Carbon cycling of Lake Kivu (East Africa)
Borges, Alberto V.; Morana, Cédric; Bouillon, Steven; Servais, Pierre; Descy, Jean Pierre; Darchambeau, François

Published in:
PLoS ONE

DOI:
10.1371/journal.pone.0109500

Publication date:
2014

Document Version
Publisher's PDF, also known as Version of record

Link to publication
Citation for published version (HARVARD):
Borges, AV, Morana, C, Bouillon, S, Servais, P, Descy, JP & 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, vol. 9, no. 10, e109500. https://doi.org/10.1371/journal.pone.0109500

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.
Carbon Cycling of Lake Kivu (East Africa): Net Autotrophy in the Epilimnion and Emission of CO₂ to the Atmosphere Sustained by Geogenic Inputs

Alberto V. Borges¹, Cédric Morana², Steven Bouillon², Pierre Servais³, Jean-Pierre Descy⁴, François Darchambeau¹

¹ Chemical Oceanography Unit, Université de Liège, Liège, Belgium, ² Department of Earth and Environmental Sciences, KU Leuven, Leuven, Belgium, ³ Ecologie des Systèmes Aquatiques, Université Libre de Bruxelles, Bruxelles, Belgium, ⁴ Research Unit in Environmental and Evolutionary Biology, University of Namur, Namur, Belgium

Abstract

We report organic and inorganic carbon distributions and fluxes in a large (>2000 km²) oligotrophic, tropical lake (Lake Kivu, East Africa), acquired during four field surveys, that captured the seasonal variations (March 2007–mid rainy season, September 2007–late dry season, June 2008–early dry season, and April 2009–late rainy season). The partial pressure of CO₂ (pCO₂) in surface waters of the main basin of Lake Kivu showed modest spatial (coefficient of variation between 3% and 6%), and seasonal variations with an amplitude of 163 ppm (between 579±23 ppm on average in March 2007 and 742±28 ppm on average in September 2007). The most prominent spatial feature of the pCO₂ distribution was the very high pCO₂ values in Kabuno Bay (a small sub-basin with little connection to the main lake) ranging between 11213 ppm and 14213 ppm (between 18 and 26 times higher than in the main basin). Surface waters of the main basin of Lake Kivu were a net source of CO₂ to the atmosphere at an average rate of 10.8 mmol m⁻² d⁻¹, which is lower than the global average reported for freshwater, saline, and volcanic lakes. In Kabuno Bay, the CO₂ emission to the atmosphere was on average 500.7 mmol m⁻² d⁻¹ (~46 times higher than in the main basin). Based on whole-lake mass balance of dissolved inorganic carbon (DIC) bulk concentrations and of its stable carbon isotope composition, we show that the epilimnion of Lake Kivu was net autotrophic. This is due to the modest river inputs of organic carbon owing to the small ratio of catchment area to lake surface area (2.15). The carbon budget implies that the CO₂ emission to the atmosphere must be sustained by DIC inputs of geogenic origin from deep geothermal springs.

Introduction

Freshwater ecosystems are frequently considered to be net heterotrophic, whereby the consumption of organic carbon (C) is higher than the autochthonous production of organic C, and excess organic C consumption is maintained by inputs of allochthonous organic C [1]. Net heterotrophy in freshwater ecosystems promotes the emission of carbon dioxide (CO₂) to the atmosphere [2], [3], [4], [5], [6], [7], [9], [10], with the global emission from continental waters estimated at ~0.75 PgC yr⁻¹ [4] (0.11 PgC yr⁻¹ from lakes, 0.28 PgC yr⁻¹ from reservoirs, 0.23 PgC yr⁻¹ from rivers, 0.12 PgC yr⁻¹ from estuaries, and 0.01 PgC yr⁻¹ from ground waters). More recent studies provided even higher CO₂ emission estimates. Tranvik et al. [7] revised the CO₂ emission from lakes to 0.53 PgC yr⁻¹, while Battin et al. [6] estimated CO₂ emission from streams at 0.32 PgC yr⁻¹.

Aufdenkampe et al. [8] estimated a total CO₂ emission of 0.64 PgC yr⁻¹ for lakes and reservoirs, a total of 0.56 PgC yr⁻¹ for rivers and streams, and a massive 2.08 PgC yr⁻¹ for wetlands. Raymond et al. [10] estimated an emission of 1.8 PgC yr⁻¹ for streams and rivers and 0.32 PgC yr⁻¹ for lakes and reservoirs. Such emissions of CO₂ from continental waters exceed the net sink of C by terrestrial vegetation and soils of ~1.3 PgC yr⁻¹ [4] as well as the sink of CO₂ in open oceans of ~1.4 PgC yr⁻¹ [11].

However, our present understanding of the role of lakes on CO₂ emissions could be biased because most observations were obtained in temperate and boreal (humic) systems, and mostly in medium to small sized lakes, during open-water (ice-free) periods. Much less observations are available from hard-water, saline, large, or tropical lakes. Tropical freshwater environments are indeed under-sampled compared to temperate and boreal systems in terms of C dynamics in general, and specifically in terms of CO₂...
dynamics. In an extensive compilation of CO₂ concentration data from 4902 lakes globally [12], there were only 140 data entries for tropical systems (~3%). Yet, about 50% of freshwater and an equivalent fraction of organic C is delivered by rivers to the oceans at tropical latitudes [13]. Tropical lakes represent about 16% of the total surface of lakes [14], and Lakes Victoria, Tanganyika, and Malawi belong to the seven largest lakes by area in the world. Current estimates assume that areal CO₂ fluxes are substantially higher in tropical systems than in temperate or boreal regions (often ascribed to higher temperatures) [8]. Thus, according to the zonal distribution given by Aufdenkampe et al. [8], tropical inland waters account for ~60% of the global emission of CO₂ from inland waters (0.45 Pg C yr⁻¹ for lakes and reservoirs, 0.39 Pg C yr⁻¹ for rivers and streams, and 1.12 Pg C yr⁻¹ for wetlands). It is clear that additional data are required to verify and re-evaluate more accurately the CO₂ fluxes from tropical systems.

Pelagic particulate primary production (PP) of East African great lakes, as reviewed by Darchambeau et al. [15], ranges from ~30 mmol m⁻² d⁻¹ for the most oligotrophic conditions (north basin of Lake Tanganyika) to ~525 mmol m⁻² d⁻¹ for the most eutrophic conditions (Lake Victoria). The comparatively fewer data on bacterial production (BP), available only for Lake Tanganyika, suggest that PP and BP are seasonally closely coupled [16]. However, with an average pelagic BP of ~25 mmol m⁻² d⁻¹ [16], the bacterial C demand would exceed the production of particulate organic C (POC) by phytoplankton in Lake Tanganyika. This has led to speculate about additional C supply to bacteria from dissolved organic C (DOC) exudation by phytoplankton [16].

Lake Kivu (2.50°S 1.59°E 29.37°E 28.83°E) is one of the East African great lakes (2370 km² surface area, 550 km³ volume). It is a deep (maximum depth of 485 m) meromictic lake, with anoxic mixolimnion down to 70 m maximum, and a deep monolimnion rich in dissolved gases and nutrients [17], [18], [19]. Deep layers receive heat, salts, and CO₂ from deep geothermal springs [19]. Seasonality of the physical and chemical vertical structure and biological activity in surface waters of Lake Kivu is driven by the oscillation between the dry season (June-September) and the rainy season (October-May), the former characterized by a deepening of the mixolimnion [20]. This seasonal mixing favours the input of dissolved nutrients and the development of diatoms, while, during the rest of the year, the phytoplankton assemblage is dominated by cyanobacteria, chrysophytes and cryptophytes [15], [21], [22]. Surface waters of Lake Kivu are oligotrophic, and, consequently, PP is at the lower end of the range for East African great lakes (on average ~50 mmol m⁻² d⁻¹) [15].

Extremely high amounts of CO₂ and methane (CH₄) (300 km³ and 60 km³, respectively, at 0°C and 1 atm) [19] are dissolved in the deep layers of Lake Kivu. This is due to a steep density gradient at 260 m depth that leads to residence times in the order of 1000 yr in the deepest part of the lake [19], [23]. Stable isotope and radiocarbon data suggest that the CO₂ is mainly geogenic [24]. While the risk of a limnic eruption is minimal [25], large scale industrial extraction of CH₄ from the deep layers of Lake Kivu is planned [26], [27] which could affect the ecology and biogeochemical cycling of C of the lake and change for instance the emission of greenhouse gases such as CH₄ and CO₂. The net emission of CH₄ to the atmosphere from Lake Kivu was quantified by Borges et al. [28], and was surprisingly low - among the lowest ever reported in lakes globally - considering the large amounts of CH₄ stored in deep waters. Here, we report a data-set obtained during four surveys covering the seasonality of CO₂ dynamics and fluxes, in conjunction with mass balances of C, and process rate measurements (PP and BP) in the epilimnion of Lake Kivu, with the aim of quantifying the exchange of CO₂ with the atmosphere and determining the underlying drivers, in particular, the net metabolic status.

Materials and Methods

Official permission was not required for sampling in locations where measurements were made and samples acquired. The field studies did not involve endangered or protected species. The full data-set is available in Table S1.

2.1 Field sampling and chemical analysis

In order to capture the seasonal variations of the studied quantities, four cruises were carried out in Lake Kivu on 15/03-29/03/2007 (mid rainy season), 28/08-10/09/2007 (late dry season), 21/06-03/07/2008 (early dry season), 21/04-05/05/2009 (late rainy season), and 19/10-10-27/10/10 (early rainy season) for a selection of variables. Sampling was carried out at 15 stations distributed over the whole lake and in Kabuno Bay, and at 12 rivers draining into Lake Kivu and representing the outflow of the lake (Ruzizi River, Fig. 1). The core of data presented hereafter was obtained in 2007-2009, while from the 2010 cruise only vertical DOC and POC data obtained at two stations are presented.

