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Cyanobacterial carbon concentrating mechanisms facilitate sustained CO_2 depletion in eutrophic lakes

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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 the $\delta^{13}C$ signatures of dissolved inorganic carbon ($\delta^{13}C_{DIC}$) and phytoplankton particulate organic carbon ($\delta^{13}C_{phyto}$) in 16 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 nonlinear 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 that active HCO_3^- uptake via CCMs may be an important mechanism in 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.

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 that 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 the 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 in 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 through the 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 also the collectively active transport across the cell membrane, the partitioning of Rubisco into carboxysomes, and the elevation of CO_2 around enzyme complexes (Price et al., 2008). 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 cyanobacteria, corresponding to a large decrease in atmospheric CO_2 and a 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 cyanobacterial CCMs that are not fully resolved, so it is unclear whether or not cyanobacterial CCMs represent a more efficient competitive advantage over other phytoplankton taxa (Moroney and Ynalvez, 2007).

The cyanobacterial CCM facilitates the 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 cyanobacterial 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., 2008). In an optimal CCM, the 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 fresh waters, cyanobacteria use form 1B Rubisco, which facilitates the 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 essential to increase both the photosynthetic efficiency and local bioavailability of inorganic carbon when CO_2 is depleted. In addition to inorganic carbon availability, cyanobacterial CCMs are triggered by photosynthetically active radiation (PAR) and nitrogen availability. Because CCMs are energetically costly (Raven and Beardall, 2016), decreased PAR lowers cellular affinity for inorganic carbon (Giordano et al., 2005). Affinity increases with the depletion of nitrate and iron, but decreases with the depletion of NH_4^+, and does not have a consistent response to phosphorus limitation (Raven et al., 2008). CCM activation under carbon and nutrient stress may thus confer a competitive advantage to cyanobacteria via efficient carbon fixation when CO_2 is low (Badger and Price, 2003; Price et al., 2008).

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

Sixteen lakes were chosen based on the Iowa State Limnology Laboratory long-term survey data (total phosphorus and phytoplankton community composition, 2000–2010; data publicly available via the Iowa Department of Natural Resources Iowa Lakes Information System: http://limnology.eeob.iastate.edu/lakereport/) along an orthogonal gradient of watershed permeability (Fraterrigo and Downing, 2008) and interannual variability in cyanobacterial 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 during May–July, biweekly in August, and monthly during 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 (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. The aqueous carbon dioxide concentration was measured at 1 m using a Vaisala GMT2220 (Vantaa, Finland) probe modified for water measurements (Johnson et al., 2009). The partial pressure of carbon dioxide (pCO_2) was determined using the 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 hPa increase 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, which is 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 (Yellow Springs, OH, USA) multiparameter probe.

The 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 Iowa Lakes Information System. Samples were counted to 150 natural units of the most abundant genera, and biovolume was determined following Hillebrand et al. (1999). Biomass was determined from biovolume assuming a cell density of $1.1\,g\,cm^{-3}$ (Filstrup et al., 2014; Holmes et al., 1969).

Samples collected for the 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, Maidstone, UK) and a 0.2 µm polycarbonate membrane filter (Millipore, Billerica, MA, USA). 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). The $\delta^{13}C_{DIC}$ samples were measured via a Finnigan MAT Delta Plus XL mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) in continuous flow mode connected to a gas bench with a CombiPAL autosampler (Sigma-Aldrich, St. Louis, MO, USA). 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, or VPDB, for carbonates). Average analytical uncertainty (analytical uncertainty and average correction factor) was ±0.06 ‰ (1σ VPDB). Samples were analyzed with standard isotope ratio mass spectrometry methods (IRMS) and reported relative to VPDB in ‰ (Eq. 1):

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

To determine the isotopic composition of phytoplankton organic carbon ($\delta^{13}C_{phyto}$), samples were filtered onto precombusted 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, 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 ‰ (1σ VPDB). For all isotopic measurements, at least one reference standard was used for every six samples.

