

SUPPORTING INFORMATION

Biodegraded peat and ultrafine calcium carbonate treatment results in retained metals and higher microbial diversities in boreal acid sulfate soil

E. Högfors-Rönholm¹, S. Christel², T. Lillhonga¹, S. Engblom¹, P. Österholm³ and M. Dopson²

¹ Research and Development, Novia University of Applied Sciences, Vaasa, Finland

² Centre for Ecology and Evolution in Microbial Model Systems (EEMiS), Linnaeus University, Kalmar, Sweden

³ Department of Geology and Mineralogy, Åbo Akademi University, Turku, Finland

Correspondence

Eva Högfors-Rönholm, Research and Development, Novia University of Applied Sciences, 65200 Vaasa, Finland.

E-mail: eva.hogfors-ronnholm@novia.fi

Table S1. Details of 16S rRNA gene amplicon sequencing of triplicate aerobic (A) and anaerobic (AN) acid sulfate soil zero time point (ZTP) samples, untreated control samples (Con), and samples treated with EnrichBio (EB), peat fraction H1 mixed equally with CaCO₃ (P1/CaCO₃), peat fraction H5 mixed equally with CaCO₃ (P5/CaCO₃), sodium acetate (NaAc) and sodium lactate (NaLa).

Sample	DNA conc. (ng/ul)	Unmerged reads	Merged reads	Filtered reads	Number of OTUs
ZTP-1	7,05	199357	6538	4012	131
ZTP-2	4,37	202984	7509	4624	139
ZTP-3	5,20	185165	7273	4526	132
Con-A-1	6,97	234388	15661	9946	188
Con-A-2	4,04	403221	25118	16009	223
Con-A-3	4,80	289136	29450	18769	253
Con-AN-1	8,16	290533	12498	7209	224
Con-AN-2	4,69	153325	14396	8982	238
Con-AN-3	4,11	561799	35579	22095	249
EB-A-1	39,00	302610	23192	14803	100
EB-A-2	28,90	504638	40470	25767	158
EB-A-3	25,40	235032	29223	18178	146
EB-AN-1	34,50	202461	8256	5072	171
EB-AN-2	16,60	295401	23587	14638	202
EB-AN-3	18,50	318804	23495	14414	174
P1/CaCO ₃ -A-1	10,20	330694	22567	14178	256
P1/CaCO ₃ -A-2	7,57	119498	8893	5747	231
P1/CaCO ₃ -A-3	6,09	267583	28133	17706	259
P1/CaCO ₃ -AN-1	12,90	446416	31441	20034	211
P1/CaCO ₃ -AN-2	6,73	293321	22531	14287	212
P1/CaCO ₃ -AN-3	6,40	308890	32221	20428	258
P5/CaCO ₃ -A-1	11,90	295214	20841	12932	284
P5/CaCO ₃ -A-2	8,35	210240	11297	7232	235
P5/CaCO ₃ -A-3	5,70	462598	51635	32426	316
P5/CaCO ₃ -AN-1	9,34	613908	48760	30870	257
P5/CaCO ₃ -AN-2	5,58	274903	23917	14990	226
P5/CaCO ₃ -AN-3	5,93	439832	53694	33884	306
NaAc-A-1	4,34	437619	33365	20800	280
NaAc-A-2	4,02	195744	10387	6594	264
NaAc-A-3	3,17	559385	48131	30622	271
NaAc-AN-1	6,67	164923	12231	7825	301
NaAc-AN-2	4,19	455029	31007	19341	213
NaAc-AN-3	3,83	179456	19129	12190	294
NaLa-A-1	7,06	491777	38218	23529	171
NaLa-A-2	4,21	319689	20191	12989	261
NaLa-A-3	3,63	643754	64149	39823	286
NaLa-AN-1	9,87	251280	11935	7547	261
NaLa-AN-2	4,44	371049	29022	17943	255
NaLa-AN-3	2,93	375854	32945	19802	303

Table S2. Geochemistry data for aerobic triplicate acid sulfate soil zero time point (ZTP) samples, untreated control samples (Con), and samples treated with EnrichBio (EB), peat fraction H1 mixed equally with CaCO₃ (P1/CaCO₃), peat fraction H5 mixed equally with CaCO₃ (P5/CaCO₃), sodium acetate (NaAc) and sodium lactate (NaLa).

