Iron and sulfate reduction structure microbial communities in (sub-)Antarctic sediments

Running title: Iron and sulfate reduction in South Georgia sediments

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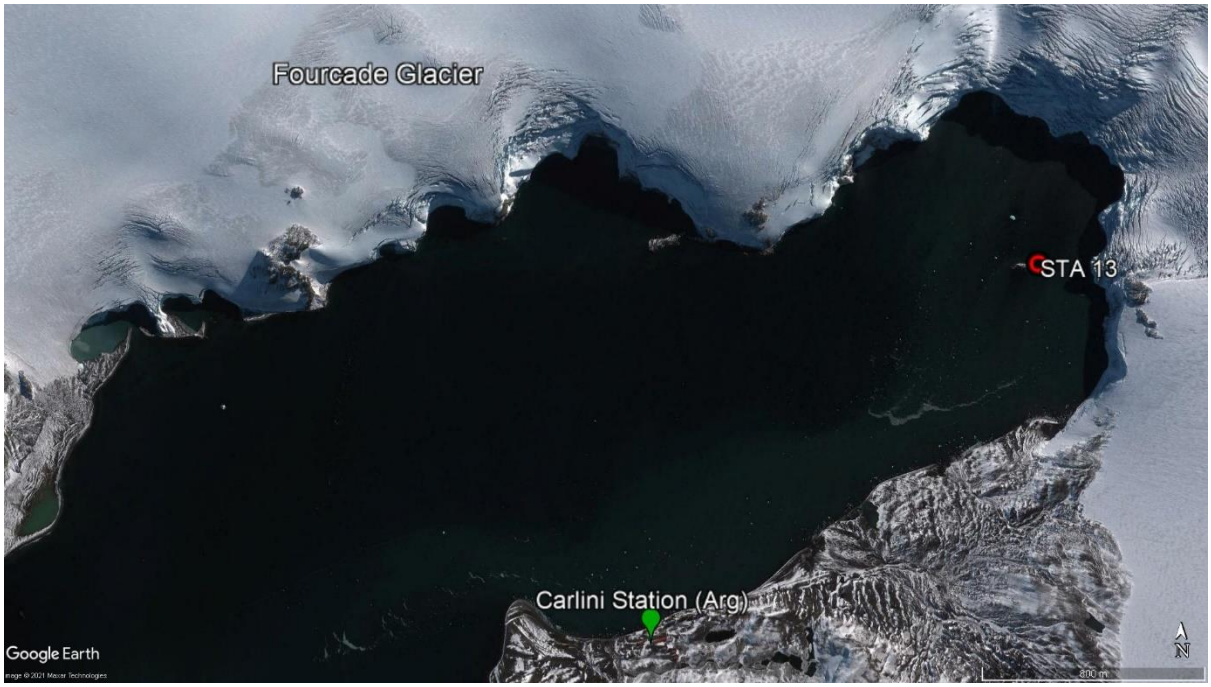

Fig. S1: Sampling location of Station 13 in Potter Cove (King George Island/Isla 25 de Mayo, Antarctic Peninsula). Produced with Google Earth.

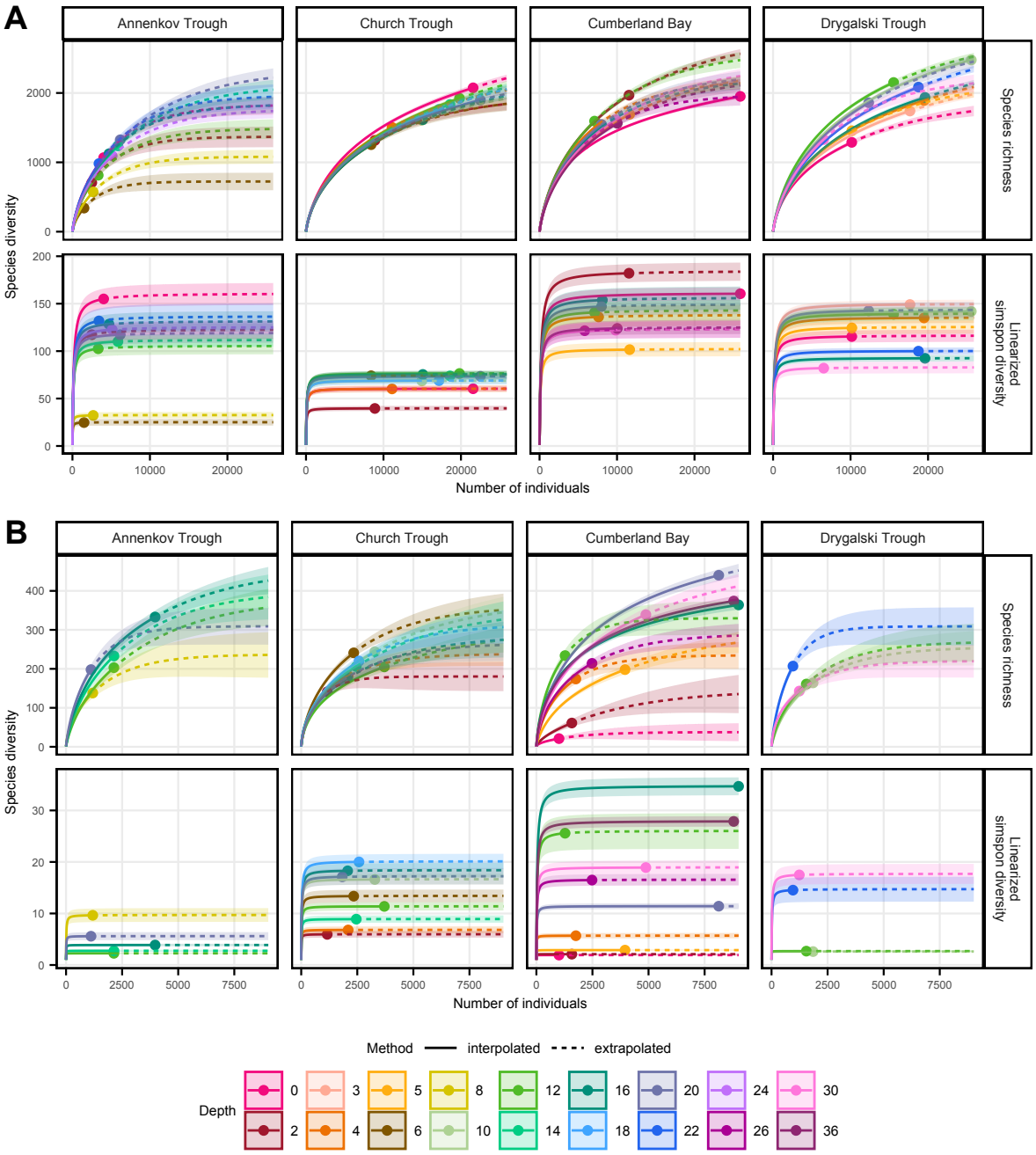

Fig. S2: Rarefaction curves of 16S rRNA sequencing of South Georgia surface sediment for bacteria (**a**) and archaea (**b**). Depth in cm below seafloor. **b** Samples after removing low read samples (see Table S2) leaving Annenkov Trough n = 5, Church Trough n = 9, Cumberland Bay n = 10, Drygalski Trough n = 4.

