

Supplementary Material: Seasonal Microbial Community Shifts in a Bioremediation System Treating Metal and Sulfate-Rich Seepage

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Table S1. Water chemistry properties in the vicinity of the organics over the monitoring period (concentrations are in mg/L).

Data Sampled	pH	Dissolved Oxygen	Temperature (°C)	Sulphide	Nitrate-N	Nitrite-N	Ammonium-N	Sulphate	Dissolved Arsenic	Dissolved Zinc	Dissolved Iron	Dissolved Manganese
11 August 2008	6.7	1.74	17	1.00	na	na	71	300	0.17	0.02	0.060	5.8
10 September 2008	6.8	0.92	16	na	na	na	83	300	0.26	0.005	0.160	6.4
8 October 2008	6.7	2.25	14	na	na	na	100	370	0.13	0.018	0.160	5.2
14 November 2008	6.7	1.62	9	0.00	0	na	84	420	0.09	0.016	0.100	5.7
10 December 2008	6.7	1.59	9	0.14	0.004	na	76	400	0.09	0.031	0.087	6.4
15 January 2009	6.9	2.23	6	0.17	0.011	na	87	440	0.08	0.022	0.180	6.9
13 February 2009	7.5	3.38	2	na	0.10	na	81	440	0.12	0.015	0.120	6.7
12 March 2009	7.0	3.58	0	0.03	0.90	0.015	74	480	0.07	0.032	0.130	7.4
8 April 2009	6.8	1.28	6	0.01	1.30	0.015	82	480	0.46	0.033	3.00	7.3
7 May 2009	6.8	1.62	10	0.10	1.00	0.009	65	460	0.29	0.02	1.40	7.6
12 June 2009	6.9	3.71	16	0.02	0.70	0.012	76	460	0.20	0.01	0.150	7.2
7 July 2009	7.5	0.10	15	0.00	1.30	0.014	82	450	0.32	0.027	0.350	6.8
Average	6.92	2.00	10.03	0.16	0.59	0.01	80.08	416.67	0.19	0.021	0.49	6.6
Std. Dev.	0.29	1.10	5.77	0.32	0.56	0.00	8.83	63.01	0.12	0.009	0.87	0.7

na = not available.

Table S2. Pyrolysis characteristics of the organic matter.

Data Sampled	TOC (wt %)	mg HC/g-material		mg CO ₂ /g-material	Total PC	PC/TOC	T _{max} (°C)
		S1	S2	S3			
11 August 2008	11.9	16.79	27.27	8.87	52.93	4.45	347
10 September 2008	11.6	15.35	27.15	8.76	51.26	4.42	353
8 October 2008	11.7	17.75	28.21	10.94	56.90	4.86	347
14 November 2008	11.9	15.51	25.91	10.53	51.95	4.37	344
15 January 2009	11.1	14.40	26.6	8.36	49.36	4.45	348
13 February 2009	12.8	19.78	35.29	11.44	66.51	5.20	345
12 March 2009	9.5	15.70	29.03	9.05	53.78	5.66	344
8 April 2009	10.6	16.44	26.21	10.73	53.38	5.04	347
7 May 2009	11.2	15.95	26.38	10.87	53.20	4.75	348
12 June 2009	11.4	17.29	26.82	9.06	53.17	4.66	347
Average	11.4	16.50	27.89	9.86	54.24	4.78	347
Std. dev.	0.8	1.44	2.63	1.08	4.48	0.39	2

TOC = total organic carbon

S1 = volatile hydrocarbon (HC) content

S2 = remaining hydrocarbon generative potential

S3 = CO₂ released during pyrolysis

Total PC = total pyrolysable carbon (sum of S1, S2 and S3) (mg/g-material)

