



# Methane paradox in tropical lakes? Sedimentary fluxes rather than pelagic production in oxic conditions sustain methanotrophy and emissions to the atmosphere

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**Abstract.** Despite growing evidence that methane (CH<sub>4</sub>) formation could also occur in well-oxygenated surface fresh waters, its significance at the ecosystem scale is uncertain. Empirical models based on data gathered at high latitude predict that the contribution of oxic CH<sub>4</sub> increases with lake size and should represent the majority of CH<sub>4</sub> emissions in large lakes. However, such predictive models could not directly apply to tropical lakes, which differ from their temperate counterparts in some fundamental characteristics, such as year-round elevated water temperature. We conducted stable-isotope tracer experiments, which revealed that oxic CH<sub>4</sub> production is closely related to phytoplankton metabolism and is a common feature in five contrasting African lakes. Nevertheless, methanotrophic activity in surface waters and CH<sub>4</sub> emissions to the atmosphere were predominantly fuelled by CH<sub>4</sub> generated in sediments and physically transported to the surface. Indeed, CH<sub>4</sub> bubble dissolution flux and diffusive benthic CH<sub>4</sub> flux were several orders of magnitude higher than CH<sub>4</sub> production in surface waters. Microbial CH<sub>4</sub> consumption dramatically decreased with increasing sunlight intensity, suggesting that the freshwater “CH<sub>4</sub> paradox” might be also partly explained by photo-inhibition of CH<sub>4</sub> oxidizers in the illuminated zone. Sunlight appeared as an overlooked but important factor determining the CH<sub>4</sub> dynamics in surface waters, directly affecting its production by photoautotrophs and consumption by methanotrophs.

## 1 Introduction

Emissions from inland waters are an important component of the global methane (CH<sub>4</sub>) budget (Bastviken et al., 2011), in particular from tropical latitudes (Borges et al., 2015). While progress has been made in evaluating the CH<sub>4</sub> emission rates, much less attention has been given to the underlying microbial production (methanogenesis) and loss (methane oxidation) processes. It is generally assumed that CH<sub>4</sub> in lakes originates from the degradation of organic matter in anoxic sediments. Because most methanogens are considered to be strict anaerobes, and net vertical diffusion of CH<sub>4</sub> from anoxic bottom waters is often negligible (Bastviken et al., 2003), physical processes of CH<sub>4</sub> transport from shallow sediments are usually invoked to explain patterns of local CH<sub>4</sub> concentration maxima in surface waters (Fernández et al., 2016; Peeters et al., 2019; Martinez-Cruz et al., 2020). Indeed, CH<sub>4</sub>-rich pore water is regularly released from littoral sediment into the water column during resuspension events associated with surface waves (Hofmann et al., 2010).

The view that CH<sub>4</sub> is formed under strictly anaerobic conditions has been challenged by several recent studies, which have proposed that acetoclastic methanogens directly attached to phytoplankton cells are involved in epilimnetic CH<sub>4</sub> production (Grossart et al., 2011; Bogard et al., 2014) and are responsible for distinct near-surface peaks of CH<sub>4</sub> concentration in certain thermally stratified, well-oxygenated waterbodies (Tang et al., 2016). It has also been shown that Cyanobacteria (Bižić et al., 2020) and widespread marine

phytoplankton (Klitzsch et al., 2019) are able to release a substantial amount of  $\text{CH}_4$  during a culture study, and this  $\text{CH}_4$  production mechanism might be linked to photosynthesis. From a model-based approach, epilimnetic  $\text{CH}_4$  production was shown to sustain most of the  $\text{CH}_4$  oxidation in 14 Canadian lakes (DelSontro et al., 2018) and would even represent up to 90 % of the  $\text{CH}_4$  emitted from a temperate lake (Donis et al., 2017). Further, empirical models based on data gathered in boreal and temperate lakes predict that the contribution of oxic  $\text{CH}_4$  increases with lake size (Günthel et al., 2019) and should represent the majority of  $\text{CH}_4$  emissions in lakes larger than  $1 \text{ km}^2$ . Still, aerobic  $\text{CH}_4$  production has so far only been documented in temperate and boreal lakes so that such predictive models could not directly apply to tropical lakes, which differ from their temperate counterparts in some fundamental characteristics, such as year-round elevated water temperature. Primary production, methanogenic and methanotrophic activities, and cyanobacterial dominance are potentially much higher in tropical lakes due to favourable temperature (Lewis, 1987; Kosten et al., 2012). It has also been shown that  $\text{CH}_4$  emissions are positively related to temperature at the ecosystem scale (Yvon-Durocher et al., 2014).

Here, we tested the hypothesis that phytoplankton metabolism could fuel  $\text{CH}_4$  production in well-oxygenated waters in five contrasting tropical lakes in East Africa covering a wide range of sizes, depths, and productivity (Lake Edward, Lake George, Lake Katinda, Lake Nyamusingere and Lake Kyambura). Phytoplankton activity could provide diverse substrates required for  $\text{CH}_4$  production mediated by methanogenic Archaea, or alternatively  $\text{CH}_4$  could be directly released by phytoplankton cells. Additionally, the significance of epilimnetic  $\text{CH}_4$  production at the scale of the aquatic ecosystem was assessed by quantifying  $\text{CH}_4$  release from sediments,  $\text{CH}_4$  production and oxidation rates in the water column, and  $\text{CH}_4$  diffusive and ebullitive emissions to the atmosphere.

## 2 Material and methods

### 2.1 Site description

The sampled lakes cover a wide range of size ( $<1$  to  $2300 \text{ km}^2$ ), maximum depth (3–117 m), mixing regimes, phytoplankton biomass and primary productivity (Table S1 in the Supplement). Oligotrophic Lake Kyamwanga ( $-0.18054^\circ \text{ N}$ ,  $30.14625^\circ \text{ E}$ ) and eutrophic Lake Katinda ( $-0.21803^\circ \text{ N}$ ,  $30.10702^\circ \text{ E}$ ) are stratified, small but deep tropical lakes located in western Uganda. Neighbouring Lake Nyamusingere ( $-0.284364^\circ \text{ N}$ ,  $30.037635^\circ \text{ E}$ ) is a small but shallow and polymictic eutrophic lake. Lake George is a larger ( $250 \text{ km}^2$ ), hypereutrophic, shallow lake located at the Equator ( $-0.02273^\circ \text{ N}$ ,  $30.19724^\circ \text{ E}$ ). A single outlet (Kazinga Channel) flows from Lake George to

the neighbouring Lake Edward ( $-0.28971^\circ \text{ N}$ ,  $29.73327^\circ \text{ E}$ ), a holomictic, mesotrophic large lake ( $2325 \text{ km}^2$ ).

Water samples from pelagic stations of Lake Katinda (0.3 km from shore, 14 m deep), Lake George (2 km from shore, 2.5 m deep) and Lake Edward (15 km from shore, 20 m deep) were collected in April 2017 (rainy season) and January 2018 (dry season). Pelagic sites of Lake Kyamwanga (0.5 km from shore, 40 m deep) and Lake Nyamusingere (0.2 km from shore, 3 m deep) were sampled only once, in April 2017 and January 2018, respectively.

### 2.2 Environmental setting of the study sites

Conductivity, temperature and dissolved-oxygen concentration measurements were performed with a Yellow Spring Instrument EXO II multi-parametric probe. Samples for particulate organic carbon (POC) concentration were collected on glass fibre filters ( $0.7 \mu\text{m}$  nominal pore size) and analysed with an elemental analyser coupled to an isotope ratio mass spectrometer (EA-IRMS; Morana et al., 2015a). Pigment concentrations were determined by high-performance liquid chromatography (Descy et al., 2017) after filtration of water samples through glass fibre filters ( $0.7 \mu\text{m}$  nominal pore size).

Water samples for determination of dissolved  $\text{CH}_4$  concentration were transferred with tubing from the Niskin bottle to 60 mL borosilicate serum bottles that were poisoned with  $200 \mu\text{L}$  of a saturated solution of  $\text{HgCl}_2$ , closed with a butyl stopper and sealed with an aluminium cap. The concentrations of dissolved  $\text{CH}_4$  were measured with the headspace equilibration technique (20 mL headspace) using a gas chromatograph with flame ionization detection (GC-FID, SRI8610C).

