Supplementary Figures and Tables for:

Novel microbial assemblages dominate weathered sulfide-bearing rock from copper-nickel deposits in the Duluth Complex, Minnesota, USA

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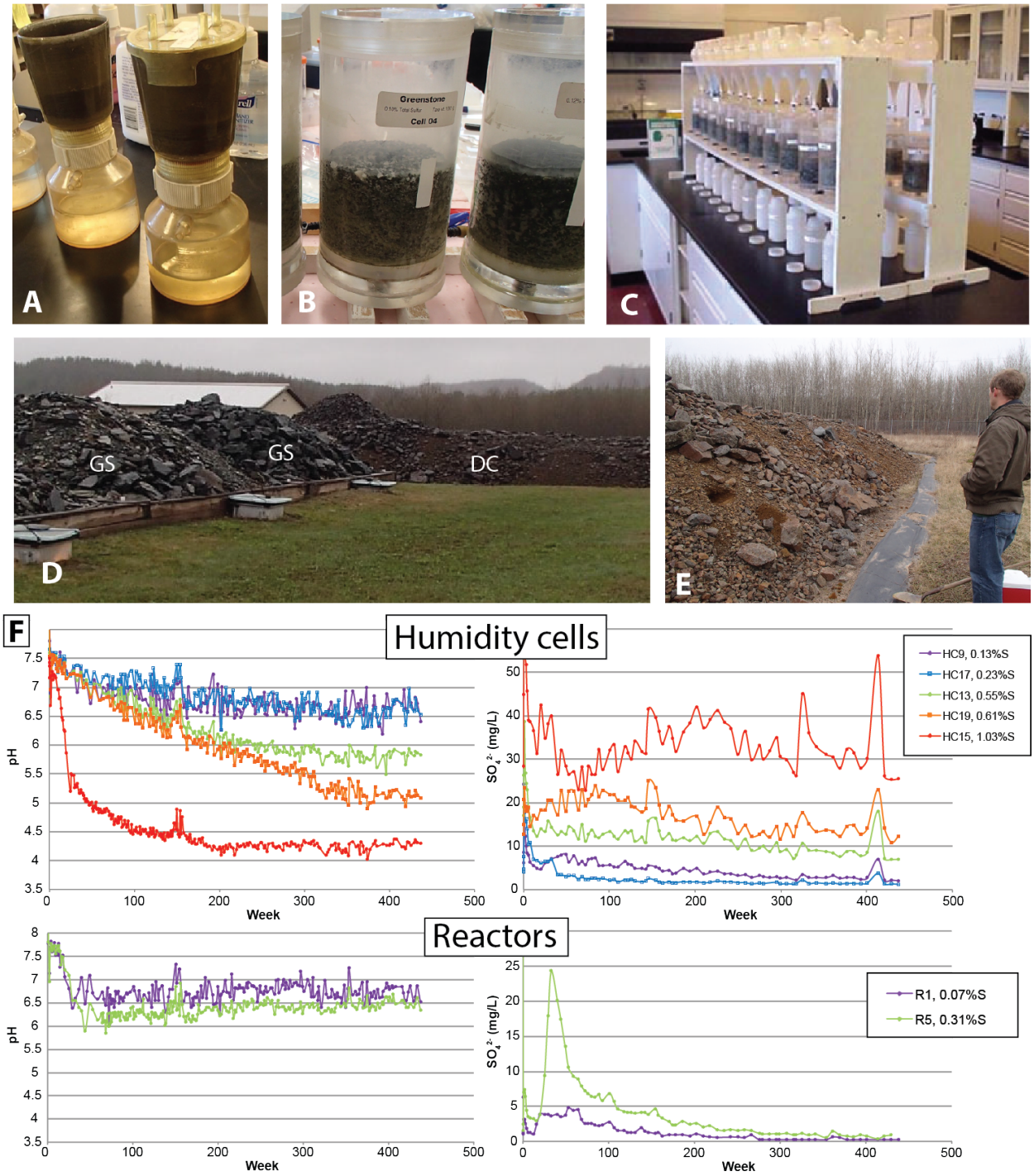
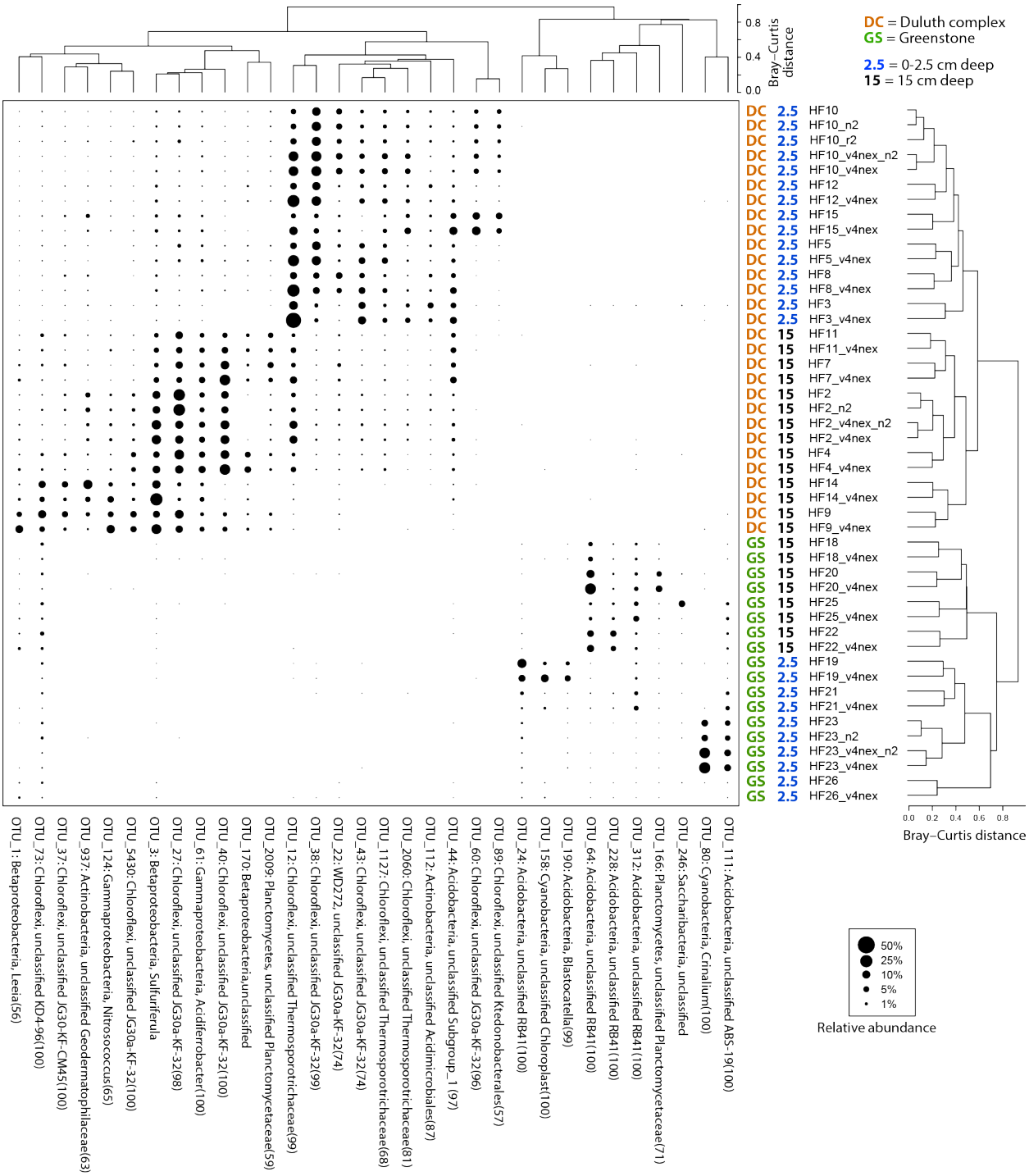
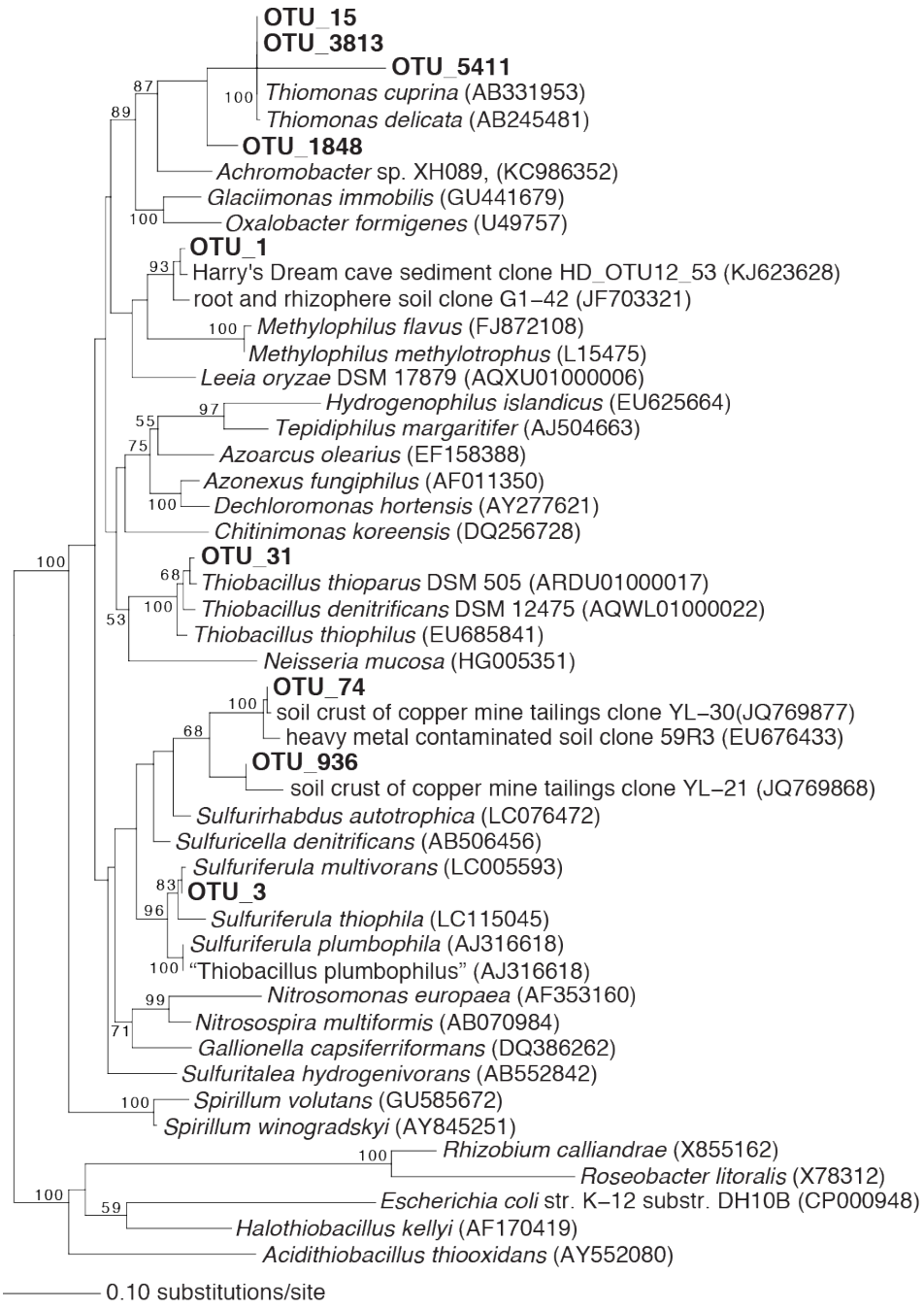


