Thank you again to all reviewers whose comments have substantially improved our manuscript. Final revisions are summarized below. Authors' responses to reviews are in italics.

Reviewer 1

I think the manuscript has improved a lot in clarity and focus after the revision. I am satisfied with the response to the issues I raised, I only have a few suggestions for technical corrections stated below.

1. Throughout the text, the word 'cyanobacteria' is sometimes written with a capital C and sometimes with a lower case c. Please be consistent.

   Resolved

2. Line 83: "HCO3-" misses sub- or superscripts.

   Resolved

3. Line 117: the use of the word 'monoculture' is incorrect here. A monoculture implies a single species. Please adapt this sentence.

   Wording changed to: "...as community becomes dominated by phytoplankton using CCM."

4. Line 171/174: please give the definition of VPDB the first time it is used in the text.

   Resolved

5. Figs 3-5: Could you please provide the equations of the regression curves in these figures?

   We have included equations for figures 4 and 5 on the figures. We did not include a linear equation for Figure 3 because it is a correlation.

6. Lines 233-234: The regression curve through the data in Fig. 5 looks like a parabolic curve that shows a negative relationship between the stable isotopic composition of the DIC pool and photosynthetic fractionation at high delta 13DIC. This must be one of the risks of using a nonlinear dynamic regression. Why not use a saturation function, or a linear regression?

   We have updated this analysis, plot (Figure 5), and corresponding methods (L209) & results (L237-239) using a linear regression.

7. Supporting Information: Could you please use the same range for each x-axis and y-axis (preferably consistent with the top panel of Fig. 4 of the main text)?

   We have edited the axes in the Supporting Information to be consistent with Fig. 4 in the main text.

Reviewer 2

I thank the authors for revising this MS. The topic on isotope fractionation by freshwater algae is a complex one by itself and a multi-location study like the one presented combined with d13C discussion does not make it easy for the reader to follow. Although I'm not an expert on freshwater microalgae and fractionation processes I feel that some important aspects are missing in the discussion.

1) Lakes are like semi-closed systems and the photosynthetic fractionation affects the seawater d13C values and vice versa. The reason why fractionation is minimal when CO2 gets depleted (no source material left). The authors should elaborate on these processes.

   We have added a discussion of diffusive limitation at L309-317.

2) Another general aspect should be addressed: In line 266-272 as well as in the following discussion the authors describe the effect of biotic processes on the source C (CO2/HCO3). How can one conclude on CCM fractionation processes and CO2 sources when the d13C values might be changing constantly due to the multitude of other processes affecting the system?

   We have updated the Discussion section to clarify these points. In this manuscript version and previous ones, we have attempted to constrain potential sources of DIC by documenting literature ranges of sources and processes (i.e., atmosphere, mineral dissolution, methanogenisis, microbial respiration of terrestrially-derived DOM) that could be responsible for our measured range of d13C values. We have clarified in the paragraph beginning at L287 that regardless of the source of DIC -- which cannot be definitively identified, only constrained – a heavy DIC pool combined with decreased photosynthetic fractionation points to active uptake of bicarbonate when CO_2 is depleted from the water column, particularly because in this pH range, geochemical processes dictate that the dominant inorganic carbon species should be bicarbonate, which cannot be taken up by passive diffusion.

Other than these two general remarks/questions and a request to maybe simplify the paper (which seems hard) I see no issue for publication.

In addition to the changes described above, we have revised and attempted to simplify the Discussion by reorganizing the order of paragraphs to improve the flow. Specifically, we moved the paragraph previously beginning at line 285 to (new) line 281 so that the discussion of patterns in northern temperate lakes follows more logically from discussion of other studies.

Cyanobacterial carbon concentrating mechanisms facilitate sustained CO_2 depletion in eutrophic lakes

Ana M. Morales-Williams[1,2,3], Alan D. Wanamaker[4], Jr., and John A. Downing[1,5]

[1]Department of Ecology, Evolution, and Organismal Biology, Iowa State University, 251 Bessey Hall, Ames, IA, 50011, USA

[2]Department of Ecology, Evolution, and Behavior, University of Minnesota-Twin Cities, 1475 Gortner Ave., Saint Paul, MN, 55108, USA

[3]Rubenstein School of Environment and Natural Resources, University of Vermont, 81 Carrigan Drive, Burlington, VT, 05405

[4]Department of Geological and Atmospheric Science, Iowa State University, 12 Science 1, Ames, IA, 50011, USA

[5]Minnesota Sea Grant, University of Minnesota-Duluth, 141 Chester Park, 31 West College St., Duluth, MN, 55812, USA

Correspondence: Ana M. Morales-Williams, ana.morales@uvm.edu

Formatted: Numbering: Restart each section

Abstract

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

Key words: Eutrophication, carbon cycling, cyanobacteria, CCM, stable isotopes

Deleted: C

1. Introduction

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

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

Deleted:

enzyme complexes (Price et al., 2008b). When water column pH exceeds 8.5, CO_2 is negligible and HCO_3^- is the dominant carbon species. HCO_3^- cannot passively diffuse across phytoplankton cell membranes, and therefore requires an active transport system. CCMs are present in many groups of aquatic photoautotrophs including green algae (Spalding, 2008) and diatoms (Hopkinson et al., 2016), as well as some higher plants. These mechanisms are thought to have evolved independently in eukaryotic algae and the cyanobacteria, corresponding to a large decrease in atmospheric CO_2 and doubling of O_2 approximately 400 million years ago (Badger and Price, 2003; Raven et al., 2008). There are, however, many similarities between eukaryotic and cyanobacteria CCMs which are not fully resolved, so it is unclear whether or not cyanobacteria CCMs represent a more efficient, competitive advantage over other phytoplankton taxa (Moroney and Ynalvez, 2007).

The cyanobacterial CCM mechanism facilitates active transport of HCO_3^- across the plasma membrane, where it is accumulated in the cytosol, transferred to Rubisco-containing carboxysomes, and converted to CO_2 via carbonic anhydrases (Raven et al., 2008). Carboxysome structures, unique to cyanobacteria CCMs, are thought to decrease CO_2 leakage rates via low permeability for uncharged species (i.e., CO_2) across the carboxysome protein shell (Kaplan and Reinhold, 1999; Price et al., 2008a). In an optimal CCM, diffusion of HCO_3^- across the carboxysome shell is fast, and leakage of converted CO_2 is slow (Mangan and Brenner, 2014). This results in reduced isotopic discrimination and an intracellular composition approaching that of source material (Fielding et al., 1998).

