

**MIDWEST**



## **2017 Midwest Geobiology Symposium**

**September 30, 2017 – Indianapolis, Indiana**

**Hosted by: Indiana University Purdue  
University Indianapolis (IUPUI)**

**Planning team: Dr. Greg Druschel, Dr. Bill Gilhooly, Dr. Alice Santos, John Shukle, Martin Kurek, Fotis Fouskas**



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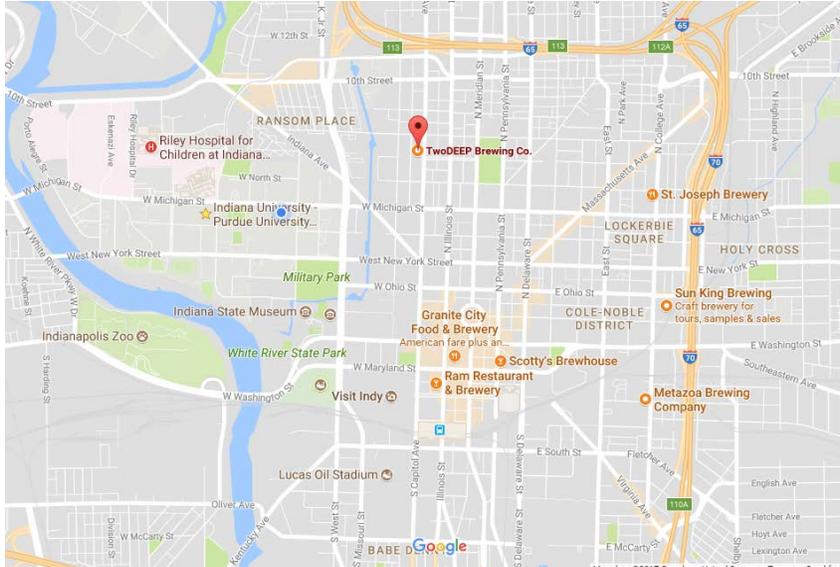
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# Midwest Geobiology Symposium 2017 Program

**Friday, September 29, 2017 – 7:00 p.m. to 11:00 p.m.** - Icebreaker event at Two Deep Brewing, 714 N Capitol Ave, Indianapolis, IN. Parking available at brewery and streets nearby. Walkable from many downtown hotels.



**Roast Pig by the Smoking Goose starts at 7pm,  
vegetarian options will also be available!**



**Saturday, September 30, 2017, IUPUI Campus Center – 420 University Boulevard.** Parking available at Vermont Street Garage (attached to Campus Center Building), take a ticket on way in, grab a validated ticket from us at registration desk!



We will be located on the 4<sup>th</sup> floor of the Campus Center, easiest way is to take the escalators all the way up – Registration will be at the top of Escalators – pick up your badge at the table! Coffee starts at 8:45a.m.



The Oral sessions will be held in room 450 C, the poster session will be held next door in room 450 B. Coffee and lunch will be served in the space outside these rooms and the outdoor terrace on the 4<sup>th</sup> floor (weather permitting)

## **Oral Session I – 450 C**

9:00: Welcoming Remarks: Greg Druschel, Bill Gilhooly

9:10: Harry McClelland, Washington University in St. Louis - Microbial Heterogeneity in clonal microbial populations

9:30: Jamie McFarlin, Northwestern University - Sedimentary lipids as a paleoclimate proxy in Greenland

9:50: Mary Sabuda, University of Minnesota - Insight into Biogenic Methane and Sulfur Cycling Within Serpentinite-hosted Groundwater

10:10: Jordan Angle, The Ohio State University - Methanogenesis in oxygenated soils is a substantial fraction of wetland methane emissions

10:30: Coffee Break

10:50: Casey Saup, The Ohio State University - Just Around the Riverbend: Seasonal hydrologic controls on dynamic hyporheic zone redox biogeochemistry

11:10: Dean Horton, Central Michigan University - Soil physicochemistry may impact microbial communities in coastal wetlands of the Great Lakes

11:30: Alex Kugler, Miami University - Protecting Filamentous Cyanobacteria from UV Irradiation using Phyllosilicate Micas

11:50: Qiang Yu, Notre Dame University - Sulfhydryl Binding Sites within Bacterial Cell Envelopes: Characterization, Environmental Implications and Applications

12:10: Clayton Johnson, Notre Dame University - Visualization and Quantification of Cd Sorption to Bacteria Using Confocal Laser Scanning Microscopy and Cd-specific Fluorescent Probes

**Lunch** (provided) – 12:30 – 1:30 – Terrace at top of escalators & outdoor terrace (weather permitting)

## **Oral Session II – 450 C**

1:40: Elliott Arnold, University of Pittsburgh - Sediment Biomarkers Elucidate the Holocene Ontogeny of a Shallow Lake

2:00: Benjamin Bates, University of Cincinnati - Transport and distribution of terrestrial plant biomarkers in modern lake sediments: implications for paleoenvironmental reconstructions

2:20: Erika Freimuth, University of Cincinnati - Controls on the production and  $\delta D$  composition of two plant biomarkers in a temperate forest and implications as proxies for precipitation  $\delta D$

2:40: Nick Lambrecht, Iowa State University - Two newly documented ferruginous lakes in the Midwest, USA are Archean ocean analogs

3:00: Coffee Break

3:20: Alice Santos, Indiana University Purdue University Indianapolis - Evidence of oxygen production in the early Neoproterozoic in the São Francisco craton, Brazil

3:40: Jocelyn Richardson, Washington University in St. Louis - New data from the Viki Drillcore, Estonia, provide insights into the veracity of  $d_{34}S_{SCAS}$  as a proxy for ancient seawater sulfate

4:00- Keynote: Lisa Pratt, IU Bloomington: Biogeochemical approaches to the search for evidence of life on Mars and Europa

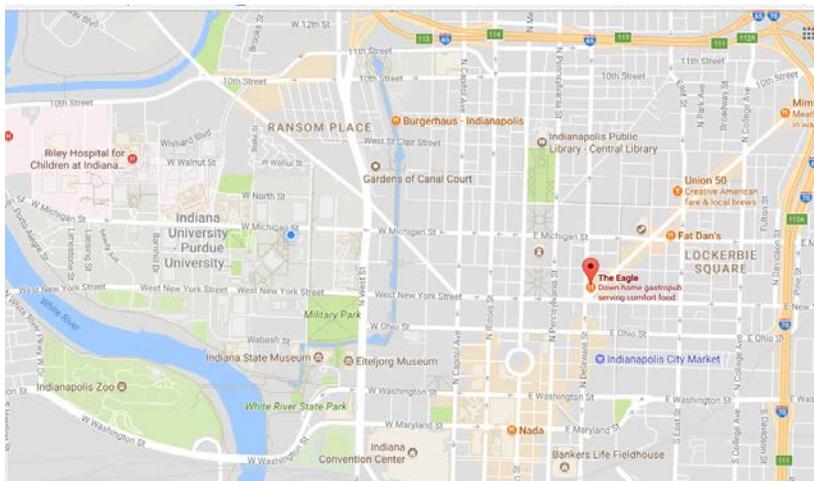
4:30: Discussion

## Poster Session – 450 B

4:45 – 7:30 – with cash bar & hors d'ouvres (see posters for drink tickets!!)

## Informal Pub Crawl

8:00 Start at: The Eagle, 310 Massachusetts Ave.



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# **Abstracts**

**(in alphabetical order)**

# Methanogenesis in oxygenated soils is a substantial fraction of wetland methane emissions

J.C. ANGLE<sup>1\*</sup>, T.H. MORIN<sup>2,3</sup>, L.M. SOLDEN<sup>1</sup>, A.B. NARROWE<sup>4</sup>, G.J. SMITH<sup>1</sup>, M.A. BORTON<sup>1,3</sup>, C. REY-SANCHEZ<sup>2,3</sup>, R.A. DALY<sup>1</sup>, G. MIRFENDERESGI<sup>2</sup>, D.W. HOYT<sup>5</sup>, W.J. RILEY<sup>6</sup>, C.S. MILLER<sup>4</sup>, G. BOHRER<sup>2,3</sup>, AND K.C. WRIGHTON<sup>1,3</sup>

<sup>1</sup> Department of Microbiology, The Ohio State University, Columbus, OH 43210 (\*presenting author)

<sup>2</sup> Department of Civil, Environmental and Geodetic Engineering, The Ohio State University, Columbus, OH 43210

<sup>3</sup> Environmental Science Graduate Program, The Ohio State University, Columbus, OH 43210

<sup>4</sup> Department of Integrative Biology, University of Colorado Denver, Denver, CO 80217

<sup>5</sup> Pacific Northwest National Laboratory, Richland, WA 99352

<sup>6</sup> Lawrence Berkeley National Laboratory, Berkeley, CA 94720

Temperate freshwater wetlands are currently the greatest source of the potent greenhouse gas methane. Predicting wetland methane emissions is highly uncertain, partially confounded by limited knowledge about methanogenic physiology in these systems. Cultivated methanogens are known to be extremely oxygen sensitive and thus environmental methanogens are expected to be inactive in oxic habitats, an assumption incorporated into global biogeochemical models. To better understand methanogen physiology in freshwater wetland soils we sampled microbial communities from Old Woman Creek, a wetland adjacent to Lake Erie. We examined soils underlying three land-coverage types (emergent *Typha* vegetation –“plant”, seasonally exposed mud flats –“mud”, and continuously flooded open water channels – “water”), conducting geochemical, metabolite, single marker gene, metagenomics, and metatranscriptomics analyses at both shallow (0-5 cm) and deep (25-30 cm) soil depths during the Fall and Summer seasons. Surprisingly, methanogen activity (*mcrA* transcript abundance) was 9-fold greater in oxygenated surface soils as compared to deep soils, correlating significantly and positively to *in situ* porewater methane and acetate concentrations but not to other methanogenic substrates. Consistent with these data, metagenomics recovered the first near complete (>90%) environmental *Methanotherix* genomes (obligate acetoclastic methanogens), which constituted a new species here named *Candidatus Methanotherix paradoxum*. Phylogenetic and functional genes indicative of *Ca. Methanotherix paradoxum* were found in numerous environmental metagenomes, demonstrating a widespread natural distribution. Mapping and normalizing transcript reads to metagenome-recovered *mcrA* sequences revealed that, across both season and land coverage type, the acetoclastic *Ca. Methanotherix paradoxum* account for 84% of the *mcrA* transcripts in surface soils. *Ca. Methanotherix paradoxum* were represented in the top 3% most abundant metatranscripts from surface soils, reinforcing their presumed activity. Highly resolved porewater gas measurements quantified methane dynamics monthly at 2 cm depth intervals, indicating that in August when *Ca. Methanotherix paradoxum* transcripts were highly expressed, oxic surface soils produced 3.6 times more methane than could be accounted for through emission measurements alone. Furthermore, up to 80% of the site-wide methane emissions were determined to be generated within oxic soils. Our results demonstrate that *Ca. Methanotherix paradoxum* is active in the surface soils of many methane-emitting ecosystems, thus representing an extremely important, yet previously unaccounted for source of soil greenhouse gas emissions.

## Sediment Biomarkers Elucidate the Holocene Ontogeny of a Shallow Lake

T. ELLIOTT ARNOLD<sup>1\*</sup>, WILLIAM F. KENNEY<sup>2</sup>, T. S. BIANCHI<sup>3</sup>, J. H. CURTIS<sup>2</sup>, M. BRENNER<sup>2</sup>

<sup>1</sup>Department of Geology and Environmental Science, University of Pittsburgh, Pittsburgh, USA, tea27@pitt.edu  
(\*presenting author)

<sup>2</sup>Department of Geological Sciences, University of Florida, Gainesville, USA, kenney@ufl.edu

<sup>3</sup>Department of Geological Sciences, University of Florida, Gainesville, USA, tbianchi@ufl.edu

<sup>4</sup>Department of Geological Sciences, University of Florida, Gainesville, USA, curtisj@ufl.edu

<sup>5</sup>Department of Geological Sciences, University of Florida, Gainesville, USA, brenner@ufl.edu

We carried out geochemical analyses on a sediment core from Lake Harris, Florida (USA) to identify sources of organic matter to the sediment throughout the Holocene, and relate changes in those sources to shifts in past climate and environmental conditions. We hypothesized that the sources of organic matter changed in response to regional hydrologic shifts following deglaciation, and to human population expansion in the state during the 20<sup>th</sup> century. Hydroclimate shifts in Florida were related to: 1) a steady rise in relative sea level and the fresh water table that began in the early Holocene, 2) wetland formation and expansion ca. 5,000 cal yrs BP, and 3) the onset of the modern El Niño (ENSO) cycle ~3,000 cal yrs BP. Stratigraphic changes in sediment variables from Lake Harris reflect each of these hydroclimate periods. Early in the Holocene, Lake Harris was a marsh-like system in a relatively dry, open-prairie environment. Organic sediments deposited at that time were derived largely from terrestrial sources, as inferred from high TOC/TN ratios, a dominance of longer-chain of *n*-alkanes (*n*-C<sub>29-31</sub>), relatively negative organic carbon isotope value ( $\delta^{13}\text{C}_{\text{TOC}}$ ), and low biogenic silica concentrations. In the middle Holocene, a positive shift in  $\delta^{13}\text{C}_{\text{TOC}}$  coincided with the onset of wetter conditions in Florida. Submerged macrophyte biomarkers (*n*-C<sub>21-23</sub>) dominated and during this period, bulk organic carbon isotope values are most similar to  $\delta^{13}\text{C}$  values of mid-chain length *n*-alkanes. In the late Holocene,  $\delta^{13}\text{C}_{\text{TOC}}$  values declined, CaCO<sub>3</sub> levels decreased to trace amounts, organic carbon concentrations increased and diatom biogenic silica concentrations increased from 10 to 120 mg g<sup>-1</sup>. Around 2,900 cal yrs BP, the effects of ENSO intensified and many Florida lakes deepened to their current limnetic state. Concentrations of algal and cyanobacterial biomarkers in the Lake Harris core increased by orders of magnitude after about AD 1940, in response to human-induced eutrophication, an inference supported by values of  $\delta^{15}\text{N}$  that fluctuate around zero.

# Sulfur and Carbon isotopic data from Palaeoproterozoic marbles of northern São Francisco Craton, Brazil: environmental and tectonic implications.

*Baldim, M.R.<sup>1,2</sup>; Gilhooly III, W.P.<sup>2</sup>; Santos, A.B.<sup>1,2</sup>; Fouskas, F.<sup>2</sup>; Oliveira, E.P.<sup>1</sup>*

<sup>1</sup>University of Campinas - UNICAMP  
(Post graduate program Geoscience - Department of Geology and Natural Resources)

<sup>2</sup>Indiana University-Purdue University Indianapolis - IUPUI  
(Post graduate program - Department of Earth Sciences)

**Abstract:** During the Archean, 3.85 to 2.5 billion years ago (Ga), the Earth experienced major chemical, environmental and geodynamical changes. However, it was during the Archean-Paleoproterozoic transition that significant environmental changes happened associated with the increase in the atmospheric oxygen (Great Oxygenation Event, ~2.4 Ga) as indicated by the loss of the mass independent sulfur isotope signal (S-MIF), and the pronounced carbon isotope ( $\delta^{13}\text{C}$ ) excursion during the Lomagundi-Jatulian Event (~2.40 - 2.04 Ga). The use of sulfur and carbon stable isotopes can reveal the past environmental conditions (e.g. redox state and presence of organic matter), and provide temporal information (e.g., the S-MIF occurred prior to ~2.4 Ga and the positive  $\delta^{13}\text{C}$  anomaly happened after ~2.4 Ga). Provided these are global events, the sulfur and carbon isotopic data can be used as geochronological tools combined with other more traditional approaches such as detrital zircons.

In the São Francisco Craton, Brazil, there are several supracrustal sequences with chemical sediments, including carbonates, cherts and banded iron formations. Our study site is the Archean-Palaeoproterozoic Serrinha Block, located in the northern part of the São Francisco craton. The Serrinha Block is an Archean to Paleoproterozoic terrane composed of (1) two high-grade Mesoarchean gneiss complexes, (2) mafic-ultramafic complexes, (3) Neoarchean mafic dike swarms, (4) Palaeoproterozoic volcano-sedimentary sequences of the Itapicuru River greenstone belts and Capim River Arc and (5) and granitic intrusions. Several marble (this study) are hosted by ca. 3.08 Ga gray gneiss and exposed along a 150 km transect that trends N-S. The marbles are dolomites ranging from pure to aluminosilicate-rich with 0.005% to 2% content of sulfur, which were obtained by pyrite extraction method. Field relationships and geochronological data show the minimum deposition age of 2.7 Ga while data from detrital zircon grains reveals the maximum deposition age of 2.1 Ga. Our preliminary  $\Delta^{33}\text{S}$  values range from -0.42 to -0.57‰, which are within the range for mass dependency (no S-MIF) given the analytical errors of our measurements by SO-SO<sub>2</sub>. The lack of a S-MIF signal suggests that the marbles could be younger than the Great Oxygenation Event (*ca.* 2.4 Ga). Sulfur and carbon isotope data combined with geochemical and geochronological data are improving the age models of the São Francisco craton, which in turn improves the tectonic models and global correlations of Paleoproterozoic continent assembly. The results reported here are a contribution to the global Palaeoproterozoic database.

# Transport and distribution of terrestrial plant biomarkers in modern lake sediments: implications for paleoenvironmental reconstructions

BENJAMIN R. BATES<sup>1\*</sup>, THOMAS V. LOWELL<sup>1</sup>, AARON F. DIEFENDORF<sup>1</sup>, ERIKA J. FREIMUTH<sup>1</sup>,  
ALEXANDER K. STEWART<sup>2</sup>

<sup>1</sup>Department of Geology, University of Cincinnati, Cincinnati, USA, [batesbr@mail.uc.edu](mailto:batesbr@mail.uc.edu) (\*presenting author)

<sup>2</sup>Department of Geology, St. Lawrence University, Canton, USA, [astewart@stlawu.edu](mailto:astewart@stlawu.edu)

Terrestrial plant biomarkers preserved in lake sediments are commonly used in paleoenvironmental reconstructions. Basin-specific transport pathways and distribution controls of plant biomarkers, however, are poorly understood. This study mapped the distribution of sedimentary *n*-alkanes sourced from vascular plant waxes to delineate possible transport pathways and quantified the contribution of terrestrial and aquatic input. We combine these data with existing leaf and pollen taphonomy literature and sediment focusing models to develop a better understanding of the controls on plant biomarker transport within lake basins.

Here, we report the spatial distribution of sedimentary *n*-alkanes, the carbon isotope values and C:N ratios of bulk sediment, and percent organic matter from three lakes in the Adirondack Mountains, NY. Preliminary carbon isotope data and *n*-alkane concentrations within each lake suggests a large terrestrial input. Bulk sediment carbon isotope values ranged from -26‰ to -32‰ consistent with carbon isotope values of modern terrestrial vegetation. The concentrations of long-chain *n*-alkanes (indicative of higher land plants), moreover, are much higher than short-chain *n*-alkanes (indicative of aquatic and microbial activity) by almost two times. By contrast, C:N ratios range from 11-14 indicating a mix of aquatic and terrestrial contribution to the lake's total organic matter.

We combined high-resolution sonar data with the sediment analyses to identify basin-specific controls on the distributions of *n*-alkanes and bulk sediment carbon isotopes. The statistical categorization of sediment zones based on relative hardness and roughness along the lake bottom delineates where organic material is concentrated. For the terrestrially sourced plant waxes, we measured low *n*-alkane concentrations in sandy littoral sediments relative to deeper sediments towards the main depo-center. Together, this information validates sediment focusing models and suggests that terrestrial carbon and *n*-alkanes are preferentially transported to the main depo-center of the lake. These observations highlight important relationships between basin-specific sediment properties and processes controlling the transport and deposition of *n*-alkanes.

# **Investigating the controls of microbialite morphogenesis using GIS-based approaches**

SCOTT R. BEELER<sup>1\*</sup>, FERNANDO J. GOMEZ<sup>2</sup>, ALEXANDER S. BRADLEY<sup>1</sup>

<sup>1</sup>Dept. of Earth and Planetary Sciences, Washington University in St. Louis, St. Louis, USA, beeler@wustl.edu

(\*presenting author)

<sup>2</sup>Laboratoria de Análisis de Cuencas, CICTERRA-CONICET, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina, fgomez@cicterra-conicet.gov.ar

Microbialites are sedimentary structures that arise from the interaction of microbial and geological processes. These structures can record information regarding microbial evolution and/or environmental changes through geologic time. A wide range of microbialite morphologies have been recognized in the geologic record, however in most cases an understanding of the controls of this morphological variability is lacking. The hypersaline lake Laguna Negra (Catamarca Province, Argentina) contains a zone of carbonate precipitation consisting of morphologically diverse structures including those interpreted as microbialites whose abundance varies across the lake edge. Gradients in physico-chemical conditions and the community structure of microbial mats and sediments associated with the carbonates occur across the zone of carbonate association making it an ideal location to study the controls of microbialite morphogenesis.

