
**Methane Filtration and Metabolic Cooperation of Microbial
Communities in Cold Seep Water Columns from South China Sea**

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20 **Supplementary materials**

21 **Supplementary Figure 1** KEGG annotation of the metagenomic reads indicating
22 relative abundance of key genes for important metabolic processes.

23 **Supplementary Table 1** The information of samples in this study.

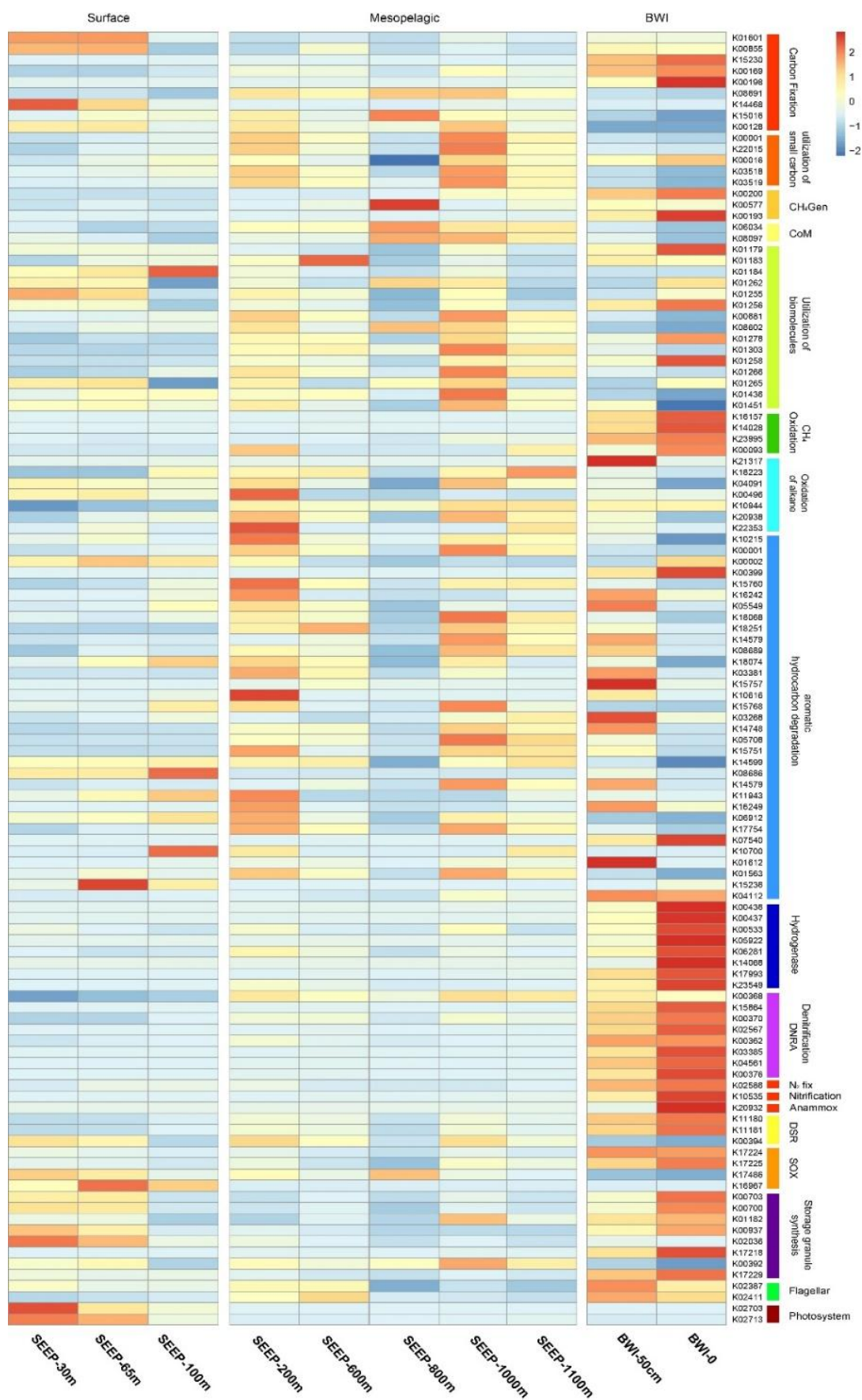
24 **Supplementary Table 2** Profiles of environmental factors in the water columns of
25 cold seep.

26 **Supplementary Table 3** The relative abundance (%) of the TOP 20 abundant phyla in
27 cold seep water columns.

28 **Supplementary Table 4** The relative abundance (%) of the TOP 20 abundant orders
29 in cold seep water columns.