T_{max} = temperature of maximum S2 peak

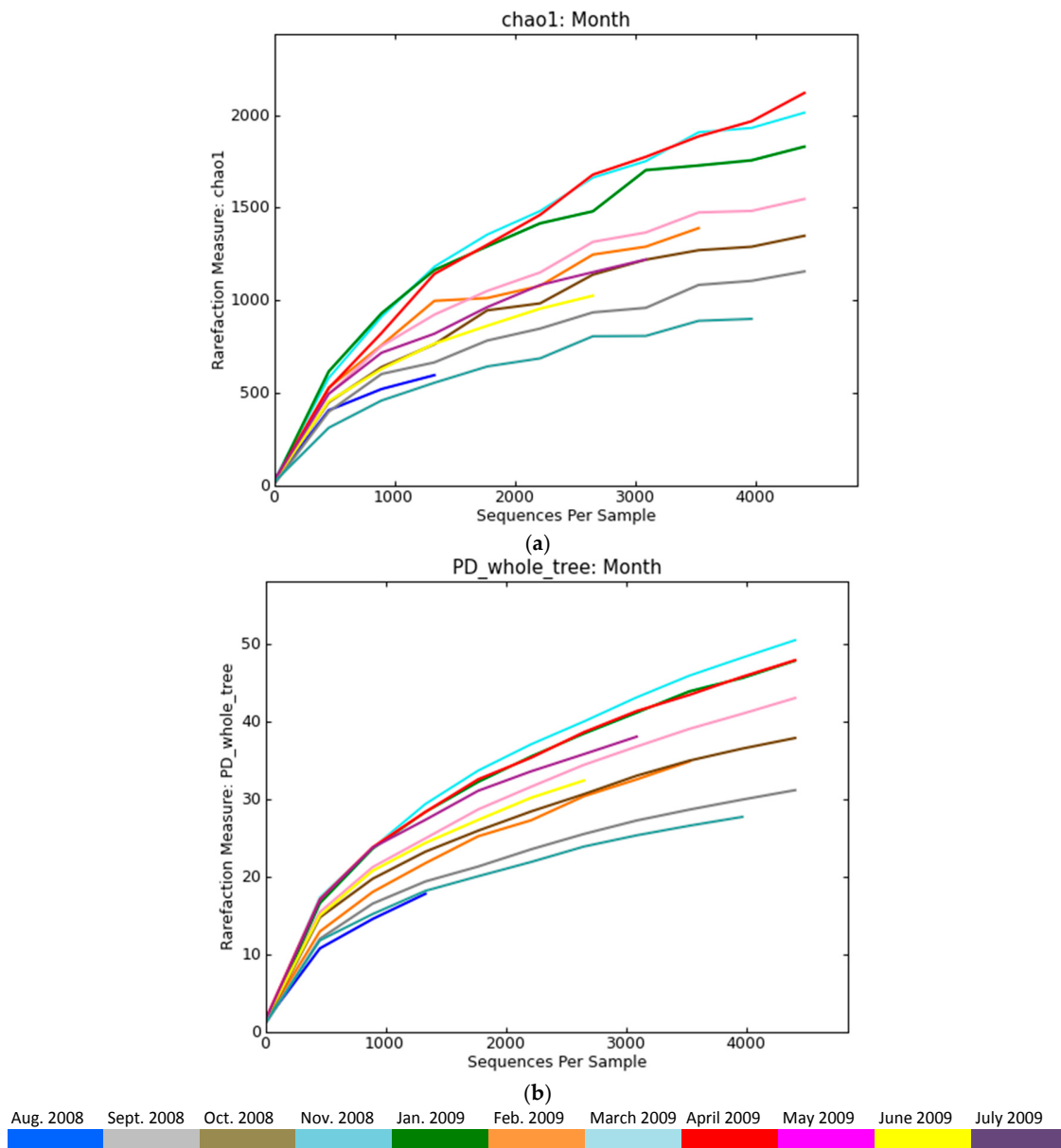


Figure S1. Rarefaction curves calculating diversity indices ((a) chao1 and (b) phylogenetic diversity (PD) using the whole tree) produced from the alpha_diversity.py script within Qiime. Curves are coloured according to month that the sample was collected in. Curves calculating higher diversity indices indicate that those samples consist of more diverse microbial populations (*i.e.*, a greater number of different types of microorganisms).

