

23rd July 2015

Dear Dr. Naqvi,

We have gone through the reviewer's evaluation of our revised version. He/she opposes our conclusion that the surface waters of the Lake Kivu are net autotrophic. First of all, we would like to stress that this is only one conclusion of our manuscript, and that the main discussion of the dataset does not focus on the metabolic status of L. Kivu, but rather on the sources and cycling of different C pools. Hence, we can conclude that, in line with the 2nd reviewer who only evaluated the first version of the ms, he/she agrees with the main body of the manuscript and its conclusions, as there are no pending comments other than the issue of the lakes metabolic status.

However, we feel the reviewer does not provide any strong and objective arguments to justify why the lake should be net heterotrophic, instead of net autotrophic. He/she only states that the variation in the $\delta^{13}\text{C}$ -DIC signatures are not significant, yet we have previously addressed this by showing that these changes are significant considering the precision of the method, and for instance, variations of similar magnitude are used to infer anthropogenic CO_2 uptake in the ocean.

Furthermore, we also included in the discussion several elements from our earlier papers that converge to the conclusion that the surface waters of Lake Kivu are net autotrophic, this conclusion has been drawn in earlier papers based on different approaches. The new data presented in this study merely confirm this conclusion, hence we do not see why the metabolic state of lake Kivu should be questioned.

While we acknowledge that our conclusions may not please the reviewer's point of view, we have addressed the reviewer's comments in the most objective and detailed possible way. We have nevertheless toned down the text in the latest version of the manuscript, as explained in more detail in the reply to the reviewer.

On behalf of all co-authors,

Best regards

Cédric Morana

Reply to referee comments

Referee #2:

I would like to first thank the authors for their responses to the other reviewer and my own initial comments. Both reviews had similar concerns regarding the main conclusion of the study i.e., that the lake is net autotrophic in light of the evidence provided in this study. Unfortunately, I was not convinced by the authors' response. In their response, the authors cited many evidence from other studies to support their point (P9L7-20), not many of which were based on the novel data presented in this study. In fact, I found it a bit odd that the authors strongly based their arguments and calculations on Borges et al. (2014) (P9L7-16), and later stated that this conclusion (i.e., net autotrophy) is supported by the same study. How couldn't it be? And how exactly the novel material presented in this study allows one to perform the calculations required to conclude on the metabolic status of the lake? Their main new argument for the support of net autotrophy probably comes from the very small change in $\delta^{13}\text{C}$ -DIC noted seasonally (Fig. 2b), but I still do not think that a change smaller than 1 per mill is enough to safely conclude that the lake is indeed autotrophic. I still believe that the study would have strongly beneficieate from other metabolic measurements or from a more detailed mass balance. I am sorry for not being more positive at this point, but I believe that even after reading authors' clarifications, the isotopic evidence provided still fall short in convincingly addressing the question of the trophic status of Lake Kivu.

Authors reply:

First of all, we would like emphasize that the main objective of this study was to assess the seasonal dynamics of a variety of C reservoirs by means of stable isotope analyses, therefore the $\delta^{13}\text{C}$ -DIC data and the discussion on the metabolic status of the Lake form only one part of the data-set and a minor aspect of the conclusions of the paper. In short, we conclude that the reviewer agrees with the rest and hence the majority of our conclusions.

While the reviewer opposes our conclusion that the surface waters of the Lake Kivu are net autotrophic, we do not find a strong argumentation to justify why it should be net heterotrophic. The reviewer only states that the variation in the $\delta^{13}\text{C}$ -DIC signature is not significant, but we find this a rather subjective statement. We feel that we have already addressed this comment, as :

- (1) Analytically, we have shown that the gradual, linear increase of $\delta^{13}\text{C}$ -DIC (1 ‰ during the rainy season) is real and significant considering the precision of the method (better than 0.2 ‰). For instance, changes of a similar magnitude over time are used to document and quantify the degree of anthropogenic CO_2 uptake by the global ocean.
- (2) We demonstrated that this isotopic enrichment can not result of physical process (ie, equilibration with atmospheric CO_2 , DIC inputs from the catchment or inputs from the monimolimnion), as this would have resulted into a decrease, and not an increase, of the $\delta^{13}\text{C}$ -DIC value. Therefore, this isotopic enrichment should have a biological origin (net CO_2 uptake).
- (3) It should also be kept in mind that the size of the DIC pool in the mixed layer is extremely high (11.9 mmol L^{-1}) compared to the majority of lakes, and especially compared with the mean phytoplanktonic primary production rates (2.6 $\mu\text{mol L}^{-1} \text{d}^{-1}$)

reported in the mixed layer of Lake Kivu (Darchambeau et al. 2014). This large “background” DIC pool further implies that an isotopic enrichment of 1‰ in the DIC over the rainy season represents a substantial change.