sample	pHsoil	EC ($\mu\text{S/cm}$)	Fe(II) (mg l^{-1})	TRS (%S)	S ($\mu\text{g l}^{-1}$)	Al ($\mu\text{g l}^{-1}$)	Ca ($\mu\text{g l}^{-1}$)	Mn ($\mu\text{g l}^{-1}$)	Co ($\mu\text{g l}^{-1}$)	Ni ($\mu\text{g l}^{-1}$)	Cu ($\mu\text{g l}^{-1}$)	Zn ($\mu\text{g l}^{-1}$)	As ($\mu\text{g l}^{-1}$)	Cd ($\mu\text{g l}^{-1}$)	Pb ($\mu\text{g l}^{-1}$)
ZTP1	4,2	55	0,21	0,00	32000	1158	16000	298	10,4	37,8	97,9	258,6	0,45	0,192	15,81
ZTP2	3,8	94	0,83	0,05	51200	2906	19840	768	23,6	64,6	63,4	248,3	0,32	0,384	7,17
ZTP3	3,8	81	1,65	0,04	38400	2144	17920	598	18,8	51,2	105,6	334,7	0,26	0,320	14,34
Con1	4,1	51	0,62	0,03	25600	960	14720	261	9,0	25,0	52,5	277,1	0,26	0,128	4,16
Con2	3,9	95	0,41	0,02	51200	3315	21760	800	25,2	67,8	67,8	450,6	0,32	0,384	7,23
Con3	3,8	93	0,21	0,08	51200	2918	22400	736	23,0	61,4	48,6	1107,2	0,32	0,384	6,98
EB1	6,6	205	0,21	0,02	166400	58	184320	22	0,8	3,8	41,0	300,8	0,83	0,001	2,69
EB2	6,0	227	0,41	0,04	179200	32	206080	47	0,8	5,1	30,1	159,4	1,47	0,002	3,33
EB3	6,5	225	0,01	0,03	179200	19	209920	54	0,8	4,5	28,8	729,6	0,70	0,001	1,41
P1/CaCO ₃ 1	5,4	120	0,83	0,05	83200	26	97920	110	2,1	6,4	48,0	160,0	0,26	0,005	8,00
P1/CaCO ₃ 2	5,4	163	0,41	0,04	121600	51	133760	332	4,9	11,5	39,7	203,5	0,45	0,064	6,98
P1/CaCO ₃ 3	5,3	169	0,41	0,03	115200	64	122880	328	5,0	11,5	37,1	851,2	0,32	0,128	4,74
P5/CaCO ₃ 1	5,4	73	0,41	0,02	83200	38	90880	109	1,8	6,4	26,2	183,0	0,26	0,004	2,62
P5/CaCO ₃ 2	5,7	189	0,62	0,05	108800	38	117120	280	3,6	8,3	36,5	121,0	0,32	0,005	6,08
P5/CaCO ₃ 3	5,2	170	2,27	0,06	108800	32	117120	281	3,7	7,7	21,8	69,1	0,26	0,064	0,51
NaAc1	5,3	61	0,41	0,00	38400	147	5120	98	3,1	10,2	42,2	302,7	0,19	0,064	4,86
NaAc2	4,5	109	0,01	0,00	64000	557	8960	356	10,0	25,6	39,0	195,2	0,19	0,128	4,74
NaAc3	4,1	88	0,21	0,08	57600	358	7680	248	7,0	17,3	20,5	142,7	0,32	0,128	4,42
NaLa1	4,2	113	0,62	0,01	44800	186	5760	106	3,5	13,4	74,9	314,2	0,38	0,192	9,34
NaLa2	4,3	91	0,01	0,03	64000	922	12160	481	14,2	36,5	53,8	218,9	0,32	0,192	7,23
NaLa3	4,0	98	1,45	0,06	64000	550	9600	322	9,7	23,7	30,1	158,1	0,32	0,128	1,66

