

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. 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 freshwaters 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

1 1996). Also, the contribution of dissolved primary production in oligotrophic tropical lake has
2 been found to substantially more important than in their temperate counterparts (Morana et al.
3 2014).

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

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

31 **2. Material and methods**

32 Lake Kivu (East Africa) is a large (2370 km²) and deep (maximum depth of 485 m)
33 meromictic lake located at the border between the Democratic Republic of the Congo and

1 Rwanda. Its vertical structure consists of an oxic and nutrient-poor mixed layer down to a
2 maximum of 70 m, and a permanently anoxic monimolimnion rich in dissolved gases (CH_4 ,
3 and CO_2) and inorganic nutrients. Seasonal variation of the vertical position of the oxic-
4 anoxic transition is driven by contrasting air humidity and incoming long-wave radiation
5 between rainy (October-May) and dry (June-September) season (Thiery et al. 2014). The
6 euphotic zone, defined at the depth at which light is 1% of surface irradiance, is relatively
7 shallow (annual average : 18 m, Darchambeau et al. 2014).

8 Sampling was carried out in the Southern Basin (02°20'S, 28°58'E) of Lake Kivu between
9 January 2012 and May 2013 at a monthly or fortnightly time interval. Vertical oxygen (O_2),
10 temperature and conductivity profiles were obtained with a Hydrolab DS5 multiprobe. The
11 conductivity cell was calibrated with a 1000 $\mu\text{S cm}^{-1}$ (25°C) Merck standard and the O_2
12 membrane probe was calibrated with humidity saturated ambient air. Water was collected
13 with a 7 L Niskin bottle (Hydro-Bios) at a depth interval of 5 m from the lake surface to the
14 bottom of the mixolimnion, at 70 m. Additionally, zooplankton was sampled with a 75-cm
15 diameter, 55- μm mesh plankton net hauled along the whole mixolimnion (0-70m).

16 Samples for CH_4 concentrations were collected in 50 ml glass serum bottles from the Niskin
17 bottle with a tube, left to overflow, poisoned with 100 μl of saturated HgCl_2 and sealed with
18 butyl stoppers and aluminium caps. Concentrations of CH_4 were measured by headspace
19 technique using gas chromatography (Weiss 1981) with flame ionization detection (SRI
20 8610C), after creating a 20 ml headspace with N_2 in the glass serum bottles, and then
21 analyzed as described by Borges et al. (2011).

22 Samples for stable C isotopic composition of dissolved inorganic carbon ($\delta^{13}\text{C-DIC}$) were
23 collected by filling with water directly from the Niskin bottle 12 mL headspace vials (Labco
24 Exetainer) without bubbles. Samples were preserved with the addition of 20 μL of a saturated
25 HgCl_2 solution. Prior to the analysis of $\delta^{13}\text{C-DIC}$, a 2 ml helium headspace was created and
26 100 μL of phosphoric acid (H_3PO_4 , 99%) was added in the vial in order to convert all
27 inorganic C species to CO_2 . After overnight equilibration, 200 μL of gas was injected with a
28 gastight syringe into a EA-IRMS (Thermo FlashHT with Thermo DeltaV Advantage). The
29 obtained data were corrected for isotopic equilibration between dissolved and gaseous CO_2 as
30 described in Gillikin and Bouillon (2007). Calibration of $\delta^{13}\text{C-DIC}$ measurement was
31 performed with the international certified standards IAEA-CO1 and LSVEC. The
32 reproducibility of $\delta^{13}\text{C-DIC}$ measurement was typically better than ± 0.2 ‰. Measurements of
33 total alkalinity (TA) were carried out by open-cell titration with HCl 0.1 mol L^{-1} according to

1 Gran (1952) on 50 mL water samples, and data were quality checked with certified reference
2 material obtained from Andrew Dickinson (Scripps Institution of Oceanography, University
3 of California, San Diego, USA). Typical reproducibility of TA measurements was better than
4 $\pm 3 \mu\text{mol L}^{-1}$. DIC concentration was computed from pH and TA measurements using the
5 carbonic acid dissociation constants of Millero et al. (2006).

6 Samples for DOC concentration and stable C isotopic composition ($\delta^{13}\text{C}$ -DOC) were filtered
7 through pre-flushed $0.2 \mu\text{m}$ syringe filters, kept in 40ml borosilicate vials with Teflon-coated
8 screw caps and preserved with $100 \mu\text{L}$ of H_3PO_4 (50%). Sample analysis was carried out with
9 a IO Analytical Aurora 1030W coupled to an IRMS (Thermo delta V
10 Advantage). Quantification and calibration of DOC and $\delta^{13}\text{C}$ -DOC was performed with IAEA-
11 C6 and an internal sucrose standard ($\delta^{13}\text{C} = -26.99 \pm 0.04 \text{ ‰}$) calibrated against international
12 reference materials.