Nevertheless, we are willing to accommodate the reviewers reservation and toned down the text. First, we have removed the statement on autotrophy from abstract, as we feel this conclusion has been previously arrived at and is hence not the most crucial message of our manuscript. We have also modified the discussion, by arguing that conclusions on metabolic functioning based on studies in boreal and temperate lakes do not necessarily apply to tropical meromictic lakes, which should not be a surprising statement for the readers of Biogeosciences. However, we feel we should keep our statement that the present data-set contributes to a body of evidence published elsewhere that converges to show that this particular lake is net autotrophic. Indeed, we now more explicitly mention that our results are consistent with or confirm this statement, rather than present it as a conclusion of our dataset. The referee feels that “more detailed mass balance and process measurements” would be needed, but we would like to stress that such data have already been published in Borges et al. (2014) and Morana et al. (2014). Both papers are available online (open access) and were already referenced to in the initial version.

Reference

Borges, A.V., Morana, C., Bouillon, S., Servais, P., Descy, J-P., & Darchambeau, F. (2014). Carbon cycling of Lake Kivu (East Africa) : Net autotrophy in the epilimnion and emission of CO₂ to the atmosphere sustained by geogenic inputs. PLoS ONE 9(10): e109500. doi:10.1371/journal.pone.0109500

Morana C., Sarmiento H., Descy, J-P., Gasol, J. M., Borges, A. V., Bouillon, S., & Darchambeau, F. (2014). Production of dissolved organic matter by phytoplankton and its uptake by heterotrophic prokaryotes in large tropical lakes. Limnology and Oceanography, 59: 1364-1375.

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

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Abstract

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

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

1. Introduction

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

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

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

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

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

2. Material and methods

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

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

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

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

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

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

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

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

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

3. Results

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

DIC concentrations in the mixed layer were very high (annual average at 10 m : $11.9 \pm 0.2 \text{ mmol L}^{-1}$, n = 24) and did not show any consistent seasonal pattern (not shown). The $\delta^{13}\text{C}$ -DIC values were vertically homogeneous in the mixed layer but gradually decreased in the oxycline to reach minimal values at 70 m (Fig. 2a). $\delta^{13}\text{C}$ -DIC values in the mixed layer 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). Taking into account the analytical precision of $\delta^{13}\text{C}$ -DIC
3 measurement (better than $\pm 0.2 \text{ ‰}$), this small but linear ^{13}C enrichment with time was
4 significant. The DOC concentration ($142 \pm 20 \mu\text{mol C L}^{-1}$, $n = 304$) and $\delta^{13}\text{C}$ -DOC signature
5 ($-23.2 \pm 0.4 \text{ ‰}$, $n = 304$) did not show any consistent variations with depth or time in the
6 mixolimnion during all the sampling period. A vertical profile performed down to the lake
7 floor revealed that the $\delta^{13}\text{C}$ -DOC did not vary significantly neither in the monimolimnion
8 (vertical profile average : $-23.0 \text{ ‰} \pm 0.2$, $n = 18$, Fig. 3), however an important increase in
9 DOC concentrations was observed starting at 260 m (Fig. 3), to reach a maximum near the
10 lake floor (350 m, $301 \mu\text{mol C L}^{-1}$).

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

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

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

4. Discussion

Stable isotope analysis of DIC is a useful tool for understanding the fate of C in aquatic ecosystems and could provide information on the lake metabolism, defined as the balance between gross primary production and community respiration of OM. Primary producers preferentially incorporate the lighter isotope (¹²C) into the biomass with the consequence that the heavier isotope (¹³C) accumulates into the DIC pool, whereas mineralization releases ¹³C-depleted CO₂ from the OM being respired, into the DIC pool. Therefore, increasing primary production leads to higher δ¹³C-DIC but increasing respiration should tend to decrease δ¹³C-DIC (Bade et al. 2004). For instance, several studies conducted in temperate lakes have reported a significant increase in δ¹³C-DIC during summer, resulting from primary production (Herczeg 1987, Hollander & McKenzie 1991). In Lake Kivu, the δ¹³C-DIC increased linearly with time during the stratified rainy season, deviating gradually from the δ¹³C-DIC value expected if the DIC pool was at equilibrium with the atmospheric CO₂ (~ 0.49 ‰). It appears unlikely that this linear isotopic enrichment of the DIC pool would be due to physical processes : the δ¹³C-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 δ¹³C-DIC in the mixed layer. Therefore, biological processes (i.e. photosynthetic CO₂ uptake) would be responsible of the isotopic enrichment of the DIC pool observed during the stratified rainy season. Nevertheless, a small decrease in δ¹³C-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 δ¹³C-DIC revealed that the DIC pool was isotopically lighter in the bottom of the mixolimnion, the measurement of lower δ¹³C-DIC values during the dry season could have resulted from the seasonal vertical mixing of surface waters with bottom waters containing relatively ¹³C-depleted DIC.

