

27th March 2015

Dear Editor,

Please find herewith a revised version of our manuscript (bg-2014-511), entitled “*Biogeochemistry of a large and deep tropical lake (Lake Kivu, East Africa): insights from a stable isotope study covering an annual cycle*”.

This updated manuscript provides a thorough revision addressing the issues raised by the reviewers. A point-by-point responses to all reviewer comments is enclosed below. Sections substantially modified in the revised manuscript are highlighted and annotated so that the corrections to the initial manuscript can be easily identified.

We sincerely hope that the present version of the manuscript can be considered for publication in *Biogeosciences* and thank the associate editor, and the two anonymous reviewers for their constructive comments.

Best regards,

Cédric Morana

First of all, we would like to thank the two anonymous referee for their insightful revision of the initial version of this manuscript.

Referee 1

Q1 : The authors derive conclusions about lake Kivu being autotrophic from the seasonal pattern in ^{13}C -DIC and other limnological concentration data. I am concerned with their definition of and proof for autotrophy in lake Kivu. Autotrophy means the ratio of primary production (PP)/respiration (R) > 1. Because $\text{PP} + \text{I} = \text{O} + \text{B} + \text{R}$, this means that in autotrophic lakes $\text{PP} - \text{R} = \text{O} + \text{B} - \text{I} > 0$, which means $\text{O} + \text{B} > \text{I}$, with O = organic carbon out through outflow, B = sediment burial of organic carbon, I = organic carbon inputs from catchment (allochthonous carbon). Please note that from the equations it follows that autotrophy means that the ratio of PP / I (= autochthonous / allochthonous carbon inputs) > $\text{R} / (\text{O} + \text{B})$. Therefore, true autotrophy cannot be demonstrated (or refuted) by the results shown in this paper. At best you can say from the results, it seems likely the lake is autotrophic.

REPLY : The seasonal pattern in $\delta^{13}\text{C}$ -DIC we reported in this study is additional evidence that adds to other arguments (gathered from DIC mass balance calculation, see Borges et al. 2014 ; or phytoplankton and bacterial processes measurements, see Morana et al. 2014). Together, these multiple lines of evidences strongly support the conclusion that the mixolimnion of Lake Kivu is net autotrophic.

According to the reviewer's equation, the lake would be autotrophic ($\text{PP} - \text{R} > 0$) if $\text{O} + \text{B} > \text{I}$.

The organic carbon inputs from the catchment (I) in Lake Kivu have been estimated at different periods of the year (rainy or dry season). These data were recently published in Borges et al. (2014). We cited this paper and provided an estimation of I ($0.7\text{-}3.3 \text{ mmol m}^{-2} \text{ d}^{-1}$) line 25 of page 17237 in the initial version of our manuscript. Sediment trap mooring (with traps at 50, 90, 130, and 172 m ; i.e. at the bottom and below the mixolimnion) have been deployed in Lake Kivu (see Pasche et al. 2010) to measure the rate of organic carbon burial (B). In their study, Pasche et al. (2010) reported an averaged B of $9.4 \text{ mmol m}^{-2} \text{ d}^{-1}$ for the period 2006-2008, that would be much higher than I ($0.7\text{-}3.3 \text{ mmol m}^{-2} \text{ d}^{-1}$). Preliminary data gathered in 2012-2013 in parallel to this study appear to support the estimate of Pasche et al. (2010). The only outflow of Lake Kivu is the Ruzizi river. Based on the long term discharge average of Ruzizi ($83.2 \text{ m}^3 \text{ s}^{-1}$), the average POC and DOC in surface waters of (0.04 and 0.2 mmol/L) and lake's surface area (2322 km²), O can be evaluated at $0.7 \text{ mmol m}^{-2} \text{ d}^{-1}$. Hence, $\text{O} + \text{B} = 0.7 + 9.4 > 0.7\text{-}3.3 \text{ (mmol m}^{-2} \text{ d}^{-1})$.

We tried to improve the clarity of text in the revised version of our manuscript, the discussion section dealing with the $\delta^{13}\text{C}$ -DIC dynamic now reads : *“Primary producers preferentially incorporate the lighter isotope (^{12}C) into the biomass with the consequence that the heavier isotope (^{13}C) accumulates into the DIC pool, whereas mineralization releases ^{13}C -depleted CO_2 from the OM being respired, into the DIC pool. Therefore, increasing primary production leads to higher $\delta^{13}\text{C}$ -DIC but increasing respiration should tend to decrease $\delta^{13}\text{C}$ -DIC (Bade et al. 2004). For instance, several studies conducted in temperate lakes have*

reported a significant increase in $\delta^{13}\text{C-DIC}$ during summer, resulting from primary production (Herczeg 1987, Hollander & McKenzie 1991). In Lake Kivu, the $\delta^{13}\text{C-DIC}$ increased linearly with time during the stratified rainy season, deviating gradually from the $\delta^{13}\text{C-DIC}$ value expected if the DIC pool was at equilibrium with the atmospheric CO_2 (~ 0.49 ‰). It appears unlikely that this linear isotopic enrichment of the DIC pool would be due to physical processes : the $\delta^{13}\text{C-DIC}$ signature of the DIC input from the inflowing rivers (Borges et al. 2014) and deep waters (Fig. 3a) was indeed lower than the measured $\delta^{13}\text{C-DIC}$ in the mixed layer. Therefore, biological processes (i.e. photosynthetic CO_2 uptake) would be responsible of the isotopic enrichment of the DIC pool observed during the stratified rainy season. Nevertheless, a small decrease in $\delta^{13}\text{C-DIC}$ was recorded at the beginning of the dry season (early in July 2012), but was concomitant with the characteristic deepening of the mixed layer observed during the dry season. As the depth profile of $\delta^{13}\text{C-DIC}$ revealed that the DIC pool was isotopically lighter in the bottom of the mixolimnion, the measurement of lower $\delta^{13}\text{C-DIC}$ values during the dry season could have resulted from the seasonal vertical mixing of surface waters with bottom waters containing relatively ^{13}C -depleted DIC. Overall, the data revealed that the input of DIC originating from the monimolimnion during the dry season provided the dominant imprint on $\delta^{13}\text{C-DIC}$ in the mixolimnion, but the seasonal variability of $\delta^{13}\text{C-DIC}$ observed in the mixed layer hold information on biological processes. The gradual increase with time of the $\delta^{13}\text{C-DIC}$ in the mixed layer suggests that photosynthetic CO_2 fixation exceeded the respiration of OM, implying that the surface waters of Lake Kivu were net autotrophic, and hence, the microbial food web was supported by autochthonous organic C sources. In Lake Kivu, riverine inputs of allochthonous OM from the catchment ($0.7 - 3.3 \text{ mmol m}^{-2} \text{ d}^{-1}$, Borges et al. 2014) are minimal compared to primary production ($49 \text{ mmol m}^{-2} \text{ d}^{-1}$; Darchambeau et al. 2014) and the export of organic carbon to the monimolimnion of $9.4 \text{ mmol m}^{-2} \text{ d}^{-1}$ reported by Pasche et al. (2010). The outflow of organic carbon through the Ruzizi River is also relatively low and was computed to be $0.6 \text{ mmol m}^{-2} \text{ d}^{-1}$, based on the long term discharge average of Ruzizi ($83.2 \text{ m}^3 \text{ s}^{-1}$, Borges et al. 2014), the average POC and DOC in surface waters (0.052 and $0.142 \text{ mmol L}^{-1}$) and the lake surface area (2322 km^2). This nevertheless implies that the outputs of OM ($9.4 + 0.7 = 10.1 \text{ mmol m}^{-2} \text{ d}^{-1}$) are higher than the inputs of OM from the catchment ($0.7-3.3 \text{ mmol m}^{-2} \text{ d}^{-1}$) suggesting a net autotrophic status. This conclusion is supported by the parallel study of Borges et al. (2014) who reported, based on a DIC (bulk concentration and isotopic) mass balance approach, that the mixed layer of Lake Kivu was net autotrophic while acting as a source of CO_2 to atmosphere. Indeed, CO_2 emissions to the atmosphere from Lake Kivu are sustained by CO_2 inputs of geogenic origin from deep geothermal springs (Borges et al. 2014)''.

Q2 : Lastly, indicate whether the % cyanobacteria is a % of abundance (cell numbers) or of biomass (Figs 5 and 7)

This is percentage of biomass. This information has been added into the caption of the figures 5 and 7.

Referee 2

Q1 : The authors present a fairly well written manuscript reporting on the seasonal and spatial variations of the concentration and isotopic signature of different carbon and nitrogen pools of a large African lake (Lake Kivu), which shown contrasting patterns across both space and time. While the study presents extensive data on the major C pools of the lake, I was left wondering however if many of the isotopic patterns described were of any significance. For example, there was only a 1 per mill change in the DIC isotopic signature, both across season and lake depth, and the authors make a series of inference on the lake functioning based on such little variation. What is the actual experimental error on these values, and why the authors suggest that the change in $\delta^{13}\text{C}$ -DIC is significant, but not the change in $\delta^{13}\text{C}$ -DOC although the latter pool also varied by one per mill?

REPLY : The reproducibility of the $\delta^{13}\text{C}$ -DIC measurement in our lab is typically better than 0.2 ‰, therefore we the seasonal pattern that shows a gradual enrichment of the DIC pool during the stratified season is significant, and does not result from analytical artefacts. This is now clearly stated in the material and methods section, that reads : *“The reproducibility of $\delta^{13}\text{C}$ -DIC measurement was typically better than ± 0.2 ‰.”*

It should also be noted that Lake Kivu is an alkaline lake with very high DIC concentration (12 mmol L⁻¹). Because of the size of the DIC pool in Lake Kivu, the effect of biological processes (for instance, photosynthetic CO₂ uptake) on the isotopic signature of the residual DIC pool is lower than in other lakes with a lower DIC concentration. The $\delta^{13}\text{C}$ signature of the DOC also showed minor variations but did not follow any seasonal, or depth-related patterns.

Q2: I also had a hard time reconciling the conclusion that the lake is net autotrophic, yet a net C source to the atmosphere based on the isotopic evidence presented. The authors suggest that allochthonous inputs are of minor importance, so my question is then where is the excess C coming from?

