LIMNOLOGY
and
OCEANOGRAPHY

ASLO

Limnol. Oceanogr. 00, 2016, 00–00

doi: 10.1002/lno.10304

Chemoautotrophy and anoxygenic photosynthesis within the water column of a large meromictic tropical lake (Lake Kivu, East Africa)

Cédric Morana,*[1] Fleur A. E. Roland,[2] Sean A. Crowe,[3] Marc Llirós,[4,5] Alberto V. Borges,[2] François Darchambeau,[2] Steven Bouillon[1]

[1]Department of Earth and Environmental Sciences, KU Leuven, Leuven, Belgium
[2]Chemical Oceanography Unit, University of Liège, Liège, Belgium
[3]Departments of Microbiology and Immunology, and Earth, Ocean, and Atmospheric Sciences, University of British Columbia, Vancouver, Canada
[4]Research Unit in Environmental and Evolutionary Biology, University of Namur, Namur, Belgium
[5]Institut des Sciences de la Vie, Université Catholique de Louvain, Louvain-la-Neuve, Belgium

Abstract

We quantified chemoautotrophic and anoxygenic photosynthetic microbial production in the water column of Lake Kivu, a permanently stratified tropical lake situated amidst volcanic activity, and aimed to identify the microorganisms involved in these processes through the analysis of their phospholipid fatty acid (PLFA) content and stable isotope (^{13}C) labelling of PLFA in a set of incubation experiments. Data demonstrate the existence of a biogeochemically active chemoautotrophic bacterial community in the redoxcline of Lake Kivu (50–70 m). PLFA data indicate that the bacterial communities are structured vertically in the water column, with a large dissimilarity between the oxic and anoxic waters. Maximum volumetric dark CO_2 fixation rates measured in Lake Kivu were in the same range as values reported from H_2S-rich marine redoxclines, such as the Black and Baltic Seas, and the Cariaco Basin. Similarly, maximal chemoautotrophic activities in Lake Kivu were observed in sulfidic waters, just below the oxycline. Anoxygenic photosynthetic production was never observed in the main basin of Lake Kivu. However, anoxygenic phototrophs largely dominated CO_2 fixation in the illuminated redoxcline of Kabuno Bay, a shallower ferruginous sub-basin. Overall, this study supports the idea that chemoautotrophs and/or anoxygenic photoautotrophs might play an important role in the flow of carbon and energy in permanently stratified tropical ecosystems. In Lake Kivu, these processes significantly contribute to organic matter biosynthesis and exert an indirect control on oxygenic photoautotrophs by shortcircuiting the vertical transport of nutrients to the illuminated and oxygenated surface waters.

The biosynthesis of organic molecules from carbon dioxide (CO_2) constitutes the base of the food web in many aquatic ecosystems, constraining their overall productivity. In modern aquatic environments, biosynthesis of organic matter is to a large extent carried out by algae and cyanobacteria through oxygenic photosynthesis. Other prokaryotes, however, have the ability to produce organic matter from CO_2 without the energy of sunlight, but using the energy generated by the oxidation of various reduced species (Enrich-Prast et al. 2009). These chemoautotrophic organisms greatly impact the biogeochemical cycles of diverse elements, such as carbon (C), nitrogen (N), and sulfur (S), especially in euxinic systems (Jost et al. 2008). In a recent literature review, the global rate of oceanic C fixation by chemoautotrophs has been estimated at $\sim$ 0.77 Pg C yr^{-1} (Middelburg 2011). According to this study, the chemoautotrophic production would represent, at the global scale, a non-negligible input of organic C in the ocean, being similar in magnitude to the supply of organic C through rivers (estimated at 0.45 Pg C yr^{-1} by Cole et al. 2007).

Some photoautotrophic prokayotes are also able to incorporate CO_2 under anoxic conditions (anoxygenic photosynthesis), using hydrogen sulfide (H_2S) or reduced iron (Fe^{2+}) as an electron donor in photosynthesis (Sirevåg et al. 1977; Heising et al. 1999). These prokaryotes are particularly well adapted to low-light levels and have been found to grow in sulfidic waters in the Black Sea at irradiance levels as low as 5×10^{-4}% of surface light intensity (Overmann et al. 1992). Even if their contribution to the biosynthesis of organic

*Correspondence: cedric.morana@ees.kuleuven.be

Additional Supporting Information may be found in the online version of this article.

molecules is usually considered to be small in modern aquatic environments compared with oxygenic photoautotrophs, it has been suggested that anoxygenic photoautotrophs played a key role in the global C cycle in the early history of Earth, before the emergence of oxygenic photosynthesis (Canfield et al. 2006; Crowe et al. 2008).

In modern aquatic environments, particularly high abundances of chemoautotrophs or anoxygenic photoautrophs have been documented in permanently stratified water bodies, such as the Black Sea (Grote et al. 2008) and the Cariaco Basin (Taylor et al. 2001), several coastal upwelling regions (Farías et al. 2009), and meromictic freshwater lakes (Hadas et al. 2001; García-Cantizano et al. 2005). All these systems are characterized by the presence of a pelagic redox gradient (redoxcline) that separates the oxygenated upper waters rich in electron acceptors from the anoxic bottom waters where inorganic electron donors are abundant. As these microorganisms derive their energy from the oxidation of reduced species, or light in the case of anoxygenic phototrophs, the redoxclines of stratified environment are usually an area of intense biogeochemical activity. Earlier studies reported net dark CO_2 fixation rates in the redoxcline of Black Sea (May 1988) reaching up to 15% of the depth-integrated phytoplankton net primary production (Jørgensen et al. 1991). Areal net chemoautotrophic production have been found to even exceed by a significant margin (333%, in July 1998) the areal net photosynthetic production in the Cariaco basin (Taylor et al. 2001). In the freshwater oligotrophic karstic Lake Cisó (Spain), chemoautotrophic organisms are the main contributors to the annual primary production (56–61%), followed by anoxygenic photosynthesis (19–35%), and lastly by oxygenic photosynthesis (4–25%) (García-Cantizano et al. 2005). Besides the role they play in the C biogeochemistry, chemoautotrophs and anoxygenic photoautotrophs could affect also the biogeochemical cycle of inorganic nutrients in stratified environments. For instance, a large population of anoxygenic photoautotrophs has been found to be the major source of phosphorus for the growth of aerobic heterotrophs in the mixolimnion of Lake Mahoney, where seasonal upwelling events of anoxygenic photoautotrophs represents an effective recycling mechanism for inorganic nutrients that minimizes nutrient loss due to sedimentation (Overmann et al. 1996). Overall, all these studies showed that chemoautotrophs and anoxygenic photoautotrophs could play a key role in the functioning of stratified ecosystems in two important ways. First, they could contribute significantly to the autochtonous production of organic matter, and second, they could exert an indirect control on oxygenic photoautotrophs by shortcircuiting the vertical transport of inorganic nutrients to the illuminated and oxygenated surface waters (Haberyan and Hecky 1987; Kilham and Kilham 1990).

The aim of this study was to quantify chemoautotrophic and anoxygenic photosynthetic microbial production within the pelagic redoxcline of Lake Kivu, a permanently stratified tropical lake located in an East Africa volcanic area, where aerobic methanotrophy has already been recognized as an important process in C and energy flow (Borges et al. 2011; Morana et al. 2015; Zigah et al. 2015). We also attempted to identify the organisms involved in these processes through analyses of their phospholipid fatty acid (PLFA) contents. Additionaly, tracing the incorporation of CO_2 into the PLFA by stable isotope (^{13}C) labeling experiments allowed to directly link the CO_2 fixation process with the identity of the microorganisms involved.

Material and methods

Study site description and sampling

The meromictic Lake Kivu (2.50°S 1.59°S 29.37°E 28.83°E) is one of the East African great lakes (2370 km^2 surface area, 550 km^3 volume). Its vertical structure consists of an oxic and nutrient-poor mixed layer (seasonally variable depth, down to 70 m), and a permanently anoxic monimolimnion rich in dissolved gases (CH_4, CO_2) and inorganic nutrients (Schmid et al. 2005). Seasonal variations of the vertical position of the thermocline are driven by contrasting heat exchange with the atmosphere between rainy (October–May) and dry (June–September) seasons (Thiery et al. 2014), the latter being characterized by a deepening of the oxic zone, and an increased input of dissolved gases and inorganic nutrients into the mixed layer (Borges et al. 2011). In constrast to the main lake, Kabuno Bay, a small and shallower sub-basin almost isolated from the rest of the lake, is characterized by a shallower, very sharp and stable stratification, and therefore the vertical position of the oxic-anoxic transition zone ($\sim$ 10.5 m) does not vary seasonally (Borges et al. 2011; Llirós et al. 2015).

Sampling was carried out in the main basin of Lake Kivu once during rainy (February 2012, 01°43′S, 29°14′E) and once during dry (September 2012, 02°20′S, 28°58′E) seasons, and once in Kabuno Bay during the rainy season (February 2012, 01°37′S, 29°02′E). Temperature and conductivity profiles were obtained with a Yellow Springs Instrument (YSI) 6600 V2 probe. The conductivity cell was calibrated with a 1000 μS cm^{-1} (25°C) YSI standard. O_2 concentration was measured with a YSI-proODO probe with a optical O_2 sensor calibrated using air saturated water. In the main basin of Lake Kivu, water samples were collected with a 7 L Niskin bottle (Hydro-Bios) at a depth interval of 5 m from the lake surface to 80 m depth. Alternatively, in Kabuno Bay, water samples were collected at a minimal depth interval of 0.25 m in the redoxcline (10.00–12.00 m) using a weighted-double conus connected to a battery-driven peristaltic pump through PTFE tubing, allowing for laminar water sampling and minimal vertical disruption (Jørgensen et al. 1979).

Chemical analyses

Hydrogen sulphide (HS^-) concentrations were determined spectrophotometrically according to Cline (1969) on syringe-filtered (0.2 μm pore size) samples preserved with Zn acetate (10 μL 20% per 1 mL sample). Sulfate (SO_4^{2-}) concentrations were determined by ion chromatography (Dionex with suppressor column) on filtered (0.2 μm pore size) samples preserved frozen. Detection limits for HS^- and SO_4^{2-} measurements were 0.25 μmol L^{-1} and 0.5 μmol L^{-1}, respectively. Fe speciation was determined spectrophotometrically using the ferrozine method (Viollier et al. 2000), on unfiltered samples preserved with 0.5 N HCl (1 : 1 ratio with sample water). Detection limits for Fe(II) are about 1 μmol L^{-1}, and detection limits for Fe(III) are about 10% of the Fe(II) concentration at Fe(III) concentrations above about 0.5 μmol L^{-1}.