Supplementary Figure S1. Representative images of the laboratory and field leaching experiments established by the Minnesota Department of Natural Resources (MN DNR) (A-E). Examples of covered (right) and uncovered (left) reactors are shown in (A), and humidity cells are pictured in (B) and (C). Photo (C) courtesy of the MN DNR Division of Lands and Minerals. Field leaching experiments are shown in panels (D) and (E). In panel (D), two greenstone (GS) piles are in the foreground, left, and the large Duluth Complex (DC) pile is in the background. Panel (E) is a close up of the Duluth Complex pile, Kipp Sande for scale. Panel (F) shows examples of long term data from five of the Duluth Complex humidity cells (top) and two of the

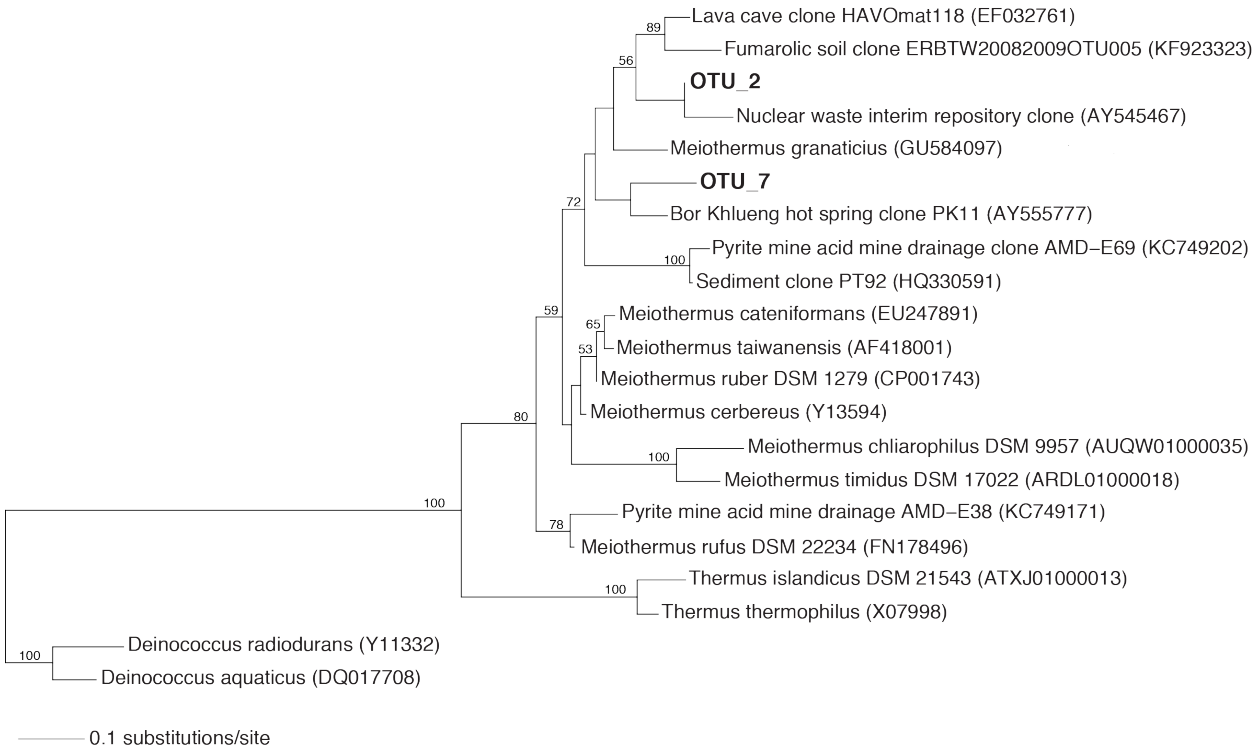
reactors (bottom) that were sampled here. Data through 2011 is shown. Samples for microbiological analysis were collected at the termination of the experiment (Dataset S1). See the MN DNR reports referenced in the table in Dataset S1 for complete geochemical and other information on all the weathering experiments sampled in this study.



