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Methane sources and sinks in Lake Kivu

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[1] Unique worldwide, Lake Kivu stores enormous amounts of CH_4 and CO_2. A recent study reported that CH_4 concentrations in the lake have increased by up to 15% in the last 30 years and that accumulation at this rate could lead to catastrophic outgassing by ~2100. This study investigates the present-day CH_4 formation and oxidation in Lake Kivu. Analyses of ^{14}C and ^{13}C in CH_4 and potential carbon sources revealed that below 260 m, an unusually high ~65% of the CH_4 originates either from reduction of geogenic CO_2 with mostly geogenic H_2 or from direct inflows of geogenic CH_4. Aerobic CH_4 oxidation, performed by close relatives of type X CH_4-oxidizing bacteria, is the main process preventing CH_4 from escaping to the atmosphere. Anaerobic CH_4 oxidation, carried out by CH_4-oxidizing archaea in the SO_4^{2-}-reducing zone, was also detected but is limited by the availability of sulfate. Changes in $^{14}C_{CH4}$ and $^{13}C_{CH4}$ since the 1970s suggest that the amount of CH_4 produced from degrading organic material has increased due to higher accumulation of organic matter. This, as well as the sudden onset of carbonates in the 1960s, has previously been explained by three environmental changes: (1) introduction of nonnative fish, (2) amplified subaquatic inflows following hydrological changes, and (3) increased external inputs due to the fast growing population. The resulting enhancement of primary production and organic matter sedimentation likely caused CH_4 to increase. However, given the large proportion of old CH_4 carbon, we cannot exclude an increased inflow of geogenic H_2 or CH_4.

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1. Introduction

[2] Methane (CH_4) and carbon dioxide (CO_2) are major end products of organic matter decomposition in water bodies. In lacustrine sediments, the most important CH_4-producing pathways are acetoclastic methanogenesis and CO_2 reduction [*Conrad*, 2005]:

$$CH_3COOH \rightarrow CO_2 + CH_4 \tag{1}$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O. \tag{2}$$

Once produced, CH_4 can be oxidized either in the sediment, at the sediment-water interface, or in the open water, depending on the availability of oxidants. Two different biological processes are currently considered to be the main contributors to CH_4 oxidation in aquatic systems: oxidation by aerobic methanotrophic bacteria and anaerobic CH_4 oxidation by syntrophic consortia of CH_4-oxidizing archaea and SO_4^{2-}-reducing bacteria [*Hinrichs and Boetius*, 2002; *Schubert et al.*, 2010]. CH_4 that is not consumed is eventually transported to shallower waters and can be released to the atmosphere where it acts as a potent greenhouse gas. It has been estimated that lakes contribute 6 to 16% to global CH_4 emissions to the atmosphere [*Bastviken et al.*, 2004]. CH_4 oxidation therefore plays an important role in controlling lacustrine CH_4 emissions.

[3] Lake Kivu is an African Rift lake (Figure 1) with a permanently stratified hypolimnion that contains an estimated 60 km^3 of CH_4 and 300 km^3 of CO_2 (gas volume at 0°C and 1 atm [*Schmid et al.*, 2005]). CH_4 accumulation in the lake bears the risk of catastrophic outgassing similar to the limnic eruptions in Lakes Nyos [*Kling et al.*, 1987] and Monoun [*Sigurdsson et al.*, 1987]. Conversely, extraction of the lake's CH_4 could provide enough energy to supply the lake's bordering countries with electricity for a decade [*Jones*, 2003]. The origin of these gases, however, has been a matter of controversy [*Deuser et al.*, 1973; *Tietze et al.*, 1980]. Most authors agree that the CO_2 has a primarily geogenic origin, whereas the CH_4 is biogenic [*Schoell et al.*,

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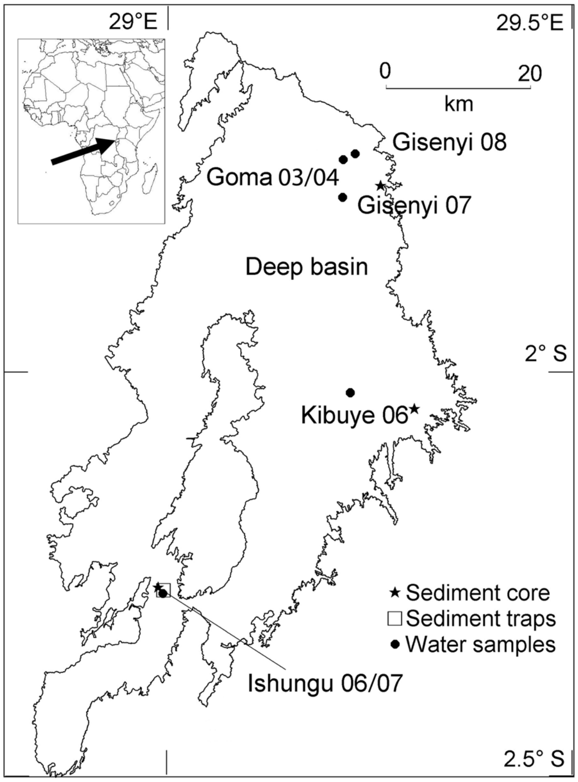

Figure 1. Map of Lake Kivu showing the locations of water sampling, sediment cores, and the mooring with sediment traps. In the deep basin, the names of the sampling locations (Goma 03/04 (6–11 November 2003; 26 February 2004), Kibuye 06 (5 May 2006), Gisenyi 07 (12 May 2007), Gisenyi 08 (October 2008)) represent the nearest city followed by the sampling year. In Ishungu Basin, water samples (17 May 2007) were taken at the same site Ishungu 06/07 as the sediment traps (17 May 2006 to 7 January 2008) and the sediment core (14 May 2006).

1988; *Tietze*, 1978; *Tietze et al.*, 1980]. *Schoell et al.* [1988] reported that two thirds of CH_4 is produced by reduction of geogenic CO_2 and one third by acetoclastic methanogenesis of sedimentary organic material (biogenic material).

[4] Based on CH_4 concentrations observed by *Tietze* [1978] and recent measurements, *Schmid et al.* [2005] estimated that CH_4 concentrations in the lake may have increased by up to 15% over the last 30 years. An increase in CH_4 formation by about a factor of 3 compared to the long-term steady state formation would have been required to explain this increase. A recent study further provided evidence that Lake Kivu experienced an increase in gross and organic carbon (OC) sedimentation since the 1960s due to (1) a modified internal food web following the introduction of the nonnative Tanganyika sardine (*Limnothrissa miodon*); (2) hydrological changes that enhanced internal upwelling; and/or (3) increased external nutrient inputs caused by the fast growing human population [*Pasche et al.*, 2010]. We hypothesize that the increase in OC sedimentation is partly responsible for the increasing CH_4 concentrations in the lake. Since the risk of outgassing increases with rising gas content, our main research interest is to understand the driving forces of CH_4 formation and consumption. Further, the CH_4 formation rate is of practical interest for planning the forthcoming exploitation of CH_4.

[5] This study is part of a larger project that assessed the nutrient cycling and its relation to CH_4 in Lake Kivu. Analyses of the external inputs [*Muvundja et al.*, 2009] and the internal loading [*Pasche et al.*, 2009] of nutrients highlighted the importance of the lake internal processes for the nutrient cycling in the lake. Observations from sediment cores indicated recent changes in the nutrient cycling that might be related to the observed increase in CH_4 concentrations [*Pasche et al.*, 2010]. In the present study, the lessons learned from the analysis of the nutrient fluxes are combined with (1) measurements of the isotopic composition of different carbon pools and (2) the analysis of the microbial community in the water column, for the following three objectives: (1) to assess the rates of CH_4 formation through acetoclastic methanogenesis and CO_2 reduction; (2) to determine aerobic and anaerobic CH_4 oxidation rates and the organisms involved; and (3) to detect changes in the carbon fluxes which could explain the recent CH_4 increase.

2. Site Description

[6] Lake Kivu is located in the East African Rift Valley, between the Republic of Rwanda and the Democratic Republic of the Congo. At an elevation of 1463 m, it has an area of 2370 km^2, a volume of 580 km^3 and a maximum depth of 485 m [*Schmid et al.*, 2004; *Tietze*, 1978]. The lake is meromictic and the mesotrophic epilimnion is permanently separated from the anoxic nutrient-rich deep waters. The depth of the oxycline varies seasonally from 30 m during the rainy season (October to May) to 60–65 m in the windy dry season (June to September). The permanently stratified deep water is further characterized by a major chemocline extending from 255 to 262 m. Subaquatic springs enter the permanently stratified deep water at different depths, with an estimated total inflow of ~1.3 km^3 yr^{-1} [*Schmid et al.*, 2005]. More than 127 rivers with a total flow of ~2.4 km^3 yr^{-1} enter the lake from the catchment (5097 km^2), and 3.6 km^3 yr^{-1} flow out with the Ruzizi River [*Muvundja et al.*, 2009]. Precipitation (3.3 km^3 yr^{-1}) is nearly equal to lake surface evaporation (3.4 km^3 yr^{-1}) [*Muvundja et al.*, 2009].

[7] Water residence times in the permanently stratified deep water are two to three orders of magnitude longer than time scales for horizontal mixing. Physical and chemical properties are therefore horizontally homogeneous throughout the lake, except for the separate basins of Kabuno Bay and Bukavu Bay. Energy supply from the wind to the deep water is limited by the strong density stratification in the lake. Consequently vertical exchange by turbulent diffusivity is weak, as evidenced by the presence of double-diffusive staircases [*Schmid et al.*, 2010]. Vertical transport is therefore dominated by the upwelling caused by the inflows of the subaquatic springs. Water below the major chemocline has a residence time of 800 to 1,000 years, which has led to an enormous accumulation of dissolved gases and nutrients [*Schmid et al.*, 2005].

[8] Nutrients for primary production are supplied to the productive surface layer mainly by internal recycling by upwelling from the nutrient-rich deep waters [*Pasche et al.*, 2009]. Contrary to many other large lakes, such as Tanganyika, where

nutrient supply by upwelling at the southern end leads to horizontal gradients in primary production [*Bergamino et al.*, 2010], the internal recycling in Lake Kivu is homogeneous, as indicated by the perfectly horizontal layering. Consequently, low spatial variability of primary production and chlorophyll concentrations have been observed in the surface waters of the lake [*Kneubühler et al.*, 2007; *Sarmento et al.*, 2009].