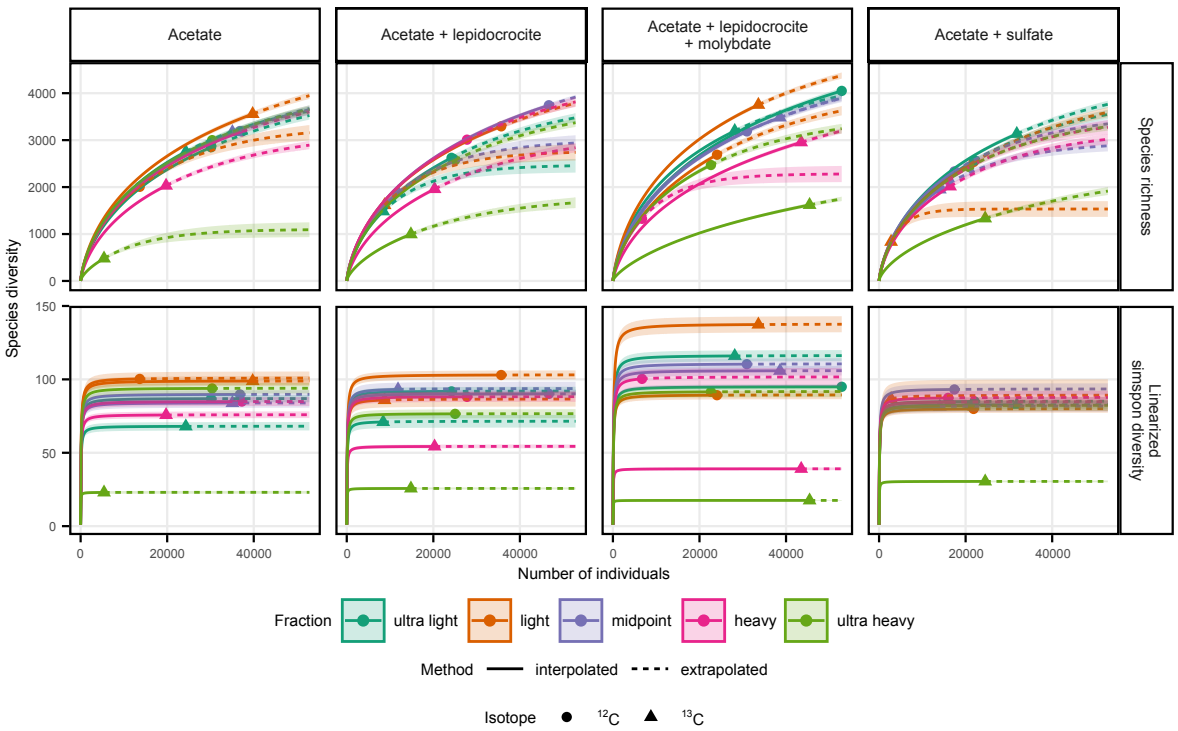

Fig. S3: Rarefaction curve of bacterial 16S rRNA sequencing of SIP incubations with Cumberland Bay sediments.

