Photosynthesis-driven methane production in oxic lake water as an important contributor to methane emission

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Abstract

Recent discovery of methane (CH4) production in oxic waters challenges the conventional understanding of strict anoxic requirement for biological CH4 production. High-resolution field measurements in Lake Stechlin, as well as incubation experiments, suggested that oxic-water CH4 production occurred throughout much of the water column and was associated with phytoplankton especially diatoms, cyanobacteria, green algae, and cryptophytes. In situ concentrations and δ13C values of CH4 in oxic water were negatively correlated with soluble reactive phosphorus concentrations. Using 13C-labeling techniques, we showed that bicarbonate was converted to CH4, and the production exceeded oxidation at day, but was comparable at night. These experimental data, along with complementary field observations, indicate a clear link between photosynthesis and the CH4 production-consumption balance in phosphorus-limited epilimnic waters. Comparison between surface CH4 emission data and experimental CH4 production rates suggested that the oxic CH4 source significantly contributed to surface emission in Lake Stechlin. These findings call for re-examination of the aquatic CH4 cycle and climate predictions.

The widely reported “methane paradox,” that is, oversaturation of dissolved methane (CH4) in oxic sea and lake waters (Tang et al. 2016), contradicts the conventional understanding that biological CH4 production occurs exclusively under anoxic conditions (Thauer 1998; Ferry and Kastead 2007).

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

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Research in recent years has shown that active CH4 production occurs in oxic sea (Karl et al. 2008; Damm et al. 2010) and lake waters (Grossart et al. 2011; Bogard et al. 2014; Tang et al. 2014). Globally, freshwaters account for about 122 ± 60 Tg yr−1 or about 20% of CH4 emission to air (Saunois et al. 2016) and their contribution is expected to increase in future climate change scenarios (Dean et al. 2018). It is therefore necessary to understand the environmental dynamics of this oxic-water CH4 source and its potential contribution to CH4 emission to the atmosphere.

We investigated oxic-water CH4 production in Lake Stechlin (Northeast Germany), an oligo-mesotrophic glacial lake in the temperate region that has been intensively monitored for decades (Casper 1985). Biological productivity in Lake Stechlin is phosphorus-limited (Casper 1985). Accordingly, the ratio of total nitrogen to total phosphorus in the epilimnion was (mean ± SD)

\[\text{mean} = 36 \pm 9 \text{ during the study period in 2016 (March–July; } n = 15)\]

(Supporting Information Fig. S1), much higher than what is considered to indicate phosphorus limitation (i.e., a ratio of > 15 to > 22; Guilford et al. 2000; Abell et al. 2010). Recurring seasonal development of CH4 oversaturation in the oxic midwater column has been observed in this lake, and previous studies have shown in situ oxic CH4 production irrespective of CH4 input from the sediment or lateral transport from the shore (Grossart et al. 2011; Tang et al. 2014). Despite skepticism (Fernandez et al. 2011; Tang et al. 2014).
2016; Peeters et al. 2019), mounting evidence for oxic-lake water CH₄ production suggests that this is a widespread phenomenon in lakes (Grossart et al. 2011; Bogard et al. 2014; Tang et al. 2014; Donis et al. 2017; DeSontro et al. 2018; Günthel et al. 2019; Khatun et al. 2019). Currently, there are two proposed pathways for oxic-lake water CH₄ production: (1) Methane as a by-product of methylphosphonate (MPN) decomposition, which is an alternative way of phosphorus acquisition when inorganic phosphorus is limited (Carini et al. 2014; Yao et al. 2016; Wang et al. 2017); and (2) a pathway independent of MPN demethylation which is thought to be based on a Coenzyme-M homologue (Tang et al. 2016). Several studies have demonstrated the involvement of photoautotrophs in CH₄ formation in oxic water, both in the field (Grossart et al. 2011; Bogard et al. 2014; Hartmann et al. 2020) and in the laboratory (Lenhart et al. 2016; Klintzsch et al. 2019; Bizic et al. 2020; Hartmann et al. 2020). The association of oxic CH₄ production to MPN degradation and autotrophic organisms suggests that phosphorus and light might be important factors driving oxic CH₄ production.

We conducted a comprehensive study of the CH₄ dynamics in the oxic water of Lake Stechlin in order to address these questions: Which environmental factors promote oxic CH₄ production? How is oxic CH₄ production connected to phytoplankton? What is the contribution of this production to water-to-air CH₄ flux? To investigate the environmental parameters that promote oxic CH₄ production, we statistically analyzed the temporal and spatial CH₄ distributions and its isotopic signatures and different biotic and abiotic factors in the lake on seasonal, weekly, and diurnal time scales. Field observations were complemented by incubation experiments manipulating light and phosphorus availability. To test for the involvement of phytoplankton, we further analyzed in situ CH₄ concentration together with chlorophyll and taxon-specific pigment fluorescence data. Additionally, we conducted incubation experiments to measure depth-specific and size-specific CH₄ production rates, as well as incubation experiments with pure diatom culture. Using ¹³C-labelling techniques, we showed that bicarbonate was converted to CH₄ in lake water, thereby establishing a direct link between phototrophic carbon fixation and oxic CH₄ production. The ¹³C-label experiment was designed to quantify CH₄ production and consumption simultaneously throughout the diurnal cycle providing information about the effect of light on the CH₄ production-consumption balance. Last, the contribution of oxic CH₄ production to the water-to-air CH₄ flux was examined by comparing oxic production rates from incubation experiments with CH₄ emission rates to the atmosphere.

