

Figure S1: Relative abundance of the eleven most dominant microbial taxa per sample detected in the metatranscriptome based on rRNA reads ($n = 98.71$ million) and mRNA reads ($n = 751.33$ million) data. Note the relative abundance of Gammaproteobacteria with increasing sediment depth in the rRNA and mRNA datasets, as well as the detection of fungal mRNA from all sediment depths.



Figure S2: The number of sequences, ORFs, and the *E*-value of ORFs (hits to the COG database) used in each of the comparisons in Figures 1, 2, 3 and S3. The corresponding figures are Division, Figure 1(b); *Dsr*, Figure 1(c); *Nar*, Figure 1(d); Amino acid metabolism, Figure 2(a); Carbohydrate metabolism, Figure 2(b); Lipid metabolism, Figure 2(c); Motility, Figure 3(a); DNA repair, Figure 3(b); *Nir*, Figure S3.

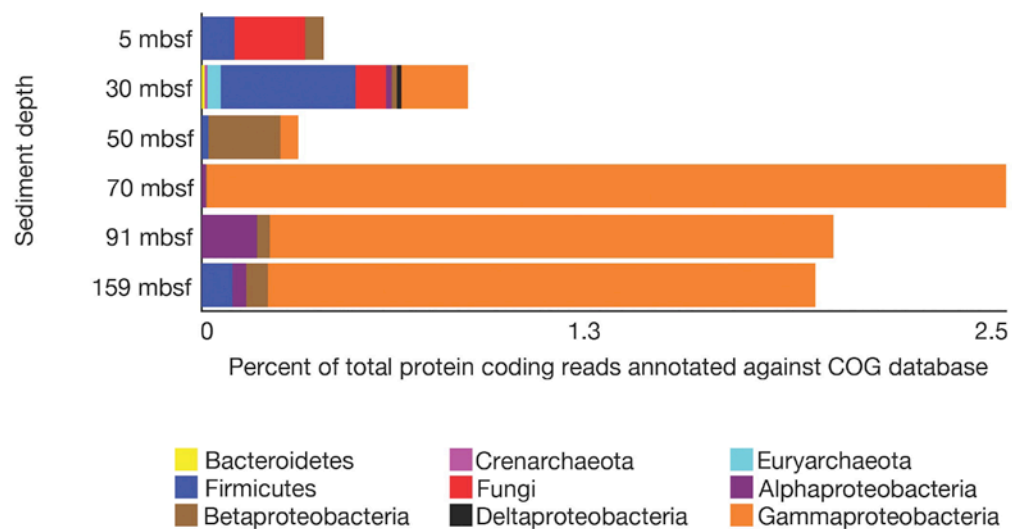


Figure S3: The relative abundance of transcripts mapping to ORFs assigned to nitrite reductase (*NirB*, COG1251). Figure S2 for the number of sequences mapping to *NirB* ORFs, the number of *NirB* ORFs, and the distribution of *E*-values for *NirB* hits in the COG database.