Samples for  $\delta^{13}\text{C}$ - $\text{CH}_4$  determination were collected in 60 mL serum bottles following the same procedure as samples for  $\text{CH}_4$  concentration determination. In the laboratory,  $\delta^{13}\text{C}$ - $\text{CH}_4$  was measured as described in Morana et al. (2015b). Briefly, a 20 mL helium headspace was created in the serum bottles, then samples were vigorously shaken and left to equilibrate overnight. The sample gas was flushed out through a double-hole needle and purified of non- $\text{CH}_4$  volatile organic compounds in a liquid  $\text{N}_2$  trap,  $\text{CO}_2$  and  $\text{H}_2\text{O}$  were removed with a soda lime and magnesium perchlorate traps, and the  $\text{CH}_4$  was converted to  $\text{CO}_2$  in an online combustion column similar to that in an elemental analyser (EA). The resulting  $\text{CO}_2$  was subsequently pre-concentrated in a custom-built cryo-focussing device by immersion of a stainless-steel loop in liquid  $\text{N}_2$ , passed through a micro-packed GC column (HayeSep Q 2 m,  $0.75 \text{ mm ID}$ ; Restek), and finally measured on a Thermo Scientific Delta V Advantage isotope ratio mass spectrometer (IRMS).  $\text{CO}_2$  produced from certified reference standards for  $\delta^{13}\text{C}$  analysis (IAEA-CO1 and LSVEC) were used to calibrate  $\delta^{13}\text{C}$ - $\text{CH}_4$  data. Reproducibility of measurements estimated based on duplicate injection of a selection of samples was typically better than

0.5‰ or better than 0.2‰ when estimated based on multiple injection of standard gas.

### 2.3 Diffusive CH<sub>4</sub> flux calculation

Surface CH<sub>4</sub> concentrations were used to compute the diffusive air–water CH<sub>4</sub> fluxes (FCH<sub>4</sub>) according to Eq. (1):

$$FCH_4 = k \times \Delta CH_4, \quad (1)$$

where  $k$  is the gas transfer velocity of CH<sub>4</sub> computed from wind speed (Cole and Caraco, 1998) and the Schmidt number of CH<sub>4</sub> in fresh water (Wanninkhof, 1992), and  $\Delta CH_4$  is the air–water gradient. Wind speed data were acquired with a Davis Instruments meteorological station located on Mweya peninsula (0.11° S, 29.53° E).

### 2.4 CH<sub>4</sub> ebullition flux

CH<sub>4</sub> ebullition flux was investigated in lakes Edward (at 20 m), George (at 2.5 m), and Nyamusingere (at 3 m) only, at the same sampling sites where biogeochemical processes were measured. Bubble traps made with an inverted funnel (24 cm diameter) connected to a 60 mL syringe were deployed for a period between 24 and 48 h at 0.5 m below the water surface (four replicates). Measurements were performed at sites with water depth of 20, 2.5 and 3 m for lakes Edward, George and Nyamusingere, respectively. After measuring the gas volume collected within the trap during the sampling period, the gas bubbles were transferred in a tightly closed 12 mL Exetainer vial (Labco) for subsequent analysis of their CH<sub>4</sub> concentration. Variability of the gas volume in the four replicates was less than 10 %. We used the SiBu-GUI software (McGinnis et al., 2006; Greinert and McGinnis, 2009) to correct for gas exchange within the water column during the rise of bubbles and thus obtained the CH<sub>4</sub> ebullition and CH<sub>4</sub> bubble dissolution fluxes. Calculations were made following several scenarios: two extreme bubble-size scenarios considering a release of many small (3 mm diameter) bubbles or fewer large (10 mm) bubbles and an intermediate scenario of release of 6 mm diameter bubbles. Delwiche and Hemond (2017) experimentally determined that a large majority (~ 90 %) of the bubbles released from lake sediments fall in this size interval.

### 2.5 Potential CH<sub>4</sub> flux across the sediment–water interface

The potential CH<sub>4</sub> flux across the sediment–water interface was determined from short-term intact core incubations in Lake Edward, Lake George and Lake Nyamusingere only, at the same sampling sites where biogeochemical processes were measured. CH<sub>4</sub> flux was quantified from the change in CH<sub>4</sub> concentration in overlying waters at five different time steps, every 2 h. Briefly, in every lake, two sediment cores (6 cm wide; ~ 30 cm of sediment and 30 cm of water) were

collected, taking care to avoid disturbance at the sediment–water interface. Cores were kept in the dark until back in the laboratory, typically 6 h later. Overlying water was carefully removed with a syringe, taking care to avoid any disturbance of the sediments. It was replaced by bottom lake water, which had been previously filtered through 0.2 µm polycarbonate filters (GSWP, Millipore) in order to remove water column methanotrophs and degassed with helium for 20 min in order to remove background O<sub>2</sub> and CH<sub>4</sub>, and the filtered and degassed water was gently returned to the core tubes on top of the sediments. Core tubes were tightly closed with a thick rubber stopper equipped with two sampling valves. A magnetic stirrer placed ~ 10 cm above the sediments was allowed to rotate gently in order to homogenize the overlying water layer during the incubation. At each time step, 60 mL of overlying water was sampled by connecting a syringe to the first sampling valve while an equivalent volume of degassed water was allowed to flow through the second valve in order to avoid any pressure disequilibrium. Subsamples of overlying water were transferred into two 20 mL serum bottles filled without headspace and poisoned with HgCl<sub>2</sub>. Determination of the dissolved CH<sub>4</sub> concentration was performed with a GC–FID following the same procedure as described above. The removal of oxygen might have inhibited the biogeochemical activity of aerobic methanotrophs present at the sediment–water interface; hence we considered that the results gathered from this experiment are representative of a potential (maximal) CH<sub>4</sub> flux.

### 2.6 Primary production and N<sub>2</sub> fixation

Primary production and N<sub>2</sub> fixation rates were determined from dual stable-isotope photosynthesis–irradiance experiments using NaH<sup>13</sup>CO<sub>3</sub> (Eurisotop) and dissolved <sup>15</sup>N<sub>2</sub> (Eurisotop) as tracers for incorporation of dissolved inorganic carbon (DIC) and N<sub>2</sub> into the biomass. The <sup>15</sup>N<sub>2</sub> tracer was dissolved in water (Mohr et al., 2010). Incident light intensity was measured by a LI-190SB quantum sensor during daytime during the entire duration of the sampling campaign. At each station a sample of surface waters (500 mL) was spiked with the tracers (final <sup>15</sup>N atom excess ~ 5 %). Three subsamples were preserved with HgCl<sub>2</sub> in 12 mL Exetainer vials (Labco) for the determination of the exact initial <sup>13</sup>C–DIC and <sup>15</sup>N–N<sub>2</sub> enrichment. The rest of the sample was divided into nine 50 mL polycarbonate flasks, filled without headspace. Eight flasks were placed into a floating incubation device, providing a range of light intensity (from 0 % to 80 % natural light) using neutral density filter screen (LEE Filters). The last one was immediately amended with neutral formaldehyde (0.5 % final concentration) and served as a killed control sample. Samples were incubated in situ for 2 h around midday just below the surface at lake surface temperature. After incubation, biological activity was stopped by adding neutral formaldehyde into the flasks, and the nine samples were filtered on pre-combusted glass fi-

bre (GF/F grade) filters when back in the lab. Glass fibre filters were decarbonated with HCl fumes overnight, dried, and their  $\delta^{13}\text{C}$ -POC and  $\delta^{15}\text{N}$ -PN (particulate nitrogen) values were determined with an EA-IRMS (Thermo Flash HT – Delta V Advantage). For the measurement of the initial  $^{15}\text{N}_2$  enrichment, a 2 mL helium headspace was created, and after 12 h equilibration, a fraction of the headspace was injected into the above-mentioned EA-IRMS equipped with a Cu column warmed at 640 °C and a  $\text{CO}_2$  trap. Initial enrichment of  $^{13}\text{C}$ -DIC was also measured.

