Widespread formation of methane by *Cyanobacteria* in aquatic and terrestrial environments

One sentence summary: Ancient and ubiquitous, *Cyanobacteria* are the first in the domain *Bacteria* shown to emit methane, paradoxically in parallel to oxygen production.

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Abstract

Evidence is accumulating to challenge the paradigm that biogenic methanogenesis, traditionally considered a strictly anaerobic process, is exclusive to Archaea. Cyanobacteria are here shown to produce methane at substantial rates under light and dark oxic conditions, demonstrating biogenic methane production within the Bacteria, the second prokaryotic domain. Biogenic methane production was enhanced during oxygenic photosynthesis and directly attributed to the cyanobacteria by stable isotope labelling. Methanogenesis by Cyanobacteria partly explains the paradox of methane production in oxygen-saturated waters. Climate change, leading to worldwide increases in cyanobacterial blooms will accordingly have a direct feedback on warming. With a ubiquitous presence on Earth for 3.5 billion years, Cyanobacteria have had and will continue to have a substantial impact on the global methane budget.

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 1,860 ppbv (Nov. 2017 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 reduce the anthropogenic contribution to 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]. Methane emission was also detected from cryptogamic covers, i.e. phototrophic assemblages on plant, rock and soil surfaces [9]. Nevertheless, thus far, members of the second prokaryotic domain, *Bacteria*, remained unexplored as to their ability to directly produce CH_4 in the absence of added external precursors.

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 [10,11] or to the association of filamentous *Cyanobacteria* with methanogenic *Archaea*, providing the latter with the necessary hydrogen for CH_4 production [12]. *Cyanobacteria* are ubiquitous, found literally in any illuminated environment. Furthermore, this phylum predominated Earth whilst the environment was still reductive and before the great oxidation event (by ca. 1.3 billion years). Therefore, we tested whether this phylum contributes to the global CH_4 budget independent of environmental precursors or the presence of

methanogenic *Archaea*. If so, their ubiquitous nature, their expected future increase in abundance [13] 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 and filamentous, freshwater, marine and terrestrial members of the prominent and ubiquitous phylum *Cyanobacteria*, a member of the domain *Bacteria*, produce methane at substantial rates under both light and dark, oxic conditions.

Results and Discussion

To test the hypothesis that *Cyanobacteria* directly produce CH_4 independent of methylated precursors (e.g. methylphosphonates) in ambient water, nine different filamentous and unicellular cyanobacterial cultures 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 formation with increasing stable isotope values ($\delta^{13}C$-CH_4 values), depending on the amount of released 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 involvement of methanogenic *Archaea* in this process can be ruled out since the oxygen concentrations during CH_4 production were in most cases above saturation level (Fig. 2 and Fig. S2). Furthermore, sequencing analysis of the cultures and quantitative real-time PCR of the *mcrA* gene showed methanogenic *Archaea* are either absent or present in negligible numbers (Fig. S3).

Demethylation of methylphosphonates is unlikely to be the mechanism involved in this instance even though some *Cyanobacteria* do possess the necessary enzymatic machinery [10,11] for the following reasons. Firstly, 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}. Indeed, publicly available transcriptomic data for *Anabaena* sp. PCC 7120 [14,15] and *Trichodesmium erythraeum* [16] show no evidence for activity of the phosphonate C-P lyase genes under standard culture conditions. Secondly, some of the *Cyanobacteria* used in this study (i.e. *Microcystis aeruginosa* PCC 7806, *Prochlorococcus marinus* MIT 9313 as well as all sequenced species of *Chroococcidiopsis* sp., *Leptolyngbia* sp. and *Phormidium* sp.) do not possess the C-P lyase genes necessary for conversion of methylphosphonates to CH_4. Thus, we consider that the mechanism by which *Cyanobacteria* readily convert fixed CO_2 to CH_4 must revolve around their central metabolism. However, the biochemical pathway(s) involved in cyanobacteria-derived CH_4 formation remain unknown and thus require further investigation.

Patterns and rates of CH_4 production were investigated 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). 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 might 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 [17]. 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.

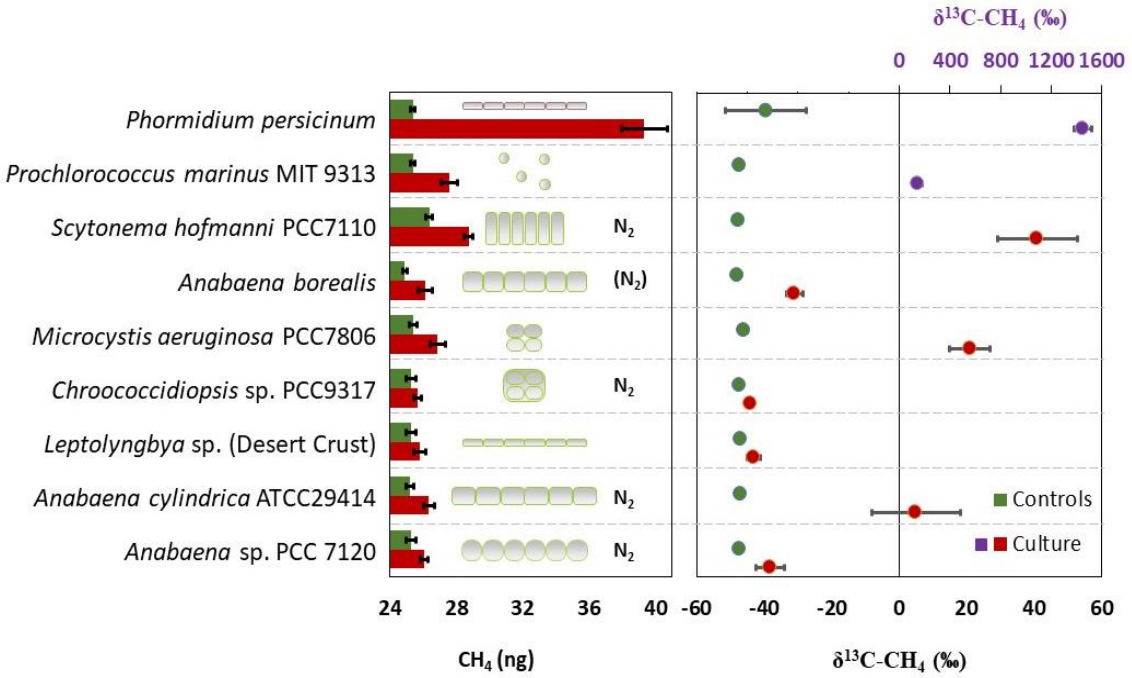

Figure 1. Amount of CH_4 produced and stable $\delta^{13}C$-CH_4 values measured during incubation experiments of nine different filamentous and unicellular (see sketches) cyanobacterial cultures with and without $NaH^{13}CO_3$ supplementation. *Cyanobacteria* that can (or likely can) fix atmospheric N_2 are labelled. All cyanobacterial cultures produced CH_4 (red bars). Using $NaH^{13}CO_3$ as carbon source resulted in increasing stable $\delta^{13}C$-CH_4 values as compared to the starting condition, as seen by the shift to the right of the red and violet circles compared to green ones. 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. Data presented in green (bars or circles) are from control experiments. Methane production was averaged across multiple experiments with and without added culture (See Fig. S4). Similarly control results of labelling experiments are the average of multiple experiments which exclude addition of culture and $NaH^{13}CO_3$ (See Fig. S5).