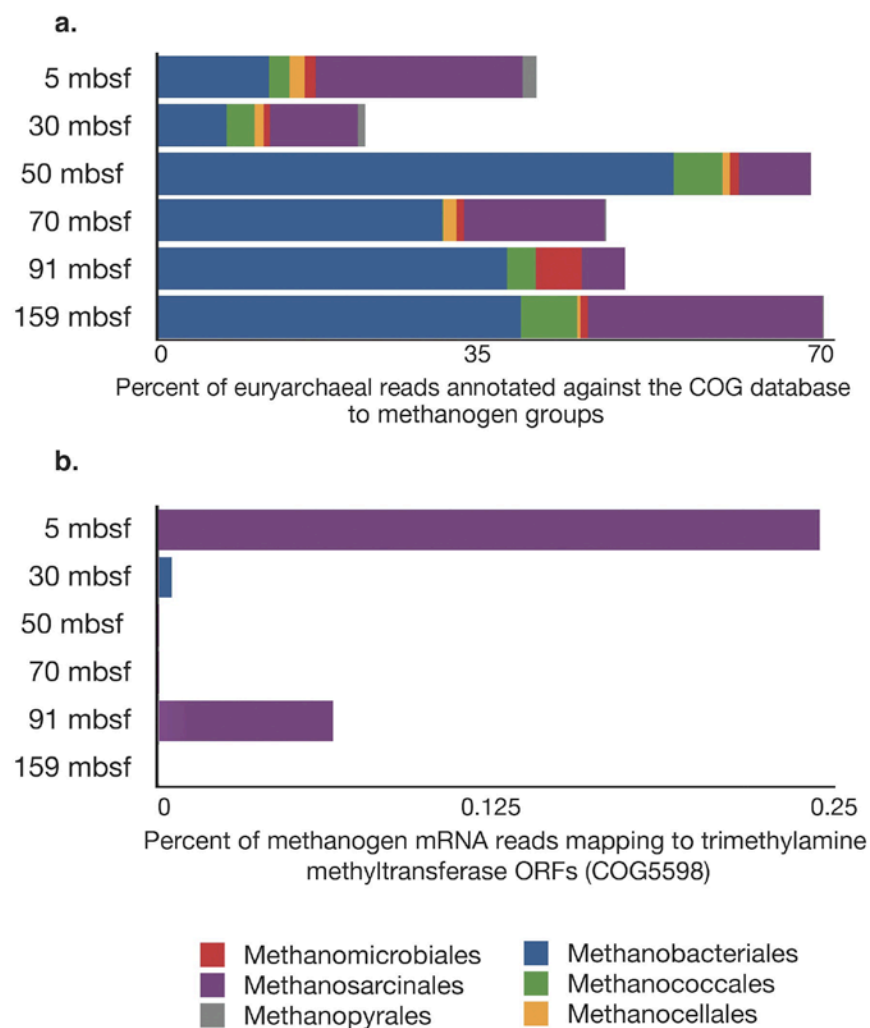


Figure S4: (a) The percentage of euryarchaeal mRNA reads ($n = 5.61$ million) assigned to known methanogenic lineages. **(b)** The relative abundance of reads ($n = 14,209$) assigned to methanogens that map to ORFs annotated as trimethylamine methyltransferase (COG5598).