In freshwaters, cyanobacteria use form 1B Rubisco, which facilitates acclimation to inorganic carbon depletion via high cellular affinity for CO_2 and HCO_3^- (Raven and Beardall, 2016; Raven et al., 2008; Shih et al., 2015). While this process is energetically costly, it is

Comment [AW1]: That way you do not have to define BP

Deleted: BP

Formatted: Subscript

Formatted: Superscript

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

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

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

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

2. Methods

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

Deleted: approaches a monoculture of

Deleted: C

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

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

Deleted: (

Deleted: ,

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

Samples collected for isotopic analysis of dissolved inorganic carbon ($\delta^{13}C_{DIC}$) were filtered to 0.2 µm in the field using a syringe filter and cartridge containing a combusted GF/F prefilter (Whatman) and 0.2 µm polycarbonate membrane filter (Millipore). Samples were then injected into helium gas-flushed septa-capped vials with H_3PO_4 to cease biological activity and to sparge CO_2 (Beirne et al., 2012; Raymond and Bauer, 2001). $\delta^{13}C_{DIC}$ samples were measured via a Finnigan MAT Delta Plus XL mass spectrometer in continuous flow mode connected to a Gas Bench with a CombiPAL autosampler. Reference standards (NBS-19, NBS-18, and LSVEC) were used for isotopic corrections, and to assign the data to the appropriate isotopic scale (Vienna Pee Dee Belemnite, VPDB, for carbonates). Average analytical uncertainty (analytical uncertainty and average correction factor) was ±0.06 ‰ (1 sigma, VPDB). Samples were analyzed by standard isotope ratio mass spectrometry methods (IRMS), and reported relative to VPDB in ‰ (Equation 1).

$$\delta^{13}C_{Sample} = [(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{VPDB} - 1] \times 1000 \quad \text{Eq. 1}$$

To determine the isotopic composition of phytoplankton organic carbon ($\delta^{13}C_{phyto}$), samples were filtered onto pre-combusted GF/C filters. Zooplankton and detritus were removed manually from filtered samples using a dissecting microscope. Samples were gently fumed in a desiccator for 24 h with 1N HCl to remove inorganic carbon, dried in a low temperature oven,

Deleted: the

Deleted: Vienna Pee Dee Belemnite

then pulverized using a mortar and pestle and analyzed with standard methods (above IRMS connected to a Costech Elemental Analyzer). Calcification is common in marine phytoplankton, but not in eutrophic freshwater lakes and was not observed in our samples. For organic isotope samples, three reference standards (Caffeine [IAEA-600], Cellulose [IAEA-CH-3], and Acetanilide [laboratory standard]) were used for isotopic corrections, and to assign the data to the appropriate isotopic scale (VPDB for carbonates). The average combined uncertainty for $\delta^{13}C$ was ± 0.17‰ (1 sigma, VPDB). For all isotopic measurements, at least one reference standard was used for every six samples.

Photosynthetic fractionation factors of biomass relative to ambient CO_2 (ε_p) were calculated using published temperature dependent fractionation factors between carbon species following methods described in Trimborn et al. 2009 (Mook, 1986; Trimborn et al., 2009), reflecting cumulative fractionation occurring during phytoplankton growth. Inorganic carbon fractions and total DIC concentration were calculated using discrete CO_2, alkalinity, and pH measurements:

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

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

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

where ε_a and ε_b are temperature dependent fractionation factors between CO_2 and HCO_3^-, and HCO_3^- and CO_2^{3-}, respectively (Trimborn et al. 2009, as referenced therein).

To test the hypothesized relationships between phytoplankton isotopic composition, photosynthetic fractionation, and ambient pCO_2 (n=196), we used a nonlinear dynamic regression and ran 199 model iterations (SigmaPlot 12, Systat Software) resulting in 100%

model convergence. We used linear regression to test the relationship between photosynthetic fractionation (ε_p) and the isotopic composition of the DIC pool. The relationship between phytoplankton biomass as chlorophyll *a* (Chl *a*) and phytoplankton isotopic composition using a Pearson correlation. Prior to analyses, data were tested for normality using a Shapiro Wilk test.

Deleted: The same approach was used

3. Results

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

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

To evaluate the predicted shift in algal carbon assimilation strategies below atmospheric equilibrium, we used a nonlinear dynamic model to analyze the relationships between ambient pCO_2 and $\delta^{13}C_{phyto}$ across lakes and sampling events. We found that while no relationship existed between these variables above atmospheric equilibrium, there was a rapid, significant increase in $\delta^{13}C_{phyto}$ (Figure 4, top; R^2=0.58, P<0.001) and decrease in fractionation (Figure 4, bottom; R^2=0.66, P<0.001) as CO_2 was depleted below atmospheric equilibrium (393 ppm, NOAA Earth System Research Laboratory, http://www.esrl.noaa.gov/). We found a significant, positive, linear relationship between the stable isotopic composition of the DIC pool and photosynthetic fractionation (ε_p, R^2=0.72, P<0.001, Figure 5). Relationships between pCO_2 and $\delta^{13}C_{phyto}$ for individual lakes can be found in supplemental information (Figures S1 and S2).

4. Discussion

Our results indicate that alternative carbon assimilation strategies may be an important mechanism sustaining cyanobacteria blooms in anthropogenically eutrophic and hypereutrophic lakes. Here we demonstrate that the relationship between pCO_2 and photosynthetic fractionation exists only when pCO_2 drops below atmospheric equilibrium during blooms. We found a similar clear breakpoint below atmospheric equilibrium between pCO_2 and phytoplankton isotopic composition, together suggesting that CCM mechanisms are switched on in phytoplankton communities when ambient water column CO_2 is depleted below atmospheric levels.

While previous models found no predictive relationship between ambient pCO_2 and photosynthetic fractionation (Bade et al., 2006), other proxy-based studies have shown long term relationships between pCO_2 and the isotopic composition of phytoplankton (Smyntek et al., 2012). The range of values measured in our study for both $\delta^{13}C_{phyto}$ and ε_p is consistent with previous laboratory and marine field studies demonstrating shifts from diffusive to active

Formatted: Indent: First line: 0.44", Add space between paragraphs of the same style

Deleted: . ... [1]

Moved down [2]: While previous studies found no predictive relationship between ambient pCO_2 and photosynthetic fractionation (Bade et al., 2006), others have shown long term relationships between pCO_2 and the isotopic composition of phytoplankton (Smyntek et al., 2012).

Moved (insertion) [2]

Deleted: studies

Deleted: s

Deleted: associated with these trends is

inorganic carbon assimilation via CCM activation (Boller et al., 2011; Cassar, 2004; Erez et al., 1998; Trimborn et al., 2009). Calculated photosynthetic fractionation was lowest during blooms, consistent with phytoplankton CCM utilization. While previous freshwater studies have demonstrated similar variability in phytoplankton isotopic composition (Vuorio et al., 2006), ours is the first to demonstrate the co-occurrence of decreased fractionation with CO_2 depletion during blooms in eutrophic and hypereutrophic lakes. The cellular mechanisms contributing to the decrease in fractionation likely provide a competitive advantage to bloom-forming taxa when high productivity depletes ambient CO_2.