Ammonia (NH_4^+) concentrations were determined using the dichloroisocyanurate-salicylate-nitroprussiate colorimetric method, whereas nitrate (NO_3^-) concentrations were determined after cadmium reduction to NO_2^- and quantified following the sulphanilamide coloration method (Llirós et al. 2010). The detection limits of these methods were 0.3 μmol L^{-1}, 0.03 μmol L^{-1}, and 0.15 μmol L^{-1} for NH_4^+, NO_2^- and NO_3^-, respectively. Samples for CH_4 concentrations were collected in 50 mL glass serum bottles from the Niskin bottle with a silicone tube, left to overflow, poisoned with 100 μL of saturated $HgCl_2$ and sealed with butyl stoppers and aluminium caps. Concentrations of CH_4 were measured by a headspace technique (Weiss 1981) using gas chromatography with flame ionization detection (GC-FID, SRI 8610C), as detailed by Borges et al. (2011).

Samples for the determination of $\delta^{13}C$ signature of dissolved inorganic carbon (DIC) were collected by gently overfilling 12 mL glass vials (Labco Exetainer), and preserved with 20 μL of a saturated $HgCl_2$ solution. For the analysis of $\delta^{13}C$-DIC, a 2 mL helium headspace was created and 100 μL of H_3PO_4 (99%) was added into each vial to transform all DIC species into CO_2. After an overnight equilibration, a variable volume of the headspace was injected into an EA-IRMS (Thermo FlashHT with Thermo DeltaV Advantage) to measure the $\delta^{13}C$-DIC. The obtained $\delta^{13}C$ data were corrected for the isotopic equilibration between gaseous and dissolved CO_2 as described in Gillikin and Bouillon (2007), and calibrated with LSVEC and NBS-19 certified standards.

Samples for particulate organic carbon concentration (POC) and its stable C isotope signature ($\delta^{13}C$-POC) were filtered on pre-combusted (overnight at 450°C) 25 mm glass fiber filters (Advantec GF-75, 0.3 μm), and dried. These filters were later decarbonated with HCl fumes for 4 h, dried and packed in silver cups. POC and $\delta^{13}C$-POC were determined on an EA-IRMS (Thermo FlashHT with Thermo DeltaV Advantage). Calibration of POC and $\delta^{13}C$-POC was performed with IAEA-C6 and acetalinide. Reproducibility of $\delta^{13}C$-POC measurements was typically better than 0.2‰.

PLFA analyses

Bacterial PLFA can be divided as typical for gram-positive bacteria and for gram-negative bacteria (Taipale et al. 2009). Generally, the iso- and anteiso-branched PLFA, such as the i15:0 and a15:0, are common in gram-positive bacteria. In contrast, C16 monounsaturated and C18 monounsaturated PLFA (hereafter C16 MUFA and C18 MUFA, respectively) are common in gram-negative bacteria and many chemoautotrophic bacteria (Imhoff 2003; Glaubitz et al. 2009; Taipale et al. 2009). Futhermore, C16 MUFA and C18 MUFA are also known to contribute to a large fraction of the PLFA composition of type I and type II methanotrophic bacteria, respectively (Le Bodelier et al. 2009). Last, C14:0, C16:0, and C16 MUFA have been found to represent 90% of all the PLFA of anoxygenic phototrophic *Chlorobium* members (Imhoff 2003).

Samples for PLFA concentration and their $\delta^{13}C$ values were filtered on pre-combusted 47 mm glass fiber filters (Advantec GF-75, 0.3 μm), and kept frozen until further processing. Extraction and derivatization of PLFA was performed following a modified Bligh and Dyer extraction, silica column partitioning, and mild alkaline transmethylation as described by Boschker (2004). Analyses were made with an Isolink GC combustion interface coupled to a Thermo DeltaV Advantage IRMS. All samples were analyzed in splitless mode, using an apolar GC column (Agilent DB-5) with a flow rate of 2 mL min^{-1}. Initial oven temperature was set at 60°C for 1 min, then increased to 130°C at 40°C min^{-1}, and subsequently to 250°C at a rate of 3°C min^{-1}. $\delta^{13}C$-PLFA were corrected for the addition of the methyl group by a simple mass balance calculation, and were calibrated using internal (C19:0) and external fatty acid methyl ester (FAME) standards (mixture of C14:0, C16:0, C18:0, C20:0, C22:0). Reproducibility was 0.6‰ or better for natural abundance samples.

CO_2 fixation rate measurements

CO_2 fixation rates by photo- and chemoautotrophic organisms were quantified at several depths within the redoxcline of the main basin and Kabuno Bay. Eight glass serum bottles (60 mL) per sampling depth were gently filled directly from the sampling bottle (main basin) or from the Teflon tubing (Kabuno Bay), overflowed, and then capped with butyl stoppers and sealed with aluminium caps. Four bottles were covered with aluminium foil to simulate dark incubations whereas the other four bottles were kept under in situ light conditions. Afterward, 1 mL of a ^{13}C-DIC solution (99.8% $NaH^{13}CO_3$ dissolved in lake water) was injected through the septa of each of the eight bottles to a final concentration of 1 mmol L^{-1} (less than 8% of total DIC pool). For controls without biological activity, 100 μL of $HgCl_2$ were immediately added to one aluminium-covered and one clear bottle. All bottles were vigorously shaken and incubated for 24 h in the lake at their corresponding depth at in situ temperature and irradiance conditions. Incubations were stopped by filtration of 40 mL of water from each bottle on

to glass fiber filters (Advantec GF-75, 0.3 μm) that were subsequently frozen to trace the incorporation of ^{13}C-DIC in the POC pool. Also, a 12 mL exetainer tube was filled and poisoned with 20 μL $HgCl_2$ to determine the exact amount of excess ^{13}C-DIC in every bottle. δ^{13}C analysis of the POC and DIC pools were performed as decribed above. Chemoautotrophic bacterial production (CBP, μmol L^{-1} d^{-1}) was calculated with data from the incubations in bottles covered with aluminium foil as follows (Hama et al. 1983):

$$\mathrm{CBP} = (\mathrm{POC_f} \times (\%^{13}\mathrm{C\text{-}POC_f} - \%^{13}\mathrm{C\text{-}POC_i}))/ (t \times (\%^{13}\mathrm{C\text{-}DIC} - \%^{13}\mathrm{C\text{-}POC_i}) \quad (1)$$

where POC_f is the particulate organic concentration at the end of the incubation (μmol L^{-1}), $\%^{13}$C-POC_f and $\%^{13}$C-POC_i is the initial and final percentage of ^{13}C in POC, t is time (d) and $\%^{13}$C-DIC is the percentage of ^{13}C in DIC after the addition of the tracer. Results from the incubations in clear bottles were used to calculate anoxygenic photoautotrophic bacterial production rates (anPBP, μmol L^{-1} d^{-1}), after correcting for the contribution of CBP to the total CO_2 fixation. No oxygenic photosynthesis inhibitor such as 3-(3,4-dichlorophenyl)−1,1-dimethylurea was used during the incubation experiments, but we assumed that the measured light-dependent CO_2 reflected anoxygenic photosynthesis as the samples were collected in anoxic waters. This assumption was confirmed by pigment (i.e., chlorophyll *a* and bacteriochlorophyll *e*) analyses that revealed the near exclusive presence of anoxygenic phototrophs in the redoxcline of Kabuno Bay (Llirós et al. 2015). AnPBP was calculated as follow (Hama et al. 1983):

$$\mathrm{anPBP} = [(\mathrm{POC_f} \times (\%^{13}\mathrm{C\text{-}POC_f} - \%^{13}\mathrm{C\text{-}POC_i}))/ (t \times (\%^{13}\mathrm{C\text{-}DIC} - \%^{13}\mathrm{C\text{-}POC_i})] - \mathrm{CBP}$$

Stable isotope labelling (^{13}C-DIC) of PLFA

At each sampling depth, and in parallel with the chemoautotrophic and anoxygenic photoautotrophic bacterial production measurements, eight large serum bottles (250 mL) were filled with water, allowed to overflow, and sealed with a butyl stopper and aluminium cap. Four of these bottles were covered with aluminium foil, then spiked with 1 mL of a ^{13}C-DIC solution (less than 8% of total DIC pool) and incubated under in situ temperature and irradiance conditions, at their corresponding sampling depth. After 24 h of incubation in the lake, the four bottles incubated in the dark were filtered on the same 47 mm glass fiber filter (Advantec GF-75, 0.3 μm) to measure the dark incorporation of the tracer (^{13}C-DIC) into bacterial PLFA. The other four bottles were directly processed in the same way to measure the phototrophic incorporation of DIC into bacterial PLFA. The filters were kept frozen until further processing. The extraction, derivatization, and analyses of PLFAs on the GC-c-IRMS were carried out as described above.

Results

Physico-chemical characteristics of the water column

During the rainy season, the water column of Lake Kivu was oxic from surface down to 45 m depth (Fig. 1a). CH_4 was abundant in deep waters but concentrations were four orders of magnitude lower in surface waters. SO_4^{2-} was abundant in oxic waters, but its concentration decreased almost linearly with depth between the oxic-anoxic transition zone at 45 m (169 μmol L^{-1}) and 80 m (11 μmol L^{-1}) (Fig. 1b). H_2S concentrations showed an opposite pattern with undetectable concentrations in the mixed layer and at the oxic-anoxic transition, H_2S concentrations concentrations increased from 55 m to reach the highest values at 80 m (114 μmol L^{-1}; Fig. 1b). Similarly, NH_4^+ concentrations were very high in deeper waters but were below the limit of detection (< 3 μmol L^{-1}) above 55 m (Fig. 1c). A maximum in NO_3^- (1.6 μmol L^{-1}) and NO_2^- (1.4 μmol L^{-1}) concentrations was measured at 50 m, 5 m above the depth where NH_4^+ became undetectable (Fig. 1c).