Vertical profiles of temperature, conductivity and oxygen were obtained with a Yellow Springs Instrument (YSI) 6600 V2 probe. Calibration of sensors was carried out prior to the cruises and regularly checked during the cruises. The conductivity cell was regularly checked during the cruises. The conductivity cell was regularly checked during the cruises.

Figure 1. Map of Lake Kivu, showing bathymetry (isobaths at 100 m intervals), catchment area (shaded in grey), rivers, and sampling stations (small circles indicate the rivers). Primary production and bacterial production measurements were made at the stations identified with a square (I = Ishungu; K = Kibuye), adapted from [29].

doi:10.1371/journal.pone.0109500.g001
calibrated with a 1000 μS cm⁻¹ (25°C) YSI standard. The oxygen membrane probe was calibrated with humidity saturated ambient air. Salinity was computed from specific conductivity according to Schmid and Wuest [29].

Sampling for the partial pressure of CO₂ (pCO₂) was carried out at 1 m depth. Measurements of pCO₂ were carried out with a non-dispersive infra-red (NDIR) analyzer coupled to an equilibrator [30] through which water was pumped with a peristaltic pump (Masterflex E/S portable sampler). In-situ temperature and temperature at the outlet of the equilibrator were determined with Li-Cor 1000-15 probes. The NDIR analyzer (Li-Cor, Li-820) was calibrated with five gas standards: pure N₂ and four CO₂:N₂ mixtures with a CO₂ molar fraction of 363, 819, 3997 and 8170 ppm (Air Liquide Belgium).

For the determination of pH, CH₄ concentrations, δ¹³C of dissolved inorganic C (DIC) (δ¹³C-DIC), and total alkalinity (TA), water was sampled with a 5 L Niskin bottle (Hydro-Bios). Samples were collected every 10 m from 10 to 60–80 m depending on the cruise and station, except for CH₄ which was only sampled at 10 m. Additional samples for pH, δ¹³CDOC and TA were collected at 5 m in Kabuno Bay. Water for CH₄ analysis was collected in 50 ml glass serum bottles from the Niskin bottle with tubing, left to overflow, poisoned with 100 μl of a saturated HgCl₂ solution, and sealed with butyl stoppers and aluminum caps. Water samples for the analysis of δ¹³CPOC were taken from the same Niskin bottle by gently overfilling 12 ml glass Exetainer vials, poisoned with 20 μl of a saturated HgCl₂ solution, and gas-tight capped. A water volume of 50 ml was filtered through a 0.2 μm pore size polyethersulfone (PES) syringe filters and was stored at ambient temperature in polyethylene bottles for the determination of TA, POC and DOC samples were obtained from surface waters in June 2008 and April 2009, and along a depth profile in October 2010. DOC was filtered on 0.2 μm PES syringe filters, stored at ambient temperature in 40 ml glass vials with polytetrafluoroethylene coated septa, and poisoned with 50 μl of H₂PO₄ (85%). POC was filtered on 0.7 μm pore 25 mm diameter Whatman GF/F glass fiber filters (pre-combusted 5 h at 500°C), stored dry. Sampling of river surface waters followed the same procedures outlined above (sampling depth ~20 cm) with the addition of water sampling for total suspended matter (TSM). Samples for TSM were obtained by filtering 50–200 ml of water on pre-combusted pre-weighted 47 mm diameter GF/F glass fiber filters, stored dry. Measurements of pH in water sampled from the Niskin bottle were carried out with a Metrohm (6.0253.100) combined electrode calibrated with US National Bureau of Standards (NBS) buffers of pH 4.002 (25°C) and pH 6.881 (25°C) prepared according to Frankignoulle and Borges [31]. Measurements of TA were carried out by open-cell titration with HCl 0.1 M according to Gran [32] on 50 ml water samples, and data were quality checked with Certified Reference Material acquired from Andrew Dickson ( Scripps Institution of Oceanography, University of California, San Diego). DIC was computed from pH and TA measurements using the carbonic acid dissociation constants of Millero et al. [33].

For the analysis of δ¹³C-DIC, a He headspace was created in 1.2 ml glass vials, and ~300 μl of H₂PO₄ (99%) was added to convert all DIC species to CO₂. After overnight equilibration, part of the headspace was injected into the He stream of an elemental analyser – isotope ratio mass spectrometer (EA-IRMS) (Thermo-Finnigan Flash1112 and ThermoFinnigan Delta+XL, or Thermo FlashEA/HT coupled to Thermo Delta V) for δ¹³C measurements. The obtained δ¹³C data were corrected for the isotopic equilibration between gaseous and dissolved CO₂ using an algorithm similar to that presented by Miyajima et al. [34], and calibrated with LSVEC and NBS-19 certified standards or internal standards calibrated with the former. Concentrations of CH₄ were determined by gas chromatography with flame ionization detection, as described by Borges et al. [29]. DOC and δ¹³C-DOC were measured with a customized Thermo HiperTOC coupled to a Delta+XL IRMS. POC and δ¹³C-POC from filters were determined on a Thermo EA-IRMS (various configurations, either Flash1112, FlashHT with Delta+XL or DeltaV Advantage). Quantification and calibration of δ¹³C data was performed with IAEA-C6 and acetanilide which was internally calibrated versus international standards.

PP and BP were measured at 2 stations: Kibuye (2.05°S 29.29°E) and Ishungu (2.34°S 28.98°E) (Fig. 1). PP was measured using the ¹³C method [35] as described by Darchambeau et al. [15] in a pooled sample prepared from discrete samples (2 L) spaced every 5 m in the mixed layer. The mixed layer depth (MLD) was determined from vertical profiles of temperature and oxygen. The ¹³C incubations were assumed to provide an estimate of net PP of the particulate phase (PNPP). Chlorophyll a (Chl-a) of the pooled sample was measured according to Descy et al. [36] by high-performance liquid chromatography analysis of extracts in 90% acetone from samples filtered on Macherey-Nagel GF5 (0.7 μm nominal pore size) filters (3–4 L). BP was estimated every 5 m in the mixed layer from tritiated thymidine (³H-Thy) incorporation rates [37]. Samples (20 mL) were incubated for 2 h in the dark at in-situ temperature in the presence of ³H-Thy (~80 Ci mmol) at saturating concentration (~50 nmol L⁻¹). After incubation, cold trichloroacetic acid (TCA) was added (final concentration 5%) and the samples were kept cold until filtration through a 0.2 μm pore-size cellulose nitrate membrane. Filters were preserved in the dark at ~20°C. The radioactivity associated with the filters was estimated by liquid scintillation using a Beckman counter LS 6000. Cell production was calculated from the ³H-Thy incorporation rate using a conversion factor of 1.2×10¹⁸ cells produced per mole of ³H-Thy incorporated into cold TGA insoluble material. This conversion factor was determined experimentally in batch experiments in which the increase of bacterial abundance and ³H-Thy incorporation were followed simultaneously (data not shown) and was similar to the one used by Stemmate et al. [16] to calculate BP in Lake Tanganyika. Celluar production was multiplied by the average bacterial C content per cell (15 fgC cell⁻¹) [16] to obtain BP data. Daily BP was estimated from the experimental values considering constant activity over 24 h, and expressed per unit area (mmol m⁻² d⁻¹), by integrating over the euphotic zone. Bacterial respiration (BR) rates were computed from BP using a bacterial growth efficiency computed from BP according to the model of Del Giorgio and Cole [38].

2.2 Bulk DIC mass balance model

TA and DIC mass balance models were constructed in order to determine the major processes controlling CO₂ dynamics in surface waters, and to evaluate the net metabolic balance of the epilimnion (net autotrophy or net heterotrophy).

The TA mass balance was constructed assuming a steady-state, according to:

\[ F_{TA_{river}} + F_{TA_{70m-10m}} + F_{TA_{upwelling}} = F_{TA_{Ruzizi}} + F_{TA} \]  

where \( F_{TA_{river}} \) is the input of TA from rivers, \( F_{TA_{Ruzizi}} \) is the output of TA by the Ruzizi river, \( F_{TA_{70m-10m}} \) is the flux of TA from the monimolimnion to the mixolimnion by eddy diffusion, \( F_{TA_{upwelling}} \) is the flux of TA from the monimolimnion to the mixolimnion by upwelling, and \( F_{TA} \) is the closing term.
\( F_{TA_{\text{river}}} \) was computed from discharge-weighted average TA in the 12 sampled rivers draining into Lake Kivu (\( TA_{\text{river}} \)) and total freshwater discharge from rivers (\( Q_{\text{river}} \)), according to:

\[
F_{TA_{\text{river}}} = TA_{\text{river}} Q_{\text{river}}
\]  

(2)

\( F_{TA_{\text{Ruzizi}}} \) was computed from TA measured in the Ruzizi River (\( TA_{\text{Ruzizi}} \)), and the flow of the Ruzizi River (\( Q_{\text{Ruzizi}} \)), according to:

\[
F_{TA_{\text{Ruzizi}}} = TA_{\text{Ruzizi}} Q_{\text{Ruzizi}}
\]  

(3)

