

Supporting information

A case study for late Archean and Proterozoic biogeochemical iron- and sulphur-cycling in a modern habitat – the Arvadi Spring

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Methods S1

Metabolism-selective growth media

All media were prepared anoxically with a N₂:CO₂ (90:10) headspace and were buffered with 30 mM NaHCO₃. FW medium contained following salts per litre: 0.6 g of KH₂PO₄, 0.3 g of NH₄Cl, 0.025 g of MgSO₄*7H₂O, 0.4 g of MgCl₂*6H₂O and 0.1 g of CaCl₂*2H₂O. BM medium contained following salts per litre: 1.0 g of NaCl, 0.4 g of MgCl₂*6H₂O, 0.15 g of CaCl₂*2H₂O, 0.2 g of KH₂PO₄, 0.5 g of KCl, 0.25 g of NH₄Cl. MWMM contained following salt per litre: 0.1 g of NH₄Cl, 0.2 g of MgSO₄*7H₂O, 0.1 g of CaCl₂*2H₂O and 0.05 g of K₂HPO₄. The pH was adjusted to 7.2 with either 1 M HCl or 0.5 M Na₂CO₃. Trace metals (selenite-tungstate solution & SL10 solution after Widdel et al., 1983) and vitamins (7-vitamine solution after Widdel & Pfennig, 1981) were added to all media in same amounts (1 ml L⁻¹), metabolism selectivity was established by the addition of different electron donors and acceptors to respective media.

MPN experiments with anaerobic Fe- and S- metabolizers

MPN counts for anaerobic Fe- and S-metabolizers were performed in 96-deep-well microtiter plates (Laufer et al., 2016). One MPN plate was prepared per sampling location and per type of bacterial metabolism. First, 900 µL of growth medium with respective metabolism-selective additives were dispersed to the test wells and inoculated with 100 µL of previously prepared sample dilutions in seven replicates. One row of negative control wells contained 1000 µL of medium without inoculum. After pipetting, plates were sealed with transparent plastic foils and inserted into incubation bags. In order to provide anoxic growth conditions, oxygen consuming catalyst bags (Anaerocult® A mini, Merck GmbH, Germany) and redox indicator stripes (Anaerotest®, Merck GmbH, Germany) were inserted to each incubation bag. The incubation bags

were sealed oxygen-tight with Anaeroclip[®] (Merck GmbH, Germany) plastic clips. Plates were prepared under anoxic conditions in a glove box (100% N₂) and were incubated afterwards for 8 to 10 weeks at 20°C under metabolism-selective conditions. MPN plates for the cultivation of phototrophic iron(II)-oxidizers were incubated under infra-red (IR) light (>730 nm) in order to prevent growth of cyanobacteria. IR light conditions were provided in a dark incubation box with an IR light filter on top, through which only the IR spectrum of a 40 W bulb could pass. MPN plates for the cultivation of nitrate-reducing iron(II)-oxidizers, iron(III)-reducers and sulphate-reducers were incubated in the dark.

MPN experiments with microaerophilic Fe- and S-metabolizers

MPN counts for microaerophilic iron(II)- and sulphide-oxidizers were performed in gradient tubes. Gradient tubes were prepared two days prior to inoculation in order to enable establishment of electron donor and acceptor gradients. For this, an electron donor (iron(II) or sulphide) and agarose-containing bottom layer was prepared either with FeS or Na₂S. After solidification in the test tube, the bottom layer was overlain by a MWMM top layer. The whole procedure was performed anoxically in order to prevent oxidation of the electron donors. Tubes were inoculated in duplicates with the same sample dilutions as used for anaerobic MPNs. Thereby, tubes were opened for 1 minute under sterile conditions to let air enter the tube headspace in order to create an oxygen gradient from the top to the bottom of each tube. Afterwards, 100 µL of sample were injected homogeneously with a syringe starting at about 0.5 cm above the bottom layer over the whole length of the top layer. Tubes were closed air-tight and incubated for 2-3 days at 20°C in the dark.

