Biogeochemical Transformations at Critical Interfaces in a Mercury Perturbed Watershed

ORNL Critical Interfaces Science Focus Area (SFA) 2021 Annual Report

Determining the mechanisms and environmental controls on mercury fate and transformation in streams

Program Overview

At a time when demand for water is dramatically increasing because of population growth, industrialization, and expansion of irrigated agriculture, freshwater resources supplied by headwater streams and their surrounding watersheds are being threatened by severe pollution from anthropogenic releases of nutrients and trace metals such as mercury (Hg). More than 9,000 waterbodies in the continental United States are impaired by Hg. Mercury is the second leading cause of impaired waters—including locations in the Tennessee River Basin—and is responsible for fish consumption advisories in all 50 states (U.S. EPA 2011, 2013).

The economic and societal importance of headwater streams and their surrounding watersheds is exemplified by the Tennessee River Basin (see Fig. 1). Located in the southeastern United States, this river basin consists of a series of nested watersheds that supports ~4.5 million people by supplying water for power generation, industry, recreation, agriculture, and human consumption (Bohac and Bowen 2012) and represents the most intensively used freshwater Water Resource Region (WRR) in the contiguous United States, with estimated withdrawals of >280,000 gallons a day per square mile. Preserving these freshwater resources for future use requires developing a deeper understanding of the structure and function of watersheds and the processes that govern pollutant transformations in aquatic ecosystems.

Research findings during Phase I of the Critical Interfaces Scientific Focus Area (SFA) project have led to the realization that transient storage zones (TSZs), and more specifically, metabolically active TSZs (MATSZs), are

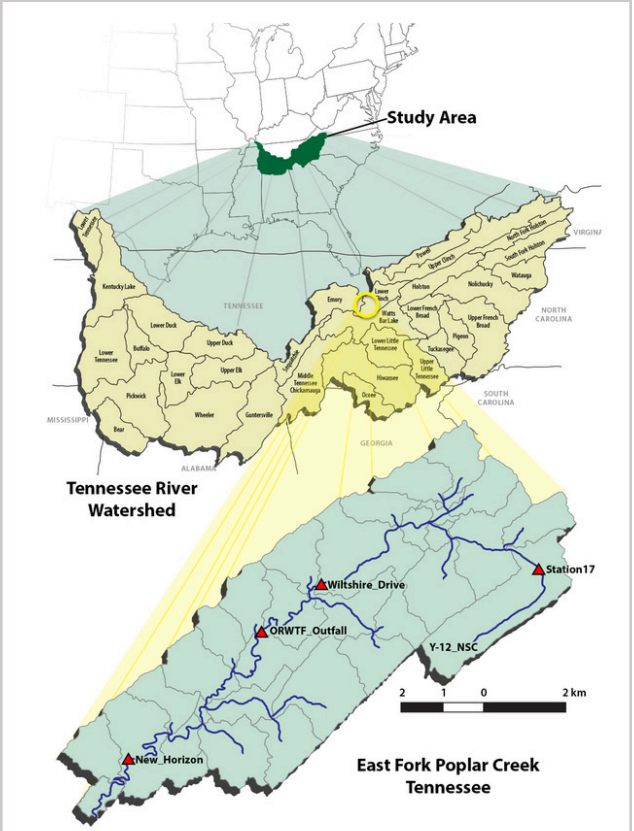

Fig. 1. SFA Study Area. East Fork Poplar Creek (EFPC), which is part of the 40,910-square-mile Tennessee River watershed, is the representative test bed system being studied. This watershed and its associated streams represent the most intensively used freshwater Water Resource Region in the contiguous United States, with estimated withdrawals of >280,000 gallons a day per square mile. The river basin is shown here as 32 segmented, 8-digit hydrologic unit codes (HUC-8). The EFPC watershed is nested in the Lower Clinch HUC8.

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important locations for further investigation. While TSZs are surface and subsurface locations (e.g., hyporheic zone) that delay the downstream flow of water in comparison to the main channel, MATSZs are microbially active TSZs, such as the interstitial spaces of hyporheic zone streambed sediments and the pore space present in the microbiome of instream biofilm. MATSZs exhibit very different biogeochemical environments (e.g., redox conditions) compared with flowing stream or streambed, making them important "hot spots" that account for a substantial proportion of the diverse and intensified biogeochemical activity in watersheds.

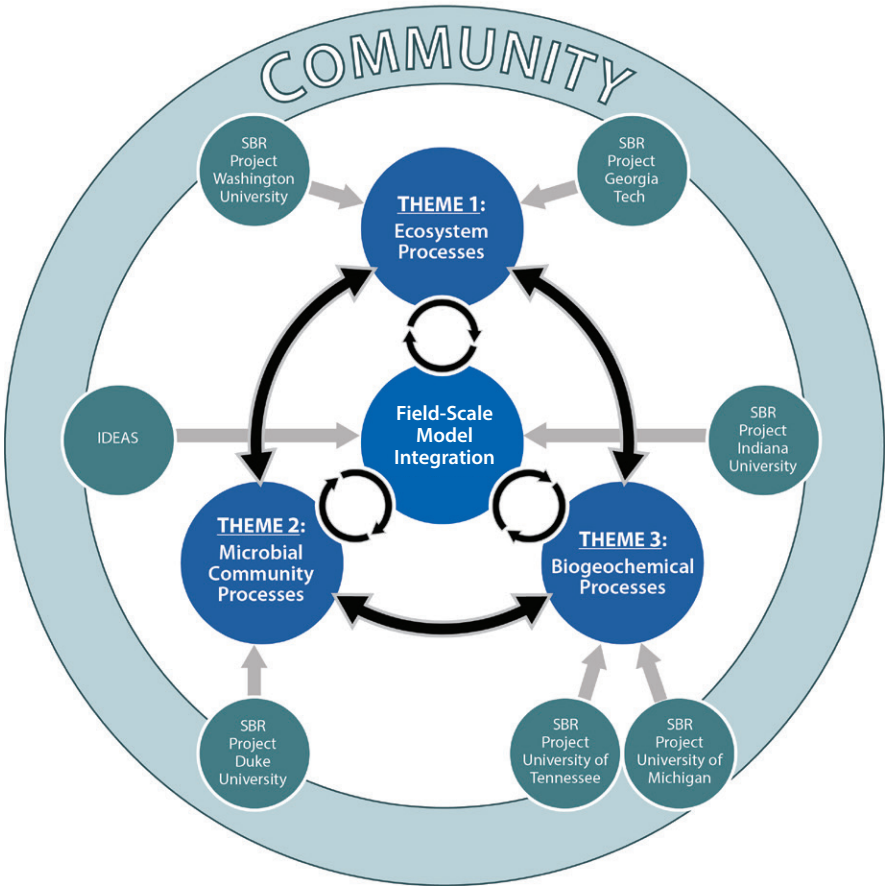

Fig. 2. Integrated, Multiscale SFA Research. Illustration showing the interconnections between themes, modeling activities, Interoperable Design of Extreme-scale Application Software (IDEAS), and various Environmental System Science (ESS) university projects.

The SFA project is progressively advancing our understanding of the factors that influence watershed structure and function using Hg and the East Fork Poplar Creek (EFPC) watershed as representative use cases. The EFPC watershed offers a unique niche to the Environmental System Science (ESS) program by being nested in the most intensively used freshwater WRR and serving as a representative low-order freshwater stream system, which is a segment of stream orders with relevance to the largest proportion of the total stream length in United States. Led by Oak Ridge National Laboratory (ORNL), this project is supported by the ESS program of the Office of Biological and Environmental Research (BER) within the Department of Energy's (DOE) Office of Science.

In FY21, the Critical Interfaces SFA team (1) added new capabilities to the Advanced Terrestrial Simulator (ATS) modeling software by extending the Advective Dispersion Equation with Lagrangian Subgrid (ADELS) model from conservative tracers to multicomponent reactive transport, (2) refined our Transient Availability Model (TAM) to include Monod-type kinetics for methylation and demethylation, (3) examined how nutrient amendments (nitrate and/or phosphate) altered periphyton community structure and function, (4) continued to explore the transcriptional regulation of *hgcA* under different conditions, and (5) used density functional theory (DFT) calculations to determine the role of metal sulfide surfaces in dimethylmercury (DMeHg) formation. We advanced our understanding of ligand exchange reactions controlling Hg (II) uptake and methylmercury (MeHg) production by determining the solution-phase configurations and metal interaction of methanobactin from *Methylocystis sp.* strain SB2 with Hg and other group 12 metals. Our results demonstrate that a linear model is consistent for Hg(II) coordination versus a tetrahedral model for zinc (II) and cadmium (II). Collectively, the aforementioned activities are providing a deeper understanding of Hg transformations in EFPC and allowing us to gain the process knowledge needed to improve predictions of Hg transformations at the scale of individual stream reaches and small watershed catchments.

Integrated, Multiscale Research Approach

Critical Interfaces SFA research encompasses three themes—ecosystem processes, microbial community processes, and biogeochemical processes—and a research activity involving field-scale model integration (see Fig. 2).

- **Ecosystem Processes.** Through a combination of field- and laboratory-scale studies, research investigates Hg biogeochemical transformations in hyporheic zone sediments and the influence of nutrient additions on net MeHg production and microbial community composition in field-derived periphyton biofilms.
- **Microbial Community Processes.** Research seeks to (1) understand the contributions of known Hg-methylating organisms to observe Hg methylation rates and extents in biofilm lifestyles, using synthetic and natural microbial communities; (2) determine the breadth and depth of Hg-methylating species; and (3) determine the biochemical roles of the proteins (HgcA and HgcB) that facilitate MeHg production.
- **Biogeochemical Processes.** Research elucidates key biogeochemical mechanisms controlling Hg bioavailability and microbial transformation of inorganic Hg to MeHg in simplified, but field-relevant, laboratory experiments. Activities include (1) investigating complex biogeochemical processes and their interactions controlling Hg species transformation and availability for cellular uptake and methylation and (2) using molecular-scale computational approaches to elucidate key biogeochemical mechanisms governing Hg speciation and microbial transformations.
- **Field-Scale Model Integration.** Improves stream reach-to-watershed reactive transport modeling of contaminant and nutrient export. Activities include estimating the volume of TSZs and MATSZs in EFPC and the mass transfer between TSZs and the creek channel, using nonreactive and reactive tracers to parameterize the field-scale model.

This annual report summarizes Critical Interfaces SFA accomplishments from June 2020 to June 2021, a period representing the third year following the program's triennial peer review in May 2018 and acceptance of the revised plan in September 2018 by BER's Environmental System Science program. The SFA received a one-year extension because of COVID-19 complications in FY20.

Scientific Progress

Theme 1: Ecosystem Features Influencing Mercury Transformation

Theme 1 research examines the biogeochemical controls on Hg methylation and demethylation within the context of the flowing creek system and its connection with the surrounding watershed. Emphasis is on field-based investigations with supporting laboratory work to elucidate mechanisms.

There are three overarching objectives:

- Identify ecosystem domains and hydro-biogeochemical conditions that govern net MeHg concentrations in EFPC.
- Identify microbial community traits and interactions that are important within periphyton and sediment ecosystems that influence Hg transformations and storage.
- Work iteratively with ongoing modeling activities to inform and support the biogeochemical modeling framework.

These objectives are addressed through a set of hypotheses-driven field and laboratory investigations and the development of a process-rich numerical model to challenge current understanding of watershed processes occurring over broad spatiotemporal scales.

FY20–FY21 Accomplishments

Over the past 12 months, Theme 1 made significant progress toward milestones and published several papers relating to the role of periphyton in Hg cycling and development of predictive models of those reactions. Additional papers have been published reporting on the effect of Hg(II) sorption on MeHg production and on improvements to predicting the equilibrium aqueous speciation of Hg.

Role of Periphyton in EFPC Mercury Cycling

Using high-throughput amplicon sequencing of the 16S rRNA gene, ITS2 region, and Hg-methylation gene pair (*hgcAB*), we characterized the archaea, bacteria, fungi, and Hg-methylating microorganisms in periphyton communities in the stream corridor of the EFPC watershed. Further, we examined how nutrient amendments (nitrate and phosphate) altered periphyton community structure and function. Overall, we found that Hg-methylation potential correlated with numerous bacterial families that do not contain *hgcAB*, suggesting that overall microbiome structure of periphyton communities influence rates of Hg transformation. Interestingly, the addition of nitrate to

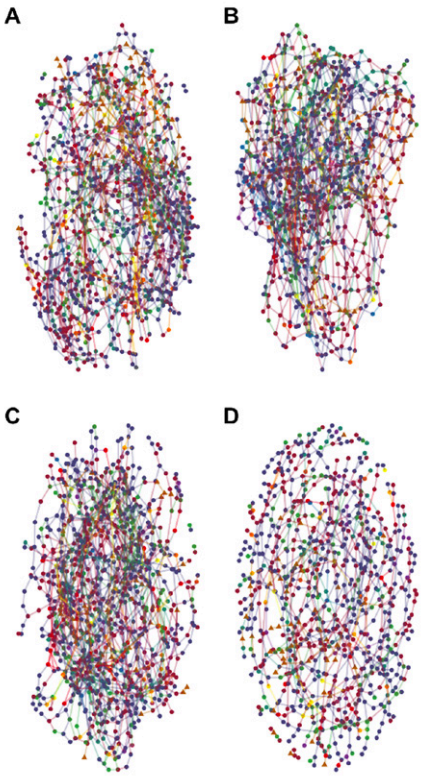

Fig. 3. Cross-Domain Correlation Networks. Charts show correlation networks of archaea, bacteria, and fungal taxa for (A) control, (B) nitrate, (C) phosphate, and (D) nitrate + phosphate nutrient exposures. Mercury methylation was highest in control and nitrate samples, suggesting these well-connected hubs may explain higher methylation.

these habitats resulted in the most connected periphyton microbial communities, which correlated with enhanced Hg-methylation potential (see Fig. 3). This research provides insight into community interactions within the periphyton microbiome that may contribute to Hg cycling and will inform future research that will focus on establishing mixed microbial consortia to uncover mechanisms driving shifts in Hg cycling within periphyton habitats (Carrell et al. 2021).

