Anaerobic methane oxidation in an East African great lake (Lake Kivu)

Fleur A. E. Roland^{1*}, François Darchambeau¹, Cédric Morana², Sean A. Crowe³, Bo
Thamdrup⁴ and Alberto V. Borges¹

5

¹ Chemical Oceanography Unit, Université de Liège, Belgium

² Department of Earth and Environmental Sciences, Katholieke Universiteit Leuven (KU Leuven),
Belgium

³ Department of Earth, Ocean and Atmospheric Sciences, University of British Columbia, Canada

10 ⁴ Institute of Biology and Nordic Center for Earth Evolution, University of Southern Denmark, Denmark

* Corresponding author: froland@ulg.ac.be

Abstract

15 This study investigates methane (CH_4) oxidation in the water column of Lake Kivu, a deep meromictic
tropical lake containing large quantities of CH_4 in the anoxic deep waters. Depth profiles of dissolved gases (CH_4
and nitrous oxide (N_2O)) and of the different potential electron acceptors for anaerobic methane oxidation (AOM)
(nitrate, sulfate, iron and manganese) were determined during six field campaigns between June 2011 and August
20 campaigns, and denitrification measurements based on stable isotopes were performed twice. Incubation
experiments were performed to quantify CH_4 oxidation and nitrate consumption rates, with a focus on AOM,
without and with an inhibitor of sulfate-reducing bacteria activity (molybdate). Nitrate consumption rates were
measured in these incubations. Substantial CH_4 oxidation activity was observed in oxic and anoxic waters, and in
the upper anoxic waters of Lake Kivu, CH_4 is a major electron donor to sustain anaerobic metabolic processes
25 coupled to AOM. The maximum aerobic and anaerobic CH_4 oxidation rates were estimated to 27 ± 2 and 16 ± 8
 $\mu\text{mol L}^{-1} \text{d}^{-1}$, respectively. We observed a decrease of AOM rates when molybdate was added for half of the
measurements, strongly suggesting the occurrence of AOM linked to sulfate reduction, but an increase of AOM
rates was observed for the other half. Nitrate reduction rates and dissolved manganese production rates tended to
be higher with the addition of molybdate, but the maximum rates of 0.6 ± 0.02 and $11 \pm 2 \mu\text{mol L}^{-1} \text{d}^{-1}$, respectively,
30 were never high enough to explain AOM rates observed at the same depths. We also put in evidence a difference
in relative importance of aerobic and anaerobic CH_4 oxidation between the seasons, with a higher importance of
aerobic oxidation when the oxygenated layer was thicker (in dry season).

1. Introduction

Due to its potential impact in global warming and its increase due to human activities, the biogeochemical cycle of methane (CH_4) raises great interest, and methanogenesis and methanotrophy have been widely studied in a large variety of environments. In natural environments, CH_4 is produced anaerobically by methanogenic archaea. The total CH_4 emission has been recently estimated to $553 \text{ Tg CH}_4 \text{ yr}^{-1}$ for the period 2000-2009, from which 64 % is emitted by tropical areas (Kirschke et al., 2013; Saunio et al., 2016). Previous studies estimated that 9.5% of CH_4 is released from tropical freshwaters and the rest from non-tropical freshwaters (13.5%), marine ecosystems (3%), human activities (63%), plants (6%), gaseous hydrates (2%) and termites (3%) (Conrad, 2009; Bastviken et al., 2011). The real amount of CH_4 produced in these systems is higher, but a significant percentage is biologically oxidized (aerobically or anaerobically) before reaching the atmosphere (Bastviken et al., 2002). Anaerobic CH_4 oxidation (AOM) has been widely observed in marine environments, where it is mainly coupled to sulfate (SO_4^{2-}) reduction (e.g. Iversen and Jørgensen, 1985; Boetius et al., 2000; Jørgensen et al., 2001). In freshwaters environments, AOM has been less studied and is often considered as negligible compared to aerobic CH_4 oxidation due to lower SO_4^{2-} concentrations than in seawater (Rudd et al., 1974). However, other potential electron acceptors for AOM, such as nitrate (NO_3^-), iron (Fe) and manganese (Mn) (Borrel et al., 2011; Cui et al., 2015), can potentially be found in non-negligible concentrations in freshwater environments. Further, in hardwater, saline and volcanic lakes SO_4^{2-} can be abundant.

Lake Kivu is a deep (maximum depth: 485 m) meromictic lake characterized by a high amount of CH_4 (60 km^3 at 0°C and 1 atm) dissolved in its deep anoxic waters. Paradoxically, this lake is a very low emitter of CH_4 to the atmosphere due to intense CH_4 oxidation (Borges et al., 2011; Roland et al., 2016a). It is divided in different basins and bays. In the water column of the main basin, SO_4^{2-} concentrations are relatively high ($100\text{--}200 \mu\text{mol L}^{-1}$; Morana et al., 2016) and a large SO_4^{2-} -reducing bacteria (SRB) community is present and co-occurs with methanotrophic archaea (İnceoğlu et al., 2015). The data based on 16S rRNA strongly suggest the occurrence of AOM coupled to SO_4^{2-} reduction (SDMO), although it remains to be demonstrated in a direct way and quantified. Also, a NO_3^- accumulation zone (nitracline) is often present during the rainy season at the oxic-anoxic interface (Roland et al., 2016a), and can thus potentially contribute to CH_4 oxidation. Based on these observations, we hypothesize that SO_4^{2-} could be the unique electron acceptor involved in AOM during the dry season, while AOM coupled with NO_3^- reduction (NDMO) could also contribute during the rainy season at a lower extent. Potential AOM linked to Fe and Mn reduction will also be investigated.

2. Material and methods

2.1 Sampling sites

Lake Kivu is an East African great lake located at the border between Rwanda and the Democratic Republic of the Congo (Fig. 1). It is divided into one main basin, two small basins and two bays: Northern Basin (or main basin), Southern Basin (or Ishungu Basin), Western Basin (or Kalehe Basin), the bay of Kabuno in the north and the bay of Bukavu in the South.

Six field campaigns were conducted in the main basin (the Northern Basin off Gisenyi; -1.72504°N , 29.23745°E) in June 2011 (early dry season), February 2012 (rainy season), October 2012 (late dry season), May 2013 (late rainy season), September 2013 (dry season) and August 2014 (dry season).

2.2 Physico-chemical parameters and sampling

Vertical profiles of temperature, conductivity, pH and oxygen were obtained with a Yellow Springs Instrument 6600 V2 multiparameter probe. Water was collected with a 7L Niskin bottle (Hydro-Bios) every 2.5 m in a ~10 m zone centered at the oxic-anoxic interface.

2.3 Chemical analyses

Samples for CH₄ and N₂O concentrations, and CH₄ oxidation measurements were collected in 60 ml glass serum bottles, filled directly from the Niskin bottle with tubing, left to overflow, and sealed with butyl stoppers and aluminium caps. Two bottles were directly poisoned with 200 µl of HgCl₂ injected through the septum with a syringe. Ten other bottles were incubated in the dark and at constant temperature close to in situ temperature (~23°C). Five of them received 250 µl of a solution of sodium molybdate (1 mol L⁻¹, hence a final concentration of 4 mmol L⁻¹), and five received no treatment. The bacterial activity of these ten bottles was stopped at 12, 24, 48, 72 and 96h by the addition of 200 µl of HgCl₂. CH₄ and N₂O concentrations were determined via the headspace equilibration technique (20 mL N₂ headspace in 50 mL serum bottles, for samples of the main basin) and measured by gas chromatography (GC) (Weiss, 1981) with electron capture detection (ECD) for N₂O and with flame ionization detection (FID) for CH₄, as described by (Borges et al., 2015). The SRI 8610C GC-ECD-FID was calibrated with certified CH₄:CO₂:N₂O:N₂ mixtures (Air Liquide, Belgium) of 1, 10, 30 and 509 ppm CH₄ and of 0.2, 2.0 and 6.0 ppm N₂O. Concentrations were computed using the solubility coefficients of Yamamoto et al. (1976) and Weiss and Price (1980), for CH₄ and N₂O, respectively. The precision of measurements was ±3.9% and ±3.2% for CH₄ and N₂O, respectively.

