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Supporting Information for

Biogeochemical Gradients in a Serpentinization-influenced Aquifer: Implications for Gas Exchange between the Subsurface and Atmosphere

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Additional Supporting Information (Files uploaded separately)

Captions for Datasets S1 to S7.

Introduction

This supporting information presents:

(1) Additional methods for aqueous sulfide (Text S1) and additional details and methods for bacterial and attempted archaeal 16S rRNA amplification (Text S2).
(2) A diagram of the CSW1.1 well and sampling scheme (Figure S1).
(3) Phylogenetic trees for organisms of interest related to *Serpentinomonas* (Figure S2), *Truepera* (Figure S3) and *Dethiobacter* (Figure S4) genera respectively.
(4) Bar plots of sample richness and evenness calculated using 16S rRNA gene sequence data (Figure S5).
(5) Table S1 – Gibbs energy full balanced reactions for abbreviations in Figures 2 and 3.
(6) Table S2 – The top 11 OTUs in the CSW1.1 depth profile samples.
(7) Captions for datasets S1-S7.

Text S1. Hydrogen sulfide determination

Fluid samples from each well were preserved immediately in the field using 2.0 mL of a 0.05M zinc acetate solution for every 0.5 mL of sample in order to preserve the volatile sulfide as zinc sulfide. In lab, the 2.5 mL triplicate aliquots of this solution were placed into individual 2 mL centrifuge tubes (Sigma-Aldrich) and vortexed. Prior to analysis, 0.2 mL of the appropriate diamine reagent (0-3 µM, 3-40 µM, 40-250 µM, or 250-1000 µM, respectively) was added to each tube to develop the characteristic blue color. For each diamine reagent used, a standard curve was created using the same method of preservation and 50 µM or 500 µM stock solutions of hydrogen sulfide, depending on the diamine reagent range being analyzed. After a 20-minute allotted time for fixation, samples and standards were immediately run in parallel to an 18 mΩ water, 0.22 µM syringe filtered, zinc acetate-preserved, 0-3 µM diamine-reacted blank on an Ultraviolet-1800 Shimadzu UV spectrophotometer at 670 nm in-house.

Text S2. Bacterial and Archaeal amplification.

Bacterial samples were amplified via quantitative Polymerase Chain Reaction (qPCR) on a BioRad C1000 instrument with a CFX96 Optics Module using the SsoAdvanced Universal SybrGreen assay, and domain-specific primers targeting the V6 region of the 16S rRNA gene. The 967F and 1046R bacterial primers were used (Sogin et al., 2006), and gene copy numbers were obtained by plotting quantification values from environmental samples onto standard curves generated by *Escherichia coli* for bacteria with the domain-specific primers. These primers were not used for amplicon sequencing. Thermal cycling for denaturation (98ºC, 2 min., 15 sec.), annealing (57ºC, 30 sec.), and extension (65ºC, 10 sec.), was run for 30 cycles total. Archaea have not been detected in CROMO fluids prior to the June 2016 field campaign (Crespo-Medina et al., 2014; Twing et al., 2017). Archaeal PCR on depth profile samples using *Methanocaldococcus jannaschii* as the positive control was unsuccessful, so 16S rRNA gene sequencing for archaea could not be completed.

