassar, 2004; Erez et al.,
252 1998; Trimborn et al., 2009). Calculated photosynthetic fractionation was lowest during blooms,
253 consistent with phytoplankton CCM utilization. While other freshwater studies have
254 demonstrated similar variability in phytoplankton isotopic composition (Vuorio et al., 2006),
255 ours is the first to demonstrate the co-occurrence of decreased fractionation with CO_2 depletion
256 during blooms in eutrophic and hypereutrophic lakes. The cellular mechanisms contributing to
257 the decrease in fractionation likely provide a competitive advantage to bloom-forming taxa when
258 high productivity depletes ambient CO_2.
259 $\delta^{13}C_{DIC}$ values presented in previous studies (e.g., Bade et al., 2006) were more negative
260 than those measured in our study, likely attributable to heterotrophic degradation of terrestrial
261 organic matter in northern temperate oligotrophic to mesotrophic lakes (Bade et al., 2007). In
262 alkaline waters, negative values in this range may also be attributable to atmospheric CO_2
263 invasion and hydroxylation accompanied by kinetic isotopic fractionation. In contrast, $\delta^{13}C_{DIC}$ in
264 our study was relatively enriched in ^{13}C across all lakes and sampling events, with values ranging
265 from -12.5 to + 5.8 ‰, within the range of previously measured values for eutrophic lakes in the
266 same region (de Kluijver et al., 2014). Values in this range can be attributable to mineral
267 dissolution and geochemical fractionation of HCO_3^- at high pH values (Mook 1986; Boutton
268 1991; Bade et al. 2004), and to biogenic methane production via acetate fermentation (Drimmie
269 et al., 1991; Simpkins and Parkin, 1993; Stiller and Magaritz, 1974). In oligotrophic/

270 mesotrophic lakes, these differences correspond to higher average photosynthetic fractionation.
271 In eutrophic/ hypereutrophic lakes, however, fractionation decreased with active uptake of
272 mineral bicarbonate (Sharkey and Berry, 1985).
273 We found a significant positive relationship between photosynthetic fractionation and
274 $\delta^{13}C_{DIC}$, which is opposite of what is generally expected in lakes. In other words, fractionation is
275 expected to increase with decreasing $\delta^{13}C_{DIC}$ values. Across trophic gradients (e.g., $\delta^{13}C_{DIC}$
276 values between -30 ~ + 5 ‰, (Bade et al. 2004; de Kluijver et al. 2014, this study), these
277 relationships would be driven by decreased $\delta^{13}C_{DIC}$ with increasing biomass (i.e., blooms), and
278 decreased fractionation as CCMs are induced (Sharkey and Berry, 1985). In eutrophic and
279 hypereutrophic lakes, however, the range of $\delta^{13}C_{DIC}$ values are enriched overall. Our results
280 suggest that CCMs are functioning and fractionation is lowest when the DIC pool is sourced
281 from mineral dissolution and HCO_3^- is the predominant species (~ -15 to 0 ‰, Boutton 1991).
282 Fractionation increased in these lakes as $\delta^{13}C_{DIC}$ became more positive, possibly indicating a
283 groundwater –sourced CO_2 generated from organic acid decomposition prior to microbial
284 methanogenisis (Simpkins and Parkin, 1993).
285 In eutrophic lakes, both phytoplankton isotopic composition and fractionation appear to be
286 strongly related to pCO_2 availability below a critical equilibrium point. In less productive
287 northern temperate lakes, however, CO_2 is a poor predictor of photosynthetic fractionation
288 (Bade et al., 2006). Our lowest modeled fractionation values reflected active uptake of HCO_3^-,
289 supported by elevated phytoplankton isotopic values. In contrast, northern temperate lakes had a
290 narrower range of phytoplankton isotopic composition (more negative on average), and much
291 higher ambient CO_2 concentrations, both attributable to heterotrophic degradation of terrestrial

