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Microbial methane production in oxygenated water column of an oligotrophic lake

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The prevailing paradigm in aquatic science is that microbial methanogenesis happens primarily in anoxic environments. Here, we used multiple complementary approaches to show that microbial methane production could and did occur in the well-oxygenated water column of an oligotrophic lake (Lake Stechlin, Germany). Oversaturation of methane was repeatedly recorded in the well-oxygenated upper 10 m of the water column, and the methane maxima coincided with oxygen oversaturation at 6 m. Laboratory incubations of unamended epilimnetic lake water and inoculations of photoautotrophs with a lake-enrichment culture both led to methane production even in the presence of oxygen, and the production was not affected by the addition of inorganic phosphate or methylated compounds. Methane production was also detected by in-lake incubations of lake water, and the highest production rate was 1.8–2.4 nM·h^{-1} at 6 m, which could explain 33–44% of the observed ambient methane accumulation in the same month. Temporal and spatial uncoupling between methanogenesis and methanotrophy was supported by field and laboratory measurements, which also helped explain the oversaturation of methane in the upper water column. Potentially methanogenic Archaea were detected in situ in the oxygenated, methane-rich epilimnion, and their attachment to photoautotrophs might allow for anaerobic growth and direct transfer of substrates for methane production. Specific PCR on mRNA of the *methyl coenzyme M reductase A* gene revealed active methanogenesis. Microbial methane production in oxygenated water represents a hitherto overlooked source of methane and can be important for carbon cycling in the aquatic environments and water to air methane flux.

epilimnic methane peak | methanogens

Although methane makes up <2 parts per million by volume (ppmv) of the atmosphere, it accounts for 20% of the total radiative forcing among all long-lived greenhouse gases (1). In the aquatic environments, methane is also an important substrate for microbial production (2). The prevailing paradigm is that microbial methanogenesis occurs primarily in anoxic environments (3, 4). A commonly observed paradox is methane accumulation in well-oxygenated waters (2, 5), which is often assumed to be the result of physical transport from anoxic sediment and water (6–8) or in situ production within microanoxic zones (9–11). Two recent studies challenged this paradigm and suggested that microbes in oligotrophic ocean can metabolize methylated compounds and release methane even aerobically (12, 13). These claims are not without caveats, because the amounts of methylated compounds added [1–10 µM methylphosphonate (12) and 50 µM dimethyl sulfoniopropionate (13)] were far higher than their environmental concentrations, and therefore, the ecological relevance remains obscure. Moreover, dissolved oxygen (DO) was not monitored during the long incubation (5–6 d), and the possibility that the experimental setups had become anoxic before methane production could not be dismissed.* Despite the uncertainty, if microbial methane production can occur in oxygenated water, it will have profound implications for carbon cycling and climate.

Here, we test if microbial methane production occurs in the well-oxygenated water column of a temperate oligotrophic lake (Lake Stechlin, Germany). To obtain conclusive evidence, we established several criteria: (*i*) verification of methane production even in unamended lake water, (*ii*) confirmation that DO remains at high levels when methane production occurs, and (*iii*) demonstration of uncoupling between methane production and consumption causing accumulation of methane in well-oxygenated water.

Results

In Situ Methane Profiles and Microbial Composition. Methane oversaturation was observed within the upper 10 m on July 21 and 22, 2010, when the lake was calm and strongly stratified (Fig. 1*A*). A methane maxima of 1.35–1.44 µM coincided with the thermocline and DO oversaturation at 6 m (Fig. 1 *B* and *C*). DO oversaturation was also present 1 mo earlier (Fig. 1*C*), coinciding with the chlorophyll (Chl) *a* peak (Fig. 1*D*). However, the Chl *a* maximum was below 10 m on July 22, and the observed DO peak was likely the result of a past phytoplankton bloom. Microbial abundance was higher in the upper 12 m than below the thermocline, and of this abundance, 11–40% were particle-associated microbes (Fig. 1*E*). The dominant autotrophs were the cyanobacterium *Aphanizomenon flos-aquae* and diatoms (*Asterionella formosa* and *Fragilaria crotonensis*) in the upper 9 m. Free-living methanogenic Archaea belonging to uncultured Methanomicrobiales were present at 6 m (Fig. S1), and particle-associated methanogenic Archaea were found at 6 (closely related to uncultured Methanomicrobiales and *Methanosaeta*) and 9 m (closely related to *Methanosaeta* and the Candidatus *Methanoregula* sp.) (Fig. S1); however, they were undetectable at 18 m. Methane-oxidizing