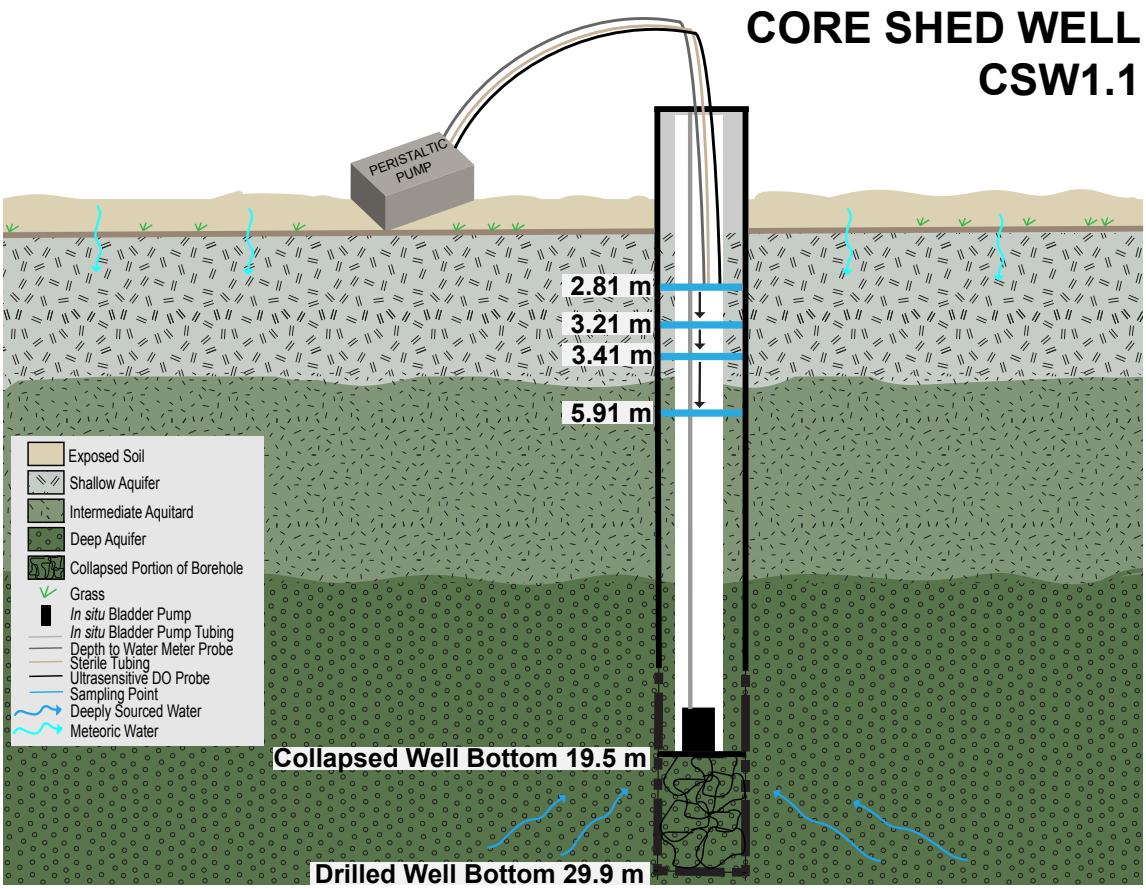

Figure S1. A schematic showing profile sampling of the CSW 1.1 well, with lithologies estimated after Ortiz et al., (2018). Note the peristaltic pump (grey box) at the ground surface was used to pump water for the profile, and the bladder pump (black rectangle, well base) was used to pump the CSW1.1 well bottom. The brown horizontal line denotes ground level. Profile samples were taken at depths of 2.81m, 3.21 m, 3.41 m, and 5.91 m depth for 100%, 15%, 50%, and 0% air saturation, respectively. Note the well is cased to 15m depth - uncased portion indicated by black dashed lines.

