

Supplementary Material

Analysis of sediment cores from 2017 & 2018

In order to assess the reproducibility of the trends observed in our fine-scale analysis of a single core, four replicate sediment cores were taken from site M5 in Aarhus Bay during both 2017 and 2018. These cores were analyzed with a broader depth resolution than the core from 2014, focusing only on the sediment surface (1-2 cmbsf), the bottom of the bioturbation zone (5 cmbsf), and below (10-50 cmbsf) in 10 cm depth intervals. Within each sample, we analyzed (1) microbial activity (SRR), (2) microbial abundances (qPCR), and (3) community composition (Illumina MiSeq sequencing).

SRR and qPCR were both performed as described in the primary text, while sequencing of the 16S rRNA gene was performed with a modified protocol and a different sequencing technology. Bacteria and Archaea were sequenced separately, using the primer pairs Bac341F/Bac805R (Herlemann et al., 2011) and Arch344Fmod/Arch915R (Casamayor et al., 2002; Xiao et al., 2017), respectively. Bacteria were sequenced in the core collected in 2017 and Archaea were sequenced in the core collected in 2018. Amplicon libraries for Bacteria and Archaea were prepared and sequenced according to Vergeynst *et al.* (2018) and Xiao *et al.* (2017), respectively. All libraries were sequenced on an Illumina MiSeq system at Aarhus University (Aarhus, Denmark). The raw sequencing reads were processed using the package DADA2 (v1.5.0) and the standard DADA2 workflow (Callahan et al., 2016) in R. Amplicon sequence variants (ASVs) were taxonomically classified in DADA2 using the SILVA SSU Ref v1.32 (Quast et al., 2013).

A					
OTU	Phylum	Class	Order	Family	Genus
8	Proteobacteria	Gammaproteobacteria	Chromatiales	Ectothiorhodospiraceae	<i>Acidiferrobacter</i>
9	Proteobacteria	Deltaproteobacteria	Myxococcales	Sandaracinaceae	unclassified
36	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	<i>Desulfobulbus</i>
11	Bacteroidetes	BD2-2	unclassified	unclassified	unclassified
100	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Sva0081 sediment group
39	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Sva1033	unclassified
82	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	OM60(NOR5) clade
14	Proteobacteria	Gammaproteobacteria	Chromatiales	Ectothiorhodospiraceae	<i>Acidiferrobacter</i>
18	Gemmatimonadetes	Gemmatimonadetes	PAUC43f_marine benthic_group	unclassified	unclassified
19	Actinobacteria	Acidimicrobiia	Acidimicrobiales	OM1 clade	unclassified
B					
OTU	Phylum	Class	Order	Family	Genus
10	Atribacteria	unclassified	unclassified	unclassified	unclassified
2	Proteobacteria	Deltaproteobacteria	Desulfarculales	Desulfarculaceae	unclassified
41	Actinobacteria	OPB41	unclassified	unclassified	unclassified
43	Planctomycetes	Phycisphaerae	MSBL9	unclassified	unclassified
12	Deinococcus-Thermus	Deinococci	Thermales	Thermaceae	<i>Oceanithermus</i>
73	Proteobacteria	Deltaproteobacteria	Desulfarculales	Desulfarculaceae	unclassified
47	Other	unclassified	unclassified	unclassified	unclassified
132	Nitrospirae	Nitrospira	Nitrospirales	Nitrospirales_Incertae Sedis	<i>Candidatus Methyloirabilis</i>
20	Spirochaetae	Spirochaetes	Spirochaetales	Spirochaetaceae	Termite Treponema cluster
1	Candidate_division OP8	unclassified	unclassified	unclassified	unclassified

Table S1. Taxonomic identity of OTUs displayed in Figure 4. (a) OTUs that decrease in absolute abundance with sediment depth, displayed in Figure 4a. (b) OTUs that increase in absolute abundance with sediment depth, displayed in Figure 4b.