Figures S1

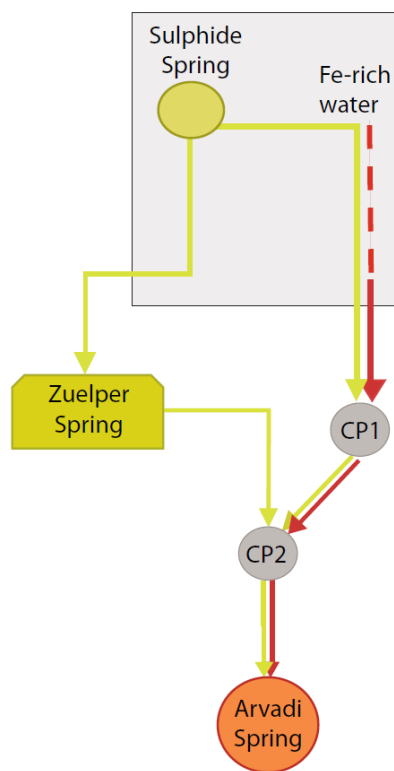


Figure S1: Water flow between Fe- and S-rich waters, the Zuelper Spring and the Arvadi Spring. The grey shaded rectangle indicates the inaccessible concrete tunnel, in which the Sulphide Spring is located that is the parental water source to Arvadi Spring sulphide. Also, Fe-rich waters with unknown source emanate in the tunnel and get mixed with sulphide-rich water. In an intermixed form, the Fe- and S-rich water is transported through a pipe system to connection points 1 and 2 (CP1, CP2). From the latter, water samples were taken for Fe(II), Fe(tot) and sulphide quantification. Fe-S-rich water further gets transported to the Arvadi Spring pond where it crops out. The Sulphide Spring water additionally flows into the Zuelper Spring, where no Fe-rich water is introduced. Zuelper Spring water flows additionally into CP2 and further to the Arvadi Spring pond.

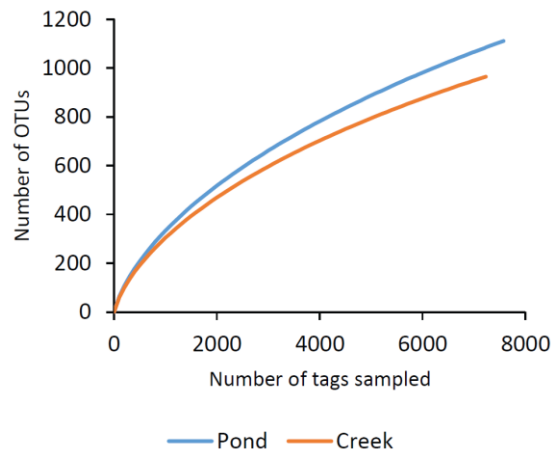


Figure S2: Species richness in the Arvadi Spring pond and creek microbial community. Rarefaction curves represent the number of observed operational taxonomic units (OTU's) in the Arvadi Spring pond and creek microbial community based on OTU clustering at 3% genetic distance.

Tables SI

Table S1: Major geochemical parameters in Arvadi Spring water at different locations.

location	pH	T [°C]	O ₂ [mg L ⁻¹]	O ₂ [%]	σ [μS cm ⁻¹]
1	7.9	7.2	10.8	101.1	1442.0
2	8.0	7.3	10.9	100.7	1450.0
3	8.0	7.0	11.0	100.8	1450.0
4	8.0	7.0	10.9	100.4	1449.0
5	8.1	6.8	11.0	101.0	1450.0
6	8.2	5.8	11.2	99.7	1436.0
7	8.1	6.7	11.0	111.1	1450.0

Numbers of different locations refer to numbers in Fig. 1

Table S2: Concentrations of major anions [μM] in Arvadi Spring water at different locations.

location	F ⁻	Cl ⁻	NO ₂ ⁻	Br ⁻	NO ₃ ⁻	PO ₄ ³⁻	SO ₄ ²⁻
1	96.7	14.0	b.d.l.	b.d.l.	b.d.l.	b.d.l.	8268.5
2	98.4	14.6	b.d.l.	b.d.l.	b.d.l.	b.d.l.	8357.2
3	97.7	15.6	b.d.l.	b.d.l.	b.d.l.	b.d.l.	8337.3
4	100.6	15.2	b.d.l.	b.d.l.	b.d.l.	b.d.l.	8307.7
5	100.7	15.5	b.d.l.	b.d.l.	b.d.l.	b.d.l.	8344.1
6	96.8	15.4	b.d.l.	b.d.l.	b.d.l.	b.d.l.	8165.7
7	100.0	15.5	b.d.l.	b.d.l.	b.d.l.	b.d.l.	8317.5

b.d.l.= below detection limit

Numbers of different locations refer to numbers in Fig. 1

Table S3: Overview of major cations [μM] in Arvadi Spring water at different locations.