Methane production rates (Fig. 3) were calculated using the slope of CH_4 profiles and were normalized on a cyanobacterial biomass dry weight basis. Daily CH_4 production rates across all cultures 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}. These rates 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 Fig. S6).

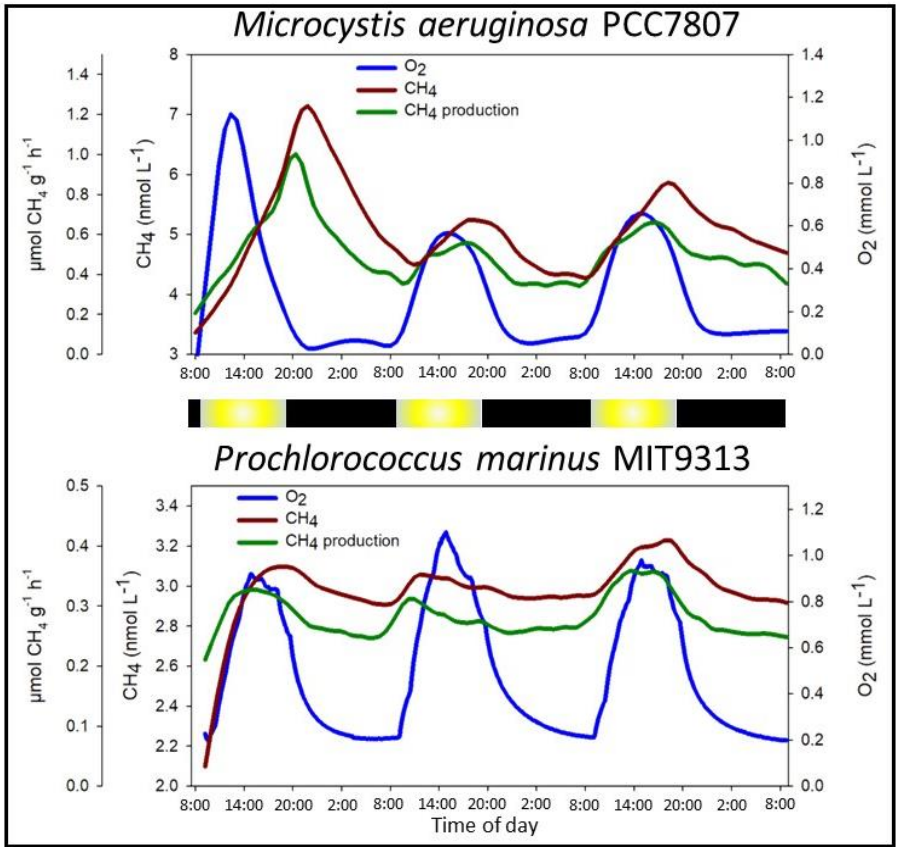

Figure 2. Continuous measurement of CH_4 and oxygen under light/dark periods using a membrane inlet mass spectrometer (MIMS). Examples are shown for two cultures but data for other cultures can be found in Fig. S2. A decrease in CH_4 concentration is a result of either reduced, or no, production coupled 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 intensity. After the maximum light period the intensity was programmed to decrease in reverse order with the same hold times until complete darkness again at 19:30.

Methane production in oxic soils has been previously discussed and attributed mainly to abiotic factors [18] or methanogenic *Archaea* [19], although the latter was thought unlikely [18,20]. Here we show that a typical desert crust *Cyanobacteria* (identified in this study as *Leptolyngbya* sp.), as well as the most common endolithic cyanobacterium *Chroococcidiopsis* [21] produce CH_4 both under light and dark conditions (Fig. 1, Fig. S2), thus inferring a new but as yet unknown mechanism for CH_4 production in oxic soils.

