

**Argonne National Laboratory Subsurface Science SFA
FY2016 Annual Progress Report
For the BER Subsurface Biogeochemical Research Program
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1. Program overview

Water is vital for sustaining life on Earth; its movement, whether through oceans, lakes, rivers, streams, or underground, is responsible for transporting most of the biologically catalyzed and many of the chemically reactive elements that cycle through the environment. The movements of water and the constituents dissolved in it, along with the biogeochemically catalyzed transformations of those constituents, determine the mobility of contaminants, atmospheric greenhouse gas (e.g., CO₂, CH₄, and N₂O) emissions, carbon cycling and sequestration in subsurface environments, nutrient (e.g., carbon and nitrogen) mobility, and the quality of water itself. Iron (Fe) is a highly abundant element in the lithosphere; its biogeochemistry in many aquatic and terrestrial environments is driven largely by microbial activity. In Fe-rich soils and sediments, particularly, Fe redox cycling by microorganisms is a significant component of major and minor elemental cycling and energy flux. Similarly, sulfur (S) is commonly found in groundwater; its transformations, often driven by microbially catalyzed redox reactions, can also greatly affect the cycling of elements. Redox-dynamic environments where Fe or S biogeochemical cycling can be a major driver of other elemental cycles include terrestrial-aquatic interfaces and subsurface environments (e.g., marshes, wetlands, floodplains, soil aggregates, plant root-soil interfaces, and locations where groundwater and surface waters mix). These environments are often part of polar, temperate, or tropical systems containing continuously or temporarily water-saturated soils and sediments. Although much is known about the respective biogeochemical cycles of Fe and S, the interplay between the two cycles is less understood. Thus, understanding the interplay of the Fe and S biogeochemical cycles with the water cycle is critical for prediction of the mobility of contaminants; atmospheric greenhouse gas emissions; carbon cycling and sequestration in subsurface environments; and nutrient mobility in near-surface and subsurface polar, temperate, or tropical systems.

The objective of the Argonne Subsurface Biogeochemical Research Program (SBR) Scientific Focus Area (SFA) is *to identify and understand coupled biotic-abiotic molecular- to core-scale transformations of Fe and S within redox-dynamic environments and understand the effects of Fe and S biogeochemistry on transformation and mobility of major/minor elements and contaminants*. To accomplish this objective, the Argonne SBR SFA integrates two unique strengths at Argonne — the Advanced Photon Source (APS) for synchrotron-based interrogation of systems and next-generation DNA sequencing and bioinformatics approaches for microbial community and metabolic pathway analysis — with biogeochemistry and microbial ecology. Drawing on these strengths, we pursue the long-term scientific goal of *elucidating the interplay, from the molecular to core scale, between specific microbial metabolic activities, solution chemistry, and mineralogy contributing to the transformations of Fe, S, nutrients, and contaminants in subsurface environments*. Addressing this objective contributes directly to the goal of the United States Department of Energy (DOE), Office of Biological and Environmental Research (BER), Climate and Environmental Sciences Division (CESD) to “advance fundamental understanding of coupled biogeochemical processes in complex subsurface environments to enable system-level environmental prediction and decision support” (<http://science.energy.gov/ber/research/cesd/>) (DOE 2012). In addition, the work of the Argonne SBR SFA specifically addresses the SBR priority of “understanding and predicting biogeochemical processes in subsurface environments” (DOE 2012). By focusing research efforts on understanding the effects of coupled Fe and S biogeochemical cycles on carbon, nutrient, and

contaminant transport, the Argonne SBR SFA will continue contributing to the CESD's identified core capability (DOE 2012) that includes "focused research on key Earth system processes that represent significant uncertainties and currently limit predictive understanding." Similarly, by continuing the well-integrated use of synchrotron radiation at the APS to investigate coupled Fe and S biogeochemical processes, the Argonne SBR SFA will contribute to CESD's identified core capabilities and needs for "exploiting synchrotron radiation light sources provided by the SC Office of Basic Energy Sciences" (DOE 2012) and providing "foundational knowledge of these molecular scale processes" (DOE 2014).

Hypotheses directed toward achieving the goal of the Argonne SBR SFA are tested by experiments that capitalize on the talents of Argonne researchers and unique Argonne capabilities. Other members of the Argonne SBR SFA are research scientists at several additional institutions; they bring diverse, relevant expertise and established track records of collaborative and integrated multidisciplinary research for experiments investigating hydrological and biogeochemical processes at multiple spatial and temporal scales, in both field and laboratory settings. All members of the Argonne SBR SFA share a long-term vision of ultimately integrating the new knowledge generated by the SFA into future multiscale modeling approaches to understand and predict relevant environmental processes. Argonne SBR SFA research addresses four critical knowledge gaps related to accomplishing this goal: *(1) an in-depth understanding of the molecular processes affecting Fe, S, and contaminant speciation in dynamic redox environments; (2) an understanding of the role of biogenic and abiotic redox-active products and intermediates in Fe, S, and contaminant transformations; (3) a mechanistic understanding of the factors controlling the mass transfer of Fe, S, and contaminants in heterogeneous media; and (4) an in-depth understanding of the relationship of microbial community dynamics and function and coupled biotic-abiotic controls and their effects on major/minor element cycling and contaminant transformations.* Addressing these knowledge gaps has driven the development of 14 specific hypotheses to be tested within the Argonne SBR SFA.

At the core of our Science Plan are sophisticated molecular speciation measurements, performed at the APS, and omics analyses, using next-generation sequencing and bioinformatics approaches, that provide insights into biogeochemical reaction mechanisms and pathways as components of larger reaction networks driven by microbial communities. This 3-yr science plan focuses on Fe and S redox transformations, driven by microbial communities, and their effects on the transformation of major and minor elements, nutrients, and contaminants at different spatial scales. Experiments at Argonne (Dion Antonopoulos, Ted Flynn, Ken Kemner, Ed O'Loughlin) — in collaboration with the Bulgarian Academy of Sciences (Max Boyanov); Illinois Institute of Technology (Bhoopesh Mishra, Carlo Segre); University of Iowa (Michelle Scherer, Drew Latta); Korean Institute of Science and Technology (Man-Jae Kwon); North Central College, Naperville, Illinois (Silvia Alvarez-Clare); and Pacific Northwest National Laboratory (Jim Fredrickson, John Zachara, Chongxuan Liu, Tim Schiebe, Kevin Rosso) — requires the integration of microbial ecology, microbiology, molecular biology, geochemistry, physics, and modeling approaches. Members of the Argonne SBR SFA team represent expertise in all of these disciplines. Research will emphasize laboratory-based experiments with single-crystalline-phase Fe oxides (including oxides, oxyhydroxides, and hydroxides) and Fe-containing clays (e.g., smectite, illite, chlorite), fabricated Fe-rich mineral assemblies designed to mimic mineralogical conditions in subsurface environments in the field, and geomaterial collected from field environments (e.g., groundwater-Columbia river water mixing zone

at the Hanford 300 Area at Richland, Washington, and stream sediments around Oak Ridge National Laboratory). Inocula for promoting bioreducing or biooxidizing conditions will include (1) monocultures of well-characterized organisms representing dominant genera with recognized roles in Fe and S redox transformations (e.g., *Geobacter* spp., *Shewanella* spp., *Desulfitobacterium* spp., *Desulfovibrio* spp., *Desulfotomaculum* spp., *Thiobacillus* spp., *Acidithiobacillus* spp.), and (2) natural microbial consortia collected from all of these sites. To improve our ability to identify cryptic biogeochemical processes within larger complex reaction networks, we will use model minerals with mixed microbial communities to reduce the complexity of the mineralogical aspects of the system. Similarly, we will use model microbial monocultures to reduce the complexity of the microbial aspects of the system.

In addition to the unique molecular-scale electronic and chemical information provided by measurements at the APS, the experimental work of the Argonne SBR SFA will drive optimization of techniques at APS beamlines, thus increasing the availability and productivity of x-ray beamlines with the characteristics required for the proposed work and benefitting the larger international synchrotron-based biogeochemistry research community. The focus will be on optimization of synchrotron-based hard x-ray capabilities for *in situ* investigations of coupled microbiological and geochemical processes in free-flowing columns. Additional developments of hard x-ray Raman approaches for bulk sample analysis of C and N chemistries in natural samples will also benefit CESD research activities.

We also have a long-term vision of work to be performed during the six years following the end of the present Science Plan. This includes (1) an ever-increasing emphasis on integrating metagenomic-based analysis approaches for understanding community structural and functional controls on fundamental Fe and S biogeochemical processes, (2) an increasing emphasis on model development to predict the transformations and mobility of nutrients and contaminants in many subsurface and near-surface environments, and (3) expansion of these studies with a greater emphasis on using minerals and microbial communities from a network of field sites encompassing many types of redox-dynamic environments, including arctic and tropical sites and a midwestern field site presently being developed by Argonne.

2. Scientific Questions

The scientific questions focusing the work done within the Argonne Subsurface Science SFA are motivated by four major knowledge gaps currently hindering our understanding of the coupled biotic-abiotic molecular- to core-scale transformations of Fe and S in redox-dynamic environments and understanding of the effects of Fe and S biogeochemistry on transformation and mobility of major/minor elements and contaminants. Providing data to fill these knowledge gaps is the central driver for this Science Plan comprising the Argonne Subsurface SFA. We list the Knowledge Gaps below. A detailed description of these Knowledge Gaps and a justification for our focus on them is contained within our Science Plan. Knowledge Gaps 4.2 and 4.3 were removed from this Science Plan because the requested \$200K/year increase in support to cover their exploration was not provided.

Knowledge Gap #1 (KG1): An in-depth understanding of the molecular processes affecting Fe, S, and contaminant speciation in dynamic redox environments.

Knowledge Gap #2 (KG2): An understanding of the role of biogenic and abiotic redox-active products and intermediates in Fe, S, and contaminant transformations.

Knowledge Gap #3 (KG3): A mechanistic understanding of the factors controlling the mass transfer of Fe, S, and contaminants in heterogeneous media.

Knowledge Gap #4 (KG4): An in-depth understanding of the relationship between microbial community dynamics and function and coupled biotic-abiotic controls and their effects on major/minor element cycling and contaminant transformations.

