Thank you to Drs. McConaughey, Verspagen, and one anonymous referee for their thorough and constructive critiques. Responses to each review and relevant changes to the manuscript are detailed below. Reviews below are indented in italics, responses are in normal font. Corresponding changing are highlighted in yellow in the attached manuscript.

Reviewer 1.

1. *Biological lipid membranes just don't retain CO2. They retain HCO3- much better, but 1000-fold accumulation factors (Line 72: Raven et al., 2008) make even an acolyte squirm. (Some papers suggest even higher accumulation ratios.) Is this necessary? Isn't it expensive?*

We agree that 1000x accumulation of internal inorganic carbon relative to ambient concentration poses an osmotic pressure problem. We do not suggest that our measurements reflect these very high internal concentrations, and were only reporting extreme literature values in this case. We have removed this statement and reference.

2. *What sorts of CCM would or wouldn't explain the isotopic results, and what can be inferred about the CCM? For example, what does the isotopic data say about internal carbon concentration factors? Leakage rates?*

L80-86. We have included a discussion of structures specific to cyanobacteria CCMs (carboxysomes) that are thought to decrease leakage via a protein shell and thus decrease isotopic discrimination and fractionation, resulting in an intracellular isotopic composition approaching that of source material.

3. *Title: "Carbon concentrating mechanisms maintain bloom biomass and CO2 depletion in eutrophic lake ecosystems" doesn't mention cyanobacteria or isotopic measurements, which are the focus of the paper.*

We have edited our title to include cyanobacteria, but did not include stable isotopes as they are only the methodology we used to characterize the system: "Cyanobacteria carbon concentrating mechanisms facilitate sustained CO_2 depletion in eutrophic lakes".

4. *Shallow surface water systems are rife with isotopic complications. Wintertime decomposition of organic matter brings springtime high CO2, low pH, low 13C-DIC. Even methane production (line 245) and methane oxidation might alter the 13C of DIC. Hydrology and groundwater inputs can be important (line 244). Carbonate rocks in the soils, like the glacial tills in Iowa, can add isotopically heavy DIC to the system. The present data is further complicated by many different ponds, with individual depths, presence or absence of macrophyte beds, farm water inputs, surface algal scums, different species of algae, blooms at different times, etc. With all this heterogeneity, focus on summertime algal bloom conditions. In the graphs, use larger or darker symbols for bloom conditions. In the table, give separate values typical of bloom conditions, and*

include representative pH, alkalinity, and chlorophyll. In text, please summarize chemical conditions during algal blooms.

We have included a table (Table 2) of average lake chemistry for each site during bloom conditions, defined as chlorophyll *a* exceeding 40 ug/L. A discussion of these data is now included in the results section text at L216-219. We have edited all figures to color points by chl a concentration (white =0-40 ug/L; gray = 41-100 ug/L; black = >100 ug/L, where all values greater than 40 ug/L indicate bloom conditions).

5. *Line 96: "decreased carbon efflux". Carbon efflux may be key to the isotopic balance. Carbon balance models for cyanobacterial CCMs (like Manger and Brennon 2014) sometimes call for large carbon effluxes, sometimes much larger than photosynthetic fluxes. CO2 efflux might leave internal HCO3- relatively enriched in C13, leading to C13 enrichment of photosynthetic products.*

We have addressed the potential for carbon efflux and referenced Manger and Brennon at L80-86.

6. *159: Phytoplankton samples fumed in HCl to remove inorganic carbon. This procedure would mainly be useful if the samples contained lots of it. Its quantity and isotopic composition would be very nice to know. Could you possibly make such measurements? Could CaCO3 or other solid phases account for some of this internal C? Many cyanobacteria do calcify. Calcification is most likely in alkaline waters with significant calcium. Please list ambient pH and alkalinity levels in table 1, and discuss this possibility. Calcification can also act as a CO2 generator (McConnaughey 2012, Mar Ecol Prog Ser doi: 10.3354/meps09776).*

While calcification is common in marine phytoplankton, it is relatively uncommon in eutrophic lakes and was not observed in our study. We have commented on this in the methods section at 181-182. Unfortunately, we are unable to make measurements of the inorganic carbon that was removed in the fuming process. We have included the requested data in Table 1.

7. *163 "appropriate isotopic scale?"*

We have clarified that this refers specifically to VPDB for carbonates at line 185.