*We estimated oxygen consumption in the experiments in the work by Karl et al. (12) as follows. According to table 2 in ref. 12, particulate carbon increased by 104 µM within 48 h. Assuming a bacterial growth efficiency of 0.33 and a respiratory quotient of one (14), the estimated O$_2$ consumption because of bacterial biomass production would have been 311 µM, which was more than the ambient O$_2$ concentration (205–215 µM) (ref. 12, table 1). Alternatively, based on table 2 in ref. 12, we calculated a dissolved organic carbon (DOC) consumption of 217 µM within 48 h. Assuming a 1:1.2 molar ratio of DOC oxygen to O$_2$ consumption by microbes (15), the incubation bottles would have become anoxic in 38–40 h. In the experiments, glucose was added (100–200 µM) to stimulate bacterial growth, which would also stimulate oxygen consumption. Assuming a molar ratio of 1 glucose to 6 O$_2$, the addition of glucose would have led to an O$_2$ consumption of 600–1,200 µM, which is three to six times the oxygen that was present in the water. Hence, using their data and estimating oxygen consumption in various ways, we came to the same conclusion. It is very likely that their incubation bottles had become anoxic before substantial methane production was observed.
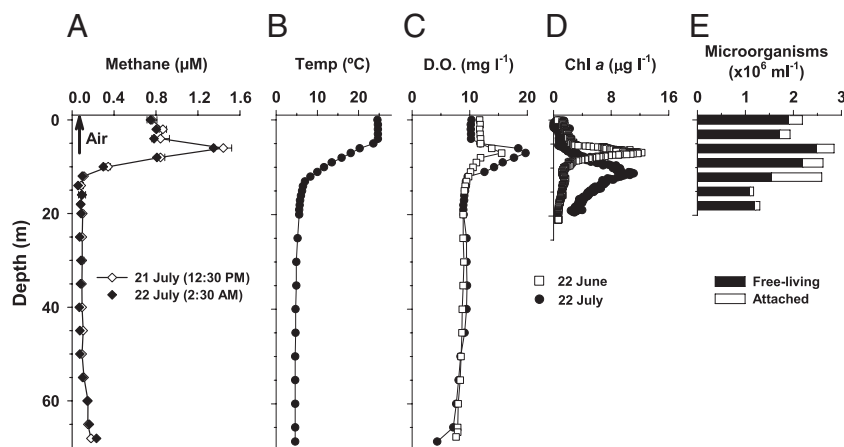
Fig. 1. Depth profiles for Lake Stechlin. (*A*) Mean methane concentrations during day and night (July 21 and 22, 2010, respectively). Error bars during the day are SDs of two to three measurements of the same bottles. Arrow indicates methane concentration in the air. (*B*) Average temperature on July 21 and 22. (*C*) Dissolved oxygen (DO) on June 22 and July 22. (*D*) Chlorophyll (Chl) *a* concentrations on June 22 and July 22. (*E*) Free-living and attached microorganism abundances on July 22.

bacteria (MOB) were not detectable in the epilimnion, whereas at 18 m, a single species of MOB type I (99% similarity to *Methylobacter tundripaludum*) was detected. MOB type II were not detectable.

Repeated measurements in September of 2010 showed temporal regression of the methane peak at 6 m, decreasing to only 0.10 μM by September 12. The reverse temporal development was observed in 2011, when the methane peak at 6 m increased from 0.18 μM in May to 1.25 μM in June (Fig. S2). As in the previous year, the methane maxima in 2011 coincided with the thermocline and DO oversaturation. We detected the active *methyl coenzyme M reductase A* (*mcrA*) gene for methanogenesis based on mRNA at 6 m in May and June of 2011. The *mcrA* gene was also present at 18–20 m, but it was undetectable at 0 m in May (Table 1).

Methane Production in Laboratory Incubation Experiments. Laboratory incubation of unamended water samples from discrete depths in July showed positive methane production (Fig. 2*A*). Significantly higher production rates were observed when daytime water samples were incubated in the dark rather than when nighttime samples were incubated in the light. Water samples from the upper 8 m where cyanobacteria and green algae were abundant showed higher methane production rates (maximum = 3.40 nM·h^{-1}), which corresponded with the high in situ methane concentrations (Fig. 2*A*). The water in the incubation bottles never became anoxic, and the DO was at 94–97% saturation at the end of the experiment. Despite the earlier reports (12, 13), addition of inorganic phosphate, methylphosphonate, or trimethylamine to lake surface water did not result in any significant increase in methane concentrations in our experiments (Table S1).

Table 1. Results of specific PCR on cDNA of the *mcrA* gene

Date	Sample (m)	PCR products
May 14, 2011	0	–/–
May 14, 2011	6	+/+
May 14, 2011	18	+/+
June 3, 2011	6	+/+
June 3, 2011	20	+/–
June 13, 2011	6	+/+

