Aquatic and terrestrial *Cyanobacteria* produce methane

***Cyanobacteria*, the most ancient and abundant photoautotrophs on Earth produce the greenhouse gas methane during photosynthesis.**

Short title

- Methanogenesis by Cyanobacteria

Authors

Bižić M.[1*#], Klintzsch T.[2#], Ionescu D.[1#], Hindiyeh M. Y.[3], Günthel M.[4], Muro-Pastor A.M.[5], Eckert W.[6], Urich T.[7], Keppler F.[2,8], Grossart H-P[1,9*]

Affiliations

1. Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), Alte Fischerhuette 2, D-16775 Stechlin, Germany
2. Institute of Earth Sciences, Biogeochemistry Group, Heidelberg University, Heidelberg, Germany
3. Department of Water and Environmental Engineering, German Jordanian University, Amman, Jordan
4. Department of Biosciences, Swansea University, SA2 8PP Swansea, United Kingdom
5. Instituto de Bioquímica Vegetal y Fotosíntesis, Consejo Superior de Investigaciones Científicas and Universidad de Sevilla, Sevilla, Spain
6. Israel Oceanographic and Limnological Research, The Yigal Alon Kinneret Limnological Laboratory, Migdal 14650, Israel
7. Institute of Microbiology, Center for Functional Genomics, University of Greifswald, Felix-Hausdorff-Str. 8, 17489 Greifswald, Germany.
8. Heidelberg Center for the Environment (HCE), Heidelberg University, 69120 Heidelberg, Germany
9. Institute of Biochemistry and Biology, Potsdam University, Maulbeerallee 2, 14469 Potsdam, Germany

# These authors contributed equally to this work
* Correspondence should be addressed to:

Mina Bižić, Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), Department of Experimental Limnology, Alte Fischerhuette 2, Neuglobsow, OT Stechlin, 16775, Germany
Email: mbizic@igb-berlin.de; Tel +49-33082-69969

Hans-Peter Grossart, Leibniz Institute of freshwater ecology and inland fisheries (IGB), Department of Experimental Limnology, Alte Fischerhuette 2, Neuglobsow, OT Stechlin, 16775, Germany
Email: hgrossart@igb-berlin.de; Tel +49-33082-69991

Abstract

Evidence is accumulating to challenge the paradigm that biogenic methanogenesis, considered a strictly anaerobic process, is exclusive to *Archaea*. We demonstrate that *Cyanobacteria* living in marine, freshwater and terrestrial environments produce methane at substantial rates under light, dark, oxic and anoxic conditions, linking methane production with light driven primary productivity in a globally relevant and ancient group of photoautotrophs. Methane production, attributed to cyanobacteria using stable isotope labelling techniques, was enhanced during oxygenic photosynthesis. We suggest that formation of methane by *Cyanobacteria* contributes to methane accumulation in oxygen-saturated marine and limnic surface waters. In these environments, frequent cyanobacterial blooms are predicted to further increase due to global warming potentially having a direct positive feedback on climate change. We conclude that this newly identified source contributes to the current natural methane budget and most likely has been producing methane since *Cyanobacteria* first evolved on Earth.

MAIN TEXT

Introduction

Methane (CH_4) is the second most important anthropogenic greenhouse gas after CO_2 and is estimated to have 28-34 times higher warming effect than the latter over a 100-year period (*1*). The mixing ratio of CH_4 in the troposphere has increased from 715 ppbv in the preindustrial era to currently 1,865 ppbv (Feb. 2019 NOAA). Estimated global CH_4 emissions to the atmosphere average at ca. 560 Tg per year (1Tg = 10^{12} g) exceeding the current estimated sinks by ca. 13 Tg per year (*2*). Thus, to mitigate the constant increase in atmospheric CH_4 a comprehensive understanding of global CH_4 sources and the environmental parameters that affect them is necessary.

Traditionally, biogenic methanogenesis is the formation of methane under strictly anoxic conditions by microbes from the domain *Archaea* (phylogenetically distinct from both eukaryotes and *Bacteria*). However, in the past decade there has been growing evidence that also eukaryotes such as algae (*3*), plants (*4*), animals (*5*), fungi (*6*) and probably humans (*7*) produce methane under oxic conditions albeit at considerably lower rates. These recent findings suggest that the phenomenon may not be solely limited to methanogenic *Archaea* and could include new metabolic pathways. For example, the conversion of methylated substrates such as methylphosphonates to CH_4 by *Bacteria* has been extensively addressed in recent years with regards to the "methane paradox" (*8*, *9*). Recently, Zheng *et al.* (2018) have shown CH_4 formation by *Rhodopseudomonas palustris* during N_2 fixation. Methane emission was also detected from cryptogamic covers, i.e. phototrophic assemblages on plant, rock and soil surfaces (*11*).

Accumulation of CH_4 in oxygenated freshwater environments has been repeatedly associated with the presence of *Cyanobacteria* (see also Fig. S1). Methane production by *Cyanobacteria* has been attributed to either demethylation of methylphosphonates (*12–14*) or to the association of filamentous *Cyanobacteria* with methanogenic *Archaea*, providing the latter with the necessary hydrogen for CH_4 production (*15*). *Cyanobacteria* are ubiquitous, found literally in any illuminated environment as well as unexpectedly in some dark subsurface ones as well (*16*, *17*). Furthermore, this phylum predominated Earth whilst the environment was still reductive and ca. 1.3 billion years prior to the great oxygenation event which occurred 2.4 billion years ago (*18*). Therefore, we tested whether this phylum contributes to the global CH_4 budget independent of naturally occurring, extra-cellular precursor substances or the presence of methanogenic *Archaea*. If so, their ubiquitous nature, their expected future increase in abundance (*19*, *20*) and their proximity to the interface with the atmosphere makes them potential key players in the global CH_4 cycle.

Here we demonstrate that unicellular as well as filamentous, freshwater, marine and terrestrial members of the prominent and ubiquitous phylum *Cyanobacteria*, a member of the domain *Bacteria*, produce CH_4 at substantial rates under light and dark as well as oxic and anoxic conditions.

Results and Discussion

To test the hypothesis that *Cyanobacteria* produce CH_4 independent of known methylated precursors (e.g. methylphosphonates) in ambient water, thirteen different filamentous and unicellular cyanobacterial cultures (for details of chosen cultures see Table 1) that are known to grow in marine, freshwater and terrestrial environments were incubated under sterile conditions with ^{13}C labelled sodium hydrogen carbonate ($NaH^{13}CO_3$) as carbon source. All investigated cyanobacterial cultures showed CH_4 production with increasing stable isotope values ($\delta^{13}C$-CH_4 values) clearly indicating that ^{13}C carbon was incorporated into CH_4, whereas no ^{13}C enrichment occurred in the control experiments (Fig. 1). These results unambiguously show that *Cyanobacteria* produce CH_4 *per se* and that the process is most likely linked to general cell metabolism such as photoautotrophic carbon fixation. The different enrichment of ^{13}C indicated by $\delta^{13}C$-CH_4 values ranging from 1.71 to 1337 ‰ observed in the different cultures is a result of different production rates as well as differences in biomass. The involvement of methanogenic *Archaea* in this process can be ruled out. First, five of the cultures were axenic. Second, the oxygen concentrations during CH_4 production were in most cases above saturation level (Fig. 2 and Fig. S2) and while methanogenic *Archaea* were recently reported from oxic environments (*21*), their activity is attributed to anoxic microniches. Peters and Conrad (*22*) have additionally shown that while methanogens are abundant in oxic environments, methanogenesis remains inactive. Furthermore, transcriptomic data from two experiments we conducted over the course of 24 h with non-axenic cultures of *Anabaena* sp. PCC 7120 and *Microcystis* sp. PCC 7806 have revealed no expression of genes known to be related to classical methanogenic activity. Third, sequencing analysis of non-axenic cultures and quantitative real-time PCR of the methyl coenzyme M reductase gene (*mcrA*) showed methanogenic *Archaea* are either absent or present in negligible numbers (Fig. S3).

Furthermore, for the following reasons demethylation of methylphosphonates from the spent growth medium is unlikely to be the mechanism involved in this instance even though some *Cyanobacteria* do possess the necessary enzymatic machinery (*12*, *13*). First, thus far, demethylation of methylphosphonates has been shown to occur only under phosphorus starvation, which was highly unlikely in this study since the culture medium contained ca. 200 µmol P L^{-1}. Publicly available transcriptomic data for *Anabaena* sp. PCC 7120 (*23*, *24*) and *Trichodesmium erythraeum* (*25*) show that the phosphonate C-P lyase genes are not expressed under standard (P-rich) culture conditions. This was further corroborated by our own transcriptomes of cultures of *Anabaena* sp. PCC 7120 and *Microcystis* sp. PCC 7806 where no expression of the C-P lyase genes was detected by the *Cyanobacteria* or the accompanying microorganisms. Second, some of the *Cyanobacteria* used in this study (i.e. *Microcystis aeruginosa* PCC 7806, *Synechococcus* WH7803 and WH8102, as well as all sequenced species of *Chroococcidiopsis* sp., *Leptolyngbya* sp., *Phormidium* sp. and *Prochlorococcus* sp.) do not possess the known C-P lyase genes necessary for conversion of methylphosphonates to CH_4. The lack of the C-P lyase genes, responsible for conversion of methylphosphonates to CH_4, was demonstrated to be a common feature of the genus *Prochlorococcus* (*26*). A recent study looking at the processing of methylphosphonates by *Prochlorococcus* (27) revealed an alternative pathway where methylphosphonates are oxidized to formate. *T. erythraeum* was shown to internally produce phosphonates as P storage later to be freed by demethylation (*28*), a process that is likely to release CH_4. Nevertheless, the same study shows, though not focusing on cyanobacteria alone, that marine unicellular organisms such as *Synechococcus* and *Crocosphaera*, do not contain any detectable phosphonate storage.

Despite the recent finding of CH_4 production during N_2 fixation by *Rhodopseudomonas palustris* (*10*) we suggest that this is not the pathway leading to CH_4 production in our experiments. First, most *Cyanobacteria* used in this study are unable (i.e marine *Synechococcus*, *Prochlorococcus*, *Microcystis aeruginosa*) or unknown (*Leptolyngbya* sp., *Phormidium persicinum*) to fix N_2. Second, all experiments were conducted in NO_3^- or NH_4^+ rich, fresh, media, and therefore N_2 fixation in capable cyanobacteria is likely to be inhibited to a certain degree (*29*). Thus, given the rapid and tight response of CH_4 production with the onset of light, we consider that the mechanism by which *Cyanobacteria* readily convert fixed CO_2 to CH_4 under light conditions must revolve around the photosynthesis process. Inhibitors of photosynthesis such as Atrazine, DBMIB (2,5-Dibromo-6-isopropyl-3-methyl-1,4-benzoquinone) and HQNO (N-oxo-2-heptyl-4-Hydroxyquinoline) inhibit the CH_4 production under light conditions (Fig. S4) and suggest a connection to the photosynthetic electron transfer chain. However, the exact biochemical pathway(s) involved in light-driven cyanobacteria-derived CH_4 formation remains so far unknown and thus requires further investigation.