3. Material and Methods

3.1. Sampling

[9] Sampling was conducted during five field campaigns in November 2003, February 2004 (site Goma 03/04), May 2006 (Kibuye 06), May 2007 (Gisenyi 07) and October 2008 (Gisenyi 08) at three different locations in the main basin (Figure 1). In May 2006 and 2007 samples were taken in the Ishungu Basin (site Ishungu 06/07, Figure 1). Water samples were collected using 5 L Niskin bottles. For depths below 200 m, the open top valve was capped with a balloon to prevent sample loss due to vigorous outgassing during bottle ascent.

[10] Gas samples were obtained using a new device consisting of a 500 mL metal cylinder for water sampling and an evacuated 250 mL metal cylinder to collect the gases. These collected gases were subsampled into evacuated 120 mL airtight glass vials.

[11] The sampling of sediment traps and cores has been described in detail by *Pasche et al.* [2010]. In May 2007, a sediment core for gas analysis was taken near Gisenyi (1°46.416′S, 29°15.796′E, 125 m depth) using an Uwitec gravity corer. Thirteen sediment samples (2 mL) were extracted between 0 and 16 cm through side ports using plastic syringes. They were transferred to 25 mL glass vials prefilled with 4 mL of 2.5% sodium hydroxide (NaOH) [*Sobek et al.*, 2009]. The vials were immediately sealed with butyl-rubber stoppers, shaken, and stored upside down until analysis for CH_4 concentration and isotopic composition. Further gravity sediment cores were taken in May 2006 at Ishungu (2°16.077′S, 28°59.374′E, 175 m depth) for total organic carbon (TOC) analyses and at Kibuye (2°02.886′S, 29°18.307′E, 190 m depth) for TOC and ^{13}C and ^{14}C analyses of the OC.

[12] A sediment trap mooring was set in Ishungu Basin from May 2006 to January 2008 (Ishungu 06/07). Sediment traps consisting of two plastic cylinders (diameter 9.2 cm, length 100 cm) were placed at four different depths (50, 90, 130 and 172 m). Trap material without any poisoning was collected monthly with overlying water in 250 mL bottles, frozen and transported to Switzerland.

3.2. Chemical Parameters

[13] In October 2008, water samples for the analyses of organic anions were taken at site Gisenyi 08 (Figure 1). Samples were preserved in the field by adding 10 M NaOH to adjust the pH to 12. Organic anions (lactate, acetate, propionate, formate, butyrate, pyruvate) were measured with a Dionex DX-320 ion chromatography system with a detection limit of 5 μmol L^{-1}. For TOC measurements, water samples were collected in 200 mL glass vials and acidified to pH 3 to remove inorganic carbon. TOC was measured within 6 days with a Total Organic Carbon Analyzer (Shimadzu TOC-V CPH).

[14] For CH_4 concentrations and isotopic ratio analyses, water samples were taken in 2006 and 2007 at sites Kibuye 06, Ishungu 06/07 and Gisenyi 07 (Figure 1) and for CO_2 isotopic ratio analyses in 2003 at site Goma 03/04. Samples were transferred to 120 mL glass vials and poisoned with cupric chloride ($CuCl_2$). All samples were measured within five weeks of collection. CH_4 concentrations were measured by a headspace (30 mL N_2) technique similar to that of *McAuliffe* [1971]. CH_4 concentrations were determined on a gas chromatograph (Agilent, 6890) equipped with a Carboxen 1010 column (30 m, Supelco) using a flame ionization detector. In order to dilute samples from below 70 m depth into the calibration range of the GC, 300 μL of headspace were transferred into 58.5 mL serum vials prefilled with N_2. Dissolved gas concentrations were calculated after *Wiesenburg and Guinasso* [1979], including the effects of salinity.

[15] Methane carbon isotopes ($^{13}C_{CH4}$) were determined using the method of *Sansone et al.* [1997]. Duplicate measurements were processed with an IsoPrime mass spectrometer connected to a trace gas preconcentrator (GV Instruments). Results are noted in the standard δ notation relative to Vienna Pee Dee Belemnite.

[16] Carbon isotopes on carbon dioxide ($^{13}C_{CO2}$) were determined using an IsoPrime mass spectrometer connected to a trace gas preconcentrator (GV Instruments). Results are noted in the standard δ notation relative to Vienna Pee Dee Belemnite.

[17] Methane radiocarbon ($^{14}C_{CH4}$), deuterium ($^{2}H_{CH4}$), and stable carbon isotope ($^{13}C_{CH4}$) analyses were performed on samples taken in 2007, at site Gisenyi 07. The methods are outlined in *Kessler and Reeburgh* [2005]. Briefly, CH_4 was extracted from samples, purified, and combusted to CO_2 and water. An aliquot of CO_2 was converted to elemental carbon by iron-catalyzed hydrogen reduction and analyzed with ^{14}C Accelerator Mass Spectrometer (AMS) at the Keck Carbon Cycle AMS Facility. ^{14}C concentrations are given as percent of the modern standard (percent modern carbon, pMC) following the conventions of *Stuiver and Polach* [1977]. A second aliquot of CO_2 was analyzed for ^{13}C by dual-inlet isotope ratio mass spectrometry at the University of California Irvine (UCI) Stable Isotope Facility. The water produced from CH_4 combustion was reduced to hydrogen with activated zinc and the $^{2}H_{CH4}$ was measured on a Finnigan MAT 252 Mass Spectrometer at UCI and is reported in delta notation relative to Standard Mean Ocean Water. The ^{14}C content of the organic matter in three samples from the Kibuye sediment core (0–0.5 cm, 0–5 cm, 15–37 cm), was measured in the Laboratory of Ion Beam Physics at the Swiss Federal Institute of Technology (ETH) in Zurich using the method described by *Hajdas et al.* [1993].

[18] For TOC in sediment, total carbon (TC) was measured using a combustion CNS elemental analyzer (VARIO Co and EuroVector Co). Total inorganic carbon (TIC) was analyzed as CO_2 by coulometry (UIC Coulometrics) after acidification with 3M hydrochloric acid (HCl). TOC was calculated as the difference between TC and TIC.

3.3. Methane Oxidation Measurements

[19] To experimentally verify CH_4 oxidation, CH_4 concentrations were monitored in vials over 5 days in 2007. At site Gisenyi 07, water from 20, 40, 60, 80, and 100 m depth

Table 1. Primers and Methods Used for PCR Amplification

Gene	Primer	Target Group	Analysis/Screening	Reference
16S rRNA	341f[a]-534r	Bacteria	DGGE, direct seq.	1
pmoA	A189f[a]-A650r	aerobic methanotrophs	DGGE, cloning and seq.	2 and 3
mcrA	Me1f-Me2r	anaerobic methanotrophs/methanogens	RFLP, cloning and seq.	2 and 4

[a]Primer also used with GC clamp [*Muyzer et al.*, 1993] for DGGE; seq., 16S rRNA DGGE bands were sequenced directly, *pmoA* and *mcrA* were cloned and screened using the given method before sequencing. See section 3. References are 1, *Muyzer et al.* [1993]; 2, *Earl et al.* [2003]; 3, *Bourne et al.* [2001]; and 4, *Hales et al.* [1996].

was sampled in five 120 mL airtight vials for each depth (Table 1). One bottle was poisoned with $HgCl_2$ immediately after sampling and then for the following four days one vial per day was poisoned and analyzed as above. For the samples from 20, 40, and 60 m depth CH_4 was further analyzed for ^{13}C on day one and day five.

3.4. Microbial Community Analysis

3.4.1. Molecular Analyses

[20] During May 2007, water samples (380 to 750 mL) from 14 depths collected at Gisenyi 07 were filtered through polycarbonate filters (0.2 μm, 47 mm). DNA was extracted from the filters as described by *Llirós et al.* [2008]. The bacterial community structure was investigated by Polymerase Chain Reaction (PCR) amplification of the partial 16S ribosomal RNA gene with primers 341f-GC and 534r (Table 1) followed by denaturing gradient gel electrophoresis (DGGE) [*Muyzer et al.*, 1993]. Selected bands were cut from the gel, reamplified, and sequenced. The particulate CH_4 monooxygenase gene (*pmoA*) served as a molecular marker for aerobic methanotrophs; *pmoA* was detected by PCR amplification with *pmoA*-specific primers A189f and A650r (Table 1) as described by *Bourne et al.* [2001] with minor modifications. As a marker for anaerobic methanotrophs (ANME) as well as methanogens, the gene for methyl coenzyme M reductase (*mcrA*) was amplified using *mcrA*-specific primers Me1f and Me2r [*Hales et al.*, 1996] (Table 1); *mcrA* genetic diversity was characterized by restriction fragment length polymorphism analysis (RFLP) [*Earl et al.*, 2003]. Clone libraries were created from amplicons from seven depths for *mcrA* and three depths for *pmoA*. Clones were screened using DGGE (*pmoA*) and RFLP (*mcrA*) and selected clones were commercially sequenced (Microsynth, Balgach, Switzerland). Sequences were submitted to the GenBank nucleotide sequence database with the following accession numbers: 16*S rRNA*, FJ952083 to FJ952140; *pmoA*, FJ952083 to FJ952102; *mcrA*, FJ952103 to FJ952140. Detailed protocols are presented in Text S1 in the auxiliary material.[1]

3.4.2. Analysis of Sequenced Data

[21] Sequences were screened, trimmed, aligned (using Clustal W [*Larkin et al.*, 2007]) and analyzed using Mega 4.1 software [*Tamura et al.*, 2007]. Reference sequences were retrieved using the BLAST network services [*Altschul et al.*, 1990] and keyword queries of the nucleotide database at the National Center for Biotechnology Information using the Entrez Database Query Tool (http://www.ncbi.nlm.nih.gov/nuccore). General bacterial 16S rRNA sequences from DGGE bands were classified using the Classifier Tool on the Ribosomal Database Project 2 Web site (http://rdp.cme.msu.edu/; release 10 [*Wang et al.*, 2007]). Phylogenetic trees for *mcrA* and *pmoA* were constructed based on translated amino acid sequences using the unweighted pair group method with arithmetic mean (UPGMA) hierarchical clustering method [*Nei and Kumar*, 2000] and the PAM distance metric [*Schwarz and Dayhoff*, 1979] as implemented in Mega 4.1, with pairwise elimination of gaps and 1000 bootstrap resamplings for tree testing.

4. Results

4.1. Chemistry of the Water Column

[22] Lake Kivu is characterized by high amounts of dissolved gases, with CO_2 concentrations five times higher than CH_4 (Figure 2a). Gas concentrations rise gradually with depth down to the major chemocline (255 to 262 m) where they abruptly increase. Gas concentrations measured in water samples compare well with published data [*Schmid et al.*, 2005] above 140 m (Figure 3c). However, below 140 m, part of the CH_4 was lost due to vigorous outgassing during sampling.