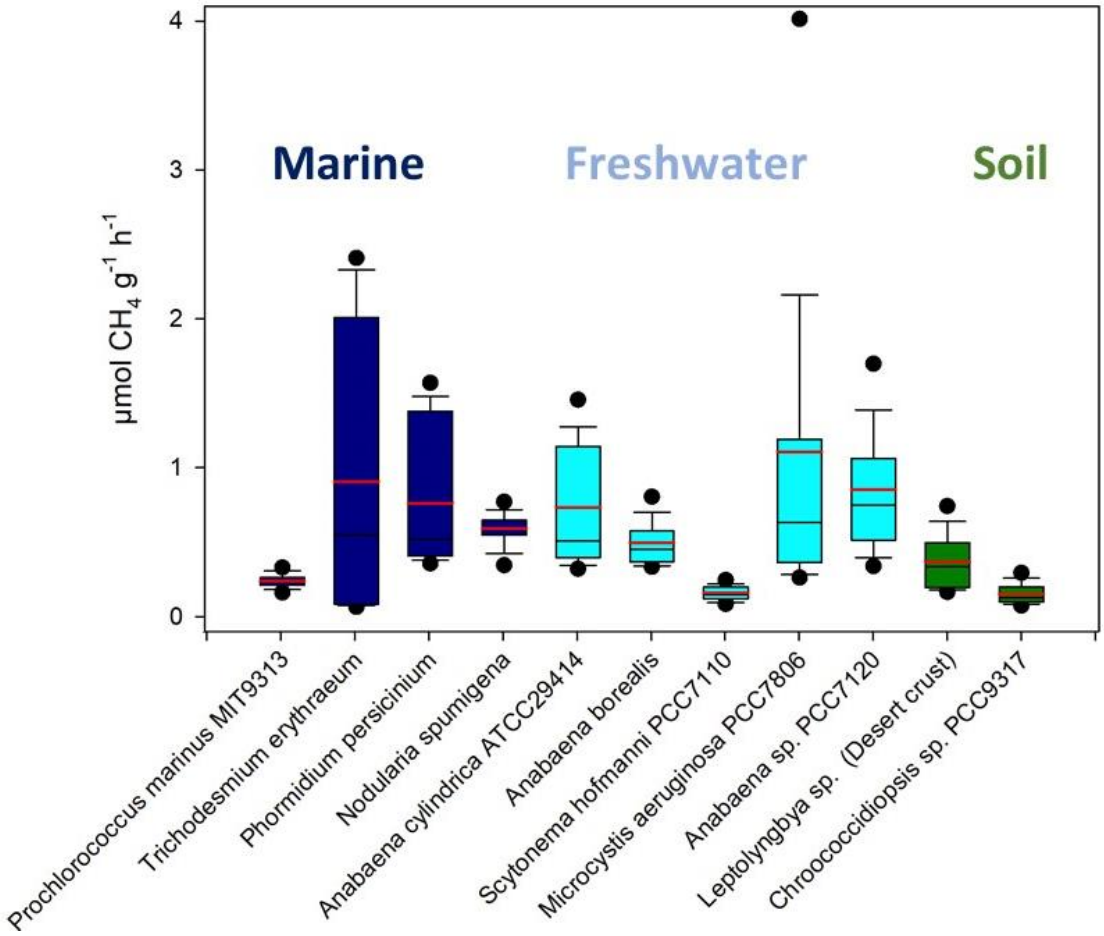

Figure 3. Average CH_4 production rates (μmol g DW^{-1} h^{-1}) obtained from multiple long-term measurements (2-5 days) using a membrane inlet mass spectrometer. The rates are designated by colour according to the environment from which the *Cyanobacteria* were originally isolated; dark blue, light blue and green for marine, freshwater and soil environments, respectively. Black and red lines represent median and mean values, respectively.

Cyanobacteria are ubiquitous in nature and their presence in aquatic systems is expected to increase with eutrophication and rising global temperatures [13]. The "methane paradox" describing the production of CH_4 in oxic water layers has been known for four decades [22] and a recent study suggests that as much as 90 % of CH_4 emitted from freshwater lakes is produced in the oxic layer [23]. As *Cyanobacteria* are often associated with elevated CH_4 concentration in oxygen

supersaturated freshwater systems [24], we used a global estimate of cyanobacterial biomass across different aquatic and terrestrial environments [21] and the production rates determined in this study to generate a conservative calculation of global cyanobacterial CH_4 production (Fig. 4). For this calculation we used hourly rates averaged over a period of 48-120 hours per experiment (see methods section for details). Minimum, maximum and average estimates for cyanobacterial production were generated for each environmental compartment. Since not all cyanobacterial biomass on Earth is present and active at the same time, specific activity periods were defined for *Cyanobacteria* from different environments (see methods section for details). We would point out that this simple approach neglects the complexity of aquatic and terrestrial ecosystems and hence our calculations should only be considered as first rough estimates.

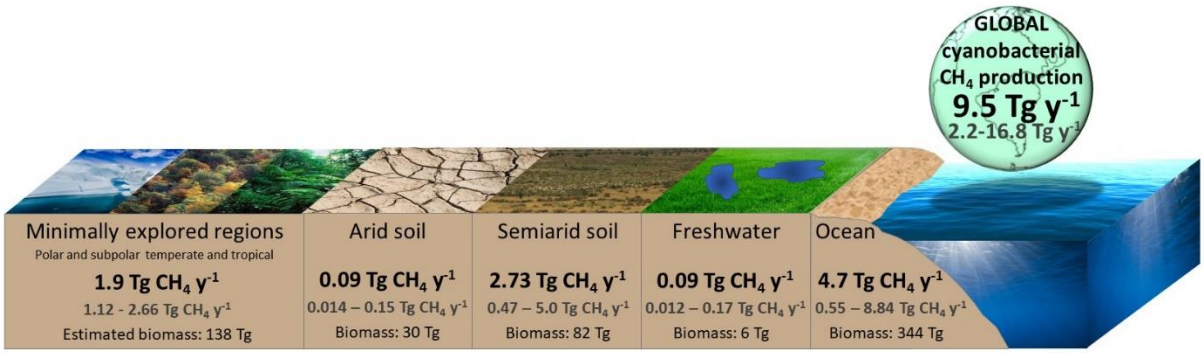

Figure 4. Estimated contribution of *Cyanobacteria* to the global CH_4 budget, calculated for different environments. The values represent estimates of production and not CH_4 emission from the different ecosystems, which most likely depend on other oxic-methane producers as well as consumers. The minimum and maximum CH_4 production values were calculated using the averaged minimum and maximum measured rates per cyanobacterial culture per environment type, alongside activity periods of different durations (see methods section).

Our calculations suggest that globally, *Cyanobacteria* thriving in aquatic and terrestrial environments produce 2.2 - 16.8 Tg CH_4 y^{-1} with a mean value of 9.5 Tg CH_4 y^{-1} (Fig. 4). Based on these estimates the largest single contribution arises from oceanic environments (4.7 Tg y^{-1}) followed by semiarid soils (2.73 Tg y^{-1}). The CH_4 emission in oceanic systems is estimated to be between 0.6-1.2 Tg y^{-1} [25], suggesting that a major portion of cyanobacterial-produced CH_4 is either consumed or accumulated within the water column alongside other potential sources of oxic-methane. Surprisingly, despite freshwater environments having a similar overall contribution as oceans to oxic-methane emission [26], the freshwater cyanobacterial component calculated here is significantly lower (0.09 Tg y^{-1}). In their global estimation, Garcia-Pichel *et al.* [21] state that freshwater cyanobacterial biomass may be underestimated since small lakes and ponds were not included. The latter study suggests the global cyanobacterial biomass may be underestimated by a total of 138 Tg of which up to 2 Tg are attributed to small lakes and ponds. Accounting for these additional 2 Tg increases the maximum freshwater contribution from 0.17 to 0.22 Tg CH_4 y^{-1} on the expense of the 1.9 Tg CH_4 y^{-1} produced by *Cyanobacteria* from understudied biomes, but it does not increase the estimated global production. However to fully account for the discrepancy

in cyanobacterial contribution to oxic-methane emission from freshwater systems would require either additional mechanisms or additional unaccounted biomass, such as under-ice blooms [27] or a significant increase in freshwater cyanobacterial biomass as reported from large lakes all over the world [28,29].

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 [10,11]. *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 global CH_4 cycle and budget. Furthermore, as phototrophic prokaryotes such as *Cyanobacteria* have been inhabiting Earth for more than 3.5 billion years 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 is needed to elucidate the biochemical pathways of CH_4 formation in *Cyanobacteria* and fully assess its possible relevance for ecology and the global CH_4 budget throughout Earth history.

Acknowledgements

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 Tim Urich from Greifswald University for 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. Funding was provided to M.B.I., D.I. and H.P.G. via the DFG-Aquameth project (GR1540-21-1) and the Human Frontiers Science project (HFSP 2039371). F.K. and T.K. were supported by the German Research Foundation (DFG; KE 884/8-2 and KE 884/11-1).

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, 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 microbial community data analysis; A.M.M.P. analyzed transcriptomics data. M.B.I., T.K., D.I., M.Y.H., M.G., A.M.M.P., F.K., H.P.G. discussed the results and wrote the paper.

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Material and Methods:

Cyanobacterial cultures

Eleven different cyanobacterial cultures were obtained from various sources and grown using the media described in Table S1.

