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Paradox reconsidered: Methane oversaturation in well-oxygenated lake waters

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Abstract

The widely reported paradox of methane oversaturation in oxygenated water challenges the prevailing paradigm that microbial methanogenesis only occurs under anoxic conditions. Using a combination of field sampling, incubation experiments, and modeling, we show that the recurring mid-water methane peak in Lake Stechlin, northeast Germany, was not dependent on methane input from the littoral zone or bottom sediment or on the presence of known micro-anoxic zones. The methane peak repeatedly overlapped with oxygen oversaturation in the seasonal thermocline. Incubation experiments and isotope analysis indicated active methane production, which was likely linked to photosynthesis and/or nitrogen fixation within the oxygenated water, whereas lessening of methane oxidation by light allowed accumulation of methane in the oxygen-rich upper layer. Estimated methane efflux from the surface water was up to 5 mmol m⁻² d⁻¹. Mid-water methane oversaturation was also observed in nine other lakes that collectively showed a strongly negative gradient of methane concentration within 0–20% dissolved oxygen (DO) in the bottom water, and a positive gradient within $\geq 20\%$ DO in the upper water column. Further investigation into the responsible organisms and biochemical pathways will help improve our understanding of the global methane cycle.

Methane accounts for 20% of the total radiative forcing among all long-lived greenhouse gases and has an estimated global warming potential 25 times that of CO₂ in the coming century (Forster et al. 2007). Balancing the global methane budget, however, remains problematic due to uncertainty in its sources and sinks (Conrad 2009; Bastviken et al. 2011). Besides geological and anthropogenic emissions, methane is also produced by methanogens via three major pathways: acetoclastic, methylotrophic, and hydrogenotrophic methane production (Mah et al. 1977). Many of the enzymes involved are believed to be sensitive to oxygen (Jarrell 1985). Accordingly, a long-standing paradigm is that biological production of methane occurs exclusively under anoxic conditions (Mah et al. 1977). Recent studies suggested that terrestrial plants may emit methane under aerobic conditions (Keppler et al. 2006), and saprotrophic fungi are able to produce methane independently of methanogenic archaea (Lenhart et al. 2012). Hence, the methane cycle appears to be more complex than previously thought.

A widely reported phenomenon in oceans and lakes is mid-water methane oversaturation (Reeburgh 2007; Conrad 2009), often referred to as the “methane paradox” because methane production is not supposed to occur in oxygen-rich water. Common explanations are input from nearby anoxic waters and sediments (Hofmann et al. 2010), or in situ production within micro-anoxic zones such as aggregates, fecal pellets, and animal guts (Oremland 1979).

Opposite to the conventional view, some methanogens are remarkably tolerant to oxygen (Jarrell 1985; Angel et al. 2011). Two research groups also reported methane production under oxic condition in the Pacific Ocean and the Arctic Ocean, respectively (Karl et al. 2008; Damm et al. 2010). In Lake Stechlin, northeast Germany, methane reached a maximum concentration in the thermocline during the summer, and incubation experiments and molecular analysis suggested the presence of active methanogens in the oxygen-rich water (Grossart et al. 2011). To better evaluate the importance of oxic lake waters as a methane source, we studied the mid-water methane peak in Lake Stechlin in relation to the water column conditions and examined whether this peak could be explained by anoxic methane sources. We also sampled other lakes to examine the commonality of mid-water methane oversaturation. For methane to accumulate in the mid-water, it requires that methane oxidation is less than production. It has been proposed that methane oxidation is decreased by light in the photic zone (Murase and Sugimoto 2005); we therefore tested for this effect in Lake Stechlin.

Ebullition is considered a major mechanism for transporting methane from anoxic sediments to the surface water and eventually to the atmosphere (Walter et al. 2007). We used a published model that describes methane bubble dissolution and stripping of dissolved gases in a stratified water column (McGinnis et al. 2006) to test if ebullition in thermally stratified Lake Stechlin could explain the mid-water methane peak. We also estimated the net flux of methane from the lake surface towards the atmosphere as a function of turbulent diffusivity, and the

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Table 1. Characteristics of lakes sampled in 2012.

Lake	Latitude (°N)	Longitude (°E)	Trophic state	Maximum depth (m)	Sampling date
Krummer	53°10'51.5"	12°51'48.2"	Eutrophic	8.5	22 Aug
Roofen	53°6'38.4"	13°2'6.1"	Meosotrophic	15	22 Aug
Dagow	53°9'3.8"	13°3'7.8"	Meso- to eutrophic	8.5	22 Aug
Nehmitz	53°7'54.8"	12°59'7.4"	Mesotrophic	11.5	22 Aug
Peetsch	53°10'6.9"	13°4'28.8"	Oligotrophic	19	29 Aug
Wittwe	53°7'37.2"	12°56'14.7"	Mesotrophic	10	29 Aug
Wumm	53°11'18.0"	12°48'1.0"	Oligotrophic	26	29 Aug
Glietzen	53°10'59.0"	13°3'44.9"	Mesotrophic	10	29 Aug
Ellbogen	53°12'38.7"	13°2'6.3"	Eutrophic	9.5	29 Aug

surface diffusive methane emission as a function of wind speed.

Methods

Field sampling—Sampling was done at the deepest point (53°9.6'N, 13°1.8'E; ~ 70 m) of Lake Stechlin in three consecutive summers (June–August 2010–2012). Water samples were taken in 2 m intervals and filled into gas-tight bottles. Within 1–2 h after sampling, dissolved methane was measured by the headspace displacement method on a Shimadzu gas chromatograph with flame ionization detector. Methane concentration in air was measured by directly injecting air samples into the gas chromatograph. Ambient dissolved oxygen (DO) and water temperature were measured by a Wissenschaftlich Technische Werkstätten (Weilheim) submersible probe. Photosynthetic pigments were measured by a biological-biophysical-engineering-Moldaenke FluoroProbe (Kiel) as proxies for photoautotroph compositions and abundances. The FluoroProbe measured the fluorescence signals from chlorophyll and accessory pigments for cryptophytes, diatoms, cyanobacteria, and green algae. In 2012 we also preserved water samples with 0.5% weight:volume ZnCl₂ for isotopic analysis of methane. Additional methane profiles were taken on three occasions along a horizontal transect that extended from the deepest point of the lake to the shore in the northwest direction. The transect was ~ 350 m long and was the shortest distance between the deepest point and the littoral zone. The transect was sampled at three locations: the deep station, the shallow littoral zone with thick vegetation, and approximately the mid-point between the two. At each location, methane samples were taken at multiple depths between the surface and close to the bottom. Methane samples were also taken in nine other lakes (Table 1) to assess the commonality of mid-water methane oversaturation.

