

Supporting information

Diversity of sediment microbial community in response to acid mine drainage pollution gradients in the Aha watershed (Southwest China)

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Table S1. Major elemental concentrations in the AMD sediment samples from AHA watershed.

Sample ID	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	MgO	CaO	Na ₂ O	K ₂ O	MnO	P ₂ O ₅	TiO ₂	LOI	TOTAL
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
SD1	24.7	6.72	8.34	0.61	28.071	0.221	0.727	0.3048	0.1655	0.921	29.9	100.68
SD2	22.72	6.53	43.72	0.59	2.129	0.131	0.652	0.2645	0.1802	0.785	22.75	100.45
SD3	40.64	11.3	13.29	1.13	7.793	0.216	1.149	0.1816	0.1765	1.728	22.16	99.76
SD4	38.46	10.89	12.58	1.04	7.595	0.247	1.132	0.1769	0.1664	1.694	26.02	100
SD5	28.36	7.97	10.67	1.39	22.754	0.095	0.65	0.3679	0.1878	0.916	26.56	99.92
SD6	33.5	12.78	26.53	0.77	1.883	0.15	0.928	0.1957	0.2141	1.49	21.56	100
SD7	36.15	13.79	22.88	0.85	2.074	0.141	1.082	0.1752	0.2261	1.621	21.19	100.18
SD9	52.44	13.56	7.31	1.64	6.749	0.092	1.699	0.7072	0.1454	1.238	14.81	100.39
B1	8.52	2.93	36.98	0.75	0.722	0.098	0.236	0.0642	1.3637	0.316	48.96	100.94
B2	1.9	0.96	59.5	0.08	0.192	0.083	0.091	0.0119	0.3444	0.11	35.77	99.04
SD13	46.44	11.12	11.79	0.81	5.728	0.193	0.712	1.6696	0.2127	0.907	20.36	99.94

Table S2. OTU number and Chao1, Shannon index in all samples.

Sample	OTU(3% cutoff)	OTU(5% cutoff)	Chao1(3% cutoff)	Chao1(5% cutoff)	Shannon(3% cutoff)	Shannon(5% cutoff)
B1	2951	1969	8895.576	5484.62449	9.168469168	7.805269494
B2	4059	2917	10922.19372	6027.053942	10.29290802	9.290636306
SD1	5197	3594	16288.33901	8169.413043	11.34798989	10.31164282
SD9	4417	3143	12658.23252	7510.315421	10.81901508	9.943651601
SD2	4810	3265	12621.03982	7293.058212	11.25947259	10.12892784
SD3	5402	3653	16910.19527	8756.5625	11.50781901	10.36412342
SD4	5130	3485	13897.668	7731.195167	11.32041861	10.21053472
SD5	5109	3476	15172.62556	8605.514344	11.35023272	10.2026151
SD6	3390	2428	11054.09391	5914.695157	9.57772413	8.52295451
SD7	3535	2466	10243.87528	6129.839623	9.754189613	8.620459058
SD13	5093	3463	15426.18224	8019.055118	11.36392189	10.25105892

Table S3. Microbial community composition as shown as relative abundance in the AMD watershed at phylum level.

Taxonomic group	B1	B2	SD13	SD1	SD2	SD3	SD4	SD5	SD6	SD7	SD9
Proteobacteria	60.7	55.73	52.79	50.44	51.33	51.81	49.28	47.22	45.25	61.85	38.09
β-proteobacteria	34.52	11.52	21.17	19.48	23.46	21.64	21.93	22.02	8.82	11.16	15.30
γ-proteobacteria	13.40	29.66	12.22	14.93	11.27	9.95	13.74	10.12	16.33	34.79	11.69
δ-proteobacteria	6.74	5.94	9.16	7.30	7.29	11.14	6.45	9.48	9.47	7.02	6.47
α-proteobacteria	4.81	6.97	5.70	7.47	8.16	7.33	5.97	4.02	9.67	7.77	3.54
ε-proteobacteria	0.25	0.52	3.67	0.22	0.32	0.71	0.32	0.51	0.19	0.27	0.35
Actinobacteria	5.73	5.99	2.6	3.38	3.78	3.21	3.4	2.79	5.24	6.09	7.72
Cyanobacteria	3.84	5.63	1.14	1.54	1.91	0.76	0.68	0.56	1.51	5.58	0.84
Firmicutes	10.12	4.96	3.24	2.69	2.31	5.18	3.97	2.77	3.03	3.09	2.41
Chloroflexi	2.27	4.37	5.41	5.2	4.79	6.33	8.58	9.91	20.13	4.58	9.59
Acidobacteria	3.95	4.29	4.9	6.04	4.9	5.96	8.36	5.76	9.92	3.85	6.66
Planctomycetes	0.88	2.98	7.26	7.43	5.62	4.97	6.64	4.29	2.51	1.89	4.75
Bacteroidetes	1	2.95	7.86	6.92	11.21	5.66	3.5	6.89	1.93	2.51	3.7
Crenarchaeota (Archaea)	0.02	1.82	0.1	0.28	0.1	0.53	0.16	0.04	0.11	0.2	0.12
Nitrospirae	0.61	1.67	1.46	1.22	1.53	1.73	1.7	1.53	0.92	0.99	1.69
Gemmatimonadetes	0.95	1.65	1.7	1.97	2	2.28	2.85	1.63	1.49	1.57	4.96
Verrucomicrobia	0.59	1.1	2.26	3.95	2.97	1.73	1.5	2.84	2.08	0.6	1.3
WPS-2	2.77	0.52	0.55	0.51	0.6	0.71	0.74	0.54	0.7	0.62	0.61
WS3	0.18	0.39	0.51	0.64	0.28	0.45	0.63	0.66	0.28	0.27	1.39
Chlorobi	0.31	0.28	0.87	0.53	0.86	0.85	0.66	1.82	0.46	0.62	1.26
Armatimonadetes	0.06	0.17	0.19	0.17	0.11	0.13	0.2	0.15	0.1	0.37	0.06
Spirochaetes	0.12	0.15	0.33	0.15	0.21	0.28	0.12	0.26	0.23	0.11	0.35
GAL15	0.03	0.12	0.01	0.07	0.01	0.11	0.11	0.06	0.06	0.04	3.39
Elusimicrobia	0.38	0.11	0.12	0.08	0.12	0.13	0.1	0.1	0.09	0.05	0.06
TM6	0.14	0.1	0.34	0.2	0.2	0.16	0.13	0.29	0.15	0.14	0.19
GN04	0.03	0.09	0.17	0.1	0.11	0.21	0.07	0.21	0.06	0.02	0.17
Chlamydiae	0.07	0.08	0.31	0.12	0.35	0.16	0.15	0.14	0.07	0.17	0.1

