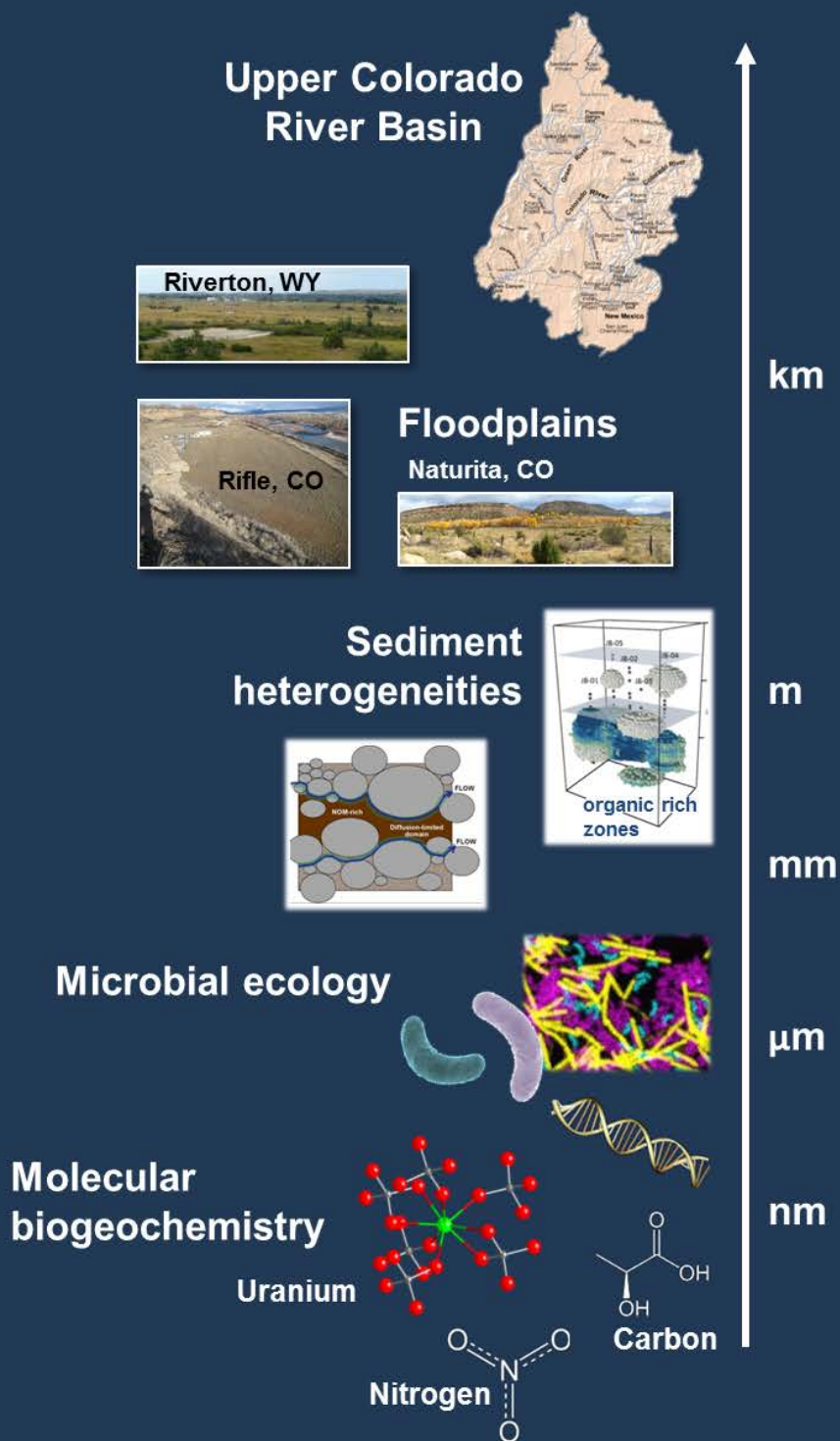


SLAC SFA

Coupled Cycling of Organic Matter, Uranium, and Biogeochemical Critical Elements in subsurface systems

2016 ANNUAL REPORT



June 30, 2016

John R. Bargar, Principal Investigator and Project Manager
*Stanford Synchrotron Radiation Lightsource, a Directorate of SLAC National Accelerator
Laboratory*

Co-principal investigators:
Scott Fendorf, Chris Francis, Kate Maher, and Alfred Spormann
Stanford University, Stanford, CA

June 30, 2016

Highlights

Knowledge produced by the SLAC SFA is transforming our understanding of uranium and carbon biogeochemistry in anoxic sediments within floodplains in the upper Colorado River Basin (CRB). Since FY 2015, we have shown that anoxic sediments are common and accumulate large deposits of both uranium and carbon *regionally* across the upper CRB. NRZs contain sufficient uranium to sustain long-term groundwater plumes under oxidizing conditions (Janot, 2016). Moreover, our recent work suggests that NRZs are regionally sensitive to climate perturbations including drought, which can trigger oxidation and release of uranium and carbon. The overall picture that is emerging from these findings is that NRZs profoundly influence uranium, organic carbon, and nutrient biogeochemistry, and likely contribute to plume persistence in the upper CRB. By studying uranium and carbon behavior at both molecular and regional scales, we are obtaining regional insights that improve the fidelity of both small- and large-scale biogeochemical models, while simultaneously obtaining dramatically improved insights about processes occurring at individual sites.

In the past year, we have made several major discoveries that advance our process understanding of uranium, carbon, and critical element biogeochemistry and the impacts of climate perturbations. At the time of writing, we are 60% through the 3-year Science Plan, but have made significant progress toward addressing all of the hypotheses proposed in the Plan. We are completing research tasks on-schedule and on-budget. In the past year, we submitted **8 manuscripts for publication** (5 accepted or published, 3 in review) and **presented 22 talks** (10 invited) at scientific meetings. Key scientific discoveries include:

1. **Discovery that U(IV) behavior in anoxic sediments is controlled by post-reduction processes, particularly sorption, and not by reduction processes** (Bone *et al.*, 2016). This discovery transforms our process understanding of U(IV) biogeochemistry in natural sediments. (page 7)
2. **Demonstration that the stability of organic carbon in anoxic sediments is dictated by composition and oxidation state** (Boye *et al.*, 2016). This finding provides a new, thermodynamic basis to predict the vulnerability of preserved carbon inventories in anoxic sediments that are subject to forcing from climate perturbations. (Page 8)
3. **NRZs exhibit diverse physicochemical properties that profoundly impact biogeochemistry and are expected to mediate response to climate perturbations.** This finding will ultimately lead to improved predictive capability of anoxic sediment behavior. (Page 13)

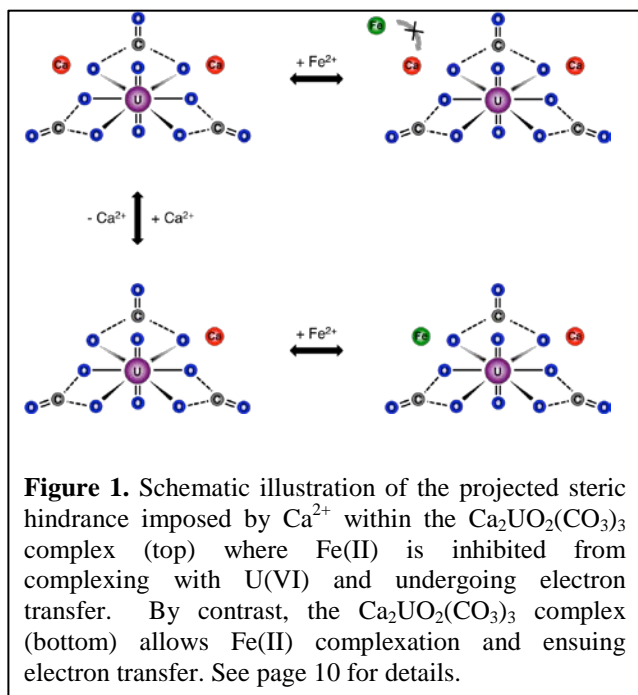


Figure 1. Schematic illustration of the projected steric hindrance imposed by Ca^{2+} within the $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$ complex (top) where Fe(II) is inhibited from complexing with U(VI) and undergoing electron transfer. By contrast, the $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$ complex (bottom) allows Fe(II) complexation and ensuing electron transfer. See page 10 for details.

4. **Reduction of aqueous U(VI) is inhibited by physical protection of the metal center by coordinating carbonate and Ca²⁺ ligands (Fig. 1)** (Jones *et al.*, 2016). This finding provides a molecular basis to explain observed U(VI) reduction rates. (Page 10)
5. **The first basin-wide study of ammonia-oxidizing microbial diversity and abundance, along a nearly 900 km north-south transect, shows that the *Thaumarchaeota* are pervasive and more abundant than ammonia-oxidizing bacteria throughout the subsurface regionally in the CRB. Moreover, they exhibit reduced abundance patterns within NRZs.** These findings provide an unprecedented view of abundance and diversity patterns for terrestrial ammonia oxidizers and help provide the scientific foundation to understand nitrogen cycling in NRZs. (Page 13)
6. A JGI community sequencing proposal was funded, which is providing ‘deep’ Illumina 16S iTag sequencing for more than 330 sediment samples from across the upper CRB, to characterize *regional* in-situ ammonia-oxidizing community structure and diversity.

