Supplementary Material "Thiotrophic mats at polar cold seeps"

Supplement to: 2 Materials and Methods

Cell counts (AODC)

Sediment sections were fixed onboard in 4% formaldehyde/seawater and stored at 4°C. Total single cells were counted after staining with acridine orange according to a modified protocol (Boetius and Lochte, 1996) of Meyer-Reil (Meyer-Reil, 1983). For each sample, at least 2 replicate filters and a minimum of 30 random grids per filter were counted.

Clone library construction, sequencing and phylogenetic analyses (modified after Girnth *et al.*, 2011 and Grünke *et al.*, 2011)

Universal bacterial primers GM3F and GM4R (5'-AGAGTTTGATCMTGGC-3' and 5'-TACCTTGTTACGACTT-3'; Muyzer *et al.*, 1995) were used for amplification of nearly full length 16S rRNA gene sequences. Replicate amplification reactions conducted at the institute's laboratories were set up as follows: 0.5 µM of each primer, 250 µM of each dNTP (Roche, Mannheim, Germany), 0.3 mg mL^{-1} BSA (Sigma-Aldrich Biochemie GmbH, Hamburg, Germany), 1 × Master*Taq* Buffer with 1.5 mM Mg^{2+} (5Prime, Hamburg, Germany), 0.625 U Master*Taq* (5Prime) and 2 µL template in a total volume of 25 µL. Replicate PCRs onboard, as well as for the fourth position of the transect at the institute's laboratories, were set up with 25 µL of PCR Master Mix (Promega Corporation, Madison, WI) and 0.5 µM of each primer in a final volume of 50 µL. PCR conditions for all samples included an initial denaturation step of 15 min at 95°C, 30 cycles of 95°C/1 min, 42°C/1 min and 72°C/3 min, prior to a final

elongation at 72°C for 10 min. Onboard conducted PCRs were subsequently stored at -20°C until further processing in the institute's laboratories.

In order to circumvent known difficulties in the amplification of *Thiomargarita*-related 16S rRNA gene sequences (Schulz, 2006), here a specialized approach was used: Three mat samples were subjected to DNA extraction with the UltraClean Soil DNA Isolation Kit (Mo Bio, Carlsbad, CA). After resuspending each mat sample in 100 µL of the provided buffer, the sample-buffer suspensions were transferred into bead-tubes and the DNA was extracted following the manufacturer´s guide. For partial 16S rRNA gene PCR the primers VSO233F (5'-CCT ATG CCG GAT TAG CTT G-3'; Salman *et al.*, 2011) and VSO673R (5'-CGC TTC CCT CTA CTG TAC-3'; Kalanetra *et al.*, 2004) were used. Replicate PCRs of 25 µL included 1 µM of each primer, 200 µM dNTPs (Invitrogen, Darmstadt, Germany), 1 × Fermentas High Fidelity PCR enzyme mix reaction buffer with 1.5 mM $MgCl_2$ (Fermentas, St. Leon-Rot, Germany), 1.25 U Fermentas High Fidelity enzyme mix (Fermentas) and 2 µL DNA. The PCR steps were performed according to (Girnth *et al.*, 2011).

For all samples, replicate PCR products were pooled and purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany), ligated into pGEM-T Easy (Promega Corporation) and transformed into One Shot TOP10 Chemically Competent *Escherichia coli* (Invitrogen) following the given specifications. After partial sequencing with vector primers, representative clones associated with the cluster of giant sulfur bacteria (*Beggiatoaceae*), as identified with the ARB software package (Ludwig *et al.*, 2004), were selected for plasmid preparation and nearly full-length sequencing (assembly with Sequencher 4.6 software; Gene Codes Corporation, Ann Arbor, MI). Examination for chimeric signals was done by using either the Mallard program (partial sequences; Ashelford *et al.*, 2006), or the Pintail

program (assembled full lengths; Ashelford *et al.*, 2005). Sequences with genuine chimeric signals were excluded from further analyses. Finally, representative 16S rRNA gene sequences were used for tree reconstruction in the ARB software package (Ludwig *et al.*, 2004).