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

28 Pigment concentrations were determined by high performance liquid chromatography
29 (HPLC). 2-4 L of waters were filtered through Macherey-Nägel GF-5 filter (average retention
30 of $0.7 \mu\text{m}$). Pigment extraction was carried out in 10 mL of 90% HPLC grade acetone. After
31 two sonication steps of 15 min separated by an overnight period at 4°C , the pigments extracts
32 were stored in 2 mL amber vials at -25°C . HPLC analysis was performed following the
33 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 decreased at the start of the dry season due to the vertical mixing with ¹³C-depleted DIC from deeper waters (Fig. 2b). Taking into account the analytical precision of $\delta^{13}\text{C}$ -DIC measurement (better than $\pm 0.2 \text{ ‰}$), this small but linear ¹³C enrichment with time was

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

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

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

31 Chlorophyll *a* concentrations exhibited little variation during the rainy season (average $74 \pm$
32 $15 \text{ mg Chl } a \text{ m}^{-2}$, $n = 16$) but increased significantly during the dry season to reach a maximal

value ($190 \text{ mg Chla m}^{-2}$) 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 ; $p < 0.01$, $\text{mean}_{\text{jan-jun}} = 23.4 \pm 5.5\%$, $\text{mean}_{\text{jul-sep}} = 9.4 \pm 1.3\%$) and the following (t -test ; $p < 0.05$, $\text{mean}_{\text{oct-may}} = 14.6 \pm 3.8\%$, $\text{mean}_{\text{jul-sep}} = 9.4 \pm 1.3\%$) 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 (^{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 ($\sim 0.49\text{‰}$). 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.

Overall, the data suggest 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

1 variability of $\delta^{13}\text{C}$ -DIC observed in the mixed layer hold information on biological processes.
2 The gradual increase with time of the $\delta^{13}\text{C}$ -DIC in the mixed layer supports the conclusions of
3 other studies carried out in Lake Kivu (Morana et al. 2014, Borges et al. 2014) which showed,
4 based on a detailed DIC and DI^{13}C mass balance approach and several microbial processes
5 measurements, that photosynthetic CO_2 fixation should exceed the respiration of OM. Indeed,
6 in Lake Kivu, riverine inputs of allochthonous OM from the catchment ($0.7 - 3.3 \text{ mmol m}^{-2} \text{ d}^{-1}$;
7 ¹, Borges et al. 2014) are minimal compared to primary production ($49 \text{ mmol m}^{-2} \text{ d}^{-1}$;
8 Darchambeau et al. 2014) and the export of organic carbon to the monimolimnion (9.4 mmol
9 $\text{m}^{-2} \text{ d}^{-1}$) reported by Pasche et al. (2010). The outflow of organic carbon through the Ruzizi
10 River is also relatively low and was computed to be $0.6 \text{ mmol m}^{-2} \text{ d}^{-1}$ (this study) based on the
11 long term discharge average of Ruzizi ($83.2 \text{ m}^3 \text{ s}^{-1}$, Borges et al. 2014), the average POC and
12 DOC in surface waters (0.052 and $0.142 \text{ mmol L}^{-1}$, this study). It implies that the outputs of
13 OM ($9.4 + 0.7 = 10.1 \text{ mmol m}^{-2} \text{ d}^{-1}$) are higher than the inputs of OM from the catchment
14 ($0.7\text{-}3.3 \text{ mmol m}^{-2} \text{ d}^{-1}$) suggesting a net autotrophic status of Lake Kivu.

15 However, these results are in contradiction with the commonly held view that oligotrophic
16 lacustrine and marine systems tend to be net heterotrophic (Del Giorgio et al. 1997, Cole
17 1999). Net heterotrophy implies that heterotrophic prokaryotes rely on a substantial amount of
18 allochthonous OM, however in Lake Kivu, riverine inputs of allochthonous OM from the
19 catchment ($0.7 - 3.3 \text{ mmol m}^{-2} \text{ d}^{-1}$, Borges et al. 2014) are minimal. Indeed, the magnitude of
20 allochthonous OM inputs relative to phytoplankton production depends strongly on the
21 catchment to surface area ratio (Urban et al 2005), that is particularly low (2.2) in Lake Kivu.
22 Therefore, Lake Kivu is relatively poor in organic C, with DOC concentrations of ~ 0.15
23 mmol L^{-1} in contrast to smaller boreal humic lakes which show DOC concentrations of on
24 average $\sim 1 \text{ mmol L}^{-1}$ (Sobek et al. 2007), and with values up to $\sim 4.5 \text{ mmol L}^{-1}$ (Weyhenmeyer
25 & Karlsson 2009). Humic substances are usually low quality substrates for bacterial growth
26 (Castillo et al. 2003), but limit primary production by absorbing incoming light. Hence,
27 heterotrophic production in the photic zone of humic lakes usually exceeds phytoplankton
28 production and DOC concentrations, despite the low substrate quality of humic substance,
29 have been found to be a good predictor of the metabolic status of lakes in the boreal region,
30 with a prevalence of net heterotrophy in organic-rich lakes (Jansson et al. 2000). However,
31 low allochthonous OM inputs and low DOC concentration do not necessary cause a system to
32 be net autotrophic. For instance, Lake Superior has a lower catchment to surface area ratio
33 (1.6), is subsidized by a similar amount of allochthonous OM ($\sim 3 \text{ mmol m}^{-2} \text{ d}^{-1}$) and the DOC

