U.S. Department of Energy
Office of Biological and Environmental Research
Subsurface Biogeochemical Research

Final Report

Coupled Biological and Micro-XAS/XRF Analysis of *In Situ* Uranium Biogeochemical Processes Exploratory Project

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Requested Funding:

Institution	FY 2012	FY 2013	No Cost Extension	Total
Colorado School of Mines	$73,325	$76,662	-0-	$149,987

Introduction: Biogeochemical processes play a critical role in reductive metal immobilization. While microbiological and molecular studies have brought insights regarding enzymes and candidate microbes involved in bioimmobilization, we still know very little about the actual subsurface distribution of organisms, functionality of extracellular proteins in these systems, and complex mineral-microbe processes influencing uranium immobilization at the micrometer scale. This is the relevant size range for both microorganisms and biogenic precipitates; hence, it is highly relevant to understanding fundamental processes that will contribute to macro-level dynamics in groundwater. The original *objective* of this exploratory proposal was to develop an interdisciplinary approach to query microbial functionality, phylogeny and spatial distribution in conjunction with geochemical alterations through a combination of molecular biology and synchrotron-based analyses.

In aligning with changing Subsurface Biogeochemical Research (SBR) priorities, the project's focus was extended to more broadly explore coupled biogeochemical analysis of metal (im)mobilization processes beyond uranium with a foundation in integrating microbial ecology with geochemical analyses. The impact of the original exploratory funding was substantially increased through a no cost extension that enabled us to leverage (A) a postdoctoral fellowship granted to Dr. Robert Almstrand during this award timeframe (Marie Curie Postdoctoral Fellowship), (B) parallel funds from a synergistic project supported by the National Science Foundation and (C) a collaboration with DOE-funded investigators (PI's Ranville and Williams) on field efforts investigating metal mobility at the Rifle Field site. This included investigations of arsenic and zinc in microbially-active sulfate reducing conditions. Complementary work with NSF funding and collaborative DOE interactions further increased the project scope to investigate metal (im)mobilization coupled to biogeochemical perturbations in forest ecosystems with an emphasis on coupled carbon and metal biogeochemistry.

Results and Accomplishments: The development of a comprehensive tool that queries functionality, phylogeny and spatial distribution in conjunction with geochemical alterations represents a significant contribution to subsurface science and is a valuable capability for understanding and engineering remediation strategies in larger hydrologic systems. In publication #5 (Almstrand et al 2015), we developed an approach to simultaneously label phylogenetically distinct bacteria using gold nanoparticles