We utilized an approach combining aerial imagery, geochemical measurements, and field observation into a GIS-based data model to investigate the spatial distribution of and relationship between environmental parameters and carbonate morphology. High resolution aerial images (1.76 cm/px resolution) were generated and utilized to map changes in the morphology, abundance, and size of carbonates at Laguna Negra as well as visual changes in microbial communities. Additionally, geochemical measurements and ground truth observations were taken at locations across the lake edge. Integration of these results into a GIS data model in ArcMap was performed to enable recognition and statistical analysis of the spatial distribution of carbonates, geochemical parameters, and microbial communities

Spatial analysis reveals that the occurrence of carbonate morphologies does vary across the lake edge, but the contact between morphologies are gradational. Non-carbonate cobbles and boulders are also abundant within the zone of carbonate formation, and typically occur in areas without the presence of large carbonate structures. Morphologies interpreted as microbialitic were more commonly associated with microbial mats than non-microbial carbonates and non-carbonates consistent with a microbial contribution to their formation. However, not all microbialites were associated with microbial mats and in some cases non-microbialites were associated with microbial mats confounding this interpretation. Large geochemical gradients due to the mixing of waters, degassing, and evaporation were measured at the lake edge and are also associated with the morphological changes. Ongoing work aims to better quantify the relationship between these environmental changes and morphology to develop a model for microbialite morphogenesis at Laguna Negra as an analog for ancient examples.

## Potential Repercussions Associated with *Halanaerobium* Colonization of Hydraulically Fractured Shales

ANNE ELIZABETH BOOKER<sup>1\*</sup>, MIKAYLA BORTON<sup>2</sup>, DAVID HOYT<sup>3</sup>, SHIKHA SHARMA<sup>4</sup>, KELLY C. WRIGHTON<sup>5</sup> AND MICHAEL WILKINS<sup>6</sup>

<sup>1</sup>Ohio State University Main Campus, Columbus, OH, United States, [booker.132@osu.edu](mailto:booker.132@osu.edu) (\*presenting author)

<sup>2</sup>Ohio State University Main Campus, Columbus, OH, United States, [borton.62@osu.edu](mailto:borton.62@osu.edu)

<sup>3</sup>Pacific Northwest National Laboratory, Richland, WA, United States, [david.hoyt@pnnl.gov](mailto:david.hoyt@pnnl.gov)

<sup>4</sup>West Virginia University, Morgantown, WV, United States, [shikha.sharma@mail.wvu.edu](mailto:shikha.sharma@mail.wvu.edu)

<sup>5</sup>Ohio State University Main Campus, Columbus, OH, United States, [wrighton.1@osu.edu](mailto:wrighton.1@osu.edu)

<sup>6</sup>Ohio State University Main Campus, Columbus, OH, United States, [wilkins.231@osu.edu](mailto:wilkins.231@osu.edu)

Hydraulic fracturing of black shale formations has greatly increased U.S. oil and natural gas recovery. Bacterial *Halanaerobium* strains become the dominant microbial community member in produced fluids from many fractured shales, regardless of their geographic location. *Halanaerobium* is not native to the subsurface, but is inadvertently introduced during the drilling and fracturing process. The accumulation of biomass in pipelines and reservoirs is detrimental due to possible well souring, microbially-induced corrosion, and pore clogging. Here, we used *Halanaerobium* strains isolated from a hydraulically fractured well in the Utica Shale, proteogenomics, isotopic and geochemical field observations, and laboratory growth experiments to identify detrimental effects associated with *Halanaerobium* growth. Analysis of *Halanaerobium* isolate genomes and reconstructed genomes from metagenomic datasets revealed the conserved presence of rhodanese-like proteins and anaerobic sulfite reductase complexes that can convert thiosulfate to sulfide. Furthermore, laboratory growth curves confirmed the capability of *Halanaerobium* to grow across a wide range of pressures (14-7000 PSI). Shotgun proteomic measurements were used to track the higher abundance of rhodanese and anaerobic sulfite reductase enzymes present when thiosulfate was available in the growth media. This technique also identified a higher abundance of proteins associated with the production of extracellular polymeric substances when *Halanaerobium* was grown under increasing pressures. *Halanaerobium* culture based assays identified thiosulfate-dependent sulfide production, while pressure incubations revealed higher cellular attachment to quartz surfaces. Increased production of sulfide and organic acids during stationary growth phase suggests that fermentative *Halanaerobium* use thiosulfate to remove excess reductant, aiding in NAD<sup>+</sup> recovery. Additionally, the increased cellular attachment to surfaces under pressure indicates *Halanaerobium* has the capability of forming cellular clusters that could clog the shale fracture network and limit natural gas recovery. These findings bring awareness to the detrimental effects that could arise from *Halanaerobium* growth in hydraulically fractured shales throughout the U.S.

# Pyrite sulfur isotopes depleted and stable under Pacific Deep Western Boundary Current in Pleistocene but variability linked to winnowing of fines

ROGER N. BRYANT<sup>1\*</sup>, STEPHANIE MOORE<sup>1</sup>, DAVID A. FIKE<sup>1</sup>

<sup>1</sup>Department of Earth & Planetary Sciences, Washington University in St. Louis, St. Louis, USA,  
rnbryant@wustl.edu (\*presenting author)

Recent research suggests that depositional environmental factors can exert control on sulfur isotope ratios in sedimentary pyrite over glacial-interglacial timescales, at least in shelf/slope settings. The implications of this revelation for the interpretation of pyrite sulfur isotopes in the geologic record are great. However, there has been little evidence to suggest that pyrite formed in deeper marine sediments should be affected by such transient changes in depositional factors. Here, we present a 350 kyr chromium reducible sulfur ( $\approx$  pyrite sulfur) isotope record from ODP Site 1123, a locality positioned at a water depth of  $\sim$ 3300m, in the vicinity of the Pacific Deep Western Boundary Current (DWBC). We demonstrate that although the sulfur isotope composition of pyrite at the site is commonly depleted in  $^{34}\text{S}$  and mainly stable (mean  $\delta^{34}\text{S} = -52.2 \pm 1.8 \text{‰}$  ( $1\sigma$ )), there is a negative correlation ( $R^2 = 0.55$ ) between pyrite- $\delta^{34}\text{S}$  and pyrite abundance. In addition, the intervals with the most  $^{34}\text{S}$ -enriched and least abundant pyrite typically have a high abundance ( $\sim$ 10 wt.%) of terrigenous grains  $>63 \mu\text{m}$  in diameter. We suggest that in discrete time intervals, either coarse terrigenous material was deposited by the DWBC (as supported by lowered  $\delta^{13}\text{C}_{\text{org}}$  values in these intervals), or selective winnowing of fine ( $<63 \mu\text{m}$ ) terrigenous material by strong currents associated with the DWBC resulted in coarse, iron-poor sediments. As result of either scenario, less hydrogen sulfide produced by dissimilatory sulfate reduction (DSR) would have reacted with iron species, instead diffusing upwards and being reoxidized or reacting with organic matter. In these intervals, pyrite growth was largely inhibited and – due to the scarcity of iron – was likely spread more evenly over a greater range of depths in the sediment, thus recording a signal of hydrogen sulfide slightly enriched in  $^{34}\text{S}$  by Rayleigh fractionation during DSR. This record serves to demonstrate the importance of iron availability in determining pyrite- $\delta^{34}\text{S}$  in deep marine (organic matter-poor, slowly-accumulating) sediments. In this instance at a site near a major ocean current, iron availability was likely controlled by the entrainment and size-sorting ability of the current. These results suggest that while the sulfur isotope composition of pyrite formed in deep ocean sediments is mainly stable over glacial-interglacial timescales, even pyrite formed in these environments can be susceptible to small ( $<10 \text{‰}$ ) isotopic enrichments resulting from transient depositional changes.

## **Design, Construction, and Application of an Inexpensive, High-Resolution Water Sampler**

W. MATTHEW SATTLEY<sup>1</sup>, BRAD M. BURCHELL<sup>1\*</sup>, STEPHEN D. CONRAD<sup>1</sup>, MICHAEL T. MADIGAN<sup>2</sup>

- 1 Division of Natural Sciences, Indiana Wesleyan University, Marion, IN 46953, USA;  
brad.burchell@myemail.indwes.edu (B.M.B.) \*(presenting author);  
matthew.sattley@indwes.edu (W. M. S.); stephen.conrad@indwes.edu (S.D.C.)
- 2 Department of Microbiology, Southern Illinois University, Carbondale, IL 62901, USA;  
madigan@siu.edu

The cost of high-resolution water sampling devices for ecological studies and water quality analyses can be prohibitive. Moreover, the potential for operator error in the use of complicated sampling equipment can lead to inaccuracies. Here we describe the construction and operation of an inexpensive and easy-to-use water sampler that achieves a water column sampling resolution of approximately 1 cm. The device is driven by a peristaltic pump and is constructed entirely of non-corrosive and non-reactive materials. The sampler has no moving parts and was completely reliable in fieldwork on temperate and Antarctic lakes. The device is especially suited for the collection of water samples from calm or stagnant surface waters, such as lakes, ponds, reservoirs, and deep swamps or other wetlands. In addition, because its components are unaffected by corrosive salts and sulfides, the device is suitable for sampling calm inlet waters, including shallow bays and estuaries. Because of its low cost, simple construction, compact design, and precision performance, this water sampler is an excellent option for studying and monitoring shallow to moderately deep (<50 m) natural waters.

# Cultivating the Deep Subsurface Microbiome

C. P. CASAR<sup>1\*</sup>, M. R. OSBURN<sup>1</sup>, T. M. FLYNN<sup>2</sup>, A. L. MASTERSON<sup>1</sup>, B. R. KRUGER<sup>3</sup>

<sup>1</sup>Northwestern University, Evanston, USA, casar@u.northwestern.edu (\*presenting author),  
maggie@northwestern.edu

<sup>2</sup>Argonne National Laboratory, Argonne, USA, tflynn@anl.gov

<sup>3</sup>Desert Research Institute, University, Las Vegas, USA, brittany.kruger@dri.gov

Subterranean ecosystems are poorly understood because many microbes detected in metagenomic surveys are only distantly related to characterized isolates [1]. Cultivating microorganisms from the deep subsurface remains a significant challenge due to its inaccessibility and potential for contamination [2]. The Deep Mine Microbial Observatory (DeMMO) at the former Homestake gold mine in Lead, SD, however, offers access to deep microbial life via pristine fracture fluids in bedrock to a depth of 1,478 meters. The metabolic landscape of DeMMO was previously characterized via thermodynamic modelling and genomic [3] and metagenomic assays [4]. These have illustrated the metabolic potential of the microbial inhabitants of DeMMO, particularly their ability to utilize mineral substrates as energy sources. Here, we seek to validate these predictions and directly link phylogeny to metabolism through field and lab based cultivation approaches using iron and manganese-bearing minerals.

Fracture fluids were directed through reactors filled with Fe<sub>3</sub>O<sub>4</sub>, Fe<sub>2</sub>O<sub>3</sub>, FeS<sub>2</sub>, MnO<sub>2</sub>, and FeCO<sub>3</sub> at two sites located at depths of 610 and 1,478 meters for two months prior to harvesting for subsequent analyses. We examined mineralogical, geochemical, and microbiological composition of the reactors via DNA sequencing, microscopy, lipid biomarker characterization, and bulk C and N isotope ratios to determine the influence of mineralogy on biofilm community development. Pre-characterized mineral chips were imaged via SEM to assay microbial growth; preliminary results suggest MnO<sub>2</sub>, Fe<sub>3</sub>O<sub>4</sub>, and Fe<sub>2</sub>O<sub>3</sub> were most conducive to colonization and the morphologies of colonizers were diverse. Additionally, we observed microbial sheath and stalk structures, cells within pits, and a diversity of mineral crystals precipitated on chip surfaces. Solid materials from the reactors were used to inoculate batch cultivation experiments. Media designed to mimic fracture fluid chemistry was supplemented with mineral substrates targeting metal reducers.

Both DNA sequence data and microscopy of iron oxide-rich biofilms and fracture fluids suggest iron oxidation is a major energy source at redox transition zones where anaerobic fluids meet more oxidizing conditions. We utilized these biofilms and fluids as inoculum in gradient cultivation experiments targeting microaerophilic iron oxidizers [5]. Differential interference contrast imaging of iron oxides in the gradient cultures indicates the growth of twisted-stalk forming *Gallionella*.

Cultivation of microbes endemic to DeMMO, a system locally dominated by unclassified and candidate phyla, has the potential to yield novel subsurface organisms with unique physiologies. We intend to further utilize subsurface isolates to probe the effects of geochemical perturbations on biosignatures in future studies, thus broadening our understanding of subterranean ecosystems.

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# Trace Metal Limitations on Methane Production in Freshwater Wetlands

JEFFREY G. CATALANO<sup>1\*</sup>, NYSSA M. CROMPTON<sup>1</sup>, ALEXANDER S. BRADLEY<sup>1</sup>, ELIZABETH A. HASENMUELLER<sup>2</sup>, LISA G. CHAMBERS<sup>3</sup>

<sup>1</sup>Department of Earth and Planetary Sciences, Washington University, Saint Louis, MO, USA, catalano@wustl.edu (\*presenting author)

<sup>2</sup>Department of Earth and Atmospheric Sciences, Saint Louis University, Saint Louis, MO, USA

<sup>3</sup>Department, University, City, USA

Freshwater wetlands are one of the largest natural sources of atmospheric methane (CH<sub>4</sub>), a powerful greenhouse gas. Global climate models predict increased CH<sub>4</sub> emissions from wetlands in the future because of an expansion in wetland area and the enhancement of CH<sub>4</sub> production as temperature increases. The large radiative forcing induced by higher atmospheric CH<sub>4</sub> concentrations makes it is critical to understand the environmental controls on its production. While models of methane biogeochemistry in wetlands account for temperature, pH, and soil redox state, a potentially important limitation that has been largely overlooked is trace metal availability. Methanogens have high enzymatic requirements for trace metals, most notably nickel (Ni), cobalt (Co), and zinc (Zn), which play key roles in all metabolic pathways to produce CH<sub>4</sub>. Studies of pure cultures of methanogens demonstrated that limited availability of trace metals inhibits methanogenesis, but such limitations have not been investigated in natural systems. To evaluate the prevalence of metal limitations in freshwater wetlands, we have characterized the content and speciation of trace metals critical to methanogenesis in the peat layers from two wetland soils from Missouri and Florida. We then investigated CH<sub>4</sub> production in soil microcosms to which differing amount of trace metals were added. At both sites investigated, the shallow surface waters overlying peat soils contained dissolved concentrations of Ni, Co, and Zn generally <0.05 μM, well below the 1 to 5 μM concentrations shown in pure culture studies to be optimal for methanogenesis. Soil digestions revealed that while both sites had low concentrations of trace metals in the peat layers, concentrations at the Missouri site were ~5 times greater than at the Florida site. The Missouri soil also contained 50-100 times more iron and sulfur than the peat from Florida. Addition of trace metals to microcosms using the Missouri soil produced up to 10 times more CH<sub>4</sub> per gram of soil than control studies lacking such additions. In contrast, addition of trace metals to microcosms using the Florida soil produced no enhancement in CH<sub>4</sub> production. The Missouri soil displayed a high metal binding capacity associated with abundant reduced sulfur groups on organic matter and iron sulfide minerals, while the Florida soil had no sulfide minerals, low organic sulfur abundance, and weak metal binding. We hypothesize that this strong intrinsic trace metal binding at the Missouri site renders the native trace metal content poorly available for methanogens, whereas the limited metals complexed to organic matter at the Florida site are likely labile and bioavailable. These studies suggest that trace metal limitations on methanogenesis in wetlands are not universal but will be important in sites with elevated sulfur contents. In addition, anthropogenic inputs of metals to wetlands may increase CH<sub>4</sub> emission from aquatic systems with high-sulfur peat soils.

# **Viral and microbial key players in wetlands that support extremely high methane emissions and sulfate reduction rates**

PAULA DALCIN MARTINS<sup>1\*</sup>, MARIE BURRIS<sup>2</sup>, ROBERT DANCZAK<sup>3</sup>, MICHAEL J. WILKINS<sup>4</sup>

<sup>1</sup>Microbiology Department, Ohio State University, Columbus, USA, dalcinmartins.1@osu.edu (\*presenting author)

<sup>2</sup>Microbiology Department, Ohio State University, Columbus, USA, burris.183@osu.edu

<sup>3</sup>Microbiology Department, Ohio State University, Columbus, USA, danczak.6@osu.edu

<sup>4</sup> Microbiology Department and School of Earth Sciences, Ohio State University, Columbus, USA, wilkins.231@osu.edu

The Prairie Pothole Region (PPR) of North America is the tenth largest wetland ecosystem in the world and contains millions of small wetlands that are very rich in dissolved organic carbon (DOC) and sulfur-containing compounds such as sulfate, sulfide, and polysulfides. Given that inland waters are increasingly recognized as critical sites of methane emissions, a previous study from our group investigated methane production and emissions from PPR wetlands with an emphasis on linked carbon and sulfur cycles using a suite of geomicrobiological tools. We observed the highest sulfate reduction rates ever reported, as well as extremely high porewater methane concentrations and methane emissions from these wetlands. Higher microbial activities correlated to increases in the "biodegradability" of DOC inferred from mass spectrometry and nuclear magnetic resonance data. Based on these results, we hypothesized that the high amounts of labile organic compounds and non-competitive substrates may alleviate the thermodynamic inhibition of sulfate reduction on methanogenesis, allowing for the co-occurrence of both processes in these wetlands at large scales.

In this study, we have identified which microorganisms are likely responsible for such intensive sulfate reduction and methane cycling, and have investigated the impact of viruses on these cycles. This work represents one of the few studies exploring sediment viruses with metagenomics. We identified ~600 *dsrA* sequences, which are marker genes for sulfur oxidation and sulfate reduction. Out of those, ~100 were reductive and present in reconstructed microbial genomes affiliated to 5 phyla, highlighting the diversity of candidate sulfate reducers. We also recovered 33 *mcrA* sequences, marker genes for methanogenesis, mostly affiliated to Methanomicrobiales, and 10 *pmoA* sequences, marker genes for aerobic methanotrophy, all affiliated to the gammaproteobacteria.

Moreover, we identified 1080 unique viral sequences and were able to link these sequences to reconstructed microbial genomes, suggesting that viruses target key microbial players and thus can impact sulfur and methane cycling. Most of these viruses could not be taxonomically classified and none were identified in published databases. Therefore, we conclude that PPR wetland sediments harbor novel viruses that can impact the ecosystem, as well as a richness of sulfur and methane-cycling microorganisms. This study provided evidence for the molecular mechanisms that control these processes and will serve as basis for future ecological modelling to predict the effects of climate change on the region, which is poorly recognized but extremely important for greenhouse gas emissions.

## Constraining Lipid Biomarker Proxies in a Small Arctic Watershed

HANNAH DION-KIRSCHNER<sup>1\*</sup>, JAMIE MCFARLIN<sup>2</sup>, YARROW AXFORD<sup>3</sup>, MAGDALENA OSBURN<sup>4</sup>

<sup>1</sup>Earth and Planetary Sciences, Northwestern University, Evanston, IL, USA,  
hannahdionkirschner2018@u.northwestern.edu (\*presenting author)

<sup>2</sup>Earth and Planetary Sciences, Northwestern University, Evanston, IL, USA,  
jamiemcfarlin2018@u.northwestern.edu

<sup>3</sup>Earth and Planetary Sciences, Northwestern University, Evanston, IL, USA, axford@northwestern.edu

<sup>4</sup>Earth and Planetary Sciences, Northwestern University, Evanston, IL, USA, maggie@northwestern.edu

Arctic amplification of climate change renders arctic environments unusually sensitive to changes in climatic conditions, particularly warming (Serreze and Barry, 2011). Lipid biomarkers, and their hydrogen and carbon isotopic compositions, can yield valuable paleoclimatic and paleoecological information. However, many variables affect the production and preservation of lipids and their constituent isotopes, including precipitation, local aridity and evapotranspiration, plant growth conditions, and biosynthesis mechanisms (Sachse et al., 2012). These variables are particularly poorly constrained for high-latitude environments, where trees are sparse or not present, and plants grow under continuous summer light and cool temperatures during a short growing season.

Here we present a study of plant-specific lipid chain lengths and carbon and hydrogen isotopic values from within a single watershed in the Kangerlussuaq region of southwest Greenland. Our analytes from in and around ‘Little Sugarloaf Lake’ (LSL) include terrestrial plants, modern lake water, and precipitation samples, as well as publicly available water isotope data. This diverse sample set allows us to fulfill two goals: 1) We evaluate the production of lipids and  $\delta^2\text{H}$  and  $\delta^{13}\text{C}$  signatures in terrestrial plants. Our data exhibit genus-level trends in leaf wax production and isotopic composition, and may help clarify the difference between terrestrial and aquatic signals. 2) We evaluate the isotopic signatures of local precipitation and lake water to determine to what extent these signals are reflected by the isotopic signatures of nearby terrestrial plants. Researchers have commonly assumed that the isotopic values of shorter lipid chain lengths reflect lake water isotopes and those of longer lipid chain lengths reflect precipitation isotopes, and we evaluate the accuracy of this assumption.

Upon the completion of this modern calibration, we will apply our findings to the analysis of a 65 cm sediment core record. Our core is organic-rich and shows evidence of high deposition rates, which will facilitate a high-resolution climate reconstruction. Our work will test the veracity of three common paleoclimate and paleoecological proxies—hydrogen isotopes, carbon isotopes, and lipid chain length assemblages—specifically for research in southwest Greenland, and will enable an accurate, high-resolution watershed-level reconstruction of Holocene climate.