Equal amounts (5 μL) of the respective PCR products were added to the 2% agarose gel together with negative and positive controls plus the DNA size standard. Samples were tested in parallels; presence (+) and absence (–) of PCR products are given for each parallel.
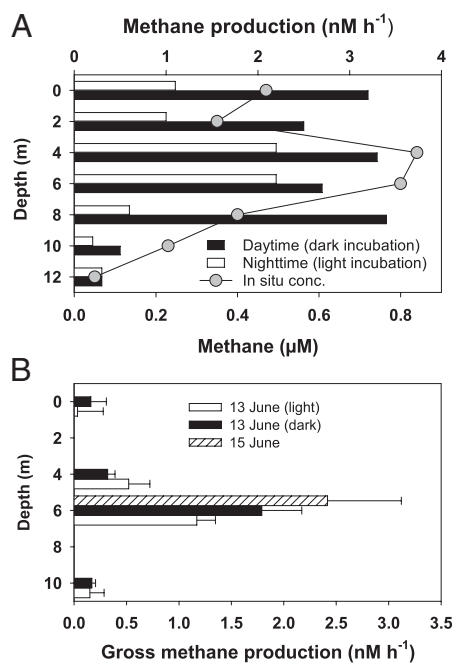
Methane Production in In-Lake Incubation Experiments. Methane production was observed in the first in-lake incubation experiment (June 13) (Fig. 2*B*). The gross methane production rate varied significantly among water samples suspended at different depths but not between the light and dark treatments. The highest average production rates were at 1.2–1.8 nM·h^{-1} at 6 m. Methane production was observed again in the second experiment (June 15) at an even higher average rate of 2.4 nM·h^{-1} at 6 m (Fig. 2*B*) when in situ methane concentrations had further increased.

Uncoupled Methane Oxidation in Laboratory Experiments. When unamended Lake Stechlin surface water samples were incubated over a longer time, methane concentrations in all three replicates increased at an average rate of 52 nM·h^{-1} (Fig. S3). Two of the replicates were killed for DO measurement, which was at ≥80% saturation. Methane concentration in the remaining replicate subsequently decreased at a rate of 52 nM·h^{-1} (Fig. S3). Threshold-dependent methane oxidation was observed in another experiment. In unamended lake water and water with added methane and oxidation inhibitor, methane concentrations did not change significantly over time; in the sample augmented with >50 μM methane, methane oxidation proceeded at a rate of 0.19 μM·h^{-1} (Fig. S4).

Enrichment Culture Experiments. Microbial enrichment culture established from lake water was used to inoculate cultures of three photoautotrophs: (*i*) *A. flos-aquae* (SAG 31.87), (*ii*) axenic *Microcystis aeruginosa* (HUB W333), and (*iii*) a xenic *Chlorella*-like green alga from Lake Stechlin. In all cases, inoculation led to significantly higher methane production under well-oxygenated conditions compared with those conditions without the inoculum or the inoculum alone (Table S2). FISH revealed direct attachment of potentially methanogenic Archaea to the photoautotrophic cells (Fig. 3).

Discussion

Our study showed that epilimnetic methane oversaturation is a recurring phenomenon in Lake Stechlin during the stratified season. Similar temporal development of epilimnetic methane oversaturation has also been observed in Lake Constance, which was assumed to be caused by anoxic aggregates within the water column (11). Here, we showed positive methane production in both laboratory and in-lake incubation experiments with unamended lake water excluding any external methane sources. Although internal sources caused by microanoxic zones could
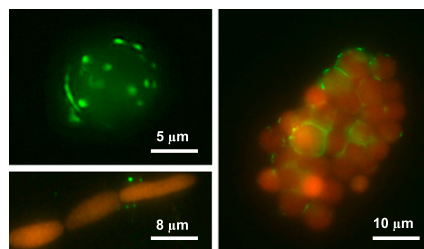
Fig. 2. (*A*) Methane production by Lake Stechlin water collected from different depths in laboratory experiments in July of 2010. Excess methane was stripped by vigorous shaking before incubation. Daytime water samples were incubated in the dark, and nighttime water samples were incubated in the light (43 μmol photons·m^{-2} s^{-1}) for 8 h at room temperature. Final DO was 7.4–7.7 mg·L^{-1} (94–97% saturation). Methane production in dark incubation was significantly higher than production in light incubation (paired *t* test; $n = 7$, $P = 0.022$). Ambient methane concentration profiles are included for comparison. (*B*) Methane production by Lake Stechlin water during in-lake incubation experiments. Water was collected from different depths on June 13, 2011 and stripped of excess methane. Afterward, the water was used to fill up three sets of gas-tight bottles in triplicate ($n = 3$). One set was used as the light treatment, one set was wrapped in aluminum foil as the dark treatment, and one set was spiked with >10^{-4} M 2-bromoethanesulfonic acid (BES) to inhibit methanogenesis. The three sets of bottles were then suspended at their original depths for 8.5 h. At the end of the incubation, dissolved methane concentrations in the bottles were measured by headspace analysis. Gross methane production rates were calculated as the difference between the light or dark treatment and the BES treatment divided by the incubation time. Two-way ANOVA test indicated that methane production rate varied significantly with depth ($n = 8$ $P < 0.0001$) but not between the light and dark treatments ($n = 8$, $P = 0.188$). The in-lake incubation experiment was repeated on June 15 at the 6-m depth with light and BES treatments, and methane production was calculated in the same manner.