During the dry season, significant concentrations of O_2 (> 3 μmol L^{-1}) were measured down to 65 m, where the conductivity increased sharply (Fig. 2a). CH_4 concentrations in bottom waters were similar to those measured during the rainy season but decreased abruptly at the bottom of the oxycline (65 m) (Fig. 2a). H_2S concentrations sharply decrease between 80 m and 65 m, to become undetectable above, in oxic waters (Fig. 2b). The NH_4^+ concentrations were below the detection limit in the mixed layer (down to 60 m), but increased with depth between 60 m (1.7 μmol L^{-1}) and 80 m (281.6 μmol L^{-1}). NO_3^- showed the opposite trend, being undetectable in anoxic waters, but relatively abundant in oxic waters. A maximum in NO_3^- concentrations was measured at the oxic-anoxic transition (4.5 μmol L^{-1} at 62.5 m) while a maximum in NO_2^- concentration was measured slightly deeper in the water column (0.4 μmol L^{-1} at 65 m).

The physico-chemical structure of the water column in Kabuno Bay differed in some important aspects with respect to the main basin of Lake Kivu. First, the mixed layer and the oxic zone were considerably shallower (down to 10.50 m) and the vertical stratification of the water column was much sharper, as indicated by the strong increase of conductivity recorded between 10.50 m and 11.50 m (Fig. 3a). Consequently, Kabuno Bay was characterized by a very sharp and illuminated redoxcline. H_2S and SO_4^{2-} showed the same opposite trend as observed at the oxic-anoxic transition in the main basin (Fig. 3b). Oxidized and reduced iron species were distributed along a redox gradient between 11.00 m and 12.00 m (Fig. 3c).

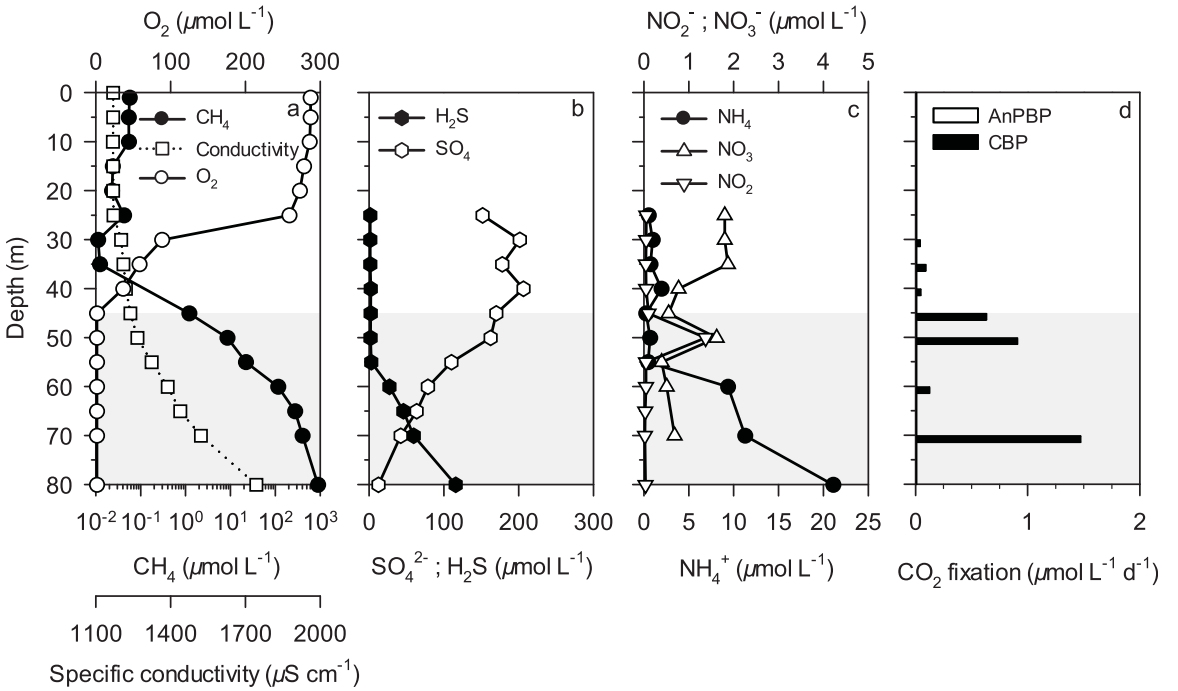

Fig. 1. Vertical depth profiles of (**a**) the conductivity (μS cm^{-1}) and the concentration of dissolved O_2 and CH_4 (μmol L^{-1}), (**b**) the concentration of H_2S and SO_4^{2-} (μmol L^{-1}), (**c**) the concentration of NH_4^+, NO_3^- and NO_2^- (μmol L^{-1}), (**d**) the daily chemoautotrophic bacterial production rates (μmol L^{-1} d^{-1}) during rainy season (February 2012) in the main basin. Grey zone corresponds to anoxic waters. AnPBP and CBP stand for anoxygenic photoautotrophic bacteria production and chemoautotrophic bacterial production, respectively.

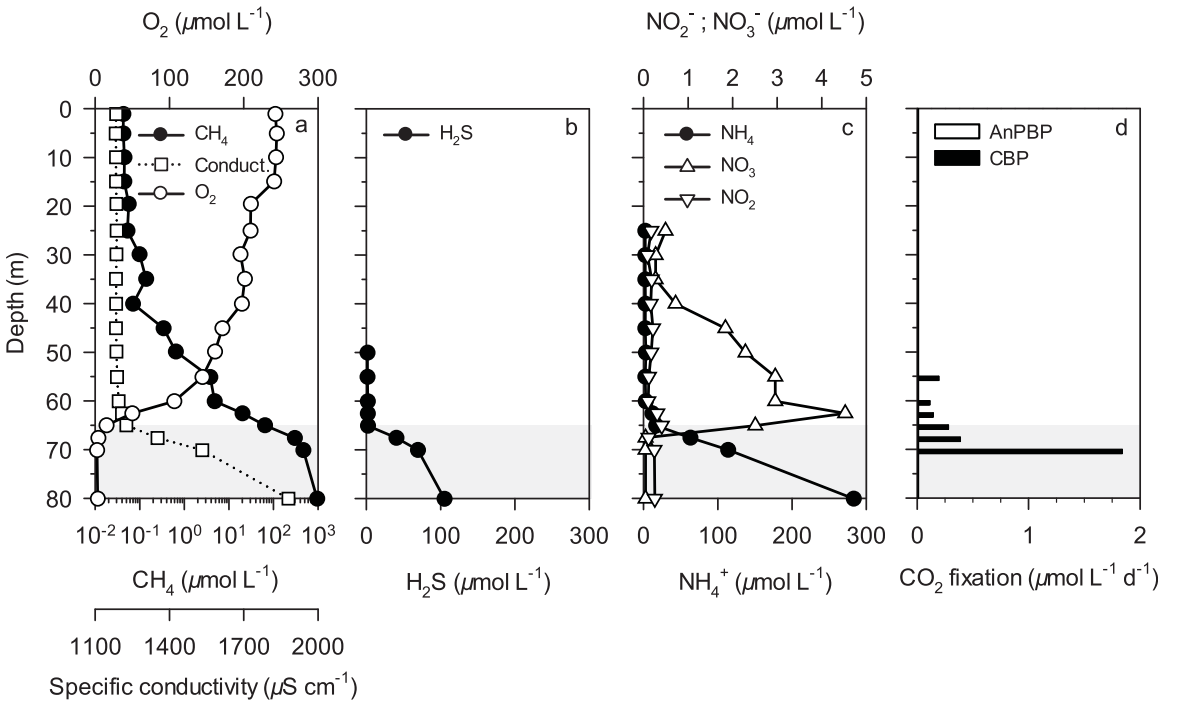

Fig. 2. Vertical depth profiles of (**a**) the conductivity (μS cm^{-1}) and the concentration of dissolved O_2 and CH_4 (μmol L^{-1}), (**b**) the concentration of NH_4^+, NO_3^- and NO_2^- (μmol L^{-1}), (**c**) the daily chemoautotrophic bacterial production rates (μmol L^{-1} d^{-1}) during dry season (September 2012) in the main basin. Grey zone corresponds to the anoxic waters. AnPBP and CBP stand for anoxygenic photoautotrophic bacteria production and chemoautotrophic bacterial production, respectively.

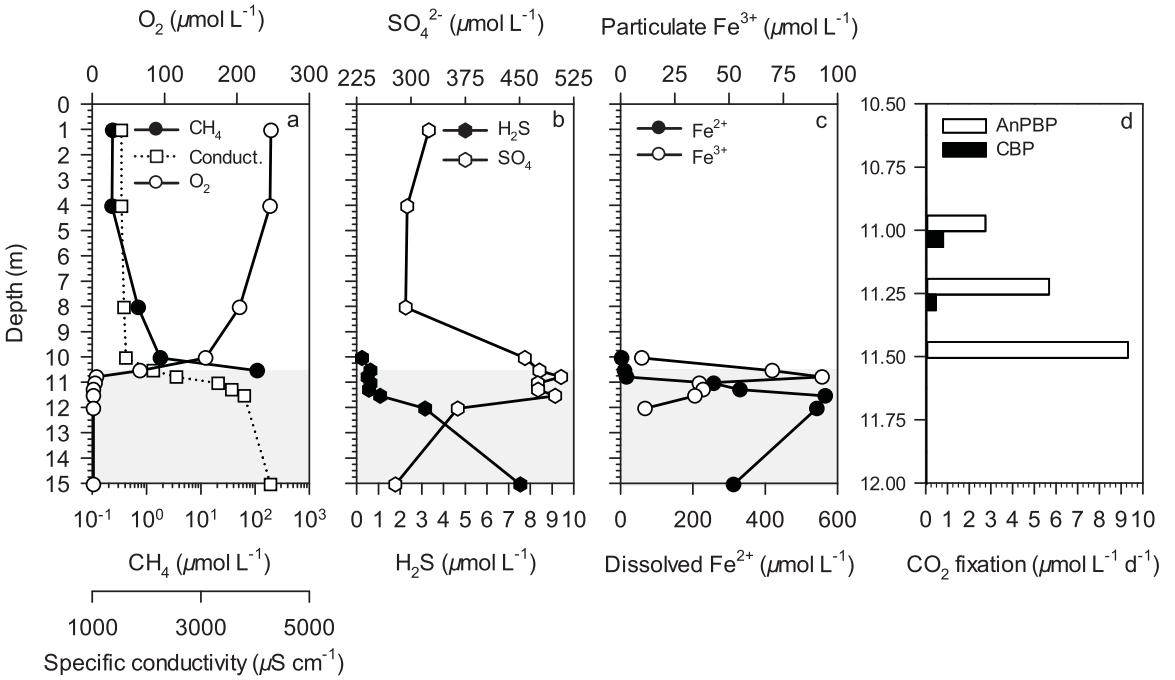

Fig. 3. Vertical depth profiles of (**a**) conductivity ($\mu S\ cm^{-1}$) and concentration of dissolved O_2 and CH_4 ($\mu mol\ L^{-1}$), (**b**) of the concentration of H_2S and SO_4^{2-} ($\mu mol\ L^{-1}$), (**c**) of the concentration of Fe^{2+} and Fe^{3+} ($\mu mol\ L^{-1}$), (**d**) of the daily chemoautotrophic bacterial production rates (black bars, $\mu mol\ L^{-1}\ d^{-1}$) and anoxygenic photosynthetic bacteria production rates (green bars, $\mu mol\ L^{-1}\ d^{-1}$). Note the different depth scale in (**c**). Grey zone in (**a**), (**b**), and (**c**) corresponds to the anoxic waters.