**Materials and methods**

**Field measurements**

**Lake characteristics and sampling sites**

Lake Stechlin is a dimictic meso-to-oligotrophic lake in Northeastern Germany. The lake has three basins with a combined surface area of 4.25 km² (volume ca. 0.09 km³), a shoreline of 16.1 km and a catchment area of about 12.4 km² (26 km² subsurface catchment area). With 69.5 m maximum depth and 22.8 m mean depth, it is one of Germany’s deepest lakes. More details can be accessed online via the monitoring station of the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (https://www.igb-berlin.de/en/monitoring/stechlin) or the World Lake Database hosted by the International Lake Environment Committee (http://www.lwb.de/Lake/EUR-31).

Two locations in Lake Stechlin were sampled: (1) the northeast basin (69.5 m deep site; 53°09'20.2N 13°01'51.5E) and (2) the south basin (20.5 m deep site; 53°08'36.6N 13°01'43.0E). Data obtained for the northeast basin include environmental parameters (YSI probe data, partial BBE probe data, nutrients), CH₄ concentration profiles (0–20 m depth; March–July 2016), carbon isotope signatures of water column CH₄, and CH₄ surface emission rates. Data for the south basin sampling site include environmental parameters (YSI probe data, complete BBE probe data) and CH₄ concentration profiles (0–20 m depth; April–July 2016). Weather data were provided by the German Environment Agency (Neuglobsow weather station located directly next to the lake). Water samples collected for incubation experiments were collected in the south basin.

**Environmental parameters**

A YSI sonde Model 6600V2 was used to record temperature, dissolved oxygen, photosynthetically active radiation (PAR), and chlorophyll fluorescence. Concentrations of taxon-specific phytoplankton pigments were measured by a BBE Molaerike Fluorprobe. Parameter profiles were taken weekly during March–July 2016 at the deepest point (at 0, 2, 4, 6, 7, 8, 9, 10, 12, 14, 16, 18, and 20 m depth; YSI) and continuously every hour in the southern basin (0.5–20 m depth in 0.5 m intervals; YSI and BBE).

Nutrient concentrations were measured photometrically using a Foss Analytical FIAstar 5000 Analyzer with DDW detector and common standards: total phosphate (AN 5241, ISO 15681-1), soluble reactive phosphate (AN 5240, ISO 15681-1), total nitrogen (AN 5202D, DIN EN ISO 13395, ISO 11905), and NH₄-nitrogen (AN 5220, ISO 11732). Nutrient data were obtained weekly (May–July 2016) at 4, 7, 8, 10, and 12 m depth (deepest point).

**Methane concentrations**

Water was transferred from a Limnos Water Sampler to 50 mL serum bottles (clear borosilicate glass, > 88% transmission of PAR spectrum), which were flushed three times then crimp-closed (PTFE-butyl septa, aluminum caps) without gas bubbles. Dissolved CH₄ was extracted using head space (Helium) displacement method and measured by a Shimadzu 14A GC/FID (35°C Permabond FFAP column on N₂, split-less injection, and detection at 140°C). Headspace CH₄ was converted to dissolved CH₄ concentrations based on Henry’s Law and standard conditions. Seasonal CH₄ data were obtained in a 1-week interval; diurnal CH₄ data were collected
in 6-h intervals (only the last data point delayed by 1 h) from 8th July 2016 at 14:00 h local time until 11th July 2016 at 15:00 h (14:00 + n*6 h; 13 profiles). The standard deviations of measurements (averaged over depths) were ± 0.004 (north-east basin, triplicates) and ± 0.009 (south basin, duplicates) for the seasonal data, and ± 0.016 μmol L⁻¹ (south basin, duplicates) for the diurnal data.

Methane emission

Emitted CH₄ was captured by a 15-liter floating chamber that was submerged at the perimeter by 3 cm and had a tube (butyl septum) at the center for gas sampling. Over 1–2 h, nine gas samples of each 20 mL were withdrawn by syringe, transferred into 50 mL serum bottles (prefilled with NaCl-saturated distilled water; PTFE septa enclosed), and the CH₄ concentrations were measured with GC/FID as described earlier. The CH₄ surface flux was then derived from linear regression over time.

Carbon isotope signature of water column methane

Anoxic sediment methanogenesis produces CH₄ with δ¹³C values typically less than −55‰ (Whiticar 1999; Conrad et al. 2007). Methane oxidation at the sediment-water interface and within the water column preferentially enriches the ¹³C content in the CH₄ pool, leading to δ¹³C values greater than −55‰ in the water column (i.e., Whiticar 1999; Tang et al. 2014). Accordingly, changing water column δ¹³C signatures to more negative values are commonly attributed to CH₄ production whereas changes to more positive values are attributed to CH₄ oxidation (Donis et al. 2017; DelSontro et al. 2018). The discovery of oxic CH₄ production, however, requires re-evaluation of this assumption. We used the water column ¹³C signatures of CH₄ to statistically analyze its relation to environmental parameters.