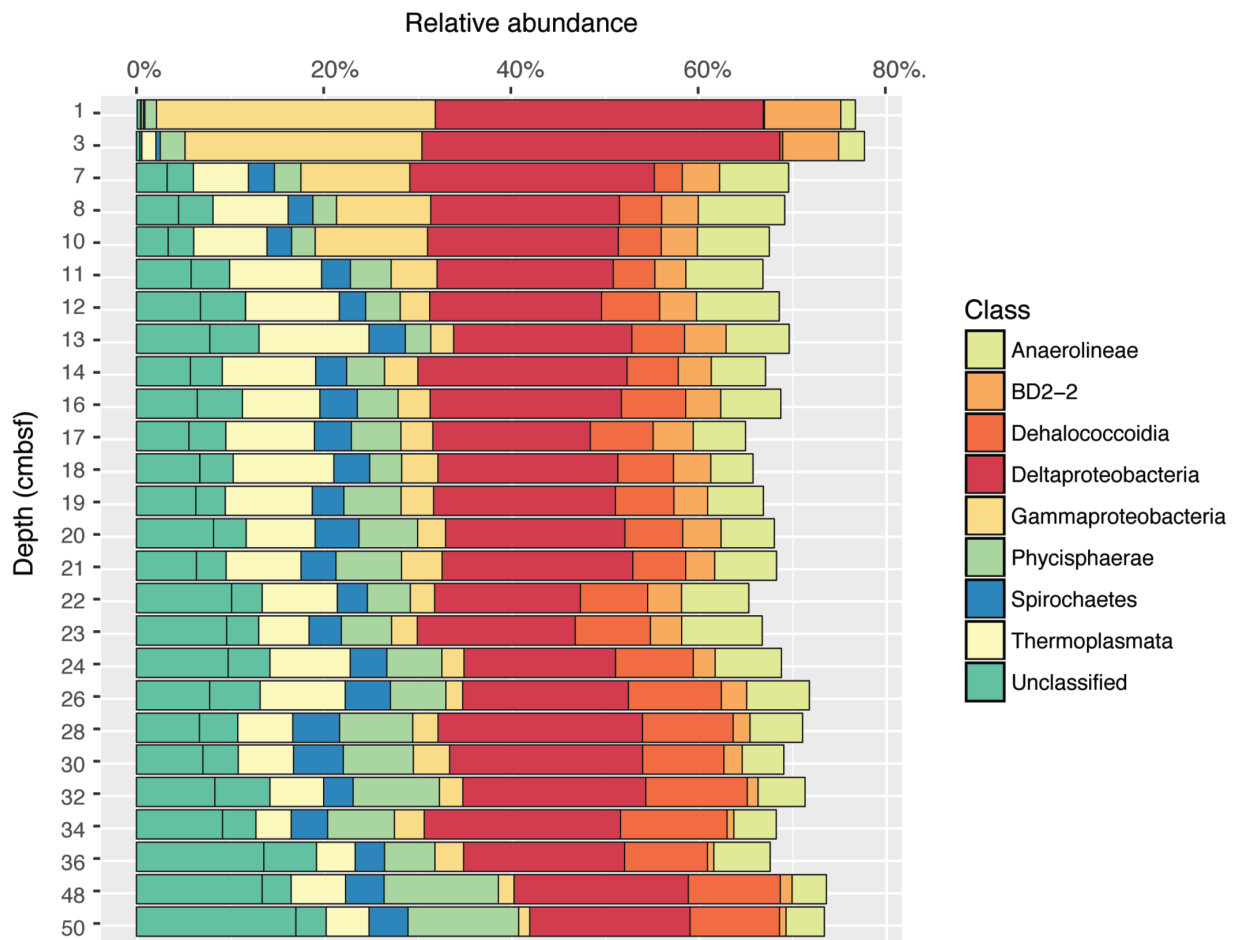


Figure S1. Relative abundance of the top 10 most abundant microbial classes identified by 16S rRNA gene sequencing. Colors correspond to the taxonomic identity at the class level and bar lengths represent the relative abundances of the classes out of the total number of sequencing reads at each depth.