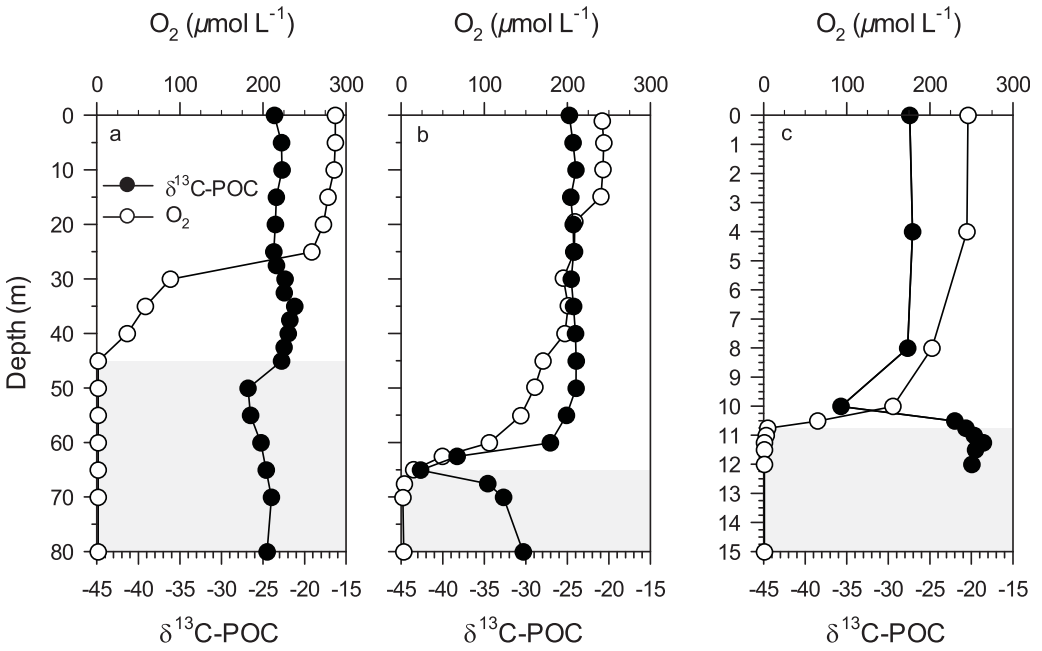

Fig. 4. Vertical depth profile of the δ^{13}C-POC in the main basin during (**a**) the rainy and (**b**) the dry season, and (**c**) in Kabuno Bay. Grey zone corresponds to the anoxic waters.

The stable isotope composition of the POC in the main basin was almost constant from surface waters to the oxic-anoxic transition but showed an excursion toward more negative values in the oxycline, with a minimum δ^{13}C of $-26.9‰$ measured at 50 m during the rainy season (Fig. 4a), and a more abrupt decrease (down to $-42.8‰$) at 65 m depth during the dry season

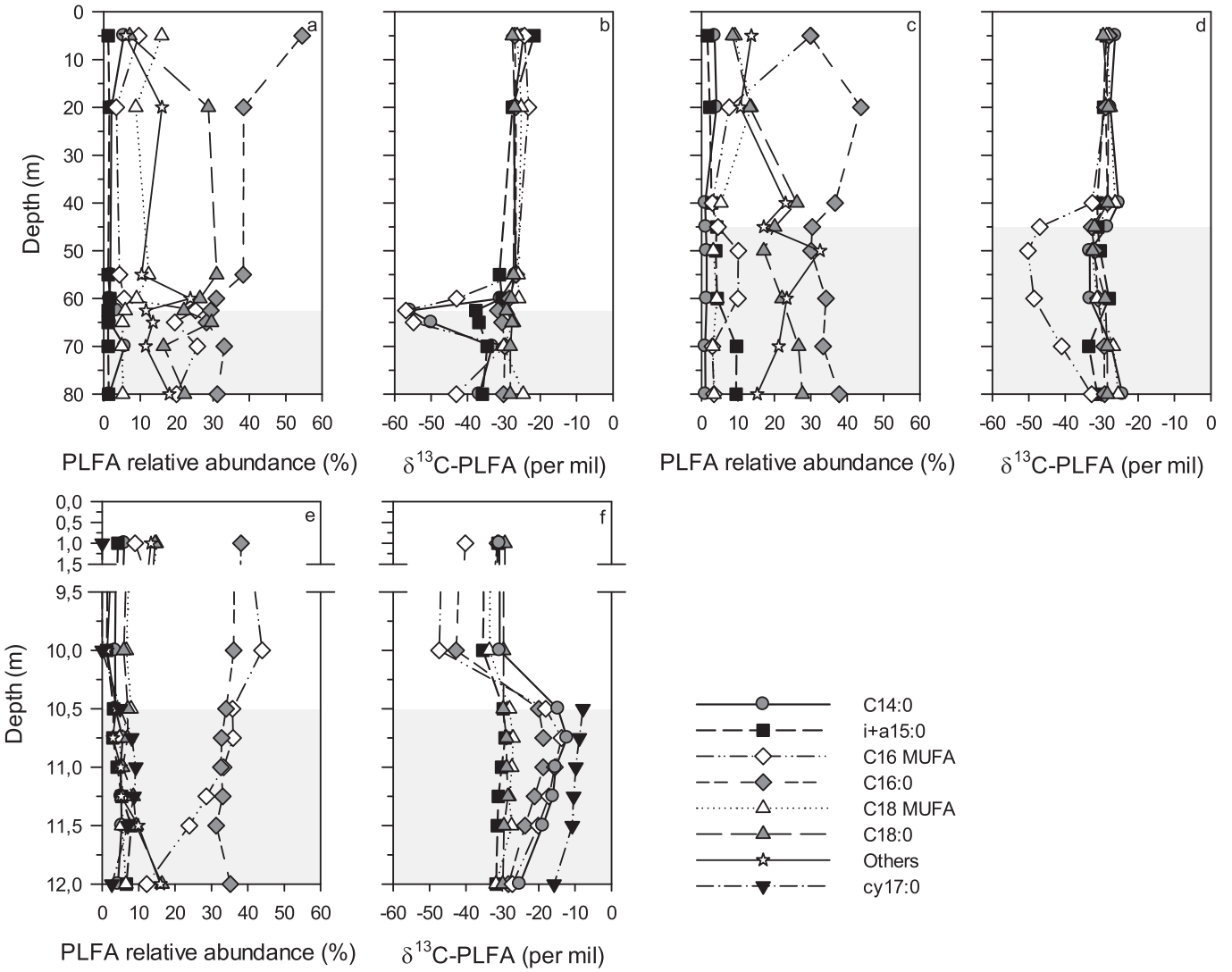

Fig. 5. Vertical variability of the relative abundance (%) and the stable isotope composition of the most common PLFA (δ^{13}C-PLFA) identified in the water column of Lake Kivu during dry (**a**,**b**) and rainy (**c**,**d**) seasons and Kabuno Bay (**e**,**f**). Grey zone corresponds to the anoxic waters.

(Fig. 4b). Deeper in the water column, the δ^{13}C-POC values slightly increased at the bottom of the mixolimnion (−24.7‰ and −30.2‰ at 80 m during the rainy and the dry season, respectively) but remained lower than in the mixed layer for both sampled periods (−22.9 ± 0.8‰ and −24.4 ± 0.3‰ during the rainy and the dry season, respectively) (Fig. 4a,b). In the shallower Kabuno Bay, δ^{13}C-POC values were constant (on average, −27.1 ± 0.3‰, $n = 3$) from the surface to the oxycline, where a sharp minimum (−35.8‰ at 10 m) was measured (Fig. 4c). However δ^{13}C-POC abruptly increased (on average, −20.1‰ ± 1.3, $n = 5$) below the oxycline (10.75–12.00 m), being even higher than in surface waters (Fig. 4c).

Depth profile of phospholipid fatty acid concentrations and their stable isotope composition

Figure 5 illustrates the vertical variation of the relative abundance and the stable isotope composition of the most common PLFA identified in the water column of Lake Kivu and Kabuno Bay. The various C16 and C18 monounsaturated fatty acids were grouped into C16 MUFA and C18 MUFA, respectively. Together, the C14:0, i+a15:0, C16 MUFA, C16:0, C18 MUFA, and C18:0 always accounted for more than 67.4% of the total PLFA pool in the water column of the main basin of Lake Kivu and Kabuno Bay (Fig. 5a,c,e). Depth profiles revealed that the relative abundance of the C16 MUFA increased in the oxycline during both the dry season and the rainy season (Fig. 5a,c). The δ^{13}C signature of C16 MUFA in oxic waters was comparable to the δ^{13}C signature of C16:0, oscillating around −27‰ or −29‰ during the rainy and the dry season, respectively. However, C16 MUFA were strongly depleted in ^{13}C in the oxycline, with minimal δ^{13}C values recorded in the oxycline as low as −55.3‰ during the dry season, and −49.5‰ during the rainy season (Fig. 5b,d). C14:0 and C18 MUFA were relatively more abundant in oxic waters

(3.4% ± 1.5% and 11.5% ± 3.0%, respectively) than in the oxycline or below (1.3% ± 0.2% and 4.1% ± 0.9%). Their isotopic composition varied with depth following the same vertical pattern as for C16 MUFA, but with a lower amplitude (Fig. 5b,d). The relative abundance of iso- and anteiso-branched 15:0 PLFA was constantly low (1–5%) and did not follow any depth pattern, their $\delta^{13}C$ signature was however slightly lower below the oxic zone.