In anoxic environments, anaerobic microorganisms carrying the *hgcAB* gene cluster can mediate Hg transformation to monomethylmercury (MMHg). The kinetics of Hg transformation to MMHg in periphyton from EFPC have previously been modeled using a transient availability model (TAM). The TAM for Hg methylation combines kinetic expressions for processes that reduce Hg and MMHg availability for methylation and demethylation (multisite sorption of Hg and MMHg, Hg(II) reduction/Hg(0) oxidation) with methylation/demethylation

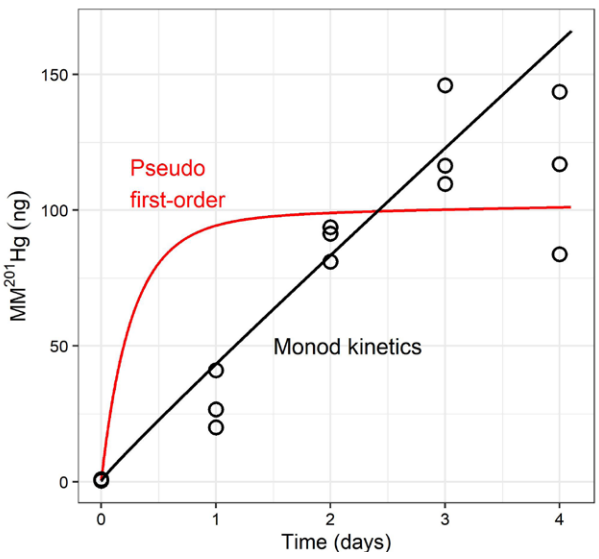

Fig. 4. Transient Availability Model (TAM). Production of $MM^{201}Hg$ over time in sediment microcosm experiments. The lines indicate the TAM using either pseudo first-order (red) or Monod (black) kinetic expressions of MMHg production.

kinetics. In this study, the TAM is used for the first time to describe MMHg production in sediment. We assessed MMHg production in sediment microcosms using two different sediment types from EFPC: a carbon-rich sediment with lower, more anoxic redox potential and a sandy, carbon-poor sediment with a higher redox potential. Based on 16s rRNA sequencing, the overall microbial community structure in the two sediments was retained during the incubations. However, the *hgcA* containing methanogenic *Euryarchaeota* communities differed between sediment types, and their growth followed different trajectories over the course of incubations, potentially contributing to the distinct patterns of MMHg production observed. The general TAM paradigm performed well in describing MMHg production in the sediments. However, the MMHg production and ancillary data suggested the need to revise the model structure to incorporate terms for variable microbial activity. We modified the TAM to include Monod-type kinetics for methylation and demethylation and observed an improved fit for the carbon-rich, microbially active sediment (see Fig. 4). Overall, our work shows that the TAM can be applied to describe Hg methylation in sediments. In some cases, including expressions that account for variable microbial activity can improve the accuracy of the model description of the data.

EFPC stream restoration activities included the initiation of a flow management program in 1996 in which water

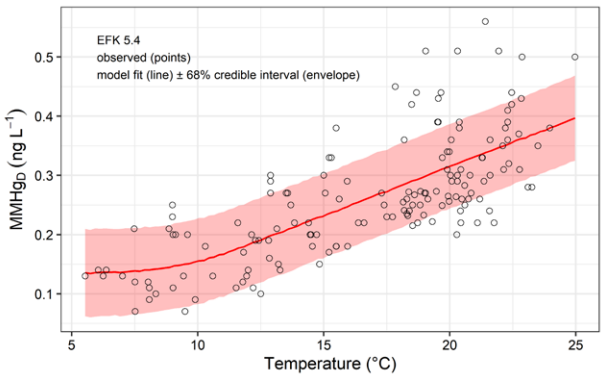

Fig. 5. Dissolved MMHg Concentration vs. Water Temperature. MMHg concentration is low, relatively constant at ~0.13 ng/L. It is independent of temperature below 10°C and significantly positively correlated with temperature above that threshold.

from a nearby lake was pumped to the head of the creek. We conducted regular water sampling for two years along the length of EFPC during active flow management and for five years after flow management stopped. Total Hg and total MMHg concentration and flux decreased in the uppermost reaches of EFPC that were closest to the point of water addition. Most water quality parameters, including dissolved organic carbon (DOC) concentration, remained unchanged after flow management termination. Nevertheless, $SUVA_{254}$, a measure of dissolved organic matter (DOM) composition, increased and coincided with increased dissolved Hg (Hg_D) concentration and flux and decreased Hg solid-water partitioning coefficients throughout EFPC. Higher $SUVA_{254}$ and Hg_D concentration have potential implications for bioavailability and MMHg production. Total and dissolved MMHg concentrations increased in lower reaches of EFPC after the end of flow management, and these increases were most pronounced during spring and early summer when biota are more susceptible to exposure and uptake. After active flow management ended, a general warming trend in the creek likely acted in concert with higher Hg_D concentration to promote higher MMHg concentration. Total and dissolved MMHg concentrations were positively correlated with water temperature above a threshold value of 10°C (see Fig. 5). Concentration changes for Hg and MMHg could not be accounted for by changes in creek discharge that accompanied the cessation of flow management. In addition to the changing DOM composition in-stream, other watershed-scale factors likely contributed to the observed patterns, as these changes occurred over months, rather than instantaneously, after flow management stopped. Nevertheless, similar changes in MMHg have not been observed in a tributary to EFPC.

Status of FY21 Milestones

Milestones 1c and d: *In situ* and *ex situ* translocation experiments and community analyses. We have completed the winter and initiated the spring *in situ* translocation experiments. The experiments for summer and autumn will be completed in early FY22. For the *ex situ* translocation experiments, we have initated flume testing in the Aquatic Ecology Laboratory (AEL) at ORNL. The start of these experiments was postponed by construction delays in the AEL and site access restrictions related to the COVID-19 pandemic.

We are also actively working with the field-scale modeling activity led by Scott Painter in the development of TAM and in data sharing and conceptual model development.

FY22 Plans

In FY22, Theme 1 planned activities include:

- Continue long-term stream gauging and material flux measurements.
- Conduct seasonal *in situ* translocation experiments to quantify the effects of nutrient exposure on periphyton composition and function.
- Initiate controlled *ex situ* nutrient exposure experiments in large stream mesocosms to study the effects of nutrient concentration on Hg cycling by periphyton biofilms.
- Assess the effects of season and nutrient concentrations on algal, archaeal, bacterial, fungal, and Hg-methylating organisms within periphyton habitats in EFPC.
- Examine biogeographic patterns in microbial diversity and Hg-methylating organisms across global montane regions.
- Examine the response of Hg-methylating and demethylating organisms to warming in the Spruce and Peatland Responses Under Changing Environments (SPRUCE) experiment.

Manuscripts

Published or In Press

Brooks, S. C., A. L. Riscassi, and K. A. Lowe. 2021. "Stream discharge and water quality data for East Fork Poplar Creek Beginning." *Hydrological Processes, Research and Observatory Catchments: The Legacy and the Future*. **35**(3):e14103. DOI:10.1002/HYP.14103.

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watershed." *Frontiers in Microbiology.* **12**:543(647861). DOI:10.3389/fmicb.2021.647861.

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Lian, P., Z. Mou, C. J. Cooper, R. C. Johnston, S. C. Brooks, B. Gu, N. Govind, S. Jonsson, and J. M. Parks. 2021. "Mechanistic investigation of dimethylmercury formation mediated by a sulfide mineral surface." *The Journal of Physical Chemistry A*. **125**(24):5397–5405. DOI:10.1021/acs.jpca.1c04014.

Mohamed, R. A. M., S. C. Brooks, C.-H. Tsai, T. Ahmed, D. F. Rucker, A. L. Ulery, E. M. Pierce, and K. C. Carroll. 2021. "Geostatistical interpolation of streambed hydrologic attributes with addition of left censored data and anisotropy." *Journal of Hydrology*. **599**:(126474). DOI:10.1016/j.jhydrol.2021.126474.

Murphy, S. A., G. E. Schwartz, and S. C. Brooks. 2021. "Demethylation or sorption? The fate of methylmercury in the presence of manganese dioxide." *Environmental Engineering Science*. **38**(3):224–30. DOI:10.1089/ees.2020.0068.

Pathak, A., R. Jaswal, X. Xu, J. R. White, B. Edwards III, J. Hunt, S. C. Brooks, R. S. Rathore, M. Agarwal, and A. Chauhan. 2020. "Characterization of bacterial and fungal assemblages from historically contaminated metalliferous soils using metagenomics coupled with diffusion chambers and microbial traps." *Frontiers in Microbiology*. **11**:1024. DOI:10.3389/fmicb.2020.01024.

Rucker, D. F., C.-H. Tsai, K. C. Carroll, S. C. Brooks, E. M. Pierce, A. Ulery, and C. DeRolph. 2021. "Bedrock architecture, soil texture, and hyporheic zone characterization combining electrical resistivity and induced polarization imaging." *Journal of Applied Geophysics*. **188**:(104306). DOI:10.1016/j.jappgeo.2021.104306.

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Brooks, S. C., A. L. Riscassi, and C. L. Miller. "Diel mercury concentration variations in a mercury-impacted stream." *In preparation*.

Brooks, S. C., C. L. Miller, A. L. Riscassi, K. A. Lowe, J. O. Dickson, and G. E. Schwartz. "Increasing temperature and flow management alter mercury dynamics in East Fork Poplar Creek." *Hydrological Processes, Research and Observatory Catchments: The Legacy and the Future. In revision.*

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Schwartz, G. E., K. A. Muller, S. Rathore, R. L. Wilpiszeski, A. A. Carrell, M. A. Cregger, D. A. Elias, M. Podar, S. L. Painter, and S. C. Brooks. "Incorporating variable sediment microbial activity into methylmercury production kinetics modeling." *Science of the Total Environment. In review.*

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Yan, J., N. Sharma, E. D. Flynn, D. E. Giammar, G. E. Schwartz, S. C. Brooks, P. B. Weisenhorn, K. M. Kemner, E. J. O'Loughlin, D. I. Kaplan, and J. G. Catalano. "Consistent controls on trace metal micronutrient speciation in wetland soils and stream sediments." *Geochimica et Cosmochimica Acta. In review*.

Data Products Released

1. Brooks, Scott C. and Kenneth A. Lowe. 2021. [Data Set] East Fork Poplar Creek Discharge at Kilometer 5.4 Water Year 2020. DOI:10.12769/1779632.
2. Brooks, Scott C. and Kenneth A. Lowe. 2021. [Data Set] East Fork Poplar Creek Sonde Data at Kilometer 5.4 Water Year 2020. DOI:10.12769/1779674.
3. Brooks, Scott C. and Kenneth A. Lowe. 2021. [Data Set] East Fork Poplar Creek Discharge at Kilometer 16.2 Water Year 2020. DOI:10.12769/1779680.
4. Brooks, Scott C. and Kenneth A. Lowe. 2021. [Data Set] East Fork Poplar Creek Sonde Data at Kilometer 16.2 Water Year 2020. DOI:10.12769/1779683.
5. Brooks, Scott C., Carrie L. Miller, Ami L. Riscassi, Kenneth A. Lowe, Johnbull O. Dickson, Grace E. Schwartz. 2020. [Data Set] Water quality response to flow management in East Fork Poplar Creek. DOI:10.12769/1664390.

Theme 2: Microbial Community Processes

The overall goals of Theme 2 are to (1) understand the mechanisms of Hg methylation at the molecular scale and the consequences to the cell in planktonic and biofilm lifestyles, whether in isolation, synthetic, or natural microbial communities; (2) determine the breadth and depth of Hg-methylating species; and (3) elucidate the biochemical roles of HgcA and HgcB. Our research is designed to answer the following questions:

- How widespread is the ability to methylate Hg, and what are the relative contributions from different microbial clades to the overall net pool of MeHg generated in different types of environments, specifically in EFPC?
- What genes and metabolic traits are required for function and maintenance of *hgcAB*?
- What environmental conditions alter HgcAB expression?
- What is the biochemical (native) function of HgcA and HgcB in the absence of Hg?
- Can sequence-inferred HgcAB structural models provide a mechanistic framework for testing structure-function hypotheses of Hg binding, methylation, and potential involvement of other proteins in the methylation process?
- Do mutations to *hgcAB* affecting Hg methylation also change organismal fitness under certain environmental conditions?
- Does the overall cellular metabolism and MeHg generation change in multispecies cultures versus single-organism cultures?

FY20–FY21 Accomplishments

Over the past 12 months, Theme 2 has published a number of manuscripts and made significant progress toward our milestones, including (1) determining alternative (native) functions of HgcAB, (2) identifying and attempting to isolate novel Hg methylators from EFPC sediments, (3) developing model microbial communities to reflect the EFPC community, (4) determining the role sulfide mineral surfaces have in mediating dimethylmercury (DMeHg) formation.