292 carbon. These results indicate inorganic carbon availability drives photosynthetic fractionation in
293 eutrophic lakes, but that other processes likely control it (e.g., temperature) in low-nutrient ones.
294 Our results have important implications for how cyanobacteria blooms may be sustained
295 in anthropogenically eutrophic systems. It is well established that high nutrient concentrations
296 result in high phytoplankton biomass (Heisler et al., 2008). It is less clear, however, what
297 mechanisms cause variability in timing and duration of blooms among eutrophic and
298 hypereutrophic lakes. CCMs may provide a competitive advantage to Cyanobacteria when high
299 primary productivity depletes ambient CO_2. This mechanism may allow blooms to be sustained
300 for weeks to months at a time with negligible concentrations of CO_2 in the water column
301 (Cotovicz et al., 2015). While nutrient reduction is ultimately critical in the prevention of blooms
302 (Heisler et al., 2008; Rigosi et al., 2014), the mechanism presented here provides insight into
303 causes of bloom duration and intensity at high nutrient concentrations.
304 Our results show that eutrophic lakes function substantially differently than less impacted
305 surface waters. Temperate lakes are generally considered sources of CO_2 to the atmosphere
306 (Tranvik et al., 2009). We demonstrate that phytoplankton CCM use allows dense phytoplankton
307 to grow at low CO_2 and may facilitate extended periods of high primary production, CO_2
308 depletion, and atmospheric CO_2 uptake in surface waters. These processes may increase
309 sediment C burial and the export of autochthonous organic C (Heathcote and Downing, 2011;
310 Pacheco et al., 2014), and may have the potential to increase methane emissions from anoxic
311 sediments (Hollander and Smith, 2001). Our work demonstrates fundamental differences in
312 inorganic carbon utilization between northern temperate and agricultural, eutrophic lakes.
313 Because the extent of impacted, high nutrient lakes is predicted to increase with the food
314 demands of a growing human population (Foley et al., 2005), understanding mechanisms driving

315 carbon cycling in these systems will be critical in evaluating the impact of cyanobacteria blooms

316 on global carbon cycles.

317

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499

500 **Author contributions** AMMW and JAD jointly conceived the study. AMMW wrote the
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510 **Figure legends**

511 **Figures 1-2.** Community composition (division level) and biomass for three summer sampling
512 points in each lake.

513 **Figure 3.** Correlation between phytoplankton $\delta^{13}C$ and chlorophyll *a,* indicating isotopic
514 enrichment increased with phytoplankton biomass. Dashed line indicates phytoplankton bloom
515 conditions, defined here as >40 µg Chl *a* L^{-1} (Bachmann et al., 2003).

516 **Figure 4. Top.** Relationship between the stable isotopic ambient pCO_2 concentration in surface
517 water and the stable carbon isotopic signature of the phytoplankton community. **Bottom**.
518 Relationship between photosynthetic fractionation (εp, biomass relative to ambient CO_2) and
519 pCO_2. The vertical line indicates atmospheric equilibrium when samples were collected (393
520 ppm). Color of points indicates Chl a concentration: white = 0-40 µg Chl *a* L^{-1}; grey = 41- 100
521 µg Chl *a* L^{-1}; black= > 100 µg Chl *a* L^{-1}. Vertical line indicates atmospheric CO_2 equilibrium
522 when study was conducted (393 ppm).

523

524 **Figure 5.** Relationship between the stable isotopic signature of the ambient DIC pool and
525 photosynthetic carbon fractionation. Color of points indicates Chl a concentration: white = 0-40
526 µg Chl *a* L^{-1}; grey = 41- 100 µg Chl *a* L^{-1}; black= > 100 µg Chl *a* L^{-1}.