Patterns and rates of CH_4 production were investigated in seventeen cultures (the above mentioned 13 and additional 4) over several days of continuous measurement of CH_4 concentration using a membrane inlet mass spectrometry system (MIMS). Our measurements, lasting 2-5 days, showed that CH_4 production occurs both under light and dark conditions (Fig. 2 and Fig. S2). This is evident by a positive production rate at almost all times in all experiments. Replicate experiments revealed that, while *Cyanobacteria* repeatedly produced CH_4, rates and patterns were not consistent, particularly so for production during the periods of darkness. Often, a period with lower rates of CH_4 production was observed between light and dark phases (Fig. 2 and Fig. S2). The latter is evidenced as a decrease in CH_4 concentration resulting from degassing of our incubation system. This suggests that different mechanisms may be involved in CH_4 production under light and dark conditions, presumably dependent on freshly generated photosynthetic products during light and on storage compounds during dark periods. Fermentation of storage compounds by *Cyanobacteria* has been previously described and known to produce among other compounds acetate and hydrogen which are known precursors of acetoclastic CH_4 formation (*30*). Interestingly, most of the genes required for methanogenesis are present in non-methanogenic organisms, including *Cyanobacteria*. Nevertheless, in this instance since the methyl-coenzyme reductase (*mcr*) gene is absent this would suggest that if *Cyanobacteria* produce CH_4 via conventional pathways, an ortholog of the *mcr* gene exists, a rather unlikely option considering current knowledge from cyanobacterial genomes.

Methane production rates (Fig. 3) were calculated using the slope of CH_4 profiles and were normalized on a cyanobacterial biomass dry weight basis for larger cyanobacteria or cell counts for small-celled marine picophytoplankton. The latter was used to obtain high accuracy for the small-cell-sized picophytoplankton, *Synechococcus* and *Prochlorococcus*. Hourly CH_4 production rates across cultures of larger cyanobacteria were in the range of 0.1 to 3.4 µmol g^{-1} h^{-1} in individual experiments and a mean of 0.51 ± 0.26 µmol g^{-1} h^{-1}. Among the marine picophytoplankton, *Synechococcus* sp. exhibited low rates ranging between 0.01 and 0.6 pmol CH_4 per 10^6 cells h^{-1}, while *Prochlorococcus* cultures produced methane at rates ranging from 0.8 to 110 pmol CH_4 per 10^6 cells h^{-1}. When compared to production rates of typical methanogenic *Archaea*, CH_4 production rates of freshwater, soil and large marine cyanobacteria are three to four orders of magnitude lower than the CH_4 production rates noted for typical methanogenic *Archaea* in culture under optimal conditions (oxygen free) but one to three orders of magnitude higher than rates observed in eukaryotes (Fig. S5). In our experiments, *Prochlorococcus* and *Synechococcus* cultures produced CH_4 only at light intensities above 20 µmol quanta m^{-2} s^{-1} and therefore, it is likely that only *Procholorococcus* and *Synechococcus* communities in the upper water layers contribute to the oceanic CH_4 flux to the atmosphere.

Methane production in oxic soils has been previously discussed and attributed mainly to abiotic factors (*31*) or methanogenic *Archaea* (*32*), although the latter was thought unlikely (*31*, *33*). Here we show that a typical desert crust *cyanobacterium* (identified in this study as *Leptolyngbya* sp.), as well as the most common endolithic cyanobacterium *Chroococcidiopsis* (*34*) produce CH_4 both under light and dark conditions (Fig. 1, Fig. S2), thus inferring a new but as yet unknown and unaccounted for source of CH_4 from oxic soils.

Cyanobacteria are ubiquitous in nature and their presence in aquatic systems is expected to increase with eutrophication and rising global temperatures (*19*). The "methane paradox" describing the production of CH_4 in oxic water layers has been known for four decades (*35*). Though values may vary between water bodies, a recent study suggests that up to 90 % of CH_4 emitted from freshwater lakes can be produced in the oxic layer (*36*) with *Cyanobacteria* often being associated with elevated CH_4 concentration in oxygen supersaturated freshwater systems (*37*). In open oceanic environments, distant from any coast, the contribution of lateral transport from anoxic environments is expected to be absent. Nevertheless, based on the emission rates from our laboratory investigations it is difficult to extrapolate the contribution of *Cyanobacteria* to marine, freshwater, terrestrial, and finally to the global CH_4 budget. First, only one attempt has been performed to estimate the global cyanobacterial biomass (*34*). That study does not account for the increase in blooms of toxic and non-toxic *Cyanobacteria* in freshwater systems (*19*, *20*, *38–40*), nor for less monitored cyanobacterial environments such as those under the ice-cover of frozen lakes (*41*). Recent estimations of *Prochlorococcus* (*42*) suggest a global biomass of 3.4×10^{27} cells globally, larger by 33 % than estimated in 2003 by Garcia-Pichel *et al* (*34*). Second, while our experiments demonstrate unambiguously the ability of all investigated *Cyanobacteria* to produce CH_4 we cannot account for the effect of nutrient concentrations and environmental factors such as light and temperature to control emissions in the natural environment. Temperature alone was shown to have a major effect on the *Prochlorococcus* growth rates (*43*). Nevertheless, considering the combined day and night average CH_4 production rates of high and low light *Prochlorococcus* species (~9 pmol CH_4 h^{-1} 10^{-6} cells) which prevail in the upper 100 m of the oceans, and an average abundance of 10^8 cells L^{-1}, the gross daily CH_4 production by *Prochlorococcus* is ~22 nmol L^{-1}.This concentration is around one order of magnitude higher than concentrations of CH_4 found in seawater that is at atmospheric equilibrium (~2 nmol L^{-1}). However, CH_4 in the mean mixed layer of the Atlantic Ocean and surface waters of the Mediterranean Sea have been reported to be often supersaturated reaching maximum CH_4 values up to 7 and 25 nmol L^{-1}, respectively (*44*, *45*). This simplified calculation demonstrates that *Prochlorococcus* alone (aside from other marine *Cyanobacteria*) might contribute substantially to the observed oceanic CH_4 supersaturation.

In this study, we show that *Cyanobacteria* can readily convert fixed inorganic carbon directly to CH_4 and emit the potent greenhouse gas under both light and dark conditions. This is in addition to the already established ability of *Cyanobacteria* to produce CH_4 by the demethylation of methylphosphonates (*12*, *13*). *Cyanobacteria* as a phylum are the most ubiquitous group of organisms on Earth, thriving in most, naturally and artificially, illuminated environments almost regardless of temperatures, salinity and nutrient concentrations. Accordingly, their ability to produce CH_4 via different pathways, likely related to their surroundings, makes them important to the present and future global CH_4 cycle and budget. Even more so, as blooms of cyanobacteria are increasing with eutrophication and rising global temperatures (*19*, *20*). A recent study, independently predicts that eutrophication resulting in a strong increase in photoautotrophs in the water column (chlorophyll a), will lead to an increase of 30-90 % in freshwater CH_4 emissions (*46*). According to Fletcher and Schafer (*47*) the drastic increase in atmospheric CH_4 concentrations since 2007 coupled to the decrease in $\delta^{13}C$-CH_4 values presumable caused by the increase in ^{12}C-enriched biogenic CH_4 sources is difficult to explain with our current understanding of known CH_4 sources and sinks of the global CH_4 budget. This further highlights the need to fully understand the

cyanobacterial contribution to the global methane budget and to identify the isotopic signatures of the various CH_4 production pathways they might harbor. Additionally, as phototrophic prokaryotes such as *Cyanobacteria* have been inhabiting Earth for more than 3.5 billion years (*48*, *49*) they may have had a major contribution to Earth's CH_4 cycle such as during the great oxygenation event or even earlier when the conditions on Earth were more reductive favoring CH_4 production.
Further research will elucidate the biochemical pathways of CH_4 formation in *Cyanobacteria* and fully assess the environmental factors affecting it, clarifying its relevance for ecology and the global CH_4 budget throughout Earth history, how it might change in the future, and how this CH_4 source can be mitigated.

Materials and Methods

As part of the routine monitoring at Lake Stechlin a YSI probe Model 6600V2 was deployed to record dissolved oxygen, and other physico-chemical parameters which are not displayed here. Concentrations of taxon-specific phytoplankton pigments were measured by a BBE Moldaenke Fluoroprobe.

Methane Concentrations in the Lake Stechlin were determined as follows. Water was transferred from a Limnos Water Sampler to 60 ml serum bottles (clear borosilicate glass, ≥ 88 % transmission of PAR spectrum), 3 times flushed and crimp-closed (PTFE-butyl septa, aluminum caps) without gas bubbles. Dissolved CH_4 was extracted using head space (He) displacement method and measured by a Shimadzu 14A GC/FID (35 °C Permabond FFAP column on N_2, split-less injection and detection at 140 °C). Headspace CH_4 was converted to dissolved CH_4 concentrations based on Henry's Law and standard conditions.

Cyanobacterial cultures

Seventeen different cyanobacterial cultures were obtained from various sources and grown using the media described in Table 1. All the cultures were used for membrane inlet mass spectrometry measurements while 13 cultures were used for stable isotope labelling experiments.

Stable isotope labeling experiments

Culturing and treatments

To investigate the production of *Cyanobacteria*-derived CH_4, 60 ml vials with 40 ml liquid and 20 ml head space volume (laboratory air) were used and sealed with septa suitable for gas sampling. For the ^{13}C labelling experiments $NaH^{13}CO_3$ (99 % purity, Sigma-Aldrich, Germany) was added amounting to 10 % of the initial dissolved inorganic carbon (DIC) in BG11 (*50*) (DIC = 0.4 mM, enriched by added $NaHCO_3$; pH ≈ 7.0) and 4.5 % of the DIC in f/2 medium (*51*) (DIC = 2.01 mM; pH ≈ 8.2) and 1 % of the DIC in the Pro99 (*52*) based medium used for axenic *Synechococcus* and *Prochlorococcus* cultures. Four different examination groups were used: (1) Sterile medium; (2) Sterile medium with $NaH^{13}CO_3$; (3) Sterile medium with culture; (4) Sterile medium with culture and $NaH^{13}CO_3$; Four replicates of each cyanobacteria culture (n = 4).
The cultures were grown under a light–dark cycle of 16 and 8 hours at 22.5 °C at a light intensity of ≈ 30 µmol quanta m^{-2} s^{-1} for a total period of 3 days.

Continuous-flow isotope ratio mass spectrometry (CF-IRMS)

CF-IRMS was employed for measurement of the $\delta^{13}C$-CH_4 values in the head space gas above the cultures. Head space gas from exetainers was transferred to an evacuated sample loop (40 mL) and interfering compounds were then separated by GC and CH_4 trapped on Hayesep D. The sample was then transferred to the IRMS system (ThermoFinniganDeltaplus XL, Thermo Finnigan, Bremen,

Germany) via an open split. The working reference gas was carbon dioxide of high purity (carbon dioxide 4.5, Messer Griesheim, Frankfurt, Germany) with a known $\delta^{13}C$ value of -23.64 ‰ relative to Vienna Pee Dee Belemnite (V-PDB). All $\delta^{13}C$-CH_4 values were corrected using three CH_4 working standards (isometric instruments, Victoria, Canada) calibrated against IAEA and NIST reference substances. The calibrated $\delta^{13}C$-CH_4 values of the three working standards were -23.9 ± 0.2 ‰, -38.3 ± 0.2 ‰, and -54.5 ± 0.2 ‰. The average standard deviations (n = 3) of the CF-IRMS measurements were in the range of 0.1 to 0.3 ‰. All ^{13}C / ^{12}C-isotope ratios are expressed in the conventional δ notation in per mille (‰) vs. V-PDB, using the equation 1.

$$\delta = \left[\frac{(^{13}C/^{12}C)_{sample}}{(^{13}C/^{12}C)_{standard}}\right] - 1 \qquad (1)$$

Membrane inlet mass spectrometer experiments

Experiments were conducted using a Bay Instruments (MD, USA) Membrane Inlet Mass Spectrometer (MIMS) consisting of a Pfeiffer Vacuum HiCube 80 Eco turbo pumping station connected to a QMG 220 M1, PrismaPlus®, C-SEM, 1-100 amu, Crossbeam ion source mass spectrometer (Pfeiffer Vacuum, Germany). Culture samples were pumped (Minipuls3, peristaltic pump, Gilson) through a capillary stainless tubing connected to Viton® pump tubing as described in Kana *et al*. (*53*). The coiled stainless-steel tubing was immersed in a water bath to stabilize the sample temperature. Temperatures were set according to the growth conditions of the different cultures. Inside the vacuum inlet the sample passed through an 8 mm long semipermeable microbore silicone membrane (Silastic®, DuPont) before exiting the vacuum and returning to the culture chamber forming a closed system with respect to liquids. This required a 3.5 ml experimental chamber which consisted of an inner chamber where cultures were placed, and an isolated outer chamber connected to a water bath to maintain the culture at a constant temperature. The experimental chamber was placed on a magnetic stirrer and the cultures were continuously stirred for the duration of the experiments to prevent the formation of concentration gradients.