OP3	0.04	0.08	0.08	0.02	0.05	0.08	0.06	0.23	0.08	0.06	0.11
NC10	0.02	0.08	0.15	0.09	0.05	0.1	0.19	0.21	0.04	0.02	0.5
WS1	0.02	0.06	0.05	0.01	0.03	0.06	0.05	0.11	0.04	0.08	0.13
GOUTA4	0.01	0.06	0.04	0	0.02	0.04	0.02	0.03	0	0.08	0.02
OP8	0.04	0.05	0.08	0.07	0.04	0.07	0.12	0.34	0.06	0.09	0.12
Euryarchaeota (Archaea)	0.01	0.04	0.02	0.12	0.01	0.13	0.02	0.06	0	0.08	0.03
Rare phyla	0.56	0.36	0.71	0.56	0.63	0.66	0.32	1.19	0.26	0.58	0.94
Unclassified	4.58	4.14	4.79	5.48	3.88	5.53	5.68	7.35	3.22	3.79	8.72

*relative abundances of α -, β -, γ -, δ -, ϵ -Proteobacteria were provided. Rare phyla stand for the phyla with relative abundances less than 5%.

Unclassified stands for the reads that cannot be classified with any known phyla.

Table S4. Fe and S related bacteria detected in the present study and their relative abundances (%) in the Aha watershed.

Taxonomic group	B1	B2	SD13	SD1	SD2	SD3	SD4	SD5	SD6	SD7	SD9	Microbial metabolism	Reference
<i>"Ferrovum myxofaciens"</i>	13.820	2.625	2.955	2.918	3.212	2.716	2.726	2.753	1.982	2.267	1.744	Acidophilic, autotrophic, Fe-oxidation	(Johnson et al., 2014)
<i>Alicyclobacillus</i>	6.974	1.083	0.835	0.863	0.789	1.221	1.083	0.890	1.404	0.964	0.853	Acidophilic, autotrophic, Fe- and S-oxidation	(Jiang et al., 2008)
<i>Thiobacillus</i>	0.340	0.193	0.606	0.239	0.376	0.450	0.596	0.844	0.202	0.092	0.688	Halotolerant, acidophilic, autotrophic, Fe- and S-oxidation	(Temple and Colmer, 1951)
<i>Acidithiobacillus</i>	0.477	0.128	0.165	0.156	0.165	0.193	0.156	0.248	0.147	0.138	0.092	Acidophilic, autotrophic, Fe- and S-oxidation	(Hallberg et al., 2010)
<i>Gallionella</i>	0.028	0.028	0.018	0.037	0.083	0.037	0.046	0.046	ND	0.018	ND	Neutrophilic, autotrophic, Fe-oxidation	(Hallbeck et al., 1993)
<i>Thermomonas</i>	0.028	0.064	0.101	0.092	0.073	0.119	0.119	0.028	0.046	0.138	0.046	Thermophilic, Fe-oxidation	(Alves et al., 2003)
<i>Geothrix</i>	ND	0.037	0.037	0.055	0.147	0.037	0.073	0.018	0.055	ND	0.018	Heterotrophic, Fe-reduction	(Coates et al., 1999)
<i>Aquabacterium</i>	0.018	0.000	0.037	0.055	0.083	0.037	0.046	0.018	0.009	0.009	0.009	Heterotrophic, Fe-reduction	(Kalmbach et al., 1999)
<i>Aciditerrimonas</i>	3.194	1.000	0.596	0.826	0.743	0.808	0.899	0.817	1.055	0.853	0.909	Heterotrophic, acidophilic, Fe-reduction	(Itoh et al., 2011)
<i>Metallibacterium</i>	2.670	0.954	1.248	1.120	1.046	0.826	1.138	0.863	0.964	0.863	0.789	Facultatively, acid-tolerant, Fe-reduction	(Ziegler et al., 2013)
<i>Shewanella</i>	1.285	3.873	1.358	1.193	1.019	0.936	0.918	1.009	1.074	4.469	0.826	Facultatively, halotolerant, Fe-reduction	(Lies et al., 2005)
<i>Geobacter</i>	0.174	0.211	0.707	0.367	0.477	1.303	0.312	0.514	0.239	0.321	0.330	Anaerobic, Fe-and sulfate reduction	(Lovley et al., 1993)
<i>Anaeromyxobacter</i>	0.138	0.229	0.156	0.229	0.138	0.459	0.184	0.239	0.174	0.138	0.294	Anaerobic, Fe-reduction	(Sanford et al., 2002)
<i>Acidiphilium</i>	0.018	0.028	0.009	0.009	0.000	0.018	0.000	0.000	0.028	0.000	0.009	Acidophilic, heterotrophic, Fe-reduction	(Küsel et al., 1999)
<i>Acidocella</i>	0.193	0.018	0.073	0.064	0.055	0.046	0.046	0.009	0.018	0.028	0.037	Acidophilic, heterotrophic, Fe-reduction	(Johnson et al., 2006)
<i>Desulfomonile</i>	0.284	0.358	0.541	0.395	0.459	0.551	0.358	1.377	1.055	0.376	1.211	Anaerobic, dehalogenating, sulfate reduction	(DeWeerd et al., 1990)
<i>Syntrophobacter</i>	0.991	0.275	0.358	0.367	0.275	0.413	0.220	0.431	0.753	0.367	0.257	Anaerobic, syntrophic, sulfate reduction	(Boone and Bryant, 1980)