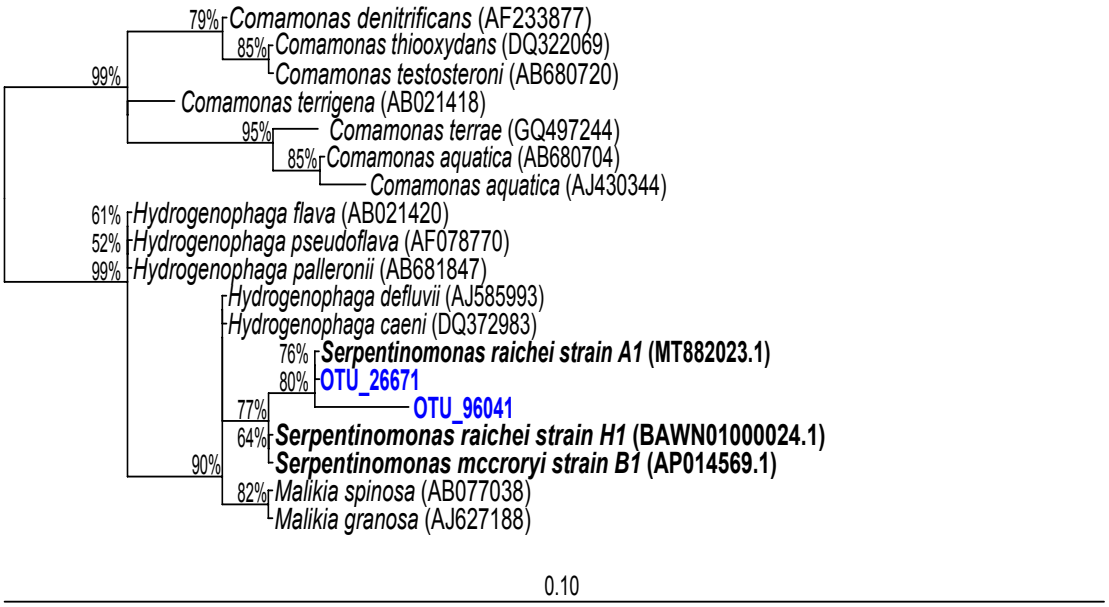

Figure S2. A 1000-bootstrap maximum likelihood phylogenetic tree of CROMO sequences was aligned and constructed using RAxML in ARB. CROMO OTUs closely matching *Serpentinomonas* and >0.1% abundance in the dataset are bold and blue. Bold and black sequences are organisms also compared in MAG analyses within this study. The scale bar indicates 0.1 inferred amino acid substitutions per site and bootstrap values >50% are shown at branch nodes.