Cultures were transferred to fresh medium before the onset of each experiment after which 3.5 ml of the culture were transferred to the experimental chamber and an equal volume was used for determination of cell counts or dry weight. The latter was determined by filtering the samples on pre-weighed combusted GFF filters (Millipore) and drying at 105 °C for 48 h. In the case of non-homogenous cultures, the biomass from the experimental chamber was used at the end of the experiment for determination of dry weight. Marine picophytoplankton cultures were normalized by cell counting using a FACSAria II flow cytometer (BD Biosciences, Heidelberg, Germany) at a flow rate of 23.5 µl / min for 2.5 min. Autofluorescence was used to separate cells from salt precipitates in the medium. Cells for counting were collected from the same batch used in the experimental chamber.

The light regime for the experiments was as follows: dark from 19:30 to 09:00 then light intensity was programmed to increase to 60, 120, 180, 400 µmol quanta m^{-2} s^{-1} with a hold time of 1.5 h at each intensity. After maximum light the intensity was programmed to decrease in reverse order with the same hold times until complete darkness again at 19:30. Experiments lasted a minimum of 48 h with at least one replicate longer than 72 h. A minimum of 3 replicate experiments were conducted for each culture.

As negative controls ultrapure water as well as autoclaved cyanobacterial biomass were measured to test for non-biogenic methane production by the experimental system (Fig. S2).

Experiments using photosynthesis inhibitors

Three photosynthesis inhibitors were used to observe their effect on methane production by *Cyanobacteria*. All three were dissolved in water rather than ethanol as the latter results has an ionization pattern in the masking that of CH4. Atrazine (Cat: 45330-250MG-R, Sigma-Aldrich) has

a solubility of 33 mg L^{-1} (153 µM) in water and was used at final concentrations of 5 and 10 µM. DBMIB (2,5-Dibromo-6-isopropyl-3-methyl-1,4-benzoquinone) (Cat: 271993-250MG, Sigma-Aldrich) has a solubility 132 mg L^{-1} (410 µM) and was used at final concentrations of 5, 10 and 40 µM. HQNO (2-n-Heptyl-4-hydroxyquinoline N-oxide) (Cat: sc-202654A, Santa Cruz Biotechnology) is described as only very slightly soluble in water without any numerical information. We used 0.1 mg mL^{-1} culture yet have noticed that the powder grains remained nearly intact. Nevertheless, given an observed effect on the culture, we conclude that part of the material dissolved in the culture media. For the experiments, cultures of *Anabaena* sp. PCC 7120 were concentrated and resuspended in fresh BG11 medium and incubated in a 10 mL analysis chamber connected via circular flow to the MIMS. Inhibitors were introduced via injection of a concentrated solution at 11:00 after 24 h from the experiment start to allow the culture to acclimate. The light regime used was the same as described above.

Methane calculations

Methane (and oxygen) concentrations were calculated using the ratio relative to the inert gas Ar (m/z 40). Methane concentration was deduced from mass 15 (see Fig. S6 for the ionization pattern of CH_4) which does not overlap with other gases in the sample (*54*). The CH_4, O_2 and Ar concentration in the different media were calculated based on known solubility constants (*55*) and were calibrated to the measured signals in MQ water and growth media at different temperatures. To further calibrate the $CH_{4(mz15)}$/Ar ratio, measurements were conducted on air saturated water at different salinities (Fig. S7).

Methane production rates were calculated as the 1st derivative of the Savizky-Golay (*56*) smoothed data using the sgolay function in the R package signal (http://r-forge.r-project.org/projects/signal/). To account for the continuous degassing from the CH_4 supersaturation experimental chamber, the degassing rate was determined experimentally using rapid heating of air-saturated water from 18 to 30 °C leading to an instant (super)saturation of 127 % and 130 % for CH_4 and Ar, respectively. This procedure was repeated under two mixing conditions: I) mixing was applied via magnetic stirring as conducted for most cultures; II) mixing occurred only via the cyclic pumping of sample through the MIMS membrane as applied to *Synechococcus* and *Prochlorococcus* cultures. The change in concentration of CH_4 was monitored and a linear ($R^2 = 0.95$) saturation degree dependent rate was determined. The determined rate given in Equations 2 and 3 for type I and type II mixing, respectively, was similar to that determined by comparing the most negative slopes of the culture experiments, when cyanobacterial production rates are expected to be minimal or zero, and the supersaturation state of the culture. Final rates were calculated by adding the absolute values of the local CH_4 slope (1st derivative) and the local degassing rate (equations. 2,3).

$$R_{degassing} = -2.2365 \times 10^{-12} \times Sat_{(CH_4)} + 2.12656 \times 10^{-12} \quad (2)$$

$$R_{degassing} = -8.8628 \times 10^{-14} \times Sat_{(CH_4)} + 3.5819 \times 10^{-14} \quad (3)$$

Where: $R_{degassing}$ is the degassing rate in mol CH_4 sec^{-1} and $Sat_{(CH4)}$ is the fraction CH_4 saturation state >1 (and <1.3) determined by measured concentration vs. calculated solubility.

Transcriptome experiment

Samples for transcriptome analyses were collected in triplicates at 5 time points during a 24 h experiment with *Anabaena* sp. PCC 7120 and *Microcystis aeruginosa* PCC 7806. For this experiment a non-axenic culture of *Anabaena* sp. PCC7120 was used. The cultures were divided into 16, 60 mL bottles. One of the bottles was connected to the MIMS as described above while the remaining fifteen were incubated under identical light and temperature conditions. The bottles were

incubated for 24-36 h of acclimation before T0 was collected on the day of the experiment. T1 was sampled at 09:30, half an hour after the first light was turned on (60 µmol quanta m^{-2} s^{-1}). T2 was sampled at 12:30, half an hour after the third light was turned on (180 µmol quanta m^{-2} s^{-1}). T3 was sampled at 20:00, half an hour after all lights were turned off. T4 was collected at 00:00. Collected samples were immediately filtered on polycarbonate filters (pore size 0.8 µm; Millipore) and frozen at -80 °C till further processing.

DNA and RNA extraction and sequencing

To evaluate the presence of methanogenic *Archaea* in non-axenic cultures DNA was extracted as described in Nercessian *et al*. (*57*). The resulting DNA was sent for Illumina sequencing at MrDNA (Shallowater, TX, USA) on a Miseq platform (2x300 bp) using the Arch2A519F (CAG CMG CCG CGG TAA) and Arch1041R (GGC CAT GCA CCW CCT CTC) primers (*58*). These primers with a barcode on the forward primer were used in a PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94 °C for 3 minutes, followed by 30 cycles of 94 °C for 30 seconds, 56 °C for 40 seconds and 72 °C for 1 minute, after which a final elongation step at 72 °C for 5 minutes was performed. After amplification, PCR products were checked in 2% agarose gel to determine the success of amplification and the relative intensity of bands. The samples were pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated Ampure XP beads. Then the pooled and purified PCR product were used to prepare an Illumina DNA library. Sequencing was performed on a MiSeq following the manufacturer's guidelines. Archaeal community composition was analyzed using the SILVA-NGS pipeline (*59*) (Fig. S3). After a standard PCR for the *mcrA* gene resulted in no visible products from any of the cultures, a qPCR assay was conducted as well. For RNA extraction a similar protocol as above was used followed by DNA removal using the Turbo DNA Free kit (Cat: AM1907, Thermo Fisher Scientific). Ribosomal RNA was removed from the RNA samples using the Ribominus kit (Thermo Fisher Scientific) following the manufacturer's instructions. The mRNA enriched samples were sequenced at MrDNA (Shallowater, TX, USA). There the RNA samples were resuspended in 25 ul of nuclease free water and further cleaned using RNeasy PowerClean Pro Cleanup Kit (Qiagen). The concentration of RNA was determined using the Qubit® RNA Assay Kit (Life Technologies). 60-500 ng of RNA was used to remove the DNA contamination using Baseline-ZERO™ DNase (Epicentre) following the manufacturer's instructions followed by purification using the RNA Clean & Concentrator columns (Zymo Research). DNA free RNA samples were used for library preparation using the KAPA mRNA HyperPrep Kits (Roche) by following the manufacturer's instructions. The concentration of double strand cDNA was evaluated using the Qubit dsDNA HS Assay Kit (Life Technologies). 25 ng DNA was used to prepare the libraries. The protocol starts with enzymatic fragmentation to produce dsDNA fragments followed by end repair and A-tailing to produce end-repaired, 5'-phosphorylated, 3'-dA-tailed dsDNA fragments. In the adapter ligation step, dsDNA adapters are ligated to 3'-dA-tailed molecules. The final step is library amplification, which employs high fidelity, low-bias PCR to amplify library fragments carrying appropriate adapter sequences on both ends. Following the library preparation, the final concentration of all the libraries was measured using the Qubit® dsDNA HS Assay Kit (Life Technologies), and the average library size was determined using the Agilent 2100 Bioanalyzer (Agilent Technologies). The libraries were then pooled in equimolar ratios of 2nM, and 8pM of the library pool was clustered using the cBot (Illumina) and sequenced paired end for 125 cycles using the HiSeq 2500 system (Illumina).

The resulting raw read data is deposited at the European Nucleotide Archive (ENA) under project number PRJEB32889.

Transcriptome analysis

The fifteen paired end libraries generated from each experiment were trimmed for quality and sequencing adapters using Trimommatic V 0.39 (*60*). For purposes of annotation two strategies were used. For the first approach, all trimmed sequences from a single culture were co-assembled using Trinity V2.8.4 (*61*). The assembled transcripts were then annotated following the Trinotate pipeline (*62*) replacing BLAST with Diamond V0.9.22 (*63*). For the second approach, the trimmed reads from each sample were mapped to the genes of either *Anabaena* sp. PCC 7120 (GCA_000009705.1) or *Microcystis aeruginosa* PCC 7806 (GCA_002095975.1).
The full results of these experiments including differential expression will be eventually published separately.

Quantitative Real Time PCR assay

The abundance of methanogens was measured with quantitative PCR specific for the *mcrA* gene encoding the alpha subunit of methyl coenzyme M reductase, the key enzyme of archaeal methanogenesis. The primer pair mlas-mod for (GGY GGT GTM GGD TTC ACM CAR TA) / mcrA- rev (CGT TCA TBG CGT AGT TVG GRT AGT) (*64*) was used. The assay was performed on a qTOWER 2.2 instrument (Analytic Jena, Germany). Assays were performed in 15 µl volume containing 7.5 µl of innuMIX qPCR MasterMix SyGreen (Analytic Jena), 0.75 µl of each primer (10 pmol/µl), 5 µl of ddH2O and 1 µl of template DNA. Assay conditions were 95°C initial denaturation 5 min, 35 cycles of denaturation (95 °C) 30 s, annealing (60 °C) 45 s and elongation (72 °C) 45 s, followed by melting curve analysis. Triplicate assays were performed for each sample, with template DNA concentrations of 10ng/µl. A parallel standard curve of *mcrA* genes was recorded. The standard curve DNA fragment consisted of the *mcrA* gene of a *Methanomassiliicoccus* sp. from cow rumen fluid cloned into the pGEM®-Teasy vector system (Promega, Mannheim, Germany), amplified with vector-specific primers sp6 and T7. The qPCR assay parameters were as follows: slope 3.55, efficiency 0.91, R2 > 0.99.