Determining Alternative hgcAB Functions

We have continued work related to physiological experiments for determining the native biochemical function of HgcAB. In FY21, we completed numerous batch culture bottle experiments with *Desulfovibrio desulfuricans* ND132 wild-type and mutant strains grown in defined media with various substrates (e.g., pyruvate, fumurate, lactate, sulfate, formate, and acetate). We chose mutant strains related to carbon and Hg cycling that exhibited differences in Hg-methylation capability compared to the wild-type (e.g., 0 to 246%). Additionally, we continue to analyze large omic datasets obtained from the Environmental Molecular Sciences Laboratory (EMSL; user proposal 50174). Results showed differences in substrate consumption, acetate production, and transcription of one-carbon (C1) metabolism genes between mutant strains and wild-type under fermentative and sulfate-reducing conditions. However, results were not consistently within a given pathway but rather seemed to be random. Most notably, mutants deficient in methylation also did not express genes for motility, including cilia and flagella. Reasons for the lack of motility gene expression are not immediately clear.

We have hypothesized that the native physiological function of HgcAB may be related to C1 metabolism for acetyl-CoA and methionine biosynthesis, metal resistance, or metalloid methylation. Clues to the native biochemical function of HgcAB may lie in determining the environmental conditions that control expression and translation of *hgcA*. Therefore, for the last year, we have been investigating transcriptional regulation of *hgcA* under different growth parameters using *D. desulfuricans* ND132 as a model organism. We utilized both molecular reverse transcription–quantitative polymerase chain reaction (RT-qPCR) and metaomic (RNA-seq) methods to test whether changes in *hgcA* expression occurred when wild-type ND132 cells were grown in conditions that require the postulated biochemical functions of HgcAB (e.g., +/– formate, methionine, arsenate, and Hg). Indeed, our results show that *hgcA* expression is significantly regulated across the growth stages of *D. desulfuricans* ND132

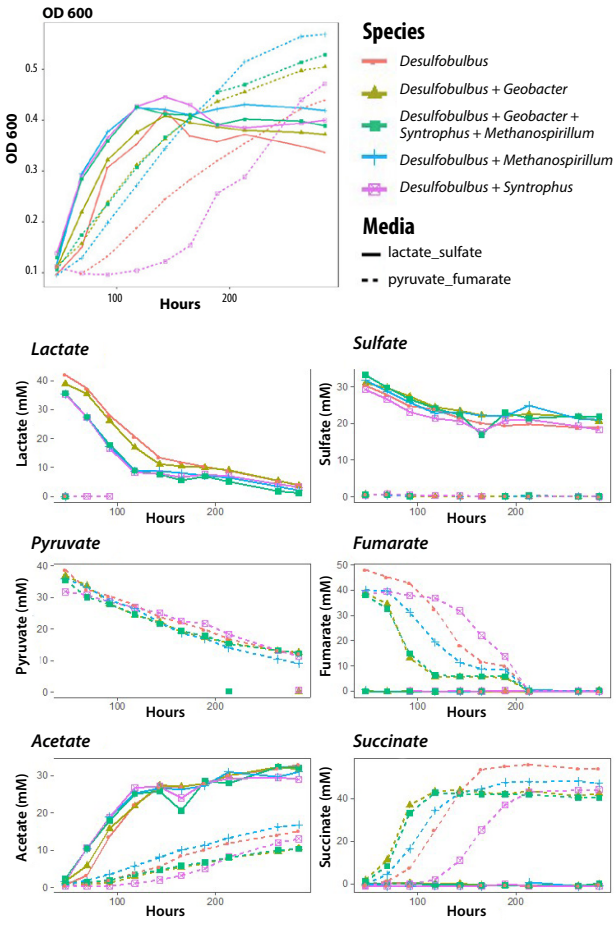

Fig. 6. Assessing *hgcAB* Alternative Function. *Left:* Growth curves of the individual and various multicultures used in this study. *Right:* The temporal loss of electron donors and acceptors, lactate/sulfate or pyruvate/fumarate, and the production the donor and acceptor degradation products, acetate and succinate, respectively. Solid lines represent lactate/sulfate cultures and broken lines represent pyruvate/fumarate cultures.

under some conditions tested. Specifically, we found that there is an *arsR*-like gene upstream of *hgcAB* and have hypothesized that, as in As metabolism, this gene may control *hgcAB* expression. To date, we have tested *hgcAB* expression with different As forms and precursors for methionine production. These tests have revealed that both *hgcAB* expression and Hg methylation were altered in several of the conditions, including with arsenate and arsenite.

Developing Model Microbial Communities and Fitness Assay

In FY21, we have made steady progress toward understanding the effect of multispecies interactions on MeHg generation and developing model microbial communities to reflect the EFPC community. Large-volume (1- to 4-L), single-species and dual-species batch cultures have been completed in bottles and bioreactors to characterize growth and chemical profiles for methylating and nonmethylating sulfate-reducing bacteria and methanogens, including *Desulfobulbus propionicus*, *Desulfobulbus oligotrophicus*, *Methanospirillum hungatei*, and *Methanococcus maripaludis*. We measured a suite of analytical parameters throughout the growth curve for each culture, including growth rates based on optical density (OD) and protein content, Hg methylation, MeHg demethylation, *hgcA* expression, qPCR and fluorescence *in situ* hybridization (FISH) determination of relative cell abundance, simple metabolites (anions/cations/organic acids), sulfide concentration, and pH (see Fig. 6). Methylation/demethylation analyses are ongoing due to the pandemic, but the pairings of methylating and nonmethylating organisms allow us to disentangle the specific effect of syntrophic interactions on MeHg generation in organisms with very different metabolisms (i.e., sulfate reduction versus methanogenesis). To date, all culturing and analysis, with the exception of the Hg analyses, are complete. Continuing efforts are underway in FY21 to increase the complexity of multispecies cultures to recapitulate the range of metabolisms present in EFPC sediment, including the addition of *Syntrophus aciditrophicus* and *G. sulfurreducens*.

With respect to cell fitness under laboratory conditions, deletion of *hgcAB* does not significantly change the growth rate of *D. desulfuricans* ND132. However, maintenance of these genes across evolutionary time scales suggests that they provide an important fitness benefit in the environment. We hypothesize that the fitness effects of an *hgcAB* deletion are environmentally dependent and that these genes are conditionally dispensable under standard laboratory conditions. In conditions where *hgcAB* are providing their native function, we expect to see a fitness defect in a Δ*hgcAB* strain compared to the wild-type. So far, we have measured competitive fitness between wild-type and Δ*hgcAB* ND132 in more than 20 different media compositions. Although additional experiments are needed, our current results show that the strains grow differently when mixed together compared to when grown separately, despite minimal genetic differences between the two strains. In turn, this result suggests that HgcAB is involved in interactions between cells of ND132. Ongoing work is exploring these interactions in more detail.

Dimethylmercury Formation Mediated by a Sulfide Mineral Surface

Previous experimental work has shown that abiotic formation of dimethylmercury (DMeHg) from methylmercury (MeHg) can be enhanced on sulfide mineral surfaces. In

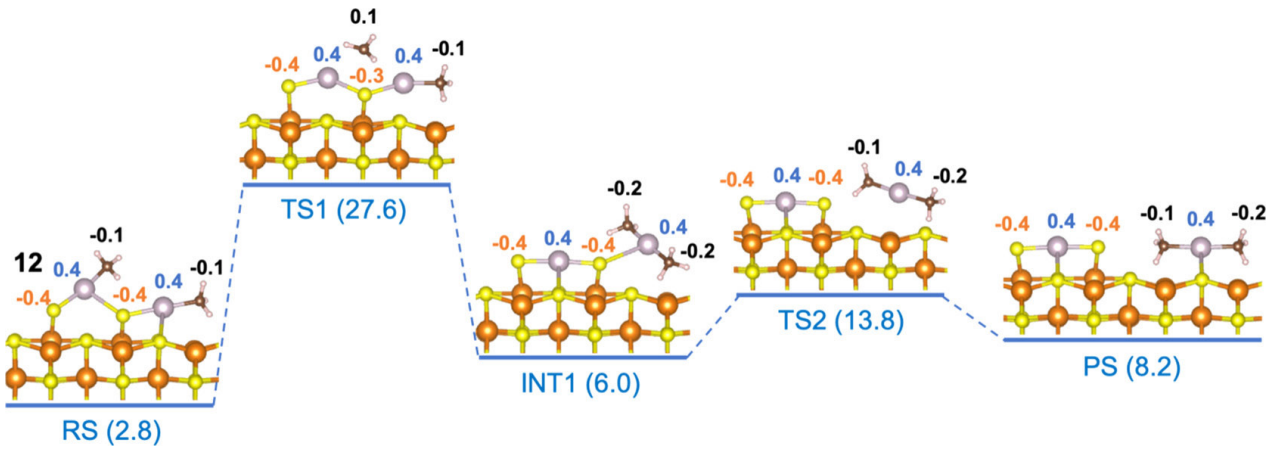

Fig. 7. Dimethylmercury Formation on Cadmium Sulfide Surfaces. Calculated reaction pathway for DMeHg formation on a model CdS nanoparticle. Charges on selected atoms are labeled. [Reprinted with permission from Lian, P., et al. 2021. "Mechanistic investigation of dimethylmercury formation mediated by a sulfide mineral surface." *Journal of Physical Chemistry*. DOI: 10.1021/acs.jpca.1c04014. Copyright 2021 American Chemical Society.]

collaboration with Niri Govind at Pacific Northwest National Laboratory (PNNL) and Sofi Jonsson at Stockholm University, we led a computational study that explored the mechanisms of DMeHg formation on the surface of a hawleyite nanoparticle. We showed that coordination of MeHg substituents to adjacent reduced sulfur groups protruding from the surface facilitate DMeHg formation and that the reaction proceeds through direct transmethylation from one MeHg substituent to another (see Fig. 7). Coordination of Hg by multiple S atoms provides transition state stabilization and activates a C-Hg bond for methyl transfer. Calculated energetics are in good agreement with available experimental data, providing confidence in the models. These findings fill a knowledge gap in our understanding of environmental Hg cycling.

Status of FY21 Milestones

Milestone 2a: Identified existing neutral, single-nucleotide mutations for fitness and performed several fitness assays using various metals, both higher and lower, as well as absent from growth media. This led to the discovery of an *arsR*-like gene that is likely influencing transcription of *hgcAB*. This hypothesis is currently being assessed jointly at SERC and ORNL.

Milestones 2b and g: Utilized the new antibodies and performed several cell sorter runs toward isolating EFPC Hg-methylating species. Thus far no Hg-methylators have been successfully isolated.

Milestone 2c: Completed use of comparative genomics for elucidating *hgcAB* pathways. This milestone, in combination with Milestone 2a, has led us to the likely promotor/repressor for *hgcAB* expression.

Milestone 2d: Have not yet begun transformation of *hgcAB* into naïve hosts to mimic HGT.

Milestones 2e and f: Completed characterization of microbial growth and Hg methylation in monocultures and two co-cultures, and are working toward characterizing a synthetic community. Cultivations and all analyses, except MeHg quantification, are complete, and the latter is well underway.

Milestones 2h and i: Submitted manuscript (under review) on metagenomics-enabled co-evolution and protein-protein interaction studies for HgcA and HgcB. We are also initiating protein-protein interaction studies for HgcAB-associated proteins for biochemical pathway elucidation.

FY22 Plans

In FY22, Theme 2 planned activities include:

- Continue to elucidate the influence of *arsR* on *hgcAB* expression.
- Continue sequence analysis efforts to determine diversity of Hg-methylating microbes in EFPC in collaboration with Theme 1.
- Continue to develop protocols for identifying, isolating, and characterizing novel EFPC methylators and demethylators.
- Initiate more complex tri- and quad-community cultures to assess the effect of syntrophic relationships

on Hg methylation for all major microbial functional groups in an anaerobic environment.

- Initiate effort to search genomes of known Hg-methylating microorganisms for additional proteins that exhibit a coevolutionary signature indicative of binding to HgcA, HgcB, or both.

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Published or In Press

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Theme 3: Biogeochemical Complexity and Molecular Mechanisms of Hg Transformations

The overarching goal of Theme 3 is to gain a fundamental understanding of complex biogeochemical processes and their interactions [e.g., dissolved organic matter (DOM), microbes, particulate organic matter (POM) and minerals, and water chemistry in EFPC] controlling Hg species transformation and availability for cellular uptake and methylation. Our specific objectives are to address the following scientific questions:

- What are the dominant Hg-binding organic ligands or molecular compositions (e.g., thiolates in DOM), and how do they competitively interact and control Hg speciation?
- What are the Hg-binding domains on cell membrane and cytosols, and how do cells competitively interact with extracellular organic and inorganic ligands for Hg binding, uptake (either passive or active), and ultimately methylation?
- How does environmental complexity (e.g., DOM, microbes, and minerals) influence Hg species distribution and availability for cell sorption, uptake, and methylation?

FY20–FY21 Accomplishments

Theme 3 made significant progress toward milestones over the past 12 months and has published 10 papers, with 10 additional manuscripts currently under review or in preparation. These studies are mostly focused on understanding a complex, yet finite set of geochemical and biomolecular processes controlling behavior, transformations, and net MeHg production in the environment. A robust predictive understanding of Hg biogeochemical transformations requires knowledge about the underlying molecular mechanisms and coupled interactions between Hg-binding organic and inorganic ligands, minerals, and methylating and demethylating microorganisms.