Menez Gwen filaments

In sense of expanding our knowledge on the global distribution of members of the family *Beggiatoaceae*, filamentous sulfur bacteria were sampled with the help of the ROV *Quest 4000* (Marum, University Bremen) at the mid-Atlantic hot vent site Menez Gwen during cruise 82/3 on RV *Meteor* in 2010. A single attached filament was directly transferred to the PCR reaction tube containing a 25 µL reaction mix including the primers VSO233F and ITSReub, amplified according to (Salman *et al*., 2011) and directly sequenced. For phylogenetic analyses, only the 16S rRNA gene region was considered.

Automated Ribosomal Intergenic Spacer Analysis (modified after Ramette, 2009)

To investigate bacterial community patterns associated with the two different types of mats, ARISA was conducted with several samples representing the upper layer of sediment (up to 2.5 cm depth). DNA from approx. 1 g of sediment was extracted with the UltraClean Soil DNA Isolation Kit (Mo Bio) by following the manufacturer's specifications for maximum yield. Final elution of the DNA was in 100 µL 1 × TE buffer (Promega Corporation). If a DNA concentration below 5 ng μL^{-1} was determined (NanoQuant infinite M200; Tecan, Crailsheim, Germany), the respective sample was further subjected to a modified DNA precipitation protocol (GlycoBlue;

Ambion Inc., Austin, TX) and finally eluted in 50 µL 1 × TE buffer to increase the yield if possible.

ARISA-PCR was conducted with primers ITSF (FAM-5'-GTC GTA ACA AGG TAG CCG TA-3'; Cardinale *et al.*, 2004) and ITSReub (5'-GCC AAG GCA TCC ACC-3'; Cardinale *et al.*, 2004), and by adding DNA amounts of 19-25 ng to a total volume of 50 µL. Final concentrations of PCR ingredients were 0.4 µM of each primer (Biomers), 250 µM of each dNTP (peqGOLD Kit; Peqlab, Erlangen, Germany), 0.1 mg mL^{-1} BSA (Sigma-Aldrich Biochemie GmbH), 1 × Buffer S with 1.5 mM $MgCl_2$ (Peqlab), 1.0 mM extra $MgCl_2$ (Peqlab) and 2.5 U peqGOLD *Taq*-DNA-Polymerase (Peqlab). Cycling conditions included a first denaturation step at 94°C for 3 min, followed by 30 cycles of 94°C/45 s, 55°C/45 s and 72°C/90 s before a final elongation step at 72°C for 5 min. Three PCR replicates were set up for each sample (except MSM16/2_863-1: only two replicates). After sephadex purification, 98-107 ng of each PCR product was mixed with 0.5 µL MapMarker *1000* (BioVentures, Inc., Murfreesboro, TN) and 14.5 µL Hi-Di Formamide (Applied Biosystems, Darmstadt, Germany), denatured for 3 min at 95°C before cooling on ice for 5 min and subsequent capillary electrophoresis (ABI Prism 3130x Genetic Analyzer; Applied Biosystems). Quality assessment of the ARISA profiles as well as subsequent binning were conducted as described by (Ramette, 2009).

Geochemical analyses

Elemental sulfur concentrations were measured by high-performance liquid chromatography (Zopfi *et al.*, 2004) from sediment sections fixed in ZnAc and extracted for 12 h in methanol.

Supplement to: 3 Results and Discussion

Microscopic identification of mat-forming thiotrophs

The identification of the different sulfide oxidizers based on their morphology was according to previously described standards: *Beggiatoa* spp. (Teske and Nelson, 2006), "*Candidatus* Marithrix spp." (Kalanetra *et al.*, 2004; Grünke *et al.*, 2011; nomenclature from Salman *et al.*, 2011), *Thiomargarita* spp. (Schulz, 2006; Salman *et al.*, 2011), *Thiobacterium* spp. (Kuenen, 2005; La Rivière and Schmidt, 2006) and *Arcobacter* spp. (Sievert *et al.*, 2007).

