

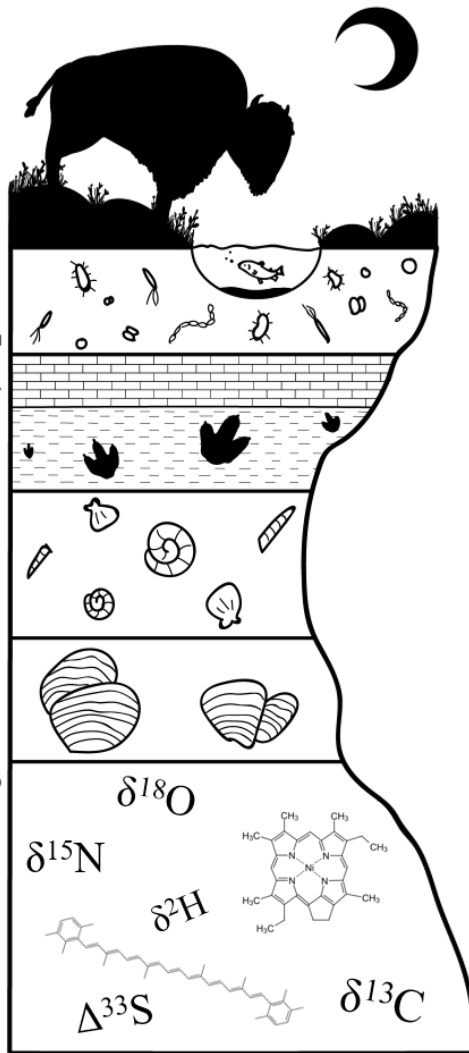
# Rocky Mountain Geobiology Symposium

April 6, 2019

University of Colorado Boulder | Boulder, CO

## Rocky Mountain Geobiology Symposium

University of Colorado Boulder, April 6<sup>th</sup> 2019



Logo by Ellie Hara

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# Schedule

## Saturday, April 6, 2019

8:00 AM	Registration begins in JSCBB; breakfast and coffee available
9:00 AM	Opening remarks
<b>Oral session #1: Isotope applications for environmental research</b> Moderator: Ben Johnson	
9:15 AM	<b>Greg Connock:</b> Terrigenously-induced photic zone euxinia in the Late Devonian midcontinent of North America
9:45 AM	<b>Ciara Asamoto:</b> Untangling the roles of enzymatic variation in nitrate reduction isotope effects
10:15 AM	<b>Joep van Dijk:</b> Is the IPCC perhaps underestimating Earth's climate sensitivity? Insights from clumped isotopes in pedogenic siderites from the PETM
10:45 AM	Coffee break
<b>Oral session #2: Evolution</b> Moderator: Sarah Hurley	
11:00 AM	<b>Andrea Halling:</b> Snowball Earth: The effect of viscosity on the multicellularity of <i>Chlamydomonas reinhardtii</i>
11:30 AM	<b>Amanda Garcia:</b> Evolution of nitrogenases: Phylogenetic analysis and structural implications for metal binding
12:00 PM	<b>Jen Reeve:</b> Salinity tolerance in cyanobacteria
12:30 PM	Group photo, followed by lunch
1:15 PM	Poster session, coffee
<b>Oral session #3: Life in extreme environments</b> Moderator: Jesse Colangelo	
2:30 PM	<b>Emily Kraus and Daniel Nothhaft:</b> Biological methane cycling in subsurface serpentinization-impacted waters
3:00 PM	<b>Mikayla Borton:</b> Coupled laboratory and field investigations resolve microbial interactions that underpin persistence in hydraulically fractured shales
3:30 PM	<b>Adam Solon:</b> Does increased nutrient availability allow microbes to avoid water limitation in extreme environments?
4:00 PM	Coffee break
<b>Invited speakers</b>	
4:15 PM	<b>Jim Howell:</b> Ecological regeneration of grasslands through properly managed livestock
5:00 PM	<b>Betül Kacar,</b> Keynote address: Recapitulating ancient history in the laboratory with the methods of evolutionary synthetic biology
6:00 PM	Reception begins at Twisted Pine Brewery