Overall, the data ~~revealed-suggests~~ 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 supports the conclusions of other studies carried out in Lake Kivu (Morana et al. 2014, Borges et al. 2014) which showed, based on a detailed DIC and D^{13}C mass balance approach and several microbial processes measurements, that that~~ photosynthetic CO_2 fixation ~~should 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. Indeed, In-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 $0.6 \text{ mmol m}^{-2} \text{ d}^{-1}$ (this study); 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 $0.142 \text{ mmol L}^{-1}$, this study)) and the lake surface area (2322 km^2). It 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 of Lake Kivu.

~~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).~~

However, these results are in contradiction with the commonly held view that oligotrophic lacustrine and marine systems tend to be net heterotrophic (Del Giorgio et al. 1997, Cole 1999). Net heterotrophy implies that heterotrophic prokaryotes rely on a substantial amount of allochthonous OM, however 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. Indeed, the magnitude of allochthonous OM inputs relative to phytoplankton production depends strongly on the catchment to surface area ratio (Urban et al 2005), that is particularly low (2.2) in Lake Kivu. Therefore, Lake Kivu is relatively poor in organic C, with DOC concentrations of $\sim 0.15 \text{ mmol L}^{-1}$ in contrast to smaller boreal humic lakes which show DOC concentrations of on

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1 average $\sim 1 \text{ mmol L}^{-1}$ (Sobek et al. 2007), and with values up to $\sim 4.5 \text{ mmol L}^{-1}$ (Weyhenmeyer
2 & Karlsson 2009). Humic substances are usually low quality substrates for bacterial growth
3 (Castillo et al. 2003), but limit primary production by absorbing incoming light. Hence,
4 heterotrophic production in the photic zone of humic lakes usually exceeds phytoplankton
5 production and DOC concentrations, despite the low substrate quality of humic substance,
6 have been found to be a good predictor of the metabolic status of lakes in the boreal region,
7 with a prevalence of net heterotrophy in organic-rich lakes (Jansson et al. 2000). However,
8 low allochthonous OM inputs and low DOC concentration do not necessary cause a system to
9 be net autotrophic. For instance, Lake Superior has a lower catchment to surface area ratio
10 (1.6), is subsidized by a similar amount of allochthonous OM ($\sim 3 \text{ mmol m}^{-2} \text{ d}^{-1}$) and the DOC
11 concentration is even lower than in Lake Kivu ($\sim 0.1 \text{ mmol L}^{-1}$), but it has been found to be
12 net heterotrophic despite the limited allochthonous OM inputs (Urban et al. 2005). Lake
13 Superior, as the majority of the lakes of the world, is holomictic, meaning that the mixing of
14 its water column can seasonally reach the lake floor, and a substantial amount of sediments,
15 including OM, could then be resuspended during these mixing events and hence re-exposed to
16 microbial mineralization in well-oxygenated waters (Meyers and Eadie 1993, Cotner 2000,
17 Urban et al. 2005). The resuspension of benthic sediments could be important in the
18 ecological functioning of these systems. In contrast, Lake Kivu, as other East African large
19 lakes such as Lake Tanganyika and Malawi, are particularly deep meromictic lakes, so that
20 their water column is characterized by an almost complete decoupling between the surface
21 and deep waters, avoiding any resuspended benthic sediment to reach the surface waters in
22 this system. In consequence, the coupling between the phytoplankton production of DOC and
23 its heterotrophic consumption by prokaryotes in the clear, nutrient-depleted waters of Lake
24 Kivu was found to be high throughout the year (Morana et al. 2014).