All mats

Total single cell numbers were usually highest in the upper 2 cm of sediment (Storegga: 4.5-13.8 × 10^9 cells cm^{-3}; Nyegga: 1.5-16.6 × 10^9 cells cm^{-3}; HMMV gray mats: 1.4-3.1 × 10^9 cells cm^{-3}; HMMV white mats: 0.2-2.6 × 10^9 cells cm^{-3}), and were mostly elevated compared to cell numbers counted in non-seep reference sediment (0.7-2.1 × 10^9 cells cm^{-3}). CARD-FISH was conducted with probes targeting the archaeal (anaerobic methanotrophs; ANME) and bacterial (Deltaproteobacteria of the genera *Desulforsarcina*/*Desulfococcus* or *Desulfobulbus*) partners in the anaerobic oxidation of methane (AOM), the sulfide-delivering process at the seeps (for probes see Supplementary Table 3). HMMV white mats were previously found to be dominated by ANME3 (Niemann *et al.*, 2006; Lösekann *et al.*, 2007). In this study, we also detected some ANME1 in HMMV white mats (Table 2). While HMMV white and gray mats resembled each other in the composition of the methanotrophs, Storegga and Nyegga gray mats were characterized by a dominance of ANME1, or ANME1 and ANME2, respectively (Table 2). Recently published findings for a Nyegga white

mat even suggested a niche-specific stratification of ANME1 and ANME2 that may be the result of an increased tolerance towards higher sulfide concentrations (Roalkvam *et al.*, 2011). Sulfate reducers of the *Desulfosarcina*/*Desulfococcus* cluster comprised <1% of the bacterial single cells in Storegga and Nyegga mats (in the upper 5 cm of sediment) and were not detected below HMMV mats. Elevated concentrations of elemental sulfur, as compared to non-sulfidic reference sediment, were detected in Storegga and Nyegga surface sediments (1.2-2.9 µmol cm^{-3}) and had previously also been reported for both gray and white HMMV mats (Lichtschlag *et al.*, 2010).

Supplementary information to Table 2

Microprofiles: For the Nyegga mats, averages based on 1 (reference sulfide flux) or 2 replicates (mat DOU and sulfide flux, reference DOU) measured in the same spot are presented. Values given for the Storegga mats are based on replicate profiles (n) recorded in 2 different push cores: n = 3 (DOU) and n = 2 (sulfide flux). *Nitrate uptake*: Nitrate uptake by the mat-forming community was determined *ex situ* in recovered push cores by adding defined amounts of nitrate to the overlying water and measuring nitrate loss from the water column. Values for the Nyegga mats were determined for 2 separate push cores. Further, a single reference experiment was conducted. *Sulfate reduction rates (SR)*: Average values were calculated from replicate measurements (n): n =4 for Storegga mats (2006), n = 2 for Nyegga mats (2006), n = 6 for HMMV gray mats (2006, 2007), and n = 21 for HMMV white mats (2006, 2007, 2009).

Supplementary information to Supplementary Table 6

During cruise ARK-XXII/1b in 2007, an extensive white mat discovered in the southeastern area of the HMMV was sampled along a North-South gradient (transect). Samples were taken at four different positions: (i) the northern end (N-end) of the mat that was characterized by the occurrence of only partially connected mat spots, (ii) an area ~ 40 m away where the mat started to become more dense (2^{nd} station), (iii) an area ~ 100 m away where we observed a dense, thick coverage of the seafloor (3^{rd} station), and (iv) the southern end (S-end) ~ 158 m away from the N-end where the mat was less thick and increasingly patchy. *AODC*: Total cell numbers were retrieved from one push core each. Presented data are the average of two values, i.e. of 0-1 cm and 1-2 cm, each being obtained by counting at least 2 replicate filters. *Sulfate reduction rates (SR)*: Integrated SR rates were determined for samples obtained during the ARK-XXII/1b cruise in 2007. Average values were calculated from replicate measurements (n), retrieved from single push cores recovered for each site: n = 1 for N-end, n = 3 for 2nd station, 3rd station and S-end. Data are a subset of SR rates in (Felden *et al.*, 2010). *Temperature gradients*: Presented gradient values resulted from single measurements.

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Supplementary Table 1 - Target sites visited within this study included gas chimneys CNE5.7 and CNE03 at the Storegga Slide, pockmarks G11 and G12 in the Nyegga area, and various sites at the Håkon Mosby mud volcano (HMMV).