To summarize the importance of these Knowledge Gaps, *a fundamental knowledge of the specific mechanisms of microbial redox processes and the links between microbial community function/dynamics and redox transformations of C, Fe, and S is needed to understand the effects of Fe and S biogeochemistry on transformation and mobility of major/minor elements and contaminants.*

We are leveraging decades of total experience in synchrotron-based Molecular Environmental Science (MES) research among members of the Argonne SFA team, the unique capabilities of the APS, and our unique local association with the APS to pursue the development and advancement of a number of synchrotron-based capabilities at the APS, particularly capabilities focused on x-ray absorption spectroscopy (XAS) and x-ray microscopy, that are critical to the Argonne SFA and are of service to the SBR scientific community. In addition, we are leveraging unique capabilities at Argonne that enable the use of DNA sequencing technologies and bioinformatics approaches to determine microbial community structure and function in our samples. The further development and integrated use of synchrotron-based and DNA sequencing and bioinformatics approaches with standard laboratory-based biogeochemical experimental approaches for characterizing microbiological and geochemical processes controlling transformations of major/minor elements and contaminants in the subsurface is central to the Argonne SBR SFA and represents unique capabilities that Argonne contributes to the SBR mission.

Fourteen hypotheses related to these knowledge gaps guide the experimental design of the Argonne Subsurface SFA so as to investigate transformations over a range of spatial scales. Experiments are done using mixed batch reactors (MBRs) and columns.

3. Argonne Subsurface SFA Program Structure

The figure below shows the organizational structure of the Argonne Subsurface Science SFA. The *research manager*, with support from the PI, is responsible for communicating with program managers in the SBR office regarding program direction and progress. The research manager is responsible for transmitting information from the SBR program office to the CELS ALD and the Laboratory Director, ensuring that SBR mission needs are represented in the Argonne strategic plan, and for transmitting information from the SBR program office to the SBR SFA PI and Co-PI. The *PI* is responsible for communicating with the SFA team members to integrate the experimental work efficiently. This communication occurs through regular group meetings (at least monthly and often weekly) at which the PI, Co-PI, and local members of the SFA discuss research progress in the context of the stated research objectives and deliverables. The research manager attends these meetings when possible; the PI is responsible for providing reports on program progress to the research manager quarterly and assists the research manager with presentations to the SBR program managers, as well as to the CELS ALD.

The key Argonne scientific staff responsibilities for FY2016 are detailed below.

Dr. Ken Kemner's efforts (50%) are focused on synchrotron beamline development and applications, and as a secondary lead on subsurface biogeochemistry and spectroscopic characterizations. Dr. Kemner also assists with the MBR and column experiments and serves as SFA PI.

Dr. Ed O'Loughlin's efforts (50%) are focused on subsurface biogeochemistry, and as a secondary lead on metagenomics-enabled science. Dr. O'Loughlin also serves as the SFA Co-PI.

Dr. Antonopoulos efforts (10%) are focused on metagenomics-enabled science.

Dr. Flynn, an early-career scientist, serves as a secondary lead on biogeochemistry and metagenomics-enabled science. He brings value to the SFA at no cost as his effort on projects related to the SBR SFA is supported under a strategic hire LDRD at Argonne.

Key non-Argonne SFA members are described below.

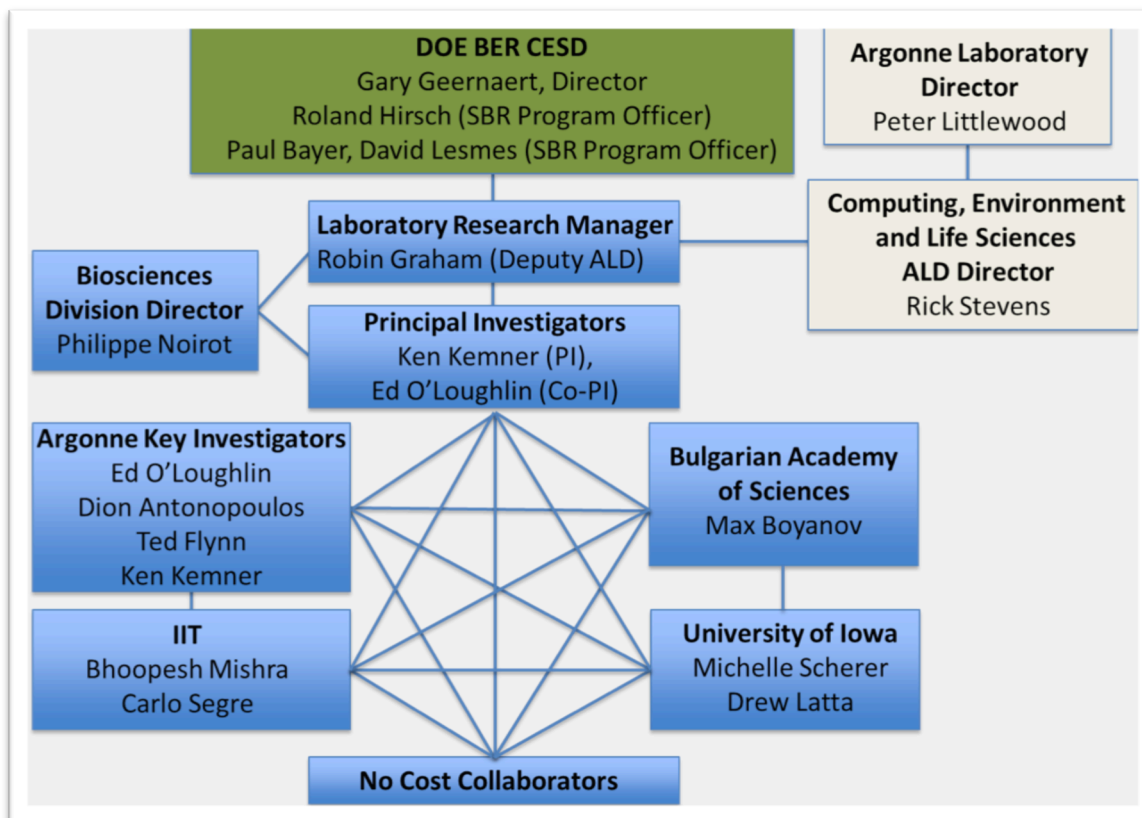
Dr. Boyanov (Bulgarian Academy of Science) is the co-lead for spectroscopic characterization and a secondary lead for beamline development and applications. Dr. Boyanov travels from Bulgaria to Argonne so that he may participate in experimental beam runs at the APS. He remains at Argonne for approximately an additional week after each of the six beam runs per year to participate in face-to-face collaborative discussions with other members of the Argonne SBR SFA team.

Dr. Mishra (Illinois Institute of Technology) is a co-lead on spectroscopic characterization and secondary lead for beamline development and applications through an Argonne Guest Appointment.

Dr. Scherer (University of Iowa) originally was identified as a secondary lead for spectroscopic characterization, and a lead for Mössbauer characterization. Similarly, Dr. Latta (University of Iowa) was identified as a secondary lead on spectroscopic characterization, particularly as it relates to investigations involving clays and U. However, since the funds to cover their efforts were not provided, the contribution from University of Iowa collaborators has been eliminated from this work. Limited contributions from the University of Iowa collaborators in the form of sample preparation have been provided as no-cost collaborations.

The roles of additional no-cost collaborators relate to supplying field materials (Fredrickson, Zachara, and Brooks), model development and integration with experiments (Liu, Schiebe), investigation of fundamental biogeochemical processes related to clays (Rosso, Zachara, Fredrickson), biogeochemical experimental work in the laboratory (Kwon), and operation of the MRCAT/EnviroCAT beamlines at the APS (Segre).

In FY2017, the responsibilities of team members will remain essentially the same as described above for FY2016. However, Dr. Mishra primarily will be stationed at the University of Leeds and will no longer be supported by Argonne SBR SFA programmatic funds. Although not supported financially in FY2017, it is expected that Dr. Mishra will finish development and submission of manuscripts related to electron transfer reactions between Hg and Mn. We will investigate the potential to replace Dr. Mishra's efforts via the partial support of a postdoctoral appointee.



4. Performance milestones and metrics

a. FY 2016 Scientific progress

i. Progress toward milestones

The milestones proposed for FY2016, including the knowledge gap and hypotheses numbers associated with that knowledge gap that the milestones address, along with the names of the SFA team members responsible for that milestone, are listed below in bold letters followed by a brief description of our progress to date. We have met all 8 of our milestones. Finally, in addition to the work described in our original Science Plan, we have continued to make progress in the development of x-ray Raman approaches to characterize the bonding environment of carbon in soil and sediment samples. A description of the opportunity to establish x-ray Raman capabilities at the MRCAT/EnviroCAT at the APS will be presented to DOE program officers during Kemner's and Flynn's visit to DOE Headquarters on July 11th. A discussion of the results associated with each FY 2016 milestone is included below.

Examine the effects of the structural environment of Fe^{II} in clays on the reduction of U^{VI}; prepare manuscript (KG1.1). Boyanov, O'Loughlin, Kemner

Several of the clays that have been identified as sediment constituents (e.g., SYn-1 and SWy-2 montmorillonites, the nontronites Nau-1 and Nau-2, or synthetic hectorite SYnH-1) show a dramatic difference in Fe content (from <0.1% to 32% w/w). Studies have shown that clays are redox active (i.e., can affect contaminant transport) and that the redox-active Fe atoms can be present in the octahedral and/or tetrahedral sites of the clay structure, depending on Fe content and solution conditions. We hypothesize that Fe in the different sites will have different redox reactivity and overall role in redox transformations. To test that we have conducted experiments evaluating the interactions of U^{VI} with environmentally-relevant clay minerals under reducing groundwater conditions. The speciation of U in the solid phase was determined using synchrotron x-ray spectroscopy (XANES and EXAFS). We find that the Fe^{II} atoms in reduced clays (SWy-2, SYnH-1, NAu-1 and NAu-2) are all able to transfer electrons to aqueous U^{VI} , but the extent of U reduction depends on the type and amount of clay. These data are currently being interpreted to relate the results to differences in the speciation of Fe in the clay, which may require additional experiments and measurements. A manuscript describing the results is in preparation.