To analyze the carbon isotope signature of water column CH₄, 5 mL gas samples extracted from lake water at different depths were stored in 12 mL Exetainer (prefilled with NaCl-saturated distilled water) and analyzed with a GC/C-IRMS unit composed of Agilent 7890A GC, GC IsoLink, a ConFlo IV interface to a MAT 253 IRMS (Thermo Fisher) and a programmed temperature vaporizer (MMI G3510A/G3511A, Agilent Technologies) with glass liner (1 cm CarboSieve SIII 60/80 packing). Compressed sample injection was done at −90°C (for 5.20 min, ramp 600°C min⁻¹ until 225°C). A MolSieve 5A column (50 m × 0.32 mm × 30 μm) running on 2 mL min⁻¹ He (35°C for 10 min, ramp 20°C min⁻¹ until 250°C, final hold for 5 min) enabled separation. Carbon isotope signatures (of CH₄) are expressed in conventional δ¹³C notation (%) relative to Vienna-PeeDee Belemnite and were correlated to field parameter together with CH₄ concentration.

Linear modeling

R-software (R v3.3.1, RStudio v1.0.153) (RStudio Team, 2016) was used to test for linear relationships between parameters using general linear models, which were set up for γ-distribution and log-link, without interaction term unless stated otherwise. Regression results shown in scatter plots are based on LM models. Supporting Information Table S1 summarizes the correlations.

Experimental setups

Size fractionation experiment

A substantial part of the phytoplankton community was larger than freshwater methanogenic Archaea (commonly < 20 μm; Lyu and Liu 2019), and included cyanobacteria, diatoms, and green algae (Supporting Information Fig. S2). In this experiment, we examined which size fraction was responsible for CH₄ production in the oxic water, considering the fractions < 20 μm, > 20 μm, and nonfractionated.

One liter of an integrated lake water sample (4–8 m depth), taken on 3rd July 2018, was filtered through a 20 μm net creating the < 20 μm size fractions. Afterward, the filter was inverted and > 20 μm particles were resuspended in 1 L of sterile-filtered (0.2 μm) lake water, creating the > 20 μm size fraction. Unfiltered lake water was used as control. Water samples were added to 50 mL serum bottles (clear borosilicate glass, ≥ 88% transmission of PAR spectrum), air-saturated by bubbling atmospheric air for 10 min through a 0.2 μm filter and crimp-closed. Two bottles per size fraction were incubated for 24 h under natural day light exposure in the laboratory. Methane concentration was measured by a GC/FID unit and net CH₄ production was calculated as changing CH₄ concentration divided by incubation time.

Depth-specific methane production

Lake water from different depths was sampled in duplicates on 13th June 2016 and incubated in serum bottles (clear borosilicate glass, ≥ 88% transmission of PAR spectrum) in the laboratory exposed to natural daylight (on a shaker; room temperature). The CH₄ concentration was measured by GC/FID on day 0, 14, and 21. The average CH₄ production was calculated as changing CH₄ concentration divided by incubation time for two periods: day 0–14 (using CH₄ concentrations recorded on day 0 and 14) and day 0–21 (using CH₄ concentrations recorded on day 0 and 21).

¹³C labeling experiment

To investigate the role of phytoplankton in oxic CH₄ production, lake water samples were spiked with dissolved inorganic carbon (DIC) as ¹³C-labeled bicarbonate. The conversion of DIC to CH₄ was then measured as the incorporation of the ¹³C label into the CH₄ product pool (IRMS analysis). Additionally, to quantify the CH₄ production-consumption balance in oxic water at different time of day, CH₄ oxidation was measured simultaneously by measuring the conversion of ¹³C-labeled CH₄ to DIC.

Lake water (from 7 m depth) was collected on 1st September 2016, transferred to 12-mL Exetainer (clear soda lime glass, ≥ 91% transmission of PAR spectrum). To measure CH₄ production, three treatment groups were set up: (1) no DI¹³C addition, (2) addition of DI¹³C, and (3) addition of DI¹³C and 1.4 mmol L⁻¹ methyl fluoride (inhibitor of CH₄ oxidation; Chan and Parkin 2000) for control. DI¹³C was added in the
form of $^{13}$C-labeled NaHCO$_3$ to a final labeling percentage of 21%. To measure CH$_4$ oxidation, three additional treatment groups were set up: (4) no $^{13}$CH$_4$ addition, (5) addition of $^{13}$CH$_4$, and (6) addition of $^{13}$CH$_4$ and 1.4 mmol L$^{-1}$ methyl fluoride for control. The $^{13}$CH$_4$ was added to a final labeling percentage of 92.5%. Each treatment group included exetainers $^{*}$. To measure CH$_4$ oxidation, three additional treatment