1 concentration is even lower than in Lake Kivu ($\sim 0.1 \text{ mmol L}^{-1}$), but it has been found to be
2 net heterotrophic despite the limited allochthonous OM inputs (Urban et al. 2005). Lake
3 Superior, as the majority of the lakes of the world, is holomictic, meaning that the mixing of
4 its water column can seasonally reach the lake floor, and a substantial amount of sediments,
5 including OM, could then be resuspended during these mixing events and hence re-exposed to
6 microbial mineralization in well-oxygenated waters (Meyers and Eadie 1993, Cotner 2000,
7 Urban et al. 2005). The resuspension of benthic sediments could be important in the
8 ecological functioning of these systems. In contrast, Lake Kivu, as other East African large
9 lakes such as Lake Tanganyika and Malawi, are particularly deep meromictic lakes, so that
10 their water column is characterized by an almost complete decoupling between the surface
11 and deep waters, avoiding any resuspended benthic sediment to reach the surface waters in
12 this system. In consequence, the coupling between the phytoplankton production of DOC and
13 its heterotrophic consumption by prokaryotes in the clear, nutrient-depleted waters of Lake
14 Kivu was found to be high throughout the year (Morana et al. 2014).

15 Besides morphometrical features, the net autotrophic status of Lake Kivu might also be
16 related to general latitudinal and climatic patterns. Due to the warmer temperature in the
17 tropics, phytoplankton production is comparatively higher in the East African large lakes
18 compared with the Laurentian Great lakes, despite similar phytoplankton abundance
19 (Bootsma & Hecky 2003). Alin and Johnson (2007) reviewed phytoplankton primary
20 production and CO_2 emission to the atmosphere fluxes in large lakes of world ($>500 \text{ km}^2$). At
21 the global scale, they found a statistically significant increase of the areal phytoplankton
22 production in large lakes with the mean annual water temperature and the insolation ; and in
23 consequence, a significant decrease of phytoplankton production with latitude. Also, they
24 report a significant decrease of the CO_2 emission to the atmosphere with the mean annual
25 water temperature and therefore an increase of the CO_2 emission with the latitude. According
26 to their estimations, less than 20% of the phytoplankton primary production would be
27 sufficient to balance the carbon loss through CO_2 evasion and OM burial in sediments in large
28 lakes located between the equator and the latitude 30° , but the CO_2 emission and OM
29 accumulation in sediments would exceed the phytoplankton primary production in systems
30 located at latitude higher than 40° (Alin and Johnson 2007). Overall, in morphometrically
31 comparable systems, this global analysis suggests a trend from autotrophic to increasingly
32 heterotrophic conditions with increasing latitude and decreasing mean annual water
33 temperature and insolation (Alin and Johnson 2007). Therefore, our study supports the view

1 that paradigms established with data gathered in comparatively small temperate and boreal
2 lakes may not directly apply to larger, tropical lakes (Bootsma & Hecky 2003). It also
3 highlights the need to consider the unique limnological characteristics of a vast region of the
4 world that harbours 16% of the total surface of lakes (Lehner & Döll 2004), and would
5 account for 50% of the global inputs of OM from continental waters to the oceans (Ludwig et
6 al. 1996).

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

21 The concentration of the POC pool varied largely with depth, being the highest in the 0-20m
22 layer, i.e. roughly the euphotic zone. However, during the dry season, POC concentrations
23 was almost as high in the oxycline than in surface waters. High POC concentrations in deep
24 waters have frequently been observed in lakes, usually as a result from the resuspension of
25 benthic sediments near the lake floor or to the accumulation of sedimenting material in
26 density gradients (Hawley and Lee 1999). However, in the deep Lake Kivu, this maximum
27 POC zone is located approximately 300 m above the lake floor and is characterized by a
28 strong depletion in ^{13}C of the POC pool. While DIC would be the major C source of the POC
29 pool in the mixed layer, the important decrease of $\delta^{13}\text{C}$ -POC values observed in the oxycline
30 suggests that another ^{13}C -depleted C source was actively incorporated into the biomass at the
31 bottom of the mixolimnion. Slight depletion in ^{13}C of the POC pool in oxyclines, such as in
32 the Black Sea, has sometimes been interpreted as a result of to the heterotrophic
33 mineralization of the sedimenting OM (Coban-Yildiz et al. 2006), but it seems unlikely that,