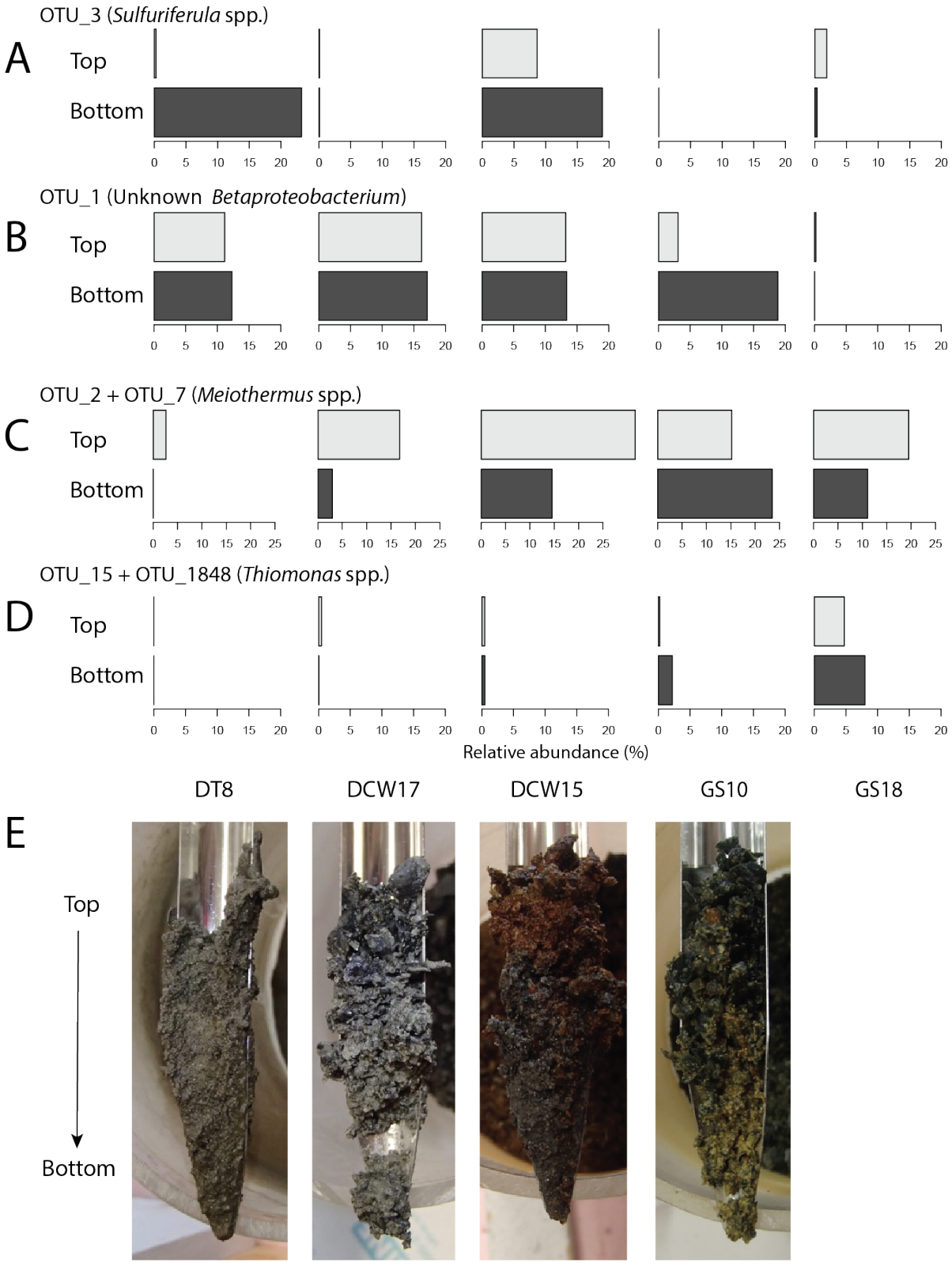
Supplementary Figure S2. Two-way cluster analysis of all DNA libraries from field samples. The libraries with "_v4nex" in the name are the in-house libraries, and the others were generated using the full service amplicon sequencing protocol. Replicates are included to show the variability among libraries. The size of the point scales with the relative abundance of each OTU. The Q-mode cluster analysis was calculated with all OTUs, while R-mode clustering only includes OTUs that were present at >5% in at least one field sample. The taxonomic affiliation of each OTU includes its phylum- and genus-level classifications, if available. OTUs that are unclassified at the genus level are indicated as such, and the highest available taxonomic classification is provided. Confidence scores >50 are provided in parentheses.



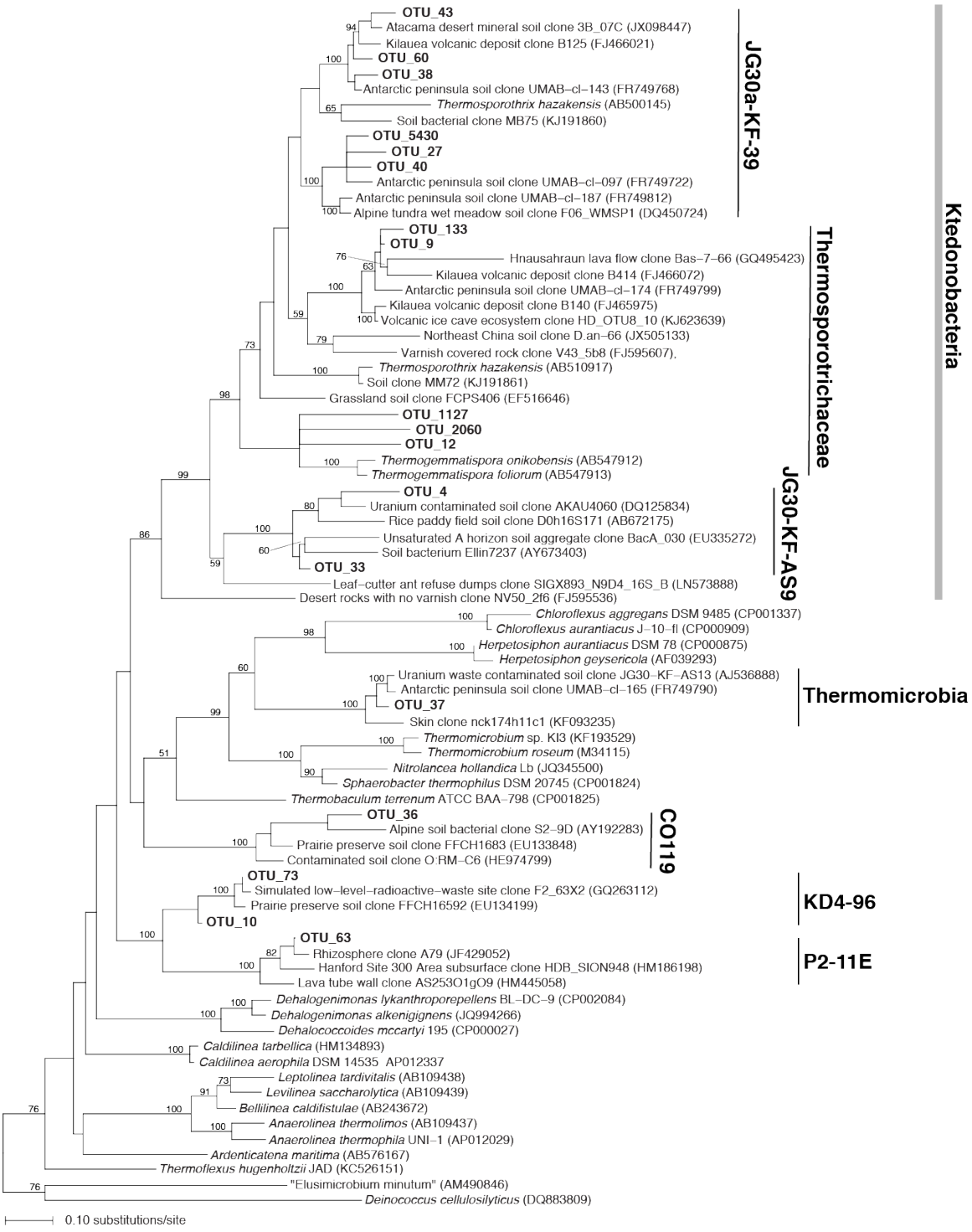
Supplementary Figure S3. Phylogenetic tree showing the placement of OTUs that classify as sulfur-oxidizing genera in the *Betaproteobacteria*, as well as one of the most abundant OTUs (OTU_1) that was unclassified. The base tree is a maximum likelihood phylogram created with nearly full-length sequences, with shorter amplicon sequences (bold) placed after the fact using the EPA algorithm. Numbers indicate bootstrap support for nodes from the maximum likelihood analysis of nearly full-length sequences.



