

Supporting Information

Denaturing gradient gel electrophoresis (DGGE)

The amplification of the bacterial 16S rRNA genes for DGGE was performed with the primer set GM5F (GC-clamp at the 5'-end) (Muyzer et al. 1993) and 907 RM (Muyzer et al. 1998) using a touchdown protocol (Muyzer et al., 1995). The reaction mixture of 100 μ l included 10-100 ng DNA, 1 μ M of each primer, 100 mM of dNTPs, 1 x buffer (Eppendorf, Hamburg, Germany), 1 x enhancer (Eppendorf) and 1.25 U of the Taq DNA Polymerase (Eppendorf). DGGE was carried out using a Bio-Rad D-Code system (Bio-Rad Laboratories). The following conditions were applied: 1 mm thick, 6% (w/v) polyacrylamide gels, 1x TAE electrophoresis buffer (pH 8.3), 20-80% denaturant. The gels were run at 60°C and a constant voltage of 200 V for 3.5 h. DGGE gels were stained with ethidiumbromide and bands were visualized under UV light.

Statistical sequence analysis

The software Distance-Based OTU and Richness (DOTUR) was applied to ARB distance matrices generated with the Jukes-Cantor correction to estimate operational taxonomic units (Schloss and Handelsman, 2005).

Table S1: Cell and CARD-FISH counts, number of 16S rRNA gene sequences, and estimated Chao1 richness for all four sediments.

Site	Cell counts [cell/g]	CARD-FISH [%]		No. of 16S rRNA gene sequences analyzed ^b		Richness estimator Chao1 ^c	
		EUB338 I-III ^a	ARCH 915	<i>Bacteria</i>	<i>Archaea</i>	<i>Bacteria</i>	<i>Archaea</i>
Anya's Garden Site F	2.9×10^9	78	2	137 84 F/53 P	13 13 F/0 P	107 (88, 147)	10 (8, 20)
Quest	4.2×10^9	75	6	111 74 F/37 P	52 46 F/6 P	173 (115, 301)	64 (35, 165)
Oceanic sediment	3.6×10^8	69	4	93 62 F/31 P	84 56 F/28 P	102 (74, 170)	13 (11, 27)
	6.8×10^7	70	8	154 78 F/76 P	81 55 F/26 P	139 (117, 181)	14 (13, 21)

^a equimolar mixture of probes EUB338, EUB338-II, and EUB338-III covering about 90% of all members of *Bacteria* (Amann and Fuchs, 2008)

^b total numbers of sequences as well as number of full-length (F) and partial (P) sequences

^c Chao1 richness with lower and upper bound of 95% confidence interval

Table S2: Oligonucleotide probes and hybridization conditions used in this study.