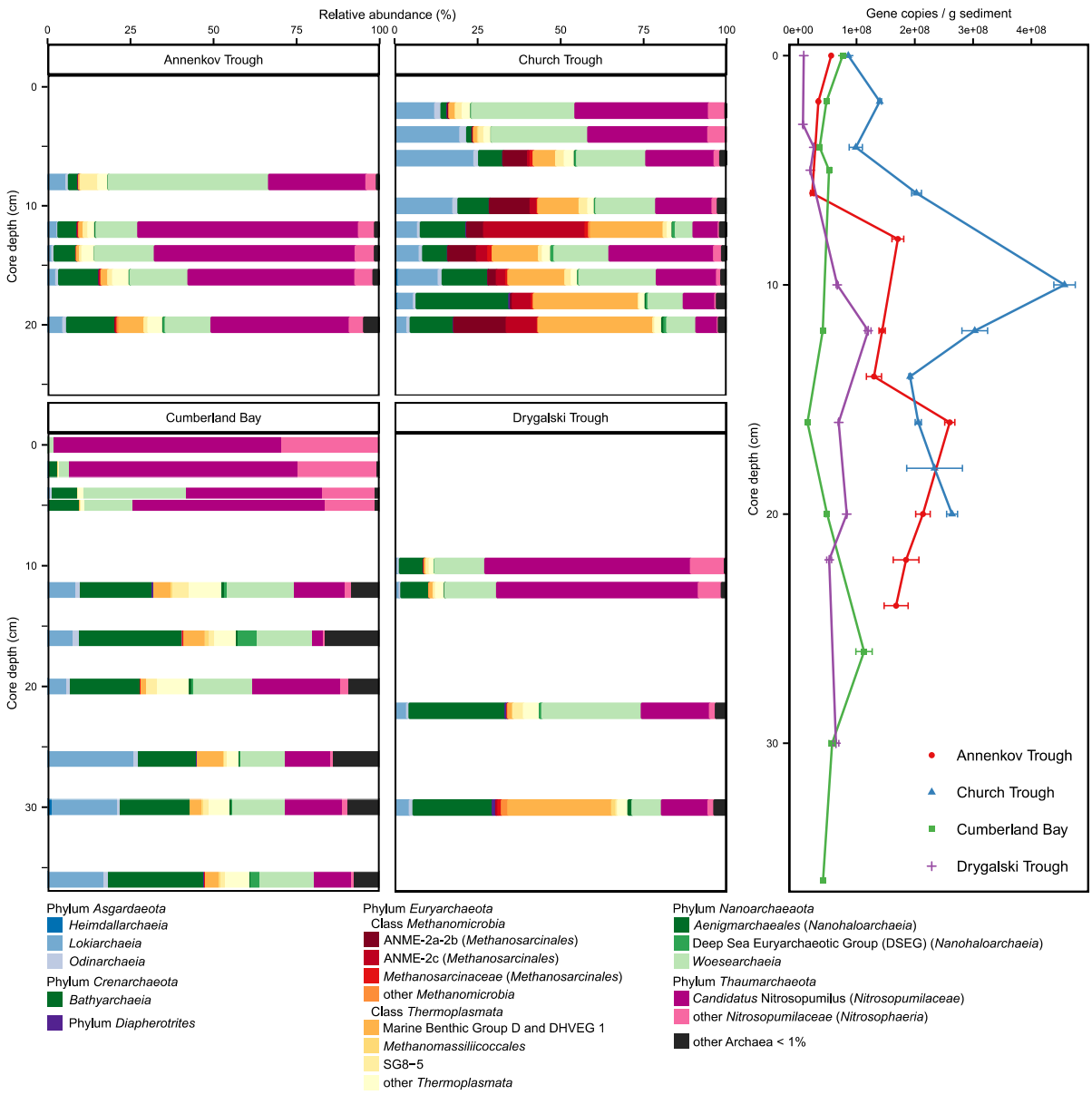

Fig. S4: Archaeal community composition and gene copy numbers in South Georgia surface sediments. **a** Relative abundance of bacterial 16S rRNA genes in Annenkov Trough, Church Trough, Cumberland Bay and Drygalski Trough. From the originally sequenced 10 samples per site, some were removed due to insufficient coverage (see Table S2). **b** Archaeal 16S rRNA gene copies per gram wet sediment of 10 samples per site with error bars displaying SD of technical qPCR replicates (n = 3).

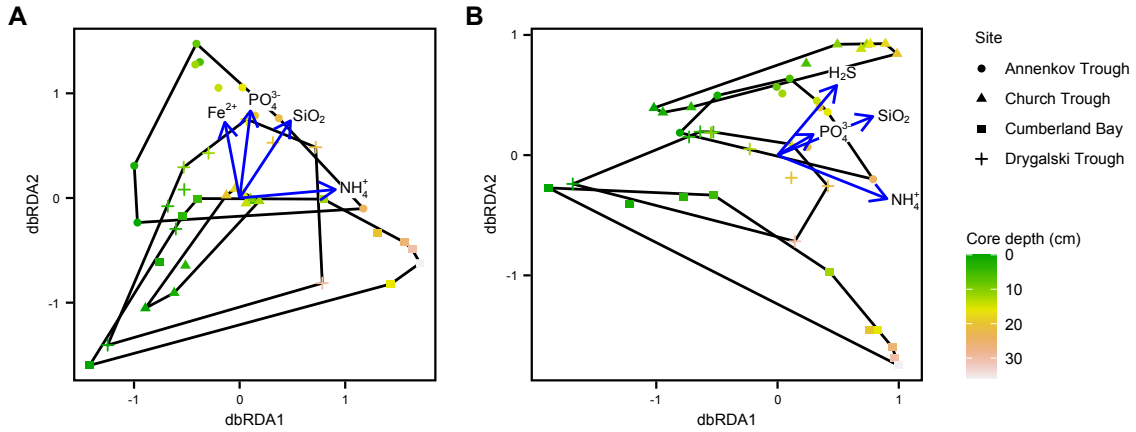

Fig. S5: Partial distance-based redundancy analysis (dbRDA) ordination plot of bacterial communities in surface sediments of South Georgia. Variation explained by (**a**) H_2S or (**b**) Fe^{2+} were removed from the model. Sample points are distinguished by site and core depth by shape and color respectively. dbRDA1 and dbRDA2 axes are displayed which constrain the Bray Curtis distance matrix with geochemical parameters PO_4^{3-}, NH_4^+, SiO_2 and Fe^{2+} or H_2S. The total model (**a** $F = 4.13$, $p < .01$, Df 4, 34; **b** $F = 4.57$, $p < .01$, Df 4, 34) and each individual parameter ($p < 0.05$) was significant.

