

Supporting Information

Role of nitrate in conditioning aquifer sediments for technetium bioreduction

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1.1. Sample collection and Storage

Sediments, upon collection, were transferred directly into sterile HDPE sample containers, sealed, and stored at 5 °C in darkness. Experiments then began within one month of sediment sampling.

1.2. Geochemical methods

Carbonate-free total sediment organic carbon content was measured by combustion according to the method of Verardo et al., (1990). Total dissolved porewater Fe was measured photometrically using the Ferrozine test (Stookey, 1970), according to the method of Viollier et al., (2000). Porewater manganese was measured photometrically with the PAN indicator method (Brewer and Spencer, 1971). Porewater nitrite was measured photometrically using the method of Harris and Mortimer, (2002).

1.3. XAS Sample Preparation

After 10 days of incubation, pre-reduced sediment microcosms were geochemically monitored prior to XAS sampling. Approximately 40 μM and 70 μM of added Tc (ca. 325 μM) remained in solution in the carbonate buffered and high-nitrate systems, respectively. After geochemical analysis, sediment slurries were centrifuged (12 minutes, 2000 g) and approximately 0.5 g of sediment (water content < 50 %) was mounted for XAS analysis on an air-tight XAS Perspex sample cell with Kapton windows. The sample cell was then double contained in heat sealed plastic bags and stored at -80 °C under argon until analysis. All sample manipulations were conducted in an O₂-free atmosphere.

1.4. XAS Analysis

Techneium K-edge spectra were collected in fluorescence mode on a 30 element solid-state Ge detector on the ultra dilute spectroscopy line (16.5) at the UK CLRC Daresbury SRS, using focusing optics and a Si-220 double crystal monochromator, calibrated from the K-edge of a Mo foil. A total of 10-12 scans were collected for each sample and averaged to improve the signal-to-noise ratio. The EXAFS spectra were background subtracted using EXPLINE and analyzed with EXCURV98 using full curved wave theory (Gurman et al., 1995). Phase shifts were determined from *ab initio* calculations using Hedin-Lundqvist potentials and von Barth ground states (Binsted, 1998). The data were fitted in k^3 space by defining a theoretical model which was informed by the relevant literature (Wharton et al., 2000; Lukens et al., 2002; Maes et al., 2004; Morris et al., 2008) and used whole integer values for shell of back-scatters around the central atom. Shells of backscatter atoms were added around a central ^{99}Tc atom and by refining an energy correction E_f (the fermi energy), the absorber-scatter distance, the number of atoms in each shell, and the least square residual (the R factor; Binsted, 1992) was minimized (Morris et al., 2008). Shells were only included in the model fit if the overall R-factor was improved by $> 5\%$.

1.5. PCR analysis

A standard curve for the qPCR reaction was created by plotting the cycle threshold values of the qPCR performed on a dilution series of DNA obtained from *Geobacter sulfurreducens* (GenBank Accession Number U13928) against the log of DNA template concentration. Dilution series concentrations ranging from $0.1 \text{ ng } \mu\text{l}^{-1}$ to $0.88 \text{ pg } \mu\text{l}^{-1}$,

were analysed in triplicate and had an R^2 value of 0.98. PCR amplification was performed in triplicate on a Stratagene MX3000P qPCR machine using 0.25 μ M of each primer and qPCR SYBR Green Master Mix (Stratagene) with an initial step of 94 °C for 10 minutes followed by 40 cycles of 94 °C for 30 seconds, 58 °C for 30 seconds and 72°C for 30 seconds. A dissociation curve was run between 94 °C and 58 °C to check primer specificity. Cycle threshold was determined automatically.

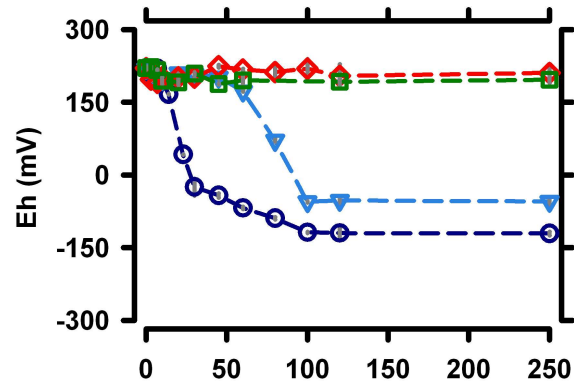


Figure 1. Microbially active microcosm Eh incubation time-series data (day 0-250). Dark blue circles = carbonate buffered system, Green squares = unamended system, Red diamonds = low-nitrate system, Light blue inverted triangles = high-nitrate system. Error bars represent 1 σ experimental uncertainty from triplicate microcosm experiments (where not visible, error bars are within the symbol size). Eh did not change in sterile controls.