Fig. 3. Direct attachment of methanogenic Archaea (green, FITC-labeled oligonucleotide probe) to autotrophs (red, autofluorescence) observed by FISH. (*Upper Left*) A single *Chlorella*-like algal cell. (*Right*) A colony of *Chlorella*-like green alga. (*Lower Left*) A filament of the cyanobacterium *A. flos-aquae*.

not be completely dismissed, metazoans, fecal pellets, or large phytoaggregates were not present in our incubations, and the short incubation time (<1 d) should have prevented oxygen depletion even for large (>1 mm) aggregates (16). The overlapping of the methane peak, *mcrA* gene transcription, and strong DO oversaturation at 6 m also implies that the methane production was positively, not negatively, related to oxygen availability. Our results, therefore, provide direct empirical evidence of microbial methane production under well-oxygenated conditions.[†]

The depth-specific differences in methane production rates in the incubation experiments corresponded well with the vertical variations in ambient methane concentrations (Fig. 2), indicating that the ambient profile was strongly influenced by depth-specific methane production and consumption processes. The methane

production rate was highest at 6 m in both in-lake incubation experiments, consistent with the strong expression of the *mcrA* gene for methanogenesis (Table 1). Based on the ambient profiles, the methane concentration at 6 m increased from 0.59 to 1.25 μM between June 10 and 15, 2011 (Fig. S2), which is equivalent to an average accumulation rate of 5.5 nM·h^{-1}. The estimated average production rates (1.8–2.4 nM·h^{-1} at 6 m) (Fig. 2*B*), therefore, suggest that in situ methane production could account for 33–44% of the observed methane accumulation at that depth in mid-June when assuming the total absence of methane oxidation.

In the presence of oxygen, methane production needs to be uncoupled from methane oxidation to produce the observed methane accumulation. Our longer-term incubation experiment with surface water showed that average methane increase rate during the first 51 h was the same as the average methane decrease rate in the subsequent 42 h. This finding suggests that, although methane oxidation could potentially balance methane production, there was a temporal uncoupling between the two processes, which could result from a time lag in growth of methanotrophs, inhibitory effects of initially high DO concentration (17), or a threshold methane concentration required by methanotrophs (18). Threshold-dependent methane oxidation was also supported by the additional experiment in which methane oxidation was detectable only in the water spiked with >50 μM methane, suggesting that methane oxidation only proceeds at an appreciable rate when the ambient methane concentration has reached a high level. In situ methane oxidation may also be inhibited by nitrate, nitrite, and ammonium released by grazing and microbial degradation of organic matter in water layers with high algal and cyanobacterial biomass (19).

Spatial uncoupling between methanogenesis and methanotrophy within the water column was supported by our molecular analysis. Methanogenic Archaea were present, but MOB were not detectable in the epilimnion in July of 2010 when methane oversaturation was observed. Active methanogens, especially at 6-m depth, were also confirmed by our mRNA data. MOB were detectable only below the thermocline (e.g., at 18- and 20-m depth), where ambient methane concentrations were low but *mcrA* gene expression was still detectable. This spatial separation between methanogenic Archaea and MOB could also contribute to the accumulation of methane in the epilimnion. Our field measurements showed that the methane peak at 6 m tended to develop in May to July and then decline in September (Fig. S2). This wax and wane pattern may be related to temporal changes in the abundance and activities of methanogens (production) and methanotrophs (consumption). Understanding what factors determine these temporal variations will be an important topic for future studies.

Several mechanisms have been proposed for methanogenesis in oxygenated waters (20): cleavage of methyl-esters by UV radiation, hypoxia-induced methane production in mitochondria,

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[†]The term aerobic methane production strictly means that the production process requires oxygen, which has not been shown in this study or earlier studies (12, 13); hence, we describe our results as methane production under oxygenated conditions.

methane release by organisms under oxidative stress, and microbial decomposition of methylated compounds. In oligotrophic lakes such as Lake Stechlin, UV penetration is limited to <2.0 m (21), much shallower than the observed methane peak. UV was also absent in our laboratory experiments where methane production was observed. The overlapping between the methane peak and the oxygen peak in situ suggests that hypoxia is not a factor. It is also unlikely that epilimnetic organisms would suffer from strong oxidative stress, because they frequently experience high oxygen concentration and have multiple mechanisms to cope with it (22). Although conversion of methylated compounds such as methanethiol to methane can be bioenergetically favorable for methanogenic Archaea (13), neither methanethiol nor other methylated compounds are commonly found in high concentrations in oxygenated epilimnion in lakes (23–25). In our experiments, the addition of neither inorganic phosphate nor methylated compounds affected methane production; hence, pelagic methanogenesis in Lake Stechlin did not seem to depend on phosphate or methylated substrates like other systems (12, 13).