Target area/ Mat type	Cruise	Year	Target site Latitude	Target site Longitude	Depth
Storegga gray mats	Vicking	2006	64°45'15''N (CNE5.7) 64°45'15''N (CNE03)	4°58'52''E (CNE5.7) 5°04'09''E (CNE03)	~ 720-740 m
Nyegga gray mats	Vicking	2006	64°40'00''N (G11) 64°39'47''N (G12)	05°17'30''E (G11) 05°17'18''E (G12)	~ 720-740 m
HMMV gray mats	Vicking ARK-XXII/1b ARK-XXIV/2b	2006 2007 2009	72°00'15''N (HMMV)	14°43'30''E (HMMV)	~ 1250 m
HMMV white mats	Vicking ARK-XXII/1b ARK-XXIV/2b MSM16/2	2006 2007 2009 2010	72°00'15''N (HMMV)	14°43'30''E (HMMV)	~ 1250 m
Reference	Vicking	2006	64°45'17''N	5°6'13''E	~ 720 m

Vicking: RV *Pourquoi Pas? and* ROV *Victor 6000* (ROV: Ifremer, France). ARK-XXII/1b, ARK-XXIV/2b: RV *Polarstern* and ROV *Quest 4000* (ROV: Marum, University Bremen, Germany). MSM16/2: RV *Maria S. Merian.*

Supplementary Table 2 - Sampling and measurements conducted in this study.

Target area/Mat type	Measurement/Analysis	PANGAEA event label[a]
Storegga gray mats	*Ex situ* microprofiling	VKGD275/PC-1, VKGD275/PC-11
	SR	VKGD275/PC-1, VKGD275/PC-3, VKGD275/PC-10, VKGD275/PC-11
	AODC	VKGD275/PC-1, VKGD275/PC-2, VKGD275/PC-3, VKGD275/PC-4
	CARD-FISH	VKGD275/PC-2, VKGD275/PC-11
	16S rRNA gene clone libraries	VKGD275/PC-2, VKGD275/PC-11
	ARISA	VKGD275/PC-1, VKGD275/PC-2
	Microscopic analyses	VKGD275/PC-1, VKGD275/PC-2, VKGD275/PC-11
Nyegga gray mats	*In situ* microprofiling	VKGD272/MIC-1
	SR	VKGD272/PC-4, VKGD272/PC-34
	Elemental sulfur	VKGD272/PC-11, VKGD272/PC-24, VKGD272/PC-28
	Nitrate uptake experiment	VKGD272/PC-11, VKGD272/PC-32
	AODC	VKGD272/PC-3, VKGD272/PC-4, VKGD272/PC-29, VKGD272/PC-31, VKGD272/PC-32, VKGD272/PC-34
	CARD-FISH	VKGD272/PC-32
	16S rRNA gene clone libraries	VKGD272/PC-11, VKGD272/PC-32
	ARISA	VKGD272/PC-3,VKGD272/PC-29, VKGD272/PC-34
	Microscopic analyses	VKGD272/PC-11, VKGD272/PC-31, VKGD272/PC-32
Reference Storegga/Nyegga	*In situ* microprofiling	VKGD272/MIC-2
	Elemental sulfur	VKGMTB2
	Nitrate uptake experiment	VKGMTB2
	AODC	VKGMTB1, VKGMTB2
	ARISA	VKGMTB1; PS70/132-1_PUC-16; PS74/136-1
HMMV gray mats	AODC	VKGD277/PC-1, VKGD277/PC-3, VKGD277/PC-10; PS70/046-1_PUC-27, PS70/052-1_PUC-16
	CARD-FISH	VKGD277/PC-10
	16S rRNA gene clone libraries	VKGD277/PC-4, VKGD277/PC-8, VKGD277/PC-10; PS74/176-1_PUC-118, PS74/176-1_PUC-121, PS74/176-1_PUC-122
	ARISA	VKGD277/PC-1, VKGD277/PC-3, VKGD277/PC-10; PS70/046-1_PUC-27, PS70/052-1_PUC-16
	Microscopic analyses	VKGD277/PC-3, VKGD277/PC-4, VKGD277/PC-8, VKGD277/PC-10; PS70/046-1_PUC-27, PS70/052-1_PUC-16; PS74/176-1_PUC-118, PS74/176-1_PUC-121, PS74/176-1_PUC-122, PS74/176-1_PUC-127, PS74/176-1_PUC-130, PS74/176-1_PUC-138
Reference HMMV gray mats	AODC	VKGMTB1, VKGMTB2
	ARISA	VKGMTB1; PS70/132-1_PUC-16; PS74/136-1
HMMV white mats	AODC	VKGD276/PC-1, VKGD276/PC-2, VKGD276/PC-3, VKGD276/PC-13; PS70/112-1_PUC-4 (N-end), PS70/112-1_PUC-1 (2nd), PS70/112-1_PUC-27 (3rd), PS70/112-1_PUC-17 (S-end)
	CARD-FISH	VKGD276/PC-2, VKGD276/PC-13
	16S rRNA gene clone libraries	VKGD276/PC-3, VKGD276/PC-13; PS70/112-1_PUC-29 (N-end), PS70/112-1_PUC-7 (2nd), PS70/112-1_PUC-28 (3rd), PS70/112-1_PUC-36 (S-end); PS74/172-1_PUC-116
	ARISA	VKGD276/PC-3, VKGD276/PC-13; PS70/112-1_PUC-4 (N-end), PS70/112-1_PUC-1 (2nd), PS70/112-1_PUC-27 (3rd), PS70/112-1_PUC-17 (S-end); PS74/172-1_PUC-116; MSM16/2_831-1, MSM16/2_839-1, MSM16/2_841-1, MSM16/2_856-1, MSM16/2_857-1, MSM16/2_858-1, MSM16/2_862-1, MSM16/2_863-1
	Microscopic analyses	VKGD276/PC-1, VKGD276/PC-2, VKGD276/PC-3, VKGD276/PC-13; PS70/096-1_PUC-34, PS70/112-1_PUC-7, PS70/112-1_PUC-28, PS70/112-1_PUC-29, PS70/112-1_PUC-36; PS74/172-1_PUC-116, PS74/172-1_PUC-119, PS74/172-1_PUC-129; MSM16/2_831-1, MSM16/2_839-1, MSM16/2_841, MSM16/2_856-1, MSM16/2_857-1,MSM16/2_858-1, MSM16/2_862-1, MSM16/2_863-1
Reference HMMV white mats	AODC	VKGMTB1, VKGMTB2; PS70/132-1_PUC-16
	ARISA	VKGMTB1; PS70/132-1_PUC-16; PS74/136-1