Samples for nutrients analyses were collected in 50 ml plastic vials after being filtered through a 0.22 µm syringe filter. 200 µl of H₂SO₄ 5N were added at each vial for preservation. Samples were then frozen. NO₂⁻ and NO₃⁻ concentrations were estimated by spectrophotometry. NO₂⁻ concentrations were determined by the sulfanilamide coloration method (APHA 1998), using a 5-cm light path on a spectrophotometer Thermo Spectronic Genesys 10vis. NO₃⁻ concentrations were determined after vanadium reduction to NO₂⁻ and quantified under this form with a Multiskan Ascent Thermo Scientific multi-plates reader (APHA, 1998; Miranda et al., 2001). The detection limits for these methods were 0.03 and 0.15 µmol L⁻¹ for NO₂⁻ and NO₃⁻, respectively. When making the headspaces for CH₄ measurements as described above, the excess water was collected and used to quantify the evolution of NO₃⁻ concentrations in the incubations (reported as NO₃⁻ consumption rates), according to the method previously described.

Samples for sulfide (HS⁻) concentrations were collected in 50 ml plastic vials, after being filtered on a 0.22 µm syringe filter. Samples were preserved with 200 µl of 20% zinc acetate (ZnAc) and were stored frozen. HS⁻ concentrations were quantified using a 5-cm light path on a spectrophotometer, according to the method described by Cline (1969). Samples for SO₄²⁻ analyses were filtered on a 0.22 µm syringe filter and collected in 5 ml Cryotube vials. Samples were preserved with 20 µl of 20% ZnAc and were stored frozen. SO₄²⁻ concentrations were determined by ion chromatography (Dionex ICS-1500, with an autosampler Dionex AS50, a guard column Dionex AG22 and an analytical column Dionex IonPac AS22). The detection limits of these methods were 0.25 and 0.5 µmol L⁻¹ for HS⁻ and SO₄²⁻, respectively. In August 2014, the decrease of SO₄²⁻ concentrations in CH₄ incubations was measured by spectrophotometry, using a 5-cm light path on a spectrophotometer Thermo

Spectronic Genesys 10vis, according to the nephelometric method described by Rodier et al. (1996), after precipitation of barium sulfate in an acid environment. The detection limit of this method was $52 \mu\text{mol L}^{-1}$.

In May 2013, September 2013 and August 2014, samples for Fe and Mn measurements were collected into 50 ml-plastic syringes directly from the Niskin bottle. Water was rapidly transferred from the syringe to the filtration set and was passed through 25 mm glass fiber filters. Filters were collected in 2 ml Eppendorf vials and preserved with 1 ml of a HNO_3 2% solution, while filtrates were collected into four 2 ml Eppendorf vials and preserved with 20 μl of a HNO_3 65% solution. The filters, for particulate Fe and Mn determination, were mineralized in specific Teflon bombs into a microwave digestion labstation (Ethos D, Milestone Inc.), after digestion with nitric acid. They were finally diluted into milli-Q water to the volume of 50 ml. Filtrates were directly diluted into milli-Q water to the volume of 50 ml. In August 2014, dissolved Mn and Fe concentrations were also determined in CH_4 incubations in order to measure the evolution of concentrations through time. Fe and Mn concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS) using dynamic reaction cell (DRC) technology (ICP-MS SCIEX ELAN DRC II, PerkinElmer inc.). Analytical accuracy was verified by a certified reference material (BCR 715, Industrial Effluent Wastewater).

2.4 CH_4 oxidation, NO_3^- and SO_4^{2-} consumption and Mn^{2+} production rates calculations

CH_4 oxidation, NO_3^- and SO_4^{2-} consumption and Mn^{2+} production rates were calculated as a linear regression of CH_4 , NO_3^- , SO_4^{2-} and Mn^{2+} concentrations over time during the course of the incubation. Rates reported here are maximum rates, as they were calculated based on the maximum slopes. Table 1 shows standard deviations, initial CH_4 concentrations, percentage of CH_4 consumed and the time laps during which the CH_4 oxidation rates were calculated for each depth. Table 2 shows standards deviations for NO_3^- and SO_4^{2-} consumption and Mn^{2+} production rates.

CH_4 oxidation rates with molybdate were corrected taking into account the oxygen supplied by the addition of the solution, which was not anoxic. As molybdate solution was at saturation with respect to O_2 , we considered here that $1.25 \mu\text{mol L}^{-1}$ of O_2 were added to each bottle (250 μl of the solution were added to 60 ml of water). The O_2 concentration required to be responsible for each CH_4 oxidation rate was calculated according to the following stoichiometric equation Eq. (1):

The part of each oxidation rate due to O_2 was then calculated according to Eq. (2):

$$(2) R_o = \frac{R_m}{O_{2r}} * O_{2a}$$

where R_o is the part of each oxidation rate due to O_2 supply, R_m is the measured oxidation rate, O_{2r} is the O_2 concentration required to be responsible for the measured CH_4 oxidation rate and O_{2a} is the O_2 concentration added by the molybdate solution.

The final CH_4 oxidation rates with molybdate were obtained by subtraction (Eq. 3):

$$(3) \text{Final CH}_4 \text{ oxidation rate with Mo} = R_m - R_o$$

All rates with molybdate reported here are final CH₄ oxidation rates.

2.5 N stable isotope labelling experiments

145 In May and September 2013, parallel denitrification experiments were conducted in order to link this process with CH₄ oxidation. Water was collected in duplicate in amber 250 ml borosilicate bottles from the Niskin bottle with tubing, left to overflow, and sealed with Teflon-coated screw caps. Before the injection of ¹⁵N-labeled solutions, a 12h pre-incubation period in the dark and at 25°C was observed in order to allow the consumption of oxygen eventually introduced in the bottles during sampling.

150 N stable isotope labelling experiments were based on Thamdrup and Dalsgaard (2002). Heterotrophic denitrification was determined by the injection of a Na¹⁵NO₃ solution in amber bottles, through the stopper (final concentration of 5 μmol L⁻¹). Six 12 ml vials (Labco Exetainer) were then filled from each of the duplicate bottles and placed in the dark in an incubator at ambient temperature (24°C), which was close to the in situ temperature (~23°C). Microbial activity in two Exetainers was immediately arrested through the addition of 500 μl 20% ZnAc.
 155 A time course was established by arresting two further Exetainers at 6, 12, 18, 24 and 48 h. While injecting ZnAc solution to stop the incubations of the Exetainers, the excess water was collected in 2 ml-Eppendorf vials, and stored frozen, to determine the evolution of the NO_x⁻ concentrations through time. NO_x⁻ were then analyzed by chemiluminescence, after reduction with vanadium chloride (VCl₃), with an NO₂⁻, NO₃⁻ and NO_x analyzer (Thermo Environmental Instruments), according to the method described by Braman and Hendrix (1989) (detection limit:
 160 2-3 ng NO_x).

²⁹N₂ and ³⁰N₂ concentrations in the Exetainers were measured with a gas source isotope ratio mass spectrometer (Delta V Plus, ThermoScientific) after creating a 2 ml helium headspace (volume injected in the mass spectrometer: 50 μl). Potential denitrification rates (detection limits of 2.7 nmol L⁻¹ h⁻¹) in the incubations with ¹⁵NO₃⁻ were calculated according to Eq. (4) (Thamdrup and Dalsgaard, 2002):

165
$$(4) \text{ Potential } N_2 \text{ denitrification} = {}^{15}N^{15}N_{\text{excess}} * (F_{NO_3})^{-2}$$

where N₂ denitrification is the production of N₂ by denitrification during the incubations with ¹⁵NO₃⁻. ¹⁵N¹⁵N_{excess} is the production of excess ¹⁵N¹⁵N and F_{NO₃} is the fraction of ¹⁵NO₃⁻ added in the NO_x⁻ pool. NO_x⁻ concentrations of the NO_x⁻ pool were measured in the incubations as described above. ¹⁵N¹⁵N is the excess relative to the mass 30: mass 28 ratio in the time zero gas samples.

170 Only natural denitrification rates are reported here. Natural denitrification rates were calculated on the base on potential denitrification rates, according to Eq. (5):

$$(5) \text{ Natural } N_2 \text{ denitrification} = \text{Potential } N_2 \text{ denitrification} * (1 - F_{NO_3}),$$

which assumes that the rate obeys 1st order kinetics with respect to NO₃⁻.

