

## Genome-centered analysis of biogeochemical cycling

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With the goal of understanding the microbiological and geochemical processes impacting subsurface environments, iron-reducing groundwater samples were collected from the Rifle Integrated Field Research Challenge site and characterized using community proteogenomics. We reconstructed a total of 87 genomes, with most genomes comprising < 1% of the community. 79 of these genomes were Bacterial, the remaining were Bacterial associated phage and mobile elements, with no evidence for Archaea or Eukaryotes in the samples. 49 genomes sampled (57%) were from members of the Bacterial candidate divisions (CD) lineages for which we have none to minimal prior genomic sampling: 21 OD1, 19 OP11, 6 genomes in which UGA (stop) is translated as tryptophan, 5 of which are affiliated with BD1-5, and three that represent a new division (PER). All CD are predicted to ferment, but some augment fermentation with archaeal-like type II/III RuBisCO inferred to couple AMP salvage with CO<sub>2</sub> fixation; others pump protons and reduce sulfur using archaeal-type hydrogenases. Proteomics indicates that fermentative CD lineages convert refractory sediment carbon compounds to acetate, ethanol, and hydrogen, stimulating the activity of respiratory iron, sulfate, and nitrate reducing bacteria. We detected the expression of uptake hydrogenases in the dominant iron (*Geobacter*) and sulfate (*Desulfotalea*-like) organisms, suggesting hydrogen cycling between these trophic groups occurs *in situ*. Other Proteobacterial and Bacterioidetes members have the capacity to oxidize ferrous iron and sulfide produced from these respiratory organisms. Moreover, dissimilatory sulfate reducing and OD1 sulf-hydrogenase proteins were detected throughout the iron-reducing samples, suggesting that biogenic aqueous sulfide produced by sulfate and sulfur reducing metabolisms contributes to abiotic iron reduction in the aquifer during secondary stimulation. The results provide a glimpse into vast phylogenetic diversity, metabolic variety, and integrated physiological networks that persist in subsurface terrestrial anaerobic niches. Rifle IFRC research is supported by U.S. Department of Energy under Contract No. DE-AC02-05CH11231.