Supporting Information

Grossart et al. 10.1073/pnas.1110716108

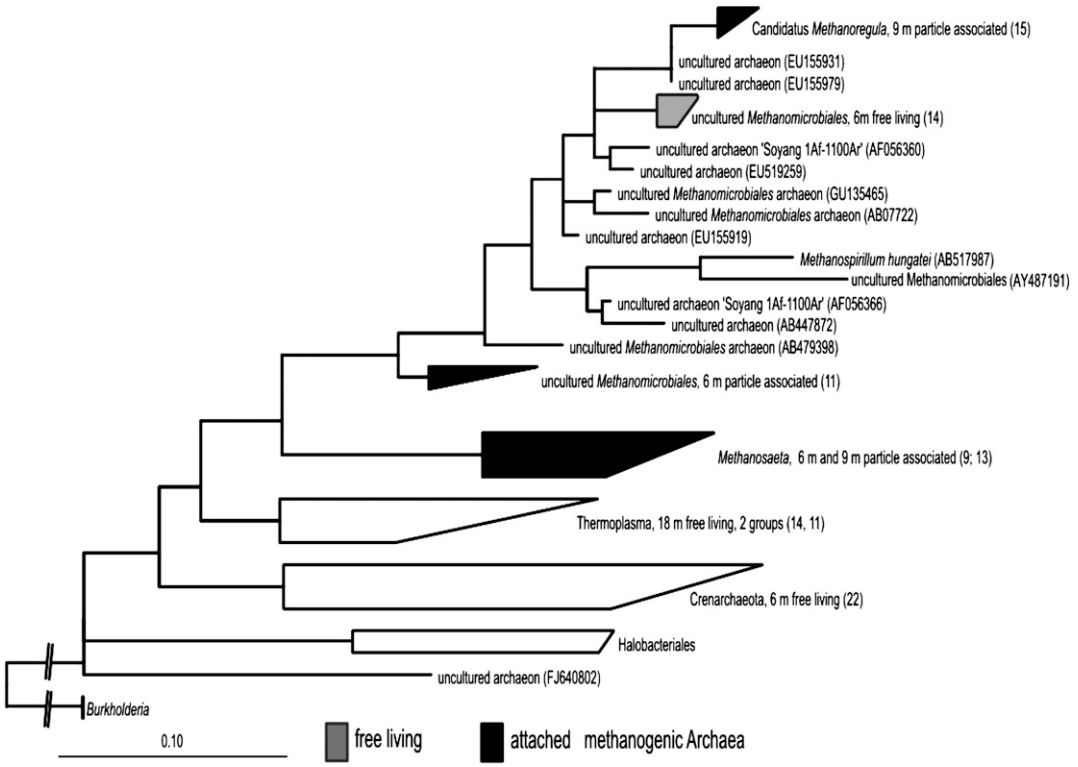

Fig. S1. Phylogenetic tree of methanogenic Archaea in Lake Stechlin. Sequences were obtained from three clone libraries (6-, 9-, and 18-m depth) using primers that are specific for Archaea including methanogenic Archaea (Table S3). The tree is constructed using the software ARB (http://www.arb-home.de/).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

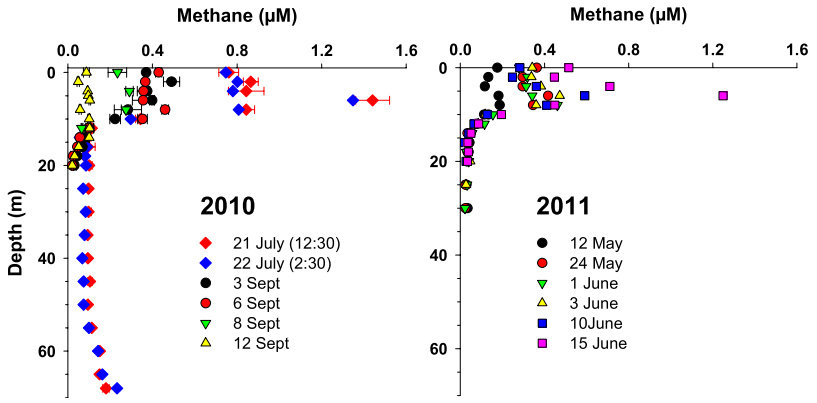

Fig. S2. In situ methane concentrations in Lake Stechlin. Each data point represents a single sample or the average of duplicate samples. In 2010, the epilimnion methane concentrations decreased over time from July to September. In 2011, the epilimnion methane concentrations increased over time from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

