Supporting Information

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SI Text

SI Results and Discussion

Factors Limiting the Absolute Quantification of Newly Synthesized Proteins. BONCAT has the theoretical potential to detect all de novo synthesized proteins that contain at least one Met, i.e., ~99% of proteins in an average archaeal and bacterial proteome. However, in practice, several factors reduce this sensitivity and prohibit the absolute quantification of the amount of new protein, and, in consequence, of cell doubling times, from fluorescence data. (*i*) The process(es) by which HPG enters the cell is currently unknown and might depend on the physiological state of the cell or differ between different taxonomic groups; (*ii*) the rate at which HPG substitutes Met during protein synthesis has, so far, only been studied for *E. coli* (17) and might deviate in other organisms; (*iii*) due to varying contents of Met and contrasting copy numbers of proteins, distinct peptides contribute differently to overall fluorescence; and (*iv*) the extent by which protein recycling and posttranslational modification affect the stability of HPG (in particular its alkyne group) is currently unknown.

CARD-FISH vs. FISH. Our CARD-FISH experiments revealed marked discrepancies in the efficiencies to permeabilize and fluorescently detect different ANME subgroups. Most importantly, although ANME-1 constituted only 0.6% of all taxonomically identified consortia in our FISH experiments on sample #3730 (114 d sample; n = 167 consortia), the same subgroup represented 81.3% of detected ANME consortia in our CARD-FISH dataset (n = 48) (Table 1). This result is mainly explained by the inability of the used CARD-FISH protocol to detect ANME-2a and -2c consortia in our samples. This finding is in contrast to two recent studies that used near-identical permeabilization protocols for the successful visualization of these ANME groups in other methane seep sediments (36, 63).

Comparing ANME Community Structure in Sediment #3730 to Previous Studies. The very low proportion of aggregated ANME-1 in our Hydrate Ridge sediment (#3730) is consistent with a previous study on this methane seep, which found that ANME-1 occurred mostly as planktonic cells rather than in multicellular associations (26, 37). Because of our focus on syntrophic consortia in this study, we initially separated microbial aggregates of >3 μm from sediment particles. Planktonic ANME-1 cells thus might have partly evaded FISH detection. It should, however, be noted that our filtered samples contained large numbers of individual cells and that in no #3730 sample were individual cells found to bind the ANME-1-specific probe.

ANME-2a and -2c probes used in this study have been successfully used in previous mono-FISH studies of geographically distinct Hydrate Ridge sediment samples with hybridization rates of 20–80% of all DAPI-stained AOM consortia (37, 42). Because of the high spatial variance in ANME community structure (37, 38), it is, however, possible that our specific sediment samples hosted a unique combination of ANME clades. Alternatively, ANME community structure might have diverged from its original composition during the nearly 4 y of incubation in the laboratory before the experiments described herein were conducted.

Considerations on the Environmental Application of BONCAT. In contrast to the well-established stable isotope probing approach, the universal applicability of BONCAT is currently untested, and several questions demand rigorous investigation in future studies: (*i*) The mechanism(s) by which bioorthogonal amino acids are taken up by cells is currently unknown. If active transporters are required for their uptake, their absence would prohibit the application of BONCAT to that particular cell. To that effect, the recent report of up to 100% BONCAT labeling efficiency of planktonic microbes in surface seawater is encouraging (14). (*ii*) HPG and AHA, the bioorthogonal amino acids that have been used in environmental studies so far (refs. 9 and 14 and this study), have to compete with intracellular Met for incorporation into newly being made proteins. The preference of the translational machinery for Met over its synthetic surrogates (17) might therefore restrict the use of BONCAT in habitats featuring high concentrations of free Met. (*iii*) Lastly, substituting proteins with synthetic amino acids bears a high risk of interfering with the cellular machinery. We recently demonstrated that the addition of up to 1 mM of HPG or AHA had no detectable effect on the growth of several different, physiologically distinct archaeal and bacterial pure cultures for at least one cell generation. At longer incubation times, however, inhibition of growth could be observed at these high concentrations (9, 46). For environmental applications, we thus recommend that low concentrations of bioorthogonal amino acid should be used, incubation times be kept to a minimum, and complementary experiments testing for potential community shifts be performed (for details, see ref. 46).

SI Materials and Methods

Environmental Sampling and Storage. Sediment sample #3730 was obtained from Hydrate Ridge South methane seep field (R/V *Atlantis* cruise AT-15-68, Alvin Dive 4635; push-core 16; 44°34.09 N, 125°9.14 W; 775 m water depth; sediment horizon 0–6 cm; 4 °C in situ temperature) on 7 August 2010. Sediment was stored under argon headspace in a Mylar bag for 5 wk before being transferred to a 1-L glass bottle with a 1.38 bar 100% methane headspace, which was stored at 4 °C for ~4 y. Seawater and headspace were exchanged at regular intervals to prevent the accumulation of inhibitory compounds.

Sediment samples #7135 and #7136-37/37 were collected from Santa Monica Basin on 9 May 2013 (R/V *Western Flyer* MBARI Cruise 2013; dive 463; push-core 43; 33°47.34 N, 118°40.10 W; 860 m water depth; horizons 6–9 cm (#7135) and 9–15/15–22 cm (#7136-37/37, pooled) below a pink and white microbial mat; 4 °C). Sediment slurry was stored in a glass bottle for ~1 y under argon at 4 °C before BONCAT experiments were carried out.

Sediment sample #7142 was collected from Santa Monica Basin on 7 May 2013 (R/V *Western Flyer* MBARI Cruise 2013; dive 459; push-core 74; 33°47.34 N, 118°40.09 W; 863 m depth; sediment horizon 4–6 cm; 4 °C in situ temperature). The sediment was sealed under argon and stored at 4 °C. After 40 d of storage, the sediment was suspended in anaerobic natural bottom seawater from the site in an anaerobic chamber (3% H_2 in N_2) and aliquots were overpressured with 1.5 bar methane. The sediment was kept for 12 mo under 1.5 bar 100% methane in natural bottom seawater that was exchanged every 3 mo.