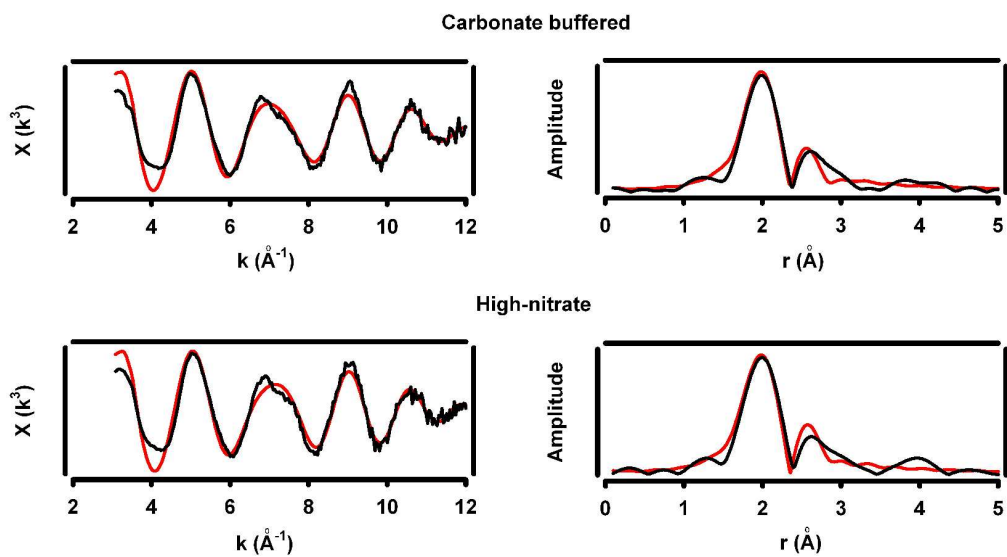


Figure 2. Background subtracted, normalized, and k^3 weighted ^{99}Tc K edge EXAFS spectra (left) and corresponding Fourier transforms (right) for reduced sediments from the carbonate buffered and high nitrate systems (black) and for the best modeled fit obtained in supporting information Table 3 (red).

Table 1. Synthetic groundwater media base composition (Wilkins et al., 2007).

Compound	g L⁻¹
KCl	0.0066
MgSO ₄ ·7H ₂ O	0.0976
MgCl ₂ ·6H ₂ O	0.0810
Na ₂ SiO ₃	0.0829
NaCl	0.0094

Table 2. Synthetic groundwater media compositions.

Microcosm	Base media	NO₃⁻ (mM)	SO₄²⁻ (mM)	Na-acetate (mM)	pH
Unamended	Wilkins et al, 2007	0.3	0.4	10.0	5.5
Low-nitrate	Wilkins et al, 2007	2.0	0.4	10.0	5.5
High-nitrate	Wilkins et al, 2007	10.0	0.4	10.0	5.5
Carbonate buffered*	Wilkins et al, 2007	0.3	0.4	10.0	7.0

* 3 mM NaHCO₃ and 1.7 mM CaCO₃ were added to the carbonate buffered groundwater media.

Table 3. Sediment major and minor elemental composition

Element	Concentration
Si	35 wt %
Al	5.8 wt %
Fe	3.1 wt %
K	2.7 wt %
Na	1.0 wt %
Mg	0.5 wt %
Ti	0.4 wt %
Ca	0.2 wt %
Ba	0.2 wt %
Mn	0.1 wt %
P	0.02 wt %
Ba	0.2 wt %
Zr	251 ppm
Rb	119 ppm
V	81 ppm
Sr	58 ppm
Zn	51 ppm
Pb	42 ppm
Cr	30 ppm
Cu	<30 ppm
Nd	21 ppm
La	18 ppm
Ni	17 ppm
Co	<10 ppm
LOI	4.07 wt %

Table 4. Summary of EXAFS modelling results for reduced sediments representative of the Sellafield site.

Sample	Shell	O-T	r (Å)	2σ ² (Å ²)	R
Carbonate buffered	1	6-O	1.99	0.016	42
	1	6-O	2.01	0.016	29
	2	1-Tc	2.52	0.014	
	1	6-O	2.00	0.016	32
	2	1-Fe	2.69	0.008	
	High-nitrate	1	6-O	2.00	0.018
1		6-O	2.00	0.016	28
2		1-Tc	2.53	0.011	
1		6-O	2.00	0.016	34
2		1-Fe	2.69	0.008	

O-T is the modelled occupancy and type, r (Å) is the interatomic distance, 2σ² (Å²) is the Debye-Waller factor, and R is the least squares residual.

Table 5. Phylogenetic affiliation of 16S rRNA gene sequences detected in the clone library from the carbonate buffered system at 0 days.