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# Reconstructing hydroclimatic variations using compound-specific Hydrogen isotope analysis of plant leaf waxes from a maar lake in the Central Highlands, Vietnam

KELSEY E. DOIRON<sup>1\*</sup>, L.R. STEVENS<sup>2</sup>, PETER E. SAUER<sup>3</sup>

<sup>1</sup>Geological Sciences, California State University - Long Beach, Long Beach, CA, USA,  
Kelsey.Doiron@student.csulb.edu (\*presenting author)

<sup>2</sup>Geological Sciences, California State University - Long Beach, City, USA, Lora.Stevens@csulb.edu

<sup>3</sup>Geological Sciences, Indiana University - Bloomington, Bloomington, IN, USA, pesauer@indiana.edu

Monsoonal variation in Southeast Asia affects a significant portion of the global population, but knowledge regarding response of the monsoon system to changing climate is limited. The paleoclimatic tool of compound-specific isotope analysis (CSIA) provides the ability to reconstruct past precipitation using a diverse set of biomarkers preserved in the sedimentary record. Limited proxies in tropical southeast Asia and difficult site access have led to a deficit in paleoclimate records. Ia M<sup>1</sup>He (14°10'45" N, 107°52' E) is a shallow volcanic crater (maar) lake, approximately 57 ha, located in the Central Highlands of Vietnam. Precipitation in the Central Highlands is sensitive to reorganizations of major climatic features, such as the migration of the ITCZ and coupled strengthening of the Indo-Asian monsoon, ENSO and related shifts in the Pacific Walker Circulation and typhoon frequency. To examine this complex behavior, this pilot study aims to provide a 500-year record of effective moisture inferred from CSIA of hydrogen isotopes on biomarkers. Carbon/nitrogen ratios and carbon isotope ratios indicate that bulk organic matter is a combination of algae and C<sub>3</sub> vegetation, offering the potential to use compound-specific hydrogen isotopes of aquatic and terrestrial organic matter in tandem. Preliminary analysis of the core shows dominant alkane chain lengths of C<sub>27</sub> and C<sub>29</sub>, associated with terrestrial plant leaf waxes. The hydrogen isotope ratios of the plant wax components provide a proxy for paleo precipitation in a region where rainfall and droughts heavily influence population dynamics and create social discord. The CSIA record is expected to correlate with records from northern Vietnam, the South China Sea and Indonesia, with greater precipitation during the Little Ice Age. The degree to which evaporative modification of lake water (i.e., seasonal drying) occurs will be estimated by comparing the terrestrial CSIA values indicative of meteoric water with aquatic CSIA values, which will capture the hydrologic balance of the lake itself.

# Influence of Extracellular Polymeric Substances on the Adsorption of Cadmium onto Three Bacterial Species

BUTZEN, M.L.<sup>1\*</sup>, FEIN, J.B.<sup>2</sup>

<sup>1</sup>Civil & Environmental Engineering & Earth Science, University of Notre Dame, Notre Dame, USA, mbutzen@nd.edu (\*presenting author)

<sup>2</sup>Civil & Environmental Engineering & Earth Science, University of Notre Dame, Notre Dame, USA, fein@nd.edu

The fate and bioavailability of heavy metals in the environment can be influenced by bacteria through surface adsorption. Bacteria in soils and groundwater systems exist primarily in biofilms which contain bacterial cells and extracellular polymeric substances (EPS) including DNA, proteins, and other organic molecules. Most previous studies of metal adsorption onto bacterial biomass have focused on bacterial cell adsorption, with the bacteria treated to remove associated EPS molecules. Few studies have attempted to quantify metal binding onto EPS molecules due to the difficulty of isolating intact EPS molecules, and the studies that have been conducted on metal-EPS binding yield inconsistent results on whether there is a need to differentiate between bacterial cell adsorption behavior and EPS binding behavior when predicting mass transport of heavy metals in aqueous geologic systems. In this study, we measured the effect of EPS on Cd adsorption by measuring the extent of adsorption onto biomass with, and without, the EPS molecules removed. We studied three common, EPS-producing bacterial species: one Gram positive (*Bacillus subtilis*) and two Gram negative (*Shewanella oneidensis*, *Pseudomonas putida*), exposing each separately to dissolved Cd in a 0.01 M sodium perchlorate solution to buffer ionic strength. The experiments were conducted from pH 3 to 8, and at three metal loadings: 1.0  $\mu\text{mol}$  Cd per gram wet bacteria, 2.0  $\mu\text{mol/g}$ , and 5.0  $\mu\text{mol/gram}$ . The experiments were allowed to react for 2 hours to attain adsorption equilibrium, and the extent of adsorption was determined by subtracting the measured Cd concentration after the experiment from the known initial concentration of the starting solution. The after-equilibration Cd concentration in solution was measured using inductively coupled plasma optical emission spectroscopy with matrix-matched standards and blanks. In order to create EPS free biomass for the experiments, we treated the biomass with a cation exchange resin in the same 0.01 M sodium perchlorate electrolyte at a ratio of 30 g of resin per gram of biomass.

In all experiments, the extent of adsorption increased with increasing pH, approaching 100% Cd adsorption at higher pH values. As the metal loading increased, the percentage of total Cd that was adsorbed decreased. Under most of the conditions studied, removing the EPS had a minimal effect on the extent of Cd adsorption for both the Gram negative and Gram positive species. Under the highest metal loading conditions, removal of the EPS molecules caused the extent of mass-normalized Cd adsorption onto *P. putida* between pH 4 and 6 to increase somewhat, and the extent of Cd adsorption onto *S. oneidensis* to decrease over this same pH range. These results imply that in general Cd-EPS binding behavior is similar to the Cd-cell binding behavior. Therefore, removal of the EPS has a minimal effect on the mass-normalized adsorption behavior of a biofilm. Our results suggest that while EPS binding likely plays a role in metal binding within a biofilm, because of the similarity between the two types of binding, metal binding onto EPS and EPS-cell mixtures can be reasonably well estimated using thermodynamic binding constants that have been determined from cell-only experiments.

# Interactions Between Colonizing Microbes and Biocides in Hydraulically Fractured Wells

David Morgan<sup>1</sup>, Joseph Moore<sup>2</sup>, Anne Booker<sup>1</sup>, Ken Wunch<sup>2</sup>, Michael Wilkins<sup>1</sup>

<sup>1</sup>Department of Microbiology, Ohio State University, Columbus, OH, USA

<sup>2</sup>Dow Microbial Control, Collegeville, PA, USA

In the past decade, hydraulic fracturing has become an increasingly popular form of energy extraction in the United States, responsible for roughly two-thirds of U.S. natural gas production. Although 300,000 wells have been drilled in the U.S., relatively little is known about the microorganisms that persist in these environments and the chemical reactions they catalyze. Despite efforts to reduce biological activity, microbes can often thrive in these hydrocarbon-rich, hypersaline (up to 150 g/L TDS) environments that reach pressures of ~345 bar. Members of the genus *Halanaerobium* are cosmopolitan, often constituting the majority of the microbial population across geographically distinct shales. *Halanaerobium* possesses the ability to produce corrosive sulfide, and forms potentially yield-reducing biofilms by clogging newly-induced fractures that are essential for hydrocarbon recovery. Biocides for microbial control are traditionally applied to hydraulic fracturing fluids on the surface, while only some persist down-well for longer-lasting activity. We tested the efficacy of multiple biocides on *Halanaerobium* growth under varying conditions representative of the subsurface, including high pressures (345 bar). Our results indicated that biomass is often inhibited (a halt in growth) or decreased (due to cell lysis) when amended with biocides at standard use concentrations. Cells cultivated under high pressure conditions were neither more resistant nor more susceptible to biocide activity. Most of the tested biocides were effective at cell concentrations much higher than those present in hydraulically fractured wells. Previous studies have observed cell counts of  $6 \times 10^4$  cfu/mL in injected fluids at the time of injection. In our study we tested cell concentrations of around  $7 \times 10^7$  cfu/mL, indicating that these biocides were effective at bacterial concentrations over 1,000 times higher than those observed in wells. In summary, the majority of biocides were effective against this representative *Halanaerobium* strain, suggesting that dominant microbial populations can be controlled through the targeted application of specific chemistries. Future studies will assess impacts of additional reactive species (e.g., sulfur compounds) on biocide efficiency in flowback fluids and produced water from hydraulically fractured systems.

# Pyrite and organo-sulfur formation and their pathways in modern euxinic lakes

FOTIOS FOUSKAS<sup>1\*</sup>, WILLIAM P. GILHOOLY III<sup>1</sup>, ALICE BOSCO-SANTOS<sup>1,2</sup>, MOLLY D. O'BEIRNE<sup>3</sup>, JOSEF P. WERNE<sup>3</sup>, MARTIN KUREK<sup>1</sup>, GREGORY K. DRUSCHEL<sup>1</sup>

<sup>1</sup>Department of Earth Sciences, Indiana University-Purdue University Indianapolis, Indianapolis, IN 46202, USA, [ffouskas@iupui.edu](mailto:ffouskas@iupui.edu) (\*presenting author); [wgilhool@iupui.edu](mailto:wgilhool@iupui.edu); [alicebosco@gmail.com](mailto:alicebosco@gmail.com)

<sup>2</sup>Department of Geology and Natural Resources, University of Campinas, Campinas, São Paulo 13083-970, Brazil

<sup>3</sup>Department of Geology & Environmental Science, University of Pittsburgh, Pittsburgh, PA 15260, USA, [jwerne@pitt.edu](mailto:jwerne@pitt.edu); [mdobeirne@pitt.edu](mailto:mdobeirne@pitt.edu)

Mahoney Lake, Canada and Green Lake, NY, are modern lakes ideal for studying the redox cycle of sulfur (S). As they are thought to be analogous to Proterozoic euxinic ocean, we are able to isolate the biogeochemical processes that are associated with the early earth environment and its prevalent microbial life. High productivity of phototrophic S-oxidizers (purple S bacteria) and sulfate-reducers in the lake's chemocline drive the active S cycle that sustain high levels of sulfide (~35 mM in Mahoney and ~2 mM in Green Lake). Sulfide thus is being incorporated into the sediments for pyrite and organo-sulfur production. The unknown relative pathways of sulfide into the inorganic and organic sedimentary phases, together with the variability of iron (Fe) and organic matter between the two lakes, lead us to analyze quantitatively and isotopically the S species within the water column and the sediments, and quantify the reactive Fe pools that are precursors for pyritization and can also show the redox conditions of the water column through time.

The S isotope composition ( $\delta^{34}\text{S}$ ; V-CDT) of sulfate and sulfide in the water column of Mahoney Lake reveals an offset of ~52‰ ( $\delta^{34}\text{S}_{\text{SO}_4}=28\text{‰}$ ,  $\delta^{34}\text{S}_{\text{H}_2\text{S}}=-24\text{‰}$ ) that indicates dissimilatory sulfate reduction as the dominant process of S isotope fractionation. In order to examine the sulfide pathway during diagenesis and how Fe availability can drive pyrite formation (syngenetic vs. diagenetic), sediment cores were subsampled (up to 3.2 mblf which represents ~7000 years BC). Total organic S (TOS), acid-volatile S (AVS), and pyrite were extracted from each sample. Isotope analysis of pyrite-S revealed low  $\delta^{34}\text{S}$  values (-15 to -33‰ in Mahoney and -24 to -28‰ in Green Lake) similar to those of sulfide in the respective water column, suggesting that pyrite was formed syngenetically. The isotope profiles of each S phase recorded identical S isotope signatures of pyrite and TOS through the sedimentary record of Mahoney Lake. In contrast, the respective S isotope signatures for Green Lake show the expected offset between pyrite and TOS (TOS has higher  $\delta^{34}\text{S}$  values of ~10‰), suggesting that the different geochemical parameters of these two systems, such as sulfide and organic matter concentrations, influence the source and timing of inorganic and organic pathway of reduced S. In particular, the higher level of sulfide and/or organic matter in Mahoney Lake could be the trigger of a faster sulfurization of organic matter similar to pyritization.

Ongoing research includes kinetic studies of pyritization and sulfurization of organic matter in order to map out the rate dynamics of these two competitive pathways and explain the different isotope results observed in Mahoney and Green Lake. Water samples above, below, and at the chemocline of Mahoney Lake are being analyzed by Fourier Transform Ion Cyclotron Resonance-Mass Spectroscopy in order to examine the nature of the organic matter and

# Controls on the production and $\delta\text{D}$ composition of two plant biomarkers in a temperate forest and implications as proxies for precipitation $\delta\text{D}$

ERIKA FREIMUTH<sup>\*1</sup>, AARON DIEFENDORF<sup>1</sup>, TOM LOWELL<sup>1</sup>

<sup>1</sup>Department of Geology, University of Cincinnati, Cincinnati, OH, USA, freimuej@mail.uc.edu (\*presenting)

An improved understanding of biomarker production by source organisms can lead to more accurate interpretations of the climate information they contain. The hydrogen isotopic composition of plant waxes ( $\delta\text{D}_{\text{wax}}$ ) in sediments reflects the  $\delta\text{D}$  of past precipitation ( $\delta\text{D}_{\text{p}}$ ) and is thus used to study changes in hydroclimate over geologic timescales. While both *n*-alkanes and *n*-alkanoic acids are applied as  $\delta\text{D}_{\text{p}}$  proxies, the latter are understudied in plants. We therefore have limited information about how the two compounds may differ with respect to their abundance, timing of synthesis and  $\delta\text{D}$  fractionation of source water. In addition, apparent fractionation ( $\epsilon_{\text{app}}$ ) between  $\delta\text{D}_{\text{p}}$  and  $\delta\text{D}_{\text{wax}}$  can vary widely at the plant level, contributing to high uncertainty in  $\epsilon_{\text{app}}$  values applied to sedimentary  $\delta\text{D}_{\text{wax}}$  to reconstruct past  $\delta\text{D}_{\text{p}}$ .

To address these uncertainties, we tracked the production and  $\delta\text{D}$  values of *n*-C<sub>29</sub> alkanes and *n*-C<sub>28</sub> alkanolic acids, as well as xylem ( $\delta\text{D}_{\text{xw}}$ ) and leaf water ( $\delta\text{D}_{\text{lw}}$ ) in the five tree species (*Prunus serotina*, *Acer saccharinum*, *Quercus rubra*, *Quercus alba*, and *Ulmus americana*) that dominate a temperate forest at Brown's Lake Bog, Ohio, USA over the 2014 growing season. *n*-Alkanes were generally produced in the 2-3 weeks following leaf emergence and at up to 30-fold higher concentrations than *n*-alkanoic acids, which were produced over the entire growing season. Despite these differences in the timing and amount of wax produced, the  $\delta\text{D}_{\text{wax}}$  values of both compound classes were constant from the spring onset of leaf maturation until autumn leaf fall. Meanwhile,  $\delta\text{D}_{\text{xw}}$  was stable over the growing season and closely resembled the modeled mean annual precipitation  $\delta\text{D}$  value for the site. This indicates that trees are accessing a well-mixed soil water pool that is buffered from monthly changes in  $\delta\text{D}_{\text{p}}$ , and validates the utility of  $\delta\text{D}_{\text{wax}}$  as a proxy for annually-integrated local  $\delta\text{D}_{\text{p}}$  in temperate settings.

During spring leaf emergence and expansion, approximately 50-70% of the changes in *n*-C<sub>29</sub> alkane  $\delta\text{D}$  could be explained by synchronous changes in  $\delta\text{D}_{\text{lw}}$ . Bulk foliar  $\delta^{13}\text{C}$  data indicate that a shift in metabolic strategy between expanding and mature leaves was an important secondary driver of  $\delta\text{D}_{\text{wax}}$  during leaf development. During this metabolic shift, an offset in  $\epsilon_{\text{app}}$  between the two compound classes was established, with  $\epsilon_{\text{app}}$  approximately 19‰ greater for *n*-C<sub>29</sub> alkanes (-107‰) than for *n*-C<sub>28</sub> alkanolic acids (-88‰). Comparisons with prior studies suggest that this  $\epsilon_{\text{app}}$  offset may be specific to temperate settings. Therefore, application of  $\epsilon_{\text{app}}$  values calibrated for *n*-alkanes to *n*-alkanoic acid records from temperate sites may overestimate paleoprecipitation  $\delta\text{D}$  by 20-30‰.

# Diversity and Physiology of Proteorhodopsin-Containing Bacteria in Aquatic Systems

GWENDOLYN GALLAGHER<sup>1\*</sup>, JACOB WALDBAUER<sup>2</sup>

<sup>1</sup>Department of the Geophysical Sciences, University of Chicago, Chicago, USA, gegallagher@uchicago.edu  
(\*presenting author)

<sup>2</sup>Department of the Geophysical Sciences, University of Chicago, Chicago, USA, jwal@uchicago.edu

Proteorhodopsin (PR), a light-activated transmembrane proton pump, has been found to be widespread and abundant in metagenomes of microbial communities of the surface ocean. PR generates a proton gradient that microbes can potentially use for ATP synthesis, flagellar movement, or nutrient transport, but its physiological role in marine microbes and its contribution to the overall energy budget of marine microbial communities is not yet clear.

To help understand how marine microbes utilize PR, we are exploring the photophysiology of model PR-bearing marine bacterial isolates using mass spectrometry-based proteomics. Growth of several PR-containing microbes was monitored under a range of carbon and light conditions to determine if PR is used as a survival mechanism to generate energy under starvation stress. To better understand the distributions of PR genes, which appear to be frequently horizontally transferred, in natural marine communities, we have looked at PR sequence diversity in oceanic metagenomic datasets. Additionally, we are expressing PR heterologously in *E. coli* both to assess the fitness benefits of a newly acquired PR gene and to improve techniques for protein-level quantification of PR expression using mass spectrometry.

# Understanding carbon and electron flow during phototrophic electron uptake in purple nonsulfur bacteria

MICHAEL S. GUZMAN<sup>1\*</sup>, ARPITA BOSE<sup>2</sup>

<sup>1</sup>Biology, Washington University in St. Louis, St. Louis, MO, USA, [abose@wustl.edu](mailto:abose@wustl.edu)

<sup>3</sup>Biology, Washington University in St. Louis, St. Louis, MO, USA, [email@univ.edu](mailto:email@univ.edu)

Bacteria that donate or accept electrons from solid electron donors or acceptors substantially influence geochemical cycles on a global scale. This phenomenon is broadly termed extracellular electron transfer (EET). Recently, EET has been linked to autotrophic metabolisms where extracellular electron donors are hypothesized to provide reductants for CO<sub>2</sub> fixation. This process has been studied in several pure cultures using poised electrodes, including chemoautotrophic and photoautotrophic bacteria, however, to date no studies have examined the mechanisms underlying the energy yielding and carbon assimilation pathways in photoelectrotrophic microbes. Only one study in a purple nonsulfur bacterium (*Rhodospseudomonas palustris* TIE-1) has examined the genetic loci involved in electron uptake from solid electron donors, however, in TIE-1 and other electrotrophs, electron uptake has not been linked genetically to CO<sub>2</sub> fixation. Furthermore, electrotrophs display modest growth rates suggesting that either these organisms are unable to capitalize on electrons from an extracellular source to generate NAD(P)H for CO<sub>2</sub> fixation, electrons are being utilized primarily for maintenance energy, or that the detection limit of previous methods used to measure cell growth were inadequate. Using *R. palustris* TIE-1 as a model system we examined if CO<sub>2</sub> fixation is the primary electron sink during photoelectrotrophic metabolism. We determined that the key CO<sub>2</sub> fixation enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (ruBisCO), is necessary and sufficient for electron uptake using a ruBisCO conditional knockdown system. Furthermore, transcriptomic analysis shows that Calvin-Benson-Bassham (CBB) cycle enzymes are upregulated during electron uptake. This data suggests that the CBB cycle is the primary sink for reduced cofactors. Understanding the mechanisms underlying electron uptake in autotrophic microbes will further biotechnological efforts in these organisms, including microbial electrosynthesis, and improve our understanding of the biogeochemical impact of these organisms on carbon and metal cycling.

# **Microbial Adaptations to Extremely High pH: Insights from Serpentinization-Associated Ecosystems**

LYDIA HAYES<sup>1\*</sup>, LAUREN M. SEYLER<sup>2</sup>, MATTHEW O. SCHRENK<sup>3</sup>

<sup>1</sup>Microbiology and Molecular Genetics, Michigan State University, East Lansing, USA, hayeslyd@msu.edu  
(\*presenting author)

<sup>2</sup>Microbiology and Molecular Genetics, Michigan State University, East Lansing, USA, email@univ.edu

<sup>3</sup>Microbiology and Molecular Genetics, Michigan State University, East Lansing, USA, email@univ.edu

Serpentinization is a widespread geochemical process that is found worldwide, particularly along continental margins. A common outcome of serpentinization is the generation of high pH (>10) groundwater. The purpose of this study is to gather a better understanding of cultivable alkaliphiles, how they develop a tolerance to hyperalkaline conditions, and how they compare with known alkaliphilic strains. Nearly 200 strains of microorganisms from water and soil samples from high pH, geographically diverse environments associated with serpentinization have been isolated and characterized by our lab. Through 16S rRNA gene sequencing we have found that 30% of these strains are related to the genus *Bacillus*, 9% are related to the genus *Dietzia*, and others are related to diverse genera including *Hydrogenophaga*, and *Microbacterium*. Results from genomic sequencing and laboratory experiments are being compared to data from well-characterized alkaliphilic *Bacillus* strains OF4 and *B. horikoshii*. These data are being used to evaluate the physiological potential of our strains and to compare their evolutionary history through phylogenetic analysis. Ongoing laboratory work is examining the differences amongst our isolates in terms of their pH optima, pigment content, oxygen tolerance, and other physiological features. Taken together, these results will provide insight into how microorganisms have come to develop an affinity or tolerance to highly alkaline environments associated with serpentinization and if they share commonalities with each other and with known alkaliphilic strains.

