

## **Final Report: Trajectories of microbial community function in response to accelerated remediation of subsurface metal contaminants**

Remediation of subsurface metal contaminants at DOE sites involves microbial mechanisms of oxidation/reduction or complexation; which is controlled in large part by the ecology of the microbial community. Recognizing and quantifying the relationships between community structure, function, and key environmental factors may yield quantitative understanding that can inform future decisions on remediation strategies.

The objectives of our project were to: (1) Determine if the trajectories of microbial community structure, composition and function following organic carbon amendment can be related to, and predicted by, key environmental determinants. (2) Assess the relative importance of the characteristics of the indigenous microbial community, sediment, groundwater, and OC supply rate as the major determinants of microbial community functional response and bioremediation capacity. We used sediments from three DOE sites: Oak Ridge, TN; Rifle, CO; Ringold formation, Hanford, WA. In order to provide a solid foundation for this experiment we extensively characterized the three subsurface bacterial assemblages using G3 16S rRNA gene PhyloChips, G4 GeoChips, and analyzed the physical-chemical properties of the three sediments. Standard physical-chemical analysis of sediment material was performed on the bulk sediments (< 2.0 mm) as well as three particle size fractions (PSF) (< 20  $\mu\text{m}$ , 20-200  $\mu\text{m}$ , 0.2-2.0 mm).

The three sediments had markedly different particle-size distributions; which affected the assemblage structure and composition between sediments and thus the trajectories of the transplanted assemblages. The majority of microbial cells were located on the smallest PSF in all sediments. Sediment type and PSF were significant determinants of detected richness at the sub-family level by PhyloChip. However, sediment type was more important than PSF for explaining bacterial assemblage composition overall.

The degree of compositional overlap at the operational taxonomic unit (OTU) level was low between the sediments with 44.4% of 16,584 detected OTUs common to the three sediments. The mean detected (OTU) richness differed by site (Hanford: 5,145  $\pm$  663; Oak Ridge: 8,390  $\pm$  604; Rifle: 11,508  $\pm$  747) (mean  $\pm$  1 s.d.) by PhyloChip analysis. Strikingly, the opposite pattern was observed for detected functional gene richness (Hanford: 16,953  $\pm$  1,486; Oak Ridge: 12,880  $\pm$  1,309; Rifle: 10,380  $\pm$  956) (mean  $\pm$  1 s.d.) by GeoChip analysis. Ordination using presence/absence data at different taxonomic ranks (by PhyloChip) showed that the assemblages in each sediment were significantly different and distinguishable at the phylum-level (Anosim  $R=0.786$ ,  $P < 0.001$ ). We used a combination of detected richness patterns, taxa-accumulation curves and a measure of  $\beta$  diversity,  $\beta_{\text{sim}}$ , to choose a seed inoculum mass of 2.0 g for the reciprocal-transplant experiment.  $\beta_{\text{sim}}$  analysis indicated that a 2.0 g mass resulted in seed inoculums that were highly similar (Hanford: 88.8  $\pm$  1.8%; Oak Ridge: 92.2  $\pm$  1.5%; Rifle: 91.7  $\pm$  1.1%) (mean  $\pm$  1 s.d.).

Although the assemblages differed markedly in detected richness and composition, use of a taxa-sampling relationship allowed us to quantify the variability, or patchiness, of the assemblages. This information enabled us to optimize our experimental design by identifying the minimum inoculum mass required to accurately represent the assemblages. More broadly, these data demonstrated that subsurface assemblages at these DOE sites harbor a high amount of diversity and significantly differ in structure to the extent that they are distinguishable at the phylum-level.