Table S3. Geochemistry data for anaerobic triplicate acid sulfate soil zero time point (ZTP) samples, untreated control samples (Con), and samples treated with EnrichBio (EB), peat fraction H1 mixed equally with CaCO₃ (P1/CaCO₃), peat fraction H5 mixed equally with CaCO₃ (P5/CaCO₃), sodium acetate (NaAc) and sodium lactate (NaLa).

sample	pHsoil	EC ($\mu\text{S/cm}$)	Fe(II) (mg l^{-1})	TRS (%S)	S ($\mu\text{g l}^{-1}$)	Al ($\mu\text{g l}^{-1}$)	Ca ($\mu\text{g l}^{-1}$)	Mn ($\mu\text{g l}^{-1}$)	Co ($\mu\text{g l}^{-1}$)	Ni ($\mu\text{g l}^{-1}$)	Cu ($\mu\text{g l}^{-1}$)	Zn ($\mu\text{g l}^{-1}$)	As ($\mu\text{g l}^{-1}$)	Cd ($\mu\text{g l}^{-1}$)	Pb ($\mu\text{g l}^{-1}$)
ZTP1	4,2	55	0,21	0,00	32000	1158	16000	298	10,4	37,8	97,9	258,6	0,45	0,192	15,81
ZTP2	3,8	94	0,83	0,05	51200	2906	19840	768	23,6	64,6	63,4	248,3	0,32	0,384	7,17
ZTP3	3,8	81	1,65	0,04	38400	2144	17920	598	18,8	51,2	105,6	334,7	0,26	0,320	14,34
Con1	4,2	43	0,83	0,03	25600	819	14080	232	7,9	21,1	55,0	215,0	0,01	0,128	7,36
Con2	3,7	108	0,01	0,02	44800	2874	19200	736	21,7	62,1	28,8	444,8	0,32	0,320	5,82
Con3	3,8	90	0,41	0,06	51200	2912	21760	730	15,7	60,8	40,3	1088,0	0,26	0,320	6,14
EB1	6,5	195	0,41	0,03	153600	19	178560	19	0,7	3,8	39,0	146,6	0,96	0,001	2,37
EB2	6,0	204	0,19	0,02	160000	19	186240	97	1,0	5,1	34,6	147,2	0,64	0,002	3,39
EB3	6,6	252	0,01	0,04	198400	19	232320	54	1,2	5,1	39,7	940,8	0,90	0,001	1,34
P1/CaCO ₃ 1	5,3	128	0,83	0,04	89600	32	105600	104	1,9	6,4	36,5	170,2	0,26	0,002	1,92
P1/CaCO ₃ 2	5,1	150	0,62	0,03	102400	58	108800	319	5,4	12,2	37,8	355,8	0,70	0,064	2,82
P1/CaCO ₃ 3	5,2	159	0,41	0,05	96000	90	99200	333	5,9	13,4	46,1	838,4	0,01	0,128	3,52
P5/CaCO ₃ 1	5,3	66	1,03	0,01	83200	26	95360	102	1,6	6,4	25,0	153,0	0,19	0,002	4,42
P5/CaCO ₃ 2	5,4	137	0,01	0,02	89600	58	98560	285	4,4	9,6	37,1	138,9	0,32	0,128	4,74
P5/CaCO ₃ 3	4,8	116	0,21	0,06	76800	102	81280	319	5,8	12,2	22,4	81,9	0,01	0,064	1,47
NaAc1	4,2	59	1,45	0,02	38400	147	5120	101	3,2	10,2	44,8	243,2	0,01	0,128	4,74
NaAc2	4,4	94	0,01	0,00	57600	550	8960	344	9,9	25,0	45,4	710,4	0,32	0,128	4,16
NaAc3	4,1	99	0,83	0,07	57600	346	7680	255	7,2	17,3	32,0	144,0	0,26	0,128	1,98
NaLa1	4,2	169	1,03	0,01	38400	262	7040	129	4,4	17,3	89,0	261,1	0,19	0,256	11,33
NaLa2	4,3	131	0,01	0,02	57600	800	10880	426	13,2	32,6	34,6	197,8	0,38	0,128	4,35
NaLa3	3,9	89	0,41	0,05	57600	1011	13440	465	14,1	35,8	42,9	204,2	0,32	0,192	4,16