Sediment sample #5119 was collected from the Hydrate Ridge methane field during R/V *Atlantis* Cruise AT-18-10 on 1 September 2011 (44°40.02 N, 125°6.00 W; dive J2-593 E4A; push-core 36 through a yellow microbial mat; water depth 600 m; sediment horizon 9–12 cm). Sediment #5202 was collected during the same cruise on 3 September 2011, dive J2-593 E6B (44°40.02 N, 125°7.51 W; push-core 18 through a pink and white microbial mat; water depth 601 m; horizon 3–6 cm).

Carbonate #3439 was collected from atop an active seep at the Hydrate Ridge methane field during cruise AT-15-68 on 1 August 2010 (44°34.09 N, 125°9.14 W; dive AD4629; water depth 775 m). Carbonate sample #2450 was retrieved from sediment sample #2450 collected at Eel River Basin on 27 July 2005 (40°48.68 N, 124°36.73 W; dive T-864; push-core 49; horizon 0–2 cm; water depth 516 m).

Information on the geochemical characteristics of the sampling sites may be requested from the corresponding authors.

Setup of Incubations. All samples were kept in an ice bath at all times during handling. Artificial seawater (ASW) consisted of 10.9 g of $MgCl_2{\cdot}6H_2O$, 0.2 g of $NaHCO_3$, 0.76 g of KCl, 25.9 g of NaCl, 1.47 g of $CaCl_2{\cdot}2H_2O$, 3.98 g of Na_2SO_4, and 26.73 mg of NH_4Cl per liter of ddH_2O at pH 7.4. One milliliter of vitamin solution (see medium 141, https://www.dsmz.de) and 1 mL of trace element solution SL-10 (see https://www.dsmz.de) were also added. Before use, ASW was filtered through a 0.2-μm filter and N_2-bubbled for 10 min. ASW was kept on ice during handling.

Approximately 50 mL of wet sediment #3730 was resuspended in ASW, yielding a total volume of ∼130 mL; 20-mL aliquots of homogenized slurry were transferred into 160-mL serum bottles, and 30 mL of ASW was added. Bottles were sealed with rubber stoppers, and headspaces were flushed with either 100% methane or 100% N_2 for 5 min before being pressurized with 2 bar 100% methane or 100% N_2. Sediment was allowed to equilibrate overnight (∼18 h) at 4 °C in the dark; 0.2 μm-filtered HPG (Click Chemistry Tools) in ddH_2O was added to reach a final concentration of 50 μM. Control incubations without HPG were supplemented with sterile ddH_2O to reach equal volumes. All bottles were then flushed for 5 min, pressurized with 2 bar methane or N_2, and incubated in the dark at 4° C. In total, six incubations were performed: two times without HPG plus methane, two times with 50 μM HPG plus methane, and two times with 50 μM HPG plus N_2.

Approximately 100 mL of wet sediment #7135 was resuspended in ASW, yielding a total volume of ∼300 mL, and incubated for 15 d under 2 bar 100% methane. After this preincubation, 100 mL of ASW was added, and the slurry was homogenized. Under constant N_2 flushing, 35-mL aliquots were transferred into 160-mL serum bottles, bottles were sealed with rubber stoppers, and headspaces were flushed for 5 min with either 100% methane or 100% N_2, depending on incubation setup. HPG was added to reach a final concentration of either 5 μM or 50 μM. In addition, controls without HPG were supplemented with sterile ddH_2O to reach equal incubation volumes. Then, all bottles were flushed for 5 min, overpressurized with 2 bar methane or N_2, and incubated in the dark at 4 °C. In total, 12 incubations were performed: four times without HPG plus methane, four times with 50 μM HPG plus methane, two times with 50 μM HPG plus N_2, and two times with 5 μM HPG plus methane.

Twenty milliliters of wet sediment #7136-37 was resuspended in ASW, yielding a total volume of ∼50 mL. The slurry was bubbled with N_2 for 10 min (this was repeated after 5 d) before the bottle was incubated for 124 d under 2 bar N_2 (detectable but nonquantifiable amount of methane, 1–10 ppm). After this prestarving, 10-mL aliquots were transferred into 75-mL bottles. HPG was added to reach a final concentration of 50 μM, and 2 bar 100% methane or 100% N_2 (2 bar) was added to the headspace of two aliquots each. In addition, a control mesocosm was incubated without HPG under 100% methane (2 bar). All bottles were incubated at 4 °C in the dark for 31 d.

One-milliliter aliquots of wet sediment #7142 in 5 mL ASW containing 25 mM Hepes buffer (pH 7.5), 5 mM sulfide, and 5 mM DIC were resuspended in serum vials, which were then sealed with rubber stoppers (12.9 mL final volume). The headspace was flushed with $^{12}CH_4$ before 1.0 mL $^{13}CH_4$ (99% ^{13}C, containing 0.05 vol% $^{13}CO_2$ as impurity; Cambridge Isotope Laboratories) was added. After ∼5 d of preincubation, HPG was added to two of the four bottles to reach a concentration of 50 μM. In addition, one incubation was performed at 250 μM HPG for 25 d and later used for activity-based cell sorting.

Sampling for Molecular and Geochemical Analyses. Sampling of sediment microcosms was undertaken at incubation start as well as after 30 d, 73 d, and 114 d and 7 d, 14 d, 41 d, 56 d, and 171 d for sediment #3730 and #7135, respectively. Samples for molecular, cellular, and geochemical analyses were removed using sterile syringes while the incubation bottles were kept in an ice bath.