## Posters

**Kaela Amundson**, Colorado State University (Poster #6)

Strain-level community dynamics in the deep biosphere: Insights from shale ecosystems

**Mackenzie Best**, New Mexico Institute of Mining and Technology (Poster #17)

Genomic and physiological properties of extremely acidophilic *Acidithiobacillus* spp. from hydrogen sulfide-rich caves

**Gregory de Wet**, University of Colorado Boulder (Poster #1)

The potential influence of bottom-water oxygenation levels on the brGDGT temperature proxy: What are we really reconstructing in Lakes CF8 and Brother-of-Fog, Baffin Island, Canadian Arctic?

**Anne Fetrow**, University of Colorado Boulder (Poster #8)

Determining the timing and driving forces of carbonate precipitation in palustrine environments to inform paleoclimate and paleoelevation estimates

**Zoe Havlena**, New Mexico Institute of Mining and Technology (Poster #19)

Preventing problematic photosynthesis in caves: Do lighting methods and substrate affect the development of destructive lampenflora in Carlsbad cavern?

**Isaac Hinz**, University of Michigan (Poster #11)

Recreating the ancient ocean in a borosilicate bottle

**Alex Honeyman**, Colorado School of Mines (Poster #4)

Megafire microbial ecology: Impact on soils immediately after variable intensity wildfire

**Laura Leonard**, Colorado School of Mines (Poster #10)

Needle litter decomposition induces soil biogeochemical shifts and selective pressures

**James Leong**, Arizona State University (Poster #3)

Predicting compositions of fluids and geochemical energies for microbes in the deep continental serpentizing subsurface

**Sarah Leventhal**, University of Colorado Boulder (Poster #2)

Marine bivalve aspect ratio, shell size, and their relationship to a latitudinal gradient

**Ashley Maloney**, Princeton University (Poster #16)

Effect of growth rate on the carbon and hydrogen isotopic composition of biomass and lipids in *Saccharomyces cerevisiae*

**Kalen Rasmussen**, Colorado School of Mines (Poster #5)

Evaluating Hydrothermal Microbial Communities and Associated Insights into the Rock Record

**Josué Rodríguez-Ramos**, Colorado State University (Poster #12)

More than meets the eye: Microbial communities and their viral predators govern carbon and nitrogen transformations in the hyporheic zone

**Olivia Salmon**, Colorado School of Mines (Poster #14)

Rare Earth element interactions with wastewater treatment microbial communities

**Danielle Saxer**, Weber State University (Poster #18)

Precipitation of carbonate by Great Salt Lake microbialite communities: Geochemical modeling and laboratory experiments

**Craig A. Stricker**, United States Geological Survey (Poster #21)

Carbon chemistry of intact versus chronically drained peatlands in the southeastern USA

**Kasdi Sujono**, University of Colorado Boulder (Poster #20)

Growth characteristics of planktonic and particle-associated states by sulfate-reducing bacteria

**Patrick Thieringer**, Colorado School of Mines (Poster #13)

Temporal insights into subsurface microbial ecology and rock-equilibrated fluid geochemistry at Edgar Experimental Mine

**Jonn van Oosten-Edl**, University of Colorado Boulder (Poster #7)

Nitrogen cycle dynamics during the Cretaceous Oceanic Anoxic Event 2 (OAE2) in the Western Interior Seaway

**Michael Vega**, Colorado School of Mines (Poster #9)

Influence of diatom photosynthesis on denitrification activity within a benthic, open water wetland biomat

**Corinne Walsh**, University of Colorado Boulder (Poster #15)

Soil microbial communities and seedling success

## Abstracts

### Strain-level community dynamics in the deep biosphere: Insights from shale ecosystems (Poster #6)

**Kaela K. Amundson**, Mikayla A. Borton, Rebecca A. Daly, Kelly C. Wrighton, Michael J. Wilkins  
Colorado State University