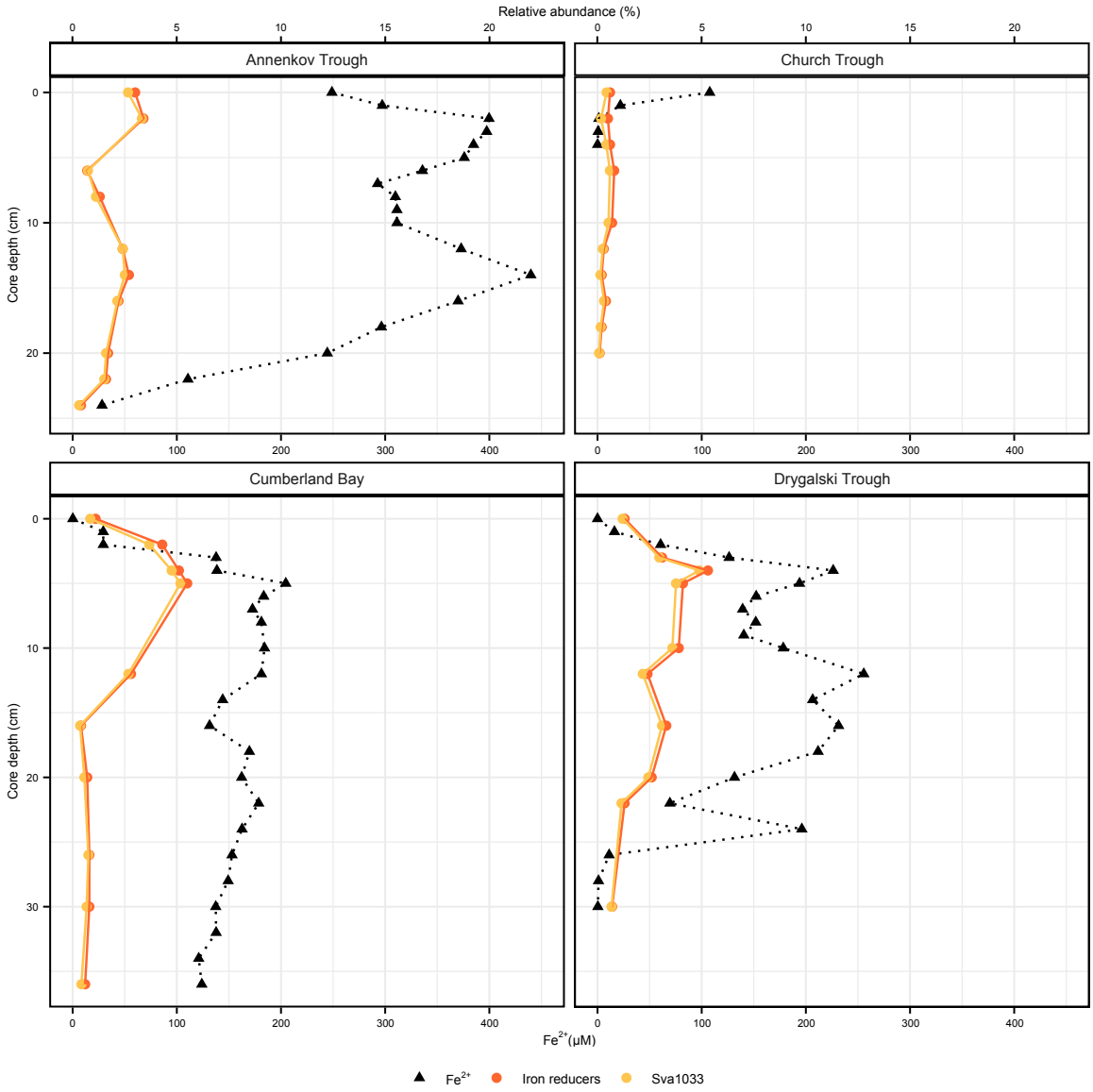

Fig. S6: Depth profile of contribution of iron reducing microorganisms in *Deltaproteobacteria* and family Sva1033 to bacterial 16S rRNA gene community in South Georgia surface sediments. Fe^{2+} profile from Fig. 2 was displayed.

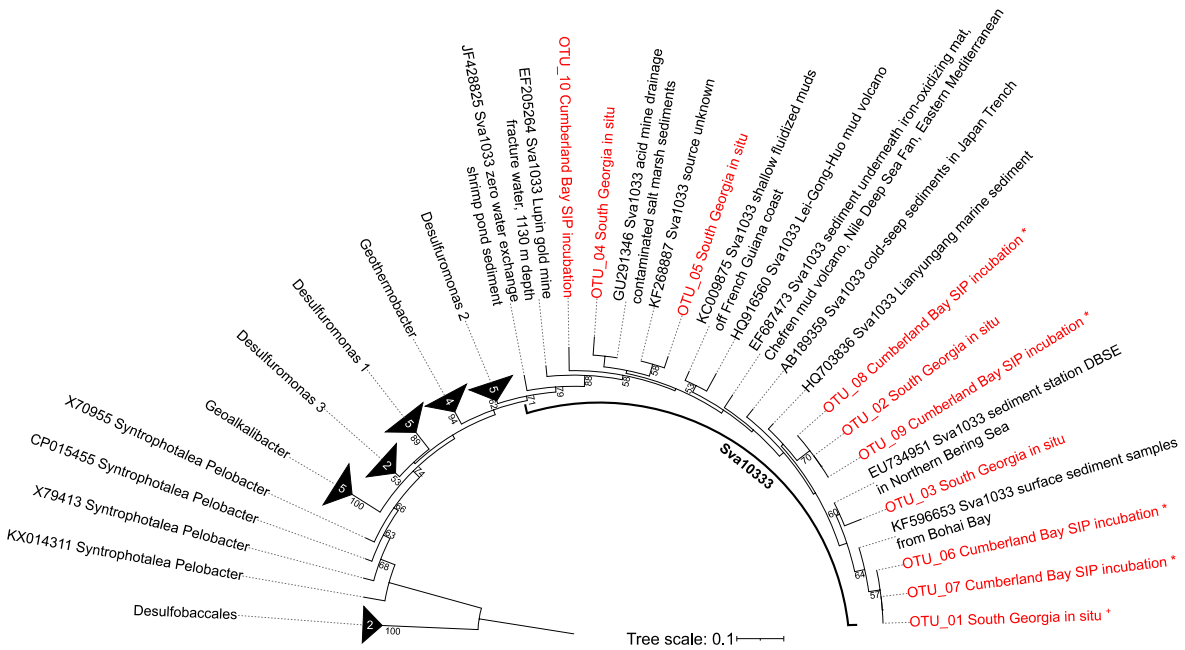

Fig. S7: Phylogenetic tree of family Sva1033 and closest sister clusters including the most abundant OTUs of this study (in red). Bootstrap values > 50% are shown in the tree. The reference sequences were exported from the ARB tree of SILVA release 138, Quast *et al.* (1). Accession numbers of sequences in collapsed nodes: Desulfobaccales FJ437876, AF002671; Geoalkalibacter CP010311, KJ817771, KT699114, DQ309326, MG602814; Desulfuromonas 3 JQ801020, JF727697; Desulfuromonas 1 JX223285, MF806540, JX224539, JX222942, HM141856; Geothermobacter KF741402, AY155599, GQ433952; Desulfuromonas 2 EU052234, KC470887, JX391250, KC471166, KM203496.

* : most abundant OTUs from SIP incubations representing together 96% of all Sva1033 sequences

+ : most abundant OTU from *in situ* sediments representing 90% of all Sva1033 sequences