\( F_{TA_{\text{70m,10m}}} \) was computed from the gradient of TA across the pycnocline (\( \delta TA_{\text{70m,10m}} / \delta z \)), where \( \delta z \) represents the depth interval) and the eddy diffusion coefficient (\( F \)) according to:

\[
F_{TA_{\text{70m,10m}}} = - F \frac{\delta TA_{\text{70m,10m}}}{\delta z}
\]  

(4)

\( F_{TA_{\text{upwelling}}} \) was computed from the TA at 70 m (\( TA_{\text{70m}} \)) and the upwelling flow (\( Q_{\text{upwelling}} \)), according to:

\[
F_{TA_{\text{upwelling}}} = TA_{\text{70m}} Q_{\text{upwelling}}
\]  

(5)

A DIC mass balance was constructed assuming a steady-state, according to:

\[
F_{DIC_{\text{river}}} + F_{DIC_{\text{70m,10m}}} + F_{DIC_{\text{upwelling}}} = F_{DIC_{\text{Ruzizi}}} + F_{CO2} + F_{\text{CaCO3}} + F_{\text{POC}}
\]  

(6)

where \( F_{DIC_{\text{river}}} \) is the input of DIC from rivers, \( F_{DIC_{\text{Ruzizi}}} \) is the output of DIC by the Ruzizi river, \( F_{DIC_{\text{70m,10m}}} \) is the flux of DIC from the monimolimnion to the mixolimnion by eddy diffusion, \( F_{DIC_{\text{upwelling}}} \) is the flux of DIC from the monimolimnion to the mixolimnion by upwelling, \( F_{CO2} \) is the exchange of CO2 with the atmosphere, \( F_{\text{CaCO3}} \) is the precipitation and subsequent export to depth of CaCO3, and \( F_{\text{POC}} \) is the closing term and represents the export of POC from surface to depth.

\( F_{DIC_{\text{river}}} \) was computed from discharge-weighted average DIC in the 12 sampled rivers draining into Lake Kivu (\( DIC_{\text{river}} \)), and \( Q_{\text{river}} \), according to:

\[
F_{DIC_{\text{river}}} = DIC_{\text{river}} Q_{\text{river}}
\]  

(7)

\( F_{DIC_{\text{Ruzizi}}} \) was computed from DIC measured in the Ruzizi River (\( DIC_{\text{Ruzizi}} \)), and \( Q_{\text{Ruzizi}} \), according to:

\[
F_{DIC_{\text{Ruzizi}}} = DIC_{\text{Ruzizi}} Q_{\text{Ruzizi}}
\]  

(8)

\( F_{DIC_{\text{70m,10m}}} \) was computed from the gradient of DIC across the metalimnion (\( \delta DIC_{\text{70m,10m}} / \delta z \)) and \( F \) according to:

\[
F_{DIC_{\text{70m,10m}}} = - F \frac{\delta DIC_{\text{70m,10m}}}{\delta z}
\]  

(9)

\( F_{DIC_{\text{upwelling}}} \) was computed from DIC at 70 m (\( DIC_{\text{70m}} \)) and \( Q_{\text{upwelling}} \), according to:

\[
F_{DIC_{\text{upwelling}}} = DIC_{\text{70m}} Q_{\text{upwelling}}
\]  

(10)

\( F_{CO2} \) was computed according to:

\[
F_{CO2} = k z \Delta p_{CO2}
\]  

(11)

where \( k \) is the gas transfer velocity, \( \alpha \) is the CO2 solubility coefficient, and \( \Delta p_{CO2} \) is the air-water gradient of pCO2 computed from water pCO2 (1 m depth) and an atmospheric pCO2 value ranging from \( \sim 372 \) ppm to \( \sim 376 \) ppm (depending on the cruise), corresponding to the monthly average at Mount Kenya (Kenya, 0.05°S 37.30°E) obtained from GLOBALVIEW-CO2 (Carbon Cycle Greenhouses Group of the National Oceanic and Atmospheric Administration, Earth System Research Laboratory), and converted into wet air using the water vapour algorithm of Weiss and Price [39].

\( \alpha \) was computed from temperature and salinity using the algorithm of Weiss [40], \( k \) was computed from wind speed using the parameterization of Cole and Caraco [41] and the Schmidt number of CO2 in fresh water according to the algorithm given by Wanninkhof [42]. Wind speed data were acquired with a Davis Instruments meteorological station in Bukavu (2.51°S 28.86°E). The wind speed data were adjusted to be representative of wind conditions over the lake by adding 2 m s\(^{-1}\) according to Thiery et al. [20]. \( F_{CO2} \) was computed with daily wind speed averages for a time period of one month centred on the date of the middle of each field cruise. Such an approach allows to account for the day-to-day variability of wind speed, and to provide \( F_{CO2} \) values that are seasonally representative.

\( F_{\text{CaCO3}} \) was computed according to:

\[
F_{\text{CaCO3}} = \frac{F_{TA_{u}}}{2}
\]  

(12)

The average value of \( Q_{\text{river}} \) (76.1 m\(^3\) s\(^{-1}\)) was given by Muvundja et al. [43], the average \( Q_{\text{Ruzizi}} \) for 2007–2009 (87.8 m\(^3\) s\(^{-1}\)) measured at Ruzizi I Hydropower Plant was provided by the Société Nationale d’Electricité. A value of \( Q_{\text{upwelling}} \) of 42 m\(^3\) s\(^{-1}\) was given by Schmid et al. [19].

### 2.3 \( \delta^{13} \)DIC mass balance model

The combination of the DIC and \( \delta^{13} \)DIC budget for the mixed layer allows to estimate independently the total DIC vertical input by upwelling and by eddy diffusion (\( F_{DIC_{\text{upward}}} \)) and \( F_{\text{POC}} \) [44]. At steady-state, DIC and \( \delta^{13} \)DIC mass balances are given by the following equations:

\[
F_{DIC_{\text{river}}} + F_{\text{upward}} = F_{DIC_{\text{Ruzizi}}} + F_{CO2} + F_{\text{CaCO3}} + F_{\text{POC}}
\]  

(13)

\[
F_{DIC_{\text{river}}} \left( C^{12}C \right) + F_{\text{upward}} \left( C^{12}C \right) = F_{DIC_{\text{Ruzizi}}} \left( C^{12}C \right) + F_{\text{CaCO3}} \left( C^{12}C \right) + F_{\text{POC}} \left( C^{12}C \right)
\]  

(14)
$F_{\text{upward}} = F_{\text{DIC}_z,z} + F_{\text{DIC}_z,\text{upwelling}}$ (15)

The $\left(^{13}\text{C}/^{12}\text{C}\right)$ ratio in the equation (14) represents the $^{13}\text{C}$ to $^{12}\text{C}$ ratio of net C fluxes, and can be expressed using the classical $\delta^{13}\text{C}$ notation [45]. Out of the 12 different terms in equations (13) and (14) were measured or can be computed from measured variables, and then the two equations can be solved in order to estimate the $F_{\text{DIC}_z,\text{upwelling}}$ and $F_{\text{POC}}$ fluxes. $F_{\text{DIC}_z,\text{river}}$, $F_{\text{DIC}_z,\text{rain}}$, $F_{\text{CaCO}_3}$ and $F_{\text{CO}_2}$ were calculated as described above. $\left(^{13}\text{C}/^{12}\text{C}\right)_{\text{DIC}_z,\text{river}}$, $\left(^{13}\text{C}/^{12}\text{C}\right)_{\text{DIC}_z,\text{lake}}$ and $\left(^{13}\text{C}/^{12}\text{C}\right)_{\text{POC}}$ were measured during the 4 field surveys. $\left(^{13}\text{C}/^{12}\text{C}\right)_{\text{CaCO}_3}$ was computed from the measured $\delta^{13}\text{C}_{\text{DIC}}$ in surface and the fractionation factor $e_{\text{CaCO}_3,\text{HCO}_3}$ of 0.88% [46].

The $\left(^{13}\text{C}/^{12}\text{C}\right)_{\text{DIC}_z,\text{upwelling}}$ which represents the $\delta^{13}\text{C}$ signature of the net upward DIC input, was estimated from the $\delta^{13}\text{C}$-DIC vertical gradient as follows:

$$\left(^{13}\text{C}/^{12}\text{C}\right)_{\text{DIC}_z,\text{upwelling}} = \frac{\text{DIC}_z \cdot \text{DIC}_{z+1} - \left(^{13}\text{C}/^{12}\text{C}\right)_{\text{DIC}_z}}{\text{DIC}_{z+1} - \text{DIC}_z}$$ (16)

where $z$ is the depth, DIC$z$ and DIC$z+1$ are the DIC concentration at the depth $z$ and $z+1$, $\left(^{13}\text{C}/^{12}\text{C}\right)_{\text{DIC}_z}$ and $\left(^{13}\text{C}/^{12}\text{C}\right)_{\text{DIC}_z,\text{upwelling}}$ are the $\delta^{13}\text{C}$ signature of DIC at the depth $z$ and $z+1$.