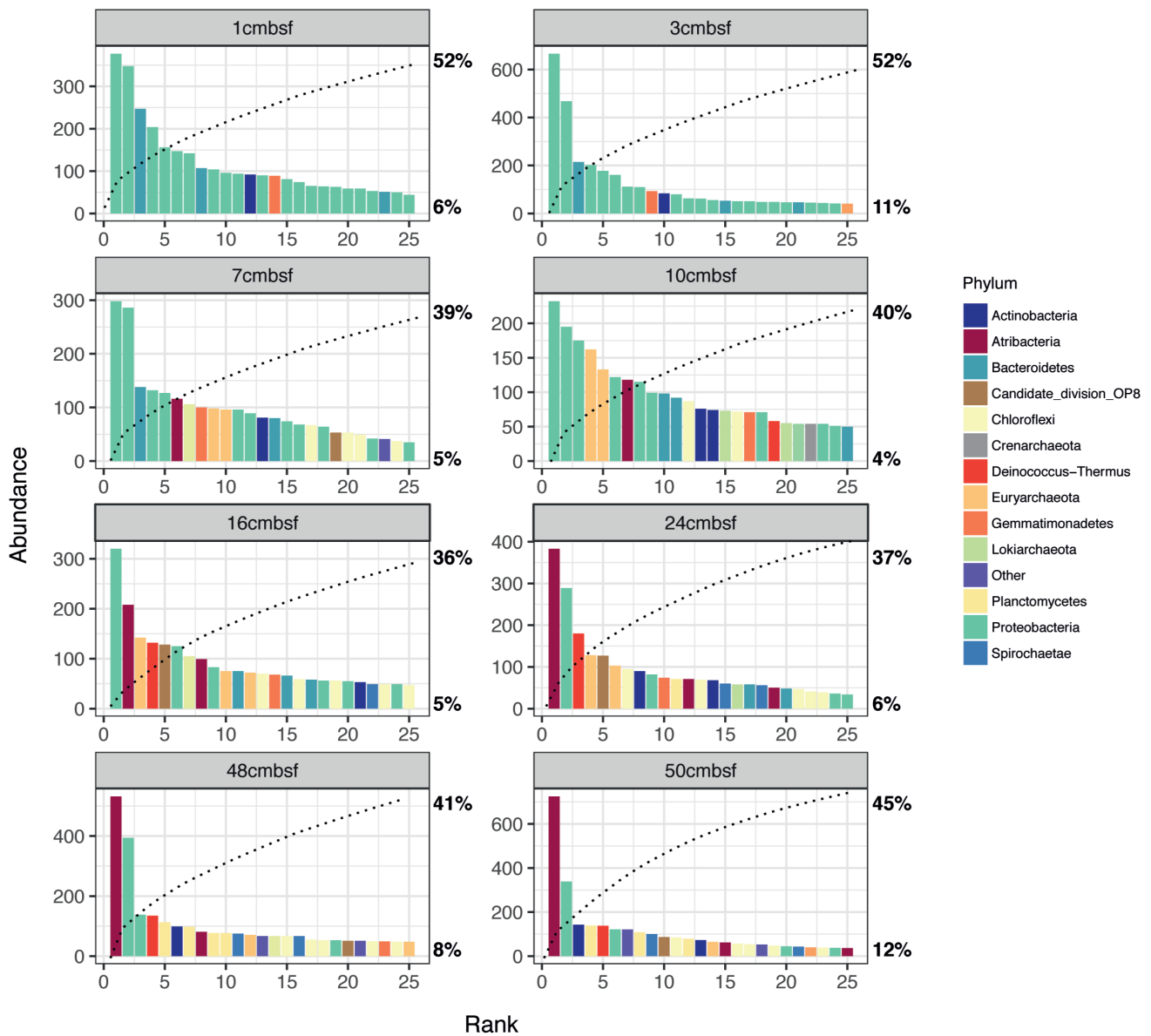


Figure S2. Rank abundance curves of the 25 most abundant OTUs identified by 16S rRNA gene sequencing throughout the sediment depth profile. Each panel displays the 25 most abundant OTUs at the indicated depth. The x-axis shows the rank of each OTU and the primary y-axis shows the number of sequencing reads assigned to each OTU. The colors of the bars indicate the taxonomic identity of each OTU on the phylum level. The dotted lines show the cumulative relative abundance (shown on the secondary y-axis) for the OTUs out of the total number of sequencing reads at that depth.