# Soil physicochemistry may impact microbial communities in coastal wetlands of the Great Lakes

DEAN J. HORTON<sup>1\*</sup>, DONALD G. UZARSKI<sup>2</sup>, DERIC R. LEARMAN<sup>3</sup>

<sup>1</sup>Institute of Great Lakes Research and Department of Biology, Central Michigan University, Mount Pleasant, MI, USA, horto2dj@cmich.edu (\*presenting author)

<sup>2</sup>Institute of Great Lakes Research and Department of Biology, Central Michigan University, Mount Pleasant, MI, USA, uzars1dg@cmich.edu

<sup>3</sup>Institute of Great Lakes Research and Department of Biology, Central Michigan University, Mount Pleasant, MI, USA, deric.learman@cmich.edu

Wetlands host a unique suite of chemical cycles while providing ecosystem services to bordering environments. These biogeochemical processes within wetlands are facilitated by microbial communities, which are often nested within the soil. Coastal wetlands bordering the Laurentian Great Lakes are essential in sustaining the proper functioning of the Great Lakes themselves. Despite this, relationships between soil physicochemistry and microbial communities within Great Lakes coastal wetlands remain unexplored. Sediment cores were collected from coastal wetlands bordering Saginaw Bay of Lake Huron, northern Lake Michigan, and the western basin of Lake Erie to provide an initial characterization of biogeochemistry and microbial community structure at these sites. Here, the data shows that environmental conditions (e.g. pH, organic carbon, nutrients, etc.) act as potential drivers of microbial structure among and within wetlands. Lake Erie coastal wetland microbial communities were significantly different in structure than all other wetland regions explored. When comparing geochemical and physical parameters of each wetland, Lake Erie wetlands were relatively more neutral in pH and higher in nutrient levels than all other sites, which could be driving differences in microbial diversity between wetlands in Lake Erie and other Great Lakes coastal wetlands. Additionally, microbial community structure changed as samples were collected at different depths within a wetland core, suggesting a connection to changing redox zones. However, this connection to sampling depth was not evident in Lake Erie wetlands. The data suggests that high carbon and nutrient levels, as well as pH levels, within Lake Erie wetlands could be influencing microbial community structure, possibly due to anthropogenic pressure (such as agricultural practices in the Lake Erie watershed). Network analyses unearthed several microbial subcommunities found to be significantly related to environmental variables, including pH, organic matter, S, and C:N. Unique taxonomic groups, such as *Anaerolineaceae*, were central within these subcommunities and were highly predictive of environmental conditions, suggesting that certain taxa may be niche-dependent and could be potentially utilized as indicator taxa of nutrient levels within Great Lakes coastal wetlands. The data here provides an initial characterization of microbial communities within Great Lakes coastal wetlands and thus generates baseline data on what microbes exist within these systems. This research also provides insight into how these microbes could be interacting, and to what extent microbial diversity may already be impacted by potential anthropogenic disturbance.

# Photographic film-based capture of the spatial and temporal heterogeneity in aqueous $\delta^{34}\text{S}_{\text{H}_2\text{S}}$

J.L.HOUGHTON<sup>1\*</sup>, S.MOORE<sup>1</sup>, C.JONES<sup>1</sup>, D.A.FIKE<sup>1</sup>

<sup>1</sup>Department of Earth and Planetary Sciences, Washington University in St. Louis, St. Louis, USA,  
jhoughton@wustl.edu (\*presenting author); s.moore@wustl.edu; clivejones@wustl.edu; dfike@wustl.edu

The ability to easily deploy, subsample and analyze photographic film makes it an ideal tool for capturing the spatial and temporal variability of hydrogen sulfide ( $\text{H}_2\text{S}$ ) in complex natural environments. Using bulk extractions, we can resolve variability in sulfide  $\delta^{34}\text{S}$  in exposed films in areas as small as  $1.5 \text{ cm}^2$ ; using secondary ion mass spectrometry (SIMS),  $\delta^{34}\text{S}_{\text{H}_2\text{S}}$  can be analyzed over distances as small as  $100 \text{ }\mu\text{m}$ . The captured sulfide is depleted in  $^{34}\text{S}$  relative to the sulfide in solution by 1.5‰, a fractionation that is independent of salinity, pH and sulfide concentration in solution.

Isotopic analysis using SIMS produces reproducible values ( $2\sigma < 0.7 \text{ ‰}$ ;  $n = 8$ ) that match the bulk  $\delta^{34}\text{S}$  for a wide range of sulfide abundance ( $8\text{-}55 \text{ }\mu\text{mol S/ g film}$ ) using a  $35 \text{ }\mu\text{m}$  raster ( $3\text{nA Cs}^+$ , FC detectors). The calibration of  $\delta^{34}\text{S}$  using SIMS is not sensitive to variable Cl concentration, indicating minimal matrix effects from halides within the film during this analysis. However, due to the gradual diffusion/capture process within the film during exposure to sulfide, a correction must be applied to account for the variable S counts with depth. The minimum sulfide abundance that can be reliably analyzed with the FC method is  $\sim 8 \text{ }\mu\text{mol S/ g film}$ . For abundances below this threshold, the analytical conditions need to be modified to a  $50 \text{ }\mu\text{m}$  raster ( $200\text{pA Cs}^+$ , EM detector). Sulfide images can also be produced as large as  $100 \text{ }\mu\text{m} \times 100 \text{ }\mu\text{m}$  using the EM method for films that display either high  $\delta^{34}\text{S}$  heterogeneity in the bulk analysis or a patchy/speckled appearance on the surface of the film related to shifts in flux and subsurface microbial activity, capturing evidence of diurnal shifts in the microbial sulfur cycle.

# Visualization and Quantification of Cd Sorption to Bacteria Using Confocal Laser Scanning Microscopy and Cd-specific Fluorescent Probes

CLAYTON R. JOHNSON<sup>1\*</sup>, JULIANE HOPF<sup>1</sup>, JOSHUA D. SHROUT<sup>1</sup>, JEREMY B. FEIN<sup>1</sup>

<sup>1</sup>Department of Civil & Environmental Engineering & Earth Sciences, University of Notre Dame, Notre Dame, IN, USA, cjohns42@nd.edu (\*presenting author)

Understanding metal sorption to bacteria is important for developing bioremediation strategies, for determining the fate of contaminants in ground water systems, and for defining controls on microbial metabolic processes in the environment. X-ray absorption spectroscopy (XAS) has been the only technique available for quantifying and mapping metal sorption to bacteria on an individual cell scale, but the XAS approach is time consuming and expensive, and quantitative mapping of the distribution of metals in complex samples is not possible with XAS.

The development of metal-specific fluorescent probes, in conjunction with confocal laser scanning microscopy (CLSM), represents a novel and more effective approach for visualizing and quantifying biosorption. In this study, we test and calibrate the use of a commercially available Cd- and Pb-specific probe, Leadmium Green (LG), to quantify the distribution of Cd in bacteria-bearing systems. We have developed a treatment technique that enables us to quantify Cd sorbed to individual bacterial cells as well as Cd sorbed to a bacteria population as a whole. Untreated LG is neutrally charged and passes through bacterial cell membranes easily. Inside the cell, cytoplasm enzymes de-esterify the LG, creating a negatively-charged de-esterified LG (DELG) molecule that cannot pass back out of the cell due to the electronegativity of the cell envelope. The DELG molecule has a high affinity to bind Cd (and Pb), which dramatically increases its fluorescence response. Therefore, we hypothesize that fluorescence of a sample that had an application of untreated LG enables quantification of the concentration of Cd internalized by the bacterial cells. Alternatively, de-esterifying the LG molecule prior to application to the biomass sample limits the location of the DELG molecule to the exterior of the cell, enabling us to quantify the concentration of externally-adsorbed Cd. Correlating the green fluorescence from LG and DELG with the fluorescence at a different wavelength of SYTO 63 cell stain enables us to accurately visualize and quantify bacterial cell Cd sorption on both a community and individual cell level. This approach, for the first time, yields a means of easily quantifying and visualizing metal adsorption onto individual bacterial cells which allows us to directly measure the distribution of Cd in bacteria-bearing environmental systems.

In this study, we report the results of a range of control experiments that demonstrate the efficacy of this approach. We demonstrate that our chemical de-esterification of LG results in 100% conversion of the LG to the DELG form that is fluorescently active, and that untreated LG is not fluorescently active until it enters the cell. We also demonstrate that calibration curves for samples containing cells, LG, DELG, SYTO 63, and Cd can be developed. Our tests also demonstrate that neither LG nor DELG cause significant changes in sorption of Cd to bacterial cells. We present results from experiments showing that Cd sorption to bacteria is heterogeneous amongst cells which differs from the previously held assumption of homogeneous sorption. The results of this study yield new insights into metal adsorption behavior in bacteria-bearing systems, and our approach represents a powerful tool for quantifying the distribution of metals in complex realistic environmental samples.

# Protecting Filamentous Cyanobacteria from UV Irradiation using Phyllosilicate Micas

ALEX KUGLER<sup>1\*</sup>, HAILIANG DONG<sup>2</sup>

<sup>1</sup>Department of Geology and Environmental Earth Science, Miami University, Oxford, USA, kugleraj@miamioh.edu (\*presenting author)

<sup>2</sup>Department of Geology and Environmental Earth Science, Miami University, Oxford, USA, dongh@miamioh.edu

Cyanobacteria are considered to be one of the major causes of the Paleoproterozoic Great Oxidation Event, which led to the oxygenation of Earth. Through photosynthesis, oxygen was released into the hydrosphere and atmosphere by cyanobacteria, changing the planet from a reducing environment to an oxidizing one. Prior to oxygenation, 3.5 Ga, the environment was reducing in nature and had high levels of UVR reaching the planet's surface unfiltered, as well as higher temperatures, and increased meteoric collisions. Photoautotrophic cyanobacteria are limited to living near the surface, and would have had to find means of survival in those harsh environments. While cyanobacteria do possess certain biological adaptations to cope with the stress of radiation, there are also means to minimize exposure by developing small scale habitats within or below minerals and rocks. The phyllosilicate minerals muscovite, biotite, and phlogopite are predicted to have been in existence dating back to the Hadean, >4.0 Ga, predating all known lifeforms indicating that they could have been used as a small-scale habitat. These minerals have all also been found in the soils of Martian samples and meteorites, indicating a pervasiveness throughout planetary systems.

These small-scale habitats provide a place for cellular growth which can protect them from harmful radiation while allowing them to undergo the photosynthetic process. In this study, laboratory incubation experiments demonstrated that filamentous cyanobacteria are able to colonize the phyllosilicates of interest. Colonies were exposed to ultraviolet radiation for up to 24 hours, and survival rates were determined using chlorophyll a, carotenoid, and phycocyanin-C assays. Samples were imaged using fluorescent and light microscopy, and recovery time was investigated as a function of protection as well. Of the three micas investigated, muscovite provided the least attenuation of ultraviolet radiation, however it transmits the most visible light of the photosynthetically active wavelengths 680 and 700 nm, which are necessary for photoautotrophs to survive. When protected by biotite and phlogopite the cyanobacteria were able to tolerate longer exposure to ultraviolet radiation however, these minerals greatly lessen the quantity of transmitted visible light. Phlogopite attenuates UV-B radiation, even when the sheets are less than one millimeter in thickness, can still transmit visible light, and were present on the early earth as well as on other planets, making it the most ideal small-scale habitat of the investigated micas. These small scale-habitats could have provided an explanation for how early photoautotrophs survived difficult conditions, as well as potential habitats for past and future Mars.

# Synthesis and Characterization of Trioctahedral Ferrous Smectites: Potential Electron Donors on the Early Earth

ROBERT J. KUPPER<sup>1\*</sup>, JEFFREY G. CATALANO<sup>2</sup>

<sup>1</sup>Dept. of Earth and Planetary Sciences, Washington University, St. Louis, USA, rkupper@wustl.edu (\*presenting author)

<sup>2</sup>Dept. of Earth and Planetary Sciences, Washington University, St. Louis, USA, catalano@eps.wustl.edu

Smectites are a chemically diverse group of phyllosilicate minerals that commonly form during alteration and weathering of silicate rocks. Iron- and magnesium-rich smectites are a major alteration product of basalt, including in large tracks of the oceanic crust. Such clays are also observed on Mars and are predicted to occur beneath the surface of the icy moons of Jupiter and Saturn. Smectites exposed today on the continents as well as on islands and island arcs contain ferric iron ( $\text{Fe}^{3+}$ ), such as in the dioctahedral smectite mineral nontronite. However, under less oxidizing conditions, such as those expected on the early Earth or in the subsurface of the modern oceanic crust, ferrous iron ( $\text{Fe}^{2+}$ ) is a major component of smectites, occurring in trioctahedral clays such as ferrosaponite.

Trioctahedral ferrous smectites represent an abundant source of  $\text{Fe}^{2+}$  potentially available as an electron donor for microbial life on the early earth, in the modern subsurface, and extraterrestrially. It is not currently known whether iron oxidizing microorganisms are able to oxidize the  $\text{Fe}^{2+}$  structurally bound within these minerals. Past work indicates that microbial oxidation of chemical or biologically reduced nontronites is possible. However, structural differences between dioctahedral and trioctahedral smectites and the essential role of atom migration through dioctahedral vacancies during nontronite redox cycling suggests that natively ferrous, trioctahedral smectites may display distinct redox properties and susceptibility to microbial oxidation. Unfortunately, it is impractical to obtain naturally occurring samples of trioctahedral iron rich smectites because of their subsurface occurrence and susceptibility to oxidation upon air exposure.

To better enable further study of these minerals, we have synthesized trioctahedral ferrous smectites that represent clay compositions observed in anoxically-altered basalt. Syntheses were performed using a hydrothermal sol-gel procedure under anoxic conditions. Powder X-ray diffraction confirmed that all synthetic clays were single-phase turbostratic trioctahedral smectites. Compositions obtained by lithium metaborate digestion followed by inductively-coupled plasma optical emission spectrometry generate structural formulae consistent with trioctahedral  $\text{Fe}^{2+}$ -bearing smectites. Preliminary data on the rate of abiotic oxidation of these minerals in the presence of air and nitrite ( $\text{NO}_2^-$ ) will also be presented.

# Ultra-high resolution mass spectrometry as a tool to study freshwater phosphate cycling

MARTIN KUREK<sup>1\*</sup>, MOURAD HARIR<sup>2</sup>, JOHN SHUKLE<sup>1</sup>, MEAGAN LEDUC<sup>3</sup>, ANDREW SCHROTH<sup>3</sup>,  
PHILIPPE SCHMITT-KOPPLIN<sup>2</sup>, GREGORY K DRUSCHEL<sup>1</sup>

<sup>1</sup>Department of Earth Sciences, Indiana University-Purdue University Indianapolis, Indianapolis, IN USA,  
markurek@indiana.edu (\*presenting author)

<sup>2</sup>Helmholtz Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt, Munich, Germany

<sup>3</sup>Department of Geology, University of Vermont, Burlington, VT USA

Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) is a powerful analytical technique that can measure atomic masses with remarkable precision, yielding a look at thousands of different molecules in a single sample. Recently, this technique has been optimized for characterizing unique organic molecular signatures of dissolved organic matter (DOM) collected from various environments, making the method robust. Traditionally used to investigate organic molecules containing only N and S as possible heteroatoms, this study demonstrated the ability to reveal multiple classes of organic phosphorus molecules. Water samples were collected from 3 eutrophic freshwater lakes that experience seasonal redox variations affecting phosphorus mobility to better understand how organic phosphorus cycling in the water column affects algal blooms. Water was sampled in early summer from Lake Champlain and Shelbourne pond, located in Vermont, and from Eagle Creek reservoir in Indianapolis at various depths. Initial analyses reveal hundreds of phosphorus-containing molecules containing H/C and O/C ratios characteristic of lipid and protein compositions, indicative of biotic origin. Molecules with low H/C ratios less than 1 are also present as possible condensed aromatics. Furthermore, the percent of organic phosphorus molecules generally decrease with depth across all 3 lakes suggesting greater microbial activity at the surface and less at the sediment-water interface. When coupled with other analytical techniques such as <sup>31</sup>P NMR and voltammetry, FTICR-MS allowed a greater understanding of aquatic phosphorus cycling and the mobility of organic phosphorus pools across sediment and water columns of these systems.

## Two newly documented ferruginous lakes in the Midwest, USA are Archean ocean analogs

NICK LAMBRECHT<sup>1\*</sup>, BETSY SWANNER<sup>1</sup>, SERGEI KATSEV<sup>2</sup>, CHAD WITTKOP<sup>3</sup>, CODY SHEIK<sup>2</sup>

<sup>1</sup>Department of Geological and Atmospheric Sciences, Iowa State University, Ames, Iowa, USA, nlambrec@iastate.edu (\*presenting author)

<sup>2</sup>Large Lakes Observatory, University of Minnesota, Duluth, MN, USA

<sup>3</sup>Department of Chemistry and Geology, Minnesota State University, Mankato, MN, USA

Recent monitoring of Brownie Lake in Minneapolis, MN (N44° 58' 4.483", W93° 19' 26.677") and Canyon Lake in Powell, Michigan (N46° 49' 58.069", W 87° 55' 14.858") reveals two lakes that are permanently stratified year round with an iron-rich, anoxic monimolimnion layer.

Brownie Lake has a surface area of ~7 hectare and a maximum depth of 15 meters. Prior analysis of Brownie Lake reveals that it has been ferruginous and meromictic since 1917. There is a shallow mixed layer where oxygen declines to <1 ppm around 3-4 meters and a sharp chemocline at ~5 meters. In addition to high iron concentrations, seasonal monitoring of Brownie Lake has revealed a nutrient-rich reservoir. Maximum total phosphorus analysis exceeds 65  $\mu\text{mol L}^{-1}$  in the bottom waters and ammonium can reach 34.5 ppm  $\text{L}^{-1}$ . Canyon Lake has a smaller surface area of 1-2 hectare with a maximum depth of ~24 meters. It features a shallow oxycline at 12 meters and deeper chemocline around 17.5 meters. This is consistent with a limnological investigation of Canyon Lake in the late 1930's, suggesting it has been permanently stratified for an extended period of time. Compared to Brownie Lake, Canyon is relatively oligotrophic. Chemocline measurements of dissolved  $\text{Fe}^{2+}$  of both lakes fall into the estimated range of dissolved iron in Archean oceans (40-120  $\mu\text{mol L}^{-1}$ ).

In Brownie, light penetrates the shallow chemocline between opposing gradients of  $\text{Fe}^{2+}$  and oxygen. This promotes growth of  $\text{Fe}^{2+}$ -oxidizing photosynthetic bacteria. The bacterial family *Chlorobiaceae*, which is known to contain these types of bacteria, make up 12% of the microbial community at the chemocline. An isolate of the *Chlorobiaceae* found at this depth is capable of oxidizing numerous electron donors in addition to  $\text{Fe}^{2+}$ , suggesting a mechanism by which it can deal with fluctuating chemical conditions in the lake. For example, enhanced  $\text{H}_2\text{S}$  in mid-summer strips much  $\text{Fe}^{2+}$  from the water column.

Methane captured by a static flux chamber from Brownie Lake in May 2017 reached 83.5  $\text{mmol m}^{-2} \text{ day}^{-1}$ , and an even larger flux was observed (335.5  $\text{mmol m}^{-2} \text{ day}^{-1}$ ) in July 2017. By contrast, a methane flux out of Canyon Lake reached ~1.1  $\text{mmol m}^{-2} \text{ day}^{-1}$  suggesting a more robust methane oxidation. We hypothesize that this results from the extended suboxic zone, which provides optimal conditions for aerobic, and possibly anaerobic, methane-oxidizing organisms. We are currently performing amplicon sequencing to assess abundances of methanogens and methanotrophs at depth in each lake, as well as measuring carbon isotopes of dissolved inorganic carbon (DIC) and methane.

# Does benthic iron shuttling promote the occurrence of cyanobacterial Harmful Algal Blooms (cyanoHABs)?

LEUNG, TANIA<sup>1\*</sup>, SWANNER, ELIZABETH<sup>1</sup>,

<sup>1</sup> Department of Geological and Atmospheric Sciences, Iowa State University, Ames, IA., tleung1@iastate.edu  
(\*presenting author)

As predominant members of harmful algal blooms (HABs), certain cyanobacteria species such as *Microcystis* produce microcystin, a toxin affecting the liver and kidney. The presence of microcystin and other cyanotoxins pose a risk to drinking and recreational waters and therefore strategies to mitigate cyanoHAB are essential. To this end, greater efforts are needed to better understand the circumstances leading to cyanobacteria dominance and toxin release during a bloom event.

CyanoHAB occurrences have been linked with climate change, increase atmospheric temperatures, and high nitrogen to phosphorus [N:P] ratios. However, the role of the critical micronutrient iron is unclear. Still, cyanobacteria have a higher bioavailable iron requirement than eukaryotic algae, suggesting that iron availability may stimulate cyanobacterial growth. Iron is a vital micronutrient during photosynthesis, as it is necessary for biosynthesis of cytochromes and other heme molecules. The redox reaction of Fe(II)/Fe(III) is controlled by oxygen availability. As such, hypoxic bottom waters coupled with high P concentrations could create a strongly favorable condition for remobilized benthic Fe(II) to be released into the water column.

