# Microbial Community Trajectories in Response to Accelerated Remediation of Subsurface Metal Contaminants

Rebecca A. Daly[1], Katerina Estera[1], Hsiao Chien Lim[2], Ping Zhang[3], Yongman Kim[2], Zhili He[3], Tetsu K. Tokunaga[2], Jiamin Wan[2], Jizhong Zhou[2,3], Eoin L. Brodie[2], and Mary K. Firestone[1,2]

*[1]University of California, Berkeley, CA [2]Lawrence Berkeley National Laboratory [3]University of Oklahoma, Norman, OK*

## Introduction

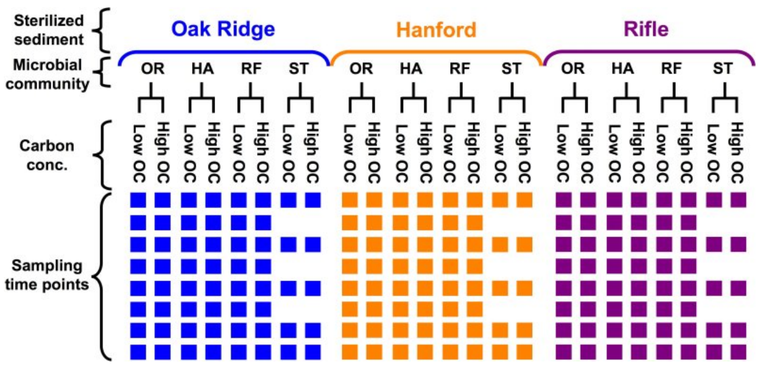
### Objectives

1. Determine if the trajectories of microbial community composition and function following organic carbon (OC) amendment can be related to, and predicted by, key environmental determinants or characteristics of the microbial community.
2. Assess the relative importance of the native microbial community, sediment and groundwater properties, and the concentration of OC amendment as determinants of microbial community functional response and bioremediation capacity.

### Experimental Design

- Sediments sterilized by γ-irradiation
- Original sediment-U was near crustal abundance (~ 0.3 ppm)
- Incubated with 1.05 mM uranyl-acetate solution
- Final U concentrations ranged from 6 - 9 ppm

**Hanford, WA 300 Area (Ringold formation)** | **Oak Ridge, TN (background)** | **Rifle, CO (no prior OC addition)**



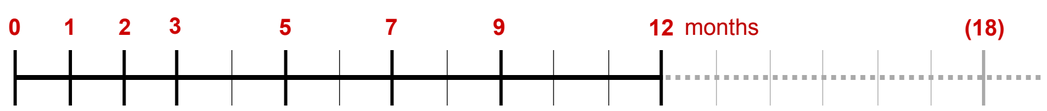

- Full-factorial reciprocal-transplant experiment
- Flow-cells contain sterilized sediment inoculated with microbial communities from each site (except sterile controls)
- OC supplied as sodium-acetate at two concentrations (0.1 and 1.0 mmol OC/kg/day)
- n = 174

### Analyses

| Community | Effluent | Sediment |
|---|---|---|
| 16S PhyloChip (DNA) | U (ICP-MS) | Uranium (ICP-MS) |
| 16S PhyloChip (RNA) | pH | extractable Fe(II) & Fe(III) |
| GeoChip (DNA) | acetate, inorganic and organic C | redox potential |
| qPCR & cell counts | cations (ICP-MS), anions (IC) | cations (ICP-MS) |
| metatranscriptomics | gases ($CO_2$, $CH_4$, $H_2$) | |

Analyses being performed at each sampling timepoint. Effluent is being collected and analyzed every two weeks. Sediment and community analyses take place after flow cell sampling according to the destructive sampling timeline below.

### Timeline for Destructive Sampling




Flow cells are being destructively sampled at the intervals indicated in red. The first sampling occurred after delivery of several pore volumes of synthetic groundwater, but before OC addition. The original 18-month timeline has been shortened to 10-12 months due to logistic constraints.

