

Microbial Population Dynamics and the Role of Sulfate Reducing Bacteria Genes in Stabilizing Pb, Zn, and Cd in the Terrestrial Subsurface

Ranju R. Karna ^{1,2,*}, Ganga M. Hettiarachchi ¹, Joy Van Nostrand ³, Tong Yuan ³, Charles W. Rice ⁴, Yared Assefa ¹ and Jizhong Zhou ^{3,5,6}

¹ Soil and Environmental Chemistry Laboratory, Department of Agronomy, Kansas State University, Manhattan, KS 66506, USA; ganga@ksu.edu (G.M.H.); yareda@ksu.edu (Y.A.)

² Oak Ridge Institute for Science and Education, Oak Ridge, TN 37830, USA

³ Institute of Environmental Genomics and Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK 73019, USA; joy.vannostrand@ou.edu (J.V.N.); 15838348600@163.com (T.Y.); jzhou@rccc.ou.edu (J.Z.)

⁴ Soil Microbial Ecology Laboratory, Department of Agronomy, Kansas State University, Manhattan, KS 66506, USA; cwrice@ksu.edu

⁵ Earth Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

⁶ School of Environment, Tsinghua University, Beijing 100084, China

* Correspondence: karna.ranju@epa.gov; Tel.: +1-513-487-2449

The supplementary information includes 6 figures and 3 tables.

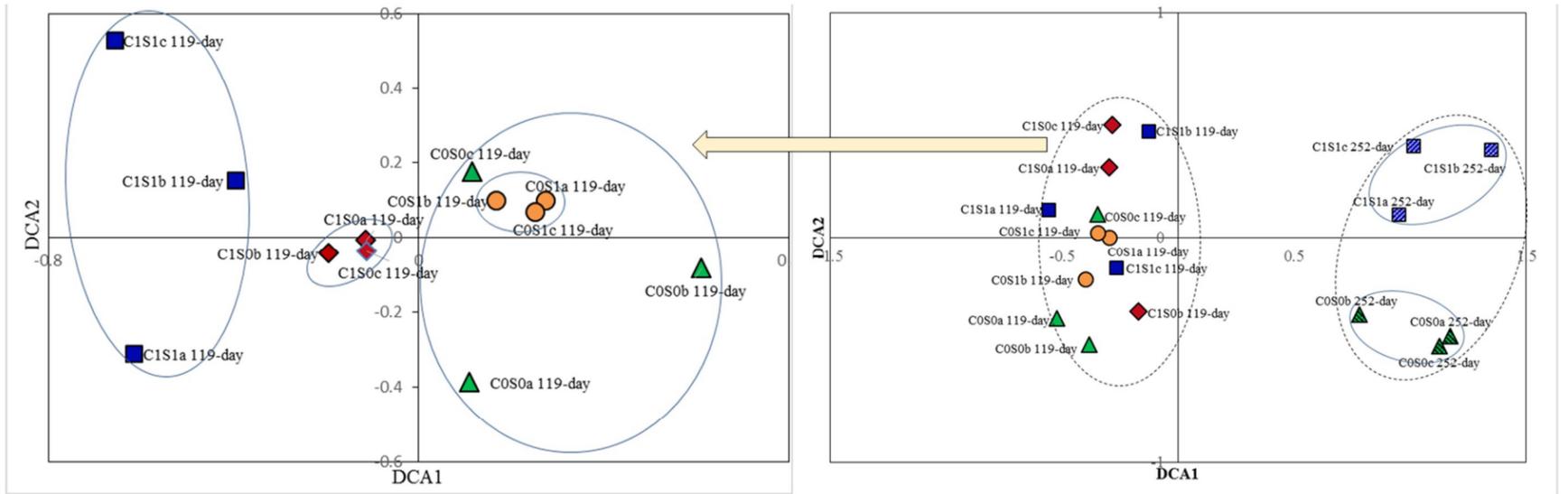


Figure S1: Detrended correspondence analysis (DCA) for the total number of detected genes under medium- (119-day) and long-term (252-day) submergence indicating community structure changes. All the treatments; COS0, COS1, C1S0, and C1S1 (solid filled markers) from medium-term submergence, and only COS0, and C1S1 (pattern filled markers) from long-term submergence are plotted.