175

3. Results

3.1 Physico-chemical characteristics of the water column

Vertical profiles of physico-chemical variables differed strongly between stations and campaigns (Fig. 2). In the dry season, the water column was anoxic from 47.5, 57.5, 55 and 60 m in June 2011, October 2012, September 2013 and August 2014, respectively. In the rainy season, it was anoxic from 45 and 55 m in February 2012 and May 2013, respectively. At each date, the thermocline mirrored the oxycline and temperature at the oxic-anoxic interface averaged $23.5 \pm 0.2^\circ\text{C}$ (mean $\pm$ standard deviation). The chemocline (based on conductivity and pH) was relatively stable and started at ~ 50 m.

CH_4 concentrations were low ($0.3 \pm 0.5 \mu\text{mol L}^{-1}$) from the surface to 45–55 m where they started to increase; at 70 m, CH_4 concentrations were $385 \pm 43 \mu\text{mol L}^{-1}$ (Fig. 3). For all campaigns, N_2O concentrations were higher in oxic waters ($7.0 \pm 0.4 \text{ nmol L}^{-1}$ from 0–40 m) than in anoxic waters ($1.3 \pm 0.8 \text{ nmol L}^{-1}$ below 60 m depth). In February 2012 and September 2013, peaks of N_2O up to 15 nmol L^{-1} were observed at 50 m (anoxic waters) and at 45 m (oxic waters), respectively.

NO_x profiles also reflected the seasonal variations of the water column characteristics. A zone of NO_x accumulation (nitracline) was not observed in June 2011 and May 2013, and a small one ($< 2 \mu\text{mol L}^{-1}$ at 52.5 m) was observed in August 2014. In February 2012, October 2012 and September 2013, NO_x maxima of $3 \mu\text{mol L}^{-1}$ (at 50 m), $4 \mu\text{mol L}^{-1}$ (at 50 m) and $4 \mu\text{mol L}^{-1}$ (at 47.5 m), respectively, were observed. For all campaigns, NH_4^+ concentrations were low ($< 1 \mu\text{mol L}^{-1}$) in oxic waters, and increased in anoxic waters ($82 \pm 59 \mu\text{mol L}^{-1}$ at 70 m). SO_4^{2-} and H_2S concentrations did not show high fluctuations between the different campaigns, with means of $153 \pm 21 \mu\text{mol L}^{-1}$ in oxic waters (from 0 to 50 m depth) and $42 \pm 25 \mu\text{mol L}^{-1}$ in anoxic waters (at 70 m depth), respectively. SO_4^{2-} concentrations strongly decreased at the oxic-anoxic interface (until $\sim 0 \mu\text{mol L}^{-1}$ at 80 m depth), while HS^- concentrations increased.

Fe concentrations were very low (less than $2 \mu\text{mol L}^{-1}$ all along the vertical profiles, not shown). Particulate Mn concentrations were low (less than $2 \mu\text{mol L}^{-1}$), with a maximum concentration peak located just above the oxic-anoxic interface, for the three campaigns. Dissolved Mn concentrations increased with depth, until maximum concentrations of $10 \mu\text{mol L}^{-1}$ in anoxic waters.

3.2 Microbial process rate measurements

CH_4 oxidation was detected during all field campaigns (Fig. 4). Aerobic CH_4 oxidation rates tended to be faster than anaerobic ones, since aerobic CH_4 oxidation consumed on average 0.9 % of initial CH_4 per hour, while anaerobic CH_4 oxidation consumed 0.2 % of initial CH_4 per hour. With molybdate added, 0.2 % of initial CH_4 concentrations were also consumed per hour on average.

The dry season was characterized by higher maximum CH_4 oxidation rates in oxic waters compared to anoxic waters. The maximum oxic and anoxic oxidation rates were observed in August 2014 and were 27 ± 2 (at 55 m) and 16 ± 8 (at 75 m) $\mu\text{mol L}^{-1} \text{ d}^{-1}$, respectively. This high anoxic rate was accompanied by a high SO_4^{2-} consumption rate of $18 \pm 6 \mu\text{mol L}^{-1} \text{ d}^{-1}$ observed at 70 m depth. CH_4 oxidation rates, especially those located in anoxic waters, were lower during the other field campaigns, but were detectable and non-negligible. Maximum

oxic CH_4 oxidation rates were 2 ± 0.04 and $13.9 \pm 0.0 \mu\text{mol L}^{-1} \text{d}^{-1}$, while maximum anoxic rates were 0.8 ± 0.01 (at 47.5 m) and 3.5 ± 0.3 (at 65 m) $\mu\text{mol L}^{-1} \text{d}^{-1}$, in June 2011 and September 2013, respectively. In October 2012, no CH_4 oxidation rate was observed in anoxic water, but a high rate of $10.2 \pm 0.4 \mu\text{mol L}^{-1} \text{d}^{-1}$ was observed in
 215 oxic waters. NO_3^- consumption rates tended to be low during all the campaigns, but a non-negligible natural denitrification rate of $1.5 \mu\text{mol L}^{-1} \text{d}^{-1}$ was observed at 65 m in September 2013, in parallel incubations.

During the rainy season, maximum CH_4 oxidation rates in anoxic waters were higher than in oxic waters. In February 2012, the maximum anoxic CH_4 oxidation rate of $7.7 \pm 0.4 \mu\text{mol L}^{-1} \text{d}^{-1}$ was observed at 50 m and co-occurred with the maximum NO_3^- consumption rate of $0.4 \pm 0.1 \mu\text{mol L}^{-1} \text{d}^{-1}$. In May 2013, the maximum anoxic
 220 CH_4 oxidation rate of $3.2 \mu\text{mol L}^{-1} \text{d}^{-1}$ was observed at 65 m, which was close to a NO_3^- consumption rate of $0.03 \pm 0.01 \mu\text{mol L}^{-1} \text{d}^{-1}$ observed at 70 m depth. Also, higher rates of natural denitrification (based on ^{15}N) were observed between 60 and 70 m depth, with a maximum of $0.7 \mu\text{mol NO}_3^- \text{L}^{-1} \text{d}^{-1}$ at 60 m depth. No oxic CH_4 oxidation rate was observed in February 2012, while a maximum rate of $1.5 \pm 0.2 \mu\text{mol L}^{-1} \text{d}^{-1}$ was observed in May 2013.

In February 2012 and August 2014, CH_4 oxidation rates decreased when molybdate was added, while rates tended to increase when molybdate was added during the other field campaigns. In October 2012 and May 2013 in particular, CH_4 oxidation rates strongly increased when molybdate was added, from 0 ± 0 to 23 ± 0.4 $\mu\text{mol L}^{-1} \text{d}^{-1}$ (at 80m) and from 3 to 18 $\mu\text{mol L}^{-1} \text{d}^{-1}$ (at 65 m), respectively. In September 2013, CH_4 oxidation rates increased when molybdate was added up to $11 \pm 0.5 \mu\text{mol L}^{-1} \text{d}^{-1}$ at 55 m. In August 2014, the addition of
 230 molybdate was also accompanied by a strong increase of dissolved Mn (Mn^{2+}) production rates. NO_3^- consumption rates also tended to increase when molybdate was added during all field campaigns.

4. Discussion

High CH_4 oxidation rates were observed in oxic and anoxic waters (maximum 27 ± 2 and $16 \pm 8 \mu\text{mol L}^{-1} \text{d}^{-1}$, respectively). Aerobic CH_4 oxidation rates were sometimes very high when considering the initial CH_4
 235 concentrations (Table 1). For example, the maximum aerobic CH_4 oxidation rate of $27 \pm 2 \mu\text{mol L}^{-1} \text{d}^{-1}$ observed at 55 m depth in August 2014 occurred at CH_4 concentrations of $42 \pm 2 \mu\text{mol L}^{-1}$. However, as shown in Table 1, this rate applied on a period of 24h, and 68 % of the initial CH_4 was consumed after 24h. The same observation is made, for example, in June 2011 (at 42.5 and 45 m), February 2012 (at 50 m) and October 2012 (at 53 m).