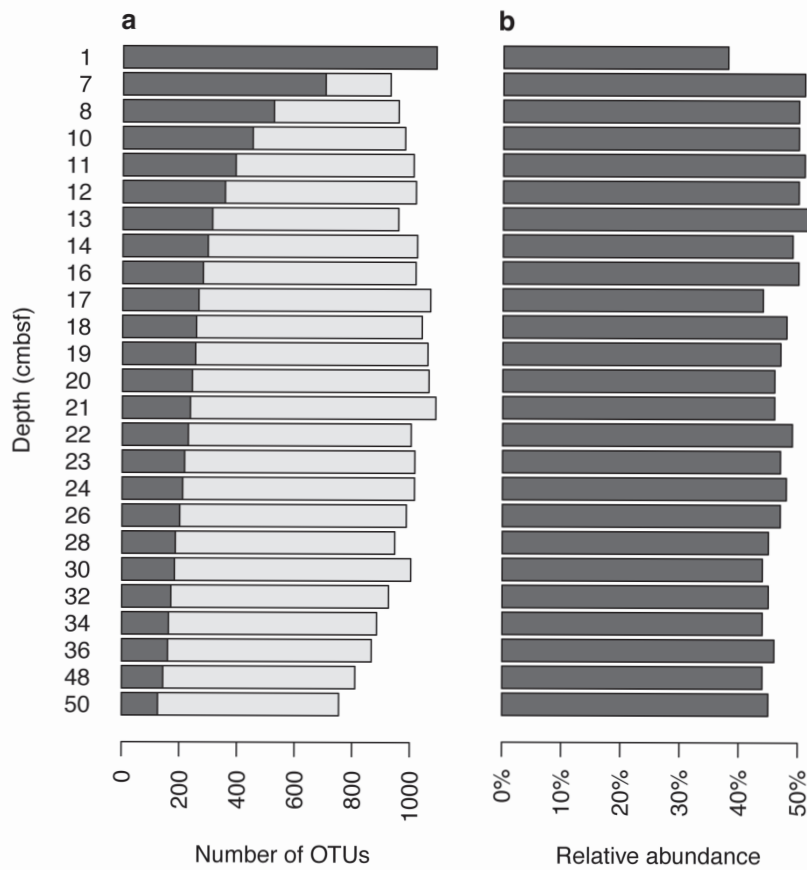


Figure S3. OTUs persisting across sediment depths. (a) Abundance of 16S rRNA gene sequence OTUs that persist across all depths in the sediment core. Light bars show the total number of OTUs present within a depth interval. Dark grey bars show the number of OTUs present within a depth that were also present in all of the above depths. (b) Relative abundance of persisting OTUs at each depth.

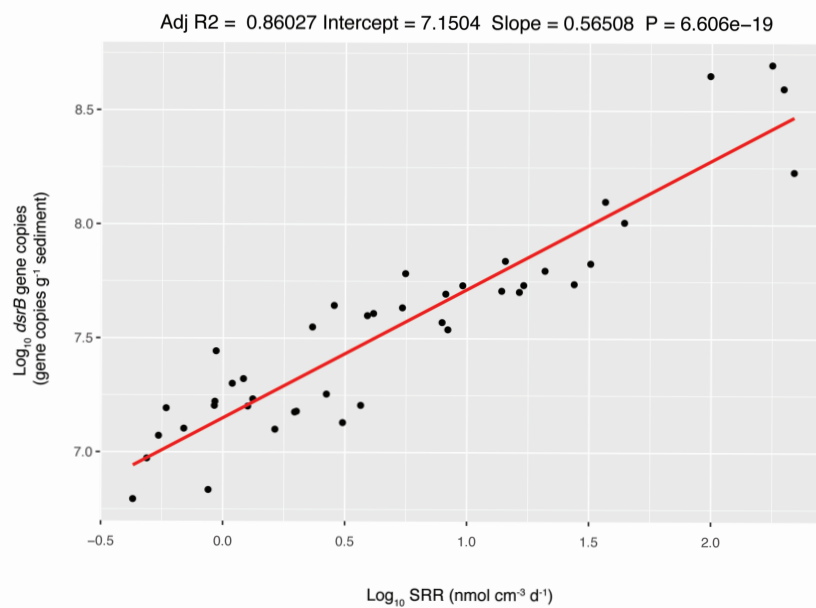


Figure S4. Correlation of *dsrB* gene copy numbers to measured sulfate reduction rates (SRR). P value is Pearson correlation coefficient. The correlation is based on comparing measured values for corresponding sediment depths between 1 and 50 cmbfs.