Photosynthetic ( $P_i$ ; Hama et al., 1983) and  $\text{N}_2$  fixation ( $\text{N}_2\text{fix}_i$ ; Montoya et al., 1996) rates in individual bottles were calculated and corrected for any abiotic tracer incorporation by subtraction of the killed control value. For each experiment, the maximum photosynthetic and  $\text{N}_2$  fixation rates ( $P_{\text{max}}$ ,  $\text{N}_2\text{fix}_{\text{max}}$ ) and the irradiance at the onset of light saturation ( $I_{k\_PP}$ ,  $I_{k\_N2fix}$ ) were determined by fitting  $P_i$  and  $\text{N}_2\text{fix}_i$  to the light intensity gradient provided by the incubator ( $I_i$ ) using Eq. (2) for photosynthesis activity (Vollenweider, 1965) and (Eq. 3) for  $\text{N}_2$  fixation (Mugidde et al., 2003).

$$P_i = 2P_{\text{max}} \left[ \frac{I_i/2I_{k\_PP}}{1 + (I_i/2I_{k\_PP})^2} \right] \quad (2)$$

$$\text{N}_2\text{fix}_i = 2\text{N}_2\text{fix}_{\text{max}} \left[ \frac{I_i/2I_{k\_N2fix}}{1 + (I_i/2I_{k\_N2fix})^2} \right]. \quad (3)$$

## 2.7 Determination of $\text{CH}_4$ oxidation rates

$\text{CH}_4$  oxidation rates in surface waters (1 m depth) were determined from the decrease in  $\text{CH}_4$  concentrations measured during short (typically <24 h) time course experiments. Samples for  $\text{CH}_4$  oxidation rate measurement were collected in 60 mL glass serum bottles filled directly from the Niskin bottle with tubing, left to overflow, and immediately closed with butyl stoppers previously boiled in Milli-Q water and sealed with aluminium caps. The first bottle was then poisoned with a saturated solution of  $\text{HgCl}_2$  (100  $\mu\text{L}$ ) injected through the butyl stopper with a polypropylene syringe and a steel needle and corresponded to the initial  $\text{CH}_4$  concentration at the beginning of the incubation ( $T_0$ ).

The remaining bottles were incubated in the dark at in situ ( $\sim 26^\circ\text{C}$ ) temperature for  $\sim 12$  h or  $\sim 24$  h except in lakes George and Nyamusingere, where the incubation was shorter ( $\sim 6$  h). At four different time steps, one bottle was poisoned with 100  $\mu\text{L}$  of  $\text{HgCl}_2$  and stored in the dark until measurement of the  $\text{CH}_4$  concentrations with the above-mentioned GC-FID.  $\text{CH}_4$  oxidation rates were calculated as a linear regression of  $\text{CH}_4$  concentrations over time ( $r^2$  generally better than 0.80) during the course of the incubation.  $\text{O}_2$  consumption was followed in a parallel incubation of water samples in 60 mL biological oxygen demand bottles (Wheaton) to ensure the samples were incubated under oxic conditions during the full course of this short-term experiment.

## 2.8 Sunlight inhibitory effect on $\text{CH}_4$ oxidation

The influence of light intensity on methanotrophy was investigated in Lake Edward (April 2017 and January 2018), Lake Nyamusingere and Lake George (January 2018) by means of a stable-isotope ( $^{13}\text{CH}_4$ ) labelling experiment. For each experiment, 12 serum bottles (60 mL) were filled with lake surface waters (1 m) as described above. All bottles were spiked with 100  $\mu\text{L}$  of a solution of dissolved  $^{13}\text{CH}_4$  (50  $\mu\text{mol L}^{-1}$  final concentration, 99 % enrichment) added in excess. In Lake Edward in January, half of the bottles were amended with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU; 0.5  $\text{mg L}^{-1}$ ) in order to inhibit photosynthesis (Bishop, 1958) and investigate the hypothetical inhibitory effect of dissolved  $\text{O}_2$  production by phytoplankton. Two bottles were poisoned immediately with pH-neutral formaldehyde (0.5 % final concentration) and served as killed controls. The 10 others were incubated for 24 h at  $26^\circ\text{C}$  in a floating device providing five different light intensities (from 0 % to 80 % of natural light using neutral density filter screens; LEE Filters). For every bottle at the end of the incubation, one 12 mL vial (Labco Exetainer) was filled with the water sample and preserved with 50  $\mu\text{L}$   $\text{HgCl}_2$ . The rest of the sample ( $\sim 50$  mL) was filtered on a pre-combusted glass fibre (GF/F grade) filter for subsequent  $\delta^{13}\text{C}$ -POC measurement.

$\delta^{13}\text{C}$ -DIC and  $\delta^{13}\text{C}$ -POC were determined with an EA-IRMS following the method described in Gillikin and Bouillon (2007) and Morana et al. (2015), respectively. The methanotrophic bacterial production (MBP), defined as the  $\text{CH}_4$ -derived  $^{13}\text{C}$  incorporation rates into the POC pool, was calculated as in Eq. (4) (Morana et al., 2015):

$$\text{MBP} = \frac{\text{POC}_t \times (\%^{13}\text{CPOC}_t / \%^{13}\text{CPOC}_i)}{t \times (\%^{13}\text{CCH}_4 / \%^{13}\text{CPOC}_i)}, \quad (4)$$

where  $\text{POC}_t$  is the concentration of POC after incubation,  $\%^{13}\text{C}$ - $\text{POC}_t$  and  $\%^{13}\text{C}$ - $\text{POC}_i$  are the final and initial percentage of  $^{13}\text{C}$  in the POC,  $t$  is the incubation time, and  $\%^{13}\text{C}$ - $\text{CH}_4$  is the percentage of  $^{13}\text{C}$  in  $\text{CH}_4$  after the inoculation of the bottles with the tracer. Similarly, the methanotrophic bacterial respiration (MBR) rates, defined as the  $\text{CH}_4$ -derived  $^{13}\text{C}$  incorporation rates into the DIC pool, were calculated as in Eq. (5):

$$\text{MBR} = \frac{\text{DIC}_t \times (\%^{13}\text{CDIC}_t / \%^{13}\text{CDIC}_i)}{t \times (\%^{13}\text{CCH}_4 / \%^{13}\text{CDIC}_i)}, \quad (5)$$

where  $\text{DIC}_t$  is the concentration of DIC after the incubation,  $\%^{13}\text{C}$ - $\text{DIC}_t$  and  $\%^{13}\text{C}$ - $\text{DIC}_i$  are the final and initial percentage of  $^{13}\text{C}$  in DIC, and  $\%^{13}\text{C}$ - $\text{CH}_4$  is the percentage of  $^{13}\text{C}$  in  $\text{CH}_4$  after the inoculation of the bottles with the tracer.

Potential  $\text{CH}_4$  oxidation rate (MOX) was calculated as the sum of MBP and MBR rates. The fraction (%) of MOX inhibited by light was calculated at every light intensity as

(Eq. 6):

$$\text{MOX}_{\text{inhibition}}(\%) = (1 - \text{MOX}_i / \text{MOX}_{\text{dark}}) \times 100, \quad (6)$$

where  $\text{MOX}_i$  is the potential  $\text{CH}_4$  oxidation for a given light treatment, and  $\text{MOX}_{\text{dark}}$  is the potential  $\text{CH}_4$  oxidation in the dark.

## 2.9 Determination of pelagic $\text{CH}_4$ production rates

Time course  $^{13}\text{C}$  tracer experiments were carried out in well-oxygenated surface waters at every sampling site. Measurement of the isotopic enrichment of the  $\text{CH}_4$  during this experiment allowed us to estimate production rates of  $\text{CH}_4$  issued from three different precursors:  $^{13}\text{C}$ -DIC ( $\text{NaH}^{13}\text{CO}_3$ ),  $^{13}\text{C}_{(1,2)}$ -acetate and  $^{13}\text{C}_{\text{methyl}}$ -methionine. Serum bottles (60 mL) were spiked with 1 mL of  $^{13}\text{C}$  tracer solution or with an equivalent volume of distilled water for the control treatment.  $\text{NaH}^{13}\text{CO}_3$  was added in the bottles at a tracer level (less than 5 % of ambient  $\text{HCO}_3^-$  concentration), while  $^{13}\text{C}_{(1,2)}$ -acetate and  $^{13}\text{C}_{\text{methyl}}$ -methionine were added largely in excess (>99 % of ambient concentration). Therefore, we assume the  $\text{CH}_4$  production rates measured from  $^{13}\text{C}$ -DIC could be representative of in situ rates, but the production rates measured from  $^{13}\text{C}$ -acetate and  $^{13}\text{C}$ -methionine should instead be viewed as potential rates. The exact amount of  $^{13}\text{C}$ -DIC added in the bottles was determined filling borosilicate 12 mL Exetainer vials preserved and analysed for  $\delta^{13}\text{C}$ -DIC as described above.