At each sampling point, 0.25 mL of sediment slurry was transferred into a sterile 1.5-mL tube and centrifuged at 16,100 g for 10 s at RT. The supernatant (SN) was removed, mixed in a 1:1 ratio with 0.5 M Zn-acetate solution, and stored for later sulfide analysis. The pellet was flash-frozen using liquid N_2 and stored at −20 °C for DNA extraction; 0.25 mL of sediment slurry was removed, centrifuged as described above, the SN was wasted, and the pellet was resuspended in a 1:1 mix of 1× PBS and absolute ethanol. Another 0.25 mL was processed in the same way but resuspended in 3% paraformaldehyde (PFA; Electron Microscopy Sciences) in 1× PBS and incubated for 1 h at RT for chemical fixation of cells. Afterward, biomass was harvested by centrifugation, the SN was wasted, and the pellet was washed with 1.5 mL of 1× PBS. Finally, sediment was centrifuged, the SN was wasted, and the biomass was resuspended in a 1:1 mix of 1× PBS and ethanol. All ethanol- or PFA-fixed samples were kept at −20 °C until further processing. After sampling (30 d, 73 d, and 114 d and 7 d, 14 d, 41 d, 56 d, and 171 d for #3730 and #7135, respectively), the headspace of bottles was flushed for 3 min with either methane or N_2 before the sediment was again incubated at 4 °C with 2 bar of either 100% methane or 100% N_2, depending on incubation setup. In addition, after 73 d (#3730) and 41 d and 130 d (#7135), ∼90% of artificial seawater overlying sediment was exchanged. The slurry volume and sediment-to-water ratio of all incubations was identical at all times for each sediment type (#3730 or #7135, respectively). When appropriate, newly added seawater was then supplemented with 5 μM or 50 μM (final) HPG.

Geochemical Analyses. Sulfide (H_2S plus HS^-) concentrations were determined via the cline assay (82). Samples were analyzed for statistically relevant differences via Student's t test. Differences were considered to be significant at $P \leq 0.05$. Methane oxidation rates for sediment #7142 were determined as described by Scheller et al. (29) by measuring the formation of DIC from $^{13}CH_4$ over time. Succinctly, 0.25 mL of ASW overlying settled sediment was removed and centrifuged (16,000 × g for 5 min). The SN was transferred into 0.6-mL tubes, flash frozen in N_2, and stored at −20 °C until further processing; 150 μL of thawed SN was then added to He-flushed vials containing 100 μL H_3PO_4 (85%). The resulting CO_2 was analyzed for isotopic enrichment on a GC-IR-MS GasBench II (Thermo Scientific).

Extraction of Microbial Aggregates. To separate microbial aggregations and individual cells from sediment particles, 50 μL of sediment slurry was resuspended in 450 μL of 1× PBS in a 2-mL tube. This solution was chilled in an ice bath for 15 min before being sonicated three times for 10 s at 3–6 W output using a Branson Sonifier 150 (Branson Ultrasonics Corporation). Between pulsing intervals, the sample was allowed to cool for 10–30 s. After sonication, the sample was applied on top of 500 μL Percoll (Sigma-Aldrich) and centrifuged at 16,100 × g for 20 min at 4 °C. To remove Percoll particles and the majority of individual planktonic cells, the entire SN was resuspended in 15 mL 1× PBS and filtered through a 3-μm TSTP white polycarbonate filter (EMD Millipore) using a filter tower at ∼0.3 bar under pressure. Each filter was washed with a total volume of 50 mL 1× PBS without letting the filter run dry. Then, particles and biomass that had been retained by the filter were transferred into a 2-mL tube using 1× PBS by repeatedly and vigorously pipetting up and down using a 1-mL

pipette. DAPI staining confirmed that this protocol leads to the near-complete transfer of microbial aggregates from the filter into solution (99–100% of DAPI-stained consortia), without selecting for or against a particular type of consortium morphology or size. After transfer into 1× PBS, biomass was harvested via centrifugation (16,100 × *g*, RT), resuspended in either 1× PBS (for nonfixed BONCAT analyses) or a 1:1 ratio of 1× PBS and ethanol (fixed biomass), and stored at either 4 °C (nonfixed) or −20 °C (fixed).

List of Oligonucleotide Probes for FISH and CARD-FISH. In FISH experiments, monolabeled and dual-labeled (indicated with ** in the list below) probes were used in different combinations (Dataset S2): Arch915, specific for most members of the domain *Archaea* (83), used at 35% formamide (FA); EUB338, -II, and -III (also known as EUB338mix), which together cover most of the known bacterial diversity (72, 84), used at 35% FA; EUB338-III, specific for most members of the *Verrucomicrobia* (72), used at 35% FA, in combination with EUB338-I and -II as competitor probes; Delta495a** together with its competitor probe, specific for most delta-proteobacteria (85), used at 35% FA; Gam42a, together with its competitor, specific for most gamma-proteobacteria (86), used at 35% FA; Ver47**, specific for *Verrucomicrobia* (73) together with its helper probe H64 (74) at 15% FA; and ANME-1-350 (26) (40% FA), ANME-2-932 (also known as EelMS-932; 26) (40% FA), ANME-2a-647 (50% FA) (37), and ANME-2c-760** (60% FA) (37), specific for different subpopulations of ANME. In addition, a new probe, ANME-2b-729, was designed, which detects >93% of all ANME-2b-affiliated 16S rRNA sequences in online and laboratory internal databases. The new probe has at least two mismatches to all other archaeal or bacterial 16S rRNA sequences [tested using probeCheck (87)]. After careful evaluation, ANME-2b-729 was used at 20% formamide concentration. Note that this probe has a one-nucleotide overlap with probe ANME-2-712 (37) and should thus not be used in conjunction with this probe. Hybridizations without probe addition or probe NONEUB388 (88) were used as negative controls.

With the exception of probe ANME-2a-647, which was used at 40% FA (rather than 50%), all probes used in CARD-FISH were used at the same FA concentrations as in FISH experiments. For CARD-FISH, hybridizations with probe NONEUB388 were used as negative controls.