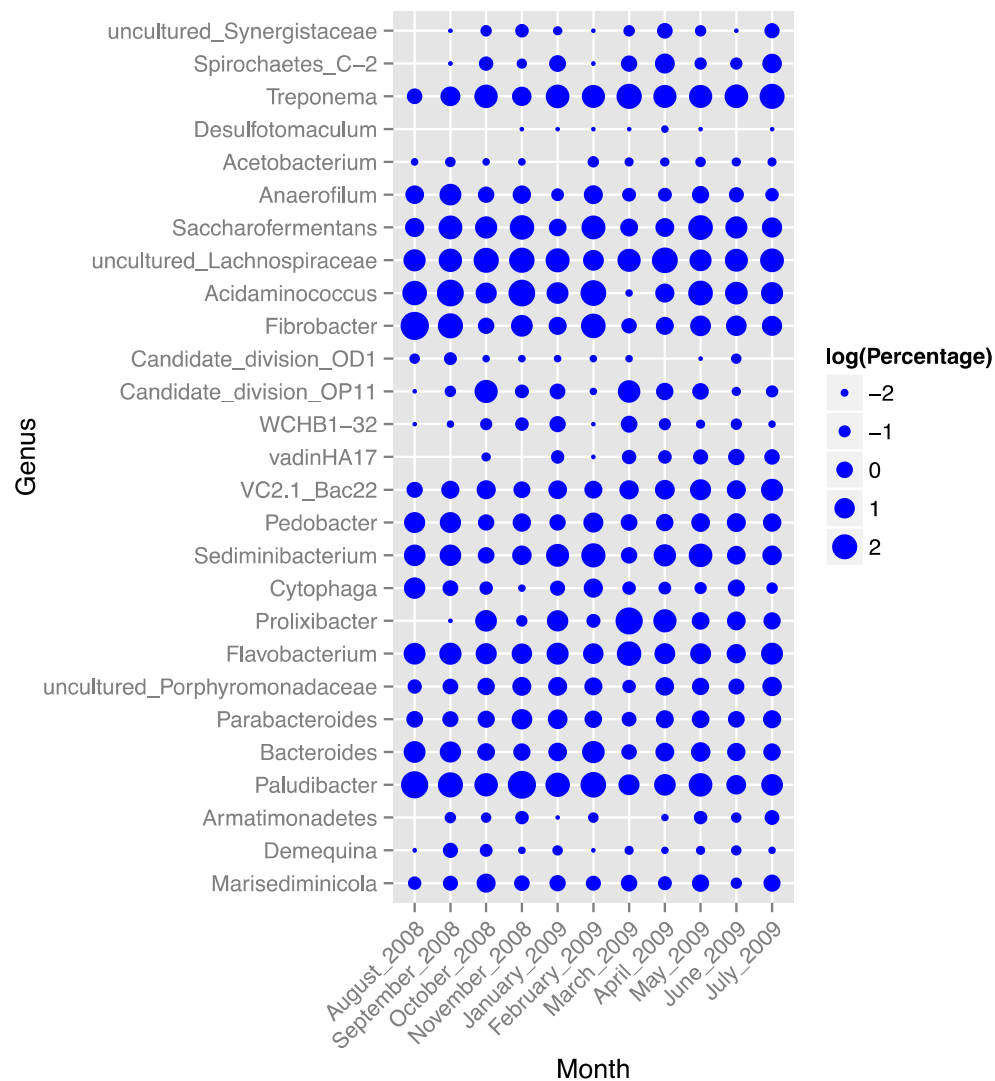


Figure S2. Relative abundance of predominant genus-level microbial taxa in each monthly sample (excluding *Proteobacteria*, which are shown in Figure S3). Dot area is proportional to log₁₀ percentage of reads assigned to that genus.