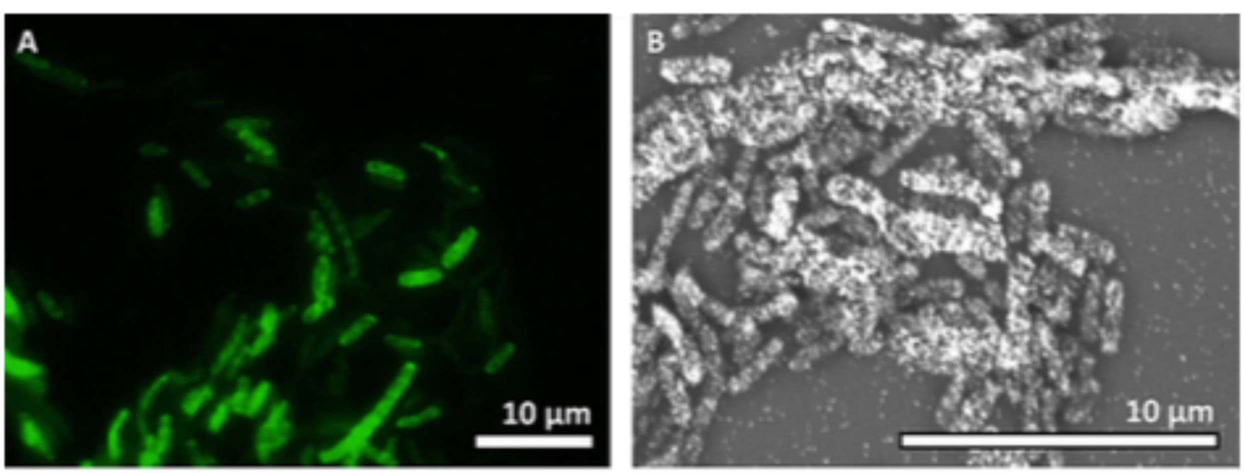

Figure 1: Fluorescent (A) and scanning electron microscope (B) images showing successful incorporation of fluoronanogold conjugates in *E. coli* cells. The polygold FISH approach uses four unique probes to achieve increased fluorescent (A) and gold amplification (B) during hybridization and was successfully applied to the commonly studied metal reducing bacterium *Shewanella oneidensis* CN32. Figure adapted from Almstrand et al 2015.

in conjunction with fluorescent primers (Polygold FISH). The use of four unique probes (poly-) enhanced signal amplification. This approach enabled targeted visualization of samples using multiple visualization tools including fluorescent microscopes, electron microscopes and synchrotron microfocused XAS as shown in **Figure 1**. Citations for this publication and others resulting from the project are given in the last section of this document “Publications supported by this Grant”. The 16S rRNA-based Polygold FISH approach proved favorable to our originally proposed independent coupling of nucleotide-based fluorescence *in situ* hybridization (FISH) with antibody-enabled metallic *in situ* hybridization (immuno-ISH) due to challenges in the potential to oxidize reduced metals during immunolabeling. We were able to successfully apply the technique to planktonic cultures in a way that minimizes the potential oxidation of metals during this process; however, background interference and nonspecific binding still present challenges should this method be employed in subsurface soils. This is the next obstacle to applying this developing technology to investigate the direct association of microorganisms of interest with biogenically reduced metals – in essence the spatial component of our original project goal. With that in mind, we chose to address our broader biogeochemical research questions beyond the confines of this developmental approach.

Complementary work exploring the outcomes of microbial bioreduction in sediment columns as well as in field-extracted pore waters from the site in Rifle, CO during in situ bioimmobilization resulted in publications #6 (Stucker et al 2014) and #9 (Sharp et al 2011). The latter study revealed that reduced molecular uranium was more likely to form in the subsurface than biogenic uraninite during dissimilatory metal reduction. Bioaugmentation with the common dissimilatory metal reducer (*S. oneidensis* MR-1) in conjunction with biostimulation using lactate, while helping to establish rapid metal reducing conditions, did not result in a microbial community containing many of these introduced microorganisms. The column sampling system and uranium effluent profiles from that study are depicted in **Figure 2**.