A great variability in oxidation rates was observed between the different campaigns. The main pathway
 240 of CH_4 oxidation was aerobic in June 2011, October 2012 and September 2013 (dry season) and anaerobic in February 2012 and May 2013 (rainy season) (Table 3). In August 2014, aerobic and anaerobic oxidation rates were quite equivalent. As shown by Figure 5, aerobic oxidation rates tended to depend on the oxygenated layer depth. Aerobic CH_4 oxidation rates tended to be higher when the mixed surface water was deeper, as usually observed in dry season. This observation confirms hypothesis by Roland et al. (2016a) who suggested, based on the seasonal evolution with depth of CH_4 concentrations, that during the dry season, the oxic layer deepens and integrated
 245 aerobic CH_4 oxidation on the oxic water column is higher. On the contrary, during the rainy season, the oxic layer is thinner, and a greater amount of CH_4 can be anaerobically oxidized before reaching the oxic part of the water column. While aerobic CH_4 oxidation is probably limited by CH_4 concentrations, AOM is probably limited by the availability of electron acceptors due to competition with more favorable processes (such as heterotrophic

denitrification, sulfate reduction etc.). Also episodic fluctuations in water column characteristics influence bacterial communities and small variations in the water column structure may influence the abundance and/or distribution of bacterial communities, and thus contribute to differences observed. Anyway, the relatively high aerobic and anaerobic CH₄ oxidation rates measured during this study and estimated from ¹³C-CH₄ production by Morana et al. (2015a) explain the low CH₄ fluxes observed throughout the year in Lake Kivu (Borges et al., 2011; Roland et al., 2016a).

Aerobic and anaerobic CH₄ oxidation rates are also high compared with most other lakes (Table 4). Large differences observed can be easily explained by the different characteristics of the environments, such as vertical structure of the water column, CH₄ concentrations, O₂ and other electron acceptors concentrations, or water temperature. Lake Kivu is a tropical lake, so high water temperatures enhance bacterial activity, contrary to temperate and boreal lakes. Also, the water column of Lake Kivu allows the accumulation of high CH₄ concentrations in anoxic waters, which can slowly diffuse to the oxic compartment, allowing the occurrence of both aerobic and anaerobic CH₄ oxidation. Very high AOM rates observed in Lake Matano compared with Lake Kivu may be explained by higher CH₄ concentrations in anoxic waters and greater concentrations of the highly favorable electron acceptor Fe (Sturm et al., 2016).

Different depth profile patterns were observed when molybdate, the inhibitor of sulfate-reducing bacteria activity, was added. About half of the measurements gave lower rates with molybdate added, and the other half gave higher rates (Fig. 6). In February 2012 and August 2014, AOM rates were lower with molybdate, suggesting the occurrence of AOM coupled with SO₄²⁻ reduction. Measurement of the SO₄²⁻ consumption rate during the incubations were performed in August 2014. The highest rate of SO₄²⁻ consumption (18 ± 6 μmol L⁻¹ d⁻¹) was observed at 70 m, what was close to the higher AOM peak of 16 ± 8 μmol L⁻¹ d⁻¹ observed at 75 m depth. In terms of stoichiometry, Fig. 7a shows that one AOM peak can be easily explained by the SO₄²⁻ reduction rate, since 1 mole of SO₄²⁻ is needed to oxidize 1 mole of CH₄, according to Eq. (6):

SO₄²⁻ consumption rates were calculated from the change in time of SO₄²⁻ concentrations measured with the nephelometric method, which might not be precise enough, since the detection limit was 52 μmol L⁻¹. Vertical profiles of SO₄²⁻ concentrations, measured by ion chromatography (detection limit of 0.5 μmol L⁻¹), show that SO₄²⁻ is present in enough quantity to explain AOM rates observed, for all campaigns (Table 5).

In February 2012, the maximum AOM peak of 7.7 ± 0.4 μmol L⁻¹ d⁻¹ co-occurred with the maximum NO₃⁻ consumption peak of 0.4 ± 0.1 μmol L⁻¹ d⁻¹, at 50 m depth, suggesting that a part of AOM might have been due to NO₃⁻ reduction. NO₃⁻ consumption rates do not clearly determine if denitrification occurs in Lake Kivu, since the NO₃⁻ consumption recorded during the incubations might reflect the incorporation of N into the biomass, or reduction to ammonium. However, in a companion paper, we showed that natural denitrification occurred in the Northern Basin in 2011 and 2012, at rates ranging between 1.2 and 2232 nmol NO₃⁻ L⁻¹ d⁻¹, during the same field campaigns, at depths close to those where we observed AOM rates (Roland et al., 2016b). Moreover, in May and September 2013, we also observed natural denitrification rates in parallel incubations, and higher denitrification rates co-occurred with higher AOM rates. Fig. 7b and 7c show AOM rates calculated based on the NO₃⁻ consumption rates measured in the incubations and based on natural denitrification rates measured in parallel

incubations (only in May and September 2013), respectively, and according to the stoichiometry of Eq. (7), where 8 moles of NO_3^- are needed for 5 moles of CH_4 (Raghoebarsing et al., 2006):

All AOM rates cannot be solely explained by NO_3^- consumption rates. Even natural denitrification rates are not sufficient to explain AOM rates observed. This discrepancy suggests that NO_3^- is not an important electron acceptor for AOM in Lake Kivu, which is not surprising when considering low natural NO_3^- concentrations. Indeed, the majority of AOM rates observed cannot be explained by the NO_x concentrations (Table 3). Only very
295 low AOM rates can be fully explained by NO_x concentrations. MnO_2 and Mn^{2+} concentrations were also measured in May 2013, September 2013 and August 2014 (Fig. 3). AOM can occur with MnO_2 as electron acceptor, and produce Mn^{2+} according to Eq. (8) (Beal et al., 2009):

According to this relationship, 1 mole of CH_4 consumes 4 moles of MnO_2 and produces 4 moles of Mn^{2+} .
300 In May 2013 and September 2013, we can see that MnO_2 can fully contribute to AOM at depths near the oxic-anoxic interface (Table 5). At 65 m in May 2013, we can see that MnO_2 can only contribute for 45% of AOM rate. Particulate Mn concentrations were very low compared with dissolved Mn concentrations, with a peak located just above the oxic-anoxic interface, for each campaign. Jones et al. (2011) showed the same profile in Lake Matano, and concluded that Mn is recycled at least 15 times before sedimentation. Mn^{2+} is probably oxidized in presence
305 of small quantities of O_2 , precipitates and is directly reduced in anoxic waters. The same profile is probably observed in Lake Kivu, and MnO_2 can thus probably significantly contributes to AOM only at depths close to the oxic-anoxic interface. A significant part of AOM could be due to MnO_2 reduction for each depth if we take into account Mn^{2+} concentrations (Table 5), and considering two hypotheses: 1) All the Mn^{2+} measured at each depth come from the reduction of precipitated MnO_2 , and 2) all the Mn^{2+} come from MnO_2 reduction with CH_4 . However,
310 these hypotheses are unlikely, since Mn^{2+} present at each depth can come from diffusion from upper depths, and MnO_2 can be reduced by other electron donors than CH_4 . Also, for SO_4^{2-} and NO_x , other processes such as SO_4^{2-} reduction with organic matter and heterotrophic denitrification can take place. The percentages of AOM reported in Table 5 and the calculated AOM rates reported in Fig. 7 are thus potential maximum percentages and rates. Nevertheless, CH_4 has the potential to be the major electron donor in anoxic waters of Lake Kivu based on
315 consideration of the standing stocks and fluxes of carbon. Indeed, CH_4 concentration at 70 m is $\sim 385 \mu\text{mol L}^{-1}$ which is distinctly higher than typical dissolved organic carbon concentrations of $142 \mu\text{mol L}^{-1}$ that is very refractory anyway (Morana et al., 2014; 2015b) and particulate organic carbon (POC) concentrations in anoxic waters typically lower than $30 \mu\text{mol L}^{-1}$ (Morana et al., 2015b). In terms of supply of carbon, the CH_4 vertical flux of $9.4 \text{ mmol m}^{-2} \text{ d}^{-1}$ (Morana et al., 2015a) is also higher compared to the downward flux of POC from the mixed
320 layer of $5.2 \pm 1.7 \text{ mmol m}^{-2} \text{ d}^{-1}$ (average value of 24 month-deployment of sediment traps in the Northern Basin from November 2012 to November 2014, unpublished data). This is in general agreement with the high methanotrophic production in Lake Kivu ($8.2 - 28.6 \text{ mmol m}^{-2} \text{ d}^{-1}$) estimated by parallel study (Morana et al., 2015a).