References

1. Intergovernmental Panel on Climate Change, Ed., *Climate Change 2013 - The Physical Science Basis* (Cambridge University Press, Cambridge, 2014).
2. M. Saunois *et al.*, The global methane budget 2000-2012. *Earth Syst. Sci. Data*. **8**, 697–751 (2016).
3. K. Lenhart *et al.*, Evidence for methane production by the marine algae *Emiliania huxleyi*. *Biogeosciences*. **13**, 3163–3174 (2016).
4. F. Keppler, J. T. G. Hamilton, M. Braß, T. Röckmann, Methane emissions from terrestrial plants under aerobic conditions. *Nature*. **439**, 187–191 (2006).
5. E. Tuboly *et al.*, Methane biogenesis during sodium azide-induced chemical hypoxia in rats. *Am. J. Physiol. Physiol.* **304**, C207–C214 (2013).
6. K. Lenhart *et al.*, Evidence for methane production by saprotrophic fungi. *Nat. Commun.* **3**, 1046 (2012).
7. F. Keppler *et al.*, Stable isotope and high precision concentration measurements confirm that all humans produce and exhale methane. *J. Breath Res.* **10**, 016003 (2016).
8. D. J. Repeta *et al.*, Marine methane paradox explained by bacterial degradation of dissolved organic matter. *Nat. Geosci.* **9**, 884–887 (2016).
9. Q. Wang, J. E. Dore, T. R. McDermott, Methylphosphonate metabolism by *Pseudomonas* sp. populations contributes to the methane oversaturation paradox in an oxic freshwater lake. *Environ. Microbiol.* **19**, 2366–2378 (2017).
10. Y. Zheng *et al.*, A pathway for biological methane production using bacterial iron-only nitrogenase. *Nat. Microbiol.* **3**, 281–286 (2018).

11. K. Lenhart *et al.*, Nitrous oxide and methane emissions from cryptogamic covers. *Glob. Chang. Biol.* **21**, 3889–3900 (2015).
12. L. J. Beversdorf, A. E. White, K. M. Björkman, R. M. Letelier, D. M. Karl, Phosphonate metabolism by *Trichodesmium* IMS101 and the production of greenhouse gases. *Limnol. Oceanogr.* **55**, 1768–1778 (2010).
13. M. R. Gomez-Garcia, M. Davison, M. Blain-Hartnung, A. R. Grossman, D. Bhaya, Alternative pathways for phosphonate metabolism in thermophilic *Cyanobacteria* from microbial mats. *ISME J.* **5**, 141–149 (2011).
14. M. Yao, C. Henny, J. A. Maresca, Freshwater bacteria release methane as a by-product of phosphorus acquisition. *Appl. Environ. Microbiol.* **82**, 6994–7003 (2016).
15. A. Berg, P. Lindblad, B. H. Svensson, *Cyanobacteria* as a source of hydrogen for methane formation. *World J. Microbiol. Biotechnol.* **30**, 539–545 (2014).
16. F. Puente-Sánchez *et al.*, Viable cyanobacteria in the deep continental subsurface. *Proc. Natl. Acad. Sci. U. S. A.* **115**, 10702–10707 (2018).
17. V. Hubalek *et al.*, Connectivity to the surface determines diversity patterns in subsurface aquifers of the Fennoscandian shield. *ISME J.* **10**, 2447–2458 (2016).
18. A. P. Gumsley *et al.*, Timing and tempo of the Great Oxidation Event. *Proc. Natl. Acad. Sci. U. S. A.* **114**, 1811–1816 (2017).
19. P. M. Visser *et al.*, How rising CO_2 and global warming may stimulate harmful cyanobacterial blooms. *Harmful Algae*. **54**, 145–159 (2016).
20. J. Huisman *et al.*, Cyanobacterial blooms. *Nat. Rev. Microbiol.* **16** (2018), pp. 471–483.
21. J. C. Angle *et al.*, Methanogenesis in oxygenated soils is a substantial fraction of wetland methane emissions. *Nat. Commun.* **8**, 1567 (2017).
22. V. Peters, R. Conrad, Methanogenic and other strictly anaerobic bacteria in desert soil and other oxic soils. *Appl. Environ. Microbiol.* **61**, 1673–6 (1995).
23. B. L. Flaherty, F. Van Nieuwerburgh, S. R. Head, J. W. Golden, Directional RNA deep sequencing sheds new light on the transcriptional response of *Anabaena* sp. strain PCC 7120 to combined-nitrogen deprivation. *BMC Genomics*. **12**, 332 (2011).
24. J. Mitschke, A. Vioque, F. Haas, W. R. Hess, A. M. Muro-Pastor, Dynamics of transcriptional start site selection during nitrogen stress-induced cell differentiation in *Anabaena* sp. PCC7120. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 20130–5 (2011).
25. U. Pfreundt, M. Kopf, N. Belkin, I. Berman-Frank, W. R. Hess, The primary transcriptome of the marine diazotroph *Trichodesmium erythraeum* IMS101. *Sci. Rep.* **4**, 6187 (2014).
26. C. Luo, K. T. Konstantinidis, Phosphorus-related gene content is similar in *Prochlorococcus* populations from the North Pacific and North Atlantic Oceans. *Proc. Natl. Acad. Sci. U. S. A.* **108**, E62-3; author reply E64-6 (2011).
27. O. A. Sosa, J. R. Casey, D. M. Karl, *Appl. Environ. Microbiol.*, in press, doi:10.1128/aem.00289-19.
28. S. T. Dyhrman, C. R. Benitez-Nelson, E. D. Orchard, S. T. Haley, P. J. Pellechia, A microbial source of phosphonates in oligotrophic marine systems. *Nat. Geosci.* **2**, 696–699 (2009).
29. A. N. Knapp, The sensitivity of marine N_2 fixation to dissolved inorganic nitrogen. *Front. Microbiol.* **3**, 374 (2012).
30. L. J. Stal, R. Moezelaar, Fermentation in *Cyanobacteria*. *FEMS Microbiol. Rev.* **21**, 179–211 (1997).
31. A. Jugold *et al.*, Non-microbial methane formation in oxic soils. *Biogeosciences*. **9**, 5291–5301 (2012).
32. W. M. Hao, D. Scharffe, P. J. Crutzen, E. Sanhueza, Production of N2O, CH4, and CO2 from soils in the tropical savanna during the dry season. *J. Atmos. Chem.* **7**, 93–105 (1988).
33. C. Kammann, S. Hepp, K. Lenhart, C. Müller, Stimulation of methane consumption by

endogenous CH_4 production in aerobic grassland soil. *Soil Biol. Biochem.* **41**, 622–629 (2009).
34. F. Garcia-Pichel, J. Belnap, S. Neuer, F. Schanz, Estimates of global cyanobacterial biomass and its distribution. *Arch. Hydrobiol. Suppl. Algol. Stud.* **109**, 213–227 (2003).
35. M. I. Scranton, J. W. Farrington, Methane Production in the Waters off Walvis Bay. *J. Geophys. Res.* **82**, 4947–4953 (1977).
36. D. Donis *et al.*, Full-scale evaluation of methane production under oxic conditions in a mesotrophic lake. *Nat. Commun.* **8**, 1661 (2017).
37. H.-P. Grossart, K. Frindte, C. Dziallas, W. Eckert, K. W. Tang, Microbial methane production in oxygenated water column of an oligotrophic lake. *Proc. Natl. Acad. Sci.* **108**, 19657–19661 (2011).
38. H. W. Paerl, J. Huisman, Blooms Like It Hot. *Science.* **320**, 57–58 (2008).
39. P. M. Glibert, R. Maranger, D. J. Sobota, L. Bouwman, The Haber Bosch–harmful algal bloom (HB–HAB) link. *Environ. Res. Lett.* **9**, 105001 (2014).
40. L. C. Bowling, S. Blais, M. Sinotte, Heterogeneous spatial and temporal cyanobacterial distributions in Missisquoi Bay, Lake Champlain: An analysis of a 9 year data set. *J. Great Lakes Res.* **41**, 164–179 (2015).
41. M. Bižić-Ionescu, R. Amann, H.-P. Grossart, Massive regime shifts and high activity of heterotrophic bacteria in an ice-covered lake. *PLoS One*. **9**, e113611 (2014).
42. P. K. Lange *et al.*, Scratching beneath the surface: A model to predict the vertical distribution of *Prochlorococcus* using remote sensing. *Remote Sens.* **10**, 847 (2018).
43. F. Ribalet *et al.*, Light-driven synchrony of *Prochlorococcus* growth and mortality in the subtropical Pacific gyre. *Proc. Natl. Acad. Sci. U. S. A.* **112**, 8008–12 (2015).
44. G. Forster *et al.*, Nitrous oxide and methane in the Atlantic Ocean between 50°N and 52°S: Latitudinal distribution and sea-to-air flux. *Deep Sea Res. Part II Top. Stud. Oceanogr.* **56**, 964–976 (2009).
45. R. Grilli *et al.*, Sub-Ocean: Subsea Dissolved Methane Measurements Using an Embedded Laser Spectrometer Technology. *Environ. Sci. Technol.* **52**, 10543–10551 (2018).
46. J. J. Beaulieu, T. DelSontro, J. A. Downing, Eutrophication will increase methane emissions from lakes and impoundments during the 21st century. *Nat. Commun.* **10**, 1375 (2019).
47. S. E. M. Fletcher, H. Schaefer, Rising methane: A new climate challenge. *Science.* **364**, 932–933 (2019).
48. R. Frei *et al.*, Oxidative elemental cycling under the low O2 Eoarchean atmosphere. *Sci. Rep.* **6**, 21058 (2016).
49. L. I. Falcón, S. Magallón, A. Castillo, Dating the cyanobacterial ancestor of the chloroplast. *ISME J.* **4**, 777–783 (2010).
50. R. Y. Stanier, J. Deruelles, R. Rippka, M. Herdman, J. B. Waterbury, Generic assignments, strain histories and properties of pure cultures of *Cyanobacteria*. *Microbiology*. **111**, 1–61 (1979).
51. R. R. L. Guillard, J. H. Ryhter, Studies of marine planktonic diatoms. *Can. J. Microbiol.* **8**, 229–239 (1962).
52. L. R. Moore *et al.*, Culturing the marine cyanobacterium *Prochlorococcus*. *Limnol. Oceanogr. Methods*. **5**, 353–362 (2007).
53. T. M. Kana, J. C. Cornwell, L. Zhong, Determination of denitrification in the Chesapeake Bay from measurements of N_2 accumulation in bottom water. *Estuaries and Coasts*. **29**, 222–231 (2006).
54. M. Schlüter, T. Gentz, Application of membrane inlet mass spectrometry for online and in situ analysis of methane in aquatic environments. *J. Am. Soc. Mass Spectrom.* **19**, 1395–402 (2008).