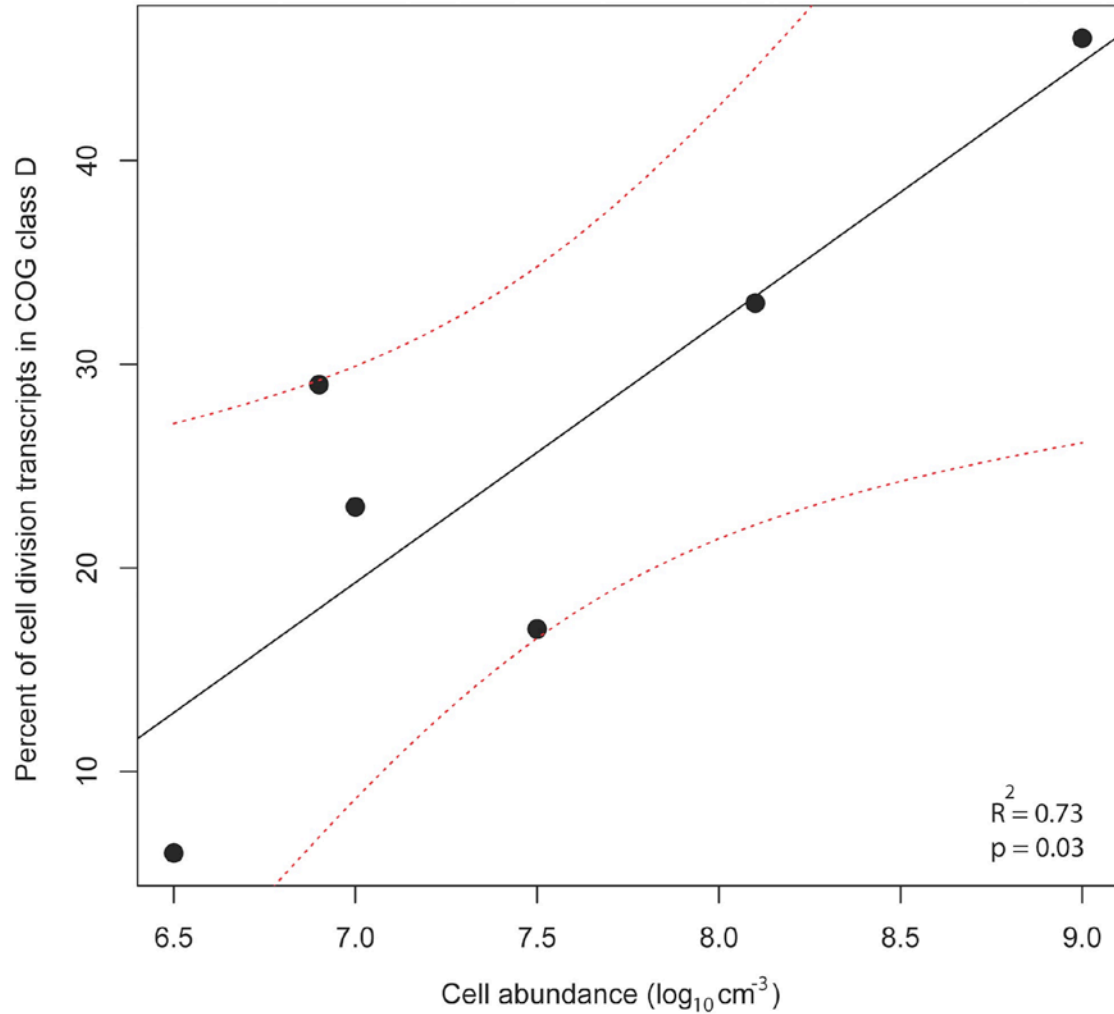


Figure S5: Correlation of cell division transcripts versus cell concentrations ($R^2 = 0.73$, $p = 0.03$) and 95% prediction interval (red dotted lines). The COG class D refers to all transcripts mapping to ORFs annotated to cell cycle control/cell division/chromosome partitioning categories ($n = 30.22$ million) in the COG database. See Table S3 for a list and description of the cell division proteins and Figure S2 for the number of sequences mapping to cell division ORFs, the number of cell division ORFs, and the distribution of *E*-values for hits in the COG database.