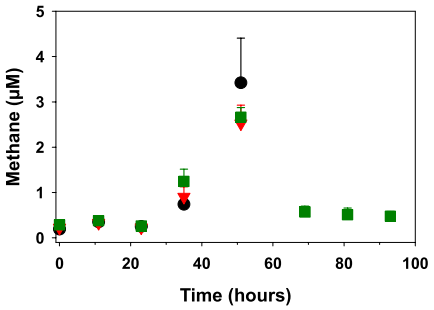

Fig. S3. Methane concentrations in unamended Lake Stechlin surface water incubated over time. Different symbols represent different replicate bottles. Error bars are SDs of triplicate measurements of the same bottles. After 51 h, two bottles were killed for oxygen measurements, and dissolved oxygen concentrations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

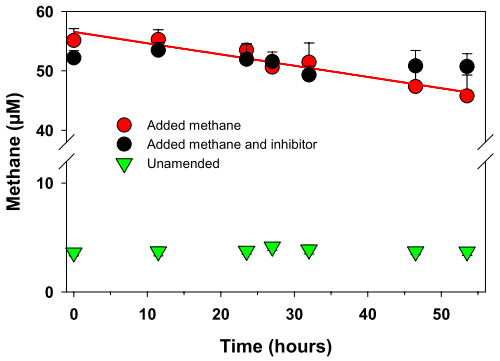

Fig. S4. Methane concentrations in Lake Stechlin surface water with or without added methane and inhibitor (0.03 kPa difluoromethane). Error bars are SDs of triplicate measurements of the same bottles. There was no significant change in methane concentration in unamended water or water + methane + inhibitor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

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

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

*No significant effect because of the addition of phosphate ($P = 0.131$, two-way ANOVA).

†Control is significantly higher than treatments ($P \ll 0.001$, two-way ANOVA). Addition of MPn and TMA did not increase methane production.

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

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

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

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

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

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

1. Muyzer G, de Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol* 59:695–700.
2. Teske A, Sigalevich P, Cohen Y, Muyzer G (1996) Molecular identification of bacteria from a coculture by denaturing gradient gel electrophoresis of 16S ribosomal DNA fragments as a tool for isolation in pure cultures. *Appl Environ Microbiol* 62:4210–4215.
3. Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 173:697–703.
4. Wise MG, McArthur JV, Shimkets LJ (1999) Methanotroph diversity in landfill soil: Isolation of novel type I and type II methanotrophs whose presence was suggested by culture-independent 16S ribosomal DNA analysis. *Appl Environ Microbiol* 65:4887–4897.
5. DeLong EF (1992) Archaea in coastal marine environments. *Proc Natl Acad Sci USA* 89:5685–5689.
6. Stackebrandt E, Goodfellow M (1991) *Nucleic Acid Techniques in Bacteria Systematics* (Wiley, New York).
7. Raskin L, Poulsen LK, Noguera DR, Rittmann BE, Stahl DA (1994) Quantification of methanogenic groups in anaerobic biological reactors by oligonucleotide probe hybridization. *Appl Environ Microbiol* 60:1241–1248.
8. Casamayor EO, et al. (2002) Changes in archaeal, bacterial and eukaryal assemblages along a salinity gradient by comparison of genetic fingerprinting methods in a multipond solar saltern. *Environ Microbiol* 4:338–348.
9. Watanabe T, Asakawa S, Nakamura A, Nagaoka K, Kimura M (2004) DGGE method for analyzing 16S rDNA of methanogenic archaeal community in paddy field soil. *FEMS Microbiol Lett* 232:153–163.