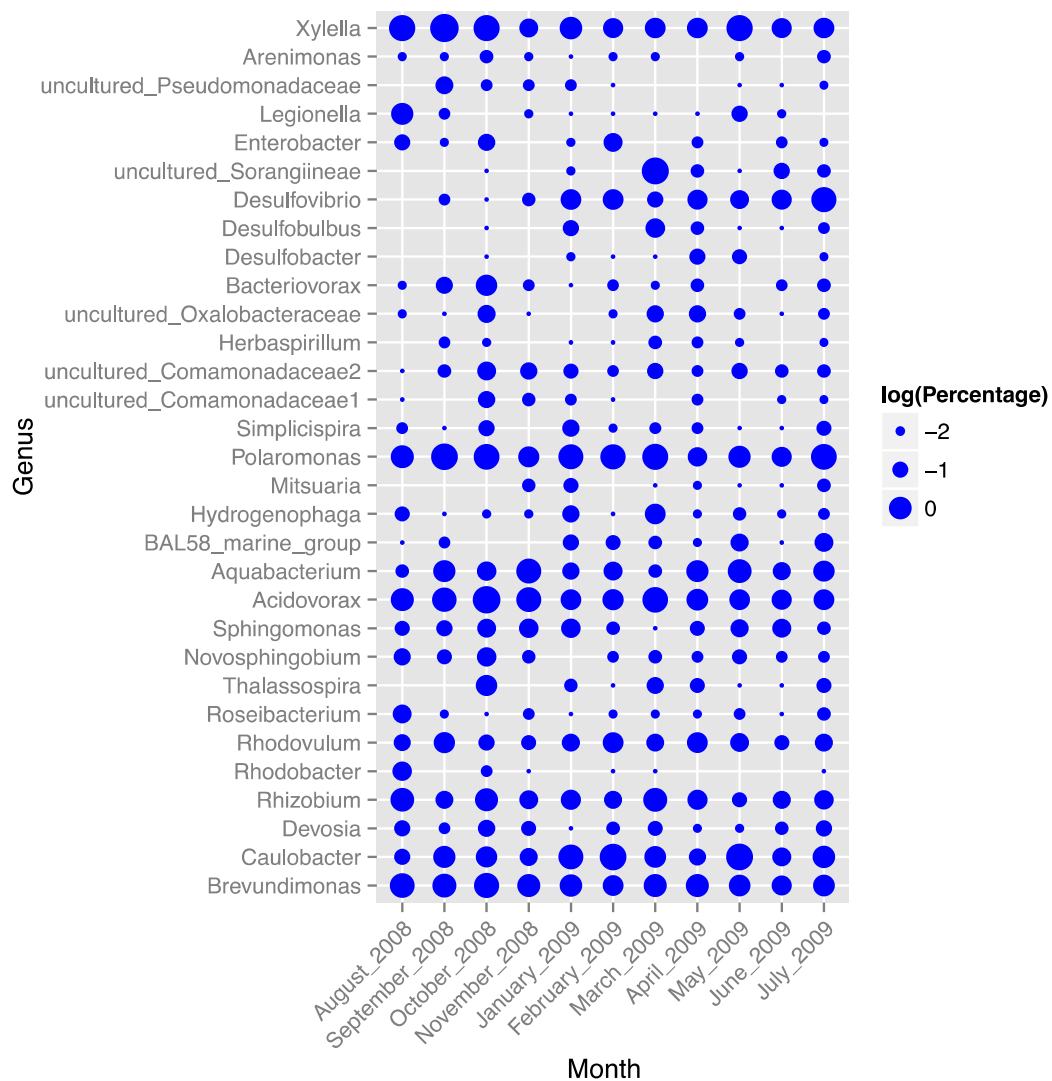


Figure S3. Genus-level taxonomic assignments to the top 30 most prevalent *Proteobacteria* genera. Dot area is proportional to $\log_{10}$ percentage of reads assigned to that.