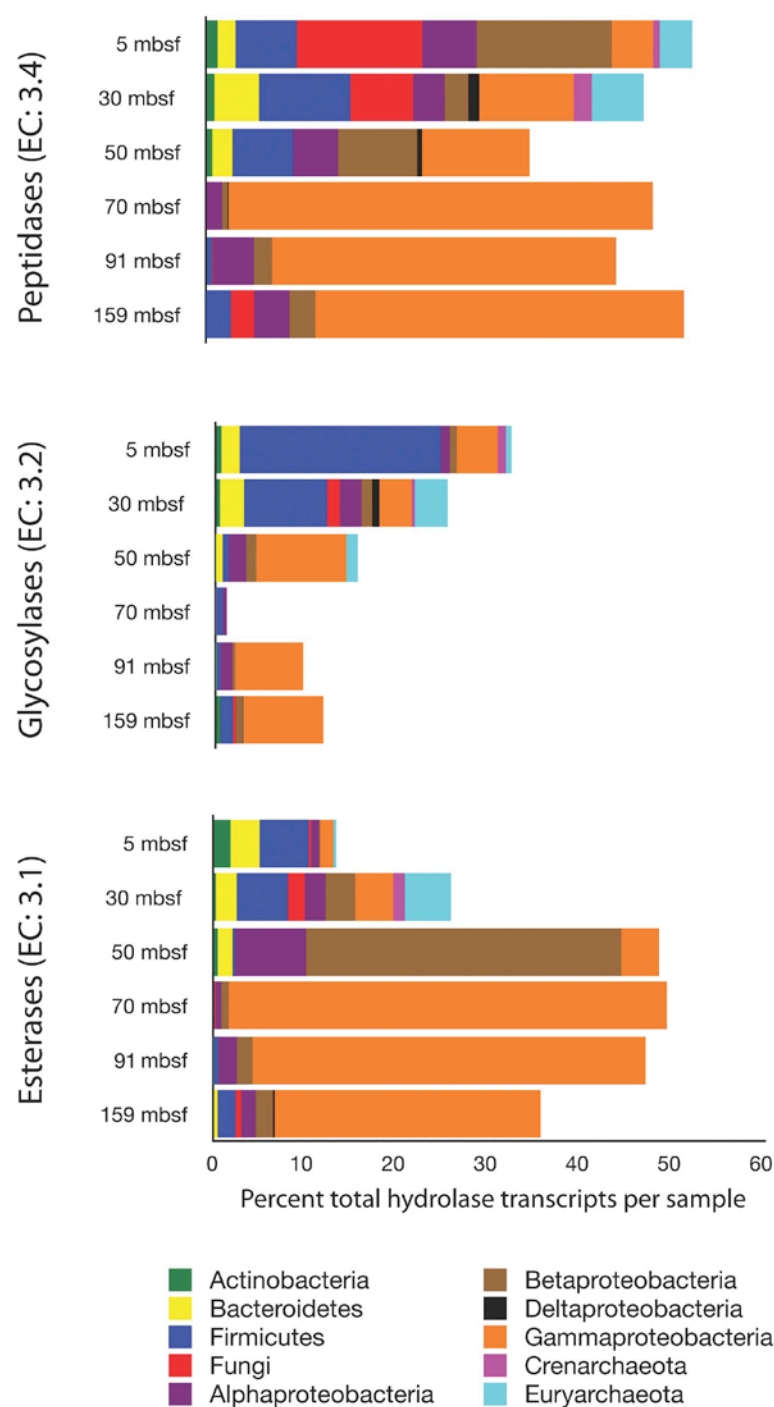


Figure S6: Relative abundance of reads mapping to ORFs coding for hydrolases including glycosylases (n = 2.39 million), esterases (n = 9.38 million), and peptidases (n = 11.39 million). Only the 10 most abundant taxa are presented.

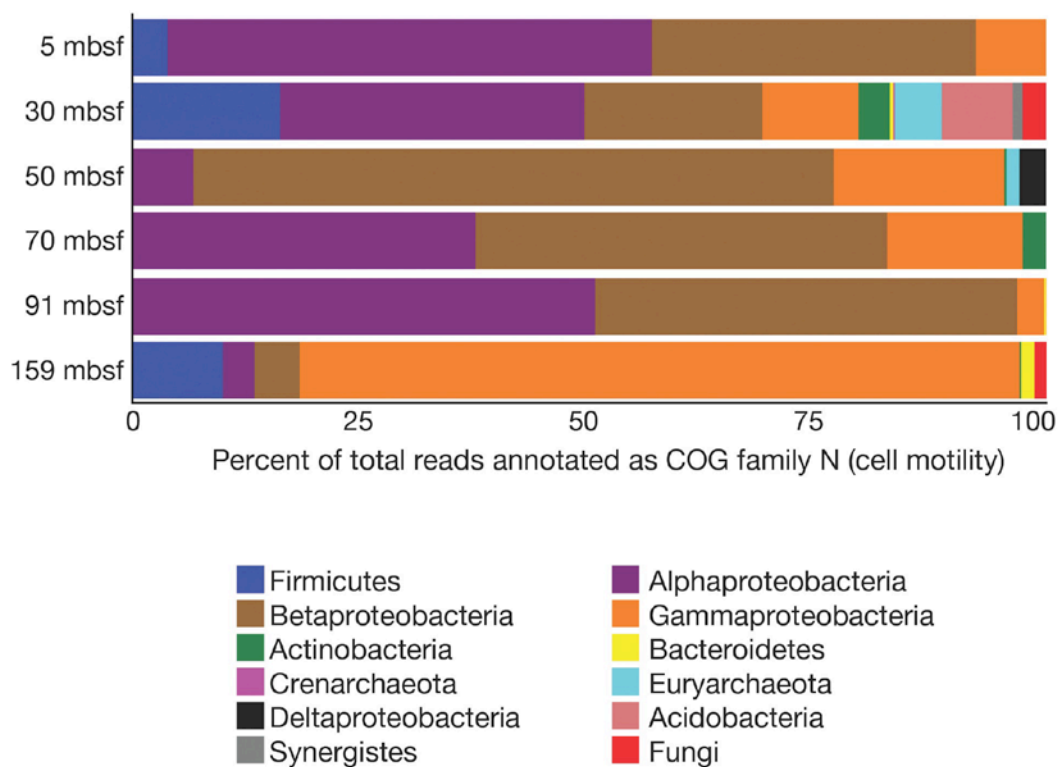
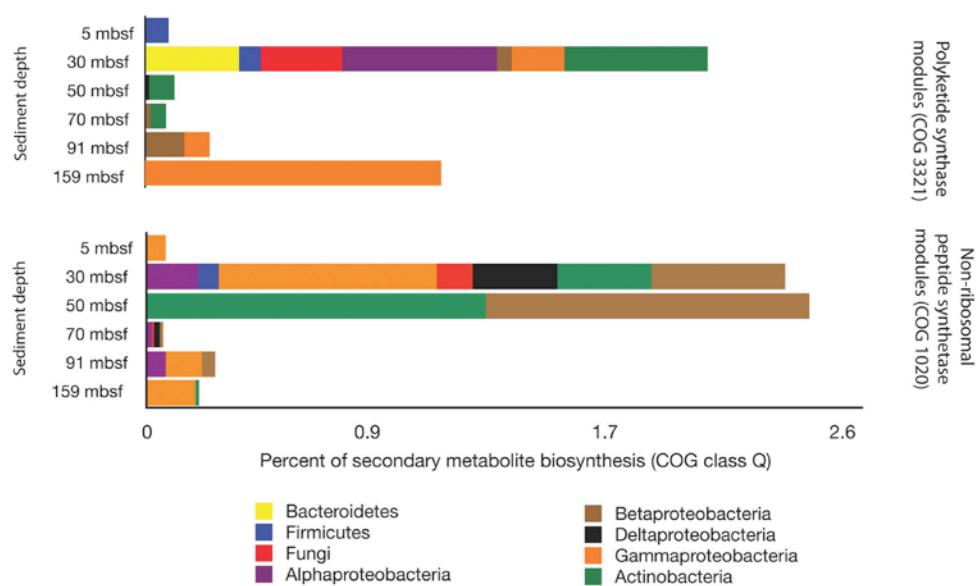