- **Initiate MBR experiments comparing the rates of U^{IV} oxidation with crystalline uraninite, nanoparticulate UO_2 , or monomeric U^{IV} as the starting phase; analyze the XAFS and chemical data, prepare manuscript (KG1.2). Boyanov, Kemner**

Despite the fact that the Uraninite mineral (UO_2) is the thermodynamically predicted and the most desirable end product of uranium bioremediation, there is now ample x-ray spectroscopy evidence that reduced sediments contain mostly complexed forms of U^{IV} (termed mononuclear U^{IV}). The properties of the latter are not well understood. We compared the oxidation kinetics and mechanisms of two potential products of U^{VI} reduction in natural systems, a nanoparticulate UO_2 phase and an amorphous U^{IV} -Ca- PO_4 phase. The valence and molecular structure of U was tracked by synchrotron x-ray absorption spectroscopy. We observed similar oxidation rates for the two phases in solutions equilibrated with atmospheric O_2 and CO_2 . However, when ubiquitous environmental ions such as Calcium and Phosphate are present in the groundwater the transformation rates and pathways change. Addition of up to 400 μM Ca and PO_4 decreased the oxidation rate by an order of magnitude for both UO_2 and U^{IV} -phosphate. In the absence of Ca or PO_4 , the product of UO_2 oxidation was Na-uranyl oxyhydroxide, whereas the product of U^{IV} -Ca- PO_4 oxidation was an U^{VI} -phosphate phase (autunite). In the presence of Ca or PO_4 the oxidation proceeded to U^{VI} -phosphate for both pre-oxidation forms of U^{IV} . Addition of Ca or PO_4 changed the mechanism of oxidation by causing the formation of a passivation layer on the surfaces of the particles. These new reaction pathways need to be included in Reactive Transport Models to improve the accuracy of uranium transport predictions in the field. The findings of this work were published recently in “Effects of calcium and phosphate on uranium(IV) oxidation: Comparison between nanoparticulate uraninite and amorphous U(IV)-phosphate” D. E. Latta, K. M. Kemner, B. Mishra, M. I. Boyanov, *Geochimica et Cosmochimica Acta*, 174 (2016) 122-142.

- **Initiate MBR studies of U^{VI} reduction in the presence of clays: establish protocols for clay purification and preparation; set up reactors with U^{VI} , clays, and reductants; perform synchrotron x-ray measurements (KG1.3). Boyanov, O’Loughlin, Kemner**

Different protocols for the purification of clay minerals have been tested and established for the preparation of clay suspensions with known particle size and extent of Fe reduction. We have begun experiments to evaluate the interactions of synthetic and natural montmorillonite clays, SYn-1 and SWy-2, as well as nontronite NAu-1, with U^{VI} under reducing groundwater conditions (2 mM bicarbonate, pH 7.2, presence or absence of a reduced electron shuttle, AH₂DS). The speciation of U in the solid phase is probed using spectroscopic techniques (XANES and EXAFS). Previous work by our group found that reduction of U^{VI} to U^{IV} in the presence of magnetite and TiO₂ resulted in the predominance of adsorbed U^{IV} complexes or the formation of 2-5 nm uraninite particles (UO₂), depending on whether a mineral-specific surface coverage threshold was exceeded. In contrast to these results, the studied clay minerals were unable to stabilize adsorbed U^{IV} species at any of the studied U surface coverages, suggesting that clay surfaces do not provide sites for the stabilization of adsorbed U^{IV} in natural sediments. Interestingly, the speciation of uranium in the clay reactors depends on the reducing capacity in the system (i.e., on Fe^{II} concentration), with uraninite being predominant in systems with high loadings of reduced Fe-containing clays and a mixed valence U^V - U^{VI} mineral forming at low clay loading. Ongoing work is defining the conditions leading to the mixed valence U^V - U^{VI} mineral. The observed stabilization of U^V - U^{VI} species by clays presents new challenges to the inclusion of appropriate reactions for reduced U in the reactive transport models that are used to model U migration at the field-scale. The submission of a manuscript is planned for the end of FY2016.

Investigate the reduction of Hg^{II} to Hg^0 by Mn^{II} in the presence of complexing ligands (e.g., SO_4^{2-} and Cl^-) (KG1.4). Mishra, O'Loughlin, Boyanov, Kemner

Our studies over the past few years demonstrated that Hg^{II} is reduced to Hg^0 by many forms of Fe^{II} due to the low redox potential of Hg^{II} reduction to Hg^0 . Hence we expanded our research and tested the ability of Mn to reduce Hg^{II} . Our results show that at pH 7.5, the majority of Hg^{II} is reduced to Hg^0 within the first three hours, followed by significantly slower rates of reaction. Hg^{II} reduction is coupled with oxidation of soluble Mn^{II} to highly insoluble Mn^{IV} oxide. Formation of a Mn^{IV} oxide mineral auto-catalyzes the reaction for the first few hours, followed by significantly slower rates of reaction concomitant with changes in the Mn^{IV} mineral phase. In contrast to our hypothesis that the presence of minerals would surface catalyze reduction of Hg^{II} to Hg^0 , our results suggest that the presence of redox inactive mineral surfaces (e.g. Al₂O₃ and TiO₂) does not enhance the rate of Hg^{II} reduction. Additionally, Hg^{II} reduction by Mn^{II} in the presence of complexing ions (e.g., Cl^- and SO_4^{2-}) results in the formation of meta-stable Hg^I species, which slowly transforms to Hg^0 over an extended period of time. It is worth mentioning that synchrotron x-ray techniques are uniquely suitable for detection of meta-stable Hg^I species, which has been previously thought to quickly disproportionate to Hg^{II} to Hg^0 .

Hg-Mn interactions could have important implications in redox dynamic environments due to coupled reduction of Hg by Mn under circumneutral pH environments. Incorporating these newly found Hg redox pathways in global Hg cycle models may improve the predictive capabilities of Hg transport, and estimation of the size of the pool of bioavailable Hg for biochemical processes like methylation. These results are currently being drafted as a manuscript to be submitted to Environmental Science and Technology before the end of this fiscal year.

- **Initiate MBR studies of U^{IV} reoxidation in redox-buffered systems: set up U^{IV} oxidation reactors in the presence of iron oxides (magnetite, green rust); perform x-ray measurements over time; analyze XAFS data (KG2.1). Boyanov, O'Loughlin, Kemner**

We have conducted experiments to evaluate the capacity of reduced Fe^{II} -containing phases in the environment (e.g., magnetite) to buffer the reoxidation of U^{IV} and thus prevent U mobility. Results show that U^{IV} oxidizes more slowly in the presence of magnetite. We are continuing with additional experiments aimed at gaining a detailed understanding of the mechanism, i.e. to discern between magnetite consuming dissolved O_2 and thus preventing it from reacting with U^{IV} , or magnetite coming in contact with the U^{IV} phase as a semiconductor and resupplying the electrons consumed during the oxidation of U^{IV} . The submission of a manuscript describing these results is planned for the end of calendar year 2016.

- **Conduct experiments on the effects of electron shuttles on the microbial oxidation of Fe^{II} in clays in systems with one Fe^{II} -oxidizing bacterial species; prepare manuscript (KG2.3). O'Loughlin, Flynn, Boyanov, Kemner**

We are currently preparing and characterizing (Fe XAFS and Mössbauer spectroscopy) more than 20 candidate clays representing a diverse range of mineralogical forms for use in studies to determine the effects of the structural location of Fe in these minerals on their redox properties. A subset of these clays will then be used in experiments examining the effects of electron shuttles on the microbial oxidation of Fe^{II} in clays by both single species of Fe^{II} -oxidizing bacteria and by native microbial communities in subsurface sediments. We are currently finalizing the experimental design and analytical approaches and anticipate that the experimental work will be completed early in FY2017.

- **Perform reactive transport simulations for F and S dynamics in the x-ray accessible columns; initiate column experiments based on the conditions determined from the simulations; perform x-ray measurements to determine the speciation of Fe and S along the column; refine the reactive transport model per the experimental results; iterate (KG3.1). Boyanov, O'Loughlin, Kemner**

We are continuing to use flow-through columns with x-ray-transparent windows (developed during prior years of the Argonne SBR SFA) to characterize the coupled biological, chemical, and physical factors that influence subsurface biogeochemical processes and the transport of contaminants. In these columns, we monitor key parameters including Fe and S mineral transformations, as well as contaminant spatial distribution and speciation (e.g., adsorbed or precipitated species) in heterogeneous regions of the sediments. We developed a method for flat-field correction of the geometric effects in the x-ray fluorescence intensity coming from the column (e.g., from bulging of the window or from shadowing near its edge). We have examined the transformations in columns constructed with ferrihydrite-coated quartz sand through which sulfide-containing solutions at pH 7.2 were passed. A collaborative relationship with Dr. Chongxuan Liu and his group at PNNL provided simulations of the spatiotemporal profiles of Fe and S species based on published reactions and the defined physical conditions in the column. We found significant differences between the predictions of the model and the progression of FeS formation in the column, as well as differences in the effluent Fe concentration profiles. We observed release of Fe^{II} from the column in the form of colloidal ferrous polysulfides, which are typically not accounted for in reactive transport models of Fe-S interactions. Work is continuing to define the differences between model and experiment and the dependency of these differences on experimental conditions. A manuscript describing these findings is expected to be submitted during FY2017.

