

Supporting Information for:

Enhancing microbial reduction in hyperalkaline, chromium contaminated sediments by pH amendment

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This section consists of 10 pages, 3 tables and 2 figures.

Section 1. XAS analysis.

Soil slurry from one *pH amended* microcosm bottle on day 153 was centrifuged (10 min, 8,000 g), and the soil was stored in the dark at -80°C under a nitrogen atmosphere. The sample was prepared by centrifugation to form a moist pellet, and mounted at 1 mm thickness in aluminium holders with a Kapton™ widow. Potassium chromate and chromium(III) hydroxide standards were prepared as 50 mg pressed pellets and diluted with BN as required.

Cr K-edge spectra were collected on beamline I18 at the Diamond Light Source in November 2009, operating at 3 GeV with a typical current of 200 mA, using a nitrogen cooled Si(111) double crystal monochromator and focussing optics. A pair of plane mirrors was used to reduce the harmonic content of the beam and microfocusing was done using Kirkpatrick-Baez mirrors. The beam size at the sample was approximately 150 μm. For standards the Cr K-edge spectra were collected in transmission mode at room temperatures (~295 °K). For the *pH amended* microcosm sample data were collected in fluorescence mode using a 9 element solid state Ge detector. Experiments were performed at liquid nitrogen temperatures (approximately 78 °K) and multiple scans averaged to improve the signal to noise ratio using Athena version 0.8.056 [1]. XANES spectra were also normalised in Athena over the full data range and plotted from 5975 eV to 6042 eV with no correction required for drift in E₀.

Section 2 Microbial community analysis.

2.1. DNA extraction and cloning procedure

Microbial DNA was extracted from a 400 mg sample of B2-310 initial soil using a FastDNA spin kit (Qbiogene, Inc.) and FastPREP instrument (unless explicitly stated, the manufacturer's protocols supplied with all kits employed were followed precisely). In order to extract microbial DNA from the *pH amended* and *unamended* control microcosm incubation experiments bottles were opened and sediment collected via centrifugation (3 mins, 16,000 x g). DNA was then extracted from 400 mg of the retrieved soil pellet using the FastDNA spin kit and FastPREP instrument. DNA fragments in the size range 3 kb ~20 kb were isolated on a 1% "1x" Tris-borate-EDTA (TBE) gel stained with ethidium bromide to enable viewing under UV light (10x TBE solution from Invitrogen Ltd., UK). The DNA was extracted from the gel using a QIAquick gel extraction kit (QIAGEN Ltd., UK.).

A fragment of the 16s rRNA gene of approximately 500bp was amplified using using broad-specificity bacterial primers 8f (5'-AGAGTTTGATCCTGGCTCAG-3') [2] and 519r (5'-GWATTACCGCGGCKGCTG-3') where K = G or T, W = A or T [3]. Each PCR reaction mixture contained 20 µl of purified DNA solution, GoTaq DNA polymerase (5 units), 1x PCR reaction buffer, MgCl₂ (1.5mM), PCR nucleotide mix (0.2 mM), T4 Gene 32 Protein (100 ng/µl) and 8f and 519r primers (0.6 µM each) in a final volume of 50 µl. The reaction mixtures were incubated at 95°C for 2 min, and then cycled 30 times through three steps: denaturing (95°C, 30 s), annealing (50°C, 30s), primer extension (72°C, 45 s). This was followed by a final extension step at 72°C for 7min. The PCR products were purified using a QIAquick PCR Purification Kit. Amplification product sizes were verified by electrophoresis of 10 µl samples in a 1.0% agarose TBE gel with ethidium bromide staining.