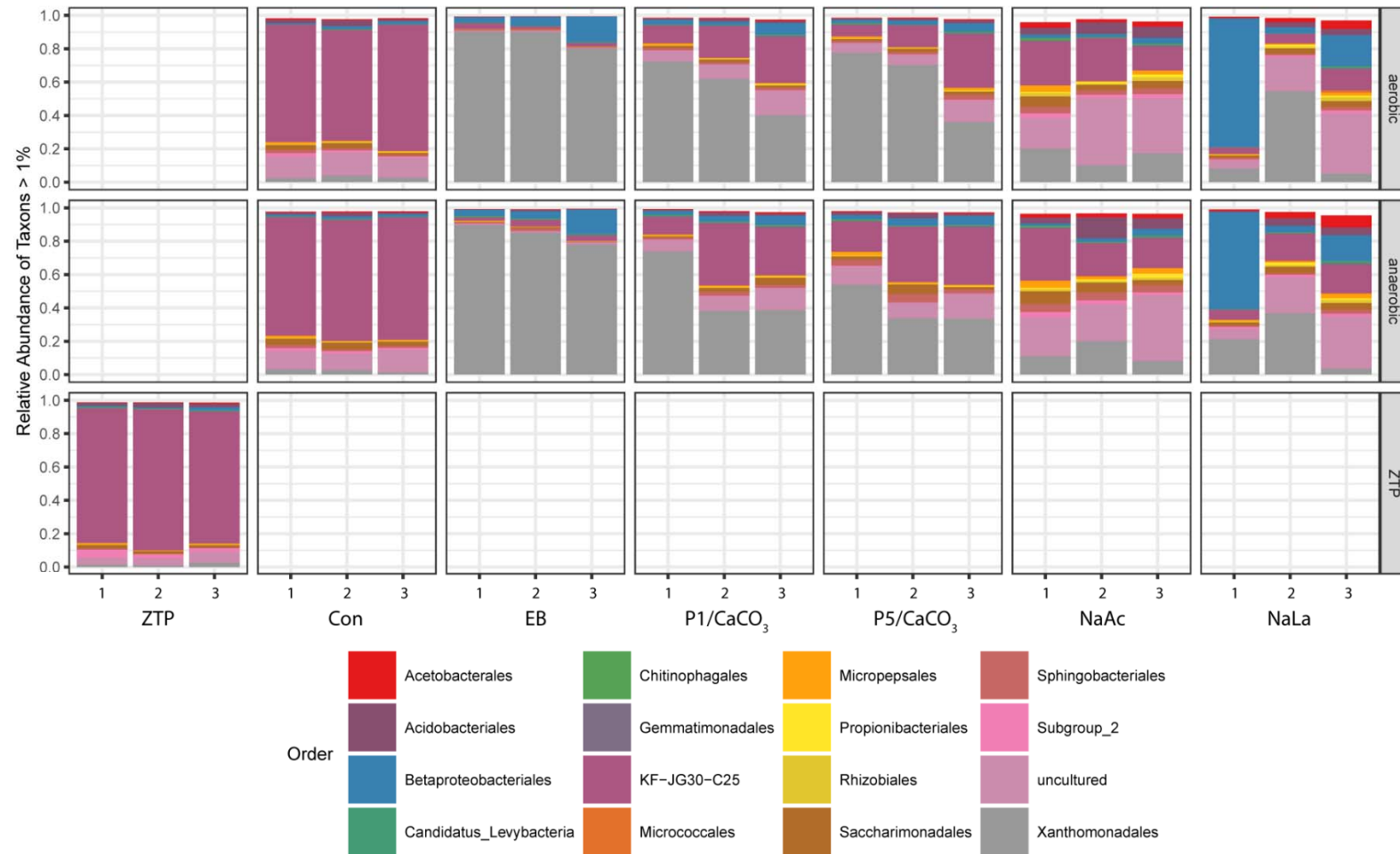


Figure S1. Microbial community structure (>1% relative abundance) on order level in triplicate acid sulfate soil zero time point (ZTP) samples, untreated control samples (Con), and samples treated with EnrichBio (EB), peat fraction H1 mixed equally with CaCO_3 (P1/ CaCO_3), peat fraction H5 mixed equally with CaCO_3 (P5/ CaCO_3), sodium acetate (NaAc) and sodium lactate (NaLa).