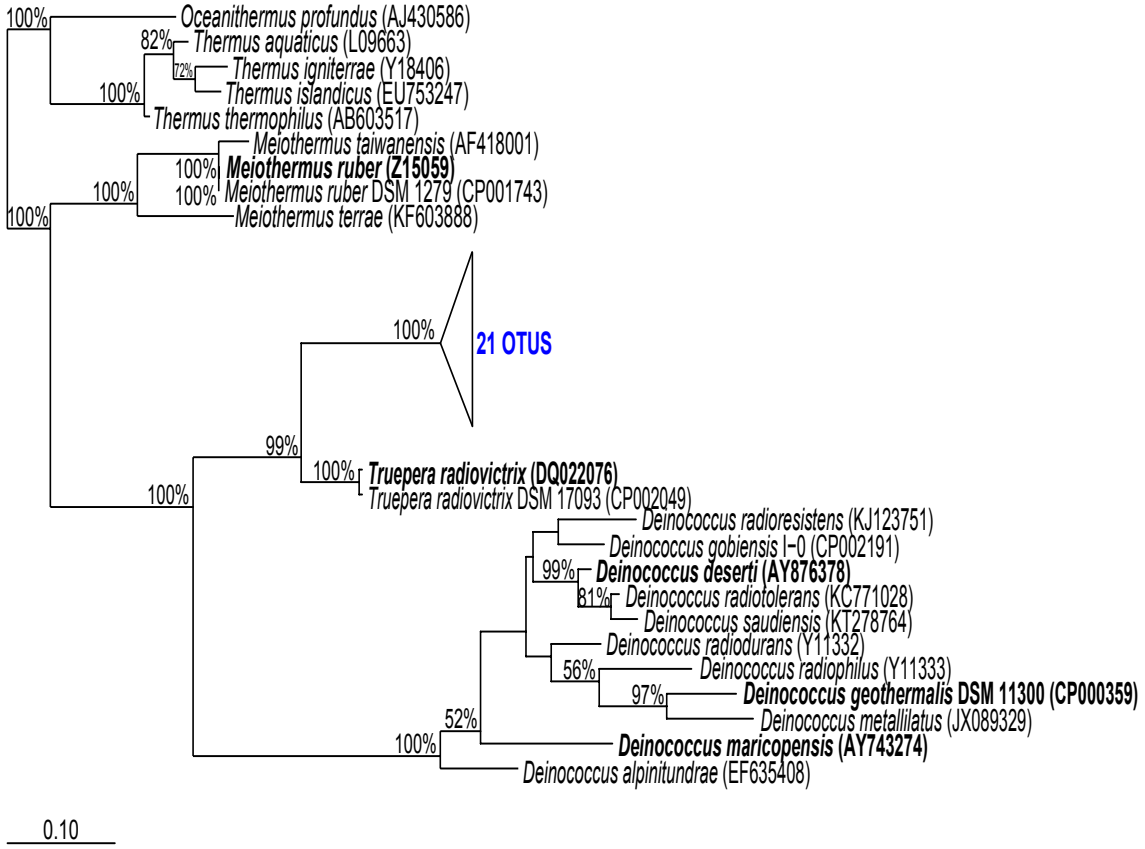

Figure S3. A 1000-bootstrap maximum likelihood phylogenetic tree of CROMO sequences was aligned and constructed using RAxML in ARB. CROMO OTUs closely matching *Truepera radiovictrix* and >0.1% abundance in the dataset are bold and blue. Bold and black sequences are organisms also compared in MAG analyses within this study. The scale bar indicates 0.1 inferred amino acid substitutions per site and bootstrap values >50% are shown at branch nodes. Collapsed OTUs are OTU_176747, OTU_262514, OTU_42509, OTU_226777, OTU_221402, OTU_243611, OTU_192810, OTU_157292, OTU_33632, OTU_54697, OTU_186428, OTU_175848, OTU_75957, OTU_100660, OTU_165475, OTU_77527, OTU_129834, OTU_127364, OTU_123719, OTU_168729, and OTU_231982.