To test this hypothesis, we have been monitoring several sites within East and West Okoboji lakes in northwestern Iowa during Summer 2017. The shallow East Okoboji (max. depth 6.7m) has higher nutrients (N, P), and is more prone to cyanoHABs than West Okoboji (max. depth 41m.) In addition to N and P measurements, temperature, dissolved oxygen, and total dissolved iron were measured from varying depths at several sites throughout the two lakes. Major phytoplankton groups were distinguished and quantified based on their fluorescence fingerprint from chlorophyll *a* and accessory pigments using a fluorometer; benthic iron fluxes were quantified by profiling sediment cores using voltammetric microelectrodes.

We noticed brown (e.g. diatoms and dinoflagellates) and green algae were dominant during early bloom period in May and June, but then transitioned to cyanobacteria dominance as water temperatures and P concentrations increased into July. This finding contradicts the direct association between cyanoHABs and high N:P ratios, as cyanoHABs in these lakes seem to correlate with low N:P ratios in July. The bloom event corresponded well with the increase in iron and P concentrations in the water column during that same period. Hypoxic conditions were periodically present even in shallow sites (<5 m), and we detected iron in sediment cores from these sites, although, iron concentrations fluctuated in the water column through the season. Our findings present further questions surrounding the extent to which benthic iron fluxes trigger cyanoHAB events. Further studies are necessary to better understand the role of benthic iron during a bloom event. In this process, our goal will be then to estimate the dispersion of Fe(II) into the water column from lake bottom sediments.

# Identification of 22 Selenium-Tolerant Bacteria and 4 Selenium-Tolerant Fungi

ELIZABETH A. MACDONALD<sup>1\*</sup>, CARLA E. ROSENFELD<sup>2</sup>, CARA M. SANTELLI<sup>2</sup>

<sup>1</sup>College of Biological Sciences, University of Minnesota-Twin Cities, Minneapolis, MN, USA, macdo323@umn.edu (\*presenting author)

<sup>2</sup>Department of Earth Sciences and BioTechnology Institute, University of Minnesota-Twin Cities, Minneapolis, MN, USA, carlar@umn.edu

<sup>3</sup>Department of Earth Sciences and BioTechnology Institute, University of Minnesota-Twin Cities, Minneapolis, MN, USA, santelli@umn.edu

Selenium (Se) contributes to air, water, and soil pollution in diverse environments globally. It enters the environment in the form of water-soluble selenate (Se(VI)) and selenite (Se(IV)), often as byproducts from human activities including mining, agriculture, and metal smelting. Trace amounts of selenium are essential for nutrition in many organisms, but the margin between nutritionally required and toxic levels is one of the smallest of all the micronutrients. Selenium also biomagnifies in the food chain, making it detrimental to ecosystem health.

Microorganisms play a crucial role in redox transformations of selenium in the environment, which influence its solubility and bioavailability. Identifying organisms that are capable of mediating these transformations is important for understanding selenium cycling and isolating organisms that could be used in bioremediation. Currently, there are no known genetic markers to identify microorganisms capable of aerobic Se(IV) or Se(VI) reduction, therefore culture-based methods are essential for identifying novel organisms that may be useful in bioremediation settings.

Bacteria and fungi were isolated from selenium-contaminated surface soils from two reclaimed phosphate mines in Southeastern Idaho. Organisms were isolated using three different media types amended with either 100  $\mu$ M Se(IV) [as Na<sub>2</sub>SeO<sub>3</sub>] or Se(VI) [as Na<sub>2</sub>SeO<sub>4</sub>]. To identify the isolates, the 16S ribosomal RNA (rRNA) gene region from bacteria and the internal transcribed spacer (ITS) region from fungi were amplified and DNA was sequenced using Sanger sequencing. Contigs were assembled using Benchling, and neighbor joining trees were made using ARB to determine the phylogenetic relationship of the isolates among well-characterized and closely related microorganisms. Isolates were also tested for their ability to reduce Se(IV) or Se(VI) to Se(0). Of 50 bacterial isolates, 22 unique strains were identified and phylogenetically characterized. Out of 10 fungal isolates, 4 were identified to genus level. The most abundant bacterial genus was *Pseudomonas* with 9 unique strains. Seventeen out of the 22 bacterial isolates could reduce Se(IV), though no isolates were able to reduce Se(VI). No fungal isolates were observed to reduce either Se(IV) or Se(VI).

# Metabolic Heterogeneity in Clonal Microbial Populations

H.L.O MCCLELLAND<sup>1</sup>, A.S. BRADLEY<sup>1</sup>, D. A. FIKE<sup>1</sup>,  
& C. JONES<sup>1</sup>

<sup>1</sup>Department of Earth and Planetary Sciences, Washington University in St. Louis, St. Louis, MO, USA.

Diauxic growth is a widely observed phenomenon where, under certain conditions, heterotrophic microbial populations undergo separate phases of exponential growth when provided with multiple substrates. Though first described in the 1940s, the phenotypic response at the level of individual cells remains unclear [1].

In the traditional interpretation, all cells within a population make a synchronous shift in substrate specialisation via the regulation of pathway-specific enzymes [1]. However, in recent years a number of competing hypotheses have emerged to describe behaviour at the single-cell level that may manifest as diauxic growth at the population level [2]. These include metabolic heterogeneity, where distinct subpopulations specialize early and deplete substrates at different rates [3], and “bet hedging” where a small subset of the population maintains the ability to metabolize alternative substrates when required [4].

Using two bacterial species (*E. coli* and *M. extorquens*) as model organisms, we employ secondary ion mass spectrometry (SIMS) to analyse individual cells grown on a mixture of isotopically labelled substrates, enabling a direct measurement of substrate specialisation and growth rate. We are able to detect metabolic phenotype of single-cells throughout the period of growth, and in each scenario, resolve the nature of the diauxic shift.

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# Sedimentary lipids as a paleoclimate proxy in Greenland

McFARLIN, JM<sup>\*</sup>, OSBURN, M<sup>2</sup>, AXFORD, Y<sup>3</sup>, LASHER, GE<sup>4</sup>

<sup>1</sup>Department of Earth and Planetary Sciences, Northwestern University, Evanston, USA,  
[jamie@earth.northwestern.edu](mailto:jamie@earth.northwestern.edu) (\*presenting author)

<sup>2</sup>Department of Earth and Planetary Sciences, Northwestern University, Evanston, USA,  
[maggie@earth.northwestern.edu](mailto:maggie@earth.northwestern.edu)

<sup>3</sup>Department of Earth and Planetary Sciences, Northwestern University, Evanston, USA,  
[axford@northwestern.edu](mailto:axford@northwestern.edu)

<sup>4</sup>Department of Earth and Planetary Sciences, Northwestern University, Evanston, USA,  
[everett@earth.northwestern.edu](mailto:everett@earth.northwestern.edu)

The hydrogen isotope ratios of sedimentary lipids in lacustrine archives are a valuable tool for reconstructing paleohydrology<sup>1</sup>. Compounds with identifiable sources allow for differentiation between aquatic and terrestrial plant lipids<sup>2,3</sup>, which offer insight into integrated (lake) water balance vs. growth-season precipitation. The hydrogen isotope ratio,  $\delta^2\text{H}$ , of precipitation varies primarily with temperature and moisture source<sup>4,5</sup>, with substantial seasonal differences observed in the Arctic<sup>6</sup>. Documenting changes in precipitation from sedimentary archives is particularly significant in Greenland where comparisons of past warm periods to the present are needed to better constrain the impacts of ongoing warming on the hydrologic cycle<sup>7</sup>. However, few paleoclimate studies of Arctic Holocene records include hydrologic reconstruction<sup>8</sup> largely due to limitations in available proxies. The emerging use of plant lipid  $\delta^2\text{H}$  as a proxy for paleo lake-water and paleoprecipitation in the Arctic is promising. However, further calibration and application of this proxy is needed, in part because plants at high latitudes endure extremes in growth-season daylight and climate<sup>9,10</sup>.

Here we present ongoing work to calibrate and apply sedimentary lipid isotope proxies in Greenland. We discuss the application of hydrogen isotope ratios in sedimentary short and long-chain *n*-alkanes in a down-core record ~2 km from the present margin of the Greenland Ice Sheet. This archive includes both the Holocene and the Last Interglacial and provides direct comparison of two warm periods. We compare plant lipid  $\delta^2\text{H}$  to other proxy work from the same core, including independent proxies for summer temperature and lake water oxygen isotope ratios. This synthesis allows us to place archival lake water isotopes onto local evaporation lines and the global meteoric water line. We also discuss our ongoing efforts to document  $\delta^2\text{H}$  of sedimentary lipids along a latitudinal transect on the western coast of Greenland, that spans from low to high Arctic climate zones. We investigate the chain-length profile and  $\delta^2\text{H}$  of sedimentary lipids from a suite of ~20 lakes, including proglacial and non-glacial lakes. Together, these studies will inform the modern relationship between  $\delta^2\text{H}$  of sedimentary lipids and their source waters in Greenland as well as the application of this proxy in conjunction with other climate proxies on lacustrine archives.

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## ***DNA Sequencing of Visually Layered Microbial Mats: Relationships between Microbes and Minerals***

Tori Moore<sup>1</sup>, Patrick Cavanagh<sup>2</sup>, Mingxu Fang<sup>3</sup>, Lisa Pratt<sup>4</sup>, Lennon Lab<sup>5</sup>

<sup>1</sup>Department of Earth and Atmospheric Sciences, Indiana University, Bloomington, USA, tormoore@indiana.edu

<sup>2</sup>Department of Earth and Atmospheric Sciences, Indiana University, Bloomington, USA, pdcavana@uemail.iu.edu

<sup>3</sup>Department of Molecular and Cellular Biochemistry, Indiana University, Bloomington, USA, fangm@uemail.iu.edu

<sup>4</sup>Associate Executive Dean of College of Arts and Sciences, Provost Professor of Department of Earth and Atmospheric Sciences, Indiana University, Bloomington, USA, prattl@indiana.edu

<sup>5</sup>Department of Biology, Indiana University, Bloomington, USA, lennonj@indiana.edu

Greenland is an accessible analogue environment for Mars based on extended periods of subfreezing temperature, periods, arid to semiarid climate, and thick perennial permafrost. However, Greenland and Mars are not equivalent from the perspective of Earth's oxygen-rich atmospheric and much higher atmospheric pressure (1000 compared to 10 millibars). After six years of field work in western Greenland, Professor Lisa Pratt detected a terrestrial, algal/bacterial mat that is visually layered with distinguishable patchy crusts of orange and yellow minerals near the margin of a small acidic pond. Closer observation revealed a vertically layered structure of brightly colored mineral deposits similar to known algal/bacterial mats from less extreme environments. We are testing to confirm that samples from visually layered mineralized crusts will yield distinctive communities of microbes in relation to mineralogical organization using the method of DNA extraction and 16s rRNA gene amplicon sequencing.

Our goal is to verify the presence of diverse microbes in interdependent layered communities and determine the identity of aforementioned microbes. If the present microbes are indeed interdependent communities and are spatially associated with the visually layered structures of orange and yellow minerals, then we will pursue further investigation into the metabolic activity of each layered community in relationship to the minerals that are precipitated in order to establish a depth profile. One mineral already identified is jarosite which is an iron-sulfate mineral already identified at several locations on Mars but rarely found on Earth except at sites of acid mine drainage.

Studying microbes in a Mars-analog environment will provide the knowledge and experience for scientists to assist with planning of future missions aimed at the search for evidence of past or present life on Mars. In addition, the Greenland microbiology study serves as a prime opportunity to monitor climate change over the next several decades, monitor the effects of predicted climate warming on the change in organisms, and monitor the structure of microbial communities in a periglacial environment.

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<sup>1</sup>Department of Earth and Atmospheric Sciences, Indiana University, Bloomington, USA, tormoore@indiana.edu

<sup>2</sup>Department of Earth and Atmospheric Sciences, Indiana University, Bloomington, USA, pdcavana@uemail.iu.edu

<sup>3</sup>Department of Molecular and Cellular Biochemistry, Indiana University, Bloomington, USA, fangm@uemail.iu.edu

<sup>4</sup>Associate Executive Dean of College of Arts and Sciences, Provost Professor of Department of Earth and Atmospheric Sciences, Indiana University, Bloomington, USA, prattl@indiana.edu

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# Interactions Between Colonizing Microbes and Biocides in Hydraulically Fractured Wells

David Morgan<sup>1</sup>, Joseph Moore<sup>2</sup>, Anne Booker<sup>1</sup>, Ken Wunch<sup>2</sup>, Michael Wilkins<sup>1</sup>

<sup>1</sup>Department of Microbiology, Ohio State University, Columbus, OH, USA

<sup>2</sup>Dow Microbial Control, Collegeville, PA, USA

In the past decade, hydraulic fracturing has become an increasingly popular form of energy extraction in the United States, responsible for roughly two-thirds of U.S. natural gas production. Although 300,000 wells have been drilled in the U.S., relatively little is known about the microorganisms that persist in these environments and the chemical reactions they catalyze. Despite efforts to reduce biological activity, microbes can often thrive in these hydrocarbon-rich, hypersaline (up to 150 g/L TDS) environments that reach pressures of ~345 bar. Members of the genus *Halanaerobium* are cosmopolitan, often constituting the majority of the microbial population across geographically distinct shales. *Halanaerobium* possesses the ability to produce corrosive sulfide, and forms potentially yield-reducing biofilms by clogging newly-induced fractures that are essential for hydrocarbon recovery. Biocides for microbial control are traditionally applied to hydraulic fracturing fluids on the surface, while only some persist down-well for longer-lasting activity. We tested the efficacy of multiple biocides on *Halanaerobium* growth under varying conditions representative of the subsurface, including high pressures (345 bar). Our results indicated that biomass is often inhibited (a halt in growth) or decreased (due to cell lysis) when amended with biocides at standard use concentrations. Cells cultivated under high pressure conditions were neither more resistant nor more susceptible to biocide activity. Most of the tested biocides were effective at cell concentrations much higher than those present in hydraulically fractured wells. Previous studies have observed cell counts of  $6 \times 10^4$  cfu/mL in injected fluids at the time of injection. In our study we tested cell concentrations of around  $7 \times 10^7$  cfu/mL, indicating that these biocides were effective at bacterial concentrations over 1,000 times higher than those observed in wells. In summary, the majority of biocides were effective against this representative *Halanaerobium* strain, suggesting that dominant microbial populations can be controlled through the targeted application of specific chemistries. Future studies will assess impacts of additional reactive species (e.g., sulfur compounds) on biocide efficiency in flowback fluids and produced water from hydraulically fractured systems.

# The role of “Oxic Fe(III) reduction” in the Archean iron cycle

ELIZABETH D. SWANNER<sup>1\*</sup>

<sup>1</sup>Department of Geological and Atmospheric Sciences, Iowa State University, Ames, IA, USA,  
eswanner@iastate.edu (\*presenting author)

It is increasingly recognized that anoxic and iron-rich conditions (i.e. ferruginous) predominated in the deep oceans throughout much of the Precambrian. Late Archean carbonates from the ca. 2.5 Gy old Campbellrand-Malmani carbonate platform in South Africa contain iron, whose isotopic composition reflects oxidation of Fe(II) from an upwelling deep water mass<sup>1</sup>. Synchrotron-based X-ray absorption spectroscopy document primary incorporation of seawater Fe<sup>2+</sup> into carbonate (Fig. 1), consistent with the isotopic results. Numerous studies have previously characterized this setting as an oxygen oasis, existing before the widespread oxygenation of the atmosphere at ca. 2.3 Gy ago. One question arising from these observations is: how do we reconcile evidence for Fe<sup>2+</sup>-containing seawater in a late Archean oxygen oasis?

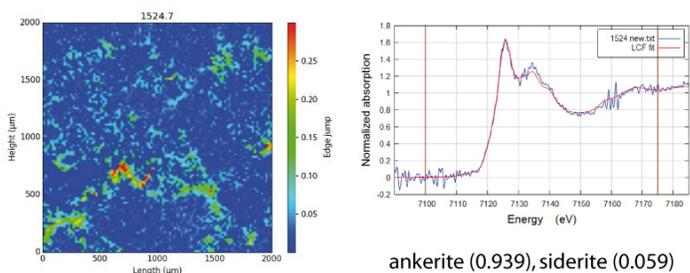


Fig. 1. Pure carbonates from the Campbellrand-Malmani platform contain ankerite, which records the presence of Fe<sup>2+</sup> in an oxygen oasis.

and is even enhanced within the photic zone, consistent with a steady-state production of Fe<sup>2+</sup>. Further experiments suggest this “oxic Fe(III) reduction” resulted from light-independent enzymatic reduction of Fe(III) at the cell surface, common among cyanobacteria.

The persistence of Fe<sup>2+</sup> in an oxygen oasis prompts re-evaluation of shallow water iron cycling at the interface of deep ferruginous oceans oxygenated surface oceans, as well as the application of redox proxies contingent upon the absence of Fe<sup>2+</sup> in oxygenated waters.

A laboratory-scale column<sup>2</sup> has been designed to simulate in 1-D a shallow-water carbonate platform exposed to upwelling ferruginous water (Fig. 2). Light at the top of the column allows growth of the cyanobacterium *Synechococcus* PCC 7002, creating an oxygen oasis. Despite the presence of O<sub>2</sub> in the water column, Fe<sup>2+</sup> persists

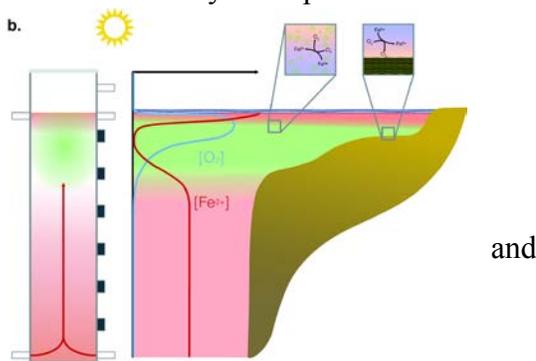


Fig. 2. The “column” simulates in 1-D the upwelling of ferruginous waters (pink) into oxic surface waters. “Oxic Fe(III) reduction” by cyanobacteria regenerates Fe<sup>2+</sup> following oxidation.

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# Lake Superior Sediments Record Anthropogenic Nitrogen Deposition

MOLLY D. O'BEIRNE<sup>1,2\*</sup>, JOSEF P. WERNE<sup>1,2</sup>, ROBERT E. HECKY<sup>1,3</sup>, THOMAS C. JOHNSON<sup>1,4</sup>,  
SERGEI KATSEV<sup>1,5</sup>, EUAN D. REAVIE<sup>6</sup>

<sup>1</sup>Large Lakes Observatory (LLO), University of Minnesota Duluth (UMD), Duluth, MN 55812, USA

<sup>2</sup>Department of Geology and Environmental Science, University of Pittsburgh, Pittsburgh, PA, 15260, USA,  
mdobeirne@pitt.edu, jwerne@pitt.edu (\*presenting author; current address)

<sup>3</sup>Department of Biology, UMD, Duluth, MN 55812, USA, rehecky@gmail.com (Professor Emeritus)

<sup>4</sup>Department of Earth and Environmental Sciences, UMD, Duluth, MN 55812, USA, tcj@d.umn.edu (Regents  
Professor Emeritus)

<sup>5</sup>Department of Physics, UMD, Duluth, MN 55812, USA, skatsev@d.umn.edu

<sup>6</sup>Center for Water and the Environment, Natural Resources Research Institute, UMD, 55811 USA,  
ereavie@d.umn.edu

Reactive nitrogen (Nr) is an important component of the nitrogen cycle. Since 1800AD, humans have more than doubled the amount of Nr within the biosphere through activities such as agriculture (e.g. Haber-Bosch process) and industrialization (e.g. burning of fossil fuels). Organic nitrogen ( $N_{\text{org}}$ ) stable isotope ratios ( $^{15}\text{N}/^{14}\text{N}$ ) in eight sediment multicores, taken from across Lake Superior's basin, encompassing the last 200 years show a signal of an isotopically distinct ( $^{15}\text{N}$ -depleted) source of nitrogen to the lake when compared to the last 9,000 years. The timing of  $^{15}\text{N}$ -depletion in Lake Superior sediments coincides with increases in agriculture (fertilizer production) and industrialization ( $\text{CO}_2$  emissions) processes, as well as the timing of  $^{15}\text{N}$ -depletion observed in remote northern hemisphere lake sediments and the  $^{15}\text{N}$ -depletion of nitrate in the Greenland Summit ice core. Our observations show not only the influence of humans on one of Earth's largest, most pristine, freshwater ecosystems, but also extend the global impacts of human activities on the nitrogen cycle.