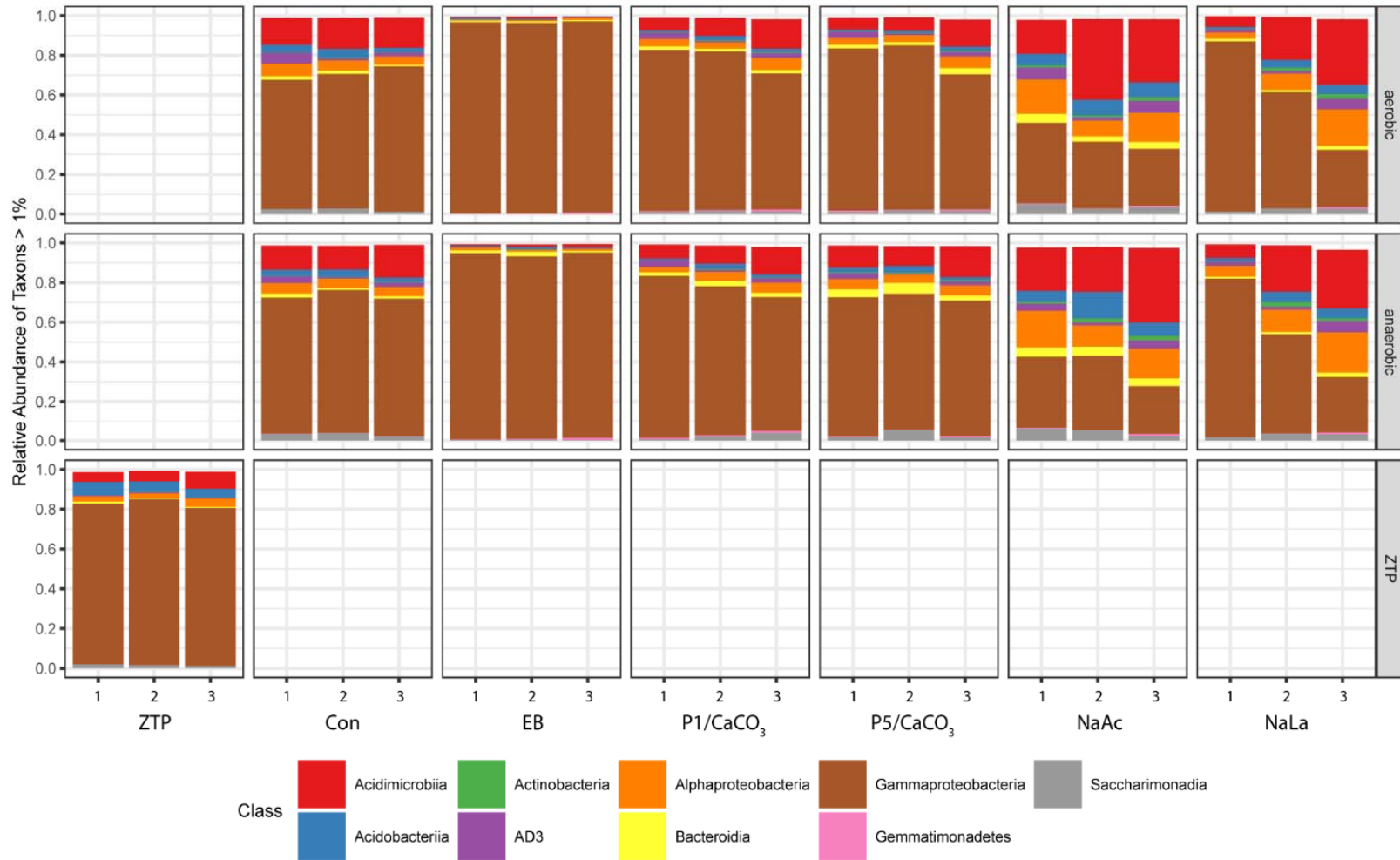


Figure S2. Microbial community structure (>1% relative abundance) on class level in triplicate acid sulfate soil zero time point (ZTP) samples, untreated control samples (Con), and samples treated with EnrichBio (EB), peat fraction H1 mixed equally with CaCO₃ (P1/CaCO₃), peat fraction H5 mixed equally with CaCO₃ (P5/CaCO₃), sodium acetate (NaAc) and sodium lactate (NaLa).