Conduct experiments examining the effects of electron shuttles on the microbial reduction of Fe^{III} oxides and Fe^{III} in clays and microbial community development in systems inoculated with native microbial communities in soils/sediments; prepare manuscript (KG4.1). O'Loughlin, Flynn, Boyanov, Kemner

Iron redox cycling by microorganisms is a significant component of C cycling and energy flux in many aquatic and terrestrial environments. Dissimilatory iron-reducing bacteria (DIRB) are phylogenetically diverse microorganisms that obtain energy by coupling oxidation of organic compounds or H₂ to reduction of Fe^{III} to Fe^{II}. Because of the relative insolubility of most Fe^{III}-bearing minerals, many DIRB use soluble electron shuttles (e.g., quinones, flavins, phenazines, and reduced sulfur species) to transfer electrons from the cell to external electron acceptors. Studies investigating effects of electron shuttles on microbial Fe^{III} reduction have typically been conducted under axenic conditions. To better understand how electron shuttles influences microbial Fe^{III} reduction in the presence of a diverse microbial community, we examined the effects of different electron shuttles (9,10-anthraquinone-2,6-disulfonate acid (AQDS), 9,10-anthraquinone-2-carboxylic acid (AQC), and 5-hydroxy-1,4-naphthoquinone (lawsone, NQL)) on the bioreduction of Fe^{III} oxide and methanogenesis in microcosms inoculated with wetland sediment. Our results show no significant enhancement of Fe^{III} reduction in the presence of NQL or AQC relative to the no shuttle (NS) control; however both the rate and extent of Fe^{II} production were enhanced in the presence of AQDS. The onset of methanogenesis was earlier in the presence of AQDS compared to NQL and NS, but in each case methane production was not evident until Fe^{II} production plateaued. Methanogenesis was completely inhibited in the presence of AQC, highlighting the potential for electron shuttles to influence microbial processes not involving microbial respiration using insoluble electron acceptors. Systems amended with AQC were dominated by microorganisms classified in the family *Pelobacteraceae* (avg. 45.4% total abundance), while *Geobacteraceae* dominated in microcosms amended with AQDS (30.48%), NQL (51.9%), or NS (37%). *Geobacteraceae* sequences were of much lower abundance in the AQC enrichments, accounting for only 8% of the total abundance on average and only 3–4% in two of the three replicates. While closely related to the *Geobacteraceae*, organisms in *Pelobacteraceae* lack c-type cytochromes and are unable to transfer electrons directly to ferric iron. This suggests AQC may inhibit direct reduction of ferric iron by organisms such as *Geobacter*, allowing *Pelobacter* spp., which would otherwise be outcompeted, to dominate. The submission of a manuscript describing these results is planned for the end of calendar year 2016.

ii. Science Highlights

Argonne SFA team members have given at least 50 presentations, including 19 invited talks and 31 contributed talks (see Appendix B). Contributed presentations included four international conferences or facilities (*Goldschmidt* 2015 in Prague, Czech Republic; *Goldschmidt* 2016 in Yokohama, Japan; XAFS 16 in Karlsruhe, Germany; The Canadian Light Source). In addition to national scientific conferences, SFA scientific staff also presented invited talks at various universities and national laboratories, as well as invited talks for local community outreach opportunities.

Since our last SFA Science Plan review (April 27, 2015), 9 papers related to the SFA research efforts were accepted for publication (see Appendix A). Two additional manuscripts are presently in review. Specific Argonne SFA scientific highlights summarized from seven of these publications are given below to illustrate some of the foci of the SFA. The names of authors who are members of the Argonne SBR SFA are listed in bold letters.

Impact of organic carbon electron donors on microbial community development under iron- and sulfate-reducing conditions. **M. J. Kwon**, **E. J. O'Loughlin**, **M. I. Boyanov**, J. M. Brulc, E. R. Johnston, **K. M.**

Kemner, and D. A. Antonopoulos. (2016) *PLOS ONE*. **11**(1): e0146689. doi:10.1371/journal.pone.0146689. To better understand the effects of electron donor on microbial community development under Fe(III)- and sulfate-reducing conditions, we created bicarbonate-buffered batch systems with acetate, lactate, or glucose as the electron donor and ferrihydrite and sulfate as the electron acceptors. The batch systems were inoculated with the native microbial community in sediment from the Rifle, Colorado, IFRC site. Mineral transformations were monitored by x-ray diffraction (XRD) and XAFS, and changes in the microbial communities were determined from 16S rRNA-based sequence inventories. All electron donors tested promoted ferrihydrite reduction to varying extents: glucose >> lactate > acetate. The rates and extents of sulfate reduction were faster with lactate than with acetate, while glucose did not stimulate sulfate reduction. 16S rRNA-based sequence analysis of the communities over time revealed that *Desulfotomaculum* was the major driver for sulfate reduction coupled with propionate oxidation in lactate-amended incubations. The reduction of sulfate resulted in sulfide production and subsequent abiotic reduction of ferrihydrite. In acetate-amended incubations, no evidence of Fe^{III} and sulfate reduction was observed until day 28. Members of the family *Peptococcaceae* (a family within the Firmicutes), including *Desulfotomaculum* spp., were dominant, and acetate oxidation was coupled with Fe^{III} and sulfate reduction. Additionally, an increase in a population in the order Desulfuromonadales (class Deltaproteobacteria) was observed between days 28 and 50, though the ability to reduce sulfate is not recognized among species in this order. In contrast, glucose promoted faster reduction of ferrihydrite, but without reduction of sulfate. Interestingly, the glucose-amended incubations led to two different biogeochemical trajectories among replicate bottles. The two outcomes in geochemical evolution might be due to the stochastic evolution of the microbial communities or subtle differences in the initial composition of the fermenting microbial community and its development via the use of different glucose fermentation pathways available within the community. Synchrotron-based x-ray analysis indicated that siderite and amorphous Fe(II) were formed in the replicate bottles with glucose, while ferrous sulfide and vivianite were formed with lactate or acetate. These data sets reveal that use of different C utilization pathways projects significant changes in microbial community composition over time that in turn lead to significant differences in Fe(II) production and secondary mineral formation.

Effects of calcium and phosphate on uranium(IV) oxidation: Comparison between nanoparticulate uraninite and amorphous U(IV)-phosphate. D. E. Latta, **K. M. Kemner, B. Mishra, M. I. Boyanov.** *Geochimica et Cosmochimica Acta*, 174 (2016) 122-142. This study compares the oxidation kinetics and mechanisms of two potential products of U^{VI} reduction in natural systems, a nanoparticulate UO₂ phase and an amorphous U^{IV}-Ca-PO₄ analog to ningyoite (CaU^{IV}(PO₄)₂·1-2H₂O). The valence of U was tracked by x-ray absorption near-edge spectroscopy (XANES), showing similar surface-normalized oxidation rate constants for U^{IV}O₂ and U^{IV}-phosphate in solutions equilibrated with atmospheric O₂ and CO₂ at pH 7.0 ($k_{\text{obs,UO}_2} = 0.17 \pm 0.075 \text{ h}^{-1}$ vs. $k_{\text{obs,U}^{\text{IV}}\text{PO}_4} = 0.30 \pm 0.25 \text{ h}^{-1}$). Addition of up to 400 μM Ca and PO₄ decreased the oxidation rate constant by an order of magnitude for both UO₂ and U^{IV}-phosphate. The intermediates and products of oxidation were tracked by electron microscopy, powder x-ray diffraction (pXRD), and extended x-ray absorption fine-structure spectroscopy (EXAFS). In the absence of Ca or PO₄, the product of UO₂ oxidation is Na-uranyl oxyhydroxide (under environmentally relevant concentrations of sodium, 15 mM NaClO₄ and low carbonate concentration), resulting in low concentrations of dissolved U^{VI} ($<2.5 \times 10^{-7} \text{ M}$). Oxidation of U^{IV}-phosphate produced a Na-autunite phase (Na₂(UO₂)PO₄·xH₂O), resulting in similarly low dissolved U concentrations ($<7.3 \times 10^{-8} \text{ M}$). When Ca and PO₄ are present in the solution, the EXAFS data and the solubility of the U^{VI} phase resulting from oxidation of UO₂ and U^{IV}-phosphate are consistent with the precipitation of Na-autunite. Bicarbonate extractions and Ca K-edge x-ray absorption spectroscopy of oxidized solids indicate the formation of a Ca-U^{VI}-PO₄ layer on the UO₂ surface and suggest a passivation layer mechanism for the decreased rate of UO₂ oxidation in the presence of Ca and PO₄. Interestingly, the extractions were unable to remove all of

the oxidized U from partially oxidized UO_2 solids, suggesting that oxidized U is distributed between the interior of the UO_2 nanoparticles and the labile surface layer. Accounting for the entire pool of oxidized U by XANES is the likely reason for the higher UO_2 oxidation rate constants determined here relative to prior studies. Our results suggest that the natural presence or addition of Ca and PO_4 in groundwater could slow the rates of U^{IV} oxidation, but that the rates are still fast enough to cause complete oxidation of U^{IV} within days under fully oxygenated conditions.

Reaction of U(VI) with green rusts: Effect of interlayer anion, D. E. Latta, **M. I. Boyanov, K. M. Kemner, E. J. O'Loughlin**, M. M. Scherer. *Current Inorganic Chemistry* 5, 156-168 (2015). Iron is the fourth most abundant element in the Earth's crust and green rusts are widely recognized as important intermediates in the biogeochemical cycling of iron. They can be produced by bacteria from iron contained in sediments or by the corrosion of metallic iron. Green rusts can come in contact with uranium when contaminated water propagates through the ground or when spent nuclear fuel interacts with compromised storage canisters. Understanding the effect of iron cycling on uranium mobility is therefore important for protecting public safety and for the continued use of uranium in a balanced energy production portfolio. Here, we investigated the interaction between dissolved uranium and several types of chemically-synthesized green rust (carbonate, sulfate, and chloride). All of the uranium was removed from solution and attached to the iron oxide solids in less than two hours, regardless of the type of green rust. Similar kinetics of U^{VI} uptake on green rusts and pyroaurite suggested that the observed uptake kinetics reflect a sorption step rather than reduction of U^{VI} by structural Fe^{II} in the green rusts. However, using advanced synchrotron x-ray techniques at the Advanced Photon Source we found that removal was due to a reductive transformation of the more soluble U^{VI} form to the less soluble U^{IV} form. Surprisingly, the reduced U^{IV} atoms did not aggregate to form the least soluble and most stable mineral uraninite (UO_2), but were instead dispersed as individual atoms bound to the iron oxide surface. This finding is important because until recently the formation of such non-uraninite U^{IV} phases was thought to be purely a biological process. In addition, the formation of surface-bound U^{IV} presents new challenges in describing the movement of U in natural environments, as the stability of this U^{IV} form relative to the currently assumed uraninite is not known.