1 in Lake Kivu, heterotrophic processes could have caused an abrupt excursion of $\delta^{13}\text{C}$ -POC to
 2 values as low as -41.6 ‰ (65 m, 22/08/12). Such large isotopic depletion of the POC pool in
 3 the water column have been reported by Blees et al. (2014), who measured $\delta^{13}\text{C}$ -POC as low
 4 as -49‰ in Lake Lugano, and they were related to high methanotrophic activity. In Lake
 5 Kivu, CH_4 concentrations were found to decrease sharply at the oxic-anoxic transition
 6 (Borges et al. 2011), and the dissolved CH_4 that reached the oxycline via turbulent diffusivity
 7 and vertical advection (Schmid et al. 2005) is known to be isotopically light, with a $\delta^{13}\text{C}$
 8 signature of approximately -60 ‰ (Pasche et al. 2011, Morana et al. 2014). Therefore, the
 9 vertical pattern in CH_4 concentrations and $\delta^{13}\text{C}$ -POC values observed during this study
 10 suggests that a substantial part of CH_4 was consumed and incorporated into the microbial
 11 biomass in the oxycline. Indeed, experiments carried out in Lake Kivu in February 2012 and
 12 September 2012 showed that microbial CH_4 oxidation was significant in the oxycline, and
 13 phospholipid fatty acids analysis revealed high abundance of methanotrophic bacteria of type
 14 I at the same depths (Morana et al. 2014). With estimates of the isotope fractionation factor
 15 during microbial CH_4 oxidation (1.016, Morana et al. 2014), and of the $\delta^{13}\text{C}$ - CH_4 at each
 16 sampling point, it is possible to estimate the theoretical $\delta^{13}\text{C}$ signature of methanotrophic
 17 organisms at each depth. Note that the $\delta^{13}\text{C}$ - CH_4 was not directly measured during this study
 18 but a very strong linear correlation between the log-transformed CH_4 concentrations and $\delta^{13}\text{C}$ -
 19 CH_4 was found along vertical profiles performed in February and September 2012 in Lake
 20 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).
 21 Hence the $\delta^{13}\text{C}$ - CH_4 at each sampling point between January 2012 and May 2013 can be
 22 approximated from the measured CH_4 concentrations, using this empirical relationship. Then,
 23 a simple isotope mixing model with the calculated $\delta^{13}\text{C}$ signature of methanotrophs and the
 24 average $\delta^{13}\text{C}$ -POC in the mixed layer as end-members allowed to determine the contribution
 25 of CH_4 -derived C to POC at each sampling depth. It appears that $4.4 \pm 1.9 \%$ ($n = 13$) and 6.4
 26 $\pm 1.6 \%$ ($n = 5$) of the depth-integrated POC pool in the mixolimnion was derived from CH_4
 27 incorporation into the biomass during the rainy and dry season, respectively, and these
 28 percentages did not significantly differ between seasons (two-tailed t -test, $p = 0.055$).
 29 Nevertheless, the low $\delta^{13}\text{C}$ signatures measured locally in the oxycline indicate that the
 30 contribution of CH_4 -derived C could be episodically as high as 50 % (65 m, 22/08/12). We
 31 hypothesized that microbial CH_4 oxidation could play an important role in the ecological
 32 functioning of Lake Kivu. Along with heterotrophic mineralization of the sinking OM, and
 33 presumably other chemoautotrophic processes occurring in the oxycline such as nitrification
 34 (Llirós et al. 2010), CH_4 oxidation would have contributed substantially to O_2 consumption in

1 the water column and was partly responsible for the seasonal uplift of the oxycline observed
2 after the re-establishment of the thermal stratification during the rainy season. Furthermore,
3 the methanotrophs in the oxycline would actively participate to the uptake of dissolved
4 inorganic phosphorus (DIP), and hence would contribute to exert an indirect control on
5 phytoplankton by constantly limiting the vertical DIP flux to the illuminated surface waters
6 (Haberyan and Hecky 1987). Indeed, phytoplankton in Lake Kivu suffers of a severe P
7 limitation throughout the year as pointed out by the relatively high sestonic C:P ratio ($256 \pm$
8 75 ; Sarmiento et al. 2009 ; Darchambeau et al 2014).