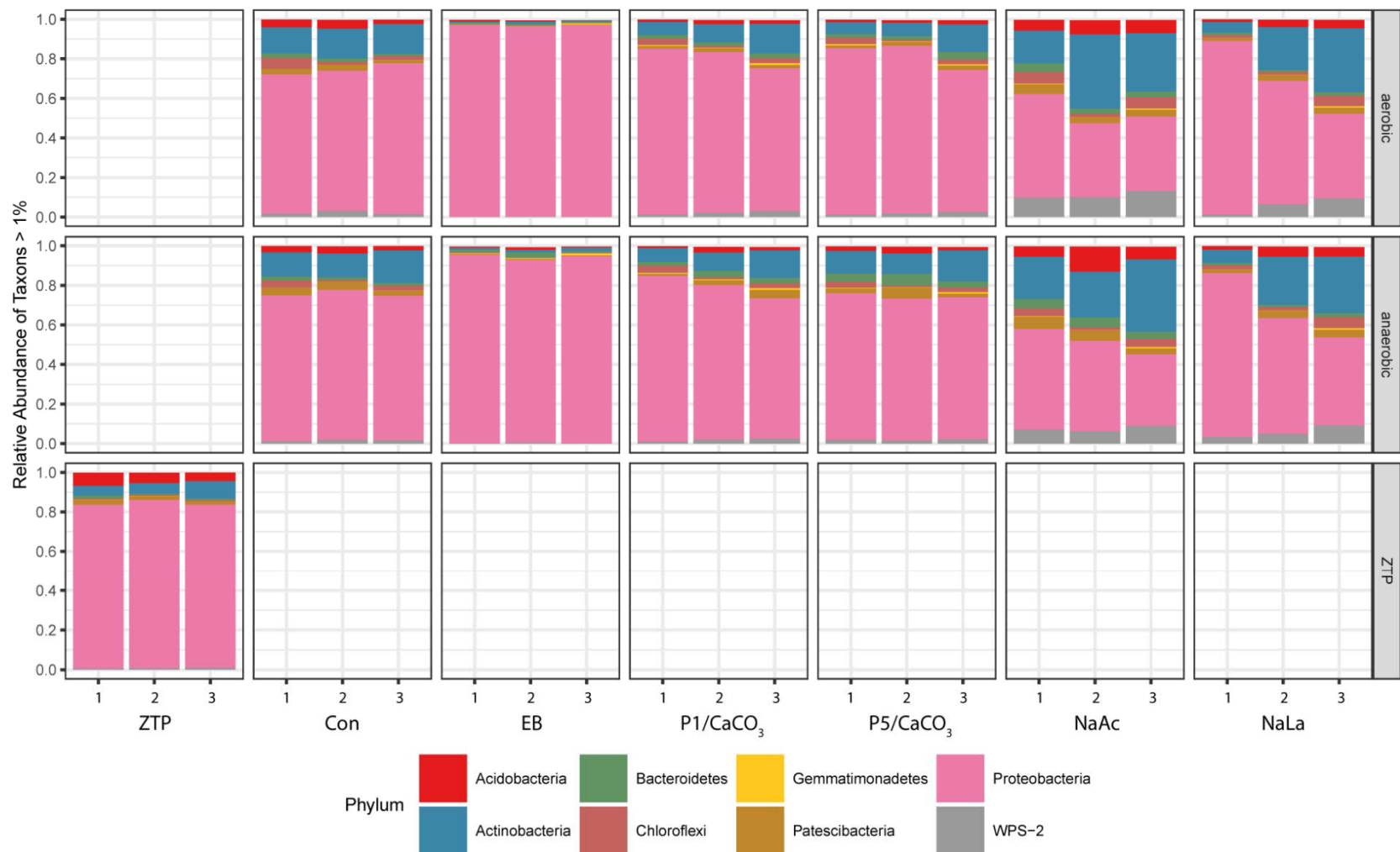


Figure S3. Microbial community structure (>1% relative abundance) on phylum level in triplicate acid sulfate soil zero time point (ZTP) samples, untreated control samples (Con), and samples treated with EnrichBio (EB), peat fraction H1 mixed equally with CaCO₃ (P1/CaCO₃), peat fraction H5 mixed equally with CaCO₃ (P5/CaCO₃), sodium acetate (NaAc) and sodium lactate (NaLa).