The PCR product was ligated into the standard cloning vector (pGEM-T Easy; Promega Corp., USA), and transformed into E. coli competent cells (XL1-Blue; Agilent Technologies UK Ltd). Transformed cells were grown on LB-agar plates containing ampicillin (100 µg.ml⁻¹) at 37°C for 17 hours. The plates were surfaced dressed with IPTG and X-gal (as per the XL1-Blue protocol) for blue-white colour screening. 48 colonies from soil B2-310 and the *unamended* control microcosm, and 72 colonies from the *pH amended* microcosm containing a plasmid with the 16s rRNA gene insert were restreaked on LB-ampicillin agar plates and incubated at 37°C. Single colonies for each condition

from restreaked plates were stab inoculated into an LB-agar ampicillin 96 well plate and sent for sequencing (GATC Biotech Ltd). In order to increase confidence that a suitable representation of the highly diverse B2 310 soil population had been collected, a further 16 plasmids were sent for sequencing. Single colonies from restreaked plates were incubated overnight at 37°C in LB media. Bacterial plasmids were then extracted from the resulting broth using the Promega Pureyield™ Plasmid Miniprep Purification system (Promega Corp., USA). The concentration of plasmid DNA was then determined using UV spectroscopy, and adjusted to 50 ng.µl⁻¹ with deionised water. 12µl of each plasmid mixture was then sent for sequencing at the Faculty of Biological Sciences, University of Leeds, Leeds, UK.

2.2 Phylogenic assignment.

16s rRNA bacterial sequence fragments of cloned DNA (~500 bp) from B2 310 initial soil sample and microcosm experiments were processed with both Bellerophon[4] and Mallard v 1.02[5] online chimera checkers in order to exclude putative chimeras from subsequent analyses. Sequences were then assigned to bacterial phyla using the online Ribosomal Database Project (RDP) naïve Bayesian Classifier version 2.2 (available online: <http://rdp.cme.msu.edu/classifier/classifier.jsp>) in August 2010. The sequences were assigned to the taxonomical hierarchy: RDP training set 6, based on nomenclatural taxonomy and Bergey's Manual, with a confidence threshold of 95% (assignments to a phylum reported in Figure 4 are based on >98% confidence). Full details of this algorithm, its development and its performance can be found in Wang et al., (2007)[6]

Supporting Information References

- [1] B. Ravel, M. Newville, ATHENA, ARTEMIS, HEPHAESTUS: data analysis for X-ray absorption spectroscopy using IFEFFIT, *J. Synchrot. Radiat.*, 12 (2005) 537-541.
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- [3] D.J. Lane, B. Pace, G.J. Olsen, D.A. Stahl, M.L. Sogin, N.R. Pace, Rapid determination of 16S ribosomal RNA sequences for phylogenetic analysis, *P. Natl. Acad. Sci. USA*, 82 (1985) 6955-6959.
- [4] T. Huber, G. Faulkner, P. Hugenholtz, Bellerophon: a program to detect chimeric sequences in multiple sequence alignments, *Bioinformatics*, 20 (2004) 2317-2319.
- [5] K.E. Ashelford, N.A. Chuzhanova, J.C. Fry, A.J. Jones, A.J. Weightman, New screening software shows that most recent large 16S rRNA gene clone libraries contain chimeras, *Appl. Environ. Microbiol.*, 72 (2006) 5734-5741.
- [6] Q. Wang, G.M. Garrity, J.M. Tiedje, J.R. Cole, Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy, *Appl. Environ. Microbiol.*, 73 (2007) 5261-5267.

Table S1. RDP classification with 95% confidence threshold and OTU assignment for sequences obtained from B2 310 initial sample.