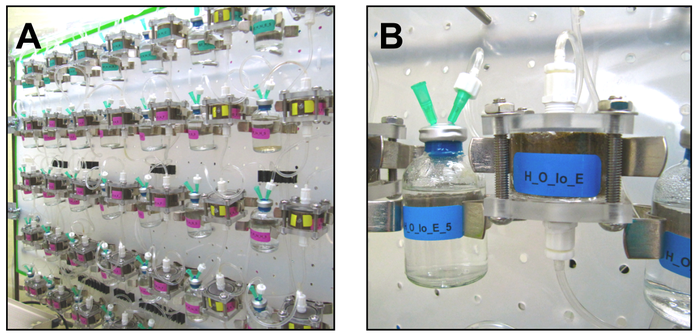
## Trajectories

- 174 flow-cells operating in an standard Coy anaerobic glovebox
- Flow-cells receive anaerobic, synthetic groundwater via peristaltic pumps
- Effluent and gas are collected from each flow-cell
- Each flow-cell (~16 $cm^3$) contains sterile sediment pre-incubated with uranyl-acetate, with a 2.0 g of "live" sediment as inoculum
- *For clarity, flow-cells are shown without gas collection bags*

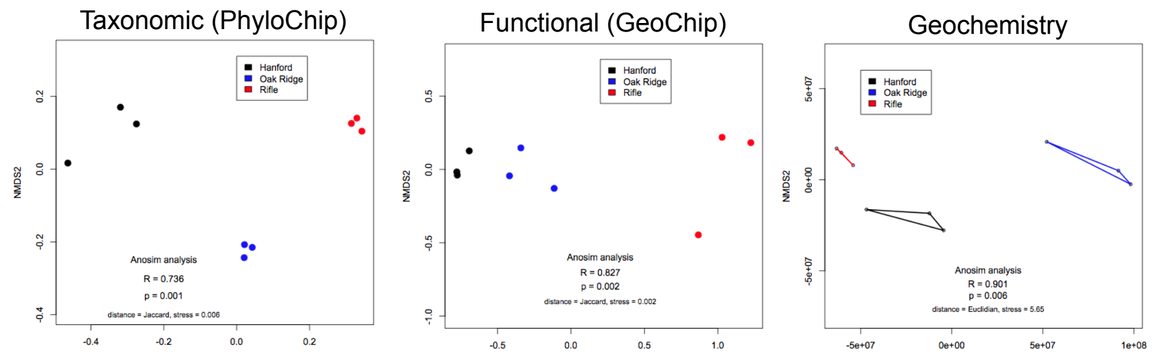
**A)** One of 5 panels holding flow-cells

**B)** Single flow-cell with effluent collection vial (gas bag removed for clarity). Cell contains Hanford inoculum in Oak Ridge sediment, receiving low carbon, designated replicate 'E'

- Trajectory analyses are being performed as communities evolve over the months of biostimulation



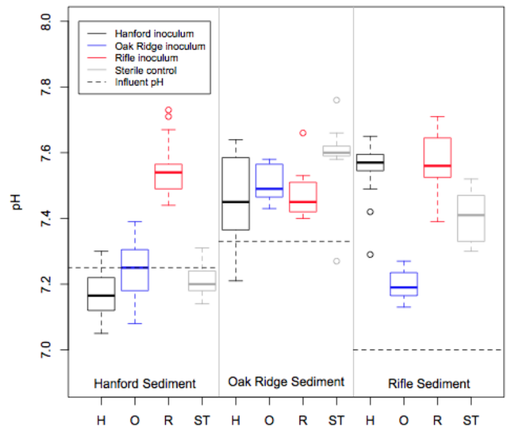

### Taxonomic, Functional & Geochemistry Characterization




The starting three sediments and their native microbial communities were characterized by PhyloChip (taxonomic/phylogenetic), GeoChip (functional genes), and sediment/groundwater geochemistry. NMDS ordinations show that the 16S OTU composition, functional gene composition, and sediment/groundwater geochemistry are significantly different by site.

**With distinct community structure and geochemistry at each site, what is the primary driver of realized function?**

- Community taxonomy/phylogeny?
- Community functional gene potential?
- Sediment and groundwater chemistry?
- Example at right: effluent pH (prior to OC addition) shows community-specific differences in some cases (Hanford and Rifle sediments) while Oak Ridge seems driven by geochemistry

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