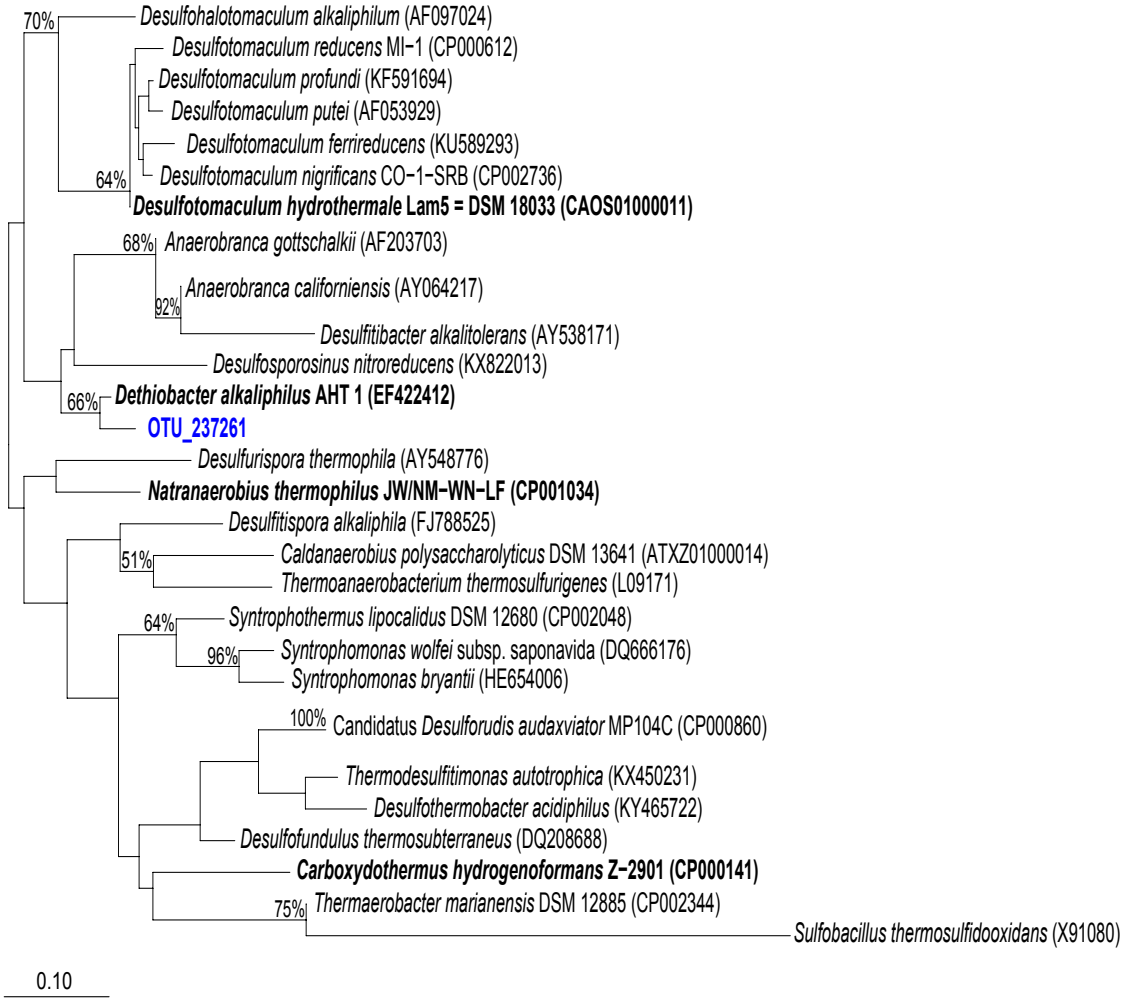

Figure S4. A 1000-bootstrap maximum likelihood phylogenetic tree of CROMO sequences was aligned and constructed using RAxML in ARB. CROMO OTUs closely matching *Dethiobacter alkaliphilus* and >0.1% abundance in the dataset are bold and blue. Bold and black sequences are organisms also compared in MAG analyses within this study. The scale bar indicates 0.1 inferred amino acid substitutions per site and bootstrap values >50% are shown at branch nodes.