REPLY : This is an interesting question that was extensively addressed in a parallel paper by Borges et al. (2014). DIC (bulk and isotopic) mass balance calculations allow to conclude that the CO₂ emission to the atmosphere is sustained by DIC inputs of geogenic origin from geothermal springs located in the monimolimnion. Our interpretation is that geogenic inputs provide the dominant imprint on the $\delta^{13}\text{C}$ -DIC in the mixolimnion, but seasonal (such as the gradual increase of the $\delta^{13}\text{C}$ -DIC values during the rainy season ; Fig. 2b) and depth (higher $\delta^{13}\text{C}$ in the mixolimnion than in the monimolimnion ; Fig. 2a) variation hold information on biological processes. The revised text now reads : *“Overall, the data revealed that the input of DIC originating from the monimolimnion during the dry season provided the dominant imprint on $\delta^{13}\text{C}$ -DIC in the mixolimnion, but the seasonal variability of $\delta^{13}\text{C}$ -DIC observed in the mixed layer hold information on biological processes. The gradual increase with time of the $\delta^{13}\text{C}$ -DIC in the mixed layer suggests that photosynthetic CO₂ fixation exceeded the respiration of OM, implying that the surface waters of Lake Kivu were net autotrophic, and hence, the microbial food web was supported by autochthonous organic C sources. In Lake*

Kivu, riverine inputs of allochthonous OM from the catchment ($0.7 - 3.3 \text{ mmol m}^{-2} \text{ d}^{-1}$, Borges et al. 2014) are minimal compared to primary production ($49 \text{ mmol m}^{-2} \text{ d}^{-1}$; Darchambeau et al. 2014) and the export of organic carbon to the monimolimnion of $9.4 \text{ mmol m}^{-2} \text{ d}^{-1}$ reported by Pasche et al. (2010). The outflow of organic carbon through the Ruzizi River is also relatively low and was computed to be $0.6 \text{ mmol m}^{-2} \text{ d}^{-1}$, based on the long term discharge average of Ruzizi ($83.2 \text{ m}^3 \text{ s}^{-1}$, Borges et al. 2014), the average POC and DOC in surface waters (0.052 and $0.142 \text{ mmol L}^{-1}$) and the lake surface area (2322 km^2). This nevertheless implies that the outputs of OM ($9.4 + 0.7 = 10.1 \text{ mmol m}^{-2} \text{ d}^{-1}$) are higher than the inputs of OM from the catchment ($0.7-3.3 \text{ mmol m}^{-2} \text{ d}^{-1}$) suggesting a net autotrophic status. This conclusion is supported by the parallel study of Borges et al. (2014) who reported, based on a DIC (bulk concentration and isotopic) mass balance approach, that the mixed layer of Lake Kivu was net autotrophic while acting as a source of CO_2 to atmosphere. Indeed, CO_2 emissions to the atmosphere from Lake Kivu are sustained by CO_2 inputs of geogenic origin from deep geothermal springs (Borges et al. 2014)”.

Q3 : Also, I wouldn't be surprised if the gradual and small enrichment of the DIC isotopic signature during the rainy season may simply reflect a return to the equilibrium of the DIC pool isotopic signature with the atmosphere after the intrusion of a more depleted pool during the dry season, which would have little to do with biological processes (PP>R), but more with simple physical mixing. In this regard, the study would have greatly beneficieate from gas evasion (or invasion) or metabolism (PP vs. R) measurements.

REPLY : At equilibrium with the atmosphere, the $\delta^{13}\text{C}$ -DIC in surface waters would be $\sim 0.49 \text{ ‰}$ (estimated with the isotopic carbon fractionation factor measured in distilled water by Zhang et al. 1995). Therefore, the $\delta^{13}\text{C}$ -DIC expected at the equilibrium with the atmosphere would in any case be much lower than the minimal $\delta^{13}\text{C}$ -DIC signature measured during this study (2.34 ‰ in July 2012), so that a “return to the equilibrium of the DIC pool isotopic signature with the atmosphere after the intrusion of a more depleted pool during the dry season”, as suggested by the reviewer, would actually have produced the opposite pattern (i.e. a gradual isotopic depletion of the DIC pool instead of a gradual enrichment). Therefore, our view is that biological processes (i.e. photosynthetic CO_2 uptake, and not CO_2 production through mineralization of OM as the dominance of this process would also have lowered the $\delta^{13}\text{C}$ -DIC) would be responsible of the seasonal isotopic enrichment of the DIC pool. The value expected at isotopic equilibrium with the atmosphere is now given in the revised version of the manuscript, to improve the clarity of our argumentation. The discussion now reads : “*In Lake Kivu, the $\delta^{13}\text{C}$ -DIC increased linearly with time during the stratified rainy season, deviating gradually from the $\delta^{13}\text{C}$ -DIC value expected if the DIC pool was at equilibrium with the atmospheric CO_2 ($\sim 0.49 \text{ ‰}$). It appears unlikely that this linear isotopic enrichment of the DIC pool would be due to physical processes : the $\delta^{13}\text{C}$ -DIC signature of the DIC input from the inflowing rivers (Borges et al. 2014) and deep waters (Fig. 3a) was indeed lower than the measured $\delta^{13}\text{C}$ -DIC in the mixed layer. Therefore, biological processes (i.e. photosynthetic CO_2 uptake) would be responsible of the isotopic enrichment of the DIC pool observed during the stratified rainy season. Nevertheless, a small decrease in $\delta^{13}\text{C}$ -DIC was recorded at the beginning of the dry season (early in July 2012), but was concomitant*

with the characteristic deepening of the mixed layer observed during the dry season. As the depth profile of $\delta^{13}\text{C-DIC}$ revealed that the DIC pool was isotopically lighter in the bottom of the mixolimnion, the measurement of lower $\delta^{13}\text{C-DIC}$ values during the dry season could have resulted from the seasonal vertical mixing of surface waters with bottom waters containing relatively ^{13}C -depleted DIC”.

Q4 : Finally, I was not totally convinced by the importance of the methane-based biomass production for the lake food web as suggested by the authors. Per the authors calculation, about 5% only of the oxidized methane could be incorporated into biomass, in line with other lake studies (Jones and Lennon. 2010. AME 58:45-53), and I would thus suggest toning down the conclusions accordingly.

REPLY : We agree with the reviewer and have modified our text accordingly. It now reads :
“It appears that $4.4 \pm 1.9 \%$ ($n = 13$) and $6.4 \pm 1.6 \%$ ($n = 5$) of the depth-integrated POC pool in the mixolimnion was derived from CH_4 incorporation into the biomass during the rainy and dry season, respectively, and these percentages did not significantly differ between seasons (two-tailed t-test, $p = 0.055$). Nevertheless, the low $\delta^{13}\text{C}$ signatures measured locally in the oxycline indicate that the contribution of CH_4 -derived C could be episodically as high as 50 % (65 m, 22/08/12). We hypothesized that microbial CH_4 oxidation could play an important role in the ecological functioning of Lake Kivu. Along with heterotrophic mineralization of the sinking OM, and presumably other chemoautotrophic processes occurring in the oxycline such as nitrification (Llirós et al. 2010), CH_4 oxidation would have contributed substantially to O_2 consumption in the water column and was partly responsible for the seasonal uplift of the oxycline observed after the re-establishment of the thermal stratification during the rainy season. Furthermore, the methanotrophs in the oxycline would actively participate to the uptake of dissolved inorganic phosphorus (DIP), and hence would contribute to exert an indirect control on phytoplankton by constantly limiting the vertical DIP flux to the illuminated surface waters (Haberyan and Hecky 1987). Indeed, phytoplankton in Lake Kivu suffers of a severe P limitation throughout the year as pointed out by the relatively high sestonic C:P ratio (256 ± 75 ; Sarmiento et al. 2009 ; Darchambeau et al 2014)”.

Q5: P17236L219: “Primary producers preferentially incorporate the lighter isotopes (^{12}C) into the biomass with the consequence that the heavier isotopes (^{13}C) accumulate into the DIC pool, whereas mineralization releases ^{13}C -depleted from the organic matter being respired, into the DIC pool. Therefore, increasing primary production leads to higher $^{13}\text{C-DIC}$ but increasing respiration should tends to decrease $^{13}\text{C-DIC}$ (Bade et al., 2004). For instance, several studies conducted in temperate lakes have reported a significant increase in $^{13}\text{C-DIC}$ during summer, resulting from increased primary production (Herczeg, 1987; Hollander and McKenzie, 1991). In Lake Kivu, a linear increase of $^{13}\text{C-DIC}$ with time was observed during the stratified rainy season”. Maybe this has been observed in temperate lakes, but here the data show a complete opposite pattern: more depleted $\text{d}^{13}\text{C-DIC}$ values were found at times i.e., during the dry season (Fig.2b) when the Chla (and potentially PP) values peaked in the lake, suggesting only a limited impact of PP on the seasonal dynamics of the DIC pool isotopic signature.

REPLY : We indeed found lower $\delta^{13}\text{C}$ -DIC values during the dry season, when the phytoplankton biomass and primary production is higher (see Darchambeau et al. 2014 for a detailed discussion of the seasonality of PP in L. Kivu). However, the seasonal increase in primary production during the dry season is triggered by the deepening of the mixing zone which brings up a large amount of inorganic nutrients, but also an important quantity of ^{13}C -depleted DIC (figure 2a) in the mixed layer. Our interpretation is that the abrupt decrease of $\sim 1 \text{ ‰}$ of the $\delta^{13}\text{C}$ -DIC value at the beginning of the dry season reflects the mixing of the DIC pool with more ^{13}C -depleted DIC originating from bottom waters. However, rainy season conditions induce the development of a thermal stratification within the mixolimnion, which reduces the inorganic nutrient (and ^{13}C -depleted DIC) supply to the mixed layer. See replies above for more details about the modification we have made in text to improve the clarity of our argumentation.

Q6: P17236L23: “It appears unlikely that this linear isotopic enrichment of the DIC pool in the mixed layer would be due to physical processes: the ^{13}C -DIC signature of the DIC input from the inflowing rivers (Borges et al., 2014) and deep waters (Fig. 3a) was lower than the ^{13}C -DIC in the mixed layer”. Again, perhaps I am missing something, but it seems to me that the enrichment of the ^{13}C -DIC during the rainy season could simply be reflecting an equilibration with atmospheric CO_2 after the DIC became depleted due to deeper water mixing (P17234L24 and Fig.2b). Did the authors take this into account at all? What would be the expected $\delta^{13}\text{C}$ -DIC values if fully equilibrated with the atmosphere?