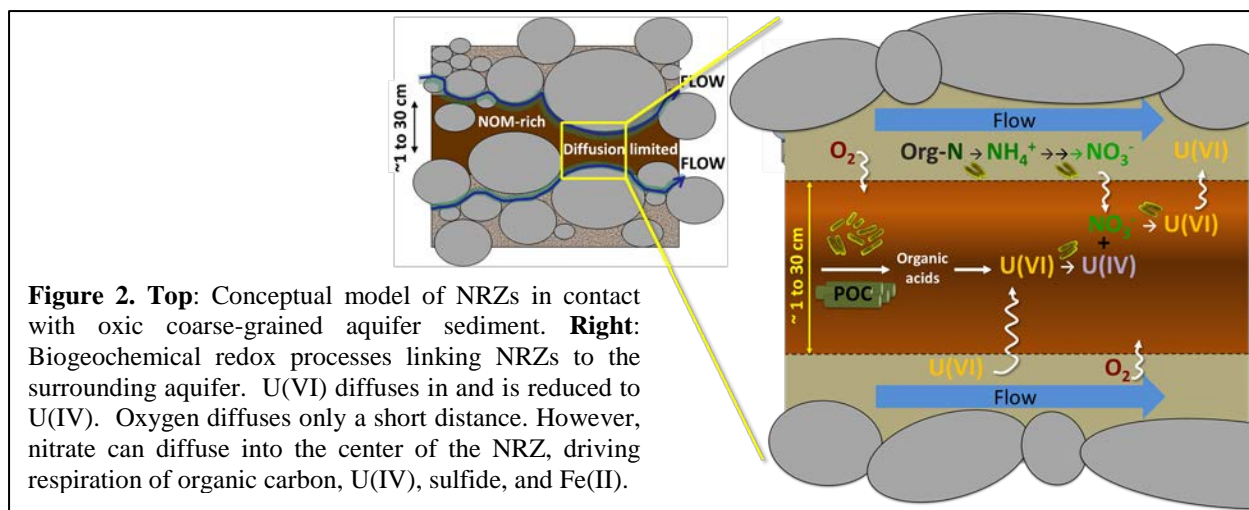
1. Program overview

Uranium is a major contaminant of concern in groundwater at contaminated legacy DOE mill sites across the upper Colorado River Basin (CRB). The mobility and fate of uranium and carbon in these subsurface settings is controlled by four primary biogeochemical and hydrological factors: (i) *molecular speciation* (chemical form and oxidation state), (ii) chemical *reactions*, many of which are controlled by *microbial metabolisms*, that transform uranium and carbon, (iii) the intrinsic *rates* of these reactions, and (iv) *transport processes* that further control the overall progress of reactions and lead to the creation of redox gradients. The first three factors are intrinsically *molecular* in nature, whereas transport occurs at the scale of sediment pores and larger scales. These factors are profoundly influenced by the *mineralogical/chemical composition of sediments* as well as the *saturation state*, which is governed by the frequency and duration of annual or long term *drought/saturation cycles*.

Knowledge of biogeochemical processes and their variability in dynamic natural systems is critical to improving earth system predictability. This knowledge rests firmly upon field- and laboratory based experimental observations that penetrate to the molecular scale.

The mission of the SLAC SFA is to unravel the biogeochemical processes occurring at molecular to pore and field scales that control the behavior of uranium and intimately coupled biogeochemical critical elements (‘BCEs’) in highly variable natural subsurface systems. These processes *regionally* impact uranium mobility and carbon release in the upper CRB.

Anoxic organic-rich sediments are globally important as repositories of uranium, organic carbon, and nutrients, and as drivers of redox cycles. Drought and flooding can cause anoxic sediment bodies to become oxidized, releasing their contaminant and nutrient loads, with major impacts on downgradient aquifer quality. NRZs are particularly important within the upper CRB as biogeochemical hot spots that host carbon, accumulate uranium, and have the potential to resupply uranium to groundwater plumes. Significantly, organic carbon mediates uranium and BCE behavior by sustaining anoxia within pore environments, through the binding of uranium on surfaces of sediment-associated microbial biomass and, we posit, by forming aqueous U(IV)-organic matter complexes. Uranium also accumulates in evaporite-bearing unsaturated sediments in the upper CRB.



The SLAC SFA research program is guided by three overarching hypotheses:

- **Overarching hypothesis I:** U(IV) behavior is strongly influenced by its binding to biomass functional groups in NRZ sediments.
- **Overarching hypothesis II:** the “Rifle model” of NRZ biogeochemistry (*cf.*, Janot, 2016) is scalable to floodplain deposits across the upper CRB regionally.
- **Overarching hypothesis III:** N cycling regionally drives floodplain-aquifer interactions because of the unique ability of nitrate to perfuse into NRZs and oxidatively mobilize U, while concurrently leading to greenhouse gas production (**Figure 2**).

Approach: Field and molecular observations are essential for hypothesis testing. We are vigorously pursuing a field research program to evaluate anoxic and unsaturated sediment biogeochemical characteristics and microbial communities in a transect across the upper CRB (from south to north) at the Shiprock, NM; Naturita, Grand Junction, and Rifle, CO; and Riverton WY sites. These activities are coupled to investigations of simplified models that provide greater control and depth of interpretation. Our integrative approach includes molecular microbiology, molecular geochemistry, geochemical and transport modeling, synchrotron and lab-based spectroscopic techniques, and x-ray, isotope, and electron microscopy imaging.

Impact and relevance to BER: We are providing new process models for uranium, carbon, and BCE behavior in complex subsurface environments subject to hydrologic forcings (meltwater discharge, drought, flooding) at molecular to regional scales. Major products include:

- Process models for uranium-organic carbon, -iron, -sulfur, and -nitrogen interactions
- Advances in our understanding of organic carbon reactivity in anaerobic sediments
- Regional-scale knowledge of N-cycling organism diversity and abundance
- Input parameters for modeling biogeochemical behavior and function of NRZs
- New insights into methods for manipulating subsurface biogeochemical processes and remediation

Developing process-level understanding of terrestrial ecosystems, from bedrock to the vegetative canopy. The SLAC SFA program is directly interrogating biogeochemical processes

occurring *in-situ* in the subsurface. Knowledge produced by these capabilities strongly complements other CESD programs.

Characterizing coupled biogeochemical processes in complex subsurface environments. We are in the process of publishing two new process models that substantially expand our understanding of the coupling between redox cycles of uranium and carbon.

Molecular to ecosystem science. SLAC SFA research aligns with decadal research goals identified in the 2014 Molecular Science Challenges workshop, including: (i) molecular biogeochemical processes occurring in hotspots (NRZs), (ii) mechanisms by which molecular processes link to floodplain scales, and (iii) constructs for scaling molecular processes to the regional level.

Crucial role of synchrotron approaches. Synchrotron x-ray absorption spectroscopy (XAS) provides unique capabilities to study molecular and electronic structure around uranium and BCEs in complex, dilute natural materials. Synchrotron x-ray microscopy allows biogeochemical reactions to be imaged in 2D and 3D at spatial resolutions down to 30 nm. These techniques are crucial to providing molecular perspectives to support process knowledge at pore to regional scales.

2. Outline of scientific objectives

This project is focused on four major gaps in our knowledge of uranium and carbon biogeochemistry that limit our ability to quantitatively predict their fate and transport in the subsurface. Research is organized into four questions, one for each knowledge gap:

Question 1. What is the speciation of uranium in organic-rich sediments? *Knowledge gap: The speciation of U(IV) in NRZ sediments remains poorly understood.* Speciation controls the behavior of uranium in the subsurface and is required to inform predictive uranium transport models. The underlying hypothesis for this module is that U(IV) behavior is controlled by binding to biomass functional groups in NRZ sediments. Model U(IV)-microbe-organic matter-mineral systems are being studied using synchrotron x-ray and microscopy techniques.

Question 2. How do molecular- to millimeter-scale processes govern U(VI) reduction pathways and products? *Knowledge gap: Microbial degradation of NOM is intimately linked to reductive transformations of uranium, iron, sulfate, and nitrate in NRZs. Yet, knowledge of the mechanistic coupling between NOM decomposition and metal reduction is largely absent.* This section is exploring three major factors controlling carbon behavior and U(VI) reduction in NRZs: (i) factors controlling the stability of organic carbon; (ii) the linkage between microbial degradation of organic carbon and U(VI) reduction, and (iii) ligand controls on uranium speciation and reduction rates/mechanisms. The underlying hypotheses are that each of these factors specifically influences uranium reduction and retention within NRZs.

Question 3. How is uranium released from organic-rich sediments? *Knowledge gap: The mechanisms and rates of uranium release from NRZ sediments are not known.* Such knowledge is critical for modeling the fate and transport of uranium and carbon across NRZ-aquifer interfaces and to understand potential roles of NRZs in plume persistence. The central hypothesis for this portion of the project is that uranium oxidation and release is tied to denitrification in organic-rich sediments. A series of diffusion-limited and flow-through column laboratory

experiments is being performed to detect the presence of these elemental cycles and their control over uranium release rates under varying conditions. We are combining synchrotron-based and aqueous-phase measurements with ‘functional gene’ markers (*e.g.*, *nirK/ nirS* encoding nitrite reductase) to specifically target microbial metabolic processes associated with nitrogen cycling.

Question 4. Are NRZs regionally important to plume-persistence in the upper CRB?

Knowledge gap: Experience at the Rifle site suggests that NRZs may be regionally important to floodplain biogeochemistry and accumulate uranium basin-wide. Regional knowledge would provide highly valuable insights to uranium behavior at the many sites where little geochemical information is available. With support from partners at the DOE Offices of Legacy Management (DOE-LM), we have collected NRZ samples at field sites across this region: Rifle, Naturita, and Grand Junction, CO; Shiprock, NM; and Riverton, WY. We are studying the distribution and speciation of uranium in NRZ sediments at these sites and investigating oxidation-reduction cycles and the underlying microbial communities as regional drivers for uranium release.

Knowledge of processes controlling NRZ-floodplain interactions supports improved *basin-wide* understanding of the basic drivers and treatment strategies for plume persistence. Conversely, knowledge of basin-wide processes provides insights crucial to understanding processes occurring at individual sites. This field component provides a larger context for molecular-level understanding produced in Questions 1-3.

3. National Laboratory program and management structure

Organizational structure. The SLAC SFA program resides within the Chemistry and Catalysis division at the Stanford Synchrotron Radiation Lightsource (SSRL), a Directorate of SLAC. The lead PI and technical research manager, John Bargar, reports to the SSRL Science Director and head of the Chemistry and Catalysis Division, Britt Hedman. Oversight and management decisions regarding the SLAC-SFA are made by Bargar, the Co-PI team, and Hedman.

Communications. Research and strategic planning, and information exchange are conducted at postdoctoral/graduate student meetings, separate all-hands monthly meetings, and monthly leadership meetings.