The control bottles and the bottles amended with the different  $^{13}\text{C}$  tracer were incubated under constant temperature conditions (26 °C) following three different treatments: (1) one-third were incubated under constant light (PAR of  $\sim 200 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ ); (2) another third were incubated under the same light intensity conditions but were first amended with DCMU ( $0.5 \text{ mg L}^{-1}$ ; final concentration), an inhibitor of photosynthesis; (3) and the last third were incubated in the dark.

At each time step (typically every 6–12 h, five time steps), the biological activity was stopped by adding 100  $\mu\text{L}$  of a saturation solution of  $\text{HgCl}_2$ . Bottles were kept in the dark until  $\text{CH}_4$  concentration measurement and  $\delta^{13}\text{C}$ - $\text{CH}_4$  determination as described above.  $\text{O}_2$  consumption was followed in a parallel incubation of water samples in 60 mL biological oxygen demand bottles (Wheaton) to ensure the samples were incubated under oxic conditions during the full course of the experiment.

The term  $\text{CH}_{4,\text{prod}}$  ( $\text{nmol L}^{-1} \text{ h}^{-1}$ ), defined as the amount of  $\text{CH}_4$  produced from a specific tracer during a time interval  $t$  (h), was calculated following this equation (Eq. 7) derived from Hama et al. (1983):

$$\text{CH}_{4,\text{prod}} = \frac{\text{CH}_{4,t} \times (\%^{13}\text{CCH}_{4,t} / \%^{13}\text{CCH}_{4,i})}{t \times (\%^{13}\text{C}_{\text{tracer}} / \%^{13}\text{CCH}_{4,i})}, \quad (7)$$

where  $\text{CH}_{4,t}$  and  $\%^{13}\text{CCH}_{4,t}$  represents the  $\text{CH}_4$  concentration ( $\text{nmol L}^{-1}$ ) and the  $\%^{13}\text{C}$  atom of the  $\text{CH}_4$  pool

at a given time step, respectively.  $\%^{13}\text{CCH}_{4,i}$  represents the  $\%^{13}\text{C}$  atom of the pool of  $\text{CH}_4$  at the beginning of the experiment.  $\%^{13}\text{C}_{\text{tracer}}$  represent the  $\%^{13}\text{C}$  atom of the isotopically enriched pool of the precursor molecule tested ( $\text{NaHCO}_3$ , methionine or acetate, depending on the treatment).  $\%^{13}\text{C}_{\text{tracer}}$  was assumed constant during the full course of the incubation given the high concentration of ambient DIC in the sampled lakes ( $\sim 2 \text{ mmol L}^{-1}$  in Lake George,  $>6 \text{ mmol L}^{-1}$  in the other lakes) and that acetate and methionine were spiked in large excess (>99 %).

## 2.10 DNA extraction

Surface water samples for DNA analysis (between 1 and 0.15 L, depending on the biomass) were first filtered through 5.0  $\mu\text{m}$  pore size polycarbonate filters (Millipore). The eluent was then subsequently filtered through 0.2  $\mu\text{m}$  pore size polycarbonate filters (Millipore) to retain free living prokaryotes. Filters were stored frozen ( $-20^\circ\text{C}$ ) and immersed in a lysis buffer until processing in the laboratory. Total DNA was extracted from the 0.2 and 5.0  $\mu\text{m}$  47 mm filters using DNeasy PowerWater kit (Qiagen) following the manufacturer's instructions. Quality and quantity of the extracted DNA were estimated using the NanoDrop ND-1000 spectrophotometer (Thermo Fisher) and the Qubit 3.0 fluorometer (Life Technologies). Extracted DNA was stored at  $-20^\circ\text{C}$  until further use.

## 2.11 Quantification of *mcrA* via qPCR

Quantification of *mcrA* gene copies was performed by quantitative polymerase chain reaction (qPCR) on the total extracted DNA. The used primer pair consisted of forward primer *qmcrA-F* 5'-TTCGGTGGATCD CARAGRGC-3' and *qmcrA-R* 5'-GBARGTCGWAWCCGTAGAAATCC-3' (Denman et al., 2007). The reaction mixture contained 3  $\mu\text{L}$  of total community DNA extract, 7.5  $\mu\text{L}$  of ABsolute qPCR SYBR Green Mix (Thermo Fisher, Cat. AB1158B), 0.3  $\mu\text{L}$  of 10  $\mu\text{M}$  forward primer *mcrF*, 0.3  $\mu\text{L}$  of 10  $\mu\text{M}$  reverse primer *mcrR*, 1.5  $\mu\text{L}$  of a 1 % *w/v* bovine serum albumin solution (Amersham Bioscience), and 2.4  $\mu\text{L}$  of nuclease- and DNA-free water. The qPCR was performed in a RotorGene 3000 (Corbett Research) using the following conditions: 95 °C (15 min) followed by 40 cycles of 20 s at 95 °C, 20 s at 58 °C, 20 s at 72 °C and a final extension step of 5 s at 80 °C. Standard curves were prepared from serial dilutions of a pre-quantified *mcrA* PCR fragment amplified using primers *mcrF* and *mcrR* from a plasmid extract carrying the complete *mcrA* gene using concentrations ranging from  $1 \times 10^2$  to  $1 \times 10^8$  copies  $\mu\text{L}^{-1}$ . Samples were analysed in triplicates.

## 2.12 16S rRNA gene amplicon sequencing

Sequencing of the 16S rRNA gene was done on the total extracted DNA to assess community composition. The 16S rRNA gene sequencing was done with the Il-

lumina MiSeq v3 chemistry following the “16S Metagenomic Sequencing Library Preparation” protocol with the following universal 16S rRNA gene primers targeting the V4 region, forward UniF/A519F-(S-D-Arch-0519-a-S-15) 5'-CAGCMGCCGCGGTAA-3' and reverse UniR/802R-(S-D-Bact-0785-b-A-18) 5'-TACNVGGGTATCTAATCC-3' (Klindworth et al., 2013). Sequenced read quality was checked using FastQC v0.11 (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>, last access: 4 June 2018). Short reads were trimmed to 250 bp with FastX Toolkit v0.0.13 ([http://hannonlab.cshl.edu/fastx\\_toolkit/](http://hannonlab.cshl.edu/fastx_toolkit/), last access: 4 June 2018) in order to remove trailing Ns and low-quality bases. Operational taxonomical units (OTUs) for each analysed sample were obtained from the quality-trimmed reads using mothur v1.39.5 (Kozich et al., 2013) and following the online MiSeq SOP ([https://mothur.org/wiki/MiSeq\\_SOP](https://mothur.org/wiki/MiSeq_SOP), last access: 4 June 2018) using the Silva v128 16S rRNA database with the following parameters: maxambig=0 bp, maxlength=300 bp, maxhomop=8 and classify OTUs to 97 % identity. The generated OTU table was used to calculate relative abundances of each OTU per sample.

### 3 Results

#### 3.1 Environmental settings

The sampled lakes cover a wide range of sizes (<1 to 2300 km<sup>2</sup>), maximum depths (3–117 m), mixing regimes, phytoplankton biomass and primary productivity (Table S1, Fig. S1). Phytoplankton biomass (chlorophyll *a* from 3.6 to 190.2 µg L<sup>-1</sup>) was dominated by Cyanobacteria (>95 %) in the most productive lakes, while diatoms (<20 %) and chrysophytes (<40 %) also contributed in the less productive ones (Fig. S2). Maximum potential photosynthetic activity ( $P_{\max}$ ) varied from 1.5 µmol CL<sup>-1</sup> h<sup>-1</sup> in Lake Edward to 199.0 µmol CL<sup>-1</sup> h<sup>-1</sup> in Lake George and was linearly related to chlorophyll *a* concentration. Light-dependent N<sub>2</sub> fixation was detected in every lake with the exception of Lake Kyamwanga. No significant N<sub>2</sub> fixation rates were measured in the dark. Maximum potential N<sub>2</sub> fixation rates ( $N_2\text{fix}_{\max}$ ) ranged between 1 and 128 nmol L<sup>-1</sup> h<sup>-1</sup> and were positively related to  $P_{\max}$  (Fig. S1).