REPLY : At equilibrium with the atmosphere, the $\delta^{13}\text{C}$ -DIC in surface waters would be $\sim 0.49 \text{ ‰}$ (estimated with the isotopic carbon fractionation factor measured in distilled water by Zhang et al. 1995). Therefore, the $\delta^{13}\text{C}$ -DIC expected at the equilibrium with the atmosphere would be in any case lower than the minimal $\delta^{13}\text{C}$ -DIC signature measured during this study, so that equilibration with the atmosphere during rainy season would have caused a decrease, and not an increase, of the $\delta^{13}\text{C}$ -DIC. See replies above for more details about the modification we have made in text to improve the clarity of our argumentation.

Q7 P17237L17: “Due to the warmer temperature in the tropics phytoplankton production is comparatively higher in the East African large lakes compared with the Laurentian Great lakes, despite similar phytoplankton abundance (Bootsma and Hecky, 2003)”. Yet, total or bacterial respiration may also be enhanced (for example, see Amado et al. Front Microbiol. 2013; 4: 167.), and perhaps even more so than primary production, resulting in a higher probability of experiencing net heterotrophy in tropical systems. I am not saying that it will be the case, but the authors’ argument should be better supported.

REPLY : In the revised version of our manuscript, we have considerably extended the discussion of this point to better support our argumentation. The text now reads : “Besides morphometrical features, the net autotrophic status of Lake Kivu might also be related to general latitudinal and climatic patterns. Due to the warmer temperature in the tropics, phytoplankton production is comparatively higher in the East African large lakes compared with the Laurentian Great lakes, despite similar phytoplankton abundance (Bootsma & Hecky

2003). Alin and Johnson (2007) reviewed phytoplankton primary production and CO₂ emission to the atmosphere fluxes in large lakes of world (>500 km²). At the global scale, they found a statistically significant increase of the areal phytoplankton production in large lakes with the mean annual water temperature and the insolation ; and in consequence, a significant decrease of phytoplankton production with latitude. Also, they report a significant decrease of the CO₂ emission to the atmosphere with the mean annual water temperature and therefore an increase of the CO₂ emission with the latitude. According to their estimations, less than 20% of the phytoplankton primary production would be sufficient to balance the carbon loss through CO₂ evasion and OM burial in sediments in large lakes located between the equator and the latitude 30°, but the CO₂ emission and OM accumulation in sediments would exceed the phytoplankton primary production in systems located at latitude higher than 40° (Alin and Johnson 2007). Overall, in morphometrically comparable systems, this global analysis suggests a trend from autotrophic to increasingly heterotrophic conditions with increasing latitude and decreasing mean annual water temperature and insolation (Alin and Johnson 2007). Therefore, our study supports the view that paradigms established with data gathered in comparatively small temperate and boreal lakes may not directly apply to larger, tropical lakes (Bootsma & Hecky 2003). It also highlights the need to consider the unique limnological characteristics of a vast region of the world that harbours 16% of the total surface of lakes (Lehner & Döll 2004), and would account for 50% of the global inputs of OM from continental waters to the oceans (Ludwig et al. 1996).

Q8: P17240L6: “Nevertheless, the low 13C signatures measured locally in the oxycline indicate that the contribution of CH₄-derived C could be episodically as high as 50 % (65 m, 22 August 2012). Overall, this illustrates that, whatever the season, CH₄-derived organic C accounted for a significant part of the POC pool, and highlight the ecological importance of microbial CH₄ oxidation processes in the water column of Lake Kivu”. I would not call a 4-6% a significant part of the POC pool. Also, I am not sure what is meant by POC here as it seems to be either referring to algae or bacteria alternatively thorough the manuscript. Please be consistent for clarity

REPLY : We agree with the reviewer and we changed the text accordingly. See replies above for detailed modification we have made in text concerning this point.

POC refer to the organic carbon pool collected on filters of a 0.3 µm porosity. Then, this pool includes both bacteria and phytoplankton.

Q9 : Fig.2b: While the patterns as present are quite clear, I find that expending the Y axis to show a 1 per mill difference a bit misleading.

REPLY : The difference of 1 per mil is much higher than the possible analytical error on the determination of δ¹³C-DIC with our equipment. Therefore we don't see a problem to adapt the Y-axis to illustrate this pattern.

Q10: P17232L21: The IO analytical Aurora 1030W does not provide d13C values, only organic and inorganic C concentrations. Please specify which instrument was used for isotopes.

REPLY : The text now reads : “*Sample analysis was carried out with a IO Analytical Aurora 1030W coupled to an IRMS (Thermo delta V Advantage)*”.

Q11: P17237L4: Please correct “could have resulted

REPLY : Done

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1 **Biogeochemistry of a large and deep tropical lake (Lake**
2 **Kivu, East Africa): insights from a stable isotope study**
3 **covering an annual cycle**

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12

13 **Abstract**

14 During this study, we investigated the seasonal variability of the concentration and the stable
15 isotope composition of several inorganic and organic matter (OM) reservoirs in the large,
16 oligotrophic and deep tropical Lake Kivu (East Africa). Data were acquired during one year at
17 a fortnightly temporal resolution. The $\delta^{13}\text{C}$ signature of the dissolved inorganic carbon (DIC)
18 increased linearly with time during the rainy season, then suddenly decreased during the dry
19 season due to vertical mixing with ^{13}C -depleted DIC waters. This pattern reflects the net
20 autotrophic status of the mixed layer of Lake Kivu, contrary to the common observation that
21 oligotrophic aquatic ecosystems tend to be net heterotrophic. The $\delta^{13}\text{C}$ signature of the
22 particulate organic carbon pool (POC) revealed the presence of a consistently abundant
23 methanotrophic biomass in the oxycline throughout the year. We also noticed a seasonal shift
24 during the dry season toward higher values in the $\delta^{15}\text{N}$ of particulate nitrogen (PN) in the
25 mixed layer and $\delta^{15}\text{N}$ -PN was significantly related to the contribution of cyanobacteria to the
26 phytoplankton assemblage, suggesting that rainy season conditions could be more favourable
27 to atmospheric nitrogen-fixing cyanobacteria. Finally, zooplankton were slightly enriched in
28 ^{13}C compared to the autochthonous POC pool, and the $\delta^{15}\text{N}$ signature of zooplankton followed
29 well the seasonal variability in $\delta^{15}\text{N}$ -PN, being consistently 3.0 ± 1.1 ‰ heavier than the PN
30 pool. Together, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis suggests that zooplankton directly incorporate algal-
31 derived OM in their biomass, and they would rely almost exclusively on this source of OM

1 throughout the year in general agreement with the very low allochthonous OM inputs from
2 rivers in Lake Kivu.

3

4 **1. Introduction**

5 Stable carbon (C) and nitrogen (N) isotope analyses of diverse inorganic and organic
6 components have been successfully used to assess the origin of organic matter (OM) and
7 better understand its cycling in aquatic systems (Lehmann et al. 2004). For instance, an
8 extensive sampling of diverse C and N pools during an annual cycle in the Loch Ness showed
9 important seasonal variation of the $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios in the crustacean zooplankton
10 biomass, reflecting a diet switch from allochthonous to autochthonous OM sources (Grey et
11 al. 2001). In small humic, boreal lakes with permanently anoxic waters, stable C isotope
12 analyses allowed also to establish that methanotrophic bacteria could be an important food
13 source for crustacean zooplankton, and hence methane-derived C contributed to fuel a large
14 fraction of the lake food web (Kankaala et al. 2006). Analyses of the stable C isotope
15 composition of carbonates and OM in sedimentary records of stratified lakes can also provide
16 reliable information about past land use of the catchment (Castañeda et al. 2009), or be used
17 to infer changes in lake productivity and climate (Schelske & Hodell 1991). However, a
18 detailed understanding of the stable isotope dynamics in the water column is a prerequisite for
19 a good interpretation of isotope data from sedimentary archives (Lehmann et al. 2004).

20 A new paradigm progressively emerged during the last decade, proposing that freshwaters
21 ecosystems are predominantly net heterotrophic, as respiration of OM exceeds autochthonous
22 photosynthetic production (Del Giorgio et al. 1997, Cole 1999, Duarte & Prairie 2005). This
23 concept seems to hold especially true for oligotrophic, unproductive ecosystems (Del Giorgio
24 et al. 1997), where the C cycle would be dominated by substantial inputs of allochthonous
25 OM of terrestrial origin, which support the production of heterotrophic organisms. Net
26 heterotrophy has been recognised as one of the main cause for the net emission of carbon
27 dioxide (CO_2) emissions from freshwater ecosystems to the atmosphere (Prairie et al. 2002),
28 although there is growing evidence of the contribution from external hydrological CO_2 inputs
29 from the catchment (Stets et al. 2009; Finlay et al. 2010; Borges et al. 2014; Marcé et al.
30 2015). However, the current understanding of the role of inland waters on CO_2 and emissions
31 could be biased because most observations were obtained in temperate and boreal systems,
32 and mostly in medium to small-sized lakes, during open-water (ice-free) periods, but tropical
33 and temperate lakes differed in some fundamental characteristics. Among them, the constantly

1 high temperature and irradiance have strong effects on water column stratification and
2 biological processes (Sarmiento 2012). For instance, primary production in tropical lakes has
3 been recognised to be twice higher than in temperate lakes, on a given nutrient base (Lewis
4 1996). Also, the contribution of dissolved primary production in oligotrophic tropical lake has
5 been found to be substantially more important than in their temperate counterparts (Morana et al.
6 2014).

7 East Africa harbours the densest aggregation of large tropical lakes (Bootsma & Hecky 2003).
8 Some of them are among the largest (lakes Victoria, Tanganyika, Malawi), or deepest lakes in
9 the world (lakes Tanganyika, Malawi, Kivu) and consequently remain stratified all year
10 round. Due to the size and the morphometric traits of the East African large lakes, pelagic
11 processes are predominant in these systems, with the microbial food web playing a
12 particularly essential role in OM transfer between primary producers and higher levels of the
13 food web, as well as in nutrient cycling (Descy & Sarmiento 2008). Most of them are also
14 characterized by highly productive fisheries that provide an affordable food source to local
15 populations (Descy & Sarmiento 2008). However, while these lakes are potentially important
16 components of biogeochemical cycles at the regional scale (Borges et al. 2011), and their
17 significance for local populations from an economic perspective (Kaningini 1995), the East
18 African large lakes are relatively poorly-studied, most probably because of their remote
19 location combined to frequent political unrest.