Overlooked Mercury Isotope Exchange in Environmental Tracer Studies and Implications

Enriched Hg-stable isotopes have been widely used as tracers in field and laboratory investigations of Hg

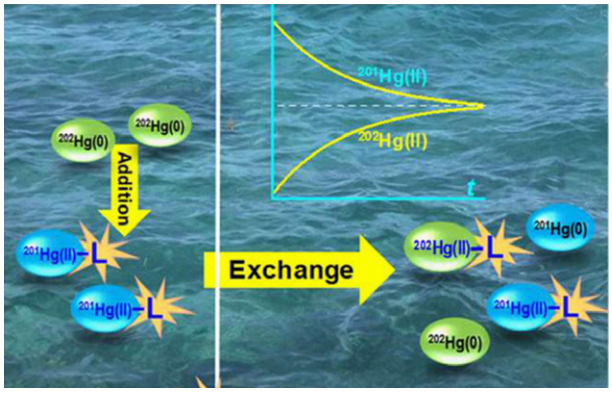

Fig. 8. Spontaneous Mercury (Hg) Isotope Exchange. Illustration of spontaneous Hg isotope exchange between enriched ^{202}Hg(0) spike and ^{201}Hg bound to various environmental matrices, such as thiols and dissolved organic matter (DOM), resulting in rapid redistributions of Hg isotopes bound to the ligand. [Reprinted with permission from Wang, Q., et al. 2020. "Rates and Dynamics of Mercury Isotope Exchange between Dissolved Elemental Hg(0) and Hg(II) Bound to Organic and Inorganic Ligands." *Environmental Science & Technology Technology*. **54**(23):15534–45. DOI:10.1021/acs.est.0c06229. See also Zhang, L., et al. 2021.]

biogeochemical transformations, such as methylation and demethylation. Few studies, however, have considered concurrent isotope exchange reactions between newly spiked and preexisting ambient Hg in environmental matrices. Using enriched Hg (as mercuric $HgCl_2$), we examined isotope exchange between enriched Hg and ambient Hg(II) bound to various environmental matrices, including soil minerals, low-molecular-weight (LMW) thiols, and DOM. The impact of isotope exchange on MeHg production in the presence of organic ligands was evaluated with an iron-reducing bacterium, *Geobacter sulfurreducens* PCA. Surprisingly, we found that the spiked Hg(II) rapidly exchanged with ligand- or mineral-bound ambient Hg(II), resulting in redistribution of Hg isotopes bound to the ligands or minerals. We also observed an apparently similar methylation rate and magnitude of the spiked Hg(II) and ambient Hg(II) by PCA cells. These observations underscore the importance of isotope exchange when an enriched Hg isotope is applied in environmental matrices, as the exchange could potentially lead to biased rate calculations of Hg transformation and bioaccumulation and thus result in biased risk assessments of new Hg input to the natural ecosystems.

Additionally, we investigated the rates and dynamics of isotope exchange between dissolved elemental $^{202}Hg(0)_{aq}$ and ^{201}Hg(II) bound to organic and inorganic ligands with varying chemical structures and binding affinities (see Fig. 8). Time-dependent exchange reactions were followed by isotope compositional changes using both inductively coupled plasma mass spectrometry and Zeeman cold vapor atomic absorption spectrometry. Rapid, spontaneous isotope exchange (<1 h) was also observed between $^{202}Hg(0)_{aq}$ and ^{201}Hg(II)-bound to chloride (Cl^-), ethylenediaminetetraacetate (EDTA), and thiols, such as cysteine (CYS), glutathione (GSH), and 2,3-dimercaptopropanesulfonic acid (DMPS). Without external reductants or oxidants, the exchange resulted in transfers of two electrons and redistribution of Hg isotopes bound to the ligand but no net changes of chemical species in the system. However, an increase in the thiol:Hg(II) ratio decreased the exchange rates due to the formation of 2:1 or higher thiol:Hg(II) chelated complexes, but it had no effects on exchange rates with ^{201}Hg(II) bound to EDTA or Cl^-. The exchange between $^{202}Hg(0)_{aq}$ and ^{201}Hg(II) bound to DOM showed an initially rapid exchange rate followed by a slower exchange rate, likely resulting from Hg(II) complexation with both low- and high-affinity binding functional groups on DOM (e.g., carboxylates vs bidentate thiolates). These results demonstrate that $Hg(0)_{aq}$ readily exchanges with Hg(II) bound to various ligands and highlight the importance of considering exchange reactions in experimental enriched Hg isotope tracer studies or in studies where natural abundance Hg isotope are present in environmental matrices.

Roles of Methanobactin in the Biogeochemical Cycling of Hg and Transition Metals

Methanotropic bacteria and the chalkophore methanobactin have been implicated in the biogeochemical cycling of Hg. Methanobactins facilitate the acquisition of Cu(II) ions in some methanotrophic bacteria, analogous to siderophores and iron. Methanobactins are also known to form strong complexes with other late transition metals, including Hg. Thus, methanobactins influence the bioavailability of Hg(II) for microbial methylation (Yin et al. 2020) and MeHg for degradation by methanotrophs (Lu et al. 2017). The influence of methanobactins on MeHg production by anaerobic bacteria is considerable. Recent studies revealed significant differences between methanobactins produced by different methanotrophs, e.g. *Methylosinus trichosporium* OB3b and *Methylocystis sp.* strain SB2. We hypothesize that the chemistry and configuration of functional groups with the methanobactin metal binding site controls Hg(II) complexation, ligand exchange reactions involved in Hg(II) uptake by methylators, and ultimately methylation of Hg(II) by HgcAB.

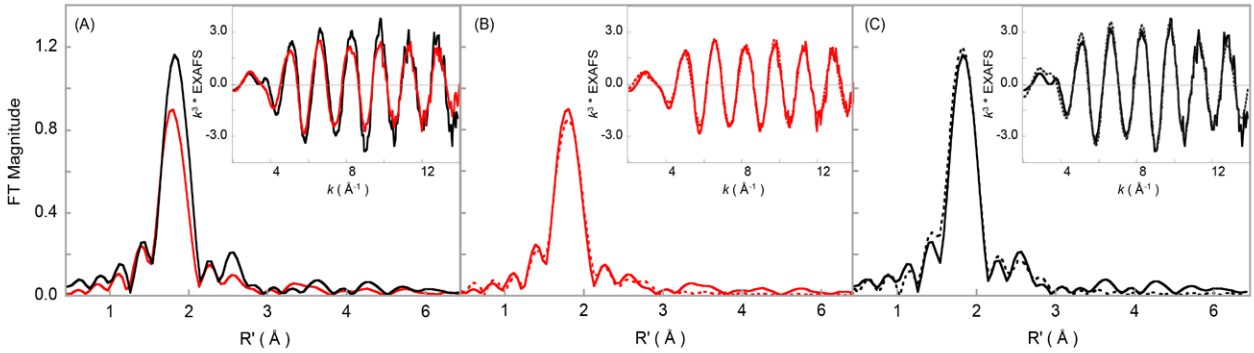

Fig. 9. Methanobactin SB2-Mercury (Hg) X-ray Absorption Spectroscopy. (A) A comparison of the non-phase shift corrected Fourier transforms of the Hg L3-edge EXAFS data for 1:1 (black) and 2:1 (red) mixtures of mb-SB2:Hg^{2+}. The inset shows the corresponding EXAFS comparison. (B) and (C) show the best structural fits (derived using FEFF) to the data for 1:1 and 2:1 mixtures of mb-SB2:Hg^{2+}, respectively. [Reprinted from Eckert P., et al. 2021. "Spectroscopic and computational investigations of organometallic complexation of group 12 transition metals by methanobactins from *Methylocystis* sp. SB2." *Journal of Inorganic Biochemistry*. *In press.* DOI:10.1016/j.jinorgbio.2021.111496. Copyright 2021, with permission from Elsevier.]

To better understand the interplay between solution-phase configurations and metal interactions of methanobactins, we compared spectroscopic signatures of methanobactin with Hg(II) and several transition metal complexes. We studied the complexation of Zn, Cd, and Hg ions by methanobactin from *Methylocystis* sp. strain SB2 using a combination of absorbance, fluorescence, and extended X-ray absorption fine structure (EXAFS) spectroscopy, complemented by time-dependent density functional theory (TD-DFT) calculations. Characteristic changes in absorbance and fluorescence spectra occur over a wide range of experimental timescales and arise from a stoichiometric complexation with thiolate functional groups in methanobactin. Hg L3-edge EXAFS and TD-DFT calculations suggest a linear model for Hg-S coordination (see Fig. 9), while TD-DFT calculations are consistent with a tetrahedral model for Zn(II) and Cd(II). Enhancement of the fluorescence emission of methanobactin observed upon interaction with transition metals indicates a mechanism of complexation-hindered isomerization of the intrinsic fluorophores in methanobactin. Investigating the biomolecular basis of metal complexation advances our understanding of ligand exchange reactions controlling Hg(II) uptake and MeHg production.

Isolation of Two Methanotrophs from EFPC and Impact on Methanotrophic-Mediated MeHg Degradation

In collaboration with Prof. Jeremy Semrau at University of Michigan and Theme 1, two novel methanotrophs of the Gammaproteobacteria class, *Methylomonas* sp. EFPC1 and *Methylococcus* sp. EFPC2, were isolated from the Hg-contaminated EFPC biofilm samples. The 16S rRNA sequence analyses of *Methylomonas* sp. strain EFPC1 indicated that it was phylogenetically similar to *Methylomonas* sp. LW13, and *Methylococcus* sp. strain EFPC2 was most similar to *Methylococcus geothermalis* $IM1^T$. Average nucleotide identity (ANI) values between *Methylomonas* sp. strain EFPC1 and *Methylomonas* sp. LW13 and between *Methylococcus* sp. strain EFPC2 and *Methylococcus* sp. $IM1^T$ were ~95% and 73%, respectively. Genes for particulate methane monooxygenase (pMMO) were found in both *Methylomonas* sp. strain EFPC1 and *Methylococcus* sp. strain EFPC2, while evidence of a divergent form of pMMO (pXMO) and soluble methane monooxygenase was found in only *Methylomonas* sp. strain EFPC1. Genes for the central pathway of methane oxidation were found in both methanotrophs, as well as for the ribulose monophosphate pathway and tricarboxylic acid cycle (Kang-Yun et al. 2021a).

Additionally, we found evidence for methanobactin "theft" among methanotrophs, which could potentially impact on methanotrophic-mediated MeHg degradation (Kang-Yun et al. 2021b). Aerobic methanotrophy is strongly controlled by copper (Cu), and methanotrophs are known to have multiple mechanisms for Cu uptake. Some methanotrophs secrete a MB that binds Cu with extremely high affinity, while others utilize a surface-bound protein (MopE) and a secreted form (MopE*) for Cu collection. Cu competition may therefore significantly impact methanotrophic community composition and activity. We show that *Methylomicrobium album* BG8, *Methylocystis* sp. strain Rockwell,

and *Methylococcus capsulatus* Bath, all lacking genes for MB biosynthesis, are not limited for Cu by multiple forms of MB. Interestingly, *Mm. album* BG8 and *Methylocystis* sp. strain Rockwell were found to have genes similar to *mbnT* that encodes for a TonB-dependent transporter required for MB uptake, indicating that these methanotrophs may "steal" Cu-MB complexes and degrade MeHg. Indeed, substantial demethylation was observed by these strains in the presence of MB. *Mc. Capsulatus* Bath, however, lacks anything similar to *mbnT*, and it was unable to degrade MeHg either in the presence or absence of MB. Similarly, when *mbnT* was deleted in *Mm. album* BG8, MeHg degradation in the presence of MB was indistinguishable from when MB was not added. These results indicate that methanotrophic-mediated MeHg degradation may be more widespread than previously thought and thus play an important role in net production of MeHg in the environment.

Status of FY20 Milestones

Milestones 3a and b: Completed spectroscopic characterization of methanobactin complexes with Hg(II) and other transition metals (Eckert et al. 2021). Additional studies to investigate the thermodynamics and dissociation constants for complexes of methanobactin with Hg(II) and other transition metals are ongoing. Completed studies of competitive interactions between divalent metal ions and Hg(II) bound to various organic ligands and the effects of mixed thiol ligands on microbial methylation of Hg. Two manuscripts have been submitted for review (Zhang et al. 2021; Liang et al. 2021).

Milestones 3c, d, and e: Completed studies of mixed organic ligands–Hg(II)–cell interactions in controlling Hg uptake and methylation (Liang et al. 2021, in revision). We also completed experiments on MeHg uptake and interactions with methanotrophs and found evidence of methanobactin theft and its impact on methanotrophic-mediated MeHg degradation. Two manuscripts are in preparation (Zhang et al. 2021; Kang-Yun et al. 2021b). We also continued our collaboration with the Ragsdale Lab at the University of Michigan on HgcAB purification and biochemical and structural characterization.

Milestones 3f, g, and h: Completed studies of the rates and dynamics of isotope exchange between spiked enriched Hg isotopes and ambient Hg and its impact on environmental Hg tracer studies and risk assessments of new Hg input to natural ecosystems (Zhang et al. 2021; Wang et al. 2020). We concluded experiments on the effects of green alga and phytoplankton on Hg methylation and MeHg degradation under varying conditions. Two manuscripts are currently in preparation (Liang et al. 2021; Wang et al. 2021). We also isolated two novel methanotrophs from EFPC biofilm samples (Kang-Yun et al. 2021a) and characterized their role in MeHg uptake and degradation (Zhang et al. 2021).