In eutrophic lakes, both phytoplankton isotopic composition and fractionation appear to be strongly related to pCO_2 availability below a critical equilibrium point. In less productive northern temperate lakes, however, CO_2 is a poor predictor of photosynthetic fractionation (Bade et al., 2006). Our lowest modeled fractionation values reflected active uptake of HCO_3^-, supported by elevated phytoplankton isotopic values. In contrast, northern temperate lakes had a narrower range of phytoplankton isotopic composition (lower on average), and overall higher ambient CO_2 concentrations, both attributable to heterotrophic degradation of terrestrial carbon. These results suggest an important distinction in carbon cycling between these two regions, where inorganic carbon availability appears to drive photosynthetic fractionation in eutrophic lakes, but is likely controlled by other processes (e.g., temperature) in low-nutrient ones.

Phytoplankton stable isotopic composition is dependent on both on the isotopic composition of DIC source material and fractionation during cellular uptake and assimilation. In our study, the DIC source material ($\delta^{13}C_{DIC}$) was enriched in ^{13}C across all lakes and sampling events, with values ranging from -12.5 to + 5.8 ‰, within the range of previously measured values for eutrophic lakes in the same region (de Kluijver et al., 2014). Source values in this

Deleted: other

range are likely attributable to dissolution of mineral bicarbonate (Mook 1986; Boutton 1991; Bade et al. 2004), but could also be sourced from the atmosphere or biogenic methane production via acetate fermentation (Drimmie et al., 1991; Simpkins and Parkin, 1993; Stiller and Magaritz, 1974). In northern temperate lakes, $\delta^{13}C_{DIC}$ values are generally lower than those measured in our study (e.g., < -25 ‰; Bade et al., 2006), attributable to heterotrophic degradation of terrestrial organic matter (Bade et al., 2007), which is negligible relative to autochthonous organic matter in the eutrophic surface waters of our study sites (authors' unpublished data; in review). Collectively, the active uptake by phytoplankton of DIC source material enriched in ^{13}C combined with decreased photosynthetic fractionation due to CCM processes result in an increase in the carbon stable isotopic signature of the phytoplankton community.

We found a significant positive relationship between photosynthetic fractionation and $\delta^{13}C_{DIC}$. Across trophic gradients (i.e., $\delta^{13}C_{DIC}$ values between -30 ~ + 5 ‰, Bade et al. 2004; de Kluijver et al. 2014, this study), these relationships are driven by decreases in $\delta^{13}C_{DIC}$ values with increasing biomass (i.e., blooms), and decreased fractionation as CCMs are induced (Sharkey and Berry, 1985). Our results suggest that CCMs are functioning and fractionation is lowest when the DIC pool is enriched in ^{13}C (~ -15 to 0 ‰, Boutton 1991). In addition to CCMs, it is possible that observed decreases in photosynthetic fractionation are attributable in part to diffusive limitation, i.e., photosynthetic fractionation decreases because ^{12}C is depleted from the water column and predominantly ^{13}C remains (Raven et al., 2005). During blooms in these very productive systems, however, pH consistently exceeds 8.3 (Table 1), making the dominant inorganic carbon species HCO_3^- due to geochemical carbonate equilibria processes. While rapid diffusive uptake of atmospheric CO_2 near the air-water interface is possible for surface blooms,

Moved (insertion) [1]

Deleted: more negative

Formatted: Superscript

Formatted: Indent: First line: 0"

Deleted: $\delta^{13}C_{DIC}$ values presented in previous studies (e.g., Bade et al., 2006) were more negative than those measured in our study, likely attributable to heterotrophic degradation of terrestrial organic matter in northern temperate oligotrophic to mesotrophic lakes (Bade et al., 2007). In alkaline waters, negative values in this range may also be attributable to atmospheric CO_2 invasion and hydroxylation accompanied by kinetic isotopic fractionation. In contrast, $\delta^{13}C_{DIC}$ in our study was relatively enriched in ^{13}C across all lakes and sampling events, with values ranging from -12.5 to + 5.8 ‰, within the range of previously measured values for eutrophic lakes in the same region (de Kluijver et al., 2014). Values in this range can be attributable to mineral dissolution and geochemical fractionation of HCO_3^- at high pH values (Mook 1986; Boutton 1991; Bade et al. 2004), and to biogenic methane production via acetate fermentation (Drimmie et al., 1991; Simpkins and Parkin, 1993; Stiller and Magaritz, 1974). In oligotrophic/ mesotrophic lakes, these differences correspond to higher average photosynthetic fractionation. In eutrophic/ hypereutrophic lakes, however, fractionation decreased with active uptake of mineral bicarbonate (Sharkey and Berry, 1985).

Moved up [1]: biogenic methane production via acetate fermentation (Drimmie et al., 1991; Simpkins and Parkin, 1993; Stiller and Magaritz, 1974).

Deleted: , which is opposite of what is generally expected in lakes. In other words, fractionation is expected to increase with decreasing $\delta^{13}C_{DIC}$ values.

Deleted: e.g.

Deleted: (

Deleted: would be

Deleted: d

Deleted: In eutrophic and hypereutrophic lakes, however, the range of $\delta^{13}C_{DIC}$ values are enriched overall.

Deleted: sourced from mineral dissolution and HCO_3^- is the predominant species

Formatted: Superscript

Deleted: . Fractionation increased in these lakes as $\delta^{13}C_{DIC}$ became more positive, possibly indicating a groundwater –sourced CO_2 generated from organic acid decomposition prior to microbial methanogenisis (Simpkins and Parkin, 1993)

Formatted: Superscript

Formatted: Superscript

Formatted: Subscript

Formatted: Superscript

Formatted: Subscript

an active uptake mechanism (CCM) is necessary for HCO_3^- utilization and to sustain blooms for weeks to months at a time, as was observed in our study.

Our results have important implications for how cyanobacteria blooms may be sustained in anthropogenically eutrophic systems. It is well established that high nutrient concentrations result in high phytoplankton biomass (Heisler et al., 2008). It is less clear, however, what mechanisms cause variability in timing and duration of blooms among eutrophic and hypereutrophic lakes. CCMs may provide a competitive advantage to cyanobacteria when high primary productivity depletes ambient CO_2. This mechanism may allow blooms to be sustained for weeks to months at a time with negligible concentrations of CO_2 in the water column (Cotovicz et al., 2015). While nutrient reduction is ultimately critical in the prevention of blooms (Heisler et al., 2008; Rigosi et al., 2014), the mechanism presented here provides insight into causes of bloom duration and intensity at high nutrient concentrations.