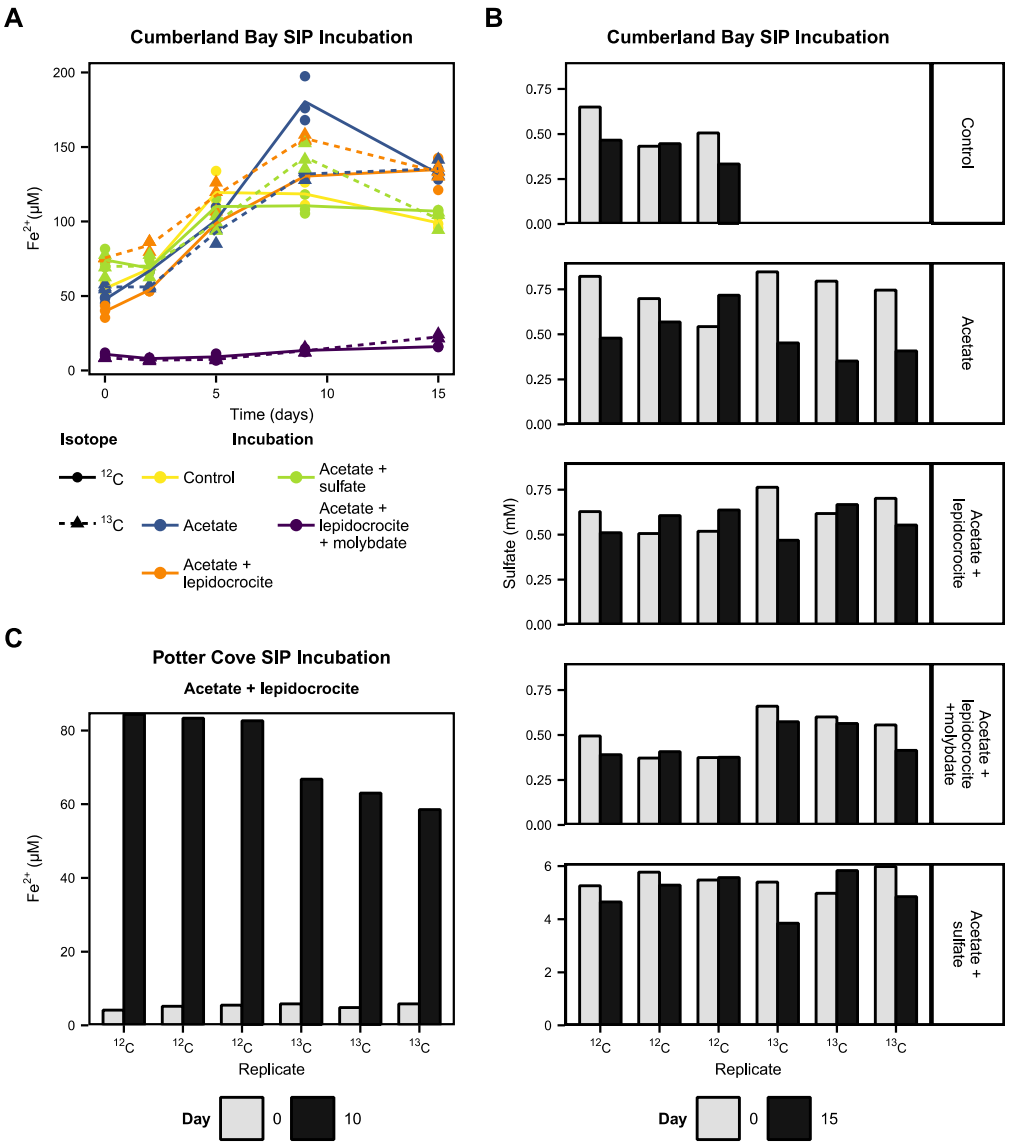

Fig. S8: Time course of Fe^{2+} and sulfate concentrations in SIP incubations of Cumberland Bay and Potter Cove sediments. **a** Fe^{2+} concentration of Cumberland Bay SIP incubations over time separated by treatment. Lines connect mean of triplicates of each treatment, separate for ^{12}C and ^{13}C acetate. **b** Sulfate concentration of Cumberland Bay SIP incubations of each replicate at start and end time point (day 0 – 15). The technical measurement error for sulfate measurements was 2%. **c** Fe^{2+} concentration of single Potter Cove SIP incubation treatment at start and end time point (day 0 – 10).

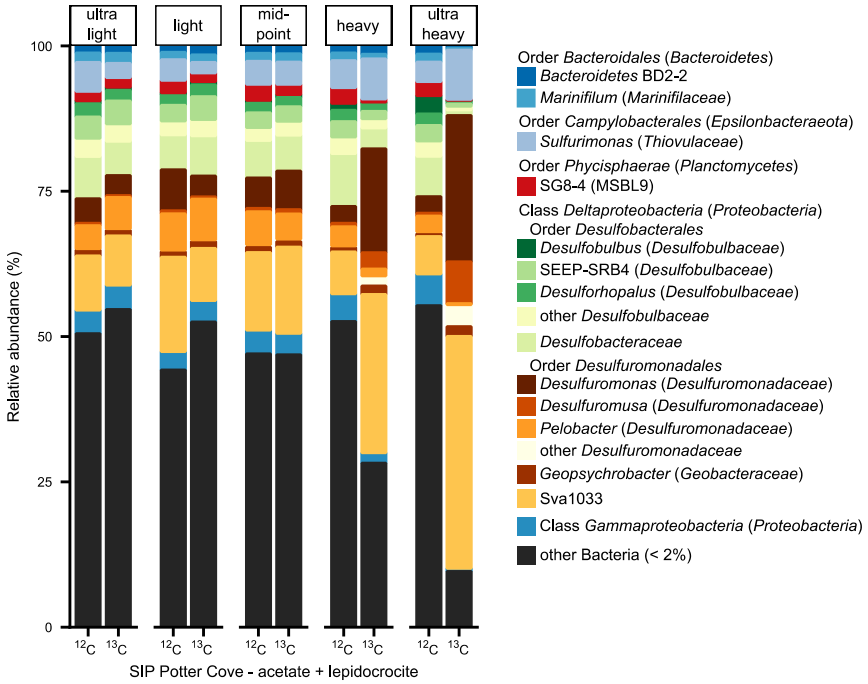

Fig. S9: SIP incubation of Potter Cove sediments. Density separated bacterial 16S rRNA community composition of taxa with > 2% relative abundance.

