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 CO₂ depletion in surface waters. Cyanobacteria and other groups of phytoplankton have the ability to actively transport bicarbonate (HCO₃⁻) across their cell membrane when CO₂ 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 CO₂ depletion, we measured δ¹³C signatures of dissolved inorganic carbon (δ¹³C_DIC) and phytoplankton particulate organic carbon (δ¹³C_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 CO₂ stress. We found a significant positive relationship between phytoplankton biomass and phytoplankton δ¹³C signatures, as well as a significant non-linear negative relationship between water column ρCO₂ and isotopic composition of phytoplankton, indicating a shift from diffusive uptake to active uptake by phytoplankton of CO₂ or HCO₃⁻ during blooms. Calculated photosynthetic fractionation factors indicated that this shift occurs specifically when surface water CO₂ drops below atmospheric equilibrium. Our results indicate active HCO₃⁻ uptake via CCMs may be an important mechanism maintaining phytoplankton blooms when CO₂ 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
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
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
essential to both increase photosynthetic efficiency and local bioavailability of inorganic carbon
when CO₂ 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 depletion of nitrate and iron, but
decreases with depletion of NH₄⁺, and does not have a consistent response to phosphorus
limitation (Raven et al., 2008). CCM activation under carbon and nutrient stress thus may confer
a competitive advantage to cyanobacteria via efficient carbon fixation when CO₂ is low (Badger
and Price, 2003; Price et al., 2008b).

Shifts to alternative carbon assimilation strategies result in measureable changes in
isotopic fractionation. 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 CO₂, photosynthetic fractionation
resembles that of C3 terrestrial plants (Yoshioka, 1997), resulting in typical mean δ¹³C signatures
between -27‰ to -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 HCO₃⁻ uptake can have elevated δ¹³C 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 HCO₃⁻
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
(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.
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\(_{\text{DIC}}\)) 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\(_3\)PO\(_4\) to cease biological activity and to sparge CO\(_2\) (Beirne et al., 2012; Raymond and Bauer, 2001). \(\delta^{13}\)C\(_{\text{DIC}}\) 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).

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

To determine the isotopic composition of phytoplankton organic carbon (\(\delta^{13}\)C\(_{\text{phyto}}\)), 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,
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 δ¹³C 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 CO₂ (εₚ) 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 CO₂, alkalinity, and pH measurements:

\[
δ^{13}C_{HCO_3^-} = \frac{δ^{13}C_{DIC}[DIC]-(ε_a[CO_2]+ε_b[CO_3^{2-}])}{(1+ε_a*10^{-3})[CO_2]+[HCO_3^-]+(1+ε_b*10^{-3})[CO_3^{2-}]}
\]  
Eq. 2

\[
δ^{13}C_{CO_2} = δ^{13}C_{HCO_3^-} (1 + ε_a x 10^{-3}) + ε_a
\]  
Eq. 3

\[
ε_p = (δ^{13}C_{CO_2} - δ^{13}C_{phyto}) / (1 + (δ^{13}C_{phyto} / 1000))
\]  
Eq. 4

where εₐ and εₐ are temperature dependent fractionation factors between CO₂ and HCO₃⁻, and HCO₃⁻ and CO₃²⁻, respectively (Trimborn et al. 2009, as referenced therein).

To test the hypothesized relationships between phytoplankton isotopic composition, photosynthetic fractionation, and ambient pCO₂ (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 ($\varepsilon_p$) and the isotopic composition of the DIC pool. The relationship between
phytoplankton biomass as chlorophyll $a$ ($\text{Chl} \, a$) and phytoplankton isotopic composition using a
Pearson correlation. Prior to analyses, 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 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 $-25.26 \pm 2.8$ ‰. 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, $\mu g$
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 $> 40$
$\mu g$ 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 ($\varepsilon_p$, $R^2=0.72$, $P<0.001$, 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 $\varepsilon_p$ 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, 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$_{\text{DIC}}$) 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 dissolution of mineral bicarbonate (Mook 1986; Boutron 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$_{\text{DIC}}$ values are generally lower than those measured in our study (e.g., $<-25\,\%_{\text{o}}$; 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$_{\text{DIC}}$. Across trophic gradients (i.e., $\delta^{13}$C$_{\text{DIC}}$ values between $-30\sim+5\,\%_{\text{o}}$, Bade et al. 2004; de Kluijver et al. 2014, this study), these relationships are driven by decreases in $\delta^{13}$C$_{\text{DIC}}$ 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 ($\sim-15$ to $0\,\%_{\text{o}}$, Boutron 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 $\text{HCO}_3^-$ due to geochemical carbonate equilibria processes. While rapid diffusive uptake of atmospheric CO$_2$ near the air-water interface is possible for surface blooms,
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
carbon cycling in these systems will be critical in evaluating the impact of cyanobacteria blooms on global carbon cycles.