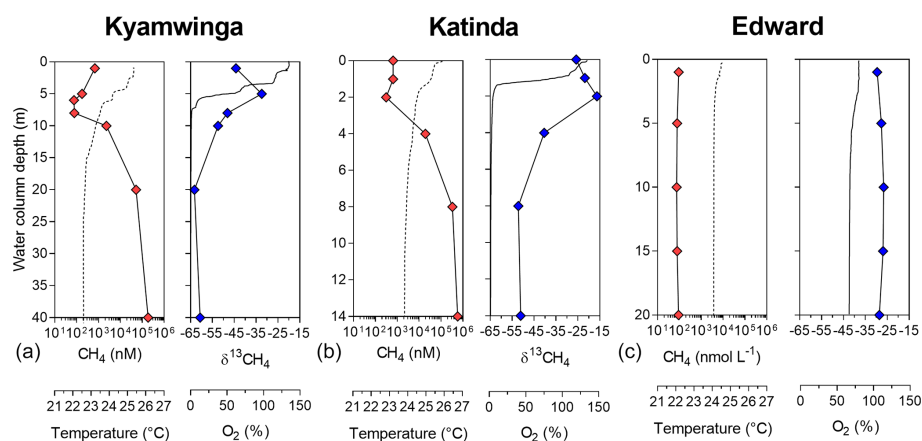
We detected and quantified the abundance of the archaeal alpha subunit of methyl-coenzyme M reductase gene (*mcrA*), a proxy for methanogens, in the surface waters of each lake. *mcrA* gene copy abundance (*mcrA* copy ng DNA<sup>-1</sup>) ranged between 319 ± 41 (Lake Edward) and 7537 ± 476 (Lake Katinda) in the fraction of seston <5 µm and between 541 ± 19 (Lake Edward) and 7968 ± 167 (Lake Katinda) in the fraction of seston >5 µm (Fig. S3). Illumina 16S rRNA gene amplicon sequencing indicated that methanogens accounted for a small fraction of the prokaryotic community in the surface waters of Lake Edward (0.01 %), Lake Kyamwanga (0.03 %) and Lake Nyamusingere (0.08 %). They repre-

sented a substantially higher fraction of the community in Lake Katinda (0.38 %) and Lake George (0.57 %; Fig. S4). In all lakes, hydrogenotrophic (Methanomicrobiales and Methanobacteriales) were always more abundant than acetoclastic (Methanosarcinales) microorganisms, representing at least 65 % of the methanogens (up to 95 % in Lake Katinda; Fig. S4).

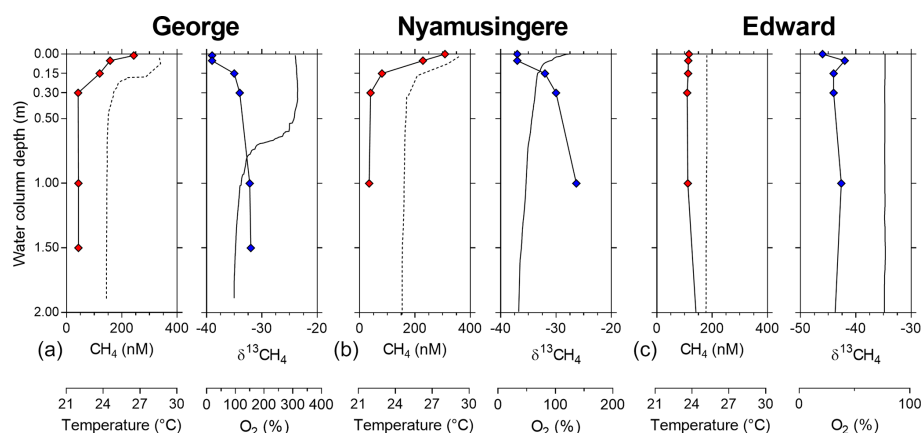
#### 3.2 Water column CH<sub>4</sub> concentration and $\delta^{13}\text{C}$ -CH<sub>4</sub> patterns

Surface waters were supersaturated in CH<sub>4</sub> in all lakes, with surface concentrations (at 1 m) ranging between 78 and 652 nmol L<sup>-1</sup> (atmospheric equilibrium ~ 2 nmol L<sup>-1</sup>). Diffusive CH<sub>4</sub> emissions varied between 0.05 (Lake Edward) and 0.40 mmol m<sup>-2</sup> d<sup>-1</sup> (Lake Katinda). The benthic CH<sub>4</sub> flux across the sediment–water interface was elevated in comparison with the diffusive CH<sub>4</sub> emissions in the three lakes where it was measured: Lake Edward (0.96 mmol m<sup>-2</sup> d<sup>-1</sup>), Lake George (9 mmol m<sup>-2</sup> d<sup>-1</sup>) and Lake Nyamusingere (5 mmol m<sup>-2</sup> d<sup>-1</sup>). CH<sub>4</sub> ebullition was the dominant pathway of CH<sub>4</sub> evasion to the atmosphere in the three lakes (Lake Edward, Lake George, Lake Nyamusingere), where ebullitive fluxes were investigated. Ebullitive flux in Lake Edward ranged between 0.16 and 0.24 mmol m<sup>-2</sup> d<sup>-1</sup>, depending on the bubble-size scenario considered (see material and methods), being at least 4 times higher than the diffusive CH<sub>4</sub> flux. This discrepancy was even larger in the shallower Lake George (13.26–13.9 mmol m<sup>-2</sup> d<sup>-1</sup>) and Lake Nyamusingere (19.03–19.09 mmol m<sup>-2</sup> d<sup>-1</sup>) where CH<sub>4</sub> ebullition appeared ~ 100 and ~ 50 times higher than diffusive CH<sub>4</sub> emissions. During the ascent of CH<sub>4</sub> bubbles to the surface, gas exchange occurs, and we estimated that, depending on the CH<sub>4</sub> bubble size considered, between 0.04 and 0.21 mmol CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup> dissolved in the water column of Lake Edward during bubble ascent. This bubble dissolution flux ranged between 0.70 and 3.30 and between 1.21 and 5.55 mmol m<sup>-2</sup> d<sup>-1</sup> in Lake George and Lake Nyamusingere, respectively.

Vertical patterns of CH<sub>4</sub> and stable carbon isotope composition of CH<sub>4</sub> ( $\delta^{13}\text{C}$ -CH<sub>4</sub>) were variable among the different lakes. In lakes Kyamwanga and Katinda, higher CH<sub>4</sub> concentrations and lower  $\delta^{13}\text{C}$ -CH<sub>4</sub> values were observed in the well-oxygenated epilimnion compared to the metalimnion showing a source of relatively <sup>13</sup>C-depleted CH<sub>4</sub> to the epilimnetic CH<sub>4</sub> pool (Fig. 1). The CH<sub>4</sub> concentrations and  $\delta^{13}\text{C}$ -CH<sub>4</sub> were homogeneous in the water column of Lake Edward, which is much larger than the other studied lakes (2300 km<sup>2</sup>; Table S1) and characterized by a higher wind exposure and a substantially weaker thermal stratification (Fig. 1). However, a clear horizontal gradient in CH<sub>4</sub> concentration and  $\delta^{13}\text{C}$ -CH<sub>4</sub> occurred between the littoral and pelagic zones (Fig. S5). Vertical gradients were also observed at a much smaller scale near the sub-



**Figure 1.** Depth profile. Depth profile of the temperature ( $^{\circ}\text{C}$ ; dashed line),  $\text{CH}_4$  concentration ( $\text{nmol L}^{-1}$ ; red symbols), dissolved-oxygen saturation (%; solid line) and stable-isotope carbon composition of  $\text{CH}_4$  ( $\delta^{13}\text{C}-\text{CH}_4$ ; ‰; blue symbols) in Lake Kyamwinda (a), Lake Katinda (b) and Lake Edward (c).



**Figure 2.** Depth profile, focus on the surface. Depth profile of the temperature ( $^{\circ}\text{C}$ ; dashed line),  $\text{CH}_4$  concentration ( $\text{nmol L}^{-1}$ ; red symbols), dissolved-oxygen saturation (%; solid line) and stable-isotope carbon composition of  $\text{CH}_4$  ( $\delta^{13}\text{C}-\text{CH}_4$ ; ‰; blue symbols) in Lake George (a), Lake Nyamusingere (b) and the surface waters (0–2 m) of Lake Edward (c).

surface (top 0.3 m) and in the shallow and entirely well-oxygenated Lake George and Lake Nyamusingere (Fig. 2). In both lakes  $\text{CH}_4$  concentrations were relatively modest in the hypolimnion ( $<50 \text{ nmol L}^{-1}$ ) but increased abruptly in the thermal gradient (0.3 m interval) to reach a surface maximum  $>240 \text{ nmol L}^{-1}$  (Fig. 2).  $\delta^{13}\text{C}-\text{CH}_4$  mirrored this pattern with significantly lower values at the surface than at the bottom of the water column, indicating that a source of relatively  $^{13}\text{C}$ -depleted  $\text{CH}_4$  contributed to the higher epilimnetic  $\text{CH}_4$ .