# Genomic Insights into a Freshwater Ammonia Oxidizing Archaeon

JUSTIN PODOWSKI<sup>1\*</sup>, SARA PAVER<sup>2</sup>, MAUREEN COLEMAN<sup>3</sup>

<sup>1</sup>Department of Geophysical Sciences, University of Chicago, Chicago, USA, jpodowski@uchicago.edu  
(\*presenting author)

<sup>2</sup>Department Of Geophysical Sciences, University of Chicago, Chicago, USA, spaver@uchicago.edu

<sup>3</sup>Department of Geophysical Sciences, University of Chicago, Chicago, USA, mlcoleman@uchicago.edu

Lake Superior represents one of the least productive aquatic environments on Earth, with primary production rates near or lower than the North Atlantic gyre (Sterner 2010) and one of the lowest concentrations of phosphorus among freshwater lakes (Hecky 2000). Lake Superior's nitrogen cycle presents a challenge to limnologists, as nitrate concentration in Lake Superior has been increasing since it was first reliably measured, increasing five-fold since 1906 (Sterner 2007). Isotopic evidence suggests that a major source of this increased nitrate is in-lake nitrification (Finlay 2007) rather than allochthonous inputs. Hence understanding the dynamics of microbial nitrogen cycling, and characterizing the taxa performing nitrification, is paramount to understanding the unusual biogeochemistry of Lake Superior

Prior to the use of environmental DNA sequencing as a means to understand microbial communities, bacteria were thought to dominate the first step of nitrification, the conversion of ammonia to nitrite. The first shotgun sequencing project of the Sargasso Sea (Venter 2004) suggested that ammonia monooxygenase, which catalyzes the conversion of ammonia to nitrite, was present in archaea. Subsequent isolation of an ammonia oxidizing archaeon from an aquarium (Könneke 2005) and evidence that archaeal, not bacterial, abundance in the ocean correlated with higher rates of ammonia oxidation (Wuchter 2006) turned this convention on its head. Further DNA sequencing studies of aquatic (Biller 2012) and soil environments (Zhang 2012) implicated ammonia oxidizing archaea of the phylum Thaumarchaeota as the largest contributors of ammonia oxidization, especially in oligotrophic environments (Hatzenpichler 2012).

Since the initial isolation of an ammonia oxidizing archaea in 2005, isolation and subsequent genome sequencing have found diverse representatives from the open ocean (Santoro 2015), soil (Tourna et al. 2011) and hot springs (De La Torre et al 2008). Most efforts have focused on marine ammonia oxidizing archaea, and thus far study on freshwater ammonia oxidizing archaea has been limited to marker gene studies of abundance. Here, we present the first genome of a freshwater ammonia oxidizing archaeon by way of a metagenome assembled genome (MAG), assembled from whole community DNA from Lake Superior. This genome, putatively in the genus *Nitrosoarchaeum*, is 98% complete according to CheckM (Parks 2015) but merely 1.2 Mb, among the smallest free living microbes. Its genome contains signatures consistent with adaptation to oligotrophic conditions like a reduced number of transcription factor binding proteins and a higher number of transporters per megabase than any other ammonia oxidizing archaeon. Additional genomic evidence suggests the possibility of urea utilization, a potential unforeseen contribution to nitrate levels in Lake Superior. This genome represents an abundant member (up to 9%) of Lake Superior's microbial community and a key contributor to nitrogen cycling.

# Early Earth's Oxygenic and Anoxygenic Producers

RACHEL RATLIFF<sup>1</sup>, ANTHONY WEINKE<sup>2</sup>, BOPI BIDDANDA<sup>3</sup>

<sup>1</sup>Annis Water Resources Institute, Grand Valley State University, Muskegon, USA, [ratliff@mail.gvsu.edu](mailto:ratliff@mail.gvsu.edu)

<sup>2</sup>Annis Water Resources Institute, Grand Valley State University, Muskegon, USA, [weinkean@gvsu.edu](mailto:weinkean@gvsu.edu)

<sup>3</sup>Annis Water Resources Institute, Grand Valley State University, Muskegon, USA, [biddandb@gvsu.edu](mailto:biddandb@gvsu.edu)

Long before humans walked the Earth's surface, the atmosphere was nearly void of oxygen. It has been hypothesized that cyanobacteria evolving in the Precambrian were the first to contribute significant amounts of oxygen to Earth's oceans and atmosphere.

Off the shore of Alpena, Michigan's Thunder Bay is Middle Island Sinkhole. Here, karst hydrogeology has created a unique system where low-oxygen and high sulfur groundwater vents into a basin 23 meters below the surface of Lake Huron. Filamentous cyanobacteria, chemosynthetic bacteria/archaea, diatoms, and micro-animals weave together to create mats along the sediment surface. Dissolved oxygen concentration fluctuates both daily and seasonally. Primary production is achieved with a combination of chemosynthetic sulphide oxidation, anoxygenic photosynthesis, and oxygenic photosynthesis. We envision that these mat communities could provide a rare peek into Earth's early pre-oxygenated history- closer to the Great Oxidation Event.

Our poster features a profile of each major microorganism group found in Middle Island Sinkhole's mat environment. Intimately knowing the key organisms interacting within the community will help us ask more specific questions about how oxygen and organic matter is produced in this ecosystem. Thus, we can continue to chip away at the mystery of Earth's early biogeochemical history and how life evolved on land.

# New Data from the Viki Drillcore, Estonia, Provide Insights into the Veracity of $\delta^{34}\text{S}_{\text{CAS}}$ as a Proxy for Ancient Seawater Sulfate

JOCELYN A. RICHARDSON<sup>1\*</sup> & DAVID. A. FIKE<sup>1</sup>

<sup>1</sup>Department of Earth and Planetary Sciences, Washington University in St. Louis, St. Louis, USA,  
j.richardson@wustl.edu (\*presenting author)

The early Silurian is characterised by rapid environmental change, including numerous global sea level changes and biotic turnover events. The Llandovery-Wenlock carbonate-marl succession from the Viki drill core, Saaremaa Island, Estonia were analysed for carbonate carbon ( $\delta^{13}\text{C}_{\text{carb}}$ ), organic carbon ( $\delta^{13}\text{C}_{\text{org}}$ ), carbonate-associated sulfate ( $\delta^{34}\text{S}_{\text{CAS}}$ ) and pyrite ( $\delta^{34}\text{S}_{\text{pyr}}$ ) isotopes, as well as trace element concentrations. This is the highest resolution sulfur isotope dataset for the early Silurian that we know of. Carbon isotopes record the end-Ordovician, Hirnantian glaciation and global isotope excursion decline, as well as two early Llandovery carbon isotope maxima (2‰). The most prominent feature is the large magnitude (~ 4.5‰)  $\delta^{13}\text{C}_{\text{carb}}$  excursion, that is associated with a shallowing and biotic turnover known as the Ireviken Event. Through the early Llandovery,  $\delta^{34}\text{S}_{\text{CAS}}$  gradually decreases from 35 to 25‰ before increasing back to 35‰ throughout the latter part of the Llandovery. Two small magnitude (5 to 10‰), transient depletions in  $\delta^{34}\text{S}_{\text{CAS}}$  are observed at the end of the Llandovery and within the Ireviken event. However,  $\delta^{34}\text{S}_{\text{pyr}}$  values do not follow these trends; such behaviour would be anticipated during global changes in pyrite burial or  $\delta^{34}\text{S}_{\text{in}}$ . Instead, during the initial drawdown in  $\delta^{34}\text{S}_{\text{CAS}}$ ,  $\delta^{34}\text{S}_{\text{pyr}}$  remain relatively invariant and during the two depletions in  $\delta^{34}\text{S}_{\text{CAS}}$ ,  $\delta^{34}\text{S}_{\text{pyr}}$  shows large magnitude, rapid, excursions of up to ~ 45‰.

Often,  $\delta^{34}\text{S}_{\text{CAS}}$  datasets from ancient carbonates show high magnitude, high frequency, variability that cannot be readily explained by global changes to the marine sulfate reservoir (based on the residence time of marine sulfate). Such variability is muted in the dataset presented here, especially with respect to other sections of a similar age. Despite broad recrystallization and dolomitization, concentrations of Fe, Sr, Mn and Mg, as well as isotope cross-plots, indicate no large-scale alteration of proxies, particularly from meteoric fluids, within the predominantly carbonate lithofacies. Increased alteration is indicated where carbonate is inter-bedded with marlstones. The antithetical isotope excursions within this section, combined with the comparison to a similar dataset from a coeval section within the same depositional basin (Gotland, Sweden), call into question the reliability of using the proxies to infer changes to the global sulfur cycle (such as changes in pyrite burial) without first considering how such signals may be variable impacted by local processes within a basin.

# **Metal bioremediation potential of microbial Mn oxidation in the Soudan Underground Iron Mine, MN**

ELIZABETH ROEPKE<sup>1\*</sup>, ELIZABETH CARLSON<sup>2</sup>, CARA SANTELLI<sup>3</sup>

<sup>1</sup>Department of Earth Sciences, University of Minnesota – Twin Cities, Minneapolis, USA, roep0043@umn.edu  
(\*presenting author)

<sup>2</sup>College of Food, Agricultural and Natural Resource Sciences, University of Minnesota – Twin Cities, Minneapolis, USA, carl4732@umn.edu

<sup>3</sup>Department of Earth Sciences; BioTechnology Institute, University of Minnesota – Twin Cities, Minneapolis, USA, santelli@umn.edu

Water flowing through the Soudan Underground Iron Mine in Northern Minnesota contains high concentrations of many metals and salts. Since mining operations ceased in 1962, briny groundwater continues to flow through the mine drifts, boreholes, and surrounding fractured bedrock, moving water and metals around the ~2400' deep mine. This water is pumped out of the mine in order to keep it dry, but treatments are necessary to decrease metal concentrations to regulation levels before being released into the surrounding area. Treatment methods including standard and peat-based ion exchange systems, chemical applications, and constructed wetlands have all been considered for the discharged mine water, but all are expensive and may not guarantee effective long-term metal removal.

Innovative metal removal strategies may be more efficient and less expensive than previously proposed methods. One possibility is through sorption of metal cations onto manganese oxide minerals (Mn oxides). Mn oxides have a high sorption capacity for metal cations including copper (Cu), cobalt (Co), nickel (Ni), and zinc (Zn), which are all abundant in the Soudan Mine. Mn oxides do not form abiotically under ambient mine conditions, but bacteria and fungi that form Mn oxides can be found throughout the mine. These microorganisms are capable of living in briny, metal-rich waters, and are good candidates for a Mn oxide bioremediation strategy to remove Cu, Co, Ni, and Zn from the mine water discharge. Before a bioremediation strategy can be proposed, more must be understood about Mn oxides formed by microorganisms, such as their stability under changing oxidation/reduction potentials, pH, crystal structure in the short- and long-term, and the effect of these properties on metal cation sorption capacity.

Multiple strains of Mn-oxidizing bacteria and fungi have been isolated from the inside the mine. Preliminary experiments will evaluate metal removal efficiency via Mn oxide formation. Future work will explore Mn oxide stability under changing conditions, and synchrotron-based investigations of microbial Mn oxide structure and metal sorption mechanisms. These results can inform bioremediation strategies based on microbial Mn oxidation in the Soudan Mine and other waste streams with high metal concentrations.

# Insight into Biogenic Methane and Sulfur Cycling Within Serpentinite-hosted Groundwater

M. SABUDA<sup>1\*</sup>, T. HOEHLER<sup>2</sup>, M. KUBO<sup>3</sup>, L. PUTMAN<sup>4</sup>, D. CARDACE<sup>5</sup>, M. SCHRENK<sup>4</sup>

<sup>1</sup>Department of Earth Sciences, University of Minnesota, Minneapolis, MN, USA, sabud001@umn.edu (\*presenting author)

<sup>2</sup>Exobiology Branch, NASA Ames Research Center, Moffett Field, CA, USA, tori.m.hoehler@nasa.gov

<sup>3</sup>SETI Institute, Mountain View, CA, USA, michael.d.kubo@nasa.gov

<sup>4</sup>Department of Earth and Environmental Sciences, Michigan State University, East Lansing, MI, USA, will3129@msu.edu; schrenkm@msu.edu

<sup>5</sup>Department of Geosciences, University of Rhode Island, Kingston, RI, USA, cardace@uri.edu

Serpentinization is the hydration of ultramafic rock, which can occur in ancient ocean crust emplaced along continental margins in the form of an ophiolite. During this process, secondary reactions can generate methane, which allows for its widespread occurrence within the serpentinite subsurface. Essential information about carbon cycling, redox reactions, and microbial activity in this system can be revealed by understanding the origins and fate of methane. To this end, a methane oxidation microcosm experiment was assembled using pH 12 serpentinite-hosted fluids extracted from a monitoring well, CSW1.1, at the Coast Range Ophiolite Microbial Observatory, CA. Water was extracted directly into 18 sterile, N<sub>2</sub>-flushed, stoppered bottles from the top of the well using a peristaltic pump, when dissolved oxygen levels were 15% of oxygen concentration in the atmosphere (the “oxic” set). A pre-installed bladder pump at the bottom of the well pumped fluid into another 18 bottles, where oxygen concentrations were equal to 3% of atmospheric oxygen (the “anoxic” set). In the field, the bottles were filled to the top with zero gas headspace. In the lab, all microcosms were inoculated with 50 cc of 20%<sup>13</sup>CH<sub>4</sub>: 80%<sup>12</sup>CH<sub>4</sub> gas, with parallel experiments run following the addition of iron-oxyhydroxide or thiosulfate respectively. Bottles from the top of the well received 2mL oxygen gas at each sampling point to maintain their “oxic” status. The evolution of <sup>13</sup>CO<sub>2</sub> -- the presumed product of methane oxidation -- was quantified using isotope ratio mass spectrometry (IRMS). Cell abundances were quantified throughout the 190-day experiment, and microbial community composition was determined through 16S rRNA gene sequencing and analysis. Evidence for methane cycling is apparent in both the thiosulfate + <sup>13</sup>CH<sub>4</sub> and <sup>13</sup>CH<sub>4</sub>-only inoculated experiments, while iron-amended bottles reveal minimal results in terms of biogenic <sup>13</sup>DIC concentrations. Thiosulfate- amended cultures show not only the highest cell growth, but the highest measured biological <sup>13</sup>DIC production of all treatments. While neither an organism capable of facilitating this entire reaction nor an ANME organism have been isolated and characterized, it is apparent sulfate-reducing and thiosulfate-utilizing bacteria are abundant in the CSW1.1 experiment. Truepera and Comamonadaceae are two groups that are ubiquitous throughout these cultures, and while Comamonadaceae are known to be endemic to serpentine systems, Truepera require further investigation to understand if their role in this system is purely survival, or if they are active contributors to the biologic <sup>13</sup>DIC quantities observed. This study reveals the potentially vital role of coupled methane and sulfur cycling in guiding community diversity within this serpentinite-hosted environment.

# Evidence of oxygen production in the early Neoproterozoic in the São Francisco craton, Brazil

ALICE BOSCO-SANTOS<sup>1,2\*</sup>, WILLIAM P. GILHOOLY III<sup>1</sup>, FOTIOS FOUSKAS<sup>1</sup>, MAURICIO RIGONI BALDIM<sup>2</sup>, ELSON PAIVA OLIVEIRA<sup>2</sup>

<sup>1</sup>Department of Earth Sciences, Indiana University-Purdue University Indianapolis, Indianapolis, USA, alicebosco@gmail.com (\*presenting author)

<sup>2</sup>Department of Geology and Natural Resources, University of Campinas, Campinas, Brazil

Oxygen generation prior to the Great Oxidation Event (GOE ~2.45 Ga) has been described as localized and transient events with important consequences for the onset of certain biological pathways, as sulfate reduction, sulphur disproportionation and oxygenic photosynthesis. The present study, provides one of the oldest evidence of the presence of oxygen in the atmosphere still associated with sulphur mass-independent fractionation (S-MIF) in a very well constrain age sequence of carbonaceous phyllites from Pitangui greenstone belt (maximum depositional age of 2.7 Ga), southern São Francisco craton, Brazil. The observed systematic negative anomaly of Ce ( $0.5 \pm 0.1$ ) throughout the entire length of the drillcore (1,809 m) combined with flat or slight enrichment in heavy rare earth elements (HREE) patterns indicate the presence of oxygen in the environment. Iron speciation also pointed to the presence of oxygen and possible development of euxinic conditions. The ratio between Fe in the pyrite and the reactive iron fractions ( $Fe_{py}/Fe_{hr}$ ) are higher than 0.6 as a consequence of 60% to 80% of the total iron being associated with pyrite burial along the sequence.

A modest but nearly uniform S-MIF ( $2.4\text{‰} < \Delta^{33}\text{S} < 4.4\text{‰}$ ) combined with low variability in  $\delta^{34}\text{S}$  (between 8.7‰ and 6.1‰) of pyrite from the lower 1570 m of core could reflect conditions with low sulfate, limiting microbial sulphur reduction. These results support a long-term stability of seawater sulfate abundance. However within the upper 240 m of core, the S-MIF and  $\delta^{34}\text{S}$  values systematically decrease up-section. This trend can be interpreted as a intensification of oxidative weathering with time, that resulted in  $\Delta^{33}\text{S}$  values closer to zero (between 1.4‰ and 1.0‰) in the top of the core. Concurrent with this trend, the sulphur isotope values of bulk organic sulphur become systematically  $^{34}\text{S}$ -enriched by ~10 permil, consistent with an increasing seawater sulfate reservoir. The carbon isotope excursion at the top of the core ( $\delta^{13}\text{C} \sim -24\text{‰}$ ) further suggests higher rates of carbon burial at the top of the core. Our data, from a Brazilian Archean terrane, indicate the presence of oxygen and the transition to a biologically mediated sulphur cycle at least 300 Ma before the GOE

# **Just Around the Riverbend: Seasonal hydrologic controls on dynamic hyporheic zone redox biogeochemistry**

CASEY M. SAUP<sup>1\*</sup>, AUDREY H. SAWYER<sup>2</sup>, KENNETH H. WILLIAMS<sup>3</sup>, MICHAEL J. WILKINS<sup>4,5</sup>

<sup>1</sup>School of Earth Sciences, The Ohio State University, Columbus, USA, saup.4@osu.edu (\*presenting author)

<sup>2</sup>School of Earth Sciences, The Ohio State University, Columbus, USA, sawyer.143@osu.edu

<sup>3</sup>Climate and Ecosystem Sciences Division, Lawrence Berkeley National Lab, Berkeley, USA, khwilliams@lbl.gov

<sup>4</sup>School of Earth Sciences, The Ohio State University, Columbus, USA, wilkins.231@osu.edu

<sup>5</sup>Department of Microbiology, The Ohio State University, Columbus, USA, wilkins.231@osu.edu

Upland rivers host exceptionally strong linkages between the terrestrial and aquatic elemental cycles. The weathering of mineral phases, coupled with degradation of organic matter and anthropogenic influences can result in the export of carbon, metals, and nutrients in upland fluvial systems, often decreasing downstream water quality with negative impacts on both human usage and ecosystem functioning. Within these fluvial networks, zones of hyporheic mixing—regions within the riverbed where surface water and groundwater mix—are thought to represent hotspots of biogeochemical activity, thus exerting significant control over elemental cycling and solute export. To investigate how the deeper exchange of oxic river water into the riverbed during snowmelt-driven peak discharge affects microbial degradation (oxidation) of carbon pools, depth resolved pore water samples were recovered from multiple locations around a representative meander on the East River near Crested Butte, CO. At each location, a series of temperature and redox probes were installed in the riverbed to track the extent of hyporheic mixing and the impact of this process on riverbed biogeochemistry. We complemented this real-time data with discrete samples collected during peak flow, intermediate flow, and base flow at a 10 cm resolution over 70 cm vertical profiles for a suite of microbiological and geochemical analyses. Results revealed elevated pore fluid concentrations of dissolved metals and recalcitrant DOC species under reducing conditions induced by base flow, while regions that were more influenced by down-welling oxic surface water hosted distinct microbial communities and lower metal concentrations. Overall, our results indicate that mixing-driven vertical redox gradients exert a strong control on biogeochemical processing in riverbeds, with implications for downstream water quality and solute export from watersheds.

# Plant carbon isotope fractionation from the Late Cretaceous through the Eocene

KRISTEN M. SCHLANSER<sup>1\*</sup>, AARON F. DIEFENDORF<sup>2</sup>

<sup>1</sup>Department of Geology, University of Cincinnati, Cincinnati, USA, schlankn@mail.uc.edu (\*presenting author)

<sup>2</sup>Department of Geology, University of Cincinnati, Cincinnati, USA, diefenan@ucmail.uc.edu

Decades of research on plant carbon isotopes ( $\delta^{13}\text{C}$ ) have shown that, during photosynthesis, water availability, light intensity, photosynthetic pathway, growth strategy, and physiological differences among species are the primary controls on leaf carbon fractionation ( $\Delta_{\text{leaf}}$ ). Recent growth chamber studies have found  $\Delta_{\text{leaf}}$  in  $\text{C}_3$  plants is also sensitive to changes in  $p\text{CO}_2$ , suggesting  $\Delta_{\text{leaf}}$  could be used as a proxy for paleo  $p\text{CO}_2$ . However, field studies conducted on longer times scales (years to decades) and those that use the geologic record (millennia), indicate  $\Delta_{\text{leaf}}$  is not sensitive to changes in  $p\text{CO}_2$ . To test the response of  $\Delta_{\text{leaf}}$  to  $p\text{CO}_2$ , we utilize the geologic record as a natural laboratory on timescales in which plants evolve. We measured carbon isotope ( $\delta^{13}\text{C}$ ) values of  $n\text{-C}_{29}$  alkanes (leaf waxes) from localities across the western US and Canada that capture a range of  $p\text{CO}_2$  levels from  $\sim 300$  ppmV to over 800 ppmV during the Late Cretaceous through the Eocene. Sample age, atmospheric  $p\text{CO}_2$ , paleovegetation, mean annual temperature (MAT) estimates, and mean annual precipitation (MAP) estimates are reasonably constrained for these sites. Preliminary results find that Late Cretaceous, Paleocene, and Eocene  $\Delta_{\text{leaf}}$  values do not respond to changes in  $p\text{CO}_2$ . However, a correlation to paleoprecipitation is apparent, although minor given the uncertainties in plant-based paleoprecipitation estimates. It appears that on very short time scales, plants optimize leaf-gas exchange to increase assimilation and water use efficiency. However, on geologic time scales, plants seem to evolve with respect to  $p\text{CO}_2$ , possibly by modifying leaf gas exchange properties, indicating that  $\Delta_{\text{leaf}}$  may not be a reliable paleo proxy for  $p\text{CO}_2$ .