The $\delta^{13}\text{C}$ signature of the net flux of CO$_2$ at the air-water interface was calculated from the $\delta^{13}\text{C}$ signature of the different DIC species in surface water and the atmospheric $^{12}\text{CO}_2$ ($-8.00\%$) according to:

$$\left(^{13}\text{C}/^{12}\text{C}\right)_{\text{CO}_2,\text{water}} = \frac{\text{pCO}_2 \cdot \text{DIC}_{\text{DIC}_z} - \text{pCO}_2 \cdot \text{DIC}_{\text{DIC}_z,\text{upwelling}}}{\text{pCO}_2 \cdot \text{DIC}_{\text{DIC}_z} - \text{pCO}_2 \cdot \text{DIC}_{\text{DIC}_z,\text{upwelling}}}$$ (17)

where $\left(^{13}\text{C}/^{12}\text{C}\right)_{\text{CO}_2,\text{water}}$ and $\left(^{13}\text{C}/^{12}\text{C}\right)_{\text{DIC}_z}$ are the $\delta^{13}\text{C}$ signature of atmospheric $\text{CO}_2$ and lake surface DIC, respectively, $\text{pCO}_2$ and $\text{DIC}_{\text{DIC}_z}$ are respectively the kinetic fractionation effect during $\text{CO}_2$ gas transfer, and the equilibrium fractionation during $\text{CO}_2$ dissolution measured by Zhang et al. [47] in distilled water. $\text{DIC}_{\text{DIC}_z}$, $\text{DIC}_{\text{DIC}_z,\text{upwelling}}$ and $\text{DIC}_{\text{DIC}_z,\text{upwelling}}$ are the equilibrium fractionation factor between aqueous DIC and gaseous $\text{CO}_2$ and is defined by:

$$\text{DIC}_{\text{DIC}_z,\text{upwelling}} = \frac{\left(^{13}\text{C}/^{12}\text{C}\right)_{\text{CO}_2,\text{water}} - \left(^{13}\text{C}/^{12}\text{C}\right)_{\text{DIC}_z}}{\left(^{13}\text{C}/^{12}\text{C}\right)_{\text{DIC}_z,\text{upwelling}}}$$ (18)

where $\left(^{13}\text{C}/^{12}\text{C}\right)_{\text{DIC}_z,\text{upwelling}}$ is the air-water $\delta^{13}\text{C}$ disequilibrium, that is the difference between the $\delta^{13}\text{C}$-DIC expected at equilibrium with atmospheric $\text{CO}_2$ minus the measured $\delta^{13}\text{C}$-DIC in surface water of the lake.

2.4 Bulk DIC mixing models

A mixing model was developed to compute the theoretical evolution of TA, DIC, and pCO$_2$ between March 2007 and September 2007, when the mixed layer deepened. The aim of this model is to compare theoretical evolution considering conservative mixing (no biology or other in/outputs) with observational data to infer the importance of certain processes. The model was computed by daily time steps assuming the conservative mixing (no biological activity) of surface waters with deep waters for TA and DIC. At each time step, pCO$_2$ was calculated from TA, DIC, salinity and temperature, allowing the computation of FCO$_2$ and correcting DIC for FCO$_2$. The mixing model was also run without correcting DIC for FCO$_2$. The ML, salinity and temperature were interpolated linearly between March and September 2007.

At each time step, TA was computed according to:

$$\text{TA} = \frac{\text{TA}_{\text{deep}} (\text{MLD} + 1) - \text{TA} (\text{MLD})}{\text{MLD} + 1}$$ (19)

where TA is TA in the mixed layer at time step $i$, TA$_{\text{MLD}+1}$ is TA in the mixed layer at time step $i+1$, MLD is the ML at time step $i$, MLD$_{i+1}$ is the MLD at time step $i+1$, and TA$_{\text{deep}}$ is TA in the deep waters.

At each time step, DIC corrected for FCO$_2$ was computed according to:

$$\text{DIC} = \frac{\text{DIC}_{\text{deep}} (\text{MLD} + 1) - \text{DIC}_{\text{MLD}} + \text{DIC}_{\text{MLD}+1}}{\text{MLD} + 1}$$ (20)

where DIC$_{\text{MLD}}$ is DIC in the mixed layer at time step $i$, DIC$_{\text{MLD}+1}$ is DIC in the mixed layer at time step $i+1$, FCO$_2$ is CO$_2$ at time step $i$, $\delta t$ is the time interval between each time step (1 d), and DIC$_{\text{deep}}$ is DIC in the deep waters.

At each time step, DIC not corrected for FCO$_2$ was computed according to:

$$\text{DIC} = \frac{\text{DIC}_{\text{deep}} (\text{MLD} + 1) + \text{DIC}_{\text{MLD}+1}}{\text{MLD} + 1}$$ (21)

Results

In surface waters (1 m depth) of the main basin of Lake Kivu (excluding Kabuno Bay), pCO$_2$ values were systematically above atmospheric equilibrium (~372 ppm to ~376 ppm depending on the cruise), and varied within narrow ranges of 534–605 ppm in March 2007, 701–781 ppm in September 2007, 597–640 ppm in June 2008, and 583–711 ppm in April 2009 (Fig. 2). The most prominent feature of the spatial variations was the much higher pCO$_2$ values in September 2007 (14.2 mmol m$^{-2}$ d$^{-1}$) and DIC values (Fig. 3). Comparing DIC and TA profiles shows that the relative contribution of CO$_2$ to DIC was more important in Kabuno Bay than in the main lake, since TA is mainly as HCO$_3^-$, and if the CO$_2$ contribution to DIC is low, then DIC and TA should be numerically close. At 60 m depth, CO$_2$ contributes ~30% to DIC in Kabuno Bay, and only ~1% in the main basin. Kabuno Bay was also characterized by a very stable chemocline (salinity, pH) and oxygen at ~11 m irrespective of the sampling period [28]. In the main basin of Lake Kivu, the oxycline varied seasonally between ~35 m in March.
and September 2007 and ~60 m in June 2008 [28]. The deepening of the mixed layer and entrainment of deeper waters to the surface mixed layer was shown to be main driver of the seasonal variations of CH4 [29]. The positive correlations between pCO2 and CH4 and between pCO2 and the ML depth also suggest that the mixing of deep and surface waters was a major driver of the seasonal variability of pCO2 (Fig. 4). This is also consistent with the negative relation between pCO2 and δ13C-DIC (Fig. 4), as DIC in deeper waters is more δ13C-depleted than that in surface waters (Fig. 3).

DIC concentrations in surface waters averaged 13.0 mmol L−1 and 16.9 mmol L−1 in the main basin of Lake Kivu and in Kabuno Bay, respectively, but were much lower in the inflowing rivers (on average ~0.5 mmol L−1). The comparison with the lake values shows that the δ13C-DIC were always more negative in rivers (mean −7.0±2.1‰) than in the main basin (mean 3.4±0.3‰) and Kabuno Bay (mean 0.8±0.5‰) (Fig. 5). This difference suggests that the DIC in surface waters of Lake Kivu originates from a different source than that in the rivers. POC concentrations in surface waters of the main basin averaged 32 μmol L−1 in June 2008, 24 μmol L−1 in April 2009 and 42 μmol L−1 in October 2010. In the rivers, POC concentration was higher, 358 μmol L−1 in June 2008 and 499 μmol L−1 in April 2009. However, POC in the rivers never contributed more than 4.4% of TSM. δ13C-POC and δ13C-DIC signatures appeared uncoupled in rivers (Fig. 6), but a positive relationship between δ13C-DIC and δ13C-POC was found in the lake when combining the data from the main lake and Kabuno Bay (model I linear regression, p<0.001, r²=0.71, n=15). Furthermore, the δ13C-POC in the main basin and Kabuno Bay (mean −24.1±2.0‰, n=15) was significantly lower than the δ13C-POC in rivers (mean −22.9±1.5‰, n=21) (t-test; p<0.05), but the δ13C-DIC in the main lake (mean −23.1±1.1‰, n=15) did not differ from the δ13C-DIC in rivers (mean −23.9±1.4‰, n=21) (Fig. 7) and was vertically uncoupled from δ13C-POC (Fig. 8).

In order to test if vertical mixing was the only driver of seasonal variations of pCO2, we applied the mixing model to the March 2007 data in order to predict the evolution of TA, DIC, and pCO2 up to September 2007 and we compared the predicted values to the actual data obtained at that time (Fig. 9). The TA value predicted by the mixing model was higher than the observations in September 2007 by 108 μmol L−1. We assumed that the process removing TA was CaCO3 precipitation in the mixolimnion and subsequent export to depth (FCaCO3). In order to account for the difference between the mixing model prediction and the observations, FCaCO3 was estimated to be 14.2 mmol m−2 d−1 between March and September 2007.

The DIC value predicted by the mixing model was higher than the observations in September 2007 by 108 μmol L−1. The emission of CO2 to the atmosphere only accounted for 19% of the DIC removal. We assumed that the remaining DIC was removed by the combination of FCaCO3 and POC production in the epilimnion and export to depth (FPOC) that was estimated to be 11.5 mmol m−2 d−1, using the FCaCO3 value estimated above from the TA data. The modeled pCO2 was above the observed pCO2 and the CO2 emission only accounted for 27% of the difference. This implies that the decrease of pCO2 was mainly related to FPOC.

To further investigate the drivers of CO2 dynamics in Lake Kivu, we computed the TA and DIC whole-lake (bulk concentration) mass balances based on averages for the cruises (Fig. 10). The major flux of TA was the vertical input from deeper waters (50.9 mmol m−2 d−1) and the outflow from the Ruzizi (42.6 mmol m−2 d−1), which was higher than the inputs from rivers by one order of magnitude (1.2 mmol m−2 d−1). The closing term of the TA mass balance was 9.5 mmol m−2 d−1. We assume that this was related to FCaCO3 (4.7 mmol m−2 d−1). Similarly, the major fluxes of DIC were the vertical input (63.5 mmol m−2 d−1) and the outflow of the Ruzizi (39.3 mmol m−2 d−1), which were higher than the inputs from rivers by one order of magnitude (1.3 mmol m−2 d−1), and than the emission of CO2 to the atmosphere (10.8 mmol m−2 d−1). The closing term of the DIC mass balance was 14.8 mmol m−2 d−1. We assume that this was related to the sum of FCaCO3 and FPOC allowing to compute FPOC using the FCaCO3 values computed from the TA mass balance. The estimated FPOC values was 10.0 mmol m−2 d−1. The whole-lake DIC stable isotope mass balance provided a FPOC of 25.4 mmol m−2 d−1, and vertical inputs of DIC of 78.0 mmol m−2 d−1.