25 Besides morphometrical features, the net autotrophic status of Lake Kivu might also be
26 related to general latitudinal and climatic patterns. Due to the warmer temperature in the
27 tropics, phytoplankton production is comparatively higher in the East African large lakes
28 compared with the Laurentian Great lakes, despite similar phytoplankton abundance
29 (Bootsma & Hecky 2003). Alin and Johnson (2007) reviewed phytoplankton primary
30 production and CO_2 emission to the atmosphere fluxes in large lakes of world ($>500 \text{ km}^2$). At
31 the global scale, they found a statistically significant increase of the areal phytoplankton
32 production in large lakes with the mean annual water temperature and the insolation ; and in
33 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).

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

The concentration of the POC pool varied largely with depth, being the highest in the 0-20m layer, i.e. roughly the euphotic zone. However, during the dry season, POC concentrations

1 was almost as high in the oxycline than in surface waters. High POC concentrations in deep
 2 waters have frequently been observed in lakes, usually as a result from the resuspension of
 3 benthic sediments near the lake floor or to the accumulation of sedimenting material in
 4 density gradients (Hawley and Lee 1999). However, in the deep Lake Kivu, this maximum
 5 POC zone is located approximately 300 m above the lake floor and is characterized by a
 6 strong depletion in ^{13}C of the POC pool. While DIC would be the major C source of the POC
 7 pool in the mixed layer, the important decrease of $\delta^{13}\text{C}$ -POC values observed in the oxycline
 8 suggests that another ^{13}C -depleted C source was actively incorporated into the biomass at the
 9 bottom of the mixolimnion. Slight depletion in ^{13}C of the POC pool in oxyclines, such as in
 10 the Black Sea, has sometimes been interpreted as a result of to the heterotrophic
 11 mineralization of the sedimenting OM (Coban-Yildiz et al. 2006), but it seems unlikely that,
 12 in Lake Kivu, heterotrophic processes could have caused an abrupt excursion of $\delta^{13}\text{C}$ -POC to
 13 values as low as -41.6 ‰ (65 m, 22/08/12). Such large isotopic depletion of the POC pool in
 14 the water column have been reported by Blees et al. (2014), who measured $\delta^{13}\text{C}$ -POC as low
 15 as -49‰ in Lake Lugano, and they were related to high methanotrophic activity. In Lake
 16 Kivu, CH_4 concentrations were found to decrease sharply at the oxic-anoxic transition
 17 (Borges et al. 2011), and the dissolved CH_4 that reached the oxycline via turbulent diffusivity
 18 and vertical advection (Schmid et al. 2005) is known to be isotopically light, with a $\delta^{13}\text{C}$
 19 signature of approximately -60 ‰ (Pasche et al. 2011, Morana et al. 2014). Therefore, the
 20 vertical pattern in CH_4 concentrations and $\delta^{13}\text{C}$ -POC values observed during this study
 21 suggests that a substantial part of CH_4 was consumed and incorporated into the microbial
 22 biomass in the oxycline. Indeed, experiments carried out in Lake Kivu in February 2012 and
 23 September 2012 showed that microbial CH_4 oxidation was significant in the oxycline, and
 24 phospholipid fatty acids analysis revealed high abundance of methanotrophic bacteria of type
 25 I at the same depths (Morana et al. 2014). With estimates of the isotope fractionation factor
 26 during microbial CH_4 oxidation (1.016, Morana et al. 2014), and of the $\delta^{13}\text{C}$ - CH_4 at each
 27 sampling point, it is possible to estimate the theoretical $\delta^{13}\text{C}$ signature of methanotrophic
 28 organisms at each depth. Note that the $\delta^{13}\text{C}$ - CH_4 was not directly measured during this study
 29 but a very strong linear correlation between the log-transformed CH_4 concentrations and $\delta^{13}\text{C}$ -
 30 CH_4 was found along vertical profiles performed in February and September 2012 in Lake
 31 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).
 32 Hence the $\delta^{13}\text{C}$ - CH_4 at each sampling point between January 2012 and May 2013 can be
 33 approximated from the measured CH_4 concentrations, using this empirical relationship. Then,
 34 a simple isotope mixing model with the calculated $\delta^{13}\text{C}$ signature of methanotrophs and the