Table 1. Specific roles of senior personnel

Senior personnel	Expertise	Leadership	Co-lead
John Bargar	Biogeochemistry, X-ray spectroscopy	Question 1, 4	Question 3
Scott Fendorf	Biogeochemistry, soils science	Question 2	Question 3
Chris Francis	Molecular microbial ecology	Question 3	Question 4
Kate Maher	Geochemistry, reactive transport modeling		Questions 3, 4
Alfred Spormann	Microbial metabolism		Question 2

4. Performance milestones and metrics toward accomplishing objectives

The SLAC SFA program made strong progress on all of the major scientific questions in FY 2016. The Question 1 experimental program was completed, along with portions of the programs for Questions 2, 3, and 4. Manuscripts for a significant fraction of this work FY 2015 have been submitted or are in preparation. **Table 2** presents a summary of current status, milestones, and

Table 2. Summary of FY 2016 progress and goals for FY 2017

Subtask	Hypothesis	FY16 Status	FY17 Goal	Pages	Deliverables
Question 1					
Hypothesis 1.1	U(IV) is associated with bacterial cells and proximal organic matter in NRZ sediments.	Confirmed. Experiments complete.	Publish paper.	7-8	Manuscript: Bone (2016). This report.
Hypothesis 1.2	Organic functional groups outcompete mineral surfaces for U(IV);, some U(IV) complexes may form with clay minerals.	Partially confirmed. Experiments complete.	Publish paper.	7-8	Manuscript: Bone (2016). This report.
Question 2					
Hypothesis 2.1	Uranium reductive immobilization requires coupled fermentative and anaerobic respiratory organisms acting on POC to mediate U(VI) reduction.	In progress.	Complete experiments.	9-10	This report.
Hypothesis 2.2	Uranium-binding ligands control the products of U(VI) reduction, leading to formation of ligand-complexed U(IV)	Partially refuted. Experiments complete.	Publish paper.	7-8 10-11	Manuscripts: Bone (2016) Jones (2016). This report.
Hypothesis 2.3	Diffusion limitations lead to a distribution of biogeochemical conditions, with U(VI) reduction progressively dominating in interior regions and oxidation in exterior portions of NRZs.	Confirmed. Measurements complete.	-----	-----	Manuscript: Janot (2016)
Hypothesis 2.4	Thermodynamic constraints mediate the stability of organic carbon in anoxic sediments.	Confirmed	Complete experiments. Publish.	8-9	Manuscript: Boye (2016) This report.
Question 3					
Hypothesis 3.1	The oxidation rate of U(IV) is diffusion-limited.	Partially confirmed. Experiments in progress.	Complete experiments. Publish paper.	11-13	This report. Manuscript in preparation.
Hypothesis 3.2	Nitrate is a more effective oxidant than O ₂ .	Refuted. Experiments in progress.	Complete experiments. Publish.	11-13	This report. Manuscript in preparation.
Question 4					
Activity 4.1	Interrogate NRZ characteristics at Upper CRB sites.	Fieldwork complete.	Analyze data. Publish.	13-15	Manuscript in preparation. This report.
Hypothesis 4.1	Uranium accumulates in NRZs as U(IV), dominantly as non-crystalline species.	Partially confirmed. In progress.	Analyze data. Publish.	13-14	Manuscript in preparation. This report.
Hypothesis 4.2	Annual variations in water tables and pore water O ₂ concentrations drive regional microbial nitrogen cycling, and subsequently, oxidative uranium release from NRZs.	In progress	Collect seasonal pore water data set, Riverton	14-15	This report.
Develop new science plan	Several, TBA	In progress	Science Plan	18	

Notes: Hypotheses and activities listed above are from the 2014 Science Plan, with the following exceptions; (i) Hypothesis 2.4 was added to the project management plan in FY 2015 in response to guidance provided in the 2014 Triennial review that we should add a carbon characterization activity; (ii) Effort corresponding to hypotheses 3.3 and 3.4 were cut from the management plan to accommodate addition of the carbon characterization effort.

4.a. Review of scientific progress toward achieving program objectives

Question 1. What is the speciation of uranium in organic-rich sediments? (S.E. Bone (lead), J.R. Bargar, external collaborators: J. Dynes, CLS, J. Cliff, EMSL). Naturally reduced sediments accumulate elevated concentrations of U(IV) at uranium-contaminated sites in the upper CRB¹. While we have shown that U(IV) occurs in a ‘noncrystalline’ form in NRZs, a detailed molecular model of this form of U(IV) has remained elusive because of the complexity of the sediments. Laboratory research suggests that U(IV) forms complexes with organic functional groups, particularly phosphorus-containing ligands². To gain a more in-depth understanding of U(IV) speciation under environmentally relevant conditions, this subtask has investigated U(IV) in simplified model systems containing partially decomposed particulate organic matter using a combination of extended X-ray absorption fine structure (EXAFS) spectroscopy to investigate the molecular-scale coordination environment of U(IV) in combination with scanning transmission X-ray microscopy (STXM) and nano-secondary ion mass spectrometry (NanoSIMS) to investigate the solids to which uranium was associated.

U(VI) reduction rates demonstrated that both microbial and surface catalyzed reduction of U(VI) occurred, which was consistent with the observed nano-scale association of U and solid-phase Fe and S. More significantly, we found that U(IV) was adsorbed to functional groups on particulate organic carbon (POC) organic carbon-coated clays (**Fig. 3**). This finding is highly significant because it reveals that *the formation of non-crystalline U(IV) relative to UO₂ can be favored simply by changing the U:solid ratio*. This conclusion is consistent with reports that U(IV) can adsorb to rutile and magnetite surfaces³ and implies that processes occurring *after* the reduction step control the obtained U(IV) products, *regardless of the reduction pathway*. The number of surface sites available to bind U(IV) is particularly important. This conceptual model represents a major paradigm shift away from the previous assumption by the community at large that U(VI) reduction processes (*e.g.*, by sulfate reducing bacteria or their inorganic products (FeS)⁴ or by Gram positive or negative bacteria⁵) control the U(IV) products. We further conclude that aggregates of microbial cells, POC, clay particles and FeS create micro-hotspots where U(VI) is reduced to U(IV) via mixed abiotic-biotic pathways; subsequently, U(IV) adsorbs to POC and mineral-associated organics. We have submitted this work for publication in Nature Geosciences (Bone, 2016).

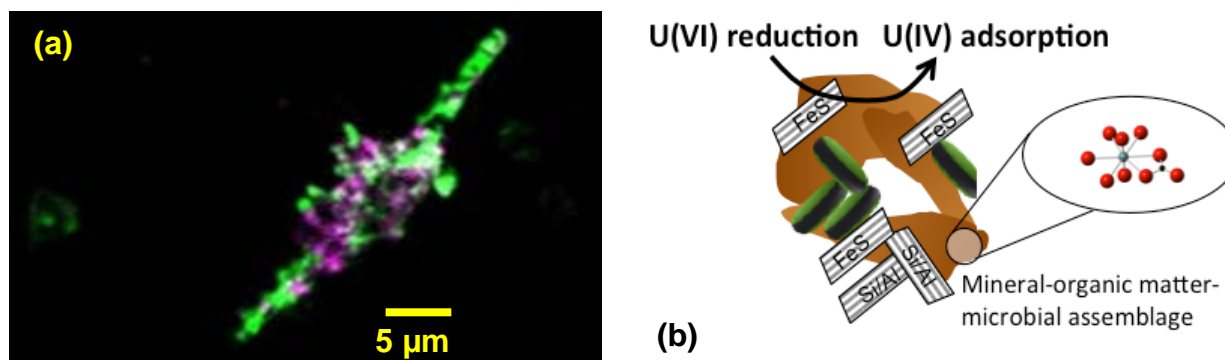


Figure 3. (a) NanoSIMS images of the locations of uranium (fuchsia) adsorbed on microbial cells (spheroidal objects) and on organic polymers between the cells (locations of carbon shown in green). The schematic (b) depicts our generalized process model; U(VI) is reduced by a mixed biotic-abiotic mechanism that occurs only in microbial-mineral aggregates such as those in (a). After reduction, U(IV) diffuses and adsorbs to functional groups on the matrix surfaces, particularly to organics.

Impact: Adsorption is concluded to dominate U(IV) speciation in many anoxic sediments; consequently, models must incorporate adsorption phenomena, regardless of the reduction pathway. Adsorbed U(IV) will be more kinetically labile than crystalline UO_2 , making it far more vulnerable to redox cycling initiated by environmental perturbations such as drought.

Question 2. How do molecular- to millimeter-scale processes govern U(VI) reduction pathways and products? The reductive immobilization of uranium in NRZs is closely linked to the sustained degradation of organic matter carried out by consortia of microorganisms. Under anaerobic conditions, extracellular hydrolytic enzymes of bacterial origin break down high molecular weight organic polymers. Fermentative bacteria subsequently use the lower-molecular weight organic compounds and generate metabolites that are used by anaerobic respiratory organisms linking carbon oxidation to metal (*e.g.*, Fe(III) and U(VI)) and sulfate reduction. We are examining the spatial and thermodynamic constraints on these fundamental metabolic processes, which ultimately control the fate of uranium, carbon, and other BCEs. Additionally, we are examining how U(VI) complexation influences the reduction to U(IV).

Thermodynamic constraints on carbon degradation (K. Boye (lead), V. Noël, S.E. Bone, S. Fendorf, J. Bargar, external collaborators: M.M. Tfaily, EMSL, K.H. Williams, LBNL) In anaerobic environments, the metabolic energy yield for microbial carbon degradation often approaches the thermodynamic limit for microbial growth. The thermodynamics are governed by available terminal electron acceptors (TEAs); oxygen, nitrate, iron oxides/uranium and sulfate provide successively less energy in the metabolic respiratory reaction.