8. *191 fractionation of biomass compared to external CO2. (Eq 4 line 173): "p=(13CCO2 - 13Cphyto) / (1 + (13Cphyto / 1000)). Text line 191 (as is figure 2 caption) should specify that you are talking about fractionation of biomass relative to ambient CO2 to prevent confusion (for example, confusion with ambient DIC, internal DIC pool, or internal CO2.) Note that this fractionation factor is a result of the cumulative fractionations that have occurred as the plankton grew. It is not an instantaneous fractionation that occurs at the time of harvest, during the bloom. Can you estimate an instantaneous fractionation?*

We have edited the text and figure caption, now L188 and 191, and Figures 4 and 5 to specify that we are talking about fractionation of biomass relative to ambient CO2.

9. *23, 204: "Harmful" and HCB: This may be true from a human or fish perspective, but this study doesn't address harm.*

We have removed the term "harmful" and all instances of the acronym "HCB" from the manuscript and replaced with "cyanobacteria bloom" throughout.

10. *234, 252: Isotopically light aquatic DIC often comes from decomposition of organic matter, especially in early spring, accompanied by high total DIC and low pH. However, CO2 invasion from air and hydroxylation in alkaline waters during summertime bloom, accompanied by kinetic isotope fractionations, might also cause isotopic enlightenment of DIC.*

We have clarified in the text (now 262-263) that these processes may occur in alkaline waters.

Reviewer 2.

11. *I have two major concerns (detailed below): 1) There is a strong emphasis on cyanobacteria and cyanobacterial blooms in the Introduction section, which is not reflected by the results section, in which only chlorophyll a concentrations are shown. The authors should either reduce the emphasis on cyanobacterial blooms in the Introduction section, or proof that the blooms they sampled were dominated by cyanobacteria.*

We have included community composition and phytoplankton biomass data (Figures 1 and 2, text L208-213.

12. *2) I have a problem with the use of a nonlinear dynamic regression to fit the patterns in Figs 2-4: these regressions do not test an expected relation. However, in Smyntek et al (2012), an isotopic fractionation model is presented that probably fits the data in Fig.3 and 4. I recommend to fit the Smyntek model to your data, it would make the results much stronger.*

After consideration, we do not feel that we have data appropriate to fit all parameters of the Smyntek model. Fitting the model would require several assumptions that we feel would weaken rather than strengthen our results. The purpose of the dynamic regression in our study is to demonstrate the sharp change point and change in the slope of these relationships with the depletion of CO2. Our results using this approach do, however, closely resemble the best fit of the Smyntek model.

13. *The title suggests that CCMs maintain (phytoplankton) bloom biomass. Yet, no evidence is presented that shows a direct relation between CCM activity (i.e. photosynthetic fractionation or delta 13 POC values) and phytoplankton biomass, and no evidence is presented that the use of CCMs maintain phytoplankton biomass. In the Introduction section and in the Discussion section, there is a strong emphasis on cyanobacteria and cyanobacterial blooms. Yet, in the title, the material and methods section, and the results section, there is no mention of cyanobacterial blooms, only of phytoplankton blooms and/or phytoplankton biomass. Are the blooms that you sampled cyanobacterial blooms? Do you have any information on the bloom composition in the lakes you sampled?*

Yes, these blooms are consistently dominated by cyanobacteria. As above, biomass and community composition data have been added to the manuscript. The title has been edited to include cyanobacteria as suggested by Reviewer 1.

14. *Line 70, and lines 259-260: It is assumed here that eukaryotic CCMs are, by definition, less efficient than cyanobacterial CCMs. I'm not convinced. Firstly, recent research suggests that the key components of eukayotic CCMs (although not fully resolved) are very similar to cyanobacterial CCMs (Moroney and Ynalvez 2007, Wang et al 2011, Meyer and Griffiths 2013). Secondly, there is experimental evidence that some chlorophytes can outcompete cyanobacteria at low CO2 concentrations, even when these cyanobacteria have a complete CCM (i.e. they have all known bicarbonate uptake systems). For competition experiments between a cyanobacterium and a chlorophyte, see Verschoor et al (2013) and Li et al (2016), for cyanobacterial CCM gene composition of Synechocystis PCC 6803, see Price et al (2008).*

We have included a discussion of this uncertainty in the Introduction at 74-77, as well as a discussion of carboxysomes structures unique to the cyanobacteria CCM that are thought to decrease leakage and provide efficiency relative to eukaryotic CCMs (L88-86).