55. R. J. Powell, Solubility of 16 gases in heptacosafluorotributylamine and carbon disulfide. *J. Chem. Eng. Data*. **17**, 302–304 (1972).
56. A. Savitzky, M. J. E. Golay, Smoothing and differentiation of data by simplified least squares procedures. *Anal. Chem.* **36**, 1627–1639 (1964).
57. O. Nercessian, E. Noyes, M. G. Kalyuzhnaya, M. E. Lidstrom, L. Chistoserdova, Bacterial populations active in metabolism of C1 compounds in the sediment of Lake Washington, a freshwater lake. *Appl. Environ. Microbiol.* **71**, 6885–99 (2005).
58. M. A. Fischer, S. Güllert, S. C. Neulinger, W. R. Streit, R. A. Schmitz, Evaluation of 16S rRNA Gene Primer Pairs for Monitoring Microbial Community Structures Showed High Reproducibility within and Low Comparability between Datasets Generated with Multiple Archaeal and Bacterial Primer Pairs. *Front. Microbiol.* **7** (2016), doi:10.3389/fmicb.2016.01297.
59. D. Ionescu *et al.*, Microbial and chemical characterization of underwater fresh water springs in the Dead Sea. *PLoS One*. **7**, e38319 (2012).
60. A. M. Bolger, M. Lohse, B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. **30**, 2114–20 (2014).
61. M. G. Grabherr *et al.*, Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat. Biotechnol.* **29**, 644–52 (2011).
62. D. M. Bryant *et al.*, A Tissue-Mapped Axolotl De Novo Transcriptome Enables Identification of Limb Regeneration Factors. *Cell Rep.* **18**, 762–776 (2017).
63. B. Buchfink, C. Xie, D. H. Huson, Fast and sensitive protein alignment using DIAMOND. *Nat. Methods*. **12**, 59–60 (2015).
64. L. M. Steinberg, J. M. Regan, mcrA-targeted real-time quantitative PCR method to examine methanogen communities. *Appl. Environ. Microbiol.* **75**, 4435–42 (2009).
65. Y.-B. Chen, J. P. Zehr, M. Mellon, Growth and nitrogen fixation of the diazotrophic filamentous nonheterocystous cyanobacterium trichodesmium sp. IMS 101 in defined media: evidence for a circadian rhythm. *J. Phycol.* **32**, 916–923 (1996).
66. D. O. Mountfort, R. A. Asher, Effect of inorganic sulfide on the growth and metabolism of Methanosarcina barkeri strain DM. *Appl. Environ. Microbiol.* **37**, 670–675 (1979).
67. L. Kröninger, J. Gottschling, U. Deppenmeier, Growth characteristics of Methanomassiliicoccus luminyensis and expression of methyltransferase encoding genes. *Archaea*. **2017**, 1–12 (2017).
68. E. Gerhard, B. M. Butsch, I. W. Marison, S. von, U, Improved growth and methane production for *Methanobacterium thermoautotrophicum*. *Appl. Microbiol. Biotechnol.* **40**, 432–437 (1993).
69. N. Brüggemann *et al.*, Nonmicrobial aerobic methane emission from poplar shoot cultures under low-light conditions. *New Phytol.* **182**, 912–918 (2009).
70. A. Wishkerman *et al.*, Enhanced formation of methane in plant cell cultures by inhibition of cytochrome c oxidase. *Plant. Cell Environ.* **34**, 457–464 (2011).
71. M. M. Qaderi, D. M. Reid, Methane emissions from six crop species exposed to three components of global climate change: Temperature, ultraviolet-B radiation and water stress. *Physiol. Plant.* **137**, 139–147 (2009).

Acknowledgments

In-situ probe data was obtained from the LakeLab (www.lakelab.de) as part of the routine monitoring of Lake Stechlin run by the Leibniz Institute of Freshwater Ecology and Inland Fisheries. We thank the technical assistants of department III for making this data available. We thank the members of the MIBI group and particularly Jason Woodhouse for assistance in sampling, analysis and discussion of the data. We thank Diana Münch, Institute of

Microbiology, University of Greifswald for the technical assistance with the qPCR analysis. We thank Claudia Steglich and Wolfgang Hess from Freiburg University for the *Prochlorococcus* culture. We thank Falk Pollehne from the Institute of Baltic Sea Research for the *Nodularia* culture. We thank Meri Eichner from the Max Planck Institute for Marine Microbiology for the *Trichodesmium* culture. We thank Daniel Sher and Dalit Roth from Haifa University for the cultures of *Synechococcus* WH7803 and WH8102 and Prochlorococcus MIT9312, NATL2A, MIT0604, MED4. We thank Katharina Makower and Jason Woodhouse for assistance with the transcriptomic experiment. Funding was provided to M.B.I., D.I. and H.P.G. via the DFG-Aquameth project (GR1540-21-1), the BMBF-BIBS project (01LC1501G) and the Human Frontiers Science project (HFSP 2039371). M.B.I. was additionally funded through DFG eigene Stelle project (BI 1987/2-1) F.K. and T.K. were supported by the German Research Foundation (DFG; KE 884/8-2, KE 884/11-1 and KE 884/16-2). T.U. acknowledges financial support from ESF and Ministry of Education, Science and Culture of Mecklenburg-Western Pomerania project WETSCAPES (ESF/14-BM-A55-0032/16).

Author contributions: M.B.I., T.K., D.I., F.K., H.P.G., conceived the study and designed the experiments; M.B.I., D.I., M.Y.H., M.G., A.M.M.P., W.E., performed MIMS experiments and in-situ measurements and analyzed the data; T.K. performed stable isotope measurements and together with F.K. analyzed the data. M.B.I., D.I., performed the transcriptomic experiments and microbial community analysis; A.M.M.P. analyzed transcriptomics data; T.U. performed the qPCR analysis; M.B.I., T.K., D.I., M.Y.H., M.G., A.M.M.P., W.E., T.U., F.K., H.P.G. discussed the results and wrote the paper.

Competing interests: The authors declare no conflict of interest.

Data and materials availability: The sequences were submitted to the European Nucleotide Archive under project numbers: PRJEB25851 and PRJEB32889.

Figures and Tables

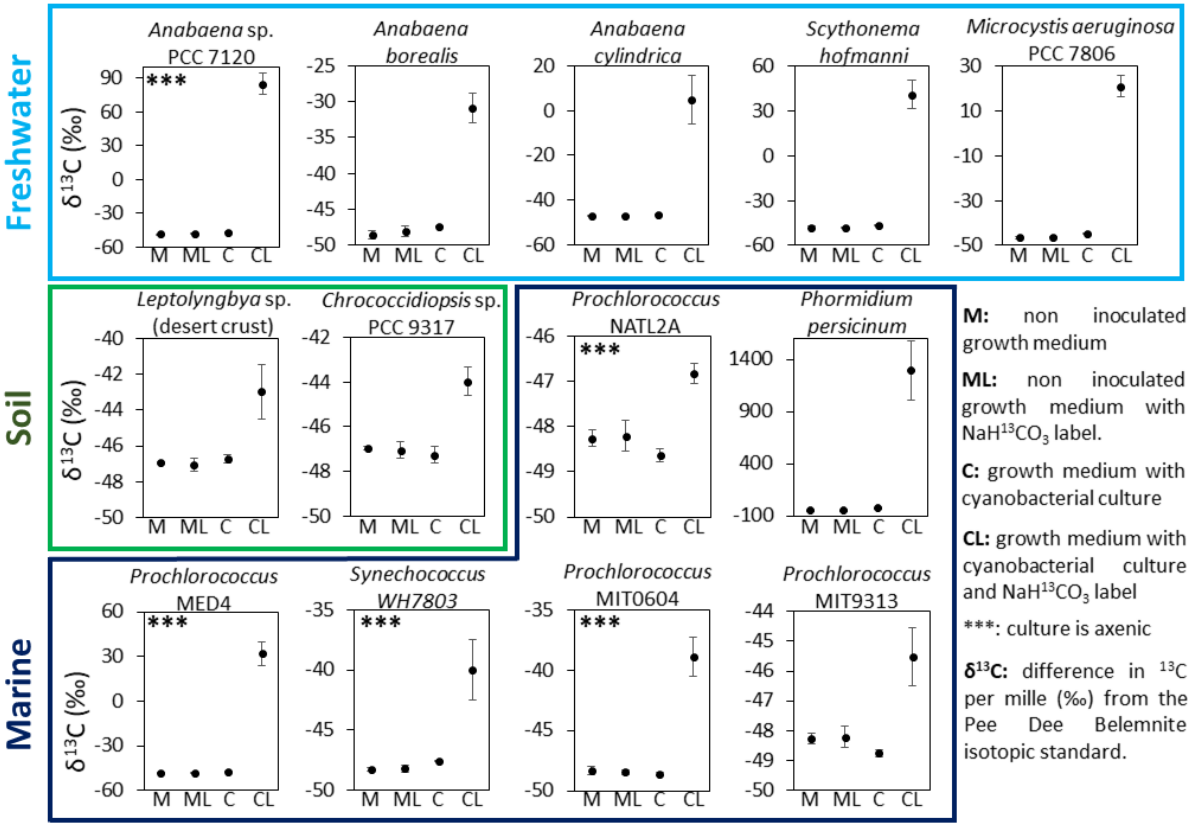

Figure 1. $\delta^{13}C$-CH_4 values measured during incubation experiments of thirteen different filamentous and unicellular freshwater, soil and marine cyanobacterial cultures with and without $NaH^{13}CO_3$ supplementation. All cyanobacterial cultures produced CH_4. Using $NaH^{13}CO_3$ as carbon source (CL) resulted in increasing stable $\delta^{13}C$-CH_4 values as compared to the starting condition. This establishes the direct link between carbon fixation and CH_4 production. The ^{13}C enrichment is not quantitative and thus not comparable between cultures. Error bars represent standard deviation (n=4).

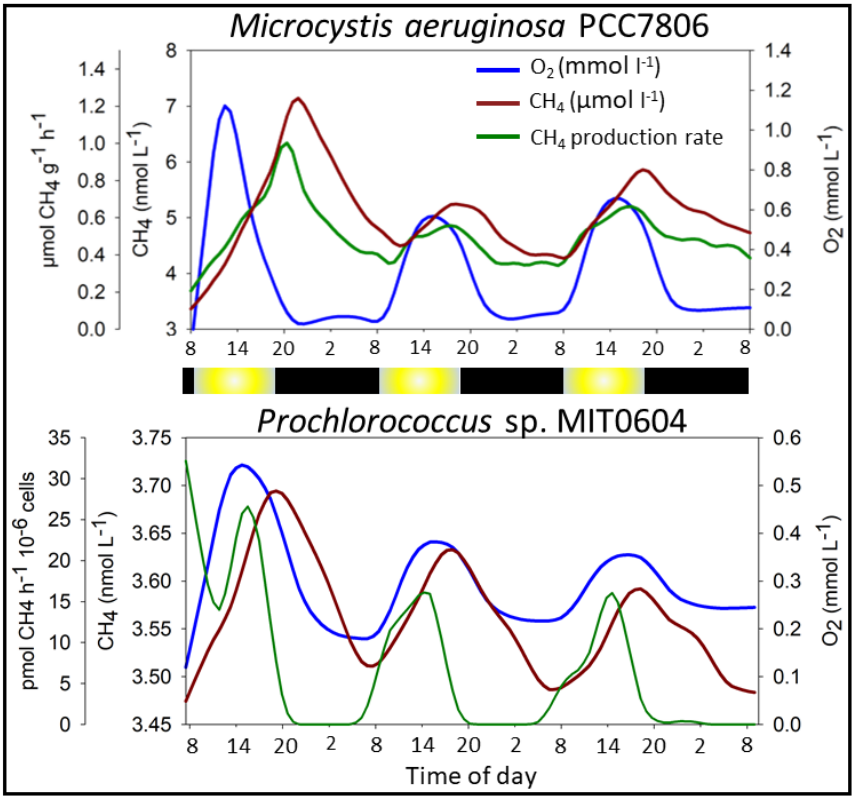

Figure 2. Continuous measurements of CH_4 and oxygen under light/dark periods using a membrane inlet mass spectrometer (MIMS). Examples are shown for two cultures. Data for other cultures can be found in Fig. S2. A decrease in CH_4 concentration is a result of either reduced, or no, production coupled with degassing from the supersaturated, continuously-mixing, semi-open incubation chamber towards equilibrium with atmospheric CH_4 (2.5 nM and 2.1 nM for freshwater and seawater, respectively). Calculated CH_4 production rates account for the continuous emission of CH_4 from the incubation chamber for as long as the CH_4 concentrations are supersaturated. The light regime for the experiments was as follows: dark (black bar) from 19:30 to 09:00 then light intensity (yellow bar) was programmed to increase to 60, 120, 180, 400 µmol quanta m^{-2} s^{-1} with a hold time of 1.5 h at each light intensity. After the maximum light period the light intensity was programmed to decrease in reversed order with the same hold times until complete darkness again at 19:30.