By 2015, hydraulic fracturing (HF) of deep subsurface shales was responsible for >60% of the total natural gas output in the United States. As a result of the HF process, microorganisms are introduced into this engineered ecosystem and can persist for extended periods of time (>500 days). Recent research by our group and others have demonstrated that a single *Halanaerobium* species, *H. congolense*, becomes dominant in these systems over time, often accounting for up to 90% of the microbial community. While *Halanaerobium* have potential to contribute to well souring, corrosion, and pore clogging, we have little understanding of the biotic and abiotic factors that might explain *H. congolense* dominance over a well's lifetime. Here, we used a combination of cultivation-dependent and independent methods in a single well to investigate the genomic basis for their persistence in this newly engineered habitat. Produced fluid chemistry shows that salinity increased from freshwater (chloride <0.008 g/L) to brine concentrations (chloride >108 g/L) over 302 days after HF. To capture the strain diversity of *Halanaerobium* through time with increasing salinity, we isolated and sequenced 21 *H. congolense* genomes throughout early (86-98 days), middle (154 days), and late (204 days) time points. Comparative genomics of these isolates revealed 5 phylogenetically distinct clades that exhibited between 97.3%-99.9% average nucleotide identity. Temporal abundance of these genomes demonstrated that members within some clades displayed consistent abundance patterns, while strains in other clades showed more variable behavior. Of the 4180 gene clusters across all isolates, 1668 genes made up the core, 2,106 genes made up the accessory genome (present in at least two genomes), and 406 unique genes (present in only one genome). Fine-scale microdiversity was observed in CRISPR-Cas systems, with 13 of the 21 isolates being capable of this viral defense mechanism, with nearly identical isolates distinguished only by unique spacer sequences. Strain-level differences in viral resistance, osmoprotectant strategies, and metabolism may explain how *H. congolense* are able to colonize and exploit different ecological niches during the lifetime of a well. The genomic tractability of this system, coupled with our ability to isolate representative microorganisms, demonstrates that fractured shales are a model system for investigating the relationship between microdiversity and biogeochemistry in the subsurface.

### Untangling the roles of enzymatic variation in nitrate reduction isotope effects (Oral presentation)

**Ciara Asamoto**  
University of Colorado Boulder

Stable nitrogen and oxygen isotopes ( $^{15}\text{N}$ ,  $^{18}\text{O}$ ) are essential tools for monitoring nitrogen fluxes in modern and ancient ecosystems. Nitrate reduction causes an enrichment in  $^{15}\text{N}$  and  $^{18}\text{O}$  in ecosystems, making it a useful indicator for nitrogen fluxes. However, the ratio at which oxygen and nitrogen isotopes fractionate ( $\epsilon^{18}\text{O} / \epsilon^{15}\text{N}$ ) varies between environments, with marine systems having  $\epsilon^{18}\text{O} / \epsilon^{15}\text{N}$  trajectories of ~1.0, and terrestrial systems closer to ~0.6. Several hypotheses have been proposed to explain this variation, and for this talk I will focus on the enzyme specific effects of nitrate reductases on the isotopic biosignature of nitrate reduction. I have collected isotopic data from physiological experiments of nitrate reducing bacteria that highlights the differential effects of the Nar and Nap nitrate reductases. My data strongly suggests that the nitrate reducing enzyme used by bacteria is a significant influence on  $\epsilon^{18}\text{O} / \epsilon^{15}\text{N}$  trajectories, underscoring the importance of combining biologic and geochemical methodology to more fully understand this process.