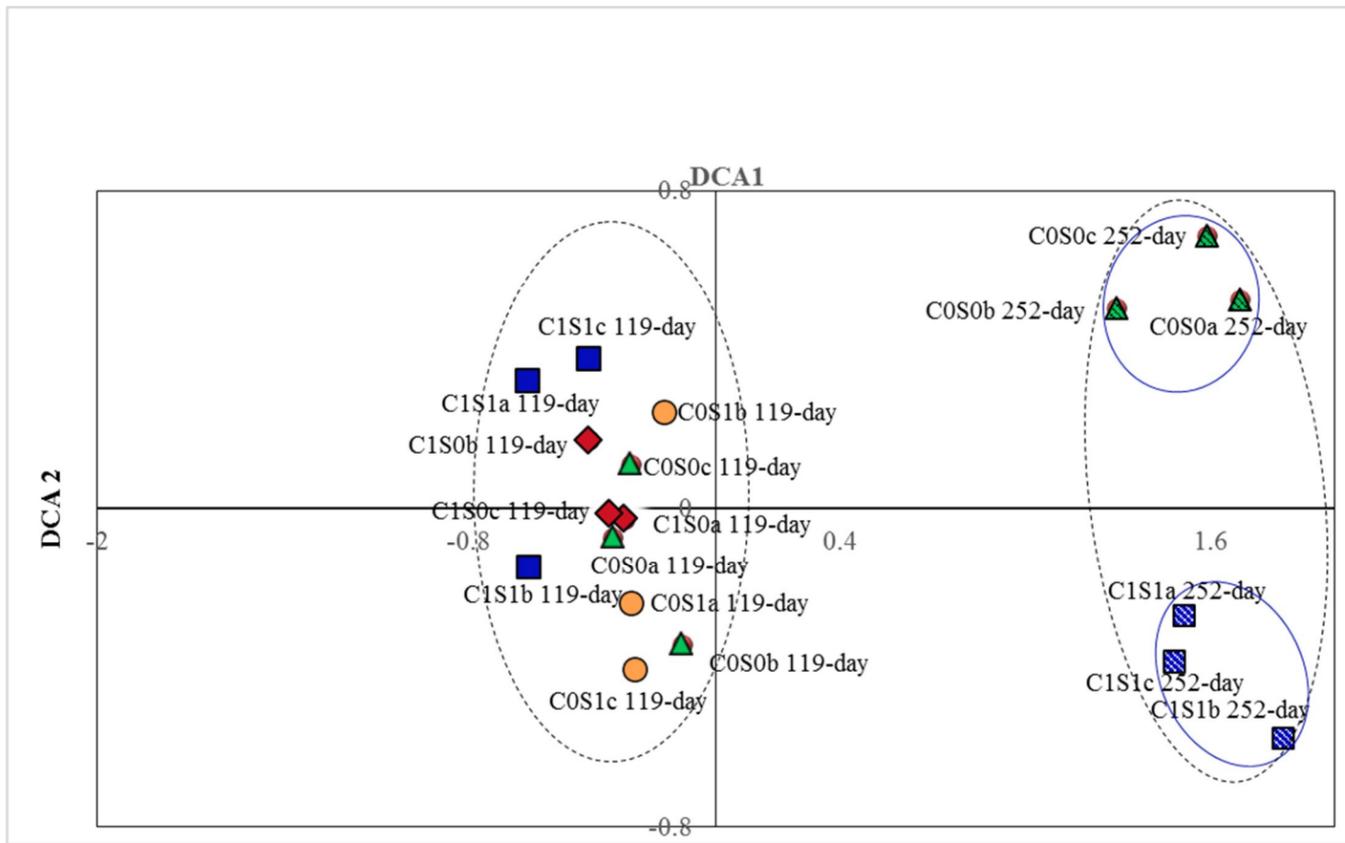


Figure S2: Detrended correspondence analysis (DCA) of functional genes in the metal resistance category showing change in community structure under medium- (119-day) and long-term (252-day) submergence. All the treatments; COS0, COS1, C1S0, and C1S1 (solid filled markers) from medium-term submergence, and only COS0, and C1S1 (pattern filled markers) from long-term submergence are plotted.