Figure S7: Relative abundance of taxa expressing transcripts that map to ORFs coding for proteins involved in cellular motility (COG family N). See Figure S2 for the number of sequences and ORFs used in the comparison, as well as *E*-values for hits in the COG database.

a.



b.

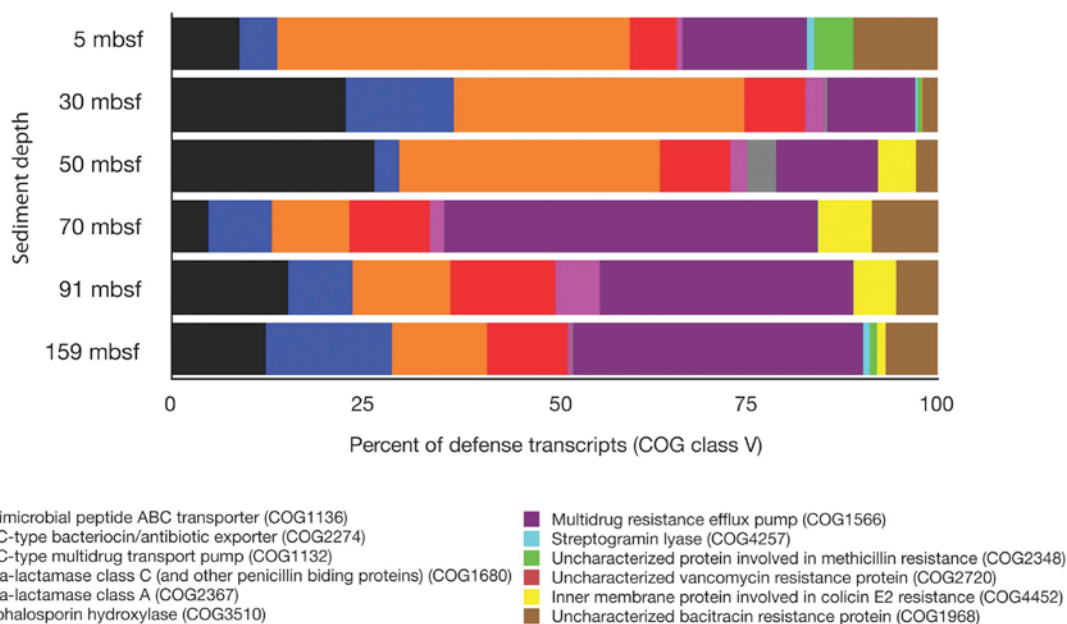


Figure S8: (a) Relative abundance of reads mapping to ORFs coding for polyketide synthase (n = 0.98 million) modules and non-ribosomal peptide synthetases (n = 0.21 million). **(b)** Relative abundance of reads (n = 7.22 million) involved in microbial defense mechanisms.