**Genomic and physiological properties of extremely acidophilic *Acidithiobacillus* spp. from hydrogen sulfide-rich caves** (Poster #17)

**Mackenzie Best**, Diana Northup, Rodolfo Gomez Cruz, Daniel Jones  
New Mexico Institute of Mining and Technology

Some of the world's largest and most spectacular limestone caves, including Carlsbad Cavern and Lechuguilla Cave, have a "sulfidic" origin. These sulfidic cave systems develop when anoxic fluids bearing hydrogen-sulfide encounter air-filled pores or oxygenated groundwater, producing sulfuric acid. Substantial cave enlargement occurs above the water table where hydrogen sulfide degasses into the cave atmosphere and oxidizes to sulfuric acid on the moist cave walls. This sulfide gas represents the primary energy source for extreme acidophiles that colonize subaerial surfaces, where they produce pendulous, highly acidic (pH 0-2) "snottite" biofilms and contribute to cave formation by rapidly oxidizing sulfide to sulfuric acid.

The numerically dominant microorganisms in these biofilms are members of the genus *Acidithiobacillus*. Previous research has shown that sulfidic caves in Italy contain diverse strains of *At. thiooxidans* that have distinct sulfur oxidation pathways and exhibit growth at pH values as low as 0.2 (Jones et al., 2016). However, detailed physiological studies of these strains have not yet been performed. We will be evaluating the pH tolerance and sulfur utilization capabilities of extremely acidophilic *At. thiooxidans* from the Frasassi and Acquasanta cave systems, and relating observed physiological differences to genomic properties of the strains. Additionally, similar snottite biofilms from sulfidic caves in Mexico are also dominated by *Acidithiobacillus* spp., but contain different microbial assemblages. We used single molecule long-read sequencing (Pacific Biosciences) to generate a metagenomic dataset from a snottite sample collected from Cueva de Villa Luz in 2013, and will present preliminary results on how *Acidithiobacillus* spp. from this North American cave system differ from snottite-forming *At. thiooxidans* from the Italian caves.

References:

Jones, D. S., Schaperdoth, I., & Macalady, J. L. (2016). Biogeography of sulfur-oxidizing *Acidithiobacillus* populations in extremely acidic cave biofilms. *ISME J*, v. 10, p. 2879.

**Coupled laboratory and field investigations resolve microbial interactions that underpin persistence in hydraulically fractured shales** (Oral presentation)

**Mikayla A. Borton**, David W. Hoyt, Simon Roux, Rebecca A. Daly, Susan A. Welch, Carrie D. Nicora, Samuel Purvine, Elizabeth K. Eder, Andrea J. Hanson, Julie M. Sheets, David M. Morgan, Richard A. Wolfe, Shikha Sharma, Timothy R. Carr, David R. Cole, Paula J. Mouser, Mary S. Lipton, Michael J. Wilkins, and Kelly C. Wrighton  
Colorado State University

Hydraulic fracturing of shale is the industrial process behind the surging natural gas output in the United States. This technology inadvertently creates an engineered microbial ecosystem thousands of meters below Earth's surface. Here, we show that persisting microbial communities in fractured shales converge to a similar membership and structure, despite differences in operators, shale formations, and input communities/chemistries. Based on the detection of the metabolite glycine betaine across all Appalachian Basin produced fluids sampled to date, we used laboratory reactors to manipulate persisting shale microbial communities that are currently not feasible in field scenarios. Metaproteomic and metabolite findings from the laboratory were then corroborated using regression-based modeling performed on field metagenomic and metabolite data from more than 40 produced fluid samples from five hydraulically fractured shale wells. Collectively, our findings show that *Halanaerobium*, *Geotoga*, and *Methanohalophilus* strain abundances predict a significant fraction of carbon and nitrogen metabolites at the field scale. Our combined laboratory and field results revealed that microorganisms persisting in hydraulically fractured shales must maintain osmotic balance in hypersaline fluids, gain energy in the absence of electron acceptors, and acquire carbon and nitrogen to synthesize cell building blocks. We provide evidence that co-fermentation of amino acids and their derivatives, like glycine betaine, meets these organismal needs and thus Stickland fermentations function as a keystone metabolism conserved across hydraulically

fractured shale communities. Our proposed ecological framework extends to the human gut and other protein-rich ecosystems, where the role of Stickland fermentations and their derived syntrophies play unrecognized roles in carbon and nitrogen turnover.