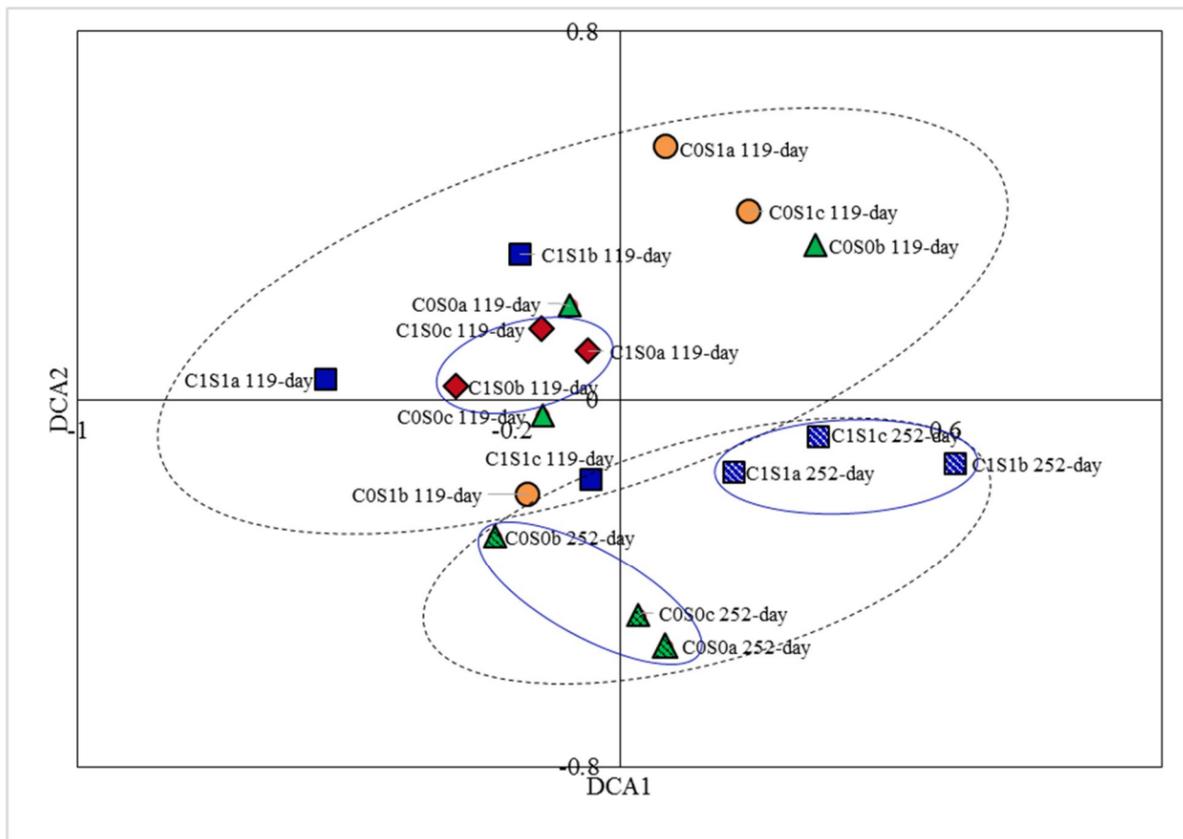


Figure S3: Detrended correspondence analysis (DCA) of functional genes in the C-cycling category showing change in community structure under medium- (119-day) and long-term (252-day) submergence. All the treatments; C0S0, C0S1, C1S0, and C1S1 (solid filled markers) from medium-term submergence, and only C0S0, and C1S1 (pattern filled markers) from long-term submergence are plotted.

