

## ***Interactive comment on “Carbon concentrating mechanisms maintain bloom biomass and CO<sub>2</sub> depletion in eutrophic lake ecosystems” by Ana M. Morales-Williams et al.***

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Thank you to Drs. McConaughey, Verspagen, and one anonymous referee for their thorough and constructive critiques. Responses to each review are below.

Reviewer 1.

Response to general comments:

Reviewer 1 expresses skepticism regarding the existence of CCMs, comparing them to cosmological “dark energy”, and noting the irony of their reports primarily from small phytoplankton. There is a substantial body of literature documenting this system, its molecular components, and evolutionary origins, which are summarized in our intro-

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duction and several reviews on the topic (e.g., Badger et al., 2006; Hopkinson et al., 2016; Kaplan and Reinhold, 1999; Raven et al., 2008). Many of the reports of this mechanism are from small phytoplankton because much of the early work on this topic was done in culture using model organisms. It has, however, been demonstrated in larger diatoms (Trimborn et al., 2009) and larger colonial cyanobacteria (Eichner et al., 2015).

Reviewer 1 asks if CCMs are necessary and whether they are energetically expensive. CCMs are not just the accumulation of inorganic carbon, but collectively the (1) active transport of CO<sub>2</sub> or HCO<sub>3</sub><sup>-</sup> across the cell membrane, (2) partitioning of Rubisco into carboxysomes, and (3) elevation of CO<sub>2</sub> around these enzyme complexes (Price et al., 2008). Yes, this is an energetically costly process, but it is necessary in that it both increases photosynthetic efficiency and local bioavailability of inorganic carbon when CO<sub>2</sub> is depleted (e.g., rapid growth and high biomass during blooms). When water column pH exceeds 8, CO<sub>2</sub> is negligible and HCO<sub>3</sub><sup>-</sup> is the dominant inorganic carbon species. HCO<sub>3</sub><sup>-</sup> cannot passively diffuse across phytoplankton cell membranes, and therefore requires some sort of active transport system, which falls under the umbrella of CCMs. We agree that 1000x accumulation of internal inorganic carbon relative to ambient concentration poses an osmotic pressure problem. We do not suggest that our measurements reflect these very high internal concentrations, and are only reporting extreme literature values in this case. We will remove this reference from our introduction if it is contentious.

Reviewer 1 asks if high phytoplankton <sup>13</sup>C levels require CCMs, or if this could be attributed to something else. No, high phytoplankton <sup>13</sup>C levels alone could be attributable to any process that elevates <sup>13</sup>C of their inorganic carbon source. CO<sub>2</sub> generated from methanogenic fermentation and carbonate dissolution could cause this. However, we demonstrate that the highest values of phytoplankton <sup>13</sup>C correspond to periods of both peak biomass, severe CO<sub>2</sub> depletion (approaching 0 ppm), and elevated pH. pH across sites and sampling events ranged from 7.6 +/- 0.2 in the fall to 10.1

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+/- 0.1 in July. These data demonstrate that CO<sub>2</sub> is both biologically and chemically depleted, and that to support bloom biomass, phytoplankton would need an active uptake mechanism (i.e., part of CCM process) to access HCO<sub>3</sub><sup>-</sup> and convert it to CO<sub>2</sub>. In addition to the discrete data presented here, we collected continuous pH and temperature sensor data (every 15 minutes) and have calculated time series of CO<sub>2</sub> concentration and flux for all sites with discrete alkalinity and conductivity measurements. These data are in review at another journal but can be referenced in a revised version of this manuscript.

Title: We will edit the title to better reflect the focus of the paper.

Reviewer 1 comments on the complicated nature of shallow surface waters and suggests focusing our study only on summertime bloom conditions. We agree that there is considerable heterogeneity among our sampling sites. We feel that information would be lost if we remove data from the shoulder seasons (temporal CO<sub>2</sub> depletion and the shift to bloom conditions). We can clarify heterogeneity among sites by analyzing lakes separately with corrections for multiple tests. We will highlight the bloom season as suggested by using larger or darker symbols for bloom conditions in plots.

We will provide separate values of bloom conditions in the table, include representative pH, alkalinity, and chlorophyll a, and summarize chemical conditions during blooms in text as requested.

Specific comments:

Line 96 “decreased carbon efflux”: This statement specifically refers to cyanobacterial CCMs, which are less leaky and have lower efflux than eukaryotic CCMs. We will clarify this in the text.

Line 159: Unfortunately, we do not have material that has not been fumed to take these measurements. We do have early trial data collected from 3 of our study sites the year preceding this study that compares fumed and not fumed samples. There

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was a measurable but not statistically significant difference between the two – these data can be included for reference if useful. It is true that many marine and benthic cyanobacteria calcify, but this is not as common in eutrophic, freshwater lakes, and has not been observed in our samples.

We will include discrete pH and alkalinity values in Table 1.

Line 163- We will clarify the meaning of “appropriate isotopic scale”.

-Line 191- We will specify that we are referring to fractionation of biomass relative to ambient CO<sub>2</sub> to prevent confusion. We recognize that this fractionation factor is a result of cumulative fractionations that occurred as the plankton grew, but because their growth and turnover time during a bloom is rapid (on the order of days), we feel that these values adequately reflect fractionation values of interest

Lines 23 and 204. We will remove the word “harmful”.

234, 252: We will clarify this statement and include CO<sub>2</sub> invasion and hydroxylation in alkaline waters as processes producing lighter DIC.

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Reviewer 2

Response to general comments:

Reviewer 2 major concerns are that (1) emphasis is placed on cyanobacteria, but only chlorophyll data is presented as a measure of bloom biomass, and that (2) nonlinear dynamic regression does not test an expected relation. To address these concerns, we will include phytoplankton community composition data, and test the Smyntek model as suggested.