9 The $\delta^{15}\text{N}$ signature of the autochthonous OM in the mixed layer of Lake Kivu oscillated
10 around 0 ‰ during the rainy season in Lake Kivu but was significantly higher during the dry
11 season (3 – 4 ‰). Also, the $\delta^{15}\text{N}$ -PN in the mixed layer correlated negatively with the
12 proportion of cyanobacteria in waters (Fig. 7, Pearson's r : -0.65, p = 0.004, n = 17). This
13 pattern may highlight the seasonal importance of N_2 -fixing cyanobacteria in Lake Kivu during
14 the rainy season. Indeed, the $\delta^{15}\text{N}$ signature of atmospheric N_2 is close to 0 ‰ and isotope
15 fractionation during cyanobacterial N_2 -fixation is known to be small (Fogel & Cifuentes
16 1991). Several studies carried out in marine (Pacific Ocean and Gulf of Mexico) and
17 lacustrine (Lake Lugano) systems have shown that $\delta^{15}\text{N}$ -PN varied between -2 ‰ and +1 ‰
18 when N_2 -fixing cyanobacteria were dominating the phytoplankton assemblage (Wada 1976,
19 Macko et al. 1984, Lehmann et al. 2004). Moreover, a good relationship between the $\delta^{15}\text{N}$ -PN
20 and the abundance of N_2 -fixing cyanobacteria has already been reported for others systems,
21 such as coastal lagoon (Lesutiene et al. 2014). In Lake Victoria, biological N_2 fixation has
22 been identified as the largest input of N, exceeding atmospheric deposition and river inputs,
23 and N_2 fixation has been found to increase with light availability (Mugidde et al. 2003). This
24 suggests that during the rainy season, when thermal stratification of the mixolimnion leads to
25 reduced nitrogen supply combined with exposure to high light levels, N_2 -fixing cyanobacteria
26 would have a competitive advantage which may explain their seasonally higher contribution
27 to the autochthonous OM pool (Sarmiento et al., 2006). Indeed, the significantly higher molar
28 C:N ratio during the rainy season than the dry season indicates that N-limitation in the mixed
29 layer was stronger during the rainy season (this study, Sarmiento et al. 2009). By contrast, the
30 deepening of the mixed layer during the dry season leads to increased nutrients input and
31 reduced light availability that favours alternative phytoplankton strategies (Hecky & Kling,
32 1987; Reynolds, 2006; Sarmiento et al. 2006; Darchambeau et al. 2014), and consequently the
33 proportion N_2 -fixing cyanobacteria decreases. A similar seasonal pattern of N_2 fixation was

1 reported in Lake Victoria by Mugidde et al. (2003). In contrast with the rather constant $\delta^{13}\text{C}$
2 signature of zooplankton ($-22.9 \pm 0.8 \text{ ‰}$), the $\delta^{15}\text{N}$ analysis revealed that the $\delta^{15}\text{N}$ of
3 zooplankton varied importantly, following well the seasonal change in $\delta^{15}\text{N}$ -PN in the mixed
4 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
5 $\pm 1.0 \text{ ‰}$ throughout the year while it was on average enriched in ^{13}C ($\Delta^{13}\text{C}_{\text{Zoo-POC}}$) by $0.9 \pm$
6 0.8 ‰ . In nature, comparison of the $\delta^{15}\text{N}$ signature of consumers and their diet indicates that
7 the $\delta^{15}\text{N}$ value increases consistently with the trophic level, because of the preferential
8 excretion of the isotopically lighter ^{14}N (Montoya et al. 2002). However the C isotope
9 fractionation between consumers and diet is usually considered to be less than 1 ‰ (Sirevag
10 et al. 1977). The constant $\Delta^{15}\text{N}_{\text{Zoo-PN}}$ value found in Lake Kivu is within the range of trophic
11 level enrichment between algae and *Daphnia magna* ($\sim 2 \text{ ‰}$ to 5 ‰) estimated in laboratory
12 experiment (Adams and Sterner 2000), and very close to the cross-system trophic enrichment
13 value ($3.4 \pm 1.0 \text{ ‰}$) proposed by Post (2002). Together with the slight enrichment in ^{13}C
14 compared with the autochthonous POC pool, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis suggests that
15 zooplankton directly incorporate phytoplankton-derived OM in their biomass (Masilya 2011),
16 and they would rely almost exclusively on this source of OM throughout the year. This is in
17 general agreement with the very low allochthonous OM inputs from rivers in Lake Kivu
18 (Borges et al. 2014).

19 In conclusion, stable isotope data revealed large seasonal variability in the $\delta^{15}\text{N}$ signature of
20 the PN pool, most likely related to changes in the phytoplankton assemblage and to N_2 -
21 fixation. In contradiction with the common observation that oligotrophic aquatic ecosystems
22 tend to be net heterotrophic, the seasonality of $\delta^{13}\text{C}$ -DIC supports the view that the mixed
23 layer of Lake Kivu is net autotrophic, as demonstrated by Borges et al. (2014) based on DIC
24 and DI^{13}C mass balance considerations. The $\delta^{13}\text{C}$ -POC showed an important variation with
25 depth due to the abundance of methanotrophic bacteria in the oxycline that fixed the lighter
26 CH_4 -derived C into their biomass. The $\delta^{13}\text{C}$ -POC and $\delta^{13}\text{C}$ -DOC appeared to be uncoupled
27 vertically and temporally, which could indicate that most of the DOC pool was composed of
28 relatively refractory compounds. Finally, the $\delta^{13}\text{C}$ of zooplankton mirrored the $\delta^{13}\text{C}$ signature
29 of the autochthonous POC pool, and its $\delta^{15}\text{N}$ signature followed the seasonal variability of the
30 $\delta^{15}\text{N}$ -PN pool in good agreement with the expected consumer-diet isotope fractionation. This
31 suggests that zooplankton would rely throughout the year on phytoplankton-derived biomass
32 as a organic C source.