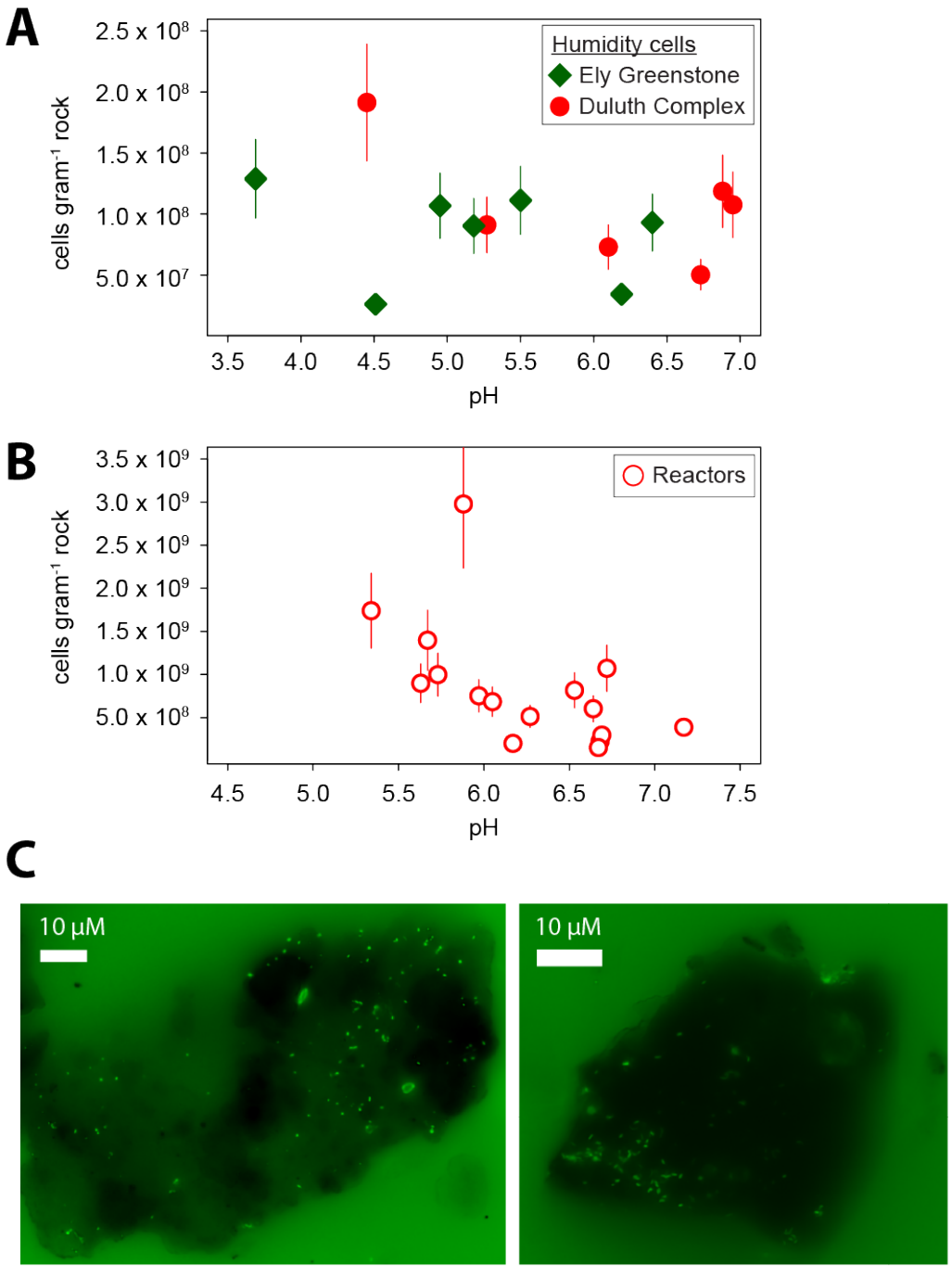
Supplementary Figure S4. Phylogenetic tree showing the placement of OTUs classified as *Meiothermus* spp. The base tree is a maximum likelihood phylogram created with nearly full-length sequences, with shorter amplicon sequences (bold) placed after the fact using the EPA algorithm. Numbers indicate bootstrap support for nodes from the maximum likelihood analysis of nearly full-length sequences.