Multiple Displacement Amplification. Individual sorted consortia were lysed and subjected to WGA as previously described (65) with the following modifications: WGA was performed with a REPLI-g Single Cell Kit (Qiagen) with a scaled-down reaction volume of 2 μL and DNA dye SYTO-13 added at 1× for real-time tracking. The cell lysis procedure followed a recently described protocol (65), which was modified by lysozyme treatment. This step included a 15-min RT incubation with 300 nL of 50 U/μL ReadyLyse lysozyme (Epicentre R1810M), which was followed by the addition of 50 nL concentrated DLB buffer (65). Lysis and stop reagents were UV-treated as described (65), and the Master Mix was used as obtained from the manufacturer (Qiagen). The amplification reaction was incubated for 6 h at 30 °C.

The 16S rRNA Gene Tag Sequencing. Sediment DNA was extracted using the Power Soil DNA Isolation Kit according to the manufacturer's protocol (MoBio), and diluted DNA from genome-amplified sorted consortia was used directly. The V4 region of the 16S rRNA gene was amplified from each extract using archaeal and bacterial primers 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) (67, 68). Sediment samples were amplified in duplicate. The nonbarcoded primers were used with Q5 Hot Start High-Fidelity 2× Master Mix (New England Biolabs) according to the manufacturer's directions, using annealing conditions of 54 °C for 30 cycles and 58 °C for 32 cycles for sediments and MDAs, respectively. Duplicates of sediment sample amplifications were then pooled. The barcoded 806R primer (CAAGCAGAAGACGGCATACGAGAT XXXXXXXXXXXX AGTCAGTCAG CC GGACTACHVGGGTWTCTAAT) was paired with 515F in a reconditioning reaction (same conditions as above except for five cycles of PCR) to barcode the PCR products. Samples were mixed together in equimolar amounts and purified in bulk through a Qiagen PCR purification kit before submission to Laragen for analysis on an Illumnia MiSeq platform. The resulting paired-end sequence data, 2× 250 bp, was demultiplexed, and sequences with >1 bp mismatch on the 12-bp barcode were removed. The resulting sequences were passed through Illumina's MiSeq Recorder software to assign quality scores to each base call and remove adapter, barcode, and primer sequence.

Analysis of 16S rRNA Gene Tag Sequences. Sequence data were processed in QIIME (Quantitative Insights Into Microbial Ecology) version 1.8.0 (89) following a recently published protocol (90). Raw sequence pairs were joined and quality-trimmed using the default parameters in QIIME. Sequences were clustered into de novo OTUs with 99% similarity using UCLUST open reference clustering protocol (91). Then, the most abundant sequence was chosen as representative for each de novo OTU (92). Taxonomic identification for each representative sequence was assigned using the SILVA-115 database (93, 94) clustered at 99% similarity. This SILVA database had been appended with 1,197 in-house high-quality, methane-seep derived bacterial and archaeal clones. Any sequences with pintail values >75 were removed. The modified SILVA database is available upon request from the corresponding authors. OTUs were then filtered to remove singletons from the combined MDA dataset. A threshold filter was used to remove any OTU that occurred below 0.01% of the entire combined sediment samples dataset. Known contaminants in PCR reagents as determined by the analysis of negative and positive controls run with each MiSeq set were also removed (95). For the sediment samples, the sequence data were rarified by random subsampling to equal the sample with the least amount of sequence data, resulting in 12,115 and 3,707 sequences per sample for sediments #3730 and #7135, respectively. Tables of both absolute and relative abundances were generated at the family level for each sample. For statistical and similarity percentage analyses [Nonmetric Multidimensional Scaling (NMDS), Analysis of Similarity (ANOSIM), and Similarity Percentage], family-level abundance tables were square-root-transformed before generation of Bray Curtis similarity matrices and analyzed using Primer-E software (www.primer-e.com). Differences were considered to be significant at $P \leq 0.05$.

Phylogenetic Analysis. The 16S rRNA gene tag sequences from each consortium as well as closely related sequences from online databases (identified via the BLAST algorithm of National Center for Biotechnology Information) were imported into and analyzed via the ARB software package (96). Sequences were automatically aligned to reference sequences of all ANME subpopulations as well as relevant bacterial clades contained within the SILVA-115 database that had been amended with 1,197 in-house seep derived clones. Sequences from cultured representatives of archaeal phyla *Thaumarchaeota* and *Euryarchaeota* were used as outgroup for reconstruction of the archaeal tree. Members of the *Planctomycetes* where chosen as outgroup for the bacterial tree. Both alignments were manually curated, and termini filters were created. During the phylogenetic reconstruction of archaeal (all >1,100 nt in length) and bacterial (all >1,000 nt in length) sequences, 958 and 1,255 positions were considered, respectively. Phylogenies were modeled using a Randomized Axelerated Maximum Likelihood (RaxML) algorithm, and short tag sequences were individually added to the resulting tree using the parsimony interactive tool in ARB without changing the overall topology of the tree. In addition, maximum parsimony (100× replications) and Neighbor Joining (1,000× replications) trees were calculated, and bootstrap values were projected onto the RaxML tree.