In-lake incubations—To measure methane production, water was collected from 6 m at the deep station on 11 July 2012 and was partitioned into total (no filtration) and < 5 μm fractions (passing through 5 μm filter). Water from each fraction was filled into nine gas-tight bottles, three of which were used to measure the initial methane concentrations. Of the remaining bottles, three were untreated and three were treated with 2-bromoethane sulfonic acid (BES;

> 10⁻⁴ mol L⁻¹ final concentration) to inhibit methanogenesis (Miller et al. 1998). The bottles were suspended in the lake at 6 m for 24 h before measuring the final methane concentrations. Difference in change in methane concentrations between the untreated and BES-treated bottles gave the gross methane production. The size-fractionated incubation was repeated on 26 July to measure the methane carbon isotope composition.

To test for lessening of methane oxidation by light, water rich in methane and methanotrophs but depleted in oxygen (K. Frindte unpubl.) was taken from the hypolimnion (6 m) of Lake Dagow, a shallow eutrophic lake adjacent to Lake Stechlin. Oxygen was introduced by mixing 1:1 with oxygen-rich (0.24 mmol O₂ L⁻¹) water from 30 m in Lake Stechlin. The mixed water was filled into nine gas-tight clear glass bottles; three were used to measure the initial methane concentrations. Of the remaining bottles, three were covered by aluminum foil as the dark treatment and three remained uncovered as the light treatment. The bottles were suspended at 6 m in Lake Stechlin for 48 h before measurement of the final methane concentrations. Secchi depth was 6.8 m.

Isotope analysis—The stable carbon isotope composition of dissolved methane was analyzed on a ThermoScientific Delta V isotope ratio mass spectrometer coupled to a ThermoScientific gas chromatograph (GC) IsoLink, a Trace GC Ultra gas chromatograph, and a custom-built pre-concentration system modifying a Conflo IV interface. Briefly, 120 mL water samples were preserved with 0.5% weight:volume ZnCl₂ and stored upside down in gas-tight bottles without headspace until analysis. One day before analysis, 20 mL of the water was exchanged at ambient pressure with helium gas (grade 5.0), and the bottles were shaken for 1 h to equilibrate the headspace. Low-concentration samples (< 40 nmol L⁻¹) were analyzed by loading 10 mL of the headspace gas into a 10 mL loop. Higher concentration samples were injected into a 2 mL loop or analyzed by direct injection, bypassing the pre-concentration system. The low-concentration samples were pre-concentrated; and water, CO₂, and other condensable contaminants were removed by passing the gas mixture through three sequential freeze traps: a capillary immersed in liquid N₂, a 60 cm HayeSep D column immersed in liquid N₂-ethanol to remove water vapor and CO₂, and another capillary immersed in liquid N₂. The cleaned gas

was finally injected into the gas chromatograph, and remaining CO₂ was separated from methane on a 25 m Poraplot Q column. The eluted methane was combusted with CuO at 1030°C to CO₂ and analyzed for its isotopic composition by monitoring the ratios 45:44 and 46:44 on the Delta Plus Advantage isotope ratio mass spectrometer. Two reference gas standards were run before each gas analysis. Precision of the system and linearity were checked with an in-house gas standard that was cross-calibrated through analysis at the University of California Davis. Linearity of the system was checked by running gas standards with signal strengths between 1.5 and 5 V (0.09 ± 0.07‰). Accuracy based on repeated analysis of the in-house gas standard (−42.8‰) was ± 0.6‰ at concentrations of 0.22 μmol L^{−1} and 3.53 μmol L^{−1}. Isotope composition was reported in the standard delta notation $\delta^{13}\text{C} = (^{13}\text{C}:^{12}\text{C})_{\text{sample}} / (^{13}\text{C}:^{12}\text{C})_{\text{standard}} - 1) \times 1000$ using Vienna Pee Dee Belemnite (VPDB) as primary reference standard.

Modeling—We used the model of McGinnis et al. (2006), which simulates a single rising bubble in water and tracks the stripping and dissolution of CH₄, O₂, and N₂ (the major dissolved gases present in the water column) and which has been calibrated for shallow, freshwater conditions (McGinnis et al. 2011). The amount of gas transferred is primarily a function of partial pressure (P_i, where i denotes a gas species), initial bubble radius (r), and bubble–water contact time (i.e., bubble rise velocity, v_b). The rate of change with depth (z) of the mass of gas in the bubble M_i is given as:

$$\frac{dM_i}{dz} = -K_{Li}(H_i P_i - C_i) \frac{4\pi r^2}{v_b} \quad (1)$$

where H_i is the Henry's Law coefficient and C_i is the water-side concentration. The evolving bubble size with depth is predicted as a function of ambient temperature and hydrostatic pressure, as well as gas transport into and out of the bubble. v_b and the mass transfer coefficients K_{Li}, both bubble-size dependent, are continually adjusted with changing conditions. The model was written in Fortran and numerically integrated using the Euler method.

To constrain the vertical transport within the surface layer, we considered the methane gradient between 0 and 2 m and calculated the flux (F) using Fick's Law:

$$F = K_z \frac{\partial \text{CH}_4}{\partial z} \quad (2)$$

where the vertical turbulent diffusivity K_z at 1 m depth is ca. 6 × 10^{−3} m² s^{−1} for Lake Stechlin (Kirillin et al. 2012). We also estimated the diffusive loss at the water surface using the boundary layer equation:

$$F_{\text{diff}} = k(\text{CH}_{4\text{aq}} - C_{\text{atmEq}}) \quad (3)$$

where the mass transfer coefficient (k) is taken from the literature (Crusius and Wanninkhof 2003), CH_{4aq} is the water-side measured concentration, and C_{atmEq} is the atmospheric equilibrium methane concentration. The diffusive mass transfer flux from the water surface to the atmosphere is given as a function of wind speed (Crusius and Wanninkhof 2003). Wind speed data were provided by

the Neuglobsow weather station of the German Federal Environmental Agency (Umweltbundesamt).