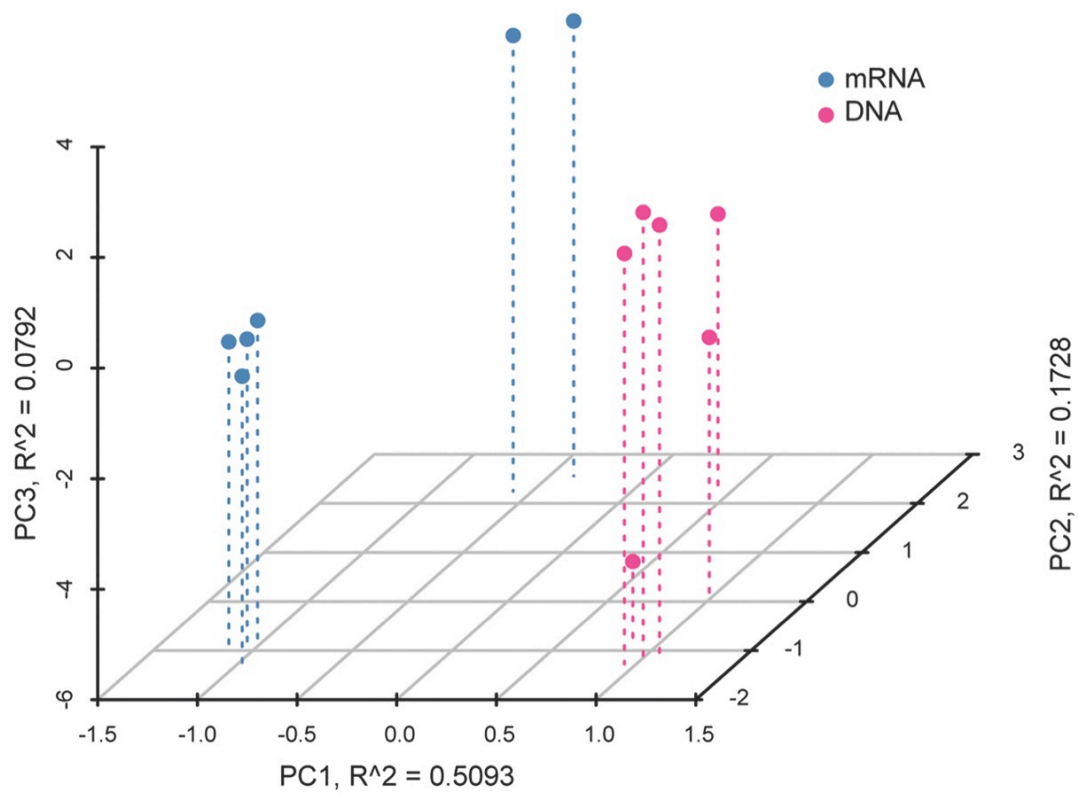


Figure S9: A principal component analysis of functional gene representation (based on hits against the COG database) in gene expression data (blue) and metagenomic data (pink) from two previous studies^{13, 29} from the same site.

Depth (mbsf)	5	30	50	70	91	159
Number of reads (millions)	179.5	224.1	227.6	323.7	340.7	252.5
Number of reads passing QC (millions)	122.7	159.5	157.3	225.1	229.2	181.5
Number of reads (millions) mapping to contigs	89.7	118.8	108.3	183.2	177.1	142.9
Number of contigs assembled	13,571	96,935	18,858	22,732	21,791	26,860
Average contig length	466	582	587	1,075	1,126	914
Number of ORFs annotated against COG database	6436	58024	11459	23094	23245	25015
Annotated protein coding reads	44%	65%	43%	68%	61%	70%
Annotated rRNA reads	6%	5%	31%	7%	5%	14%
Annotated tRNA reads	>1%	>1%	1%	>1%	1%	1%
Total reads annotated	50%	70%	75%	75%	67%	85%

Table S1: A summary of the sequencing effort, quality control (QC), de novo assembly, and annotation of the metatranscriptomic data.