33

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1 Figures

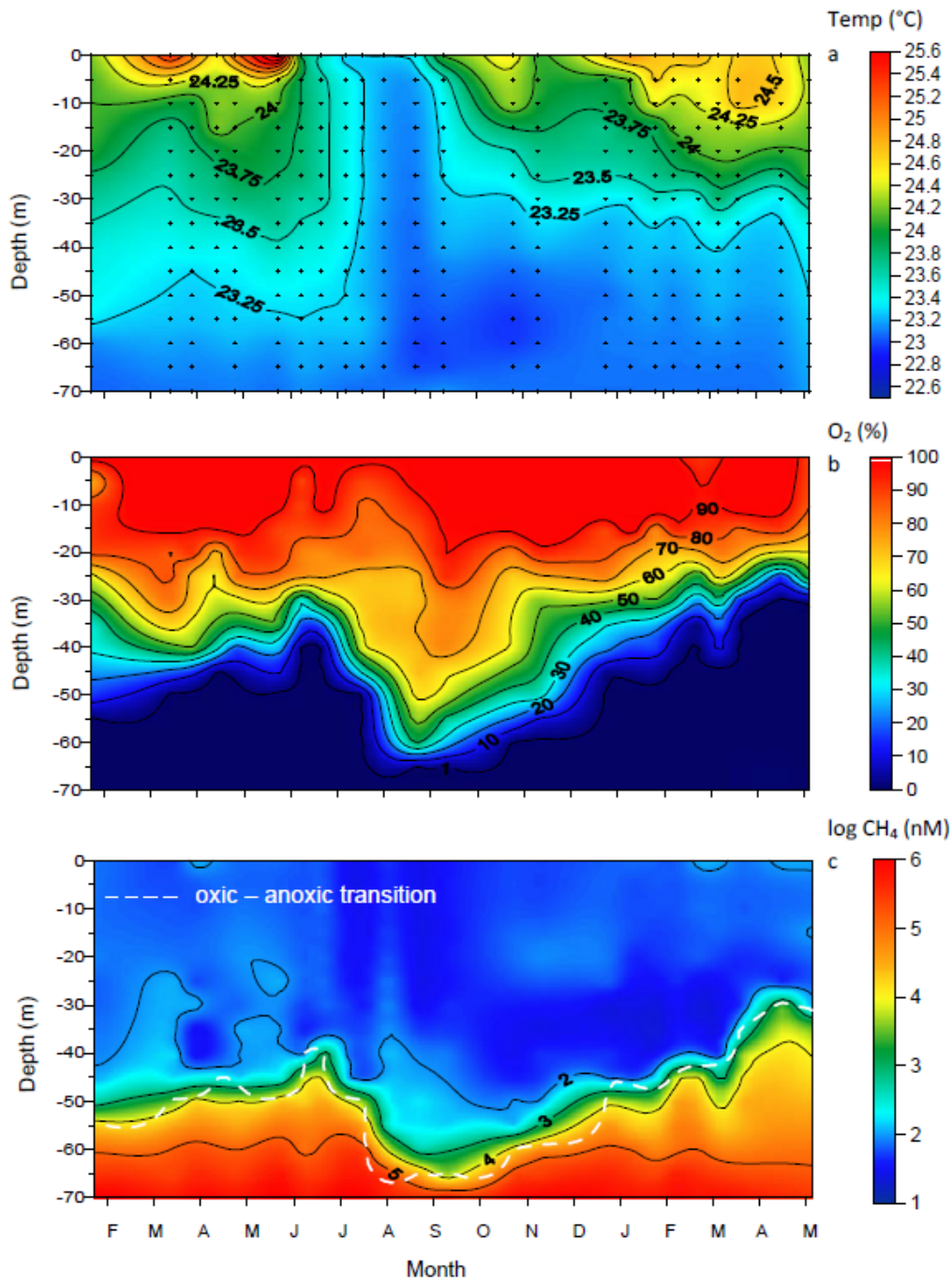


Figure 1. Temporal variability of (a) temperature (°C), (b) oxygen saturation (%), and (c) the log-transformed CH₄ concentration (nmol L⁻¹) in the mixolimnion of Lake Kivu, between February 2012 and May 2013. Small crosses in the figure (a) represent each sampling points.