Our results show that eutrophic lakes function substantially differently than less impacted surface waters. Temperate lakes are generally considered sources of CO_2 to the atmosphere (Tranvik et al., 2009). We demonstrate that phytoplankton CCM use allows dense phytoplankton to grow at low CO_2 concentrations and may facilitate extended periods of high primary production, CO_2 depletion, and atmospheric CO_2 uptake in surface waters. These processes may increase sediment C burial and the export of autochthonous organic C (Heathcote and Downing, 2011; Pacheco et al., 2014), and may have the potential to increase methane emissions from anoxic sediments (Hollander and Smith, 2001). Our work demonstrates fundamental differences in inorganic carbon utilization between northern temperate and agricultural, eutrophic lakes. Because the extent of impacted, high nutrient lakes is predicted to increase with the food demands of a growing human population (Foley et al., 2005), understanding mechanisms driving

Formatted: Subscript

Formatted: Superscript

Deleted: an

Deleted: In eutrophic lakes, both phytoplankton isotopic composition and fractionation appear to be strongly related to pCO_2 availability below a critical equilibrium point. In less productive northern temperate lakes, however, CO_2 is a poor predictor of photosynthetic fractionation (Bade et al., 2006). Our lowest modeled fractionation values reflected active uptake of HCO_3^-, supported by elevated phytoplankton isotopic values. In contrast, northern temperate lakes had a narrower range of phytoplankton isotopic composition (more negativelower on average), and much higher ambient CO_2 concentrations, both attributable to heterotrophic degradation of terrestrial carbon. These results indicate inorganic carbon availability drives photosynthetic fractionation in eutrophic lakes, but that other processes likely control it (e.g., temperature) in low-nutrient ones. ... [2]

Formatted: Indent: First line: 0.44"

Deleted: C

carbon cycling in these systems will be critical in evaluating the impact of cyanobacteria blooms on global carbon cycles.

References

Anneville, O., Domaizon, I., Kerimoglu, O., Rimet, F. and Jacquet, S.: Blue-Green Algae in a Greenhouse Century? New Insights from Field Data on Climate Change Impacts on Cyanobacteria Abundance, Ecosystems, (February), doi:10.1007/s10021-014-9837-6, 2015.

Anon: APHA Standard Methods for the examination of waste and wastewater, 22nd ed., American Public Health Association, Washington D.C., 2012.

Arar, E. J. and Collins, G. B.: Method 445.0 In vitro determination of chlorophyll a and pheophyton a in marine and freshwater algae by fluorescence: Revision 1.2. [online] Available from: c:%5CDocuments and Settings%5Cbwolfend%5CMy Documents%5CElectronic References%5CLibrary%5CArar and Collins 1997.pdf, 1997.

Bachmann, R., Hoyer, M. V. and Canfield, D. E. J.: Predicting the frequencies of high chlorophyll levels in Florida lakes from average chlorophyll or nutrient data, Lake Reserv. Manag., 19(3), 229–241.

Bade, D. L., Carpenter, S. R., Cole, J. J., Hanson, P. C. and Hesslein, R. H.: Controls of delta 13 C-DIC in lakes : Geochemistry , lake metabolism , and morphometry, Limnol. Oceanogr., 49(4), 1160–1172, 2004.

Bade, D. L., Pace, M. L., Cole, J. J. and Carpenter, S. R.: Can algal photosynthetic inorganic carbon isotope fractionation be predicted in lakes using existing models?, Aquat. Sci., 68(2), 142–153, doi:10.1007/s00027-006-0818-5, 2006.

Bade, D. L., Carpenter, S. R., Cole, J. J., Pace, M. L., Kritzberg, E., Bogert, M. C., Cory, R. M. and McKnight, D. M.: Sources and fates of dissolved organic carbon in lakes as determined by whole-lake carbon isotope additions, Biogeochemistry, 84(2), 115–129, doi:10.1007/s10533-006-9013-y, 2007.

Badger, M. R. and Price, G. D.: CO2 concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution, J. Exp. Bot., 54(383), 609–622, doi:10.1093/jxb/erg076, 2003.

Balmer, M. B. and Downing, J. A.: Carbon dioxide concentrations in eutrophic lakes : undersaturation implies atmospheric uptake, Inl. Waters, 1, 125–132, doi:10.5268/IW-1.2.366, 2011.

Beirne, E. C., Wanamaker, A. D. and Feindel, S. C.: Experimental validation of environmental controls on the δ13C of Arctica islandica (ocean quahog) shell carbonate, Geochim. Cosmochim. Acta, 84, 395–409, doi:10.1016/j.gca.2012.01.021, 2012.

Boller, A. J., Thomas, P. J., Cavanaugh, C. M. and Scott, K. M.: Low stable carbon isotope fractionation by coccolithophore RubisCO, Geochim. Cosmochim. Acta, 75(22), 7200–7207,

Deleted: [online] Available from: http://www.tandfonline.com/doi/full/10.1080/07438140309354088 (Accessed 14 July 2015), 2003.

435 doi:10.1016/j.gca.2011.08.031, 2011.

436 Boutton, T. W.: Stable carbon isotope ratios of natural materials: Atmospheric, terrestrial,
437 marine, and freshwater environments, in Carbon Isotope Techniques, edited by D. C. Coleman
438 and B. Fry, pp. 173–183, San Diego., 1991.

439 Brooks, B. W., Lazorchak, J. M., Howard, M. D. A., Johnson, M.-V. V., Morton, S. L., Perkins,
440 D. A. K., Reavie, E. D., Scott, G. I., Smith, S. A. and Steevens, J. A.: Are harmful algal blooms
441 becoming the greatest inland water quality threat to public health and aquatic ecosystems?,
442 Environ. Toxicol. Chem., 35(1), 6–13, doi:10.1002/etc.3220, 2016.

443 Cassar, N.: Bicarbonate uptake by Southern Ocean phytoplankton, Global Biogeochem. Cycles,
444 18(2), 1–10, doi:10.1029/2003GB002116, 2004.

445 Cotovicz, L. C., Knoppers, B. A., Brandini, N., Costa Santos, S. J. and Abril, G.: A strong CO2
446 sink enhanced by eutrophication in a tropical coastal embayment (Guanabara Bay, Rio de
447 Janeiro, Brazil), Biogeosciences, 12(20), 6125–6146, doi:10.5194/bg-12-6125-2015, 2015.

448 Crumpton, W. D., Isenhart, T. M. and Mitchell, P. D.: Nitrate and organic N analyses with
449 second-derivative spectroscopy, Limnol. Oceanogr., 37(4), 907–913, 1989.

450 Drimmie, R. J., Aravena, R., Wassenaar, L. I., Fritz, P., James Hendry, M. and Hut, G.:
451 Radiocarbon and stable isotopes in water and dissolved constituents, Milk River aquifer, Alberta,
452 Canada, Appl. Geochemistry, 6(4), 381–392, doi:10.1016/0883-2927(91)90038-Q, 1991.