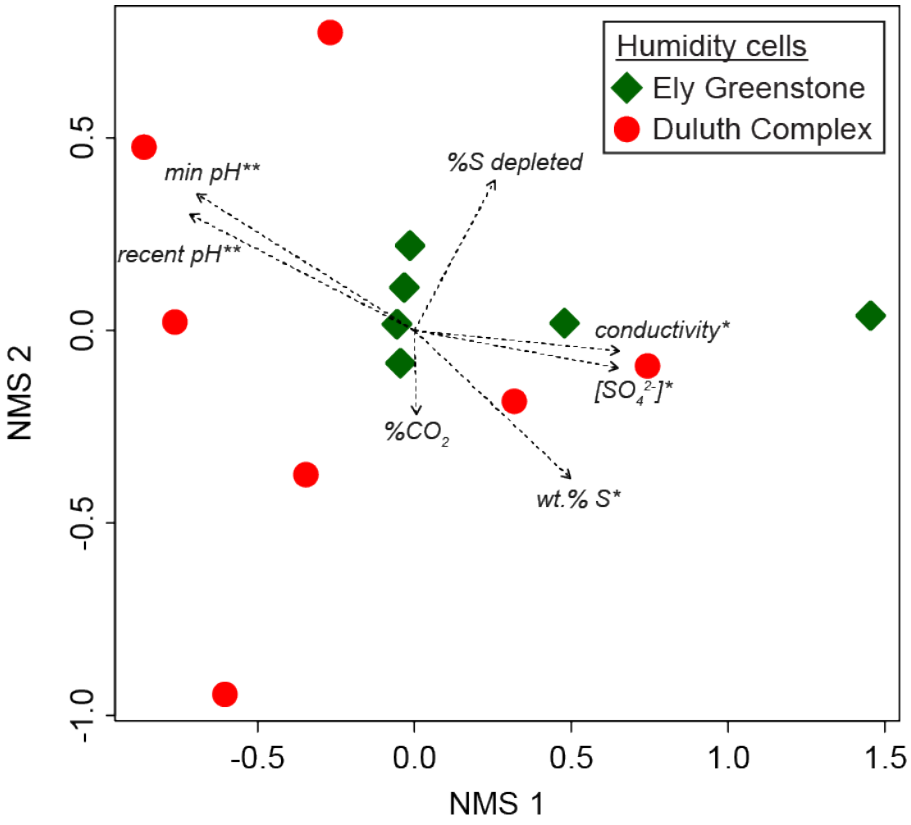
Supplementary Figure S5. (A-D) Relative abundance of select abundant OTUs in the surface and deeper layers of five humidity cells. Photos of vertical profiles of the humidity cells are shown in (E). (Photo not available for GS18.) Note that an abrupt redox horizon is apparent in some cells (DWC15) but not in others.



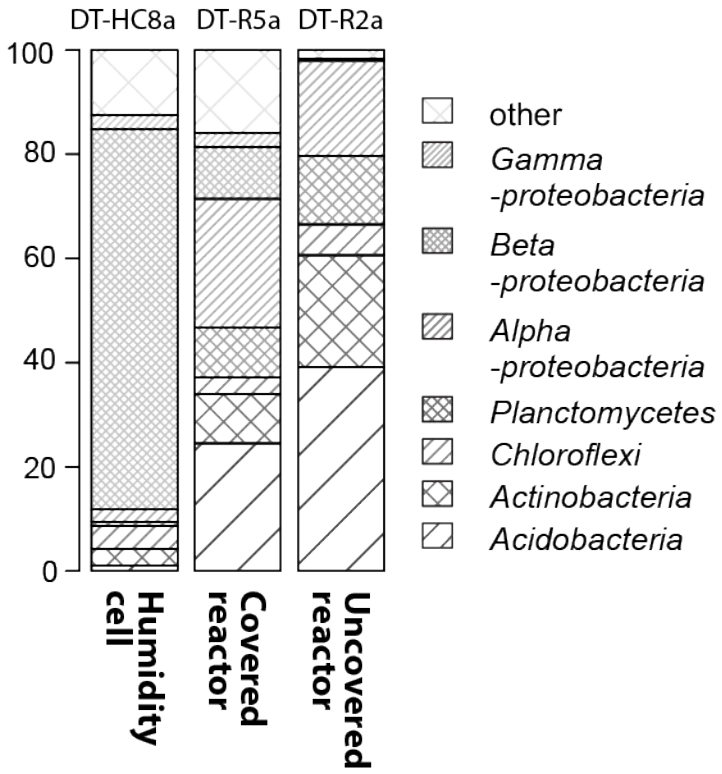
Supplementary Figure S6. Phylogenetic tree showing the placement and associations of OTUs from the phylum *Chloroflexi*. The base tree is a maximum likelihood phylogram created with nearly full-length sequences, with shorter amplicon sequences (bold) placed after the fact using the EPA algorithm. Numbers indicate bootstrap support for nodes from the maximum likelihood analysis of nearly full-length sequences. The group CO119 is classified as *Ktedonobacteria*, but does not group with it in this reconstruction.