Target group	Probe	Sequence (5' to 3')	Label	FA [%] ^a	Hybridization Temp (°C)	Reference
Most Archaea	ARCH915	GTGCTCCCCGCCAATTCCT	HRP, Cy3	35	46	Stahl and Amann, 1991
Most Bacteria	EUB338	GCTGCCTCCCGTAGGAGT	HRP, Cy3	35	46	Amann et al., 1990
	EUB338-II	GCAGCCACCCGTAGGTGT	HRP, Cy3	35	46	Daims et al., 1999
	EUB338-III	GCTGCCACCCGTAGGTGT	HRP, Cy3	35	46	Daims et al., 1999
control probe complementary to EUB338	NON338	ACTCCTACGGGAGGCAGC	HRP, Cy3	35	46	Wallner et al., 1993
<i>Epsilonproteobacteria</i>	EPSY549	CAGTGATCCGAGTAACG	HRP, Cy3	35	46	Lin et al., 2006
<i>Epsilonproteobacteria</i>	EP404	AAAKGYGTCATCCTCAA	Cy3	30	46	Macalady et al., 2006
<i>Arcobacter</i> spp.	Arc1430	TTAGCATCCCCGCTTCGA	HRP, Cy3	20	46	Snaidr et al., 1997
<i>Arcobacter</i> spp.	Arc94	TGCGCCACTTAGCTGACA	HRP	20	46	Snaidr et al., 1997
Most <i>Deltaproteobacteria</i> and <i>Gemmatimonadetes</i>	Delta495a ^b	AGTTAGCCGGTGCTTCCT	HRP	35	46	Loy et al., 2002
Competitor for Delta495a	cDelta495a	AGTTAGCCGGTGCTTCTT	-	-	-	Macalady et al., 2006
Some <i>Deltaproteobacteria</i>	Delta495b ^b	AGTTAGCCGGCGCTTCCT	HRP	35	46	Loy et al., 2002
Competitor for Delta495b	cDelta495b	AGTTAGCCGGCGCTTC(T/G)T	-	-	-	Lücker et al., 2007
Some <i>Deltaproteobacteria</i>	Delta495c ^b	AATTAGCCGGTGCTTCCT	HRP	35	46	Loy et al., 2002
Competitor for Delta495c	cDelta495c	AATTAGCCGGTGCTTCTT	-	-	-	Lücker et al., 2007
<i>Desulfosarcina</i> -related bacteria	DSS658	TCCACTTCCCTCTCCCAT	HRP, Cy3	60	46	Manz et al., 1998
Most <i>Desulfovibrio</i> spp.	DSV698	GTTCTCCAGATATCTACGG	HRP	40	46	Manz et al., 1998
Gammaproteobacteria	GAM42a ^b	GCCTTCCCACATCGTTT	HRP, Cy3	35	46	Manz et al., 1992
Competitor for GAM42a	BET42a	GCCTTCCCCTTCGTTT	-	-	-	Manz et al., 1992
Potential sulfur-oxidizing Gammaproteobacteria	GAM660	TCCACTTCCCTCTAC	HRP	35	46	Ravenschlag et al., 2001
most <i>Flavobacteria</i> , some <i>Bacteroidetes</i> , some <i>Sphingobacteria</i> , some <i>Epsilonproteobacteria</i>	CF319a	TGGTCCGTGTCTCAGTAC	HRP, Cy3	35	46	Manz et al., 1996

^a Formamide (FA) concentration in hybridization buffer.^b Competitor probes are required.

Table S3: Accession numbers of 16S rRNA gene sequences affiliated to the uncultivated *Gammaproteobacteria* JTB255/BD3-6.

16S rRNA sequences	ACC
Logatchev sediment clone Quest_014	FN 553598
Logatchev sediment clone Quest_015	FN 553599
Logatchev sediment clone Quest_028	FN 553611
Logatchev sediment clone Quest_030	FN 553613
Logatchev sediment clone Quest_031	FN 553614
Logatchev sediment clone Quest_036	FN 553618
Logatchev sediment clone Quest_037	FN 553619
Logatchev sediment clone Quest_043	FN 553623
Logatchev sediment clone Quest_054	FN 553629
Logatchev sediment clone Quest_055	FN 553630
Logatchev sediment clone Quest_071	FN 553644
Logatchev sediment clone Quest_074	FN 553646
Logatchev sediment clone Quest_020	FN 553666
Logatchev sediment clone OC_004	FN 553444
Logatchev sediment clone OC_014	FN 553454
Logatchev sediment clone OC_017	FN 553457
Logatchev sediment clone OC_025	FN 553465
Logatchev sediment clone OC_026	FN 553466
Logatchev sediment clone OC_041	FN 553480
Logatchev sediment clone OC_043	FN 553482
Logatchev sediment clone OC_047	FN 553486
Logatchev sediment clone OC_048	FN 553487
Logatchev sediment clone OC_050	FN 553489
Logatchev sediment clone OC_055	FN 553494
Logatchev sediment clone OC_069	FN 553508
Logatchev sediment clone OC_070	FN 553509
Logatchev sediment clone OC_p006	FN 553767
Logatchev sediment clone OC_p012	FN 553772
Logatchev sediment clone OC_p014	FN 553774
Logatchev sediment clone OC_p031	FN 553790
Logatchev sediment clone OC_p036	FN 553794
Logatchev sediment clone OC_p037	FN 553795
Logatchev sediment clone OC_p056	FN 553814
Logatchev sediment clone OC_p060	FN 553817
Logatchev sediment clone OC_p062	FN 553819
Logatchev sediment clone OC_p063	FN 553820
Logatchev sediment clone OC_p064	FN 553821
Logatchev sediment clone OC_p067	FN 553823
Logatchev sediment clone OC_p073	FN 553828
Logatchev sediment clone OC_p079	FN 553833
Logatchev sediment clone OC_p080	FN 553834
Logatchev sediment clone OC_p082	FN 553836
Logatchev sediment clone OC_p083	FN 553837

