

Interactive comment on “Biogeochemistry of a large and deep tropical lake (Lake Kivu, East Africa): insights from a stable isotope study covering an annual cycle” by C. Morana et al.

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Received and published: 27 March 2015

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

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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

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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 ($0.7\text{-}3.3 \text{ mmol m}^{-2} \text{ d}^{-1}$) 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 benefited 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

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$\sim 0.49 \text{ ‰}$ (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

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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 CH₄ 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 CH₄-derived C could be episodically as high as 50 % (65 m, 22/08/12). We hypothesized that microbial CH₄ 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 (Lirós et al. 2010), CH₄ oxidation would have contributed substantially to O₂ 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 (¹²C) into the biomass with the consequence that the heavier isotopes (¹³C) accumulate into the DIC pool, whereas mineralization releases ¹³C-depleted from the organic matter being respired, into the DIC pool. Therefore, increasing primary production leads to higher ¹³C-DIC but increasing respiration should tend to decrease ¹³C-DIC

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(Bade et al., 2004). For instance, several studies conducted in temperate lakes have reported a significant increase in ¹³C-DIC during summer, resulting from increased primary production (Herczeg, 1987; Hollander and McKenzie, 1991). In Lake Kivu, a linear increase of ¹³C-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 $\delta^{13}\text{C}$ -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 ¹³C-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 ¹³C-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 ¹³C-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 ¹³C-DIC signature of the DIC input from the inflowing rivers (Borges et al., 2014) and deep waters (Fig. 3a) was lower than the ¹³C-DIC in the mixed layer". Again, perhaps I am missing something, but it seems to me that the enrichment of the $\delta^{13}\text{C}$ -DIC during the rainy season could simply be reflecting equilibration with atmospheric CO₂ after the DIC became depleted due to deeper water mixing (P17234L24 and Fig.2b). Did the

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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 tempera-

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ture 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 $\delta^{13}\text{C}$ 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 through 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

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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 expanding 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 $\delta^{13}\text{C}$ -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 $\delta^{13}\text{C}$ 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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Interactive comment on Biogeosciences Discuss., 11, 17227, 2014.

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