

Title: Comparison of Multiple Ecogenomics Methods for Determining Ecosystem Function in Uranium-Contaminated Environments

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Background: Bioremediation may offer the only feasible strategy for the nearly intractable problem of metal and radionuclide contamination of soil and groundwater. To understand bioremediation in contaminated environments, it is critical to determine the organisms present in these environments, analyze their responses to stress conditions, and elucidate functional position in the environment.

Methods: We used multiple molecular techniques on both sediment and groundwater to develop a better understanding of the functional capability and stress level within the microbial community in relationship to over one hundred geochemical parameters. Due to the low pH (3.5-4.5) and high contaminant levels (e.g., uranium) microbial densities and activities were low. We used a phage polymerase amplification system to construct large and small insert DNA libraries, performed metagenome sequencing, constructed clonal libraries of select functional genes (SSU rRNA gene, *nirK*, *nirS*, *amoA*, *pmoA*, and *dsrAB*), used a SSU rDNA Phylochip microarray (9,000 taxa), and a functional gene array (23K genes). A complete comparison for community differences and similarities between the different techniques was assessed using several bioinformatics techniques.

Results: SSU rDNA analysis revealed the presence of distinct bacterial phyla, including proteobacteria, acidobacteria, and planctomycetes along the contaminant gradient. Metagenome analysis identified many of the same organisms, and diversity was lower in water than sediment. Analysis with functional gene arrays, phylochip, and specific probes for genes and organisms involved in biogeochemical cycling of C, N, and S, metal resistance, stress response, and contaminant degradation suggested that the dominant species could be biostimulated during *in situ* uranium reduction. Several other findings of difference and similarities between methods are presented.

Conclusion: These systems biology field studies could be enabling for strategies to attenuate metal and radionuclide contamination.