15. *Lines 93-104: In this section the authors suggest that cyanobacteria that use CCMs to take up bicarbonate have elevated delta 13C signatures: how about the delta 13C signature of eukaryotic phytoplankton (particularly chlorophytes) that use a CCM to take up bicarbonate? According to the references in lines 215-216, marine eukaryotic phytoplankton also have elevated delta 13C signatures.*

We fully agree with this statement and did not intend to imply that only cyanobacteria would be isotopically heavier with CCM utilization. The focus was on cyanobacteria in this section because our systems specifically had cyanobacteria blooms, not eukaryotic blooms. We have clarified this in the text at L106.

16. *Line 113: "16 lakes were chosen based on . . . survey data". What were the selection criteria?*

Lakes were chosen along an orthogonal gradient of interannual variability in cyanobacteria dominance and watershed permeability. This has been clarified in the text at L129-131.

17. *Line 120-124: Here a listing is given of standard physical, chemical and biological parameters measured at each sampling event. Many of these parameters are not referred to in the results section. Please remove these parameters from the text, or present and discuss them in the results/discussion section. Also, please add alkalinity and pH to Table 1.*

Alkalinity and pH have been added to Table 1. We have removed mention of meteorological data and depth profiles that were not discussed in the results.

18. *Lines 171-173 (equations 2-4). Please explain the parameters in these equations, e.g. in particular, what do epsilon(a) and epsilon(b) mean?*

These are temperature dependent fractionation factors. This has been clarified at 198-199.

19. *I have some concerns about the statistical analysis of the dataset. 1) I wonder whether one has to control for the different lakes. The reason for my concern is that the shape of the fits of the nonlinear regressions of Figs 2, 3 and 4 rely heavily on 6-7 points at low pCO2/low photosynthetic fractionation/low delta 13C of POC.*

We have addressed this by 1) providing plots of the individual lake relationships for del13C-POC and pCO2 in Supplemental Information. Additionally, we have binned the points in each plot by chlorophyll a concentration as suggested by Reviewer 1.

20. *Note that low delta 13C of POC does not necessarily imply high chl a concentrations (Fig. 1). These 6-7 points might come from 1 outlier lake. For this reason, I'm not sure whether a nonlinear dynamic regression (as presented in Figs 2-4) is an appropriate statistical procedure to analyze the dataset. If I understand correctly, nonlinear dynamic regression is an iterative process that may converge to find the best possible curve that fits the dataset. It does not test an expected relation between a dependent and an independent parameter. In Smyntek et al (2012), an isotopic fractionation model is presented (in Eqs 1 and 2, plotted in Fig. 2 of Smyntek et al 2012) that shows relations between pCO2 and delta 13C of POC, and between pCO2 and the photosynthetic fractionation that look remarkably similar to the shape of the curves that were derived in this study by nonlinear dynamic regression (i.e. Fig. 3 and 4). The Smyntek model should also predict the relation between delta 13DIC and the photosynthetic fractionation in Fig. 2. It makes perfect sense to test whether the fractionation model by Smyntek et al (2012) fits your dataset.*

Please see comment above regarding the Smyntek model. Regarding the iterative fit process, the Smyntek model would also be an iterative process resulting in the best fit for the data.

21. *Line 198-199: what kind of regressions are given here? Linear regressions of data with a pCO_2 < 393? Please be more precise: give the name of the regression and the statistical parameters: e.g. Linear regression, $R^2 = 0.90$, $P < 0.01$, $N = 10$*

These are dynamic regression models described in our methods. The model parameters are stated in the text at L229-230.

22. *Table 1: please add two extra columns, one with the averaged alkalinity, and one withthe number of observations per lake (N).*

We have edited Table 1 as suggested.

23. *Fig. 1: x-axis label should be "Chl a (ug L-1)"*

Figure 1 (now Figure 3) axis label has been corrected.

Reviewer 3.

24. *A simple correlation of d13C values with Chl a concentration cannot be used in this study to predict CCM activity.*

This was not our intent. The purpose of this figure was only to visualize an increase in phytoplankton community d13C values with chlorophyll a concentration, which is commonly used as a proxy for phytoplankton biomass. We have edited this figure to only show the correlation between these two variables, rather than a linear regression.