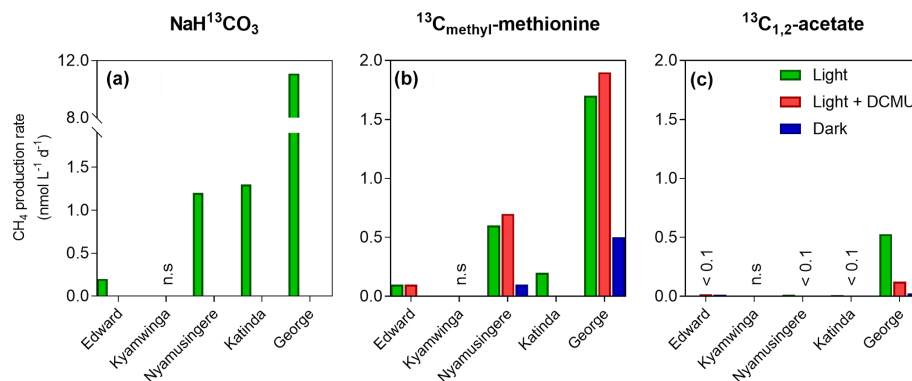
### 3.3 Occurrence of microbial $\text{CH}_4$ production in surface waters under oxic conditions

Despite the prevalence of oxic conditions,  $^{13}\text{C}$ -labelling experiments revealed that  $\text{CH}_4$  was produced in surface waters of each lake with the exception of Lake Kyamwinda (Fig. 3). The kinetic of incorporation of  $\text{NaH}^{13}\text{CO}_3$  into the  $\text{CH}_4$  pool

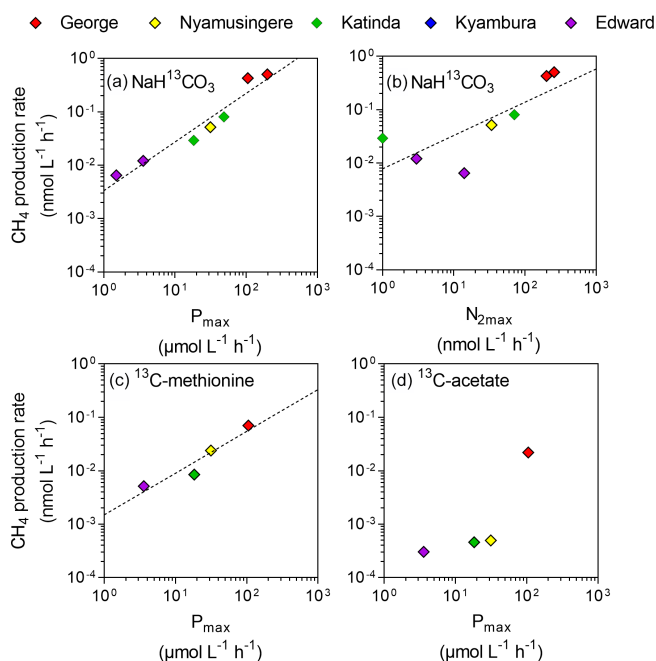
revealed that a substantially higher amount of  $\text{CH}_4$  was produced from dissolved inorganic carbon (DIC) in illuminated waters, and this mechanism of  $\text{CH}_4$  formation appears to be related to photosynthesis as no or only modest quantities of  $\text{CH}_4$  were produced from  $^{13}\text{C}$ -labelled DIC in darkness or when photosynthesis was inhibited by DCMU (Figs. 3a and S6). Furthermore,  $\text{CH}_4$  production from DIC appeared strongly correlated ( $r^2 = 0.91$ ) to the photosynthetic activity (Fig. 4a) and  $\text{N}_2$  fixation rates (Fig. 4b), supporting the view that  $\text{CH}_4$  formation in oxic waters was directly linked to phytoplankton metabolism (Bizic et al., 2020).

Aside from DIC, an appreciable amount of  $\text{CH}_4$  was generated in all lakes from the sulfur-bonded methyl group of methionine when bottles were incubated under light, irrespective of the addition of DCMU (Figs. 3b and S6).  $\text{CH}_4$  production rates were approximately 4 times higher in the bottles incubated in the light than in the dark. In addition, a





**Figure 3.** Tracer experiments show  $\text{CH}_4$  production in well-oxygenated surface waters.  $\text{CH}_4$  production rates ( $\text{nmol L}^{-1} \text{d}^{-1}$ ) from dissolved inorganic carbon (a), the methyl group of methionine (b) and acetate (c) measured in the surface waters (0.3 m) of a variety of African tropical lakes. Green, grey and dark bars, respectively, represent rates measured under light, light in the presence of a photosynthesis inhibitor (DCMU) and darkness. Values showed for Lake Edward, Lake George and Lake Katinda are the average of 2017 and 2018 sampling campaign measurement; n.s. = not significant,  $<0.1$  = below  $0.1 \text{ nmol L}^{-1} \text{d}^{-1}$ .



**Figure 4.** Direct link between  $\text{CH}_4$  production and phytoplankton metabolism. Relationship between the maximum photosynthetic activity ( $P_{\text{max}}$ ;  $\mu\text{mol CL}^{-1} \text{h}^{-1}$ ) or maximum nitrogen fixation rates ( $N_{2\text{max}}$ ;  $\text{nmol L}^{-1} \text{h}^{-1}$ ) and surface  $\text{CH}_4$  production rates ( $\text{nmol CL}^{-1} \text{h}^{-1}$ ) from dissolved inorganic carbon (a, b), methyl group of methionine (c) and acetate (d).

positive relationship between  $\text{CH}_4$  production from methionine in the light and the photosynthetic activity was found (Fig. 4c).

$^{13}\text{C}$ -labelled acetate, the substrate of acetoclastic methanogenesis, supported the production of  $\text{CH}_4$  in all lakes with the exception of Lake Kyamwinda but at much lower rates compared to light-dependent  $\text{CH}_4$  production

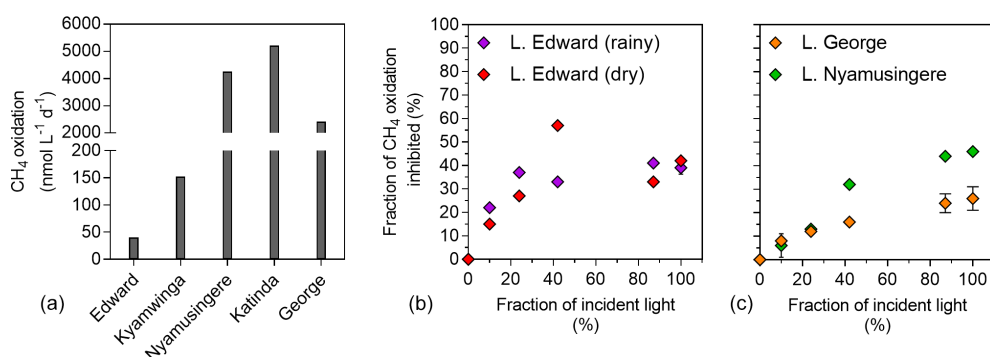
from DIC (50 times lower,  $n = 7$ ) or methionine (10 times lower,  $n = 4$ ; Figs. 3c and S6).  $\delta^{13}\text{C}$  analysis of the DIC in the bottles spiked with  $^{13}\text{C}$ -labelled acetate showed that the acetate was mineralized at rates of 5–6 orders of magnitude higher than acetoclastic methanogenesis so that added acetate appeared to be used almost exclusively by heterotrophic microorganisms other than methanogens. Patterns of acetate-derived production of  $\text{CH}_4$  were similar in light and dark treatments (Figs. 3c and S6), and this mode of  $\text{CH}_4$  production appeared to be unrelated to phytoplankton activity (Fig. 4d).