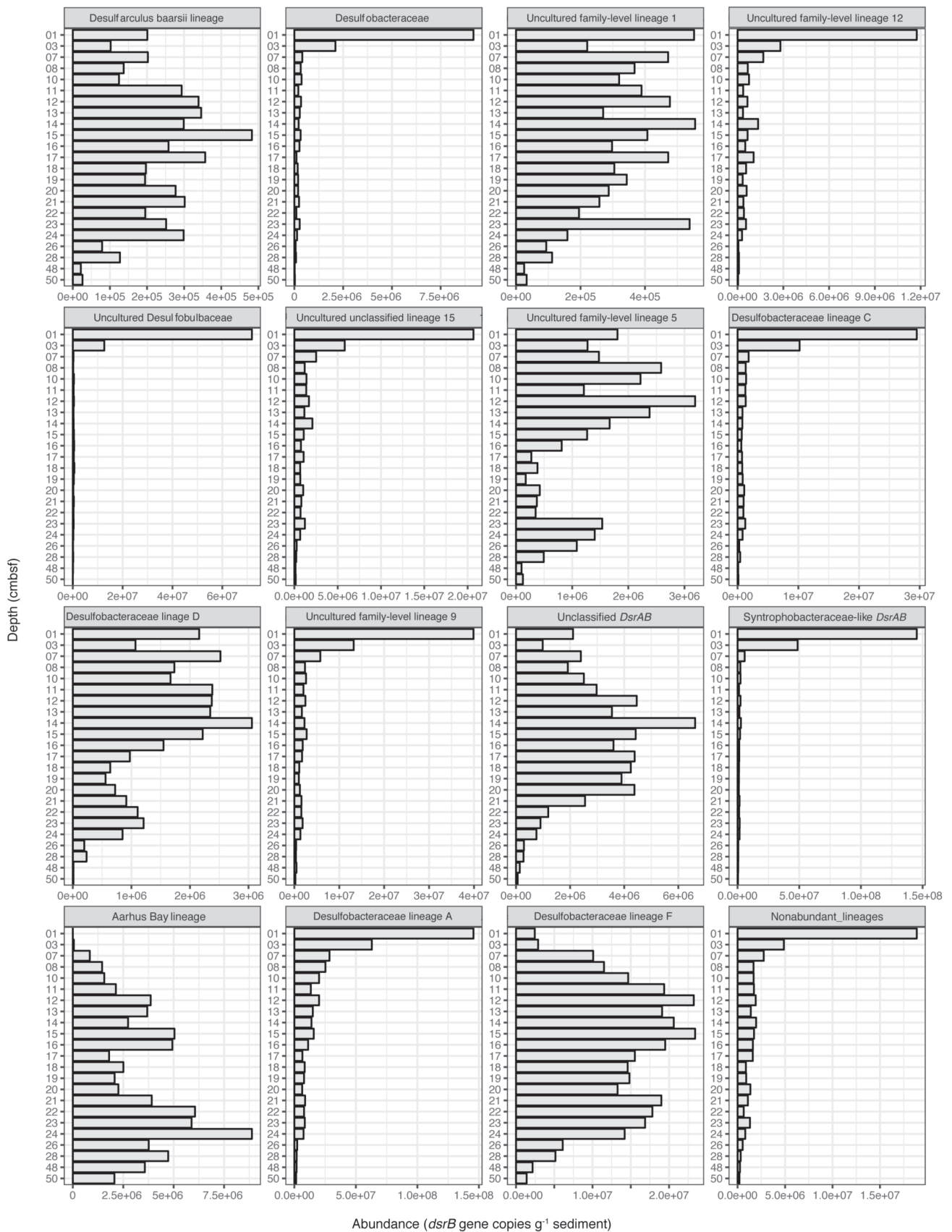


Figure S5. Absolute abundances of dominant lineages of sulfate reducing microorganisms (SRM). Absolute abundances were estimated by multiplying the relative abundances of *dsrB* gene sequences classified within each lineage by total *dsrB* gene copy numbers obtained from qPCR. Pooled nonabundant lineages comprised less than 20% of total sequences at each depth.