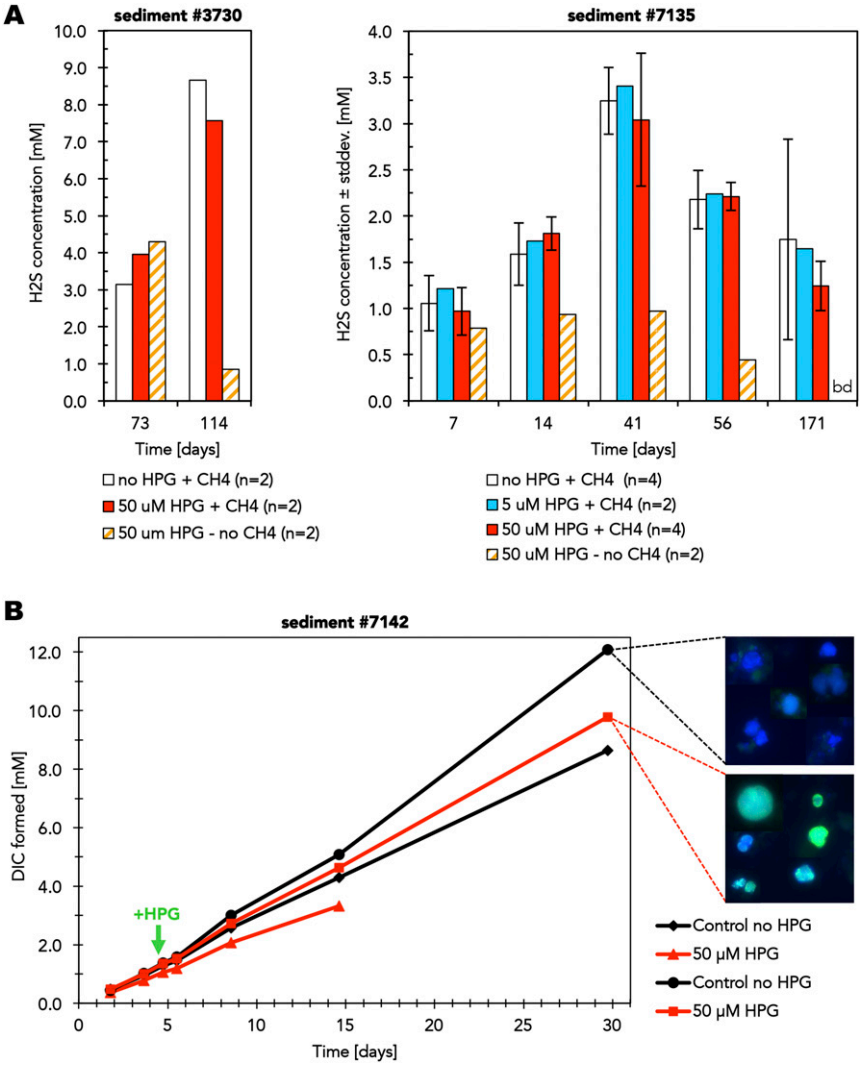

Fig. S1. Sediment sulfide production rates and methane oxidation rates are not affected by the presence of HPG. (*A*) Sulfide [H_2S + HS^-] levels cannot be directly compared between different time points, because seawater and headspace of incubations were refreshed at regular intervals. In contrast to HPG, methane has a statistically significant effect on sulfide production ($P = 0.0183$ and $P = 0.0063$ for #3730 and #7135 after 114 d and 56 d of incubation, respectively). Sulfide levels in sediment #7135 samples #09 and #10 (both without methane) were below detection limit (bd) after 171 d of incubation. (*B*) Sediment methane oxidation rates are not affected by the presence of HPG over a course of up to 25 d. Four separate aliquots of sediment #7142 were incubated in the absence of HPG for ∼5 d, after which 50 µM HPG (final concentration) was added to two incubations. Note that one of the HPG-containing incubations exhibited low rates of AOM from the start of the experiment on. Because of this, the experiment was stopped after ∼10 d of incubation. Compilations of representative AOM consortia from the endpoints of two incubations are shown on the right. Green fluorescence indicates that cells have been translationally active during time of incubation. DAPI-stained DNA is in blue. Methane oxidation rates were measured as ^{13}C DIC formed from $^{13}CH_4$. Sampling on day 5 was performed immediately after addition of HPG.

A

3730			HPG	NO	NO	NO	50	50	50	50
			CH4	Y	Y	Y	Y	Y	NO	NO
			Time	0	114	114	114	114	114	114
HPG	CH4	Time	Sample	#0	#1	#2	#3	#4	#5	#6
NO	Y	0	#0							
NO	Y	114	#1	91.7						
NO	Y	114	#2	92.0	94.5					
50	Y	114	#3	90.8	94.3	94.2				
50	Y	114	#4	91.0	94.4	93.1	94.8			
50	NO	114	#5	88.4	92.6	91.8	93.2	92.2		
50	NO	114	#6	91.1	93.6	92.8	93.6	92.7	92.8	