Effects of phosphate on secondary mineral formation during the bioreduction of akaganeite ($\beta\text{-FeOOH}$): Green rust versus framboidal magnetite, **E. J. O'Loughlin**, C. A. Gorski, M. M. Scherer. *Current Inorganic Chemistry* 5, 214-224 (2015). The activity of microorganisms plays a key role in the biogeochemical cycling of Fe in aquatic and terrestrial environments, where green rusts are often observed as intermediates or products of microbially driven oxidative and reductive processes. To further our understanding of the factors controlling the formation of green rust as a secondary mineral during microbial reduction of Fe^{III} oxides, we examined the effects of the presence of an electron shuttle [9,10-anthraquinone 2,6 disulfonate (AQDS)] and phosphate on the bioreduction of the Fe^{III} oxide akaganeite by the iron(III)-reducing bacterium (IRB) *Shewanella putrefaciens* CN32. Framboidal magnetite (named for the raspberry-like appearance of the nominally spherical aggregates of fine-grained magnetite particles) was the principal secondary mineral formed during akaganeite bioreduction in the absence of phosphate; this is the first report of formation of framboidal magnetite as a product of microbial Fe^{III} oxide reduction. Framboidal magnetite was less crystalline when formed in the presence of AQDS than without AQDS and over time was further reduced to chukanovite. Carbonate green rust was the dominant secondary mineral observed from akaganeite bioreduction in the presence of phosphate, with and without AQDS; however, siderite was also observed in the presence of AQDS. This first report of green rust as a product of akaganeite bioreduction expands the range of Fe^{III} oxides that can be transformed to green rust by IRB, suggesting that the bioreduction of Fe^{III} oxides such as ferrihydrite, lepidocrocite, and akaganeite by IRB is a key process leading to the presence of green rusts in aquatic and terrestrial environments.

Solute concentrations influence microbial methanogenesis in coal-bearing strata of the Cherokee basin, USA. M. F. Kirk, B. H. Wilson, K. A. Marquart, L. H. Zeglin, D. S. Vinson, **T. M. Flynn**. *Frontiers in Microbiology* Vol. 6(1287), p 1–14 (2015). Microorganisms have contributed significantly to subsurface

energy resources by converting organic matter in hydrocarbon reservoirs into methane, the main component of natural gas. In this study, we consider environmental controls on microbial populations in coal-bearing strata of the Cherokee basin, an unconventional natural gas resource in southeast Kansas, USA. Pennsylvanian-age strata in the basin contain numerous thin (0.4-1.1 m) coalbeds with marginal thermal maturities (0.5-0.7% R_o) that are interbedded with shale and sandstone. We collected gas, water, and microbe samples from 16 commercial coalbed methane wells for geochemical and microbiological analysis. The water samples were Na-Cl type with total dissolved solids (TDS) content ranging from 34.9 to 91.3 g L⁻¹. Gas dryness values averaged 2640 and carbon and hydrogen isotope ratios of methane differed from those of carbon dioxide and water, respectively, by an average of 65 and 183‰. These values are thought to be consistent with gas that formed primarily by hydrogenotrophic methanogenesis. Results from cultivation assays and taxonomic analysis of 16S rRNA genes agree with the geochemical results. Cultivable methanogens were present in every sample tested, methanogen sequences dominate the archaeal community in each sample (avg. 91%), and few archaeal sequences (avg. 4.2%) were classified within *Methanosarcinales*, an order of methanogens known to contain methylotrophic methanogens. Although hydrogenotrophs appear dominant, geochemical and microbial analyses both indicate that the proportion of methane generated by acetoclastic methanogens increases with the solute content of formation water, a trend that is contrary to existing conceptual models. Consistent with this trend, beta diversity analyses show that archaeal diversity significantly correlates with formation water solute content. In contrast, bacterial diversity more strongly correlates with location than solute content, possibly as a result of spatial variation in the thermal maturity of the coalbeds.

Thiosulfate oxidation by *Thiomicrospira thermophila*: metabolic flexibility in response to ambient geochemistry. J. L. Houghton, D. Foustoukos, **T. M. Flynn**, C. Vetriani, A. S. Bradley, D. A. Fike (2016) *Environmental Microbiology*, doi: 10.1111/1462-2920.13232. Previous studies of the stoichiometry of thiosulfate oxidation by colorless sulfur bacteria have failed to demonstrate mass balance of sulfur, indicating that unidentified oxidized products must be present. Here we present reaction stoichiometry and kinetics under variable pH conditions during the growth of *Thiomicrospira thermophila* strain EPR85. At pH 8.0, thiosulfate is stoichiometrically converted to sulfate. At lower pH, the products of thiosulfate oxidation are extracellular elemental sulfur and sulfate. We were able to replicate previous experiments and identify the missing sulfur as tetrathionate, consistent with previous reports of the activity of thiosulfate dehydrogenase. Tetrathionate was formed under slightly acidic conditions. Genomic DNA from *T. thermophila* strain EPR85 contains genes homologous to those in the Sox pathway (*soxXYZBCDL*), as well as rhodanese and thiosulfate dehydrogenase. No other sulfur oxidizing bacteria containing *sox(CD)*₂ genes have been reported to produce extracellular elemental sulfur. If the apparent modified Sox pathway we observe in *T. thermophila* is present in marine *Thiobacillus* and *Thiomicrospira* species, production of extracellular elemental sulfur may be biogeochemically important in marine sulfur cycling.

Transhydrogenase and growth substrate influence lipid hydrogen isotope ratios in *Desulfovibrio alaskensis* G20. W. D. Leavitt, **T. M. Flynn**, M. K. Suess, A. S. Bradley (2016) *Frontiers in Microbiology*, Vol. 7(918), p. 1–14. Microbial fatty acids preserve metabolic and environmental information in their hydrogen isotope ratios (²H/¹H). This ratio is influenced by parameters that include the ²H/¹H of water in the microbial growth environment, and biosynthetic fractionations between water and lipid. In some microbes, this biosynthetic fractionation has been shown to vary systematically with central energy metabolism, and controls on fatty acid ²H/¹H may be linked to the intracellular production of NADPH. We examined the apparent fractionation between media water and the fatty acids produced by *Desulfovibrio alaskensis* G20. Growth was in batch culture with malate as an electron donor for sulfate respiration, and with pyruvate and fumarate as substrates for fermentation and for sulfate respiration. A larger fractionation was observed as a consequence of respiratory or fermentative growth on pyruvate than growth on fumarate or malate. This difference correlates with opposite apparent flows of electrons through the electron bifurcating/confurcating transhydrogenase NfnAB. When grown on malate or fumarate, mutant strains of *D. alaskensis* G20 containing transposon disruptions in a copy of *nfnAB* show different fractionations than the wild type strain. This phenotype is muted during fermentative growth on

pyruvate, and it is absent when pyruvate is a substrate for sulfate reduction. All strains and conditions produced similar fatty acid profiles, and the $^2\text{H}/^1\text{H}$ of individual lipids changed in concert with the mass-weighted average. Unsaturated fatty acids were generally depleted in ^2H relative to their saturated homologs, and anteiso-branched fatty acids were generally depleted in ^2H relative to straight-chain fatty acids. Fractionation correlated with growth rate, a pattern that has also been observed in the fractionation of sulfur isotopes during dissimilatory sulfate reduction by sulfate-reducing bacteria.

b. Future scientific goals

FY2017

- . Investigate the effects of structural Fe^{II} in clays on the reduction of Hg^{II} ; prepare manuscript (KG1.1).
- . Complete MBR studies of U^{VI} reduction in the presence of clays: explore the effects of ligands and surface:U ratio on U^{IV} speciation; complete synchrotron x-ray measurements, analyze data, prepare manuscript (KG1.3).
- . Investigate the reduction of Hg^{II} to Hg^0 by Mn^{II} in the presence of redox-inactive minerals. Prepare manuscript detailing results of ligand and mineral surface effects on Hg^{II} reduction by Mn^{II} (KG1.4).
- . Complete MBR studies of U^{IV} reoxidation in redox-buffered systems: set up oxidation reactors with reduced clays, perform x-ray measurements, analyze XAFS and chemical data, prepare manuscript (KG2.1).
- . Initiate MBR studies of redox cycling of clays and sulfur species: incubate Fe-rich clays with sulfide; characterize Fe/S speciation; cycle oxidation conditions and characterize the changes in Fe speciation (KG2.2).
- . Conduct experiments examining the effects of electron shuttles on the microbial oxidation of Fe^{II} in clays and microbial community development in systems inoculated with native microbial communities in soils/sediments (KG2.3 and KG4.1).
- . Investigate the reduction of Hg^{II} by biogenic Mn^{II} species; prepare manuscript (KG2.4).
- . Perform reactive transport simulations for F, S, and contaminant (U, Hg, Cr) dynamics in x-ray accessible columns; initiate column experiments based on the conditions determined from the simulations; perform x-ray measurements to determine the speciation along the column; refine the reactive transport model as needed; iterate. Finish experiments from year 1; prepare manuscript. (KG3.1).
- . Initiate column studies of U^{IV} remobilization under anoxic conditions: compare columns with uraninite or non-uraninite starting U^{IV} phases, with or without ferric iron oxides present; explore the effect of carbonate and phosphate; characterize the spatiotemporal species profiles by using x-ray techniques; characterize the effluents (KG3.2).
- . Continue MBR experiments examining microbial community evolution under Fe/S reducing conditions; expand to include addition of oxidant (O_2 , NO_3^-) following the consumption of added donor and onset of steady-state reducing conditions (KG4.2).

- . Analyze 16S rRNA-based microbial community profiles created over the course of MBR experiments; identify potential candidates for whole-genome shotgun metagenome analysis of potentially novel organisms (KG4.2).
- . Run replicate column experiments on specific oxidizing vs. reducing condition impacts over time; determine microbial community composition and structural changes over time via rRNA targeted surveys (KG4.3).
- . Obtain and analyze metagenomes of steady-state microbial communities (KG4.3).

c. Scientific results that may shift current research focus and/or identify new knowledge gaps

Results obtained to this point are on track with anticipated outcome and, in some cases, ahead of schedule.