In the shallower Kabuno Bay (Fig. 5e,f), C16:0 and C16 MUFA were the dominant PLFA in the water column, accounting together for 47–80% of the total PLFA pool. Their $\delta^{13}C$ signature varied widely with depth, following the same trend as for $\delta^{13}C$-POC. They were relatively depleted in ^{13}C in the mixed layer (−31.3‰ and −40.2‰ for the C16:0 and C16 MUFA, respectively) and even more so in the oxycline (−42.7‰ and −47.4‰ for the C16:0 and C16 MUFA, respectively). However, in contrast with the results gathered in the main basin, the C16:0 and C16 MUFA were considerably enriched in ^{13}C in anoxic waters ($\delta^{13}C$ of −20.5 ± 2.1‰ or −17.0 ± 2.4‰ for C16:0 and C16 MUFA, respectively). This strong natural isotopic enrichment in anoxic waters was also measured for C14:0 and C17 cyclopropane ring PLFA (cy17:0), the latter being detected only in the redoxcline of Kabuno Bay (relative abundance: 7.6% ± 1.7%) (Fig. 5e,f). Together, C14:0, C16:0, C16:1 and cy17:0 accounted for 77% of the total PLFA pool in anoxic waters in Kabuno bay (10.50–11.50 m interval).

Hierarchical clustering analyses of normalized (i.e., log-transformed) relative concentration of PLFA (%) as parameters allowed the grouping of bacterial communities from different depths by their similarities in PLFA composition. Dendrograms (Supporting Information Fig. S1a,b) show that the PLFA composition of the bacterial community was structured following a vertical pattern in the main basin, differing largely between the oxic waters and the rest of the water column. Furthermore, the bacterial community in the upper 10.0 m of Kabuno Bay water column appeared to be very different from that of the anoxic waters (10.5–11.5 m; Supporting Information Fig. S1c).

Chemoautotrophic and anoxygenic phototrophic bacterial production

Significant CBP rates were measured at the oxic-anoxic transition and in anoxic waters. In the main basin, the highest rates were always observed at the bottom of the mixolimnion (70 m) during the dry and rainy seasons (1.43 μmol C L^{-1} d^{-1} for both seasons), in the gradient of H_2S/SO_4^{2-} (Figs. 1d, 2d). During the rainy season, an additional maximum in CBP rates (0.9 μmol C L^{-1} d^{-1} at 45–50 m) was observed above the sulfidic zone, in a nitrogenous zone characterized by the accumulation of NO_3^-. Significant anPBP rates were not measured in the water column of the main basin. In contrast, anPBP rates were relatively high in the sharp redoxcline of Kabuno bay, ranging between 2.7 μmol C L^{-1} d^{-1} and 9.3 μmol C L^{-1} d^{-1} while CBP rates never exceeded 0.8 μmol C L^{-1} d^{-1} (Fig. 3d).

Integrated over the upper water column (0–80 m) of the main basin of Lake Kivu, the CBP rate was estimated at 19.0 mmol C m^{-2} d^{-1} and 13.9 mmol C m^{-2} d^{-1} during the rainy and the dry season, respectively. In Kabuno Bay, integrated anPBP (2.9 mmol C m^{-2} d^{-1}) was largely higher than CBP (0.2 mmol C m^{-2} d^{-1}). It should be noted that these areal rates might be underestimations due to the limited depth range sampled (i.e., 11.00–11.50 m) in the redoxcline of Kabuno Bay, and we cannot rule out the possibility that appreciable CBP and anPBP took place in deeper waters.

Stable isotope labelling of PLFA

The PLFA labelling experiment showed that the CO_2 fixed in the dark by the chemoautotrophs in the main basin was incorporated almost exclusively in C16 MUFA and C16:0. Indeed, between 43% and 98% of the total amount of CO_2 fixed into PLFA in anoxic waters (50–70 m) was incorporated into C16 MUFA and C16:0 during the rainy season, and between 53% and 88% (62.5–70.0 m) during the dry season (data not shown). A similar incorporation pattern was found below the oxycline in Kabuno Bay, where 76% ± 5% of the total amount of CO_2 fixed in the dark into PLFA was also incorporated into C16 MUFA and C16:0 (data not shown).

In the main basin, at the depths where significant CBP rates were recorded, the highest specific CO_2 incorporation rate in the dark (i.e., incorporation rates expressed in ng C L^{-1} d^{-1} normalized to PLFA concentration expressed in ng C L^{-1}) were always observed for C16 MUFA during the rainy (0.037 d^{-1}) and the dry (0.051 d^{-1}) season (Fig. 6). In contrast, during incubations in Kabuno Bay under in situ light conditions, CO_2 was predominantly fixed into C14:0, C16:0, C16 MUFA and C18 MUFA (Fig. 7).

Discussion

Our study provides evidences for the existence of a biogeochemically active and dynamic chemoautotrophic bacterial community in the redoxcline of Lake Kivu. Additionally, PLFA analyses shows that the bacterial community composition was structured vertically in the water column, with a large dissimilarity between oxic and anoxic waters, in accordance with previous reports in Lake Kivu based on pyrosequencing (İnceoğlu et al. 2015a,b). The strong isotopic depletion of the POC pool and C16 MUFA in the oxycline, where the CH_4 concentration decreased sharply, indicates that a substantial part of CH_4 was consumed and incorporated into the biomass. Indeed, measurements of the CH_4 stable isotopic composition and of the methanotrophic bacterial production rates (MBP) carried out with the same set of samples revealed that type I methanotrophs oxidized most of the upward flux of CH_4, and vertically integrated MBP values (8.2–29.5 mmol C m^{-2} d^{-1}) were equivalent to

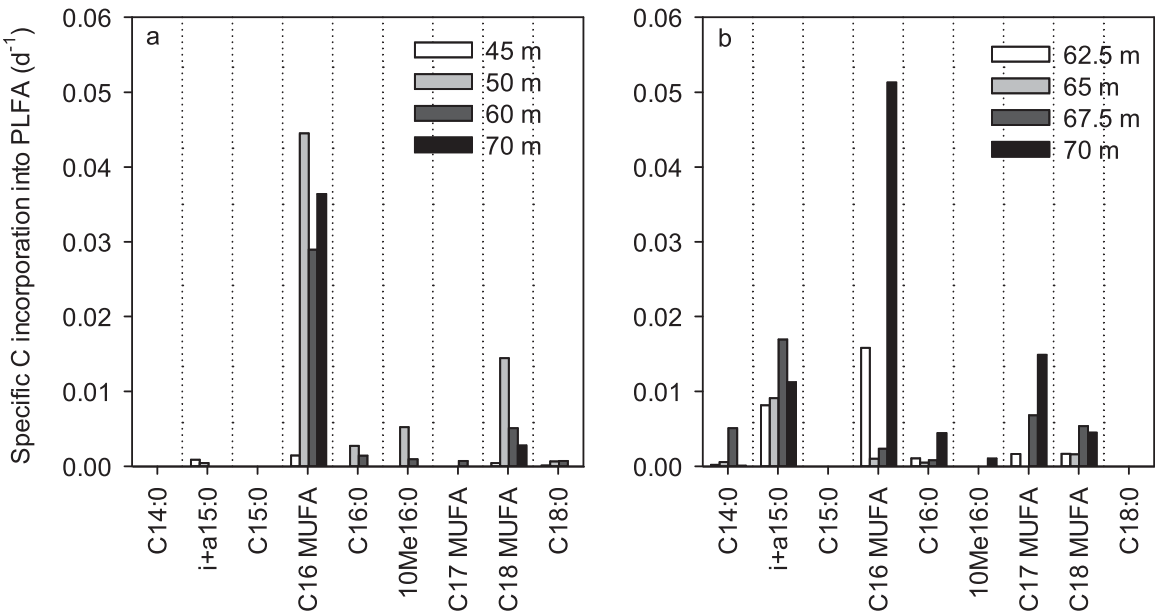

Fig. 6. Specific C incorporation patterns into PLFA (incorporation rates normalized to PLFA concentration) during (**a**) rainy season (February 2012) and (**b**) dry season (September 2012) in the main basin of Lake Kivu.

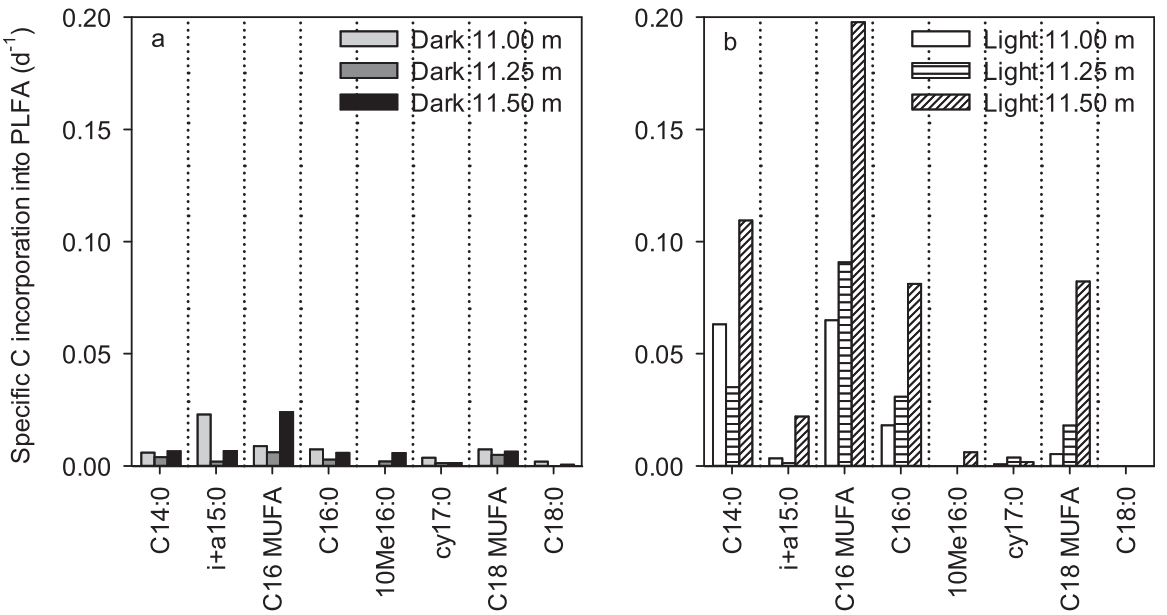

Fig. 7. Specific C incorporation pattern into PLFA in Kabuno Bay, (**a**) and (**b**) correspond to chemoautotrophic bacterial production and anoxygenic phototrophic bacterial production, respectively.