The alternatives are hydrogenotrophic and acetoclastic methanogenesis, both of which are common among methanogenic Archaea (26). The Archaea in Lake Stechlin belong to groups that have the ability to perform hydrogenotrophic or acetoclastic methanogenesis (27–29). Many photoautotrophs can produce H_2 through both direct and indirect photolysis (30). Cyanobacteria can also increase H_2 yield through nitrogen-fixing activities at night (31). Indeed, the slightly higher methane production rates in our dark vs. light incubations (Fig. 2A) suggest that H_2 production through intracellular fermentation using storage photosynthates (32) or nitrogen fixation in the dark may be particularly important for pelagic methanogenesis in Lake Stechlin. Contrary to common belief, many methanogens can tolerate and survive oxygen exposure (33), which helps explain their methanogenic activity within the water column. It has been shown that *Methanosarcina* and *Methanocella* in desert soils can transcribe the *mcrA* gene even under oxic/oxygenic conditions and at the same time, actively transcribe the gene for oxygen-detoxifying catalase (34). It is also noteworthy that particle-associated *Methanosaeta* were present in the epilimnion in Lake Stechlin (Fig. S1). This group of methanogens is known to tolerate oxygen exposure (35) and is particularly well-adapted to low acetate environments (28). The co-occurrence of high numbers of cyanobacteria, algae, and attached Archaea within the epilimnion in our study may enable a direct transfer of H_2 or acetate from the autotrophs to the methanogenic Archaea to support methane production. Although our field observations suggest that, in Lake Stechlin, this methane production was closely associated with cyanobacteria, our laboratory experiments confirmed that methane production could also be supported by other photoautotrophs in the presence of methanogenic Archaea (Table S2). Given the ubiquitous distribution of Archaea and photoauthotrophs in the aquatic environments, this methane production process may be more widespread than previously recognized.

Inverse modeling has been used to explain the observed atmospheric methane concentrations by constraining the uncertainties in known sources and sinks of methane, but this approach does not consider methane production in oxygenated water bodies (36). It is estimated that freshwater environments contribute >70% of the natural source of methane to the atmosphere (37). Because of the traditional emphasis on anaerobic methanogenesis, this methane is assumed to originate from anoxic sediments and bottom waters (38). Our study provides compelling evidence that microbial methane production can also take place within the oxygenated water and is high enough to contribute substantially to epilimnetic methane accumulation. Quantifying the fate of this methane source—whether through water to air

flux or carbon cycling within the water column—may improve our understanding of the global methane budget and climate.

Materials and Methods

Study Site. Lake Stechlin is a dimictic oligotrophic lake in northeastern Germany (53° 10′ N, 13° 02′ E) with low anthropogenic impact (39). The lake has been continuously studied for almost 50 y, and it serves as a reference lake for the European Water Framework Directive. The lake has a maximum depth of 69.5 m and an area of 4.3 km², with a hypolimnetic oxygen saturation level of up to 60%.

In Situ Methane and Hydrographical Profiles. Water samples for dissolved methane were taken in 2-m depth intervals filled into 120-mL preweighed gas-tight crimp bottles. Within 1–2 h after sampling, dissolved methane was determined by headspace analysis (40) on a gas chromatograph with flame ionization detector (Shimadzu). Concurrent DO and water temperature were measured with an in situ probe (Wissenschaftlich Technische Werkstätten). Chl a profiles were measured by a fluorescence probe (Hardt). Attached and free-living microbes were operationally separated by a 5.0-μm polycarbonate membrane and measured by DAPI direct count (41) at 1,000× magnification with an epifluorescence microscope (Zeiss).

Laboratory Incubation Experiments. Laboratory incubation experiments were conducted to test for methane production in July of 2010 (Fig. 2). To test for the effects of inorganic phosphate and methylated compounds, surface water samples were incubated with or without the addition of 3 μM inorganic phosphate, 1 μM methylphosphonate, or 1 μM trimethylamine, and the methane concentrations were monitored over 55–69 h. To test for temporal uncoupling between methanogenesis and methanotrophy, methane concentrations in triplicate bottles of unamended surface lake water samples were monitored over a longer time, and the average rate of methane increase in the first 51 h was compared with the average rate of methane decrease in the next 42 h (Fig. S3). To test for threshold-dependent methane oxidation, surface lake water samples were incubated for 58 h with added methane or methane plus the inhibitor difluoromethane to prevent methane oxidation (42). Unamended lake water sample was used as the control (Fig. S4).

In-Lake Incubation Experiments. The first in-lake incubation experiment was conducted on June 13, 2011 to test for methane production under near in situ condition. Water samples collected from different depths were stripped of excess methane by shaking and incubated at the original depth with light, without light, or with added 2-bromoethane sulfonic acid (BES; final concentration >10⁻⁴ M) to inhibit methanogenesis (43). Gross methane production rates were calculated as the difference between the light or dark treatment and the BES treatment divided by the incubation time. The experiment was repeated on June 15 at 6-m depth with light and BES treatments (Fig. 2).

