

Supplementary Materials for

Fermentation, methanotrophy and methanogenesis influence sedimentary Fe and As dynamics in As-affected aquifers in Vietnam

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Project details: <http://gepris.dfg.de/gepris/projekt/320059499?language=en>

Table S1. List of primers, primer sequences and thermal programs used for quantification of bacterial and archaeal 16S rRNA genes, particulate methane monooxygenase genes (*pmoA*) and methyl-coenzyme M reductase subunit alpha genes (*mcrA*).

Specificity	Primer	Primer sequence (5' → 3')	Thermal program	References
16S rRNA genes of <i>Bacteria</i>	341f	CCTACGGGAGGCAGCAG	98°C - 2'; (98°C - 5''; 60°C - 12'')	(Muyzer et al., 1993)
	534r	ATTACCGCGGCTGCTGG	95°C - 1'; 60°C - 1') x 40; 60 - 95°C - 10''	
16S rRNA genes of <i>Archaea</i>	Ar109f	ACK GCT GAG TAA CAC GT GTG CTC CCC CGC CAA	98°C - 3'; (98°C - 5''; 52°C - 12'')	(Großkopf et al., 1998)
	Ar915r	TTC CT	72°C - 15') x 40; 98°C - 1'; 52°C - 1'; 52 - 95°C - 10''	
<i>pmoA</i>	A189f	GGNGACTGGGACTTCTGG	96°C - 5'; (94°C - 1';	(Holmes et al., 1995)
	A682r	GAASGCNGAGAAGAASGC	56°C - 1'; 72°C - 1') x 38; 72°C - 5'	
<i>mcrA</i>	ME1f	GCMATGCARATHGGWATG	95°C - 5'; (95°C - 50'');	(Hales et al., 1996)
	ME1r	TC TCATKGCRTAGTTDGGRTA GT	54°C - 50'' 72°C - 50') x 34; 72°C - 10'	

Table S2. Volatile fatty acids compositions in the sedimentary porewater. In order to evaluate whether fatty acids were present in the aquifer, pore water from sandy sediments (piston core collected in 2017 at the same field site in Van Phuc) was collected by centrifugation and subjected to volatile fatty acid (VFA) analyses with a detection limit of 0.2 µM, as described previously by Laufer et al., 2016. The sufficient amount of porewater was obtained only for 9 samples.

Nr.	Depth [m]	Concentration µM					
		EtOH	Acetate	Formate	Lactate	Propionate	
1	20.4	--	--	--	--	--	Reduced sand
2	22.5	--	--	--	--	--	
3	24.8	--	--	--	59.7	--	
4	26.5	--	15.4	--	--	--	
5	27.2	--	--	--	--	209	
6	28.6	--	--	--	--	--	
7	29.8	--	--	--	--	--	
8	30.9	--	--	--	--	--	Oxidized sand
9	33.9	--	--	3.85	4.29	--	

Table S3. Hydrogeochemistry of water from wells located at the redox transition zone in Van Phuc. Water from the upper gray aquifer is characterized by high dissolved As, while water from lower orange aquifer contains low As concentrations. Modified from Glodowska et al., 2020.