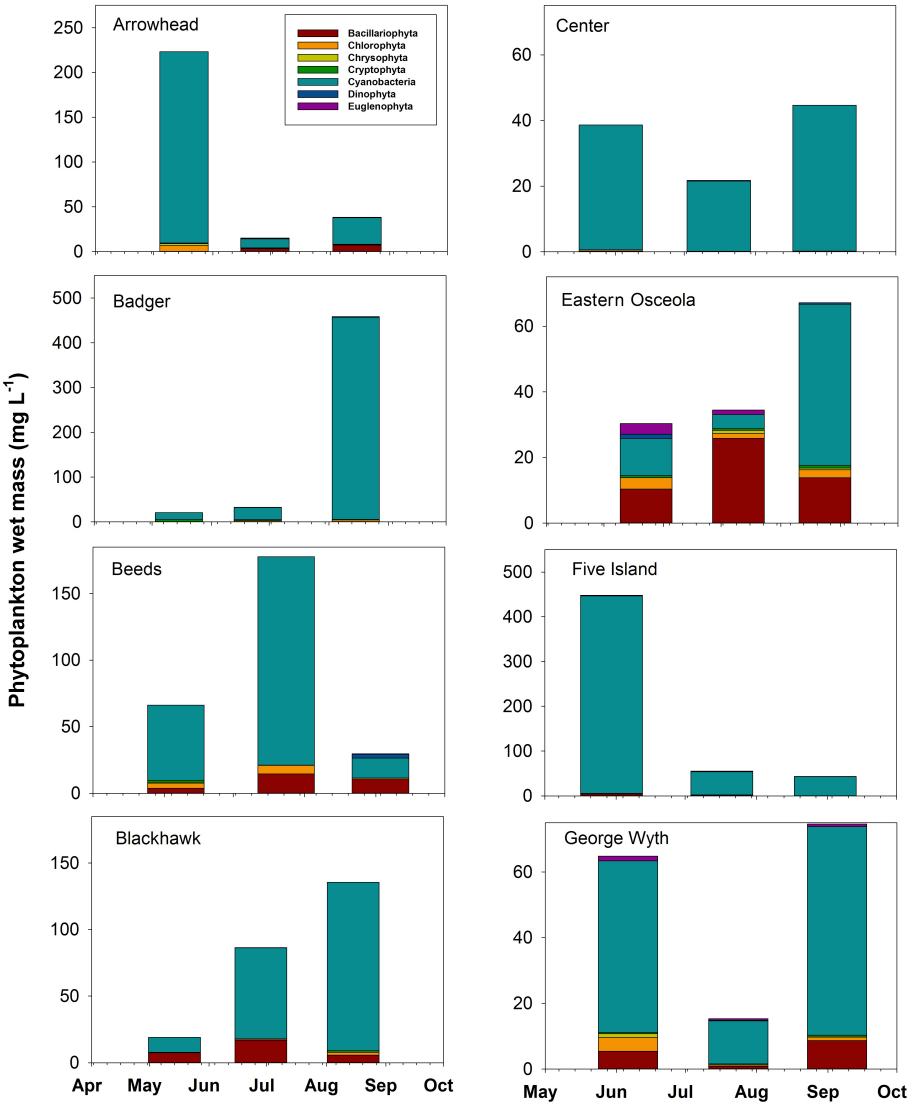

Figure 1. Community composition (division level) and biomass for three summer sampling points in the first 8 of 16 lakes.