Enrichment Culture Experiments. A microbial enrichment culture was established by incubating Lake Stechlin water with Z-Medium at 26 °C and 72 μmol photons·m⁻² s⁻¹ with a 16:8 light:dark cycle. The enrichment culture was then used to inoculate A. flos-aquae (31.87; SAG), axenic M. aeruginosa HUB W333 (Humboldt University), and a green alga isolate from Lake Stechlin (Chlorella-like unicellular, round green alga). Inoculated cultures were kept in gas-tight crimp bottles, and methane production rates were calculated based on methane concentrations measured after 2 and 6 d. Untreated photoautotrophic cultures and inoculum were used as controls, where no methane production was detectable.

Molecular Analysis. For molecular analysis, water samples were taken between July 21 and 26, 2010 at 0-, 3-, 6-, 9-, 12-, 16-, and 18-m depth; 500 mL were prefiltered through a 5.0-μm Nuclepore polycarbonate membrane, and genomic DNA of free-living microbes was subsequently collected by filtering 500 mL prefiltered sample onto a 0.2-μm Nuclepore polycarbonate filter. DNA was extracted with chloroform-phenol-isoamylalcohol and zirconium beads (44). The reaction mixtures for PCR amplification contained 2 μL template DNA, 200 nM each of the appropriate primers (Table S3), 250 μM each deoxyribonucleoside triphosphate, 3 mM MgCl₂, 5 μL 10× PCR buffer, and 0.5 U BIOTAQ Red DNA polymerase (Bioline) in a total volume of 50 μL. Cloning of partial 16S rRNA genes was done using primers specific for Archaea including methanogenic Archaea (Table S3) and the pGEM-T-Easy Vector System II (Promega) according to the manufacturer's protocol. Insert length was checked by primers T7 and SP6. Of each clone library, 20–30 clones were picked and sequenced. PCR products of clones were purified using an established protocol (45). Sequences are deposited in GenBank

under the accession numbers JF510050–JF510160. To test for active methanogenesis as indicated by mRNA expression, samples for RNA extraction were taken in May and June of 2011; 1 L water from selected depths (0, 6, 18, or 20 m) was filtered over a PES filter (Millipore). The samples were stored at −80 °C. Extraction of DNA and RNA used an established protocol (46). DNA was then removed by digestion with a Turbo DNA-Free Kit (Ambion) using the manufacturer's instructions. The digestion was proofed by a PCR with universal primers. cDNA synthesis from extracted RNA was performed with random oligohexamers and the Array Script (Ambion) using the manufacturer's instructions. Specific primers (Table S3) were then used in a nested PCR approach to target cDNA from mRNA for *mcrA*. PCR conditions were the same as above. For FISH, photoautotrophic cells were embedded on slides (47) and fixed with 70% ethanol for 1.5 min. Hybridization was done for 2 h using the probe MG3 for predominantly methanogens (48) and used here to target potentially methanogenic Archaea. The probe was labeled with fluorescein-isothiocyanate (FITC). Afterward, hybridized cells were analyzed with an epifluorescence microscope using the FITC filter set (Leica).

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Supporting Information
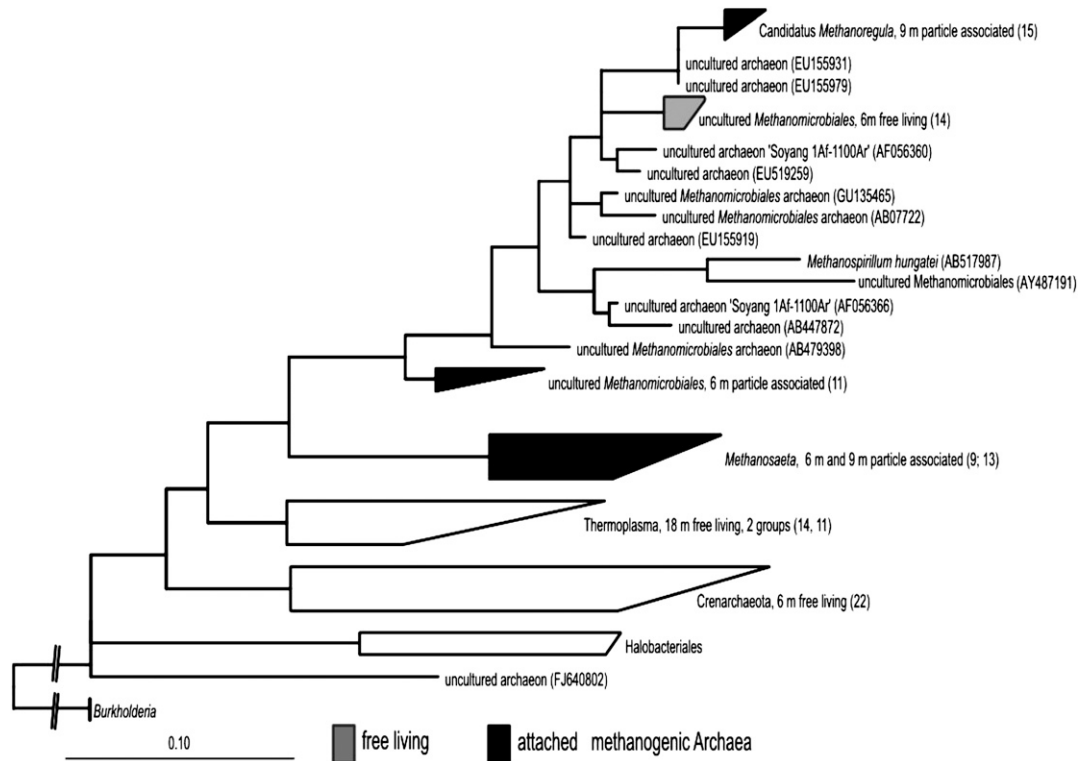
Grossart et al. 10.1073/pnas.1110716108