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consumption balance. An integrated lake water sample (4-8 m depth) was taken on 29th June 2018, filtered through a 100 μm net, mixed carefully by shaking, then added to serum bottles (clear borosilicate glass, ≥ 88% transmission of PAR spectrum) without disturbing the ambient gas conditions and crimp-closed (Teflon coated septa). The water sample was either untreated or enriched with 2.1 mmol (per 50 mL) K$_2$HPO$_4$ daily, and was incubated in the laboratory under natural day light exposure or in the dark. Microbial activity was stopped after incubation by adding ZnCl$_2$ to a final concentration of 0.5%. δ$^{13}$C of CH$_4$ (GC/IRMS; 2–5 measurements), soluble reactive phosphorus (SRP; Foss Analytical FlAstar 5000 Analyzer, one bottle sacrificed per time point), and oxygen saturation (PreSense sensor, one bottle sacrificed per time point) were measured at the start, and after 5 and 10 d of incubation.

### Pure culture experiment

We measured the CH$_4$ production by two diatom cultures: *Navicula* sp. (isolated from Lake Stechlin) and *Leptocylindrus danicus* (marine), using a membrane inlet mass spectrometer (MIMS) and following the procedure of Bizić et al. (2020). The MIMS device (Bay Instruments, MD, U.S.A.) consisted of a crossbeam ion source mass spectrometer (Pfeiffer Vacuum, Germany), HiCube 80 Eco turbo pumping station (Pfeiffer Vacuum, Germany), QMG 220 M1, PrismaPlus®, C-SEM, 1-100 amu, and an experimental chamber. The experimental chamber (3.5 mL) contained an inner chamber for the culture and an outer chamber connected to a water bath to stabilize the temperature. The cultures were continuously mixed by a magnetic stirrer, kept at constant temperature, and incubated for 3 or 4 d under the following light regime: 19:30–09:00 h no light, gradually increasing to 60, 120, 180, and 400 μmol photon m$^{-2}$ s$^{-1}$ (1.5 h hold) then gradually decreasing in reverse order. A peristaltic pump (MiniPuls3, Gibson) circulated the diatom cultures (constant temperature) continuously through a capillary linked to Viton pump tubing (Kana et al. 2006) kept in a water bath to stabilize the temperature and back to the culture (semi-closed system: no liquid loss, only gas loss). Throughout the circulation, the culture passed a microbore silicone membrane (8 mm, Silastic®, DuPont) permeable only to gases. The surrounding vacuum removed dissolved gases from the culture medium and fed it to the mass spectrometer where corresponding $m/z$ ratios were recorded. The MIMS setup was calibrated to measure mass/charge ratio ($m/z$) in MilliQ water and growth media at different temperatures. Further calibration was realized by making measurements on air-saturated MilliQ water at different salinities.

To start, 3.5 mL culture were transferred to the experimental chamber and the $m/z$ ratios representative of CH$_4$ (15) and oxygen (32) were recorded in a 9 s interval relative to the $m/z$ ratio of argon ($m/z = 40$). Concentrations of CH$_4$, oxygen, and argon were calculated using published solubilities (Powell 1972). Production rates of CH$_4$ were calculated by first smoothing the raw data (sgolay function of the R package signal; 20 min interval; signal developers) (Signal Developers 2013) and then applying the 1$^{st}$ derivate. The degassing rate resulting from gas

### Phosphorus addition experiment

In this experiment, we tested whether the addition of inorganic phosphorus to the lake water would affect the CH$_4$ production-rates and percentage of 92.5%. To measure CH$_4$ oxidation, three additional treatment groups were set up: (4) no $^{13}$CH$_4$ addition, (5) addition of $^{13}$CH$_4$, and (6) addition of $^{13}$CH$_4$ and 1.4 mmol L$^{-1}$ methyl fluoride for control. The $^{13}$CH$_4$ was added to a final labeling percentage of 92.5%. Each treatment group included exetainers...
consumption by the MIMS unit was determined experimentally and added to the absolute values of the 1st derivative. Final CH₄ production rates are presented relative to dry weight of the diatom biomass. To determine the dry weight, 3.5 mL diatom culture aliquot was filtered through a GF/F filter (combusted and preweighed; Millipore); the filter cake was subsequently dried (105°C) for 48 h and weighed at room temperature.

Results

Environmental setting

Seasonal scale

Seasonal CH₄ and environmental data for the south basin are presented in Fig. 1a–d and Supporting Information Fig. S3. Preliminary measurements prior to the seasonal study showed low CH₄ concentrations in the upper 20 m in the northeast basin, on average 0.02 and 0.03 μmol L⁻¹ in February and March, respectively. As the lake began to stratify, upper water CH₄ concentration increased along with water temperature, reaching up to 1.4 μmol L⁻¹ in late June at the thermocline depth (Fig. 1a). Strong CH₄ accumulations were consistently found in waters with oversaturated dissolved oxygen (up to 167%; 17 mg L⁻¹). Methane concentrations at 0–20 m depth were positively correlated with temperature (p < 0.001), PAR (p < 0.001), and oxygen saturation (up to 167% saturation) (p < 0.001). The concurrent total phosphorus was < 20 μg L⁻¹, SRP < 8 μg L⁻¹, total nitrogen < 1 mg L⁻¹, and ammonium < 0.05 mg L⁻¹. Further positive correlations were found between CH₄