Clone number	Accession number	Closest relative (accession number)	Similarity (% Match)	% present in clone library	Phylogenetic Division
AT0-1	GQ903133	Uncultured bacterium TSC131 (EU360041)	409/443 (92%)	2.3 (1/43)	Alphaproteobacteria
AT0-2	GQ903134	Uncultured bacterium 5V79 (DQ663849)	400/425 (94%)	6.97 (3/43)	Acidobacteria
AT0-3	GQ903135	Uncultured <i>Nitrosovibrio</i> sp. B4_14 (AM773598)	387/395 (97%)	4.65 (2/43)	Unknown
AT0-5	GQ903136	Uncultured bacterium AH31 (AY963324)	358/395 (90%)	4.65 (2/43)	Acidobacteria
AT0-7	GQ903137	Uncultured bacterium GASP-MA3W3_H06 (EF663817)	381/399 (95%)	9.3 (4/43)	Acidobacteria
AT0-10	GQ903138	Uncultured <i>Planctomyces</i> sp. BD1-23 (AB015527)	330/356 (92%)	2.3 (1/43)	Planctomycetes
AT0-11	GQ903139	Uncultured bacterium SX2-19 (DQ469209)	320/352 (90%)	2.3 (1/43)	Betaproteobacteria
AT0-14	GQ903140	Uncultured bacterium C33.24SM (AF432814)	386/398 (96%)	2.3 (1/43)	Acidobacteria
AT0-15	GQ903141	Uncultured bacterium S15-3 (EU680463)	324/360 (90%)	2.3 (1/43)	Unknown
AT0-17	GQ903142	Uncultured alpha proteobacterium GASP-45KA-43-C06 (EU044312)	409/415 (98%)	6.97 (3/43)	Alphaproteobacteria
AT0-18	GQ903143	Unidentified bacterium H02_SGPO01(EF221060)	471/504 (93%)	4.65 (2/43)	Acidobacteria
AT0-19	GQ903144	Uncultured bacterium isolate High.60.F3.HB13 (DQ195631)	403/433 (93%)	4.65 (2/43)	Unknown
AT0-20	GQ903145	Uncultured Verrucomicrobia bacterium A07_WMSP1 (DQ450782)	451/474 (95%)	2.3 (1/43)	Verrucomicrobia
AT0-21	GQ903146	Uncultured bacterium anNA12 (EF034689)	330/361 (91%)	2.3 (1/43)	Unknown
AT0-24	GQ903147	Uncultured bacterium Amb_16S_1165 (EF018714)	402/416 (96%)	2.3 (1/43)	Acidobacteria
AT0-25	GQ903148	Uncultured bacterium FCPU671 (EF516884)	378/420 (90%)	4.65 (2/43)	Acidobacteria
AT0-26	GQ903149	Uncultured Acidobacteria bacterium WSD-032 (EF588358)	345/382 (90%)	2.3 (1/43)	Acidobacteria
AT0-28	GQ903150	Uncultured bacterium FAC55 (DQ451494)	516/523 (98%)	2.3 (1/43)	Unknown
AT0-29	GQ903151	Uncultured bacterium JG30-KF-C37(AJ536864)	379/393 (96%)	6.97 (3/43)	Acidobacteria
AT0-30	GQ903152	Uncultured bacterium S15-143 (EU680411)	494/497 (99%)	2.3 (1/43)	Betaproteobacteria
AT0-32	GQ903153	Uncultured bacterium GASP-16KA-214-F03 (EU043920)	393/399 (98%)	4.65 (2/43)	Acidobacteria

AT0-33	GQ903154	Uncultured bacterium 3-33 (EU637706)	488/497 (98%)	2.3 (1/43)	Unknown
AT0-34	GQ903155	Uncultured Verrucomicrobia bacterium A07_WMSP1 (DQ450782)	478/524 (91%)	2.3 (1/43)	Verrucomicrobia
AT0-37	GQ903156	Uncultured gamma proteo-bacterium N41.113PG (AF431365)	466/498 (93%)	4.65 (2/43)	Unknown
AT0-43	GQ903157	<i>Variovorax</i> sp. KS2D-23 (AB196432)	489/498 (98%)	2.3 (1/43)	Betaproteobacteria
AT0-50	GQ903158	Uncultured bacterium FCPP450 (EF516894)	455/461 (98%)	2.3 (1/43)	Acidobacteria
AT0-53	GQ903159	Uncultured Verrucomicrobiales bacterium AhedenT24 (FJ475385)	421/451 (93%)	2.3 (1/43)	Verrucomicrobia

Table 6. Phylogenetic affiliation of 16S rRNA gene sequences detected in 16S rRNA clone library from the high nitrate system at 0 days.

Clone name	Accession number	Closest relative (accession number)	Similarity (% Match)	% present in clone library	Phylogenetic Division
CT0-1	GQ903176	Uncultured bacterium FCPT606 (EF516172)	485/506 (95%)	2.0 (1/51)	Verrucomicrobia
CT0-2	GQ903177	Uncultured alpha proteobacterium glen99_6 (AY150881)	417/445 (93%)	21.6 (11/51)	Alphaproteobacteria
CT0-3, CT0-55	GQ903178	Uncultured bacterium 9S26 (DQ664128)	479/480 (99%)	15.7 (8/51)	Acidobacteria
CT0-5	GQ903179	Uncultured bacterium FCPP437 (EF516340)	468/492 (95%)	2.0 (1/51)	Unknown
CT0-6	GQ903180	Uncultured bacterium FAC71 (DQ451510)	489/492 (99%)	2.0 (1/51)	Acidobacteria
CT0-8	GQ903181	Uncultured bacterium S22.41WL (AF432729)	464/470 (98%)	3.9 (2/51)	Unknown
CT0-9	GQ903182	Unidentified bacterium D01_SGPO01 (EF220972)	451/480 (93%)	3.9 (2/51)	Unknown
CT0-14	GQ903183	Uncultured bacterium DOK_CONFYM_clone792 (DQ828993)	438/448 (97%)	3.9 (2/51)	TM7
CT0-16	GQ903184	Uncultured alpha proteobacterium AKYG981 (AY921934)	431/438 (98%)	7.8 (4/51)	Alphaproteobacteria
CT0-17	GQ903185	Uncultured forest soil bacterium DUNssu227 (AY913429)	470/473 (99%)	7.8 (4/51)	Acidobacteria
CT0-18	GQ903186	Uncultured bacterium 38 (EF474280)	475/484 (98%)	3.9 (2/51)	Acidobacteria
CT0-20	GQ903187	Unidentified bacterium AI-2M_E11 (EF219790)	454/470 (96%)	11.8 (6/51)	Unknown
CT0-28	GQ903188	Uncultured bacterium AS51 (AY963415)	459/463 (99%)	2.0 (1/51)	Acidobacteria
CT0-29	GQ903189	Uncultured bacterium FFCH12595 (EU133712)	461/493 (93%)	2.0 (1/51)	Bacteroidetes
CT0-31	GQ903190	Uncultured bacterium DOK_CONFYM_clone440 (DQ828690)	443/451 (98%)	2.0 (1/51)	Alphaproteobacteria
CT0-40	GQ903191	Uncultured bacterium DUNssu007(+1B)(OTU#078) (AY724094)	450/452 (99%)	2.0 (1/51)	Unknown
CT0-43	GQ903192	Uncultured proteobacterium TSC21 (EU359932)	469/496 (94%)	2.0 (1/51)	Unknown
CT0-51	GQ903193	Uncultured Acidobacteria bacterium WSD-032 (EF588358)	459/485 (94%)	2.0 (1/51)	Acidobacteria
CT0-53	GQ903194	Uncultured bacterium FCPP453 (EF516137)	446/453 (98%)	2.0 (1/51)	Acidobacteria