### 3.4 Microbial methane oxidation

Net  $\text{CH}_4$  oxidation was detected in all five investigated lakes ranging from 11 to  $5212 \text{ nmol L}^{-1} \text{d}^{-1}$  (Fig. 5), and was by far the largest loss term of dissolved  $\text{CH}_4$  at the ecosystem scale (8 to 46 times higher than the diffusive emission to the atmosphere). Surface water  $\text{CH}_4$  turnover times were particularly short in the shallow and eutrophic Lake George (2 h) and Lake Nyamusingere (3 h) and slightly longer in the deeper and less productive Lake Katinda (11 h), Lake Kyamwinda (77 h) and Lake Edward (100 h). In all studied lakes, the volumetric  $\text{CH}_4$  oxidation rates were always much higher than the volumetric  $\text{CH}_4$  production we measured during the stable-isotope tracer experiments, regardless of the  $\text{CH}_4$  precursors tested. Pelagic  $\text{CH}_4$  production rates represented 8.5 %, 2.6 %, 0.2 % and 0.1 % of  $\text{CH}_4$  oxidation rates in lakes Edward, George, Katinda and Nyamusingere, respectively.

The influence of light on methanotrophy was investigated in the deep Lake Edward and shallow Lake George and Lake Nyamusingere, revealing that  $\text{CH}_4$  oxidation rates decreased dramatically with increasing light intensity (Fig. 5). For instance, when exposed to full sunlight intensity, methanotrophs consumed only 42 % (Lake Edward),





**Figure 5.** Light inhibition patterns of CH<sub>4</sub> oxidation in surface waters. (a) CH<sub>4</sub> oxidation rates (nmol L<sup>-1</sup> d<sup>-1</sup>) measured in the dark in the surface waters (0.3 m) of a variety of African tropical lakes. (b) Relationship between illumination (fraction of incident sunlight irradiance; %) and CH<sub>4</sub> oxidation inhibition (fraction of CH<sub>4</sub> oxidation in the dark, inhibited at a given irradiance; %) in Lake Edward, Lake George and Lake Nymusingere. Symbols represent the mean, and error bars represent the maximum and minimum of duplicate experiments.

54 % (Lake Nymusingere) or 74 % (Lake George) of the CH<sub>4</sub> they were able to oxidize in the dark. The magnitude of this sunlight-induced inhibition decreased substantially with decreasing sunlight intensities (Fig. 5). In Lake Edward (April 2017) sunlight inhibition of methanotrophy followed the same pattern of lower rates at high sunlight intensities in the bottles where O<sub>2</sub> production (via photosynthesis) was stopped by DCMU addition.

## 4 Discussion

### 4.1 Mechanisms of CH<sub>4</sub> production under aerobic conditions

The results from the stable-isotope labelling experiment highlight that only a minimal fraction of the CH<sub>4</sub> produced under aerobic conditions originated from acetate in contrast with several earlier studies (Bogard et al., 2014; Donis et al., 2017), which proposed, based on the apparent fractionation factor of  $\delta^{13}\text{C}-\text{CH}_4$ , that acetoclastic methanogenesis linked to phytoplankton production of organic matter would be the dominant biochemical pathway of pelagic CH<sub>4</sub> production in oxic fresh waters. Instead, our results support the study of Bizic et al. (2020) and suggest that epilimnetic CH<sub>4</sub> production in well-oxygenated conditions was related to DIC fixation by photosynthesis (Fig. 3) and correlated to primary production (Fig. 4a) and N<sub>2</sub> fixation (Fig. 4b). When normalized to POC concentrations, the average DIC-derived CH<sub>4</sub> production rates ( $0.08 \pm 0.05$  nmol mmol<sub>POC</sub><sup>-1</sup> h<sup>-1</sup>  $n = 7$ ) were remarkably similar to the CH<sub>4</sub> production rates recently reported in Cyanobacteria cultures ( $0.04 \pm 0.02$  nmol mmol<sub>POC</sub><sup>-1</sup> h<sup>-1</sup>) grown at 30 °C, among which the freshwater *Microcystis aeruginosa* (Bizic et al., 2020), the dominant Cyanobacterium species in the tropical lakes investigated in our study (see Fig. S2). These CH<sub>4</sub> production rates are 2 orders of magnitude higher than rates reported in an axenic culture of the eukaryote *Emilia-*

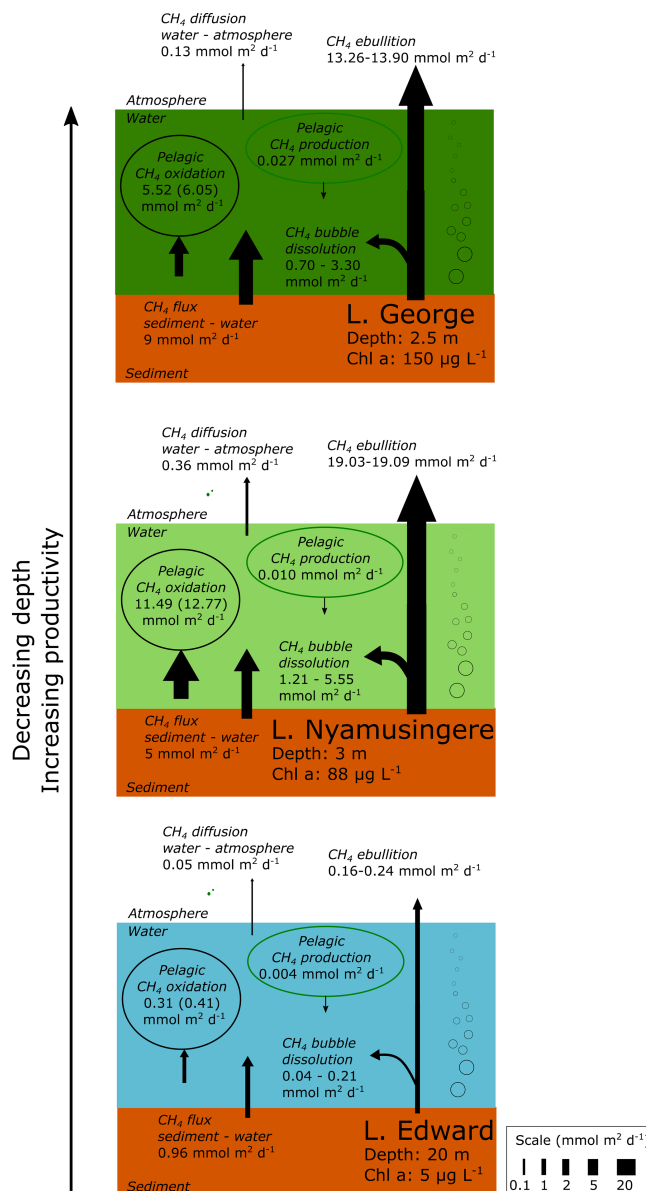
*nia huxleyi* ( $0.19 \pm 0.07$  pmol mmol<sub>POC</sub><sup>-1</sup> d<sup>-1</sup>; Lenhart et al., 2016), but they are 4 orders of magnitude lower than typical anoxic CH<sub>4</sub> production rates by methanogenic Archaea (Mountfort and Asher, 1979). Although it seems improbable that <sup>13</sup>C–DIC acted as a direct precursor molecule for the CH<sub>4</sub> released by phytoplankton (Lenhart et al., 2016; Klintzsch et al., 2019), <sup>13</sup>C–DIC could have been taken up by phytoplankton cells and then used as a C source for the synthesis of many different organic molecules that may serve as the actual CH<sub>4</sub> precursors. Indeed, healthy phytoplankton cells actively release a variety of low-molecular-weight molecules, which are generally highly labile and rapidly consumed (Baines and Pace, 1991; Morana et al., 2014). Phytoplankton metabolism could have fuelled CH<sub>4</sub> production pathways, at least partially, excreting substrates involved in CH<sub>4</sub> production via biochemical processes such as demethylation of a variety of organic molecules like methionine, one of the S-bonded methylated amino acids (Lenhart et al., 2016), trimethylamine (Bizic et al., 2018) or methylphosphonate (Yao et al., 2016).