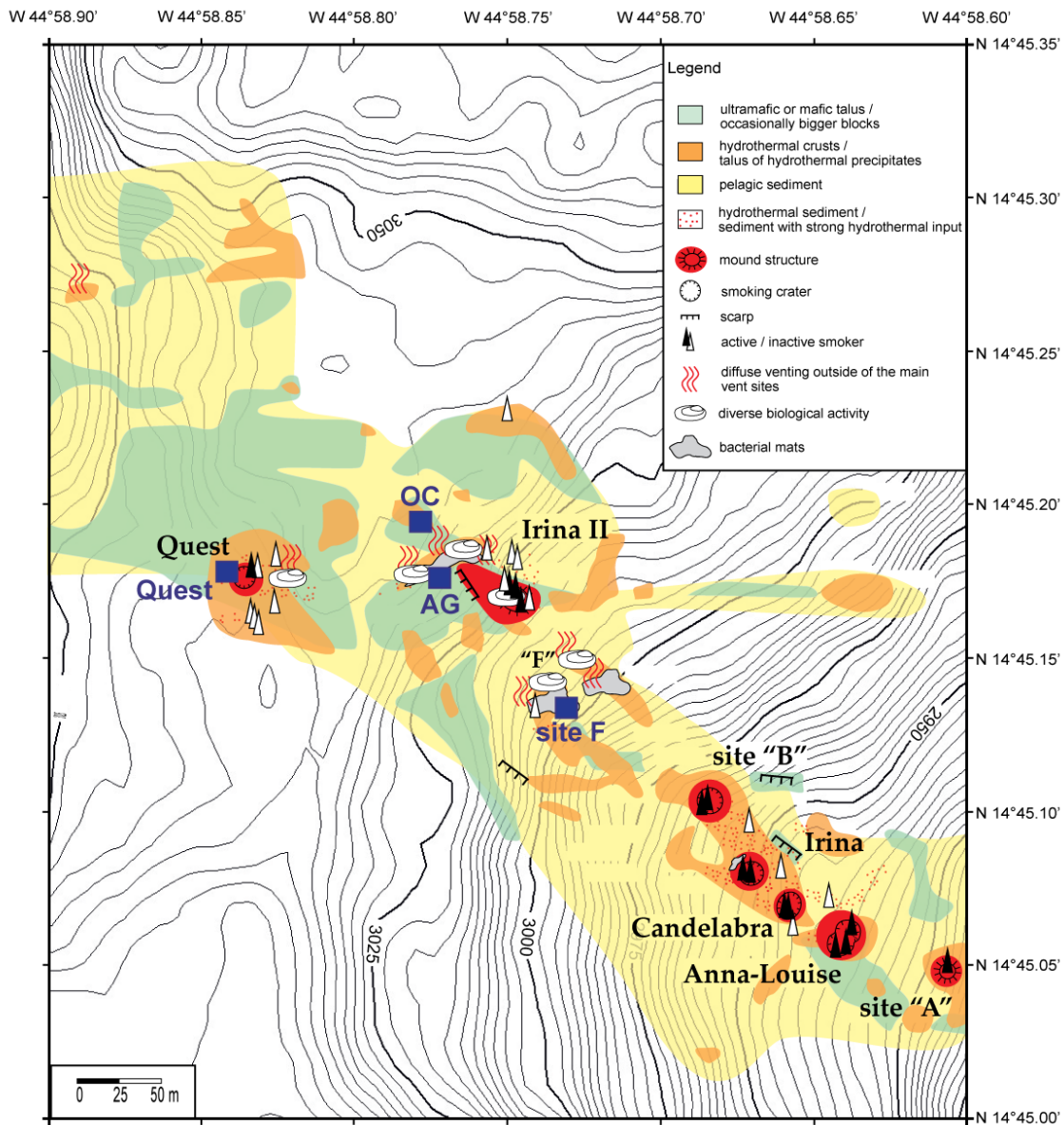


Figure S1: Active black smoker and diffuse venting sites at the Logatchev hydrothermal vent field (modified after Petersen et al., 2009). Sampling sites are indicated by blue coloured squares.

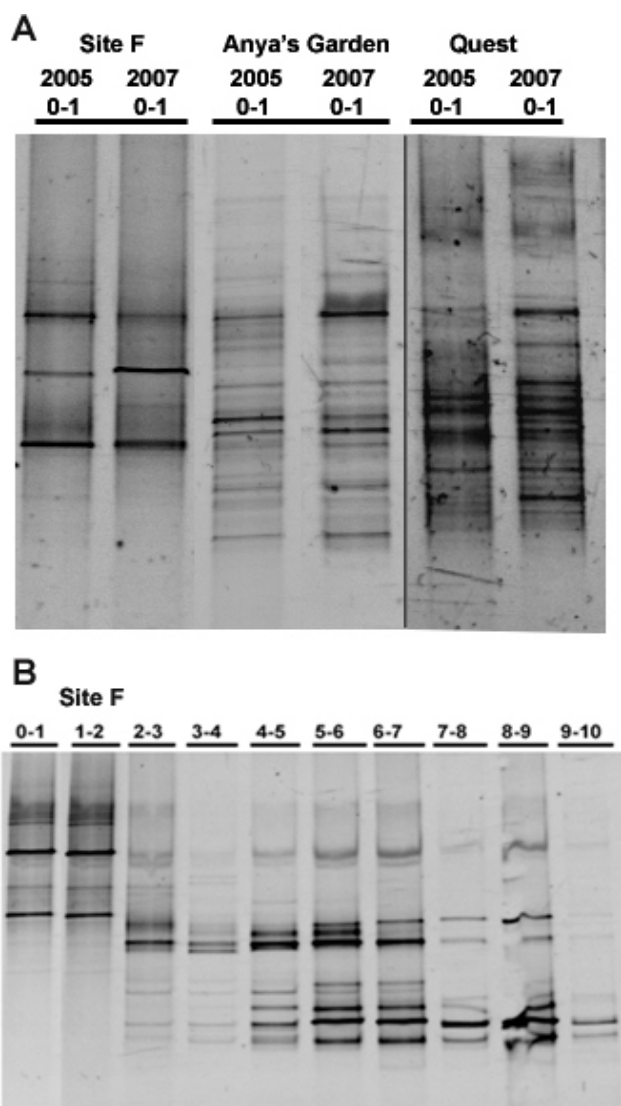


Figure S2: DGGE fingerprints of PCR-amplified bacterial 16S rRNA sequences from the surface sediments (0-1 cm) of site F, Anya's Garden and Quest sampled in 2005 and 2007 (A) and depth profiles of site F sediment to a depth of 10 cm sampled in 2007 (B).