Planktonic metabolic rates in the epilimnion (PNPP and BP) were measured during each cruise (Table 2). The PNPP values ranged from 14.2 to 49.7 mmol m−2 d−1 and were relatively similar in March 2007, September 2007 and June 2008, but distinctly lower in April 2009. The BP values ranged from 3.9 to 49.8 mmol m−2 d−1. This range encompasses the one reported for BP in the euphotic layer (~40 m) of Lake Tanganyika (3.0 to 20.0 mmol m−2 d−1) [16]. The BR values estimated from BP ranged from 13.6 to 61.2 mmol C m−2 d−1, PNPP was markedly in excess of BR only in June 2008. In March 2007 and September 2007, BR was balanced by PNPP or slightly in excess of PNPP. In April 2009, BR was markedly in excess of PNPP.

**Discussion**

The amplitude of the seasonal variations of mean pCO2 across the main basin of Lake Kivu was 163 ppm (between 579±23 ppm on average in March 2007 and 742±28 ppm on average in September 2007). Such pCO2 seasonal amplitude is low compared to temperate and boreal lakes, where it is usually between ~500 ppm [48] and >1000 ppm [49],[50],[51],[52],[53],[54], and even up to ~10,000 ppm in small bog lakes [53]. The lower amplitude of seasonal variations of the pCO2 in Lake Kivu might be related to the tropical climate leading only to small surface water temperature seasonal variations (from 23.6°C in September 2007 to 24.6°C in March 2007 on average), and also for relatively modest variations in mixing (MLD changed from 20 m to 70 m). Hence, compared to temperate and boreal lakes, the seasonal variations of biological activity are less marked (due to relatively constant temperature and light, and modest changes in mixing), and also there is an absence of large episodic CO2 inputs to surface waters such as those occurring in temperate or boreal systems during lake overturns or of CO2 accumulation during ice covered periods.

The spatial variations of pCO2 in the main basin of Lake Kivu were also low. The coefficient of variation of pCO2 in surface waters of the main basin ranged for each cruise between 3% and 6%, below the range reported by Kelly et al. [54] in five large boreal lakes (range 5% to 40%). The relative horizontal homogeneity of pCO2 could be in part related to the absence of extensive shallow littoral zones, owing to the steep shores [18], and also due to very small influence of C inputs from rivers in the overall DIC budget (Fig. 10). The most prominent spatial feature in Lake Kivu was the much larger pCO2 values in surface waters of Kabuno Bay compared to the main basin. Furthermore, surface and deep waters of Kabuno Bay were characterized by higher salinity, DIC and TA values and by lower pH and δ13C-DIC values. These vertical patterns indicate that there is a much larger contribution of subaquatic springs to the whole water column.
including surface waters in Kabuno Bay than in the main basin of Lake Kivu relative to their respective volumes. This is related to the different geomorphology, since Kabuno Bay is shallower than the main basin (maximum depth of 110 m versus 485 m) and exchanges little water with the main basin (narrow connection ~10 m deep). Also, Kabuno Bay is smaller (~48 km²) than the main basin (~2322 km²). Hence, there is a stronger fetch limitation of wind induced turbulence that also contributes to the stability of the vertical water column structure in Kabuno Bay irrespective of the season [28].

The overall average of pCO2 for the 4 cruises in the main basin of Lake Kivu (646 ppm) is lower than the average of 41 large lakes (>500 km²) of the world (850 ppm) [5], than the global average for freshwater lakes (1287 ppm) [12], than the average of tropical freshwater lakes (1804 ppm) [55], and than the average for tropical freshwater lakes (2296 ppm) [49]. Lake Kivu corresponds to a saline lake according to the definition of Duarte et al. [56] (specific conductivity >1000 µS cm⁻¹; salinity >0.68) that collectively have a global average pCO2 of 1900 ppm (derived from carbonic acid dissociation constants for freshwater) or 3040 ppm (derived from carbonic acid dissociation constants for seawater). Kabuno Bay, in contrast, was characterized by an exceptionally high average pCO2 value (12994 ppm) compared to other freshwater lakes, tropical (African) freshwater lakes, and saline lakes globally.

The average FCO2 of the 4 cruises in the main basin of Lake Kivu was 10.8 mmol m⁻² d⁻¹, which is lower than the global average for freshwater lakes of 16.0 mmol m⁻² d⁻¹ reported by Cole et al. [49], and the average for saline lakes ranging between 81 and 105 mmol m⁻² d⁻¹ reported by Duarte et al. [56]. The average FCO2 in Kabuno Bay (500.7 mmol m⁻² d⁻¹) is distinctly higher than the FCO2 global averages for freshwater and saline lakes globally.

**Table 1.** Average wind speed (m s⁻¹), air-water gradient of the partial pressure of CO2 (ΔpCO2, ppm), and air-water CO2 flux (FCO2, mmol m⁻² d⁻¹) in the main basin of Lake Kivu and Kabuno Bay in March 2007, September 2007, June 2008, and April 2009.

|          | March 2007 |         |         |
|----------|------------|---------|---------|
|          | (m s⁻¹)    | (ppm)   | (mmol m⁻² d⁻¹) |
| Main basin | 3.3±0.4    | 207±22  | 8.0±1.3  |
| Kabuno Bay | 3.2±0.4    | 370±27  | 14.2±2.0 |
| September 2007 |      |         |         |
| Main basin | 3.6±0.2    | 245±15  | 10.5±1.0 |
| Kabuno Bay | 3.6±0.2    | 267±41  | 10.3±1.8 |
| June 2008 |          |         |         |
| Main basin | 3.3±0.2    | 267±41  | 10.3±1.8 |
| Kabuno Bay | 3.3±0.2    | 301.6   | 504.5±36.7 |
| April 2009 |          |         |         |

**Figure 2.** Spatial distribution of the partial pressure of CO2 (pCO2, ppm) in the surface waters of Lake Kivu (1 m depth) in March 2007, September 2007, June 2008 and April 2009. doi:10.1371/journal.pone.0109500.g002
Figure 3. Vertical profiles in March 2007 of pH, oxygen saturation level (%O₂, %), total alkalinity (TA, mmol L⁻¹), salinity, dissolved inorganic carbon (DIC, mmol L⁻¹), δ¹³C signature of DIC (δ¹³C-DIC, %) in Kabuno Bay and in the three northernmost stations in the main basin of Lake Kivu.

doi:10.1371/journal.pone.0109500.g003
Figure 4. Average partial pressure of CO₂ (pCO₂, ppm) in the surface waters of the main basin of Lake Kivu (1 m depth) versus mixed layer depth (MLD, m), δ¹³C signature of dissolved inorganic carbon (DIC) (δ¹³C-DIC, ‰), and methane concentration (CH₄, nmol L⁻¹) in March 2007, September 2007, June 2008, and April 2009. Vertical and horizontal bars represent standard deviations.

doi:10.1371/journal.pone.0109500.g004

Figure 5. Relation between δ¹³C signature of dissolved inorganic carbon (DIC) (δ¹³C-DIC, ‰) and DIC concentration (mmol L⁻¹), in the mixed layer of the main basin of Lake Kivu, Kabuno Bay, and various inflowing rivers, in March 2007, September 2007, June 2008, and April 2009.

doi:10.1371/journal.pone.0109500.g005

Figure 6. Relation between δ¹³C signature of particulate organic carbon (POC) (δ¹³C-POC, ‰) and δ¹³C signature of dissolved inorganic carbon (DIC) (δ¹³C-DIC, ‰), in the mixed layer of the main basin of Lake Kivu, Kabuno Bay and various inflowing rivers, in June 2008, April 2009, and October 2010.

doi:10.1371/journal.pone.0109500.g006
lakes. However, the average \( F_{CO2} \) in Kabuno Bay is equivalent to average of \( F_{CO2} \) value of alkaline volcanic lakes (458 mmol m\(^{-2}\) d\(^{-1}\)) but lower than average of \( F_{CO2} \) of acid volcanic lakes (5183 mmol m\(^{-2}\) d\(^{-1}\)) reported by Pérez et al. [57].

Cross system regional analyses show a general negative relationship between \( pCO2 \) and lake surface area [5], [54], [58], [59], [60] and a positive relationship between \( pCO2 \) and DOC [61] (and reference therein). The low \( pCO2 \) and \( F_{CO2} \) values in Lake Kivu are consistent with these general patterns, since this is a large (>2000 km\(^2\)) and organic poor (DOC ~0.2 mmol L\(^{-1}\)) system. However, the low seasonal amplitude of \( pCO2 \) and relative horizontal homogeneity of \( pCO2 \) in Lake Kivu are not necessarily linked to its large size. Indeed, spatial and temporal variability of \( pCO2 \) within a single lake have been found to be no greater nor smaller in larger lakes than in smaller lakes, in cross system analyses in Northwest Ontario [54] and Northern Quebec [60].

Borges et al. [28] reported diffusive \( CH4 \) emissions of 0.04 mmol m\(^{-2}\) d\(^{-1}\) and 0.11 mmol m\(^{-2}\) d\(^{-1}\) for the main basin of Lake Kivu and Kabuno Bay, respectively. Using a global warming potential of 72 for a time horizon of 20 yr [62], the \( CH4 \) diffusive emissions in CO\(_2\) equivalents correspond to 0.26 mmol m\(^{-2}\) d\(^{-1}\) and 0.77 mmol m\(^{-2}\) d\(^{-1}\) for the main basin of Lake Kivu and Kabuno Bay, respectively, hence 41 to 650 times lower than the actual \( F_{CO2} \) values.