Results

Water column methane in Lake Stechlin—The lake was thermally stratified and well-oxygenated during our study. The methane profile assumed a similar shape in all three summers such that methane concentrations were very low in the hypolimnion, increased to maximum in the thermocline, and remained high in the epilimnion (Fig. 1). Methane concentration in air was 0.06 μmol L^{−1}. The minimum surface water temperature recorded during the field study was 17°C, giving a dissolved methane concentration of ~ 0.002 μmol L^{−1} at equilibrium. The measured surface-water methane concentrations were 0.09–0.76 μmol L^{−1}, which were at least 1–2 orders of magnitude higher than the atmospheric saturating concentration. Mid-water methane oversaturation was an annually recurring phenomenon, and the methane peak repeatedly overlapped with oxygen oversaturation and the thermocline (Fig. 2). The peak was particularly strong in 2010 during a pronounced bloom of cyanobacteria (*Aphanizomenon flos-aquae*) when DO reached 0.58 mmol L^{−1} (205% saturation). However, we found no significant relationships between photoautotroph abundances and methane concentrations in the upper 20 m (maximum cable length for the FluoroProbe) or the upper 7 m (Secchi depth = 6.8 m).

In our transect study, there was no distinct concentration gradient that would suggest lateral input of methane (Fig. 3). In contrast, the water column methane concentrations in the littoral zone were either comparable to or lower than those in the middle and deep stations.

In the in-lake incubation experiment, gross methane production in the total fraction was 90.6 ± 0.1 nmol L^{−1} d^{−1} (mean ± standard deviation (SD)), comparable to an earlier report (Grossart et al. 2011). The production in the < 5 μm fraction was 38.7 ± 9.2 nmol L d^{−1}, accounting for 42.7% of the total production.

In the experiment testing for light effect on methane oxidation, methane concentrations decreased in all bottles (1-way ANOVA; p < 0.001; Fig. 4), but the rate of decrease was significantly higher in the dark bottles (0.103 μmol L^{−1} d^{−1}) than in the light bottles (0.089 μmol L d^{−1}; t-test, t = 6.318, degrees of freedom [df] = 4, p = 0.003).

Methane isotope signature—The starting isotope composition of methane at the beginning of the experiment was −47.5‰, which is the isotope composition of methane shown at 6 m depth on 26 July 2012. Over the course of the experiment the concentration of methane increased from 0.5 μmol L^{−1} to 1.3 μmol L^{−1}, but the isotope composition of methane did not change significantly. This indicates that reservoir effects relative to the putative source compound of carbon, possibly dissolved inorganic carbon, were insignificant. The isotope composition of methane in the incubation experiments was −47.5 ± 0.3‰ (n = 3) for the total fraction and −46.5 ± 1.0‰ (n = 3) for the < 5 μm fraction. Depth profiles showed the lowest δ¹³C_{methane} at the surface (−49.2 ± 0.8‰, n = 6). In the methane maximum, δ¹³C increased to −48.6 ± 1.0‰ (n = 6); below

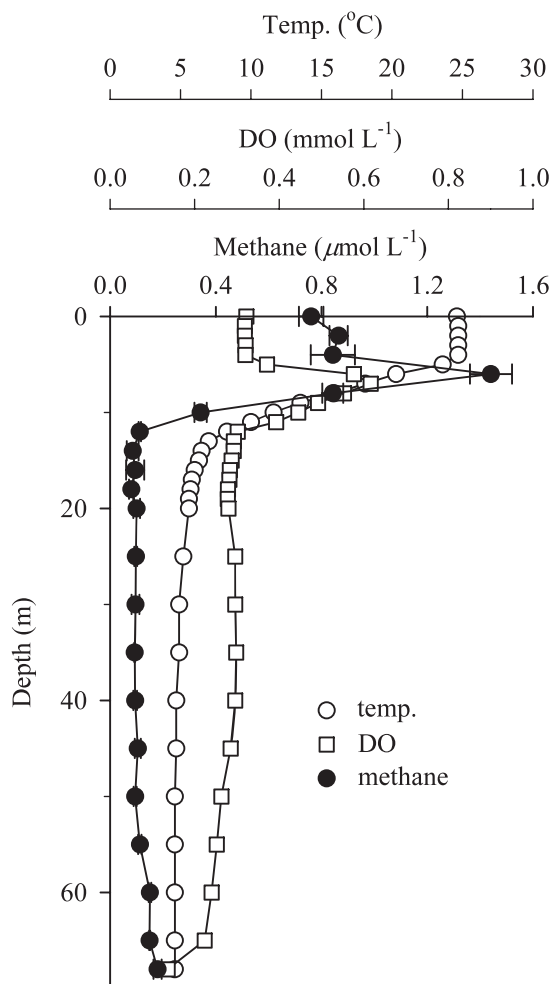


Fig. 1. Typical water column profiles of temperature (temp.), DO, and methane (mean \pm SD of duplicates) at Lake Stechlin deep station during the summer. The shown profiles were taken on 21 July 2010 (Grossart et al. 2011).

this depth, $\delta^{13}\text{C}$ increased to as high as $-29.5 \pm 3\text{‰}$ ($n = 6$) at 60 m. Methane below the thermocline was heavily enriched in ^{13}C and averaged $-31.5 \pm 0.02\text{‰}$ (Fig. 5).