527

528

Lake	*n*	*Latitude*	*Longitude*	*TP (μg L^{-1})*	*TN (mg L^{-1})*	*Chl a (μg L^{-1})*	*TA (mg $CaCO_3$ L^{-1})*	*pH*	*δ^{13}DIC (‰ VPBD)*
Arrowhead	13	42.297218	-95.051228	25 ± 8	0.8 ± 0.1	10 ± 6	190 ± 8	8.4 ± 0.1	-1.68 ± 1.08
Badger	13	42.586161	-94.192562	58 ± 35	9.4 ± 5.7	33 ± 34	166 ± 33	8.3 ± 0.4	-2.60 ± 1.96
Beeds	12	42.770320	-93.236436	75 ± 48	7.4 ± 4.5	48 ± 40	193 ± 37	8.4 ± 0.3	-3.12 ± 1.31
Big Spirit	11	43.479377	-95.083424	46 ± 22	1.1 ± 0.3	22 ± 22	168 ± 7	8.6 ± 0.1	0.51 ± 1.03
Black Hawk	12	42.296334	-95.029191	225 ± 118	2.4 ± 0.5	78 ± 35	188 ± 12	8.8 ± 0.2	2.61 ± 1.25
Center	13	43.412607	-95.136293	104 ± 50	1.8 ± 0.2	41 ± 36	163 ± 4	8.5 ± 0.2	2.97 ± 1.70
East Osceola	11	41.032548	-93.742649	195 ± 77	1.9 ± 0.4	80 ± 47	111 ± 27	8.8 ± 0.6	-4.92 ± 2.00
Five Island	14	43.145274	-94.658204	106 ± 50	2.1 ± 0.3	67 ± 37	165 ± 10	8.4 ± 0.2	2.58 ± 1.48
George Wyth	13	42.534834	-92.400362	62 ± 22	1.0 ± 0.2	26 ± 7	141 ± 26	8.4 ± 0.2	-1.63 ± 1.54
Keomah	13	41.295123	-92.537482	106 ± 105	1.4 ± 0.6	44 ± 52	117 ± 15	8.6 ± 0.4	-4.70 ± 1.44
Orient	12	41.196669	-94.436084	397 ± 286	2.3 ± 1.2	144 ± 105	98 ± 22	9.4 ± 0.4	-5.01 ± 5.36
Lower Gar	11	43.352299	-95.120186	95 ± 35	1.6 ± 0.2	50 ± 23	186 ± 14	8.6 ± 0.1	0.19 ± 1.59
Rock Creek	12	41.736936	-92.851859	115 ± 44	1.7 ± 0.4	52 ± 49	148 ± 7	8.5 ± 0.2	-1.43 ± 1.64
Silver-D	12	43.439162	-95.336799	161 ± 85	2.1 ± 0.9	35 ± 58	174 ± 17	8.4 ± 0.2	-2.52 ± 1.23
Silver-PA	12	43.030775	-94.883701	339 ± 206	2.5 ± 0.6	117 ± 60	163 ± 32	8.8 ± 0.3	3.25 ± 1.62
Springbrook	12	41.775930	-94.466736	38 ± 25	1.8 ± 0.9	17 ± 14	181 ± 20	8.3 ± 03	-3.66 ± 1.08

529 Table 1. Summary data for lakes included in this study. Total phosphorus (TP), total nitrogen (TN), chlorophyll *a* (Chl *a*), total

530 alkalinity (TA), pH, and **δ**^{13}DIC are reported as average values of all sampling events (ice free season, April to November 2012) ±

531 standard deviation; n represents the number of observations per lake.