We foresee new opportunities to apply synchrotron x-ray radiation produced at the APS to investigate the chemical and spatial characteristics of carbon within sediments and soils. Specifically, we have investigated the utility of non-resonant inelastic x-ray scattering (often referred to as x-ray Raman) for determining the chemical bonds associated with carbon atoms in soils. Results from the measurement of organic and inorganic standards, Alaskan Permafrost material, and soils from the Spruce Site indicate that this technique is very promising. We have also performed experiments at the APS to determine the feasibility of developing these capabilities at the MRCAT/EnviroCAT. Developing these capabilities at the MRCAT/EnviroCAT will provide the necessary access to this technique that we will need to address biogeochemical studies related to carbon chemistry within soils and sediments. We are requesting support in FY2017 from DOE BER to establish these capabilities at the MRCAT/EnviroCAT insertion device beam line.

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

Integration of scientific and technical expertise, plus communication among all of the researchers who are part of the Argonne Subsurface Biogeochemical Research (SBR) SFA, is critical for the success of the Argonne SBR SFA science program. Active participation by external collaborators with the Argonne staff provides complementary expertise essential to the productivity of the research program. The following activities between Argonne and external SFA researchers since March 2015 are worthy of note:

- . Beginning in June 2016, a graduate student (Jackie Mejia) from the lab of Dr. Eric Roden at the University of Wisconsin, Madison is spending 3-6 months with members of the SBR SFA to integrate biogeochemical and 'omics approaches into her research on Fe and C biogeochemistry.
- . Although presently not funded by the Argonne SBR SFA, collaborative research efforts with Drew Latta and Michelle Scherer from the University of Iowa in the study of the interactions of U with clays is continuing. Dr. Latta has participated in the preparation of samples for characterization at the Advanced Photon Source and will be coming to Argonne to participate in future beamruns.
- . Dr. Kemner met with Dr. Scott Brooks (ORNL) at the SBR PI meeting in 2016 to discuss the use of x-ray microspectroscopic imaging to determine the spatial distribution of the Hg valence state in biofilms collected from Hg contaminated streams on the Oak Ridge site. Synchrotron experiments are being planned for late summer or fall of 2016.
- . In August of 2015 and March of 2016, Dr. Kemner travelled to PNNL and met with Drs. Liu, Fredrickson, and Zachara to discuss collaborations between the PNNL and Argonne SBR SFAs. Although the frequency of these collaborative discussions have decreased during the past few years, the bonds that have been formed will enable the development of future collaborations when they are mutually beneficial to both SFAs.
- . Dr. Rob Sanford (University of Illinois, Urbana-Champaign (UIUC) has been overseeing a postdoctoral researcher (Yiran Dong) at UIUC who is collaborating with the SFA team. She is testing

the metabolic potential of deep subsurface metal-reducing bacteria isolated from groundwater collected from the Illinois Basin and their impact on the formation of secondary iron minerals. In addition to the two manuscripts that are presently in review, we expect an additional manuscript to result from this work that will be submitted for publication.

- Dr. Man-Jae Kwon, a research scientist at the Korean Institute of Science and Technology (KIST) Gangneun, has continued an active no-cost collaboration with the Argonne SBR SFA. He works closely with Drs. O'Loughlin, Boyanov, and Kemner on projects of mutual interest involving Fe and S biogeochemistry.
- Drs. O'Loughlin, Boyanov, Mishra, and Kemner worked closely with Dr. Sen Yan, an Assistant Professor in the Department of Geochemistry at the China University of Geosciences-Wuhan. As a visiting scholar at Argonne in 2015, Dr. Yan worked with the Argonne SBR SFA team to investigate U^{VI} interactions with biotic and abiotic green rusts and is currently working with the SFA team to finalize the results of his research and the preparation of a manuscript.

The role each external collaborator plays in the ANL SFA is shown below. Their contributions to specific milestones within the Argonne SBR SFA in the second half of FY2015 and 2016 were noted in the "Progress on Milestones" discussion above.

Maxim Boyanov: Physicist (stationed at the Bulgarian Academy of Science); Beginning in February 2014 Dr. Boyanov was no longer a local Argonne member of the Argonne SBR SFA. Experimental design and synchrotron data collection and analysis related to biogeochemical transformations of S, Fe, and U.

Bhoopesh Mishra: Physicist (Illinois Institute of Technology); synchrotron data collection and analysis related to abiotic transformations of Fe, Mn, S, and Hg and development of x-ray Raman capabilities for measurement of carbon chemistry in soils.

Jim Fredrickson/John Zachara: Environmental Microbiologist/Geochemist (PNNL); Consult on experimental design for material from the Columbia River-groundwater interface.

Chongxuan Liu: Reactive transport modeling (PNNL); Collaborate on reactive transport modeling of experimental column studies.

Scott Brooks: Environmental Engineer (ORNL); Collaborate on experimental design related to spatial distribution of Hg chemistry within biofilms.

Carlo Segre: MRCAT/EnviroCAT Associate Director (Illinois Institute of Technology); Operates MRCAT/EnviroCAT at the Advanced Photon Source.

5) Staffing and budget summary

a/b. Allocation by program tasks and to support external collaborations

The allocation of FY2016 funds is shown below.

| Institution | FY2016 Funding (\$K) | Status FY2017 (\$K) |
|--|----------------------|---------------------------|
| Argonne | | |
| Kemner | 280 | Active (300) |
| O'Loughlin | 280 | Active (300) |
| Antonopoulos | 50 | Active (50) |
| University of Iowa | 0 | Tentatively Active (50) |
| University of Illinois | 0 | 0 |
| Boyanov (Bulgarian Academy of Science) | 150 | Active (150) |
| Illinois Institute of Technology | 240 | Active (150) ¹ |

¹The subcontract to IIT will be decreased to \$150K to cover beam line support. Dr. B. Mishra's efforts will no longer be supported.

c. Personnel actions and procedures

Argonne SBR SFA staffing changes that occurred or will occur in FY2016 include the following: Dr. Bhoopesh Mishra primarily will be stationed at the University of Leeds instead of the Illinois Institute of Technology and will no longer be supported by Argonne SBR SFA programmatic funds. Although not supported financially in FY2017, it is expected that Dr. Mishra will finish development and submission of manuscripts related to electron transfer reactions between Hg and Mn. We will investigate the potential to replace Dr. Mishra's efforts. However, because of the rather small size of funding originally provided for Dr. Mishra's efforts and now available for other efforts, we can only partially support a postdoc to continue that line of research. In light of that, we are investigating the potential of supporting Dr. Drew Latta (University of Iowa) to participate in APS beam runs and to continue collaborations with the Argonne SBR SFA.

Argonne encourages new/young investigators to pursue innovative research ideas through LDRD support as well as proposals to funding agencies. Dr. Flynn (previous Argonne Director's Fellow) has been supported by Argonne LDRD funds to support his efforts after the expiration of his Director's Fellowship at the end of September 2013. Our new and young investigators are also provided with the opportunity to present their work at local, national, and international scientific conferences. Dr. Flynn's effort on the Argonne SBR SFA has been supported at no cost to the SFA. He presented the results of his work at the 24th International Goldschmidt Conference during the summer of FY2014 and at many other conferences (see appendix). The Laboratory views attendance and presentations at such meetings as providing both continuing education and networking opportunities to these staff members, which strengthens the SFA effort.

d. National Laboratory investment in the program

Argonne's Director's Post-doctoral Fellowship program provided support for Dr. Flynn's position in FY2012 and FY2013. This program is a highly competitive national program (<http://www.dep.anl.gov/postdocs/directorpostdoc.htm>) that requires an Argonne sponsor who will work together with the candidate to propose a project as the justification for the requested position. Dr. Kemner

recruited Dr. Flynn from the University of Illinois and, together, they produced a successful proposal that was also relevant to topics being addressed by the Argonne SBR SFA. Joint Argonne and University of Chicago funds were used to support Dr. Flynn in FY2014 and FY2015. Since the beginning of FY2016, Dr. Flynn has been supported as an Argonne Strategic Hire with LDRD funds.

Argonne provided LDRD funds (~\$225K/year) in FY2013-FY2015 to a project entitled “Identifying novel pathways for anaerobic microbial oxidation of methane to carbon dioxide” that included support for Dr. Ed O’Loughlin (Lead PI), Dr. Dion Antonopoulos, and Dr. Kemner. Argonne provided \$50K of support in FY2016 to a project entitled “Developing Remote Automated Sensors to Direct Sampling of Aerobic-Anaerobic Switching in Floodplain Ecosystems” that includes support for Dr. O’Loughlin. Argonne also provided \$85K/year of LDRD support in FY2013-2015 to a project entitled “Imaging “ecological engineers”: a novel quantum dots approach to map microbes in complex soil structures with x-rays” that included support for Drs. Kemner and O’Loughlin. This project sought to develop x-ray tomographic methods to image the three-dimensional distribution of microbial cells and their metabolic state in microenvironments within opaque soil aggregates and is expected to be helpful for research related to the biogeochemical cycling of carbon as well as iron and sulfur. This work is now supported by DOE BER BSSD under the Argonne Small Worlds Project.

Argonne provided LDRD funds (\$300K) in FY2014 to Dr. Kemner to investigate the feasibility of developing a scanning transmission x-ray microscope at a bending magnet beamline at the APS to image the spatial heterogeneities of carbon with ~50 nm spatial resolution within soils and sediments.

Capital Equipment needs (FY2016)

\$260K is requested for the additional beam line components and effort required to develop an x-ray Raman spectrometer at the MRCAT/EnviroCAT insertion device beam line (sector 10) at the APS for investigations of carbon chemistries in bulk environmental samples. Besides providing a unique capability for the Argonne SBR SFA and the research community in general, this spectrometer will also enable higher throughput of x-ray Raman measurements for a project recently funded by DOE BER TES entitled “Resolving conflicting physical and biochemical feedbacks to climate in response to long-term warming” that includes the efforts of two of the members of the Argonne SBR SFA (Drs. Mishra and Kemner).