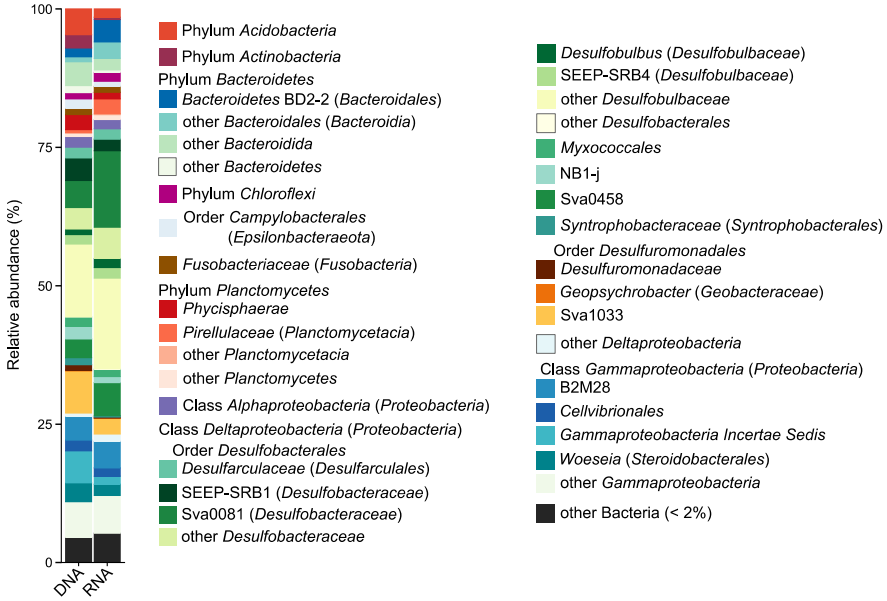

Fig. S10: SIP incubation of Cumberland Bay sediments bacterial 16S rRNA starting community on RNA and DNA level.

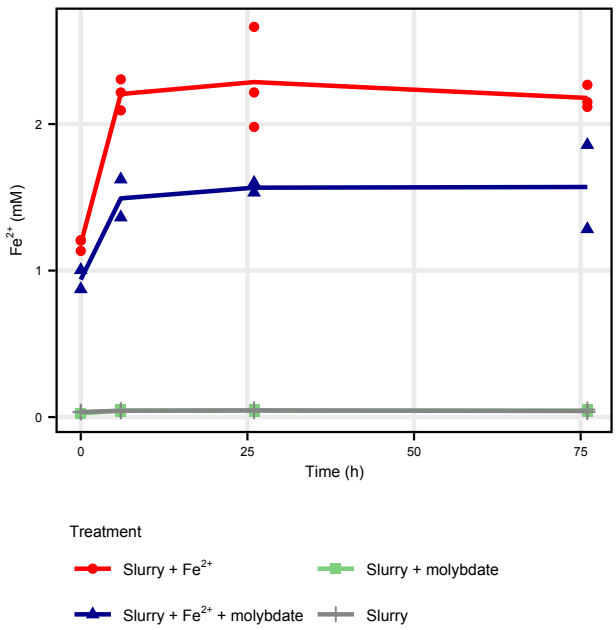

Fig. S11: Measured Fe^{2+} of abiotic ferrous iron. The line connects the mean for replicates of each treatment with n = 3 for all except Slurry + Fe^{2+} + molybdate with n = 2. For details see text below.

Fig. S12: Serum bottles of abiotic control experiment. One replicate of each treatment, from left to right: slurry + molybdate, slurry + Fe^{2+}, slurry + Fe^{2+} + molybdate, slurry only.

Supplementary tables

Table S1: Sampling location and sample details

Sampling objective	Site Core ID	Core depth (cm)*	Coordinates	
			Latitude	Longitude
Archaeal and bacterial community composition analysis and quantification, *dsrA* gene quantification	Annenkov Trough GeoB22054-2 (MUC-12)	0 – 1 2 – 3 6 – 7 8 – 9 12 – 14 14 – 16 16 – 18 20 – 22 22 – 24 24 – 26	54°26.169 S	37°21.094 W
Geochemical measurements on pore water		0 – 10 every 1 cm 10 – 34 every 2 cm		
Archaeal and bacterial community composition analysis and quantification, *dsrA* gene quantification	Church Trough GeoB22031-1 (MUC-5)	0 – 1 2 – 3 4 – 5 6 – 7 10 – 12 12 – 14 14 – 16 16 – 18 18 – 20 20 – 22	53°46.209 S	38°08.413 W
Geochemical measurements on pore water		0 – 10 every 1 cm 10 – 30 every 2 cm		
Archaeal and bacterial community composition analysis and quantification, *dsrA* gene quantification	Cumberland Bay GeoB22046-1 (MUC-8)	0 – 1 2 – 3 4 – 5 5 – 6 12 – 14 16 – 18 20 – 22 26 – 28 30 – 32 36 – 38	54°17.270 S	36°27.710 W
Geochemical measurements on pore water		0 – 10 every 1 cm 10 – 40 every 2 cm		
Archaeal and bacterial community composition analysis and quantification, *dsrA* gene quantification	Drygalski Trough GeoB22015-1 (MUC-4)	0 – 1 3 – 4 4 – 5 5 – 6 10 – 12 12 – 14 16 – 18 20 – 22 22 – 24 30 – 32	54°51.269 S	35°54.667 W
Geochemical measurements on pore water		0 – 10 every 1 cm 10 – 32 every 2 cm		
SIP incubation	Cumberland Bay GeoB22024-1 (GC-6)	0 – 14	54°15.885 S	36°26.225 W
SIP incubation	Potter Cove Station 13-04	0 – 29 (whole core)	62°13.523 S	58°38.470 W

* In the text, other tables and figures only start depth is displayed

Table S2: Sequencing details surface sediments, South Georgia

Red labelled archaea samples were removed from the analyses due to insufficient sequencing depth

Site Core ID	Core depth (cm)	Total reads		Total OTUs	
		Bacteria	Archaea	Bacteria	Archaea
Annenkov Trough GeoB22054-2	0	4025	423	1067	74
	2	2497	334	707	80
	6	1494	670	340	133
	8	2680	1192	573	140
	12	3326	2132	814	204
	14	5870	2138	1236	235
	16	4712	3973	1123	336
	20	6165	1113	1325	199
	22	3420	502	982	121
	24	5129	618	1093	133
Church Trough GeoB22031-1	0	21567	761	2074	111
	2	8887	1167	1316	146
	4	11103	2093	1504	182
	6	8405	2342	1255	243
	10	14952	3284	1607	246
	12	19810	3706	1923	206
	14	18582	2456	1833	207
	16	15080	2071	1616	177
	18	17165	2579	1764	222
	20	22506	1829	1898	170
Cumberland Bay GeoB22046-1	0	25910	1009	1951	21
	2	11546	1581	1968	61
	4	7618	1761	1548	174
	5	11630	3952	1693	198
	12	7098	1280	1594	235
	16	8106	9021	1516	367
	20	7996	8132	1546	442
	26	5828	2481	1263	214
	30	9828	4883	1616	341
	36	10034	8799	1562	375
Drygalski Trough GeoB22015-1	0	10154	122	1288	37
	3	17665	142	1740	41
	4	19482	568	1892	90
	5	10165	805	1455	103
	10	25580	1853	2476	166
	12	15554	1551	2151	161
	16	19605	732	1936	129
	20	12337	451	1859	137
	22	18759	955	2084	207
	30	6532	1239	1328	144