### **Terrigenously-induced photic zone euxinia in the Late Devonian midcontinent of North America** (Oral presentation)

**Gregory T. Connock**; Thanh X. Nguyen; R. Paul Philp; Xiaolei Liu  
University of Oklahoma

The  $C_{30}$  diaromatic carotenoids, fossil pigments derived exclusively from anoxygenic phototrophic sulfur oxidizing bacteria (SOB), are becoming an increasingly common observation in ancient rock and oil samples, punctuating nearly every geologic period back to the Precambrian. By extension, photic zone euxinia (PZE), not observed in open marine settings on modern Earth, was a geologically frequent process in our planet's history irrespective of global climate and continental configuration. Many studies document the occurrence and sustainment of PZE but lack tangible evidence to substantiate the speculated mechanisms. Recently, we documented the development and temporal extent of PZE in immature core samples of the Woodford Shale from the Lawrence uplift in the Arkoma Basin, OK. Samples exhibited favorable bulk geochemical characteristics (e.g. 3.5-16.9 wt.% TOC) and high  $C_{40}$  diaromatic carotenoid concentrations (detected via GC-MS and GC-MSMS). Paleorenieratane and isorenieratane were the dominant carotenoids in all samples, with renieratane and renierapurpurane identified in lesser amounts. Compound-specific isotopic analysis of paleorenieratane revealed a highly enriched carbon value ( $\delta^{13}C = -12\text{‰} \pm 1.5\text{‰}$ ) indicative of carbon fixation via the reverse tricarboxylic acid cycle, a diagnostic trait of SOB (specifically, the Chlorobiaceae or green sulfur bacteria). Additional molecular parameters sensitive to changes in redox corroborated interpretations founded on fossil carotenoids. The severity of PZE was inferred by the effects this environmental state imposed on the overlying microbial community. Polyaromatic hydrocarbons (PAHs) of terrigenous origin were utilized to investigate causal mechanisms of PZE. Covariance of terrestrially-derived aromatic compounds, such as perylene and dihydro-ar-curcumene, methyltrimethyltridecylchromans (MTTCs), and some PAHs with compound suites directly linked to water column chemistry and structure (e.g.  $C_{30}$  diaromatic carotenoids, gammacerane, 28,30-dinorhopane) suggests direct land-ocean interactions at least partially responsible for the development, maintenance, and ultimate collapse, of PZE during Woodford deposition.

### **The potential influence of bottom-water oxygenation levels on the brGDGT temperature proxy: what are we really reconstructing in Lakes CF8 and Brother-of-Fog, Baffin Island, Canadian Arctic?** (Poster #1)

**Gregory de Wet**, Lucy Camuti, Jonathan Raberg, Sarah Crump, Christopher Florian, Gifford Miller, Julio Sepúlveda  
University of Colorado, Boulder

The Arctic has proven to be one of the most climatically sensitive regions on Earth and is predicted to warm two to three times more than the global average by the year 2100. To accurately capture possible feedbacks in future climate projections, it is necessary to constrain the expected ecological response to warming at high latitudes. One of the main goals of the PACEMAP project (Predicting Arctic Change through Ecosystem Molecular Proxies) is to utilize newly developed molecular techniques to reconstruct temperature variations through the Holocene (past ~11 ka) and the Last Interglacial period (~130 - 115 ka) using lake sediment archives from Baffin Island. Here we present preliminary biomarker temperature data from Lakes CF8 and Brother-Of-Fog in the Canadian Arctic. Interestingly, it appears that water column and sediment oxygenation levels may be exerting a strong signal on reconstructed temperatures based on bacterial membrane lipids known as branched glycerol dialkyl glycerol tetraethers (brGDGTs). Here we present a comparison of brGDGT based reconstructed temperatures with other proxy records from the region along with oxygenation proxies from the same sediment archives. Our data suggests that variations in brGDGT temperatures may be recording variations in bottom-water oxygenation in addition to environmental temperature.