Considering very high SO_4^{2-} concentrations compared with other potential electron acceptors (mean of 103,
325 0.40 and $0.42 \mu\text{mol L}^{-1}$ for SO_4^{2-} , NO_x and particulate Mn, respectively, at depths where AOM was observed), it

is likely that AOM in Lake Kivu is mainly coupled to SO_4^{2-} reduction. Moreover, half of the measurements showed that the inhibition of SRB activity by molybdate induced a decrease of AOM rates. However, the other half of the measurements showed that AOM rates were higher when molybdate was added. These results were surprising and difficult to explain. We firstly considered if we artificially induced aerobic oxidation by injecting molybdate, since the solution was not anoxic. As described in Sect. 2.4, we calculated the impact of O_2 supply for each CH_4 oxidation rate, which was clearly limited, since the median value of relative standard deviations (between rates with molybdate and rates with molybdate if no O_2 was added) was 2.5 %, and thus did not strongly influence CH_4 oxidation rates. Even if a significant artificially-induced aerobic oxidation can be ruled out, the O_2 supply could potentially induce NO_3^- and SO_4^{2-} production, and thus increase AOM linked to these electron acceptors. However, no increased NO_3^- production was observed during the incubations with molybdate (data not shown). We were not able to directly measure the SO_4^{2-} concentrations in incubations with molybdate with the nephelometric method, due to a reaction between molybdate and reagents inducing absorbance higher than the maximum absorbance measurable for the specific wavelength. Since HS^- oxidation is very fast (Canfield et al., 2005), it is very likely that the artificially introduced O_2 was directly consumed by this way, and thus that SO_4^{2-} concentrations were higher in incubations with molybdate. However, the increase of AOM in presence of molybdate was always observed in samples collected at depth where SO_4^{2-} concentrations were higher than $100 \mu\text{mol L}^{-1}$, so it seems unlikely that the addition of $1.25 \mu\text{mol L}^{-1}$ of O_2 , which would produce $0.62 \mu\text{mol L}^{-1}$ of SO_4^{2-} (according to Eq. (9); Lens, 2009) could be responsible for a significant increase of AOM rates, in a context where SO_4^{2-} were not limiting.

We can thus hypothesize that a modification in competitive relationships among the bacterial community in presence of molybdate, such as a decrease of competition between denitrifying bacteria and/or Mn-reducing bacteria and SRB, would explain the higher NO_3^- consumption rates observed with molybdate added. Also, Mn^{2+} production rates increased with molybdate in August 2014. Competitive relationships for electron donors among bacterial communities have already been observed in literature (e.g. Westermann and Ahring, 1987, Achtnich et al., 1995). However, in Lake Kivu, it is unlikely that the strong increase in AOM rates was only due to a change in competition between SRB and denitrifying bacteria and/or SRB and Mn-reducing bacteria, since NO_3^- and MnO_2 concentrations are in any way insufficient to be responsible for all AOM rates.

In conclusion, we put in evidence and measured AOM in the water column of a meromictic tropical lake. We were not able to clearly identify the main electron acceptor of AOM based on this dataset, but considering the high SO_4^{2-} concentrations, it is likely that AOM could be mainly coupled to SO_4^{2-} reduction. A seasonal variability in the respective importance of aerobic and anaerobic CH_4 oxidation rates was observed, with a higher importance of aerobic oxidation in dry season and of AOM in rainy season. This can be linked to the position of the oxygenated layer depth, which is located deeper during the dry season, due to the seasonal mixing of the mixolimnion. At this period of the year, the oxic-anoxic interface is located close to the chemocline, below which the concentration of CH_4 are typically 5 orders of magnitude larger than in the upper part of the mixolimnion. By contrast, during the rainy season, when the thermal stratification within the mixolimnion is well established, the volume of the oxic compartment is smaller than the volume of the anoxic compartment, and hence CH_4 can only reach the oxic waters

by diffusion, after that a significant fraction of the CH₄ upward flux has been oxidized by AOM, which limit the
365 aerobic CH₄ oxidation.

Author contributions

FAER, FD and AVB designed the experiments. FAER and AVB were responsible for manuscript preparation. FAER was also responsible for the fieldwork, the data acquisition, analysis and interpretation.

370 CM, FD, SC participated in fieldwork. CM, FD, SC and BT were involved in scientific discussions, data interpretation and manuscript preparation. SC was also involved in measurements of nutrients, sulfate and sulfide concentrations. BT also organized denitrification, nutrients, sulfate and sulfide measurements in the Institute of Biology and Nordic Center for Earth Evolution (University of Southern Denmark).

Acknowledgements

375 We thank the Rwanda Energy Company for the access to their platform for the sampling, Renzo Biondo (University of Liège), Laura Bristow, Dina Holmgaard Skov and Heidi Grøn Jensen (University of Southern Denmark) for help in measurements. This study was funded by the Belgian Federal Science Policy Office (BELSPO, Belgium) under the EAGLES (East African Great lake Ecosystem Sensitivity to Changes, SD/AR/02A) project, by the Fonds National de la Recherche Scientifique (FNRS) under the MICKI (Microbial diversity and processes in Lake Kivu, 1715859) project, and contributes to the European Research Council (ERC) starting grant
380 project AFRIVAL (African river basins: Catchment-scale carbon fluxes and transformations, 240002). GC was acquired with funds from the FNRS (contract n°2.4.598.07). AVB is a senior research associate at the FNRS. FAER has a PhD grant from FNRS («Fonds pour la formation à la Recherche dans l'Industrie et dans l'Agriculture» - FRIA).