Strain name	Source	Morphology	Growth medium	Incubation temperature °C
Anabaena sp. PCC7120	IBVF	Filamentous	BG11 [30]	30
Anabaena cylindrica ATCC29414	IBVF	Filamentous	BG11 [30]	30
Anabaena borealis	CCALA	Filamentous	BG11[30]	30
Scytonema hofmanni PCC7110	IBVF	Filamentous	BG11 [30]	30
Leptolyngbya sp. (desert crust)	HUJI	Filamentous	BG11 [30]	30
Phormidium persicinum	IBVF	Filamentous	f/2 [31]	26
Trichodesmium erythraeum	MPI-MM	Filamentous	YBCII [32]	26
Nodularia spumigena	IOW	Filamentous	f/2 [31] (8 psu)	20
Chroococcidiopsis sp. PCC9317	IBVF	Unicellular	BG11 [30]	30
Microcystis aeruginosa PCC7806	IGB	Unicellular	BG11 [30]	30
Prochlorococcus marinus MIT9313	Uni Freiburg	Unicellular	AMP1 [33]	22

Table S1. Cyanobacterial cultures used in this study and their growth conditions. 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.

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 (ambient 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 5 to 10 % of the initial dissolved inorganic carbon (DIC) in BG11 (DIC = 0.4 mM, enriched by added $NaHCO_3$; pH ≈ 7.0) and 4.5 % of the DIC in f/2 medium (DIC = 2015 µmol L^{-1}; pH ≈ 8.2). 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 $NaH^{13}CO_3$; Three replicates of each cyanobacteria culture (n = 3) were investigated, except for *Prochlorococcus marinus* MIT 9313 and *Phormidium persicinum* where four replicates were used (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}. The initial inoculated dry weight was specific for each species and ranged between 0.03 and 0.28mg dw ml^{-1}. The incubation time was between 3 and 11 days.

CF-IRMS

CF-IRMS was employed for measurement of the $\delta^{13}C$ values of the CH_4 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 $\delta^{13}C$ values of CH_4 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.

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

Determination of CH_4 mole fraction

The head space pressure was measured with a sensor (GMSD 1,3 BA, Greisinger, capped by a needle). Afterwards 5 ml gas volume was sampled from the head space using a Hamilton gas syringe needle and analyzed by a gas chromatograph (GC; column: 2 m, Ø = 3.175 mm inner diameter, high-grade steel tube packed with Molecular Sieve 5A 60/80 mesh from Supelco) equipped with a GC–flame ionization detector (FID) (Shimadzu GC-14B). The system was calibrated by a reference standard containing 2192 ppb_v CH_4.

The CH_4 mole fraction (n_{CH_4}) was determined by its mixing ratio (x_{CH_4}) and the ideal gas law (eq. 2).

$$n_{CH_4} = x_{CH_4} \frac{p\,V}{RT} \quad (2)$$

Where p = pressure, T = temperature, R = ideal gas constant, V = volume.

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*. [34]. 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 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.

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.

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 which does not overlap with other gases in the sample [35]. The CH_4, O_2 and Ar concentration in the different media were calculated based on known solubility constants [36] 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 [37] 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. 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 Equation 1 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 (eq. 3).

$$R_{degassing} = -2.2365 \times 10^{-12} \times Sat_{(CH_4)} + 2.12656 \times 10^{-12} \quad (3)$$

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

Global budget parameters and calculations

Global cyanobacterial biomass estimates were taken from Garcia-Pichel *et al.* [21] and were used to calculate the discussed compartments: Marine (Oceanic), Freshwater, Arid soil, Semiarid soil and minimally explored areas. The *Cyanobacteria* were divided to the different compartments based on their source as shown in Fig. 2. The CH_4 production rates derived from the two soil *Cyanobacteria* was applied to both soil compartments. Minimum and maximum contributions per compartment were calculated by defining minimum and maximum CH_4 production rates as well as a short or long activity period. To calculate the minimum or maximum contribution of each compartment the minimum or maximum average daily rates from all experiments conducted with a given *Cyanobacteria* were averaged across the *Cyanobacteria* in the compartment. These parameters are provided in Table S2.

Minimum rates	**Biomass (g C)**	**Biomass Dry weight (g)**	**Daily rates (µmol h^{-1} g^{-1})**	**Annual max production (µmol CH_4)**	**h/day active**	**weeks /year active**	**Fraction / year**	**Yearly sum (µmol CH_4)**	**Yearly sum (mol CH_4)**	**g CH_4 y^{-1}**	**Tg CH_4 y^{-1}**
Oceans	1.720E+14	3.440E+14	0.299	9.02E+17	24	2	0.038	3.47E+16	3.47E+10	5.55E+11	0.555
Lakes	3.000E+12	6.000E+12	0.383	2.01E+16	24	2	0.038	7.75E+14	7.75E+08	1.24E+10	0.012
Arid	1.500E+13	3.000E+13	0.164	4.30E+16	2	13	0.021	8.95E+14	8.95E+08	1.43E+10	0.014
Semiarid	4.100E+13	8.200E+13	0.164	1.17E+17	12	26	0.250	2.94E+16	2.94E+10	4.70E+11	0.470
Unknown	6.900E+13	1.380E+14	0.252	3.05E+17	12	24	0.231	7.04E+16	7.04E+10	1.13E+12	1.127
Global	3.000E+14	6.000E+14								**Global**	**2.18**
Maximum rates											
Oceans	1.720E+14	3.440E+14	0.733	2.21E+18	24	13	0.250	5.52E+17	5.52E+11	8.84E+12	8.836
Lakes	3.000E+12	6.000E+12	0.789	4.15E+16	24	13	0.250	1.04E+16	1.04E+10	1.66E+11	0.166
Arid	1.500E+13	3.000E+13	0.435	1.14E+17	4	26	0.083	9.54E+15	9.54E+09	1.53E+11	0.153

Semiarid	4.100E+13	8.200E+13	0.435	3.13E+17	24	52	1.000	3.13E+17	3.13E+11	5.00E+12	5.004
Unknown	6.900E+13	1.380E+14	0.598	7.23E+17	12	24	0.231	1.67E+17	1.67E+11	2.67E+12	2.670
Global	3.000E+14	6.000E+14								**Global**	**16.82**

Table S2. Parameters used to calculate the estimated global CH_4 production by *Cyanobacteria* and the calculation outcome.

DNA extraction and sequencing

Cyanobacterial cultures are known to be rarely axenic. To evaluate the presence of methanogenic *Archaea* in the cultures DNA was extracted as described in Nercessian *et al*. [38]. The resulting DNA was sent for Illumina sequencing at MrDNA (Shallowater, TX, USA) on a Miseq platform 2x300 bp using the Arch2A519F (CAGCMGCCGCGGTAA) and Arch1017R (GGCCATGCACCWCCTCTC) primers [39]. Archaeal community composition was analyzed using the SILVA-NGS pipeline [40] (Fig. S5). 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 resulting in low copy numbers of the gene (Fig. S3). The sequences were submitted to the European Nucleotide Archive under project number: PRJEB25851.