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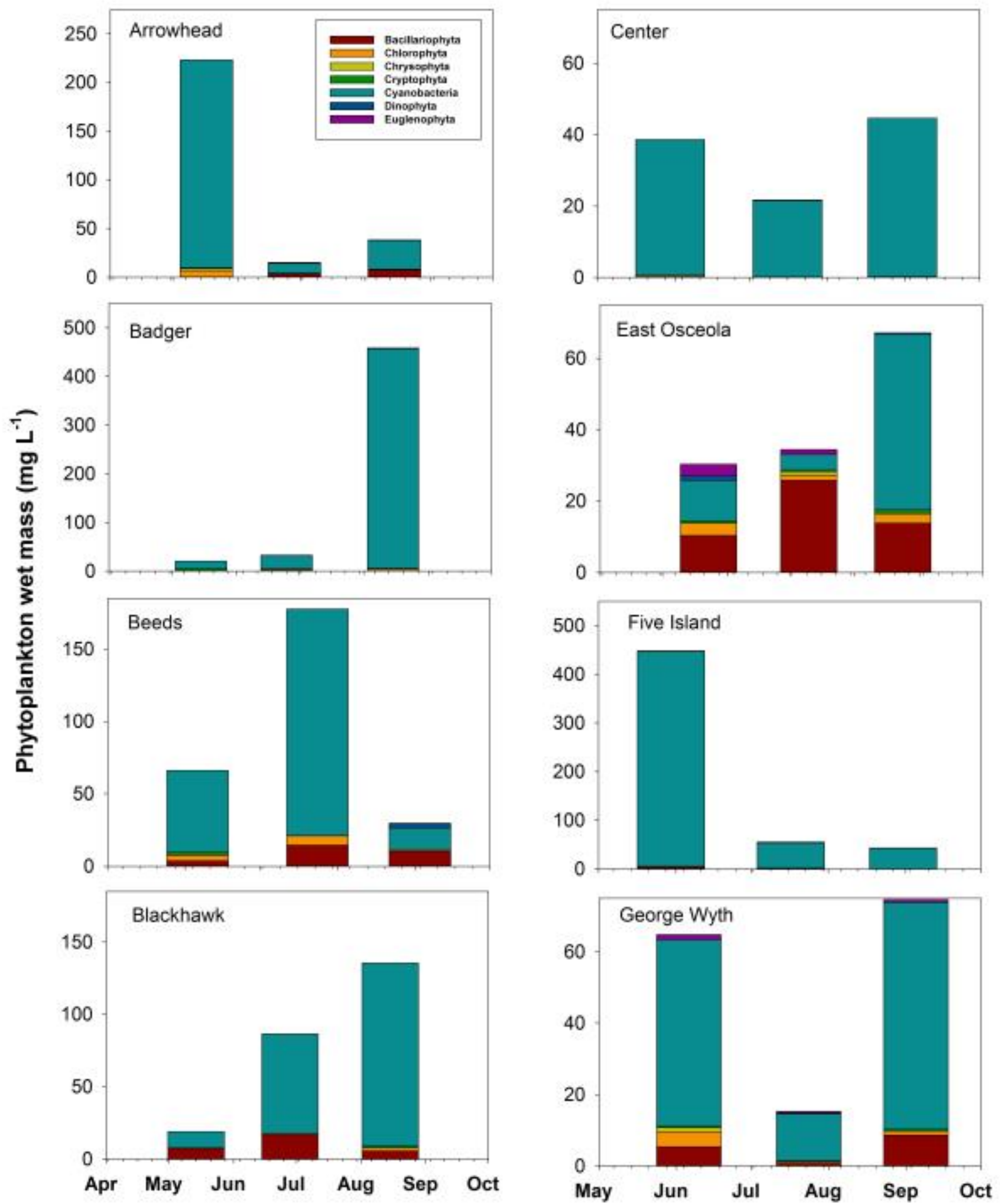
533