Table 7. Phylogenetic affiliation of 16S rRNA gene sequences detected in 16S rRNA clone library from the carbonate buffered system at 24 days.

Clone name	Accession number	Closest relative (accession number)	Similarity (% Match)	% present in clone library	Phylogenetic Division
AT24-1	GQ903160	<i>Sphingomonas</i> sp. EC-K085 (AB264174)	346/361 (95%)	4.5 (2/44)	Alphaproteobacteria
AT24-2	GQ903161	Uncultured bacterium RS 8-Bact23 (AJ867667)	476/494 (96%)	13.6 (6/44)	Betaproteobacteria
AT24-3	GQ903162	<i>Janthinobacterium lividum</i> GA01 (DQ473538)	469/489 (95%)	2.3 (1/44)	Unknown
AT24-4	GQ903163	Uncultured bacterium Elev_16S_1406 (EF020015)	466/485 (96%)	2.3 (1/44)	TM7
AT24-5	GQ903164	Uncultured candidate division TM7 bacterium AI-2M_C01 (EF219756)	444/446 (99%)	6.8 (3/44)	Betaproteobacteria
AT24-6	GQ903165	<i>Burkholderia sediminicola</i> strain HU2-65W (EU035613)	476/482 (98%)	6.8 (3/44)	Betaproteobacteria
AT24-8	GQ903166	Uncultured gamma proteobacterium S-A1 (AY622264)	483/488 (98%)	25.0 (11/44)	Gammaproteobacteria
AT24-12	GQ903167	Uncultured bacterium M14C24 (EU331388)	477/488 (97%)	4.5 (2/44)	Betaproteobacteria
AT24-13	GQ903168	Uncultured Bacteroidetes bacterium CrystalBog5D8 (AY792301)	480/483 (99%)	2.3 (1/44)	Bacteroidetes
AT24-14	GQ903169	Uncultured soil bacterium L1A.13F01 (AY989483)	439/458 (95%)	11.4 (5/44)	Betaproteobacteria
AT24-18	GQ903170	Uncultured bacterium KuyT-ice-74 (EU263768)	469/492 (95%)	2.3 (1/44)	Bacteroidetes
AT24-19	GQ903171	Unidentified bacterium C05_SGPL01 (EF221128)	464/481 (96%)	6.8 (3/44)	Acidobacteria
AT24-25	GQ903172	<i>Desulfosporosinus</i> sp. A10 (AJ582756)	499/514 (97%)	2.3 (1/44)	Firmicutes
AT24-27	GQ903173	Uncultured Acidobacteria bacterium WSD-037 (EF588363)	471/481 (97%)	4.5 (2/44)	Acidobacteria
AT24-31	GQ903174	Uncultured bacterium BANW605 (DQ264563)	448/474 (94%)	2.3 (1/44)	Betaproteobacteria
AT24-32	GQ903175	<i>Dyella koreensis</i> strain BB4 (AY884571)	475/489 (97%)	2.3 (1/44)	Gammaproteobacteria
		<i>Ralstonia</i> sp. PHD-12 (DQ374437)	463/482 (96%)	2.3 (1/44)	Betaproteobacteria

Table 8. Phylogenetic affiliation of 16S rRNA gene sequences detected in 16S rRNA clone library from the carbonate buffered system at 250 days.