ID*	Accession number	Sequence length	Classification using the RDP classifier[6] (95% Confidence threshold)
BHL3-310I-1	FR695903	536	Firmicutes, Bacilli, Bacillales, Bacillaceae
BHL3-310I-2	FR695904	527	Proteobacteria, Betaproteobacteria
BHL3-310I-3	FR695905	547	Verrucomicrobia, Subdivision3, Subdivision3_genera_incertae_sedis
BHL3-310I-4	FR695906	470	Proteobacteria, Alphaproteobacteria
BHL3-310I-5	FR695907	517	Actinobacteria, Actinobacteria, Rubrobacteridae, Solirubrobacterales
BHL3-310I-6	FR695908	527	Proteobacteria, Betaproteobacteria
BHL3-310I-7	FR695909	535	Firmicutes, Bacilli, Bacillales, Bacillaceae, Bacillus
BHL3-310I-8	FR695910	527	Proteobacteria, Betaproteobacteria
BHL3-310I-9	FR695911	527	Proteobacteria, Betaproteobacteria
BHL3-310I-10	FR695912	499	Firmicutes, Clostridia, Clostridiales
BHL3-310I-11	FR695913	545	Verrucomicrobia, Subdivision3, Subdivision3_genera_incertae_sedis
BHL3-310I-12	FR695914	510	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
BHL3-310I-14	FR695915	526	Nitrospira, Nitrospira, Nitrospirales, Nitrospiraceae, Nitrospira
BHL3-310I-15	FR695916	527	Bacteroidetes, Sphingobacteria, Sphingobacteriales, Chitinophagaceae
BHL3-310I-16	FR695917	510	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
BHL3-310I-17	FR695918	531	Proteobacteria
BHL3-310I-18	FR695919	510	Firmicutes, Clostridia, Clostridiales, IncertaeSedisXIV, Anaerobranca
BHL3-310I-19	FR695920	517	Bacteroidetes, Flavobacteria, Flavobacteriales, Flavobacteriaceae, Flavobacterium
BHL3-310I-20	FR695921	525	Proteobacteria, Betaproteobacteria
BHL3-310I-21	FR695922	509	Actinobacteria, Actinobacteria, Actinobacteridae, Actinomycetales, Micrococccineae, Microbacteriaceae
BHL3-310I-22	FR695923	523	Bacteroidetes, Sphingobacteria, Sphingobacteriales, Chitinophagaceae
BHL3-310I-23	FR695924	549	Firmicutes, Clostridia, Clostridiales, Peptococcaceae, Peptococcaceae1, Desulfosporosinus
BHL3-310I-24	FR695925	525	Proteobacteria, Gammaproteobacteria
BHL3-310I-25	FR695926	522	-
BHL3-310I-26	FR695927	527	Proteobacteria, Betaproteobacteria, Burkholderiales
BHL3-310I-27	FR695928	532	Proteobacteria
BHL3-310I-28	FR695929	525	Proteobacteria, Betaproteobacteria
BHL3-310I-29	FR695930	517	Bacteroidetes, Flavobacteria, Flavobacteriales, Flavobacteriaceae, Flavobacterium
BHL3-310I-30	FR695931	500	Planctomycetes, Planctomycetacia, Planctomycetales, Planctomycetaceae, Gemmata
BHL3-310I-31	FR695932	536	Acidobacteria, Acidobacteria_Gp6, Gp6
BHL3-310I-32	FR695933	515	Bacteroidetes, Flavobacteria, Flavobacteriales, Flavobacteriaceae, Flavobacterium
BHL3-310I-34	FR695934	542	Nitrospira, Nitrospira, Nitrospirales, Nitrospiraceae, Leptospirillum
BHL3-310I-35	FR695935	509	Actinobacteria, Actinobacteria
BHL3-310I-36	FR695936	525	Proteobacteria, Betaproteobacteria
BHL3-310I-37	FR695937	507	Actinobacteria, Actinobacteria, Actinobacteridae, Actinomycetales, Micrococccineae
BHL3-310I-38	FR695938	528	Bacteroidetes, Sphingobacteria, Sphingobacteriales, Chitinophagaceae, Ferruginibacter
BHL3-310I-39	FR695939	543	
BHL3-310I-40	FR695940	536	Proteobacteria, Deltaproteobacteria, Myxococcales, Nannocystineae
BHL3-310I-41	FR695941	526	Nitrospira, Nitrospira, Nitrospirales, Nitrospiraceae, Nitrospira
BHL3-310I-42	FR695942	526	Proteobacteria, Betaproteobacteria
BHL3-310I-43	FR695943	508	Planctomycetes, Planctomycetacia, Planctomycetales, Planctomycetaceae
BHL3-310I-44	FR695944	531	Actinobacteria, Actinobacteria, Rubrobacteridae, Solirubrobacterales
BHL3-310I-45	FR695945	549	Firmicutes, Clostridia, Clostridiales, Peptococcaceae, Peptococcaceae1, Desulfosporosinus
BHL3-310I-46	FR695946	541	
BHL3-310I-47	FR695947	541	