Modeling of methane ebullition—We simulated methane ebullition using bubbles of 4 mm and 6 mm diameters, typical sizes found in natural aquatic environments (Ostrovsky et al. 2008; Maeck et al. 2013). Bubbles were released from 20 m, 40 m, and 70 m (Fig. 6). For the 70 m case, the 4 mm bubble barely reached the surface, and its methane dissolution rate was zero by the time it reached about 30 m (Fig. 6c). The 6 mm bubble lost $> 98\%$ of its methane to dissolution before reaching the thermocline (Fig. 6b); by the time it reached the surface, most of the gas had been replaced by N_2 ($\sim 70\%$) and O_2 ($\sim 13\%$; Fig. 6d). Similar argumentation could be used for bubbles released at 40 m and 20 m. Assuming these initial bubble sizes, small bubbles (< 1 mm), if they were caught in the thermocline, would no longer contain methane.

Water column methane in other lakes—Sampling in the other lakes also showed mid-water methane oversaturation

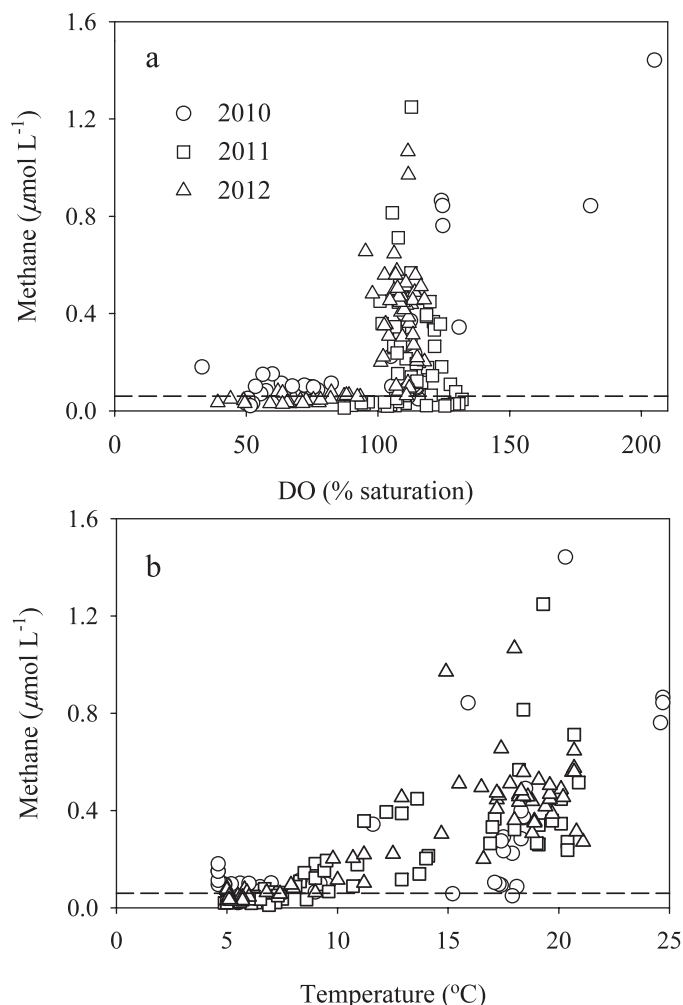


Fig. 2. Water column methane concentration in relation to (a) % DO saturation, and (b) water temperature at Lake Stechlin deep station in three consecutive summers. The dashed lines represent the atmospheric methane concentration.

(Fig. 7). Methane concentrations were very high in the hypoxic or anoxic bottom waters of the more eutrophic lakes but decreased rapidly to a minimum in the hypolimnion before increasing again in the thermocline and epilimnion. The one exception is Lake Ellbogen, which was visibly more turbid than the other lakes due to sediment delivery by the Steinhavel River and in which methane concentration remained relatively constant within 0–4 m. When we pooled all of the data and plotted log methane concentrations against % DO saturation, a tilted “L-shape” pattern emerged (Fig. 8): A steep, negative gradient within 0–20% DO in the bottom water, and a second, less pronounced, gradient where methane actually increased with DO in the upper waters.

Discussion

Possible anoxic methane sources—To explain the mid-water methane saturation in Lake Stechlin, we first considered several possible anoxic sources of methane. One explanation for the methane paradox is lateral input of

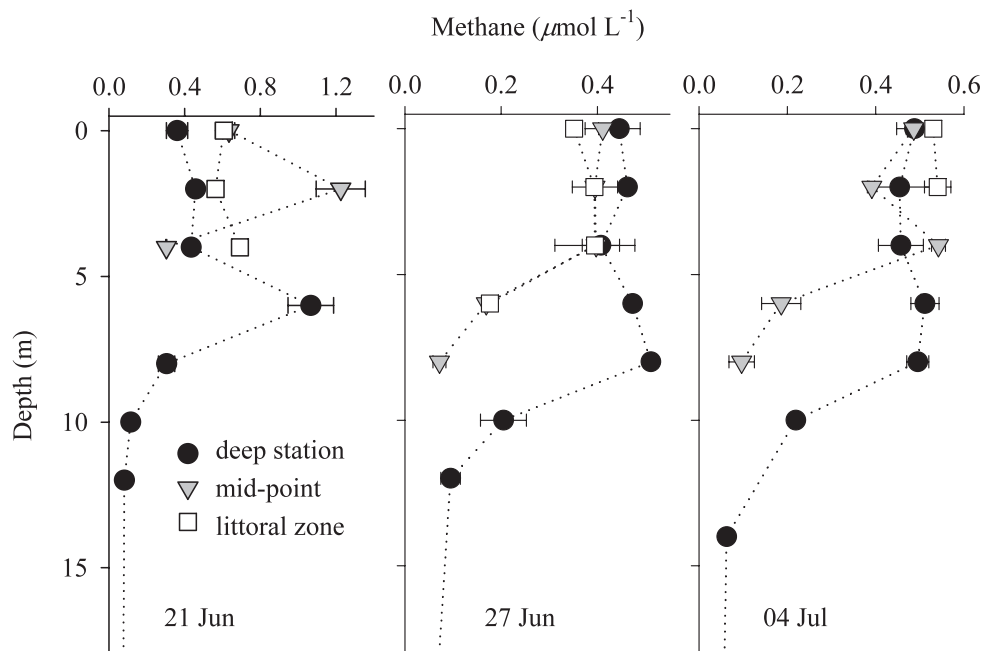


Fig. 3. Water column methane concentration (mean \pm SD of duplicates) along a transect between the deep station and the littoral zone in Lake Stechlin on three occasions in 2012. Methane profile for the deep station is shown only for the upper 17 m to aid comparison.

methane from the surrounding littoral zone, which could be important for a small lake with an organic-rich littoral zone such as Lake Stechlin. Contrary to our expectation, in our transect study we did not see a methane concentration gradient that would suggest lateral transport of methane from the littoral zone to the deep station.