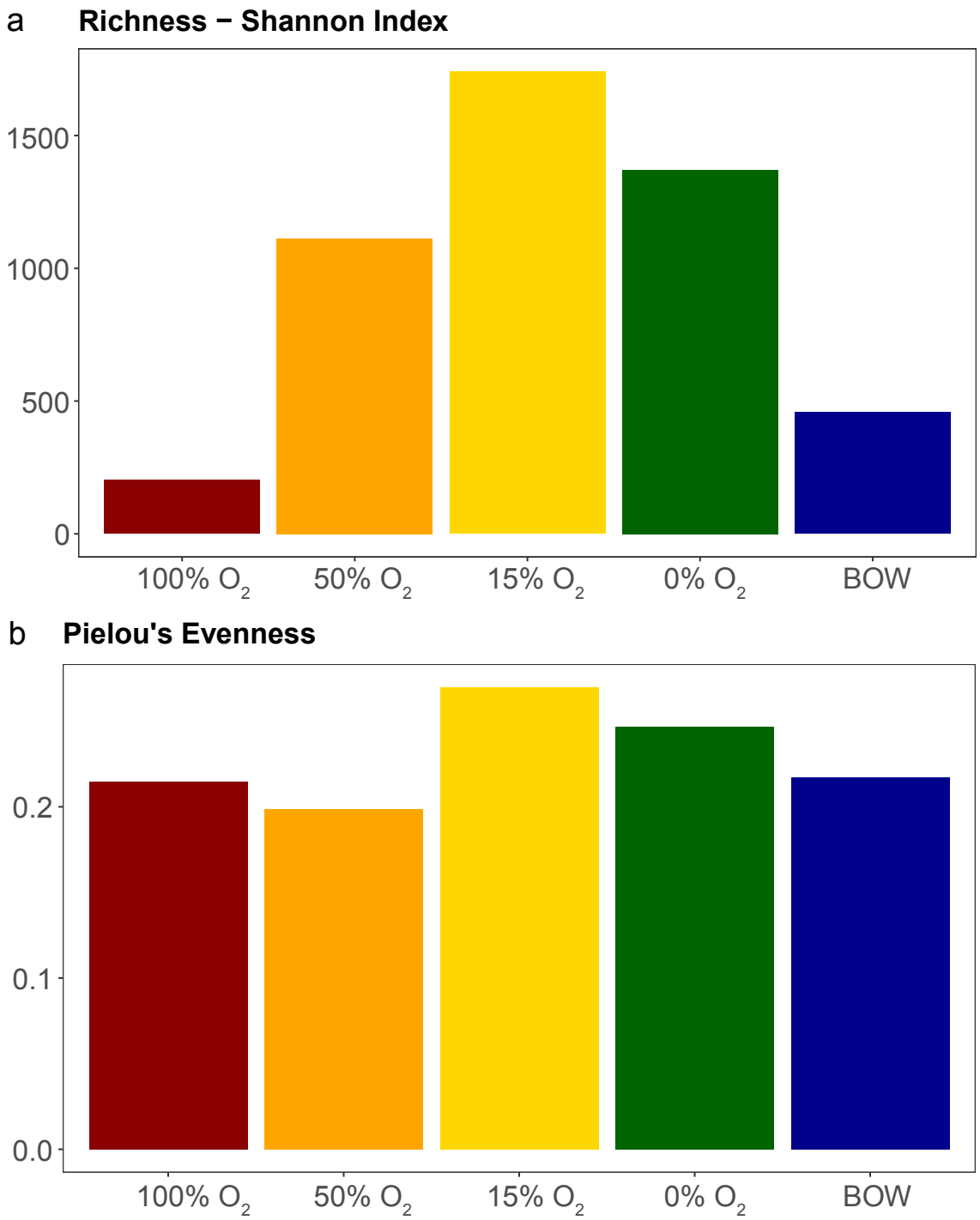

Figure S5. Sample richness (A) and sample evenness (B) calculated in R for CROMO CSW1.1 depth profile samples.

Figure Panel	Y Axis Label	Reaction
A	O_2	$CO\ (aq) + 0.5O_2(aq) \leftrightarrow CO_2(aq)$
A	$NO_3^- \rightarrow NO_2^-$	$CO(aq) + NO_3^- \leftrightarrow CO_2(aq) + NO_2^-$
A	$NO_3^- \rightarrow NH_4^+$	$4CO(aq) + NO_3^- + 2H^+ + H_2O(l) \leftrightarrow NH_4^+ + 4CO_2(aq)$
A	SO_4^{2-}	$4CO(aq) + SO_4^{2-} + H^+ \leftrightarrow HS^- + 4CO_2(aq)$
B	O_2	$CH_3COO^- + 2O_2(aq) + H^+ \leftrightarrow 2CO_2(aq) + 2H_2O(l)$
B	$NO_3^- \rightarrow NO_2^-$	$CH_3COO^- + 4NO_3^- + H^+ \leftrightarrow 2CO_2(aq) + 4NO_2^- + 2H_2O(l)$
B	$NO_3^- \rightarrow NH_3$	$CH_3COO^- + NO_3^- + 2H^+ \leftrightarrow 2CO_2(aq) + NH_3(aq) + H_2O(l)$
B	SO_4^{2-}	$CH_3COO^- + SO_4^{2-} + 2H^+ \leftrightarrow 2CO_2(aq) + HS^- + 2H_2O(l)$
C	O_2	$CH_4(aq) + 2O_2(aq) \leftrightarrow CO_2(aq) + 2H_2O(l)$
C	$NO_3^- \rightarrow NO_2^-$	$CH_4(aq) + 4NO_3^- \leftrightarrow CO_2(aq) + 4\ NO_2^- + 2H_2O(l)$
C	SO_4^{2-}	$CH_4(aq) + SO_4^{2-} + H^+ \leftrightarrow HS^-(aq) + CO_2(aq) + 2H_2O(l)$
D	O_2	$H_2(aq) + 0.5O_2(aq) \leftrightarrow H_2O(l)$
D	$NO_3^- \rightarrow NO_2^-$	$H_2(aq) + NO_3^- \leftrightarrow NO_2^- + H_2O(l)$
D	$NO_3^- \rightarrow NH_3$	$4H_2(aq) + NO^{3-} + H^+ \leftrightarrow NH_3(aq) + 3H_2O(l)$
D	SO_4^{2-}	$4H_2(aq) + SO_4^{2-} + H^+ \leftrightarrow HS^-(aq) + 4H_2O(l)$