Photosynthetic fractionation factors of biomass relative to ambient CO_2 (ε_p) were calculated using published temperature-dependent fractionation factors between carbon species following the 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:

$$\delta^{13}C_{HCO_3-} = \frac{\delta^{13}C_{DIC}\,[DIC] - (\varepsilon_a\,[CO_2] + \varepsilon_b\left[CO_3^{2-}\right])}{(1+\varepsilon_a \times 10^{-3})\,[CO_2] + \left[HCO_3-\right] + (1+\varepsilon_b \times 10^{-3})\left[CO_3^{2-}\right]}, \quad (2)$$

$$\delta^{13}C_{CO_2} = \delta^{13}C_{HCO_3^-}\left(1+\varepsilon_a \times 10^{-3}\right) + \varepsilon_a, \quad (3)$$

$$\varepsilon_p = \left(\delta^{13}C_{CO_2} - \delta^{13}C_{phyto}\right) / \left(1 + (\delta^{13}C_{phyto}/1000)\right), \quad (4)$$

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 was tested using a Pearson correlation. Prior to anal-

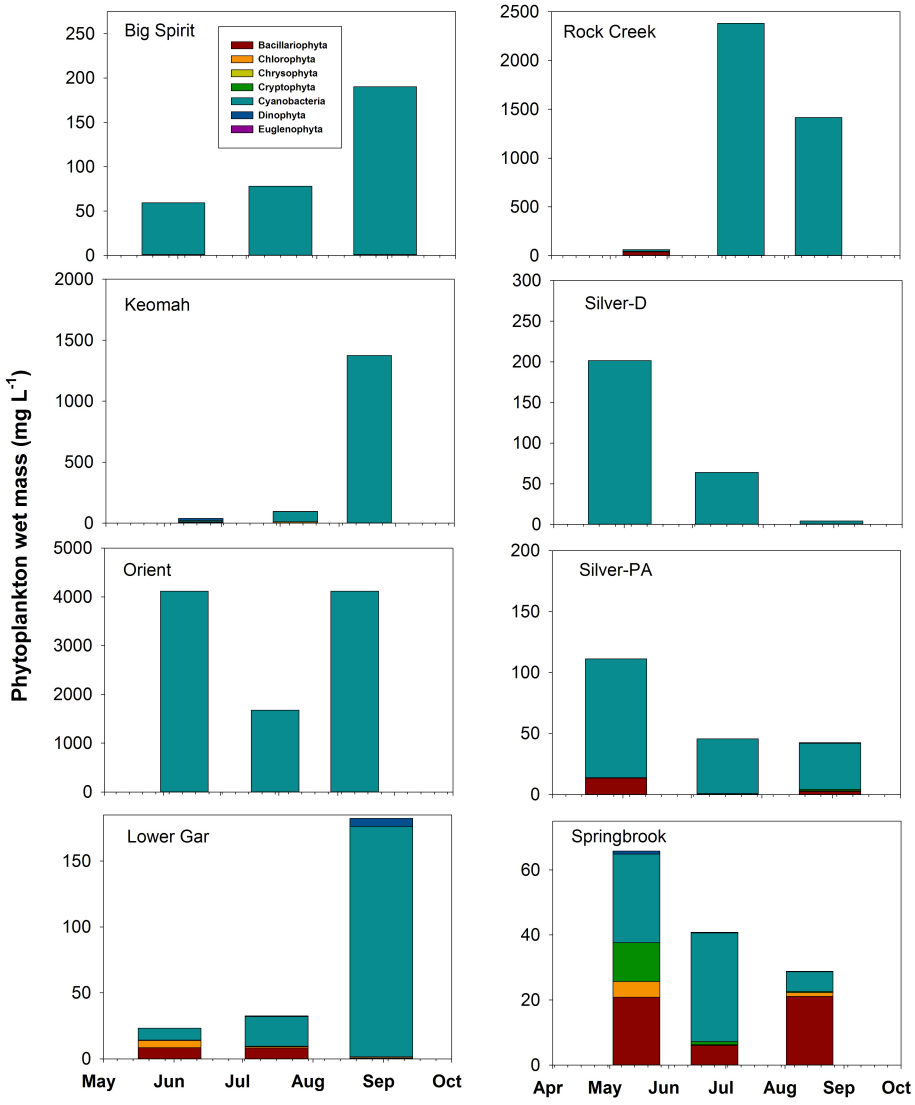

Figure 2. Community composition (division level) and biomass for three summer sampling points in the remaining 8 of 16 lakes.

yses, data were tested for normality using a Shapiro–Wilk test.