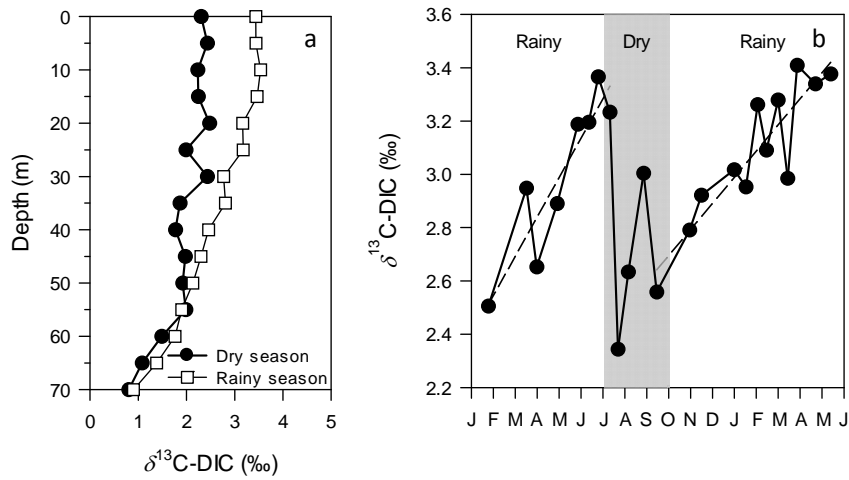


Figure 2. Depth profile of the $\delta^{13}\text{C}$ of the dissolved inorganic carbon (DIC) pool in the mixolimnion during the dry (18/07/12) and the rainy (20/03/13) season and (b) temporal variation of the $\delta^{13}\text{C-DIC}$ in the mixed layer of Lake Kivu between January 2012 and June 2013.

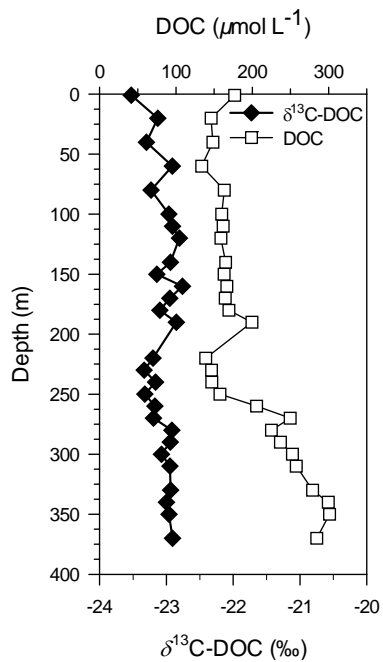


Figure 3. Vertical profile from the lake surface to the lake floor of the dissolved organic carbon (DOC) concentration ($\mu\text{mol L}^{-1}$) and the $\delta^{13}\text{C}$ signature of the DOC pool, in September 2012.