location	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}
1	38.9	b.d.l.	26.1	3251.6	6981.0
2	39.1	b.d.l.	26.4	3275.8	7038.6
3	39.5	b.d.l.	26.4	3272.7	7062.3
4	39.4	b.d.l.	27.0	3245.9	7044.3
5	39.8	b.d.l.	26.9	3269.6	7061.3
6	39.1	b.d.l.	26.8	3208.4	6909.5
7	39.5	b.d.l.	27.3	3280.4	7095.1

b.d.l.= below detection limit

Numbers of different locations refer to numbers in Fig. 1

Table S4: Overview of total carbon (TC), total inorganic carbon (TIC) and dissolved organic carbon (DOC) content [mg litre^{-1}] in Arvadi Spring water at different locations.

location	TIC	DOC	TC
1	49.9	3.5	53.4
2	49.8	3.7	53.5
3	49.8	2.4	52.2
4	49.5	3.6	53.1
5	49.2	3.6	52.7
6	48.6	3.0	51.6
7	50.3	1.8	52.1

Numbers of different locations refer to numbers in Fig. 1

Table S5: Mössbauer data collected for red flocs at 77K and 4.2K.

T [K]			CS [mm/s]	ϵ / QS [mm/s]	H [T]	σ [mm/s]	pop [%]	χ^2
77	Db1	Fe(III) (oxyhydr)oxide	0.5	1.0		0.4	58.0	0.6
	Db2	Fe(III) (oxyhydr)oxide	0.4	4.3		5.0	12.7	
	Db3	Fe(II) sulphide	0.5	0.6		0.2	21.0	
	Db4	Fe(II)	1.3	2.7		0.2	9.1	
4.2	Db1	Fe(II)	1.3	2.7		0.8	4.4	0.7
	Db2	Unknown	0.2	0.9		0.3	3.7	
	S1	Fe(III) (oxyhydr)oxide	0.5	0.0	49.1	1.7	18.9	
	S2	Fe(III) (oxyhydr)oxide	0.4	0.0	46.2	4.1	37.4	
	S3	Fe(III) (oxyhydr)oxide	0.6	-0.1	47.6	2.9	26.7	
	S4	Poorly ordered Fe oxide	0.7	-0.01	22.4	2.8	8.8	
	Db = doublet S = sextet CS = centre shift ϵ /QS = shift/quadrupole splitting H = hyperfine field σ = Gauss' sigma parameter pop = population χ^2 = goodness of fitting							

Table S6: Bacterial genera (including the number of sequences and relative sequence abundance per genus, respectively) in the Arvadi Spring pond and creek sediment samples.

Genus	Pond	Creek	Pond	Creek
	No. of sequences		Rel. sequence abundance[%]	
<i>Acetivibrio</i>	0	1	0.00	0.01
<i>Acidaminobacter</i>	2	1	0.03	0.01
<i>Acidiferrobacter</i>	26	38	0.34	0.53
<i>Acidisoma</i>	1	0	0.01	0.00
<i>Aciditerrimonas</i>	2	1	0.03	0.01
<i>Acidithiobacillus</i>	2	1	0.03	0.01
<i>Acidovorax</i>	1	0	0.01	0.00
<i>Actinomycetospora</i>	0	1	0.00	0.01
<i>Adhaeribacter</i>	0	1	0.00	0.01
<i>Afipia</i>	14	9	0.18	0.12
<i>AKYG587</i>	7	4	0.09	0.06
<i>Albidiferax</i>	55	23	0.73	0.32
<i>Amaricoccus</i>	9	0	0.12	0.00
<i>Anaerolinea</i>	1	0	0.01	0.00
<i>Anaeromyxobacter</i>	4	1	0.05	0.01
<i>Aquicella</i>	2	0	0.03	0.00
<i>Aquimonas</i>	7	13	0.09	0.18
<i>Arcobacter</i>	1	0	0.01	0.00
<i>Arcticibacter</i>	0	2	0.00	0.03
<i>Arenimonas</i>	89	49	1.17	0.68
<i>Armatimonas</i>	3	1	0.04	0.01
<i>Asticcacaulis</i>	11	2	0.15	0.03
<i>Aureimonas</i>	0	1	0.00	0.01
<i>Aureispira</i>	1	1	0.01	0.01
<i>Azotobacter</i>	0	1	0.00	0.01
<i>Bacteriovorax</i>	3	0	0.04	0.00
<i>Bacteroides</i>	0	1	0.00	0.01
<i>Bauldia</i>	5	2	0.07	0.03
<i>Bdellovibrio</i>	6	7	0.08	0.10
<i>Blastocatella</i>	70	67	0.92	0.93
<i>Blastopirellula</i>	3	1	0.04	0.01
<i>Bosea</i>	3	4	0.04	0.06
<i>Brasilonema</i>	77	1	1.02	0.01
<i>Brevundimonas</i>	87	60	1.15	0.83
<i>Bryobacter</i>	11	17	0.15	0.24
<i>Caenimonas</i>	0	4	0.00	0.05
<i>Calothrix</i>	13	0	0.17	0.00
<i>Candidatus Accumulibacter</i>	1	0	0.01	0.00
<i>Candidatus Alysiosphaera</i>	1	1	0.01	0.01
<i>Candidatus Amoebophilus</i>	10	7	0.13	0.10

