

# ***Interactive comment on “A 150-year record of phytoplankton community succession controlled by hydroclimatic variability in a tropical lake” by K. A. Yamoah et al.***

**K. A. Yamoah et al.**

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Anonymous Referee #2

The authors presented a data set of lipids abundances, compound specific hydrogen isotope, bulk carbon and nitrogen isotopes, and DNA from a sediment core, to investigate decadal variations in phytoplankton communities in a ~150 year of a tropical lake. Although the authors make an effort to establish a new methodology to evaluate the ecological changes in the lake by these biological and chemical analyses, this paper is rather descriptive and spotty discussion and lacks the in-depth discussion. As long as the authors presented a lot of data set, I think that the authors should comprehensively discuss the lake environment rather than picking up the specified topic.

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Therefore, in my opinion, a substantial revision is required to make this MS suitable for publication in BG.

BGD

Answer We sincerely thank the reviewer for taking the time to review our manuscript. As the reviewer noticed, the focus of this manuscript is the investigation of changes in phytoplankton community structure over 150 years by using key biomarkers, bulk and compound specific isotopes, quantitative PCR tied to geochemistry, all controlled by hydroclimate variability. Importantly, this study shows the advantages of combining organic geochemistry and molecular studies to constrain natural and anthropogenic influences on lake trophic state over time (i.e. oligotrophic to eutrophic) and the concurrent changes in dominant phytoplankton community dominating the lake. The methodology is likely applicable in many aquatic systems, regardless of whether it is a lake or not. Therefore, the focus is more on elucidation the physical and chemical factors that lead to successional changes in the dominant microbial community over sub-centennial timescales other than paleolimnology of the lake, whose changes are indeed directly reflected by our data. For example, our results clearly show that the nutrient structure of the lake has changed over the last 150-years because of changes in rainfall and runoff patterns. In turn, this strongly influenced the trophic organization of the lake ecosystem structure. It is not very clear to us what the reviewer has in mind when asking to discuss the lake environment more comprehensively. We have, however, made an attempt to improve on this aspect in the revised manuscript.

Detailed comments: Page 3, line 11: Meaning of the terms "external" and "internal" ecosystem should be specified. Answer: This is similar to the question posed by the anonymous reviewer #1. We have elaborated more on the external and internal factors in the revised manuscript. By external factors, we mean processes such as rainfall and anthropogenic activities that affect the lake through weathering and runoff intensity. Internal factors include the cycling of various nutrients within the lake, including nutrient regeneration, rates of primary productivity and organic carbon and nitrogen cycling, etc. These are factors, which we subsequently address by the amount of data provided.

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Page 5, line 14: Please describe the depth (m) of the sampling location, the "deepest part". Answer: The deepest part of the lake is approximately 3 m. This has been incorporated in the revised manuscript

Page 5, lines 19-22: I suggest to tone down this part. Robador et al. 2015 did not give you off-handed support nor the guarantee for storing sampled sediments without froze them for days. Organic compounds and its isotope compositions can very likely be affected. Answer: The text has been modified, and the reference cited as an example. Samples were kept cool by ice blocks, maintaining maximum temperatures of 4°C. At this temperature the hydrocarbon (the analyzed organic compounds), and let alone their isotope compositions are not likely to be affected significantly. The reviewer may not be aware that such lipid biomarkers and their isotopic compositions are routinely analyzed from million-year-old sediments. Preservation of DNA was more critical, but here we refer to Robador et al. (2015), who suggest that heterotrophic microbial activity is severely limited at such temperatures. The fact that our multiple proxies end up showing similar trends for both organic compounds and DNA specific methods substantiates the assumption that any potential degradation has not influenced the main results.

"Such" temperatures needs to be specified. Answer: The temperature has been specified as 4°C.

Page 5, line 22: It would be better to remove the word "biogeochemical". Answer: "biogeochemical" has been removed and replaced with "geochemical."

Page 6, lines 3-5: Descriptions of the standard materials for carbon and nitrogen isotope analysis (e.g. working standards) should be included. Answer: The working standards used for the analysis are: 1) Acetanilide,  $\delta^{13}\text{C} = -27.07\text{‰}$ ;  $\text{‰}^{15}\text{N} = 71.09\text{‰}$ ; 2) Methionine,  $\delta^{13}\text{C} = -26.23\text{‰}$ ;  $\text{‰}^{15}\text{N} = 40.25\text{‰}$ ; 3)  $\delta^{13}\text{C} = -24.24\text{‰}$ ;  $\text{‰}^{15}\text{N} = 9.39\text{‰}$ . These standards were calibrated against standards from IAEA. We have clarified these in the revised manuscript.