[23] The profiles of the main electron acceptors (O_2, SO_4^{2-}, and NO_3^-) indicated a typical succession of redox zones for stratified water bodies (Figure 2b) and were described in detail by *Pasche et al.* [2009]. The oxycline extended to a depth of 50 m in May 2006 and 2007. Below the oxycline, SO_4^{2-} decreased with a sharp gradient to 80 m and dropped below detection (0.05 mmol L^{-1}) at 100 m depth. In contrast, H_2S was absent above the oxycline, increased sharply between 50 and 80 m depth, and more gradually to 150 m. Below 150 m H_2S remained constant at ~0.27 mmol L^{-1} (data not shown). Other electron acceptors, namely NO_3^-, manganese (see the reduced product Mn^{2+}, Figure 2b) and Fe (III) (not shown) were present only in very low concentrations.

[24] TOC concentrations increased from 1.8 mg L^{-1} at 40 m to 4.2 mg L^{-1} at 360 m depth. Acetate and other short-chain organic anions were below the detection limit of 5 μmol L^{-1}.

4.2. Methane and Carbon Isotopic Signature

4.2.1. Water Column

[25] Above 90 m, $\delta^{13}C_{CH4}$ increased from −59.8‰ to −43‰ at the surface, with a high variability within and above the oxycline (Figure 3a). Below 90 m, $\delta^{13}C_{CH4}$ was constant at −59.8 ± 1‰. δ^2H_{CH4} below 140 m averaged at −215‰, with a considerable error margin. Values decreased in excess of the error between 140 m and 255 m. The decrease in the water column above the chemocline is supported by two separate measurements analyzed in a different laboratory (Figure 3b, open circles). A sharp increase in excess of the error was observed at the main chemocline. Below the

[1]Auxiliary materials are available in the HTML. doi:10.1029/2011JG001690.

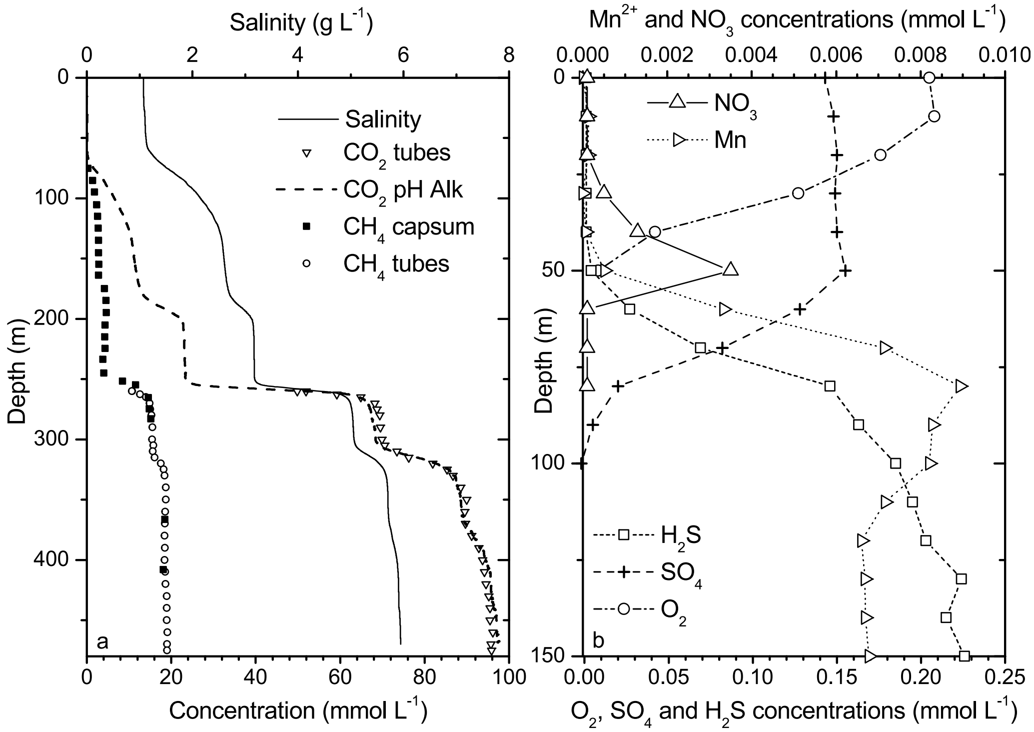

Figure 2. (a) Vertical profiles of salinity and concentrations of dissolved gases (sampled 2003 at site Goma 03/04) in Lake Kivu from *Schmid et al.* [2005]. The dashed curve is the CO_2 concentration calculated from pH and alkalinity. CO_2 was measured by slowly pumping water in polyethylene tubes and analyzing the gas and liquid flows. CH_4 concentrations [*Schmid et al.*, 2005] were measured in situ with a Capsum Mets sensor and on a platform using polyethylene tubes (see above). The salinity profile was calculated from a conductivity profile measured with a conductivity-temperature-depth (CTD) probe. (b) Top 150 m of vertical profiles of electron acceptors concentrations O_2, NO_3^-, SO_4^{2-} from *Pasche et al.* [2009] and reduced species Mn^{2+} and H_2S. These profiles are the average of three stations in the deep basin and two observations in Ishungu Basin taken in February 2004, May 2006, and May 2007.

chemocline, δ^2H_{CH4} decreased again to an average of −220‰ (Figure 3b). pMC decreased from 15.7% at 140 m depth to 12.9% at 250 m depth. Across the chemocline it decreased steeply to 11.9% at 255 m and 11.1% at 270 m. Below the chemocline pMC increased slightly to 11.6% at 440 m. Conversion to ^{14}C age [*Stuiver and Polach*, 1977] indicates an average age of 17,000 years for CH_4 (Figure 3b). In comparison, the residence time of water below 250 m has been estimated to be ~800 years [*Schmid et al.*, 2005], indicating the strong influence of geogenic CO_2 with a dead radiocarbon signal.

4.2.2. Sediment

[26] The $\delta^{13}C_{CH4}$ measured in the Gisenyi core decreased slightly from the surface (−66‰) to a minimum of −68‰ at 15 cm depth (Figure 4).

[27] In the Kibuye core, representing the last 300 years [*Pasche et al.*, 2010], isotope signatures of OC ($\delta^{13}C_{OC}$) varied slightly around −24.1‰ (−22.6 to −25.1‰). The ^{14}C content of OC ($^{14}C_{OC}$) in this core varied with depth. In the top layer (0–0.5 cm, last 2–3 years) it was 34 pMC (carbon age 8,670 years), a mixed sample of the section including the bomb peak (0.5–5 cm, last 20 years) had 43 pMC (6,780 years), while a mixed sample from before the bomb peak (15 to 37 cm, approximately representing the time between 300 and 100 years before present) was 28 pMC (10,200 years). The latter sample was assumed to be representative for the source organic matter in the sediment before the impact of bomb tests.

4.3. Estimating CH_4, CO_2, and Organic Carbon Fluxes

[28] In this section, the knowledge gained from previous studies is combined with the results of our measurements and model simulations described in the auxiliary material to create an overview of CH_4, CO_2, and OC fluxes in Lake Kivu. Error margins are calculated based on estimated error ranges for the measured quantities and standard formulas for error propagation. The fluxes estimated in the following sections are summarized in Figure 5.

4.3.1. Primary Production and CO_2 Fluxes

[29] Annual primary production (averaged over 2.5 years) was determined to be PP = 228 g C m^{-2} yr^{-1}, by *Sarmento et al.* [2009]. The total upward fluxes of CO_2 (internal loading, IL) and dissolved inorganic carbon (DIC) through the chemocline were previously estimated at $IL_{CO2} = 126 \pm 25$ g C m^{-2} yr^{-1} and $IL_{DIC} = 335 \pm 67$ g C m^{-2} yr^{-1} [*Pasche et al.*, 2009]. Since PP < IL_{DIC}, the lake is a net source of CO_2. The ^{14}C content of the sediment (Section 4.2.2) suggests that primary production uses only approximately one third to one fourth of atmospheric CO_2 as a carbon source.

4.3.2. Organic Carbon Fluxes

[30] OC analyses from sediment traps deployed at three different depths in the Ishungu Basin over two years revealed