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 eastern Lake Osceola in June and August and Springbrook Lake in August, which were both dominated by diatoms (Figs. 1 and 2). Maximum cyanobacterial biomass was measured in Lake Orient in June (4119.34 mg L^{-1}) and the minimum occurred in Silver Lake-D (in Dickinson County, Iowa, USA) 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 of -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}, $R = 0.60$, $P < 0.001$; Fig. 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

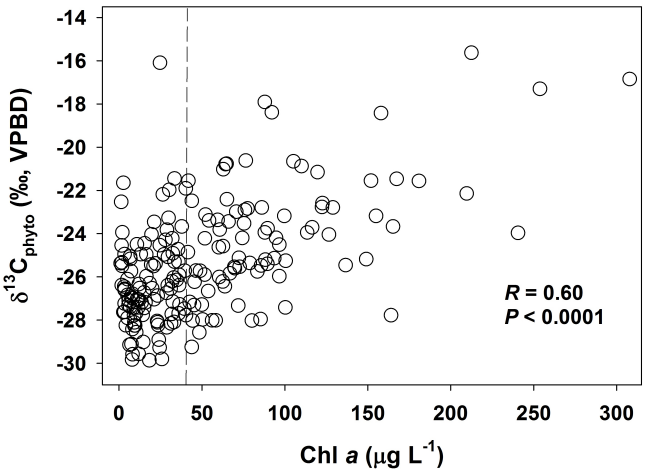

Figure 3. Correlation between phytoplankton $\delta^{13}C$ and chlorophyll a, indicating that isotopic enrichment increased with phytoplankton biomass. The dashed line indicates phytoplankton bloom conditions, defined here as $>40\,\mu g$ Chl $a\,L^{-1}$ (Bachmann et al., 2003).

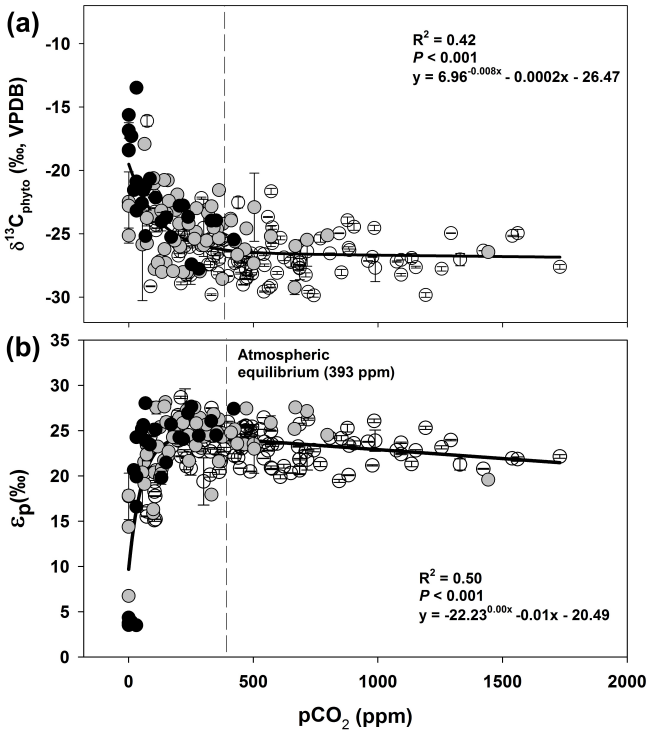

Figure 4. (a) Nonlinear relationship between the stable isotopic ambient pCO_2 concentration in surface water and the stable carbon isotopic signature of the phytoplankton community. **(b)** Nonlinear relationship between photosynthetic fractionation (ε_p; biomass relative to ambient CO_2) and pCO_2. The vertical line indicates atmospheric equilibrium when samples were collected (393 ppm). The color of the 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 $a\,L^{-1}$. The vertical line indicates atmospheric CO_2 equilibrium when the study was conducted (393 ppm).

measured across the study were in the eutrophic to hypereutrophic range on average (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 $\delta^{13}C_{phyto}$ (Fig. 4a; $R^2 = 0.58$, $P<0.001$) and a decrease in fractionation (Fig. 4b; $R^2 = 0.66$, $P<0.001$) 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; $R^2 = 0.72$, $P<0.001$; Fig. 5). Relationships between pCO_2 and $\delta^{13}C_{phyto}$ for individual lakes can be found in the Supplement (Supplement Figs. S1 and S2).