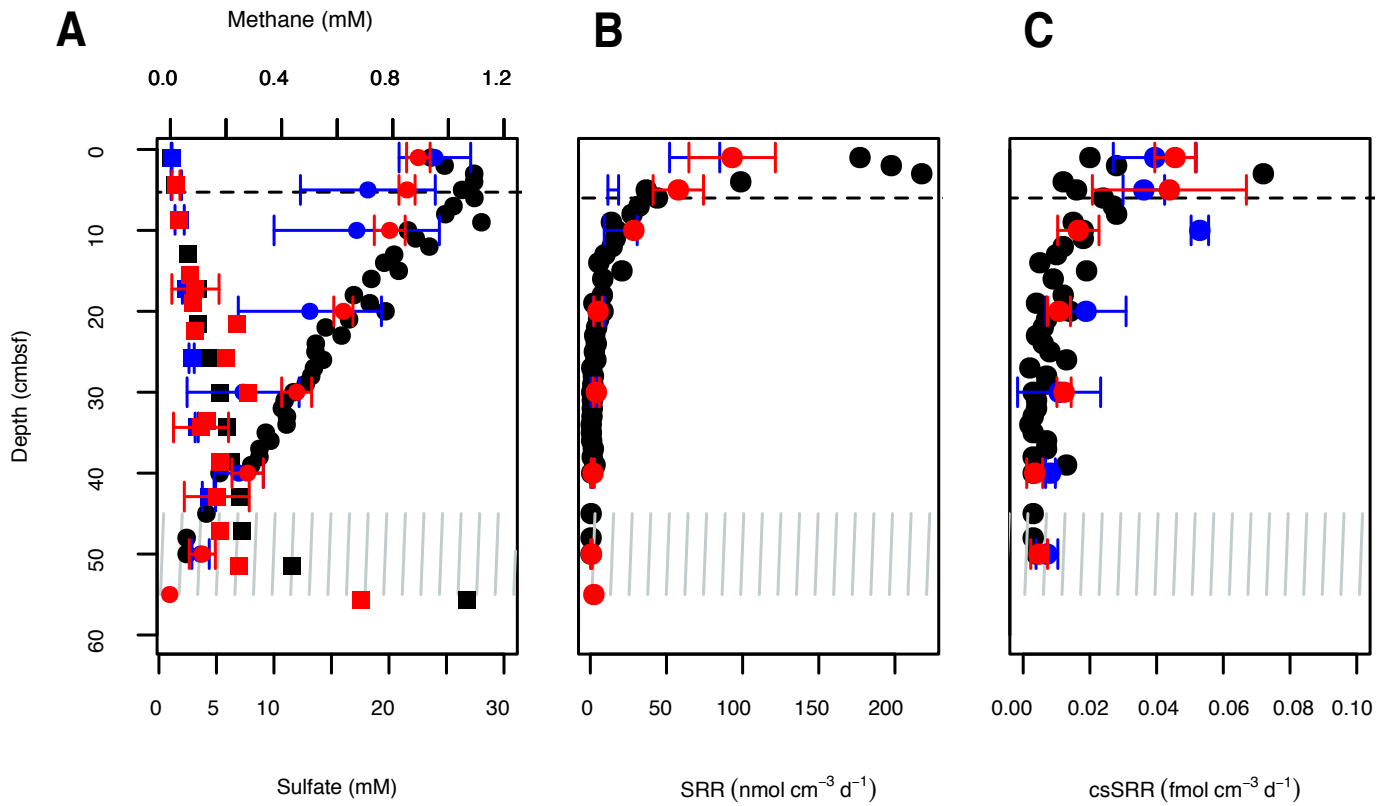