DIC concentrations in surface waters of the main basin of Lake Kivu and Kabuno Bay averaged 13.0 and 16.8 mmol L\(^{-1}\), respectively, and were well within the range of DIC reported for saline lakes by Duarte et al. [56], which range from 0.1 to 2140 mmol L\(^{-1}\), but are lower than the global average for saline lakes of 59.5 mmol L\(^{-1}\). DOC averaged in surface waters 0.15 mmol L\(^{-1}\) and 0.20 mmol L\(^{-1}\) in the main basin of Lake Kivu and in Kabuno Bay, respectively. Hence, DIC strongly dominated the dissolved C pool, with DIC:DOC ratios of 82 and 87 in the main basin of Lake Kivu and in Kabuno Bay, respectively. These DIC:DOC ratios are higher than those in six hard-water lakes of the northern Great Plains ranging from 3 to 6 [63], and higher than those in boreal lakes where DOC is the dominant form of the dissolved C pool, with DIC:DOC ratios ranging from 0.01 to 0.68 e.g. [64], [65], this range reflecting both seasonal changes [59] and differences in catchment characteristics [66]. Unlike the 6 hard-water lakes of the northern Great Plains, where the high DIC concentrations are due to river inputs [67], the high DIC concentrations in Lake Kivu were related to vertical inputs of DIC from deep waters that were on average 49 times larger than the DIC inputs from rivers (Fig. 10), as confirmed by \( ^{13}C-DIC \) values clearly more positive in the lake than in the rivers (Fig. 5). The difference in C stable isotope composition of POC between the lake and rivers indicates that these two pools of organic C do not share the same origin. In the small, turbid rivers flowing to Lake Kivu, we expect the POC and DOC pools to be derived from terrestrial inputs, as reflected by the low contribution of POC to TSM e.g. [68]. In contrast, the positive relationship between the \( ^{13}C-DIC \) and \( ^{13}C-POC \) in surface waters (Fig. 6) suggests that DIC is the main C source for POC in surface waters of Lake Kivu, implying that the whole microbial food web could be supported by autochthonous organic C. However, the \( ^{13}C \) data indicate a surprising difference between the origin of DOC and POC in the lake (Figs. 7, 8). The \( ^{13}C-POC \) signatures were constant from the surface to the oxic-anoxic interface, then showed a local and abrupt excursion to values as low as ~40%, reflecting the incorporation of a \( ^{13}C \)-depleted source in the POC (Fig. 8). Indeed, while the large pool of DIC is the main C source for POC in surface waters, it appears that \( CH4 \) with a \( ^{13}C \) signature of approximately ~60% (own data not shown) contributes significantly to C fixation at the oxic-anoxic interface, as also shown in Lake Lugano [69]. In contrast, the \( ^{13}C \) signature of the DOC pool in the mixolimnion showed little seasonal and spatial variations and appeared to be decoupled from the POC pool (Figs. 7, 8). Heterotrophic bacteria quickly mineralized the labile autochthonous DOC that reflects the \( ^{13}C \) signature of POC, produced by cell lysis, grazing, or phytoplankton excretion.
Hence, standing stocks of autochthonous DOC are small, and older refractory compounds constitute the major part of the DOC pool. The \( F_{\text{CaCO}_3} \) value computed from the whole-lake TA budget was 4.7 mmol m\(^{-2}\) d\(^{-1}\), higher than the total inorganic C (TIC) annual average fluxes in sediment traps ranging from 0.3 mmol m\(^{-2}\) d\(^{-1}\) (at 50 m depth) to 0.5 mmol m\(^{-2}\) d\(^{-1}\) (at 130 m depth) reported by Pasche et al. [71] at Ishungu. The maximum individual monthly TIC flux from sediment traps at Ishungu reported by Pasche et al. [71] was 4.0 mmol m\(^{-2}\) d\(^{-1}\). However, the \( F_{\text{CaCO}_3} \) value was closer to the TIC deposition fluxes in the top cm of sediment cores ranging from 1.4 mmol m\(^{-2}\) d\(^{-1}\) (at Ishungu) to 4.8 mmol m\(^{-2}\) d\(^{-1}\) (at Gisenyi) also reported by Pasche et al. [71]. The \( F_{\text{POC}} \) computed from the DIC whole-lake budget was 10.0 mmol m\(^{-2}\) d\(^{-1}\) close to the total organic C (TOC) average fluxes in sediment traps ranging from 8.7 mmol m\(^{-2}\) d\(^{-1}\) (at 50 m depth) to 9.8 mmol m\(^{-2}\) d\(^{-1}\) (at 172 m depth) reported by Pasche et al. [71] at Ishungu.

Due to thermodynamic equilibria of the dissolved carbonate system, CaCO$_3$ precipitation leads to a shift from the HCO$_3$- to the CO$_2$ pool according to:

\[
Ca^{2+} + 2HCO_3^- \rightleftharpoons CaCO_3 + H_2O
\]  

However, CaCO$_3$ precipitation has frequently been reported in lakes as biologically mediated by primary producers [50], [72], [73], whereby the CO$_2$ produced by the precipitation of CaCO$_3$ is fixed into organic matter by photosynthesis and does not accumulate in the water [50], [74].
Table 2. Photic depth (Ze, m), chlorophyll-a concentration in the mixed layer (Chl-α, mg m⁻²), particulate net primary production (PNPP, mmol m⁻² d⁻¹), bacterial production integrated over Ze (BP, mmol m⁻² d⁻¹), bacterial respiration (BR, mmol m⁻² d⁻¹), and percent of extracellular release (PER, %), at two stations in the main basin of Lake Kivu (Kibuye, Ishungu) in March 2007, September 2007, June 2008, and April 2009.

| Date       | Station | Ze  | Chl-α | PNPP   | BP    | BR    | PNPP-BR | PER  |
|------------|---------|-----|-------|--------|-------|-------|---------|------|
| 15/03/2007 | Kibuye  | 18  | 38.3  | 27.0   | 23.2  | 35.7  | -8.7    | 54   |
| 17/03/2007 | Kibuye  | 20  | 48.4  | 42.5   | 25.8  | 39.9  | 2.6     | 33   |
| 23/03/2007 | Ishungu | 17  | 36.1  | 49.7   | 40.5  | 49.6  | 0.1     | 32   |
| 09/09/2007 | Kibuye  | 19  | 56.4  | 42.9   | 35.9  | 47.9  | -5.0    | 42   |
| 12/09/2007 | Kibuye  | 18  | 55.1  | 45.9   | 34.0  | 45.9  | 0.1     | 36   |
| 04/09/2007 | Ishungu | 20  | n.d.  | 16.0   | 29.9  | n.d.  | n.d.    |      |
| 23/06/2008 | Kibuye  | 24  | 42.8  | 46.0   | 7.7   | 21.6  | 24.4    | 3    |
| 11/07/2008 | Kibuye  | 20  | 37.8  | 42.0   | 11.1  | 24.3  | 17.7    | 17   |
| 03/07/2008 | Ishungu | 19  | 28.1  | 40.7   | 3.9   | 13.6  | 27.1    | -4   |
| 04/05/2009 | Kibuye  | 21  | 22.9  | 14.2   | 49.8  | 61.2  | -47.0   | 82   |
| 21/04/2009 | Ishungu | 24  | 39.3  | 24.5   | 43.5  | 58.9  | -34.4   | 68   |

PNPP and BP were derived from experimental measurements. BR was computed from BP (see material and methods), and PER was computed from PNPP, BR, river inputs and vertical export of organic matter according to equation (24).

doi:10.1371/journal.pone.0109500.t002
Precipitation and preservation of CaCO$_3$ in lakes are not considered in global compilations of C fluxes in lakes, that focus exclusively on organic C and CO$_2$ fluxes e.g. [4], [7]. However, in Lake Kivu, $F_{CaCO_3}$ was found to be a major flux term in the C budgets, 3.6 times larger than the DIC inputs from rivers, and comparable to the emission of CO$_2$ to the atmosphere and $F_{POC}$ (~2 times lower). The $F_{POC}/F_{CaCO_3}$ ratio in the main basin of Lake Kivu was 2.1, which is consistent with the values reported in 6 hard-water lakes of the northern Great Plains, ranging from 1.0 to 4.0 [63], and with the values in Lake Malawi ranging from 2 to 7.3 (on average 2.5) [75]. As a comparison, the average $F_{POC}/F_{CaCO_3}$ in the ocean (so called rain ratio) has been estimated from models of varying complexity to be 4.0 [76], 3.5 to 7.5 [77], and 11.0 [78].

The export ratio (ER) is the fraction of PP that is exported from surface waters to depth and is an important metric of the net metabolism and overall C fluxes in aquatic systems. We computed ER as defined by Baines et al. [79], according to:

$$ER = \frac{F_{POC}}{PNPP} \times 100 \quad (23)$$

where $F_{POC}$ is derived from the DIC mass balance (Fig. 10), and PNPP, is the average PNPP for a given cruise $i$ measured by incubations (Table 2).

ER was 25%, 23%, 23%, and 52% in March 2007, September 2007, June 2008, and April 2009, respectively. These values are consistent with the fact that the ER in lakes is negatively related to lake primary production based on the analysis of Baines et al. [79]. These authors reported ER values as high as 50% for oligotrophic lakes such as Lake Kivu.