Another possibility is methane production within micro-anoxic zones. In our size-fraction incubation experiments, we observed methane production in the $< 5 \mu\text{m}$ fraction. This size fraction excluded any large detritus, metazoans, and fecal pellets; hence, our results showed that methane production was not restricted to known micro-anoxic zones.

One intriguing observation was that the methane maxima repeatedly overlapped with the upper boundary

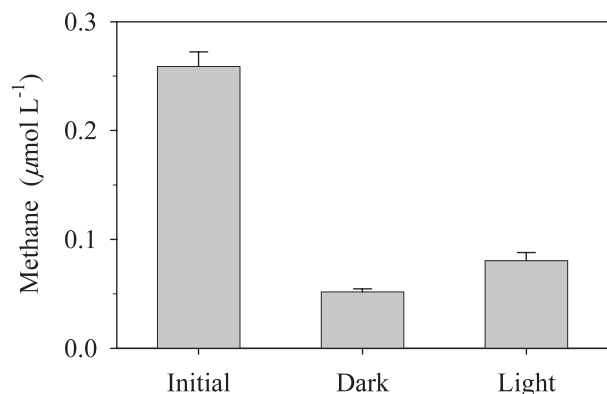


Fig. 4. Initial and final methane concentrations (mean \pm SD of triplicates) for the in-lake incubation experiment to test for light effects on methane oxidation.

of the thermocline, prompting the speculation that, if the thermocline acted as a barrier to trap small methane bubbles from the sediment, it could lead to methane accumulation there. This issue was addressed in our modeling study. Our model showed that small bubbles (4 mm) released from 70 m would have lost all of their methane before reaching the thermocline (Fig. 6b); therefore, they would not contribute methane to the thermocline peak. Large bubbles (6 mm) released from 70 m would contain mostly N_2 and O_2 and would have a methane dissolution rate of ca. 0.01 nmol s^{-1} upon reaching the thermocline. Because the methane dissolution rate was an inverse function of depth, dissolution of bubble methane should produce much (up to 40 times) higher methane concentrations below the thermocline; but this was not supported by our water column profile measurements. Very small bubbles ($< 1 \text{ mm}$ diameter) would no longer contain methane when reaching the thermocline. Overall, the model showed that ebullition was unlikely to explain the methane peak in the thermocline.

Active methane production in oxic water—As we demonstrated here, the recurring mid-water methane peak in Lake Stechlin was not dependent on lateral transport, in situ micro-anoxic zones, or bubble input from bottom sediment. Rather, our incubation experiments and isotope analysis suggested active methane production in the oxic layer, consistent with earlier observations of methyl coenzyme M gene expression within the thermocline (Grossart et al. 2011). Whereas the presence of methanotrophs in the hypolimnion had been supported by molecular assay (Grossart et al. 2011) and methane isotope analysis (this study), they were not detectable in the meta- and

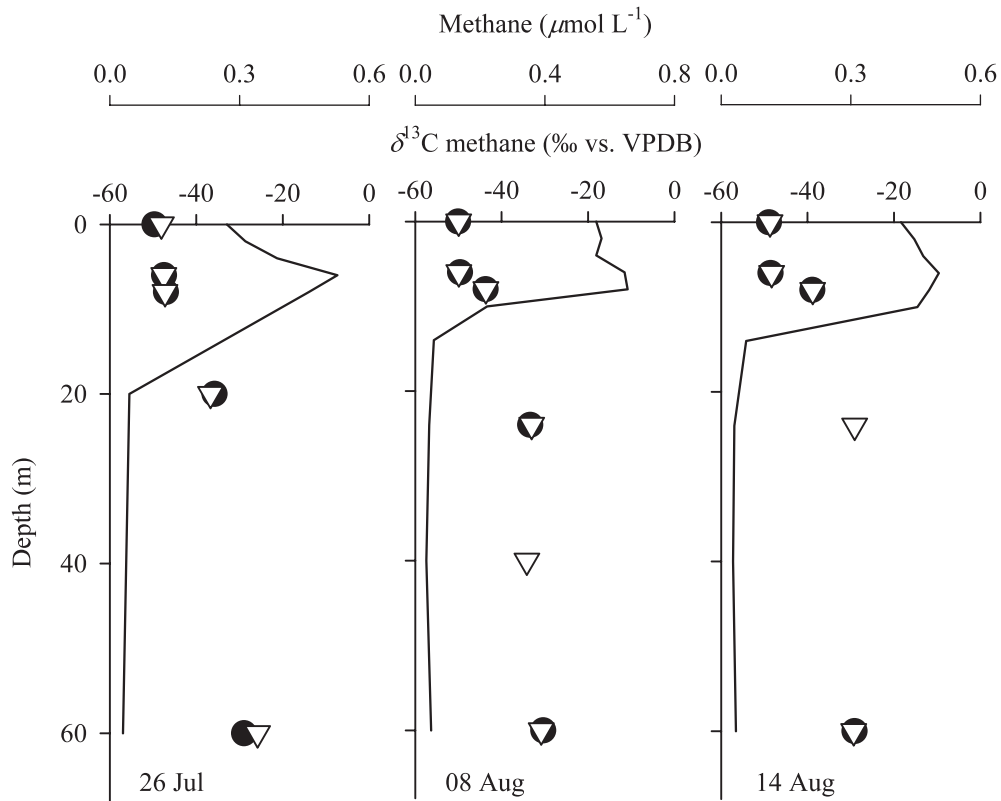


Fig. 5. Lake Stechlin water column profiles of methane (line) and $\delta^{13}\text{C}_{\text{methane}}$ (replicate samples with symbols) measured on three occasions in 2012.