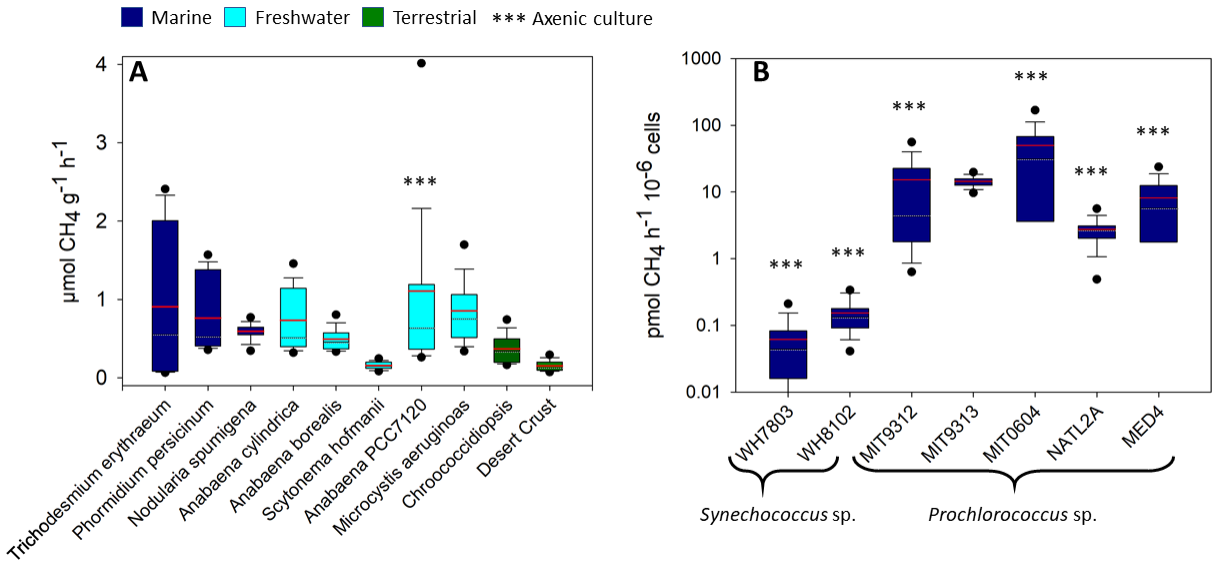

Figure 3. Average CH_4 production rates obtained from multiple long-term measurements (2-5 days) using a membrane inlet mass spectrometer. The rates are designated by color according to the environment from which the *Cyanobacteria* were originally isolated; dark blue, light blue and green for marine, freshwater and soil environments, respectively. Gray and red lines represent median and mean values, respectively. Rates for the larger cyanobacteria in panel (A) are given in μmol g DW^{-1} h^{-1} and rates for the picocyanobacterial (B) are given in pmol $h^{-1}10^{-6}$ cells.

Table 1. Cyanobacterial cultures used in this study and their growth conditions.

Strain name	**Source**	**Morphology**	**Growth medium**	**Incubation Temp. °C**
Anabaena sp. PCC7120	IBVF	Filamentous	BG11	30
Anabaena cylindrica ATCC29414	IBVF	Filamentous	BG11	30
Anabaena borealis	CCALA	Filamentous	BG11	30
Scytonema hofmanni PCC7110	IBVF	Filamentous	BG11	30
Leptolyngbya sp. (desert crust)	HUJI	Filamentous	BG11	30
Phormidium persicinum	IBVF	Filamentous	f/2	26
Trichodesmium erythraeum	MPI-MM	Filamentous	YBCII	26
Nodularia spumigena	IOW	Filamentous	f/2 (8 psu)	20
Chroococcidiopsis sp. PCC9317	IBVF	Unicellular	BG11	30
Microcystis aeruginosa PCC7806	IGB	Unicellular	BG11	30
Prochlorococcus sp. MIT9313	Uni Freiburg	Unicellular	AMP1	22
Prochlorococcus sp. MIT9312	Haifa Uni	Unicellular	ASW-Pro99	22
Prochlorococcus sp. MIT0604	Haifa Uni	Unicellular	FSW-Pro99	22
Prochlorococcus sp. NATL2A	Haifa Uni	Unicellular	ASW-Pro99	22
Prochlorococcus sp. MED4	Haifa Uni	Unicellular	ASW-Pro99	22
Synechococcus sp. WH7803	Haifa Uni	Unicellular	ASW-Pro99	22
Synechococcus sp. WH8102	Haifa Uni	Unicellular	ASW-Pro99	22

Shaded cultures are fully axenic while others are mono-algal. Sources abbreviations: IBVF: Culture collection of the Institute for Plant Biochemistry and Photosynthesis, Sevilla Spain; CCALA: Culture collection of autotrophic organisms; HUJI: Laboratory of Aaron Kaplan, Hebrew University of Jerusalem, Jerusalem Israel; IOW: Laboratory of Falk Pollehne, Leibniz Institute for Baltic Sea research, Warnemünde, Germany; MPI-MM: Max Planck Institute for Marine Microbiology, Bremen, Germany; IGB: Leibniz Institute of Freshwater Ecology and Inland Fisheries, Neuglobsow, Germany; Uni. Freiburg, Laboratory of Claudia Steglich, Freiburg University, Freiburg, Germany. Haifa University, Laboratory of Daniel Sher. Media source: BG11 (*50*); f/2 (*51*); YBCII (*65*); AMP1 (*52*); Filtered sea water (FSW) / Artificial sea water Pro99 (*52*).

Supplementary Materials

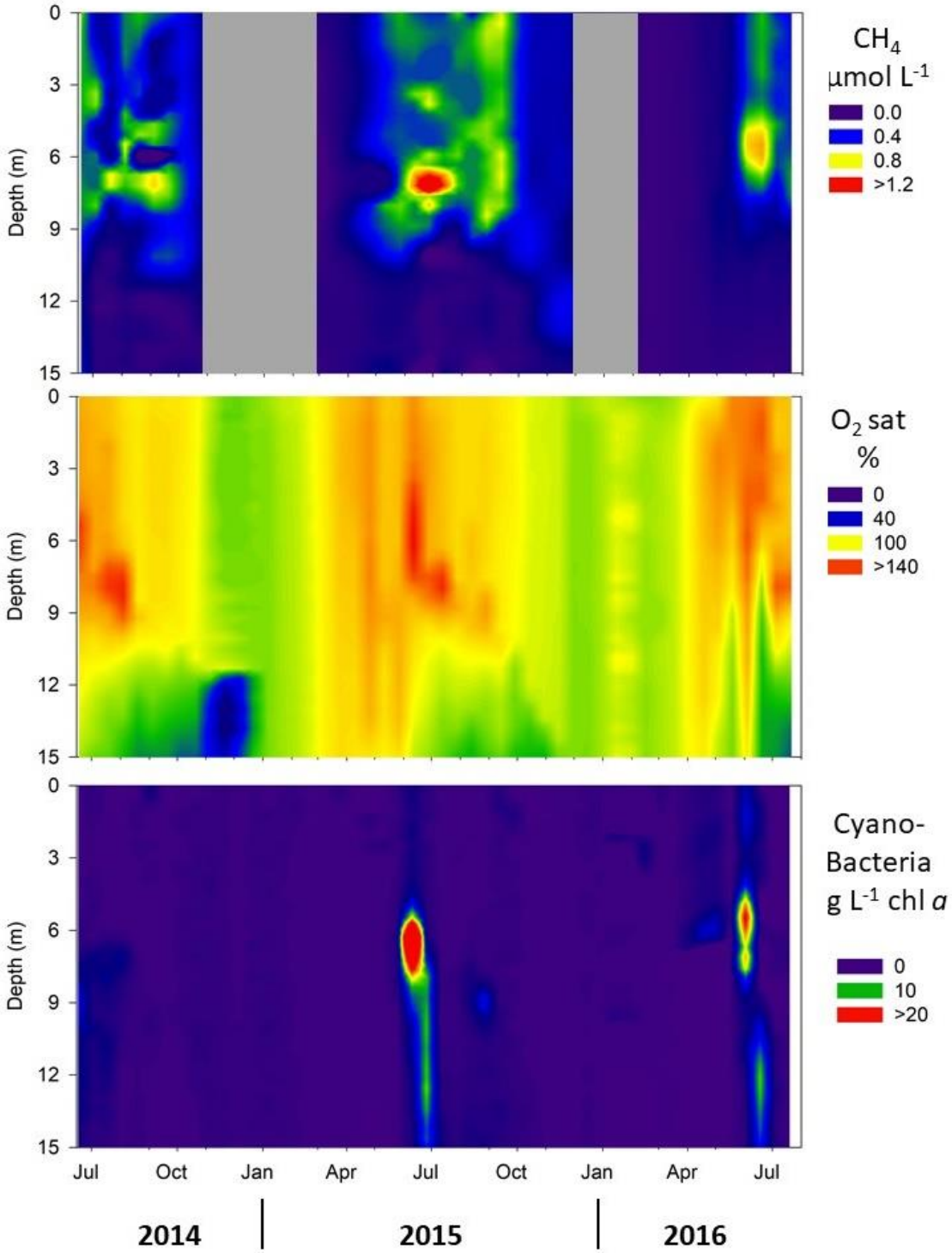

Fig. S1. Temporal profiles of CH_4, O_2 and cyanobacterial derived Chl a between July 2014 and July 2016. The CH_4 data was measured every 1 - 4 weeks depending on season using a GC-FID as described in Grossart *et al*. (*37*). O_2 and Chl a were measured hourly using a YSI and a BBE probe (see www.lake-lab.de), respectively.