The general agreement between the $F_{CaCO_3}$ computed from the TA budget and the TIC deposition fluxes derived from sediment cores reported by Pasche et al. [71], and the $F_{POC}$ computed from the DIC budget and TOC average fluxes in sediment traps reported by Pasche et al. [71], give confidence on the overall robustness of the TA and DIC whole-lake budget we computed. Also, the $F_{CaCO_3}$ and $F_{POC}$ values computed from whole-lake budget are consistent with those derived independently from a mixing model based on the March and September 2007 data (Fig. 9). The whole-lake DIC stable isotope mass balance budgets give $F_{POC}$ and upward DIC inputs estimates that are of same order of magnitude as those derived from whole-lake bulk DIC mass balance budget. The difference in the two approaches is that in the DIC stable isotope mass balance the upward DIC inputs were computed from vertical distributions of DIC and $^{6}{^1}$C-DIC while in the bulk DIC mass balance they are computed from the $E$ and $Q_{netPerturbation}$ values from the model of Schmid et al. [19] and the DIC vertical distribution. This can explain the mismatch between both approaches in the upward DIC input estimates (difference of 23%) that propagated into a relatively larger mismatch in the $F_{POC}$ estimates (difference of 61%) computed as a closing term in both approaches.

Based on the POC and DOC data acquired during the June 2008 and April 2009 cruises in 12 rivers flowing into Lake Kivu (Fig. 1), we computed an overall TOC input from rivers of 0.7 mmol m$^{-2}$ d$^{-1}$ and 3.3 mmol m$^{-2}$ d$^{-1}$, respectively. The $F_{POC}$ was 10 mmol m$^{-2}$ d$^{-1}$, implying a net organic C production in the epilimnion (net autotrophic community metabolic status). This would mean that the fraction of PNPP that does not sediment out of the epilimnion cannot meet BR, and that BR must then rely on other organic C sources. We assume these other organic C sources to be dissolved primary production (DPP), that was estimated assuming steady-state, according to:

$$DPP = F_{POC} + BR - PNPP - F_{TOC_{river}} \quad (24)$$

where $F_{TOC_{river}}$ is the input of TOC from rivers that was computed from the discharge weighted average TOC concentrations from the June 2008 and April 2009 cruises.

The percent of extracellular release (PER) allows to determine the relative importance of DPP in overall C flows in an aquatic system. PER as defined by Baines and Pace [80] was computed according:

$$PER = \frac{DPP}{DPP + PNPP} \times 100 \quad (25)$$

In June 2008, for two stations, the sum of organic C inputs ($PNPP + F_{TOC_{river}}$) exceeded the sum of organic C outputs ($F_{POC} + BR$), leading to negative DPP and PER estimates. If we exclude these values, PER estimates ranged from 3% to 80% (Table 2) encompassing the range reported by Baines and Pace [80] for freshwater lakes from ~0% to ~75%. PNPP in April 2009 was distinctly lower than during the other cruises, leading to high PER estimates. The average PER for all cruises was 32%, and if the April 2009 data are excluded, the average PER was 19%. During the April 2009 field survey, we carried 6 h incubations using the $^{13}$C incorporation method [81] in light controlled (200 µE m$^{-2}$ s$^{-1}$) conditions, allowing to measure PNPP and to compute DPP using the model of Moran et al. [82]. Experimentally-derived PER estimates were 57% at Ishungu, 62% at Kibuye, and 50% in Kabuno Bay [70]. These experimentally determined PER values are within the range of those determined from the mass balance (3% to 80%) and above the average for all cruises (32%). This confirms that a substantial part of the BR is subsidised by DPP, that part of the PNPP is available for export to depth, and consequently that the epilimnion of Lake Kivu is net autotrophic, although a source of CO$_2$ to the atmosphere.

Conclusions

Surface waters of 93% of the lakes in the compilation of Sobek et al. [12] were over-saturated in CO$_2$ with respect to the atmospheric equilibrium. Hence, the overwhelming majority of lakes globally act as a CO$_2$ source to the atmosphere. These emissions to the atmosphere have been frequently explained by the net heterotrophic nature of lakes sustained by terrestrial organic C inputs mainly as DOC [1], [6], [7], [12], [49], [83], [84], [85], [86], [87]. While this paradigm undoubtedly holds true for boreal humic lakes, several exceptions have been put forward in the literature. For instance, Balmer and Downing [88] showed that the majority (60%) of eutrophic agriculturally impacted lakes are net autotrophic and CO$_2$ sinks. Karim et al. [89] showed that surface waters of very large lakes such as the Laurentian Great Lakes are at equilibrium with atmospheric CO$_2$ and O$_2$. This is consistent with the negative relationship between pCO$_2$ and lake size reported in several regional analyses [5], [54], [58], [59], [60], and with the positive relationship between pCO$_2$ and catchment area: lake area reported for Northern Wisconsin lakes [90]. Also, in some lakes among which hard-water lakes, the magnitude of CO$_2$ emissions to the atmosphere seems to depend mainly on hydrological inputs of DIC from rivers and streams [67], [91], [92], [93] or ground-water [52], [94], [95], rather than on lake metabolism. Some of these hard-water lakes were actually found to be net autotrophic, despite acting as a source of CO$_2$ to the atmosphere [67], [91], [93].
Here, we demonstrate that Lake Kivu represents an example of a large, oligotrophic, tropical lake acting as a source of CO₂ to the atmosphere, despite having a net autotrophic epilimnion. The river inputs of TOC were modest, and were on average 11 times lower than the export of POC to depth. This is probably related to the very low ratio of catchment surface area: lake surface area \((5100:2370 \text{ km}^2: \text{km}^2)\) that is among the lowest in lakes globally [96]. We showed that BR was in part subsidized by DPP, based on mass balance considerations and incubations. Since the epilimnion of Lake Kivu is net autotrophic, the CO₂ emission to the atmosphere must be sustained by DIC inputs. The river DIC inputs are also low owing to very low ratio of catchment surface area: lake surface area, and cannot sustain the CO₂ emission to the atmosphere unlike the hard water lakes studied by Finlay et al. [67] and Stets et al. [91]. In Lake Kivu, the CO₂ emission is sustained by DIC inputs from depth, and this DIC is mainly geogenic [24] and originates from deep geothermal springs [19].

Carbonate chemistry in surface waters of Lake Kivu is unique from other points of view. The dissolved C pool is largely dominated by DIC, with DIC:DOC ratios distinctly higher than in hard-water lakes and humic lakes. The high DIC content in surface water results in CaCO₃ over-saturation, in turn leading to CaCO₃ precipitation and export to depth. This flux was found to be significant, being 4 times larger than the river inputs of DIC and of similar magnitude than the CO₂ emission to the atmosphere.

Supporting Information

Table S1  Data-set of depth (m), water temperature (°C), specific conductivity at 25°C (μS cm⁻¹), oxygen saturation level (%O₂, %), δ¹³C signature of dissolved inorganic carbon (DIC) (δ¹³C-DIC, ‰), total alkalinity (TA, mmol L⁻¹), pH, partial pressure of CO₂ (pCO₂, ppm), dissolved methane concentration (CH₄, nmol L⁻¹), particulate organic carbon (POC, mg L⁻¹), δ¹³C signature of POC (δ¹³C-POC, ‰), dissolved organic carbon (DOC, mg L⁻¹), δ¹³C signature of DOC (δ¹³C-DOC, ‰), total suspended matter (TSM, mg L⁻¹) in Lake Kivu and 12 rivers flowing into Lake Kivu, in March 2007, September 2007, June 2008, April 2009 and October 2010. (XLS)

Acknowledgments

We are grateful to Pascal Isumbisho Mwapu (Institut Supérieur Pédagogique, Bukavu, République Démocratique du Congo) and Laetitia Nyinawamwoza (National University of Rwanda, Butare, Rwanda) and their respective teams for logistical support during the cruises, to Bruno Delille, Gilles Lepoint, Bruno Leporcq, and Marc-Vincent Commmariou for help in field sampling, to an anonymous reviewer and Pirkko Kortelainen (reviewer) for constructive comments on a previous version of the manuscript.

Author Contributions

Conceived and designed the experiments: AVB SB PS JPD FD. Performed the experiments: AVB CM FD. Analyzed the data: AVB CM SB PS JPD FD. Contributed reagents/materials/analysis tools: AVB CM SB PS JPD FD. Wrote the paper: AVB CM SB PS JPD FD.