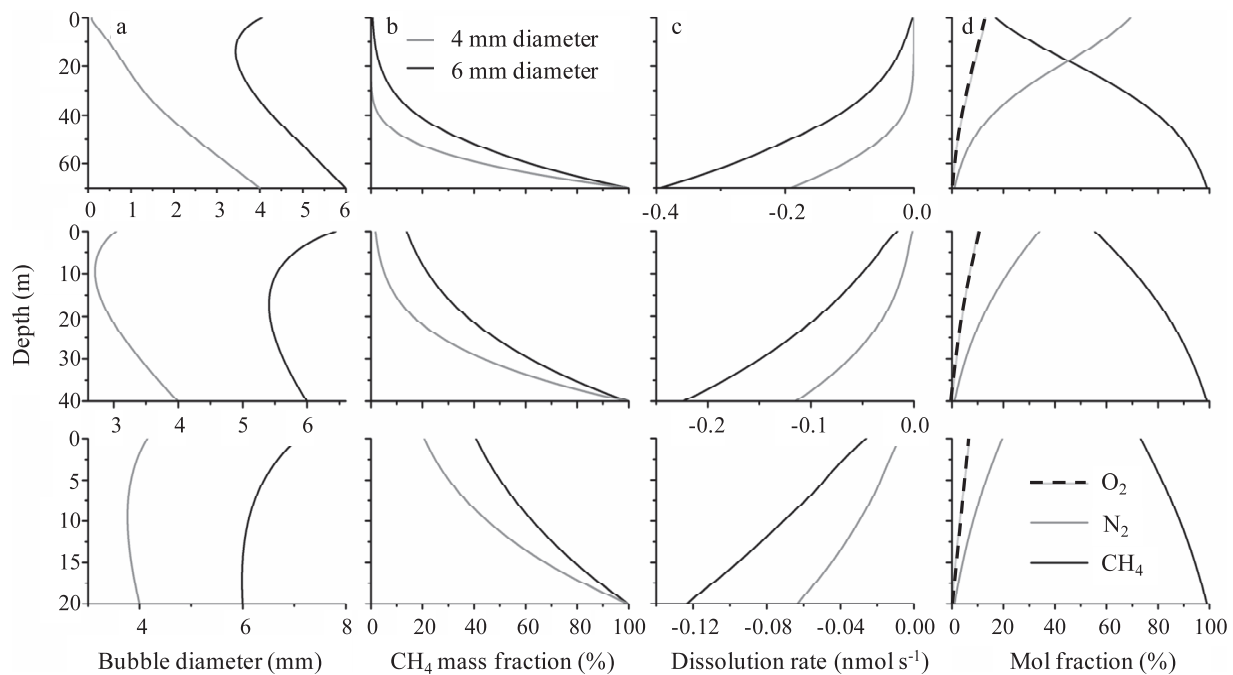


Fig. 6. Results from bubble modeling for three different depths (70 m, 40 m, and 20 m). (a) Bubble diameter as a function of depth; (b) mass fraction of methane remaining in bubble as a function of depth; (c) methane dissolution rate as a function of depth; (d) mol fraction of the dominant gas species shown for the 6 mm diameter case.