Response to specific comments:

Chlorophyll a concentration as presented here is commonly used as a metric of phyto-

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plankton biomass. As above, we will edit the manuscript to include community composition and biovolume data (microscope counts).

Regarding emphasis on cyanobacteria: The blooms we sampled were cyanobacteria blooms. We will include these community composition data and update the title, methods, and results accordingly.

Lines 70 and 259-260: Regarding the efficiency of cyanobacterial versus eukaryotic CCMs, we will update our references to include those suggested showing that, in some cases, chlorophytes can outcompete cyanobacteria in culture. However, Price 2008 (as referenced therein) supports our assertion that cyanobacteria are better competitors for inorganic carbon, attributable to the partitioning of Rubisco and elevation of CO<sub>2</sub> in carboxysomes not present in eukaryotes, as well as higher Rubisco nitrogen use efficiency and very low levels of photorespiration.

Lines 93-104: Yes, chlorophytes using a CCM would also be expected to have elevated <sup>13</sup>C signatures, but the data presented here are cyanobacteria blooms, not chlorophyte blooms. Updating our manuscript to include community composition data will clarify this.

Line 113: Lakes were chosen along an orthogonal gradient of inter-annual variability in cyanobacteria dominance and watershed permeability. We will update the manuscript to include this information.

Line 129-124: We will remove variables not essential to our results from this section, and update Table 1 to include alkalinity and pH.

Lines 171-173: a and b represent temperature-dependent fractionation factors between CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup>, and HCO<sub>3</sub><sup>-</sup> and CO<sub>3</sub><sup>2-</sup>, respectively. We will update the manuscript to include this information.

Statistical analysis: Reviewer 1 also noted that heterogeneity between lakes may complicate our results. Based on these comments, we will partition the data to highlight

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effects of individual lakes, and fit the model presented in Smyntek et al. (2012) rather than using dynamic regression.

Lines 198-199: Non-linear dynamic regression

Technical corrections: We will edit Table 1 as suggested and will fix the units on the Figure 1 axis label.

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Reviewer 3

Response to general comments:

The relationship presented in Figure 1 was not meant to predict CCM activity, but rather illustrate the correlation between an increase in biomass and elevated phytoplankton <sup>13</sup>C. We will remove the trendline and report R rather than R<sup>2</sup> here.

We will update our references to include those suggested here to better treat the issue of leakage. We are aware that the isotopic signal is influenced by leakage and the external <sup>13</sup>C DIC. We measured the external <sup>13</sup>C and have reported these data.

Regarding composition of the lake phytoplankton communities, as described in our response to Reviewer 2, we will include phytoplankton community composition data.

Reviewer 3 indicates that we do not specifically mention how we obtained the biomass measured. As presented, we have used chlorophyll a as a metric of phytoplankton biomass, which is detailed in the methods. However, when community composition data are included, this can be updated to reflect biovolume measurements from microscopy. Regarding detritus and organic matter from other sources, this is detailed in lines 156-158 of the manuscript.

Table 1: We will make these corrections to include alkalinity and pH, as also suggested by Reviewers 1 and 2. We did not include <sup>13</sup>C DIC isotopic data in tabular form because it is presented in Figure 2.

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Title: We will revise the title accordingly.

Response to specific comments:

Line 113: The criteria for choosing lakes is described above in our response to Reviewer 2. We feel that including evidence that sample sites were chosen in an informed way and not arbitrarily is useful and should remain in the manuscript.

Lines 127,132: Yes, measurements were corrected for temperature and pressure as described in lines 123-133.

Lines 143-145: We will move this paragraph as suggested.

Lines 171 and 172: Addressed in response to Reviewer 2.

Figure 1: Addressed above in general comments.

Line 220: We will rephrase as suggested.

Paragraph beginning at line 234: Specific suggestions as to what should be clarified would be helpful here, but we will attempt to clarify and better explain these discussion points.

#### References

Badger, M. R., Price, G. D., Long, B. M. and Woodger, F. J.: The environmental plasticity and ecological genomics of the cyanobacterial CO<sub>2</sub> concentrating mechanism., *J. Exp. Bot.*, 57(2), 249–65, doi:10.1093/jxb/eri286, 2006. Eichner, M., Thoms, S., Kranz, S. A. and Rost, B.: Cellular inorganic carbon fluxes in *Trichodesmium*: A combined approach using measurements and modelling, *J. Exp. Bot.*, 66(3), 749–759, doi:10.1093/jxb/eru427, 2015. Hopkinson, B. M., Dupont, C. L. and Matsuda, Y.: The physiology and genetics of CO<sub>2</sub> concentrating mechanisms in model diatoms, *Curr. Opin. Plant Biol.*, 31, 51–57, doi:10.1016/j.pbi.2016.03.013, 2016. Kaplan, A. and Reinhold, L.: *Co<sub>2</sub> Concentrating Mechanisms in Microorganisms*, 1999. Price, G. D., Badger, M. R., Woodger, F. J. and Long, B. M.: *Advances in understanding*

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the cyanobacterial CO<sub>2</sub>-concentrating-mechanism (CCM): functional components, Ci transporters, diversity, genetic regulation and prospects for engineering into plants., *J. Exp. Bot.*, 59(7), 1441–61, doi:10.1093/jxb/erm112, 2008. Raven, J. a, Cockell, C. S. and De La Rocha, C. L.: The evolution of inorganic carbon concentrating mechanisms in photosynthesis., *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, 363(1504), 2641–50, doi:10.1098/rstb.2008.0020, 2008. Trimborn, S., Wolf-Gladrow, D., Richter, K.-U. and Rost, B.: The effect of pCO<sub>2</sub> 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.

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