Biogeochemical Transformations at Critical Interfaces in a Mercury Perturbed Watershed Science Focus Area

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Project Abstract: Freshwater resources supplied by headwater streams and their surrounding watersheds are being threatened by severe pollution from anthropogenic releases of nutrients and trace metals (e.g., mercury [Hg]). Preserving these services for future use requires developing a deeper understanding of watershed structure and function. Research findings during Phase I of the Critical Interfaces Science Focus Area (SFA) project have led to the realization that transient storage zones (TSZs), and more specifically metabolically active TSZs (MATSZs) are 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 associated with the interstitial spaces of hyporheic zone streambed that are microbially active and pore space present in the microbiome of in-stream biofilm. Unlike TSZs, MATSZs exhibit very different biogeochemical environments (e.g., redox conditions) compared with the flowing stream or streambed, making them important "hot spots" that account for a substantial proportion of the diverse and intensified biogeochemical activity in watersheds.

The SFA 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 ESS program by being nested in the most intensively used freshwater Water Resource Region in the contiguous United States (Tennessee River Basin) and serving as a representative low-order freshwater stream system with relevance to the largest proportion of the total stream length in United States.

In FY21, the team (1) added new capabilities to the Advanced Terrestrial Simulator (ATS) modeling software creating tools that extend the 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 stream periphyton community structure and function, and (4) continued to explore the transcriptional regulation of methylation genes under different conditions. We also advance our understanding of metal-ligand interactions that influence Hg isotope exchange and control, at a molecular scale, how methanobactin from *Methylocystis sp*. strain SB2 interacts with group 12 metals. 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 carbon, nutrients, and trace metal cycling at the scale of individual stream reaches and small watershed catchments.

Changing Nutrient Concentration Alters Periphyton Biofilm Composition and Function

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Project Abstract: Periphyton biofilms are complex assemblages comprising algae, bacteria, fungi, detritus, extracellular polymers, invertebrates, and mineral particles. These biofilms play a central role in stream ecohydrology as they both produce (e.g., dissolved oxygen, labile carbon) and consume (e.g., nitrate, phosphate) nutrients critical to the ecosystem. They are also the site of steep geochemical gradients with conditions transitioning from fully oxic to anoxic over distances of 100 µm or less. The microbially-catalyzed production of monomethylmercury (MMHg) from inorganic mercury (Hg) is an anaerobic process. Controlled laboratory experiments have demonstrated that actively photosynthesizing biofilms may generate a significant fraction of the MMHg flux in East Fork Poplar Creek (EFPC), a Hg contaminated creek in Oak Ridge, TN.

To better understand the relationship between stream nutrient status and periphyton biofilm composition and function, we are conducting both in-stream experiments and controlled artificial stream experiments in which nutrient levels (nitrate and/or phosphate) are manipulated. The resulting biofilms are then used in experiments to quantify function (Hg methylation and MMHg demethylation kinetics) and composition (16s, 18s, ITS, and *hgcA* sequencing). Biofilms supplemented with additional nitrate and/or phosphate had lower bacterial/archaeal richness than controls in summer and higher richness in autumn. In contrast, fungal diversity in nutrient amended samples generally increased in summer and decreased in autumn relative to controls. Surprisingly, Hg methylation potential correlated with numerous bacterial families that do not contain *hgcAB*, the two-gene cluster encoding for Hg methylation ability, suggesting that overall microbiome structure of periphyton communities influence rates of Hg transformation. Microbial network analysis revealed that the nitrate amended biofilms had the highest number of hub taxa that also corresponded with enhanced Hg-methylation potential. This work provides insight into community interactions within the periphyton microbiome that may contribute to Hg cycling and will inform future research which will focus on establishing mixed microbial consortia to uncover mechanisms driving shifts in Hg cycling within periphyton habitats.

Additional experiments and development work on our transient availability model (TAM), developed for quantifying Hg methylation kinetics in biofilms, show that the TAM can be applied to describe Hg-methylation in sediments. Additionally, including expressions that

account for variable microbial activity improves model accuracy. Application of Bayesian parameter estimation methods identified model structural uncertainty and improved overall model performance.