Surprisingly, thermodynamic considerations for anaerobic respiration have largely neglected the energetics of the electron donor, *i.e.* the carbon substrate. We have investigated the composition and thermodynamics of the carbon available for microbial respiration, the water-exchangeable fraction, and related it to sulfide accumulation in sediment cores collected from the Rifle, Grand Junction, Naturita and Shiprock field sites. When samples from *all* of these sites are compared simultaneously to produce a *regional* perspective, we observe a clear *inverse* correlation between the nominal oxidation state of carbon (NOSC) in the water-exchangeable fraction and sulfide content in the solid phase (**Fig. 4**). This remarkable result indicates that thermodynamic constraints in sulfidic sediments protect against microbial oxidation of highly reduced carbon, such as in lipids and proteins, due specifically to insufficient energy yield from the reduction of sulfate^{6,7}. In support of this conclusion, we note that the relative abundance of lipids and proteins was higher in sulfidic compared to non-sulfidic sediments (**Fig. 5**). ***These findings are the first field-based and regional-scale evidence of thermodynamic constraints on microbial metabolic activity leaving an imprint on organic matter chemistry through selective preservation of highly reduced carbon. Importantly, this suggests 1) that carbon preserved under anaerobic conditions is vulnerable to perturbations leading to incursion of oxygen or nitrate, and 2) that U(VI)***

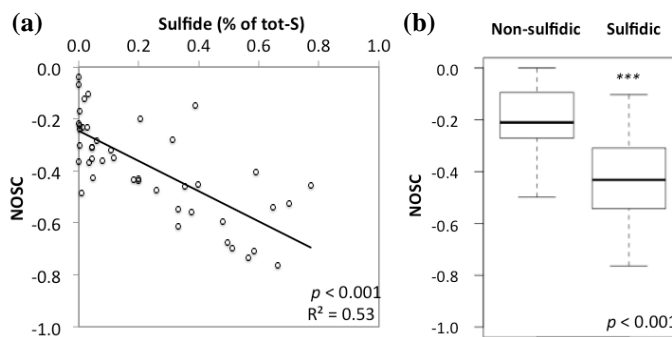


Figure 4. (a) NOSC vs. sulfide content in NRZ samples from across the upper CRB. (b) NOSC values for sulfidic and non-sulfidic sediments. Sulfidic sediments have strongly lower NOSC values, indicating a thermodynamic limitation (Boye 2016). Asterisks denote significant differences of the means ($p < 0.001$).

reduction is thermodynamically favored over sulfate reduction with the C sources currently available in NRZs.

Our investigations in FY 2017 will focus on testing the vulnerability of thermodynamically preserved C stocks to perturbations leading to changes in redox conditions (e.g. through water table fluctuations).

Impact: The composition and oxidation state of organic carbon fundamentally influences carbon stability in anoxic sediments. Moreover, thermodynamic constraints on carbon oxidation will select active metabolic pathways, in turn impacting the fate of uranium and other BCEs. These considerations must be addressed in predictive models in order to account for ecosystem perturbations initiated by climate change or human land use.

Spatial constraints on microbial metabolic activity (A. Müller (lead), S. Fendorf, C.A. Francis, J.R. Bargar, A. Spormann) In addition to thermodynamic constraints, *spatial* constraints are highly relevant to microbial metabolic activity in NRZs (and anaerobic environments in general). Particulate organic carbon and anaerobic TEAs, such as Fe(III) or U(VI), used in anaerobic respiration are often insoluble and spatially separated, creating a dilemma for metal-reducers (*how do cells physically access both substrate and TEAs to compete with sulfate reducers?*). We are evaluating how spatial constraints impact microbial syntrophy and metal/uranium fate. To this end we have developed a co-culture system with three bacteria representing key metabolic processes in NRZs (fermentation, metal reduction, and sulfate reduction). *Ruminiclostridium cellulolyticum* (formerly known as *Clostridium cellulolyticum*) is a fermentative bacterium capable of degrading cellulose via an extracellular enzymatic complex called the cellulosome and fermenting the resulting sugars to acetate, lactate, ethanol, hydrogen, and CO₂. In our triculture system, these fermentation products are used by *Geobacter sulfurreducens* which couples oxidation of acetate (the main product of *R. cellulolyticum*) to the reduction of metals (inclusive of U(VI)), as well as by *Desulfovibrio vulgaris*, a sulfate reducer that uses the other products and produces additional acetate. The co-cultures were grown in electrochemical reactor systems, where an electrode served as the electron acceptor for *G. sulfurreducens* and provided an immediate indicator for the combined

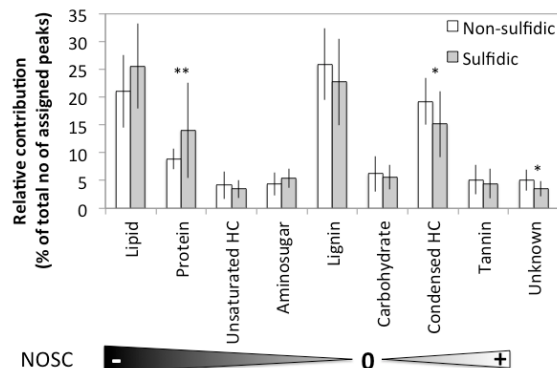


Figure 5. Relative distribution between carbon functional groups in water extracts of sediment samples with and without confirmed sulfide presence. Error bars show standard deviation of the mean. Functional groups are organized in the order of increasing NOSC from left to right. Asterisks denote significant differences between sulfidic and non-sulfidic samples (* = $p < 0.05$, ** = $p < 0.01$)

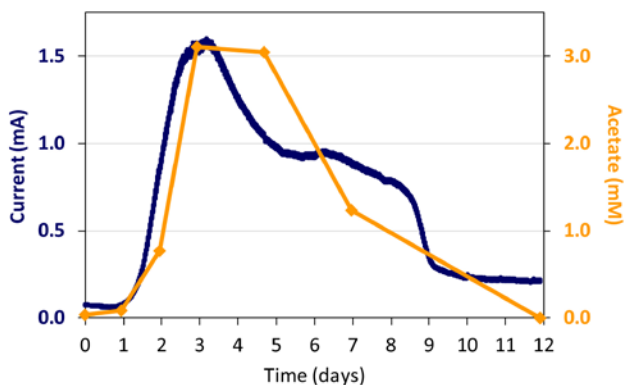


Figure 6. Current production (blue) and acetate concentration (orange) in a co-culture grown on cellulose. Acetate is initially produced from cellulose fermentation by *R. cellulolyticum* and subsequently consumed by *G. sulfurreducens* during current production on a graphite anode.

metabolic activity of the co-culture (**Fig. 6**). When grown on cellulose, a combination of both *R. cellulolyticum* and *G. sulfurreducens* was necessary to elicit current production, as *G. sulfurreducens* cannot utilize cellulose on its own, whereas *R. cellulolyticum* cannot use the anode for growth. The addition of the sulfate reducer *D. vulgaris* resulted in a shift of community dynamics that, depending on experimental conditions, either increased or decreased the *G. sulfurreducens* population within the overall reductive capability. ***With our bacterial model community, we are able to precisely monitor the flow of electrons and carbon through the system and to microscopically visualize the spatial organization of the community with high resolution. Therefore, our co-culture provides an excellent model system for investigating syntrophic and competitive interaction mechanisms during carbon degradation and their impact on the fate of uranium under reducing conditions.***

In FY 2017 we will focus on the effects of spatial constraints on the model community dynamics, using micro- to millimeter spatially separated substrates.

Impact: These experiments will help evaluate the biogeochemical relevance of spatial separation of different metabolic substrates, a constraint imposed on microbial function within NRZs (and other anaerobic environments). Such information is needed to understand the mobility of uranium, nutrients, and BCEs in NRZs.

The importance of U(VI) complexation. (M.E. Jones (lead), S. Fendorf) Dissolved concentrations and migration of uranium within subsurface environments is largely controlled by its redox state and chemical speciation, generally as minimally soluble U(IV) or more soluble U(VI) species. Accordingly, natural and stimulated attenuation of uranium through reduction of U(VI) to U(IV) plays a key role in environmental and water quality. However, in the presence of even low carbonate concentrations, uranyl-carbonato complexes become the dominant aqueous species; they are, in fact, the primary aqueous species in most groundwaters. At circumneutral and more alkaline pH values, Ca stabilizes ternary uranyl-calcium-carbonato complexes that limit the rate and extent of both U(VI) adsorption and reduction^{8,9}. The decrease in uranyl reduction has been attributed to surface processes, thermodynamic limitations, and kinetic factors — with no clarity in the governing mechanism that ultimately imparts a major fate on uranium behavior¹⁰⁻¹². We present a mechanism by which electron transfer proceeds through uranyl-carbonato complexes, and the critical role Ca plays in limiting the redox reaction. Using Fe(II) as a common reductant, we performed a series of experiments under variable Ca conditions to determine the role of Ca in limiting uranium reduction rates. Calcium ions in the $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$ complex sterically prevent the interaction of Fe(II) with U(VI), in turn preventing the Fe(II)-U(VI) distance required for electron transfer (**Fig. 1**). ***Our results reveal that Ca inhibits the reduction of U(VI) through a competitive exchange process with Fe(II), whereby the rate of electron transfer is limited with increasing Ca:Fe ratios. The blocking mechanism prevents uranyl reduction and will increase the mobility and persistence of soluble uranium even upon the onset of anaerobic aquifer conditions.***

Impact: Reaction rates are extremely important for reactive transport modeling but are poorly understood under field conditions. This finding is significant provides for the first time a demonstrated molecular basis to explain the rates observed U(VI) reduction rates.

Question 3. How is U(IV) released from organic-rich sediments? Floodplain aquifers undergo seasonal water table fluctuations that drive redox cycling. We posited that intrusion of O_2 into anoxic sediments would stimulate NO_3^- production and subsequent denitrification, which

oxidizes U(IV)¹³ leading to release of U(VI). The fine-grained texture of NRZ sediments impose diffusion limitations on the exchange of solutes with the bulk aquifer sediments, thereby slowing both the transport of oxidants and subsequent U(VI)_(aq) mobilization. Nitrate and denitrification products exhibit relatively high solubility, permitting their diffusion into NRZs, whereas molecular oxygen is less soluble and is readily consumed through a multitude of abiotic (*e.g.* sulfide oxidation) and biotic reactions (respiration). To better understand the importance of and mechanisms behind oxidative uranium mobilization from NRZs, we are examining the release of uranium from NRZ sediments through nitrate or oxygen exposure.