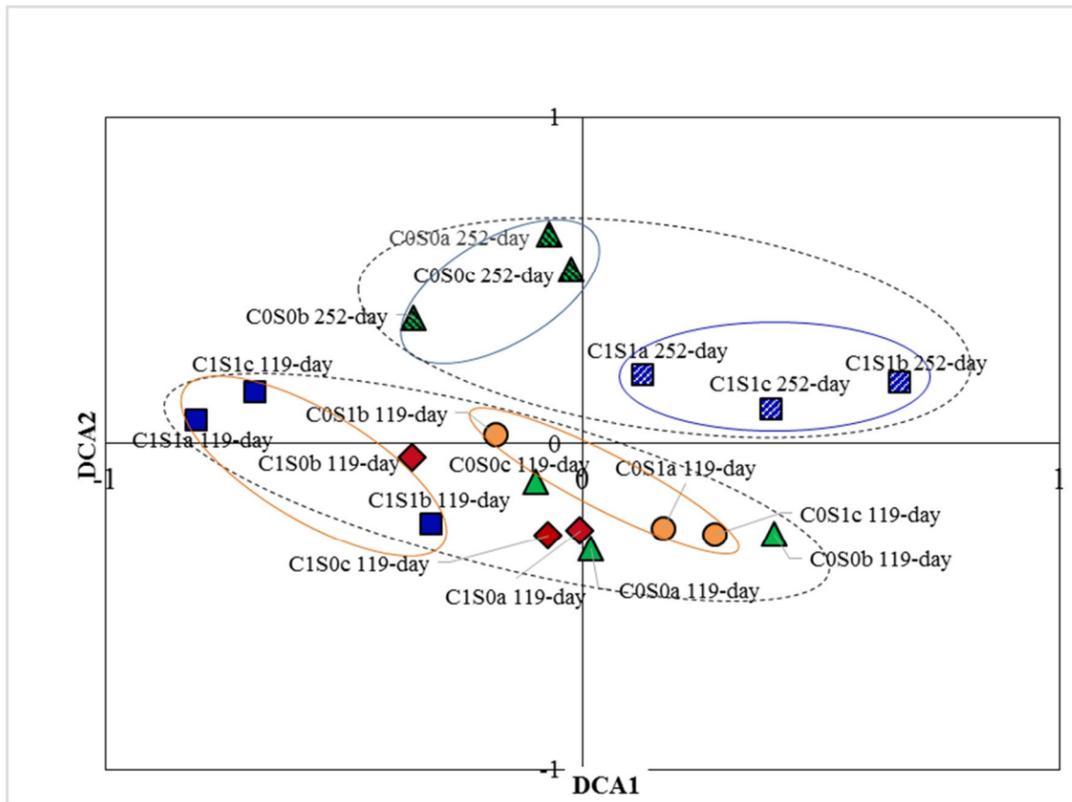


Figure S4: Detrended correspondence analysis (DCA) of functional genes in the sulfur category showing change in community structure under medium- (119-day), and long-term (252-day) submergence. All the treatments; C0S0, C0S1, C1S0, and C1S1 (solid filled markers) from medium-term submergence, and only C0S0, and C1S1 (pattern filled markers) from long-term submergence are plotted.

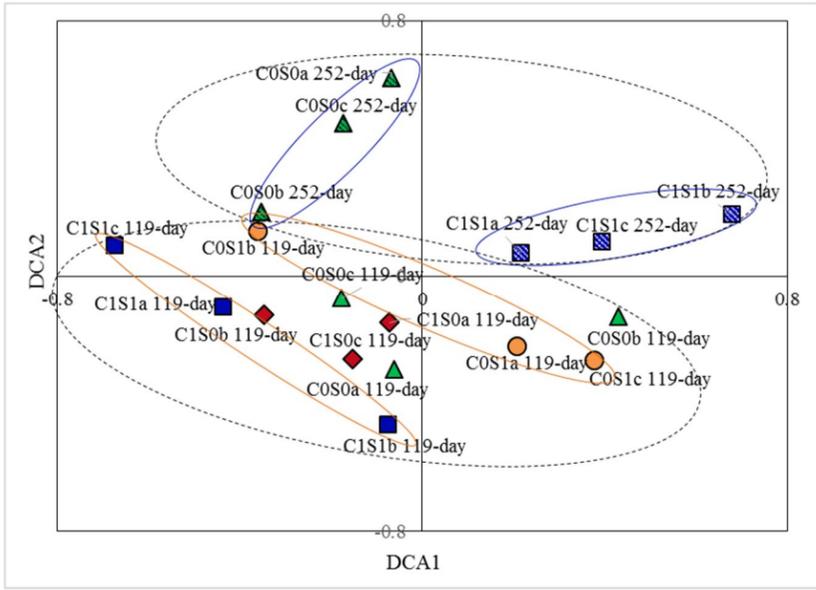


Figure S5: Detrended correspondence analysis (DCA) of *dsrB* showing a change in community structure under medium- (119-day), and long-term (252-day) submergence. All the treatments; COS0, COS1, C1S0, and C1S1 (solid filled markers) from medium-term submergence, and only COS0, and C1S1 (pattern filled markers) from long-term submergence are plotted.