Clone name	Accession number	Closest relative (accession Number)	Similarity (% Match)	% present in clone library	Phylogenetic division
AT250-1	GQ903237	<i>Geobacter psychrophilus</i> P35 (AY653549)	496/516 (96%)	2.0 (1/49)	Deltaproteobacteria
AT250-2	GQ903238	Uncultured <i>Geobacter</i> sp. NO3_C-10 (AY752772)	497/515 (96%)	8.2 (4/49)	Deltaproteobacteria
AT250-4, AT250-9	GQ903239 GQ903240	Uncultured bacterium t30d34L66 (FM956250)	503/515 (97%)	8.2 (4/49)	Bacteroidetes
AT250-5	GQ903241	Uncultured bacterium 9S26 (DQ664128)	499/500 (99%)	4.1 (2/49)	Acidobacteria
AT250-6	GQ903242	Uncultured bacterium M14C24 (EU331388)	492/503 (97%)	10.2 (5/49)	Betaproteobacteria
AT250-10	GQ903243	Uncultured bacterium FRC-A2_735 (EF508009)	477/498 (95%)	4.1 (2/49)	Bacteroidetes
AT250-14	GQ903244	Uncultured bacterium 9S54 (DQ664146)	487/507 (96%)	4.1 (2/49)	Unknown
AT250-19	GQ903245	Uncultured bacterium BS67 (AY963503)	462/465 (99%)	2.0 (1/49)	Acidobacteria
AT250-20	GQ903246	Uncultured bacterium RB355 (AB240350)	492/509 (96%)	2.0 (1/49)	Acidobacteria
AT250-22	GQ903247	Uncultured bacterium LWS-T4642 (EU546377)	480/499 (96%)	2.0 (1/49)	Unknown
AT250-23, AT250-45	GQ903248 GQ903249	Uncultured Bacteroidetes bacterium FNE11-29 (DQ501308)	476/483 (98%)	16.3 (8/49)	Bacteroidetes
AT250-25	GQ903250	Uncultured bacterium LWS-RSG-3932 (EU546744)	482/495 (97%)	2.0 (1/49)	Bacteroidetes
AT250-26	GQ903251	Uncultured bacterium FRC-A2_251 (EF507972)	499/515 (96%)	4.1 (2/49)	Deltaproteobacteria
AT250-31	GQ903252	Uncultured <i>Xylophilus</i> sp. CMIB6 (AM936163)	490/499 (98%)	4.1 (2/49)	Betaproteobacteria
AT250-35	GQ903253	Uncultured <i>Verrucomicrobia</i> bacterium HF032 (EF417621)	399/425 (94%)	2.0 (1/49)	Verrucomicrobia
AT250-37	GQ903254	Uncultured bacterium D41S30C27 (FM956915)	433/453 (95%)	2.0 (1/49)	Bacteroidetes
AT250-38	GQ903255	Uncultured bacterium D242_27F_BAC2_013 (AB447719)	321/336 (95%)	4.1 (2/49)	Bacteroidetes
AT250-41	GQ903256	Uncultured bacterium Roth04 (AY395221)	399/418 (95%)	2.0 (1/49)	Unknown
AT250-42	GQ903257	Uncultured bacterium LD_Ba_Top_B42 (EU644267)	399/423 (94%)	2.0 (1/49)	Deltaproteobacteria
AT250-43	GQ903258	Uncultured bacterium D41S30C84 (FM956960)	403/434 (92%)	2.0 (1/49)	BRC1
AT250-44	GQ903259	Uncultured bacterium FAC71(DQ451510)	264/265 (99%)	2.0 (1/49)	Acidobacteria

AT250-47	GQ903260	Uncultured bacterium X9Ba78 (AY607208)	241/260 (92%)	4.1 (2/49)	Chloroflexi
AT250-48	GQ903261	Uncultured bacterium FW42 (F524019)	374/386 (96%)	2.0 (1/49)	Chloroflexi
AT250-50	GQ903262	Uncultured delta proteo- bacterium C32.51SM (AF431389)	214/221 (96%)	2.0 (1/49)	Deltaproteobacteria
AT250-53	GQ903263	Uncultured proteo- bacterium AhedenR37 (FJ475537)	226/227 (99%)	2.0 (1/49)	Unknown

Table 9. Phylogenetic affiliation of 16S rRNA gene sequences detected in 16S rRNA clone library from the high nitrate system at 30 days.

Clone name	Accession number	Closest relative (accession number)	Similarity (% Match)	% present in clone library	Phylogenetic division
CT30-1	GQ903195	Uncultured forest soil bacterium DUNssu011 (AY913232)	450/455 (98%)	1.9 (1/52)	Alphaproteobacteria
CT30-2	GQ903196	Unidentified bacterium C05_SGPL01 (EF221128)	371/374 (99%)	1.9 (1/52)	Acidobacteria
CT30-3	GQ903197	Uncultured Acidobacteria bacterium B08_WMSP1 (DQ450707)	326/340 (95%)	1.9 (1/52)	Acidobacteria
CT30-4	GQ903198	<i>Rhodanobacter terrae</i> GP18-1 (EF166076)	409/417 (98%)	1.9 (1/52)	Gammaproteobacteria
CT30-5	GQ903199	Uncultured bacterium MBFOS-11 (EU369170)	347/385 (90%)	1.9 (1/52)	Firmicutes
CT30-6	GQ903200	<i>Herbaspirillum</i> sp. P-64 (AM411937)	290/302 (96%)	28.8 (15/52)	Betaproteobacteria
CT30-53	GQ903201	<i>Herbaspirillum</i> sp. PIV-34-1 (AJ505863)	288/302 (95%)		
CT30-7	GQ903202	Uncultured soil bacterium CWT ST03_F05 (DQ129198)	318/329 (96%)	1.9 (1/52)	Acidobacteria
CT30-8	GQ903203	<i>Bacillus senegalensis</i> RN2 (AB110415)	284/295 (96%)	13.5 (7/52)	Firmicutes
CT30-9	GQ903204	<i>Burkholderia</i> sp. SB5 (AJ971354)	337/337(100%)	1.9 (1/52)	Betaproteobacteria
CT30-10	GQ903205	Uncultured Acidobacteria bacterium WSD-032 (EF588358)	373/376 (99%)	1.9 (1/52)	Acidobacteria
CT30-11	GQ903206	Uncultured bacterium MSC49 (AJ830735)	289/294 (98%)	3.8 (2/52)	Betaproteobacteria
CT30-13	GQ903207	Uncultured bacterium M40C1 (EU331385)	493/502 (98%)	1.9 (1/52)	Betaproteobacteria
CT30-14	GQ903208	Uncultured bacterium FCPT732 (EF516752)	405/409 (99%)	1.9 (1/52)	Acidobacteria
CT30-15	GQ903209	<i>Sporolactobacillus dextrus</i> (96%)	334/345 (96%)	11.5 (6/52)	Firmicutes
CT30-47	GQ903210	IAM 12380 (D16282)			
CT30-17	GQ903211	Uncultured bacterium PA2 01_D06 (AM900227)	388/393 (98%)	11.5 (6/52)	Firmicutes
CT30-23	GQ903212	Uncultured bacterium GPT3 (AY706437)	381/388 (98%)	3.8 (2/52)	Gammaproteobacteria
CT30-27	GQ903213	Uncultured forest soil bacterium DUNssu072 (AY913295)	478/481 (99%)	1.9 (1/52)	Acidobacteria
CT30-32	GQ903214	Uncultured bacterium 9S26 (DQ664128)	491/495 (99%)	1.9 (1/52)	Acidobacteria
CT30-33	GQ903215	Uncultured <i>Bacilli</i> bacterium (95%)	400/420 (95%)	3.8 (2/52)	Firmicutes
CT30-42	GQ903216	X3Ba61 (AY607149)			