References


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.

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**Figure legends**

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

**Figure 3.** Correlation between phytoplankton δ¹³C 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 pCO₂ 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 CO₂) and pCO₂. 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 CO₂ 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 ($\varepsilon_p$, 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$^{-1}$.)
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<th>Lake</th>
<th>n</th>
<th>Latitude</th>
<th>Longitude</th>
<th>TP (µg L⁻¹)</th>
<th>TN (mg L⁻¹)</th>
<th>Chl a (µg L⁻¹)</th>
<th>TA (mg CaCO₃ L⁻¹)</th>
<th>pH</th>
<th>δ¹³DIC (% VPBD)</th>
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<td>0.8 ± 0.1</td>
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<td>67 ± 37</td>
<td>165 ± 10</td>
<td>8.4 ± 0.2</td>
<td>2.58 ± 1.48</td>
</tr>
<tr>
<td>George Wyth</td>
<td>13</td>
<td>42.534834</td>
<td>-92.400362</td>
<td>62 ± 22</td>
<td>1.0 ± 0.2</td>
<td>26 ± 7</td>
<td>141 ± 26</td>
<td>8.4 ± 0.2</td>
<td>-1.63 ± 1.54</td>
</tr>
<tr>
<td>Keomah</td>
<td>13</td>
<td>41.295123</td>
<td>-92.537482</td>
<td>106 ± 105</td>
<td>1.4 ± 0.6</td>
<td>44 ± 52</td>
<td>117 ± 15</td>
<td>8.6 ± 0.4</td>
<td>-4.70 ± 1.44</td>
</tr>
<tr>
<td>Orient</td>
<td>12</td>
<td>41.196669</td>
<td>-94.436084</td>
<td>397 ± 286</td>
<td>2.3 ± 1.2</td>
<td>144 ± 105</td>
<td>98 ± 22</td>
<td>9.4 ± 0.4</td>
<td>-5.01 ± 5.36</td>
</tr>
<tr>
<td>Lower Gar</td>
<td>11</td>
<td>43.352299</td>
<td>-95.120186</td>
<td>95 ± 35</td>
<td>1.6 ± 0.2</td>
<td>50 ± 23</td>
<td>186 ± 14</td>
<td>8.6 ± 0.1</td>
<td>0.19 ± 1.59</td>
</tr>
<tr>
<td>Rock Creek</td>
<td>12</td>
<td>41.736936</td>
<td>-92.851859</td>
<td>115 ± 44</td>
<td>1.7 ± 0.4</td>
<td>52 ± 49</td>
<td>148 ± 7</td>
<td>8.5 ± 0.2</td>
<td>-1.43 ± 1.64</td>
</tr>
<tr>
<td>Silver-D</td>
<td>12</td>
<td>43.439162</td>
<td>-95.336799</td>
<td>161 ± 85</td>
<td>2.1 ± 0.9</td>
<td>35 ± 58</td>
<td>174 ± 17</td>
<td>8.4 ± 0.2</td>
<td>-2.52 ± 1.23</td>
</tr>
<tr>
<td>Silver-PA</td>
<td>12</td>
<td>43.030775</td>
<td>-94.883701</td>
<td>339 ± 206</td>
<td>2.5 ± 0.6</td>
<td>117 ± 60</td>
<td>163 ± 32</td>
<td>8.8 ± 0.3</td>
<td>3.25 ± 1.62</td>
</tr>
<tr>
<td>Springbrook</td>
<td>12</td>
<td>41.775930</td>
<td>-94.466736</td>
<td>38 ± 25</td>
<td>1.8 ± 0.9</td>
<td>17 ± 14</td>
<td>181 ± 20</td>
<td>8.3 ± 0.3</td>
<td>-3.66 ± 1.08</td>
</tr>
</tbody>
</table>

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 δ¹³DIC 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.
Table 2. Average chemical conditions during bloom events (Chl a > 40 µg L⁻¹). Values are average ± standard deviation of n observations 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 this threshold.
Figure 1.
Figure 2.
Figure 3.
Figure 4.
Figure 5.