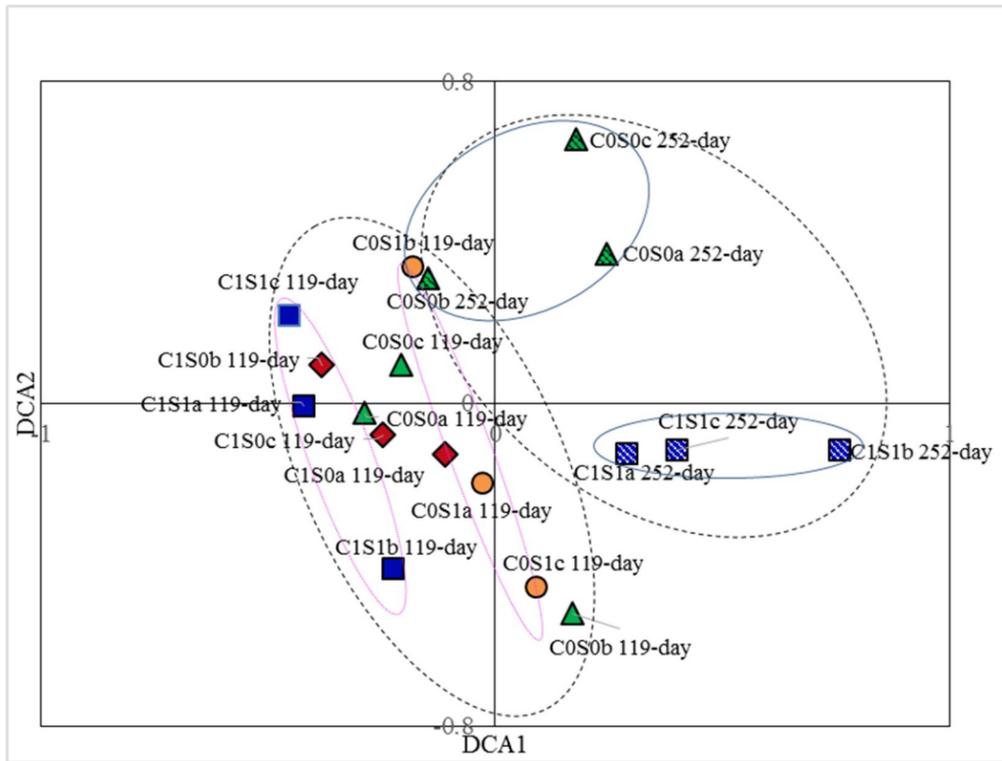


Figure S6: Detrended correspondence analysis (statistics) of *dsrA* showing a change in community structure under medium- (119-day), and long-term (252-day) submergence. All the treatments; C0S0, C0S1, C1S0, and C1S1 (solid filled markers) from medium-term submergence, and only C0S0, and C1S1 (pattern filled markers) from long-term submergence are plotted.

Table S1: Total element concentration in the mine waste fraction of <2mm collected from the Tri-State mining district.

Element	Cd	Pb	Zn	Fe	Mn	S
mg kg ⁻¹	67	5048	23468	6834	97	9458

Table S2: Summary of total phospholipid fatty acid analysis (PLFA) biomarkers detected in original mine tailings, submerged control, and submerged treated materials under 119-day (medium-term) submergence

	Common microbial group	Gram positive Bacteria	Gram negative Bacteria	Desulfovibrio	Fungi	Fungi Arbuscular Mycorrhiza	Actinomycetes	Total PLFA (nmole/g)
Mine tailings	0.76	0.42	0.29	0.02	0.73	0.13	0.08	2.42
Submerged sediment (Inoculum)	1.91	1.15	1.84	0.09	0.86	0.31	0.02	6.18
C0S0	0.82±0.004	0.67±0.001	0.35±0.005	0.02±0.002	0.35±0.008	0.06±0.001	0.04±0.002	2.31
C1S1	4.03±0.12	3.10±0.14	1.39±0.20	3.22±0.10	0.62±0.006	0.05±0.002	0.05±0.005	12.47

Table S3: Bray Curtis dissimilarity test giving q -value for each treatment during medium- (119-day) and long-term (252-day) submergence.

Group	Adonis Bray
Whole	0.001
C0S0 119-day vs C0S0 252-day	0.001
C0S0 119-day vs C1S1 119-day	0.001
C0S0 119-day vs C1S1 252-day	0.001
C0S0 119-day vs C0S1 119-day	0.001
C0S0 119-day vs C1S0 119-day	0.132
C0S0 252-day vs C1S1 119-day	0.11
C0S0 252-day vs C1S1 252-day	0.039
C0S0 252-day vs C0S1 119-day	0.09
C0S0 252-day vs C1S0 119-day	0.013
C1S1 119-day vs C1S1 252-day	0.068
C1S1 119day vs C0S1 119-day	0.001
C1S1 119-day vs C1S0 119-day	0.001
C1S1 252-day vs C0S1 119-day	0.497
C1S1 252-day vs C1S0 119-day	0.001
C0S1 119-day vs C1S0 119-day	0.305

The p -values were calculated based on total number of detected genes.