Assessing Biogeographic Survey Gaps in Bacterial Diversity Knowledge: A Global Synthesis of Freshwaters

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Project Abstract: Organismal and genetic diversity within ecosystems is important for the mediation of ecosystem processes such as carbon and nutrient cycling, climate regulation, and ecosystem provisioning. Therefore, characterizing this biodiversity is essential for predicting and mitigating the impacts of environmental change on valued ecosystem services. Biodiversity of microorganisms is especially important given the role they serve in cycling carbon, nutrients, and trace metals within terrestrial and aquatic ecosystems and supporting plant growth and productivity. While national and global biodiversity assessments have been conducted for terrestrial vertebrates, plants, and freshwater fishes, the cataloguing of microbial diversity has been limited because technologies for describing microbial diversity have only recently become available and microbial distributions have been viewed as ubiquitous and of low conservation need. New studies have indicated microbial taxa probably exhibit biogeographic patterns, thus more detailed characterization of these communities is necessary.

In this study, we identified gaps in microbial data coverage along climatic and landscape disturbance gradients and among terrestrial biomes and hydrographic regions for all freshwater ecosystems and three freshwater habitat types: lakes and reservoirs (lentic); streams and rivers (lotic); and wetlands. Freshwaters account for <1% of Earth's surface area, yet support >10% of known plant and animal species making them disproportionately biodiverse and important ecosystems to characterize. We reviewed literature on microbial diversity in freshwaters surveyed using 16S ribosomal RNA sequencing which identify microbial taxa. We georeferenced survey locations and used a geographic information system to identify and map gaps in survey coverage using open-source data for climate, landscape disturbance, terrestrial biomes, and freshwater ecoregions. We compiled 3,425 georeferenced survey locations reported from 963 studies. Our assessment revealed high climatic coverage of freshwater microbial diversity knowledge, but expansive ecoregional gaps attributable to biased sampling near research institutions in North America, western Europe, and China. Future surveys should target ecoregions in Africa, South America, Central Asia, Australia, and Antarctica. An essential next step will be to curate and disseminate sequencing efforts to facilitate the study of processes driving global diversity patterns.

Characterization of Methanobactin Interactions with Group 11 and 12 Transition Metals

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Project Abstract: 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 post-translationally 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 post-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. 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 mercury and other metals. We characterized the interactions of methanobactin from *Methylocystis sp*. SB2 (mb-SB2) with transition metals using UV-Vis absorbance, fluorescence, extended X-ray absorption fine structure spectroscopy (EXAFS), and isothermal titration calorimetry, 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 group 11 and 12 transition metals.

Rates and Dynamics of Mercury Isotope Exchange Reactions and Implications for Environmental Tracer Studies

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Project Abstract: Enriched mercury (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 pre-existing ambient Hg in environmental matrices. In this work, we describe detailed studies of isotope exchange reactions between spiked divalent ^{198}Hg(II) and ambient Hg(II), as well as between dissolved elemental ^{202}Hg(0)$_{aq}$ and Hg(II) bound to various environmental matrices, such as low-molecular-weight thiols, minerals, and dissolved organic matter (DOM) in water. The impact of isotope exchange on methylmercury production in the presence of organic ligands was also evaluated with an iron-reducing bacterium *Geobacter sulfurreducens* PCA (PCA). Surprisingly, we found that the spiked ^{198}Hg(II) rapidly exchanged 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. Similarly, rapid, spontaneous isotope exchange (<1 h) was observed between dissolved elemental ^{202}Hg(0)$_{aq}$ and ^{201}Hg(II)-bound to organic and inorganic ligands with varying chemical structures and binding affinities, including chloride (Cl^-), ethylenediaminetetraacetate (EDTA), cysteine, glutathione, and 2,3-dimercaptopropanesulfonic acid. 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-to-Hg(II) ratio decreased the exchange rates due to the formation of 2:1 or higher thiol:Hg(II) chelated complexes, but had no effects on exchange rates with ^{201}Hg(II) bound to weaker ligands, such as EDTA or Cl^-. The exchange between ^{202}Hg(0)$_{aq}$ and ^{201}Hg(II)-bound to DOM showed an initially rapid followed by a slower exchange rate due to Hg(II) complexation with both low- and high-affinity binding functional groups on DOM (e.g., carboxylates vs bidentate thiolates). Our results underscore the importance of considering isotope exchange reactions 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 risk assessments of new Hg input to the natural ecosystems.