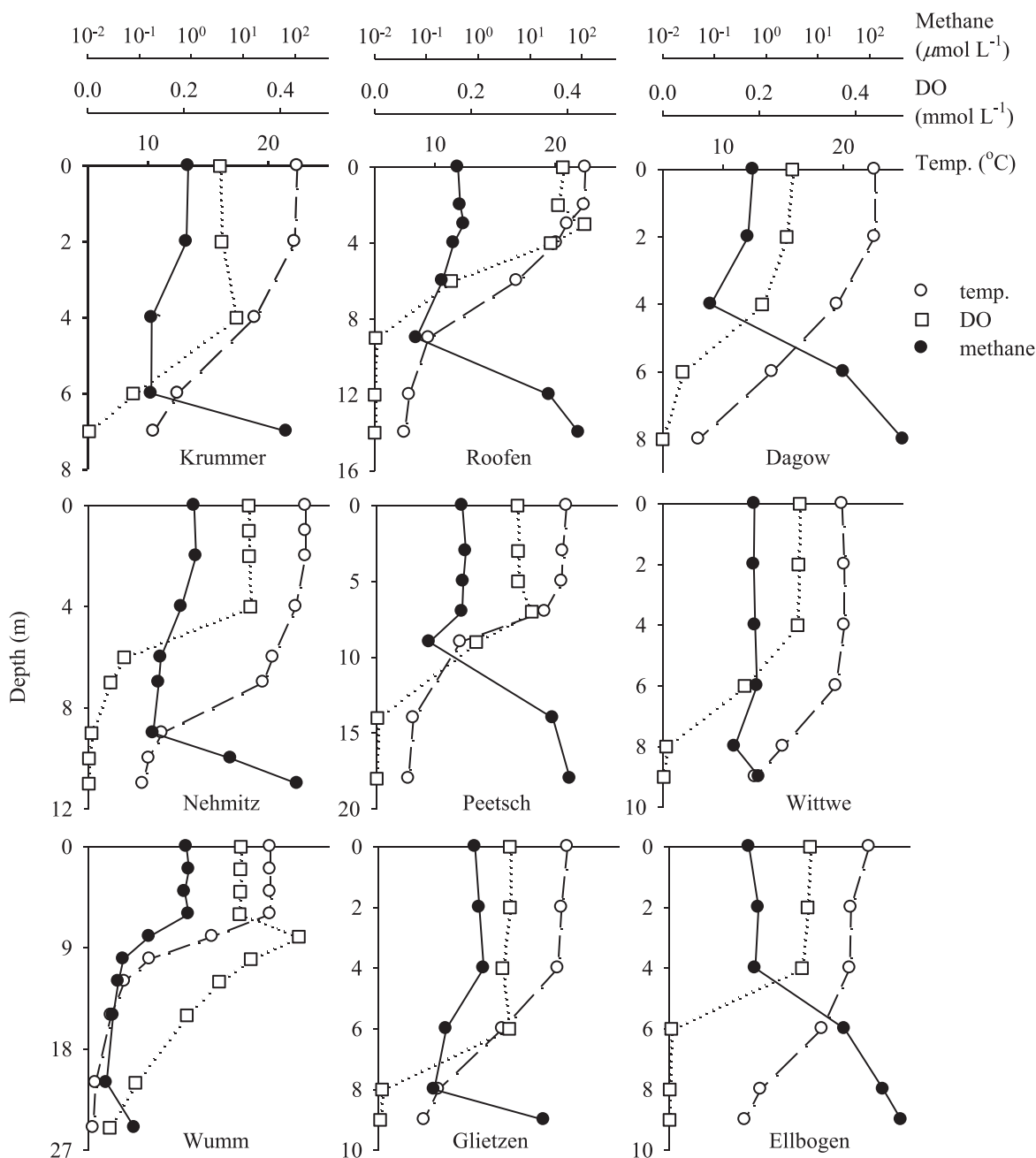


Fig. 7. Water column profiles of temperature, DO, and methane for the nine lakes (see Table 1) sampled in 2012. Panels in the same column share the same x-axes. Methane concentrations are plotted in log scale. Note the different y-axis scales for depth.

epilimnion (Grossart et al. 2011). Here we showed that lessening of methane oxidation by light within the photic zone can further uncouple methane production and oxidation, allowing methane accumulation in the upper layer.

Aerobic methane oxidation produces CO_2 depleted in ^{13}C (Templeton et al. 2006); and, therefore, the residual methane will be enriched in ^{13}C . Hence, the strong enrichment in ^{13}C observed below the thermocline is best explained by aerobic oxidation by methanotrophs. To test the hypothesis that the methane in the hypolimnion was an oxidized residue of methane formed in the thermocline that

diffused downward, we used an isotope fractionation of 20‰ (Bastviken et al. 2002) to calculate the predicted isotope composition of methane in a Rayleigh fractionation model (Mariotti et al. 1981):

$$\delta^{13}\text{C}_{\text{methane, hypolimnion}} = \delta^{13}\text{C}_{\text{methane, thermocline}} - \varepsilon(\ln f) \quad (4)$$

where ε is the isotope fractionation factor and f represents the fraction of methane remaining after oxidation. The predicted methane isotope value was between 0‰ and +20‰ in the hypolimnion, significantly more ^{13}C -enriched than observations. It is apparent that methane in the

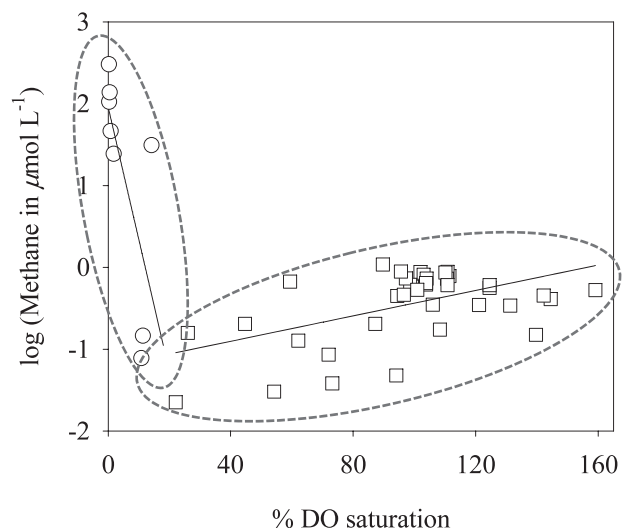


Fig. 8. Water column methane concentration (in log scale) vs. % DO saturation. Data are compiled from nine lakes (see Table 1). Two gradients are apparent from the data: A steep negative gradient within 0–20% DO in the bottom waters ($y = -0.16x + 1.96$; $r^2 = 0.50$; $p = 0.05$), and a positive gradient extending from 20% DO in the deep waters to oxygen-oversaturation in the upper layers ($y = 0.01x - 1.21$; $r^2 = 0.28$; $p < 0.01$).

hypolimnion must have an additional source, likely diffusion from the sediment.

Relationship with photoautotrophy—Methane production in association with phytoplankton has been observed in the laboratory cultures (Scranton 1977; Grossart et al. 2011). In our field study, however, the ambient methane concentration did not appear to be a simple function of photoautotroph abundances, and we suspect that primary production perhaps would be a better predictor. To explore this issue, we used 2010–2012 Lake Stechlin primary production (^{14}C -bicarbonate uptake method) data from an ongoing monitoring program, kindly provided by M. Gessner (Leibniz Institute of Freshwater Ecology and Inland Fisheries). We selected only the primary production measurements taken within ± 1 day and ± 1 m depth of our methane measurements. The in situ methane concentrations were significantly related to primary production (Fig. 9), but the modest explanatory power of the linear regression (44.9%) suggests that the in situ methane concentration was controlled by additional unknown factors. The relationship between methane concentration and primary production does not necessarily mean that the methane was produced directly by the photoautotrophs. Rather, we hypothesize that methanogens were using substrates released by the photoautotrophs to produce methane, as we discuss below.