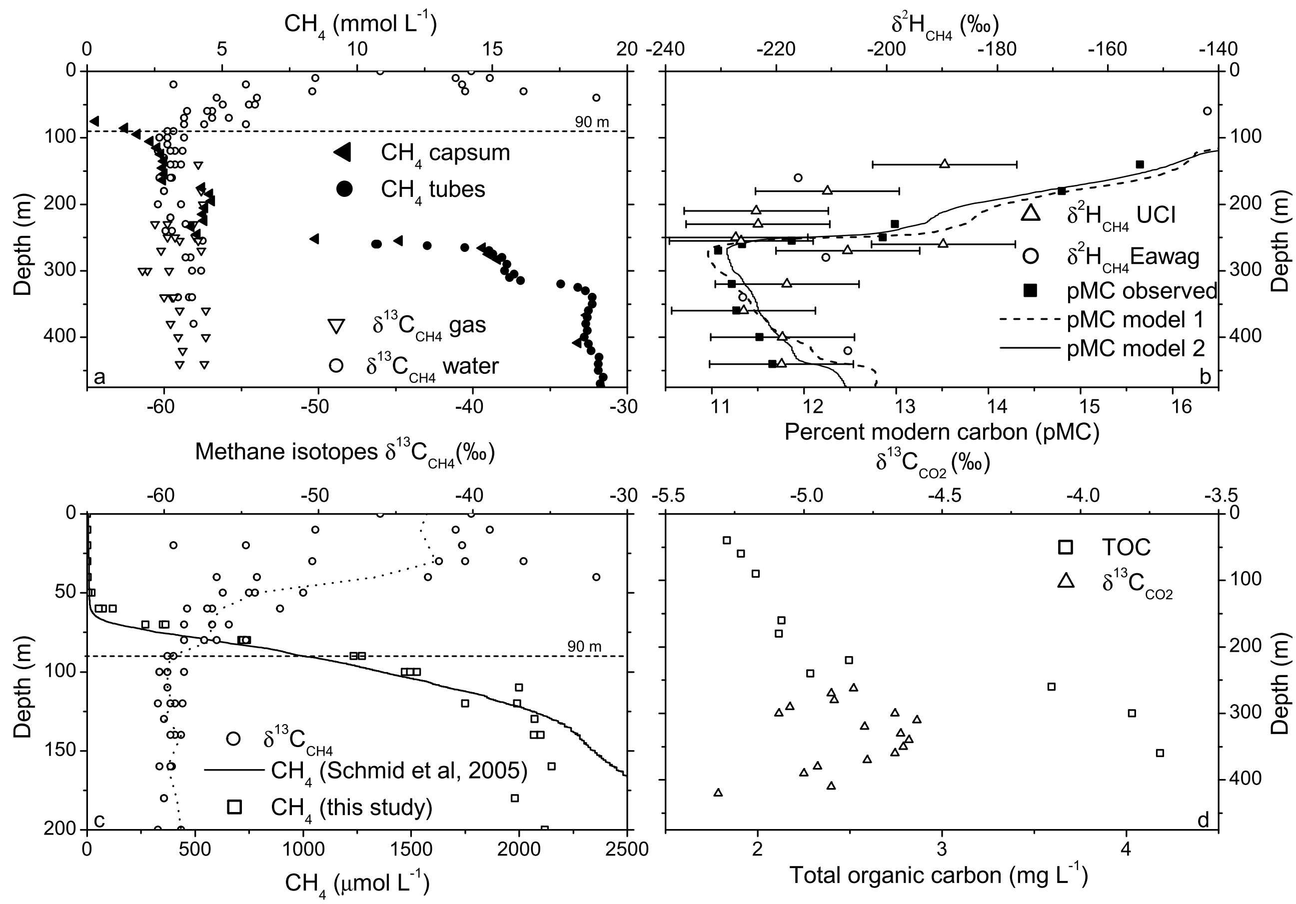

Figure 3. (a) Profiles of CH_4 concentrations [*Schmid et al.*, 2005] and ^{13}C isotopes (this study, data from samplings Kibuye 06, Ishungu 07, and Gisenyi 07). The $^{13}C_{CH4}$ was measured in gas and water samples collected during three field visits. Gas samples were fractionated by +0.7‰ compared to water samples. The dashed line represents the lower boundary of the SO_4^{2-}-reducing zone (90 m depth). (b) CH_4 deuterium isotopes (the large error bars of 13‰ represent the standard deviation of the standards, which were fractionated during sample preparation) and ^{14}C. The models used to reproduce the ^{14}C (given as percent modern carbon, pMC) depth profile (solid and dashed lines), are described in the auxiliary material. (c) Profiles between 0 and 200 m depth of CH_4 concentrations measured in this study (data from samplings Kibuye 06, Ishungu 07 and Gisenyi 07) compared with data from *Schmid et al.* [2005], as well as $\delta^{13}C_{CH4}$. The horizontal dashed line represents the lower limit of the SO_4^{2-}-reducing zone (90 m). The dotted curve is the averaged value for each depth of $\delta^{13}C_{CH4}$. (d) Profile of ^{13}C isotopes of dissolved CO_2 and total organic carbon (TOC) concentrations.

a homogeneous average gross sedimentation of $S_{OCgross}$ = 41 ± 2 g C m^{-2} yr^{-1} from 90 to 172 m depth [*Pasche et al.*, 2010]. However, comparisons with nutrient loading data and observations of phytoplankton abundance have shown that the observed gross sedimentation was approximately 60 ± 10% below the long-term average [*Pasche et al.*, 2010]. Thus, we estimate the long-term average of $S_{OCgross}$ to 110 ± 27 g C m^{-2} yr^{-1}. A sediment core taken at the same location revealed a net sedimentation of 7 g C m^{-2} yr^{-1} (top 10 cm, approx. 40 years). Higher values were found at Gisenyi (17 g C m^{-2} yr^{-1}) and Kibuye (13 g C m^{-2} yr^{-1}), providing a range of S_{OCnet} = 12 ± 5 g C m^{-2} yr^{-1} [*Pasche et al.*, 2010]. The mineralization rate of OC in the sediment was therefore estimated as

$$M_{OC\_sed} = S_{OCgross} - S_{OCnet} = 98 \pm 28 \text{ g C m}^{-2} \text{ yr}^{-1}.$$

4.3.3. Methane Fluxes From Organic Matter Decomposition in the Sediment

[31] Assuming methanogenesis proceeds to completion, the mineralized carbon partitions 1:1 between CO_2 and CH_4 [*Conrad*, 1999]. We can therefore calculate the total CH_4 formation (P_{CH4_sed}) based on the mineralization of the settling OC:

$$P_{CH4\_sed} = 0.5 \cdot M_{OC\_sed} = 49 \pm 14 \text{ g C m}^{-2} \text{ yr}^{-1}.$$

[32] Independently, the CH_4 flux from the sediment to the water column was calculated from CH_4 concentrations measured in the Gisenyi sediment core taken at 125 m water depth. Undisturbed cores could not be retrieved from greater depths, since outgassing destroyed the layering within the core. A net CH_4 flux from the sediment to the water column of

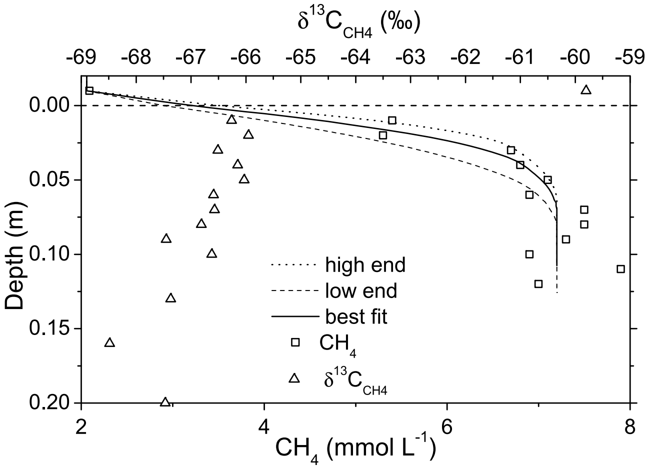

Figure 4. Profiles of CH_4 concentration and $^{13}C_{CH4}$ isotopes in the top sediment. The dashed line marks the sediment-water interface at 125 m depth. CH_4 concentration and isotope values in the water column at 125 m depth taken at a different location are plotted for comparison. The three lines, representing the best fit (solid curve, corresponding to a flux of ~80 g C_{CH4} m^{-2} yr^{-1}), the lower end (dashed curve, ~60 g C_{CH4} m^{-2} yr^{-1}), and the higher end (dotted curve, ~100 g C_{CH4} m^{-2} yr^{-1}), were determined with the one-dimensional diffusion-reaction model described by *Müller et al.* [2003].

60 to 100 g C_{CH4} m^{-2} yr^{-1} was estimated with the one-dimensional diffusion-reaction model described by *Müller et al.* [2003], by setting in situ CH_4 concentrations in the water column (2.1 mmol L^{-1}, measured at a different site) at 1 cm above the sediment. Due to uncertainty about the thickness of the diffusive boundary layer in Lake Kivu, these values are only a rough estimate based on reasonable assumptions. The upper estimate is well outside the possible rate of methanogenesis based on the reported organic matter deposition rate, but the lower estimate falls within the range for P_{CH4_sed} estimated above. The CO_2 flux is assumed to be equal to the CH_4 flux:

$$P_{CO2\_sed} = P_{CH4\_sed} = 49 \pm 14 \text{ g C m}^{-2}\text{ yr}^{-1}.$$

4.3.4. Partitioning Between CO_2 Reduction and Acetoclastic Methanogenesis

[33] In order to reproduce the $^{14}C_{CH4}$ vertical profile in the water column (Figure 3b), we used an extended version of the one-dimensional diffusion advection model described by *Schmid et al.* [2005] (see the auxiliary material). In the model it was assumed that settled OC has 28 pMC, while two different scenarios were used for the pMC of CO_2 used for methanogenesis via CO_2 reduction: pure geogenic CO_2 with 0 pMC, and a mixture of 15% (P_{CO2_sed}/IL_{DIC}) biogenic CO_2 derived from the settled OC with pMC 28 and 85% geogenic CO_2 with pMC 0. Simulations were performed for constant steady state CH_4 formation and for the increasing formation rate postulated by *Schmid et al.* [2005]. This analysis consistently showed different contributions of the CH_4 formation pathways below and above the major chemocline. Below the chemocline a large fraction of CH_4 (f_{CO2} = 65 ± 5%) is produced with old carbon probably originating from geogenic CO_2. Above the chemocline, CH_4 formation from recently settled OC with a maximum of 33% CO_2 reduction [*Conrad*, 1999] can explain the observed radiocarbon ages. The model also reproduced the finer structure of the vertical $^{14}C_{CH4}$ profile (Figure 3b and Figure S1 in Text S1), indicating that it correctly represents the interplay between CH_4 formation from the sediment and the residence times due to the vertical transport in the lake.

[34] An independent estimate of the partitioning between the two pathways below the chemocline was made from the available $\delta^{13}C$ data. In this zone, CH_4 oxidation processes are absent (see sections 4.3.6 and 4.4 for detail). Following the calculations described by *Itoh et al.* [2008], the fraction of CH_4 produced by acetoclastic methanogenesis (F_{Ac}) is calculated by

$$F_{Ac} = \frac{\delta^{13}C_{CH4} - \delta^{13}C_{CH4(CO_2)}}{\delta^{13}C_{CH4(Ac)} - \delta^{13}C_{CH4(CO_2)}}. \quad (3)$$

The isotope ratio of CH_4 from CO_2 reduction ($\delta^{13}C_{CH4(CO2)}$) is estimated by equation (4) from the average of measured $\delta^{13}C_{CO2}$ (−4.9‰, Figure 3d) and using reported ranges of fractionation coefficients for CO_2 reduction from natural bogs and lakes (α_{mc} = 1.06 to 1.073 [*Conrad*, 2005]):

$$\delta^{13}C_{CH4(CO_2)} = \frac{(\delta^{13}C_{CO2} + 1000) - (\alpha_{mc} \cdot 1000)}{\alpha_{mc}} = -61 \text{ to } -70‰. \quad (4)$$

Acetoclastic methanogenesis from organic matter comprises two steps potentially contributing to fractionation: fractionation during the formation of acetate, and fractionation during the actual acetoclastic methanogenesis. We assume that acetate is produced by fermentation and that fractionation between OC and acetate-methyl is negligible (α_{ao} = 1.0 [*Conrad*, 2005]); therefore,

$$\delta^{13}C_{Ac-methyl} = \delta^{13}C_{OC\_sed} = -24‰.$$

The isotope ratio of acetoclastic CH_4 ($\delta^{13}C_{CH4(Ac)}$) can then be estimated using reported ranges of fractionation coefficients for acetoclastic methanogenesis (α_{ma} = 1.007 to 1.027 [*Conrad*, 2005]) analogous to equation (4):

$$\delta^{13}C_{CH4(Ac)} = -31\text{to } -50‰.$$

Inserting these values into equation (3), we obtain percent contributions of CH_4 formation from CO_2 reduction in the deep water below the chemocline ranging from 43 to 94%, which supports the results of the calculation based on the ^{14}C profile (f_{CO2} = 65 ± 5%).