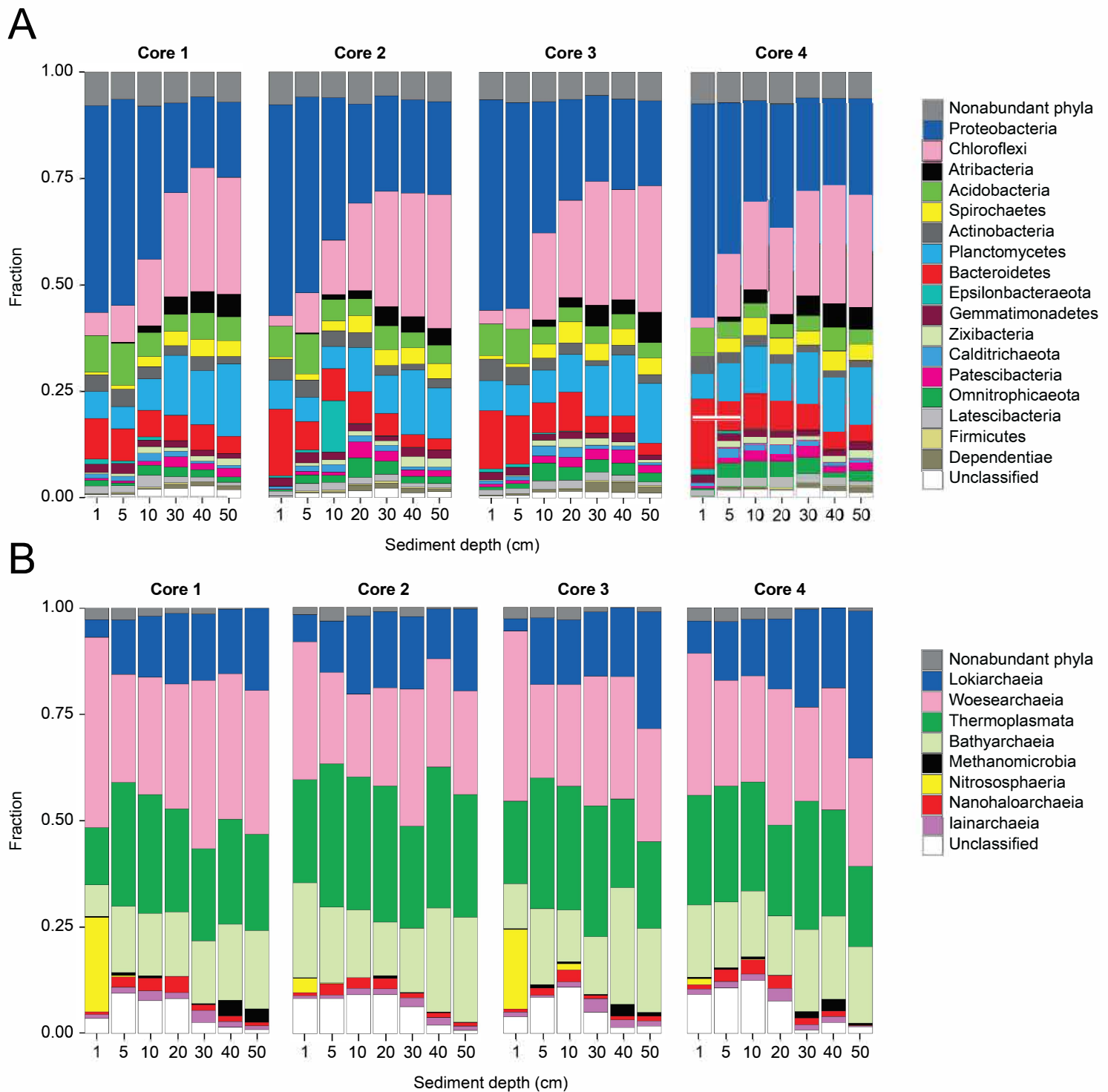