Importance of oxidative mobilization for uranium mobilization from NRZ sediments. (S.E. Bone (lead), M.E. Jones, J.R. Bargar, S. Fendorf) Whether or not oxidative mobilization of U(VI) is important for plume maintenance in NRZ-containing aquifers depends on how quickly solutes are exchanged between the bulk aquifer and the NRZ sediments via diffusion, in addition

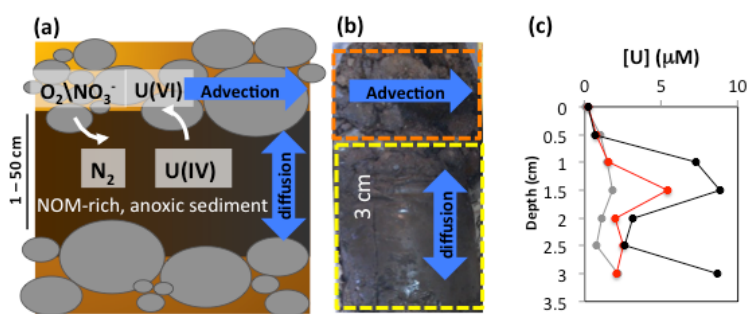


Figure 7. Diffusion-limited U(IV) oxidation by O₂ and NO₃⁻. (a) O₂ or NO₃⁻ diffuses into the reduced NRZ, causing the oxidation of U(IV). (b) Advection occurs within the surrounding, sandy aquifer sediments, whereas diffusion occurs across the NRZ-aquifer interface and within the fine-grained NRZ. (c) Depth profiles of aqueous U in the sediment, showing elevated porewater U concentrations in reactors exposed to NO₃⁻ (red) or O₂ (black) relative to the control (grey).

to the rate of U(IV) oxidation. NRZ sediments also contain many other reduced species (*e.g.* S(-II)) that could consume NO₃⁻, buffering the oxidation of U(IV). Our goal was to determine if O₂ and NO₃⁻ oxidation of U(IV) could lead to aqueous U(VI) release over time and length scales relevant to the field. To this end, we constructed diffusion-limited reactors containing NRZ sediments from Riverton, WY. The reaction cells were

submerged in circulating 1 mM NO₃⁻/artificial groundwater, O₂-saturated/artificial groundwater, or anoxic artificial groundwater solution for 85 days. Aqueous uranium was monitored daily in the circulating (“advection”) solution. Additionally, porewater concentrations of U, O₂, S(-II), NO₃⁻, NO₂⁻ and NO were measured as a function of depth and time, along with the solid-phase concentration and oxidation state of U.

Results: Diffusion of O₂ and NO₃⁻ into the sediments caused short-term oxidative release of uranium out of the sediments relative to the anoxic control: an efflux of uranium (50 – 120 ng/cm² d) was sustained only for the first few days of the experiment, after which, the efflux dropped to approximately zero. In contrast, porewater concentrations of uranium remained elevated for the first month of the experiment, suggesting that uranium oxidation continued to occur, even after the uranium efflux dropped to zero (**Fig. 7**). This suggests that uranium redox cycling within the sediments was faster than diffusional exchange with the advection solution. Nitrate was almost entirely consumed within the sediments and sulfate reduction occurred in both the anoxic control and 1 mM NO₃⁻ reactors. Additionally, O₂ was entirely consumed within the first 0.5 cm of the sediment tube. *These results suggest that the oxidative release of uranium may be limited by the high reducing capacity of the sediments, which leads to rapid*

O₂ and NO₃⁻ consumption, especially in combination with relatively high abundance of organic carbon that stimulates microbial respiration.

Impact: Oxidative release of uranium due to seasonal increases in O₂ and NO₃⁻ reduction can be buffered in anoxic sediments that are rich in bioavailable organic carbon, which stimulates rapid oxidant consumption and subsequent anaerobic respiration. Our results demonstrate the important link between C cycling and the distribution and mobility of uranium in anoxic sediments.

The role of denitrification in oxidative mobilization of U. (E. Cardarelli (lead), V. Noël, K. Boye, and C. Francis) Although both abiotic and biotic uranium oxidation has been documented, the rate of uranium oxidation coupled to nitrate reduction by bacteria far exceeds observed rates of abiotic oxidation¹⁴⁻¹⁷. Thus, N-cycling microbial communities, specifically those involved in denitrification, may be key to understanding uranium mobility and long-standing contamination; yet, there is a paucity of knowledge regarding nitrate-reducing communities in the subsurface.

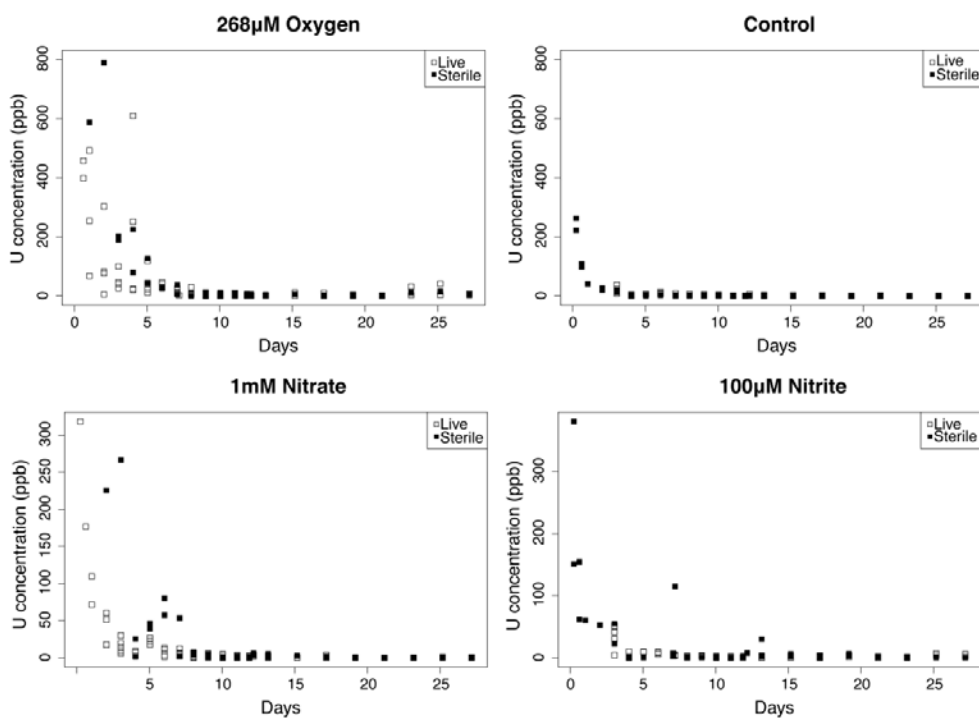


Figure 8. Effluent uranium concentrations (ppb) from the live (open symbols) and the sterile (closed symbols) columns throughout the 28-day oxidation experiment.

Here we investigated the microbial community response to oxidation of U(IV) within NRZ sediments from Riverton, WY. Using a set of flow-through column reactors, sterilized or unsterilized NRZ material was stimulated for 24 days with an oxidant (either 1 mM NO₃, 100 µM NO₂, or 268 µM O₂). Our objectives were to (1) identify oxidant-induced effects on the denitrifying microbial community, and (2) assess over what timescales and to what extent nitrate, nitrite, and oxygen oxidize uranium in reduced sediments. We found that the abundances of *nirK* and *nirS* (functional genes encoding the key denitrification enzyme, nitrite reductase) remained within the range of values observed whole sediment cores at Riverton, WY, and a number of distinct *nirK/nirS* sequence types emerged. Preliminary uranium effluent data suggests that uranium mobilization was highest in the oxygen-stimulated columns (**Fig. 8**). **Therefore, although nitrate and nitrite amended columns resulted in changes in microbial diversity and**

abundances, only a minimal increase in the rate of uranium mobilization was observed and oxygen was the most efficient uranium oxidant in this system.

Impact: Our results provide important insights into the denitrifying community dynamics and the molecular mechanisms of uranium mobilization through oxidative perturbations of NRZ sediments.

Question 4. Are NRZs regionally important to plume-persistence in the Colorado River Basin (CRB)? Organic-rich NRZs are believed to result from burial of plant material and surface sediments along active river margins. Therefore, we posit that NRZs are regionally common features on alluvial floodplains throughout the upper CRB, and that they accumulate uranium wherever it is present in the groundwater. To explore the relevance of NRZs in uranium behavior, we are linking the molecular level investigations and laboratory experiments described above to regional characterization of uranium contaminated floodplain materials in the upper CRB.

NRZ functional types and regional U, Fe, and S speciation (V. Noël (lead), K. Boye, S.E. Bone, and J.R. Bargar, external collaborators: W.L. Dam (DOE-LM), R.H. Johnson (Navarro), K.H. Williams (LBNL)) We are investigating pore and molecular scale processes and their dependence upon hydrological processes in order to understand regional functionality, vulnerability, and roles of NRZs in upper CRB floodplains.

In our 2015 Annual Report, we reported that organic-rich NRZs are regionally extant and accumulate uranium. Since then, we have completed depth-resolved field sampling from five sites, on-schedule, and completed a large fraction of the characterization of molecular structures of uranium, iron, and sulfur using chemical extractions, XAS, and X-ray mapping). Our investigations demonstrate that:

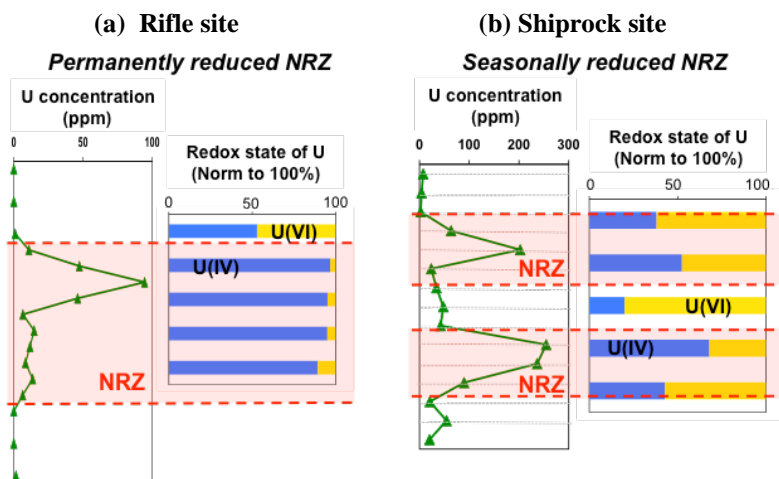


Figure 9. Profiles of sediment U concentrations (green lines) versus depth in NRZs (pink bands) at the Rifle and Shiprock sites are compared to the observed oxidation states of U. (a) Permanently saturated NRZs contain U(VI). In contrast, (b) when NRZs are exposed to annual water table variations, such as at the Shiprock site, they can become desiccated and oxidized. Because U(VI) is more mobile than U(IV), seasonally reduced NRZs are predicted to be relatively vulnerable to climatic perturbations (drought).

