Biogeosciences Discuss.,
doi:10.5194/bg-2016-350-RC2, 2016

Biogeosciences Discussions
Open Access
EGU

Interactive comment on "Carbon concentrating mechanisms maintain bloom biomass and CO_2 depletion in eutrophic lake ecosystems" *by* Ana M. Morales-Williams et al.

J. Verspagen (Referee)

J.M.H.Verspagen@uva.nl

Received and published: 23 November 2016

General comments:

In this manuscript, Morales et al. present carbon isotope data collected from 16 eutrophic lakes, and show that when dissolved CO2 concentrations become undersaturated, the isotopic composition of particulate organic carbon increases, while the photosynthetic fractionation decreases. These findings are attributed to the (increased) use of Carbon Concentrating Mechanisms (CCMs) by phytoplankton (i.e., the ability to utilize bicarbonate as an inorganic carbon source) when dissolved CO2 concentrations are depleted. These findings are not entirely novel, similar relations are described in Smyntek et al (2012), however, the data by Morales et al show that these findings apply

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to a wide(r) range of lakes.

I have two major concerns (detailed below): 1) There is a strong emphasis on cyanobacteria and cyanobacterial blooms in the Introduction section, which is not reflected by the results section, in which only chlorophyll a concentrations are shown. The authors should either reduce the emphasis on cyanobacterial blooms in the Introduction section, or proof that the blooms they sampled were dominated by cyanobacteria. 2) I have a problem with the use of a nonlinear dynamic regression to fit the patterns in Figs 2-4: these regressions do not test an expected relation. However, in Smyntek et al (2012), an isotopic fractionation model is presented that probably fits the data in Fig. 3 and 4. I recommend to fit the Smyntek model to your data, it would make the results much stronger.

Specific comments:

The title suggests that CCMs maintain (phytoplankton) bloom biomass. Yet, no evidence is presented that shows a direct relation between CCM activity (i.e. photosynthetic fractionation or delta 13 POC values) and phytoplankton biomass, and no evidence is presented that the use of CCMs maintain phytoplankton biomass.

In the Introduction section and in the Discussion section, there is a strong emphasis on cyanobacteria and cyanobacterial blooms. Yet, in the title, the material and methods section, and the results section, there is no mention of cyanobacterial blooms, only of phytoplankton blooms and/or phytoplankton biomass. Are the blooms that you sampled cyanobacterial blooms? Do you have any information on the bloom composition in the lakes you sampled?

Line 70, and lines 259-260: It is assumed here that eukaryotic CCMs are, by definition, less efficient than cyanobacterial CCMs. I'm not convinced. Firstly, recent research suggests that the key components of eukayotic CCMs (although not fully resolved) are very similar to cyanobacterial CCMs (Moroney and Ynalvez 2007, Wang et al 2011, Meyer and Griffiths 2013). Secondly, there is experimental evidence that some chloro-

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phytes can outcompete cyanobacteria at low CO2 concentrations, even when these cyanobacteria have a complete CCM (i.e. they have all known bicarbonate uptake systems). For competition experiments between a cyanobacterium and a chlorophyte, see Verschoor et al (2013) and Li et al (2016), for cyanobacterial CCM gene composition of Synechocystis PCC 6803, see Price et al (2008).

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

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

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

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

I have some concerns about the statistical analysis of the dataset. 1) I wonder whether one has to control for the different lakes. The reason for my concern is that the shape of the fits of the nonlinear regressions of Figs 2, 3 and 4 rely heavily on 6-7 points at low pCO2/low photosynthetic fractionation/low delta 13C of POC. Note that low delta 13C of POC does not necessarily imply high chl a concentrations (Fig. 1). These 6-7 points might come from 1 outlier lake. For this reason, I'm not sure whether a nonlinear dynamic regression (as presented in Figs 2-4) is an appropriate statistical procedure to analyze the dataset. If I understand correctly, nonlinear dynamic regression is an

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iterative process that may converge to find the best possible curve that fits the dataset. It does not test an expected relation between a dependent and an independent parameter. In Smyntek et al (2012), an isotopic fractionation model is presented (in Eqs 1 and 2, plotted in Fig. 2 of Smyntek et al 2012) that shows relations between pCO2 and delta 13C of POC, and between pCO2 and the photosynthetic fractionation that look remarkably similar to the shape of the curves that were derived in this study by nonlinear dynamic regression (i.e. Fig. 3 and 4). The Smyntek model should also predict the relation between delta 13DIC and the photosynthetic fractionation in Fig. 2. It makes perfect sense to test whether the fractionation model by Smyntek et al (2012) fits your dataset.

Line 198-199: what kind of regressions are given here? Linear regressions of data with a pCO2 < 393? Please be more precise: give the name of the regression and the statistical parameters: e.g. Linear regression, Rˆ2 = 0.90, P < 0.01, N = 10

Technical corrections:

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

Fig. 1: x-axis label should be “Chl a (ug L-1)”

References:

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