Depth	Reads mapping	COG number	E value	Description	Class description	Archaeal genome ²⁴
30 mbsf	1291	COG0574	1.00E-113	Phosphoenolpyruvate synthase/pyruvate phosphate dikinase	Carbohydrate transport and metabolism	MBG-D_1
	137	COG1908	1.00E-25	Coenzyme F420-reducing hydrogenase, delta subunit	Energy production and conversion	MBG-D_2
	2541	COG2086	7.00E-19	Electron transfer flavoprotein, beta subunit	Energy production and conversion	MBG-D_3
	1849	COG3383	2.00E-09	Uncharacterized anaerobic dehydrogenase	General function prediction only	MBG-D_3
	192	COG0491	4.00E-09	Zn-dependent hydrolases, including glyoxylases	General function prediction only	MCG
	186	COG3185	2.00E-05	4-hydroxyphenylpyruvate dioxygenase	Multiple classes	MCG
	1008	COG0459	3.00E-61	Chaperonin <i>GroEL</i> (HSP60 family)	Posttranslational modification, chaperones	MCG
	2382	COG1397	1.00E-08	ADP-ribosylglycohydrolase	Posttranslational modification, chaperones	MCG
5 mbsf	346	COG1088	2.00E-14	dTDP-D-glucose 4,6-dehydratase	Cell wall/membrane/envelope biogenesis	MBG-D_1
	776	COG1134	4.00E-19	ABC-type polysaccharide/polyol phosphate transport system, ATPase	Multiple classes	MBG-D_1
	306	COG0864	9.00E-34	Predicted transcriptional regulators	Transcription	MBG-D_1
	330	COG2251	7.00E-08	Predicted nuclease (<i>RecB</i> family)	General function prediction only	MBG-D_2
	672	COG1063	5.00E-23	Threonine dehydrogenase and related Zn-dependent dehydrogenases	Multiple classes	MBG-D_2
	572	COG0318	1.00E-26	Acyl-CoA synthetases	Multiple classes	MBG-D_2
	52	COG0443	5.00E-18	Molecular chaperone	Posttranslational modification, chaperones	MBG-D_2
	4576	COG0395	8.00E-20	ABC-type sugar transport system, permease component	Carbohydrate transport and metabolism	MBG-D_3
	2374	COG0279	3.00E-19	Phosphoheptose isomerase	Carbohydrate transport and metabolism	MBG-D_3
	400	COG3640	7.00E-17	CO dehydrogenase maturation factor	Cell cycle control, cell division, chromosome partitioning	MCG
	746	COG0438	2.00E-22	Glycosyltransferase	Cell wall/membrane/envelope biogenesis	MCG
	4368	COG0399	1.00E-07	Predicted pyridoxal phosphate-dependent enzyme	Cell wall/membrane/envelope biogenesis	MCG
	1016	COG2088	7.00E-05	Uncharacterized protein, involved in the regulation of septum location	Cell wall/membrane/envelope biogenesis	MCG
	159	COG0414	8.00E-10	Pantothenate synthetase	Coenzyme transport and metabolism	MCG
	11372	COG0451	4.00E-07	Nucleoside-diphosphate-sugar epimerases	Multiple classes	MCG
	980	COG0606	9.00E-21	Predicted ATPase with chaperone activity	Posttranslational modification, chaperones	MCG
	550	COG2368	9.00E-58	Aromatic ring hydroxylase	Secondary metabolites biosynthesis	MCG
	1085	COG2012	2.00E-18	DNA-directed RNA polymerase, subunit H, <i>RpoH</i>	Transcription	MCG
91 mbsf	16	COG1355	2.00E-15	Predicted dioxygenase	General function prediction only	MBG-D_1
	80460	COG1835	6.00E-48	Predicted acyltransferases	Lipid transport and metabolism	MBG-D_1
	12	COG1100	2.00E-21	GTPase SAR1 and related small G proteins	General function prediction only	MBG-D_2
	24	COG0667	2.00E-15	Predicted oxidoreductases	Energy production and conversion	MBG-D_2
	5463	COG0001	1.00E-34	Glutamate-1-semialdehyde aminotransferase	Coenzyme transport and metabolism	MBG-D_3
	24	COG5434	3.00E-15	Endopolygalacturonase	Cell wall/membrane/envelope biogenesis	MBG-D_3
	18	COG1156	3.00E-61	Archaeal/vacuolar-type H ⁺ -ATPase subunit B	Energy production and conversion	MBG-D_3
	24	COG0097	1.00E-05	Ribosomal protein L6P/L9E	Translation, ribosomal structure and biogenesis	MBG-D_3
	26	COG0031	3.00E-52	Cysteine synthase	Amino acid transport and metabolism	MBG-D_3
	70	COG0247	7.00E-10	Fe-S oxidoreductase	Energy production and conversion	MBG-D_3
70 mbsf	4942	COG0463	2.00E-05	Glycosyltransferases involved in cell wall biogenesis	Cell wall/membrane/envelope biogenesis	MBG-D_1
	946	COG1285	2.00E-24	Uncharacterized membrane protein	Function unknown	MBG-D_1
	27	COG0667	6.00E-12	Predicted oxidoreductases	Energy production and conversion	MBG-D_2
50 mbsf	40	COG0018	3.00E-17	Arginyl-tRNA synthetase	Translation, ribosomal structure and biogenesis	MCG_1

Table S2: COG annotations of ORFs (rows) from the gene expression data that were assigned to MCG and MBG partial genomes²⁴ from a shallow sediment site.