FY22 Plans

In FY22, Theme 3 planned activities include:

- Continue studies of complex environmental matrixes and processes (divalent metal ions, N_2O, and organic matter) on Hg biogeochemical transformations.
- Complete studies of the effects of mixed organic ligands on cellular Hg uptake and methylation.
- Determine influences of algae and phytoplankton on microbial Hg methylation and MeHg degradation under varying environmental conditions.
- Characterize the two novel methanotrophs isolated from EFPC biofilm samples and determine methanotrophs interactions and implications on methanotrophic-mediated MeHg degradation.
- Investigate coordination geometry of transition metal complexes with different methanobactin variants using EXAFS spectroscopy. Determine thermodynamic parameters of metal-methanobactin complexation using istothermal calorimetry.
- Continue biochemical characterization of HgcAB complex.

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Published or In Press

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Data Products Released

1. The annotated genome sequences have been deposited in Genbank under accession numbers CP070494 and CP070495 (*Methylomonas* sp. strain EFPC1) and CP070491, CP070492, and CP070493 (*Methylococcus* sp. strain EFPC2). Raw reads have been deposited in the NCBI Sequence Read Archive under accession numbers SRX10121820 and SRX10121822 (for Illumina MiSeq reads of *Methylomonas* sp. strain EFPC1 and *Methylococcus* sp. strain EFPC2, respectively).
2. Quality filtered sequence data from studies of the biogeochemical drivers of MeHg production in Arctic tundra soils were deposited at European Nucleotide Archive (PRJEB37429).

Field-Scale Modeling Activity

The field-scale modeling activity, a partnership activity with the Interactive Design of Extreme-scale Application Software (IDEAS)–Watersheds project, is developing, evaluating, and refining multiscale modeling approaches and software frameworks that allow increasingly detailed understanding of fine-scale biogeochemical processes to be used in basin-scale river network models. Central to our strategy is our new multiscale ADELS model (Painter 2018; 2021) that extends highly successful residence-time

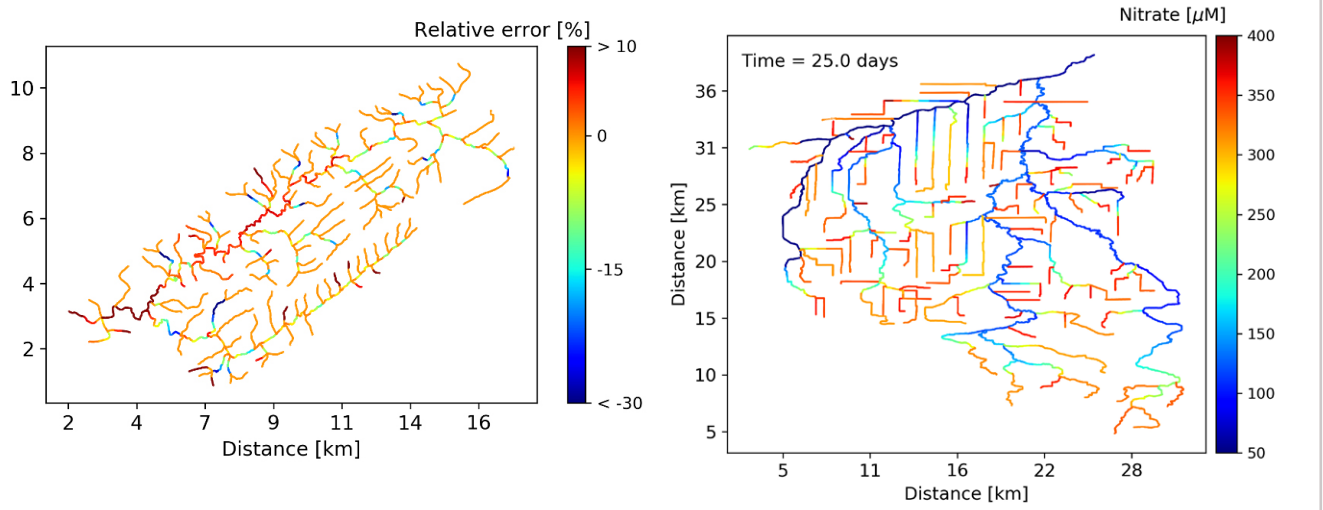


Fig. 10. Multiscale Advanced Terrestrial Simulator (ATS) Simulations. *Left*: Difference in calculated denitrification between coarse- and fine-resolution representations of the stream network in EFPC. The coarse representation, which used one channel grid cell per channel segment as in semi-distributed models like SWAT, resulted in significant spatial discretization error. *Right:* Calculated nitrate concentration in a nitrate loading experiment in a subbasin of the Portage River Basin.

models to accommodate nonlinear multicomponent reactions. The approach moves biogeochemical process representation to subgrid models that each represent an ensemble of hyporheic-zone flowpaths. Shifting to subgrid models allows biogeochemical processes to be represented in great detail at their native spatial scales without averaging over important, fine-scale variability in redox states that occurs within sediments and periphyton biofilms.

The broad objective of integrated activity on model development and parameterization is to facilitate state-of-the-art advancements in process-based modeling of reactive transport in stream systems using Hg in EFPC as a representative use case. Specific objectives are to implement and test the new multiscale model in the ATS integrated hydrology software, develop and evaluate methods for estimating model parameters from stream tracer tests, and demonstrate the capability in watershedscale reactive transport simulations.

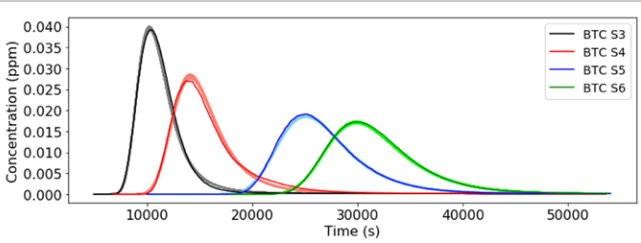


Fig. 11. Stream Tracer Test Breakthrough Curves. Predictive uncertainty plot for breakthrough curves at four locations simulated together using an ensemble of jointly estimated parameters. Observed concentrations are represented by solid lines and ensemble of simulated BTCs is shown in the corresponding semi-transparent lighter shade. [Reprinted by permission from Rathore, S. S., et al. 2021. "On the Reliability of Parameter Inferences in a Multiscale Model for Transport in Stream Corridors." *Water Resources Research*. **57**(5). DOI: 10.1029/2020WR028908. Copyright 2021. John Wiley & Sons, Inc.]

FY20–FY21 Accomplishments

The implementation of ADELS in ATS was extended from conservative tracers to multicomponent reactive transport. The reactive transport capability uses the Alquimia interface to access the general geochemical reaction capability within the PFLOTRAN software. Multiple subgrid models with different reaction models may be specified for each channel grid cell to represent, for example, metabolically active and inactive transient storage. The implementation was verified against independent solutions. Mesh convergence studies using denitrification, as an example, revealed that semi-distributed conceptualizations, like that used for reactive transport in the Soil & Water Assessment Tool (SWAT) model, can produce significant spatial discretization error (see Fig. 10, left panel). Additional simulations using a subbasin of the Portage River Basin reveal complex spatial patterns in denitrification, including spatially localized hotspots (see Fig. 10, right panel) that are difficult to represent in existing simulation tools.

Methods for estimating model parameters from stream tracer test results were developed, implemented in Python workflows, and evaluated (Rathore et al. 2021) using a public dataset from tracer tests in the Hammer Stream in West Sussex, UK. For that work, we used Bayesian inference and the Markov Chain Monte Carlo (MCMC) method, which provides estimates of uncertainty in the various model parameters. The hyporheic travel time distributions were estimated without assumptions about the distribution shape. For the first time, the shape-free distributions were estimated simultaneously with channel properties. Moreover, we demonstrated that simultaneous analysis of observations from multiple locations constrains parameters better than analyzing those observations individually. Results from that work are shown in Fig. 11. The observational correlations provide additional confidence in the ADELS model. In addition, the methods and workflow tools developed provide the basis for analyzing future tracer tests in EFPC to estimate model parameters.

A novel Bayesian joint-fitting strategy was developed (see Fig. 12) and used to reinterpret results from the sorption and methylation experiments on EFPC sediments (see Theme 1 Accomplishments). Specifically, we applied Bayesian inference and MCMC simultaneously to multiple sorption and methylation experiments instead of the traditional approach of analyzing subprocess models individually. Joint-fitting more rigorously propagates uncertainties between different subprocess models and also allows information that is shared between datasets to be more fully used. Bayesian inference and MCMC also produce the full joint distribution of parameters, thus providing global uncertainty estimates and facilitating the detection of overparameterization manifesting as null spaces in the parameter space. The identification of null spaces guided the simplification of certain subprocess

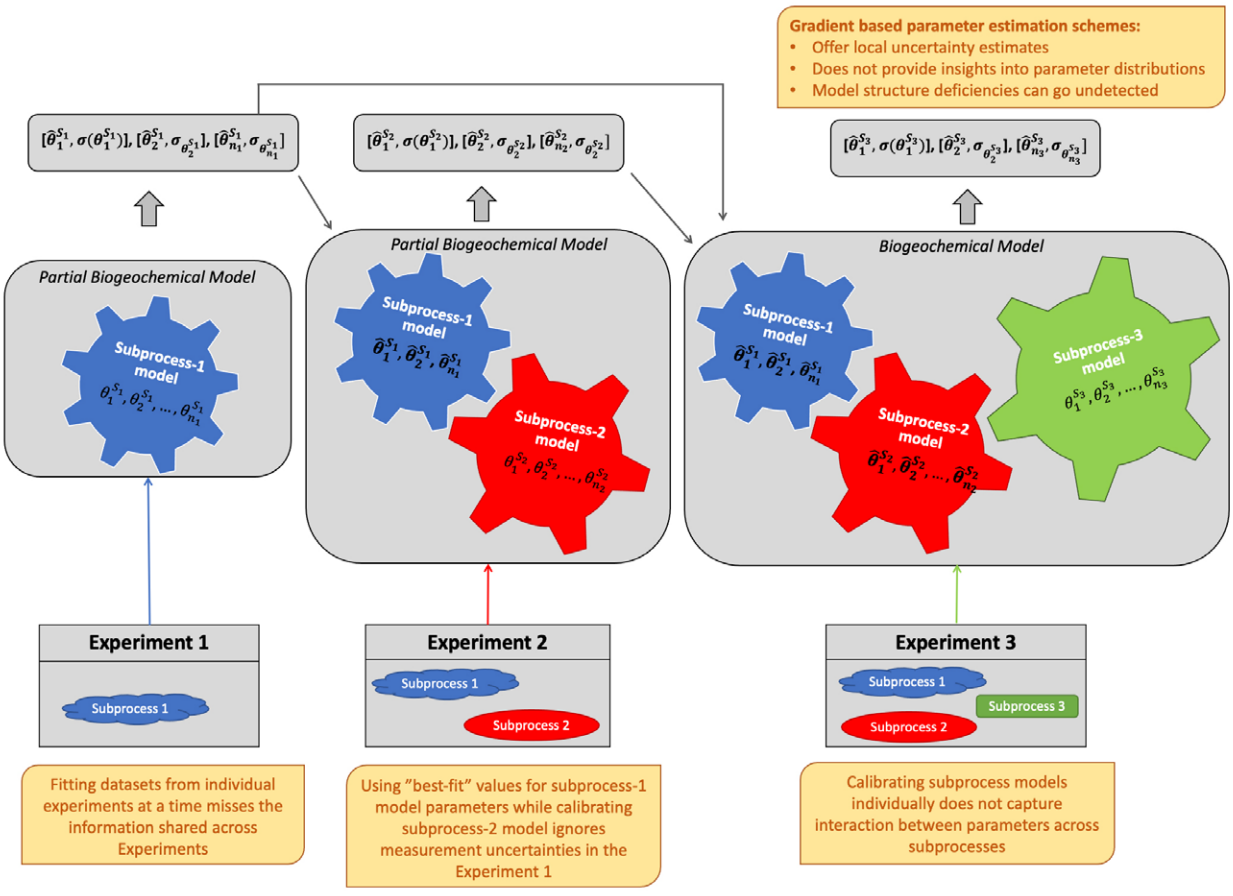


Fig. 12. Illustration of Bayesian Scheme. Schematics of Bayesian joint-fitting scheme for calibrating complex biogeochemical models offer multiple advantages with efficient utilization of datasets, provide full uncertainty propagation and robust estimation, and detect parameterization deficiencies.

models: fast kinetic sorption was replaced by equilibrium sorption, and Monod demethylation was replaced by first-order demethylation based on the analysis. The proposed scheme will benefit parameter estimation for other complex biogeochemical systems characterized through multiple experiments.

The ADELS model was compared to an alternative multirate model that conceptualizes the hyporheic zone as a large number of well-mixed transient storage zones that exchange solute directly with the channel, but not with each other. That analysis showed that ADELS and the multirate model are mathematically equivalent in the special case of nonreacting tracers. However, they produce very different predictions of reactive transport. In particular, direct exchange of oxygen between each transient storage zone and the stream channel suppresses redox zonation in the multirate model, thus limiting its potential as a general-purpose model for reactive transport in stream corridors. The comparison also emphasizes that nonreacting tracers alone are unable to constrain models for hyporheic exchange and need to be augmented by additional observations, like reactive tracer tests.

Status of FY21 Milestones

As described in the FY19 and FY20 annual reports, our original proposed work on modeling EFPC tracer tests was delayed to focus first on analysis of tracer test results from other systems. A detailed analysis of conservative tracer tests has been completed (FRA2h), and a journal article (FRA2i) has been published (Rathore et al. 2021).