Table 10. Phylogenetic affiliation 16S rRNA gene sequences detected in 16S rRNA clone library from the high nitrate system at 60 days.

Clone name	Accession number	Closest relative (accession number)	Similarity (% Match)	% present in clone library	Phylogenetic division
CT60-1	GQ903217	Uncultured beta proteobacterium LF033 (EF417722)	442/478 (92%)	2.1 (1/48)	Betaproteobacteria
CT60-2	GQ903218	Uncultured bacterium PA2 01_D06 (AM900227)	384/395 (97%)	8.3 (4/48)	Firmicutes
CT60-3	GQ903219	<i>Sporolactobacillus dextrus</i> IAM 12380(D16282)	343/356 (96%)	8.3 (4/48)	Firmicutes
CT60-4	GQ903220	Uncultured bacterium M40C1 (EU331385)	468/487 (96%)	2.1 (1/48)	Betaproteobacteria
CT60-5	GQ903221	Uncultured beta proteobacterium JG35+U3-JT43 (AM084888)	365/385 (94%)	60.4 (29/48)	Betaproteobacteria
CT60-16	GQ903222	Uncultured soil bacterium MJK38 (EF540381)	492/537 (91%)	4.2 (2/48)	TM7
CT60-20	GQ903223	Uncultured forest soil bacterium DUNssu174 (AY913381)	432/435 (99%)	2.1 (1/48)	Acidobacteria
CT60-27	GQ903224	Uncultured bacterium 1/5/6F (FJ380139)	399/418 (95%)	2.1 (1/48)	Gammaproteobacteria
CT60-31	GQ903225	Uncultured <i>Bacillus</i> sp.	304/327 (92%)	4.2 (2/48)	Firmicutes
CT60-32	GQ903226	<i>PmeaMucB8</i> (EU249961)	(92%)	(2/48)	
CT60-45	GQ903227	Uncultured bacterium LS4-200 (AB234278)	399/424 (94%)	4.2 (2/48)	Gammaproteobacteria
CT60-49	GQ903228	Uncultured <i>Rhodanobacter</i> sp. GASP-WC1S1_C08 (EF074391)	358/389 (92%)	2.1 (1/48)	Gammaproteobacteria

Table 11. Phylogenetic affiliation of 16S rRNA gene sequences detected in 16S rRNA clone library from the high nitrate system at 80 days.

Clone name	Accession number	Closest relative (accession number)	Similarity (% Match)	% present in clone library	Phylogenetic division
CT80-1	GQ903229	Uncultured <i>Geobacteraceae</i> bacterium M16_6691 (EF669008)	499/515 (96%)	28.3 (13/46)	Deltaproteobacteria
CT80-2	GQ903230	<i>Herbaspirillum</i> sp. PIV-34-1 (AJ505863)	473/484 (97%)	58.7 (27/46)	Betaproteobacteria
CT80-3	GQ903231	<i>Mucilaginibacter</i> sp. BR5-28 (EU423305)	464/484 (95%)	2.2 (1/48)	Bacteroidetes
CT80-8	GQ903232	Uncultured Acidobacteria bacterium WSD-032 (EF588358)	482/491 (98%)	2.2 (1/48)	Acidobacteria
CT80-9	GQ903233	Uncultured bacterium ph5Glu032-5C (AY527739)	480/495 (96%)	2.2 (1/48)	Firmicutes
CT80-16	GQ903234	Uncultured <i>Geobacteraceae</i> bacterium M16_6691 (EF669008)	465/501 (92%)	2.2 (1/48)	Deltaproteobacteria
CT80-39	GQ903235	Uncultured bacterium KD9-53 (AY218669)	475/484 (98%)	2.2 (1/48)	Bacteroidetes
CT80-47	GQ903236	<i>Sporolactobacillus dextrus</i> IAM 12380 (D16282)	485/491 (98%)	2.2 (1/48)	Firmicutes

Table 12. NarG sequences retrieved from the high nitrate system at day 0 and their closest matches in the GenBank.