References

- 385 Achtnich, C., Bak, F., and Conrad, R.: Competition for electron donors among nitrate reducers, ferric iron reducers, sulfate reducers, and methanogens in anoxic paddy soil, *Biol Fert Soils*, 19, 65-72, doi:10.1007/BF00336349, 1995.
- APHA: Standard methods for the examination of water and wastewater, edited by: APHA, Washington, 1998.
- 390 Bastviken, D., Ejlerthsson, J., and Tranvik, L.: Measurement of methane oxidation in lakes: A comparison of methods, *Environ. Sci. Technol.*, 36, 3354-3361, doi:10.1021/es010311p, 2002.
- Bastviken, D., Tranvik, L. J., Downing, J. A., Crill, P. M., and Enrich-Prast, A.: Freshwater methane emissions offset the continental carbon sink, *Science*, 331, 50, doi:10.1126/science.1196808, 2011.
- Beal, E. J., House, C. H., and Orphan, V. J.: Manganese- and iron-dependent marine methane oxidation, *Science*, 325, 184-187, doi:10.1126/science.1169984, 2009.
- 395 Boetius, A., Ravensschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jørgensen, B. B., Witte, U., and Pfannkuche, O.: A marine microbial consortium apparently mediating anaerobic oxidation of methane, *Nature*, 407, 623-626, doi:10.1038/35036572, 2000.
- Borges, A. V., Abril, G., Delille, B., Descy, J. P., and Darchambeau, F.: Diffusive methane emissions to the atmosphere from Lake Kivu (Eastern Africa), *J. Geophys. Res. Biogeosci.*, 116, doi:10.1029/2011JG001673, 2011.
- 400 Borges, A. V., Darchambeau, F., Teodoru, C. R., Marwick, T. R., Tammooh, F., Geeraert, N., Omengo, F. O., Guérin, F., Lambert, T., and Morana, C.: Globally significant greenhouse-gas emissions from African inland waters, *Nat. Geosci.*, 8, 637, doi:10.1038/ngeo2486, 2015.
- Borrel, G., Jézéquel, D., Biderre-Petit, C., Morel-Desrosiers, N., Morel, J.-P., Peyret, P., Fonty, G., and Lehours, A.-C.: Production and consumption of methane in freshwater lake ecosystems, *Res. Microbiol.*, 162, 832-847, doi:10.1016/j.resmic.2011.06.004, 2011.
- 405 Brame, R. S., and Hendrix, S. A.: Nanogram nitrite and nitrate determination in environmental and biological materials by vanadium(III) reduction with chemiluminescence detection, *Anal. Chem.*, 61, 2715-2718, doi:10.1021/ac00199a007, 1989.
- 410 Canfield, D. E., Kristensen, E., and Thamdrup, B.: The sulfur cycle, *Adv. Mar. Biol.*, 48, 313-381, doi:10.1016/S0065-2881(05)48009-8, 2005.
- Cline, J. D.: Spectrophotometric determination of hydrogen sulfide in natural waters, *Limnol. Oceanogr.*, 14, 454-458, doi:10.4319/lo.1969.14.3.0454, 1969.
- Conrad, R.: The global methane cycle: Recent advances in understanding the microbial processes involved, *Environ. Microbiol. Rep.*, 1, 285-292, doi:10.1111/j.1758-2229.2009.00038.x, 2009.
- 415 Cui, M., Ma, A., Qi, H., Zhuang, X., and Zhuang, G.: Anaerobic oxidation of methane: an "active" microbial process, *Microbiologyopen*, 4, 1-11, doi:10.1002/mbo3.232, 2015.
- İnceoğlu, Ö., Llírs, M., García-Armisen, T., Crowe, S. A., Michiels, C., Darchambeau, F., Descy, J.-P., and Servais, P.: Distribution of bacteria and archaea in meromictic tropical Lake Kivu (Africa), *Aquat. Microb. Ecol.*, 74, 215-233, doi:10.3354/ame01737, 2015.
- 420 Iversen, N., and Jørgensen, B.: Anaerobic methane oxidation rates at the sulfate-methane transition in marine sediments from Kattegat and Skagerrak (Denmark), *Limnol. Oceanogr.*, 30, 944-955, doi:10.4319/lo.1985.30.5.0944, 1985.
- Iversen, N., Oremland, R. S., and Klug, M. J.: Big Soda Lake (Nevada). 3. Pelagic methanogenesis and anaerobic methane oxidation, *Limnol. Oceanogr.*, 32, 804-814, doi:10.4319/lo.1987.32.4.0804, 1987.
- 425 Jones, C., Crowe, S. A., Sturm, A., Leslie, K. L., MacLean, L. C. W., Katsev, S., Henny, C., Fowle, D. A., and Canfield, D. E.: Biogeochemistry of manganese in ferruginous Lake Matano, Indonesia, *Biogeosciences*, 8, 2977-2991, doi:10.5194/bg-8-2977-2011, 2011.
- Jørgensen, B. B., Weber, A., and Zopfi, J.: Sulfate reduction and anaerobic methane oxidation in Black Sea sediments, *Deep-Sea Res. Pt. I*, 48, 2097-2120, doi:10.1016/S0967-0637(01)00007-3, 2001.
- 430 Kirschke, S., Bousquet, P., Ciais, P., Saunoy, M., Canadell, J. G., Dlugokencky, E. J., Bergamaschi, P., Bergmann, D., Blake, D. R., Bruhwiler, L., Cameron-Smith, P., Castaldi, S., Chevallier, F., Feng, L., Fraser, A., Heimann, M., Hodson, E. L., Houweling, S., Josse, B., Fraser, P. J., Krummel, P. B., Lamarque, J. F., Langenfelds, R. L., Le Qué, C., Naik, V., O'Doherty, S., Palmer, P. I., Pison, I., Plummer, D., Poulter, B., Prinn, R. G., Rigby, M., Ringeval, B., Santini, M., Schmidt, M., Shindell, D. T., Simpson, I. J., Spahni, R., Steele, L. P., Strode, S. A., Sudo, K., Szopa, S., Van Der Werf, G. R., Voulgarakis, A., Van Weele, M., Weiss, R. F., Williams, J. E., and Zeng, G.: Three decades of global methane sources and sinks, *Nat. Geosci.*, 6, 813-823, doi:10.1038/ngeo1955, 2013.
- Lens, P.: Sulfur cycle, in: *Encyclopedia of microbiology*, edited by: Schaechter, A., Elsevier, USA, 361-369, 2009.
- 440 Lopes, F., Viollier, E., Michard, G., Abril, G., Groleau, A., Prévot, F., Carrias, J.-F., Albéric, P., and Jézéquel, D.: Biogeochemical modelling of anaerobic vs. aerobic methane oxidation in a meromictic

- crater lake (Lake Pavin, France), *Appl. Geochem.*, 26, 1919-1932, doi:10.1016/j.apgeochem.2011.06.021, 2011.
- 445 Miranda, K. M., Espey, M. G., and Wink, D. A.: A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite, *Nitric Oxide-Biol. Ch.*, 5, 62-71, doi:10.1006/niox.2000.0319, 2001.
- Morana, C., Sarmiento, H., Descy, J. P., Gasol, J. M., Borges, A. V., Bouillon, S., and Darchambeau, F.: Production of dissolved organic matter by phytoplankton and its uptake by heterotrophic prokaryotes in large tropical lakes, *Limnol. Oceanogr.*, 59, 1364-1375, doi:10.4319/lo.2014.59.4.1364, 2014.
- 450 Morana, C., Borges, A. V., Roland, F. A. E., Darchambeau, F., Descy, J. P., and Bouillon, S.: 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, 2015a.
- Morana, C., Darchambeau, F., Roland, F. A. E., Borges, A. V., Muvundja, F. A., Kelemen, Z., Masilya, P., Descy, J. P., and Bouillon, S.: Biogeochemistry of a large and deep tropical lake (Lake Kivu, East Africa): insights from a stable isotope study covering an annual cycle, *Biogeosciences*, 12, 4953-4963, doi:10.5194/bg-12-4953-2015, 2015b.
- 455 Morana, C., Roland, F. A., Crowe, S. A., Llíros, M., Borges, A. V., Darchambeau, F., and Bouillon, S.: Chemoautotrophy and anoxygenic photosynthesis within the water column of a large meromictic tropical lake (Lake Kivu, East Africa), *Limnol. Oceanogr.*, doi:10.1002/lno.10304, 2016.
- 460 Raghoebarsing, A. A., Pol, A., Van de Pas-Schoonen, K. T., Smolders, A. J., Ettwig, K. F., Rijpstra, W. I. C., Schouten, S., Damsté, J. S. S., den Camp, H. J. O., and Jetten, M. S.: A microbial consortium couples anaerobic methane oxidation to denitrification, *Nature*, 440, 918-921, doi:10.1038/nature04617, 2006.
- Rodier, J., Bazin, C., Broutin, J., Chambon, P., Champsaur, H., and Rodi, L.: *L'Analyse de l'Eau* (8ème éd.), Dunod, Paris: France, 1996.
- 465 Roland, F. A. E., Darchambeau, F., Morana, C., and Borges, A. V.: Nitrous oxide and methane seasonal variability in the epilimnion of a large tropical meromictic lake (Lake Kivu, East-Africa), *Aquat. Sci.*, 1-10, doi:10.1007/s00027-016-0491-2, 2016a.
- Roland, F. A. E., Crowe, S., Borges, A. V., De Brabandere, L., Morana, C., Servais, P., Thamdrup, B., and Darchambeau, F.: Denitrification, anaerobic ammonium oxidation and dissimilatory nitrate reduction to ammonium in an East African Great Lake (Lake Kivu), *Limnol. Oceanogr.*, (in prep), 2016b.
- 470 Rudd, J. W.: Methane oxidation in Lake Tanganyika (East Africa), *Limnol. Oceanogr.*, 25, 958-963, doi:10.4319/lo.1980.25.5.0958, 1980.
- Rudd, J. W. M., Hamilton, R. D., and Campbell, N. E. R.: Measurement of microbial oxidation of methane in lake water, *Limnol. Oceanogr.*, 19, 519-524, doi:10.4319/lo.1974.19.3.0519, 1974.
- 475 Saunois, M., Bousquet, P., Poulter, B., Peregón, A., Ciais, P., Canadell, J. G., Dlugokencky, E. J., Etiope, G., Bastviken, D., Houweling, S., Janssens-Maenhout, G., Tubiello, F. N., Castaldi, S., Jackson, R. B., Alexe, M., Arora, V. K., Beerling, D. J., Bergamaschi, P., Blake, D. R., Brailsford, G., Brovkin, V., Bruhwiler, L., Crevoisier, C., Crill, P., Curry, C., Frankenberg, C., Gedney, N., Höglund-Isaksson, L., Ishizawa, M., Ito, A., Joos, F., Kim, H. S., Kleinen, T., Krummel, P., Lamarque, J. F., Langenfelds, R., Locatelli, R., Machida, T., Maksyutov, S., McDonald, K. C., Marshall, J., Melton, J. R., Morino, I., O'Doherty, S., Parmentier, F. J. W., Patra, P. K., Peng, C., Peng, S., Peters, G. P., Pison, I., Prigent, C., Prinn, R., Ramonet, M., Riley, W. J., Saito, M., Schroeder, R., Simpson, I. J., Spahni, R., Steele, P., Takizawa, A., Thornton, B. F., Tian, H., Tohjima, Y., Viovy, N., Voulgarakis, A., van Weele, M., van der Werf, G., Weiss, R., Wiedinmyer, C., Wilton, D. J., Wiltshire, A., Worthy, D., Wunch, D. B., Xu, X., Yoshida, Y., Zhang, B., Zhang, Z., and Zhu, Q.: The Global Methane Budget: 2000-2012, *Earth Syst. Sci. Data Discuss.*, 2016, 1-79, doi:10.5194/essd-2016-25, 2016.
- 485 Sturm, A., Fowle, D. A., Jones, C., Leslie, K., Nomosatryo, S., Henny, C., Canfield, D. E., and Crowe, S. A.: Rates and pathways of CH₄ oxidation in ferruginous Lake Matano, Indonesia, *Biogeosciences Discuss.*, 2016, 1-34, doi:10.5194/bg-2015-533, 2016.
- 490 Thamdrup, B., and Dalsgaard, T.: Production of N₂ through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments, *Appl. Environ. Microbiol.*, 68, 1312-1318, doi:10.1128/AEM.68.3, 2002.
- Weiss, R. F., and Price, B. A.: Nitrous oxide solubility in water and seawater, *Mar. Chem.*, 8, 347-359, doi:10.1016/0304-4203(80)90024-9, 1980.
- 495 Weiss, R. F.: 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, 1981.
- Westermann, P., and Ahring, B. K.: Dynamics of methane production, sulfate reduction, and denitrification in a permanently waterlogged alder swamp, *Appl. Environ. Microbiol.*, 53, 2554-2559, 1987.
- Yamamoto, S., Alcauskas, J. B., and Crozier, T. E.: Solubility of methane in distilled water and seawater, *J. Chem. Eng. Data*, 21, 78-80, doi:10.1021/je60068a029, 1976.