16–60% of the average phytoplankton particulate primary production (Morana et al. 2015).

During our study, the maximum volumetric CBP rates were always observed in H_2S-rich waters, i.e., below the oxic-anoxic transition zone where the methanotrophic bacterial community was highly active as reported elsewhere (Morana et al. 2015). Maximum CBP rates measured in Lake Kivu were within the range of those reported from H_2S-rich marine redoxclines, such as the Black Sea (Grote et al. 2008), the Baltic Sea (Jost et al. 2008), and the Cariaco Basin (Taylor et al 2001). Also in these marine systems, the maximal chemoautotrophic activities were observed in sulfidic waters, well below the oxic-anoxic transition zone. In Lake Kivu, oxygen was below the detection limit (< 3 μmol L^{-1}) at

most of the depths where significant chemoautotrophic production rates were measured, raising the question of which electron acceptors are used by chemoautotrophic organisms in the lower zone of the redoxcline. Recent results (Darchambeau et al. 2014) indicate that the density gradient of the mixed layer is usually weak in Lake Kivu, and the stratification of the upper water column is rather unstable. Episodical intrusion of dissolved O_2 in the deeper part of the redoxcline could therefore partly fuel aerobic NH_4^+ or H_2S oxidation. However, in the absence of O_2, it is widely assumed that prokaryotes use the thermodynamically most favourable electron acceptors available in waters (Enrich-Prast et al. 2009). NO_3^- is commonly the next most energetically favourable electron acceptor in many aquatic environments and it can be used in the anaerobic oxidation of H_2S by chemoautotrophic bacteria such as many members of the Epsilonproteobacteria group (*Sulfurimonas*, *Sulfuricurvum*), among others. In Lake Kivu, Epsilonproteobacteria were indeed observed in high abundances at the bottom of the redoxcline (İnceoğlu et al. 2015a,b). Furthermore, the PLFA labelling experiment revealed that CO_2 was almost exclusively incorporated in the dark into C16 MUFA at the depths where significant CBP rates were measured. It has actually been already suggested that MUFA are common in gram-negative bacteria (Glaubitz et al. 2009) and particularly abundant in sulphur oxidizing bacteria (Li et al. 2007; Glaubitz et al. 2009). The biogeochemical importance of chemoautotrophic Epsilonproteobacteria has been also highlighted by microautoradiography in the redoxcline of karstic lakes (Noguerola et al. 2015) and marine basins, such as the Black Sea and the Baltic Sea (Grote et al. 2008), where they have been found to contribute most to the chemoautotrophic production. Also, in the meromictic Lake Lugano, chemoautotrophic denitrification carried out by proteobacteria members has been identified as the dominant fixed N elimination process in the redoxcline (Wenk et al. 2013). We can not totally exclude the possibility that the presence of a trace-level amount of O_2 at the start of the incubation led to an overestimate of the CBP rates due the relatively high limit of detection the O_2 sensor (3 μmol L^{-1}), it nevertheless seems improbable that such a small concentration of O_2 could have sustained the relatively large CBP rates measured at the bottom the redoxcline. For instance, assuming that 2 moles of O_2 are necessary to oxidize 1 mole of HS^- ($HS^- + 2\ O_2 \rightarrow SO_4^{2-} + H^+$) and using the maximum growth efficiency for sulphide oxiders estimated experimentally found in literature (0.42 mole of CO_2 fixed in the biomass per mole of HS^- used for energy; Kelly 1990), a trace level amount of O_2 in the incubations bottles (< 3 μmol L^{-1}) could not have sustained CBP rates higher than 0.63 μmol C L^{-1} d^{-1}. This theoretical aerobic CBP rate is certainly an upper limit but is still ~ 2 times lower than the CBP rates measured at the bottom of the redoxcline of Lake Kivu, strongly suggesting that other electron acceptors than O_2 fueled the chemoautotrophic processes at the bottom of the redoxcline.

Considering theoretical stoichiometries of nitrification and sulphide oxidation, the vertical diffusive and advective fluxes of the main inorganic electron donors (NH_4^+, 1.95 mmol m^{-2} d^{-1}; H_2S, 0.61 mmol m^{-2} d^{-1}) estimated in the main basin of Lake Kivu by Pasche et al. (2009) were largely insufficient to fuel the CBP rates measured during this study (19.0 mmol C m^{-2} d^{-1} and 13.9 mmol C m^{-2} d^{-1} during the rainy and the dry season, respectively). The discrepancy between CBP rates and vertical electron donors fluxes could imply that an intensive, yet cryptic, recycling of S- and N-redox species in the redoxcline must play an important role in Lake Kivu to sustain the chemoautotrophic demand, as suggested for the Black Sea (Murray et al. 1995) and the Cariaco basin (Li et al. 2012). Indeed, despite their biogeochemical significance in the water column, these processes would not have any clear in situ chemical expression because of the tight coupling between production and consumption of the chemical species used by the chemoautotrophs.

With the exception of the Kabuno Bay basin, characterized by a more shallow and well-illuminated redoxcline, measurable rates of anoxygenic photosynthesis were never detected in Lake Kivu during this study. This might be related to the very low light availability at the oxic-anoxic transition zone in Lake Kivu and competition between different microbial communities for available resources. Indeed, considering a light attenuation coefficient of 0.26 m^{-1} (Darchambeau et al. 2014), the estimated light intensity at the oxic-anoxic transition zone was estimated at 6.66×10^{-3} μmol photon m^{-2} s^{-1} for the rainy season (in February 2012) and 3×10^{-5} μmol photon m^{-2} s^{-1} for the dry season (in September 2012), well below the values reported in the chemocline of the Black Sea ($\sim$ 0.18 μmol photon m^{-2} s^{-1}), Lake Matano ($\sim$ 0.12 μmol photon m^{-2} s^{-1}), and Kabuno Bay (0.6 μmol photon m^{-2} s^{-1}), where low-light adapted *Chlorobium* species were identified (Marschall et al. 2010; Crowe et al. 2014; Llirós et al. 2015).

Kabuno Bay was characterized by AnPBP rates an order of magnitude higher than CBP rates. The strong ^{13}C-labelling during the tracer experiments of the C14:0, C16:0, and C16 MUFA, which were also naturally enriched in ^{13}C under in situ conditions, indicates that the bacteria responsible for the anoxygenic photosynthetic CO_2 fixation were members of the *Chlorobium* genus. Indeed, these fatty acids are usually highly abundant in *Chlorobium* (Imhoff 2003), and they are known to use the reverse tricarboxylic cycle (rTCA) pathway to fix C into the cellular biomass. This alternative C fixation pathway is less discriminating against ^{13}C than other photosynthetic pathway, such as the Calvin cycle (Sirevåg et al. 1977). Considering the isotope fractionation factor for C fixation by *Chlorobium* via the rTCA pathway ($-12.2‰$, Sirevåg et al. 1977) and the measured $\delta^{13}C$-DIC values below the oxycline in Kabuno Bay during this study ($-5.4 \pm 0.3‰$,

$n = 6$), we estimated that the theoretical isotopic signature of the biomass fixed by *Chlorobium* should approximate −17‰ in Kabuno Bay. Therefore, using a simple isotope mixing model with the δ^{13}C-POC in surface as a sedimenting organic matter end-member and the δ^{13}C signature of *Chlorobium* as a second end-member, it could be estimated that 74% ± 13% of the POC pool below the oxycline originated from anoxygenic photosynthesis, with a maximum at 11.25 m (89%). Furthermore, when integrated from the surface to the bottom of the redoxcline (12.00 m), the data revealed that 23% of the POC pool of the water column was derived from anoxygenic CO_2 fixation by *Chlorobium*. Altogether, these results gathered from stable isotope analysis stress the important role played by *Chlorobium* in the C cycle of Kabuno Bay, as recently evidenced by molecular and culturing approaches (Llirós et al. 2015).

A relatively high abundance of cy17:0 (reaching 10%) was found below the oxycline of Kabuno Bay, whereas this PLFA was undetectable in the main basin of Lake Kivu. Furthermore, this PLFA was naturally enriched in ^{13}C in the water column (−9.5 ± 1.2‰, $n = 5$), reflecting its almost exclusive presence in *Chlorobium* cellular membranes; however, it was never labelled during the ^{13}C-tracer experiment (Fig. 7). It has been shown that cy17:0 is directly synthesized from C16 MUFA by modification of the *cis* double bond, but cyclopropane ring formation in bacterial membranes has a high energetic cost. The cyclopropane bond is more stable than a double bond and plays a crucial role in protection against thermal, acidic, oxidative, or salt stress (Grogan and Cronan 1997; Zhang and Rock 2008). In anoxygenic phototrophs, the cyclopropane ring has notably been found to reinforce the resistance of the light-harvesting chlorosome to different types of environmental stress (Mizoguchi et al. 2013) and its production typically occurs when bacterial cells encounter starvation or others forms of growth stasis (Grogan and Cronan 1997). Overall, it is generally assumed that the main physiological function of cyclopropane fatty acids is to improve the viability of slow-growing or quiescent cells (Zhang and Rock 2008). However, once formed, cyclopropane fatty acids appear to be extremely stable molecules, so that they remain in the cell membrane even after return to more favourable environmental conditions (Grogan and Cronan 1997). The absence of isotopic labelling of the cy17:0 during the 24 h incubation carried out during this study is consistent with this idea. Therefore, the relative increase of the proportion of cy17:0 relative to its precursor (C16 MUFA) found below the oxycline could indicate that a non-negligible fraction of the anoxygenic phototroph community had been subjected to environmental stress during their life span. For instance, we might consider that light attenuation induced by the physico-chemical structure of the water column, scattering by organic and inorganic particles, and self-shading, would affect the growth of the anoxygenic phototrophs present at the bottom of the redoxcline, as demonstrated in the ferruginous Lake Matano (Crowe et al. 2014).