20 In this study, we present a comprehensive data set covering a full annual cycle, including
21 hydrochemical data and measurements of the concentration of dissolved methane (CH_4) and
22 the concentrations and stable isotope compositions of dissolved inorganic carbon (DIC),
23 dissolved and particulate organic carbon (DOC and POC), particulate nitrogen (PN), and
24 zooplankton. Data were acquired during one full year at a fortnightly/monthly temporal
25 resolution. We aimed to assess the net metabolic status of Lake Kivu, the seasonal and depth
26 variability of sources of OM within the water column, and the relative contribution of
27 autochthonous or allochthonous OM to the zooplankton. To our best knowledge, this is the
28 first detailed study to assess the seasonal dynamics of different OM reservoirs by means of
29 their stable isotope composition in any of the large East African lakes. The detailed analysis
30 of the stable isotope composition of diverse organic and inorganic components carried out
31 during this study allowed to trace the OM dynamics in Lake Kivu during a seasonal cycle, and
32 might be useful to improve the interpretation of sedimentary archives of this large and deep
33 tropical lake.

1 2. Material and methods

2 Lake Kivu (East Africa) is a large (2370 km²) and deep (maximum depth of 485 m)
3 meromictic lake located at the border between the Democratic Republic of the Congo and
4 Rwanda. Its vertical structure consists of an oxic and nutrient-poor mixed layer down to a
5 maximum of 70 m, and a permanently anoxic monimolimnion rich in dissolved gases (CH₄,
6 and CO₂) and inorganic nutrients. Seasonal variation of the vertical position of the oxic-
7 anoxic transition is driven by contrasting air humidity and incoming long-wave radiation
8 between rainy (October-May) and dry (June-September) season (Thiery et al. 2014). The
9 euphotic zone, defined at the depth at which light is 1% of surface irradiance, is relatively
10 shallow (annual average : 18 m, Darchambeau et al. 2014).

11 Sampling was carried out in the Southern Basin (02°20'S, 28°58'E) of Lake Kivu between
12 January 2012 and May 2013 at a monthly or fortnightly time interval. Vertical oxygen (O₂),
13 temperature and conductivity profiles were obtained with a Hydrolab DS5 multiprobe. The
14 conductivity cell was calibrated with a 1000 μS cm⁻¹ (25°C) Merck standard and the O₂
15 membrane probe was calibrated with humidity saturated ambient air. Water was collected
16 with a 7 L Niskin bottle (Hydro-Bios) at a depth interval of 5 m from the lake surface to the
17 bottom of the mixolimnion, at 70 m. Additionally, zooplankton was sampled with a 75-cm
18 diameter, 55-μm mesh plankton net hauled along the whole mixolimnion (0-70m).

19 Samples for CH₄ concentrations were collected in 50 ml glass serum bottles from the Niskin
20 bottle with a tube, left to overflow, poisoned with 100 μl of saturated HgCl₂ and sealed with
21 butyl stoppers and aluminium caps. Concentrations of CH₄ were measured by headspace
22 technique using gas chromatography (Weiss 1981) with flame ionization detection (SRI
23 8610C), after creating a 20 ml headspace with N₂ in the glass serum bottles, and then
24 analyzed as described by Borges et al. (2011).

25 Samples for stable C isotopic composition of dissolved inorganic carbon ($\delta^{13}\text{C-DIC}$) were
26 collected by filling with water directly from the Niskin bottle 12 mL headspace vials (Labco
27 Exetainer) without bubbles. Samples were preserved with the addition of 20 μL of a saturated
28 HgCl₂ solution. Prior to the analysis of $\delta^{13}\text{C-DIC}$, a 2 ml helium headspace was created and
29 100 μL of phosphoric acid (H₃PO₄, 99%) was added in the vial in order to convert all
30 inorganic C species to CO₂. After overnight equilibration, 200 μL of gas was injected with a
31 gastight syringe into a EA-IRMS (Thermo FlashHT with Thermo DeltaV Advantage). The
32 obtained data were corrected for isotopic equilibration between dissolved and gaseous CO₂ as
33 described in Gillikin and Bouillon (2007). Calibration of $\delta^{13}\text{C-DIC}$ measurement was

1 performed with the international certified standards IAEA-CO1 and LSVEC. The
2 reproducibility of $\delta^{13}\text{C}$ -DIC measurement was typically better than ± 0.2 ‰. Measurements of
3 total alkalinity (TA) were carried out by open-cell titration with HCl 0.1 mol L⁻¹ according to
4 Gran (1952) on 50 mL water samples, and data were quality checked with certified reference
5 material obtained from Andrew Dickinson (Scripps Institution of Oceanography, University
6 of California, San Diego, USA). Typical reproducibility of TA measurements was better than
7 ± 3 $\mu\text{mol L}^{-1}$. DIC concentration was computed from pH and TA measurements using the
8 carbonic acid dissociation constants of Millero et al. (2006).

Comment [CMorana1]: Details on the reproducibility of the $\delta^{13}\text{C}$ -DIC measurement have been added, following the comment 1 of reviewer 2.

9 Samples for DOC concentration and stable C isotopic composition ($\delta^{13}\text{C}$ -DOC) were filtered
10 through pre-flushed 0.2 μm syringe filters, kept in 40ml borosilicate vials with Teflon-coated
11 screw caps and preserved with 100 μL of H₃PO₄ (50%). Sample analysis was carried out with
12 a IO Analytical Aurora 1030W coupled to an IRMS (Thermo delta V
13 Advantage). Quantification and calibration of DOC and $\delta^{13}\text{C}$ -DOC was performed with IAEA-
14 C6 and an internal sucrose standard ($\delta^{13}\text{C} = -26.99 \pm 0.04$ ‰) calibrated against international
15 reference materials.

Comment [CMorana2]: This section has been amended as requested by reviewer 2

16 Samples for POC and particulate nitrogen (PN) concentration and stable carbon and nitrogen
17 isotope composition ($\delta^{13}\text{C}$ -POC; $\delta^{15}\text{N}$ -PN) were obtained by filtering a known volume of
18 water on pre-combusted (overnight at 450°C) 25 mm glass fiber filters (Advantec GF-75 ; 0.3
19 μm), kept frozen until subsequent processing. The filters were later decarbonated with HCl
20 fumes for 4 h, dried and packed in silver cups prior to analysis on a EA-IRMS (Thermo
21 FlashHT with Thermo DeltaV Advantage). Calibration of $\delta^{13}\text{C}$ -POC, $\delta^{15}\text{N}$ -PN, POC and PN
22 measurements was performed with acetanilide ($\delta^{13}\text{C} = -27.65 \pm 0.05$; $\delta^{15}\text{N} = 1.34 \pm 0.04$)
23 and leucine ($\delta^{13}\text{C} = -13.47 \pm 0.07$; $\delta^{15}\text{N} = 0.92 \pm 0.06$) as standards. All standards were
24 internally calibrated against the international standard IAEA-C6 and IAEA-N1.
25 Reproducibility of $\delta^{13}\text{C}$ -POC and $\delta^{15}\text{N}$ -PN measurement was typically better than ± 0.2 ‰
26 and relative standard deviation for POC and PN measurement were always below 5%.
27 Samples for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of zooplankton were collected on precombusted 25 mm glass fiber
28 filters (Advantec GF-75 ; 0.3 μm), and dried. Subsequent preparation of the samples and
29 analysis on the EA-IRMS were performed similarly as described for the $\delta^{13}\text{C}$ -POC and $\delta^{15}\text{N}$ -
30 PN samples.

31 Pigment concentrations were determined by high performance liquid chromatography
32 (HPLC). 2-4 L of waters were filtered through Macherey-Nägel GF-5 filter (average retention
33 of 0.7 μm). Pigment extraction was carried out in 10 mL of 90% HPLC grade acetone. After

1 two sonication steps of 15 min separated by an overnight period at 4°C, the pigments extracts
2 were stored in 2 mL amber vials at -25°C. HPLC analysis was performed following the
3 gradient elution method described in Wright et al. (1991), with Waters system comprising
4 photodiode array and fluorescence detectors. Calibration was made using commercial external
5 standards (DHI Lab Products, Denmark). Reproducibility for pigment concentration
6 measurement was better than 7%. Pigment concentrations were processed with the
7 CHEMTAX software (CSIRO Marine Laboratories) using input ratio matrices adapted for
8 freshwater phytoplankton (Descy et al. 2000). Data processing followed a procedure similar
9 to that of Sarmiento et al. (2006) in Lake Kivu, that allows to estimate chlorophyll a (Chl a)
10 biomass of cyanobacteria, taking into account variation of pigment ratios with season and
11 depth.

12

13 **3. Results**

14 Analysis of the vertical and seasonal variability of temperature and dissolved O₂
15 concentrations during 18 months allow to divide the annual cycle into two distinct
16 limnological periods. Rainy season conditions resulted in a thermal stratification within the
17 mixolimnion (October-June) while the dry season was characterized by deeper vertical mixing
18 of the water column down to the upper part of the permanent chemocline at 65 m (July-
19 September) (Fig. 1a). The vertical position of the oxycline varied seasonally: the oxic-anoxic
20 transition reached its deepest point (65 m) during the dry season, then became gradually
21 shallower after the re-establishment of the thermal stratification within the mixolimnion at the
22 start of the following rainy season to finally stabilize at approximately 35m, corresponding to
23 the bottom of the mixed layer during the rainy season (Fig. 1b). The temporal variability of
24 the vertical distribution of CH₄ corresponded well with the seasonal variation of the oxycline.
25 The CH₄ concentrations were very high in the monimolimnion throughout the year (average at
26 70 m : $356 \pm 69 \mu\text{mol L}^{-1}$, n = 24) but sharply decreased at the oxic-anoxic transition, and
27 were 4 orders of magnitude lower in surface waters (annual average at 10 m : 0.062 ± 0.016
28 $\mu\text{mol L}^{-1}$, n = 24) (Fig. 1c).