Table 1: Depth (m) where CH₄ oxidation was observed, presence (+) or absence (-) of oxygen (O₂), CH₄ oxi = maximum CH₄ oxidation rates (μmol L⁻¹ d⁻¹) calculated based on a linear regression, [CH₄]_{in} = initial CH₄ concentrations (μmol L⁻¹) from which the linear regression begins, % CH₄ = percentage of initial CH₄ consumed, and time (h) required for this consumption (time lapse during which the linear regression was applied to calculate CH₄ oxidation rates), without and with molybdate added (- Mo and + Mo, respectively), for all field campaigns. N.d. = not determined.

Depth (m)	O ₂	CH ₄ oxi (μmol L ⁻¹ d ⁻¹)		[CH ₄] _{in} (μmol L ⁻¹)		% CH ₄		Time (h)	
		- Mo	+ Mo	- Mo	+ Mo	- Mo	+ Mo	- Mo	+ Mo
June 2011									
42.5	+	0.3 ± 0.0	N.d.	0.3 ± 0	N.d.	90 ± 0	N.d.	24	N.d.
45	+	2.0 ± 0.0	N.d.	2 ± 0	N.d.	97 ± 0	N.d.	24	N.d.
47.5	-	0.8 ± 0.0	N.d.	18 ± 0	N.d.	23 ± 0	N.d.	96	N.d.
50	-	0.4 ± 0.1	N.d.	21 ± 0	N.d.	10 ± 0	N.d.	96	N.d.
February 2012									
45	-	0.3 ± 0.0	0.1 ± 0.0	1 ± 0	1 ± 0	73 ± 0	66 ± 0	72	72
50	-	7.7 ± 0.4	3.0 ± 0.3	8 ± 0	8 ± 0	64 ± 1	27 ± 2	16	16
55	-	4.0 ± 0.2	4.3 ± 0.0	21 ± 0	21 ± 0	12 ± 1	13 ± 0	16	16
60	-	1.2	0.2	115	105	4	3	72	96
October 2012									
53	+	10.2 ± 0.4	10.0 ± 0.4	10 ± 0	10 ± 0	98 ± 0	97 ± 0	24	24
55	+	0.4 ± 0.4	16.9 ± 5.4	78 ± 4	78 ± 4	2 ± 6	18 ± 5	96	24
57.5	-	0.2 ± 0.2	1.3 ± 0.5	65 ± 3	65 ± 3	4 ± 5	10 ± 5	96	96
60	-	0 ± 0	2.1 ± 2.5	129 ± 17	129 ± 17	0 ± 0	10 ± 12	96	96
70	-	0 ± 0	1.3 ± 1.6	344 ± 11	344 ± 11	0 ± 0	2 ± 3	96	96
80	-	0 ± 0	23.4 ± 0.4	538 ± 2	538 ± 2	0 ± 0	26 ± 0	96	96
May 2013									
40	+	0.1 ± 0.0	N.d.	0.4 ± 0	N.d.	45 ± 2	N.d.	96	N.d.
45	+	0.1 ± 0.0	N.d.	0.7 ± 0	N.d.	63 ± 1	N.d.	72	N.d.
47.5	+	0.6 ± 0.0	0.6 ± 0.0	2 ± 0	2 ± 0	75 ± 1	75 ± 1	48	48
50	+	1.5 ± 0.2	1.0 ± 0.3	20 ± 1	20 ± 1	31 ± 4	18 ± 5	96	72
52.5	+	0.5 ± 0.1	0.3 ± 0.0	25 ± 0	25 ± 0	7 ± 1	7 ± 1	72	96
55	-	1.2	4.5 ± 4.2	45	45 ± 4	8	8 ± 7	96	24
60	-	1.0	6.0 ± 5.6	115	113 ± 4	4	4 ± 4	96	24
65	-	3.2	18.0	227	223	6	6	96	24
70	-	1.5 ± 0.3	1.0 ± 0.0	410 ± 3	410 ± 3	2 ± 1	2 ± 1	120	120
September 2013									
40	+	0.02	N.d.	0.2	N.d.	37	N.d.	48	N.d.
45	+	0.02 ± 0.0	0.0 ± 0.0	0.1 ± 0	0.1 ± 0	73 ± 3	0 ± 0	96	96

47.5	+	13.4 ± 0.3	10.5 ± 0.3	13 ± 0	13 ± 0	93 ± 0	75 ± 1	24	24
50	+	13.9 ± 0.0	12.7 ± 0.0	27 ± 0	27 ± 0	17 ± 0	16 ± 0	12	12
52.5	+	0.2 ± 0.0	1.0 ± 0.0	50 ± 0	50 ± 0	2 ± 0	11 ± 0	96	96
55	-	0.5 ± 0.0	11.3 ± 0.5	90 ± 0	90 ± 0	2 ± 0	4 ± 0	72	12
57.5	-	0.3 ± 0.0	10.2 ± 0.4	99 ± 0	99 ± 0	3 ± 0	3 ± 0	96	12
65	-	3.5 ± 0.3	2.6 ± 0.3	215 ± 1	215 ± 1	6 ± 1	5 ± 1	72	72
<i>August 2014</i>									
55	+	27.1 ± 1.6	18.0 ± 1.6	42 ± 2	42 ± 2	68 ± 1	46 ± 3	24	24
57.5	+	2.1	1.5	69	69	6	4	48	48
60	-	0.0 ± 0.0	4.7	167 ± 20	167 ± 20	0 ± 0	6	96	48
65	-	5.1 ± 0.9	1.3 ± 0.9	275 ± 1	275 ± 1	6 ± 2	3 ± 2	96	96
67.5	-	6.8 ± 7.8	0.0 ± 0.0	358 ± 6	358 ± 6	16 ± 12	0 ± 0	96	96
70	-	3.3 ± 1.3	0.0 ± 0.0	445 ± 10	445 ± 10	6 ± 2	0 ± 0	96	96
75	-	16.0 ± 8.2	0.0 ± 0.0	689 ± 58	689 ± 58	3 ± 8	0 ± 0	96	96

Table 2: Anoxic depths (m), NO_3^- and SO_4^{2-} consumption and Mn^{2+} production rates ($\mu\text{mol L}^{-1} \text{d}^{-1}$) and their standard deviation, without and with molybdate added (- Mo and + Mo, respectively), for all campaigns. N.d. = not determined. All rates were calculated based on a linear regression of concentrations through time.