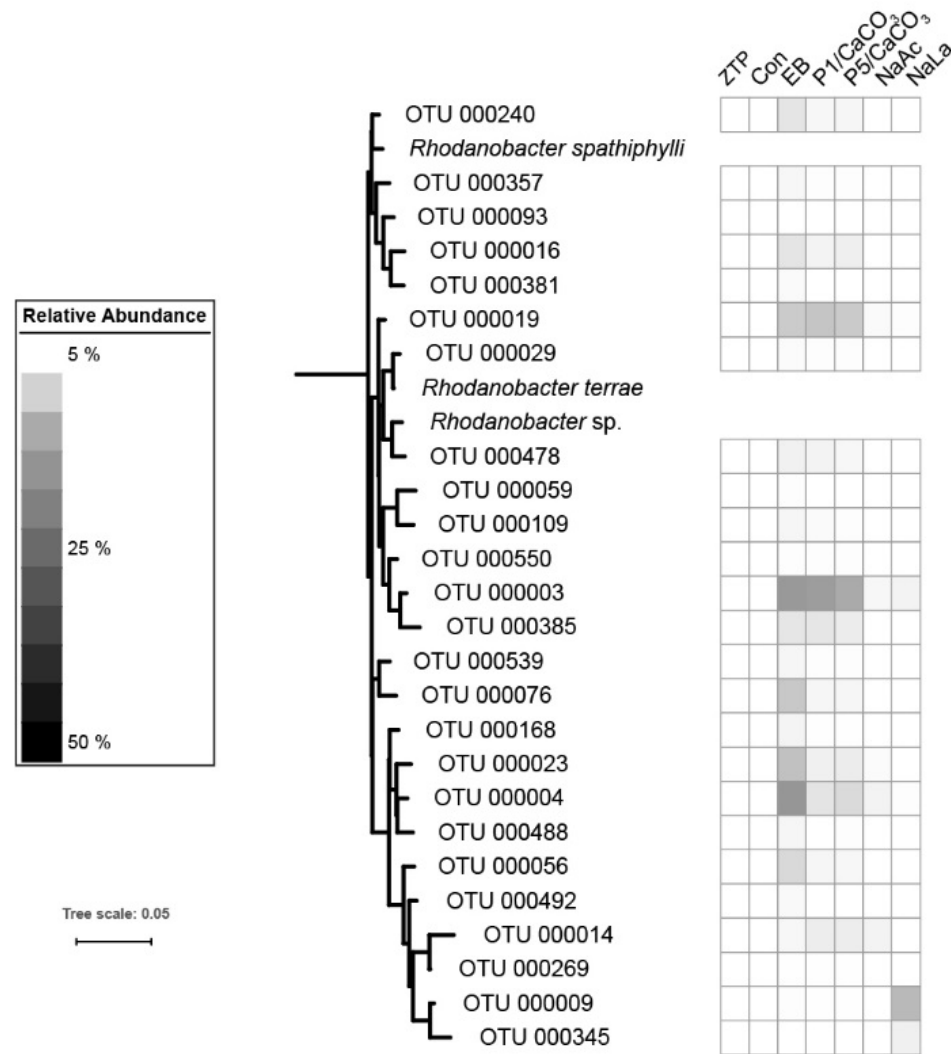


Figure S4. Maximum likelihood (unrooted) phylogenetic tree and heat map of *Rhodanobacter* OTUs (>5% relative abundance) from the 16S rRNA gene sequencing of triplicate acid sulfate soil zero time point (ZTP) samples, untreated control samples (Con), and samples treated with EnrichBio (EB), peat fraction H1 mixed equally with CaCO₃ (P1/CaCO₃), peat fraction H5 mixed equally with CaCO₃ (P5/CaCO₃), sodium acetate (NaAc) and sodium lactate (NaLa). The greyscale gives percentage relative abundance of the OTUs and the scale bar gives the number of substitutions per site.