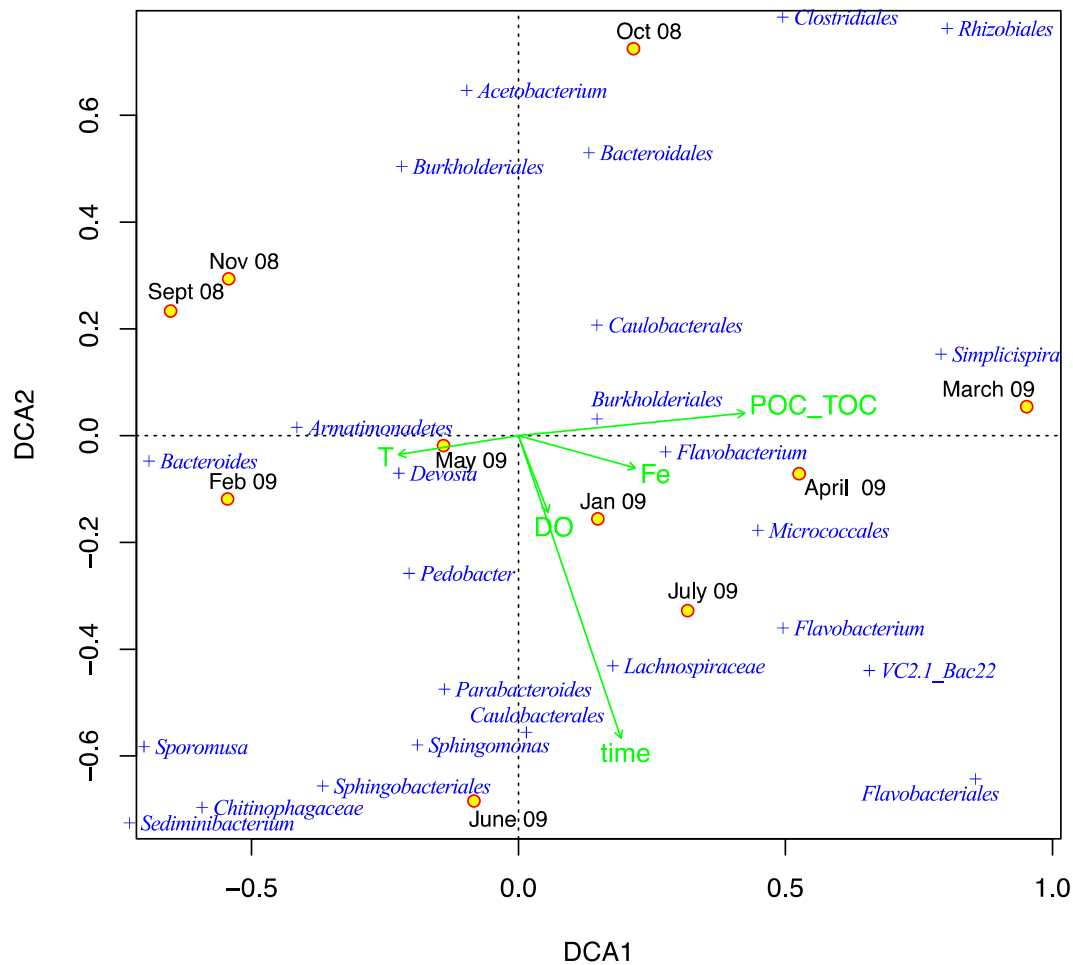


Figure S4. Detrended correspondence (DCA) plot of Bray-Curtis dissimilarities between microbial populations by month. Points represent microbial population of a particular month. When two months are close to one another that means they have very similar microbial populations. Text in blue are names of predominant taxa at the genus level close to the months in which they were more enriched. Bray-Curtis dissimilarities were fit to a linear model of environmental variables: temperature (T), dissolved oxygen (DO), time, iron concentration (Fe) and the ratio of pyrolysable carbon (PC) to total organic carbon (TOC). The green arrows represent the direction in which the variable increases. The length of the green vector represents the strength of the effect of that variable on the microbial population. The only statistically significant effect was time ($P < 0.01$).