Clone name	Accession number	Closest amino acid sequence (accession number)	Similarity (% Match)	% present
CT0-narG_1	FN554828	putative dissimilatory membrane-bound nitrate reductase [uncultured bacterium] (AAM62209) clone C71	179/203 (88.2 %)	40
CT0-narG_3	FN554829	membrane bound nitrate reductase [uncultured bacterium] (ABF20963) clone LT-250_43	181/205 (88.3%)	2
CT0-narG_4	FN554830	membrane bound nitrate reductase [uncultured bacterium] (ABF20963) clone LT-250_43	179/207 (86.5 %)	18
CT0-narG_5	FN554831	respiratory nitrate reductase 1 alpha chain [<i>Marinobacter</i> sp. ELB17] (ZP_01736799 or EBA00369)	160/208 (76.9%)	2
CT0-narG_11	FN554832	putative dissimilatory membrane-bound nitrate reductase [unidentified bacterium] (AAS59913) clone NCGC34	176/208 (84.6 %)	2
CT0-narG_12	FN554833	membrane bound nitrate reductase [unidentified bacterium] (AAY56563) clone 10g44 nitrate reductase 1, alpha subunit [<i>Herminiimonas arsenicoxydans</i>] (CAL61819)	185/204 (90.7%) 174/204 (85.3 %)	4
CT0-narG_13	FN554834	membrane bound nitrate reductase [uncultured bacterium](ABF20878) clone UT-075_46 nitrate reductase, alpha subunit [<i>Rubrobacter xylanophilus</i> DSM 9941] (YP_643983)	157/202 (77.7%) 136/202 (67.3%)	2
CT0-narG_17	FN554835	dissimilatory membrane-bound nitrate reductase [uncultured bacterium] (CAL80874) clone JG35-2-AG-narG81 nitrate reductase, alpha subunit [<i>Thiobacillus denitrificans</i> ATCC 25259] (YP_315161)	188/207 (90.8%) 177/208 (85.1%)	2
CT0-narG_15	FN554836	putative dissimilatory membrane-bound nitrate reductase [uncultured bacterium] (AAP79341) clone GA7	182/197 (92.4 %)	4
CT0-narG_38	FN554837	putative dissimilatory membrane-bound nitrate reductase [uncultured bacterium] (AAM62164) clone A8	185/200 (92.5%)	4
CT0-narG_19	FN554838	nitrate reductase [uncultured bacterium](CAL92067) clone Dbf75 nitrate reductase, alpha subunit [<i>Burkholderia cepacia</i> AMMD] (YP_777592)	168/183 (91.8%) 129/182 (70.8%)	4
CT0-narG_25	FN554839	putative dissimilatory membrane-bound nitrate reductase [unidentified bacterium] (AAS59880) clone NCGB54	177/209 (84.7%)	2
CT0-narG_32	FN554840	respiratory nitrate reductase alpha chain (NarG) [<i>Ralstonia eutropha</i> H16] (YP_841777)	127/160 (79.4%)	4
CT0-narG_33	FN554841	membrane bound nitrate reductase [unidentified bacterium] (AAY56506) clone 17g20	194/204 (95.1%)	2
CT0-narG_34	FN554842	putative dissimilatory membrane-bound nitrate reductase [unidentified bacterium](AAS59904) clone NCSF38	178/208 (85.6%)	2

CT0-narG_40	FN554843	putative dissimilatory membrane-bound nitrate reductase [uncultured bacterium] (AAX59101) clone GRAMD33	139/207 (67.1%)	2
CT0-narG_44	FN554844	putative dissimilatory membrane-bound nitrate reductase [uncultured bacterium] (AAM62255) clone D101	190/207 (91.8%)	2
CT0-narG_45	FN554845	putative dissimilatory membrane-bound nitrate reductase [uncultured bacterium] (AAX59116) clone GRAMO22		
		putative dissimilatory membrane-bound nitrate reductase [unidentified bacterium] (AAS59880) clone NCGB54	179/207 (86.5%)	2

Table 13. NarG sequences retrieved from the high nitrate system at day 30 and their closest matches in the GenBank.