Appendix A. Manuscripts published, in press, or in review, since March 2015

(Authors in bold are Argonne SFA members)

Peer-reviewed Publications:

1. **M.-J. Kwon, E. J. O'Loughlin, M. I. Boyanov**, J. M. Brulc, E. R. Johnston, **K. M. Kemner, D. A. Antonopoulos**. Impact of organic carbon electron donors on microbial community development under iron- and sulfate-reducing conditions. *PLOS ONE*, **11**(1):e0146689. (2016) doi:10.1371/journal.pone.0146689.
2. **D. E. Latta, K. M. Kemner, B. Mishra, M. I. Boyanov**. Effects of calcium and phosphate on uranium(IV) oxidation: Comparison between nanoparticulate uraninite and amorphous U(IV)-phosphate. *Geochimica et Cosmochimica Acta*, **174** (2016) 122-142
3. **M.-J. Kwon**, S. Leeb, G. Leec, B. Hama, **M. I. Boyanov, K. M. Kemner, E. J. O'Loughlin**, J.-S. Yang. Geochemical Characteristics and Microbial Community Compositions in Toxic Metal-rich Sediments Contaminated from Au-Ag Mine Tailings. *J. Hazardous Materials* **296** (2015) 147–157
4. **D. E. Latta, M. I. Boyanov, K. M. Kemner, E. J. O'Loughlin, M. Scherer**. Reaction of U(VI) with green rusts: Effect of interlayer anion. *Current Inorganic Chemistry* **5**, 156-168 (2015)
5. M. J. Shim, B. Y. Choi, G. Lee, Y. H. Hwang, Y. H. Hwang, J.-S. Yang, **E. J. O'Loughlin**, and **M. J. Kwon**. (2015). Water quality changes in acid mine drainage streams in Gangneung, Korea 10 years after treatment with limestone. *Journal of Geochemical Exploration*. **159**:234-242. DOI:10.1016/j.gexplo.2015.06.015
6. **E. J. O'Loughlin**, C. A. Gorski, and **M. M. Scherer**. (2015). Effects of phosphate on secondary mineral formation during bioreduction of akaganeite (β -FeOOH): Green rust versus framboidal magnetite. Invited paper for special issue of *Current Inorganic Chemistry*. **5**(3):214-224. DOI:10.2174/1877944105666150421001126
7. Houghton JL, Foustoukos D, **Flynn TM**, Vetriani C, Bradley AS, Fike DA (2016) "Thiosulfate oxidation by *Thiomicrospira thermophila*: metabolic flexibility in response to ambient geochemistry," *Environmental Microbiology*, doi: 10.1111/1462-2920.13232.
8. Kirk MF, Wilson BH, Marquart KA, Zeglin LH, Vinson DS, **Flynn TM** (2015) "Solute concentrations influence microbial methanogenesis in coal-bearing strata of the Cherokee basin, USA," *Frontiers in Microbiology*, Vol. 6 (1287), p. 1–14.
9. Leavitt WD, **Flynn TM**, Suess MK, Bradley AS (2016) "Transhydrogenase and growth substrate influence lipid hydrogen isotope ratios in *Desulfovibrio alaskensis* G20," in review at *Frontiers in Microbiology*, Vol 7 (918), p 1-14.

Manuscripts in Review:

1. Y. Dong, R. Sanford, **M. I. Boyanov, K. M. Kemner, T. M. Flynn, E. J. O'Loughlin**, R. Locke, J. Weber, S. Egan, B. Fouke. "Isolation and characterization of *Orenia metallireducens* sp. nov.: a novel metal-reducing Firmicute bacterium from 2.0 km deep subsurface, Illinois Basin, USA", Revised version submitted to *Applied and Environmental Microbiology* on 5/28/2016, manuscript AEM01588-16.
2. Y. Dong, R. Sanford, **M. I. Boyanov, K. M. Kemner, T. M. Flynn, E. J. O'Loughlin**, R. Locke, J. Weber, S. Egan, B. Fouke. "*Tepidibacillus decaturensis* sp. nov.: an anaerobic, moderately thermophilic iron-reducing bacterium isolated from a depth of 1.7 km in the Illinois Basin, USA", In review at *International Journal of Systematic and Evolutionary Microbiology* as of 6/2016.

Appendix B. Scientific Presentations since March 2015

Invited Presentations: (Presenting author is underlined)

1. **D.A. Antonopoulos**, “Rarity and stability within microbial communities: a spatial tale,” Department of Microbiology and Immunology, Montana State University, Bozeman, MT. April 12, 2016.
2. **K. M. Kemner**, **E. J. O’Loughlin**, **M. I. Boyanov**, **D. A. Antonopoulos**, **B. Mishra**, **T. M. Flynn**. Fe/Mn/S redox dynamics and effects on U/Hg transformations. Session on Redox Cycling and Organomineral Interactions, Environmental System Science Principal Investigator (PI) Meeting, Potomac, MD, April 27, 2016
3. **M. I. Boyanov**, D. Latta, **B. Mishra**, **E. J. O’Loughlin**, **K. M. Kemner**. Reduction and reoxidation of uranium: mechanisms, species, and implications for dispersal. Session on “Environmental Impacts and Remediation of Mine, Fuel and Energy Extraction Processes”, Goldschmidt 2015, Prague, Czech Republic, August 17, 2015.
4. **K. M. Kemner**, **E. J. O’Loughlin**, **M. I. Boyanov**, **D. A. Antonopoulos**, **B. Mishra**, **T. M. Flynn**, **D. Latta**, **M. Scherer**, **M.-J. Kwon**, T. DiChristina, K. Skinner. Biogeochemical cycling of Fe and S: Who are moving the electrons and where are the electrons going? Session on “Coupled Cycling of Biogeochemical Critical Elements and Contaminants”, ACS Meeting, Denver, CO. March 22-26, 2015.
5. **O’Loughlin, E. J.** 2016. Giving thanks, giving back, Symposium in Honor of Dr. Samuel Traina, University of California, Merced, CA, April 9, 2016.
6. **O’Loughlin, E. J.** 2016. Small worlds: Microbes are all around us, Northern Illinois University, DeKalb, IL, April 6, 2016.
7. **O’Loughlin, E. J.** 2015. Cycling of Major/Minor Elements and the Fate and Transport of Contaminants in Natural Systems, North Central College, Naperville, IL, October 27, 2015.
8. **Flynn, T. M.** “Geochemical cycling and microbial ecology in the subsurface” Department of Geology, University of Wisconsin-Eau Claire, March 6, 2015.
9. **Flynn, T. M.** “Microbial Redox Pathways in the Terrestrial Subsurface” Department of Geophysical Sciences, The University of Chicago, Chicago, IL. October 16, 2015.
10. **Y. Dong**, R. Sanford, **M. I. Boyanov**, **K. M. Kemner**, **T. M. Flynn**, **E. J. O’Loughlin**, S. George, K. Fouke, B. Fouke. Effect of Mineralogy, pH, Temperature, Salinity and Anions on the Ability of Bacteria to Reduce Ferric Iron Oxides and Drive Diagenesis. GSA North-Central 50th Annual Meeting, Champaign, IL, April 18, 2016.
11. **B. Mishra**, March 2015 “**Use of Synchrotron Techniques for Climate Change Research**” Colloquium, Physics Department, Illinois Institute of Technology, Chicago, IL,
12. **B. Mishra**, Oct 2015 “Biogeochemical Controls on Contaminant and Nutrient Cycling in Aquatic and Terrestrial Ecosystems”, University of Leeds, Leeds (United Kingdom)
13. **B. Mishra**, Nov. 2015 “Biogeochemistry of the molecular scale interactions of metals with bacteria” Colloquium, Chemistry Department, Illinois Institute of Technology, Chicago, IL
14. **B. Mishra**, May 2016 “Biogeochemical Controls on Contaminant and Nutrient Cycling”, **APS User Meeting, Argonne National Laboratory, IL**
15. **K. M. Kemner**, “Integrating hard x-ray synchrotron radiation into a biogeochemist’s toolbox,” Canadian Light Source, Saskatchewan, Saskatoon, Canada, April 7, 2016.
16. **K. M. Kemner**, “Understanding processes that drive heavy metal and radionuclide transformations: Integrating hard x-ray synchrotron radiation into a (Biogeo)chemist’s toolbox,” Washington State University, Pullman Chemistry Division Colloquium,

Pullman, Washington, February 29, 2016.

17. **K. M. Kemner**, "Synchrotron radiation and its application to biogeochemistry," North Central Illinois College Chemistry Colloquium, Naperville, Illinois, October 20, 2015.
18. **K. M. Kemner, M. I. Boyanov, B. Mishra, T. Flynn, D. Antonopoulos, E. J. O'Loughlin**, "Fe and S biogeochemistry in redox dynamic environments: progressing towards a predictive understanding of U biogeochemical transformations," US DOE BER Environmental System Science Annual PI Meeting, Washington D.C., April 29, 2015.
19. **K. M. Kemner, M. I. Boyanov, B. Mishra, E. J. O'Loughlin, S. L. O'Brien, D. Sholto-Douglas, B. Lai, M. Balasubramanian, R. A. Gordon, S. D. Kelly, V. L. Bailey**, "Novel synchrotron-based x-ray approaches to understanding controls on metal ion fate in subsurface and terrestrial environments," American Chemical Society 249th National Meeting, Denver, Colorado, March 22-25, 2015.