NO no HPG addition
5 5 μM HPG
50 50 μM HPG
HPG

NO 30 psi N2
Y 30 psi CH4
CH4

3730: 12,115 sequences
7135: 3,707 sequences

Bray Curtis similarity [%]
85.7 94.8

0 start
114 114 days
41 41 days
171 171 days
Time

B

7135			HPG	NO	NO	NO	NO	NO	NO	NO	NO	50	50	50	50	50	50	50	50	50	50	50	50	5	5	5	5
			CH4	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	NO	NO	NO	NO	Y	Y	Y	Y
			Time	41	41	41	41	171	171	171	171	41	41	41	41	171	171	171	171	41	41	171	171	41	41	171	171
HPG	CH4	Time	Sample	#01	#02	#03	#04	#01	#02	#03	#04	#05	#06	#07	#08	#05	#06	#07	#08	#09	#10	#09	#10	#11	#12	#11	#12
NO	Y	41	#01																								
NO	Y	41	#02	92.7																							
NO	Y	41	#03	92.9	92.3																						
NO	Y	41	#04	91.2	91.9	91.8																					
NO	Y	171	#01	87.9	88.2	87.7	88.5																				
NO	Y	171	#02	87.3	87.8	89.0	87.6	86.6																			
NO	Y	171	#03	87.6	88.2	87.7	88.1	91.2	88.9																		
NO	Y	171	#04	87.1	88.2	88.1	87.3	89.9	87.3	90.1																	
50	Y	41	#05	91.8	91.4	92.3	90.7	87.8	88.3	87.2	87.1																
50	Y	41	#06	92.1	92.2	93.7	90.9	87.6	89.2	87.7	87.9	90.7															
50	Y	41	#07	93.4	92.4	92.6	92.3	87.9	87.0	87.6	87.5	91.3	92.8														
50	Y	41	#08	90.9	92.7	92.5	91.7	88.8	88.8	88.5	88.7	91.4	91.5	91.6													
50	Y	171	#05	89.3	88.9	89.2	88.6	89.9	88.0	89.6	88.4	88.5	88.7	89.4	88.4												
50	Y	171	#06	88.8	89.0	89.3	88.5	90.3	87.3	90.8	90.0	87.5	88.8	88.5	88.9	90.7											
50	Y	171	#07	90.0	91.0	90.4	89.4	90.3	87.5	89.7	89.8	89.5	89.5	89.2	89.9	89.7	89.8										
50	Y	171	#08	89.9	90.6	90.5	89.6	89.2	89.8	89.1	88.8	90.9	90.3	90.5	90.4	90.9	89.1	90.1									
50	NO	41	#09	89.9	89.3	90.2	90.4	86.7	85.7	88.6	86.7	89.0	87.9	88.7	90.1	87.4	88.3	89.9	87.4								
50	NO	41	#10	91.3	91.8	92.1	89.8	86.8	89.1	86.4	86.2	90.8	91.6	91.0	91.3	88.1	87.8	89.4	90.7	89.2							
50	NO	171	#09	89.3	89.9	88.8	88.1	89.4	87.7	88.9	88.4	88.1	88.2	88.6	88.3	90.5	89.0	90.5	90.1	88.4	88.5						
50	NO	171	#10	89.2	88.9	89.3	88.2	89.4	86.8	88.8	88.9	88.0	87.7	88.7	89.0	89.0	89.5	91.2	88.5	89.4	88.3	90.4					
5	Y	41	#11	91.0	92.1	91.9	90.8	88.1	88.5	88.2	86.5	90.2	90.6	90.8	91.3	87.5	88.7	89.5	89.1	89.8	90.8	88.5	88.4				
5	Y	41	#12	92.6	92.8	93.2	92.2	88.0	88.0	88.3	87.0	92.2	91.2	92.2	91.8	89.6	88.8	90.6	89.7	91.1	91.4	88.7	89.0	91.8			
5	Y	171	#11	88.5	88.5	90.4	89.9	86.5	87.5	86.6	86.0	89.4	89.2	88.7	88.8	87.9	86.7	90.1	89.8	88.7	88.4	86.8	87.1	88.3	89.3		
5	Y	171	#12	89.4	90.1	89.6	89.6	90.9	87.9	91.2	90.7	89.1	89.3	89.3	90.2	89.0	91.1	90.1	90.2	89.1	89.6	89.6	89.5	89.3	89.2	87.2	

Fig. S2. Bray Curtis similarity indexes of the microbial communities of sediments #3730 (*A*) and #7135 (*B*) after incubation in the absence or presence of HPG for 114 d and 171 d, respectively. For statistical analyses, see Figs. S3 and S4.

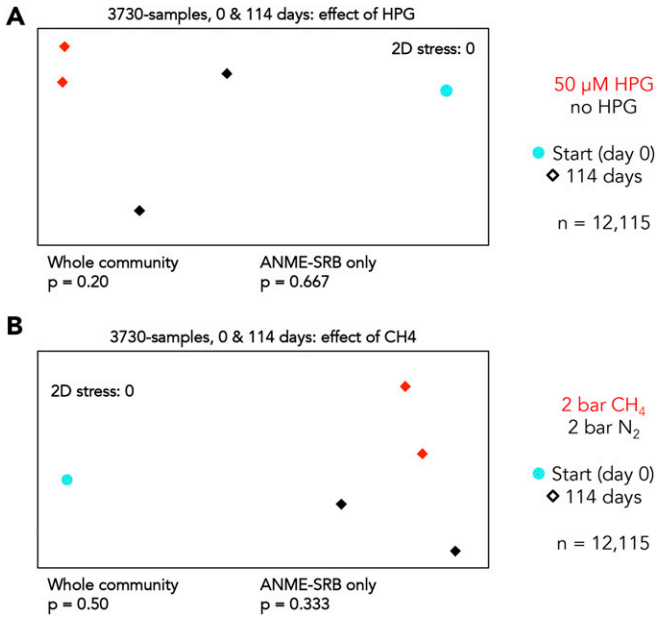

Fig. S3. NMDS ordinations of 16S rRNA gene tag sequences demonstrated that neither HPG (*A*) nor methane (*B*) have a statistically relevant effect on the microbial community of sediment #3730 after 114 d of incubation. Stress values of NMDS ordinations and *P* values of concomitant Anosim analyses for whole communities and ANME–SRB-related lineages specifically are shown next to the plots; n, number of sequences per sample. The more similar the microbial communities from two samples are, the closer they lie together. Differences between samples were considered to be significant at $P \leq 0.05$.