<i>Candidatus Captivus</i>	0	1	0.00	0.01
<i>Candidatus Methylocacidiphilum</i>	4	0	0.05	0.00
<i>Candidatus Microthrix</i>	1	3	0.01	0.04
<i>Candidatus Nostocoida</i>	1	1	0.01	0.01
<i>Candidatus Solibacter</i>	2	0	0.03	0.00
<i>Candidatus Xiphinematobacter</i>	1	0	0.01	0.00
<i>Chitinimonas</i>	3	0	0.04	0.00
<i>Chitinophaga</i>	7	15	0.09	0.21
<i>Christensenella</i>	1	0	0.01	0.00
<i>Chroococcidiopsis</i>	2	1	0.03	0.01
<i>Chryseolinea</i>	61	43	0.81	0.59
<i>Chthoniobacter</i>	1	3	0.01	0.04
<i>Chthonomonas</i>	1	1	0.01	0.01
CL500-29 marine group	13	16	0.17	0.22
CL500-3	2	2	0.03	0.03
<i>Clostridium sensu stricto</i> 1	1	2	0.01	0.03
<i>Coxiella</i>	0	1	0.00	0.01
<i>Crocinitomix</i>	1	5	0.01	0.07
<i>Cyanobium</i>	0	1	0.00	0.01
<i>Cytophaga</i>	1	1	0.01	0.01
<i>Dechloromonas</i>	1	0	0.01	0.00
<i>Deefgea</i>	0	1	0.00	0.01
<i>Defluviicoccus</i>	2	0	0.03	0.00
<i>Defluviimonas</i>	7	1	0.09	0.01
<i>Desulfatirhabdium</i>	1	1	0.01	0.01
<i>Desulfocapsa</i>	0	3	0.00	0.04
<i>Devosia</i>	3	7	0.04	0.10
<i>Dokdonella</i>	1	0	0.01	0.00
<i>Dongia</i>	2	0	0.03	0.00
<i>Dyadobacter</i>	1	5	0.01	0.07
<i>Elstera</i>	3	0	0.04	0.00
<i>Emticicia</i>	12	4	0.16	0.06
<i>Exiguobacterium</i>	1	0	0.01	0.00
<i>Falsirhodobacter</i>	2	3	0.03	0.04
<i>Ferrithrix</i>	1	1	0.01	0.01
<i>Ferruginibacter</i>	35	33	0.46	0.46
<i>Fibrella</i>	2	1	0.03	0.01
<i>Filomicrobium</i>	34	62	0.45	0.86
<i>Flaviramulus</i>	0	1	0.00	0.01
<i>Flavisolibacter</i>	1	1	0.01	0.01
<i>Flavitalea</i>	0	1	0.00	0.01
<i>Flavobacterium</i>	185	51	2.44	0.71
<i>Flectobacillus</i>	0	2	0.00	0.03
<i>Flexibacter</i>	1	0	0.01	0.00
<i>Fluviicola</i>	1	0	0.01	0.00