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*. [24]. O_2 and Chl a were measured hourly using a YSI and a BBE probe (see www.lake-lab.de), respectively.

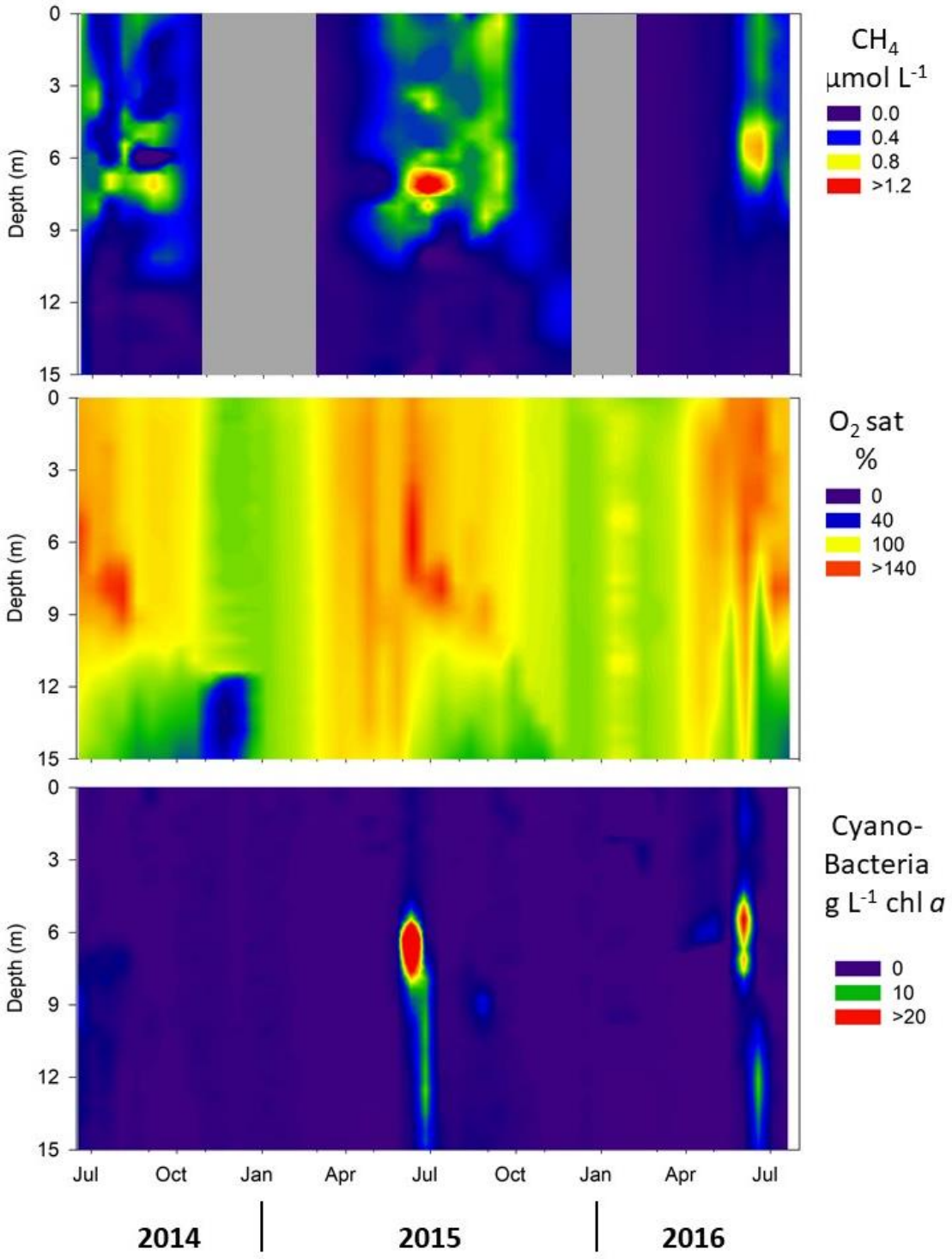
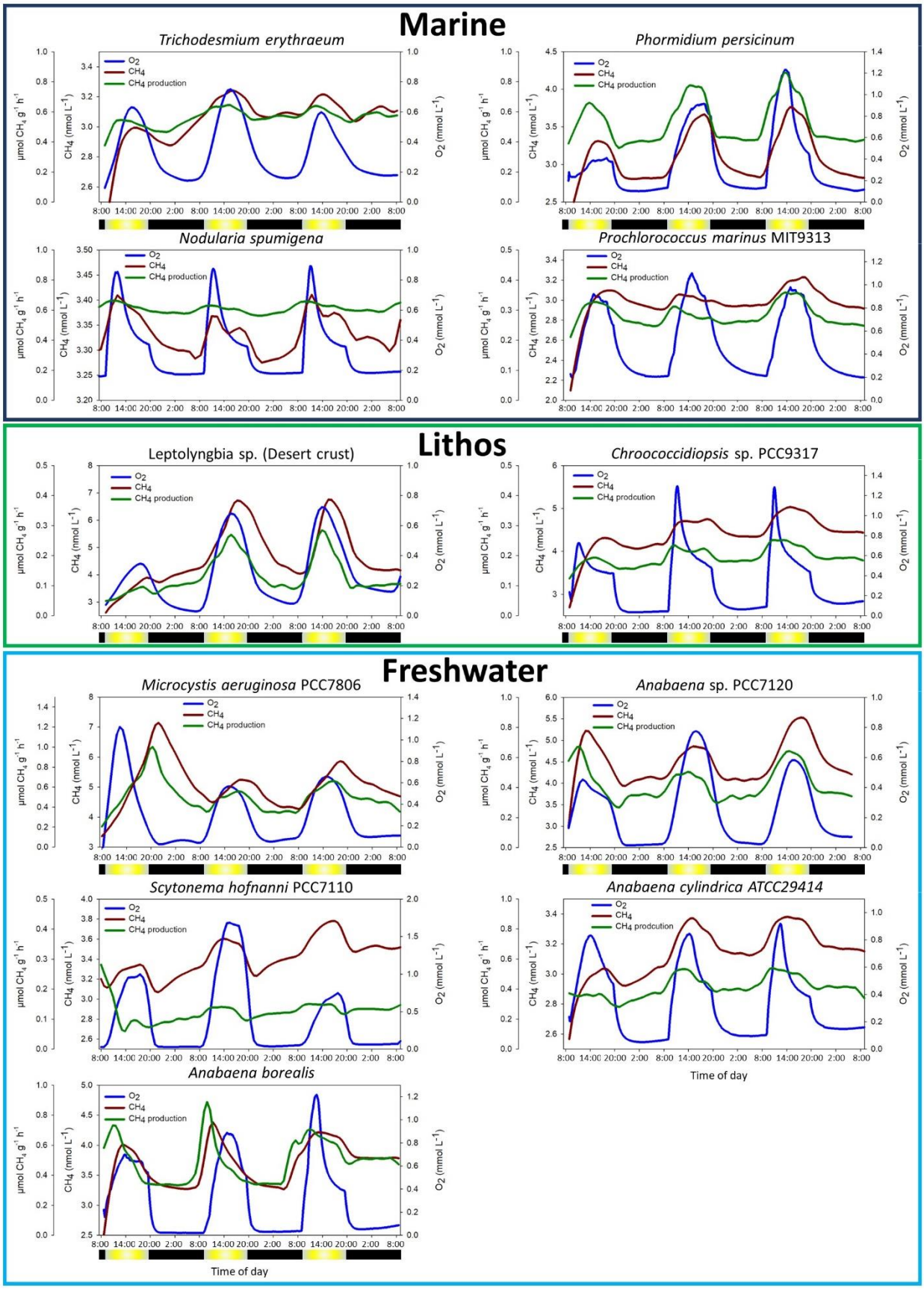