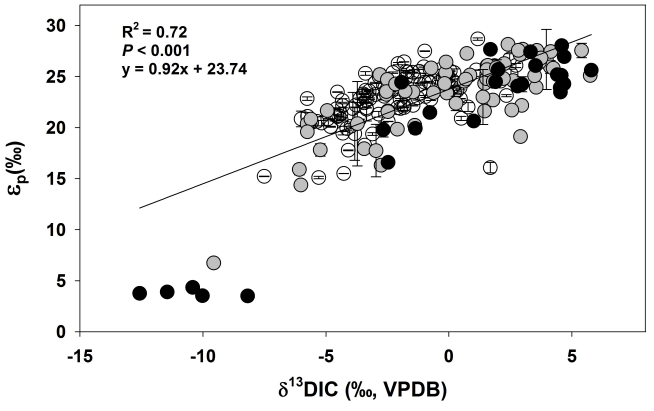

Figure 5. Linear relationship between the stable isotopic signature of the ambient DIC pool and photosynthetic carbon fractionation (ε_p; biomass relative to ambient CO_2). The color of the points indicates Chl a concentration: white = 0–40 μg Chl $a\,L^{-1}$; grey = 41–100 μg Chl $a\,L^{-1}$; black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

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 similarly clear breakpoint below atmospheric equilibrium between pCO_2 and phytoplankton isotopic composition, suggesting that CCMs are switched on in phytoplankton communities when ambient water column CO_2 is depleted below atmospheric levels.

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 δ^{13}DIC are reported as average values of all sampling events (ice-free season, April–November 2012) ± SD; *n* represents the number of observations per lake.

Lake	*n*	Latitude	Longitude	TP ($\mu g\,L^{-1}$)	TN ($mg\,L^{-1}$)	Chl *a* ($\mu g\,L^{-1}$)	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
Eastern 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 2. Average chemical conditions during bloom events (Chl $a > 40\,\mu g\,L^{-1}$). Values are average ± SD of *n* observations occurring when Chl *a* exceeded $40\,\mu g\,L^{-1}$. Values are not reported for Arrowhead and George Wyth because Chl *a* values never exceeded this threshold.

Lake	*n*	Chl *a* ($\mu g\,L^{-1}$)	TA ($mg\,L^{-1}\ CaCO_3^-$)	pH	δ^{13}DIC (‰ VPDB)	δ^{13}POC (‰ VPDB)	ε_p	pCO_2 (ppm)
Arrowhead	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a
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
Eastern 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	n/a	n/a	n/a	n/a	n/a	n/a	n/a
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

The n/a indicates not applicable.

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 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, which is 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 pro-

vide 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 the active uptake of HCO_3^-; this was 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 the 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 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 range are likely attributable to the 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 the 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). 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 and $+5$ ‰; 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 that 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 the rapid diffusive uptake of atmospheric CO_2 near the air–water interface is possible for surface blooms, 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 the 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 the 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 the mechanisms driving carbon cycling in these systems will be critical in evaluating the impact of cyanobacteria blooms on global carbon cycles.

Data availability. The dataset for this manuscript is available via Figshare: https://doi.org/10.6084/m9.figshare.5103052.v1.

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Author contributions. AMMW and JAD jointly conceived the study. AMMW wrote the paper, conducted field sampling and laboratory analysis, and analyzed data. ADW contributed stable isotope methodology and laboratory analyses. JAD supervised the project.

Competing interests. The authors declare that they have no conflict of interest.

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