ATS development activities were reprioritized to undertake implementation of the ADELS model for full river networks instead of coupling PHREEQC to ATS. ADELS in ATS is now fully operational for steady flows using PFLOTRAN for chemical reactions, and a manuscript (FRA2b) on that work has been submitted. We initiated premodeling (FRA2j) of the planned EFPC tracer test in FY21, and this effort should be completed by the end of the fiscal year.

FY22 Plans

In FY22, we plan to extend the ADELS framework to accommodate unsteady discharge. This capability will then be used to analyze tracer tests affected by unsteady flow. We will work with the developers of the Networks with EXchange and Subsurface Storage (NEXSS) model to couple it with our workflow for building stream network models in ATS. We will also continue our premodeling and optimization of EFPC tracer tests.

Manuscripts

Published or In Press

Painter, S. L. 2021. "On the representation of hyporheic exchange in models for reactive transport in stream and river corridors." *Frontiers in Water*. **2**:69. DOI:10.3389/frwa.2020.595538.

Rathore, S. S., A. Jan, E. T. Coon, and S. L. Painter. 2021. "On the reliability of parameter inferences in a multiscale model for transport in stream corridors." *Water Resoures Research*. **57**(5):e2020WR028908. DOI:10.1029/2020WR028908.

Submitted or In Preparation

Rathore, S. S., G. E. Schwartz, S. C. Brooks, and S. L. Painter. "Model selection and joint estimation of biogeochemical model parameters from multiple experiments: A Bayesian approach applied to mercury methylation." *Environmental Modeling and Software. Submitted.*

Jan, A., E. T. Coon, and S. L. Painter. "Toward more mechanistic representations of biogeochemical processes in stream and river networks: Implementation and demonstration of a multiscale model." *Environmental Modeling and Software. Submitted.*

Select Research Highlights

In FY2021, a total of 49 manuscripts were published or submitted by the Critical Interfaces SFA. Of these publications, 24 are published or in press, bringing the total to 166 for the Critical Interfaces SFA since its inception. Of these 166 publications, 151 are the result of new mercury research, and 15 represent DOE Environmental Remediation Sciences Program projects that were completed with partial SFA funding. In this section, we highlight four of the 24 published or submitted manuscripts.

Research Highlight

New Global Database Launches for Identifying Mercury-Cycling Microbes

Curated in an international collaboration led by ORNL postdoc Caitlin Gionfriddo, Hg-MATE-Db provides tools and data for expanding insights into microbially mediated mercury (Hg) transformations in the environment.

The Science

The Hg-cycling Microorganisms in Aquatic and Terrestrial Ecosystems Database (Hg-MATE-Db) contains resources for identifying microbes that can transform Hg into the airborne Hg^0 and Hg^{2+} found in streams, lakes, and oceans and the neurotoxic methylmercury (MeHg) found in fish and other aquatic life. This first release of the database has tools for measuring Hg methylator abundance and diversity and the potential of these organisms to methylate Hg. Hg-MATE-Db can be used to identify key microbial producers of MeHg, and upcoming versions will provide tools for identifying microbes that can transform MeHg into Hg^0 and Hg^{2+}.

Hg-MATE-Db—the Hg-cycling Microorganisms in Aquatic and Terrestrial Ecosystems Database—houses resources for identifying mercury-cycling microbes from diverse environments, such as the tidal salt marsh seen here on Assateague Island in Maryland. Marsh sediments harbor highly diverse communities of mercury-methylating microorganisms and can be hot spots for methylmercury production. [Courtesy Smithsonian Environmental Research Center]

The Impact

Microbes play a critical role in controlling which form of Hg is dominant in an environment. Produced by microbes, MeHg is the form most toxic to humans, especially to developing fetuses and children. It accumulates in the food web and is the predominant form found in fish and seafood. The net production of MeHg depends on the presence and activity of Hg-methylating microbes and the bioavailability of Hg species. Microbes that demethylate Hg (to Hg^{2+}) or reduce it (to Hg^0) can impact MeHg production. The Hg-MATE-Db provides resources to identify the major microbial players in Hg cycling in an environment. Understanding these microbially mediated dynamics is key to predicting how changes to an ecosystem's microbiome may impact MeHg production.

Summary

Hg-MATE-Db provides an ongoing and up-to-date collated resource of Hg-cycling genes from pure culture and environmental datasets. This resource enables researchers to identify Hg-cycling microorganisms and compare findings to other environmental settings and datasets. As the database grows with community input, so too will knowledge of the microorganisms that drive Hg cycling. Such microbes include those with the *hgcAB* gene pair that can produce MeHg, those that have genes of the *mer* operon that can demethylate or reduce Hg species as part of a detoxification pathway, and those with the *mer* operon that are resistant to Hg and can affect the form of Hg in the environment. Future versions of Hg-MATE-Db will include *hgcAB* sequences from targeted gene studies, further expanding insight into the gene pair's diversity across environmental settings. Developers also plan to add a *mer* dataset to the database, which will contain resources for identifying genes of the *mer* operon that encode for demethylation of organic Hg species (*merB*), as well as genes involved in reducing inorganic Hg (*merA*), operon regulation (*merR*), and Hg transport across the cell (*merTPC*).

Publication

Gionfriddo, C., E. Capo, B. Peterson, H. Lin, D. Jones, A. G. Bravo, S. Bertilsson, J. Moreau, K. McMahon, D. A. Elias, and C. Gilmour. 2021. "Hg-MATE-Db.v1.01142021." [Dataset]. The Smithsonian Institution. DOI:10.25573/serc.13105370.v1.

Research Highlight

Overlooked Mercury Isotope Exchange in Environmental Tracer Studies and Implications

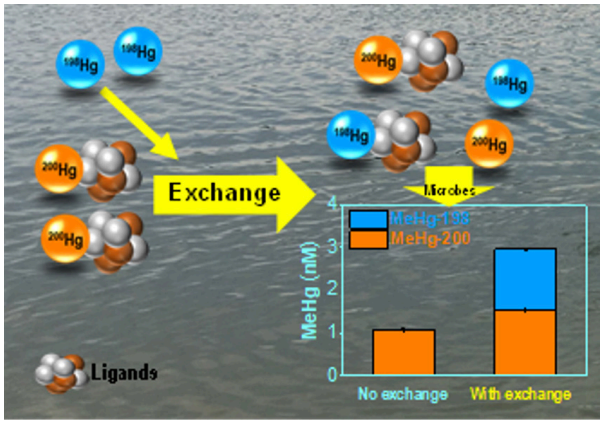
Rapid, spontaneous mercury (Hg) isotope exchange in environmental matrices alter Hg isotope redistribution and thus affects Hg bioavailability and methylmercury (MeHg) production.

The Science

Enriched Hg-stable isotopes have been widely used as tracers in field and laboratory investigations of Hg biogeochemical transformations such as methylation and demethylation. Few studies, however, have considered concurrent isotope exchange reactions between newly spiked and preexisting ambient Hg in environmental matrices, which may alter redistribution and thus transformation of the spiked and ambient Hg. Using enriched Hg [as mercuric Hg(II) or dissolved elemental $Hg(0)_{aq}$], this study investigated isotope exchange between spiked enriched Hg and ambient Hg(II) bound to various environmental matrices, including soil minerals, low-molecular-weight (LMW) thiols, and dissolved organic matter (DOM). The impact of isotope exchange on MeHg production in the presence of organic ligands was also evaluated with an iron-reducing bacterium *Geobacter sulfurreducens* PCA. Without external reductants or oxidants, the exchange between $Hg(0)_{aq}$ and Hg(II)resulted in transfers of two electrons and redistribution of Hg isotopes bound to the ligand but no net changes of chemical species in the system. Similarly, the spiked Hg(II) rapidly exchanges with ligand- or mineral-bound ambient Hg(II), resulting in redistribution of Hg isotopes bound to the ligands or minerals and an apparently similar methylation rate and magnitude of the spiked Hg and ambient Hg by PCA cells. These observations underscore the importance of isotope exchange when an enriched Hg isotope is applied in environmental matrices, as the exchange could potentially lead to biased rate calculations of Hg transformation and bioaccumulation and thus result in biased risk assessments of new Hg input to the natural ecosystems.

The Impact

The research highlights the importance of considering isotope exchange reactions in experimental enriched Hg tracer studies or in studies where natural abundance Hg isotope are present in environmental systems.



Schematic illustration of Hg isotope exchange process.

Summary

We investigated the rates and dynamics of Hg isotope exchange between spike-enriched Hg and ambient Hg bound to various environmental matrices, and found surprisingly rapid, spontaneous Hg isotope exchange reactions resulting in redistribution of Hg isotopes and changes in Hg bioavailability. Without consideration of these reactions in enriched Hg tracer studies, the estimated rates and predictions of Hg transformation and bioaccumulation could be biased in environmental systems.

Publications

Wang, Q., L. Zhang, X. Liang, X. Yin, Y. Zhang, W. Zheng, E. M. Pierce, and B. Gu. 2020. "Rates and dynamics of mercury isotope exchange between dissolved elemental Hg(0) and Hg(II) bound to organic and inorganic ligands." *Environmental Science & Technology*. **54**(23): 15534–45. DOI:10.1021/acs.est.0c06229.

Zhang, L., X. Liang, Q. Wang, Y. Zhang, X. Yin, X. Lu, E. M. Pierce, and B. Gu. 2021. "Isotope exchange between mercuric [Hg(II)] chloride and Hg(II) bound to minerals and thiolate ligands: Implications for enriched isotope tracer studies." *Geochimica et Cosmochimica Acta*. **292**:468–81. DOI:10.1016/j.gca.2020.10.013.

Research Highlight

Tracer Tests Inform a New Model for Transport in Streams

Parameters in a new multiscale model for stream transport are estimated from stream tracer tests.

The Science

The exchange of water between stream channels and adjacent groundwater is an important control on exports of carbon, nutrients, and contaminants from watersheds. That exchange is typically estimated by analyzing tracer tests using fluorescent dyes. Indirect estimates of exchange measured that way are sensitive to measurement and model uncertainties. Oak Ridge National Laboratory researchers reanalyzed previous tracer tests using uncertainty-aware inverse modeling and a new multiscale model for stream transport. The results demonstrate that the new model accurately represents transport in streams. The results also show that tracer tests can be used to estimate model parameters.

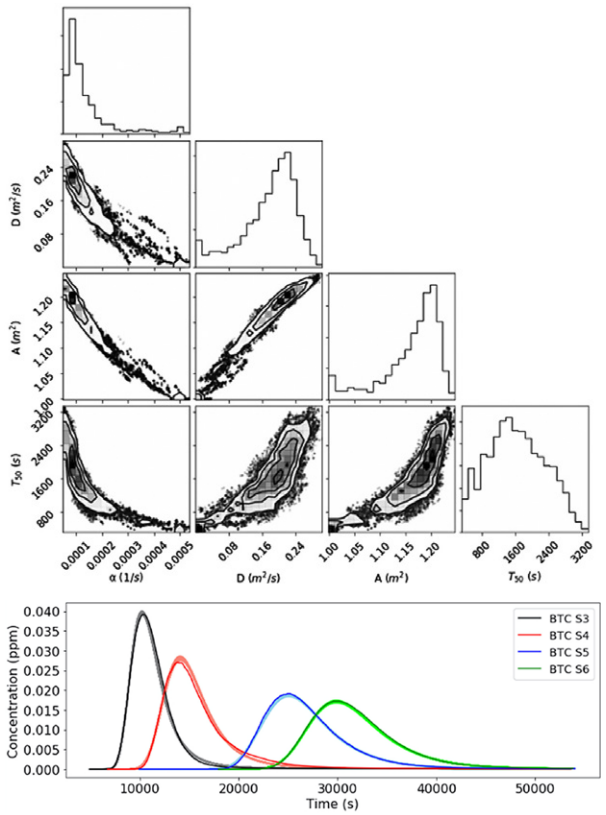
The Impact

This study provides additional confidence in a recently developed multiscale model for transport in river corridors. The model is implemented in the Advanced Terrestrial Simulator (ATS) software and provides the foundation for new watershed modeling capabilities that require fewer restrictive assumptions about stream-groundwater interactions. The analysis framework provides estimates of model parameters and their uncertainties. The study shows how different measurement locations and test durations can affect uncertainty in estimated model parameters. It also shows how data from multiple locations can be leveraged for reliable parameter estimates. That information can inform experimental design of future tracer tests including those involving reactive tracers.

Summary

Hyporheic exchange, the bidirectional movement of water and solute between stream and river channels and adjacent groundwater, is difficult to measure directly. Tracer tests offer an indirect method for estimating hyporheic exchange and constraining transport parameters in river corridor models. However, uncertainties and detection limits in measured concentrations, the indirect and ill-posed nature of inverse modeling, and model structural uncertainties present challenges for tracer test interpretations.

This study used a recently developed multiscale stream and river corridor model that represents solute flux to and from the hyporheic zone without explicitly solving three-dimensional subsurface transport. Implementation of the model in the ATS software combined with a Bayesian parameter estimation process and high-performance computing made it possible to estimate key model parameters and uncertainty in those parameters. Significantly, the hyporheic travel time distribution was estimated without assumptions on the distribution shape and simultaneously with channel transport parameters.



Estimated joint distribution of parameters representing transport in a stream channel and exchange with adjacent groundwater. Comparison between breakthrough curves observed and simulated at four measurement locations. [Reprinted by permission from Rathore, S. S., et al. 2021.]

Analyses of nonreacting tracer data from a low-gradient stream suggested that short reach length, brief measurement duration, and a time-dispersed source may impede parameter identifiability and result in broad or multimodal parameter distributions. Joint-fitting of data at multiple locations with uniform hyporheic zone and non-uniform channel parameters improve parameter identifiability with well-constrained unimodal parameter distributions.