1 average $\delta^{13}\text{C}$ -POC in the mixed layer as end-members allowed to determine the contribution
2 of CH_4 -derived C to POC at each sampling depth. It appears that $4.4 \pm 1.9 \%$ ($n = 13$) and 6.4
3 $\pm 1.6 \%$ ($n = 5$) of the depth-integrated POC pool in the mixolimnion was derived from CH_4
4 incorporation into the biomass during the rainy and dry season, respectively, and these
5 percentages did not significantly differ between seasons (two-tailed t -test, $p = 0.055$).
6 Nevertheless, the low $\delta^{13}\text{C}$ signatures measured locally in the oxycline indicate that the
7 contribution of CH_4 -derived C could be episodically as high as 50 % (65 m, 22/08/12). We
8 hypothesized that microbial CH_4 oxidation could play an important role in the ecological
9 functioning of Lake Kivu. Along with heterotrophic mineralization of the sinking OM, and
10 presumably other chemoautotrophic processes occurring in the oxycline such as nitrification
11 (Llirós et al. 2010), CH_4 oxidation would have contributed substantially to O_2 consumption in
12 the water column and was partly responsible for the seasonal uplift of the oxycline observed
13 after the re-establishment of the thermal stratification during the rainy season. Furthermore,
14 the methanotrophs in the oxycline would actively participate to the uptake of dissolved
15 inorganic phosphorus (DIP), and hence would contribute to exert an indirect control on
16 phytoplankton by constantly limiting the vertical DIP flux to the illuminated surface waters
17 (Haberyan and Hecky 1987). Indeed, phytoplankton in Lake Kivu suffers of a severe P
18 limitation throughout the year as pointed out by the relatively high sestonic C:P ratio ($256 \pm$
19 75 ; Sarmiento et al. 2009 ; Darchambeau et al 2014).

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

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

In conclusion, stable isotope data revealed large seasonal variability in the $\delta^{15}\text{N}$ signature of the PN pool, most likely related to changes in the phytoplankton assemblage and to N₂-fixation. In contradiction with the common observation that oligotrophic aquatic ecosystems tend to be net heterotrophic, the seasonality of $\delta^{13}\text{C}$ -DIC ~~suggests~~ supports the view that the

1 mixed layer of Lake Kivu is net autotrophic, ~~as demonstrated by supporting the conclusions~~
2 ~~of~~ Borges et al. (2014); based on DIC and $\delta^{13}\text{C}$ mass balance considerations. The $\delta^{13}\text{C}$ -POC
3 showed an important variation with depth due to the abundance of methanotrophic bacteria in
4 the oxycline that fixed the lighter CH_4 -derived C into their biomass. The $\delta^{13}\text{C}$ -POC and $\delta^{13}\text{C}$ -
5 DOC appeared to be uncoupled vertically and temporally, which could indicate that most of
6 the DOC pool was composed of relatively refractory compounds. Finally, the $\delta^{13}\text{C}$ of
7 zooplankton mirrored the $\delta^{13}\text{C}$ signature of the autochthonous POC pool, and its $\delta^{15}\text{N}$
8 signature followed the seasonal variability of the $\delta^{15}\text{N}$ -PN pool in good agreement with the
9 expected consumer-diet isotope fractionation. This suggests that zooplankton would rely
10 throughout the year on phytoplankton-derived biomass as a organic C source.

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12 Acknowledgements

13 We are grateful to Boniface Kaningini, Pascal Isumbisho (Institut Supérieur Pédagogique,
14 Bukavu, DRC) for logistic support during the cruises, to Georges Alunga and the staff of the
15 Unité d'Enseignement et de Recherche en Hydrobiologie Appliquée (UERHA – ISP Bukavu)
16 who carried out the field sampling in DRC. We are also grateful to Marc-Vincent Commarieu
17 who carried out the TA measurements in the University of Liège, to Stephan Hoornaert who
18 carried out part of the CH_4 measurements and Bruno Leporcq who carried out the pigment
19 analysis in the University of Namur. This work was funded by the EAGLES (East African
20 Great lake Ecosystem Sensitivity to Changes, SD/AR/02A) project from the Belgian Federal
21 Science Policy Office (BELSPO, Belgium) and contributes to the European Research Council
22 (ERC) starting grant project AFRIVAL (African river basins: Catchment-scale carbon fluxes
23 and transformations, 240002). AVB is a senior research associate at the FNRS.