1. **NRZs are not all the same! Indeed, strong textural, compositional, and hydrological differences exist between NRZs.** We have distinguished two NRZ functional type “end members”, controlled by (i) sediment texture and (ii) position relative to the water table. **Fine-grained, permanently saturated NRZs** are characterized by the presence of iron monosulfides and disulfides (S^{2-} and S^0). In contrast, **coarse-grained NRZs that are located in the zone of annual water table variation** are seasonally saturated and desiccated, exposing them to oxidation. These NRZs characteristically are rich in goethite, lack iron monosulfides, but contain iron disulfides.

2. **NRZ functional type strongly impacts the speciation and release of U.** The ability of NRZs to control uranium mobility is directly related to the presence of sustained reducing conditions. Uranium is present as U(IV) in fine-grained, permanently reduced NRZs (**Figure 9**). In contrast, coarse-grained, seasonally saturated NRZs contain both U(VI) and U(IV). Because U(VI) is more mobile than U(IV), seasonally reduced NRZs are predicted to be relatively vulnerable to climatic perturbations (drought).

Impact: Seasonal maintenance of reducing conditions is critical to the biogeochemical function of NRZs with respect to uranium and carbon. Seasonally saturated NRZs are biogeochemical hotspots that appear to be highly vulnerable to seasonal and long-term drought and have the capability to seasonally discharge oxidized uranium and carbon.

Regional diversity and abundance of ammonia oxidizers (E. Cardarelli (lead), V. Noël, K. Boye, S.E. Bone, and C. Francis) Microbial nitrogen cycling has the capability to “unlock” the biogeochemical nutrient supply stored within NRZs, drive carbon cycling, and cause uranium to be released to the aquifers. Specifically, nitrification links organic matter decomposition ($\text{Org-N} \rightarrow \text{NH}_3$) to the production of nitrate ($\text{NH}_3 \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^-$), a strong oxidant of U(IV) and the primary electron acceptor for denitrification ($\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$). Nitrification is carried out by ammonia-oxidizing archaea (AOA) and bacteria (AOB) along with nitrite-oxidizing bacteria (NOB). In spite of its expected importance to the mobility and fate of organic carbon (OC) and uranium within NRZs, virtually nothing is currently known about the N-cycling communities within NRZs.

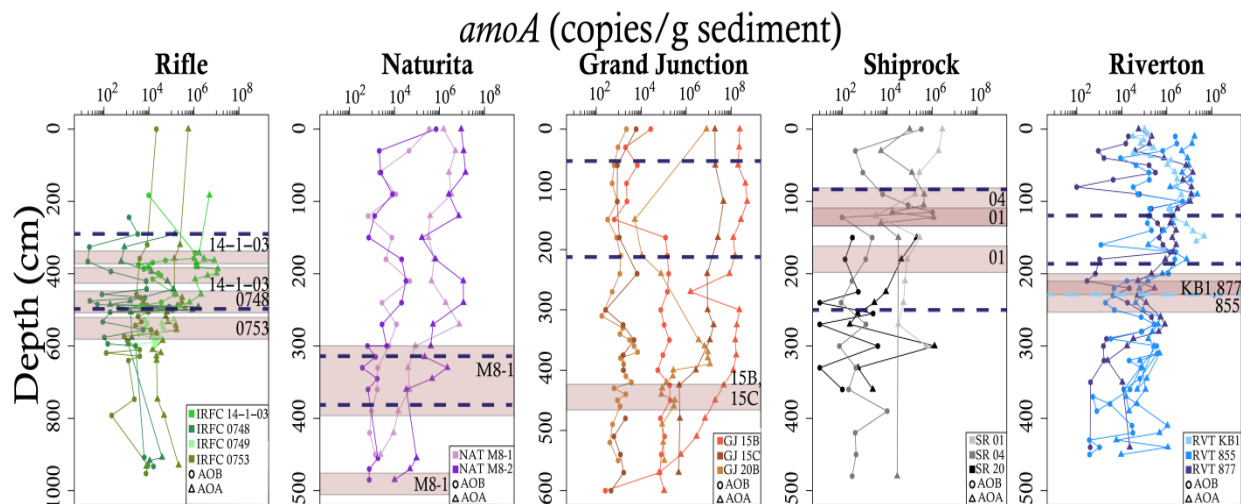


Figure 10. Vertical profiles of archaeal (triangle symbols) and bacterial (circle symbols) *amoA* abundances as a function of depth in sediment cores from five upper CRB sites: Rifle, Naturita, Grand Junction, Shiprock, and Riverton. Each panel shows the abundances of both archaeal and bacterial *amoA* per gram sediment, plotted against depth below ground surface, with identified NRZ elevations indicated in red shading. For each site, individual core locations are noted by different colors, as defined in the insets. At each location, water table elevations vary between summer high and winter low values. Dashed horizontal lines provide the upper/lower ranges observed at each core location. At Riverton, location KB1 had a different minimum water table elevation than locations 855/877. This lower level is indicated by a lower light-blue dashed line.

Here, we present the first, basin-wide insights on the diversity and abundance of ammonia-oxidizing organisms. Using Sanger sequencing and quantitative PCR for the *amoA* gene (encoding the α -subunit of ammonia monooxygenase), we have surveyed more than 330

sediment samples, across nearly 900 km, to a depth of 10m, and produced 567 AOA and 332 AOB sequences to characterize the *in-situ* ammonia-oxidizing community structure and diversity. Our results confirm *Thaumarchaeota* are pervasive throughout the subsurface in the CRB. Regionally, in every sediment sample, AOA abundances exceed AOB abundances by more than 10^2 - 10^3 copies/g sediment (**Fig. 10**). Further, throughout the upper CRB, AOA and AOB populations were found to be highly diverse, and in most cases, relatively distinct from cultured representatives. Some AOA/AOB sequences appeared to be location- or depth-specific, while other members of the AOA and AOB communities were found at multiple locations and various depths. From the phylogenetic data, the estimated community α -diversity (*i.e.*, the diversity within each sample) for AOA (Shannon Index: 3.7037, Simpson Index: 0.0479) and AOB (Shannon Index: 3.2622, Simpson Index: 0.0473) were similar. Furthermore, the β -diversity, a metric that describes the number of species shared between different environments, shows that almost all AOA and AOB sequences are non-overlapping between sites. ***Our work reveals that the ammonia oxidizing communities in the examined sediments are dominated by AOA, are highly diverse and exhibit a strong site-specificity in phylogenetic composition.***

In FY 2017 we will examine the community diversity at each site through extensive sequencing, which ultimately will facilitate prediction of responses to particular environmental perturbations into BER ecosystem-scale models.

Impact: These findings have elevated our knowledge about microbial N cycling to the *regional* scale, illuminating a critical part of the microbial ‘black box’ of subsurface BCE nutrient cycling. Our work will help drive breakthroughs in the molecular- to ecosystem-scale understanding of biogeochemical cycling and uranium transport within a geographically large and important area.

References Cited.

1. Janot, N. *et al.* Physico-Chemical Heterogeneity of Organic-Rich Sediments in the Rifle Aquifer, CO: Impact on Uranium Biogeochemistry. *Environ. Sci. Technol.* 50, 46–53 (2016);
2. Alessi, D. S. *et al.* The product of microbial uranium reduction includes multiple species with U(IV)-phosphate coordination. *Geochim. Cosmochim. Acta* 131, 115–127 (2014);
3. Latta, D. E., Mishra, B., Cook, R. E., Kemner, K. M. & Boyanov, M. I. Stable U(IV) Complexes Form at High-Affinity Mineral Surface Sites. *Environ. Sci. Technol.* 48, 1683–1691 (2014);
4. Bargar, J. R. *et al.* Uranium redox transition pathways in acetate-amended sediments. *Proc. Natl. Acad. Sci. U. S. A.* 110, 4506–4511 (2013);
5. Boyanov, M. I. *et al.* Solution and Microbial Controls on the Formation of Reduced U(IV) Species. *Environ. Sci. Technol.* 45, 8336–8344 (2011);
6. Jin, Q. & Bethke, C. M. Predicting the rate of microbial respiration in geochemical environments. *Geochim. Cosmochim. Acta* 69, 1133–1143 (2005);
7. LaRowe, D. E. & Van Cappellen, P. Degradation of natural organic matter: A thermodynamic analysis. *Geochim. Cosmochim. Acta* 75, 2030–2042 (2011);
8. Brooks, S. C. *et al.* Inhibition of bacterial U(VI) reduction by calcium. *Environ. Sci. Technol.* 37, 1850–1858 (2003);
9. Stewart, B. D., Mayes, M. A. & Fendorf, S. Impact of Uranyl–Calcium–Carbonato Complexes on Uranium(VI) Adsorption to Synthetic and Natural Sediments. *Environ. Sci. Technol.* 44, 928–934 (2010);
10. Du, X. *et al.* Reduction of Uranium(VI) by Soluble Iron(II) Conforms with Thermodynamic Predictions. *Environ. Sci. Technol.* 45, 4718–4725 (2011);
11. Stewart, B. D., Neiss, J. & Fendorf, S. Quantifying constraints imposed by calcium and iron on bacterial reduction of uranium(VI). *J. Environ. Qual.* 36, 363–372 (2007);
12. Liger, E., Charlet, L. & Van Cappellen, P. Surface catalysis of uranium(VI) reduction by iron(II). *Geochim. Cosmochim. Acta* 63, 2939–2955 (1999);
13. Senko, J. M., Istok, J. D., Suflita, J. M. & Krumholz, L. R. In-Situ Evidence for Uranium Immobilization and Remobilization. *Environ. Sci. Technol.* 36, 1491–1496 (2002);
14. Weber, K. A., Thrash, J. C., Trump, J. I. V., Achenbach, L. A. & Coates, J. D. Environmental and Taxonomic Bacterial Diversity of Anaerobic Uranium(IV) Bio-Oxidation. *Appl. Environ. Microbiol.* 77, 4693–4696 (2011);
15. Senko, J. M., Suflita, J. M. & Krumholz, L. R. Geochemical Controls on Microbial Nitrate-Dependent U(IV) Oxidation. *Geomicrobiol. J.* 22, 371–378 (2005);
16. Beller, H. R. Anaerobic, Nitrate-Dependent Oxidation of U(IV) Oxide Minerals by the Chemolithoautotrophic Bacterium *Thiobacillus denitrificans*. *Appl. Environ. Microbiol.* 71, 2170–2174 (2005);
17. Beller, H. R. *et al.* The genome sequence of the obligately