Figure S6. Spatiotemporal variation in (A) sediment porewater concentrations of sulfate and methane, (B) sulfate reduction rates (SRR), and (C) cell-specific sulfate reduction rates estimated using *dsrB* gene copy numbers as a proxy for SRM abundance. *DsrB* gene copy numbers are displayed in Figure S8. For (A) sulfate data are shown by circles and methane data are shown by squares. The color of the symbols refers to the sampling date, with cores taken from 2014 (black, n=1), 2017 (blue, n=4), and 2018 (red, n=4). The grey shaded region indicates the onset of the SMT.



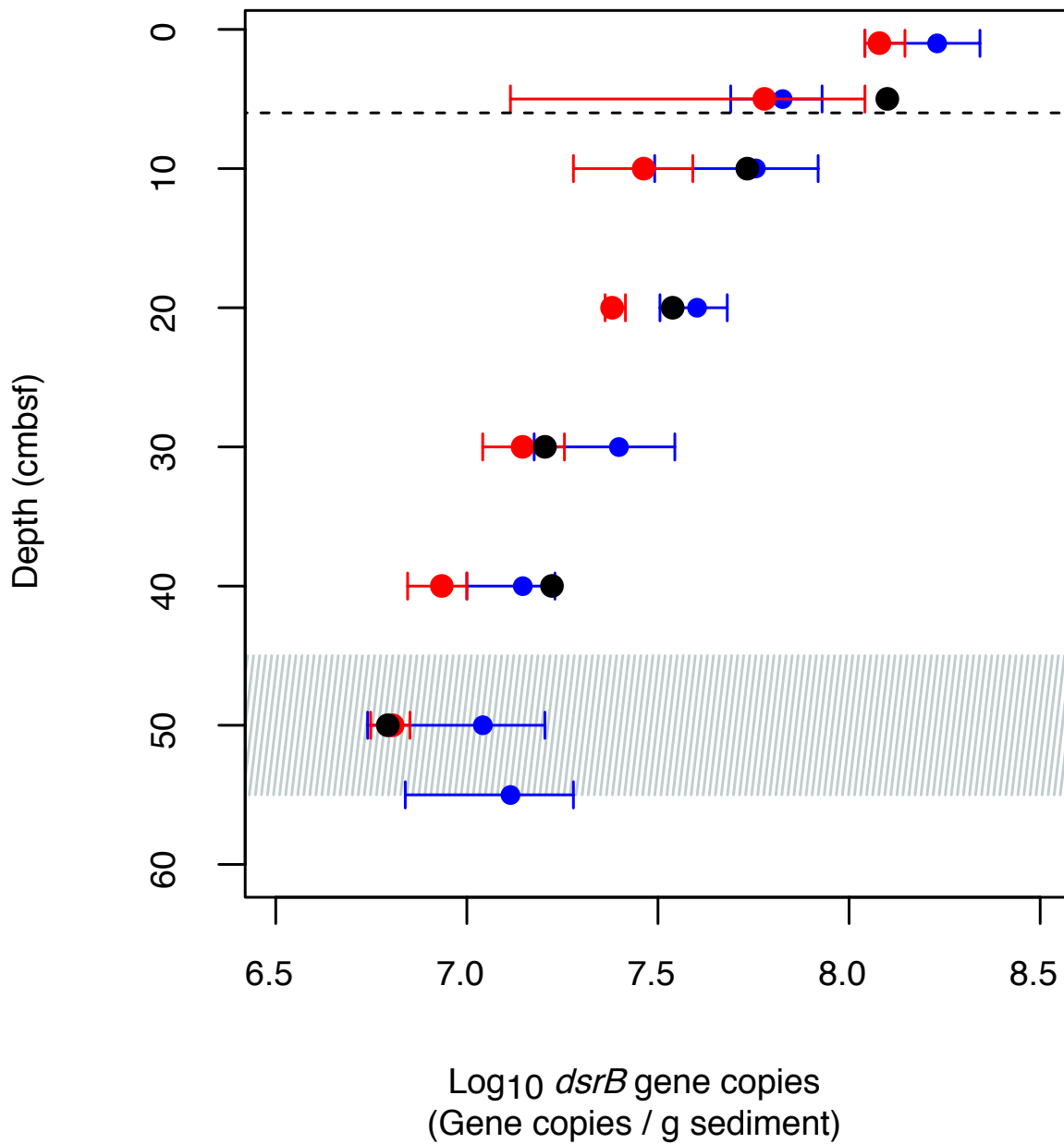


Figure S8. Spatiotemporal variation in abundance of SRM, as measured by qPCR of the *dsrB* gene. The color of the symbols refers to the sampling date, with cores taken from 2014 (black, n=1), 2017 (blue, n=4), and 2018 (red, n=4). The number of *dsrB* gene copies are presented on a log₁₀ scale.

Supplementary References

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