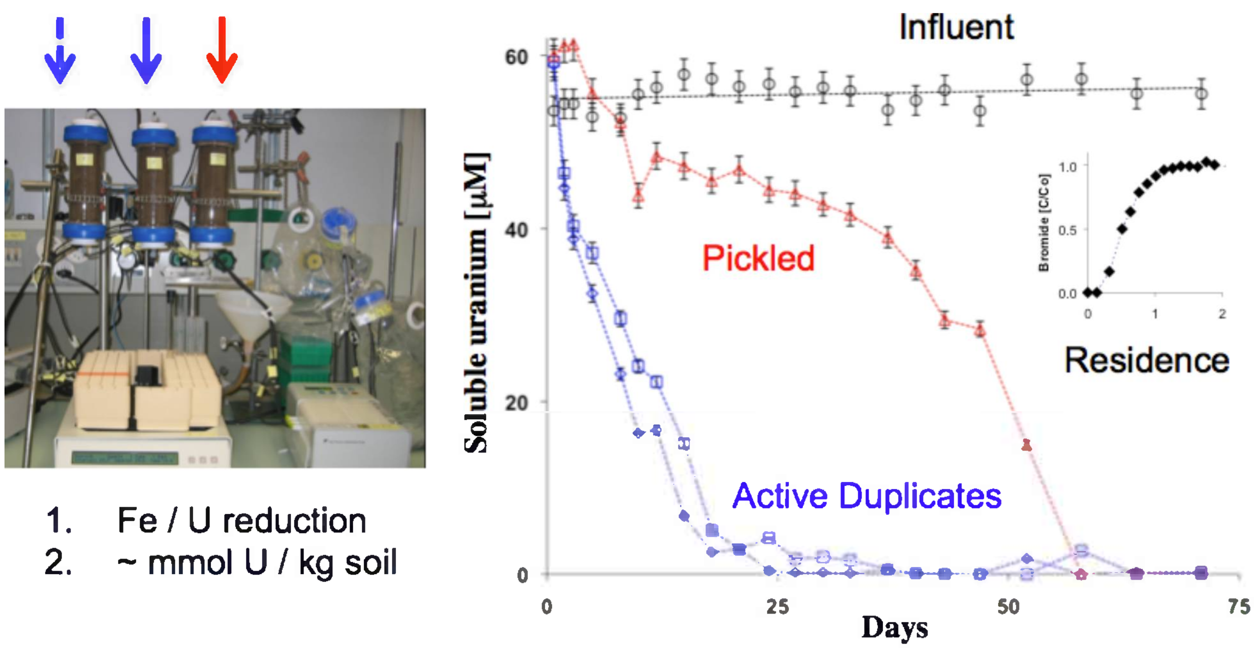

Figure 2: Laboratory sediment columns were employed to investigate the structure of uranium in association with sediments and biomass during dissimlatory metal reducing conditions. A control column (red arrow) was used to contrast biological signatures and uranium speciation from those biostimulated with *Shewanella oneidensis* CN-32 (blue arrow). Portions of the image on the right are adapted from Sharp et al 2011.

In Stucker et al (2014), we explored arsenic mobility in association with sulfur species during subsurface sulfate reducing conditions resulting from field-scale biostimulation. We were able to conclude that thioarsenate species were responsible for arsenic mobilization during these conditions. This study also served as a stimulus for further exploration of metal immobilization during sulfate reducing conditions stimulated by lignocellulytic fermentation resulting in publications #1 (Drennan et al 2016) and #3 (Almstrand et al 2016) where we investigated the interplay of fermentative microorganisms in support of the sulfate reducing community. Importantly, we were able to demonstrate that the backbone fermentative community and the establishment of lactate scavenging bacteria appeared to play a strong selective pressure on overall sulfate reducing activity (**Figure 3**). As an outgrowth of that work, publication #4 (Pinto et al 2016) was a short genome announcement where we communicated the deposition of draft genome sequences of two novel acidimicrobiaceae from an acid mine drainage biofilm in support of potential future metagenomic inquiry in these systems.