453 Erez, J., Bouevitch, A. and Kaplan, A.: Carbon isotope fractionation by photosynthetic aquatic
454 microorganisms : Experiments with Synechococcus PCC7942, a simple carbon flux model, Can.
455 J. Bot., 76, 1109–1118, 1998.

456 Fielding, A. S., Turpin, D. H., Guy, R. D., Calvert, S. E., Crawford, D. W. and Harrison, P. J.:
457 Influence of the carbon concentrating mechanism on carbon stable isotope discrimination by the
458 marine diatom Thalassiosira pseudonana, Can. J. Bot., 76, 1098–1103, 1998.

459 Filstrup, C. T., Hillebrand, H., Heathcote, A. J., Harpole, W. S. and Downing, J. A.:
460 Cyanobacteria dominance influences resource use efficiency and community turnover in
461 phytoplankton and zooplankton communities, Ecol. Lett., 17(4), 464–474,
462 doi:10.1111/ele.12246, 2014.

463 Foley, J. a, Defries, R., Asner, G. P., Barford, C., Bonan, G., Carpenter, S. R., Chapin, F. S.,
464 Coe, M. T., Daily, G. C., Gibbs, H. K., Helkowski, J. H., Holloway, T., Howard, E. a, Kucharik,
465 C. J., Monfreda, C., Patz, J. a, Prentice, I. C., Ramankutty, N. and Snyder, P. K.: Global
466 consequences of land use., Science, 309, 570–4, doi:10.1126/science.1111772, 2005.

467 Fraterrigo, J. M. and Downing, J. a.: The Influence of Land Use on Lake Nutrients Varies with
468 Watershed Transport Capacity, Ecosystems, 11(7), 1021–1034, doi:10.1007/s10021-008-9176-6,
469 2008.

470 Giordano, M., Beardall, J. and Raven, J. A.: CO $_2$ CONCENTRATING MECHANISMS IN
471 ALGAE: Mechanisms, Environmental Modulation, and Evolution, Annu. Rev. Plant Biol., 56(1),
472 99–131, doi:10.1146/annurev.arplant.56.032604.144052, 2005.

473 Gu, B., Schelske, C. L. and Coveney, M. F.: Low carbon dioxide partial pressure in a productive
474 subtropical lake, Aquat. Sci., 73(3), 317–330, doi:10.1007/s00027-010-0179-y, 2010.

475 Heathcote, A. J. and Downing, J. a.: Impacts of Eutrophication on Carbon Burial in Freshwater

Lakes in an Intensively Agricultural Landscape, Ecosystems, 15(1), 60–70, doi:10.1007/s10021-011-9488-9, 2011.

Heisler, J., Glibert, P. M., Burkholder, J. M., Anderson, D. M., Cochlan, W., Dennison, W. C., Dortch, Q., Gobler, C. J., Heil, C. a., Humphries, E., Lewitus, a., Magnien, R., Marshall, H. G., Sellner, K., Stockwell, D. a., Stoecker, D. K. and Suddleson, M.: Eutrophication and harmful algal blooms: A scientific consensus, Harmful Algae, 8(1), 3–13, doi:10.1016/j.hal.2008.08.006, 2008.

Hillebrand, H., Dürselen, C.-D., Kirschtel, D., Pollingher, U. and Zohary, T.: Biovolume calculation for pelagic and benthic microalgae, J. Phycol., 35(2), 403–424, doi:10.1046/j.1529-8817.1999.3520403.x, 1999.

Hollander, D. J. and Smith, M. A.: Microbially mediated carbon cycling as a control on the delta 13C of sedimentary carbon in eutrophic Lake Mendota (USA): New models for interpreting isotopic excursions in the sedimentary record, Geochim. Cosmochim. Acta, 65(23), 4321–4337, doi:10.1016/S0016-7037(00)00506-8, 2001.

Holmes, R., Norris, R., Smayda, T. and Wood, E.: Collection, fixation, identification, and enumeration of phytoplankton standing stock., in Recommended procedures for measuring the productivity of plankton standing stock and related oceanic properties., edited by Anonymous, pp. 17–46, National Academy of Sciences, Washington D.C., 1969.

Hopkinson, B. M., Dupont, C. L. and Matsuda, Y.: The physiology and genetics of CO2 concentrating mechanisms in model diatoms, Curr. Opin. Plant Biol., 31, 51–57, doi:10.1016/j.pbi.2016.03.013, 2016.

Jeffrey, S. W., Mantoura, R. F. C. and S.W. Wright: Phytoplankton Pigments in Oceanography., 1997.

Johnson, M., Billett, M., Dinsmore, K., Wallin, M., Dyson, K. E. and Jassal, R. S.: Direct and continuous measurement of dissolved carbon dioxide in freshwater aquatic systems—method and applications, Ecohydrology, doi:10.1002/eco, 2009.

Kaplan, A. and Reinhold, L.: CO2 Concentrating Mechanisms in Microorganisms, 1999.

de Kluijver, a., Schoon, P. L., Downing, J. a., Schouten, S. and Middelburg, J. J.: Stable carbon isotope biogeochemistry of lakes along a trophic gradient, Biogeosciences, 11(22), 6265–6276, doi:10.5194/bg-11-6265-2014, 2014.

Laas, A., Nõges, P., Kõiv, T. and Nõges, T.: High-frequency metabolism study in a large and shallow temperate lake reveals seasonal switching between net autotrophy and net heterotrophy, Hydrobiologia, 694(1), 57–74, doi:10.1007/s10750-012-1131-z, 2012.

Mangan, N. and Brenner, M.: Systems analysis of the CO2 concentrating mechanism in cyanobacteria, Elife, 2014(3), 1–17, doi:10.7554/eLife.02043, 2014.

Michalak, a. M., Anderson, E. J., Beletsky, D., Boland, S., Bosch, N. S., Bridgeman, T. B., Chaffin, J. D., Cho, K., Confesor, R., Daloglu, I., DePinto, J. V., Evans, M. a., Fahnenstiel, G. L., He, L., Ho, J. C., Jenkins, L., Johengen, T. H., Kuo, K. C., LaPorte, E., Liu, X., McWilliams, M. R., Moore, M. R., Posselt, D. J., Richards, R. P., Scavia, D., Steiner, a. L., Verhamme, E., Wright, D. M. and Zagorski, M. a.: Record-setting algal bloom in Lake Erie caused by agricultural and meteorological trends consistent with expected future conditions, Proc. Natl. Acad. Sci., 110(16), doi:10.1073/pnas.1216006110, 2013.