ID*	Accession number	Sequence length	Classification using the RDP classifier[6] (95% Confidence threshold)
BHL3-310I-48	FR695948	510	
BHL3-310I-49	FR695949	510	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
BHL3-310I-50	FR695950	527	Proteobacteria, Betaproteobacteria
BHL3-310I-51	FR695951	522	Bacteroidetes, Sphingobacteria, Sphingobacteriales, Cyclobacteriaceae
BHL3-310I-52	FR695952	531	
BHL3-310I-53	FR695953	522	Bacteroidetes, Sphingobacteria, Sphingobacteriales, Cyclobacteriaceae
BHL3-310I-54	FR695954	531	Proteobacteria
BHL3-310I-55	FR695955	527	Proteobacteria, Betaproteobacteria
BHL3-310I-56	FR695956	512	Firmicutes, Clostridia, Clostridiales, IncertaeSedisXIV, Anaerobranca
BHL3-310I-57	FR695957	523	Bacteroidetes
BHL3-310I-58	FR695958	532	Actinobacteria, Actinobacteria, Rubrobacteridae, Solirubrobacterales, Solirubrobacteraceae, Solirubrobacter
BHL3-310I-59	FR695959	513	Bacteria_incertae_sedis, Ktedonobacteria, Ktedonobacterales, Ktedonobacteraceae, Ktedonobacter
BHL3-310I-60	FR695960	510	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
BHL3-310I-61	FR695961	516	Proteobacteria, Betaproteobacteria, Burkholderiales, Comamonadaceae, Pelomonas
BHL3-310I-62	FR695962	521	Firmicutes, Clostridia, Clostridiales, IncertaeSedisXI, Tissierella
BHL3-310I-63	FR695963	537	Firmicutes, Bacilli, Bacillales, Bacillaceae, Bacillus
BHL3-310I-64	FR695964	474	Proteobacteria, Alphaproteobacteria, Rhizobiales

* BHL3 was renumbered B2 after sequences had been submitted to GenBank

Table S2. RDP classification with 95% confidence threshold and OTU assignment for sequences obtained from *pH9 amended* microcosm day 153.