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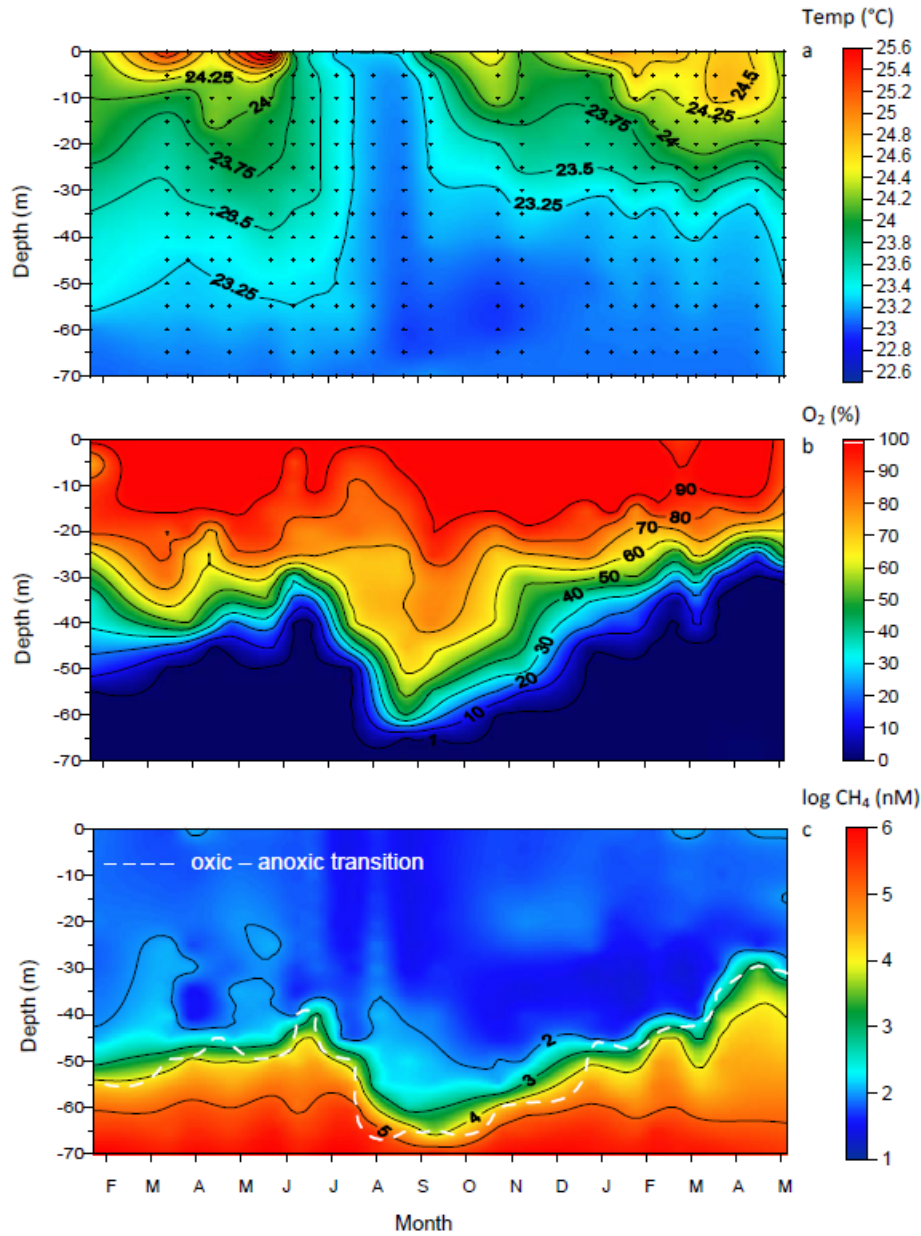
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1 Figures



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4 Figure 1. Temporal variability of (a) temperature (°C), (b) oxygen saturation (%), and (c) the
5 log-transformed CH₄ concentration (nmol 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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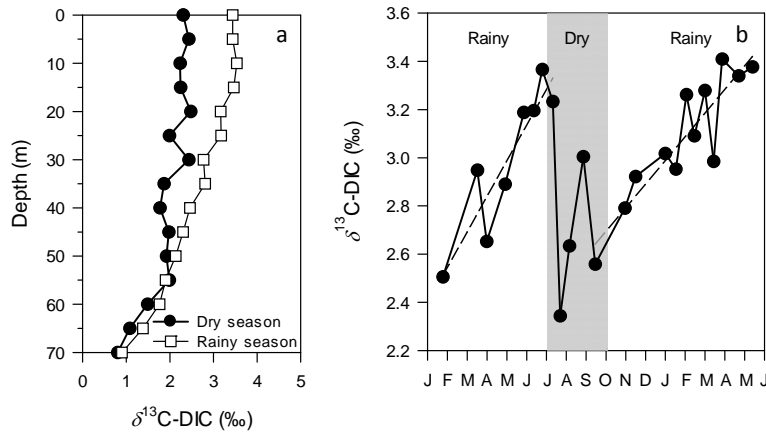


Figure 2. Depth profile of the $\delta^{13}\text{C}$ of the dissolved inorganic carbon (DIC) pool in the mixolimnion during the dry (18/07/12) and the rainy (20/03/13) season and (b) temporal variation of the $\delta^{13}\text{C-DIC}$ in the mixed layer of Lake Kivu between January 2012 and June 2013.