29 DIC concentrations in the mixed layer were very high (annual average at 10 m : 11.9 ± 0.2
30 mmol L^{-1} , n = 24) and did not show any consistent seasonal pattern (not shown). The $\delta^{13}\text{C}$ -
31 DIC values were vertically homogeneous in the mixed layer but gradually decreased in the
32 oxycline to reach minimal values at 70 m (Fig. 2a). $\delta^{13}\text{C}$ -DIC values in the mixed layer
33 increased linearly with time during the rainy season ($r^2 = 0.79$, n = 12), then suddenly

1 decreased at the start of the dry season due to the vertical mixing with ^{13}C -depleted DIC from
2 deeper waters (Fig. 2b). The DOC concentration ($142 \pm 20 \mu\text{mol C L}^{-1}$, $n = 304$) and $\delta^{13}\text{C}$ -
3 DOC signature ($-23.2 \pm 0.4 \text{‰}$, $n = 304$) did not show any consistent variations with depth or
4 time in the mixolimnion during all the sampling period. A vertical profile performed down to
5 the lake floor revealed that the $\delta^{13}\text{C}$ -DOC did not vary significantly neither in the
6 monimolimnion (vertical profile average : $-23.0 \text{‰} \pm 0.2$, $n = 18$, Fig. 3), however an
7 important increase in DOC concentrations was observed starting at 260 m (Fig. 3), to reach a
8 maximum near the lake floor (350 m, $301 \mu\text{mol C L}^{-1}$).

9 The concentration of POC was substantially higher in the mixed layer than below in the
10 mixolimnion all over the year. However during the dry season, POC concentrations in the
11 oxycline ($\sim 50\text{-}65\text{m}$) were found to be as high as in surface water (Fig. 4a). POC concentration
12 integrated over the mixolimnion (0-70 m) averaged $2157 \pm 4 \text{ mmol m}^{-2}$ ($n = 19$) and did not
13 vary between the rainy and dry seasons. The isotopic signature of the POC pool stayed almost
14 constant throughout the year in the mixed layer (at 10 m : $-23.8 \pm 0.8\text{‰}$, $n = 19$), but at the top
15 of the oxic-anoxic transition, $\delta^{13}\text{C}$ -POC values systematically decreased sharply (at the oxic-
16 anoxic transition : $-33.9 \pm 4.3\text{‰}$, $n = 19$) (Fig. 4b). The vertical position of this abrupt
17 excursion toward more negative values followed closely the oxycline, and was therefore
18 located deeper in the water column during the dry season.

19 The concentrations of the PN pool in the water column followed the same pattern than POC
20 (Fig. 4c). The PN pool was larger in the mixed layer than below in the water column during
21 most of year. However, higher PN concentrations were measured in the oxycline during the
22 dry season (Fig. 4c). The molar C:N ratio in the mixolimnion varied depending on season,
23 being significantly higher (t -test ; $p < 0.05$) during the rainy season (11.2 ± 2.4 , $n = 15$) than
24 during the dry season (8.1 ± 0.9 , $n = 4$). $\delta^{15}\text{N}$ -PN values in the mixed layer oscillated between
25 0‰ and 1‰ during the rainy season but shifted toward significantly higher values during the
26 dry season ($3\text{‰} - 4\text{‰}$) (Fig. 5a). $\delta^{15}\text{N}$ -zooplankton mirrored the seasonal variability of $\delta^{15}\text{N}$ -
27 PN in the mixed layer with a small time-shift, ranging between $3\text{‰} - 5\text{‰}$ during the rainy
28 season, then increasing at the start of dry season to reach a maximum of 7.5‰ (Fig. 5a). The
29 difference between $\delta^{15}\text{N}$ -zooplankton and $\delta^{15}\text{N}$ -PN was on average $3.0 \pm 1.1 \text{‰}$ ($n = 19$) and
30 did not follow any clear seasonal pattern. The $\delta^{13}\text{C}$ signature of the zooplankton was on
31 average $-22.9 \pm 0.8 \text{‰}$ ($n = 19$) and did not vary between seasons (not shown).

1 Chlorophyll *a* concentrations exhibited little variation during the rainy season (average $74 \pm$
2 $15 \text{ mg Chl } a \text{ m}^{-2}$, $n = 16$) but increased significantly during the dry season to reach a maximal
3 value ($190 \text{ mg Chl } a \text{ m}^{-2}$) in September 2012 (Fig. 5b). This increase corresponded with a
4 change in phytoplankton community composition. The relative contribution of cyanobacteria
5 to the phytoplankton assemblage, as assessed from the concentration of marker pigments, was
6 smaller during the dry season than in the preceding (t -test ; $p < 0.01$, $\text{mean}_{\text{jan-jun}} = 23.4 \pm$
7 5.5% , $\text{mean}_{\text{jul-sep}} = 9.4 \pm 1.3\%$) and the following (t -test ; $p < 0.05$, $\text{mean}_{\text{oct-may}} = 14.6 \pm 3.8\%$,
8 $\text{mean}_{\text{jul-sep}} = 9.4 \pm 1.3\%$) rainy seasons (Fig. 5b).

9

10 **4. Discussion**

11 Stable isotope analysis of DIC is a useful tool for understanding the fate of C in aquatic
12 ecosystems and could provide information on the lake metabolism, defined as the balance
13 between gross primary production and community respiration of OM. Primary producers
14 preferentially incorporate the lighter isotope (^{12}C) into the biomass with the consequence that
15 the heavier isotope (^{13}C) accumulates into the DIC pool, whereas mineralization releases ^{13}C -
16 depleted CO_2 from the OM being respired, into the DIC pool. Therefore, increasing primary
17 production leads to higher $\delta^{13}\text{C}$ -DIC but increasing respiration should tend to decrease $\delta^{13}\text{C}$ -
18 DIC (Bade et al. 2004). For instance, several studies conducted in temperate lakes have
19 reported a significant increase in $\delta^{13}\text{C}$ -DIC during summer, resulting from primary production
20 (Herczeg 1987, Hollander & McKenzie 1991). In Lake Kivu, the $\delta^{13}\text{C}$ -DIC increased linearly
21 with time during the stratified rainy season, deviating gradually from the $\delta^{13}\text{C}$ -DIC value
22 expected if the DIC pool was at equilibrium with the atmospheric CO_2 ($\sim 0.49 \text{ ‰}$). It appears
23 unlikely that this linear isotopic enrichment of the DIC pool would be due to physical
24 processes : the $\delta^{13}\text{C}$ -DIC signature of the DIC input from the inflowing rivers (Borges et al.
25 2014) and deep waters (Fig. 3a) was indeed lower than the measured $\delta^{13}\text{C}$ -DIC in the mixed
26 layer. Therefore, biological processes (i.e. photosynthetic CO_2 uptake) would be responsible
27 of the isotopic enrichment of the DIC pool observed during the stratified rainy season.
28 Nevertheless, a small decrease in $\delta^{13}\text{C}$ -DIC was recorded at the beginning of the dry season
29 (early in July 2012), but was concomitant with the characteristic deepening of the mixed layer
30 observed during the dry season. As the depth profile of $\delta^{13}\text{C}$ -DIC revealed that the DIC pool
31 was isotopically lighter in the bottom of the mixolimnion, the measurement of lower $\delta^{13}\text{C}$ -
32 DIC values during the dry season could have resulted from the seasonal vertical mixing of
33 surface waters with bottom waters containing relatively ^{13}C -depleted DIC. Overall, the data

1 revealed that the input of DIC originating from the monimolimnion during the dry season
2 provided the dominant imprint on $\delta^{13}\text{C}$ -DIC in the mixolimnion, but the seasonal variability
3 of $\delta^{13}\text{C}$ -DIC observed in the mixed layer hold information on biological processes. The
4 gradual increase with time of the $\delta^{13}\text{C}$ -DIC in the mixed layer suggests that photosynthetic
5 CO_2 fixation exceeded the respiration of OM, implying that the surface waters of Lake Kivu
6 were net autotrophic, and hence, the microbial food web was supported by autochthonous
7 organic C sources. In Lake Kivu, riverine inputs of allochthonous OM from the catchment
8 ($0.7 - 3.3 \text{ mmol m}^{-2} \text{ d}^{-1}$, Borges et al. 2014) are minimal compared to primary production (49
9 $\text{mmol m}^{-2} \text{ d}^{-1}$; Darchambeau et al. 2014) and the export of organic carbon to the
10 monimolimnion of $9.4 \text{ mmol m}^{-2} \text{ d}^{-1}$ reported by Pasche et al. (2010). The outflow of organic
11 carbon through the Ruzizi River is also relatively low and was computed to be 0.6 mmol m^{-2}
12 d^{-1} , based on the long term discharge average of Ruzizi ($83.2 \text{ m}^3 \text{ s}^{-1}$, Borges et al. 2014), the
13 average POC and DOC in surface waters (0.052 and $0.142 \text{ mmol L}^{-1}$) and the lake surface
14 area (2322 km^2). This nevertheless implies that the outputs of OM ($9.4 + 0.7 = 10.1 \text{ mmol m}^{-2}$
15 d^{-1}) are higher than the inputs of OM from the catchment ($0.7-3.3 \text{ mmol m}^{-2} \text{ d}^{-1}$) suggesting a
16 net autotrophic status. This conclusion is supported by the parallel study of Borges et al.
17 (2014) who reported, based on a DIC (bulk concentration and isotopic) mass balance
18 approach, that the mixed layer of Lake Kivu was net autotrophic while acting as a source of
19 CO_2 to atmosphere. Indeed, CO_2 emissions to the atmosphere from Lake Kivu are sustained
20 by CO_2 inputs of geogenic origin from deep geothermal springs (Borges et al. 2014).