ID	Accession number	Sequence length	OTU	Classification using the RDP classifier [6] (95% Confidence threshold)
pH9-153-1	FR696008	508	C1	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
pH9-153-2	FR696009	517	C2	
pH9-153-4	FR696010	510	C3	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
pH9-153-5	FR696011	510	C4	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
pH9-153-6	FR696012	531	C5	Proteobacteria
pH9-153-7	FR696013	522	C6	Firmicutes
pH9-153-8	FR696014	522	C6	Firmicutes
pH9-153-9	FR696015	514	C7	Firmicutes
pH9-153-10	FR696016	510	C3	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
pH9-153-12	FR696017	522	C6*	
pH9-153-13	FR696018	510	C3	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
pH9-153-14	FR696019	510	C8	
pH9-153-15	FR696020	510	C3	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
pH9-153-16	FR696021	510	C3	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
pH9-153-17	FR696022	533	C9	Proteobacteria
pH9-153-18	FR696023	517	C10	Firmicutes
pH9-153-20	FR696024	522	C6*	
pH9-153-22	FR696025	511	C11	Firmicutes, Clostridia, Clostridiales, Incertae Sedis XIV, Anaerobranca
pH9-153-23	FR696026	510	C8	
pH9-153-24	FR696027	507	C1	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
pH9-153-25	FR696028	511	C12	
pH9-153-26	FR696029	510	C4	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
pH9-153-28	FR696030	515	C13	Firmicutes, Clostridia, Clostridiales, Ruminococcaceae, Acetivibrio
pH9-153-30	FR696031	511	C3	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
pH9-153-31	FR696032	506	C14	Firmicutes, Clostridia
pH9-153-32	FR696033	494	C15	Proteobacteria, Alphaproteobacteria
pH9-153-33	FR696034	510	C3	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
pH9-153-34	FR696035	516	C16	Firmicutes, Clostridia
pH9-153-35	FR696036	511	C17	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
pH9-153-36	FR696037	496	C18	Firmicutes
pH9-153-37	FR696038	508	C19	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
pH9-153-38	FR696039	508	C20	
pH9-153-39	FR696040	510	C3	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
pH9-153-40	FR696041	509	C21	Firmicutes, Clostridia
pH9-153-41	FR696042	510	C22	Firmicutes, Clostridia
pH9-153-42	FR696043	512	C11	Firmicutes, Clostridia, Clostridiales, Incertae Sedis XIV, Anaerobranca
pH9-153-43	FR696044	511	C17	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
pH9-153-44	FR696045	510	C17	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
pH9-153-45	FR696046	510	C3	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
pH9-153-48	FR696047	510	C8	
pH9-153-50	HE573872	525	C23	Proteobacteria, Betaproteobacteria, Burkholderiales, Oxalobacteraceae, Herbaspirillum
pH9-153-51	HE573873	516	C16	Firmicutes, Clostridia
pH9-153-53	HE573874	496	C18	
pH9-153-56	HE573875	527	C24	Proteobacteria, Betaproteobacteria
pH9-153-57	HE573876	511	C25	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
pH9-153-59	HE573877	509	C26	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
pH9-153-60	HE573878	510	C3	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
pH9-153-61	HE573879	506	C14	Firmicutes, Clostridia
pH9-153-62	HE573880	516	C16	

ID	Accession number	Sequence length	OTU	Classification using the RDP classifier [6] (95% Confidence threshold)
pH9-153-63	HE573881	509	C26	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
pH9-153-64	HE573882	509	C8	
pH9-153-66	HE573883	522	C6	Firmicutes
pH9-153-68	HE573884	509	C26	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
pH9-153-69	HE573885	509	C27	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
pH9-153-70	HE573886	512	C28	
pH9-153-71	HE573887	519	C29	
pH9-153-72	HE573888	510	C4	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter

* Two member of OTU B6 are classified as Firmicutes while two were unclassified

Table S3. RDP classification with 95% confidence threshold and OTU assignment for sequences obtained from *unamended microcosm day 270*.

ID	Accession number	Sequence length	OTU	Classification using the RDP classifier [6] (95% Confidence threshold)
UN-270-1	FR695965	522	B1	Firmicutes,
UN-270-2	FR695966	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-3	FR695967	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-4	FR695968	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-5	FR695969	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-6	FR695970	503	B2	Deinococcus-Thermus, Deinococci
UN-270-7	FR695971	503	B2	Deinococcus-Thermus, Deinococci
UN-270-8	FR695972	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-9	FR695973	503	B2	Deinococcus-Thermus, Deinococci
UN-270-10	FR695974	509	B3	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
UN-270-11	FR695975	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-12	FR695976	507	B4	Firmicutes, Clostridia, Natranaerobiales, Natranaerobiaceae, Dethiobacter
UN-270-13	FR695977	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-15	FR695978	503	B2 ⁺	
UN-270-16	FR695979	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-17	FR695980	504	B5	
UN-270-18	FR695981	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-19	FR695982	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-20	FR695983	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-21	FR695984	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-22	FR695985	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-23	FR695986	502	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-24	FR695987	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-25	FR695988	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-28	FR695989	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-30	FR695990	512	B6	Firmicutes, Clostridia, Clostridiales, Incertae Sedis XIV, Anaerobranca
UN-270-31	FR695991	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-32	FR695992	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-33	FR695993	503	B2	Deinococcus-Thermus, Deinococci
UN-270-34	FR695994	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-35	FR695995	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-36	FR695996	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-38	FR695997	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-39	FR695998	503	B2 ⁺	
UN-270-40	FR695999	503	B2 ⁺	
UN-270-41	FR696000	503	B2	Deinococcus-Thermus, Deinococci
UN-270-42	FR696001	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-43	FR696002	498	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-44	FR696003	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-45	FR696004	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-46	FR696005	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-47	FR696006	503	B2	Deinococcus-Thermus, Deinococci, Deinococcales, Trueperaceae, Truepera
UN-270-48	FR696007	522	B1	Firmicutes