chemolithoautotrophic, facultatively anaerobic bacterium *Thiobacillus denitrificans*. *J. Bacteriol.* 188, 1473–1488 (2006).

4.b. New scientific results that may shift current research focus areas and/or identified knowledge gaps in the program

Three significant discoveries within our research program in the past year have important implications for our research activities.

1. Initial sampling of sediments and pore water from a large NRZ identified at the Riverton site, WY, suggest that large variations in redox conditions occur in the spring and fall, discharging uranium and carbon to the aquifer. These variations are believed to be tied to seasonal variation of the water table.

This discovery suggests that the Riverton site is well suited to investigating vulnerability of NRZs to perturbations by drought and biogeochemical changes that accompany seasonal redox cycling. The suitability of this site for continued study is heightened by the ease of coring Riverton sediments by hand augur, greatly facilitating the installation of sampling hardware for continuous monitoring. As described in section 4.c., we are conducting scoping studies to further evaluate the Riverton site's suitability as a study site for the 2017 Science Plan renewal.

2. The unsaturated zone at the Riverton site is also enriched in uranium, albeit at lower levels (≤ 4 mg/kg) than observed in the NRZ (≤ 22 mg/kg). A large flood at the site in late May 2016 was followed by elevated uranium concentrations in groundwater at relatively shallow depths, suggesting that uranium associated with the evaporite layer was being discharged to the aquifer.

This discovery suggests that processes in the unsaturated zone impart control on uranium mobility at the Riverton site. This subject falls within the scope of our research mission, and we are expanding activities to address the speciation of uranium in unsaturated sediments from Riverton and associated controls on uranium mobility.

3. Region-wide, NRZs exhibit considerable variability in saturation state and particle size, leading to predictions of large differences in vulnerability to drought. Fine-grained, permanently saturated NRZs (Rifle type) are expected to be relatively stable, whereas coarser-grained NRZs residing in the zone of annual groundwater table fluctuation are expected to exhibit stronger biogeochemical variability and to be relatively vulnerable to drought and flooding, leading to release of uranium and carbon.

In summary, the Riverton site provides opportunities to study the impacts of environmental perturbations on floodplain sediment ecosystems and the fate of U(IV) and carbon. As described in 4.c., we are planning to propose such work in our 2017 Science Plan proposal.

4.c. Future scientific goals, vision, and plans toward meeting program objectives

Specific goals for the FY 2017 research program are provided in Table 2. In the 2014 review of our SFA Science Plan, BER suggested that SLAC could benefit from: (i) increased modeling activity, and (ii) developing alternate hypotheses and research approaches in case the initial hypothesis regarding interactions of uranium with NRZs turn out to be incorrect. We have embraced these recommendations through the following actions:

- i. We have initiated a pore-water collection program at the Riverton site, with logistical field support from DOE-LM, which will provide unique and valuable input for a new modeling program, which will feed into the SFA science plan to be proposed in FY 2017.
- ii. We have initiated a subtask to study the chemical speciation and stability of U(VI) in evaporite-bearing sediments at the Riverton site.

Initial plans for triennial science plan renewal. We are evaluating research opportunities and developing ideas that could form the basis for the FY 2018 Science Plan (to be submitted for review in FY 2017). Based on the new scientific results described in sections 4.a and b., we are planning a new proposal to address the following overarching scientific questions:

- How vulnerable are anoxic sediment ecosystems to climate perturbations, including drought and flooding?

This question is important at sites of interest to BER, including the upper CRB, the East River, CO site, and unsaturated aquifer sediments subject that are subject to inundation during large-scale aquifer recharge using surface water. To gain comprehensive insights into the impact of redox condition fluctuations on site-specific biogeochemical processes, we are planning to investigate biogeochemical processes mediating ecosystem change during desiccation and re-wetting of anoxic sediments, and inundation of unsaturated zone sediments. Numerous studies have highlighted the importance of *frequency* and *duration* of perturbations as drivers of biogeochemical response and vulnerability. We are planning to evaluate the impact of these drivers through observations at upper CRB field sites and in model systems. A combination of geochemistry, molecular microbial ecology, and biogeochemical reactive transport modeling approaches would allow us to assess the vulnerability and adaptability of these sediment systems to different forms of change, in order to better predict the impact of perturbations.

- What controls the mobility of reduced uranium and carbon at the Riverton site?

This question would address molecular scale processes that, by controlling the reactivity of uranium and BCEs, project to the field scale. We are planning to investigate: (i) the impact of organics and evaporite phases on U(IV) mobility; (ii) the relative affinity of U(IV) on mineral and organic surfaces; and (iii) mechanisms controlling organic carbon stability and transformations.

- What are hotspots for uranium and carbon release from floodplains?

This fundamental question projects to all floodplains within the upper CRB and acknowledges the need to look beyond NRZs and unsaturated zone processes to fully understand floodplain ecosystem function and vulnerability, specifically relating to uranium and carbon mobility. For example, our work at the Riverton site has shown that uranium and carbon accumulate in the reduced phreatic zone sediments in the riparian corridor immediately proximal to the Little Wind River (*i.e.*, above and distinct from the hyporheic zone). The uranium groundwater plume flows through these zones, which act as filters and strongly impact downgradient water supplies. Climate perturbations impacting the behavior of these sediments, could have significant regional consequences to water quality and availability. In addition to the riparian corridors, there is accumulating evidence that subsurface microsites sustain ‘hot zone’ reducing conditions throughout the unsaturated zone, with the potential to broadly impact biogeochemical transformations of uranium and carbon.

We will continue to discuss these ideas with program managers and plan to submit a white paper in early October summarizing the tentative new research plan.

4.d. Collaborative research activities with external researchers in pursuit of program objectives

The SLAC-SFA has significant collaborations with the following projects and groups:

- DOE-LM: William Dam, Richard Bush, and Ray Johnson (Navarro Navarro Research and Engineering): logistical and scientific support for access and sediment sampling at DOE-LM field sites. (Question 4)
- EMSL: collaborations with Malak Tfaily on carbon characterization (FT-ICR MS), with John Cliff on NanoSIMS isotope imaging, and with Ravi Kukkadapu on Mössbauer spectroscopy. (Questions 1 and 4)
- LBNL: K.H. Williams and J.A. Davis: scientific collaborations on Questions 3 and 4.
- USGS: Collaborations with C. Fuller to investigate the speciation of U(IV) in the zero-valent iron demonstration project at the Fry Canyon, UT, field demonstration site. (related to Question 1)
- Academic collaborators: Karrie Weber (Univ. of Nebraska): research to understand the speciation of uranium in high-plains aquifer sediments; and Guillaume Morin (Univ. of Paris): research to understand the speciation and reactivity of U(IV) in reduced lake sediments. (Related to Question 4)

4.e. Leveraging

SLAC-SFA funds are strongly leveraged through collaborations with independently-funded postdoctoral and Stanford graduate students, including: C. Bobb (biogeochemical reactive transport modeling), E. Cardarelli (microbial N cycling), G. Dublet (organic carbon- Fe oxide interactions), M. Keiluweit (NOM stability in soils), P. LeFebvre (U isotope geochemistry, visiting graduate student fellow), S. Roycroft (evaporite uranium speciation), and L. Zalles (Riverton pore water analyses).

5. Staffing and budget summary

As noted in Table 2, we added a carbon characterization effort (one FTE postdoctoral) to the project in FY 2015 and 2016 in response to guidance provided in the 2014 Triennial review. To accommodate this activity, it was necessary to remove original hypotheses 3.3 and 3.4 from the plan in order to accommodate this addition.

5.a. Funding allocation by program element (task) and individual researcher

The SLAC-SFA program funded five postdoctoral researchers in the reporting period (S. Bone, K. Boye, M. Jones, V. Noël, and A. Müller). Senior personnel Bargar, Fendorf, and Francis received partial salary support to cover management activities noted in Table 1.

5.b. Funding allocation to external collaborators (if any)

No new funding allocations were made to external collaborators in FY 2016.

5.c. Personnel actions and procedures

A. Müller was recruited to join the SLAC SFA team and M. Jones departed from the team during this period. All full-time researchers in the SLAC-SFA program were postdoctorals.

5.d. National Laboratory investment in the program

SLAC/SSRL provides salary support for Bargar that covers part of the SFA management effort and Bargar's research activities. SLAC/SSRL operates the SPEAR3 synchrotron facility and x-ray beam lines, which are integral to the program.

6. List of publications and presentations

6.a. Comprehensive list of publications (Published, in press, and submitted) wholly or partly supported by this program, July 1, 2015 – June 30, 2016.