<i>Friedmanniella</i>	1	0	0.01	0.00
<i>Gaiella</i>	11	7	0.15	0.10
<i>Geitlerinema</i>	1	0	0.01	0.00
<i>Gemmata</i>	24	14	0.32	0.19
<i>Gemmatimonas</i>	31	18	0.41	0.25
<i>Gemmobacter</i>	250	180	3.30	2.49
<i>Giesbergeria</i>	1	0	0.01	0.00
<i>Gleocapsa</i>	0	2	0.00	0.03
<i>Granulicella</i>	1	1	0.01	0.01
<i>Haliangium</i>	5	13	0.07	0.18
<i>Haliscomenobacter</i>	100	45	1.32	0.62
<i>Haloferula</i>	8	1	0.11	0.01
<i>Hirschia</i>	26	36	0.34	0.50
<i>Hyalangium</i>	1	2	0.01	0.03
<i>Hydrogenophaga</i>	114	30	1.50	0.41
<i>Hymenobacter</i>	0	1	0.00	0.01
<i>Hyphomicrobium</i>	63	105	0.83	1.45
<i>Hyphomonas</i>	12	22	0.16	0.30
<i>Iamia</i>	2	0	0.03	0.00
<i>Ideonella</i>	3	1	0.04	0.01
<i>Ilumatobacter</i>	16	24	0.21	0.33
<i>Incertae Sedis</i>	1	3	0.01	0.04
<i>Inhella</i>	1	0	0.01	0.00
<i>Iodobacter</i>	1	2	0.01	0.03
<i>Leadbetterella</i>	13	1	0.17	0.01
<i>Leeia</i>	1	0	0.01	0.00
<i>Legionella</i>	7	10	0.09	0.14
<i>Leptolinea</i>	1	1	0.01	0.01
<i>Leptolyngbya</i>	338	101	4.46	1.40
<i>Leptospira</i>	6	9	0.08	0.12
<i>Leuconostoc</i>	1	0	0.01	0.00
<i>Lewinella</i>	115	96	1.52	1.33
<i>Litorilinea</i>	1	0	0.01	0.00
<i>Lysobacter</i>	2	0	0.03	0.00
<i>Mangroviflexus</i>	1	0	0.01	0.00
<i>Marmoricola</i>	0	1	0.00	0.01
<i>Megamonas</i>	0	1	0.00	0.01
<i>Meganema</i>	3	1	0.04	0.01
<i>Methylibium</i>	15	2	0.20	0.03
<i>Methylorosula</i>	0	1	0.00	0.01
<i>Microcoleus</i>	9	0	0.12	0.00
<i>Mycobacterium</i>	1	1	0.01	0.01
<i>Nakamurella</i>	0	4	0.00	0.06
<i>Nevskia</i>	2	6	0.03	0.08
<i>Niabella</i>	3	0	0.04	0.00

<i>Nitrospira</i>	21	23	0.28	0.32
<i>Nocardioides</i>	9	2	0.12	0.03
<i>Nodularia</i>	0	1	0.00	0.01
<i>Nordella</i>	10	8	0.13	0.11
<i>Nostoc</i>	113	2	1.49	0.03
<i>Novosphingobium</i>	1	1	0.01	0.01
<i>Oceanicella</i>	1	1	0.01	0.01
<i>Ohtaekwangia</i>	91	88	1.20	1.22
OM27 clade	25	43	0.33	0.59
<i>Opitutus</i>	7	0	0.09	0.00
<i>Paludibacter</i>	1	0	0.01	0.00
<i>Parasegetibacter</i>	2	0	0.03	0.00
<i>Parvularcula</i>	16	17	0.21	0.24
<i>Paucibacter</i>	2	0	0.03	0.00
<i>Pedobacter</i>	0	1	0.00	0.01
<i>Pedomicrobium</i>	10	2	0.13	0.03
<i>Pedosphaera</i>	2	1	0.03	0.01
<i>Peredibacter</i>	0	1	0.00	0.01
<i>Phormidium</i>	6	38	0.08	0.53
<i>Phycisphaera</i>	0	4	0.00	0.06
Pir4 lineage	16	17	0.21	0.24
<i>Pirellula</i>	12	16	0.16	0.22
<i>Piscinibacter</i>	1	0	0.01	0.00
<i>Planctomyces</i>	15	15	0.20	0.21
<i>Planktothrix</i>	0	2	0.00	0.03
<i>Polaromonas</i>	9	3	0.12	0.04
<i>Porphyrobacter</i>	7	3	0.09	0.04
<i>Portibacter</i>	4	7	0.05	0.10
<i>Prochlorothrix</i>	5	0	0.07	0.00
<i>Pseudanabaena</i>	32	33	0.42	0.46
<i>Pseudochrobactrum</i>	1	6	0.01	0.08
<i>Pseudofulvimonas</i>	5	3	0.07	0.04
<i>Pseudolabrys</i>	2	1	0.03	0.01
<i>Pseudomonas</i>	2	0	0.03	0.00
<i>Pseudonocardia</i>	4	1	0.05	0.01
<i>Pseudorhodoferax</i>	3	2	0.04	0.03
<i>Pseudospirillum</i>	1	0	0.01	0.00
<i>Pseudoxanthomonas</i>	7	1	0.09	0.01
<i>Reichenbachella</i>	4	1	0.05	0.01
<i>Reyranella</i>	8	7	0.11	0.10
<i>Rhizobacter</i>	55	46	0.73	0.64
<i>Rhizobium</i>	30	6	0.40	0.08
<i>Rhodobium</i>	0	3	0.00	0.04
<i>Rhodopirellula</i>	3	3	0.04	0.04
<i>Rickettsia</i>	3	3	0.04	0.04