Fig. S1. Phylogenetic tree of methanogenic Archaea in Lake Stechlin. Sequences were obtained from three clone libraries (6-, 9-, and 18-m depth) using primers that are specific for Archaea including methanogenic Archaea (Table S3). The tree is constructed using the software ARB (http://www.arb-home.de/). Numbers in parenthesis give the number of sequences in the respective clusters.
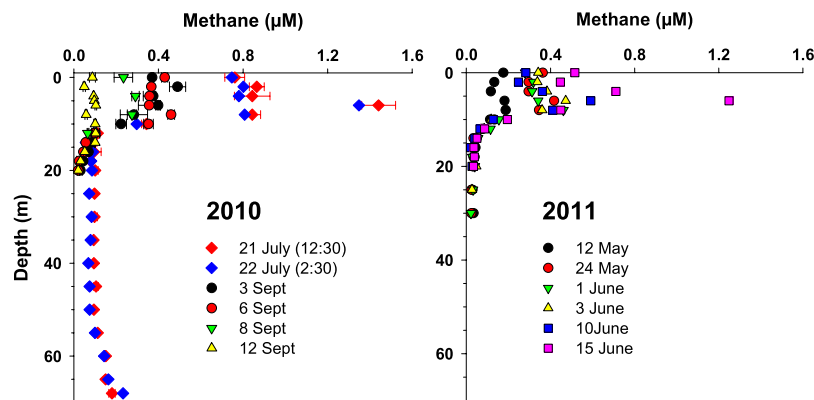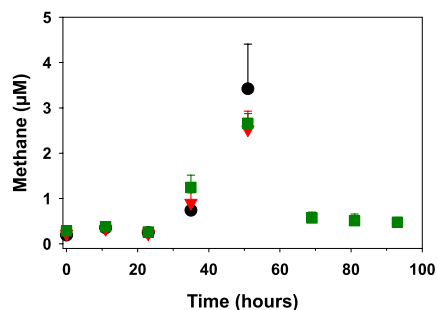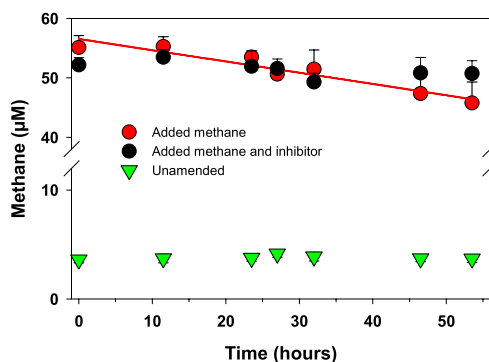

Fig. S2. In situ methane concentrations in Lake Stechlin. Each data point represents a single sample or the average of duplicate samples. In 2010, the epilimnion methane concentrations decreased over time from July to September. In 2011, the epilimnion methane concentrations increased over time from May to June. In all cases, the epilimnion was near or over 100% oxygen-saturated.

Fig. S3. Methane concentrations in unamended Lake Stechlin surface water incubated over time. Different symbols represent different replicate bottles. Error bars are SDs of triplicate measurements of the same bottles. After 51 h, two bottles were killed for oxygen measurements, and dissolved oxygen concentrations were 6.6–6.8 mg L^{-1} (80–84% saturation). The remaining bottle was incubated longer, and the average rate of increase (+52 nM h^{-1} for 0–51 h) was the same as the average rate of decrease (−52 nM h^{-1} for 51–93 h).

Fig. S4. Methane concentrations in Lake Stechlin surface water with or without added methane and inhibitor (0.03 kPa difluoromethane). Error bars are SDs of triplicate measurements of the same bottles. There was no significant change in methane concentration in unamended water or water + methane + inhibitor (linear regressions: slope not significantly different from zero; $n = 7$, $P > 0.05$). In water with added methane, methane concentration decreased significantly over time (linear regression: $y = 56.54 − 0.189x$; $n = 7$, $r^2 = 0.99$, $P = 0.008$).