[35] However, homoacetogenic formation of acetate from CO_2 could provide a source of acetate with a $\delta^{13}C$ that strongly deviates from $\delta^{13}C_{OC\_sed}$. Although this has mainly been observed under conditions of high acetate accumulation [*Heuer et al.*, 2010], such observations challenge the assumption of $\delta^{13}C_{Ac-methyl} = \delta^{13}C_{OC\_sed}$. *Conrad et al.* [2010], in a study on tropical lake sediments, found

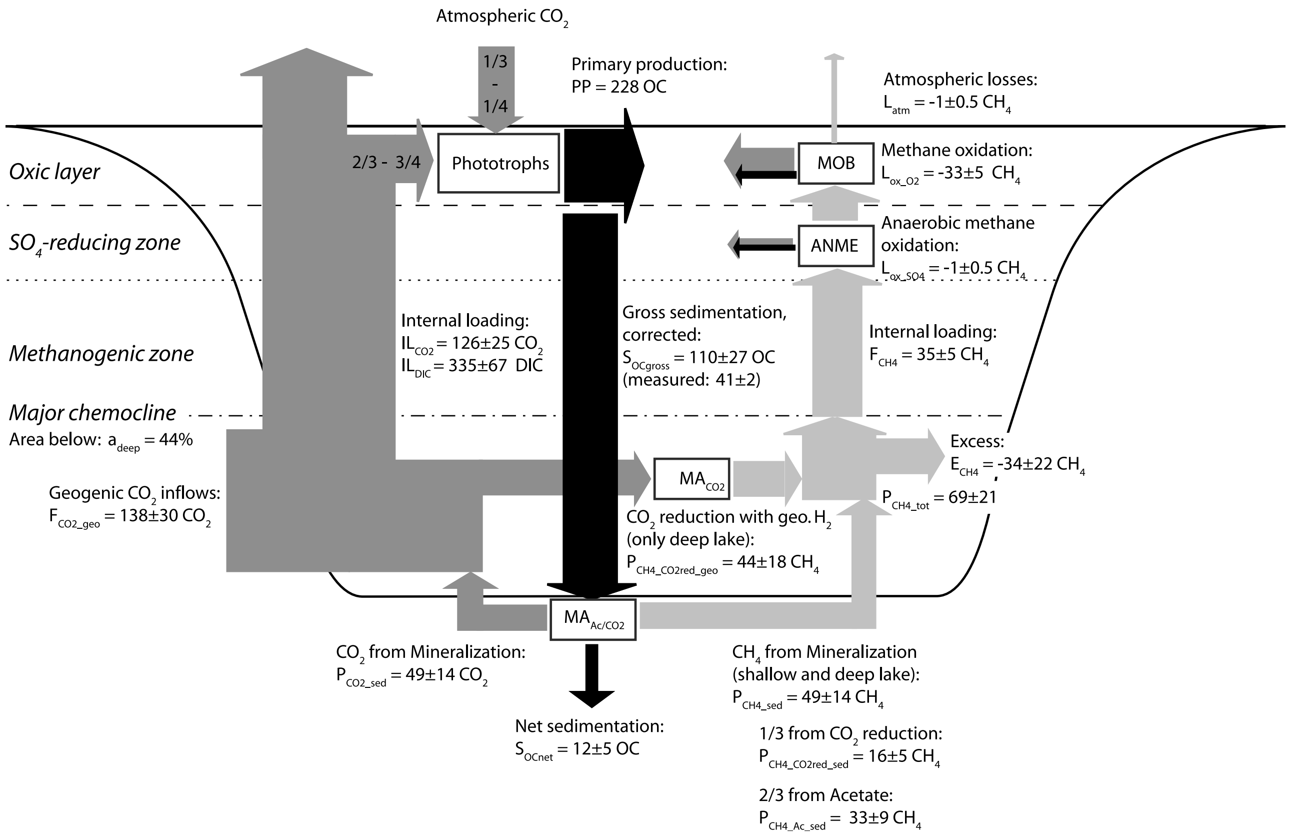

Figure 5. Overview of carbon cycling in Lake Kivu (for details, see section 4.3). CH_4 (light gray), CO_2 (dark gray), and OC (black) fluxes in g C m^{-2} yr^{-1} in Lake Kivu. The dashed line represents the oxycline, the dotted line is the lower boundary of the SO_4^{2-}-reducing zone and the dot-dashed line represents the major chemocline (255 to 262 m depth). All values are areal fluxes and thus do not always add up, as some of them relate to areas smaller than the lake surface. The abbreviations of the fluxes are explained in the text. Boxes indicate microbial populations involved in the process: MA_{Ac}, acetoclastic methanogenic archaea; MA_{CO2}, CO_2-reducing methanogenic archaea; ANME, anaerobic methane oxidizing archaea; MOB, methane oxidizing bacteria (aerobic).

only a nonsignificant correlation between $\delta^{13}C_{Ac\text{-}methyl}$ and $\delta^{13}C_{OC\_sed}$, and observed $\delta^{13}C_{Ac\text{-}methyl}$ to be between 4 and 44‰ more negative than $\delta^{13}C_{OC\_sed}$. While a slightly more negative $\delta^{13}C_{Ac\text{-}methyl}$ would not substantially affect the above estimate, strongly negative values would make it impossible to distinguish acetoclastic methanogenesis from CO_2 reduction by the isotope signature of CH_4. In general, acetogenesis seems to be favored over methanogenesis primarily at low temperatures and high H_2 concentrations [*Heuer et al.*, 2010]. Deep water temperatures in Lake Kivu are 25°C to 26°C [*Schmid et al.*, 2005]. The hydrogen (H_2) concentrations occurring in the sediments are unknown. Acetate in the sediments has not been measured, but in the water column acetate was below detection. Therefore we have no indication that high rates of acetogenesis occur in Lake Kivu. In any case, the net fractionation resulting from homoacetogenesis (using the fractionation factor of 1.06 reported by *Gelwicks et al.* [1989]) and subsequent acetoclastic methanogenesis, is very similar to that resulting from direct reduction of CO_2 by H_2. The occurrence of significant homoacetogenesis would therefore not challenge our conclusions about the fraction of CH_4 derived from CO_2 but only about the pathways for the conversion of CO_2 to CH_4.

[36] Generally, for CH_4 produced only from the degradation of organic matter in the sediment no more than 33% can derive from CO_2 reduction, due to limited amount of H_2 that can form during anaerobic OC degradation [*Conrad*, 1999]:

$$P_{CH4\_CO2red\_sed} = \frac{1}{3} P_{CH4\_sed} = 16 \pm 5 \text{ g C m}^{-2} \text{ yr}^{-1}$$

$$P_{CH4\_Ac\_sed} = \frac{2}{3} P_{CH4\_sed} = 33 \pm 9 \text{ g C m}^{-2} \text{ yr}^{-1}.$$

Below the main chemocline, we assume that 35 ± 5% of CH_4 ($f_{Ac} = 1 - f_{CO2} = 0.35 \pm 0.05$) is derived from acetoclastic methanogenesis and the remainder from CO_2 reduction. The total flux of CH_4 into the compartment below the chemocline is then

$$P_{CH4\_deep} = P_{CH4\_Ac\_sed}/f_{Ac} = 93 \pm 30 \text{ g C m}^{-2} \text{ yr}^{-1}.$$

And the total CH_4 formation from CO_2 reduction is

$$P_{CH4\_CO2red\_deep} = P_{CH4\_Ac\_sed}(1/f_{Ac} - 1) = 61 \pm 22 \text{ g C m}^{-2} \text{ yr}^{-1}.$$

Table 2. Experimental Evidence for Methane Oxidation Determined by Monitoring of Five Bottles per Depth, Kept at in Situ Conditions, 13–20 May 2007, Gisenyi

	Depth				
	20 m	40 m	60 m	80 m	100 m
Methane[a] (μM)					
13.05.2007	0.9	3.6	54	722	1528
14.05.2007	1.3	1.8	53	767	1516
15.05.2007	1.0	1.8	53	727	1571
16.05.2007	1.4	0.9	50	659	1587
17.05.2007	1.2	1.0	50	721	1528
$\delta^{13}C_{CH4}$[b]					
13.05.2007	−59.4	−54.0	−57.2	−54.7	−58.7
17.05.2007	−57.8	−32.0	−57.8		
Apparent methane oxidation rate (nM d^{-1})	ns	618 ± 173	1096 ± 287	ns	ns

[a]Error of measurement is ±1%.
[b]Error of measurement is ±2‰; ns, not significant ($p > 0.05$).

The difference between $P_{CH4\_CO2red\_deep}$ and $P_{CH4\_CO2red\_sed}$ must be produced by an additional pathway that uses old carbon. Here we assume that this pathway is CO_2 reduction with a geogenic H_2 source ($P_{CH4\_CO2red\_geo}$), although geogenic CH_4 is an alternative possibility:

$$
\begin{aligned}
P_{CH4\_CO2red\_geo} &= P_{CH4\_CO2red\_deep} - P_{CH4\_CO2red\_sed} \\
&= \left[\frac{2}{3}(1/f_{Ac} - 1) - \frac{1}{3}\right] P_{CH4\_sed} \\
&= 44 \pm 18 \text{ g C m}^{-2} \text{ yr}^{-1}.
\end{aligned}
$$

For steady state, the flux of geogenic CO_2 into the lake water column can now be estimated from internal loading, subtracting CO_2 from mineralization of OC in the sediment (P_{CO2_sed}), and adding the amount of CO_2 reduced by methanogenesis (P_{CH4_CO2red}):

$$
\begin{aligned}
F_{CO2\_geo} &= IL_{CO2} - P_{CO2\_sed} + P_{CH4\_CO2red\_deep} \\
&= 138 \pm 30 \text{ g C m}^{-2} \text{ yr}^{-1}.
\end{aligned}
$$

This parameter might be underestimated given that the lake is probably not at steady state and primary production probably increased since the 1960s [*Pasche et al.*, 2010].