Fig. 1. Methane and environmental data—seasonal scale. (a) Methane concentration in μmol L⁻¹; (b) temperature in °C; (c) oxygen saturation in %; (d) combined pigment concentration of green algae, cyanobacteria, diatoms, and cryptophytes in μg L⁻¹; (e) SRP in μg L⁻¹; and (f) total phosphorus (TP) in μg L⁻¹. Methane (a) and probe data (b–d) were recorded in the south basin and nutrient data in the northeast basin (e, f). The arrow marks the time point of water sampling for investigating depth-specific methane production rates.
concentrations and pigment concentrations of cyanobacteria \( (p < 0.001) \), diatoms \( (p < 0.001) \), with temperature as interaction term, \) and cryptophytes \( (p < 0.04) \), with temperature as interaction term) at 0–20 m depth. Correlations are summarized in Supporting Information Table S1.

From the beginning of May to early June, the carbon isotope signature of water column CH4 \( \delta^{13}C \) scattered around \(-50\% \) \((\pm 2.5\%)\) (Fig. 2a). In mid-June, the \( \delta^{13}C \) values increased to \(-45\% \) to \(-37\% \) (maximum at 7 m depth), then gradually decreased but remained greater than \(-50\% \) in July. When plotted against SRP levels (Fig. 2b), both \( \delta^{13}C \) values \( (R^2 = 0.50; p < 0.001) \) and concentrations of CH4 \( (R^2 = 0.21; p = 0.015) \) showed negative correlations.

The water-to-air CH4 flux increased by an order of magnitude between March and July (Fig. 3a), and generally followed the increasing amount of CH4 in the upper 7 m (Fig. 1a), as well as increasing wind speed \( u_{10} \) (Supporting Information Fig. S4). Both CH4 accumulation and emission showed a negative log-linear relationship with SRP concentrations in the upper 7 m (Fig. 3b). Supporting Information

![Fig. 2. Isotope characteristics of oxic methane accumulation. Methane concentrations and corresponding \( \delta^{13}C \) values in relation to (a) time and (b) ambient SRP concentrations. CH4 concentration \( (R^2 = 0.21, p = 0.015) \) and its \( \delta^{13}C \) signature \( (R^2 = 0.50, p < 0.001) \) correlated negatively with SRP concentration (CH4 concentration/\( \delta^{13}C \): mean \pm SD of triplicate measurements).](image)

![Fig. 3. Water-to-air CH4 flux and environmental parameters. Panel (a) shows the seasonal increase in surface emission. Data points are labeled with radiation values (combined direct solar and reflected/scattered radiation) recorded at 21 m height (W m \(^{-2}\)). (b) Both surface emission \( (R^2 = 0.68, p < 0.001) \) and CH4 accumulation in the upper 7 m \( (R^2 = 0.87, p < 0.001) \) significantly increased with decreasing SRP concentration (upper 7 m) in a log-linear fashion. Note that y-axes in panel (b) are in log-scale.](image)
Table S2 summarizes the seasonal surface flux data and related environmental data.  

Diurnal scale  
Diurnal measurements revealed a rise-and-fall cycle of CH₄ in the water column (Fig. 4a–d; Supporting Information Fig. S5). During daylight at about 10:00–14:00 h local time, CH₄ concentrations increased to 0.6–0.9 μmol L⁻¹, whereas at night the concentrations dropped to about 0.4 μmol L⁻¹. The highest daily CH₄ accumulation coincided with the highest daily wind speeds around noon, showing variations in the CH₄ inventory throughout a diurnal cycle. Strong CH₄ accumulation consistently occurred at 7 m depth and at the 16°C isotherm, typically coinciding with high dissolved oxygen concentration (up to 16 mg L⁻¹; 162% saturation). The δ¹³C values of CH₄ at 7 m varied between −43‰ and −47‰ over the day–night cycle (Supporting Information Fig. S6). Similar to the seasonal data, CH₄ showed a positive linear relationship with temperature (p < 0.001), PAR (p = 0.003), and oxygen saturation (p < 0.001) throughout the diurnal cycle. To account for diurnal differences in fluorescence response, we analyzed the daytime (05:00–21:30 h) and nighttime phytoplankton pigment data (21:30–05:00 h) separately, both of which gave positive relationships between CH₄ and chlorophyll (daytime: p = 0.002; nighttime: p = 0.005), green algae (daytime: p < 0.001; nighttime: p < 0.001), cyanobacteria (daytime: p = 0.003), and diatom pigment concentration (daytime: p = 0.02; nighttime: p = 0.02).