Depth (m)	NO_3^- consumption ($\mu\text{mol L}^{-1} \text{d}^{-1}$)		SO_4^{2-} consumption ($\mu\text{mol L}^{-1} \text{d}^{-1}$)		Mn^{2+} production ($\mu\text{mol L}^{-1} \text{d}^{-1}$)	
	- Mo	+ Mo	- Mo	+ Mo	- Mo	+ Mo
<i>February 2012</i>						
45	0.01 ± 0.01	0 ± 0	N.d.	N.d.	N.d.	N.d.
50	0.37 ± 0.07	0.56 ± 0.02	N.d.	N.d.	N.d.	N.d.
55	0.21 ± 0.02	0 ± 0	N.d.	N.d.	N.d.	N.d.
60	0.01 ± 0	0.33 ± 0	N.d.	N.d.	N.d.	N.d.
<i>October 2012</i>						
57.5	0 ± 0	0 ± 0	N.d.	N.d.	N.d.	N.d.
60	0	0.02	N.d.	N.d.	N.d.	N.d.
65	0	0.04	N.d.	N.d.	N.d.	N.d.
70	0	0.04	N.d.	N.d.	N.d.	N.d.
<i>May 2013</i>						
55	0 ± 0	0 ± 0	N.d.	N.d.	N.d.	N.d.
60	0.07	0	N.d.	N.d.	N.d.	N.d.
65	0 ± 0	0 ± 0	N.d.	N.d.	N.d.	N.d.
70	0.03 ± 0.01	0.25 ± 0.05	N.d.	N.d.	N.d.	N.d.
<i>September 2013</i>						
55	0 ± 0	0 ± 0	N.d.	N.d.	N.d.	N.d.
57.5	0 ± 0	0 ± 0	N.d.	N.d.	N.d.	N.d.
65	0 ± 0	0.04 ± 0.01	N.d.	N.d.	N.d.	N.d.
<i>August 2014</i>						
60	0 ± 0	0 ± 0	1.0 ± 0.5	N.d.	0 ± 0	0 ± 0
62.5	0 ± 0	0 ± 0	0.7 ± 0.3	N.d.	0 ± 0	0.5 ± 0.2
65	0 ± 0	0 ± 0	0 ± 0	N.d.	0 ± 0	0 ± 0
67.5	0 ± 0	0 ± 0	1.5 ± 0.6	N.d.	0 ± 0	10.6 ± 1.6
70	0 ± 0	0 ± 0	17.5 ± 5.8	N.d.	0 ± 0	8.1 ± 0.1
75	0 ± 0	0 ± 0	0.3 ± 0.03	N.d.	0 ± 0	0 ± 0

Table 3: Depth-integrated CH_4 oxidation rates ($\mu\text{mol m}^{-2} \text{d}^{-1}$) in Lake Kivu and the percent related to anaerobic oxidation of methane (AOM).

	Integration depth interval (m)	CH_4 oxidation ($\mu\text{mol m}^{-2} \text{d}^{-1}$)	% AOM
<i>Dry season</i>			
<i>June 2011</i>	1-65	9	30
<i>October 2012</i>	1-80	27	1
<i>September 2013</i>	1-65	81	15
<i>August 2014</i>	1-75	162	55
<i>Rainy season</i>			
<i>February 2012</i>	1-60	63	99
<i>May 2013</i>	1-80	44	81

Table 4: Aerobic and anaerobic CH₄ oxidation rates (μmol L⁻¹ d⁻¹) in Lake Kivu and other lakes in literature.

Lake	Aerobic CH ₄ oxidation (μmol L ⁻¹ d ⁻¹) (CH ₄ concentrations; μmol L ⁻¹)	AOM (μmol L ⁻¹ d ⁻¹) (CH ₄ concentrations; μmol L ⁻¹)	Source
Kivu	0.02-27 (0.2-42)	0.2-16 (65-689)	This study
Pavin (France)	0.006-0.046 (0.06-0.35)	0.4 (285-785)	Lopes et al. (2011)
Big Soda (US)	0.0013 (0.1)	0.060 (50)	Iversen et al. (1987)
Marn (Sweden)	0.8 (10)	2.2 (55)	Bastviken et al. (2002)
Tanganyika	0.1-0.96 (<10)	0.24-1.8 (~10)	Rudd (1980)
Matano (Indonesia)	0.00036-0.0025 (0.5)	4.2-117 (12-484)	Sturm et al. (2016)

Table 5: SO_4^{2-} , NO_x , Mn^{2+} and MnO_2 concentrations ($\mu\text{mol L}^{-1}$) and potential anaerobic CH_4 oxidation (%) based on these concentrations for all campaigns, for each depth where AOM rates were observed. N.d. = not determined.

Field campaign	Depth (m)	SO_4^{2-} concentrations ($\mu\text{mol L}^{-1}$) (%)	NO_x concentrations ($\mu\text{mol L}^{-1}$) (%)	Mn^{2+} concentrations ($\mu\text{mol L}^{-1}$) (%)	MnO_2 concentrations ($\mu\text{mol L}^{-1}$) (%)
June 2011	47.5	125.6 (100)	0.23 (18)	N.d.	N.d.
	50	158.8 (100)	0.24 (38)	N.d.	N.d.
February 2012	45	168.5 (100)	1.84 (100)	N.d.	N.d.
	47.5	160.9 (100)	1.08 (9)	N.d.	N.d.
	50	108.4 (100)	0.04 (1)	N.d.	N.d.
	55	77.0 (100)	0.38 (20)	N.d.	N.d.
October 2012	57.5	N.d.	0.53 (100)	N.d.	N.d.
May 2013	55	130.4 (100)	0.04 (2)	3.0 (63)	0.2 (100)
	60	135.4 (100)	0.15 (9)	5.5 (100)	0.2 (100)
	65	112.2 (100)	0.11 (2)	7.3 (57)	0.2 (45)
	70	47.5 (100)	0.2 (8)	8.0 (100)	N.d.
September 2013	55	130.5 (100)	0.91 (100)	8.7 (100)	0.7 (100)
	57.5	124.1 (100)	0.43 (90)	9.2 (100)	0.8 (100)
	60	108.1 (100)	0.43 (8)	9.5 (68)	N.d.
August 2014	65	60.6 (100)	0.20 (2)	8.8 (43)	N.d.
	67.5	45.5 (100)	0.00 (0)	9.2 (34)	N.d.
	70	25.6 (100)	0.33 (6)	9.3 (70)	N.d.
	75	10.7 (67)	0.13 (1)	9.4 (15)	N.d.

Figure 1: Map of Lake Kivu, showing the different basins and bays, and the sampling site in the main basin (black plot).

Figure 2: Vertical profiles of dissolved oxygen (μmol L⁻¹), temperature (°C), specific conductivity (μS cm⁻¹) and pH for the six field campaigns.

Figure 3: Vertical profiles of CH_4 ($\mu\text{mol L}^{-1}$), N_2O (nmol L^{-1}), NO_x ($\mu\text{mol L}^{-1}$), SO_4^{2-} ($\mu\text{mol L}^{-1}$), HS^- ($\mu\text{mol L}^{-1}$) and particulate Mn ($\mu\text{mol L}^{-1}$) and dissolved Mn ($\mu\text{mol L}^{-1}$) concentrations for the six field campaigns. Horizontal dashed lines represent the anoxic layer for each season (same color code).

500

Figure 4: Process rates (CH₄ oxidation, NO₃⁻ consumption, Natural denitrification, SO₄²⁻ consumption and dissolved Mn production; μmol L⁻¹ d⁻¹) without and with molybdate (Mo) added, during the six field campaigns (DS: dry season; RS: rainy season). Horizontal dashed lines represent the anoxic layer for each season (same color code).

Figure 4: Depth-integrated aerobic CH_4 oxidation rates ($\mu\text{mol m}^{-2} \text{d}^{-1}$) compared to the oxygenated layer depth (m).

Figure 5: Comparison between AOM rates ($\mu\text{mol L}^{-1} \text{d}^{-1}$) measured without and with molybdate (Mo) added.

Figure 6: Comparison between measured and calculated AOM rates ($\mu\text{mol L}^{-1} \text{d}^{-1}$) based on (a) SO_4^{2-} consumption rates, (b) NO_3^- consumption rates and (c) Natural denitrification. Note the log scales.