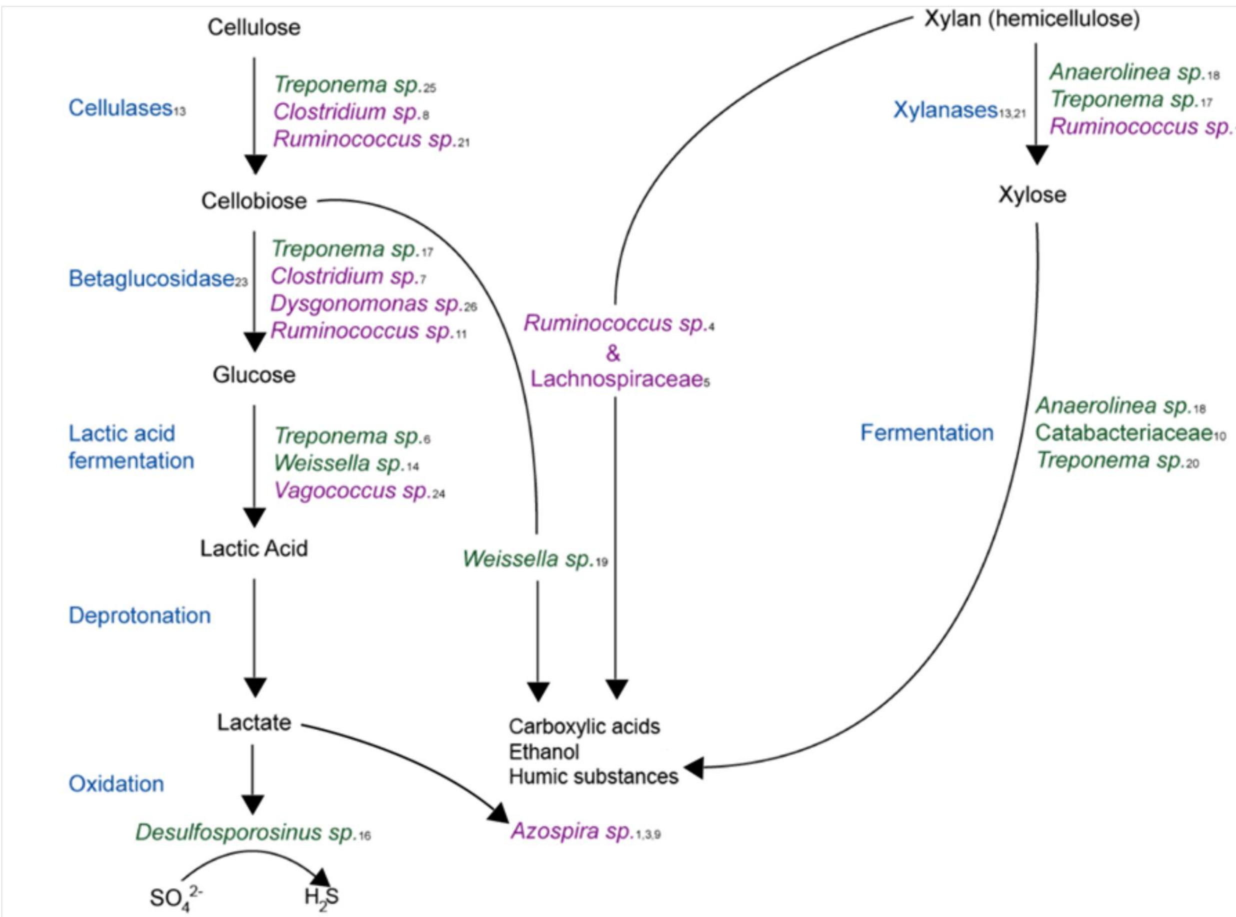

Figure 3: Putative metabolic flowchart of core microrganisms reveals potential role of *Azospira* in scavenging produced lactate from the sulfate reducer *Desulfosporosinus*. Figure reprinted from Drennan et al 2016.

The DOE Office of Science and Biological Research's increasing interests in terrestrial biogeochemical processes in natural systems led us to apply our expertise in metal biogeochemisty to explore the impact of large-scale insect-mediated forest disruptions in publications #7 (Mikkelson et al 2014) and #8 (Bearup et al 2014) on metal fate, partitioning and mobility. These publications showed that disruptions to the subsurface edaphic parameters, in part due to changing carbon inputs, led to the mobilization of copper, zinc and aluminum in adjacent soils. It also served as inspiration for exploring how these disruptions impacted terrestrial microbial ecology in montane ecosystems as documented in publication #2

(Mikkelson et al 2016). Here we discovered that extensive disruptions (i.e. >80% mortality of trees in a stand) was accompanied by shifting edaphic parameters such as water content and organic matter as well as microbial ecology shifts. Interestingly, these shifts were not observed in a less impacted (~20% tree mortality) watershed. **Figure 4** depicts how this type of ecosystem disturbance influences certain terrestrial properties relevant to biogeochemical cycling.