Cell division	Motility	DNA repair
COG2919: Septum formation initiator	COG1344: Flagellin and related hook-associated proteins	COG0249: Mismatch repair ATPase <i>MutS</i>
COG0445: NAD/FAD-utilizing enzyme involved in cell division (<i>GidA</i>)	COG1516: Flagellin-specific chaperone <i>FliS</i>	COG0323: Mismatch repair ATPase <i>MutL</i>
COG3115: Cell division protein <i>ZipA</i>	COG1868: Flagellar motor switch protein	COG0353: Recombinational DNA repair protein <i>RecF</i>
COG2917: Intracellular septation protein A	COG1536: Flagellar motor switch protein	COG0389: Nucleotidyltransferase/ DNA polymerase involved in DNA repair <i>DinP</i>
COG0851: Septum formation specificity factor <i>MinE</i>	COG1360: Flagellar motor protein	COG0419: ATPase involved in DNA repair <i>SbcC</i>
COG0772: Cell division protein <i>FtsW</i>	COG1291: Flagellar motor component	COG0420: DNA repair exonuclease <i>SbcD</i>
COG0849: Cell division ATPase <i>FtsA</i>	COG3144: Flagellar hook-length control protein	COG0497: ATPase involved in DNA repair <i>RecN</i>
COG0206: Cell division GTPase <i>FtsZ</i>	COG1256: Flagellar hook-associated protein	COG1195: DNA repair ATPase <i>RecF</i>
COG0849: Cell division ATPase <i>FtsA</i>	COG1749: Flagellar hook protein <i>FlgE</i>	COG2003: DNA repair proteins <i>RadC</i>
COG3116: Cell division protein <i>FtsL</i>	COG1843: Flagellar hook capping protein	COG3145: Alkylated DNA repair protein <i>AlkB</i>
COG3087: Cell division protein <i>FtsN</i>	COG1419: Flagellar GTP-binding protein	COG3663: Mismatch-specific DNA glycosylase
COG2177: Cell division protein <i>FtsX</i>	COG1345: Flagellar capping protein	COG3723: DNA repair protein <i>RecE</i>
COG2184: Cell division protein <i>Fic</i>	COG5443: Flagellar biosynthesis regulator <i>FlbT</i>	COG3727: DNA G:T- mismatch repair endonuclease
COG2884: Cell division ATPase <i>FtsE</i>	COG5442: Flagellar biosynthesis regulator <i>FlaF</i>	COG1107: Archaea-specific RecJ-like exonuclease
COG3599: Cell division initiation protein <i>DivIVA</i>	COG1298: Flagellar biosynthesis pathway, component <i>FlhA</i>	COG0419: ATPase involved in DNA repair
	COG1377: Flagellar biosynthesis pathway, component <i>FlhB</i>	COG0497: ATPase involved in DNA repair
	COG1338: Flagellar biosynthesis pathway, component <i>FlhP</i>	COG0178: Excinuclease <i>uvrA</i> , ATPase subunit
	COG1987: Flagellar biosynthesis pathway, component <i>FlhQ</i>	COG0648: Endonuclease IV (<i>Nfo</i>)
	COG1684: Flagellar biosynthesis pathway, component <i>FlhR</i>	COG0708: Exonuclease III (<i>XthA</i>)
	COG3190: Flagellar biogenesis protein	COG1330: Exonuclease V (<i>RecC</i>)
	COG1706: Flagellar basal-body P-ring protein	COG1570: Exonuclease VII
	COG1580: Flagellar basal body-associated protein	COG1515: Endonuclease V
	COG4787: Flagellar basal body rod protein	COG3857: ATP-dependent nuclease (<i>AddB</i>)
	COG1558: Flagellar basal body rod protein	COG4335: DNA alkylation repair enzyme
	COG4786: Flagellar basal body rod protein	COG2925: Exonuclease I
	COG1815: Flagellar basal body protein	COG0556: DNA excision repair complex
	COG2063: Flagellar basal body L-ring protein	
	COG1955: Archaeal flagella assembly protein J	
	COG2805: Pilus assembly protein, pilus retraction ATPase <i>PilT</i>	
	COG3063: Pilus assembly protein <i>PilF</i>	
	COG3167: Pilus assembly protein <i>PilO</i>	
	COG3215: Pilus assembly protein <i>PilZ</i>	
	COG3225: Gliding motility-associated lipoprotein <i>Gldg</i>	
	COG4726: Pilus assembly protein <i>PilX</i>	
	COG4961: Pilus assembly protein <i>TadG</i>	
	COG4962: Pilus assembly protein, ATPase <i>CpaF</i>	
	COG4964: Pilus assembly protein, secretin <i>CpaC</i>	
	COG4968: Pilus assembly protein <i>PilE</i>	
	COG4969: Pilus assembly protein, major pilin <i>PilA</i>	
	COG5010: Pilus assembly protein <i>TadD</i>	

Table S3: Descriptions and COG categories of proteins involved in cell division, motility, and DNA repair that were detected in the metatranscriptome.