References

1. Cole JJ, Caraco NF (2001) Carbon in catchments: connecting terrestrial carbon losses with aquatic metabolism. Mar Fresh Res 52: 101–110.
2. Kempe S (1964) Links of the anthropogenically enhanced carbon cycle in surface fresh waters. J Geophys Res 69: 4657–4676.
3. Richet JE, Melack JM, Aufdenkampe AK, Ballester VM, Hess LL (2002) Outgassing from Amazonian rivers and wetlands as a large tropical source of atmospheric CO₂. Nature 416: 617–620.
4. Cole JJ, Prairie YT, Caraco NF, McDowell WH, Tranvik LJ, et al. (2007) Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. Ecosystems 10: 171–184.
5. Alin SR, Johnson TC (2007) Carbon cycling in large lakes of the world: a synthesis of production, burial, and lake-atmosphere exchange estimates. Global Biogeochem Cycles 21(GB002): doi:10.1029/2006GB002821.
6. Rattan TJ, Kaplan LA, Findlay S, Hopkinson CS, Mari E, et al. (2008) Biophysical controls on organic carbon fluxes in fluvial networks. Nature Geosc 1: 95–100.
7. Tranvik LJ, Downing JA, Cotner JB, Loisel SA, Striegl RG, et al. (2009) Lakes and reservoirs as regulators of carbon cycling and climate. Limnol Oceanogr 54: 2298–2314.
8. Aufdenkampe AK, Mayorga E, Raymond PA, Melack JM, Doney SC, et al. (2011) Riverine coupling of biochemical cycles between land, oceans, and atmosphere, Front Ecol Environ 9: 53–60.
9. Butman D, Raymond PA (2011) Significant eflux of carbon dioxide from streams and rivers in the United States. Nature Geosc 4: 839–842.
10. Raymond PA, Hartmann J, Laurierwald R, Sohbe S, McDonald G, et al. (2013) Global carbon dioxide emissions from inland waters. Nature 503: 355–359.
11. Takahashi T, Sutherland SC, Wanninkhof R, Sweeney C, Feely RA, et al. (2009) The atmosphere, chemical and biological oceanography of the carbon dioxide system of the surface waters of the world oceans. J Geophys Res 114(C03001): doi:10.1029/2008JC004714.
12. Hoefs J (1979) Determination of Isotopic Composition of Carbon and Hydrogen in Water and Marls. Berlin: Springer. pp. 13–29.
13. Ludwig W, Probst JL, Kempe S (1996) Predicting the oceanic input of organic carbon by continental erosion. Global Biogeochem Cycles 10: 25–41.
14. Lebrer B, Doll P (2004) Development and validation of a global database of lakes, reservoirs and wetlands. J Hydrol 296: 1–22.
15. Darchambeau F, Sarmento H, Duesy J-P (2014) Primary production in a tropical large lake: The role of phytoplankton composition. Sci Total Environ 473–474: 178–188.
16. Stemuste S, Priol S, Tarbe AL, Sarmento H, Lecomte M, et al. (2009) Abundance and production of bacteria, and relationship to phytoplankton production, in a large tropical lake (Lake Tanganyika). Freshwater Biol 54: 1300–1311.
17. Dumas H (1957) La stratification thermique et chimique des lacs Kivu, Edouard et Ndalaga (Conga Belge). Verh Internat Verein Theor Angew Limnol 8: 51–68.
18. Degens ET, vos Herzer RP, Wosg H-K, Deuser WG, Jannasch HW (1975) Lake Kivu: Structure, chemistry and biology of an East African rift lake. Geol Rundsch 62: 245–277.
19. Schmid M, Halsbach M, Wehrli B, Wiest A (2005) Weak mixing in Lake Kivu: new insights indicate increasing risk of uncontrolled gas eruption. Geochim Cosmochim Acta 69(2005): doi:10.1016/j.gca.2005.06.020.
20. Thiery W, Stefanenko VM, Fang X, Jobak KD, Li Z, et al. (2014) LakeMIP Kivu: Evaluating the representation of a large, deep tropical lake by a set of one-dimensional lake models. Tellus A 66(2190): doi:10.3402/tellusa.v66i2190.
21. Sarmento H, Isumbishi M, Duesy J-P (2006) Phytoplankton ecology of Lake Kivu: Eastern Africa, J Plankton Res 28: 815–829.
22. Sarmento H, Darchambeau F, Duesy J-P (2012) Phytoplankton of Lake Kivu. In: Lake Kivu - Limnology and biogeochemistry of a tropical great lake: Springer. pp. 67–83.
23. Schmid M, Bushbridge M, Wiest A (2010) Double-diffusive convection in Lake Kivu. Limnol Oceanogr 55: 225–238.
24. Schoell M, Tietze K, Schobert SM (1988) Origin of methane in Lake Kivu (East-Central Africa). Chem Geol 71: 257–265.
25. Schmid M, Tietze K, Halsbach M, Leslie A, McGinnis D, et al. (2004) How hazardous is the gas accumulation in Lake Kivu? Arguments for a risk assessment in light of the Nyiragongo Volcano eruption of 2002. Acta Vulcanol 14/15: 115–121.
26. Nayar A (2009) A lakeful of trouble. Nature 460: 321–323.
27. Wiest A, Jarc I, Burgmann H, Pasche N, Schmid M (2012) Methane Formation and Future Extraction in Lake Kivu. In: Lake Kivu - Limnology and biogeochemistry of a tropical great lake: Springer. pp. 163–180.
28. Borges AV, Abril G, Delille B, Duesy J-P, Darchambeau F (2011) Diffusive methane emissions to the atmosphere from Lake Kivu (Eastern Africa). J Geophys Res 116(G03032): doi:10.1029/2011JG001673.
29. Schmid M, Wiest A (2012) Stratification, Mixing and Transport Processes in Lake Kivu: New insights indicate increasing risk of uncontrolled gas eruption. Geochim Cosmochim Acta 69(2005): doi:10.1016/j.gca.2005.06.020.
30. Frankignoulle M, Borges A, Boutron N, Duesy J-P (2001) A new design of equilibrator to monitor carbon dioxide in highly dynamic and turbid environments. Water Res 35: 1344–1347.
31. Frankignoulle M, Borges A (2001) Direct and indirect pCO₂ measurements in a wide range of pCO₂ and salinity values (the Scheldt estuary). Aquat Geochim 7: 267–273.
Carbon Cycling in Surface Waters of Lake Kivu

32. Gran G (1932) Determination of the equivalence point in potentiometric titrations of seawater with hydrochloric acid. Oceano Acta 5: 209–218.

33. Millero FJ, Graham TB, Huang F, Bustos-Serrano H, Pierrot D (2006) Dissociation constants of carbonic acid in sea water as a function of salinity and temperature. Mar Chem 100: 89–94.

34. Miyajima T, Yamada Y, Hanba YT, Yoshii K, Kostabashi T, et al. (1995) Determining the stable-isotope ratio of total dissolved inorganic carbon in lake water by GC/IRMS. Limnol Oceanogr 40: 994–1000.

35. Neumann-Nielsen A (1951) Measurement of carbon organic in by means of carbon-14. Nature 167: 684–685.

36. Dacey JP, Higgins HW, Mackey DJ, Hurley JP, Frost TM (2006) Pigment ratios and phytoplankton assessment in northern Wisconsin lakes. J Phycol 36: 274–285.

37. Fuhrman JA, Azam F (1982) Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: Evaluation and field results. Mar Biol 66: 109–120.

38. Del Giorgio PA, Cole JJ (1998) Bacterial growth efficiency in natural aquatic ecosystems. Annu Rev Mar Sci 29: 509–557.

39. Weiss RF (1974) Carbon dioxide in water and seawater: the solubility of a non-CSTR-reactor gas. Deep Sea Res 21: 803–808.

40. Del Giorgio PA, Cole JJ (1998) Bacterial growth efficiency in natural aquatic ecosystems. Annu Rev Mar Sci 29: 509–557.

41. Cole JJ, Caraco NF (1998) Atmospheric exchange of carbon dioxide in a low-latitude coastal lake: Application of the two-box model. Limnol Oceanogr 43: 647–656.

42. Wanninkhof R (1992) Relationship between wind speed and gas exchange over the ocean. J Geophys Res 97: 7373–7382.

43. Muvundja FA, Pasche N, Bugenyi FWB, Isumbisho M, Malaba A, et al. (2009) Observations of Lake Victoria pCO2: Implications on CO2 evasion/accumulation ratio in boreal lakes is linked to nitrogen. Global Biogeochem Cycles 1375.

44. Cole JJ, Caraco NF (1998) Atmospheric exchange of carbon dioxide in a low-latitude coastal lake: Application of the two-box model. Limnol Oceanogr 43: 647–656.

45. Quay PD, Stutzman J (2003) Surface layer carbon budget for the subtropical N. Atlantic. J Geophys Res 108:C10, 2003.

46. Emrich K, Ehhalt DH, Vogel JC (1970) Carbon isotope fractionation during the combustion of carbon containing substances. Nature 227: 684–685.

47. Atilla N, McKinley GA, Bennington V, Riedl M, Urban N, et al. (2011) Observation of Lake Superior pCO2. Limnol Oceanogr 56: 775–786.

48. Emrich K, Ehhalt DH, Vogel JC (1970) Carbon isotope fractionation during the combustion of carbon containing substances. Nature 227: 684–685.
92. Maberly SC, Barker PA, Stott AW, De Ville MM (2012) Catchment productivity controls CO₂ emissions from lakes. Nat Clim Chang doi:10.1038/NCLIMATE1748.
93. McDonald CP, Stets EG, Striegl RG, Butman D (2013) Inorganic carbon loading as a primary driver of dissolved carbon dioxide concentrations in the lakes and reservoirs of the contiguous United States, Global Biogeochem Cycles 27: doi:10.1002/gbc.20032.
94. Dubois K, Carignan R, Veizer J (2009) Can pelagic net heterotrophy account for carbon fluxes from eastern Canadian lakes? Appl Geochem 24: 988–998.
95. Humborg C, Morth C-M, Sundbo M, Borg H, Blenckner T, et al. (2010) CO₂ supersaturation along the aquatic conduit in Swedish watersheds as constrained by terrestrial respiration, aquatic respiration and weathering, Global Change Biol 16: 1966–1978.
96. Spigel RH, Coulter GW (1996) Comparison of hydrology and physical limnology of the East African Great Lakes: Tanganyika, Malawi, Victoria, Kivu and Turkana (with reference to some North American Great Lakes). In: Johnson TC, Odada EO, editors. The limnology, climatology and paleoclimatology of the East African lakes. Boca Raton, FL: Gordon and Breach Publishers. pp. 103-139.