While the source of methylphosphonate in fresh waters is obscure, and its actual natural abundance remains to be determined, dissolved free amino acids would represent up to 4 % of the DOC produced by phytoplankton and are rapidly consumed by heterotrophic bacteria (Sarmiento et al., 2013). Our incubations indeed demonstrated that the methyl group of methionine was a potential precursor of CH<sub>4</sub> in all lakes investigated, in line with recent findings showing that *Emiliana huxleyi* could act as a direct source of CH<sub>4</sub> in oxic conditions using methionine as a precursor without the involvement of any other microorganisms (Lenhart et al., 2016). We found that CH<sub>4</sub> production from methionine was clearly stimulated under light, even when photosynthetic activity was inhibited by DCMU, while little CH<sub>4</sub> from methionine was produced in darkness (Fig. 3b). DCMU notably prevents reduction in plastoquinone at photosystem II and generates singlet oxygen (Petrillo et al., 2014). The mecha-

nism of  $\text{CH}_4$  production from methionine is still unclear, but its residue in proteins is particularly sensitive to oxidation to methionine sulfoxide by radical oxygen species (ROS; Levine et al., 1996) so that methionine would act as an effective ROS scavenger and play important protective roles under photo-oxidative stress conditions, as shown in vascular plants (Bruhn et al., 2012). The side chain of methionine sulfoxide is identical to dimethyl sulfoxide, which is known to react with hydroxyl radicals (OH) to form  $\text{CH}_4$  (Repine et al., 1979). Besides its photoprotective role for phytoplankton, methionine could also be catabolized by a wide variety of microorganisms to methanethiol, which could in turn be transformed to  $\text{CH}_4$  as shown in Arctic Ocean surface waters (Damm et al., 2010). Nevertheless, occurrence of this latter mechanism in the tropical lakes investigated seems unlikely as this mode of  $\text{CH}_4$  production would be expected to be insensitive to light irradiance, and no  $\text{CH}_4$  was produced from methionine in the dark during the incubations.

#### 4.2 Relevance of pelagic $\text{CH}_4$ production compared to methanotrophy and $\text{CH}_4$ emissions at the ecosystem scale

The stable-isotope labelling experiments revealed that microbial  $\text{CH}_4$  oxidation largely exceed the pelagic  $\text{CH}_4$  production. All of the major sources and sinks of  $\text{CH}_4$  at the ecosystem scale were experimentally determined offshore in three lakes (lakes Edward at 20 m depth, George at 2.5 m and Nyamusingere at 3 m; Fig. 6). In contrast with high-latitude lakes where no ebullition could be detected in locations with a water column depth higher than 2 m (DelSontro et al., 2018), we found that the  $\text{CH}_4$  ebullition flux dominates over the  $\text{CH}_4$  diffusion flux in every sampling site. However, due to the dissolution of arising bubbles, the contribution of ebullition to the total  $\text{CH}_4$  emissions might be lower in deeper (>20 m) locations of Lake Edward (maximum depth of 113 m), as shown elsewhere (DelSontro et al., 2015). Comparison of the  $\text{CH}_4$  production, consumption and emission fluxes (Fig. 6) shows that the depth-integrated  $\text{CH}_4$  production rates determined from diverse precursor molecules were modest relative to the diffusive  $\text{CH}_4$  efflux to the atmosphere and the depth-integrated microbial  $\text{CH}_4$  oxidation. In opposition, the combined  $\text{CH}_4$  bubble dissolution flux and diffusive benthic  $\text{CH}_4$  flux were several orders of magnitude higher than  $\text{CH}_4$  production in surface waters and were sufficient to support the microbial  $\text{CH}_4$  oxidation and the emissions to the atmosphere (Fig. 6). These results gathered in tropical lakes of various size (from 0.44 to 2300 km<sup>2</sup>) and depth are in sharp contrast with the estimation of an empirical model (Guntel et al., 2019) which proposed that mechanisms of oxic  $\text{CH}_4$  production represents the majority of  $\text{CH}_4$  emissions in lakes larger than 1 km<sup>2</sup>. This discrepancy highlights the need to consider the unique limnological characteristics of a vast region of the world that harbours 16 % of the total surface of lakes (Lehner and Döll, 2004). One of the most distinc-



**Figure 6.** Epilimnetic  $\text{CH}_4$  production is a marginal source of  $\text{CH}_4$  compared to sedimentary sources and  $\text{CH}_4$  sinks in several contrasting African lakes. Summary of the different  $\text{CH}_4$  flux experimentally measured in Lake Edward, Lake George and Lake Nyamusingere. Values of  $\text{CH}_4$  oxidation in brackets are values not considering  $\text{CH}_4$  photoinhibition. Pelagic  $\text{CH}_4$  production are values determined from  $\text{NaH}^{13}\text{CO}_3$  (~5 % final enrichment) and  $^{13}\text{C}$ -acetate (99 % final enrichment), as described in the methods section.  $^{13}\text{C}$ -labelling experiment carried out under constant light irradiance.  $\text{CH}_4$  flux at the water–air and sediment–water interface was determined experimentally as described in the methods section.  $\text{CH}_4$  bubble dissolution and  $\text{CH}_4$  ebullition flux were determined using the SiBu-GUI software (Greinert and McGinnis, 2009); minimum and maximum represent the values obtained from two extreme bubble-size scenarios considering a release of many small (3 mm diameter) bubbles or fewer large (10 mm) bubbles.

tive features of the tropical aquatic environment is the persistent elevated water temperature in the hypolimnion and at the water–sediment interface, which favours methanogenic activity in sediment and decreases CH<sub>4</sub> solubility, enhancing bubble formation.

Epilimnetic CH<sub>4</sub> production appeared as a marginal flux at the ecosystem scale and could not explain alone the accumulation of <sup>13</sup>C-depleted CH<sub>4</sub> in the epilimnion of most of the lakes of our dataset (Figs. 1, 2), for which we propose a combination of two other alternative mechanisms: dissolution of arising CH<sub>4</sub> bubbles in the epilimnion combined with inhibition by light of CH<sub>4</sub> oxidation. The partial dissolution of the CH<sub>4</sub> bubbles as they rise in the epilimnion would allow a rapid transport of <sup>13</sup>C-depleted CH<sub>4</sub> from the sediment, bypassing the hotspot of CH<sub>4</sub> oxidation at the sediment–water interface and representing an alternative source of <sup>13</sup>C-depleted CH<sub>4</sub> in water column. This mechanism of bubble-mediated transport to the epilimnion would be especially important in shallow lakes as CH<sub>4</sub> ebullition is widely variable as a function of water column depth (DelSontro et al., 2015). The shallower Lake George and Lake Nyamusingere were notably characterized by sharp thermal-density gradients (Fig. 2) and extreme phytoplankton biomass largely dominated by *Microcystis aeruginosa* (chlorophyll *a* up to 190 µg L<sup>-1</sup>). *Microcystis aeruginosa* cells form large aggregates (> 1 mm) embedded in a matrix of extracellular polymeric substance that might act as a barrier to trap small CH<sub>4</sub> bubbles arising from the sediment (Fig. S7 in the Supplement). Dissolution of CH<sub>4</sub> bubbles could be enhanced at the very near surface due to the entrapments of bubbles at the air–water interface by abundant surface organic films that delay the bubble “burst”. The presence of a sharp subsurface temperature gradient would further enhance CH<sub>4</sub> accumulation during daytime near the air–water interface (by blocking vertical redistribution of CH<sub>4</sub> by mixing). We hypothesize that this process could be widespread in shallow tropical lakes, which are characterized by high productivity and are simultaneously susceptible to being large benthic CH<sub>4</sub> sources.

The stable-isotope labelling experiment carried out to investigate the inhibitory effect of light on methanotrophic activity demonstrated that CH<sub>4</sub> consumption dramatically decreased with increasing light intensities (Fig. 5), as already reported in a tropical reservoir (Dumestre et al., 1999) and Lake Biwa (Murase and Sugimoto, 2005). The inhibitory effect of light on freshwater methanotrophs remains surprisingly understudied since it was first reported 20 years ago (Dumestre et al., 1999), so the physiological mechanism of photoinhibition is still not understood. The physiological mechanism of photoinhibition of CH<sub>4</sub> oxidation could be related to the fact that the copper-containing methane mono-oxygenase enzyme and be structurally close to the ammonia mono-oxygenase enzyme, and it might be inactivated by ROS produced during photo-oxidative stress, as shown for ammonium oxidizers (French et al., 2012; Tolar et al., 2016). Altogether, our results emphasize the role of sunlight irradi-

ance as an important but frequently overlooked environmental factor driving the CH<sub>4</sub> dynamics in lake surface waters and possibly contributing to the occurrence of <sup>13</sup>C-depleted CH<sub>4</sub> in surface waters.

**Data availability.** All data included in this study are available upon request by contacting the corresponding author.

**Supplement.** The supplement related to this article is available online at: <https://doi.org/10.5194/bg-17-5209-2020-supplement>.

**Author contributions.** This study was designed by CM, AVB and SB. All authors participated in sample collection, data acquisition and analysis. The manuscript was drafted by CM and substantially improved with the input of all authors. All authors approved the final version of the paper.

**Competing interests.** The authors declare that they have no conflict of interest.

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