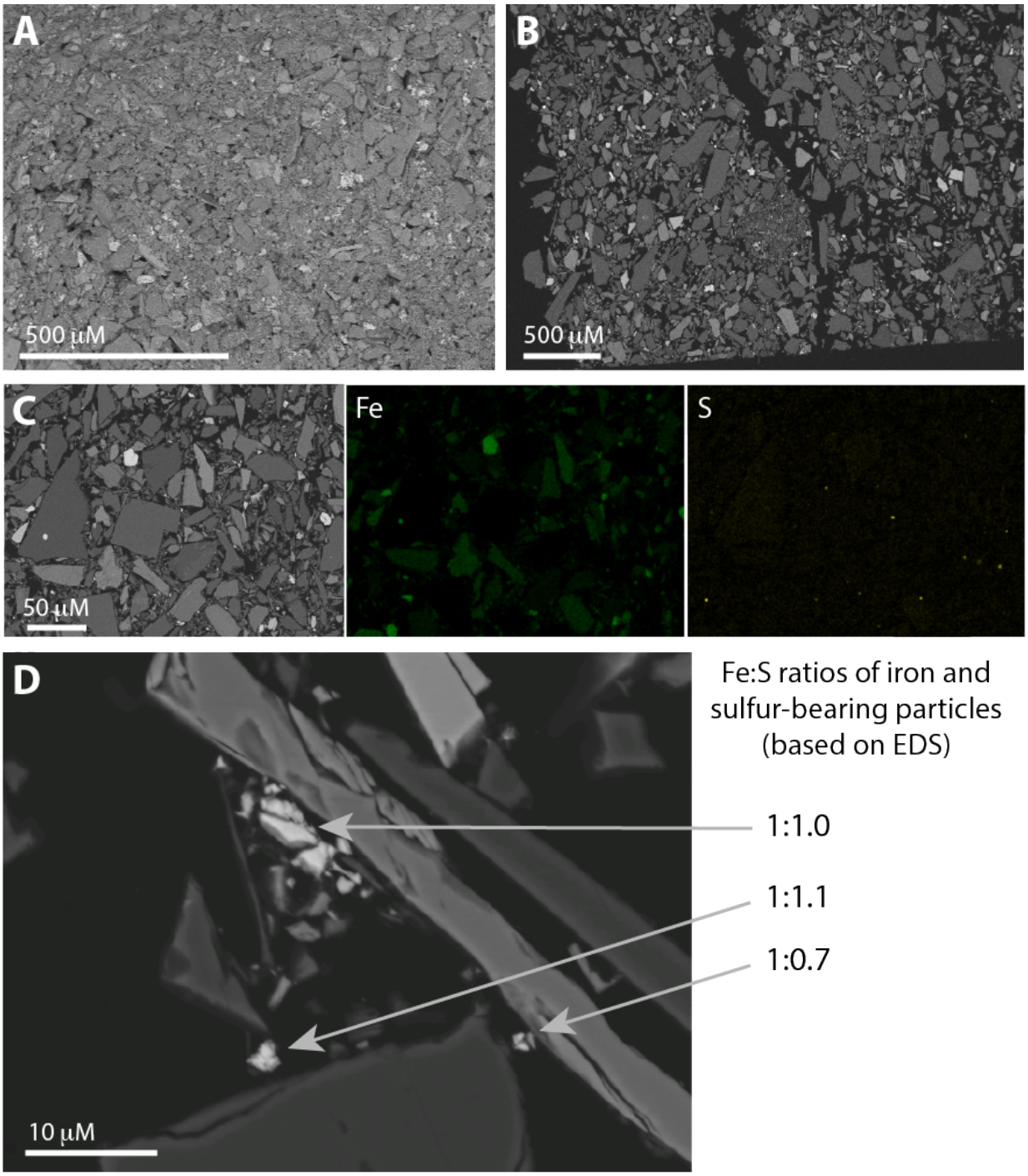
Supplementary Figure S7. Cell counts versus pH for humidity cell (A) and reactor (B) samples. pH was not statistically significantly correlated with cell abundance in humidity cells (panel A: Duluth Complex, r^2=0.38, p=0.19; greenstone, r^2=0.08, p=0.53) but it was in reactors (panel B: r^2=0.32, p=0.02). (C) Photomicrographs of CYBR Gold stained cells attached to mineral surfaces in humidity cell samples DCW15 and DCW13, respectively. The vast majority of cells in the experiments were attached to mineral surfaces, and sonication was necessary to detach cells and achieve a homogenous distribution for counting



Supplementary Figure S8. NMS ordination of 16S rRNA transcript libraries from humidity cells. Fitted vectors of variables indicated by single and double asterisks (**) are statistically significant (** = $p<0.001$, $r^2 > 0.8$; * = $p<0.02$, $r^2 > 0.5$).