Deleted: o

519 Mook, W. G.: 13C in Atmospheric CO2, Netherlands J. Sea Res., 20(2/3), 211–223, 1986.
520 Moroney, J. V. and Ynalvez, R. A.: Proposed carbon dioxide concentrating mechanism in
521 Chlamydomonas reinhardtii, Eukaryot. Cell, 6(8), 1251–1259, doi:10.1128/EC.00064-07, 2007.
522 O'Leary, M.: Carbon isotopes in photosynthesis, Bioscience, 38(5), 328–336, 1988.
523 Pacheco, F., Roland, F. and Downing, J.: Eutrophication reverses whole-lake carbon budgets,
524 Inl. Waters, 4(1), 41–48, doi:10.5268/IW-4.1.614, 2014.
525 Paerl, H. W., Hall, N. S. and Calandrino, E. S.: Controlling harmful cyanobacterial blooms in a
526 world experiencing anthropogenic and climatic-induced change, Sci. Total Environ., 409, 1739–
527 1745, doi:10.1016/j.scitotenv.2011.02.001, 2011.
528 Persaud, A. D., Paterson, A. M., Dillon, P. J., Winter, J. G., Palmer, M. and Somers, K. M.:
529 Forecasting cyanobacteria dominance in Canadian temperate lakes, J. Environ. Manage., 151,
530 343–352, doi:10.1016/j.jenvman.2015.01.009, 2015.
531 Price, G., Badger, M., Woodger, F. J. and Long, B. M.: Advances in understanding the
532 cyanobacterial CO2-concentrating-mechanism (CCM): functional components, Ci transporters,
533 diversity, genetic regulation and prospects for engineering into plants, J. Exp. Bot., 59(7), 1441–
534 1461, doi:10.1093/jxb/erm112, 2008a.
535 Price, G. D., Badger, M. R., Woodger, F. J. and Long, B. M.: Advances in understanding the
536 cyanobacterial CO2-concentrating-mechanism (CCM): functional components, Ci transporters,
537 diversity, genetic regulation and prospects for engineering into plants., J. Exp. Bot., 59(7), 1441–
538 61, doi:10.1093/jxb/erm112, 2008b.
539 Raven, J., Ball, L. A., Beardall, J., Giordano, M. and Maberly, S. C.: Algae lacking carbon-
540 concentrating mechanisms, Can. J. Bot., 83(7), 879–890, doi:10.1139/b05-074, 2005.
541 Raven, J. a, Cockell, C. S. and De La Rocha, C. L.: The evolution of inorganic carbon
542 concentrating mechanisms in photosynthesis., Philos. Trans. R. Soc. Lond. B. Biol. Sci.,
543 363(1504), 2641–50, doi:10.1098/rstb.2008.0020, 2008.
544 Raven, J. A. and Beardall, J.: The ins and outs of CO2, J. Exp. Bot., 67(1), 1–13,
545 doi:10.1093/jxb/erv451, 2016.
546 Raymond, P. A. and Bauer, J. E.: DOC cycling in a temperate estuary : A mass balance approach
547 using natural 14 C and C isotopes, Limnol. Oceanogr., 46(3), 655–667, 2001.
548 Rigosi, A., Carey, C. C., Ibelings, B. W. and Brookes, J. D.: The interaction between climate
549 warming and eutrophication to promote cyanobacteria is dependent on trophic state and varies
550 among taxa, Limnol. Oceanogr., 59(1), 99–114, doi:10.4319/lo.2014.59.01.0099, 2014.
551 Sharkey, T. and Berry, J.: Carbon isotope fractionation of algae as influenced by an inducible
552 carbon concentrating mechanism. In: Inorganic carbon uptake by aquatic photosynthetic
553 organisms., 1st ed., edited by W. Lucas and J. Berry, American Society of Plant Physiologists,
554 Rockville., 1985.
555 Shih, P. M., Occhialini, A., Cameron, J. C., Andralojc, P. J., Parry, M. A. J. and Kerfeld, C. A.:
556 Biochemical characterization of predicted Precambrian RuBisCO, Nat. Commun., 7, 1–11,
557 doi:10.1038/ncomms10382, 2015.
558 Simpkins, W. W. and Parkin, T. B.: Hydrogeology and redox geochemistry of CH4 in a Late
559 Wisconsinan Till and Loess Sequence in central Iowa, Water Resour. Res., 29(11), 3643–3657,

Deleted: [online] Available from: http://www.jstor.org/stable/10.2307/1310735 (Accessed 9 April 2013)

Deleted: 1(JANUARY)

doi:10.1029/93WR01687, 1993.

Smyntek, P. M., Maberly, S. C. and Grey, J.: Dissolved carbon dioxide concentration controls baseline stable carbon isotope signatures of a lake food web, Limnol. Oceanogr., 57(5), 1292–1302, doi:10.4319/lo.2012.57.5.1292, 2012.

Spalding, M. H.: Microalgal carbon-dioxide-concentrating mechanisms: Chlamydomonas inorganic carbon transporters., J. Exp. Bot., 59(7), 1463–73, doi:10.1093/jxb/erm128, 2008.

Stiller, M. and Magaritz, M.: Carbon-13 enriched carbonate in interstitial waters of Lake Kinneret sediments, Limnol. Oceanogr., 19(5), 849–853, 1974.

Tranvik, L. J., Downing, J. A., Cotner, J. B., Loiselle, S. A., Striegl, R. G., Ballatore, T. J., Dillon, P., Finlay, K., Fortino, K., Knoll, L. B., Kortelainen, P. L., Kutser, T., Larsen, S., Laurion, I., Leech, D. M., Mccallister, S. L., Mcknight, D. M., Melack, J. M., Overholt, E., Porter, J. A., Prairie, Y., Renwick, W. H., Roland, F., Sherman, B. S., Schindler, D. W., Sobek, S., Tremblay, A., Vanni, M. J., Verschoor, A. M., Wachenfeldt, E. Von and Weyhenmeyer, G. A.: Lakes and reservoirs as regulators of carbon cycling and climate, Most, 54(1), 2298–2314, 2009.

Trimborn, S., Wolf-Gladrow, D., Richter, K.-U. and Rost, B.: The effect of pCO2 on carbon acquisition and intracellular assimilation in four marine diatoms, J. Exp. Mar. Bio. Ecol., 376(1), 26–36, doi:10.1016/j.jembe.2009.05.017, 2009.

Visser, P. M., Verspagen, J. M. H., Sandrini, G., Stal, L. J., Matthijs, H. C. P., Davis, T. W., Paerl, H. W. and Huisman, J.: How rising CO2 and global warming may stimulate harmful cyanobacterial blooms, Harmful Algae, 54, 145–159, doi:10.1016/j.hal.2015.12.006, 2016.

Vuorio, K., Meili, M. and Sarvala, J.: Taxon-specific variation in the stable isotopic signatures (delta13C and delta15N) of lake phytoplankton, Freshw. Biol., 51(5), 807–822, doi:10.1111/j.1365-2427.2006.01529.x, 2006.