CH4
µmol L-1
0.0
0.4
0.8
>1.2
O2 sat
%
0
40
100
>140
Cyano-
Bacteria
g L-1 chl a
0
10
>20
Depth (m)
0
3
6
9
12
15
Jul
Oct
Jan
Apr
Jul
Oct
Jan
Apr
Jul
2014
2015
2016

Fig. S2. Continuous measurements of CH_4 and oxygen under light/dark cycles using a membrane inlet mass spectrometer (MIMS) in 11 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.


Marine
Trichodesmium erythraeum
Phormidium persicinum
Nodularia spumigena
Prochlorococcus marinus MIT9313
Lithos
Leptolyngbia sp. (Desert crust)
Chroococcidiopsis sp. PCC9317
Freshwater
Microcystis aeruginosa PCC7806
Anabaena sp. PCC7120
Scytonema hofnanni PCC7110
Anabaena cylindrica ATCC29414
Anabaena borealis
O2
CH4
CH4 production
µmol CH4 g-1 h-1
CH4 (nmol L-1)
O2 (mmol L-1)
Time of day

478

Fig. S3. Community composition of the cyanobacterial cultures as obtained when sequenced using *Archaea* specific primers. Methanogenic *Archaea* (red) are in most cases lower that 0.1% of the obtained sequences. In the absence of *Archaea* DNA template, the primers amplify DNA of *Bacteria*. The background presence or complete absence of methanogens was confirmed by qPCR of the *mcrA* gene.

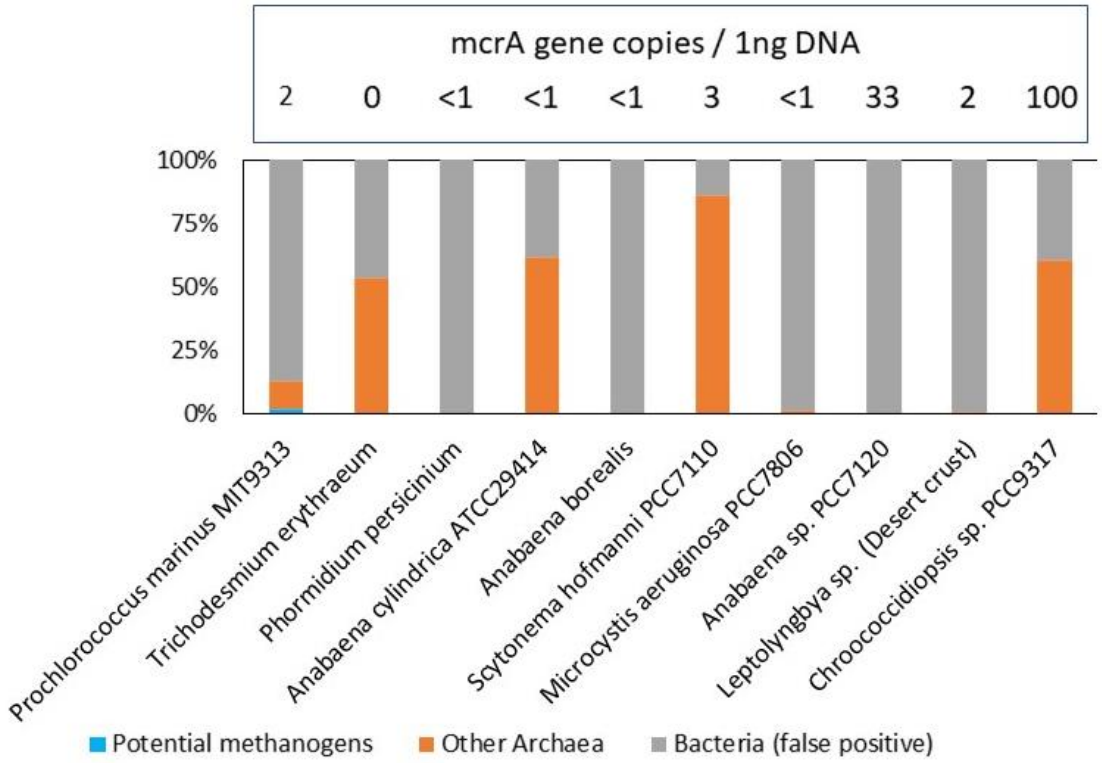


Fig. S4. Average CH_4 mass (ng) in head space of different cyanobacterial species. a) *Anabaena cylindrica*, ATCC29414; b) *Anabaena sp.*, PCC7120; c) *Chroococcidiopsis sp.*, PCC9317; d) *Scytonema hofmanni* PCC7110; e) *Microcystis aeruginosa*, PCC7806; f) *Anabaena borealis*; g) *Leptolyngbya* (desert crust); h) *Prochlorococcus marinus*, MIT9313; i) *Phormidium persicinum*. After the incubation period the CH_4 mass was significantly higher ($p<0.05$, two-way ANOVA) for each species in the culture group (grey bars) as compared to the group without cultures (white bars). P-values for this comparison are given in the graph. The addition of $NaH^{13}CO_3$ had no significant effect ($p>0.05$) on the CH_4 quantity. Error bars indicate the standard deviation (n=3-4).