Contributed Presentations

1. S. Yan, **M.I. Boyanov, B. Mishra, K.M. Kemner, E.J. O'Loughlin**. U(VI) Reduction by Biotic and Abiotic Green Rusts. Goldschmidt 2016, Yokohama, Japan, June 28, 2016.
2. **M. Boyanov, D. Latta, M. Scherer, Y. Liu, C. Liu, B. Mishra, E. O'Loughlin, K. Kemner**. The effect of surfaces and ligands on the reduction and reoxidation reactions of U studied in mixed-batch reactors and flow-through columns. Environmental System Science Principal Investigator (PI) Meeting, Potomac, MD, April 27-28, 2016
3. **K.M. Kemner, D.A. Antonopoulos, T.M. Flynn, B. Mishra, M.I. Boyanov, D.E. Latta, M.M. Scherer, C. Liu, J.K. Fredrickson, K.M. Rosso, J.M. Zachara, E.J. O'Loughlin**. The Argonne National Laboratory Subsurface Biogeochemical Research Program SFA: Fe and S Biogeochemistry in Redox Dynamic Environments. Environmental System Science Principal Investigator (PI) Meeting, Potomac, MD, April 27-28, 2016
4. **B. Mishra, E.J. O'Loughlin, M.I. Boyanov, K.M. Kemner**. Abiotic redox dynamics between Hg and Mn. Environmental System Science Principal Investigator (PI) Meeting, Potomac, MD, April 27-28, 2016
5. C.R. Johnson, **D.A. Antonopoulos, M. I. Boyanov, T. M. Flynn, J.C. Koval, K.M. Kemner, E.J. O'Loughlin**. Reduction of Sb(V) by coupled biotic and abiotic processes in sulfidogenic microcosms. ACS Meeting San Diego, March 14, 2016
6. **M. I. Boyanov, D. Latta, B. Mishra, M. Scherer, E.J. O'Loughlin, K.M. Kemner**. Influence of mineral surfaces on the speciation of uranium under reducing conditions. ACS Meeting San Diego, March 16, 2016
7. **E. J. O'Loughlin, C. R. Johnson, D. A. Antonopoulos, M. I. Boyanov, T. M. Flynn, J. Koval, K. M. Kemner**. Biogeochemistry of Antimony(V) in Microcosms under Sulfidogenic Conditions. American Geophysical Union Fall Meeting, San Francisco, USA, December 15, 2015
8. **K.M. Kemner, M.I. Boyanov, S.D. Kelly, K. Skinner, B. Mishra, S. Brooks, D. Watson, W-M Wu**. Investigating redox processes under diffusive and advective flow conditions using a coupled omics and synchrotron approach. American Geophysical Union Fall Meeting, San Francisco, USA, December 15, 2015
9. Leavitt WD, **Flynn TM**, Suess M, Bradley AS (2015) "Electron-bifurcating transhydrogenase is central to hydrogen isotope fractionation during lipid biosynthesis in sulfate reducing bacteria," American Geophysical Union Fall Meeting, San Francisco, CA. 14–18 Dec. 2015.

10. Y. Dong, R. Sanford, **M. I. Boyanov, K. M. Kemner, T. M. Flynn, E. J. O'Loughlin, S. George, K. Fouke, B. Fouke**. Effects of mineral speciation, pH, temperature, salinity and anions on the bioreduction of ferric iron oxides and the formation of secondary mineralization products. Fourth Annual Midwest Geobiology Symposium, Indiana University, Bloomington, IN, October 10, 2015.
11. **T. M. Flynn, D.A. Antonopoulos, K. Handley, M. I. Boyanov, F. Meyer, B. Mishra, W. Trimble, K. Skinner, S. Kelly, E.J. O'Loughlin, K.M. Kemner**. Structure and function of microbial communities in the subsurface. Fourth Annual Midwest Geobiology Symposium, Indiana University, Bloomington, IN, October 10, 2015.
12. C. R. Johnson, **D. A. Antonopoulos, M. I. Boyanov, T. M. Flynn, J. Koval, K. M. Kemner, E. J. O'Loughlin**. Biogeochemistry of antimony(V) in microcosms under sulfidogenic conditions. Fourth Annual Midwest Geobiology Symposium, Indiana University, Bloomington, IN, October 10, 2015.
13. **M. I. Boyanov, D. Latta, B. Mishra, E. J. O'Loughlin, K. M. Kemner**. Biological and abiotic factors affecting the solid-phase speciation of U(IV) following reduction of aqueous U(VI)". Session on "Actinides & Radionuclides", XAFS16 Conference, Karlsruhe, Germany, August 24, 2015.
14. **B. Mishra, E. J. O'Loughlin, M. I. Boyanov, K. M. Kemner**. Biogeochemical controls on the molecular scale interactions of mercury with microbes. Goldschmidt 2015, Prague, Czech Republic, August 21, 2015.
15. **M. I. Boyanov, C. R. Johnson, D. A. Antonopoulos, T. M. Flynn, J. Koval, K. M. Kemner, E. J. O'Loughlin**. Reduction of Sb(V) by coupled biotic-abiotic processes under sulfidogenic conditions. Goldschmidt2015, Prague, Czech Republic, August 19, 2015.
16. **T.M. Flynn, J.C. Koval, S.M. Greenwald, S.M. Owens, K.M. Kemner, and D.A. Antonopoulos**, "Community Level Physiological Profiling Of Diverse Environments Reveals Functional And Taxonomic Diversity Within Aerobic, Single Carbon-source Enrichments," ASM General Meeting 2015, New Orleans, LA, May 30-June2, 2015.
17. C. R. Johnson, **D. A. Antonopoulos, M. I. Boyanov, T. M. Flynn, K. M. Kemner, J. Koval, E. J. O'Loughlin**. Biogeochemistry of antimony(V) in microcosms under sulfidogenic conditions. 115th General Meeting of the American Society for Microbiology, New Orleans, LA. May 30-June 2, 2015.
18. C. R. Johnson, **D. Antonopoulos, M. I. Boyanov, T. M. Flynn, K. M. Kemner, J. Koval, E. J. O'Loughlin**. Behavior of antimony(V) under Fe(III)- and sulfate-reducing conditions. Session on "Coupled Cycling of Biogeochemical Critical Elements and Contaminants", ACS Meeting, Denver, CO. March 22-26, 2015.
19. **K. M. Kemner, M. I. Boyanov, B. Mishra, E. J. O'Loughlin, S. O'Brien, D. Sholto-Douglas, B. Lai, M. Balasubramanian, R. Gordon, S. Kelly, V. Bailey**. Novel synchrotron-based hard x-ray approaches to understanding controls on metal ion fate in subsurface and terrestrial environments. Symposium in Honor of Dr. Donald Sparks, 2015 Geochemistry Medal Recipient, ACS Meeting, Denver, CO. March 22-26, 2015.
20. **Kwon, M. J., Y. Hwang, D. Lee, B. Ham, J. Lee, and E. J. O'Loughlin**. 2016. Geochemical and microbiological dynamics in a diesel-contaminated subsurface environment during in-situ soil flushing. 251st American Chemical Society National Meeting, San Diego, CA, March 13-17, 2016.
21. **O'Loughlin, E. J., M. F. Sladek, D. A. Antonopoulos, T. M. Flynn, J. C. Koval, C. L. Marshall, and K. M. Kemner**. 2016. Effects of soluble electron shuttles on microbial Fe(III) reduction and methanogenesis in wetland sediments. 251st American Chemical Society National Meeting, San Diego, CA, March 13-17, 2016.

22. **T.M. Flynn**, J.C. Koval, **K.M. Kemner**, and **D.A. Antonopoulos**, "Niche and neutral community dynamics in parallelized aerobic, single carbon-source enrichment cultures," 7th Annual Argonne National Laboratory Soil Metagenomics Meeting, Lisle, IL, October 21-23, 2015.
23. Marshall, C. W., S. L. O'Brien, **K. M. Kemner**, **E. J. O'Loughlin**, N. R. Gottel, S. Alvarez Clare, A. A. Best, **T. M. Flynn**, and J. A Gilbert. 2015. Microbial ecology of restored floodplains. 7th Annual Argonne Soil Metagenomics Meeting, Lisle, IL, October 21-23, 2015.
24. **O'Loughlin, E. J.**, M. F. Sladek, **D. A. Antonopoulos**, **T. M. Flynn**, J. C. Koval, C. L. Marshall, and **K. M. Kemner**. 2015. Effects of soluble electron shuttles on microbial Fe(III) reduction and methanogenesis in wetland sediment microcosms. 7th Annual Argonne Soil Metagenomics Meeting, Lisle, IL, October 21-23, 2015.
25. **O'Loughlin, E. J.**, M. F. Sladek, **D. A. Antonopoulos**, **T. M. Flynn**, J. C. Koval, C. L. Marshall, and **K. M. Kemner**. 2015. Effect of soluble electron shuttles on microbial Fe(III) reduction and methanogenesis. 2015 Midwest Geobiology Symposium, Bloomington, IN, October 10, 2015.
26. **Mishra, B.**, **E. J. O'Loughlin**, W. T. Cooper, J. Jastrow, R. Gordon, M. Balasubramanian, and **K. M. Kemner**. 2015. Using X-ray Raman to Study Soil Carbon Biogeochemistry The 16th International Conference on X-ray Absorption Fine Structure, Karlsruhe, Germany, August 23-28, 2015.
27. Alvarez-Clare, S., C. C. Cleveland, **E. J. O'Loughlin**, B. W. Sullivan, S. R. Weintraub, **T. M. Flynn**, and **D. A. Antonopoulos**. 2015. Influence of nutrient availability on greenhouse gas emissions and microbial community development in a lowland tropical rainforest. 52nd Annual Meeting of The Association for Tropical Biology and Conservation, Honolulu, HA, July 12-16, 2015.
28. Marshall, C., S. O'Brien, **K. M. Kemner**, **E. J. O'Loughlin**, A. Best, J. A. Gilbert. 2015. Microbial ecology of restored wetlands at various stages of maturity and flooding frequency. 115th General Meeting, American Society for Microbiology, New Orleans, LA, May 30-June 2, 2015.
29. Leavitt WD, **Flynn TM**, Suess M, Bradley AS (2016) "Electron-bifurcating transhydrogenase is central to hydrogen isotope fractionation during lipid biosynthesis in sulfate reducing bacteria," Gordon Research Conference in Geobiology: Reconstructing Processes from Genes to the Geologic Record, Galveston, TX. 31 Jan.–5 Feb. 2016.
30. Marquart KA, Wilson BH, Zeglin LH, Vinson DS, **Flynn TM**, Kirk MF (2015) "Pathway of microbial methanogenesis varies with formation water salinity in a coalbed methane reservoir," Geological Society of America Annual Meeting, Baltimore, MD. 1–4 November, 2015.
31. **B. Mishra**, **M. I. Boyanov**, **E. J. O'Loughlin**, **K. M. Kemner**, "**Biogeochemical controls on the molecular scale interactions of metals with microbes**" 251st American Chemical Society Meeting, San Diego, March 13-17, 2016.