Yoshioka, T.: Phytoplanktonic carbon isotope fractionation: equations accounting for CO_2 concentrating _, J. Plankton Res., 19(10_ _), 1455–1476, 1997.

Deleted: [online] Available from: file:///y:/6434.pdf

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, James Cotner and

James Raich for comments on early versions of the manuscript, 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 study was funded by a grant from the National Science Foundation to John A. Downing, DEB-1021525.

Figure legends

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

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

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

Figure 5. Linear relationship between the stable isotopic signature of the ambient DIC pool and photosynthetic carbon fractionation (εp, biomass relative to ambient CO_2. Color of points indicates Chl a concentration: white = 0-40 µg Chl *a* L^{-1}; grey = 41- 100 µg Chl *a* L^{-1}; black= > 100 µg Chl *a* L^{-1}.

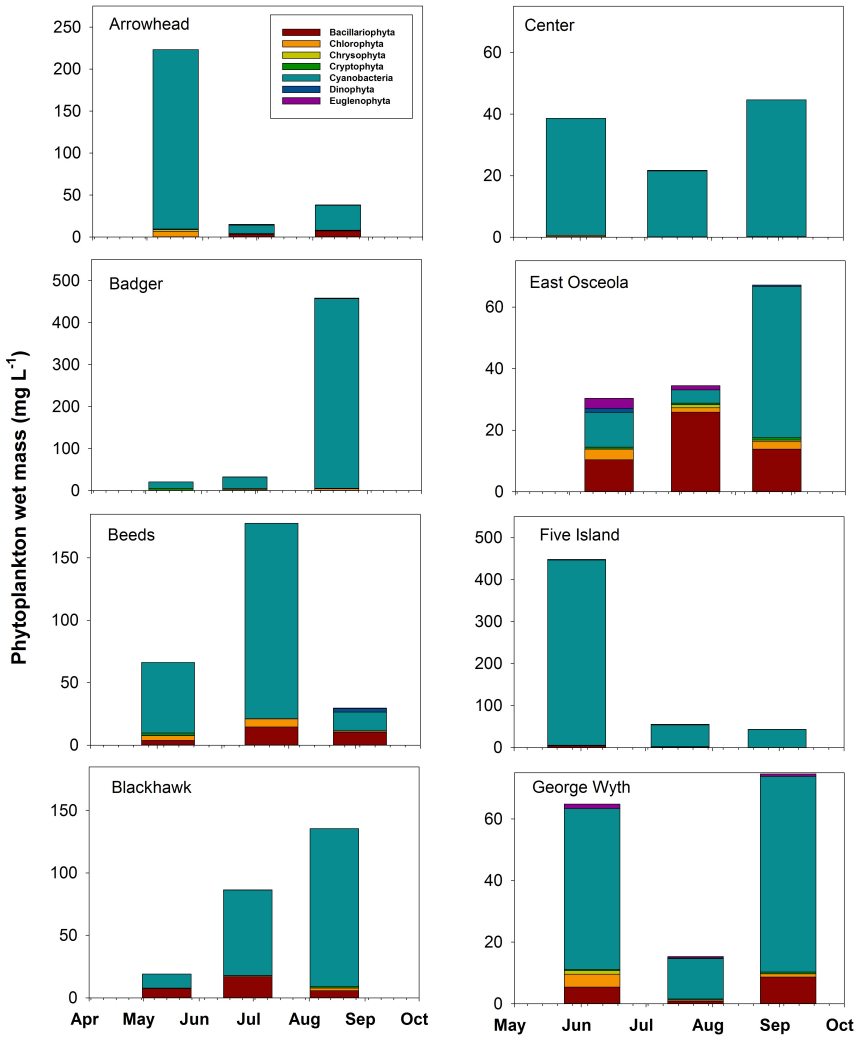
Deleted: R

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

Table 1. Summary data for lakes included in this study. Total phosphorus (TP), total nitrogen (TN), chlorophyll *a* (Chl *a*), total alkalinity (TA), pH, and $\boldsymbol{\delta}^{13}$DIC are reported as average values of all sampling events (ice free season, April to November 2012) ± standard deviation; n represents the number of observations per lake.

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

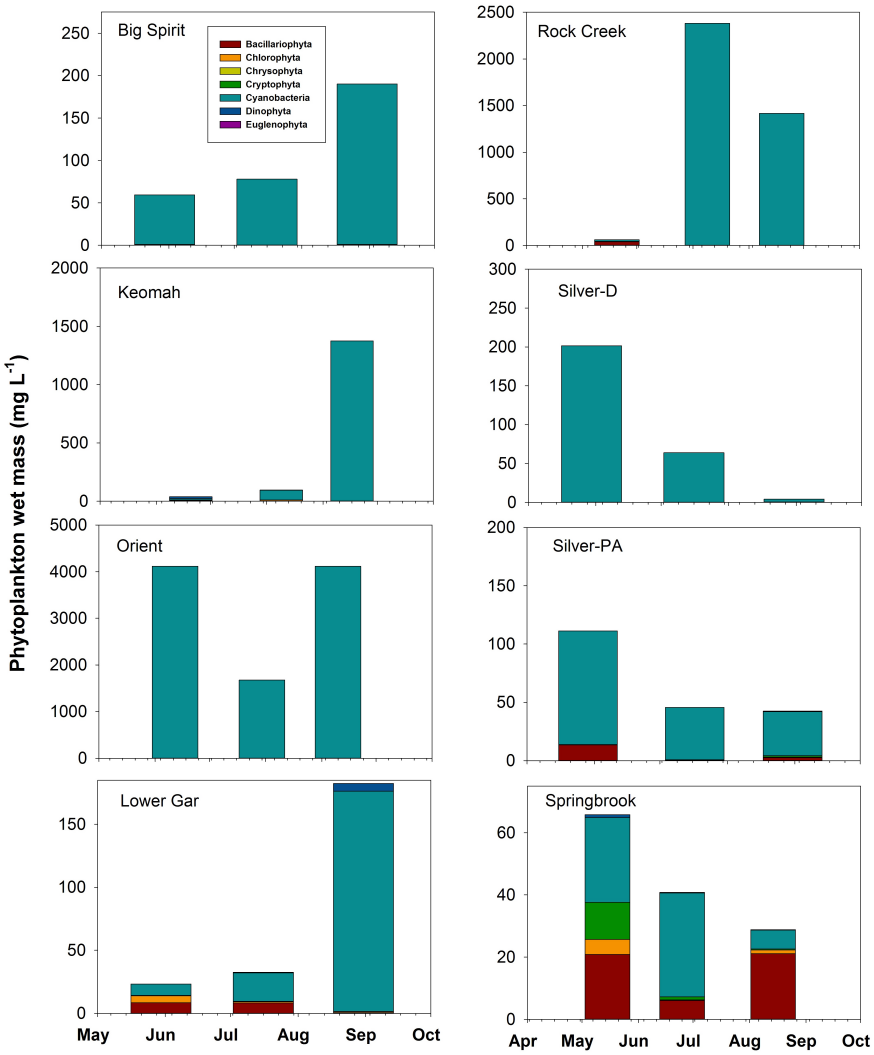
630 Table 2. Average chemical conditions during bloom events (Chl *a* > 40 µg L^{-1}). Values are average ± standard deviation of *n* observations
631 occurring when Chl *a* exceeded 40 µg L^{-1}. Values are not reported for Arrowhead and George Wyth Lakes because Chl *a* values never exceeded
632 this threshold.