Table 1. Comparison of upward electron donor (e_d; H_2S, Fe^{2+}) fluxes in Kabuno Bay and estimation of the percentage of anoxygenic phototrophic bacterial production (anPBP) that could be potentially sustained by these fluxes.

	H_2S		Fe^{2+}
Upward flux (mmol m^{-2} d^{-1})	0.02–0.14		5–37
Oxidation product	S^0	SO_4^{2-}	Fe^{3+}
Reaction stoichiometry (e_d:CO_2)	2	0.5	4
Areal anPBP (mmol m^{-2} d^{-1})	2.9	2.9	2.9
% of anPBP sustained by upward flux	0–2	1–10	43–318

Anoxygenic phototrophic bacteria are known to use H_2S as an electron donor and usually occur in sulfidic environments. However a strain of the *Chlorobium* genus (i.e., *Chlorobium ferrooxidans*) has been found to be able to oxidize Fe^{2+} in iron-rich environments (Heising et al. 1999; Llirós et al. 2015). In the water column of Kabuno Bay, the vertical upward flux of electron donors was estimated as follows:

$$F_{\text{total}} = -D_{\text{turbulent}} \times (\Delta C/\Delta z) + C \times \text{Adv} \quad (3)$$

where $D_{\text{turbulent}}$ is the turbulent diffusion coefficient (m^2 s^{-1}), $\Delta C/\Delta z$ is the vertical concentration gradient (mol m^{-4}), Adv is the vertical upwelling velocity (m s^{-1}) and C is the concentration at a given depth (mol m^{-3}). Using coefficients for turbulent diffusivity (1.4×10^{-7} – 1.0×10^{-6} m^2 s^{-1}) and vertical advection (6.3×10^{-9} – 6.3×10^{-8} m s^{-1}) estimated for Kabuno Bay (Martin Schmid pers. comm.) and the measured concentration gradient of Fe^{2+} (422 mmol m^{-4}) and H_2S (1.6 mmol m^{-4}), the upward flux of Fe^{2+} and H_2S through the redoxcline would approximate 5–37 mmol m^{-2} d^{-1}, and 0.02–0.14 mmol m^{-2} d^{-1}, respectively. It appears from these calculations that the upward H_2S inputs is at least one order of magnitude too low to support the high AnPBP rates (2.9 mmol C m^{-2} d^{-1}) measured during this study (11.0–11.5 m), but the upward Fe^{2+} flux would be sufficient to fuel the totality of the AnPBP (Table 1). Therefore, the relatively low supply of H_2S compared with Fe^{2+} implies that the anoxygenic phototrophic community in Kabuno largely relies on Fe^{2+} as an electron donor. These results are complementary to those found by molecular proxies by Llirós and co-workers, revealing the presence of a pelagic Green-Sulfur Bacteria species higly related to *Chlorobium ferrooxidans*, the unique known photoferrotrophic *Chlorobi* (Llirós et al. 2015).

In summary, chemoautotrophy in the pelagic redoxcline of Lake Kivu is significant, and may affect the ecological functioning of Lake Kivu in several ways. First, chemoautotrophs may represent alternative sources of autochtonous organic matter for higher trophic levels, besides oxygenic

photosynthesis carried out by phytoplankton in the surface waters. Second, they could exert an indirect control on phytoplankton production by limiting the amount of inorganic nutrients that reach the illuminated surface waters through diffusion from bottom waters. This shortcircuiting of the vertical nutrient transport seems to be specially important in the large East African Rift lakes where internal nutrient loading via upward fluxes is of major importance for phytoplankton growth (Kilham and Kilham 1990; Pasche et al. 2009). For instance, if chemoautotrophic uptake of dissolved inorganic phosphorus (DIP) follows Redfield stoechiometry (C : P = 106 : 1), chemoautotrophic DIP uptake in the redoxcline of the main basin would have approximated 0.18 mmol P m^{-2} d^{-1} and 0.13 mmol P m^{-2} d^{-1} in February and September 2012, respectively. This DIP uptake flux is higher than the upward DIP flux of 0.08 mmol m^{-2} d^{-1} estimated by Pasche et al. (2009) highlighting the importance of the control that chemoautotrophs might exert on nutrient availability in the mixed layer, but it also suggests that a substantial amount of inorganic nutrients has to be actively recycled within the redoxcline to sustain the chemoautotrophic demand.

References

Borges, A. V., G. Abril, B. Delille, J.-P. Descy, and F. Darchambeau. 2011. Diffusive methane emissions to the atmosphere from Lake Kivu (Eastern Africa). J. Geophys. Res. **116**: G03032. doi:10.1029/2011JG001673

Boschker, H. T. S. 2004. Linking microbial community structure and functioning: stable istope (13C) labeling in combination with PLFA analysis, p. 1673–1688. In G. A. Kowalchuk, F. J. de Bruijn, I. M. Head, A. D. L. Akkermans, & J. D. van Elsas (Eds.), Molecular Microbial Ecology Manual, 2nd edition. Kluwer Academic Publishers.

Canfield, D. E., M. T. Rosing, and C. Bjerrum. 2006. Early anaerobic metabolisms. Philos. Trans. R. Soc. B Biol. Sci. **361**: 1819–1836. doi:10.1098/rstb.2006.1906

Cline, J. D. 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol. Oceanogr. **14**: 454–458. doi:10.4319/lo.1969.14.3.0454

Cole, J. J., and others. 2007. Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. Ecosystems **10**: 171–184. doi:10.1007/s10021-006-9013-8

Crowe, S. A., and others. 2008. Photoferrotrophs thrive in an Archean Ocean analogue. Proc. Natl. Acad. Sci. **105**: 15938–15943. doi:10.1073/pnas.0805313105

Crowe, S. A., and others. 2014. Deep-water anoxygenic photosythesis in a ferruginous chemocline. Geobiology **12**: 322–339. doi:10.1111/gbi.12089

Darchambeau, F., H. Sarmento, and J.-P. Descy. 2014. Primary production in a tropical large lake: The role of phytoplankton composition. Sci. Total Environ. **473**: 178–188. doi:10.1016/j.scitotenv.2013.12.036

Enrich-Prast, A., D. Bastviken, and P. M. Crill. 2009. Chemosynthesis, p. 221–225. *In* G. Likens [ed.], Encyclopedia of inland waters. Springer.

Farías, L., C. Fernández, J. Faúndez, M. Cornejo, and M. E. Alcaman. 2009. Chemolithoautotrophic production mediating the cycling of the greenhouse gases N2O and CH4 in an upwelling ecosystem. Biogeosciences **6**: 3053–3069. doi:10.5194/bg-6-3053-2009

García-Cantizano, J., E. O. Casamayor, J. M. Gasol, R. Guerrero, and C. Pedrós-Alió. 2005. Partitioning of CO2 incorporation among planktonic microbial guilds and estimation of in situ specific growth rates. Microb. Ecol. **50**: 230–241. doi:10.1007/s00248-004-0144-9

Gillikin, D. P., and S. Bouillon. 2007. Determination of $\delta^{18}O$ of water and $\delta^{13}C$ of dissolved inorganic carbon using a simple modification of an elemental analyser-isotope ratio mass spectrometer: An evaluation. Rapid Commun. Mass Spectrom. **21**: 1475–1478. doi:10.1002/rcm.2968

Glaubitz, S., T. Lueders, W. R. Abraham, G. Jost, K. Jürgens, and M. Labrenz. 2009. ^{13}C-isotope analyses reveal that chemolithoautotrophic Gamma-and Epsilonproteobacteria feed a microbial food web in a pelagic redoxcline of the central Baltic Sea. Environ. Microbiol. **11**: 326–337. doi:10.1111/j.1462-2920.2008.01770.x

Grogan, D. W., and J. E. Cronan. 1997. Cyclopropane ring formation in membrane lipids of bacteria. Microbiol. Mol. Biol. Rev. **61**: 429–441.

Grote, J., G. Jost, M. Labrenz, G. J. Herndl, and K. Jürgens. 2008. Epsilonproteobacteria represent the major portion of chemoautotrophic bacteria in sulfidic waters of pelagic redoxclines of the Baltic and Black Seas. Appl. Environ. Microbiol. **74**: 7546–7551. doi:10.1128/AEM.01186-08

Haberyan, K. A., and R. E. Hecky. 1987. The late Pleistocene and Holocene stratigraphy and paleolimnology of Lakes Kivu and Tanganyika. Palaeogeogr. Palaeoclimatol. Palaeoecol. **61**: 169–197. doi:10.1016/0031-0182(87)90048-4

Hadas, O., R. Pinkas, and J. Erez. 2001. High chemoautotrophic primary production in Lake Kinneret, Israel: A neglected link in the carbon cycle of the lake. Limnol. Oceanogr. **46**: 1968–1976. doi:10.4319/lo.2001.46.8.1968

Heising, S., L. Richter, W. Ludwig, and B. Schink. 1999. Chlorobium ferrooxidans sp. nov., a phototrophic green sulfur bacterium that oxidizes ferrous iron in coculture with a "Geospirillum" sp. strain. Arch. Microbiol. **172**: 116–124. doi:10.1007/s002030050748

Imhoff, J. F. 2003. Phylogenetic taxonomy of the family Chlorobiaceae on the basis of 16S rRNA and fmo (Fenna–Matthews–Olson protein) gene sequences. Int. J. Syst. Evol. Microbiol. **53**: 941–951. doi:10.1099/ijs.0.02403-0

İnceoğlu, O., and others. 2015a. Distribution of bacteria and archea in meromictic tropical Lake Kivu. Aquat. Microb. Ecol. **74**: 215–233. doi:10.3354/ame01737

İnceoğlu, O., and others. 2015b. Vertical distribution of functional potential and active microbial communities in

meromictic Lake Kivu. Microb. Ecol. **70**: 596–611. doi:10.1007/s00248-015-0612-9

Jørgensen, B. B., J. G. Kuenen, and Y. Cohen. 1979. Microbial transformations of sulfur compounds in a stratified lake (Solar Lake, Sinai). Limnol. Oceanogr. **24**: 799–822. doi:10.4319/lo.1979.24.5.0799

Jørgensen, B. B., H. Fossing, C. O. Wirsen, and H. W. Jannasch. 1991. Sulfide oxidation in the anoxic Black Sea chemocline. Deep-Sea Res. Part A Oceanogr. Res. Pap. **38**: S1083–S1103. doi:10.1016/S0198-0149(10)80025-1

Jost, G., M. V. Zubkov, E. Yakushev, M. Labrenz, and K. Jurgens. 2008. High abundance and dark CO_2 fixation of chemolithoautotrophic prokaryotes in anoxic waters of the Baltic Sea. Limnol. Oceanogr. **53**: 14–22. doi:10.4319/lo.2008.53.1.0014

Kelly, D. P. 1990. Energetics of chemolithotrophs, p. 479–503. *In* T. A. Krulwich [ed.], Bacterial energetics. Academic Press.