Clone	Accession number	Closest amino acid sequence (accession number)	Similarity (% Match)	% present
CT30-narG_1	FN554846	nitrate reductase alpha subunit <i>Bacillus subtilis</i> (ABG33916)	~77%	19
CT30-narG_5	FN554847	membrane bound nitrate reductase [uncultured bacterium] clone LT-075_21	200/207 (96.6%)	15
		Respiratory nitrate reductase alpha subunit [<i>Geobacillus thermodenitrificans</i> NG80-2] (YP_001125821)	149/208 (71.6%)	
CT0-narG_2	FN554848	dissimilatory membrane-bound nitrate reductase [uncultured bacterium] clone JG35-K4-AG-narG1 (CAL80836)	200/207 (96.6 %)	11
		Respiratory nitrate reductase alpha subunit [<i>Geobacillus thermodenitrificans</i> NG80-2] (YP_001125821)	145/204 (71.1%)	
CT30-narG_25	FN554849	dissimilatory membrane-bound nitrate reductase [uncultured bacterium] clone JG35-K4-narG9 (CAL80838)	179/197 (90.9%)	6
		nitrate reductase, alpha subunit [<i>Bacillus coagulans</i> 36D1] (ZP_01695255)	153/200 (76.5%)	
CT30-narG_4	FN554850	dissimilatory membrane-bound nitrate reductase [uncultured bacterium] (AAY56563) clone JG35-K4-AG-narG3	175/205 (85.4 %)	4
		nitrate reductase, alpha subunit [<i>Bacillus coagulans</i> 36D1] (ZP_01695255)	131/206 (63.4%)	
CT30-narG_15	FN554851	dissimilatory membrane-bound nitrate reductase [uncultured bacterium] (AAY56563) clone JG35-K4-AG-narG3	168/199 (84.4%)	6
		nitrate reductase, alpha subunit [<i>Bacillus coagulans</i> 36D1] (ZP_01695255)	123/200 (61.5 %)	
CT30-narG_11	FN554852	membrane bound nitrate reductase [uncultured bacterium] clone LT-075_23(ABF20968)	129/207 (62.7%)	9
		nitrate reductase, alpha subunit [<i>Bacillus coagulans</i> 36D1] (ZP_01695255)	129/207 (62.3%)	
CT30-narG_20	FN554853	putative dissimilatory membrane-bound nitrate reductase [uncultured bacterium] (AAM62209) clone C71	182/207 (87.9%)	9
CT30-narG_32	FN554854	membrane bound nitrate reductase [unidentified bacterium] (AAY56563) clone 10g44	187/204 (91.7%)	2
		dissimilatory membrane-bound nitrate reductase [uncultured bacterium] (CAL80849) clone JG35+U1-AG-narG21	180/204 (88.2%)	
		nitrate reductase 1, alpha subunit [<i>Hermiimonas arsenicoxydans</i>] (YP_001099944)	176/204 (86.3%)	
CT30-narG_34	FN554855	putative dissimilatory membrane-bound nitrate reductase [unidentified bacterium] (AAS59880) clone NCGB54	180/207 (87.0%)	2
		nitrate reductase, alpha subunit [<i>Methylobacterium</i> sp. 4-46] (ZP_01851204)	171/207 (82.6%)	
CT30-narG_35	FN554856	membrane bound nitrate reductase [uncultured bacterium] (ABF20967) clone LT-075_21	201/207 (97.1%)	2
		Respiratory nitrate reductase alpha subunit [<i>Geobacillus thermodenitrificans</i> NG80-2]	149/208 (71.6%)	

(YP_001125821)

CT30-narG_37	FN554857	membrane bound nitrate reductase [uncultured bacterium] (ABF20963) clone LT-250_43	180/208 (86.5%)	2
CT30-narG_46	FN554858	putative dissimilatory membrane-bound nitrate reductase [uncultured bacterium](AAM62164) clone A8	189/206 (91.7%)	2
CT30-narG_47	FN554859	nitrate reductase [uncultured bacterium] (CAL92067)clone DBf75	186/205 (90.7%)	2
CT30-narG_27	FN554860	dissimilatory membrane-bound nitrate reductase [uncultured bacterium] (CAL80836) clone JG35-K4-AG-narG1	200/204 (98.0%)	2
CT30-narG_31	FN554861	Respiratory nitrate reductase, alpha subunit [<i>Geobacillus thermodenitrificans</i> NG80-2] dissimilatory membrane-bound nitrate reductase [uncultured bacterium] (CAL80836) clone JG35-K4-AG-narG1	144/204 (70.6%)	2
		Respiratory nitrate reductase, alpha subunit [<i>Geobacillus thermodenitrificans</i> NG80-2] (YP_001124778)	201/204 (98.5%)	2
CT30-narG_44	FN554862	dissimilatory membrane-bound nitrate reductase [uncultured bacterium] (CAL80836) clone JG35-K4-AG-narG1	143/204 (70.1%)	2
		Respiratory nitrate reductase, alpha subunit [<i>Geobacillus thermodenitrificans</i> NG80-2] (YP_001124778)	204/205 (99.5%)	2
CT30-narG_49	FN554863	membrane bound nitrate reductase [uncultured bacterium] (ABF20967) clone LT-075_21	145/205 (70.7%)	2
		Respiratory nitrate reductase alpha subunit [<i>Geobacillus thermodenitrificans</i> NG80-2] (YP_001125821)	201/207 (97.1%)	2
			146/208 (70.2%)	

Table 14. Quantities of *Geobacteracea* genomic DNA detected by real-time PCR with *Geobacteraceae* specific primers (Geo564f and Geo840r) and qPCR SYBR Green Master Mix (Stratagene).

Well name	Well comment	Assay	Well type	Threshold (dR)	Ct (dR)	Quantity (ng)	RSq (dR)	Slope (dR)
NTC	Negative control	SYBR	NTC	471.392	No Ct	No Ct	0.98	-3.32
NPC	Primer control	SYBR	NPC	471.392	No Ct	No Ct	0.98	-3.32
G.s 1 in 5	<i>G. sul</i> 1 in 5	SYBR	Standard	471.392	17.9	2.700	0.98	-3.32
G.s 1 in 25	<i>G. sul</i> 1 in 25	SYBR	Standard	471.392	19.37	0.540	0.98	-3.32
G.s 1 in 125	<i>G. sul</i> 1 in 125	SYBR	Standard	471.392	21.86	0.108	0.98	-3.32
G. s 1 in 625	<i>G. sul</i> 1 in 625	SYBR	Standard	471.392	24.8	0.022	0.98	-3.32
A T0	A T0	SYBR	Unknown	471.392	No Ct	No Ct	0.98	-3.32
A T24	A T24	SYBR	Unknown	471.392	24.99	0.015	0.98	-3.32
C T0	C T0	SYBR	Unknown	471.392	No Ct	No Ct	0.98	-3.32
C T30	C T30	SYBR	Unknown	471.392	No Ct	No Ct	0.98	-3.32
C T60	C T60	SYBR	Unknown	471.392	No Ct	No Ct	0.98	-3.32
C T80	C T80	SYBR	Unknown	471.392	22.57	0.080	0.98	-3.32

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