25. *The authors describe the function of the CCM and how this could potentially change the isotopic signature of the cells (see line 91). Recent papers by Eichner et al 2015 and Raven and Beardal 2015 include internal cycling and loss terms of CO_2. These two paper directly affect the interpretation of the data in this MS and should be introduced and discussed. Additional, a paper by Kranz et al 2015 showed the change in epsilon 13C during a bloom of diatoms. These authors also measured CCM parameters directly, seeing a switch from CO_2 to HCO_3^- uptake at low CO_2 conditions. However, this study used a model (Hopkinson) to predict the changes in d13C POC due to the switch to HCO_3^- uptake. The authors could contribute less than 0.5 permill change in the d13C signal to the switch in the inorganic carbon source. Together with the findings by Eichner et al 2015 and Raven and Beardall (2015). I feel that the authors have be aware that isotopic signal of organic matter are not necessarily driven by the uptake of different carbon species, but largely are affected by other cellular processes such as leakage as well as the external d13C DIC. Additionally, different species have different isotopic compositions – do the authors know if the lakes have similar phytoplankton communities?*

We have updated the Introduction to include a more extensive discussion of leakage and effects on isotopic composition (L80-86). We have included community composition data to demonstrate that these communities are dominated by cyanobacteria.

26. *In the method section the authors do not specifically mention how they obtained the biomass measured. Please be more precise in this and also mention how much of the organic material might have been detritus from other sources.*

The previous version of our manuscript used chlorophyll a as a proxy for phytoplankton biomass. We have updated the current manuscript to include biomass calculated from microscopic counts. Methods are detailed at L179-183. We manually removed zooplankton and detritus from filtered samples using a dissecting scope and are confident that the material measured was phytoplankton biomass (L177-178).

27. *The authors have to include the data of TA, DIC, d13C DIC, pH into Table 1 for the reader to understand the dataset and the correlations given.*

We have included these data in Table 1.

28. *The title of the MS is a little farfetched. Neither does the study proof that CCMs maintain biomass in the lakes not did the authors show actual CCM activity. Please revise.*

The title has been updated as suggested by Reviewers 1 and 2.

29. *Line 113: What are the criteria for which the lakes have been chosen? Wouldn't it be sufficient to just mention that 16 lakes were sampled and then briefly describe their properties?*

Lakes were chosen along an orthogonal gradient of interannual variability in cyanobacteria dominance and watershed permeability. This has been clarified in the text at L129-131.

30. *Line 143-145. I feel that this short paragraph should move behind line 160.*

The paragraph has been moved as requested, now L173-175.

31. *Line 171 and 172: describe what alpha a and alpha b means (Temperature-dependent fractionation factors between CO2 and HCO3 (a) as well as HCO3 ⍰ and CO32⍰ (b).*

This has been clarified at L198-199.

32. *Fig 1: Despite being significant, the predictive power of the dataset is relatively low! How would the dataset look like, if you use epsilon vs. Chl a. I feel that this would be more appropriate especially after reading how d13C seems to change in the different lakes.*

As mentioned above, the intent of this figure was not to show predictive power, and we agree that it may not have been appropriate to fit a regression line in this case. We have edited the figure to only show the correlation between these two variables.

33. *Line 220: Please rephrase: “This mechanism likely provides a competitive: : :” The authors refer to decreased fractionation as a mechanism, yet the fractionation calculated is the result of cellular mechanisms such as enhanced HCO_3^- uptake and/or enhanced CO_2 leakage. Maybe rephrase to: “The cellular mechanisms which led to the decrease in fractionation under low pCO_2 likely provide...”*

The text has been edited as suggested, now at 256-258.

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

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

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

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

318 **References**

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Author contributions AMMW and JAD jointly conceived the study. AMMW wrote the manuscript, conducted field sampling and laboratory analysis, and analyzed data. ADW contributed stable isotope methodology and laboratory analyses. JAD supervised the project. The authors declare no competing interests.

Acknowledgements We thank Amber Erickson, Lisa Whitehouse, Dan Kendall, Clayton Williams, and Suzanne Ankerstjerne for chemical and analytical assistance, and Adam Heathcote for his contributions to site selection and sampling design. Thank you to Drs. McConaughey, Verspagen, and one anonymous reviewer for constructive comments on the manuscript. This

508 study was funded by a grant from the National Science Foundation to John A. Downing, DEB-

509 1021525.

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 (εp, biomass relative to ambient CO_2). Color of points

526 indicates Chl a concentration: white = 0-40 µg Chl *a* L^{-1}; grey = 41- 100 µg Chl *a* L^{-1}; black= >

527 100 µg Chl *a* L^{-1}.

