

## Microbial Community Structure in Redox Dynamic Environments

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Unraveling the complexity of subsurface microbial communities and their responses to dynamic environmental conditions is essential to improving our understanding of the biogeochemical cycling of major and minor elements, including contaminants. We characterized microbial communities from microcosms designed to test the effect of ethanol diffusion into U<sup>VI</sup>-contaminated sediment from the Oak Ridge IFRC. Groundwater and sediment was amended with sulfate, then ethanol was allowed to diffuse into the underlying sediment. The spatial distribution, valence, and speciation of both U and Fe were monitored *in situ* by synchrotron x-ray spectroscopy, in parallel with measurements of solution chemistry. As the ethanol was consumed concomitant with sulfate depletion, a ~1-cm layer of sediment near the sediment-water interface became visibly dark. XANES spectra in this layer were consistent with the formation of FeS and the reduction of U<sup>VI</sup> to U<sup>IV</sup>. Sequence analysis of 16S rRNA gene amplicons showed that the microbial community had evolved from its originally homogeneous composition, becoming spatially heterogeneous. At the sediment-water interface of the ethanol-amended (EtOH+) microcosms, sequences from the dissimilatory metal-reducing bacteria *Geobacter* and *Anaeromyxobacter* were by far the most abundant, accounting for 65-68% of the total community compared to 2-5% at the same location in no-ethanol controls (EtOH-). In the EtOH- samples, sulfate reducers composed 27-29% of the bacterial community, compared to only 4-7% in the EtOH+ samples. Statistical analyses showed that while ethanol was consumed within 7 days, its impact on the bacterial community was still evident after 4 years. These results indicate that even brief, one-time amendments can result in long-term perturbations of a microbial community and that these events continue to shape the community even after the resumption of static conditions.

In a separate experiment, we found that the microbial community in sediment from the Rifle IFRC was dominated by a previously-uncultivated epsilonproteobacterium, *Candidatus Sulfuricurvum* sp. RIFRC-1. Using whole-genome shotgun sequencing, we were able to reconstruct its complete 2.4-Mb-long genome *de novo* using a complexity reduction approach. Genome-based comparisons indicated that the organism is a novel species within the *Sulfuricurvum* genus. Genomic evidence suggests that RIFRC-1 is capable of growing by microaerobic or nitrate-/nitric oxide-dependent oxidation of S<sup>0</sup>, sulfide, sulfite, or H<sub>2</sub>. Consistent with these physiological attributes, the local aquifer was microoxic with small concentrations of available nitrate, small but elevated concentrations of reduced sulfur, and limited NH<sub>4</sub><sup>+</sup>/NH<sub>3</sub>. These results provide valuable insight into the complex role of microbially-mediated sulfur cycling in the subsurface.