Table S1. Methane concentrations (μM) in Lake Stechlin surface water incubated over time with and without the addition of inorganic phosphate (PO_4^{3-}; 3 μM), methylphosphonate (MPn; 1 μM), or trimethyl amine (TMA; 1 μM)

Hour	Control	+PO_4^{3-}	+MPn	+TMA
0*	0.619	0.690	—	—
9*	0.726	0.797	—	—
21*	0.536	0.766	—	—
33*	0.523	0.574	—	—
55*	0.600	0.573	—	—
0†	0.192	—	0.181	0.160
2†	0.235	—	0.148	0.144
4†	0.181	—	0.128	0.130
6†	0.136	—	0.136	0.132
14†	0.115	—	0.102	0.099
16†	0.104	—	0.096	0.090
24†	0.105	—	0.094	0.099
35.5†	0.107	—	0.092	0.088
45.5†	0.116	—	0.095	0.091
55.5†	0.106	—	0.093	0.101
69†	0.115	—	0.083	0.090

*No significant effect because of the addition of phosphate ($P = 0.131$, two-way ANOVA).
†Control is significantly higher than treatments ($P \ll 0.001$, two-way AN-OVA). Addition of MPn and TMA did not increase methane production.

Table S2. Methane production by Lake Stechlin bacteria inoculum when incubated with different photoautotroph cultures for 4 d in the light

Culture	Methane production rate (nM h^{-1}; mean $\pm$ SD)
Aphanizomenon flos-aquae (SAG31.87)	0.346 $\pm$ 0.463
Microcystis aeruginosa (HUB W333)	0.337 $\pm$ 0.145
Chlorella-like green algae*	0.306 $\pm$ 0.105

The corresponding controls were the photoautotroph alone and the inoculum alone. No methane production was detectable in any of the controls.
*Isolated from Lake Stechlin.

Table S3. Primers and PCR conditions used for PCR amplification performed in the present study

Primer	Function	Sequence	Cycler program*	Source
341f	16S universal bacteria	CCT ACG GGA GGC AGC AG	95 °C for 3 min; 30 cycles of 1 min at 95 °C, 1 min at 55 °C, 2 min at 72 °C; 10 min at 72 °C	1
907r	16S universal bacteria	CCG TCA ATT CMT TTG AGT TT	95 °C 3 for min; 30 cycles of 1 min at 95 °C, 1 min at 55 °C, 2 min at 72 °C; 10 min at 72 °C	2
533f	16S universal bacteria	GTG CCA GCA GCC GCG GTA A	95 °C for 5 min; 30 cycles of 1 min at 95 °C, 1 min at 58 °C, 1 min at 72 °C; 10 min at 72 °C; both MOB-specific primers were used with universal 533f	3
MethT1bRgc	16S methylotrophic bacteria type 1	GAT TCY MTG SAT GTC AAG G	95 °C for 5 min; 30 cycles of 1 min at 95 °C, 1 min at 58 °C, 1 min at 72 °C; 10 min at 72 °C; both MOB-specific primers were used with universal 533f	4
MethT2Rgc	16S methylotrophic bacteria type 2	CAT CTC TGR CSA YCA TAC CGG	95 °C for 5 min; 30 cycles of 1 min at 95 °C, 1 min at 58 °C, 1 min at 72 °C; 10 min at 72 °C; both MOB-specific primers were used with universal 533f	4
21f	16S universal archaea	TCC GGT TGA TCC YGC CGG	95 °C for 5 min; 30 cycles of 1 min at 95 °C, 1 min at 56 °C, 2 min at 72 °C; 10 min at 72 °C; first PCR for a nested approach for 16S of methanogenic archaea*	5
1492r	16S universal bacteria/archaea	GGY TAC CTT GTT ACG ACT T	95 °C for 5 min; 30 cycles of 1 min at 95 °C, 1 min at 56 °C, 2 min at 72 °C; 10 min at 72 °C; first PCR for a nested approach for 16S of methanogenic archaea*	6
Arc 344f	16S archaea	TCG CGC CTG CTG CIC CCC GT	94 °C for 5 min; 19 cycles of 1 min at 94 °C, 71 °C (−0.5 °C per cycle) for 1 min, 72 °C for 2 min; 20 cycles at 94 °C for 1 min, 61 °C for 1 min, 72 °C for 2 min; 72 °C for 10 min	7
Arc 915r	16S archaea	GTG CTC CCC CGC CAA TTC CT	94 °C for 5 min; 19 cycles of 1 min at 94 °C, 71 °C (−0.5 °C per cycle) for 1 min, 72 °C for 2 min; 20 cycles at 94 °C for 1 min, 61 °C for 1 min, 72 °C for 2 min; 72 °C for 10 min	8
357f	16S methanogenic archaea	CCC TAC GGG GCG CAG CAG	95 °C for 10 min; 35 cycles of 45 s at 95 °C, 45 s at 54 °C, 45 s at 72 °C; 10 min at 72 °C	9
691r	16S methanogenic archaea	GGA TTA CAR GAT TTC AC	95 °C for 10 min; 35 cycles of 45 s at 95 °C, 45 s at 54 °C, 45 s at 72 °C; 10 min at 72 °C	9

*All PCR reactions were performed in a PT-200 gradient cycler (Biorad); the nested approach for 16S methanogenic archaea was necessary, because otherwise, there were false bands.

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