Table S3: SIP incubation set-up Cumberland Bay

Treatment (n = 3)	Acetate (500 µM)	Lepidocrocite (5 mM)	Sulfate (5 mM)	Molybdate (10 mM)	Days pre-incubation
Control					4
Acetate	^{12}C				6
Acetate	^{13}C				6
Acetate + lepidocrocite	^{12}C	x			6
Acetate + lepidocrocite	^{13}C	x			6
Acetate + sulfate	^{12}C		x		6
Acetate + sulfate	^{13}C		x		6
Acetate + lepidocrocite + molybdate	^{12}C	x		x	4
Acetate + lepidocrocite + molybdate	^{13}C	x		x	6

Table S4.1: Sequencing details SIP incubation samples

Treatment	Isotope	Fraction	Total reads	Total OTUs	Density (g/ml)
Cumberland Bay Acetate	^{12}C	3+4	30386	3000	1.818 – 1.826
		5+6	37248	3184	1.803 – 1.810
		7+8	36816	3194	1.791 – 1.799
		9+10	13656	2006	1.776 – 1.783
		11+12	30136	2849	1.760 – 1.768
	^{13}C	3+4	5410	479	1.814 – 1.822
		5+6	19743	2030	1.799 – 1.806
		7+8	35068	3172	1.783 – 1.791
		9+10	39751	3557	1.768 – 1.776
		11+12	24305	2753	1.753 – 1.760
Cumberland Bay Acetate + lepidocrocite	^{12}C	3+4	25033	2575	1.814 – 1.822
		5+6	27784	3010	1.799 – 1.806
		7+8	46695	3743	1.787 – 1.791
		9+10	35683	3293	1.772 – 1.779
		11+12	24208	2623	1.756 – 1.764
	^{13}C	3+4	14822	995	1.814 – 1.818
		5+6	20291	1958	1.799 – 1.806
		7+8	11846	1886	1.783 – 1.791
		9+10	8732	1616	1.768 – 1.776
		11+12	8416	1483	1.753 – 1.760
Cumberland Bay Acetate + sulfate	^{12}C	3+4	20815	2427	1.818 – 1.822
		5+6	15954	2151	1.799 – 1.806
		7+8	17408	2333	1.783 – 1.791
		9+10	21839	2534	1.772 – 1.776
		11+12	22078	2570	1.756 – 1.764
	^{13}C	3+4	24520	1333	1.814 – 1.822
		5+6	16403	2014	1.799 – 1.806
		7+8	14038	1950	1.783 – 1.791
		9+10	2782	834	1.768 – 1.776
		11+12	31827	3133	1.753 – 1.760

Table S4.2: Sequencing details SIP incubation samples

Treatment	Isotope	Fraction	Total reads	Total OTUs	Density (g/ml)
Cumberland Bay Acetate + lepidocrocite + molybdate	^{12}C	3+4	22646	2470	1.814 – 1.820
		5+6	6721	1324	1.799 – 1.806
		7+8	30945	3186	1.783 – 1.791
		9+10	24045	2689	1.768 – 1.776
		11+12	52866	4049	1.756 – 1.760
	^{13}C	3+4	45440	1620	1.814 – 1.818
		5+6	43504	2954	1.799 – 1.806
		7+8	38599	3482	1.783 – 1.791
		9+10	33612	3752	1.768 – 1.776
		11+12	28149	3196	1.756 – 1.764
Potter Cove Acetate + lepidocrocite	^{12}C	3+4	45543	3806	1.815 – 1.817
		5+6	44232	3689	1.803 – 1.806
		7+8	53991	3738	1.792 – 1.794
		9+10	54971	3609	1.780 – 1.783
		11+12	26062	2756	1.769 – 1.774
	^{13}C	3+4	10703	629	1.820 – 1.826
		5+6	2123	476	1.809 – 1.815
		7+8	31702	3045	1.797 – 1.803
		9+10	15678	2245	1.789 – 1.794
		11+12	20690	2647	1.783 – 1.777

Table S5: Primer details 16S rRNA gene qPCR

Primer	Sequence (5'-3')	Target	Denaturation time	Reference
Bac8Fmod	AGAGTTTGATYMTGGCTCAG	bacteria	15 s	modified from (2)
Bac338Rmod	GCWGCCWCCCGTAGGWGT	bacteria	15 s	modified from (3)
27F	AGAGTTTGATCCTGGCTCAG	bacteria		(4)
Ba1492	GGTTACCTTGTTACGACTT	bacteria		(4)
Ar806F*	ATTAGATACCCSBGTAGTCC	archaea	30 s	(3)
Ar912rt	GTGCTCCCCCGCCAATTCCTTTA	archaea	30 s	(5)
Ar109F	ACKGCTCAGTAACACGT	archaea		(6)
A1492	GGCTACCTTGTTACGACTT	archaea		(4)

* alternative name Arc787F

Supplementary material and methods

Experimental set up for stable isotope probing incubations

Anoxic slurries were prepared by homogenizing sediment with sulfate-free artificial sea water (per liter 26.4 g NaCl, 11.2 g $MgCl_2 \cdot 6\ H_2O$, 1.5 g $CaCl_2 \cdot 2\ H_2O$, 0.7 g KCl, prepared with purified water (Milli-Q)) at a ratio of 1:4 under a stream of nitrogen gas (N_2 5.0). 40 ml slurry was transferred into 120 ml serum bottles sealed with butyl rubber stoppers. The headspace gas was exchanged with N_2. The detailed set up is shown in Table S3. Both C-atoms in acetate were ^{13}C-labelled. Incubation was conducted at 5°C in the dark for a total of 15 days after substrate addition.

When samples for Fe^{2+} measurements were taken anoxically, 1 ml slurry was frozen for later analyses. These samples were subsequently used to determine aqueous sulfate concentrations by fixing 200 µl slurry supernatant in 800 µl 1% zinc acetate. The measurement was performed with a Metrohm 930 Compact IC Flex ion chromatograph (sulfate detection limit 50 µM).