## Determining the timing and driving forces of carbonate precipitation in palustrine environments to inform paleoclimate and paleoelevation estimates (Poster #8)

Anne C. Fetrow, Kathryn E. Snell, Ana María Alonso-Zarza  
University of Colorado Boulder

Palustrine (i.e. wetland) carbonates form in transitional zones between lacustrine and pedogenic environments and are characterized by highly seasonal water levels. Palustrine carbonates are important archives of geochemical signals with which we can estimate environment, climate, and elevations through geologic time<sup>1</sup>. Our ability to interpret these geochemical archives, however, is hindered by our limited understanding of how clumped and traditional single stable isotope signals ( $\Delta 47$ ,  $\delta^{13}\text{C}_{\text{carb}}$ , and  $\delta^{18}\text{O}_{\text{carb}}$ ) reflect climate in palustrine carbonates. Similar modern analogue studies that have been done in pedogenic and lacustrine settings show a common warm-season bias to carbonate formation<sup>2,3</sup>, and we predict a similar bias in palustrine systems. It is therefore necessary to better characterize palustrine carbonate formation and determine how  $\Delta 47$ ,  $\delta^{13}\text{C}_{\text{carb}}$ , and  $\delta^{18}\text{O}_{\text{carb}}$  values are preserved in a modern palustrine environment to inform paleoclimate and paleoaltimetry interpretations. With a combination of sediment and water sampling, and climate monitoring, we will address the following fundamental questions about palustrine carbonate formation: 1) What are the major drivers of carbonate precipitation in palustrine settings? 2) Subsequently, when is carbonate precipitation most likely to be induced? 3) How do  $\Delta 47$  temperature estimates relate to mean annual air temperature, mean warmest month temperature, and surface water temperatures? and 4) How do environmental parameters, such as  $\delta^{18}\text{O}$  values of surface water and precipitation, and  $\delta^{13}\text{C}$  of the dissolved inorganic carbon, correlate with  $\delta^{13}\text{C}_{\text{carb}}$  and  $\delta^{18}\text{O}_{\text{carb}}$ ?

Here we present preliminary  $\Delta 47$ ,  $\delta^{13}\text{C}_{\text{carb}}$ , and  $\delta^{18}\text{O}_{\text{carb}}$  data from the Las Tablas de Daimiel National Park, which is a series of preserved wetland environments with active carbonate precipitation located in central Spain. Preliminary  $\Delta 47$  temperatures estimates range between 11-30°C which occupy the intermediate to warmer range of the local temperature range of 3-30°C<sup>4</sup>.  $\delta^{13}\text{C}_{\text{carb}}$  and  $\delta^{18}\text{O}_{\text{carb}}$  values range between -10 to -3‰ and -8 to -4‰, respectively, and show systematic clustering based on depositional environment. Additionally, we will present preliminary results from a carbonate equilibrium model that will predict carbonate saturation states throughout palustrine environments. This model will provide a necessary framework for understanding the relationship between carbonate saturation and geochemical archives, and will estimate isotopic values based on specified environmental conditions.

- 1: Alonso-Zarza, A. M. (2003), *Earth-Science Reviews*, v. 60, p. 261-298.
- 2: Breecker, D. O., Sharp, Z. D., and McFadden, L. D. (2009), *Geological Society of American Bulletin*, v. 121, p. 630-640.
- 3: Huntington K. W., Wernicke, B. P., Eiler, J. M. (2010), *Tectonics*, v. 29, p. 1-19.
- 4: IAEA/WMO (2018) Global Network of Isotopes in Precipitation. The GNIP Database. Accessible at: [nucleus.iaea.org/wiser](https://nucleus.iaea.org/wiser).