21 However, these results are in contradiction with the commonly held view that oligotrophic
22 lacustrine and marine systems tend to be net heterotrophic (Del Giorgio et al. 1997, Cole
23 1999). Net heterotrophy implies that heterotrophic prokaryotes rely on a substantial amount of
24 allochthonous OM, however in Lake Kivu, riverine inputs of allochthonous OM from the
25 catchment ($0.7 - 3.3 \text{ mmol m}^{-2} \text{ d}^{-1}$, Borges et al. 2014) are minimal. Indeed, the magnitude of
26 allochthonous OM inputs relative to phytoplankton production depends strongly on the
27 catchment to surface area ratio (Urban et al 2005), that is particularly low (2.2) in Lake Kivu.
28 Therefore, Lake Kivu is relatively poor in organic C, with DOC concentrations of ~ 0.15
29 mmol L^{-1} in contrast to smaller boreal humic lakes which show DOC concentrations of on
30 average $\sim 1 \text{ mmol L}^{-1}$ (Sobek et al. 2007), and with values up to $\sim 4.5 \text{ mmol L}^{-1}$ (Weyhenmeyer
31 & Karlsson 2009). Humic substances are usually low quality substrates for bacterial growth
32 (Castillo et al. 2003), but limit primary production by absorbing incoming light. Hence,
33 heterotrophic production in the photic zone of humic lakes usually exceeds phytoplankton

Comment [CMorana3]: This section of the discussion has been considerably changed to improve its clarity, as requested by the reviewer 1 & 2 in several of their comments. We aimed to support better our argumentation concerning the metabolic status of the lake.

1 production and DOC concentrations, despite the low substrate quality of humic substance,
2 have been found to be a good predictor of the metabolic status of lakes in the boreal region,
3 with a prevalence of net heterotrophy in organic-rich lakes (Jansson et al. 2000). However,
4 low allochthonous OM inputs and low DOC concentration do not necessary cause a system to
5 be net autotrophic. For instance, Lake Superior has a lower catchment to surface area ratio
6 (1.6), is subsidized by a similar amount of allochthonous OM ($\sim 3 \text{ mmol m}^{-2} \text{ d}^{-1}$) and the DOC
7 concentration is even lower than in Lake Kivu ($\sim 0.1 \text{ mmol L}^{-1}$), but it has been found to be
8 net heterotrophic despite the limited allochthonous OM inputs (Urban et al. 2005). Lake
9 Superior, as the majority of the lakes of the world, is holomictic, meaning that the mixing of
10 its water column can seasonally reach the lake floor, and a substantial amount of sediments,
11 including OM, could then be resuspended during these mixing events and hence re-exposed to
12 microbial mineralization in well-oxygenated waters (Meyers and Eadie 1993, Cotner 2000,
13 Urban et al. 2005). The resuspension of benthic sediments could be important in the
14 ecological functioning of these systems. In constrast, Lake Kivu, as other East African large
15 lakes such as Lake Tanganyika and Malawi, are particularly deep meromictic lakes, so that
16 their water column is characterized by an almost complete decoupling between the surface
17 and deep waters, avoiding any resuspended benthic sediment to reach the surface waters in
18 this system. In consequence, the coupling between the phytoplankton production of DOC and
19 its heterotrophic consumption by prokaryotes in the clear, nutrient-depleted waters of Lake
20 Kivu was found to be high throughout the year (Morana et al. 2014).

21 Besides morphometrical features, the net autotrophic status of Lake Kivu might also be
22 related to general latitudinal and climatic patterns. Due to the warmer temperature in the
23 tropics, phytoplankton production is comparatively higher in the East African large lakes
24 compared with the Laurentian Great lakes, despite similar phytoplankton abundance
25 (Bootsma & Hecky 2003). Alin and Johnson (2007) reviewed phytoplankton primary
26 production and CO_2 emission to the atmosphere fluxes in large lakes of world ($>500 \text{ km}^2$). At
27 the global scale, they found a statistically significant increase of the areal phytoplankton
28 production in large lakes with the mean annual water temperature and the insolation ; and in
29 consequence, a significant decrease of phytoplankton production with latitude. Also, they
30 report a significant decrease of the CO_2 emission to the atmosphere with the mean annual
31 water temperature and therefore an increase of the CO_2 emission with the latitude. According
32 to their estimations, less than 20% of the phytoplankton primary production would be
33 sufficient to balance the carbon loss through CO_2 evasion and OM burial in sediments in large

1 lakes located between the equator and the latitude 30°, but the CO₂ emission and OM
2 accumulation in sediments would exceed the phytoplankton primary production in systems
3 located at latitude higher than 40° (Alin and Johnson 2007). Overall, in morphometrically
4 comparable systems, this global analysis suggests a trend from autotrophic to increasingly
5 heterotrophic conditions with increasing latitude and decreasing mean annual water
6 temperature and insolation (Alin and Johnson 2007). Therefore, our study supports the view
7 that paradigms established with data gathered in comparatively small temperate and boreal
8 lakes may not directly apply to larger, tropical lakes (Bootsma & Hecky 2003). It also
9 highlights the need to consider the unique limnological characteristics of a vast region of the
10 world that harbours 16% of the total surface of lakes (Lehner & Döll 2004), and would
11 account for 50% of the global inputs of OM from continental waters to the oceans (Ludwig et
12 al. 1996).

13 Despite the net autotrophic status of the mixed layer of lake Kivu, the $\delta^{13}\text{C}$ data indicate a
14 difference in the origins of the POC and DOC pools in the mixed layer. Indeed, the $\delta^{13}\text{C}$ -DOC
15 showed very little variation and appeared to be vertically and temporally uncoupled from the
16 POC pool in the mixed layer (Fig. 6). A recent study (Morana et al. 2014) demonstrated that
17 phytoplankton extracellular release of DOC is relatively high in Lake Kivu, and the fresh and
18 labile autochthonous DOC produced by cell lysis, grazing or phytoplankton excretion, that
19 would reflect the $\delta^{13}\text{C}$ signature of POC, is quickly mineralized by heterotrophic bacteria.
20 Therefore, it appears that the freshly produced autochthonous DOC would contribute less than
21 1% of the total DOC pool (Morana et al. 2014), and as the standing stock of phytoplankton-
22 derived DOC seems very small, it can be hypothesized that the bulk DOC pool is mainly
23 composed of older, more refractory compounds that would reach the mixed layer through
24 vertical advective and diffusive fluxes. Indeed, the $\delta^{13}\text{C}$ signature of the DOC in the
25 monimolimnion (80 m – 370 m, $-23.0 \pm 0.2 \text{ ‰}$, n = 24) did not differ from the $\delta^{13}\text{C}$ -DOC in
26 the mixolimnion (0 m – 70 m, $-23.2 \pm 0.2 \text{ ‰}$, n = 5), suggesting that they share the same
27 origin (Fig. 4).

28 The concentration of the POC pool varied largely with depth, being the highest in the 0-20m
29 layer, i.e. roughly the euphotic zone. However, during the dry season, POC concentrations
30 was almost as high in the oxycline than in surface waters. High POC concentrations in deep
31 waters have frequently been observed in lakes, usually as a result from the resuspension of
32 benthic sediments near the lake floor or to the accumulation of sedimenting material in
33 density gradients (Hawley and Lee 1999). However, in the deep Lake Kivu, this maximum

Comment [CMorana4]: The discussion on the metabolic status of Lake Kivu has been extended to support better one of our conclusion (i.e Lake Kivu is a net autotrophic system, despite being relatively unproductive and a source of CO₂ to the atmosphere), following the comment 7 of reviewer 2

1 POC zone is located approximately 300 m above the lake floor and is characterized by a
2 strong depletion in ^{13}C of the POC pool. While DIC would be the major C source of the POC
3 pool in the mixed layer, the important decrease of $\delta^{13}\text{C}$ -POC values observed in the oxycline
4 suggests that another ^{13}C -depleted C source was actively incorporated into the biomass at the
5 bottom of the mixolimnion. Slight depletion in ^{13}C of the POC pool in oxyclines, such as in
6 the Black Sea, has sometimes been interpreted as a result of to the heterotrophic
7 mineralization of the sedimenting OM (Coban-Yildiz et al. 2006), but it seems unlikely that,
8 in Lake Kivu, heterotrophic processes could have caused an abrupt excursion of $\delta^{13}\text{C}$ -POC to
9 values as low as -41.6‰ (65 m, 22/08/12). Such large isotopic depletion of the POC pool in
10 the water column have been reported by Blees et al. (2014), who measured $\delta^{13}\text{C}$ -POC as low
11 as -49‰ in Lake Lugano, and they were related to high methanotrophic activity. In Lake
12 Kivu, CH_4 concentrations were found to decrease sharply at the oxic-anoxic transition
13 (Borges et al. 2011), and the dissolved CH_4 that reached the oxycline via turbulent diffusivity
14 and vertical advection (Schmid et al. 2005) is known to be isotopically light, with a $\delta^{13}\text{C}$
15 signature of approximately -60‰ (Pasche et al. 2011, Morana et al. 2014). Therefore, the
16 vertical pattern in CH_4 concentrations and $\delta^{13}\text{C}$ -POC values observed during this study
17 suggests that a substantial part of CH_4 was consumed and incorporated into the microbial
18 biomass in the oxycline. Indeed, experiments carried out in Lake Kivu in February 2012 and
19 September 2012 showed that microbial CH_4 oxidation was significant in the oxycline, and
20 phospholipid fatty acids analysis revealed high abundance of methanotrophic bacteria of type
21 I at the same depths (Morana et al. 2014). With estimates of the isotope fractionation factor
22 during microbial CH_4 oxidation (1.016, Morana et al. 2014), and of the $\delta^{13}\text{C}$ - CH_4 at each
23 sampling point, it is possible to estimate the theoretical $\delta^{13}\text{C}$ signature of methanotrophic
24 organisms at each depth. Note that the $\delta^{13}\text{C}$ - CH_4 was not directly measured during this study
25 but a very strong linear correlation between the log-transformed CH_4 concentrations and $\delta^{13}\text{C}$ -
26 CH_4 was found along vertical profiles performed in February and September 2012 in Lake
27 Kivu ($\delta^{13}\text{C}\text{-CH}_4 = -7.911 \log(\text{CH}_4) - 13.027$; $r^2 = 0.87$, $n = 34$; Morana et al. submitted).
28 Hence the $\delta^{13}\text{C}$ - CH_4 at each sampling point between January 2012 and May 2013 can be
29 approximated from the measured CH_4 concentrations, using this empirical relationship. Then,
30 a simple isotope mixing model with the calculated $\delta^{13}\text{C}$ signature of methanotrophs and the
31 average $\delta^{13}\text{C}$ -POC in the mixed layer as end-members allowed to determine the contribution
32 of CH_4 -derived C to POC at each sampling depth. It appears that $4.4 \pm 1.9\%$ ($n = 13$) and 6.4
33 $\pm 1.6\%$ ($n = 5$) of the depth-integrated POC pool in the mixolimnion was derived from CH_4
34 incorporation into the biomass during the rainy and dry season, respectively, and these

1 percentages did not significantly differ between seasons (two-tailed t -test, $p = 0.055$).
2 Nevertheless, the low $\delta^{13}\text{C}$ signatures measured locally in the oxycline indicate that the
3 contribution of CH_4 -derived C could be episodically as high as 50 % (65 m, 22/08/12). We
4 hypothesized that microbial CH_4 oxidation could play an important role in the ecological
5 functioning of Lake Kivu. Along with heterotrophic mineralization of the sinking OM, and
6 presumably other chemoautotrophic processes occurring in the oxycline such as nitrification
7 (Llirós et al. 2010), CH_4 oxidation would have contributed substantially to O_2 consumption in
8 the water column and was partly responsible for the seasonal uplift of the oxycline observed
9 after the re-establishment of the thermal stratification during the rainy season. Furthermore,
10 the methanotrophs in the oxycline would actively participate to the uptake of dissolved
11 inorganic phosphorus (DIP), and hence would contribute to exert an indirect control on
12 phytoplankton by constantly limiting the vertical DIP flux to the illuminated surface waters
13 (Haberyan and Hecky 1987). Indeed, phytoplankton in Lake Kivu suffers of a severe P
14 limitation throughout the year as pointed out by the relatively high sestonic C:P ratio ($256 \pm$
15 75 ; Sarmiento et al. 2009 ; Darchambeau et al 2014).