SR (Sulfate Reduction), AODC (Acridine Orange Direct Cell counts), CARD-FISH (Catalyzed Reporter Deposition Fluorescence *In Situ* Hybridization), rRNA (ribosomal RNA), ARISA (Automated Ribosomal Intergenic Spacer Analysis). VKG (Vicking cruise 2006), PS70 (ARK-XXII/1b cruise 2007), PS74 (ARK-XXIV/2b cruise 2009), MSM16/2 (MSM16/2 cruise 2010), MIC (microprofiler), PC or PUC (push core), MTB (multicorer), HMMV (Håkon Mosby Mud Volcano).

[a]Corresponds to PANGAEA event label (www.pangaea.de).

Supplementary Table 3 - Oligonucleotide probes and hybridization conditions used for CARD-FISH analyses in this study.

Probe	Probe specificity	Probe sequence (5'-3')	Target site within 16S rRNA gene[a]	FA[b] (%)	T_h[c]/T_w[d] (°C)	Reference
EUB338(I-III)	Most Bacteria	Equimolar mixture of the following three probes	338-355	30	46/46	Daims *et al.* (1999)
EUB338	Most Bacteria	GCT GCC TCC CGT AGG AGT	338-355	30	46/46	Amann *et al.* (1990)
EUB338-II	Planctomycetales	GCA GCC ACC CGT AGG TGT	338-355	30	46/46	Daims *et al.* (1999)
EUB338-III	Verrucomicrobiales	GCT GCC ACC CGT AGG TGT	338-355	30	46/46	Daims *et al.* (1999)
DSS658	*Desulfosarcina*/*Desulfococcus*	TCC ACT TCC CTC TCC CAT	658-685	60	46/46	Manz *et al.* (1998)
660[e]	*Desulfobulbus*	GAA TTC CAC TTT CCC CTC TG	660-679	60	46/46	Devereux *et al*. (1992)
NON338	Negative control	ACT CCT ACG GGA GGC AGC	338-355	20	46/46	Wallner *et al.* (1993)
ARCH915	Most Archaea	GTG CTC CCC CGC CAA TTC CT	915-935	30	46/46	Raskin *et al.* (1994)
ANME1-350	ANME-1 archaea	AGT TTT CGC GCC TGA TGC	350-367	40	46/46	Boetius *et al.* (2000)
ANME2a-647	ANME-2 archaea	TCT TCC GGT CCC AAG CCT	647-664	55	46/46	Knittel *et al.* (2005)
ANME3-1249	ANME-3 archaea	TCG GAG TAG GGA CCC ATT	1250-1267	40	46/46	Niemann *et al.* (2006)
ANME3-1249H3	helper probe for ANME3-1249	GTC CCA ATC ATT GTA GCC GGC	1229-1249	40	46/46	Lösekann *et al*. (2007)
ANME3-1249H5	helper probe for ANME3-1249	TTA TGA GAT TAC CAT CTC CTT	1268-1288	40	46/46	Lösekann *et al*. (2007)