Methane production by phytoplankton  
Size fraction experiment  
We measured CH₄ production in the < 20 μm and > 20 μm size fractions of the lake water, as well as unfiltered lake water. The > 20 μm size fraction showed considerably higher net CH₄ production (mean ± SD) (19.2 ± 0.9 nmol L⁻¹ d⁻¹) than the smaller fraction (4.3 ± 0.3 nmol L⁻¹ d⁻¹) and unfiltered lake water (5.4 ± 0.3 nmol L⁻¹ d⁻¹), suggesting that most of the CH₄ production was associated with large phytoplankton cells, colonies, and particles (Fig. 5a). Microscopic observations revealed that cyanobacteria, diatoms, and green algae were prevalent in the > 20 μm size fraction (Supporting Information Fig. S2). In comparison, CH₄ oxidation appeared to be primarily driven by cells smaller than 20 μm.

Depth-specific methane production  
Water samples collected in the epilimnion, thermocline, and hypolimnion (0–20 m sampled; ca. 20.5 m deep site) all showed increasing CH₄ concentrations over time when incubated under natural daylight in the laboratory (Fig. 5b), and the highest production rates were found in waters collected...
from the epilimnion and thermocline depth. Methane production was significantly correlated with in situ CH₄ concentration (incubation day 0–14: R² = 0.77, p < 0.001; incubation day 0–21: R² = 0.83, p < 0.001) and chlorophyll a (Chl a) concentration in the corresponding depths (incubation day 0–14: R² = 0.68, p < 0.001; incubation day 0–21: R² = 0.75, p < 0.001) (Supporting Information Table S3). Thus, waters collected from depths with high in situ CH₄ concentration showed high CH₄ production, and vice versa.

**¹³C labeling experiment**

In this experiment, CH₄ oxidation and CH₄ production were measured using ¹³C-labeled substrates and recording the incorporation of ¹³C into the respective product pools over time. Thermocline-depth water samples treated with ¹³CH₄ showed a linear increase in incorporation of ¹³C into the DIC pool over time (Fig. 5c), whereas samples treated with methyl fluoride to inhibit CH₄ oxidation showed negligible change in DI¹³C. The difference between the two treatments gave a CH₄ oxidation of 1.9 ± 0.3 pmol L⁻¹ h⁻¹ (Eq. 1). In the parallel treatment groups, the addition of NaH¹³CO₃ resulted in incorporation of ¹³C into the CH₄ pool (Fig. 5c). There was a small decrease in the ¹³CH₄ pool at night even with added methyl fluoride, suggesting incomplete inhibition of CH₄ oxidation, whereas ¹³C incorporation into CH₄ during daytime exceeded the nighttime loss. Based on ¹³C incorporation, the net change in CH₄ varied between −1.4 (night) and 7 pmol L⁻¹ h⁻¹ (day) throughout the diurnal cycle (Fig. 5c). Using Eq. 2 and applying the average oxidation rate from the ¹³CH₄ labeling experiment, the gross CH₄ production was estimated to be 0.5 (night) and 8.9 pmol L⁻¹ h⁻¹ (day). The results are summarized in Supporting Information Tables S4 and S5.

**Phosphorus addition experiment**

An integrated lake water sample was taken from 4 to 8 m depth during the stratified and phosphorus limited season (SRP ≤ 3 µg L⁻¹) (Supporting Information Fig. S7). This lake water was incubated in the laboratory under natural daylight exposure or in darkness, with or without the addition of K₂HPO₄. All treatment groups remained oxic throughout the experiment and δ¹³C values varied among the treatment groups (Supporting Information Fig. S8 and Table S6). While the addition of phosphorus shifted δ¹³C to more negative values in the light treatment, dark incubation resulted in more positive δ¹³C values.

**Pure culture experiment**

Both the freshwater diatom *Navicula* sp. and the marine diatom *Leptocylindrus danicus* showed CH₄ production (Fig. 6). Highest CH₄ production rates were observed during the light periods, especially during highest light intensities. During dark phases production rates were low (both species) or undetectable (*Navicula* sp.). A decrease in CH₄ concentration could be the result of either decreased or no production coupled with degassing from the supersaturated, continuously...
mixing, semi-closed incubation chamber toward equilibrium with atmospheric CH4 (2.5 nmol L\(^{-1}\) and 2.1 nmol L\(^{-1}\) for freshwater and seawater, respectively).

**Discussion**

**Environmental setting**

Methane accumulation in oxic surface waters of temperate lakes often coincides with seasonal stratification (Bastviken et al. 2008; Tang et al. 2016). In our study, CH4 concentrations were up to 115 times higher in the summer months than in early spring, leading to a 29-fold increase in total CH4 in the top 7 m of the strongly oxygenated water column (> 100 % saturation; > 10 mg L\(^{-1}\)) between late March and July.