1. Boye, K., Noël, V., Tfaily, M., Cardarelli, E., Bone, S. E., Williams, K. H., Bargar, J. R. and Fendorf, S. (2016). Vulnerability of thermodynamically constrained carbon within floodplain sediments. *Nature Geoscience*. In review.
2. Bone, S. E., Dynes, J., Cliff, J., and Bargar, J. R. (2016). Uranium(IV) adsorption by natural organic matter in sediments. *Nature Geoscience*. Submitted.
3. Jones, M.E., Bargar, J. R., and Fendorf, S. (2016). Inhibition mechanism of uranyl reduction induced by calcium-carbonate complexes. *Environmental Science & Technology*. Submitted.
4. Morin, G., Mangeret, A., Othmane, G., Stetten, L., Seder-Colomina, M., Brest, J., Ona-Nguema, G., Bassot, S., Courbet, C. and Guillevic, J. (2016). Mononuclear U(IV) complexes and ningyosite as major uranium species in lake sediments. *Geochemical Perspectives Letters* 2(1): 95-105. DOI: 10.7185/geochemlet.1610
5. Janot, N., Lezama Pacheco, J. S., Pham, D. Q., O'Brien, T. M., Hausladen, D., Noël, V., Lallier, F., Maher, K., Fendorf, S. and Williams, K. H. (2016). Physico-chemical heterogeneity of organic-rich sediments in the Rifle aquifer, CO: Impact on uranium biogeochemistry. *Environmental Science & Technology* 50(1): 46-53. DOI: 10.1021/acs.est.5b03208
6. Noël, V., Morin, G., Juillot, F., Marchand, C., Brest, J., Bargar, J. R., Muñoz, M., Marakovic, G., Ardo, S. and Brown, G. E. (2015). Ni cycling in mangrove sediments from New Caledonia. *Geochimica et Cosmochimica Acta* 169: 82-98. DOI: 10.1016/j.gca.2015.07.024
7. Herndon, E. M., Yang, Z., Bargar, J., Janot, N., Regier, T. Z., Graham, D. E., Wulfschleger, S. D., Gu, B. and Liang, L. (2015). Geochemical drivers of organic matter decomposition in arctic tundra soils. *Biogeochemistry* 126(3): 397-414. DOI: 10.1007/s10533-015-0165-5
8. Gallegos, T. J., Campbell, K. M., Zielinski, R. A., Reimus, P., Clay, J., Janot, N., Bargar, J. R. and Benzel, W. M. (2015). Persistent U(IV) and U(VI) following in-situ recovery (ISR) mining of a sandstone uranium deposit, Wyoming, USA. *Applied Geochemistry* 63: 222-234. DOI: 10.1016/j.apgeochem.2015.08.017

6.b. Presentations at National and International Scientific Meetings wholly or partly supported by this program July 1, 2015 – June 30, 2016.

1. Boye, K., Noël, V., Cardarelli, E., Tfaily, M., Dam, W. L., Bargar, J. R. and Fendorf, S. (2016). Imprints of thermodynamic constraints on microbial carbon oxidation in floodplains. Presented at the 2016 Goldschmidt Conference, Yokohama, Japan. 30 Jun. [oral]
2. Bargar, J. R., Bone, S. E. and Janot, N. (2016). Redox and coordination environment: Controls over uranium chemistry in complex natural sediments. Presented at the International Symposium on Metal Complexes, Barcelona, Spain. 7-10 Jun. [invited plenary]
3. Bargar, J. R., Bone, S. E., Boye, K., Lezama-Pacheco, J., Noël, V., Cardarelli, E., Jones, M. E., Fendorf, S., Francis, C. A., Spormann, A. M., Long, P. E. and Williams, K. H. (2016). Aquifer anaerobic heterogeneities: Molecular controls over carbon fate and radionuclide cycling. Presented at the 2016 Environmental System Science (ESS) PI Meeting, Potomac, MD. 27 Apr. [invited]
4. Bargar, J. R., Bone, S. E., Jones, M. E., Dam, W. L., Johnson, R. H., Dynes, J., Cliff, J. and Fendorf, S. (2016). Molecular controls over uranium mobility in organic-rich sediments. Presented at the 2016 Environmental System Science (ESS) PI Meeting, Potomac, MD. 27 Apr. [poster]
5. Bargar, J. R., Cardarelli, E., Boye, K., Dam, W. L., Johnson, R. H., Williams, K. H. and Francis, C. A. (2016). Diversity and biogeography of subsurface nitrogen-cycling communities at uranium-contaminated DOE legacy sites in the upper Colorado River Basin. Presented at the 2016 Environmental System Science (ESS) PI Meeting, Potomac, MD. 27 Apr. [poster]

6. Bargar, J. R., Fendorf, S., Francis, C. A., Maher, K. and Spormann, A. M. (2016). Coupled cycling of organic matter, uranium, and biogeochemical critical elements in subsurface systems. Presented at the 2016 Environmental System Science (ESS) PI Meeting, Potomac, MD. 27 Apr. [poster]
7. Noël, V., Boye, K., Müller, A. L., Bone, S. E., Cardarelli, E., Lezama-Pacheco, J., Lefebvre, P., Williams, K. H., Dam, W. L., Johnson, R. H., Kukkadapu, R., Tfaily, M., Chu, R. K., Pasa-Tolic, L., Spormann, A. M., Fendorf, S. and Bargar, J. R. (2016). Mechanistic linkages between floodplain critical element and contaminant redox cycles. Presented at the 2016 Environmental System Science (ESS) PI Meeting, Potomac, MD. 27 Apr. [poster]
8. Cardarelli, E., Bargar, J. R., Williams, K. H., Dam, W. L. and Francis, C. A. (2016). Metagenomic characterization of nitrogen-cycling microbial communities impacting uranium release in the upper Colorado River Basin. Presented at the Joint Genome Institute's Annual User Meeting, Walnut Creek, CA. 22-24 Apr. [poster]
9. Lefebvre, P., Noël, V., Jemison, N., Weaver, K., Bargar, J. R. and Maher, K. A. (2016). Uranium bio-accumulation and cycling as revealed by uranium isotopes in naturally reduced sediments from the Upper Colorado River Basin. Presented at the European Geosciences Union General Assembly 2016, Vienna, Austria. 17-22 Apr. [poster]
10. Bargar, J. R., Bone, S. E., Lezama-Pacheco, J., Alessi, D. S., Cerrato, J. M., Veeramani, H., Noël, V., Suvorova, E., Bernier-Latmani, R., Giammar, D. E., Long, P. E. and Williams, K. H. (2016). Impact of redox conditions on interfacial uranium chemistry in complex natural sediments. Presented at the 251st ACS National Conference, San Diego, CA. 14 Mar. [invited]
11. Fendorf, S. (2016). Threats to groundwater quality during managed aquifer recharge. Presented at the Annual conference of Association of Ground Water Agencies and American Ground Water Trust on "Everything aquifers and groundwater management", Ontario, CA. 17-18 Feb. [invited]
12. Jones, M. E., Bargar, J. R. and Fendorf, S. E. (2015). Inhibition mechanism of uranyl reduction induced by calcium-carbonate complexes Presented at the AGU Fall Meeting, San Francisco, CA. 16 Dec. [oral]
13. Bone, S. E., Dynes, J. J., Cliff, J. and Bargar, J. R. (2015). Spectromicroscopic and spectroscopic investigation of U(IV) speciation in model mineral-organic matter assemblages. Uranium Mobility in the Environment II. Presented at the AGU Fall Meeting, San Francisco, CA. 14-18 Dec. [oral]
14. Boye, K., Noël, V., Tfaily, M., Dam, W. L., Bargar, J. R. and Fendorf, S. (2015). Carbon characteristics and biogeochemical processes of uranium accumulating organic matter rich sediments in the Upper Colorado River Basin. Presented at the AGU annual meeting, San Francisco, CA. 14-18 Dec. [oral]
15. Cardarelli, E., Bargar, J. R., Williams, K. H., Dam, W. L. and Francis, C. A. (2015). Subsurface nitrogen-cycling microbial communities at uranium contaminated sites in the Colorado River Basin. Presented at the American Geophysical Union, San Francisco, CA. 14-18 Dec. [poster]
16. Fendorf, S. (2015). Anticipating and avoiding metal contamination of groundwater during managed aquifer recharge. Presented at the Groundwater Resource Association of California's symposium on "Naturally Occurring Compounds of Regulatory Concern", Garden Grove, CA. 18-19 Nov. [invited]
17. Boye, K., Noël, V., Tfaily, M., Bone, S. E., Bargar, J. R. and Fendorf, S. (2015). Organic matter chemistry in floodplain soils and sediments – implications for uranium retention. Presented at the SSSA annual meeting, Minneapolis, MN. 15-18 Nov. [oral]
18. Jones, M. E. (2015). Diffusion-limitation on uranium cycling in organic-rich sediments contributes to plume persistence in a contaminated aquifer. Presented at the The International Symposium on Persistent Toxic Substances (ISPTS), Riverside, CA. 15-17 Nov. [invited]
19. Boye, K., Noël, V., Cardarelli, E., Tfaily, M., Bargar, J. R. and Fendorf, S. (2015). Carbon characteristics and biogeochemical processes of uranium accumulating organic matter rich sediments in the upper colorado river basin. Presented at the LCLS/SSRL Annual Users' Meeting, Menlo Park, CA. 7-10 Oct. [invited]
20. Bone, S. E., Dynes, J. J., Cliff, J. and Bargar, J. R. (2015). Nanocrystalline and non-crystalline U(IV) in the subsurface. Amorphous and Nanocrystalline Materials and their Role in Biogeochemical Element Cycling, Pollutant Remediation and Radioactive Waste Disposal. Presented at the 2015 Goldschmidt Conference, Prague, CZ. 21 Aug. [invited]
21. Bargar, J. R., Williams, K. H., Bone, S. E., Boye, K., Janot, N. and Noël, V. (2015). Uranium biogeochemistry: Nanometer to regional scales. Presented at the 2015 Goldschmidt Conference, Prague, Czech Republic. 19 Aug. [invited]
22. Bargar, J. R. (2015). Why little matters: Fluid-mineral interactions from mineral and microbial surfaces to pores. Presented at the 2015 Basic Research Needs for Environmental Management Workshop, Bethesda, MD. 8 Jul. [invited]