Well ID	LOQ	AMS	AMS	AMS	PC	AMS	PC	AMS	
		11(25)	32	31	43	11(32)	44	11(47)	
Upper gray aquifer		Lower orange aquifer							
Depth	m	-	23-24	23-24	23-24	26-27	30-31	36-37	45-46
pH	-	-	7.40	7.22	7.21	7.21	7.09	6.96	6.69
O₂	mg/L	-	0.07	0.09	0.05	0.06	0.02	0.02	0.08
E_h(SHE)	mV	-	18	8	12	18	185	125	105
SO₄²⁻	mg/L	0.25	<.25	<.25	<.25	<.25	<.25	4.3	0.26
Cl⁻	mg/L	0.05	9.8	13	18	26	31	17	12
DN	mg/L	0.5	22	14	17	14	9.1	0.7	0.5
DOC	mg/L	0.5	4.4	2.6	3.5	2.5	1.4	1.5	1.1
NH₄⁺	mgN/L	0.01	25	16	19	15	9.3	0.49	0.63
PO₄³⁻	mgP/L	0.005	0.76	0.52	0.52	0.53	0.01	0.02	0.32
As	μg/L	0.1	401	80	266	58	0.9	4.3	6.2
As(III)	μg/L	0.1	372	76	262	58	0.3	3.8	6.1
Fe	mg/L	0.05	13	8.9	10	9.9	<.05	0.44	16
Mn	mg/L	0.005	0.50	3.6	1.0	2.5	1.5	2.7	1.0
P_{tot}	mg/L	0.02	0.77	0.60	0.52	0.59	0.06	0.06	0.38
S_{tot}	mg/L	0.1	<.1	<.1	<.1	<.1	<.	1.5	<.1
Si	mg/L	1	10	8	9	9	15	13	17
Sr	μg/L	1	490	489	475	522	584	599	231
Br	mg/L	0.04	0.18	0.10	0.09	0.11	0.09	0.09	0.13
Na	mg/L	0.5	10	10	9.4	9.8	14	17	42
K	mg/L	0.1	6.0	5.0	5.3	5.0	4.5	5.8	3.9
Ca	mg/L	0.1	96	98	100	101	110	67	30
Mg	mg/L	0.01	33	26	32	34	37	67	21
Ba	μg/L	0.2	76	146	108	137	342	110	236
C-alk	mmolHCO ₃ ⁻ /L	0.1	12	9.2	10	9.3	9.7	9.8	5.8
CH₄	mg/L	<0.13	51	28	25	15	<.13	<.13	<.13

Table S4. Geochemistry of sediments across the redox transition zone including As speciation.

Depth [m]	C _{tot} [%]	S _{tot} [%]	MnO [%]	Fe ₂ O ₃ [%]	As [mg/kg]	As ^{III}	As ^V	FeAsS	As ₂ S ₃
3.4	0.11	0.014	0.055	4.27	8.26				
4.2	0.24	0.041	0.085	4.13	8.79				
4.4	1.65	0.090	0.015	1.16	14.7				
4.9	1.25	0.045	0.097	5.23	11.6				
5.9	0.43	0.013	0.072	6.10	9.88				
6.3	1.49	0.044	0.113	5.97	14.0				
6.6	1.44	0.167	0.072	5.90	15.8				
7.7	1.19	0.315	0.038	7.57	24.1				
8.4	3.43	0.559	0.176	8.81	17.4				
9	5.85	0.411	0.1	6.99	23.0				
9.9	3.43	0.441	0.093	7.24	15.4				
10.8	5.63	0.749	0.074	6.54	19.1				
10.9	7.11	0.990	0.035	5.94	22.3				
11.5	6.60	0.626	0.036	5.17	19.8				
12.3	0.66	0.015	0.01	2.04	6.01				
13.1	0.72	0.007	0.005	1.68	4.64				
13.4	0.09	0.008	0.001	1.01	6.11				
13.9	0.67	0.007	0.014	6.60	8.25				
14.9	0.17	0.005	0.005	1.94	7.51				
15.4	0.17	0.006	0.006	3.15	8.76				
16.4	0.12	0.006	0.009	3.87	6.97				
17.1	0.09	0.007	0.024	23.7	16.8				
17.7	0.06	0.005	0.016	9.18	6.78				
19.7	0.04	0.006	0.062	3.64	3.15	61	39		
20.4	0.04	0.004	0.033	2.02	4.89	64	21	15	
20.9	0.03	0.005	0.029	1.95	3.95				
22.1	0.03	0.006	0.03	1.62	1.25				
24.2	0.03	0.005	0.029	2.65	6.00	71	25		4
24.8	0.03	0.006	0.013	2.19	4.12				
25.3	0.15	0.017	0.035	2.39	3.39				
26.6	0.03	0.004	0.011	2.03	3.58				
26.9	0.03	0.007	0.016	1.94	2.18	79	19	2	
28.1	0.02	0.007	0.007	1.37	4.08				
29	0.02	0.003	0.007	1.21	4.27				
29.3	0.02	0.006	0.015	2.06	1.92	50	20	30	
30.1	0.05	0.006	0.232	2.40	5.59				
30.3	0.03	0.007	0.155	2.84	3.61				
30.6	0.03	0.005	0.031	3.31	5.86	25	75		
30.7	0.03	0.005	0.074	6.54	14.2	33	67		
31	0.02	0.005	0.024	2.77	5.27	30	70		
31.3	0.03	0.007	0.018	3.46	3.66				
31.8	0.03	0.005	0.018	1.59	1.69	27	73		
32.3	0.03	0.005	0.019	2.26	3.14				
33.5	0.03	0.007	0.025	2.34	2.41				
34.9	0.02	0.004	0.006	1.06	1.03				
35.6	0.03	0.006	0.007	1.38	3.60	85	15		
36.1	0.03	0.005	0.009	1.38	1.02	45	55		
37.7	0.02	0.006	0.009	2.23	2.91				
37.9	0.02	0.007	0.119	2.02	2.62				
38.3	0.03	0.004	0.005	2.13	2.03				
39	0.03	0.006	0.01	2.68	2.44				
39.7	0.02	0.004	0.006	1.26	2.33				
40	0.04	0.006	0.008	1.02	1.24				
40.5	0.03	0.005	0.011	1.97	1.82				
41.4	0.03	0.004	0.01	1.79	1.65				
42	0.03	0.006	0.006	2.93	2.52				
42.4	0.03	0.007	0.009	4.14	3.88				
42.5	0.05	0.008	0.011	2.83	3.46				
42.6	0.04	0.008	0.008	5.11	4.79	76	18	6	
42.6	0.04	0.006	0.01	2.41	3.12	43	57		
42.7	1.74	0.150	0.024	2.77	11.6				
42.8	0.15	0.033	0.021	2.37	4.04	77	23		
42.9	0.11	0.045	0.03	6.08	11.1				
43.6	0.13	0.013	0.051	7.35	10.3				
44.9	0.55	0.012	0.179	10.0	12.9				
45.9	0.17	0.012	0.069	4.56	4.62	7	81	12	