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Supplementary Figure 1 KEGG annotation of the metagenomic reads indicating relative abundance of key genes for important metabolic processes. In the surface zone, pathways for photosynthesis and oxidative phosphorylation were enriched, indicating the role of photosynthetic and heterotrophic bacteria. At 100m depth, genes for secondary metabolite biosynthesis suggested potential microbial resource development. The mesopelagic zone shifted from autotrophic carbon fixation to organic carbon degradation. In the BWI zone, KOs linked to methane metabolism were prevalent, reflecting high methane fluxes. Aromatic compound degradation pathways were abundant, highlighting microbial activity. Inorganic CO₂ fixation pathways, particularly rTCA and WL, and nitrogen metabolism, emphasized carbon-nitrogen coupling. Additionally, pathways for biofilm formation and microbial interactions with invertebrates were significant, showcasing the complex ecological dynamics in this layer.



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Supplementary Table 1 The information of samples in this study.

Sample	Description	Clean data accession no.	Total number of bases	GC %
BWI-0	Fluid at the venting seepage	SRR29288234	50,740,837,868	39.03
BWI-50cm	Seawater 50cm above the venting seepage	SRR29288233	47,939,762,242	41.80
SEEP-1100m	Seawater at the depth of 1100 m	SRR29288232	44,916,768,480	48.48
SEEP-1000m	Seawater at the depth of 1000 m	SRR29288231	62,575,200,078	49.74
SEEP-800m	Seawater at the depth of 800 m	SRR29288230	38,353,995,388	34.22
SEEP-600m	Seawater at the depth of 600 m	SRR29288229	41,306,383,390	47.41
SEEP-200m	Seawater at the depth of 200 m	SRR29288228	53,425,880,718	51.47
SEEP-100m	Seawater at the depth of 10 m	SRR29288227	57,446,422,664	53.85
SEEP-65m	Seawater at the depth of 65 m	SRR29288226	50,310,423,152	49.03
SEEP-30m	Seawater at the depth of 30 m	SRR29288225	55,715,461,781	47.97

Supplementary Table 2 Profiles of environmental factors in the water columns of cold seep

Sample	S (‰)	T (°C)	DO (mg/L)	CH ₄ (nmol/L)	NO ₃ ⁻ (μmol/L)	NO ₂ ⁻ (μmol/L)	NH ₄ ⁺ (μmol/L)
SEEP-30m	34.33	30.50	6.20	2.91	0.13	0.19	0.68
SEEP-65m	34.55	22.50	5.85	2.77	0.35	0.49	2.11
SEEP-100m	34.66	22.00	5.27	2.35	3.89	0.32	1.71
SEEP-200m	34.63	17.50	5.11	2.55	4.21	0.21	1.52
SEEP-600m	34.45	9.00	3.29	2.23	13.57	0.24	1.01
SEEP-800m	34.46	5.54	3.08	2.35	18.77	0.27	1.72
SEEP-1000m	34.51	5.10	3.06	2.49	20.23	0.31	1.33
SEEP-1100m	34.53	4.00	3.10	8.83	27.45	0.34	2.12
BWI-0	34.64	3.59	3.17	387.20	22.33	3.22	5.12

51 **Supplementary Table 3 The relative abundance (%) of the TOP 20 abundant**
52 **phyla in cold seep water columns.**

Phylum	SEEP- 30m	SEEP- 65m	SEEP- 100m	SEEP- 200m	SEEP- 600m	SEEP- 800m	SEEP- 1000m	SEEP- 1100m	BWI- 50cm	BWI- 0
Proteobacteria	49.26	43.05	37.01	45.18	60.10	49.87	30.90	36.68	39.73	28.13
Cyanobacteria	23.47	20.87	7.06	0.88	0.71	2.26	2.47	2.52	0.43	0.29
Marinimicrobia (SAR406)	4.48	5.43	11.26	8.26	6.88	10.10	12.28	10.00	2.60	0.46
*Thermoplasmatota	6.82	8.89	13.00	6.99	3.87	3.82	8.53	5.32	1.60	0.29
Bacteroidota	2.17	4.03	1.79	1.61	1.49	1.61	1.49	2.16	12.73	30.21
*Crenarchaeota	0.01	1.46	3.10	9.17	5.11	11.80	8.31	11.45	2.62	0.48
SAR324 clade	3.68	2.69	10.98	7.45	4.31	3.73	5.70	4.44	1.32	0.22
Actinobacteriota	4.26	6.05	2.01	3.85	2.33	1.76	5.05	3.52	0.85	0.24
Campylobacterota	0.02	0.02	0.01	0.03	0.32	0.25	0.08	0.10	21.30	18.45
Chloroflexi	0.79	1.14	2.76	3.88	2.19	1.82	6.07	3.69	1.02	0.34
Verrucomicrobiota	0.84	0.73	2.19	2.12	1.40	1.75	2.10	2.81	1.35	4.64
Planctomycetota	0.27	0.61	0.80	1.21	1.27	1.35	1.85	2.91	0.88	0.77
Firmicutes	0.20	0.14	0.23	0.28	0.55	0.45	0.62	0.51	1.37	1.59
Desulfobacterota	0.21	0.33	0.20	0.22	0.25	0.21	0.28	0.39	0.91	3.82
Nitrospinota	0.00	0.01	1.11	1.30	1.40	1.14	1.29	1.28	0.25	0.04
Bdellovibrionota	0.54	0.58	1.12	0.54	0.32	0.39	0.54	0.74	0.57	0.69
Acidobacteriota	0.01	0.01	0.03	0.75	0.73	0.59	3.33	2.20	0.36	0.08
Patescibacteria	0.02	0.02	0.02	0.01	0.06	0.16	0.09	0.12	2.69	2.08
Myxococcota	0.10	0.22	0.71	0.42	0.27	0.24	0.54	0.60	0.18	0.13
NB1-j	0.00	0.04	0.10	0.23	0.39	0.29	0.71	0.89	0.16	0.03