Publication

Rathore, S. S., A. Jan, E. T. Coon, and S. L. Painter. 2021. "On the reliability of parameter inferences in a multiscale model for transport in stream corridors." *Water Resoures Research*. **57**(5):e2020WR028908. DOI:10.1029/2020WR028908.

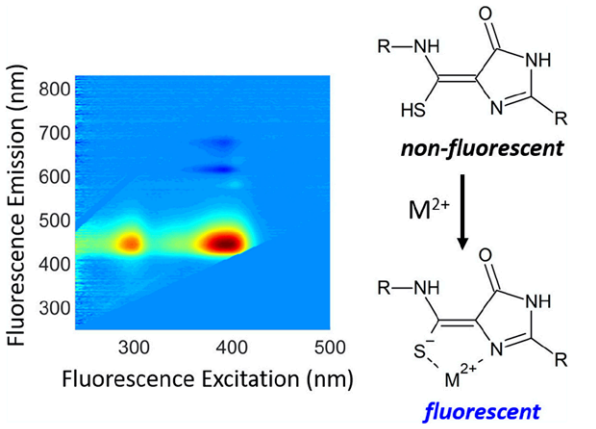
Research Highlight

Methanobactins Control Biogeochemical Cycling of Trace Metals

Organometallic interaction of group 12 metals by Methanobactin chalkophore from *Methylocystis sp.* SB2

The Science

Methanotrophic bacteria catalyze the aerobic oxidation of methane to methanol using enzymes with copper (Cu)-based active sites. To facilitate the acquisition of Cu ions some methanotrophic bacteria secrete small postranslationally modified peptides known as methanobactins. Analogous to siderophores and iron, methanobactins strongly bind Cu ions and functions as an extracellular Cu recruitment relay. In addition to binding Cu, methanobactins will bind most transition metals and near-transition metals and protect the host methanotroph as well as other bacteria from metal toxicity. Investigating the mechanisms of methanobactin-metal interactions is essential for understanding chemical speciation, competitive interactions, and biological processes involved in metal transformations.



Excitation emission spectrum and a schematic illustration of the proposed molecular mechanism for a notable fluorescence enhancement with transition metal complexation. [Reprinted with permission by Elsevier from Eckert, P. A., et al. 2021.]

The Impact

This work offers insights into mechanistic aspects of transition metal complexation by mb-SB2 and demonstrates its influence on the speciation and biogeochemical cycling of Hg and other transition metals. Results suggest that small metal-active peptides need to be considered in evaluating biological processes participating in metal transformations in natural environments and lay a groundwork for spectroscopic analysis of the peptide and its complexes.

Summary

Methanotrophic bacteria are typically found at oxic-anoxic interfaces in wetlands, soils, and aquatic systems and thus may have significant influence on the biogeochemical cycling of Hg and other metals. We characterized the interactions of methanobactin from *Methylocystis sp.* SB2 (mb-SB2) with transition metals using UV-Vis absorbance, fluorescence, and extended X-ray absorption fine structure spectroscopy (EXAFS) complemented by time-dependent density functional theory (TD-DFT) calculations. The metal binding site in mb-SB2 is comprised of two enethiolate groups, each conjugated with nitrogen-containing heterocycles, which facilitate interactions with a wide range of transition metal ions. The complexation of metal ions is reflected in the electronic structure of the conjugated system. Our spectroscopic data shows that mb–SB2–metal complexes may assume a range of intra- and intermolecular configurations that are distinct for each metal and depend on the metal to methanobactin ratio. We further report time-dependent changes in sample absorbance and fluorescence spectra, which occur on a wide range of experimental timescales. EXAFS data and TD-DFT calculations are consistent with tetrahedral coordination for Zn^{2+}, Cd^{2+} and linear coordination for Hg^{2+}. Furthermore, we propose a mechanism of complexation-hindered isomerization for a fluorescence enhancement observed upon the interaction of methanobactins with transition metals. This work represents the first combined computational and experimental spectroscopy study of methanobactins complexes with transition metals. Our results suggest that the methanobactins may influence the speciation and biogeochemical cycling of several group 11 and 12 transition metals.

Publication

Eckert, P., A. Johs, J. D. Semrau, A. A. DiSpirito, J. Richardson, R. Sarangi, E. Herndon, B. Gu, and E. M. Pierce. 2021. "Spectroscopic and computational investigations of organometallic complexation of group 12 transition metals by methanobactins from *Methylocystis sp.* SB2." *Journal of Inorganic Biochemistry.* **222**:(111496). DOI:10.1016/j.jinorgbio.2021.111496.

Postgraduate Spotlight

A key goal of the Critical Interfaces SFA and ORNL is to train the next generation of scientists and engineers. To this end, the SFA has maintained a number of outstanding graduate and postgraduate researchers since its inception 12 years ago. As part of this year's report, we highlight one of our outstanding postgraduate researchers—Spencer Washburn—who, along with others, has contributed significantly to the overall SFA goals and objectives. See website for a complete list of postgraduate researchers (www.esd.ornl.gov/programs/rsfa/alumni.shtml).

Spencer Washburn

Spencer Washburn is a biogeochemist whose main research focus is the understanding of the cycling of trace metal contaminants in natural systems, with a particular interest in mercury. Spencer received a B.S. in Environmental Science and a B.A. in Chemistry from the University of Chicago and earned his Ph.D. from the University of Michigan in 2018. His dissertation work was focused on using mercury-stable isotope ratios to understand Hg cycling within contaminated fluvial ecosystems, leading to five first-author publications and the receipt of the EESD John Dorr Graduate Academic Achievement Award. He recently completed a postdoctoral appointment at the Smithsonian Environmental Research Center, where he worked to develop an equilibrium passive sampler for methylmercury within sediment porewaters. Spencer began work as a postdoctoral research associate at ORNL in late summer of 2020, under the mentorship of Scott Brooks. At ORNL, Spencer is focused on understanding the impact of nutrient concentrations on mercury methylation within stream biofilms and periphyton. As part of this work, both field experiments in East Fork Poplar Creek and laboratory experiments with stream mesocosms are being conducted. In addition to scientific research, Spencer enjoys cycling, watching soccer, and hiking with his family.

National and International Impact

ORNL Critical Interfaces SFA team members attend strategic conferences in the United States and abroad to gain insights into the state of the science, share project findings and strategies with the broader mercury research community, and identify collaborative opportunities. From July 2020 to June 2021, SFA scientists delivered or published 9 presentations, abstracts, or posters (see Appendix C, p. 27, for details). Described below are team members' contributions to the virtual Goldschmidt 2021 conference, the 2020 American Geophysical Union (AGU) Fall Meeting, and the 2021 Environmental System Science Principal Investigators Meeting.

Goldschmidt 2021: Several Critical Interfaces SFA team members attended the Goldschmidt 2021 virtual conference held on July 4–9, 2021. Eric Pierce was a co-convener for the session, "(Bio) mineralization: Geochemical, Industrial, and Engineering Perspectives" and numerous SFA team members also gave presentations at the meeting.

American Geophysical Union Fall Meeting: Several Critical Interfaces SFA team members virtually attended the AGU Fall Meeting in San Francisco, California, on December 1–17, 2020. Eric Pierce gave an invited talk, and Baohua Gu was a convener for the session, "Mercury Biogeochemistry and Environmental Change I." SFA team members also gave a number of oral and poster presentations at the meeting.

BER's Environmental System Science Principal Investigators (PI) Meeting: SFA team members plan to attend the virtual PI meeting on August 17–19, 2021.

Ongoing Collaborative Research Activities

The ORNL Critical Interfaces SFA continues to engage a number of key collaborators in the project (see Fig. 13). In FY21, we continued to collaborate with EMSL staff to identify the proteomic and metabolomic signatures that will enable identification of the HgcAB alternative (native) biochemical function. External collaborators, including South River Science Team, Cynthia Gilmour (Smithsonian Environmental Research Center), Adam Ward (Indiana University), Marie Kurz (Drexel University), Helen Hsu-Kim (Duke University), Jeremy Smith (University of Tennessee), Jeremy Semrau (University of Michigan), and several Minority Serving Institutions: K.C. Carroll (New Mexico State University), B.K. Robinson (Alabama State University), and Victor Ibeanusi (Florida A&M University), continue to contribute to SFA milestones.

Although the SFA's primary objective is fundamental science, it is important that project personnel have the opportunity to translate scientific discovery into information relevant to DOE's Office of Environmental Management (EM) and the broader DOE complex. We continue to fulfill this need through active engagement with local Oak Ridge EM staff (Elizabeth Phillips and Brian Henry), EM headquarter staff (Kurt Gerdes), and the Oak Ridge site-specific advisory board.

Fig. 13. ORNL Critical Interfaces SFA partners.

Organizational Leadership

The scientific objectives of the Critical Interfaces SFA are aligned to the three integrated research themes and one research activity. These themes are managed across the SFA as an integrated team effort. Eric Pierce is the Laboratory Research Manager (LRM) and the point of contact with DOE Environmental System Science program managers. He speaks to Paul Bayer and Jennifer Arrigo biweekly on SFA progress and potential issues. Theme leaders are Scott Brooks, Dwayne Elias, and Baohua Gu, and the field-scale modeling activity lead is Scott Painter. These leaders, along with the broader team, meet tri-weekly to provide an update for current research directions, future plans, and staffing changes. See website for a complete SFA organization chart (www.esd.ornl.gov/programs/rsfa/contacts.shtml).

National Laboratory Investments

ORNL is committed institutionally to the success of the Critical Interfaces SFA program. In FY21, ORNL continued funding a strategic-hire Laboratory Directed Research and Development project under its Science & Technology Initiative titled "Understanding Complexity in Biological and Environmental Systems." Research under this investment is focused on understanding metal cycling in soils in Walker Branch watershed. Additional investments were made to modernize the biogeochemistry laboratories in ORNL's Environmental Sciences Division Building 1505. An estimated value of $2 million in equipment investments were made in FY21 and include (1) Sigray Quantum Leap H2000 X-ray Absorption Spectroscopy instrument, (2) two YSI EXO2 Sondes and accompanying sensor package, and (3) Labconco freeze dryer.

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Wang, L., X. Yin, X. Liang, X. Lu, L. Zhang, and B. Gu. "Opposing effects of green alga *Chlorella vulgaris* on mercury methylation by *G. sulfurreducens* PCA and *D. desulfuricans* ND132." *The Journal of Hazardous Material. In preparation*.

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Zhang, L., X. Liang, Q. Wang, Y. Zhang, X. Yin, X. Lu, E. M. Pierce, and B. Gu. 2021. "Isotope exchange between mercuric [Hg(II)] chloride and Hg(II) bound to minerals and thiolate ligands: Implications for enriched isotope tracer studies." *Geochimica et Cosmochimica Acta*. **292**:468–81. DOI:10.1016/j.gca.2020.10.013.

Appendix B. SFA Publications

See website for complete list (www.esd.ornl.gov/programs/rsfa/).

Appendix B. SFA Publications

Published Manuscripts

Abdelmageed Y., C. L. Miller, C. Sanders, T. Egbo , A. Johs, and B. K. Robertson. 2021. "Assessing microbial communities related to mercury transformations in contaminated streambank soils." *Water, Air, & Soil Pollution*. **232**:31. DOI:10.1007/s11270-020-04978-0.

Brooks, S. C., A. L. Riscassi, and K. A. Lowe. 2021. "Stream discharge and water quality data for East Fork Poplar Creek Beginning." *Hydrological Processes, Research and Observatory Catchments: The Legacy and the Future*. **35**(3):e14103. DOI:10.1002/HYP.14103.

Carrell, A. A., G. E. Schwartz, M. A. Cregger, C. M. Gionfriddo, D. A. Elias, R. L. Wilpiszeski, D. M. Klingeman, A. M. Wymore, K. A. Muller, and S. C. Brooks. 2021. "Nutrient exposure alters microbial composition, structure, and mercury methylating activity in periphyton in a contaminated watershed." *Frontiers in Microbiology*. **12**:543(647861). DOI:10.3389/fmicb.2021.647861.

Crowther, E. R., J. D. Demers, J. D. Blum, S. C. Brooks, and M. W. Johnson. 2021. "Use of sequential extractions and mercury stable isotope analysis to assess remobilization of sediment-bound legacy mercury." *Environmental Science: Processes & Impacts*. **23**(5):756–75. DOI:10.1039/D1EM00019E.

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Eckert, P., A. Johs, J. D. Semrau, A. A. DiSpirito, J. Richardson, R. Sarangi, E. Herndon, B. Gu, and E. M. Pierce. 2021. "Spectroscopic and computational investigations of organometallic complexation of group 12 transition metals by methanobactins from *Methylocystis sp.* SB2." *Journal of Inorganic Biochemistry*. **222**:(111496). DOI:10.1016/j.jinorgbio.2021.111496.

Egbo T. E., A. Johs, R. Sahu, Y. Abdelmageed, J. Ogbudu, and B. K. Robertson. 2021. "Interaction of soil microbes with organoclays and their impact on the immobilization of Hg under aerobic conditions." *Water, Air, & Soil Pollution*. **232**:157. DOI:10.1007/s11270-021-05093-4.

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Kim, M., R. L. Wilpiszeski, M. Wells, A. M. Wymore, C. M. Gionfriddo, S. C. Brooks, M. Podar, and D. A. Elias. 2021. "Metagenome-assembled genome sequences of novel species from the mercury-contaminated East Fork Poplar Creek, Oak Ridge, Tennessee, USA." *Microbiology Resource Announcements*. **10**(17):e00153–21. DOI:10.1128/MRA.00153-21.