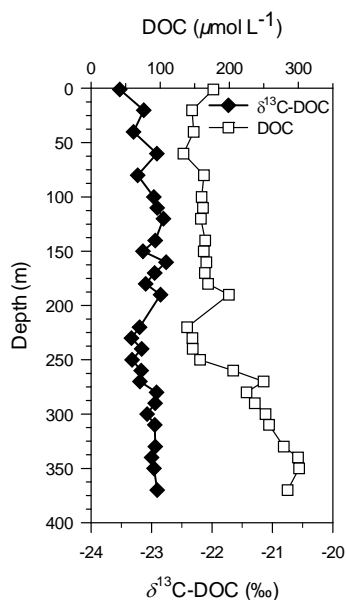


Figure 3. Vertical profile from the lake surface to the lake floor of the dissolved organic carbon (DOC) concentration ($\mu\text{mol L}^{-1}$) and the $\delta^{13}\text{C}$ signature of the DOC pool, in September 2012.

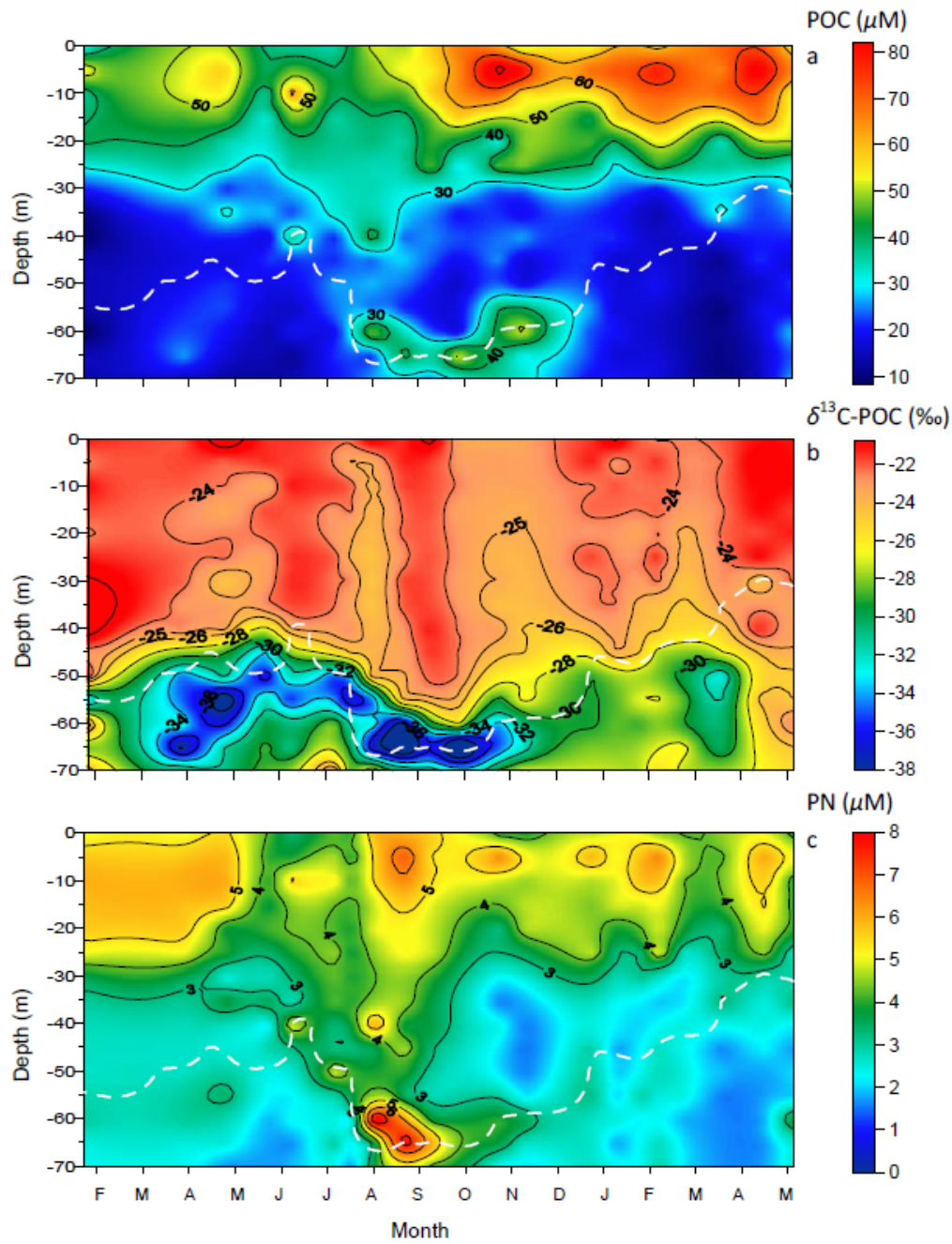


Figure 4. Temporal variability of (a) the particulate organic carbon (POC) concentration ($\mu\text{mol L}^{-1}$), (b) the $\delta^{13}\text{C}$ signature of the POC pool, and (c) the particulate nitrogen (PN) concentration ($\mu\text{mol L}^{-1}$) in the mixolimnion of Lake Kivu, between February 2012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

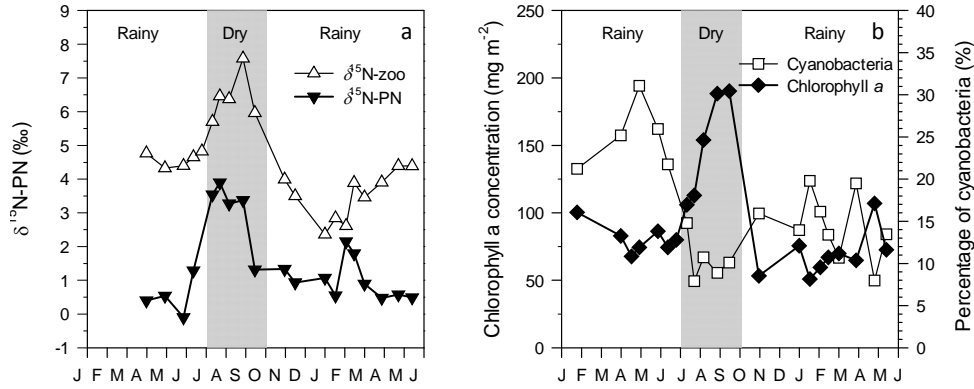