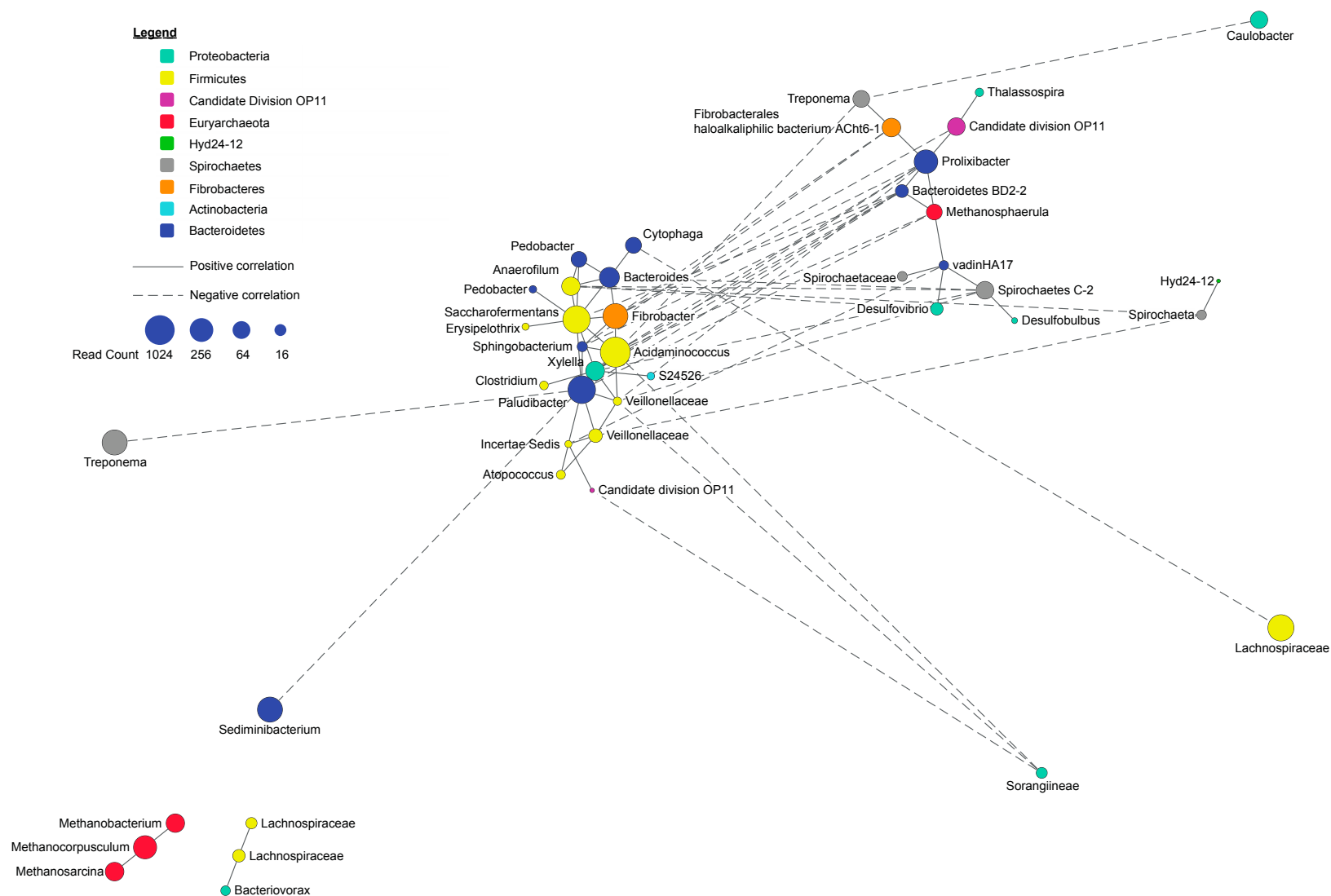


Figure S5. Network diagram of strongly correlated OTUs (>95% similarity) with Pearson correlation coefficients greater than 0.7 (solid lines) or less than −0.7 (dashed lines). Size of the vertices (OTUs) corresponds to the read count for that OTU. Vertices are coloured according to the Phylum that the OTU was classified in. Vertices are labelled according to the genus that they were classified in.