Table S4. (Cont'd)

<i>Desulfobacca</i>	0.037	0.110	0.257	0.092	0.101	0.211	0.110	0.395	0.257	0.138	0.073	Anaerobic, heterotrophic, sulfate reduction	(Elferink et al., 1999)
<i>Desulfobulbus</i>	0.046	0.028	0.073	0.037	0.092	0.211	0.055	0.046	0.018	0.055	0.028	Anaerobic, heterotrophic, sulfate reduction	(Widdel and Pfennig, 1982)
<i>Desulfococcus</i>	0.037	0.119	0.028	0.083	0.083	0.073	0.064	0.110	0.037	0.037	0.028	Anaerobic, heterotrophic, sulfate reduction	(Imhoff-Stuckle and Pfennig, 1983)
<i>Clostridium</i>	0.037	0.239	0.128	0.110	0.064	0.092	0.156	0.101	0.046	0.055	0.083	Anaerobic, heterotrophic, sulfate reduction	(Akagi and Campbell, 1962)
<i>Desulfobacterium</i>	0.037	0.046	0.083	0.037	0.055	0.055	0.046	0.037	0.046	0.073	0.073	Anaerobic, heterotrophic, sulfate reduction	(Brysch et al., 1987)
<i>Desulfomicrobium</i>	0.018	0.046	0.156	0.046	0.046	0.055	0.046	ND	ND	0.046	0.037	Anaerobic, heterotrophic, sulfate reduction	(Langendijk et al., 2001)
<i>Desulfosporosinus</i>	0.395	0.018	0.046	0.046	0.028	0.037	0.046	0.028	0.101	0.028	0.055	Spore-forming, sulfate reduction	(Robertson et al., 2001)
<i>Desulfitobacterium</i>	0.220	0.018	0.037	0.018	0.028	0.028	0.018	0.028	0.073	0.046	0.028	Anaerobic, heterotrophic, sulfate reduction	(Gerritse et al., 1996)
<i>Desulfovibrio</i>	0.018	0.037	0.064	0.018	ND	0.046	ND	0.018	0.018	ND	0.037	Anaerobic, heterotrophic, sulfate reduction	(Nicolet et al., 1999)
<i>Sulfuritalea</i>	0.202	0.128	0.229	0.211	0.239	0.294	0.459	0.505	0.037	0.174	0.128	Facultative, autotrophic, S-oxidation	(Kojima and Fukui, 2011)
<i>Sulfurimonas</i>	ND	0.092	0.138	ND	ND	ND	0.018	0.046	ND	0.018	0.018	Mesophilic autotrophic, S-oxidation	(Inagaki et al., 2003)
<i>Sulfuricurvum</i>	0.101	0.110	1.358	0.128	0.128	0.459	0.211	0.239	0.083	0.092	0.174	Facultative, autotrophic, S-oxidation	(Kodama and Watanabe, 2004)

A**B****C**

Figure S1. Photos showing the major components of zone 1. A: the acid streamer from which B1 was taken; B: the acid mine drainage in zone 1; C: the onsite bioreactor treating AMD in zone 1. The onsite bioreactor will be operational soon.

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