⁺ 26 members of OTU C2 were assigned to Deinococcus-Thermus while 3 members were unassigned

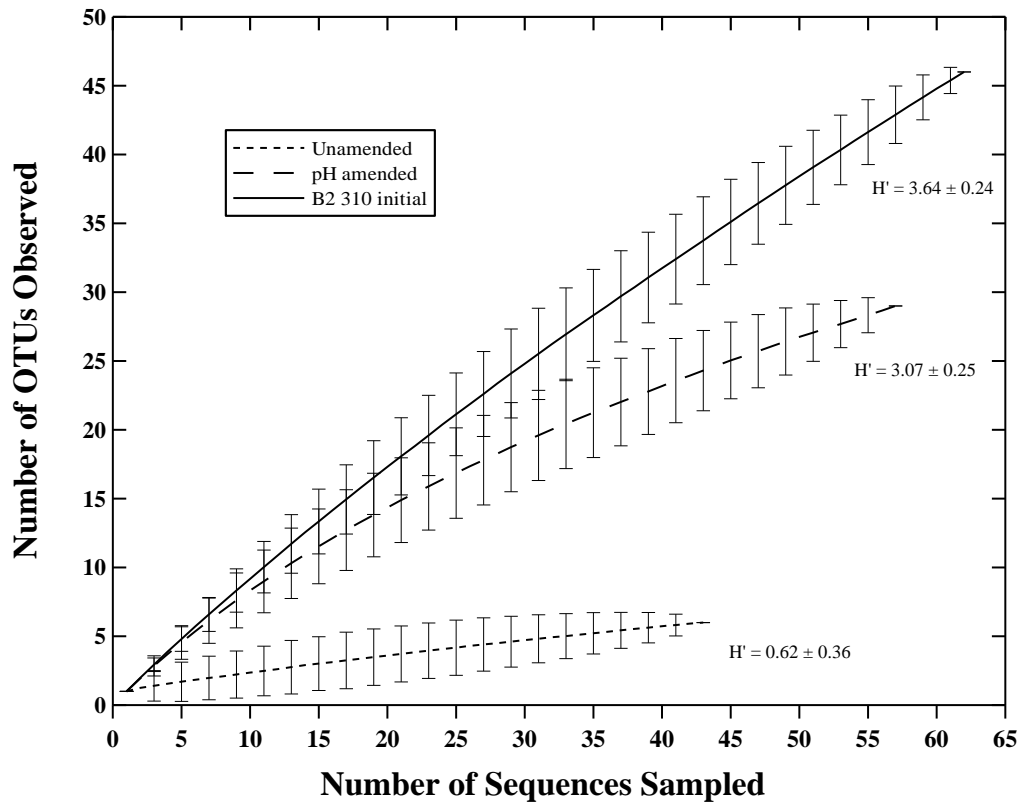


Figure S1. Rarefaction curves from MOTHUR analysis using nearest neighbour assignment with 98% similarity cut-off