Lake	*n*	*Chl a* (*μg L*$^{-1}$)	*TA* (*mg L-1 CaCO3-*)	*pH*	δ^{13}*DIC* (*‰ VPDB*)	δ^{13}*POC* (*‰VPDB*)	ε_p	*pCO2 (ppm)*
Arrowhead	0	NA	NA	NA	NA	NA	NA	NA
Badger	4	71 ± 20	133 ± 28	8.7 ± 0.4	-1.31 ± 1.40	-25.55 ± 2.66	22.70 ± 2.23	234 ± 289
Beeds	4	101 ± 49	170 ± 40	8.6 ± 0.2	-2.23 ± 1.00	-24.07 ± 1.52	20.28 ± 2.32	240 ± 195
Big Spirit	3	68 ± 28	168 ± 10	8.7 ± 0.1	1.43 ± 0.60	-27.04 ± 1.20	26.99 ± 0.83	227 ± 29
Black Hawk	9	86 ± 32	184 ± 10	8.8 ± 0.3	2.75 ± 0.91	-22.34 ± 1.32	23.56 ± 1.36	221 ± 107
Center	8	73 ± 27	164 ± 4	8.7 ± 0.2	4.11 ± 0.90	-22.51 ± 1.23	25.05 ± 1.01	172 ± 92
East Osceola	9	69 ± 24	107 ± 26	8.9 ± 0.6	-5.08 ± 2.23	-24.79 ± 3.55	18.07 ± 4.88	241 ± 457
Five Island	10	84 ± 32	163 ± 9	8.4 ± 0.1	2.92 ± 1.54	-24.65 ± 0.98	26.23 ± 1.67	451 ± 224
George Wyth	0	NA	NA	NA	NA	NA	NA	NA
Keomah	4	63 ± 22	103 ± 11	9.0 ± 0.3	-4.36 ± 1.58	-24.79 ± 1.57	18.53 ± 3.18	29 ± 34
Orient	9	175 ± 77	90 ± 20	9.5 ± 0.5	-5.80 ± 5.90	-18.38 ± 3.13	10.73 ± 8.33	42 ± 53
Lower Gar	7	66 ± 17	177 ± 7	8.7 ± 0.1	1.03 ± 0.87	-25.84 ± 1.04	25.44 ± 0.74	293 ± 86
Rock Creek	7	70 ± 19	148 ± 8	8.6 ± 0.2	-0.78 ± 1.61	-25.42 ± 2.08	23.19 ± 1.47	266 ± 146
Silver-D	3	96 ± 62	168 ± 12	8.7 ± 0.2	-0.92 ± 0.91	-27.65 ± 0.44	25.22 ± 0.71	208 ± 78
Silver-PA	11	135 ± 69	163 ± 34	8.8 ± 0.4	3.59 ± 1.24	-24.27 ± 1.90	26.32 ± 1.39	234 ± 177
Springbrook	1	48	174	8.0	-2.50	-28.57	24.71	375

534 Table 2. Average chemical conditions during bloom events (Chl *a* > 40 μg L^{-1}). Values are average ± standard deviation of *n* observations

535 occurring when Chl *a* exceeded 40 μg L^{-1}. Values are not reported for Arrowhead and George Wyth Lakes because Chl *a* values never exceeded

536 this threshold.