53 *Crenarchaeota and Thermoplasmatota belong to Archaea, others belong to Bacteria.

54 **Supplementary Table 4 The relative abundance (%) of the TOP 20 abundant**
55 **orders in cold seep water columns.**

Class; Order	SEEP- 30m	SEEP- 65m	SEEP- 100m	SEEP- 200m	SEEP- 600m	SEEP- 800m	SEEP- 1000m	SEEP- 1100m	BWI- 50cm	BWI- 0
Alphaproteobacteria; SAR11 clade	25.26	20.23	15.19	13.13	8.08	15.33	8.60	10.96	2.21	0.63
Alphaproteobacteria; Rickettsiales	1.23	1.58	1.12	0.57	0.44	1.11	0.94	0.65	0.19	0.05
Gammaproteobacteria; Pseudomonadales	9.87	7.75	5.76	10.16	5.65	9.35	5.94	7.84	5.67	1.92
Cyanobacteria; Synechococcales	21.21	14.01	4.24	0.61	0.51	0.46	1.29	2.16	0.16	0.26
Nitrososphaeria; Nitrosopumilales	0.01	1.46	3.08	8.92	4.98	11.47	8.14	11.23	2.55	0.48
Thermoplasmata; Marine Group II	5.74	8.24	10.76	5.77	3.12	2.48	6.70	3.57	1.15	0.20
Bacteroidia; Flavobacteriales	1.80	3.36	1.25	1.06	1.07	1.23	0.74	1.52	3.79	19.51
Campylobacteria; Campylobacterales	0.02	0.02	0.01	0.02	0.32	0.25	0.08	0.10	21.30	18.45
Cyanobacteria; Chloroplast	2.22	6.57	2.76	0.26	0.17	1.79	1.15	0.34	0.25	0.03
Marinimicrobia; SAR406 clade	2.15	2.55	5.23	3.05	1.85	2.49	3.16	2.51	0.68	0.15
Dehalococcoidia; SAR202 clade	0.78	1.12	2.65	3.58	2.05	1.71	4.25	3.37	0.79	0.14
Alphaproteobacteria; Rhodospirillales	2.35	2.39	2.89	3.07	1.72	2.72	2.25	2.76	0.54	0.15
Acidimicrobiia; Microtrichales	0.38	1.59	1.01	3.09	2.10	1.49	4.09	3.01	0.58	0.13
Gammaproteobacteria; Methylococcales	0.01	0.01	0.01	0.04	0.47	1.64	1.38	1.92	8.08	9.06
SAR324 clade (Marine group B)	0.74	0.61	4.38	3.26	1.27	1.28	1.15	1.08	0.36	0.05
Thermoplasmata; Marine Group III	1.08	0.63	2.19	1.19	0.73	1.31	1.79	1.74	0.43	0.08
Acidimicrobiia; Actinomarinales	3.74	4.28	0.91	0.59	0.11	0.21	0.43	0.29	0.03	0.07
Gammaproteobacteria; Thiotrichales	0.07	0.06	0.04	0.03	0.14	0.04	0.00	0.03	6.41	7.69
Verrucomicrobiae; Arctic97B-4 marine group	0.45	0.20	1.44	1.45	0.79	1.38	1.72	2.04	0.47	0.09
Alphaproteobacteria; Rhodobacterales	1.42	1.41	0.73	1.27	0.18	0.36	0.11	0.34	0.58	0.49