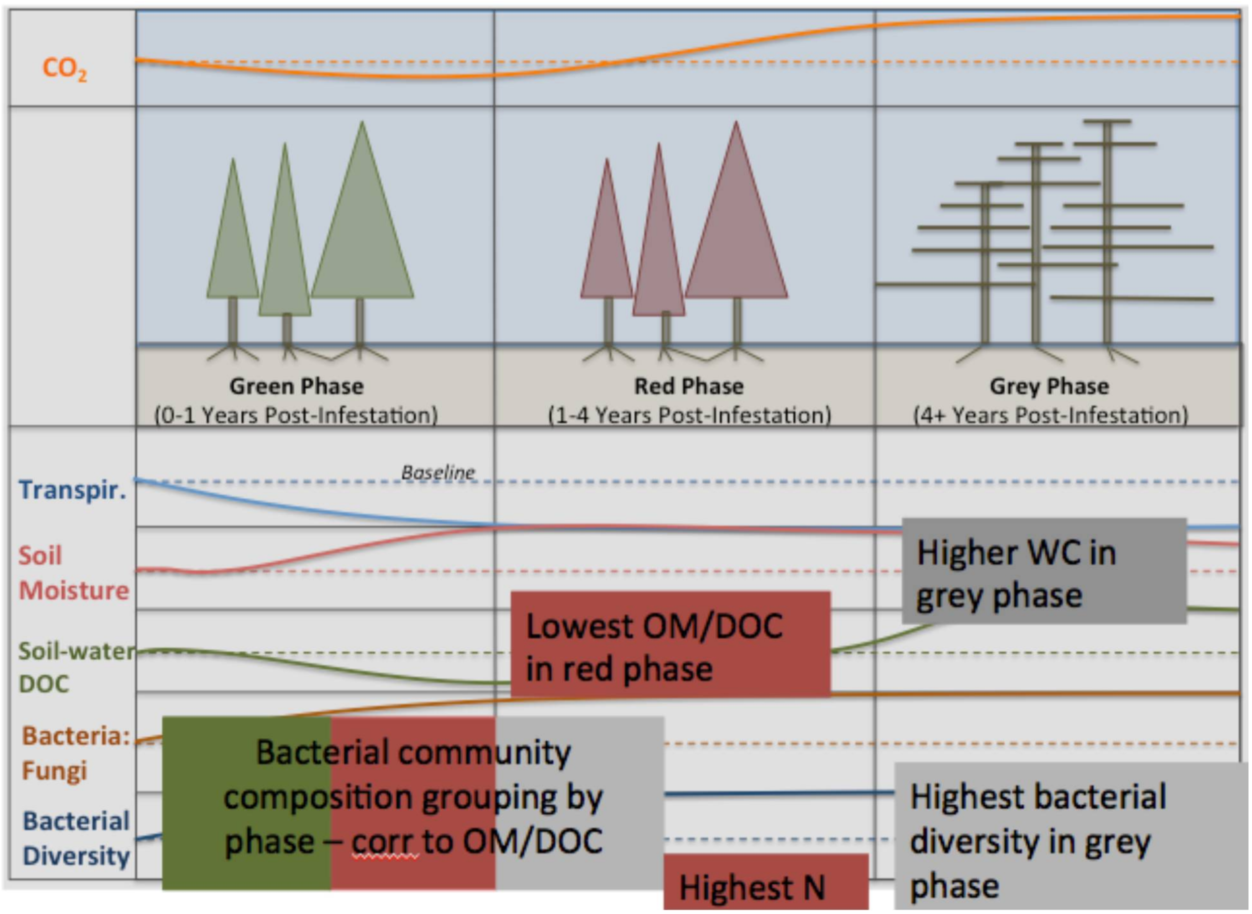

Figure 4: Conceptual depiction of shifting edaphic and terrestrial microbiology profiles resulting from bark beetle induced mortality of trees in montane ecosystems. These properties change during a succession of tree stages from healthy (green), to red (dead but still holding needles), and finally to grey (needles have senesced from the tree and are deposited on the soil below). These shifts and parameters are explored in Mikkelson et al 2016.