# Effects on Sulfur and Oxygen Fractionation in Sulfate Reducing Bacteria by the Perturbation of Sulfate Permease and Sulfate Adenylyl Transferase Expression

DEREK A. SMITH<sup>1\*</sup>, DAVID T. JOHNSTON<sup>2</sup>, ALEXANDER S. BRADLEY<sup>1,3</sup>

<sup>1</sup>Department of Earth and Planetary Sciences, Washington University in St. Louis, St. Louis, USA, derek.smith@wustl.edu (\*presenting author)

<sup>2</sup>Department of Earth and Planetary Sciences, Harvard University, Cambridge, USA, johnston@eps.harvard.edu

<sup>3</sup>Division of Biology and Biomedical Sciences, Washington University in St. Louis, St. Louis, USA, abradley@eps.wustl.edu

We examined the role of two key up-stream enzymes in microbial sulfate reduction (MSR), sulfate permease and sulfate adenylyl transferase (Sat) on the isotopic fractionations of  $^{18}\text{O}/^{16}\text{O}$  and  $^{34}\text{S}/^{32}\text{S}$  during sulfate reduction. MSR is critical for the oxidation of organic matter in modern and ancient oceans, and essential in regulating the redox state of the Earth's surface. The sulfur cycle represents one of the oldest biologically mediated chemical cycles on earth. The sulfur and oxygen isotopic composition of seawater sulfate and of sulfate minerals reflect the biogeochemical processes that cycle sulfur, of which MSR is among the most important. MSR is a multi-enzymatic reaction network that partitions the isotopes of sulfur and oxygen as a consequence of both the flux of sulfate through its enzymes and the fractionation imposed by each individual enzyme. The flux of these reactions may be partially dependent on the activity of these enzymes. Mutant strains of *Desulfovibrio vulgaris* str. Hildenborough (DvH) with perturbed sulfate permease or Sat expression were monitored throughout growth. Unexpectedly, the permease mutants produced sulfur isotopic fractionations that were indistinguishable from the wild type strain. However, the Sat mutants produced isotopic fractionations that differed from those produced by the wild type strain under identical conditions. These mutations altered Sat expression through deletion of the transcriptional repressor Rex or alteration of the Rex-binding site. The resulting isotopic phenotypes did not entirely correlate with growth rate and could not be fully explained by changes in sulfate reduction rate. We observed changes in the isotopic fractionations produced by the mutants that may have resulted from differences in the material flux through the sulfate reduction network. We intend on replicating these experiments at lower cell specific sulfate reduction rates. Collectively, this work illustrates the utility of genetic engineering in geobiological studies and the potential of this approach to target enzymatic controls, and address specific hypotheses about the effect that one enzyme can have in metabolic networks and biogeochemical cycles.

## New Methylococcaceae species, *<sup>U</sup>Methylobacter aquaesolum*, Possessing Denitrification Potential Dominates Freshwater Wetland Soils

GARRETT J. SMITH<sup>1\*</sup>, JORDAN C. ANGLE<sup>2</sup>, MIKAYLA A. BORTON<sup>3</sup>, KAY C. STEFANIK<sup>4</sup>, MICHAEL D. JOHNSTON<sup>5</sup>, TIMOTHY H. MORIN<sup>6</sup>, REBECCA A. DALY<sup>6</sup>, RICHARD WOLFE<sup>7</sup>, GIL BOHRER<sup>8</sup>, KELLY C. WRIGHTON<sup>9</sup>

<sup>1</sup>Department of Microbiology, The Ohio State University, Columbus, OH, USA, smith.10284@osu.edu (\*presenting author)

<sup>2</sup>Department of Microbiology, The Ohio State University, Columbus, OH, USA, angle.42@osu.edu

<sup>3</sup>Department of Microbiology, The Ohio State University, Columbus, OH, USA, borton.62@osu.edu

<sup>4</sup>School of Environment and Natural Resources, The Ohio State University, Columbus, OH, USA, stefanik.13@osu.edu

<sup>5</sup>School of Earth Science, The Ohio State University, Columbus, OH, USA, johnstonm101@gmail.com

<sup>6</sup>Department Civil and Environmental Engineering and Geodetic Sciences, Columbus, OH, USA, morin.37@osu.edu

<sup>7</sup>Department of Microbiology, The Ohio State University, Columbus, OH, USA, daly.130@osu.edu

<sup>8</sup>Department Civil and Environmental Engineering and Geodetic Sciences, Columbus, OH, USA, bohrer.17@osu.edu

<sup>9</sup>Department of Microbiology, The Ohio State University, Columbus, OH, USA, wrighton.1@osu.edu

Wetlands are the largest single source of atmospheric methane, but the distribution and activity of methanotrophs reducing methane emissions from these ecosystems is poorly resolved. To better understand methanotroph presence and metabolism in freshwater wetlands, we examined microbial communities and nutrient gradients in Northern Ohio freshwater wetland soils during multiple seasons and across three land coverage types (vegetated, mud-flat, channel). Here we've integrated geochemical profiling with 16S rRNA gene, metagenomic, and metatranscriptomic sequencing in surface (0-5 cm) and deep (20-30 cm) soils. When amended with oxygen and methane, oxic surface soils oxidized methane faster than anoxic deep soils – consuming 10  $\mu$ mol of methane per gram soil within 4 versus 8 days – showing that aerobic methane consumption can occur in both soil depths. Consistent with this finding, Methylococcales were the fifth most abundant microbial order across spatial and temporal gradients, composed primarily of uncultivated OTUs. Methanotroph genomes reconstructed from surface soil metagenomes at each land coverage type (~61-81% complete) represent a new species of *Methylobacter* (77-79% ANI and 73-77% AAI shared with published genomes), named *<sup>U</sup>Methylobacter aquaesolum* due to its abundance in freshwater saturated soils. *<sup>U</sup>M. aquaesolum* not only dominated soil communities, but were the most active methanotrophs as transcripts for their particulate methane monooxygenase were consistently in the top 2% most abundant genes in vegetated and mud-flat surface soil metatranscriptomes. In addition to coupling methane oxidation to aerobic respiration, *<sup>U</sup>M. aquaesolum* genomes also possessed genes for dissimilatory nitrate reduction to nitrous oxide. While nitrate reduction pathways were not expressed in oxic surface soils, utilizing nitrate as a terminal electron acceptor for energy generation may enable methane consumption in soils subject to low or fluctuating dissolved oxygen. Our research provides additional evidence for the importance of oxygen conservation in uncultivated Methylococcales, which are widely distributed active in methane emitting ecosystems throughout the northern hemisphere. These results suggest that obligate aerobic methanotrophs, like *<sup>U</sup>M. aquaesolum*, may be an underestimated methane sink and capable of nitrate removal in oxic to suboxic freshwater wetlands.

# Historical demography of a community of marine phages reveals ecological constraints on highly dynamic populations

SERGEI A. SOLONENKO<sup>1\*</sup>, ANN C. GREGORY<sup>2</sup>, MATTHEW B. SULLIVAN<sup>3</sup>, BRYAN C. CARSTENS<sup>4</sup>

<sup>1</sup>Evolution, Ecology and Organismal Biology Department, The Ohio State University, Columbus, USA, solonenko.1@osu.edu (\*presenting author)

<sup>2</sup>Microbiology Department, The Ohio State University, Columbus, USA, gregory.392@osu.edu

<sup>3</sup>Microbiology Department, The Ohio State University, Columbus, USA, sullivan.948@osu.edu

<sup>4</sup>Evolution, Ecology and Organismal Biology Department, The Ohio State University, Columbus, USA, carstens.12@osu.edu

Host-specific phage infection acts as a top-down control on the marine microbial communities responsible for significant contributions to global ocean carbon flux and primary production. While bulk measurements of phage productivity suggest a key role for phages in the oceans, how the ecology of phage infection might affect long-term phage productivity is poorly understood. Fortunately, genomic data can provide information about the ecology of the system, because ecological interactions such as competition can leave characteristic signals in phage genomes.

Here we applied the Sequentially Markov Coalescent model to genome data collected from four phage lineages that represent the major part of the phage community infecting cyanobacterial strain WH7803. We observe fluctuations in population size over more than 600,000 generations, and the pattern of these fluctuations supports negative density-dependent growth, potentially due to predator-prey cycles. A strong trend towards negative frequency-dependent growth is likely obscured by positive correlation with density in our dataset. Frequency-dependent effects are central to the “Killing the Winner” model of marine microbial community structure, which suggests a stabilizing effect of host-parasite co-evolutionary dynamics on the phage community, also confirmed in the focal phage community.

With the increase of genome data collected for communities of microbes and phages, there are ample opportunities to extend existing frameworks for demographic inference to these new systems, clarifying the role of ecological and evolutionary processes in shaping the productivity of natural microbial communities.

# Effects of molybdenum and environmental factors on abundance and taxonomic affiliation of genes that transport or use Mo

Shreya Srivastava<sup>1\*</sup>, Brandon R. Briggs<sup>2</sup>, Hailiang Dong<sup>3</sup>

<sup>1</sup>Department of Geology and Environmental Earth Science, Miami University, Oxford, OH 45056, USA, srivass3@miamioh.edu (\*presenting author)

<sup>2</sup>Department of Biological Sciences, University of Alaska-Anchorage, Anchorage, AK 99508, USA, bbriggs6@alaska.edu

<sup>3</sup>Department of Geology and Environmental Earth Science, Miami University, Oxford, OH 45056, USA, dongh@miamioh.edu

The trace element molybdenum (Mo) is an important component of enzymes known as molybdoenzymes, which are involved in nitrogen, sulfur and carbon metabolisms. Using metagenomic data from 9 hot springs in Tengchong, Yunnan Province, China, the effect of pH, temperature and spring water chemistry on the abundance and taxonomic affiliations of genes that transport or use Mo as a cofactor was studied with a special emphasis on the nitrogenase enzyme protein (*nifD*). BLAST search using custom Mo utilizing and transport protein database followed by taxonomic and functional pathway annotation suggests a positive correlation between Mo concentration and relative abundance of Mo transporting/ utilizing genes up to 0.2 ppb of Mo. Mo transport genes were predominantly annotated within the Archaeal phylum Crenarchaeota and Thaumarchaeota in low pH and high pH, respectively. The KEGG annotations for the genes involved in Mo transport at all sites were predominantly ABC transporter proteins. Similarly genes that use Mo as a cofactor were predominantly annotated within the Archaeal phylum. The functional annotation of Mo utilization was mainly genes involved in nitrogen metabolism followed by glycolysis/ gluconeogenesis and nitrotoluene degradation in most sites. The *nifD* gene was found at most sites and was detected in six bacterial phyla (Firmicutes, Cyanobacteria, Chloroflexi, Proteobacteria, Aquificae and Nitrospirae). The results suggested that temperature, pH and spring water geochemistry work intricately to affect the taxonomy and molybdoenzyme in these high temperature environments.

# Elucidating Neolithic diet with compound specific carbon isotopes

MELANIE K. SUESS<sup>1\*</sup>, RACHEL E. B. REID<sup>2</sup>, ALEXANDER S. BRADLEY<sup>3,4</sup>, XINYI LIU<sup>5</sup>

<sup>1</sup> Department of Earth & Planetary Sciences, Washington University in St. Louis, MO, USA, msuess@eps.wustl.edu (\*presenting author)

<sup>2</sup> Department of Anthropology, Washington University in St. Louis, MO, USA, rreid@wustl.edu

<sup>3</sup> Department of Earth & Planetary Sciences, Washington University in St. Louis, MO, USA, abradley@eps.wustl.edu

<sup>4</sup> Division of Biology & Biomedical Sciences, Washington University in St. Louis, MO, USA

<sup>5</sup> Department of Anthropology, Washington University in St. Louis, MO, USA liuxinyi@wustl.edu

For prehistoric human populations in Asia, broomcorn (*Panicum miliaceum*) and foxtail (*Setaria italica*) millets were expansive crops. These cereals were first cultivated in north China, some 8000 years ago and spread to regions beyond China's borders by at least the third millennium BC. These two crops potentially represent a central part of diet for peoples from central Asia to India and Europe. The importance of millet (a C4 plant) in the diets of ancient humans may be directly assessed through isotopic analysis of skeletal remains.

C isotope analysis of bulk bone collagen demonstrates that between 6000 and 5000 BC, ancient human populations in north China, particularly near the Xiliao River region, used millet as a staple food source. Domestic animal remains from these sites do not bear the signature of C4 plant consumption. C and N isotope values derived from bulk human bone collagen can constrain ancient human diets:  $\delta^{13}\text{C}$  values of collagen suggest that millet was important, but  $\delta^{15}\text{N}$  values show that humans also heavily relied on animal protein. The relative proportion of these food sources is not well resolved.

In this study, we address the following questions: ***To what extent did Neolithic humans consume millet (C4) directly? What proportion of their diets consisted of C3-derived meat?***

Partitioning the relative contributions of plant and animal resources to past human diet is difficult to address using stable isotope values of bulk collagen alone, because bulk bone collagen values of both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  disproportionately reflect dietary protein.

Therefore, we have begun applying compound specific carbon isotope analyses of individual amino acids extracted from human bone collagen.

This approach is more detailed, as the isotopic composition of essential and non-essential amino acids can be addressed separately, allowing deconvolution of some dietary sources. The principle of this approach is that in human tissues, the  $\delta^{13}\text{C}$  values of the essential amino acids reflect the  $\delta^{13}\text{C}$  values of their dietary protein, while the  $\delta^{13}\text{C}$  values of non-essential amino acids (partially synthesized *de novo* by humans) reflect all dietary sources. In our study population, dating prior to 5000 BC, millet contains a C4  $\delta^{13}\text{C}$  signature, while animal protein lacks it, which should facilitate deconvolution of these carbon sources.

# Plant wax isotope records in the Gulf of Mexico sediments are biased toward forested areas of the Mississippi River catchment

YEON JEE SUH<sup>1\*</sup>, AARON F. DIEFENDORF<sup>1</sup>, GABRIEL J. BOWEN<sup>2</sup>

<sup>1</sup>Department of Geology, University of Cincinnati, Cincinnati, USA, suhye@mail.uc.edu (\*presenting author)

<sup>2</sup>Department of Geology and Geophysics, University of Utah, Salt Lake City, USA, gabe.bowen@utah.edu

Carbon ( $\delta^{13}\text{C}$ ) and hydrogen ( $\delta\text{D}$ ) isotopic compositions of leaf waxes reflect vegetation composition (trees vs. grasses) and hydrologic conditions (precipitation  $\delta\text{D}$  and meteorological processes), respectively. Geologic leaf wax isotopes have been used extensively to address a wide range of questions including reconstructing rainfall, plant community changes, and strength of monsoon systems. However, potential variations in leaf wax isotopes during transportation from plant source to sediment sink are poorly understood, leading to challenges in quantitative interpretations of leaf wax isotope records. This study examined whether spatial variations in runoff, distance to the river mouth, net primary production (NPP), and leaf wax production within a catchment area affect wax signals in sediments discharged by an associated river.

We modeled carbon and hydrogen isoscapes of *n*-alkanes in the Mississippi River (MR) basin under current climate without human interference as a control condition. We also quantified each of the catchment factors above by weighting them on the isoscape maps. The modeled *n*-alkane  $\delta^{13}\text{C}$  and  $\delta\text{D}$  values for the catchment were then compared to the measured values in ODP 625B core from the northeastern Gulf of Mexico (GoM) for the Holocene (3-11 ka). This core is located near the MR discharge area and has been used to reconstruct ocean environments in the past. The measured *n*-alkane  $\delta^{13}\text{C}$  values were consistently more negative than the modeled values by 1-3‰. In the MR basin, *n*-alkane  $\delta^{13}\text{C}$  values are more positive in drier, western grasslands and more negative in wetter, eastern forests. More negative measured  $\delta^{13}\text{C}$  *n*-alkane values may therefore represent higher export of leaf waxes from forestlands. Comparing modeled and measured data suggests that leaf waxes from the regions that are farther from the river mouth and have lower runoff, NPP, and leaf wax production (i.e. western grasslands), were underrepresented in the GoM sediments. The magnitude of underrepresentation of grasslands varied by *n*-alkane chain-length: greater in *n*-C<sub>29</sub> and *n*-C<sub>31</sub> alkanes than in *n*-C<sub>33</sub> alkanes. Grasses generally produce more *n*-C<sub>33</sub> alkanes than other plant types, and this may have led to a better representation of grassland in *n*-C<sub>33</sub> alkanes of sediment records. This leaf wax isotopic bias toward forested areas and varying extent of bias by chain-length have important implications for quantifying the past  $\delta^{13}\text{C}$ . A simple mixing model may incorrectly estimate  $\delta^{13}\text{C}$  if plant waxes in sediment records are biased toward a certain region or plant type within the catchment area. Therefore, it is recommended to understand the source and transportation of plant waxes from the catchment area to the sediment sink for more quantitative paleovegetation and paleohydrologic reconstructions especially in marine sediments that incorporate plant waxes from spatially large and climatically dynamic area, such as the MR basin.

# **Towards sustainable bioplastic production in resource limited environments**

## **using *Rhodopseudomonas palustris* TIE-1**

TAHINA RANAIVOARISOA<sup>1\*</sup>, KARTHIK RENGASAMY<sup>1</sup>, RAJESH SING<sup>1</sup>, MICHAEL GUZMAN<sup>1</sup> ARPITA BOSE<sup>1</sup>

<sup>1</sup>Department, of biology Washington University in Saint Louis , Saint Louis, MO USA, ranaivoarisoa@wustl.edu

The increasing need to substitute conventional petroleum derived plastic is making bioplastics an attractive alternative. Polyhybutyrate (PHB) is a well-known bioplastic produced by several bacterial strains using different carbon source. We explored PHB synthesis by the bacterium *Rhodopseudomonas palustris* TIE-1 based on its extreme metabolic versatility. Here we report that TIE-1 is able to produce PHB under photoferroautotrophic and photoelectroautotrophic growth conditions using electrons from soluble iron or poised electrodes, respectively. PHB production was coherent with ferrous iron oxidation and electron uptake. PHB accumulation was observed both under nitrogen fixing where nitrogen source was N<sub>2</sub> gas or when nitrogen was supplemented as ammonium chloride salt. Under such conditions, carbon source was provided as CO<sub>2</sub> gas and light as energy source. Such growth conditions have not previously been explored for bioplastic production and are unique to TIE-1, providing an infinite carbon and energy sources for PHB biosynthesis and making a perfect carbon cycle due to its biodegradability. We also determined PHB production under other growth conditions such as chemo-, photoheterotrophy and photoautotrophy using electrons from hydrogen. Photoferro- and photoelectroautotrophic PHB production was at par with that observed from substrates such as butyrate. Our observations open new doors for sustainable bioplastic production in resource-limited environments on Earth, and potentially during space exploration and on other planets.

# **A biomarker and compound-specific hydrogen isotope record from the onset of Ocean Anoxic Event 2, Kaiparowits Plateau, Southern Utah**

JORDAN P. TODES<sup>1\*</sup>, MATTHEW M. JONES<sup>2</sup>, BRADLEY B. SAGEMAN<sup>3</sup>, MAGDALENA R. OSBURN<sup>4</sup>

<sup>1</sup>Department of Earth and Planetary Sciences, Northwestern University, Evanston, USA, jordantodes2018@u.northwestern.edu (\*presenting author)

<sup>2</sup>Department of Earth and Planetary Sciences, Northwestern University, Evanston, USA, matt@earth.northwestern.edu

<sup>3</sup>Department of Earth and Planetary Sciences, Northwestern University, Evanston, USA, brad@earth.northwestern.edu

<sup>4</sup>Department of Earth and Planetary Sciences, Northwestern University, Evanston, USA, maggie@earth.northwestern.edu

Rhythmic lithologic variations (limestone-shale couplets) interpreted to reflect Milankovitch cycles occur at the onset of Ocean Anoxic Event 2 (OAE2) in the Bridge Creek Limestone and correlative strata of the Western Interior Seaway. These couplets have been interpreted to reflect climate cycles; however, the physical mechanism(s) through which climate cycles were translated to the sedimentary record during peak greenhouse conditions remain unsettled. Although glacioeustasy has been considered, variance in surface ocean temperature, ocean circulation, or local hydrology may be more plausible options. Compound-specific hydrogen isotope ratios ( $\delta^2\text{H}$ ) of *n*-alkanes and other biomarkers may provide a means to evaluate such mechanisms. Since sedimentary *n*-alkanes are for the most part generated through consistent biosynthetic pathways in plants and in membrane lipid diagenesis, and furthermore are resistant to secondary hydrogen exchange during thermal maturation at low (<100 °C) temperatures, they have the potential to reflect the isotopic composition of primary waters.