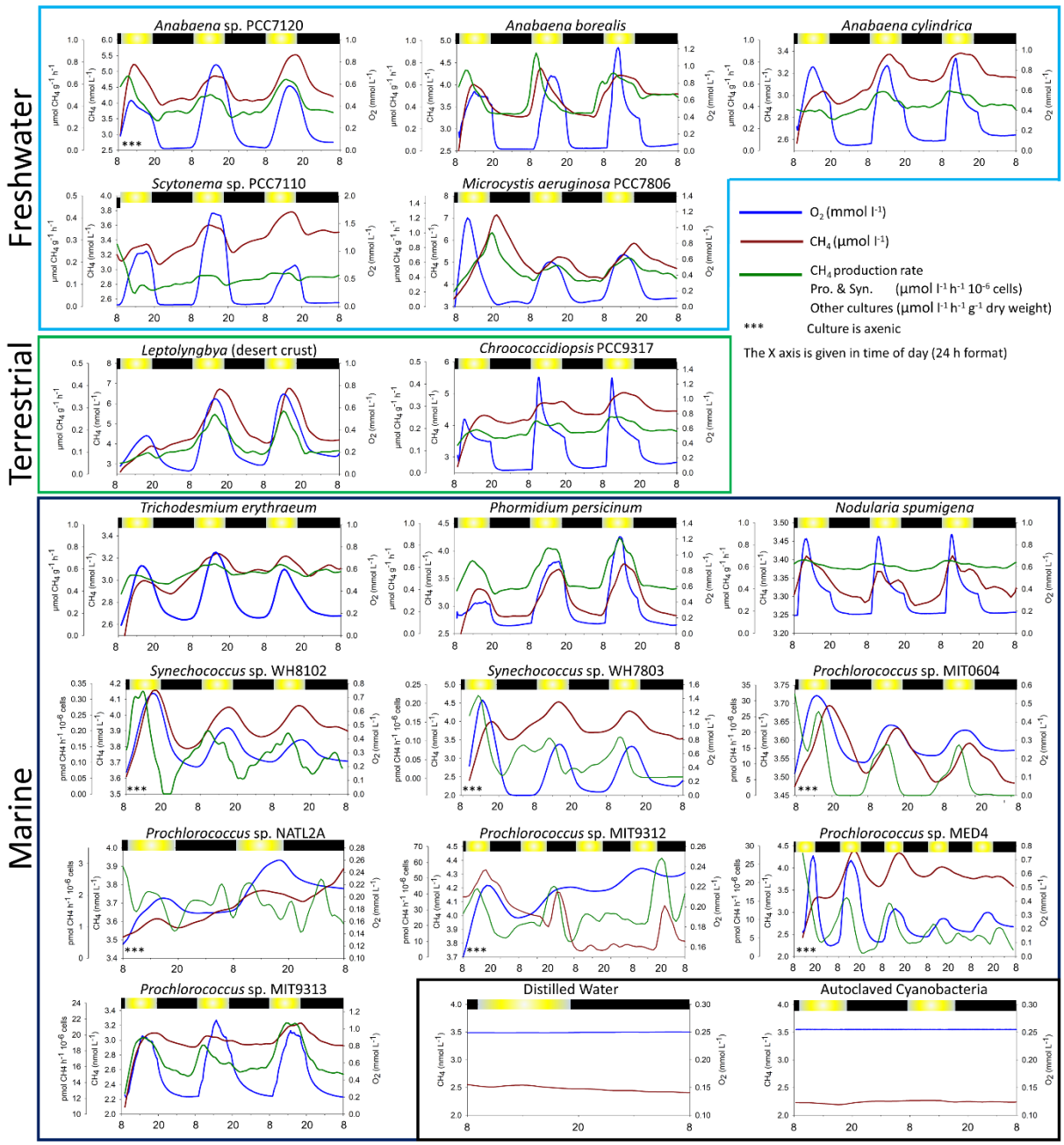

Fig. S2. Continuous measurements of CH_4 and oxygen under light/dark cycles using a membrane inlet mass spectrometer (MIMS) in 17 different cyanobacterial cultures. A decrease in CH_4 concentration is a result of reduced (or no) production coupled with degassing from the supersaturated, continuously-mixing, semi-open incubation chamber towards equilibrium with atmospheric CH_4 (2.5 nM and 2.1 nM for freshwater and seawater, respectively). Calculated CH_4 production rates account for the continuous emission of CH_4 from the incubation chamber for as long as the CH_4 concentrations are supersaturated. The light regime for the experiments was as follows: dark (black bar) from 19:30 to 09:00 then light intensity (yellow bar) was programmed to increase to 60, 120, 180, 400 µmol quanta m^{-2} s^{-1} with a hold time of 1.5 h at each intensity. After maximum light period the intensity was programmed to decrease in reverse order with the same hold times until complete darkness again at 19:30.

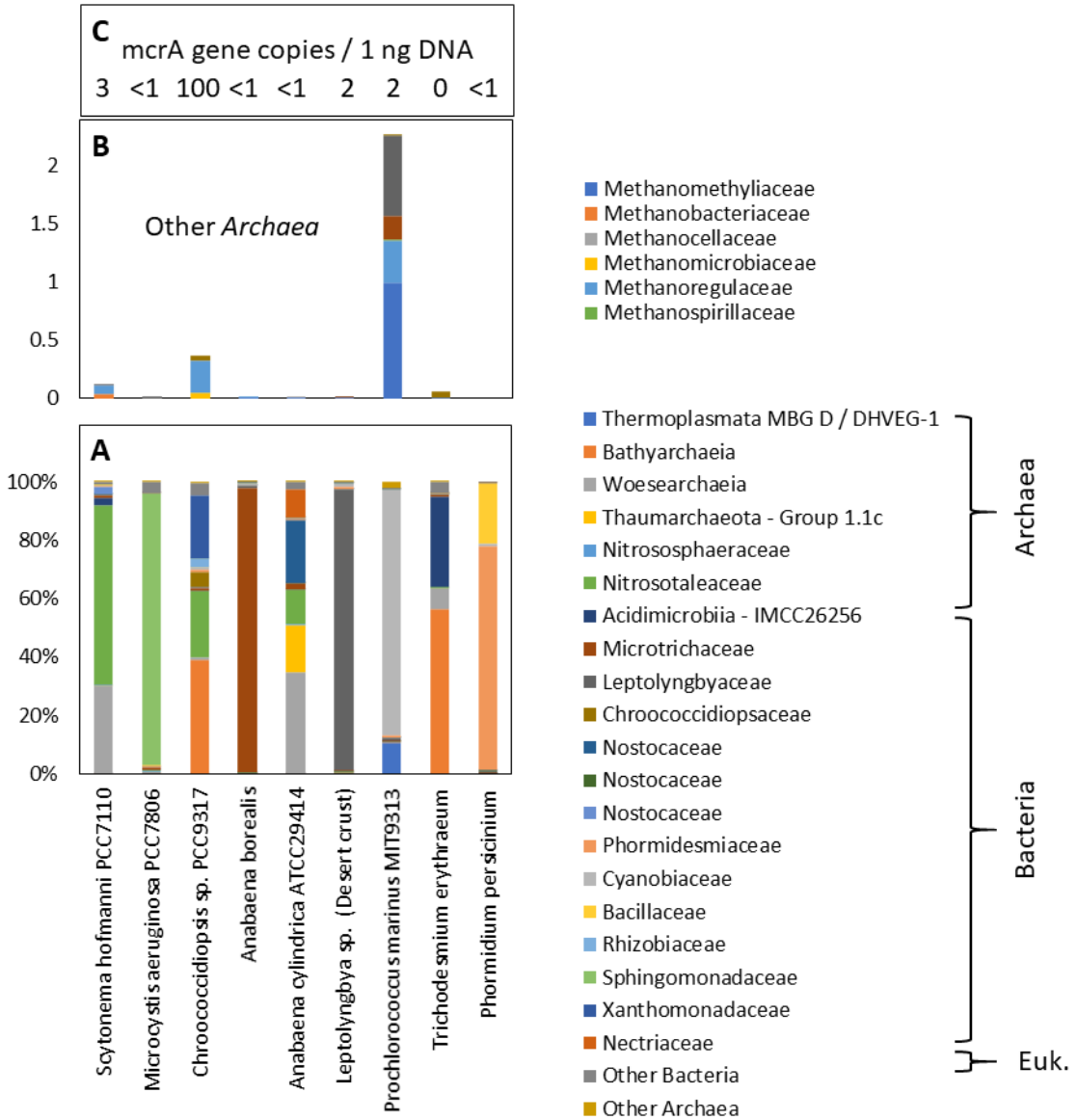

Fig. S3. Community composition of the cyanobacterial cultures as obtained when sequenced using ***Archaea* specific** primers resulting in 71981 reads for PCC7110, 5442 reads for PCC 7806, 14,489 reads for PCC 9317, 91,105 reads for *A. borealis*, 13,173 reads for ATCC29414, 133,336 reads for *Leptolyngbia* sp., 172,112 reads for MIT9313, 35,257 for *T. erythraeum* and 82,190 reads for *P. persicinum* out of 200,000 commissioned reads. The variability in reads abundance alongside false positive results, suggest a low abundance of *Archaea*. Families of *Archaea* and false positive Bacteria making up more than 2% of the reads are shown in panel (A). Low abundance *Archaea*, comprising of methanogens are shown in panel (B). The background presence or complete absence of methanogens was confirmed by qPCR of the *mcrA* gene (C).

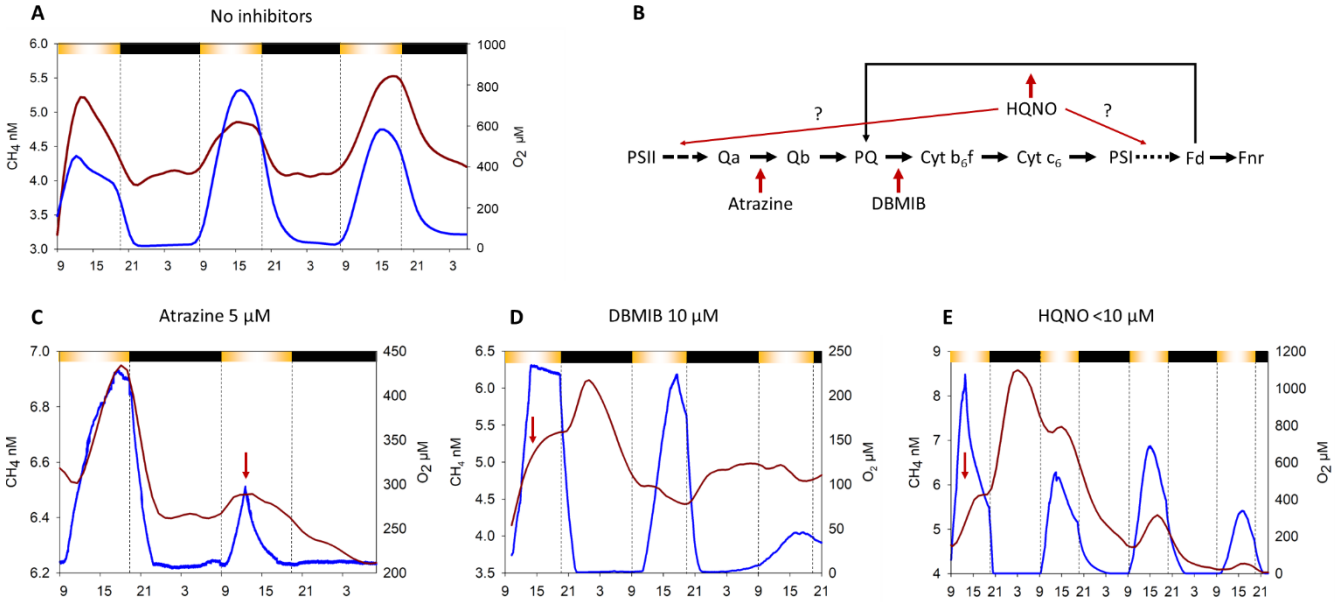

Fig S4. Under standard experimental conditions the diurnal CH_4 (red lines) and O_2 (blue lines) profiles measured in a culture of *Anabaena* sp. PCC7120 look as in panel (A). To test the link between the photosynthetic system and CH_4 production, a series of known photosynthesis inhibitors (B) were used. The inhibitors were added at 11:00 am (red arrows) after the culture was exposed to 2 h of illumination and the expected increase in CH_4 concentration was observed. Atrazine (C) blocks immediately both O_2 and CH_4 production leading to the subsequent death of the culture. DBIMB (2,5-dibromo-3-methyl-6-isopropylbenzoquinone) (D), results in an immediate decrease in CH_4 production rate under oxic conditions and a burst in CH_4 production when the culture turns anoxic. In subsequent days a decrease in methane concentration is observed immediately upon illumination. This is a result of cease of production coupled with degassing of the CH_4 from the semi-open experimental system. Since DBMIB can be reduced and it's blocking site bypassed, the culture resumes normal activity within few days. HQNO (2-Heptyl-4-hydroxyquinoline n-oxide) (E) has a similar effect as DBMIB in a dark anoxic culture. Upon re-illumination CH_4 production is delayed till stronger light intensities are applied.