While it is common to describe CH4 dynamics based on either exclusively CH4 concentration data (e.g., Juutinen et al. 2009; Li et al. 2018) or exclusively CH4 isotope data (e.g., Cadieux et al. 2016; Lecher et al. 2017), by combining both data types we revealed a new aspect of CH4 dynamics in the water column. For example, when considering CH4 concentration alone, the increase in in situ mid-water CH4 between March and July (Fig. 2a) can be interpreted as the result of a stronger or accumulating CH4 input from sediments and littoral zone, which would be accompanied by a decrease in the corresponding \(\delta^{13}C\) value indicative of anoxic CH4 (Whiticar 1999). Conversely, when only considering CH4 isotope data alone, the observed \(^{13}C\) enrichment throughout June (Fig. 2a) can be interpreted as an increase in CH4 oxidation activity, which should also lead to a decrease in the corresponding CH4 concentration (Whiticar 1999). However, by combining both data sets we showed a concurrent increase in both CH4 concentration (from ca. 0.3/0.6 to 1.0 \(\mu\)mol L\(^{-1}\)) and \(\delta^{13}C\) signature (from ca. −52‰ to −37‰). Our observations allow the alternative explanation of an internal (oxic) CH4 source with a \(\delta^{13}C\) signature distinctively higher than that of the anoxic CH4 sources. This explanation is supported by our bottle incubations showing active CH4 production under oxic conditions, and a recent study modeling CH4 carbon isotope changes in lake water (Hartmann et al. 2020).

Phosphorus deficiency has been shown to promote demethylation of MPNs with subsequent CH4 release in both marine and freshwater environments (Carini et al. 2014; Repeta et al. 2016; Yao et al. 2016; Wang et al. 2017). Methyl groups cleaved from the C-1 compounds are converted to CH4 by a reductase, potentially drawing the required reductive power via electron dumping from photosynthesis (Tang et al. 2014), especially under nutrient limitation (Hemschemeier and Happe 2011). This hypothesis is corroborated by our field and lab measurements together showing that oxic CH4 production was connected to photosynthesis as well as low SRP concentrations. Nevertheless, it remains unclear whether the MPN pathway would result in CH4 with \(\delta^{13}C\) values greater than −40‰ (kinetic isotope effects strongly depend on elemental composition and molecule configuration; therefore, incubation experiments with enriched MPN pool as CH4 precursor do not reflect the natural \(\delta^{13}C\) response in the field).

**Methane production by phytoplankton**

Several lines of evidence point to the direct role of phytoplankton in oxic CH4 production in our study. First, in situ CH4 concentrations were positively correlated to PAR, phytoplankton pigment concentrations, and oxygen (over)saturation (as a by-product of photosynthesis). Second, our diurnal measurements showed a cyclical CH4 pattern in the water.
column that aligned with the light–dark periods. Third, highest CH₄ production was observed in the > 20 μm size fraction, which contained mostly cyanobacteria, diatoms, and green algae. Our light incubations using lake water amended with K₂HPO₄ resulted in a δ¹³C shift toward more negative values, whereas dark incubation resulted in more positive values, together indicating that CH₄ production was triggered by light. The alternative explanation of light-inhibited CH₄ oxidation (Murase and Sugimoto 2005) is unlikely to be relevant because our ¹³C-labeling experiment showed constant oxidation rates throughout the diurnal cycle (Fig. 4c). Further direct evidence was provided by incubation with NaH¹³CO₃ resulting in a much higher incorporation of ¹³C into CH₄ in daytime vs. nighttime, showing that photoautotrophic carbon fixation was involved in the process, as we hypothesized earlier (Tang et al. 2014). Among the major phytoplankton groups, cyanobacteria showed the strongest and most consistent positive correlation with CH₄ concentrations in situ, implicating their key role in oxic CH₄ production. Using cyanobacteria cultures and a MIMS, Bizic et al. (2020) showed that CH₄ production follows the light–dark cycle with a small time-lag. Here, we conducted similar MIMS measurements with cultures of the freshwater diatom *Navicula* sp. (isolated from Lake Stechlin) and the marine diatom *Leptocylindrus danicus* (Fig. 6). Both species showed CH₄ production aligning with light–dark incubation periods and more CH₄ was produced at higher light intensities. Combining ours and others’ findings (Bizic et al. 2020; Hartmann et al. 2020), the results suggest that light-triggered CH₄ production may be common among phytoplankton. It is likely that the conversion of NaH¹³CO₃ to CH₄ involves multiple steps, not all being light-dependent (decreased but active CH₄ production during dark phases: Bizic et al. 2020; Fig. 6). Nevertheless, the observations that oxic CH₄ production was linked to photosynthesis indicate that the oxic and anoxic methane sources will react differently to environmental perturbations.

In the marine environment, the cyanobacterium *Trichodesmium erythraeum* has been found to carry gene cassettes encoding the enzymes for the conversion of MPNs to CH₄ (Dyhman et al. 2006; Beversdorf et al. 2010). Similar genes have been found in the freshwater cyanobacterium *Pico cyanobacterium* (Kutovaya et al. 2013; Yao et al. 2016). However, in previous studies, enrichment of Lake Stechlin water with MPNs has produced conflicting results in stimulating CH₄ production (Grossart et al. 2011; Bizic-Ionescu et al. 2018). Another possible pathway involves nitorgenase enzymes, which are common among cyanobacteria (Bothe et al. 2010). Iron-only nitorgenase of wild-type proteobacteria have been shown to convert carbon dioxide, nitrogen gas and protons to CH₄ in a single enzymatic reaction, and the CH₄ yield increases with increasing light intensity (up to 30 μmol photons m⁻² s⁻¹) (Zheng et al. 2018). Furthermore, this reaction has been found in an obligate aerobic bacterium, *Azotobacter vinelandii* (Zheng et al. 2018). However, the iron-only nitrogenase activity has never been demonstrated in CH₄ production by cyanobacteria. Diatoms are generally incapable of nitrogen-fixation; therefore, the observed CH₄ production (Fig. 6) indicates that the underlying mechanism does not necessarily require nitrogenase.