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Page 6, lines 16-29: It will be better to cite original papers for the method. Answer: The method has been revised, and citations have been added (e.g. Woszczyk et al., 2011; Chawchai et al., 2015, Yamoah, 2016).

Page 7, lines 10-25: For the delta-D analysis, please present at least one set of IRMS chromatogram from analyzed sample. Answer: A new figure has been added showing an IRMS chromatogram in the revised manuscript.

For the compound-specific isotope analysis, especially for the  $\delta$ D, single-peak baseline separation of targeted compound is essential to get reliable data. It will be better to cite original papers for the analytical method. Answer: Citations have been added (e.g. Sessions et al., 1999, 2001)

Page 9, line 10-12: Specify the reason to compare sedimentation rates between a lake and a near -by estuary? The two aquatic fields have completely different physical natures, I failed to see the reason or the necessity for their sedimentation rates should be in the same range. Answer: We agree completely with the reviewer, this comparison has been deleted.

Page11 Sec.4.1: The description of the data trend is rather difficult to understand. Please indicate specific unit name or age from each figures when discussing. Unclear description made it difficult to follow the thread of your discussion. (e.g. p.11 line 22, "the second half of the last century in Figure 3" can be addressed by year). Answer: The units have been further clarified regarding ages, as suggested by the reviewer, throughout the manuscript.

Page 11, lines 17-23: This part fails to convince the readers, as some of the discussion seems to be contradictive. I think to draw this conclusion, the  $\delta$ 15N variation in surrounding watersheds, substrate nitrogen, actual values of phytoplankton and N-fixing cyanobacteria should be considered and discussed. Especially, when the lake is small and easily affected by surrounding environments. The same thing can be said about the discussion regarding  $\delta$ 13C trends. Answer: We agree with the reviewer that the

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lake is easily affected by surrounding environments due to its size. This is, however, the main reason why we present multiple analysis to constrain the parameters influencing the dynamics in the lake. The first part of the discussion (Page 11, lines 17-23), which was mainly based on the bulk analysis, show the ambiguity associated with an interpretation based solely on bulk parameters especially when inferring biogeochemical cycles back in time. The importance of this study lies therefore in the combination of proxies and molecular data to elucidate the factors influencing the dominant phytoplankton changes instead of directly measuring the actual values of phytoplankton and N-fixation by cyanobacteria as suggested by the reviewer. In fact, it is impossible to measure such values back 150 years in time. Indeed, one could go to the present-day lake and determine the actual limnological features, however then one still does not know how these were in the past. For deeper timescales, the phytoplankton can only be identified and analyzed through proxies recovered from the lake sediments. Moreover, these proxies can be constrained with geochemistry and molecular data. Therefore, we do not think that it is necessary to do direct measurements of the dominant phytoplankton communities that we allude to especially when these factors are properly constrained. We attempt to clarify this aspect better in the revised version.

The reason why the low C/N ratio can be the direct indication of the shift in dominant plankton from diatoms to cyanobacteria should be addressed, too. Answer: The C/N ratio has generally been used in lake systems as a proxy for terrestrial versus aquatic input into lakes. However for a productive lake, changes in phytoplankton community can also change the C/N ratios since different phytoplankton community are modulated by the different degree of nutrient enrichment, and this would reflect the C/N ratios. Clarity on this will be incorporated in the revised manuscript.

Page 12 Sec.4.2: Recently, Chikaraishi et al. (2012) reported that the terrestrial insects have long-chain n-alkanes (C<sub>21</sub>–C<sub>33</sub>) with lighter  $\delta$ D ( $-195 \pm 16\text{‰}$ ) abundantly. Does this affect some of your discussion in this section, as the contribution of insect-derived n-alkanes can be one of the reasons for the negative shift in  $\delta$ D records? Answer:

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Chikaraishi et al., (2012) indeed looked at  $\delta D$  of long chain alkanes of terrestrial insects (bees, wasps, and hornets) and had lighter  $\delta D$  values ( $-195 \pm 16\text{‰}$ ). We, however, deem it unlikely that n-alkanes from insects would significantly contribute to the total amount of plant-wax derived alkanes, their total biomass is much, much smaller than that of the vegetation. There is not evidence for large amounts of insects deposited in the lake. If insects such as bees, wasps, and hornets dominated the core, one would have expected traces of these insects at least at the topmost part of the core. Also,  $\delta^{13}\text{C}$  values from this core (presented in another manuscript) show an entirely terrestrial vegetation origin of the long chain n-alkanes (C25-C33).

Supplement page5 sec 4.3: The English is difficult to understand. Answer: The text has been rephrased in the revised manuscript

Table S1: Please address units for S, O<sub>2</sub>, P. Figure S1: Captions should explain all the symbols or lines in the figure. Please remove excess notes. Answer: Units given in percentages have now been added to the revised manuscript.

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