16 The $\delta^{15}\text{N}$ signature of the autochthonous OM in the mixed layer of Lake Kivu oscillated
17 around 0 ‰ during the rainy season in Lake Kivu but was significantly higher during the dry
18 season (3 – 4 ‰). Also, the $\delta^{15}\text{N}$ -PN in the mixed layer correlated negatively with the
19 proportion of cyanobacteria in waters (Fig. 7, Pearson's r : -0.65, $p = 0.004$, $n = 17$). This
20 pattern may highlight the seasonal importance of N_2 -fixing cyanobacteria in Lake Kivu during
21 the rainy season. Indeed, the $\delta^{15}\text{N}$ signature of atmospheric N_2 is close to 0 ‰ and isotope
22 fractionation during cyanobacterial N_2 -fixation is known to be small (Fogel & Cifuentes
23 1991). Several studies carried out in marine (Pacific Ocean and Gulf of Mexico) and
24 lacustrine (Lake Lugano) systems have shown that $\delta^{15}\text{N}$ -PN varied between -2 ‰ and +1 ‰
25 when N_2 -fixing cyanobacteria were dominating the phytoplankton assemblage (Wada 1976,
26 Macko et al. 1984, Lehmann et al. 2004). Moreover, a good relationship between the $\delta^{15}\text{N}$ -PN
27 and the abundance of N_2 -fixing cyanobacteria has already been reported for others systems,
28 such as coastal lagoon (Lesutiene et al. 2014). In Lake Victoria, biological N_2 fixation has
29 been identified has the largest input of N, exceeding atmospheric deposition and river inputs,
30 and N_2 fixation has been found to increase with light availability (Mugidde et al. 2003). This
31 suggests that during the rainy season, when thermal stratification of the mixolimnion leads to
32 reduced nitrogen supply combined with exposure to high light levels, N_2 -fixing cyanobacteria
33 would have a competitive advantage which may explain their seasonally higher contribution

Comment [CMorana5]: Following comment 4 of the reviewer 2, we tone down the conclusion regarding the contribution of CH_4 derived carbon to the food web

1 to the autochthonous OM pool (Sarmiento et al., 2006). Indeed, the significantly higher molar
2 C:N ratio during the rainy season than the dry season indicates that N-limitation in the mixed
3 layer was stronger during the rainy season (this study, Sarmiento et al. 2009). By contrast, the
4 deepening of the mixed layer during the dry season leads to increased nutrients input and
5 reduced light availability that favours alternative phytoplankton strategies (Hecky & Kling,
6 1987; Reynolds, 2006; Sarmiento et al. 2006; Darchambeau et al. 2014), and consequently the
7 proportion N₂-fixing cyanobacteria decreases. A similar seasonal pattern of N₂ fixation was
8 reported in Lake Victoria by Mugidde et al. (2003). In contrast with the rather constant $\delta^{13}\text{C}$
9 signature of zooplankton ($-22.9 \pm 0.8 \text{ ‰}$), the $\delta^{15}\text{N}$ analysis revealed that the $\delta^{15}\text{N}$ of
10 zooplankton varied importantly, following well the seasonal change in $\delta^{15}\text{N}$ -PN in the mixed
11 layer. The difference between $\delta^{15}\text{N}$ -zooplankton and $\delta^{15}\text{N}$ -PN ($\Delta^{15}\text{N}_{\text{Zoo-PN}}$) was on average 3.2
12 $\pm 1.0 \text{ ‰}$ throughout the year while it was on average enriched in ^{13}C ($\Delta^{13}\text{C}_{\text{Zoo-POC}}$) by $0.9 \pm$
13 0.8 ‰ . In nature, comparison of the $\delta^{15}\text{N}$ signature of consumers and their diet indicates that
14 the $\delta^{15}\text{N}$ value increases consistently with the trophic level, because of the preferential
15 excretion of the isotopically lighter ^{14}N (Montoya et al. 2002). However the C isotope
16 fractionation between consumers and diet is usually considered to be less than 1 ‰ (Sirevag
17 et al. 1977). The constant $\Delta^{15}\text{N}_{\text{Zoo-PN}}$ value found in Lake Kivu is within the range of trophic
18 level enrichment between algae and *Daphnia magna* ($\sim 2 \text{ ‰}$ to 5 ‰) estimated in laboratory
19 experiment (Adams and Sterner 2000), and very close to the cross-system trophic enrichment
20 value ($3.4 \pm 1.0 \text{ ‰}$) proposed by Post (2002). Together with the slight enrichment in ^{13}C
21 compared with the autochthonous POC pool, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis suggests that
22 zooplankton directly incorporate phytoplankton-derived OM in their biomass (Masilya 2011),
23 and they would rely almost exclusively on this source of OM throughout the year. This is in
24 general agreement with the very low allochthonous OM inputs from rivers in Lake Kivu
25 (Borges et al. 2014).

26 In conclusion, stable isotope data revealed large seasonal variability in the $\delta^{15}\text{N}$ signature of
27 the PN pool, most likely related to changes in the phytoplankton assemblage and to N₂-
28 fixation. In contradiction with the common observation that oligotrophic aquatic ecosystems
29 tend to be net heterotrophic, the seasonality of $\delta^{13}\text{C}$ -DIC suggests that the mixed layer of Lake
30 Kivu is net autotrophic, supporting the conclusions of Borges et al. (2014), based on DIC
31 mass balance considerations. The $\delta^{13}\text{C}$ -POC showed an important variation with depth due to
32 the abundance of methanotrophic bacteria in the oxycline that fixed the lighter CH₄-derived C
33 into their biomass. The $\delta^{13}\text{C}$ -POC and $\delta^{13}\text{C}$ -DOC appeared to be uncoupled vertically and

1 temporally, which could indicate that most of the DOC pool was composed of relatively
2 refractory compounds. Finally, the $\delta^{13}\text{C}$ of zooplankton mirrored the $\delta^{13}\text{C}$ signature of the
3 autochthonous POC pool, and its $\delta^{15}\text{N}$ signature followed the seasonal variability of the $\delta^{15}\text{N}$ -
4 PN pool in good agreement with the expected consumer-diet isotope fractionation. This
5 suggests that zooplankton would rely throughout the year on phytoplankton-derived biomass
6 as a organic C source.

7

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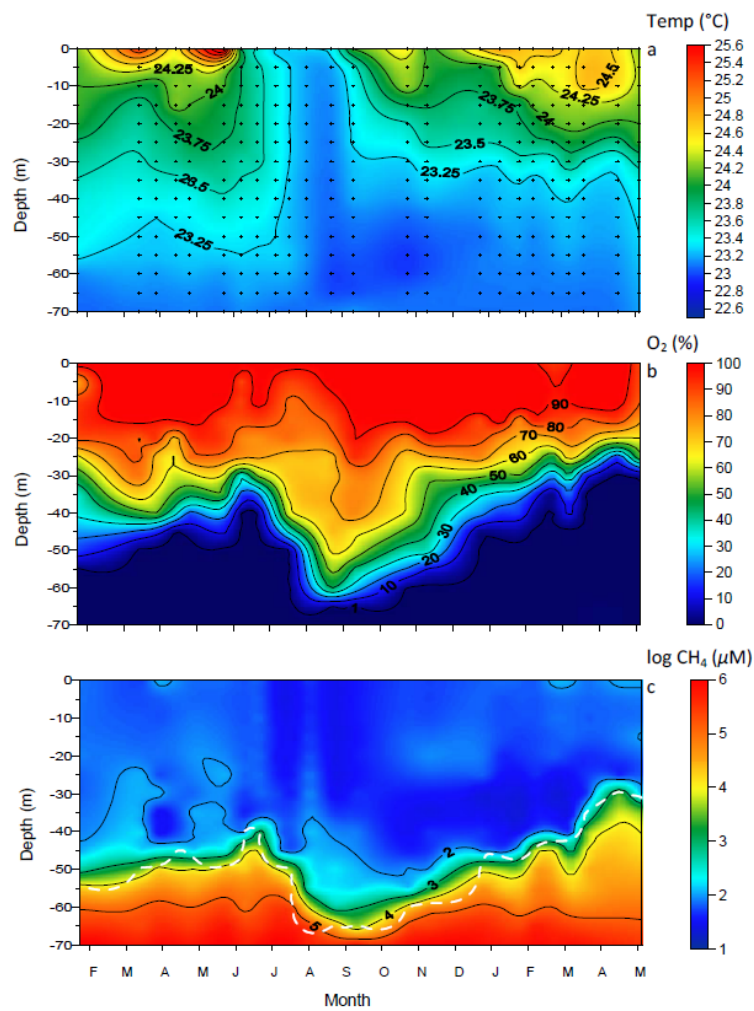
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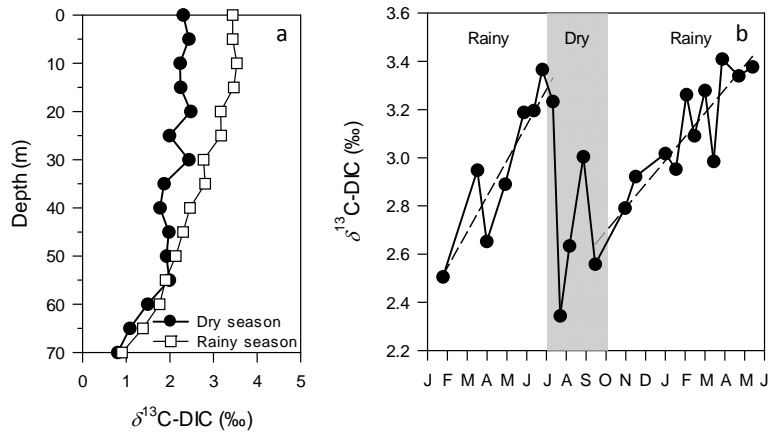
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1 Figures