528

529

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

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

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

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

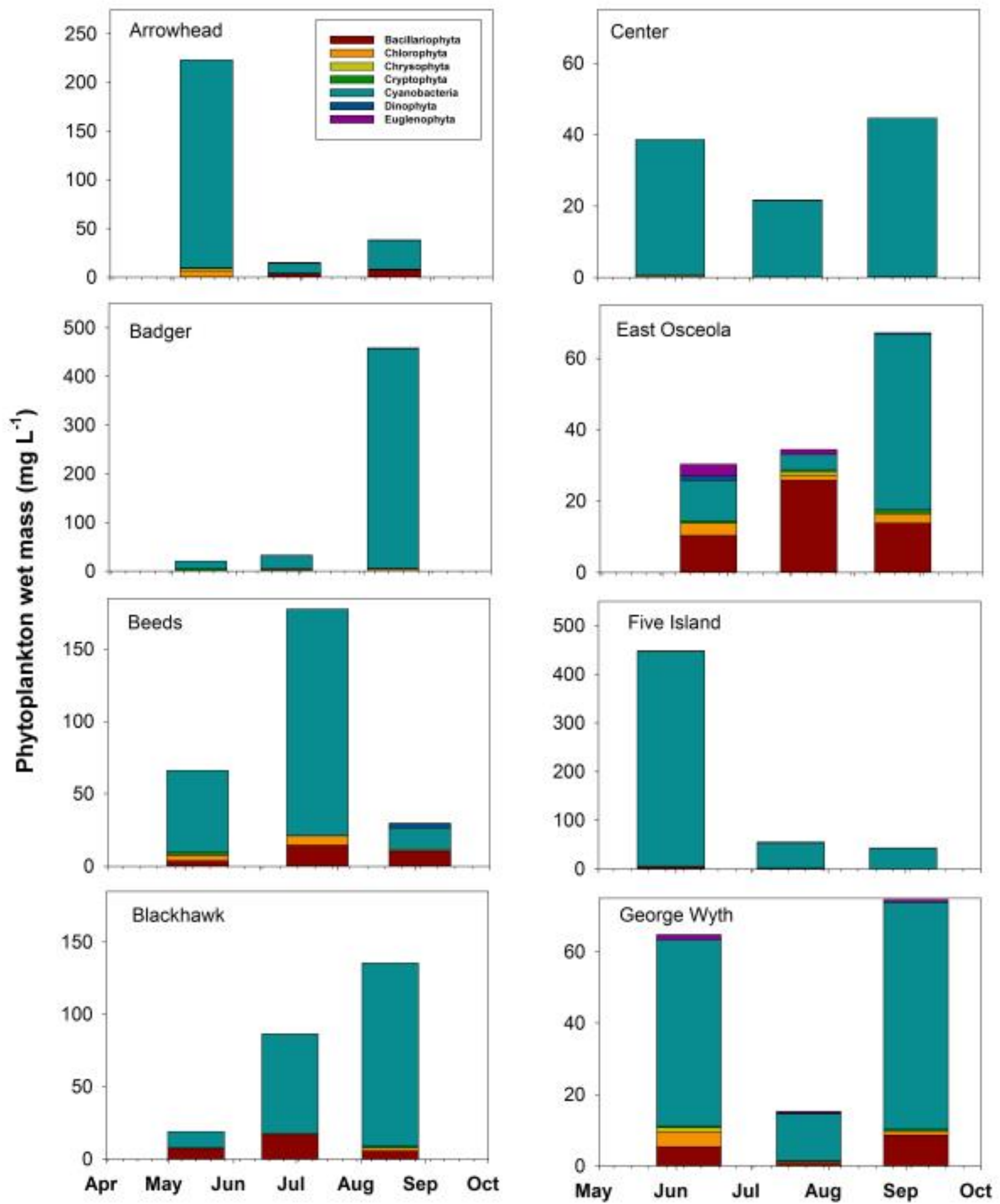
533

534

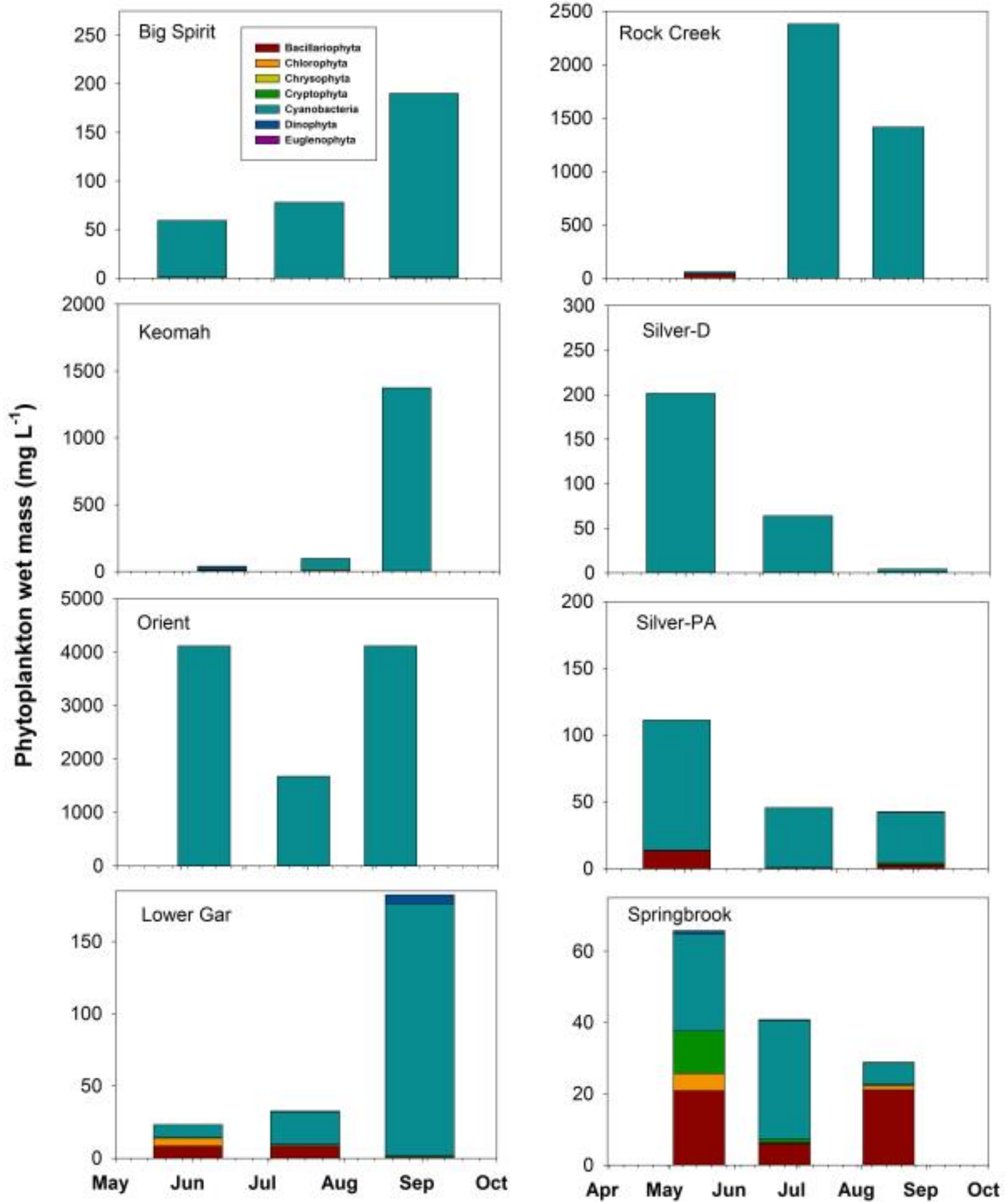
Lake	***n***	***Chl a (µg L^{-1})***	***TA (mg L-1 CaCO3-)***	***pH***	***δ^{13}DIC (‰ VPDB)***	***δ^{13}POC (‰VPDB)***	***ε_p***	***pCO2 (ppm)***
Arrowhead	0	NA	NA	NA	NA	NA	NA	NA
Badger	4	71 ± 20	133 ± 28	8.7 ± 0.4	-1.31 ± 1.40	-25.55 ± 2.66	22.70 ± 2.23	234 ± 289
Beeds	4	101 ± 49	170 ± 40	8.6 ± 0.2	-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

535 Table 2. Average chemical conditions during bloom events (Chl *a* > 40 µg L^{-1}). Values are average ± standard deviation of *n* observations
536 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
537 this threshold.