Silt-clay aquitard

Reduced gray sediment

Oxidized orange sediment

Silt-clay aquitard

Figure S1. Incubation of oxidized orange sediment retrieved from 30 m depth with artificial groundwater. In our previous study addition of CH₄ (upper image) and NOM (lower image) triggered Fe(III) reduction and change of color to brown/gray indicating the formation of Fe(II) phases.



Table S5. Amplicon sequencing variants (ASVs, a qualitative measure of community richness) and alpha diversity indices; Pielou's index (a measure of community evenness), Faith's Phylogenetic Diversity (a qualitative measure of community richness that incorporates phylogenetic relationships between the features) and Shannon's diversity index (a quantitative measure of community richness) at different depth across redox transition zone. Table below represent average and standard deviation for

	Depth [m]	ASVs	Pielou	Faith PD	Shannon
Clay/silt aquitard	3.4	1510	0.85	115	8.96
	4.9	1270	0.89	91	9.18
	6.5	996	0.85	86	8.43
	7.6	1583	0.90	102	9.55
	9	742	0.86	48	8.21
	10.8	761	0.87	49	8.31
	11.7	674	0.86	52	8.10
	17.2	585	0.86	54	6.28
	17.8	1490	0.86	111	9.05
	18.5	652	0.75	60	7.01
Reduced sand	20.3	1246	0.84	106	8.67
	20.9	831	0.77	77	7.46
	22.4	599	0.76	60	6.98
	24.1	541	0.75	60	6.85
	24.6	687	0.74	66	7.02
	25.2	1356	0.83	103	8.61
	26	601	0.72	67	6.67
	26.9	748	0.78	79	7.49
	29.8	1170	0.85	92	8.69
	30.2	1240	0.82	97	8.38
Oxidized sand	30.6	1445	0.81	102	8.53
	31	1114	0.83	96	8.35
	32.3	1091	0.84	88	8.44
	33.5	1176	0.82	86	8.40
	37.2	998	0.82	76	8.17
	37.3	766	0.80	63	7.67
	39.7	1890	0.80	138	8.74
	41.4	855	0.81	58	7.87
	41.5	812	0.82	52	7.97
	42.5	967	0.83	76	8.27
Clay	42.6	429	0.88	29	7.69
	42.9	125	0.53	24	3.68
	45.9	385	0.79	36	6.78

Depth	AVS	Pielou	Faith PD	Shannon
0-20 m Silty/clay aquitard	1026±398	0.85±0.042	76±27	8.3±1.0
20-30 m Reduced gray aquifer	901±315	0.78±0.044	80±17	7.6±0.8
30-42.5 m Orange oxidized aquifer	1111±338	0.81±0.012	83±25	8.2±0.3
42.5-46 m Silty/clay aquitard	313±164	0.73±0.182	29±6.1	6.0±2.1

each distinguished zone.

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