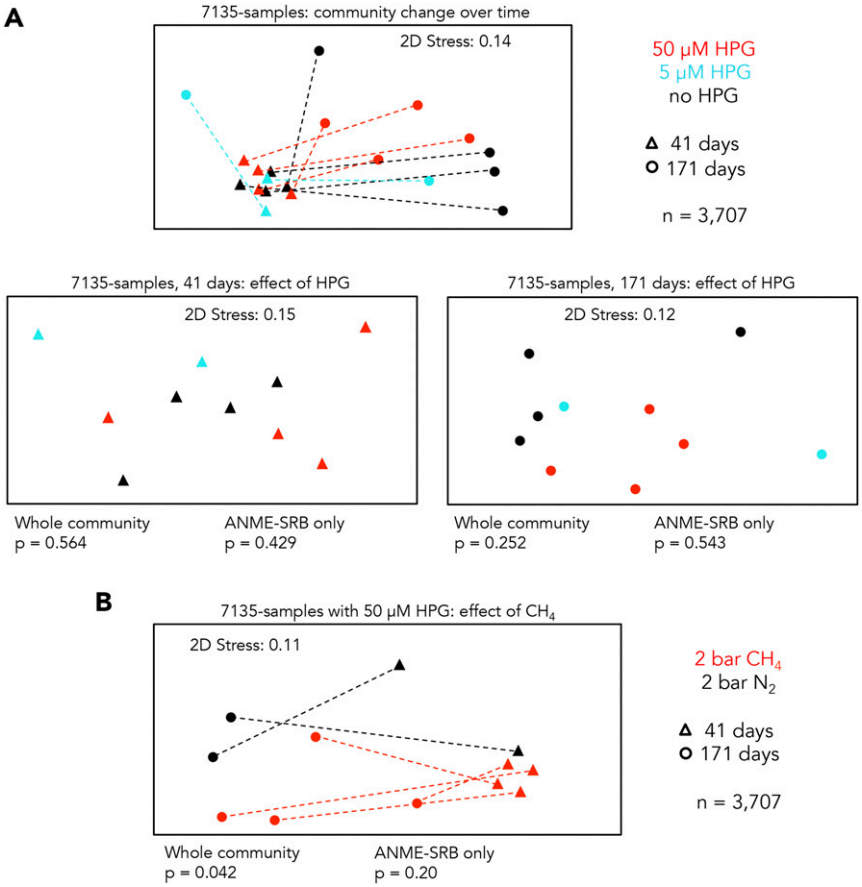

Fig. S4. (*A*) NMDS ordinations of 16S rRNA gene tag sequences demonstrated that HPG does not have a statistically relevant effect on the microbial community of sediment #7135 after 171 d of incubation. (*B*) The absence of methane, on the other hand, has a clear effect on the community composition. Stress values of NMDS ordinations and *P* values of concomitant Anosim analyses for whole communities and ANME–SRB-related lineages specifically are shown next to the plots. Dotted lines connect the individual sampling points for each incubation bottle; n, number of sequences per sample. The more similar the microbial communities from two samples are, the closer they lie together. Differences between samples were considered to be significant at $P \leq 0.05$.

A

3730	HPG	NO	NO	NO	50	50	50	50
	CH4	Y	Y	Y	Y	Y	NO	NO
	TIME	0	114	114	114	114	114	114
	Sample	#0	#1	#2	#3	#4	#5	#6
ANME-1;Other		0.7	0.3	0.4	0.4	0.4	0.2	0.3
ANME-1;ANME-1a		20.3	14.6	15.0	12.8	15.7	9.8	12.4
ANME-1;ANME-1b		1.3	1.2	1.5	1.5	0.9	1.0	0.9
Methanosarcinales;ANME-2a-2b		9.7	6.0	7.9	6.1	5.2	4.5	6.5
Methanosarcinales;ANME-2c		6.1	5.8	5.0	6.9	7.0	4.2	5.6
Methanosarcinales;Methanosaetaceae		0.0	0.0	0.0	0.1	0.0	0.0	0.0
Desulfarculales;Desulfarculaceae		0.4	0.9	0.6	0.9	0.8	1.0	0.8
Desulfobacterales;Desulfobacteraceae		11.9	12.5	10.7	10.8	11.6	14.4	16.8
Desulfobacterales;Desulfobulbaceae		3.3	4.4	5.0	4.5	4.3	5.1	4.8
Desulfuromonadales;Desulfuromonadaceae		0.2	0.2	0.5	0.3	0.3	0.5	0.2
Other archaea		0.9	1.1	1.0	1.0	1.2	1.4	0.7
Other bacteria		45.1	52.9	52.3	54.7	52.6	58.0	50.9

3730: 12,115 sequences

7135: 3,707 sequences

Relative abundance of taxon [%]

low — high

HPG
- NO no HPG addition
- 5 5 μM HPG
- 50 50 μM HPG

CH4
- NO 30 psi N2
- Y 30 psi CH4

Time
- 0 start
- 114 114 days
- 41 41 days
- 171 171 days

B

7135	HPG	NO	NO	NO	NO	NO	NO	NO	NO	50	50	50	50	50	50	50	50	50	50	50	50	5	5	5	5
	CH4	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	NO	NO	NO	NO	Y	Y	Y	Y
	Time	41	41	41	41	171	171	171	171	41	41	41	41	171	171	171	171	41	41	171	171	41	41	171	171
	Sample	#01	#02	#03	#04	#01	#02	#03	#04	#05	#06	#07	#08	#05	#06	#07	#08	#09	#10	#09	#10	#11	#12	#11	#12
ANME-1;_ANME-1a		2.3	1.7	1.9	4.2	3.2	1.3	2.1	5.4	3.3	2.8	3.4	1.8	3.3	2.1	1.5	3.6	0.7	1.6	1.5	1.2	1.2	1.9	2.6	2.4
ANME-1;_ANME-1b		0.3	0.2	0.3	0.2	0.1	0.0	0.1	0.1	0.3	0.1	0.2	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.2	0.2
Methanomicrobia;D-C06;__f		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	1.1	0.0	0.0	0.1
Methanosarcinales;ANME-2a-2b		0.7	0.7	0.9	0.7	1.3	0.5	1.5	1.0	0.4	0.8	0.5	0.9	0.6	1.4	0.8	0.5	0.3	0.2	0.3	0.4	0.7	0.5	0.7	1.1
Methanosarcinales;ANME-2c		1.1	1.8	1.4	1.6	4.9	7.8	7.5	3.4	1.5	2.0	1.2	1.7	3.0	4.8	2.4	3.3	0.4	0.6	2.1	1.3	1.1	0.8	1.8	3.5
Desulfarculales;Desulfarculaceae		1.1	0.9	1.0	0.9	0.8	1.0	0.9	0.9	1.0	1.1	0.8	0.9	0.9	1.0	1.2	1.2	1.5	1.0	0.9	1.2	0.9	1.1	2.0	0.9
Desulfobacterales;Desulfobacteraceae		3.3	3.5	3.4	3.4	4.4	6.0	5.9	5.8	3.8	3.2	3.3	3.4	4.7	4.3	5.6	4.3	4.9	4.4	4.5	3.5	3.6	3.6	6.8	5.2
Desulfobacterales;Desulfobulbaceae		3.2	2.8	2.8	2.8	2.8	2.6	2.3	2.3	2.5	3.0	3.3	2.6	2.2	3.0	2.9	2.9	2.4	3.3	2.1	2.0	2.6	2.6	3.2	2.9
Other archaea		13.6	14.1	12.8	12.8	14.2	9.1	11.9	12.6	13.2	11.3	12.8	13.7	12.1	12.3	13.3	12.2	12.8	11.7	17.3	17.1	12.1	12.0	9.6	12.4
Other bacteria		74.3	74.2	75.2	73.3	68.2	71.5	67.5	68.2	73.9	75.5	74.2	74.6	72.8	70.8	72.0	71.7	76.6	76.7	71.1	72.8	76.2	77.3	73.1	71.3