4.3.5. Total Vertical Methane Fluxes

[37] For the sediment area located above the major chemocline we assume, according to our ^{14}C based model, that degradation of organic material (P_{CH4_sed}) is the only CH_4

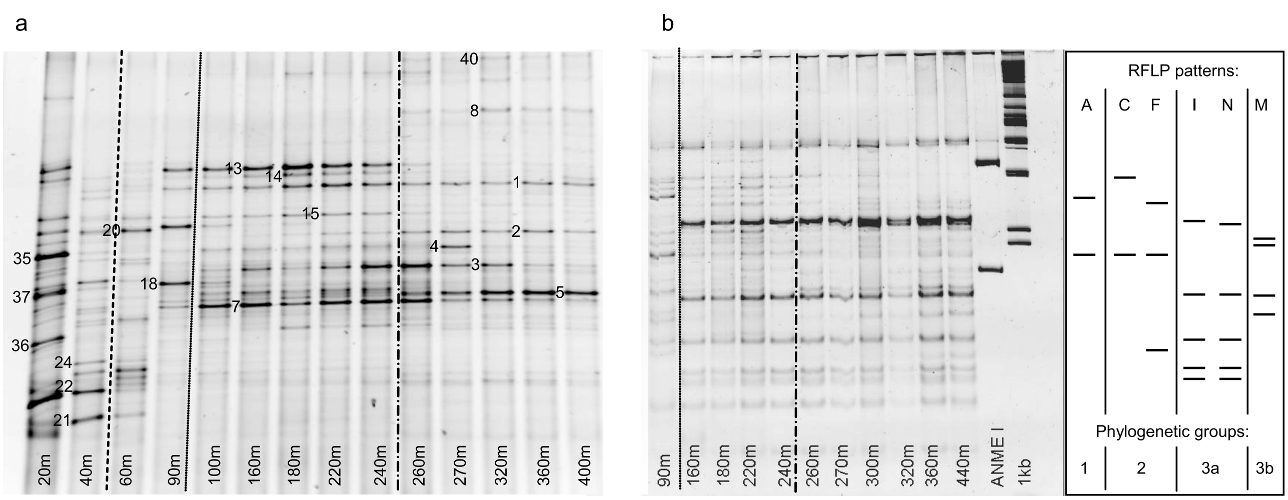

Figure 6. (a) DGGE gel photograph of PCR products obtained with universal bacterial primers 341f-GC and 534r. Samples were taken at 14 depths: in the oxic zone (20 and 40 m depth; dashed line), in the SO_4^{2-}-reducing zone (60 and 90 m; dotted line) and in the anoxic zone (>50 m) that is characterized by increased salinity (up to 6 g L^{-1}) below 260 m (dot-dashed line). Bands which were cut for sequencing are marked to the left of the band. (b) RFLP analyses of *mcrA* gene fragments amplified from the hypolimnion. The pattern of an ANME 1 clone is included as a reference. The patterns of selected RFLP fragment types found in clone libraries (capital letters) are presented to the right. The dotted and dot-dashed lines represent the lower boundary of the SO_4^{2-}-reducing zone and the major chemocline, respectively. No PCR product was obtained for samples above 90 m depth.

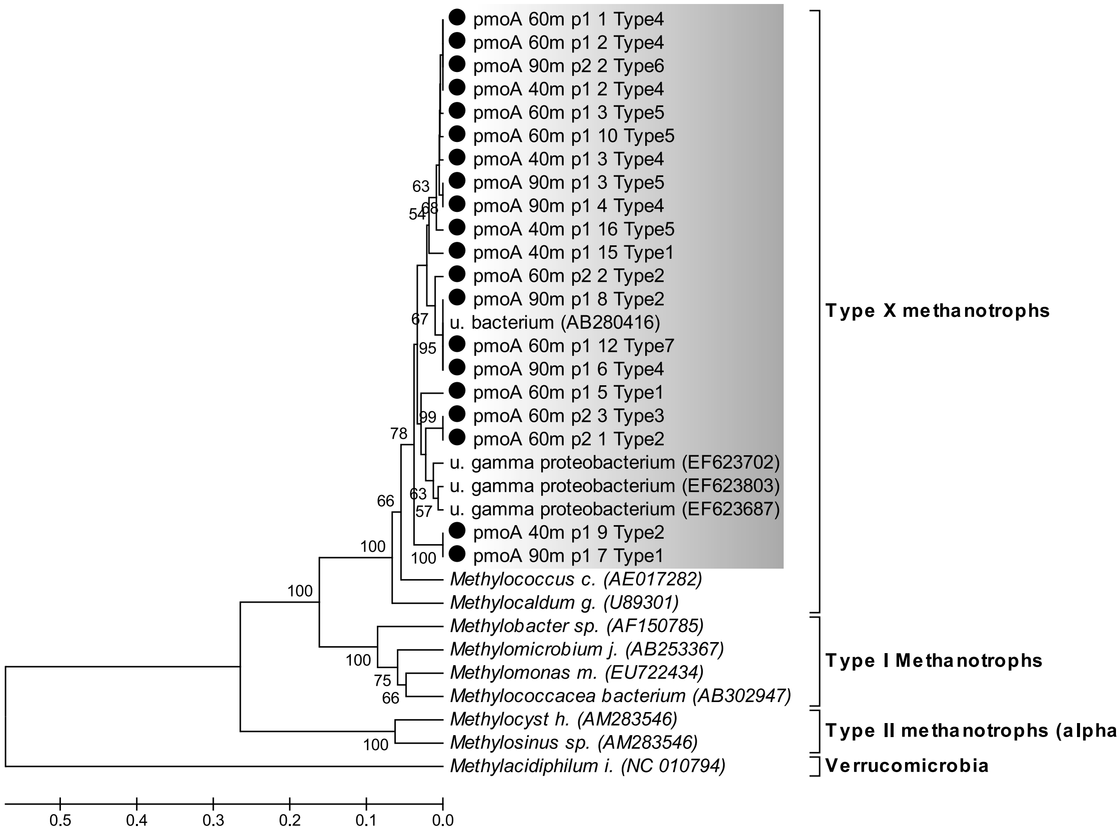

Figure 7. Phylogenetic relationships of PmoA sequences from 0 to 90 m depth (circles) and reference sequences using the UPGMA hierarchical clustering method [*Nei and Kumar*, 2000] and the PAM distance matrix [*Schwarz and Dayhoff*, 1979] as implemented in Mega 4.1, with pairwise gap elimination and 1000 bootstrap resamplings for tree testing. Clone sequences had a length of 166 amino acid residues. The cloned sequences formed two main groups without cultured representatives, which appeared most closely related to PmoA of type X gammaproteobacterial CH_4 oxidizers. Name indicates sample depth, plate number, clone number, and clone phylotype. Scale is number of amino acid substitutions per site.

source. Therefore, the total CH_4 formation averaged over the lake area is

$$P_{CH4\_tot} = a_{shallow}\ P_{CH4\_sed} + a_{deep}\ P_{CH4\_deep} = 69 \pm 21\ \mathrm{g\ C\ m^{-2}\ yr^{-1}},$$

where $a_{shallow} = 0.56$ and $a_{deep} = 0.44$ are the sediment area fractions located at depths <260 m and >260 m, respectively [*Lahmeyer International*, 1998].

[38] CH_4 internal loading, i.e., the total upward flux of CH_4 in the anoxic water column, was independently determined from flux analysis [*Pasche et al.*, 2009]:

$$F_{CH4} = 35 \pm 5\ \mathrm{g\ C\ m^{-2}\ yr^{-1}}.$$

4.3.6. Methane Sinks

[39] Based on model calculation and CH_4 surface concentrations, the loss of CH_4 to the atmosphere was estimated to approximately 1 g C m^{-2} yr^{-1}. A recent thorough analysis by *Borges et al.* [2011] found the loss to the atmosphere to be 0.16 g C m^{-2} yr^{-1}, indicating that we slightly overestimated this flux:

$$L_{atm} = 1 \pm 0.5\ \mathrm{g\ C\ m^{-2}\ yr^{-1}}.$$

The difference between internal loading and loss to the atmosphere comprises the sum of aerobic and anaerobic CH_4 oxidation. The maximum anaerobic CH_4 oxidation with SO_4^{2-} can be calculated from the SO_4^{2-} flux [*Pasche et al.*, 2009]:

$$L_{ox\_SO4} = 1 \pm 0.5\ \mathrm{g\ C\ m^{-2}\ yr^{-1}}.$$

Most of the CH_4 is therefore oxidized aerobically:

$$L_{ox\_O2} = F_{CH4} - L_{atm} - L_{ox\_SO4} = 33 \pm 5\ \mathrm{g\ C\ m^{-2}\ yr^{-1}}.$$

The excess of CH_4 formation, which is currently accumulating in the lake, can be estimated as the difference between CH_4 formation and upward flux:

$$E_{CH4} = P_{CH4\_tot} - F_{CH4} = 34 \pm 22\ \mathrm{g\ C\ m^{-2}\ yr^{-1}}.$$

4.4. Methane Oxidation Incubation Experiment

[40] CH_4 oxidation was verified experimentally with incubation experiments. Significant ($p < 0.05$) decrease of CH_4 over time was only determined at depths 40 and 60 m (Table 2). At 40 m depth, a large proportion of the CH_4 was oxidized during incubation, resulting in a shift of $\delta^{13}C_{CH4}$