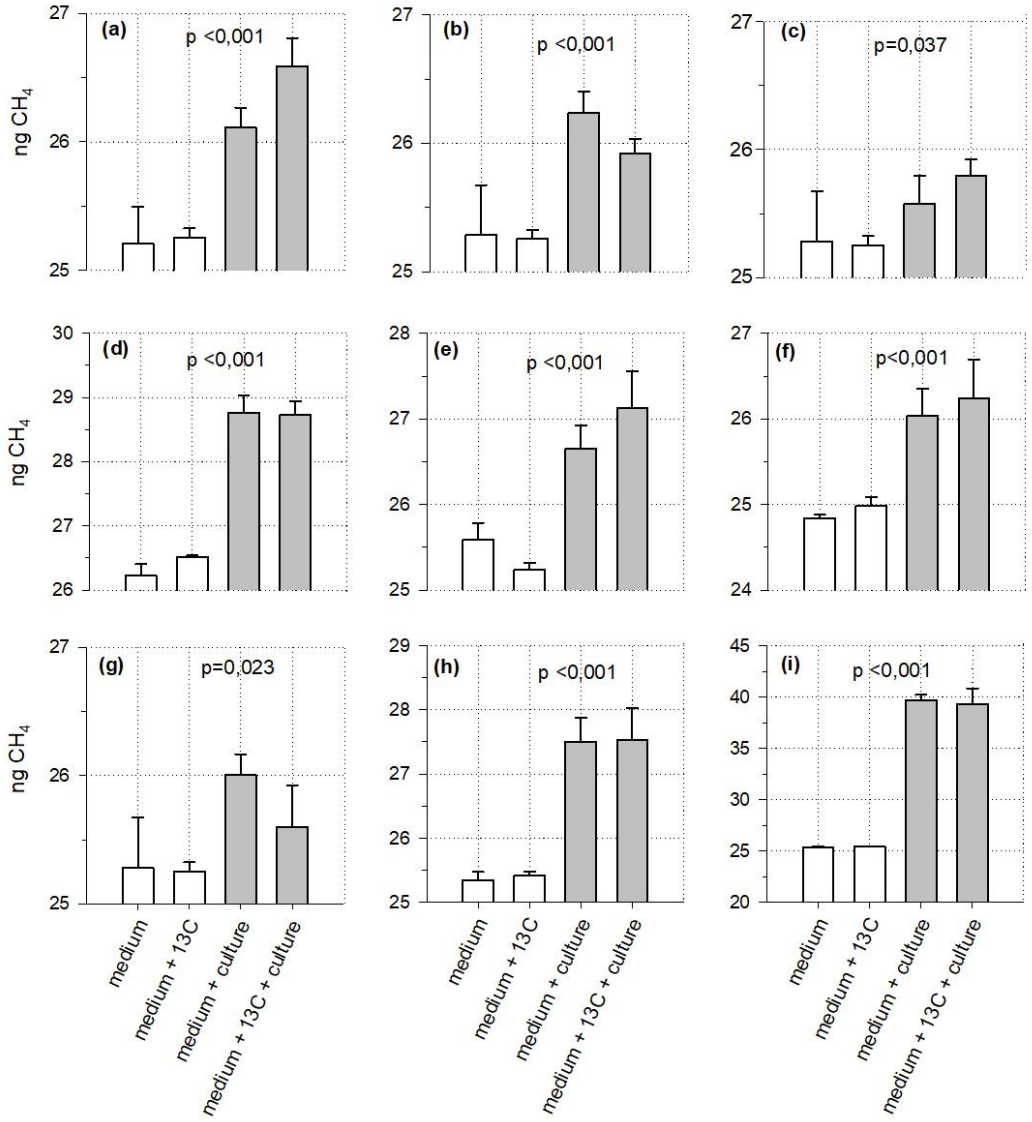


Fig. S5. Average $\delta^{13}C$-CH_4 values in head space of different cyanobacterial species. a) *Anabaena cylindrica*, ATCC29414; b) *Anabaena sp.*, PCC7120; c) *Chroococcidiopsis sp.*, PCC9317; d) *Scytonema hofmanni* PCC7110; e) *Microcystis aeruginosa*, PCC7806; f) *Anabaena borealis*; g) *Leptolyngbya* (desert crust); h) *Prochlorococcus marinus*, MIT9313; i) *Phormidium persicinum*. The $\delta^{13}C$-CH_4 values in cultures that were treated with $NaH^{13}CO_3$ (medium+ ^{13}C+ culture) are clearly different from the three control groups. Error bars mark the standard deviation.