Probable biochemical pathways—The observed methane production in oxic water calls into question the mechanism(s) involved. Here we consider several possibilities. Methanogens can remain active in oxic waters provided that they can tolerate oxygen exposure. This oxygen

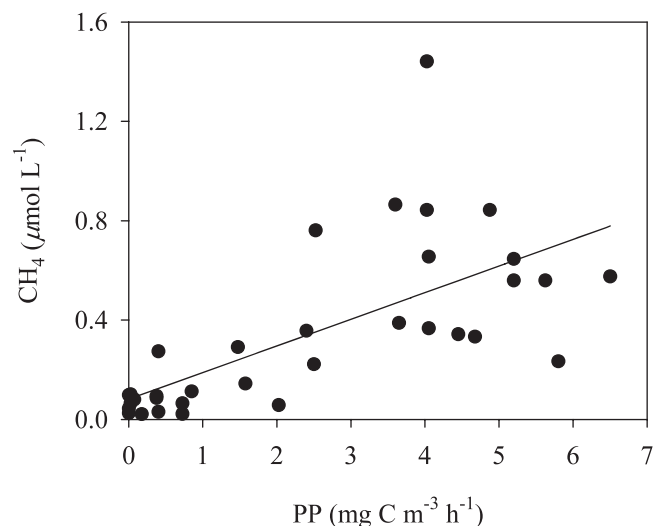


Fig. 9. Relation between methane concentration and primary production (PP) for the upper 25 m in Lake Stechlin. The linear regression is $y = 0.107x + 0.082$ ($r^2 = 0.45$; $p < 0.0001$).

tolerance may be regulated at the molecular level as recently discovered (Angel et al. 2011): The desert soil methanogens *Methanosarcina* and *Methanocella* increasingly transcribe the gene for the enzyme catalase in the presence of oxygen, which detoxifies reactive oxygen species and allows the methanogens to actively produce methane. We suggest that the methanogens in Lake Stechlin water column had similar properties.

Even if the methanogens were oxygen tolerant, questions remain as to what biochemical pathway(s) they used to produce methane. The relatively ^{13}C -enriched isotope composition of methane at the lake surface suggests a production pathway with a relatively small isotope fractionation. Stable isotope fractionation effects during acetoclastic methanogenesis range between -5% and -35% (Goevert and Conrad 2009). In comparison, the value is between -29% and -79% for hydrogenotrophic methanogenesis (Valentine et al. 2004), and in excess of -79% for methylotrophic methanogenesis (Penger et al. 2012). Phytoplankton are known to exude a wide variety of compounds, including methylphosphonate and dimethylsulfoniopropionate, the two methylated substrates shown to support methane production (Karl et al. 2008; Damm et al. 2010). However, enrichment experiments with Lake Stechlin water using dimethylphosphonate and trimethylamine failed to stimulate methane production (Grossart et al. 2011), and our observed isotope fractionation values were much lower than the typical values for a methylotrophic pathway. Instead, our isotope data were more consistent with acetoclastic methanogenesis. Low concentrations of acetate have been observed in lake surface waters ($0\text{--}0.1 \mu\text{mol L}^{-1}$; Allen 1968). Although the origin of this acetate is not known, it could partially support methanogenesis in the upper water column. Hydrogenotrophic methanogens can also produce fractionations of -25% when the H_2 partial pressure is high (Valentine et al. 2004) or when cell-specific methanogenesis rates are high (Krzycki et al. 1987). Desert soil methanogens have been

shown to switch from acetoclastic methanogenesis to hydrogenotrophic methanogenesis under oxic conditions (Angel et al. 2011). In Lake Stechlin, photosynthesis by phytoplankton could provide hydrogen to adjacent methanogens for methane production. The strong methane peak associated with a diazotroph bloom in 2010 suggests that hydrogen production via nitrogen fixation (Conrad 1980) may further promote methane production.

Methane emission from oxic lake waters—Eugster et al. (2003) showed that penetrative convective mixing (e.g., during cooling events) in the surface layer can potentially enhance fluxes to the atmosphere by nearly 5 times compared with stratified periods. Therefore, we use two estimates for methane emissions, a water-side and an air-side method.

If not consumed or dispersed, the accumulated methane in the surface layer would diffuse upwards towards the atmosphere. We calculate this as an upper estimate of potential methane emission to the atmosphere. The methane data from 2011 and 2012 (we chose not to use the very high values from 2010), which averaged (\pm SD) $0.38 \pm 0.18 \mu\text{mol L}^{-1}$ at 0 m and $0.40 \pm 0.15 \mu\text{mol L}^{-1}$ at 2 m, gives a concentration gradient (0–2 m) of $-11 \pm 34 \mu\text{mol m}^{-4}$ (the minus sign indicates an upward flux). Using Fick's Law (gradient approach) and replacing the molecular diffusion coefficient with the turbulent diffusivity (Eq. 2), the average upward flux in the surface layer was ca. $-5 \text{ mmol m}^{-2} \text{ d}^{-1}$. Given a surface lake area of 4.52 km^2 , this translates to an average efflux of $2.4 \times 10^4 \text{ mol d}^{-1}$ (380 kg d^{-1}).

Alternatively, surface emissions at the water–air interface were estimated using the mass transfer rate (Crusius and Wanninkhof 2003). Using the average wind speed on the sampling days for 2011–2012 (2.1 m s^{-1}) and the average surface methane concentration ($0.4 \mu\text{mol L}^{-1}$), the estimated diffusive flux rate was $\sim 563 \text{ mol d}^{-1}$ ($\sim 9 \text{ kg d}^{-1}$) over the entire lake surface area. While this is ~ 40 times less than the earlier estimate, this should be considered a conservative estimate using only the mean methane values. Using hourly measured wind value, fluxes were periodically up to 10 times higher. Because the wind piston velocity is nonlinear, using the integrated average on a daily basis would result in a higher flux value.

Both estimates obviously come with some uncertainty and are meant to represent the upper and lower bounds of the actual value. The fluxes will be influenced by the diffusivity value, which would increase substantially during periods of intense wind mixing, extended cool weather events, or nighttime deep-penetrating convective mixing events (Eugster et al. 2003). Further studies with, e.g., eddy towers or flux chambers are needed to better constrain the actual fluxes.

Future directions—Our study showed previously overlooked active microbial methanogenesis in the oxygen-rich Lake Stechlin water column that is likely coupled with photosynthesis and/or nitrogen fixation. While the precise biochemical pathway(s) remains unclear, isolation and cultivation of the methanogens in question is critically needed for detailed studies. Positive correlation between methane concentration and DO in the upper water column

of nine other lakes suggests that this methane production process is quite common. Mid-water methane production has also been observed in the thermocline in coastal oceans, e.g., the North Sea (Schneider von Deimling et al. 2011). The contribution of methane from oxic fresh and marine waters could be substantial but is currently excluded from the global methane budget. Further investigation into this methane source will help improve our understanding of the methane cycle.

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