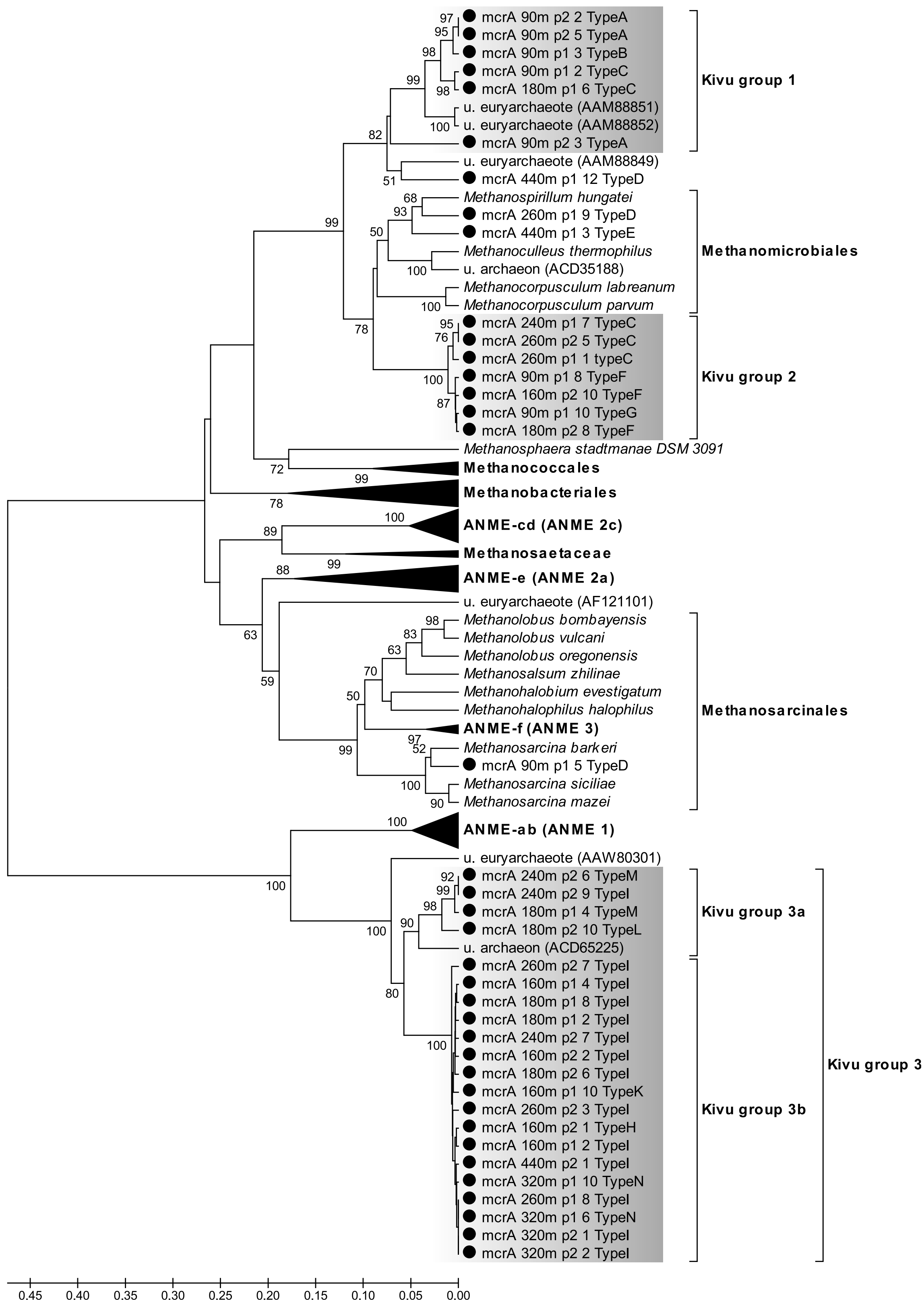

Figure 8. Phylogenetic relationships of McrA sequences from 90 to 440 m depth (circles) and reference sequences using the UPGMA hierarchical clustering method [*Nei and Kumar*, 2000] and the PAM distance matrix [*Schwarz and Dayhoff*, 1979] as implemented in Mega 4.1, with pairwise gap elimination and 1000 bootstrap resamplings for tree testing. Clone sequences had a length of 252 amino acid residues. Kivu groups 1 and 2 appeared closely related to the *Methanomicrobiales*. Group 3 was related to ANME group ab. Scale is number of amino acid substitutions per site.

from −54 to −32‰. A small increase of $\delta^{13}C_{CH4}$ was also observed at 20 m, but was within the error of measurement (±2‰). At 60 m depth, oxygen was depleted at the time of sampling, thus the measured decrease in CH concentration is likely due to anaerobic CH_4 oxidation. Based on published fractionation factors [*Holler et al.*, 2009], a corresponding shift of +1 to +3‰ to a less negative $\delta^{13}C_{CH4}$ would have been expected, which is, however, close to the measurement error, and was not observed. In addition, anaerobic CH_4 oxidation is a process for which low apparent fractionation factors have been reported for environmental samples [*Holler et al.*, 2009]. In summary, results demonstrate active CH_4 oxidation in the 40 m sample, and possibly anaerobic oxidation of CH_4 at 60 m depth. Due to relatively large variations of initial CH_4 concentrations resulting from CH_4 outgassing during filling of the bottles, especially for deep water samples, low rates of CH_4 oxidation would have been undetectable at greater depths.

4.5. Characterization of Microbial Communities

4.5.1. General Microbial Characterization

[41] The bacterial community was characterized using DGGE analysis from amplified 16S rRNA gene fragments (Figure 6a). The community in the oxic (20 and 40 m depth) and in the SO_4^{2-}-reducing (60 and 90 m depth) zones differed strongly from each other and from the deep water community. Between 100 and 260 m depth the community changed little. However, some bands disappeared and some new bands appeared and there were gradual changes in relative band intensity with depth, indicating a slow succession of microbial species in this zone. Below the main chemocline, the community again changed considerably and the number of bands decreased with depth.

[42] Sequencing of excised intense bands (Table S1 in Text S1) revealed typical freshwater bacterial clades in the 20 and 40 m depth samples, e.g., *Actinobacteria* sequences (bands 22, 35, 36). In the hypolimnion several phylotypes closely matched environmental clones from Lake Tanganyika (bands 1, 14, 20), indicating that these are typical components of stratified lakes in the region. An intense band in the SO_4^{2-}-reducing zone (90 m depth, band 18) yielded a *δ-Proteobacterial* sequence with high similarity to SO_4^{2-}-reducing *Desulfocapsa*. Band 4, occurring from 90 to 270 m depth was related to *Lactococcus*, and band 2, occurring from 160 to 400 m depth was classified as belonging to the *Clostridia*, demonstrating the presence of fermenting bacteria among the dominant phylotypes in the hypolimnion.

4.5.2. Aerobic and Anaerobic Methanotrophs

[43] The *pmoA* gene, a marker for aerobic methanotrophs, was readily amplified only from the 40 and 60 m depth samples and weakly from the 90 m sample. Screening and phylogenetic analysis of the clone library created from these amplicons revealed a considerable microdiversity, but all sequenced clones were most closely related to *Methylococcus* and likely represent type X gammaproteobacterial CH_4 oxidizers (Figure 7).

[44] The *mcrA* gene, indicative for methanogenic or anaerobic methanotrophic archaea, was absent from surface waters, but could be PCR-amplified in samples from 90 m depth and below (data not shown). However, RFLP analysis revealed that the 90 m sample (within the SO_4^{2-}-reducing zone) had a community distinct from deeper samples (Figure 6b). Screening of the *mcrA* clone libraries revealed 14 distinct RFLP patterns, and most sequenced clones belonged to three clusters (Kivu groups 1, 2 and 3, Figure 8). Kivu group 1 dominated the 90 m sample library (7 out of 11 clones, 64%) and the most frequent clone RFLP, type (A), is evident as intense bands in the 90 m community RFLP pattern (Figure 6b). Only one representative of this group was found in a library from a greater depth (180 m). Kivu group 2 (Figure 8) was found in libraries from depths 90, 180, 240 and 260 m (1 to 2 clones or ≤20% per library) and its frequent clone types C and F were readily apparent in the community fingerprint (Figure 6b). Both Kivu groups 1 and 2 were most closely related to the *Methanomicrobiales*. Kivu group 3 (with subgroups a and b) was most closely related to the *mcrA* sequences of the ANME-ab clade (associated with ANME 1 [*Hallam et al.*, 2003]) and clearly dominated all clone libraries (60 to 100%) and the community RFLP patterns (Type I and N) in all samples below 90 m depth. The clone RFLP type M was associated with subgroup 3b and appeared to become more abundant below the chemocline according to the community fingerprints. In the 90 m sample, we found no sequences related to group 3, and the clone RFLP patterns associated with this group were not evident in the community fingerprint.

5. Discussion

5.1. Spatial and Temporal Homogeneity of Lake Kivu

[45] By combining data obtained from different sites over a period of five years, we are making assumptions regarding the spatial and temporal homogeneity of Lake Kivu. The chemical and physical data taken over several years and at different locations show that the permanently stratified water column (>60 m depth) is completely homogenous in the horizontal dimension [*Pasche et al.*, 2009]. Chlorophyll and thus primary production is also uniform across the lake, although slightly higher chlorophyll concentrations were found close to the shore [*Kneubühler et al.*, 2007]. This behavior is to be expected, as most of the nutrients stem from the deep water [*Pasche et al.*, 2009], which eliminates any horizontal gradients over the several 100 years of residence. Consistently, sediment cores taken in different parts of the lake reflected the same global changes in lake chemistry [*Pasche et al.*, 2010]. Sedimentation rates and organic matter fluxes however were more variable, e.g., sedimentation rate and TOC flux were 55% and 130% higher in the Gisenyi core compared to the Ishungu core, respectively [*Pasche et al.*, 2010].

[46] Temporal changes in the deep water are notable in the lake, but are slow. The increase in methane concentrations by up to 15% within 30 years [*Schmid et al.*, 2005] corresponds to a change of <0.5% yr^{-1}. All measurements performed within this study were made within a time frame of less than five years. There is no reason to expect changes of more than 2% in the chemical or physical properties of the deep water within this time frame. The only exception from this statement are measurements that are directly related to processes in the surface waters, such as the measured gross sedimentation, which we have corrected for the long-term mean based on available primary productivity data.

5.2. Methanogenesis

5.2.1. Carbon Sources for Methanogenesis

[47] CH_4 in Lake Kivu could be produced by acetoclastic methanogenesis, using acetate produced from OC, or from CO_2 reduction using either geogenic or OC-derived CO_2. Throughout the anoxic water column the CO_2 produced by organic matter fermentation (P_{CO2_sed}, 49 ± 14 g C m^{-2} yr^{-1}) is small compared to the total upward flux of DIC (335 ± 67 g C m^{-2} yr^{-1}) or CO_2 (126 ± 25 g C m^{-2} yr^{-1}) [*Pasche et al.*, 2009]. Geogenic CO_2 (F_{CO2_geo} = 138 ± 30 g C m^{-2} yr^{-1}) is thus far more abundant than CO_2 from mineralization of OC and will be the main substrate for CO_2 reduction. Furthermore, the OC flux observed in sediment traps did not decrease with depth, indicating that mineralization in the anoxic water column is minor [*Pasche et al.*, 2010]. We therefore conclude that acetoclastic methanogenesis in the anoxic water column is limited, although analysis of the *mcrA* gene indicated the presence of a methanogenic population in the water column.

5.2.2. Partitioning Between Methanogenic Pathways

[48] The old age of geogenic CO_2 results in a dead ^{14}C signal (0 pMC). Since Lake Kivu is heavily influenced by geogenic CO_2 this dead radiocarbon strongly influences the apparent ^{14}C ages. This signature can be used to trace the impact of geogenic CO_2 in the system. We observed ^{14}C signals of 28 (15 to 37 cm), 34 (0 to 0.5 cm) and 43 (0.5 to 5 cm) pMC in the sediment OC, while previous measurements resulted in 27 pMC [*Tietze et al.*, 1980]. The observed variability in $^{14}C_{OC}$ cannot be completely explained by the bomb peak [*Manning and Melhuish*, 1994] and might reflect variation in the relative contribution of atmospheric and internally recycled CO_2 to the carbon uptake by primary producers. Modeling of the $^{14}C_{CH4}$ vertical profile in the water column (see section 4.3.4 and Text S1) showed that below 260 m, a large fraction of CH_4 (65 ± 5%) is produced from old carbon. The ^{14}C increase above 260 m results from a combination of the shorter residence time, and a higher proportion (>60%) of CH_4 produced from recently settled OC. The independent estimates of the fraction of CH_4 produced from CO_2 (43 to 94%) based on $\delta^{13}C$ data for CO_2, CH_4, and sediment OC confirmed that this is a realistic estimate. We conclude that below 260 m depth a large fraction (~65%) of CH_4 is produced by reduction of CO_2 with a dead ^{14}C signal, while above 260 m CH_4 is mainly produced from comparatively young OC originating from primary production in the surface mixed layer.