Fig. S5. Relative abundances of ANME-related archaea and SRB in sediment #3730 (*A*) or #7135 (*B*) over time. Abundance-based color-coding indicates relative abundance of taxa within a sample. Sequences summarized as "other archaea," which are slightly enriched in #7135 samples incubated in the absence of methane, were related to rRNA genes from Marine Benthic Group D/Deep Sea Hydrothermal Vent Euryarchaeotal Group 1 as well as the Marine Hydrothermal Vent Group and Miscellaneous Euryarchaeotal Group. The physiology of these uncultured, yet environmentally widely distributed, clades is currently unknown. However, recent genomic data suggest an implication of members of Marine Benthic Group D in the degradation of detrital proteins in marine sediments.

Fig. S6. Extended versions of the phylogenetic trees shown in Fig. 5. Green and white colored boxes in trees show support ≥90% and ≥70%, respectively. Values <70% are not given. Boxes in left and right trees indicate maximum parsimony (100×) and neighbor joining (1,000×) values, respectively. Numbers in boxes give the number of sequences within a group. The 16S rRNA gene tag sequences were added after tree construction without changing overall tree topology. Dotted lines indicate individual partnerships. The scales bars represent 10% estimated sequence divergence.

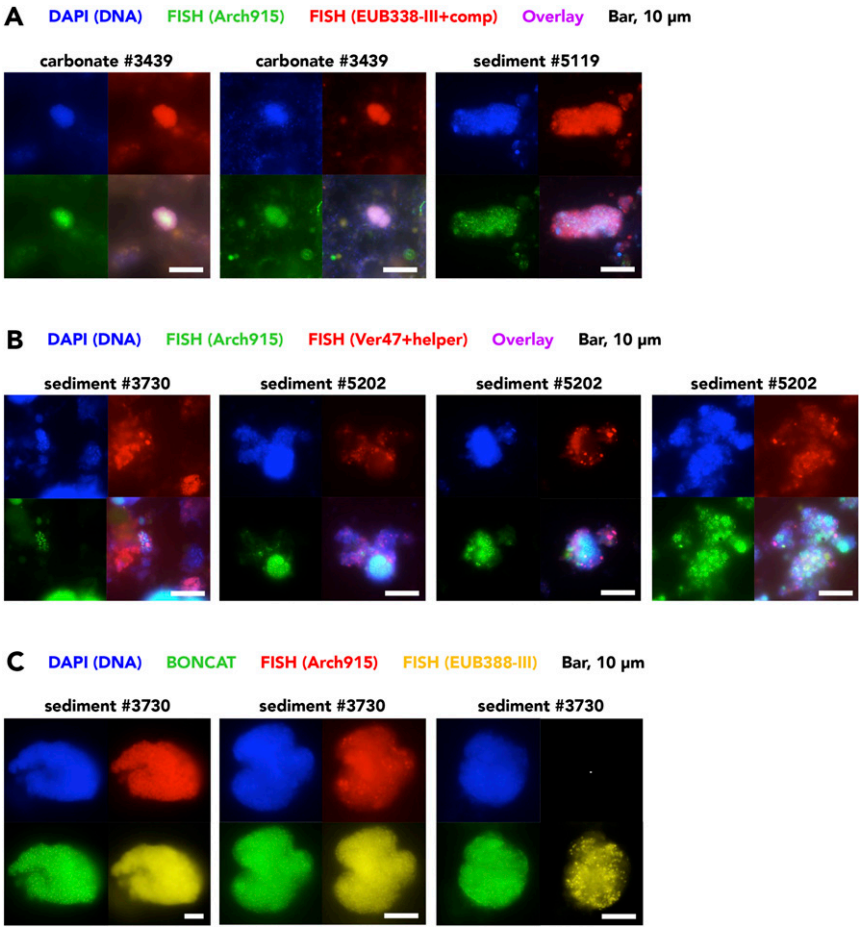

Fig. S7. Representative images of our FISH-based screening of methane seep sediment and carbonate samples for associations of *Archaea* and members of the *Verrucomicrobia*. *A–C* show results of screenings using different combinations of *Archaea*- and *Verrucomicrobia*-targeted FISH probes. For references of FISH probes and detailed aggregate-counts for specific samples refer to *SI Text* and Dataset S3. Arch915, a probe specific for archaea; EUB338-III, a probe specific for most *Verrucomicrobia*; comp, unlabeled competitor probes EUB338-I and II; Ver47, a *Verrucomicrobia*-specific probe, used together with an unlabeled helper probe (helper).

Dataset S1. Incubation setup and sampling details

Dataset S1

-, not determined; d, days of incubation; Y, yes; 5/50, 5/50 μM HPG.

Dataset S2. Details on BONCAT-FISH and FISH experiments summarized in Fig. 2

Dataset S2

For each probe set listed, the first probe had been labeled with a Cy3 dye, the second with a Cy5 dye. >, transfer to; comp, competitor probe; d, days of incubation; help, helper probe; na, not applicable; nd, not determined.

Dataset S3. Details on *Verrucomicrobia* FISH experiments

Dataset S3

For each probe set listed, the first probe had been labeled with a Cy3 dye, the second with a Cy5 dye. comp, competitor probe; cons, consortium/consortia; help, helper probe.