Figure 5. Temporal variability of (a) the $\delta^{15}\text{N}$ signature of the particulate nitrogen (PN) pool and zooplankton in the mixed layer, and (b) the chlorophyll a concentration (mg m^{-2}) and the relative contribution of cyanobacteria to the phytoplankton assemblage (% of biomass) in the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

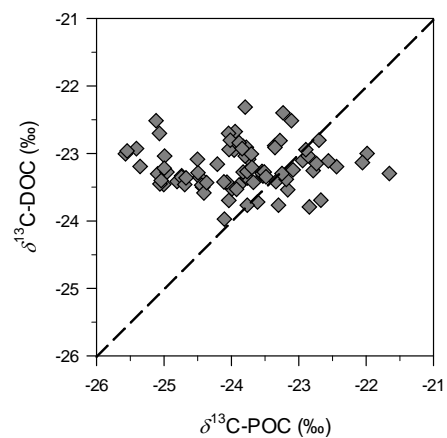


Figure 6. Relationship between the $\delta^{13}\text{C}$ signature of the particulate and dissolved organic carbon pool (POC and DOC, respectively) in the mixed layer.

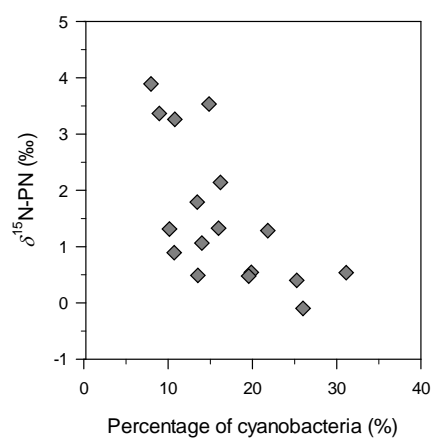


Figure 7. Relationship between the relative contribution of cyanobacteria to the phytoplankton assemblage (% of biomass) and the $\delta^{15}\text{N}$ signature of the particulate nitrogen pool in the mixed layer.