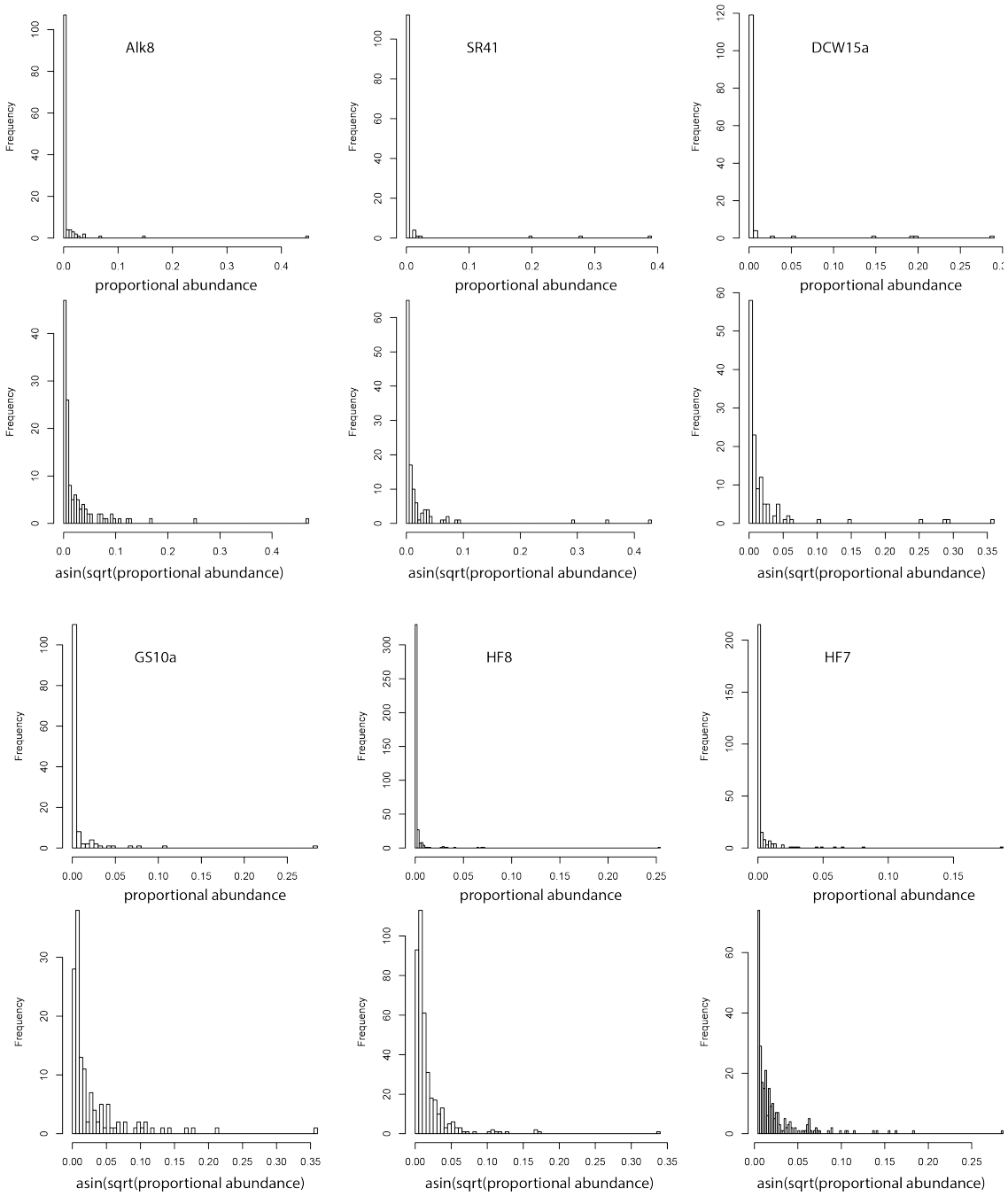


Supplementary Figure S9. Phylum-level comparison of libraries from three tailings weathering experiments: a humidity cell (DT_HC8a), a covered reactor (DT_R5a), and an uncovered reactor (DT_R2a). Phyla representing >2% in at least one of the three libraries are shown. These three experiments were all initiated with the same tailings material, and the libraries were all created with the full-service method from RNAlater-preserved samples.



Supplementary Figure S10. Representative backscatter (BSE) scanning electron microscopy (SEM) images of weathered tailings from humidity cells. Panel A is an image of loose tailings, and panels B-D are from a thin section created from an epoxy-embedded humidity cell (MN DNR, unpublished). Panel C shows elemental maps for iron and sulfur, collected from the region the BSE image to the left. Panel D is high magnification BSE image from which energy dispersive spectroscopy (EDS) analyses were collected from S-bearing particles (arrows). Iron to sulfur ratios indicate that the iron sulfides are likely pyrrhotite (the elevated iron in the bottom particle may be from the surrounding minerals, because in this case the EDS spot size is large

than the particle). Image A was collected from an uncoated sample with a Hitachi TM-1000 scanning electron microscope (Hitachi, Tokyo, Japan), and the other images were collected with a JEOL JSM-6500F scanning electron microscope, 15 keV accelerating voltage for the EDS analysis, from a carbon-coated thin section.



Supplementary Figure S11. Histograms depicting the impact of arcsine square root transformation on the structure of the data, for six representative libraries. For each library, untransformed data is in the top panel, transformed data is in the bottom panel.

Table S1. Library sizes of blank controls included with the sequencing libraries

Blank name	library size[a]	submission date	Blank type	Library type
blankDCW1v4nex	17	Nov2015	DNA extraction blank	custom V4 nextera, barcoding only[b]
blankDCW2v4nex	14	Nov2015	DNA extraction blank	custom V4 nextera, barcoding only[b]
blankHF1v4nex	13	Nov2015	DNA extraction blank	custom V4 nextera, barcoding only[b]
blankHF2v4nex	5	Nov2015	DNA extraction blank	custom V4 nextera, barcoding only[b]
BLANK1_S64_L001	494	Oct2014	Water blank	Full service
BLANK2_S8_L001	1289[c]	Oct2014	Water blank	Full service
H20-BLANK-002-A	4	Nov2015	Water blank	Full service
H20-BLANK-002-H	6	Nov2015	Water blank	Full service
H20-BLANK-H06_S	30	Nov2015	Water blank	Full service
H20-BLANK-1_S30	183	Oct2014	Water blank	Full service
H20-BLANK-2_S40	48	Oct2014	Water blank	Full service
H20-BLANK-3_S50	53	Oct2014	Water blank	Full service
H20-BLANK-4_S60	40	Oct2014	Water blank	Full service
H20-BLANK-5_S70	69	Oct2014	Water blank	Full service
H20-BLANK-6_S80	94	Oct2014	Water blank	Full service

[a]Library size is the number of sequences that remained following quality trimming, filtering, and OTU calling

[b]DNA extraction blanks were amplified using the custom V4 nextera primers (no visible PCR product) and submitted for "barcoding only" like the other custom V4 nextera libraries.

[c]Most of the sequences in this blank were from two OTUs, classified as the genera *Anaerococcus* and *Staphylococcus*