633

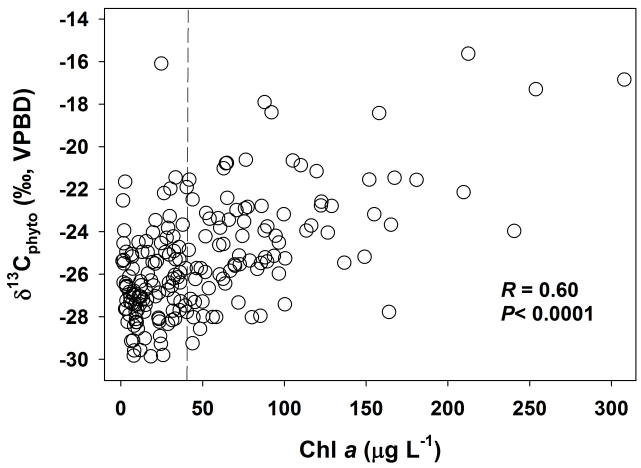
634 **Figure 1.**

0



635

636 **Figure 2.**



637

638 **Figure 3.**

639

640

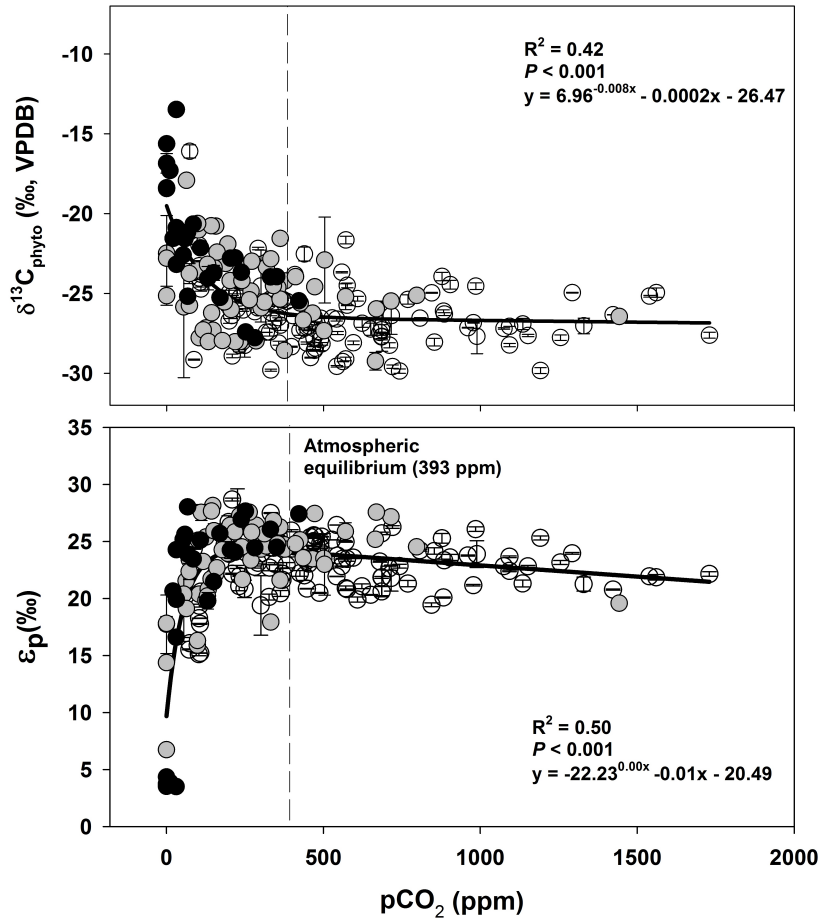
641

642

643

644

645



646

647 **Figure 4.**

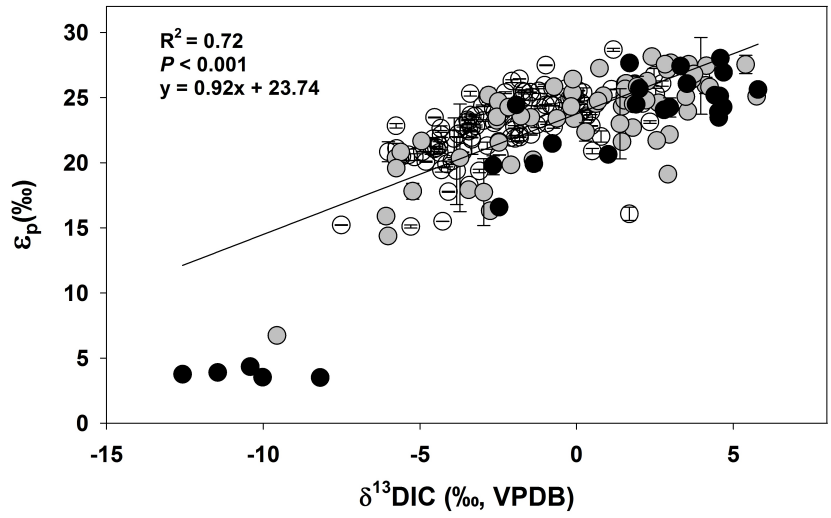

R² = 0.72
P < 0.001
y = 0.92x + 23.74
εp(‰)
δ13DIC (‰, VPDB)
30
25
20
15
10
5
0
-15
-10
-5
0
5

Figure 5.

Page 11: [1] Deleted Ana Morales 4/29/17 12:05:00 PM

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

Page 14: [2] Deleted Ana Morales 5/5/17 8:16:00 AM

In eutrophic lakes, both phytoplankton isotopic composition and fractionation appear to be strongly related to pCO_2 availability below a critical equilibrium point. In less productive northern temperate lakes, however, CO_2 is a poor predictor of photosynthetic fractionation (Bade et al., 2006). Our lowest modeled fractionation values reflected active uptake of HCO_3^-, supported by elevated phytoplankton isotopic values. In contrast, northern temperate lakes had a narrower range of phytoplankton isotopic composition (more negativelower on average), and much higher ambient CO_2 concentrations, both attributable to heterotrophic degradation of terrestrial carbon. These results indicate inorganic carbon availability drives photosynthetic fractionation in eutrophic lakes, but that other processes likely control it (e.g., temperature) in low-nutrient ones.