538



539 **Figure 1.**



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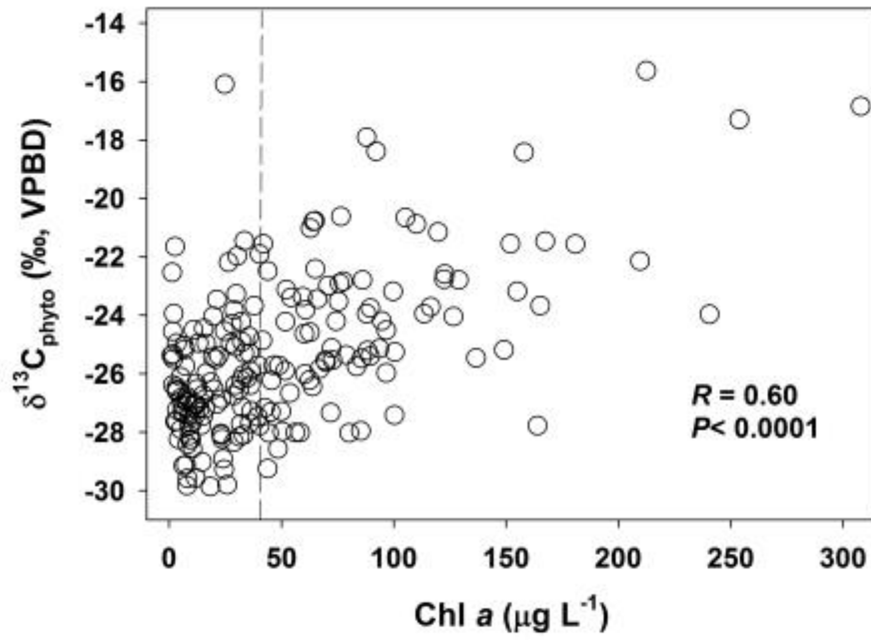
541 **Figure 2.**



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543 **Figure 3.**

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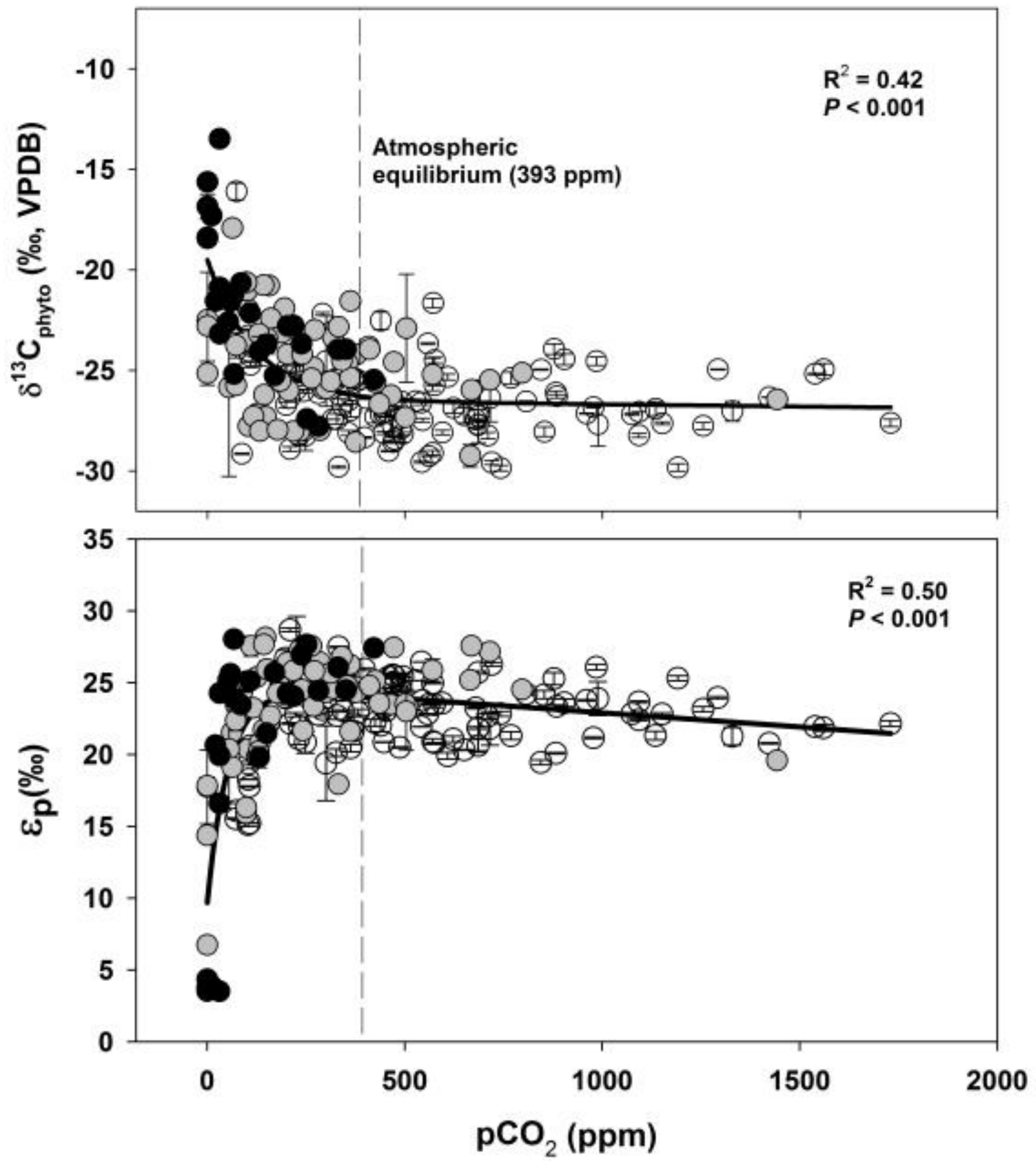