**Implication for methane emission to the atmosphere**

Measured net CH₄ production was comparable above and within the thermocline; loss of CH₄ to oxidation and diffusion within the epilimnion could result in a concentration gradient with the CH₄ maximum at the thermocline as observed. An earlier study did not detect more methane oxidizer in surface water compared to thermocline water (Grossart et al. 2011); therefore, it is more likely that the gradient was caused by diffusive loss of CH₄ across the water–air interface, which is corroborated by our surface flux measurements. To explain the observed average surface flux of 0.32 mmol m⁻² d⁻¹ during the stratified season (after 17th May), it would require an oxic production of 46 nmol L⁻¹ d⁻¹ in the upper 7 m. In our incubation experiment, water collected from the upper 7 m yielded about 8 nmol L⁻¹ d⁻¹ oxic CH₄ production. Our measurement represents only a snapshot of the system and does not fully capture the natural variability of CH₄ production. For example, Grossart et al. (2011) reported about seven times higher oxic CH₄ production rate in an earlier year based on bottle incubations. Günthel et al. (2019) and Hartmann et al. (2020), using mass balance analysis of CH₄ production and loss, arrived at oxic CH₄ production rates of up to > 200 nmol L⁻¹ d⁻¹. Therefore, our oxic CH₄ production rates should be considered conservative, which potentially explained 18% of the observed average surface flux. Similarly, recent investigations in the mesotrophic Lake Hallwil, Switzerland found that about 63–90% of CH₄ emission could be attributed to oxic CH₄ production and accumulation in the upper mixed layer (Donis et al. 2017; Günthel et al. 2019). Light and phosphorus were found in our incubation experiments to be important factors for oxic CH₄ production, which is consistent with our field observations that the water-to-air CH₄ flux correlates with both parameters. Together, our observations suggest that oxic CH₄ production in the upper layer is an important contributor to CH₄ emission, but hitherto has not been acknowledged in global methane budgets and models (IPCC 2013).

**Reconsidering the methane paradox**

The methane paradox is rooted in the paradigm that biological CH₄ production occurs strictly under anoxic conditions and therefore, oxic water CH₄ oversaturation is often exclusively attributed to physical transport from anoxic sources. However, reports of oxic CH₄ production by terrestrial flora and fauna have prompted questions of this paradigm (Keppeler et al. 2006; Ghyzy et al. 2008; Lenhart et al. 2012; Althoff et al. 2014). Similarly, the mounting evidence in both marine and freshwater environments (Tang et al. 2016) and reference therein,
including this study, has demonstrated unequivocally that active biological CH$_4$ production occurs in oxic waters. Accordingly, the methane paradox can be explained, at least partially, by internal oxic production (Grossart et al. 2011; Tang et al. 2014; Günthel et al. 2019), with or without external inputs (Fernandez et al. 2016; DelSontro et al. 2018; Peeters et al. 2019).

Pure-culture experiments demonstrated the ability of oxic CH$_4$ production in diatoms (Hartmann et al. 2020; this study), cyanobacteria (Bizic et al. 2020), green algae, and cryptophytes (Hartmann et al. 2020). Additionally, our study showed the conversion of ($^{13}$C-labeled) bicarbonate to CH$_4$ demonstrating a direct link of oxic CH$_4$ production to photosynthesis, and our field data corroborate the involvement of diatoms, cyanobacteria, green algae, and cryptophytes. While the precise biochemical pathway(s) is subject to further investigation, these findings together indicate CH$_4$ production may be a common feature among diverse phytoplankton taxa. Phytoplankton are ubiquitous in illuminated aquatic environments and are globally on the rise (Hampton et al. 2008; Duan et al. 2009; Ho et al. 2019). It is therefore necessary to understand how these potential oxic methane producers react to environmental perturbation such as widespread eutrophication and global warming, and the corresponding effect on atmospheric CH$_4$ emission. Available data for Lake Stechlin (Günthel et al. 2019, Hartmann et al. 2020; this study), Lake Crowmnell (Bogard et al. 2014) and Lake Hallwil (Donis et al. 2017, Günthel et al. 2019) show that oxic CH$_4$ production can account for 18–90% of the surface emission. Albeit limited, these findings warrant an urgent re-examination of the (oxic) CH$_4$ cycle (Kirschke et al. 2013; Saunois et al. 2016) and climate change predictions (IPCC 2013) that are presently based on almost exclusively conventional anoxic CH$_4$ sources.

Data availability statement

Manuscript related data are available in tabular form in the Supporting Information (incubation experiments, flux data) and via GLEON data repositories upon acceptance (seasonal and diurnal field data) (http://gleon.org/).

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Conflict of Interest

None declared.