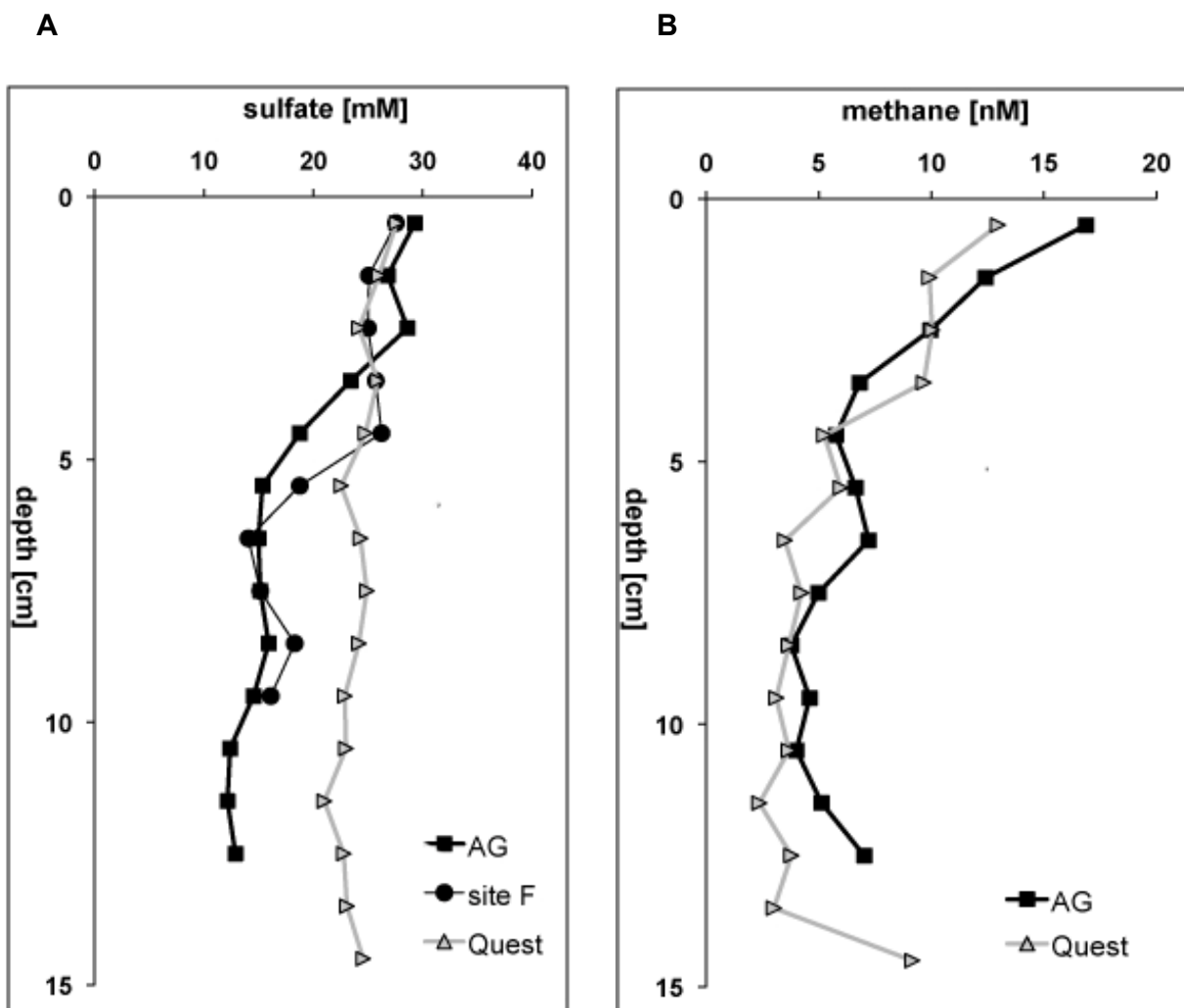


Figure S3: Depth profiles of porewater sulfate determined for sediment cores from Anya's Garden (AG), site F and Quest and of dissolved methane concentrations in AG and Quest sediment cores.

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