[49] The constant $\delta^{13}C_{CH4}$ and the absence of electron acceptors that could be used for CH_4 oxidation below 100 m, indicated that *mcrA* sequences found in these depths must originate from methanogens, despite the phylogenetic similarity of group Kivu 3 to ANME-ab sequences. The methanogenic archaea in the sediment were not studied, but the presence of unusual *mcrA* groups in the methanogenic zone of the water column indicates that Lake Kivu may harbor an unusual methanogenic community deserving further study.

5.2.3. Origin of Hydrogen

[50] Previous studies have postulated that H_2 used for CO_2 reduction originates from the decomposition of organic matter [*Schoell*, 1980], or from free geogenic H_2 [*Conrad*, 1999; *Deuser et al.*, 1973]. In Lake Kivu we found that below 260 m, only approximately 35% of CH_4 derive from acetoclastic methanogenesis. The δ^2H_{CH4} values of −220‰ in the bottom water are in the range that is typical for carbonate reduction (−250 to −150‰) [*Whiticar*, 1999], supporting the notion that CO_2 reduction is a dominant process in the deep water. A considerable amount of H_2 is required to account for the formation of the remaining 65% ($P_{CH4\_CO2red\_geo}$ = 44 ± 18 g C m^{-2} yr^{-1}) of CH_4 through CO_2 reduction. An additional source of H_2 is therefore necessary to explain our findings.

[51] At the moment we can only speculate on the source of this additional H_2. The gases from Nyiragongo volcano north of Lake Kivu contained only low amounts of H_2 (<2 vol %), also relative to CO_2 (≤1% of CO_2 [*Gerlach*, 1980; *Le Guern*, 1987; *Tedesco et al.*, 2007]). Applying this ratio to the geogenic CO_2 influx (138 ± 30 g C m^{-2} yr^{-1}), this H_2 could only account for <1.4 g C_{CH4} m^{-2} yr^{-1}. Considerably higher H_2 concentrations (23% of CO_2) have been observed in gas bubbles collected in the lake near the lava front of the 2002 Nyiragongo eruption. The source of these gases remains unclear [*Tedesco et al.*, 2007], but nevertheless this may indicate that inflows of groundwater that has been in contact with juvenile gases could be a significant source of H_2. H_2 could also be formed by chemical processes, e.g., pyrite precipitation [*Rickard*, 1997]. An alternative explanation for our observations would be a direct inflow of geogenic CH_4, formed during the cooling of magmatic gases at depth in the absence of oxygen [*Gerlach*, 1980], which would likewise have a dead ^{14}C signal and high δ^2H_{CH4} [*Whiticar*, 1999].

5.2.4. Methane Fluxes

[52] The CH_4 release estimated from the Gisenyi sediment core ranged between 60 and 100 g C_{CH4} m^{-2} yr^{-1} (Figure 5). The upper flux estimate is too high to be explained by the available sedimenting OC but the lower estimate falls within the range calculated from OC gross and net sedimentation (P_{CH4_sed} = 49 ± 14 g C_{CH4} m^{-2} yr^{-1}) after correcting for the unusually low primary productivity during the two years of our sediment trap deployment [*Pasche et al.*, 2010]. Based on the considerations in sections 4.3.4 and 5.2.2, we propose that this flux represents the total CH_4 formation above 260 m but only ~53% of the CH_4 formation below 260 m. Below 260 m, the additional geogenic CH_4 leads to a total formation of 93 ± 30 g C_{CH4} m^{-2} yr^{-1} (P_{CH4_deep}).

5.2.5. Methane Budget and Accumulation

[53] CH_4 concentrations seem to have increased by up to 15% based on measurements made in 1975 and 2004. Our estimate of CH_4 accumulation (E_{CH4} = 34 ± 22 g C_{CH4} m^{-2} yr^{-1}) is still imprecise, but strongly indicates that CH_4 currently accumulates in the lake. Several observations further support the hypothesis of a recent increase in CH_4 concentrations in Lake Kivu. The ^{14}C signal in dissolved CH_4 (11.4 pMC) has increased since 1975 (9 pMC [*Tietze*, 1978]). In the same time span, $\delta^{13}C_{CH4}$ decreased from −57‰ [*Tietze et al.*, 1980] to −59.8‰. These differences in the CH_4 isotopic values are not significant, as they are within the uncertainty range of a comparison of the historic and recent measurements, but they are both consistent with an increased formation of CH_4 with isotopic signatures observed in the surface sediment (34 pMC, $\delta^{13}C$ = −65‰). Furthermore, OC mass accumulation in the sediment has increased by 20% since the 1960s, when carbonates suddenly started to precipitate [*Pasche et al.*, 2010]. However, the calculated range of E_{CH4} (34 ± 22 g C_{CH4} m^{-2} yr^{-1}) does not allow for the

excess required (~75 g C_{CH4} m^{-2} yr^{-1}) to explain the observed CH_4 increase over the last decades [*Schmid et al.*, 2005]. In summary, the analysis supports the hypothesis that CH_4 concentrations in Lake Kivu are currently increasing, but probably at a lower rate than previously assumed.

[54] We propose that several concurrent environmental changes might have increased OC sedimentation [*Pasche et al.*, 2010]. First, an increase of the subaquatic inflows [*Schmid et al.*, 2010], potentially caused by higher rainfall observed during 1961 to 1990 in eastern Africa [*Nicholson and Yin*, 2001], might have led to stronger uplift and consequently greater supply of nutrients. Second, recent external P and N inputs might have increased due to the fast growing population [*Muvundja et al.*, 2009]. Third, the introduction of *Limnothrissa miodon* strongly altered the food web, which might have modified the exported OC [*Isumbisho et al.*, 2006]. As a large proportion of CH_4 seems to be produced from geogenic H_2, we cannot exclude that geogenic inputs have also increased, especially in this geologically active region.

5.3. Methane Oxidation

[55] CH_4 oxidation was confirmed by incubation experiments, the heavier $^{13}C_{CH4}$ in the water layers above 90 m depth, and the presence of specific microbial communities. The determined rates (618 ± 173 and 1096 ± 287 nM d^{-1}) were an order of magnitude higher than those observed in marine environments with relatively high CH_4 concentrations [*Kelley*, 2003] (up to 57 nM d^{-1}) but in the same range as reported for Lake Tanganyika [*Rudd*, 1980] (up to 1920 nM d^{-1}). Comparing $^{13}C_{CH4}$ to electron acceptor depth profiles indicates two major zones for CH_4 oxidation: the oxic layer (0 to 50 m); and the SO_4^{2-}-reducing zone (60 to 90 m). Concentrations of other potential electron acceptors for anaerobic CH_4 oxidation, i.e., nitrate [*Ettwig et al.*, 2010] and oxidized iron and manganese ions [*Beal et al.*, 2009] were too low to significantly oxidize CH_4. At 90 m depth, near the lower end of the SO_4^{2-}-reducing zone, we detected a dominant distinct clade of *mcrA* genes with closest matches to the *Methanomicrobiales*, which could potentially represent a new ANME group. A potential SO_4^{2-}-reducing partner related to *Desulfocapsa* was identified from the general bacterial DGGE (band 18). Flux analyses [*Pasche et al.*, 2009] showed that the SO_4^{2-} flux can oxidize a maximum of 3% (1 ± 0.5 g C m^{-2} yr^{-1}) of the CH_4 upward flux (35 ± 5 g C m^{-2} yr^{-1}). The 3% is probably overestimated, as sulfate reducers can also use organic matter or H_2 as electron donors. In conclusion, anaerobic CH_4 oxidation takes place in Lake Kivu and may involve unique microbial populations but represents only a minor CH_4 sink. This is in contrast to neighboring Lake Tanganyika, where anaerobic CH_4 oxidation consumes about half of the CH_4 transported upwards from the deep waters [*Durisch-Kaiser et al.*, 2011]. The reason for the higher importance of anaerobic CH_4 oxidation is the much higher relative occurrence of SO_4^{2-} to CH_4 in Lake Tanganyika (maximum concentrations 40 μM SO_4^{2-} to 150 μM CH_4) compared to Lake Kivu (150 μM SO_4^{2-} to 20,000 μM CH_4).

[56] Conversely, aerobic CH_4 oxidation is the main sink for CH_4 in Lake Kivu. In addition to the experimental verification of CH_4 oxidation at 40 and 60 m depth we also observed that the $^{13}C_{CH4}$ isotopic signal showed marked (10‰) enrichment at the oxycline (Figure 3). The *pmoA* gene, a marker for aerobic methanotrophs, was also detected in the oxycline in the 40 and 60 m depth samples. The cloned sequences were most closely related to *Methylococcus capsulatus* (type X). However, a more diverse community of CH_4 oxidizers could be present, as the used primer set was reported to have a potential bias toward type X methanotrophs [*McDonald et al.*, 2008; *Rahalkar and Schink*, 2007]. Flux analyses revealed that the O_2 flux (oxidation potential 57 g C m^{-2} yr^{-1}) was higher than the CH_4 upward flux (35 ± 5 g C $m^{-2}yr^{-1}$) and has the potential to oxidize most of the CH_4 present, even if O_2 is shared to oxidize other electron donors [*Pasche et al.*, 2009]. We estimate an oxidation rate of 33 ± 5 g C m^{-2} yr^{-1} (section 4.3.6), as the CH_4 release to the atmosphere is low. The oxidation rate agrees well with a previous estimate (31 g C m^{-2} yr^{-1}) for Lake Kivu [*Jannasch*, 1975]. Aerobic CH_4 oxidation has been estimated to 37 g C m^{-2} yr^{-1} for Lake Tanganyika [*Rudd*, 1980], which is, however, not supported by the estimated areal CH_4 formation of 6 g C m^{-2} yr^{-1} [*Durisch-Kaiser et al.*, 2011].

6. Conclusion

[57] Lake Kivu is a unique ecosystem with extremely high CH_4 concentrations. The lake's volcanic setting provides high quantities of geogenic CO_2. In the deep water, about 65% of the CH_4 originates from dead carbon, suggesting the input of geogenic H_2 or CH_4. The flux analysis as well as several changes in the hydrological and ecological dynamics of Lake Kivu qualitatively support the previously observed CH_4 increase [*Schmid et al.*, 2005]. CH_4 is mainly oxidized by type X CH_4-oxidizing bacteria at the oxycline, while anaerobic CH_4 oxidation plays a minor role, but may involve a novel cluster of ANME.

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