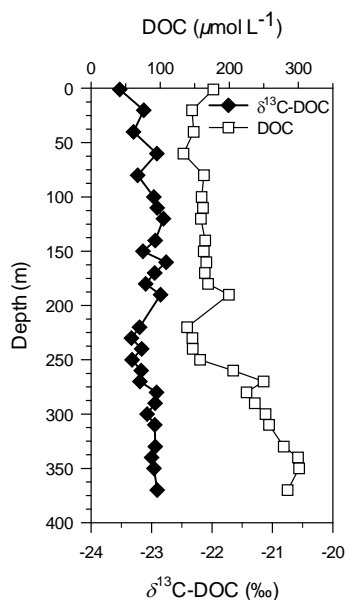


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4 Figure 1. Temporal variability of (a) temperature (°C), (b) oxygen saturation (%), and (c) the
5 log-transformed CH₄ concentration (μmol L⁻¹) in the mixolimnion of Lake Kivu, between
6 February 2012 and May 2013. Small crosses in the figure (a) represent each sampling points.
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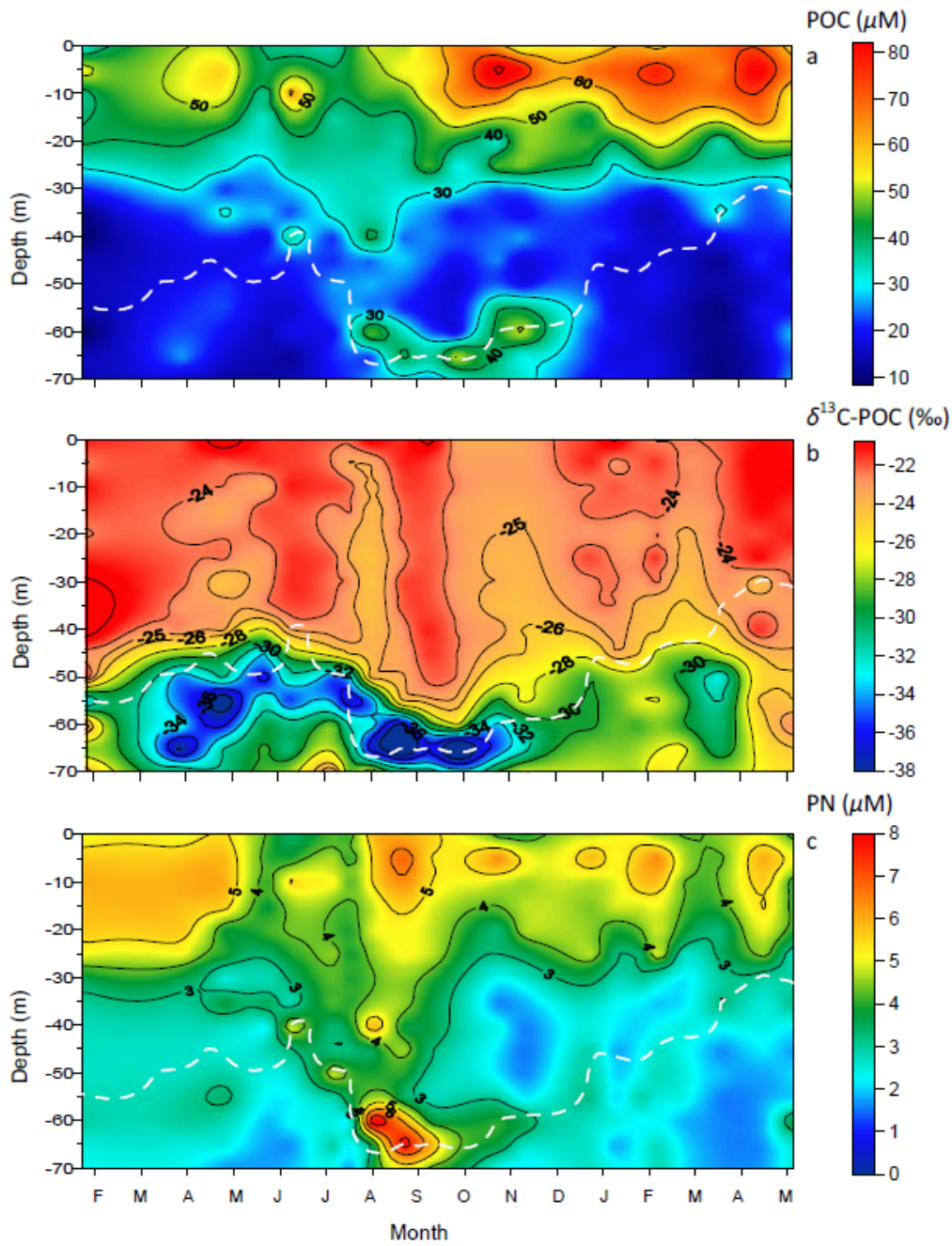
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 2 Figure 2. Depth profile of the $\delta^{13}\text{C}$ of the dissolved inorganic carbon (DIC) pool in the
 3 mixolimnion during the dry (18/07/12) and the rainy (20/03/13) season and (b) temporal
 4 variation of the $\delta^{13}\text{C-DIC}$ in the mixed layer of Lake Kivu between January 2012 and June
 5 2013.

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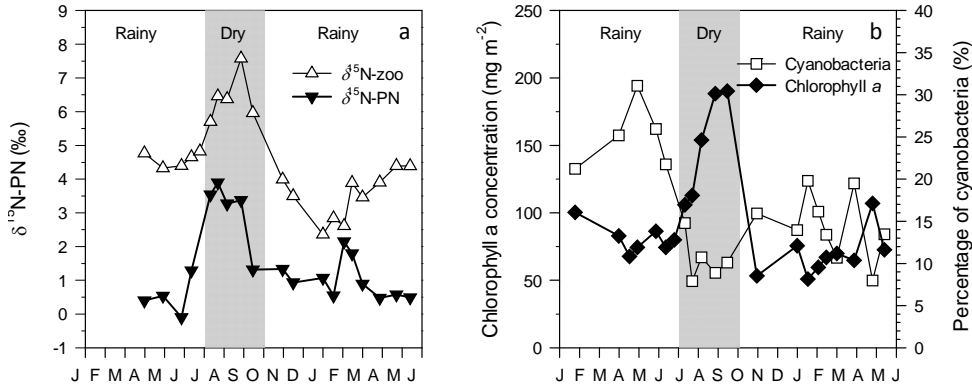


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 2 Figure 3. Vertical profile from the lake surface to the lake floor of the dissolved organic
 3 carbon (DOC) concentration ($\mu\text{mol L}^{-1}$) and the $\delta^{13}\text{C}$ signature of the DOC pool, in September
 4 2012.

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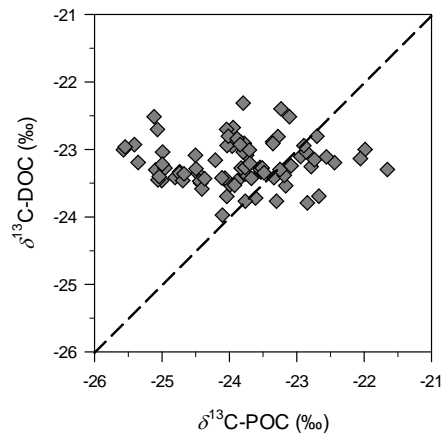
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 3 Figure 4. Temporal variability of (a) the particulate organic carbon (POC) concentration
 4 ($\mu\text{mol L}^{-1}$), (b) the $\delta^{13}\text{C}$ signature of the POC pool, and (c) the particulate nitrogen (PN)
 5 concentration ($\mu\text{mol L}^{-1}$) in the mixolimnion of Lake Kivu, between February 2012 and May
 6 2013.



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 2 Figure 5. Temporal variability of (a) the $\delta^{15}\text{N}$ signature of the particulate nitrogen (PN) pool
 3 and zooplankton in the mixed layer, and (b) the chlorophyll a concentration (mg m^{-2}) and the
 4 relative contribution of cyanobacteria to the phytoplankton assemblage (% of biomass) in the
 5 mixolimnion, assessed from pigments analyses, between February 2012 and May 2013.

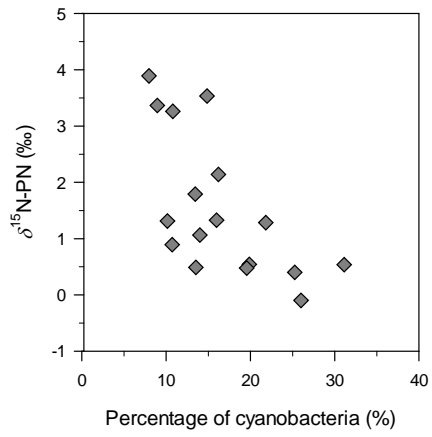
Comment [CMorana6]: Contribution of cyanobacteria is expressed as a % of biomass. It is now explicitly stated, as requested by reviewer 1 (comment 2).

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2 Figure 6. Relationship between the $\delta^{13}\text{C}$ signature of the particulate and dissolved organic
3 carbon pool (POC and DOC, respectively) in the mixed layer.

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Figure 7. Relationship between the relative contribution of cyanobacteria to the phytoplankton assemblage (% of biomass) and the $\delta^{15}\text{N}$ signature of the particulate nitrogen pool in the mixed layer.