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Rathore, S. S., A. Jan, E. T. Coon, and S. L. Painter. 2021. "On the reliability of parameter inferences in a multiscale model for transport in stream corridors." *Water Resoures Research*. **57**(5):e2020WR028908. DOI:10.1029/2020WR028908.

Rucker, D. F., C.-H. Tsai, K. C. Carroll, S. C. Brooks, E. M. Pierce, A. Ulery, and C. DeRolph. 2021. "Bedrock architecture, soil texture, and hyporheic zone characterization combining electrical resistivity and induced polarization imaging." *Journal of Applied Geophysics*. **188**:(104306). DOI:10.1016/j.jappgeo.2021.104306.

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Abdelmageed Y., C. L. Miller, A. Johs, and B. K. Robertson. "Mercury methylation by *Desulfomonile tiedjei* DCB-1 and effects of biochar on methylmercury production." *Biogeochemistry*. *In preparation*.

Barkay, T. and B. Gu. "Demethylation: The other side of the mercury methylation coin." *ACS Environmental Au*. *In preparation*.

Brooks, S. C., A. L. Riscassi, and C. L. Miller. "Diel mercury concentration variations in a mercury-impacted stream." *In preparation*.

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Jan, A., E. T. Coon, and S. L. Painter. "Toward more mechanistic representations of biogeochemical processes in stream and river networks: Implementation and demonstration of a multiscale model." *Environmental Modeling and Software. Submitted.*

Kang-Yun, C. S., X. Liang, P. Dershowitz, W. Gu, A. Schepers, A. Flatley, J. Lichtmanneger, H. Zischka, L. Zhang, X. Lu, B. Gu, J. C. Ledesma, D. J. Pelger, A. A. DiSpirito, and J. D. Semrau. "Evidence for methanobactin 'theft' and novel chalkophore production in methanotrophs: Impact on methanotrophic-mediated methylmercury degradation." *The ISME Journal*. *In preparation*.

Koenigsmark, F., C. Weinhouse, A. Berky, E. Ortiz, E. M. Pierce, W. Pan, and H. Hsu-Kim. "Efficacy of hair total mercury content as a biomarker of methylmercury exposure to communities near artisanal and small-scale gold mining in Madre de Dios, Peru." *Environmental Science & Technology. In review*.

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Liang, X., L. Zhang, A. Johs, J. Zhao, N. Zhu, E. M. Pierce, and B. Gu. "Influences of mixed thiol ligands on microbial methylation of mercury." *Environmental Science & Technology Letters. In preparation*.

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Rathore, S. S., G. E. Schwartz, S. C. Brooks, and S. L. Painter. "Model selection and joint estimation of biogeochemical model parameters from multiple experiments: A Bayesian approach applied to mercury methylation." *Environmental Modeling and Software. Submitted.*

Schwartz, G. E., K. A. Muller, S. Rathore, R. L. Wilpiszeski, A. A. Carrell, M. A. Cregger, D. A. Elias, M. Podar, S. L. Painter, and S. C. Brooks. "Incorporating variable sediment microbial activity into methylmercury production kinetics modeling." *Science of the Total Environment. In review.*

Wang, L., X. Yin, X. Liang, X. Lu, L. Zhang, and B. Gu. "Opposing effects of green alga *Chlorella vulgaris* on mercury methylation by *G. sulfurreducens* PCA and *D. desulfuricans* ND132." *The Journal of Hazardous Material. In preparation*.

Watson, D. B., D. H. Phillips, G. S. Baker, D. P. Gaines, M. I. Boyanov, K. M. Kemner, J. E. Earles, K. H. Williams, S. S. Hubbard, B. Dafflon, and S. C. Brooks. "Geophysical detection and characterization of preferential transport pathways for uranium and nitrate in interbedded fractured shale and limestone bedrock." *Water Resources Research*. *In review*.

Wilpiszeski, R. L., A. M. Wymore, C. M. Gionfriddo, M. Podar, and D. A. Elias. 2021. "Effects of syntrophic interactions on MeHg generation." *PLOS Biology*. *In preparation*.

Wilpiszeski, R. L., C. M. Gionfriddo, M. Kim, A. M. Wymore, A. Soren, M. Podar, C. C. Gilmour, and D. A. Elias. "Assessment of the influence of syntrophy on Hg-methylating bacteria and archaea in multi-cultures." *Applied and Environmental Microbiology. In preparation.*

Wilpiszeski, R. L., A. M. Wymore, A. Soren, M. Podar, C. C. Gilmour, C. M. Gionfriddo, and D. A. Elias. "Elucidation of an Ars-R like gene that controls *hgcAB* transcription and expression." *mSystems. In preparation*.

Wilpiszeski, R. L., C. M. Gionfriddo, M. Kim, A. M. Wymore, A. Soren, M. Podar, C. C. Gilmour, and D. A. Elias. "Syntrophy plays a role in the rate and extent of Hg-methylation in co-cultures." *Environmental Science & Technology. In preparation.*

Yan, J., N. Sharma, E. D. Flynn, D. E. Giammar, G. E. Schwartz, S. C. Brooks, P. B. Weisenhorn, K. M. Kemner, E. J. O'Loughlin, D. I. Kaplan, and J. G. Catalano. "Consistent controls on trace metal micronutrient speciation in wetland soils and stream sediments." *Geochimica et Cosmochimica Acta. In review.*

Zhang, L., X. Liang, C. S. Kang, S. C. Brooks, J. Semrau, and B. Gu. "Sorption and uptake of Hg(II) and MeHg by methylating bacteria and methanotrophs: Implications for Hg fate and transport in the environment." *In preparation*.

Zhang, L., M. Philben, N. Taş, A. Johs, Z. Yang, S. D. Wullschleger, D. E. Graham, E. M. Pierce, and B. Gu. "Biogeochemical drivers of methylmercury production in arctic tundra soils." *Science of the Total Environment. Submitted*.

Zhang, Y., L. Zhang, X. Liang, Q. Wang, X. Yin, E. M. Pierce, and B. Gu. "Divalent metal ions (Cu^{2+}, Zn^{2+}, Ca^{2+}) compete with Hg^{2+} bound to thiol ligands and natural organic matter." *Environmental Science & Technology*. *Submitted*.

Data Products Released

Brooks, Scott C. and Kenneth A. Lowe. 2021. [Data Set] East Fork Poplar Creek Discharge at Kilometer 5.4 Water Year 2020. DOI:10.12769/1779632.

Brooks, Scott C. and Kenneth A. Lowe. 2021. [Data Set] East Fork Poplar Creek Sonde Data at Kilometer 5.4 Water Year 2020. DOI:10.12769/1779674.

Brooks, Scott C. and Kenneth A. Lowe. 2021. [Data Set] East Fork Poplar Creek Discharge at Kilometer 16.2 Water Year 2020. DOI:10.12769/1779680.

Brooks, Scott C. and Kenneth A. Lowe. 2021. [Data Set] East Fork Poplar Creek Sonde Data at Kilometer 16.2 Water Year 2020. DOI:10.12769/1779683.

Brooks, Scott C., Carrie L. Miller, Ami L. Riscassi, Kenneth A. Lowe, Johnbull O. Dickson, Grace E. Schwartz. 2020. [Data Set] Water quality response to flow management in East Fork Poplar Creek. DOI:10.12769/1664390.

The annotated genome sequences have been deposited in Genbank under accession numbers CP070494 and CP070495 (*Methylomonas* sp. strain EFPC1) and CP070491, CP070492, and CP070493 (*Methylococcus* sp. strain EFPC2). Raw reads have been deposited in the NCBI Sequence Read Archive under accession numbers SRX10121820 and SRX10121822 (for Illumina MiSeq reads of *Methylomonas* sp. strain EFPC1 and *Methylococcus* sp. strain EFPC2, respectively).

Quality filtered sequence data from studies of the biogeochemical drivers of MeHg production in Arctic tundra soils were deposited at European Nucleotide Archive (PRJEB37429).

Appendix C. Presentations and Conferences

Ahmed, T., S. C. Brooks, D. VanLeeuwen, R. A. M. Mohamed, C. Tsai, and K. C. Carroll. "Statistical Characterization of Hyporheic Zone Properties Over Three Years." American Geophysical Union Virtual Fall Meeting, December 1–17, 2020. San Francisco, California.

Eckert, P., A. Johs, J. Semrau, A. DiSpirito, J. Richardson, R. Sarangi, B. Gu, and E. M. Pierce. "Complexation of Group 12 Transition Metals by Methanobactins from *Methylocystis sp*. strain SB2." Goldschmidt Virtual Conference, July 4–9, 2021. Lyon, France.

Gu, B., L. Zhang, Z. Yang, M. J. Philben, N. Tas, S. Wullschleger, D. E. Graham, and E. M. Pierce. "Warming Effects and Biogeochemical Drivers on Methylmercury Production in Arctic Soils." American Geophysical Union Virtual Fall Meeting, December 1–17, 2020. San Francisco, California.

Mohamed, R. A. M., C, Gabrielli, T. Ahmed, J. S. Selker, S. C. Brooks, and K. C. Carroll. "Comparison of Fiber-Optic Distributed Temperature Sensing and Mobile High-Sensitivity Temperature Probes for Stream and Hyporheic Zone Characterization." American Geophysical Union Virtual Fall Meeting, December 1–17, 2020. San Francisco, California.

Pierce, E. M., P. Eckert, B. Gu, L. Zhang, and A. Johs. "Metal-Ligand Interactions Influence on Mercury Transformations in Metabolically Active Transient Storage Zones." American Geophysical Union Virtual Fall Meeting, December 1–17, 2020. San Francisco, California. *Invited*.

Rathore, S., A. Jan, E. Coon, and S. L. Painter. "Improving Parameter Inferences in a Multiscale Model for Transport in Stream Corridors." American Geophysical Union Virtual Fall Meeting, December 1–17, 2020. San Francisco, California.

Tsai, C., S. C. Brooks, and K. C. Carroll. "Method Comparison for Hyporheic Zone Transport Model Parameter Estimation using Tracer Tests Conducted in East Fork Poplar Creek, Tennessee, USA." American Geophysical Union Virtual Fall Meeting, December 1–17, 2020. San Francisco, California.

Washburn, S. J., M. Cregger, A. Carrell, G. Schwartz, D. A. Elias, and S. C. Brooks. "Understanding the Effects of Nutrient Concentration on Mercury Cycling within Fluvial Periphyton." Goldschmidt Virtual Conference, July 4–9, 2021. Lyon, France.

Yan, J., N. Sharma, D. Giammar, G. Schwartz, P. Weisenhorn, E. O'Loughlin, E. Flynn, J. G. Catalano, S. C. Brooks, K. Kemner, and D. Kaplan. "Speciation and Availability of Trace Metal Micronutrient in Wetland Soils and Stream Sediments." Goldschmidt Virtual Conference, July 4–9, 2021. Lyon, France.

Acronyms and Abbreviations

1D, 2D, 3D	one dimensional, two dimensional, three dimensional
ADELS	Advective Dispersion Equation with Lagrangian Subgrid
AEL	Aquatic Ecology Laboratory
ANI	average nucleotide identity values
ATS	Advanced Terrestrial Simulator
BER	DOE Office of Biological and Environmental Research
Cd	Cadmium
CYS	cysteine
DFT	density functional theory
DMeHg	dimethylmercury
DMPS	2,3-dimercaptopropanesulfonic acid
DOC	dissolved organic carbon
DOE	U.S. Department of Energy
DOM	dissolved organic matter
GSH	glutathione
EDTA	ethylenediaminetetraacetate
EFPC	East Fork Poplar Creek
EMSL	DOE Environmental Molecular Sciences Laboratory
ESS	Environmental System Science program
EXAFS	Extended X-ray Absorption Fine Structure
FISH	fluorescence *in situ* hybridization
Hg	mercury
hgcAB	Hg-methylation gene pair
HgcAB	Hg-methylation protein
HGT	horizontal gene transfer
Hg-MATE-Db	Hg-cycling Microorganisms in Aquatic and Terrestrial Ecosystems Database
IDEAS	Interactive Design of Extreme-scale Application Software
LMW	low molecular weight
MATSZ	metabolically active transient storage zone
MCMC	Markov Chain Monte Carlo method
MeHg	methylmercury
MMHg	monomethylmercury
NEXSS	Networks with EXchange and Subsurface Storage model
OD	optical density
ORNL	Oak Ridge National Laboratory
PCR	polymerase chain reaction
PI	principal investigator
pMMO	particulate methane monooxygenase
PNNL	Pacific Northwest National Laboratory
POM	particulate organic matter
pXMO	a divergent form of pMMO
qPCR	quantitative polymerase chain reaction
RT-qPCR	reverse transcription–quantitative PCR
SFA	Scientific Focus Area
SPRUCE	Spruce and Peatland Responses Under Changing Environments
SUVA	specific ultraviolet absorbance
SWAT	Soil & Water Assessment Tool
TAM	Transient Availability Model
TD-DFT	time-dependent density functional theory
TSZ	transient storage zone
WRR	Water Resource Region
Zn	zinc

SFA Contact and Sponsor

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Sponsor: The ORNL Mercury SFA is sponsored by the Environmental System Science (ESS) program within the U.S. Department of Energy's Office of Biological and Environmental Research. Contact Paul Bayer, ESS Program Manager, paul.bayer@science.doe.gov and Jennifer Arrigo, jennifer.arrigo@science.doe.gov.

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