Kilham, P., and S. S. Kilham. 1990. Endless summer: Internal loading processes dominate nutrient cycling in tropical lakes. Freshw. Biol. **23**: 379–389. doi:10.1111/j.1365-2427.1990.tb00280.x

Le Bodelier, P., M.-J. Bär Gillisen, K. Hordijk, J. S. Sinninghe Damsté, I. C. Rijpstra, J. A. J. Geenevasen, and P. F. Dunfield. 2009. A reanalysis of phospholipid fatty acids as ecological biomarkers for methanotrophic bacteria. ISME J. **3**: 606–617. doi:10.1038/ismej.2009.6

Li, Y. L., A. D. Peacock, D. C. White, R. Geyer, and C. L. Zhang. 2007. Spatial patterns of bacterial signature biomarkers in marine sediments of the Gulf of Mexico. Chem. Geol. **238**: 168–179. doi:10.1016/j.chemgeo.2006.11.007

Li, X. N., G. T. Taylor, Y. Astor, R. Varela, and M. I. Scranton. 2012. The conundrum between chemoautotrophic production and reductant and oxidant supply: A case study from the Cariaco Basin. Deep-Sea Res. Part I Oceanogr. Res. Pap. **61**: 1–10. doi:10.1016/j.dsr.2011.11.001

Llirós, M., and others. 2010. Vertical distribution of ammonia-oxidizing crenarchaeota and methanogens in the epipelagic waters of Lake Kivu (Rwanda – Democratic Republic of the Congo). Appl. Environ. Microbiol. **76**: 6853–6863. doi:10.1128/AEM.02864-09

Llirós, M., and others. 2015. Pelagic photoferrotrophy and iron cycling in a modern ferruginous basin. Scientific Reports, **5**, 13803. doi:10.1038/srep13803

Marschall, E., M. Jogler, U. Henssge, and J. Overmann. 2010. Large-scale distribution and activity patterns of an extremely low-light adapted population of green sulphur bacteria in the Black Sea. Environ. Microbiol. **12**: 1348–1362. doi:10.1111/j.1462-2920.2010.02178.x

Middelburg, J. J. 2011. Chemoautotrophy in the ocean. Geophys. Res. Lett. **38**. doi:10.1029/2011GL049725

Mizoguchi, T., Y. Tsukatani, J. Harada, S. Takasaki, T. Yoshitomi, and H. Tamiaki. 2013. Cyclopropane-ring formation in the acyl groups of chlorosome glycolipids is crucial for acid resistance of green bacterial antenna systems. Bioorg. Med. Chem. **21**: 3689–3694. doi:10.1016/j.bmc.2013.04.030

Morana, C., A. V. Borges, F. A. E. Roland, F. Darchambeau, J.-P. Descy, and S. Bouillon. 2015. Methanotrophy within the water column of a large meromictic tropical lake (Lake Kivu, East Africa). Biogeosciences **12**: 2077–2088. doi:10.5194/bg-12-2077-2015

Murray, J. W., L. A. Codispoti, and G. E. Friederich. 1995. Oxidation-reduction environments: The suboxic zone in the Black Sea, p. 157–176. *In* C. P. Huang, C. R. O'Melia and J. J. Morgan [eds.], Aquatic chemistry: Interfacial and interspecies processes. American Chemical Society: Advances in Chemistry Series, v. 244.

Noguerola, I., A. Picazo, M. Llirós, A. Camacho, and C. M. Borrego. 2015. Diversity of freshwater Epsilonproteobacteria and dark inorganic carbon fixation in the sulphidic redoxcline of a meromictic karstic lake. FEMS Microbiol. Ecol. **91**: fiv086. doi: 10.1093/femsec/fiv086

Overmann, J., H. Cypionka, and N. Pfennig. 1992. An extremely low-light-adapted phototrophic sulfur bacterium from the Black Sea. Limnol. Oceanogr. **37**: 150–155. doi:10.4319/lo.1992.37.1.0150

Overmann, J., T. Beatty, and K. J. Hall. 1996. Purple sulfur bacterial control the growth of aerobic heterotrophic bacterioplankton in a meromictic salt lake. Appl. Environ. Microbiol. **62**: 3251–3258.

Pasche, N., C. Dinkel, B. Müller, M. Schmid, A. Wüest, and B. Wehrli. 2009. Physical and bio-geochemical limits to internal nutrient loading of meromictic Lake Kivu. Limnol. Oceanogr. **54**: 1863–1873. doi:10.4319/lo.2009.54.6.1863

Schmid, M., M. Halbwachs, B. Wehrli, and A. Wüest. 2005. Weak mixing in Lake Kivu: New insights indicate increasing risk of uncontrolled gas eruption. Geochem. Geophys. Geosyst. **6**: Q07009. doi:10.1029/2004GC000892

Sirevåg, R., B. B. Buchanan, J. A. Berry, and J. H. Troughton. 1977. Mechanisms of CO2 fixation in bacterial photosynthesis studied by the carbon isotope fractionation technique. Arch. Microbiol. **112**: 35–38. doi:10.1007/BF00446651

Taipale, S., R. I. Jones, and M. Tiirola. 2009. Vertical diversity of bacteria in an oxygen-stratified lake, evaluated using DNA and phospholipid analyses. Aquat. Microb. Ecol. **55**: 1–16. doi:10.3354/ame01277

Taylor, G. T., M. Iabichella, T. Y. Ho, M. I. Scranton, R. C. Thunell, F. Muller-Karger, and R. Varela. 2001. Chemoautotrophy in the redox transition zone of the Cariaco Basin: A significant midwater source of organic carbon production. Limnol. Oceanogr. **46**: 150–155. doi:10.4319/lo.2001.46.1.0148

Thiery, W., A. Martynov, F. Darchambeau, J. Descy, P. Plisnier, L. Sushama, and N. Van Lipzig. 2014. Understanding the performance of the FLake model over two African Great Lakes. Geosci. Model Dev. **7**: 317–337. doi:10.5194/gmd-7-317-2014

Viollier, E., P. W. Inglett, K. Hunter, A. N. Roychoudhury, and P. Van Cappellen. 2000. The ferrozine method revisited: Fe (II)/Fe (III) determination in natural waters. Appl. Geochem. **15**: 785–790. doi:10.1016/S0883-2927(99)00097-9

Weiss, R. F. 1981. Determinations of carbon dioxide and methane by dual catalyst flame ionization chromatography and nitrous oxide by electron capture chromatography. J. Chromatogr. Sci. **19**: 611–616. doi:10.1093/chromsci/19.12.611

Wenk, C. B., and others. 2013. Anaerobic ammonium oxidation (anammox) bacteria and sulfide-dependent denitrifiers coexist in the water column of a meromictic south-alpine lake. Limnol. Oceanogr. **58**: 1–12. doi:10.4319/lo.2013.58.1.0001

Zhang, Y. M., and C. O. Rock. 2008. Membrane lipid homeostasis in bacteria. Nat. Rev. Microbiol. **6**: 222–233. doi: 10.1038/nrmicro1839

Zigah, P. K., K. Oswald, A. Brand, C. Dinkel, B. Wehrli, and C. Schubert. 2015. Methane oxidation pathways and associated methanotrophic communities in the water column of a tropical lake. Limnol. Oceanogr. **60**: 553–572. doi: 10.1002/lno.10035

Acknowledgments

The authors are grateful to Boniface Kaningini, Pascal Isumbisho and Pascal Masilya (Institut Supérieur Pédagogique, Bukavu, DRC), Laetitia Nyinawamwiza (National University of Rwanda, Rwanda), for logistical support during the cruises; to Zita Kelemen (KU Leuven) and Stephan Hoornaert (University of Liège) for help during fieldwork or during laboratory analysis; and to Martin Schmid (Swiss Federal Institute of Aquatic Science and Technology) for providing estimates of the advection and turbulent diffusivity coefficients that were useful to calculate vertical fluxes in Kabuno Bay. We are also grateful to two anonymous reviewers and the associate editor (Mary I. Scranton) for their constructive comments and insightful suggestions that helped to improve the quality of the manuscript. This work was funded by the EAGLES (East African Great lake Ecosystem Sensitivity to Changes, SD/AR/02A) project from the Belgian Federal Science Policy Office (BELSPO, Belgium), the CAKI (Cycle du carbone et des nutriments au Lac Kivu, contract 2.4.598.07) and MICKI (Microbial diversity and processes in Lake Kivu, contract 2.4.515.11) projects from the Fonds National de la Recherche Scientifique (FNRS, Belgium), and contributes to the European Research Council (ERC) starting grant project AFRIVAL (African river basins: Catchment-scale carbon fluxes and transformations, 240002). AVB is a senior research associate at the FNRS.

Submitted 21 December 2015
Revised 16 February 2016; 14 March 2016
Accepted 15 March 2016

Associate editor: Mary Scranton