## **Evolution of nitrogenases: Phylogenetic analysis and structural implications for metal binding** (Oral presentation)

**A.K. Garcia**, H. McShea, B. Kolaczowski, B. Kacar  
University of Arizona

Nitrogenase, which catalyzes the biological reduction of  $N_2$  to bioavailable  $NH_3$ , has an ancient evolutionary history evidenced by putative  $\sim 3$  Ga geochemical signatures. The most common form of modern nitrogenase binds a Mo-containing active-site metal cofactor and rarer forms bind alternative V- or Fe-only-cofactors. Potential marine Mo scarcity prior to the  $\sim 2.3$  Ga Great Oxidation Event has led to the suggestion that alternative nitrogenases may have been ancestral due to metal-availability constraints on early nitrogenase evolution. However, recent phylogenetic and structural studies suggest that Mo-nitrogenases were ancestral and instead evolved during or after the Great Oxidation Event. To evaluate ancient nitrogenase metal-binding behavior, we reconstructed ancestral sequences of the nitrogenase protein subunits and modeled structural active site pocket volumes, previously proposed to correlate with differential metal binding. We reconstructed a maximum likelihood phylogeny from 284 modern nitrogenase protein sequences, including 28 alternative nitrogenases and 16 uncharacterized nitrogenases for which metal dependence has not been experimentally determined. Under five evolutionary models, alternative and uncharacterized nitrogenases constitute an early-evolved, monophyletic lineage relative to all Mo-nitrogenases within which cofactor differentiation towards V or Fe would be predicted. We inferred maximum likelihood ancestral sequences for well-supported ancestral nodes within this lineage across all evolutionary models. In addition, we randomly sampled 100 Bayesian sequence variants from the site posterior probability distributions of each maximum likelihood ancestor. We then generated structural homology models for each of 2,525 ancestral sequences and 66 representative modern sequences to calculate active-site pocket volume. We find that ancestral sequence features and pocket volumes generally correlate with those of their descendants. However, though modern sequence features are well correlated with distinct evolutionary clades, pocket volume is not strongly associated with the metal dependency of modern nitrogenases. Several ancestors share greatest sequence similarity with modern uncharacterized nitrogenases and share pocket volume similarity with both V- and anaerobe-hosted Mo-nitrogenases. Pocket volume variance of the Bayesian-sampled ancestors was close to that of the maximum likelihood ancestors, indicating that our structural analyses are robust to uncertainty associated with ancestral sequence inference. Our results suggest that pocket volume is not a reliable proxy for nitrogenase metal dependence and that inferences of ancestral Mo metal binding based on similar approaches may not be conclusive. Future investigations of ancient metal-binding behavior should be augmented both by experimentally determining the metal dependency of uncharacterized nitrogenases, as well as by laboratory restorations of ancestral nitrogenases.

## **Snowball Earth: The effect of viscosity on the multicellularity of *Chlamydomonas reinhardtii*** (Oral presentation)

**Andrea Halling**, Boswell Wing, Carl Simpson, Jesse Colangelo  
University of Colorado Boulder

The Snowball Earth hypothesis proposes that multiple times in Earth's history, Earth's surface froze over nearly entirely for many millions of years. The decreased temperatures of the Snowball Earth would have resulted in the increased viscosity of ocean waters under the global icecap. The most recent of these events is thought to be the trigger for the evolution of multicellularity resulting in the Cambrian explosion almost 550 million years ago. To test this hypothesis, the single-celled green algae, *Chlamydomonas reinhardtii*, was used in order to observe the effects that viscosity has on the development of multicellularity. An agar plate was created with three sections of media ranging in relative viscosities from 1X to 10X, and *Chlamydomonas* was inoculated at the low viscosity end. Growth was observed as the algae spread into the higher viscosity sections and two distinct phenotypes emerged. The phenotype in the lower viscosity tended to be diffuse and largely single celled. As the viscosity increased, discrete clumps became apparent. Microscopy was used to view the phenotypes in each section. The microscope images revealed that not only were the *Chlamydomonas* clumping as the viscosity increased, but in some cases the orientation of the flagella in these clumps were aligned in the same direction. These findings suggest that increased viscosity of ocean water as a result of Snowball Earth could have played a major role in the diversification of early life.