CARD-FISH (Catalyzed Reporter Deposition Fluorescence *In Situ* Hybridization)

[a]*E.coli* positions. rRNA (ribosomal RNA).

[b]Formamide (FA) concentration in the hybridization buffer.

[c]Hybridization temperature.

[d]Washing temperature.

[e]This probe was only applied to samples of the core VKGD276/PC-13.

Supplementary Table 4 - Microscopic diversity of thiotrophic mats at the deep Norwegian margin.

Target area/Mat type	Mat-dominating bacteria	Cores analyzed (year)
Storegga	*Arcobacter* mats	3 (2006)
gray mats	*Thiobacterium* mats and cells	
	sulfur-storing *Beggiatoa* spp.-like filaments associated with *Thiobacterium* mats and sediment	
	sulfur-storing filaments attached to worms and sediment resembling *"Candidatus* Marithrix spp."	
Nyegga	sulfur-storing filaments attached to worms	3 (2006)
gray mats	*Arcobacter* mats	
	sulfur-storing *Beggiatoa* spp.-like filaments	
HMMV	*Arcobacter* mats	4 (2006)
gray mats	unknown single cells (some pigmented)	2 (2007)
	pigmented filaments	6 (2009)
	sulfur-storing *Beggiatoa* spp.-like filaments	
	single *Thiomargarita spp.* -like cells	
	thin filamentous bacteria (chains of sulfur granules)	
HMMV	1-2 types of sulfur-storing *Beggiatoa spp.* -like filaments	4 (2006)
white mats		5 (2007)
		3 (2009)
		8 (2010)

HMMV (Håkon Mosby Mud Volcano)

Supplementary Table 5 - Results of bacterial 16S rRNA gene clone library construction for all mats.

Target area/Mat type	Total no. of sequences	Gamma-proteobacteria %	Epsilon-proteobacteria %	Delta-proteobacteria %	Alpha-proteobacteria %	Flavo-bacteria %	other %	unclassified %
Storegga gray mats	203	69	15	3	1	4	5	3
Nyegga gray mats	483	51	41	2	1	<1	1	3
HMMV gray mats	544	7	56	21	<1	3	2	11
HMMV white mats	1044	64	24	3	<1	1	2	5

HMMV (Håkon Mosby Mud Volcano). Taxonomic identification of the partial sequences was achieved by applying the RDP Naïve Bayesian rRNA Classifier (Wang *et al*., 2007). Sequences were termed "unclassified", when their classification reliability was lower than 80%. Part of the Storegga sequence data set was already previously published (Grünke *et al*., 2010).

Supplementary Table 6 - Transect along a 158 m-long white *Beggiatoa* mat at the Håkon Mosby mud volcano (2007).

Parameter	Relative transect position				
	HMMV white mat N-end	HMMV white mat 2nd station	HMMV white mat 3rd station	HMMV white mat S-end	next to mat
Mat description	thin mat patches	beginning of dense coverage	dense coverage	patchy appearance	no mat visible
AODC[a] (10^9 single cells cm^{-3})	1.98	1.19	2.16	2.51	2.70
SR[b] (mmol m^{-2} d^{-1})	3.29	14.0 ± 6.8	20.9 ± 10.2	12.2 ± 6.5	ND
T[c] (°C m^{-1})	1.52	2.20	2.73	1.11	0.94

AODC (Acridine Orange Direct Cell Counts), SR (Sulfate Reduction), T (temperature), HMMV (Håkon Mosby Mud Volcano). For additional information see Supplementary Material.

[a]Average of the upper 2 cm of sediment.

[b]Integrated SR rates were determined *ex situ* for the upper 10 cm of sediment (if available: values ± standard deviation).

[c]Temperature gradients were determined with a ROV-operated temperature probe (Felden *et al*., 2010).