The Tropic Shale of the Kaiparowits Plateau (Grand Staircase-Escalante National Monument, southern Utah) provides an exceptional opportunity to explore  $\delta^2\text{H}$  variability in this interval. Organic matter from outcrop samples of three couplets have been extracted, separated, and analyzed with Gas Chromatography – Mass Spectrometry (GC-MS) and GC – Pyrolysis - Isotope Ratio Mass Spectrometry (GC-P-IRMS) to ascertain facies-specific  $\delta^2\text{H}$  variability. Strong odd-over-even *n*-alkane chain length distributions suggest low thermal maturity and thus the possible preservation of primary  $\delta^2\text{H}$  values. Short and long chain *n*-alkanes are potentially sourced from planktonic biomass and terrestrial plants, respectively, enabling a comparison of climatic processes between marine and terrestrial settings. Biomarkers, including both steranes and hopanes, are preserved and reflect putative source organisms and local paleoenvironmental conditions. In particular, the relative abundances of steranes allude to a mixed terrestrial and marine influence in the deposited organic matter, and pristane/phytane ratios may suggest increased oxygenation at the onset of the Plenian Cold Event in the Western Interior.

Facies-specific  $\delta^2\text{H}$  analysis will allow for evaluation of changes in the dominant source of atmospheric moisture in the Western Interior during orbitally-forced climate cycles. Organic matter deposited during influxes of northerly Boreal water masses would have a  $^2\text{H}$ -depleted isotopic composition relative to those deposited during periods of more southerly Tethys influence. In this model, these variations are reflected by lithology – limestone deposition would occur during warm, evaporative Tethys-dominated times, while cooler, wetter Boreal periods would promote shale deposition.

## Considerations for Aerobic Bioremediation of Selenite and Selenate

Torgeson, Joshua<sup>1\*</sup>, Rosenfeld, Carla<sup>2</sup>, Santelli, Cara<sup>3</sup>

<sup>1</sup>Department of Earth Sciences and the BioTechnology Institute, University of Minnesota – Twin Cities, Minneapolis, USA, torge158@umn.edu (\*presenting author)

<sup>2</sup>Department of Earth Sciences and the BioTechnology Institute, University of Minnesota – Twin Cities, Minneapolis, USA, carlar@umn.edu

<sup>3</sup>Department of Earth Sciences and the BioTechnology Institute, University of Minnesota – Twin Cities, Minneapolis, USA, santelli@umn.edu

Selenium (Se) contamination is a global environmental problem, with many anthropogenic activities (e.g. coal mining and combustion, phosphate mining, and agricultural irrigation) altering its distribution and releasing potentially hazardous concentrations into soils and natural water ecosystems. Aqueous selenium species (Se(IV) and Se(VI)) are highly mobile and bioavailable relative to insoluble Se(0) and volatile Se(-II) compounds, making reduction an ideal method of remediation. While past bioremediation has relied on anaerobic mechanisms which can result in the formation of undesired products (e.g. methylmercury), novel remediation strategies plan to incorporate known aerobic Se-reducing fungi to remove aqueous Se(IV) and Se(VI) by reduction to insoluble Se(0).

Three known selenium-reducing fungi (*Alternaria alternata*, *Acremonium strictum*, and *Stagnospora sp.*) were grown on plates with selenium-containing media. First, carbon source (glucose vs. sodium acetate) and concentration (0.5mM, 5.0mM, and 50mM) were varied for each species to determine the influence of carbon source on growth and reduction. Next, sulfate concentrations were varied (0mM, 0.01mM, 0.1mM, 1.0mM, and 10mM). Growth was determined by measuring mycelium diameter while reduction was determined qualitatively by the presence of red, amorphous Se nanoparticles.

Fungal-mediated Se(IV and VI) reduction was highly dependent on carbon source (e.g. glucose vs. acetate) and concentration. Selenite reduction appeared to occur most strongly. At 5.0mM Growth in the presence of Se(VI), though not Se(IV) was also contingent upon sufficient sulfate conditions, indicating different uptake and reduction mechanisms for the different oxyanions. This research can help promote more effective removal of Se(IV and VI) by native microbial colonies allowing for passive removal of selenium.

# illuminating viral impacts on microbial carbon cycling from thawing permafrost peatlands in northern Sweden

Gareth Trubl<sup>1</sup>, Simon Roux<sup>2</sup>, Benjamin Bolduc<sup>3</sup>, Ho Bin Jang<sup>4</sup>, Joanne B. Emerson<sup>5</sup>, Natalie Solonenko<sup>6</sup>, Lindsey Solden<sup>7</sup>, Kelly Wrighton<sup>8</sup>, Matthew B. Sullivan<sup>9</sup>, & Virginia I. Rich<sup>10</sup>

<sup>1</sup>Department of Microbiology, The Ohio State University, Columbus, OH, USA, [trubl.1@osu.edu](mailto:trubl.1@osu.edu) (\*presenting author)

<sup>2</sup>United States Department of Energy Joint Genome Institute, Lawrence Berkeley National Laboratory, Walnut Creek, CA, USA, [sroux@lbl.gov](mailto:sroux@lbl.gov)

<sup>3</sup>Department of Microbiology, The Ohio State University, Columbus, OH, USA, [bolduc.10@osu.edu](mailto:bolduc.10@osu.edu)

<sup>4</sup>Department of Microbiology, The Ohio State University, Columbus, OH, USA, [hobin1976@gmail.com](mailto:hobin1976@gmail.com)

<sup>5</sup>Department of Plant Pathology, University of California, Davis, CA, USA, [joanne.b.emerson@gmail.com](mailto:joanne.b.emerson@gmail.com)

<sup>6</sup>Department of Microbiology, The Ohio State University, Columbus, OH, USA, [natalie.solonenko@gmail.com](mailto:natalie.solonenko@gmail.com)

<sup>7</sup>Department of Microbiology, The Ohio State University, Columbus, OH, USA, [solden.2@buckeyemail.osu.edu](mailto:solden.2@buckeyemail.osu.edu)

<sup>8</sup>Department of Microbiology, The Ohio State University, Columbus, OH, USA, [wrighton.1@osu.edu](mailto:wrighton.1@osu.edu)

<sup>9</sup>Departments of Microbiology and Civil, Environmental and Geodetic Engineering, The Ohio State University, Columbus, OH, USA, [sullivan.948@osu.edu](mailto:sullivan.948@osu.edu)

<sup>10</sup>Department of Microbiology, The Ohio State University, Columbus, OH, USA, [rich.270@osu.edu](mailto:rich.270@osu.edu)

Permafrost contains 30–50% of global soil carbon (C) and is rapidly thawing. While the fate of this C is unknown, it will be shaped in part by microbes and their associated viruses, which modulate host activities via mortality and metabolic control. To date, viral research in soils has been outpaced by that in aquatic environments, due to the technical challenges of accessing viruses as well as the dramatic physicochemical heterogeneity in soils. Here, we describe advances in soil viromics from our research on permafrost-associated soils, and their implications for associated terrestrial C cycling. First, we optimized viral resuspension–DNA extraction methods for a range of soil types. Second, we applied cutting-edge viral-specific informatics methods to recover viral populations, define their gene content, connect them to potential hosts, and analyze their relationships to environmental parameters. A total of 781 viral populations were recovered from size-fractionated virus samples of three soils along a permafrost thaw gradient. Ecological analyses revealed endemism as recovered viral populations were largely unique to each habitat and unlike those in aquatic communities. Genome- and network-based classification assigned these viruses into 231 viral clusters (VCs; ~genus-level taxonomy), 55% of which were novel. This increases the number of VCs by a third and triples the number of soil viral populations in the RefSeq database (currently contains 259 VCs and 316 soil viral populations). Genomic analyses revealed 85% of the genes were functionally unknown, though ~5% of the annotatable genes contained C-related auxiliary metabolic genes (AMGs; e.g. glycoside hydrolases). Using sequence-based features and microbial population genomes, we were able to *in silico* predict hosts for ~30% of the viral populations. The identified hosts spanned 3 phyla and 6 genera but suggested these viruses have species-specific host ranges as >80% of hosts for a given virus were in the same species. Several identified hosts (e.g. *Acidobacterium*) are dominant community members that play major roles in C cycling through organic matter degradation. Together these findings show that permafrost viruses play a major role in the fate of soil C through infection of key hosts and metabolic reprogramming using specific C cycling AMGs.

# Butanol Producing Pathway in *Rhodopseudomonas Palustris* TIE-1

WEI BAI<sup>1</sup>, TAHINA RANAIVOARISOA<sup>1\*</sup>, ARPITA BOSE<sup>1</sup>

<sup>1</sup>Department, of biology Washington University in Saint Louis , Saint Louis, MO USA, weibai@wustl.edu

Biobutanol, a sustainable vehicle fuel with similar properties to gasoline, has been argued to be a potential substitute for gasoline. Using photoautotrophic strains has a distinct advantage over traditionally used microbes that use sugars for bio-butanol production because they can fix carbon dioxide using the energy of light. Such a strategy would result in carbon neutral to a carbon-sequestering biofuel production. In the present study, we built a butanol production pathway in *Rhodopseudomonas palustris* TIE-1, a strain that possesses several different metabolic capabilities<sup>1</sup>. Besides being able to use inorganic carbon as carbon source, the versatile metabolisms of TIE-1 enable us to use alternative electron and nitrogen source as well. This feature has a great value for biofuel production at places where the common carbon, electron, and nitrogen sources are limited.

Our butanol synthesis pathway includes five genes that subsequently convert acetyl-coA to 1-butanol. The cassette is built in a broad-host-range vectors allowing target gene expression in Gram- negative bacteria<sup>2</sup>. Different strain backgrounds and cassette constructions were tested for butanol production under several different growth conditions.

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## **Seasonal dynamics in the fluxes and isotopic composition of settling particulate organic matter in Lake Superior: Implications for the interpretation of sedimentary records**

MARTIJN WOLTERING<sup>1</sup>, PROSPER ZIGAH<sup>2\*</sup>, JOSEF WERNE<sup>3</sup>

<sup>1</sup>Australian Resources Research Centre, Commonwealth Scientific and Industrial Research Organisation (CSIRO), Bentley, Australia, [Martijn.Woltering@csiro.au](mailto:Martijn.Woltering@csiro.au) (\*presenting author)

<sup>2</sup>Department of Geology and Environmental Science, University of Pittsburgh, Pittsburgh, USA, [pkz2@pitt.edu](mailto:pkz2@pitt.edu)

<sup>3</sup>Department of Geology and Environmental Science, University of Pittsburgh, Pittsburgh, USA, [jwerne@pitt.edu](mailto:jwerne@pitt.edu)

Settling particulate organic matter (POM) is a key pathway for the transport of organic matter produced in the euphotic zone to the surface sediments and into the sedimentary records. Therefore, constraining the sources and diagenetic alterations of POM is critical in understanding past environmental changes obtained from paleoenvironmental reconstruction using sediment cores. Lakes play important roles in the global carbon cycle with annual carbon burial of ~25-60% more than the oceans. However, POM dynamics in lakes are not well constrained. Here we examined the seasonal changes in the fluxes and carbon and nitrogen stable isotopic compositions of settling POM in the western basin of Lake Superior from 2005 to 2008. As different seasons evolved, isotopic diversity was observed in the settling POM. We will discuss these isotopic trends in the context of phototrophic microbial communities and sediment focusing and/or resuspension, as well as the implications for the interpretation of sedimentary records.

# Geochemical characterization of the late Ediacaran in the East European platform: constrained by well Kostovo-13

MING WU<sup>1\*</sup>, DAVID FIKE<sup>2</sup>, ALEXANDER BRADLEY<sup>3</sup>

<sup>1</sup>Washington University in St. Louis, St. Louis, USA, [mingwu@wustl.edu](mailto:mingwu@wustl.edu) (\*presenting author)

<sup>2</sup>Washington University in St. Louis, St. Louis, USA, [dfike@levee.wustl.edu](mailto:dfike@levee.wustl.edu)

<sup>3</sup>Washington University in St. Louis, St. Louis, USA, [abradley@eps.wustl.edu](mailto:abradley@eps.wustl.edu)

One of the significant differences in geochemical features between Proterozoic and Phanerozoic hydrocarbons is the isotopic ordering in the  $\delta^{13}\text{C}$  of *n*-alkanes, isoprenoids, and kerogen. In Phanerozoic hydrocarbons, the  $\delta^{13}\text{C}$  values of these are generally  $\delta^{13}\text{C}_{n\text{-alkanes}} < \delta^{13}\text{C}_{\text{isoprenoids}} < \delta^{13}\text{C}_{\text{kerogen}}$ , which is consistent with the expectation from biosynthesis. In contrast, Proterozoic hydrocarbons have a pattern where is  $\delta^{13}\text{C}_{\text{isoprenoids}} < \delta^{13}\text{C}_{\text{kerogen}} < \delta^{13}\text{C}_{n\text{-alkanes}}$ . One mechanism proposed to explain this difference is intensive heterotrophic reworking of organic matter in the Proterozoic. However, this explanation is still debated including preferential preservation of heterotrophic organic carbon and/or a ‘mat-seal’ effect.

We attempt to assess this using compound-specific hydrogen isotopes. Characteristic hydrogen fractionations associated heterotrophic and autotrophic metabolisms are different which could be a potential tool to test if severe heterotrophic reworking occurred in Proterozoic. The well-preserved organic matter in late Ediacaran formation in the East European Platform provides an invaluable chance to test the theory. The results show that samples in well Kostovo-13 are low in organic matter content with TOC primarily less than 1.0%. The biomarker composition indicates the organic matter are immature to mildly mature. Bacteria is the dominant organic matter source while red algae is the major component in eukaryote regime. Conspicuous UCM and low  $\text{C}_{28}/\text{C}_{29}$  sterane are consistent with Proterozoic source with no/little contamination. A covaried positive shift in  $\delta^{13}\text{C}_{\text{org}}$  and  $\delta^{34}\text{S}_{\text{pyr}}$  are observed in well Kostovo-13 which could be a representative of global carbon and sulfur cycle during late Ediacaran to early Cambrian though other scenarios cannot be completely ruled out without data on carbonate and sulfate isotopic composition. The compound specific hydrocarbon isotope analyses show primary isotopic composition might be retained with limited degree of exchange with other hydrogen pool while no systematic difference between isoprenoids and alkanes is observed.

# Effects of citrate on hexavalent chromium reduction by structural Fe(II) in nontronite

Xiaolei Liu<sup>1\*</sup>, Hailiang Dong<sup>1,2</sup>, Libor Kovarik<sup>3</sup>

<sup>1</sup>School of Earth Science and Resources, China University of Geosciences, Beijing, China, lxiaolei\_liu@126.com (\*presenting author)

<sup>2</sup>Department of Geology and Environmental Earth Science, Miami University, Oxford, USA, dongh@miamioh.edu

<sup>3</sup>Environmental Molecular Science Laboratory, Pacific Northwest National Laboratory, Richland, USA, Libor.Kovarik@pnl.gov

Previous studies have shown that organic ligands could influence Cr(VI) reduction by aqueous Fe<sup>2+</sup> and pyrite. In this study, the effects of citrate on Cr(VI) reduction by structural Fe(II) in nontronite (NAu-2) were investigated under near neutral pH condition (pH=6). Our results showed that the presence of citrate decreased the rate but increased the amount of Cr(VI) reduction by structural Fe(II) in NAu-2. The decreased reaction rate was likely due to competitive sorption of citrate and anionic dichromate (Cr<sub>2</sub>O<sub>7</sub><sup>-</sup>), because sorption of dichromate appeared to be the first step for subsequent Cr(VI) reduction. The increased amount of Cr(VI) reduction in the presence of citrate was likely because citrate provided additional reducing power through ligand-metal electron transfer in the presence of soluble Fe<sup>3+</sup>, which was possibly derived from dissolution of reduced NAu-2. Soluble Cr(III)-citrate complex was a possible form of reduced Cr(VI) when citrate was present. In contrast, nanometer-sized Cr<sub>2</sub>O<sub>3</sub> particles were the product of Cr(VI) reduction by reduced NAu-2 without citrate. Our study highlights the importance of organic ligands on Cr(VI) reduction and immobilization when iron-bearing clay minerals are applied to treat Cr(VI) contaminant in organic rich environments.

# **Sulfhydryl Binding Sites within Bacterial Cell Envelopes: Characterization, Environmental Implications and Applications**

QIANG YU<sup>\*1</sup>, JEREMY B. FEIN<sup>2</sup>

<sup>1</sup> Department of Civil & Environmental Engineering & Earth Sciences, University of Notre Dame, Notre Dame, USA, qyu@nd.edu (\*presenting author)

<sup>2</sup> Department of Civil & Environmental Engineering & Earth Sciences, University of Notre Dame, Notre Dame, USA, fein@nd.edu

Bacterial sulfhydryl binding sites can form strong complexes with chalcophilic metals such as Hg and Cd, thereby affecting the fate, transport and bioavailability of these metals in both natural and engineered systems. In order to characterize sulfhydryl sites within bacterial cell envelopes, a novel approach coupling selective sulfhydryl blocking, potentiometric titration, and surface complexation modeling was developed to measure the concentration and reactivity of sulfhydryl sites within the cell envelopes and on EPS molecules of several bacterial species.

Our results suggest that sulfhydryl sites are present in relatively low and similar concentrations over a wide range of bacterial diversity when bacteria are grown in a nutrient-rich trypticase soy broth plus yeast extract medium. However, these bacterial species contain significantly different concentrations of sulfhydryl sites when they are grown in a M9 minimal medium. Furthermore, the glucose concentration in the growth medium strongly affects the concentration of sulfhydryl sites that are present within the cell envelope, with higher glucose concentrations yielding higher bacterial sulfhydryl site concentrations. The distribution of sulfhydryl sites between EPS molecules and the cell envelope components varies significantly between *S. oneidensis* and *P. putida* biomass samples, with *S. oneidensis* having virtually all of its sulfhydryl sites located within the cell envelope and *P. putida* having virtually all of its sulfhydryl sites located on its EPS molecules. Although the measured concentrations of sulfhydryl sites within bacterial cell envelopes are lower than the concentrations of other binding site types, such as carboxyl and phosphoryl sites, we demonstrate that the concentrations of sulfhydryl sites are high enough to control the binding of chalcophilic metals onto bacteria under low metal-loading conditions, and our work suggests that the binding affinity of sulfhydryl sites for metals is orders of magnitude greater than that exhibited by non-sulfhydryl cell envelope sites. Therefore, it is important to characterize bacterial sulfhydryl sites in order to determine and model the effects of bacterial cells on metal cycling and metal bioavailability in the environment. Moreover, we also demonstrate that bacterial biomass with elevated concentrations of sulfhydryl sites caused by growth in the presence of excess glucose may have industrial and treatment applications as a low cost biosorbent for the efficient removal and recovery of chalcophilic metals from aqueous media.

# Function of outer membrane cytochrome proteins in the bioreduction of nontronite by *Shewanella oneidensis* MR-1 and *Geobacter sulfurreducens* PCA

LI ZHANG<sup>1\*</sup>, HAILIANG DONG<sup>1</sup>, LIANG SHI<sup>2</sup>

<sup>1</sup>Department of Geology and Environmental Earth Science, Miami University, Oxford, OH, USA, zhanglm@miamioh.edu (\*presenting author)

<sup>2</sup>Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA, USA, Liang.Shi@pnl.gov

Clay minerals are ubiquitous in soil environment, where bacterial metal reduction is an important biogeochemical process. Iron is an essential component in clay and the structural ferric iron in clay minerals can be reduced to ferrous iron by dissimilatory iron-reducing bacteria (DIRB), including *Shewanella oneidensis* MR-1 and *Geobacter sulfurreducens* PCA. Numerous work has been done to study the kinetics of Fe(III) bioreduction and to identify mineralogical transformation. However, the mechanisms of electron transfer between microbial cells and structural Fe(III) in clay minerals are not well understood. Several mechanisms have been proposed for metal oxides including secretion of electron shuttle or chelator, cellular appendage such as nanowires, and discovery of outer membrane cytochrome proteins. It is reported that outer membrane cytochrome proteins OmcA and MtrC from *Shewanella oneidensis* MR-1 can act as terminal reductases capable of electron transfer to iron oxides such as hematite. Similarly, OmcB of *Geobacter sulfurreducens* PCA was also proposed to mediate electron transfer across the outer membrane.

To test the function of outer membrane cytochrome proteins in the bioreduction of structural ferric iron in clay minerals and thus understanding the mechanism of electron transfer involved in clay bioreduction process, nontronite (an iron-rich smectite variety) is used as the type of clay to be bioreduced by *Shewanella oneidensis* MR-1/*Geobacter sulfurreducens* PCA wildtype, and their deletion mutants deficient in MtrC/OmcA/OmcB in separate experiments. AQDS as the electron shuttle was added to each experiment to test the effect of electron shuttling on the electron transfer pathways. The kinetics of Fe(III) reduction in nontronite is monitored using Fe(II) concentration change through time.

Our results prove that outer membrane cytochrome proteins MtrC/OmcA/OmcB could act as terminal reductases capable of electron transfer to iron containing clay minerals such as nontronite. However, they are not the only pathways for electron transfer in *Shewanella oneidensis* MR-1 and *Geobacter sulfurreducens* PCA.