Table S1. Full balanced reactions for abbreviated Figure 3 Gibbs energy y-axis labels.

OTU	Avg. Tot. Abund	100% O_2	50% O_2	15% O_2	0% O_2	BOW	Organism	% Identical Sites	% Pairwise Identity
OTU_266710	21.60	14.05	77.88	62.84	69.72	38.29	*Serpentinomonas raichei* strain A1	99.60%	99.60%
OTU_75957	12.33	76.09	4.86	18.59	9.28	52.39	*Truepera radiovictrix*	91.30%	91.30%
OTU_19770	0.92	1.84	2.45	3.73	1.65	2.94	*Hydrogenophaga soli*	99.60%	99.60%
OTU_29562	0.17	1.11	0.19	0.45	0.26	0.60	*Gracilibacter thermotolerans*	92.10%	92.10%
OTU_237261	0.13	0.00	0.00	0.25	0.20	0.44	*Dethiobacter alkaliphilus*	96.00%	96.00%
OTU_4746	0.00	0.00	0.70	0.00	0.00	0.00	*Methylobacterium bullatum*	100.00%	100.00%
OTU_6766	0.06	0.03	0.25	0.05	0.25	0.06	*Hydrogenophaga soli*	99.60%	99.60%
OTU_27194	0.07	0.00	0.19	0.09	0.29	0.05	*Hydrogenophaga soli*	99.60%	99.60%
OTU_181552	0.06	0.00	0.27	0.04	0.24	0.04	*Hydrogenophaga soli*	99.60%	99.60%
OTU_10013	0.02	0.35	0.04	0.08	0.06	0.04	*Hydrogenophaga soli*	99.60%	99.60%
OTU_163786	0.05	0.00	0.20	0.07	0.21	0.02	*Hydrogenophaga soli*	99.60%	99.60%

Organism = most closely related BLAST match
BOW = Bottom of Well

Table S2. Top 11 OTUs in the CSW1.1 profile and their top NCBI BLAST match.

Data Set S1. Thermodynamic Gibbs energy calculations.

Data Set S2. CSW1.1 depth profile final 16S rRNA taxonomy and counts table.

Data Set S3. 16S rRNA contaminants filtered out.

Data Set S4. Metagenomic data from CSW1.1 depth profile samples.

Data Set S5. MAG abundances per depth profile sample. Note CSW1.1 bottom of well is averaged between triplicate field samples.

Data Set S6. Other relevant CROMO depth profile accessory genes present in *Serpentinomonas*, *Truepera*, and *Dethiobacter* MAG bins but not necessarily listed in Figure 4 or 5.

Data Set S7. Completeness and Contamination percentages for CROMO CSW1.1 depth profile MAG bins.