Personnel: Grant funds directly supported tuition/salary for two graduate students during various periods of study: Dina Drennan (PhD anticipated December 2016) and Jeffrey Ladderud (MS in December 2014). Funds were also used to supplement operational costs for a Marie Curie Postdoctoral Fellowship for Dr. Robert Almstrand as well as those of graduate advisees Silverman and Mikkelson who were funded on other projects and are highlighted in publications (#2, 6, 7 and 8) at the end of this report. Importantly, this grant provided operational support during a critical juncture of my academic career as it was awarded during pre-tenure residence. I was promoted to Associate Professor with Tenure at the Colorado School of Mines in the spring of 2015, and I am grateful for the DOE SBR support of our mutual research mission during that time. In going forward, findings provided inspiration for a two subsequent proposals in 2016 with DOE collaborators from Lawrence Berkeley and Los Alamos National Laboratories.

Outputs: The research was productive and resulted in **9 publications to date** that acknowledge this funding source: DE-SC0006997. I further anticipate the submission of ~2 additional publications within the next year on topics related to zinc immobilization during microbially-induced sulfate reduction – one of these includes unfunded collaborator Webb (publication #9 includes collaborator Bargar). The work has also been disseminated through 15+ conference abstracts and presentations at conferences including ISME, ISSM, AGU, IMWA, and ASM. Journal article titles (and links where appropriate) are provided in the list below.

Publications Supported by this Grant (where Colorado School of Mines **student advisees** and ***postdocs*** are highlighted in addition to the **PI** and **collaborators**):

1. **Drennan DM**, ***Almstrand R***, Lee I, Landkamer L, Figueroa L, **Sharp JO** (2016). Organoheterotrophic bacterial abundance associates with zinc removal in lignocellulose-based sulfate-reducing systems. *Environ Sci Technol*. **50**(1): 378-87. *DOI 10.1021/acs.est.5b04268*
2. ***Mikkelson KM,*** Lozupone CA, **Sharp JO.** (2016) Altered edaphic parameters couple to shifts in terrestrial bacterial community structure associated with insect-induced tree mortality. *Soil Biology Biogeochem*. **95**:19-26 doi:10.1016/j.soilbio.2015.12.001
3. ***Almstrand R***, Pinto AJ Figueroa LA, **Sharp JO** (2016) Draft genome of a novel Desulfobacteraceae member from a sulfate-reducing bioreactor metagenome. *Genome Announcements* **4**(1) e01563-15
4. Pinto AJ, **Sharp JO**, Yoder MJ, ***Almstrand R*** (2016) Draft genome sequences of two novel acidimicrobiaceae from an acid mine drainage biofilm metagenome. *Genome Announcements* **4**(1) e01563-15
5. ***Almstrand R***, **Drennan D**, **Sharp JO**. (2015) Polygold FISH for signal amplification of metallo-labeled microbial cells. *J Basic Microbio*. **54**:1-5
6. Stucker VK, **Silverman DR**, Williams KH, **Sharp JO**, Ranville JF. (2014) Thioarsenic species associated with increased arsenic release during biostimulated subsurface sulfate reduction. *Environ Sci Technol*. **48**(22): 13367-75 http://pubs.acs.org/doi/abs/10.1021/es5035206
7. **Mikkelson KM**, Bearup LA, Navarre-Sitchler AK, McCray JE, **Sharp JO**. (2014) Changes in metal mobility associated with bark beetle-induced tree mortality. *Environ Sci: Processes Impacts*. **16**(6): 1318-27. http://pubs.rsc.org/en/content/articlelanding/2014/em/c3em00632h
8. Bearup L, **Mikkelson K**, Wiley J, Navarre-Sitchler A, Maxwell R, **Sharp JO,** McCray J (2014). Metal fate and partitioning in soils under bark beetle-killed trees. *Sci Total Environ*. **496**, 348-357 http://www.sciencedirect.com/science/article/pii/S0048969714010821
9. **Sharp JO,** Schofield EJ, Lezama J, Ulrich K, Veeramani H, Junier P, Roquier C, Suvorova EI, Webb S, Tebo B, Giammar DE, **Bargar JR**, Bernier-Latmani R (2011). Uranium speciation and stability after reductive immobilization in sediments. *Geochim Cosmochim Acta*. **75**(21):6497-6510. http://www.sciencedirect.com/science/article/pii/S0016703711004789