For RNA extraction the slurry of treatment triplicates were pooled and 15 ml were used in order to retrieve sufficient biomass for fractionation.

Sequencing analysis

The sequence read analysis of surface sediment samples was performed as previously described (7) with updated software, using the QIIME 1.9.0 and USEARCH 11.0. For sequencing data of the *in situ* surface sediment samples (2x 150 bp), only the forward reads were used for further analysis and truncated to a minimum sequence length of 143 bp.

For sequences of SIP incubation samples (2x 250 bp), the pipeline was modified in its first steps before de-replication: forward and reverse reads were joined with minimum overlap of 10 bases followed by de-multiplexing and quality filtering to minimum sequence length of 242 bp and expected error of < 0.5 using QIIME 1.9.0 and USEARCH 11.0. The taxonomic assignment was based on the 16S rRNA database Silva release 132 (1).

Unassigned reads or assigned as archaea, chloroplast or mitochondria were removed from the bacterial OTU tables and respectively bacterial and unassigned reads were removed from the archaeal OTU table prior to further analyses. Sequencing details are provided in Table S2 and S4. Rarefaction curves were generated (vegan package (8)) and all samples not reaching the inflection point of the rarefaction curve were removed from the dataset, as their community coverage was considered insufficient (Fig. S2, S3). Differing sample sizes were normalized by scaling OTU abundance to the observation totals in each sample ("relative data"). Separately, the relative abundance of each taxon on all available ranks was summed up, i.e. for all phyla, classes, orders and so on.

Fe^{2+} measurement in molybdate treated incubations – abiotic controls

During the course of the stable isotope probing (SIP) incubations with Cumberland Bay sediments, measured Fe^{2+} concentrations in the treatment containing acetate + lepidocrocite + molybdate was much lower compared to the other incubations, including the control (Fig. S8). However, the microbial community from the sequencing results indicated on-going iron reduction: the same known iron reducing microorganisms as in the other incubations were present and active (Fig. 6). One hypothesis for the lower concentrations of detectable Fe^{2+} concentrations was abiotic reaction of Fe^{2+} from iron reduction with molybdate. To address this hypothesis, supplementary incubations were set up and the findings are discussed below.

Material and methods

Experiments investigating the abiotic reaction of Fe^{2+} and molybdate were set up in 120 ml serum bottles with 40 ml 1:4 slurry containing 10 g Cumberland Bay sediment (gravity core, 0 – 14 cm, same as used for main SIP experiments) and 30 ml artificial sea water (ASW, see supplementary methods above and main text). The slurry was autoclaved and all oxygen removed by flushing with N_2 gas before the substrate was added. Four treatments were set-up

containing 10 mM molybdate (n = 3), 1 mM Fe^{2+} (n = 3, added as $FeCl_2$), both together (n = 2) or only sediment (n = 3).

Fe^{2+} measurements were performed following the ferrozine assay from Viollier *et al.* (9). Fe^{2+} was measured the first time directly after the substrate was added to all treatments, followed by measurements after 6, 26 and 76 h (Fig. S11). During that time, the treatments were incubated at 5°C in the dark.

Results and discussion

The Fe^{2+} concentrations showed clear differences between the treatments (Fig. S11). Lower Fe^{2+} concentrations were measured in the treatment slurry + Fe^{2+} + molybdate (7 – 13 mM) compared to the treatment slurry + Fe^{2+} (11 – 19 mM). Fe^{2+} concentrations in the other treatments slurry only and slurry + molybdate stayed very low between 0.025 – 0.038 mM and 0 – 0.006 mM respectively. An immediate color change was observed in the slurry + Fe^{2+} + molybdate treatment after adding the substrates, but not in any of the other control treatments (Fig. S12). Higher Fe^{2+} concentrations were observed than initial Fe^{2+} was added in according incubations. The addition of Fe^{2+} in the form of $FeCl_2$ lowered the pH in these treatments probably resulting in the elution of Fe^{2+} from the sediment particles.

The observations from these abiotic sediment incubations give clear indication for an abiotic reaction between the added Fe^{2+} and the molybdate, therefore limiting the possibility to measure the exact levels of iron reduction in the acetate, lepidocrocite and molybdate treatments of the initial experiments (Fig. S8A). In summary, based on the results from these abiotic sediment incubations, we argue that in the acetate, lepidocrocite and molybdate treatments (Fig. 6 main text, Fig. S8A), iron reduction was on-going but most of the Fe^{2+} formed reacted abiotically with molybdate.

References

1. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P*, et al.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 2012; **41**: D590-D596.

2. Eden PA, Schmidt TM, Blakemore RP, Pace NR. Phylogenetic analysis of *Aquaspirillum magnetotacticum* using Polymerase Chain Reaction-amplified 16S rRNA-specific DNA. *Int J Syst Evol Microbiol* 1991; **41**: 324-325.

3. Yu Y, Lee C, Kim J, Hwang S. Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. *Biotechnol Bioeng* 2005; **89**: 670-679.

4. Lane DJ. 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds). *Nucleic acid techniques in bacterial systematics*. John Wiley and Sons: New York, 1991, pp 115-175.

5. Lueders T, Friedrich MW. Effects of amendment with ferrihydrite and gypsum on the structure and activity of methanogenic populations in rice field soil. *Appl Environ Microbiol* 2002; **68**: 2484-2494.

6. Großkopf R, Janssen PH, Liesack W. Diversity and structure of the methanogenic community in anoxic rice paddy soil microcosms as examined by cultivation and direct 16S rRNA gene sequence retrieval. *Appl Environ Microbiol* 1998; **64**: 960-969.

7. Aromokeye DA, Richter-Heitmann T, Oni OE, Kulkarni A, Yin X, Kasten S*, et al.* Temperature controls crystalline iron oxide utilization by microbial communities in methanic ferruginous marine sediment incubations. *Front Microbiol* 2018; **9**: 2574.

8. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D*, et al.* vegan: Community Ecology Package, 2.5-6. 2019. Available from: https://CRAN.R-project.org/package=vegan

9. Viollier E, Inglett P, Hunter K, Roychoudhury A, Van Cappellen P. The ferrozine method revisited: Fe(II)/Fe(III) determination in natural waters. *Appl Geochem* 2000; **15**: 785-790.