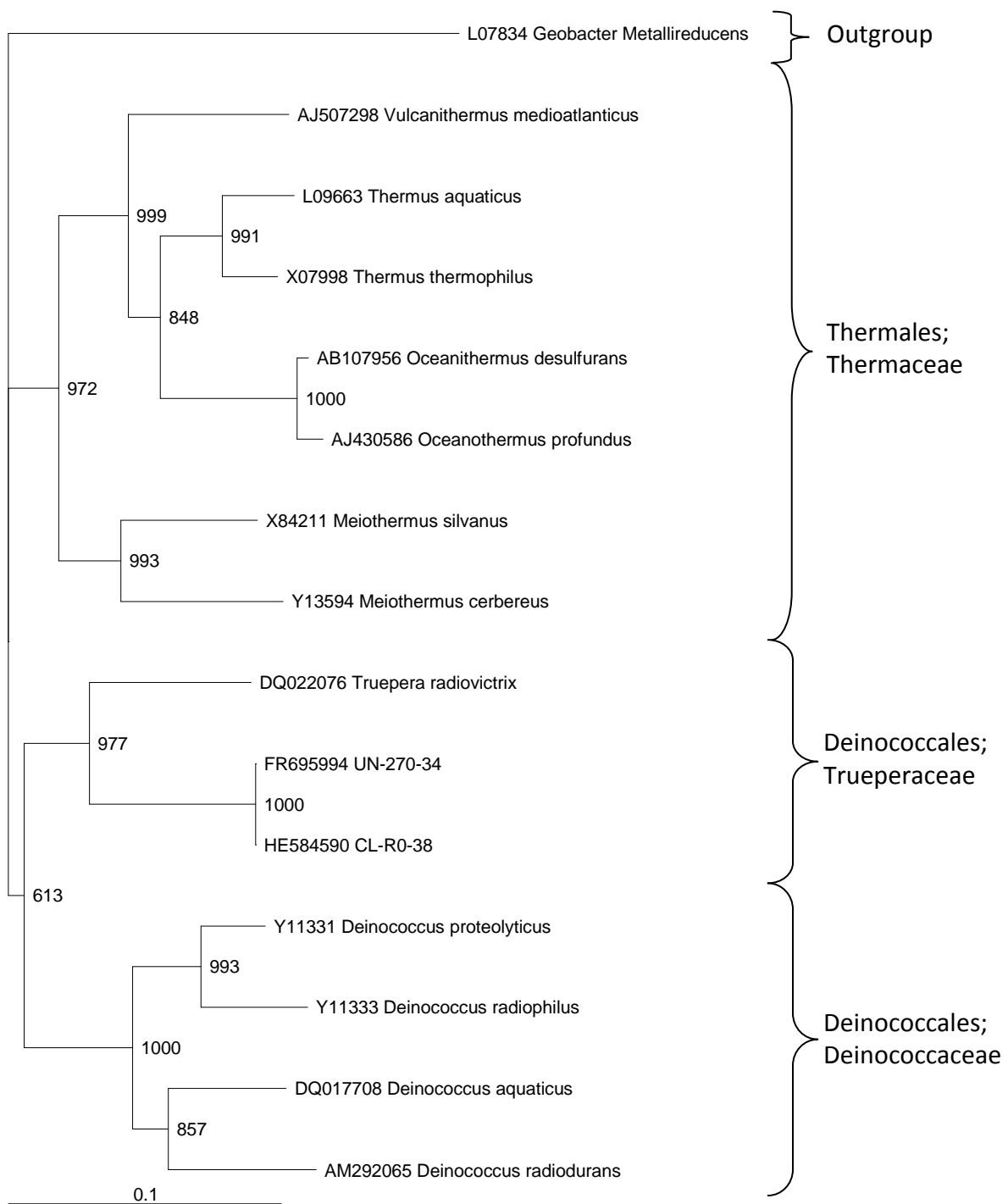


Figure S2. Phylogenetic tree showing the relationship between a representative sequence from the major OTU recovered from the *unamended* microcosm series day 270 and other members of the orders Deinococcales and Thermales of the phylum Deinococcus-Thermus. *Geobacter metallireducens* (δ -proteobacteria) is included as an out-group. The scale bar corresponds to 0.1 nucleotide substitutions per site. Bootstrap values (from 1000 replications) are shown at branch points.