537

538 **Figure 1.**

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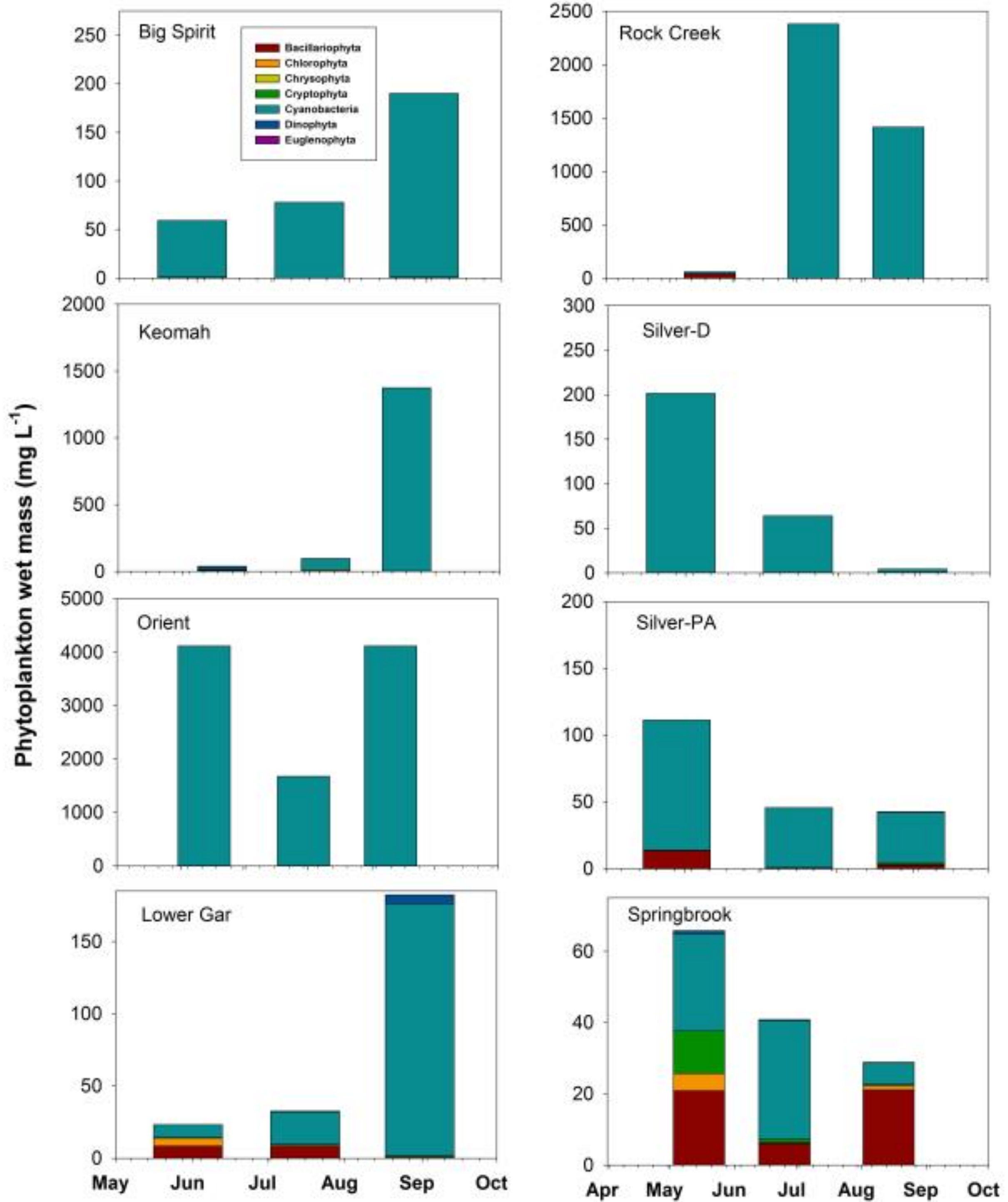

540 **Figure 2.**

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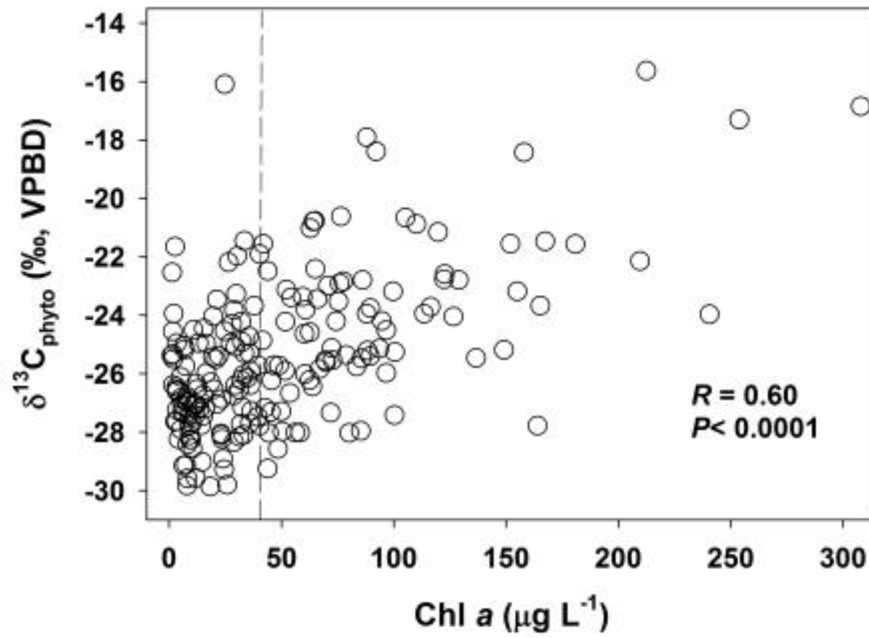