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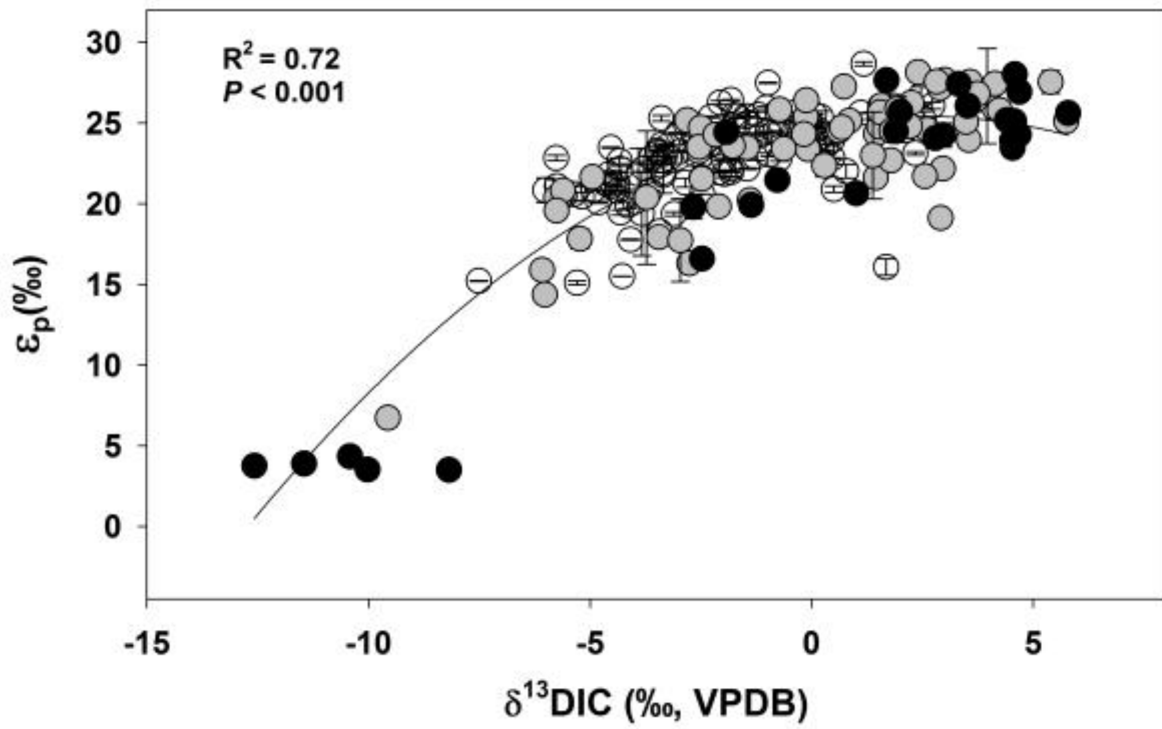
552 **Figure 4.**



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554 **Figure 5.**

555