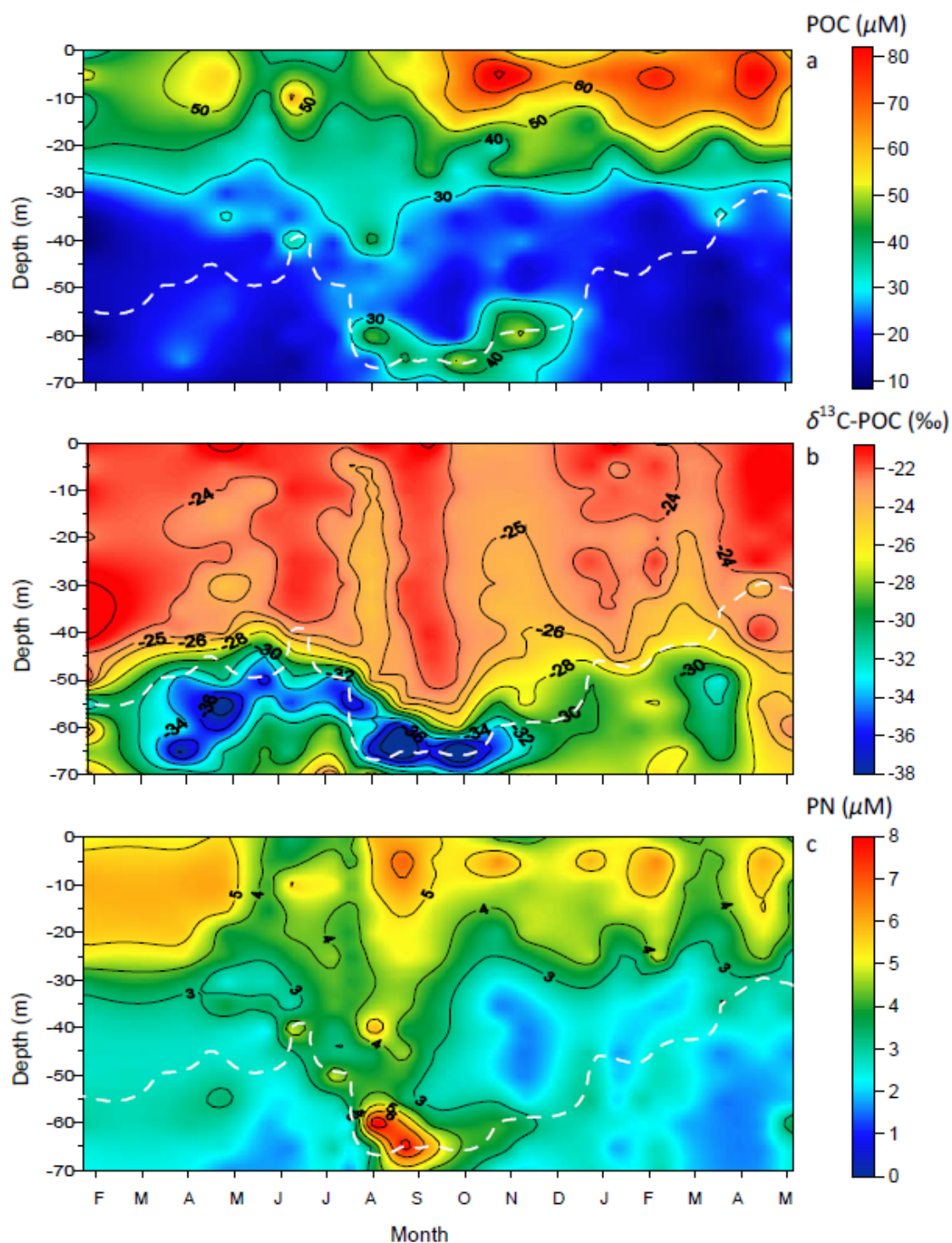


Figure 4. Temporal variability of (a) the particulate organic carbon (POC) concentration ($\mu\text{mol L}^{-1}$), (b) the $\delta^{13}\text{C}$ signature of the POC pool, and (c) the particulate nitrogen (PN) concentration ($\mu\text{mol L}^{-1}$) in the mixolimnion of Lake Kivu, between February 2012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

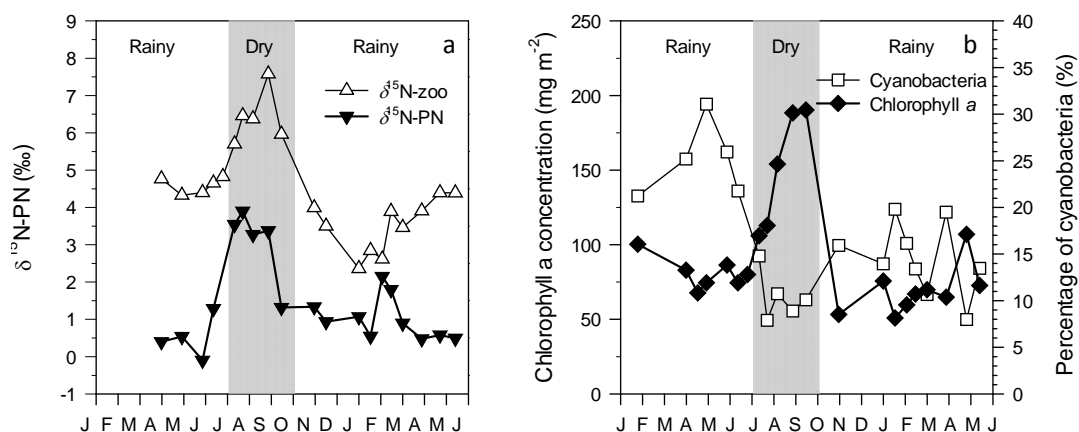


Figure 5. Temporal variability of (a) the $\delta^{15}\text{N}$ signature of the particulate nitrogen (PN) pool and zooplankton in the mixed layer, and (b) the chlorophyll a concentration (mg m^{-2}) and the relative contribution of cyanobacteria to the phytoplankton assemblage (% of biomass) in the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

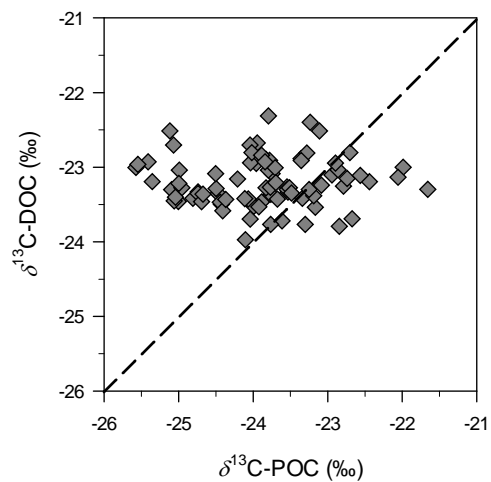


Figure 6. Relationship between the $\delta^{13}\text{C}$ signature of the particulate and dissolved organic carbon pool (POC and DOC, respectively) in the mixed layer.

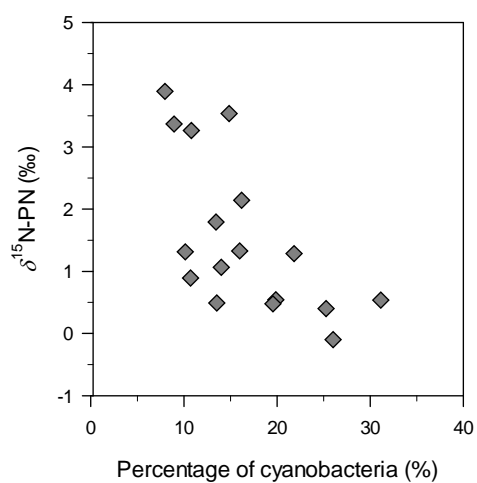


Figure 7. Relationship between the relative contribution of cyanobacteria to the phytoplankton assemblage (% of biomass) and the $\delta^{15}\text{N}$ signature of the particulate nitrogen pool in the mixed layer.