542 **Figure 3.**

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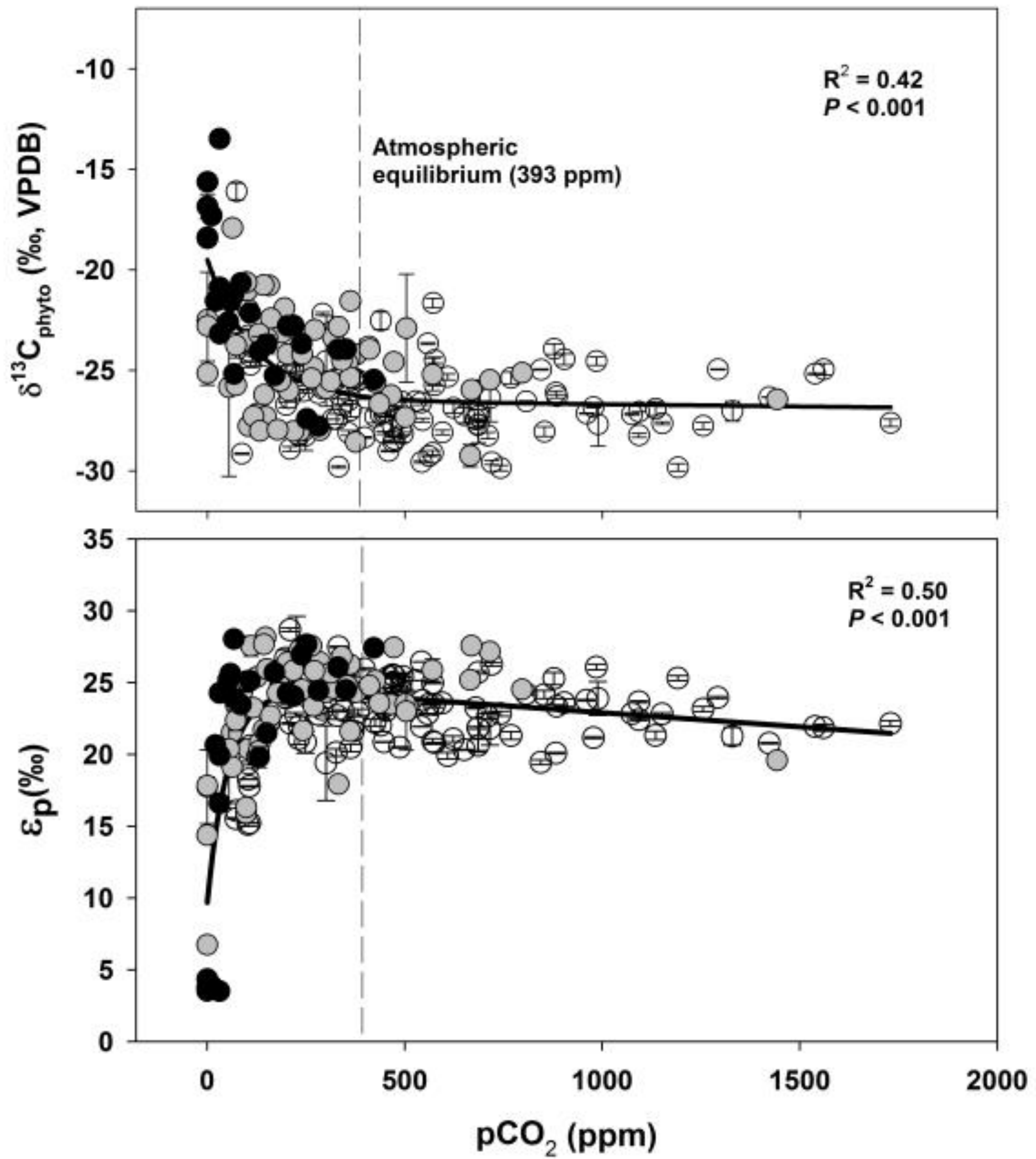