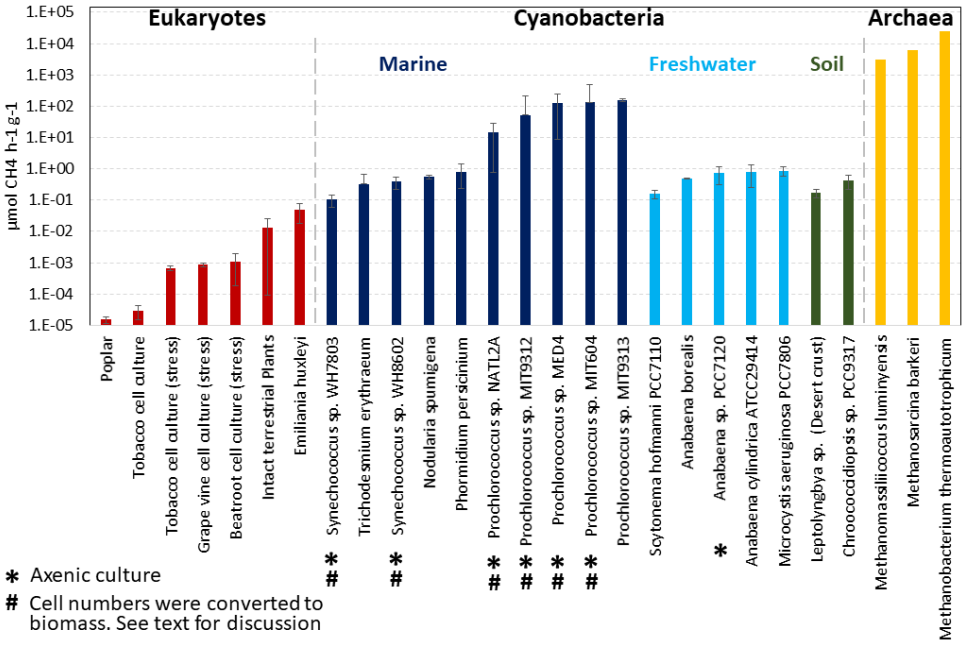

Fig. S5. Average CH_4 production rates (µmol gDW^{-1} h^{-1}) obtained from multiple long-term measurements (2-5 days) with a membrane inlet mass spectrometer. The rates are shown by color according to the environment from which the *Cyanobacteria* were originally isolated; dark blue, light blue and green for marine, freshwater and soil environments, respectively. The rates are presented in comparison to three known methanogens. Rates for the methanogens were obtained from references: Mountfort and Asher (*66*), Kröninger *et al*. (*67*) and Gerhard *et al*. (*68*). Rates for eukaryotes including marine algae and terrestrial plants were taken from Lenhart *et al*. (*3*), Keppler *et al*. (*4*), Brüggemann *et al*. (*69*), Wishkermann *et al*. (*70*) and Qaderi *et al*. (*71*) No emission rates (on a dry weight basis) are available for fungi and animals.

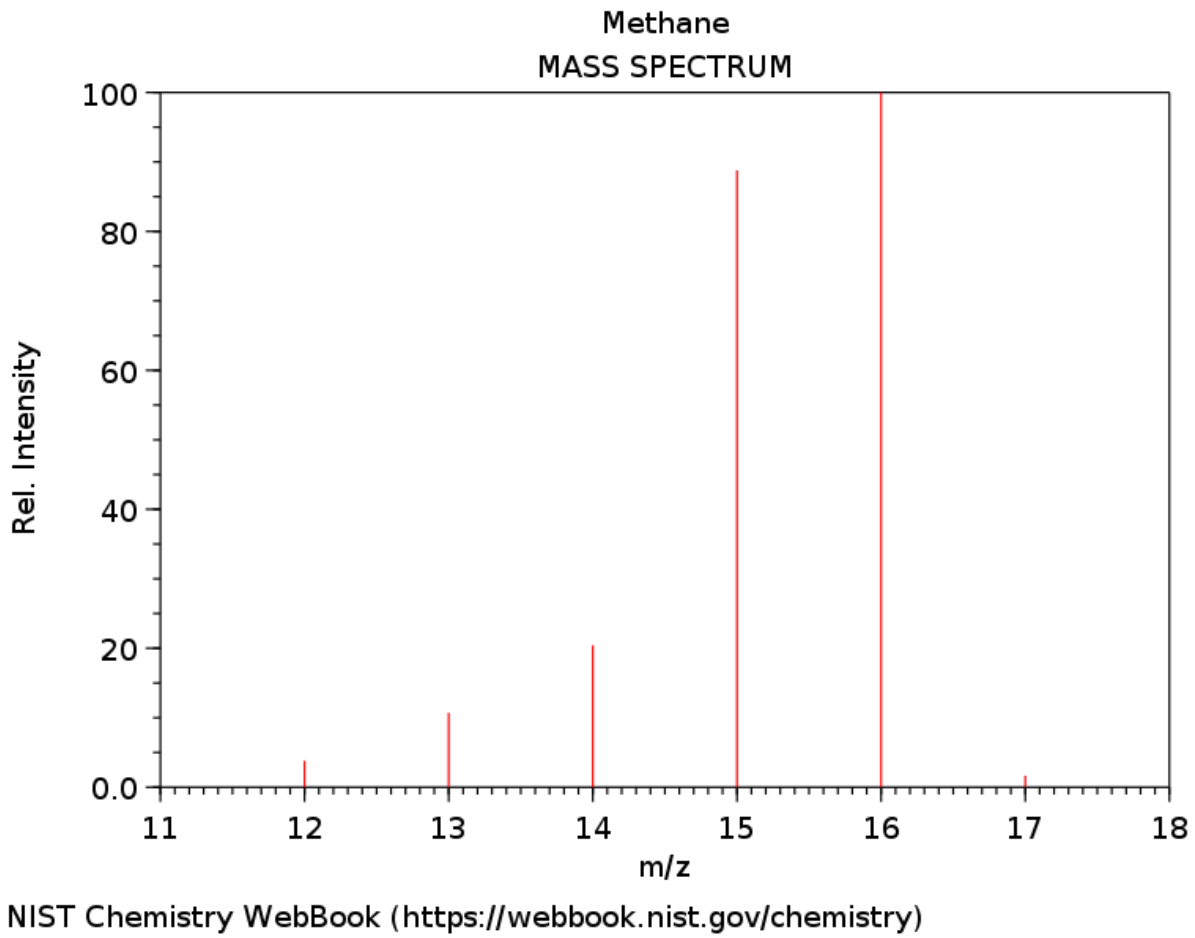

Fig S6. Ionization spectrum (mass spectrum) of methane. The peak at m/z 15 is used for analyses with the membrane inlet mass spectrometer and is ca. 90% of the main peak at m/z 16.

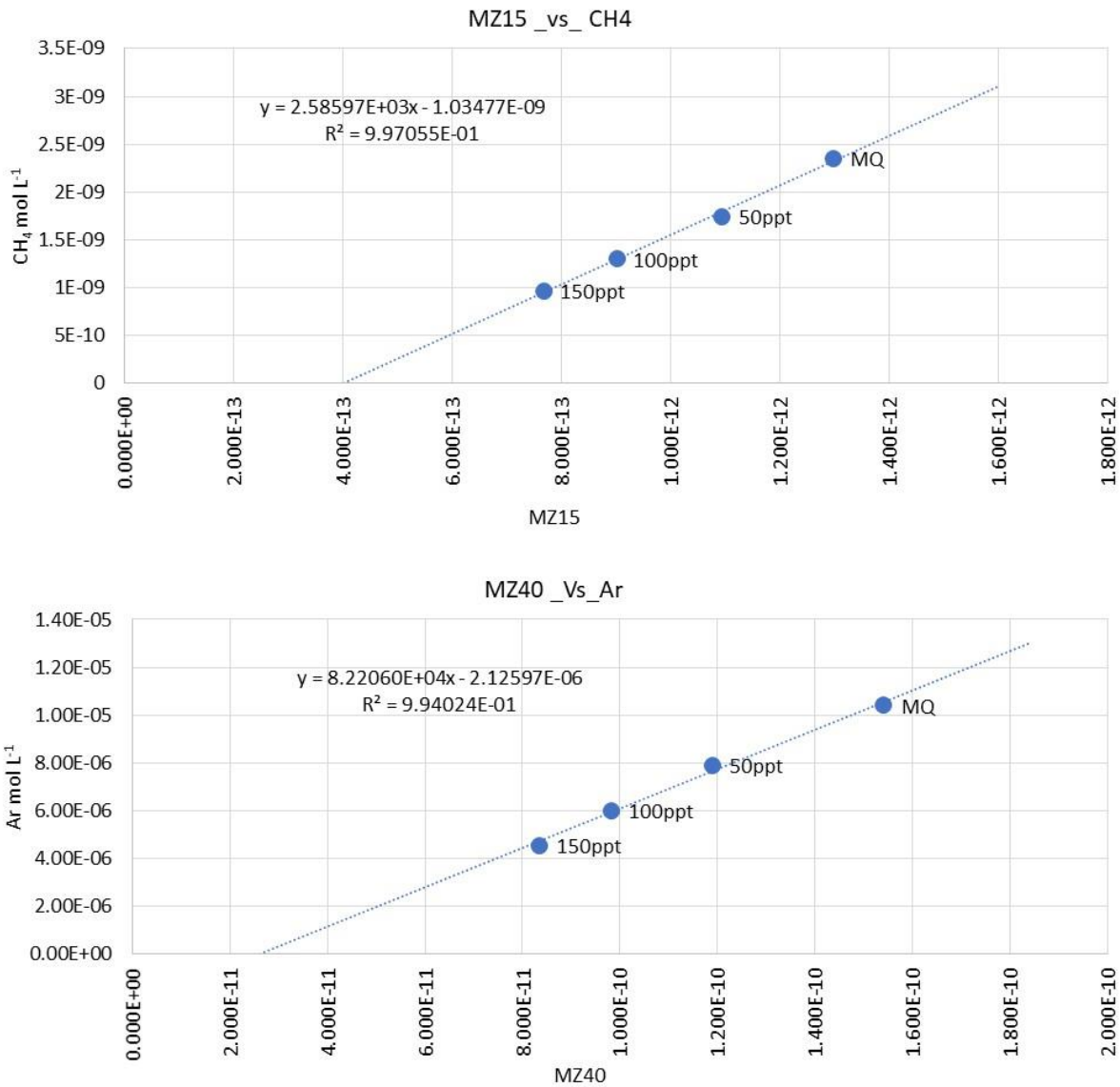

Fig. S7. Raw signal obtained from mass 15 (CH_4) and mass 40 (Argon) plotted against the calculated solubility at different salinities at 30 °C. The signal in both cases is linearly correlated to the concentration of the dissolved gas. The ratio between the two masses was extrapolated between 0 and 50 ppt and was used for calculating the CH_4 concentration.