<i>Roseiflexus</i>	3	5	0.04	0.07
<i>Roseomonas</i>	25	11	0.33	0.15
<i>Rubellimicrobium</i>	1	0	0.01	0.00
<i>Rubribacterium</i>	7	7	0.09	0.10
<i>Rubrivirga</i>	1	0	0.01	0.00
<i>Rudanella</i>	1	0	0.01	0.00
<i>Runella</i>	2	0	0.03	0.00
<i>Sandaracinus</i>	1	3	0.01	0.04
<i>Sandarakinorhabdus</i>	93	50	1.23	0.69
<i>Sediminibacterium</i>	20	3	0.26	0.04
<i>Sideroxydans</i>	2	0	0.03	0.00
<i>Silanimonas</i>	5	4	0.07	0.06
<i>Singulisphaera</i>	1	0	0.01	0.00
<i>Siphonobacter</i>	2	0	0.03	0.00
SM1A02	35	33	0.46	0.46
<i>Solibacillus</i>	1	0	0.01	0.00
<i>Solirubrobacter</i>	0	2	0.00	0.03
<i>Spirosoma</i>	5	1	0.07	0.01
<i>Sporocytophaga</i>	1	0	0.01	0.00
<i>Stenotrophomonas</i>	0	1	0.00	0.01
<i>Steroidobacter</i>	4	3	0.05	0.04
<i>Subdoligranulum</i>	0	1	0.00	0.01
<i>Sulfuricella</i>	1	0	0.01	0.00
<i>Sulfuricurvum</i>	1	3	0.01	0.04
<i>Sulfuritalea</i>	0	1	0.00	0.01
<i>Sulfurospirillum</i>	3	0	0.04	0.00
<i>Sulfurovum</i>	106	124	1.40	1.71
<i>Tabrizicola</i>	21	11	0.28	0.15
<i>Taibaiella</i>	1	1	0.01	0.01
<i>Terrimonas</i>	50	33	0.66	0.46
<i>Thermomonas</i>	5	6	0.07	0.08
<i>Thiobacillus</i>	314	428	4.14	5.92
<i>Thiothrix</i>	1063	1241	14.03	17.16
<i>Turneriella</i>	0	6	0.00	0.08
Unclassified	3113	3480	41.09	48.13
<i>Undibacterium</i>	1	2	0.01	0.03
<i>Woodsholea</i>	17	20	0.22	0.28
<i>Xanthomonas</i>	1	0	0.01	0.00
<i>Zavarzinella</i>	4	0	0.05	0.00

Table S7: Richness estimators, diversity indices and observed OTU numbers of bacterial community in pond and creek sediment samples based on OTU clustering at a genetic distance of 3%.

	Number of sequences		OTUs	Richness		Diversity	
	Raw	Quality-filtered		Chao	ACE	Shannon	Simpson
Pond	13351	7576	1111	1954	2908	5.16	0.03
Creek	13049	7231	965	1519	2122	4.88	0.04

References

Widdel F, Pfennig N (1981) Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. *Archives of Microbiology*, **129**, 396-400.

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