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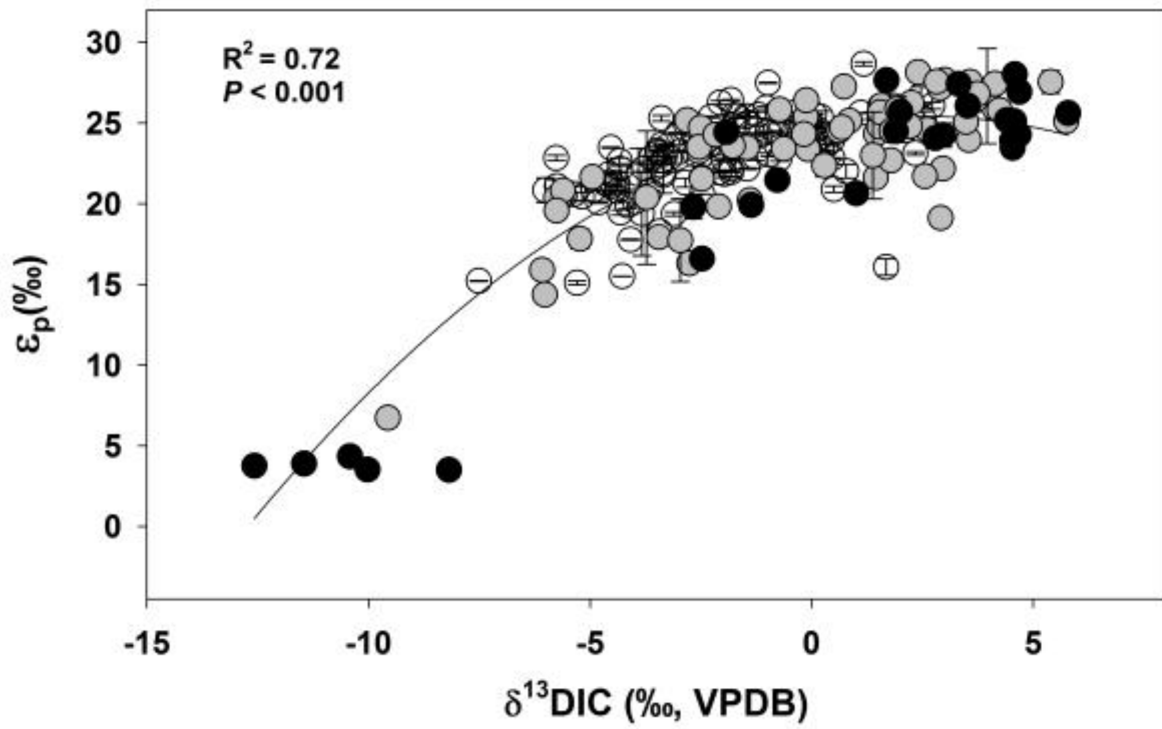
551 **Figure 4.**



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553 **Figure 5.**

554