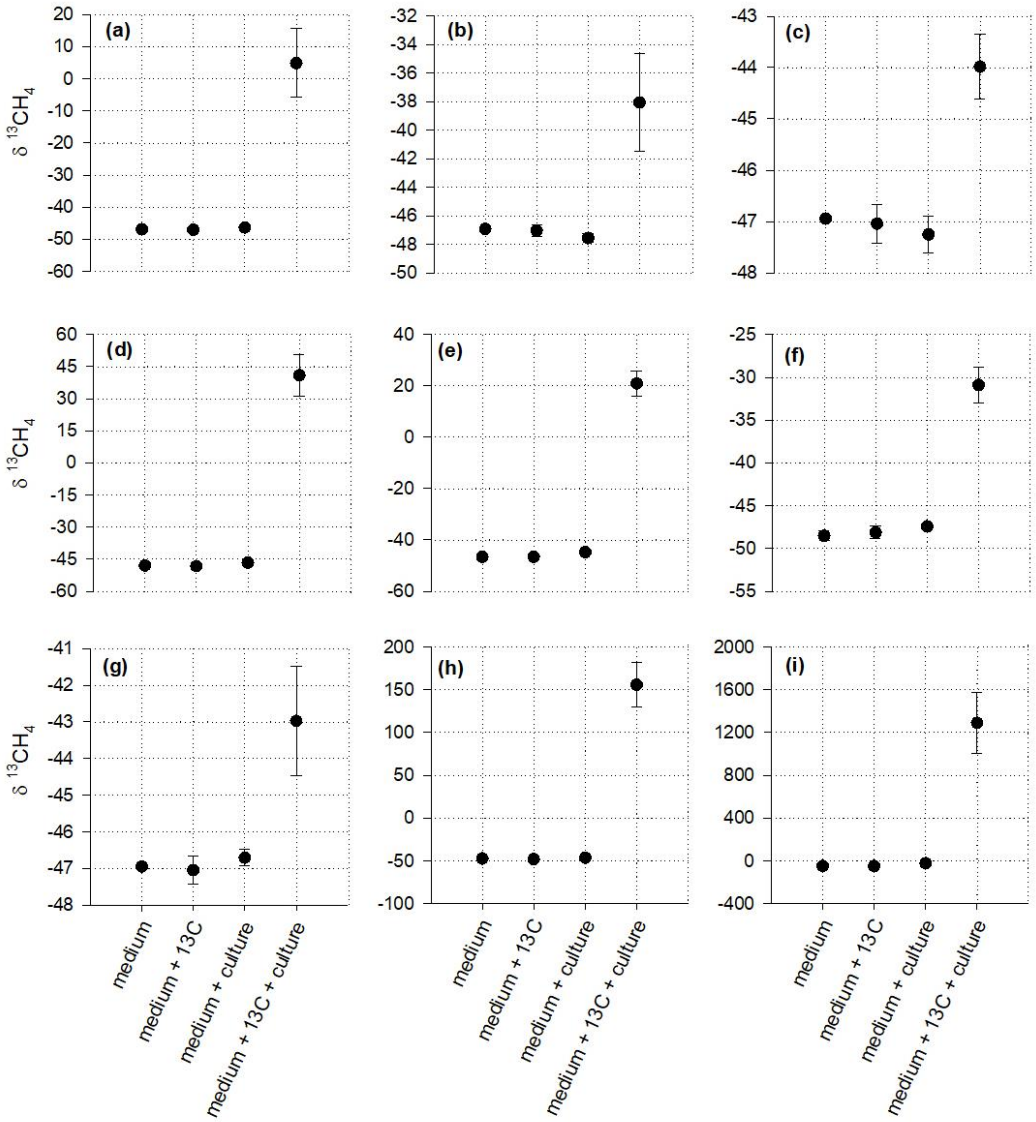


Fig. S6. Average CH_4 production rates (µmol g_{DW}^{-1} h^{-1}) obtained from multiple long-term measurements (2-5 days) with a membrane inlet mass spectrometer. The rates are shown by colour according to the environment from which the *Cyanobacteria* were originally isolated; dark blue, light blue 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 [41], Kröninger *et al*. [42] and Gerhard *et al*.[43]. Rates for

eukaryotes including marine algae and terrestrial plants were taken from Lenhart *et al*. [3], Keppler *et al*. [4], Brüggemann *et al*. [44], Wishkermann *et al*. [45] and Qaderi *et al*. [46] No emission rates (on a dry weight basis) are available for fungi and animals.

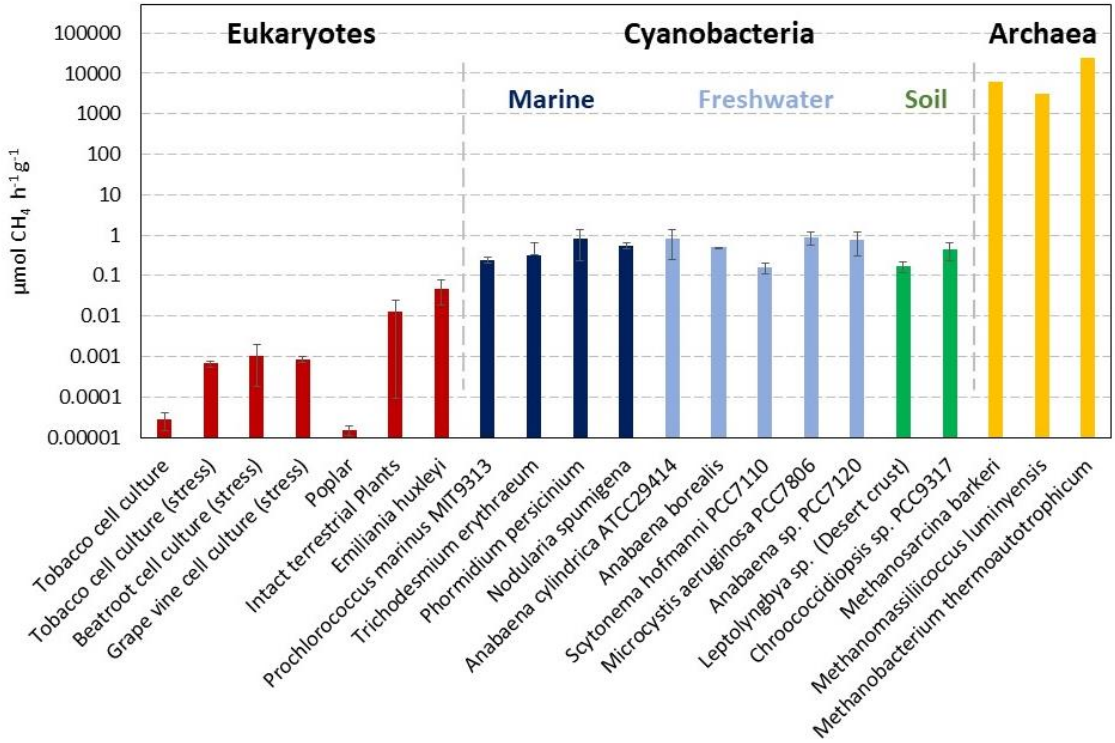
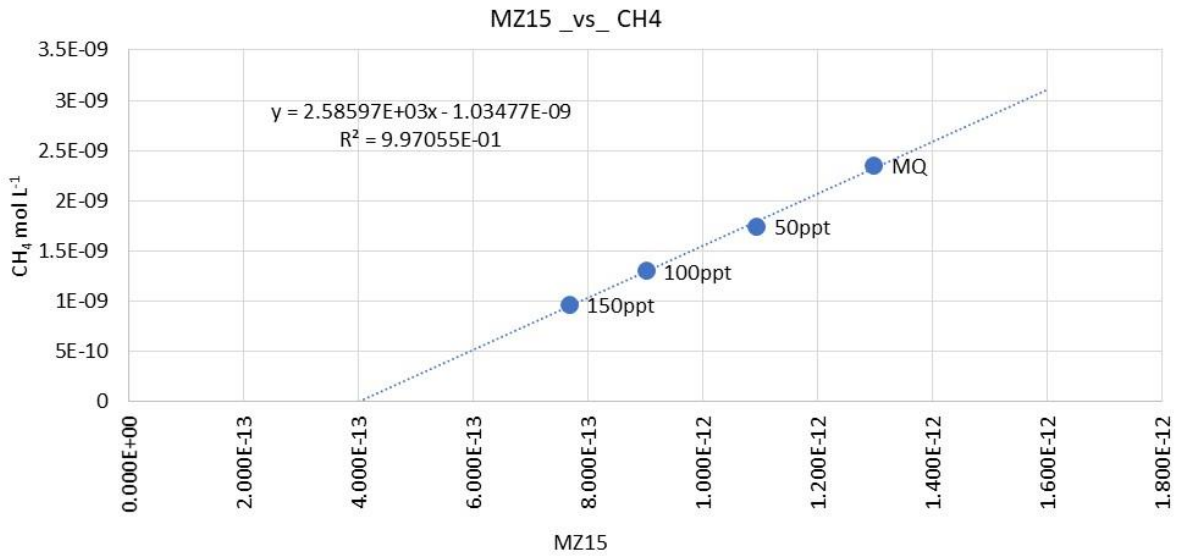



Fig. S7. Raw signal obtained from mass 15 (CH_4) and mass 40 (Argon) plotted against the calculated solubility at different salinities at 30 °C. The signal in both cases is linearly correlated to the concentration of the dissolved gas. The ratio between the two masses was extrapolated between 0 and 50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