## **Preventing problematic photosynthesis in caves: Do lighting methods and substrate affect the development of destructive lampenflora in Carlsbad Cavern? (Poster #19)**

**Zoe Havlena**, Thomas Kieft, George Veni, Rod Horrocks, Daniel Jones  
New Mexico Institute of Mining and Technology

Photosynthetic communities of microorganisms including algae and cyanobacteria, termed "lampenflora," have been proliferating in Carlsbad Caverns National Park and other show caves worldwide since the adoption of artificial lighting systems used for illumination. These suites of organisms prove a detriment to the aesthetics of the cave and can physically degrade the underlying speleothems and other surfaces on which they grow [1]. With the goal of curbing this growth, the National Park Service recently modernized the lighting in Carlsbad Cavern to an LED system that allows for a range of adjustment of color temperature and intensity. This functionality of the new system was used to lower the color temperature of the lights to a range that should be less conducive to the photosynthesis.

We compared lampenflora biofilm development at sites with lights adjusted to high versus low intensity, on different substrate types using a several methods. To assess growth, high-throughput DNA sequence analysis of the microbial communities was used in conjunction with quantitative and semi-quantitative visual assessment of biofilm growth over time. Lampenflora response to decreased color temperature output in the new LED system was monitored with a handheld reflected-light spectrophotometer. This device was used to correlate change in color to relative change in photosynthetic cell density. 16S and 18S rRNA gene amplicon sequencing provided taxonomic identities and microbial community composition data.

The low biomass conditions at many of the study locations proved challenging for recovery of material for sequencing. Despite this, taxonomic identities and relative abundance data indicated a diversity of different bacteria, eukarya, and some archaea among the study sites. Several members of the photosynthetic community were observed in the biofilms, including the green algae Chlorophyta and several cyanobacteria. Some sites were observed to shift from a cyanobacteria predominant makeup to one of more eukaryotic-based photosynthesis during the maturity period, although these changes did not consistently correlate with substrate, lighting intensity, or lighting type.

The spectrophotometric data did not indicate any clear expected trend of increasing levels of growth over time, where instead some study sites saw a decreased growth. We were also surprised to see that there was only minimal differences in the rates of biofilm development at the lower color temperature sites versus higher. In terms of cave management, this may indicate that the lowered color temperature alone is not sufficient for reduction of lampenflora growth and additional reduction methods may be necessary.

### References

[1] J. Mulec, and G. Kosi, Lampenflora algae and methods of growth control (2009) *Journal of Cave and Karst Studies* 71, 109–115.

## **Recreating the ancient ocean in a borosilicate bottle (Poster #11)**

**Isaac L. Hinz**, Alexis S. Templeton, Jena E. Johnson  
University of Michigan

Insights into the ancient ocean are preserved in Banded Iron Formations (BIFs), comprised of fine chemical precipitates from ancient seawater. The original BIF minerals can be encased and preserved in early-silicifying chert, and recent studies have found well-preserved nanoparticles of iron-silicates in BIF chert at ~2.5 Ga (Rasmussen et al. 2015, 2016, 2017). After characterizing these iron-silicates as low Fe(III)-Greenalite (Johnson et al, 2018), we have been conducting research into the chemical and biological conditions needed to produce similar iron-silicates to help constrain the ancient ocean chemical and potentially biological conditions. We began with simple abiotic experiments involving various concentrations of dissolved Fe<sup>2+</sup> and silica under anoxic conditions at environmentally relevant pHs of 7-8.3, and poorly crystalline iron-silicates formed at a pH of ~ 8.2. To mimic diagenesis and help increase the crystallinity, we subjected the precipitates to hydrothermal aging experiments and initial X-ray diffraction results indicated the presence of clay structure(s). By iron X-ray absorption spectroscopy (XAS), the abiotic iron-silicates appear very similar to those observed as nanoparticles in chert, but they did not contain the levels of Fe<sup>3+</sup> observed in the rock record. Furthermore, these abiotic ferrous silicates require higher pHs to precipitate than expected for the Archean ocean (Halevy and Bachan, 2017). Therefore, we are also investigating whether iron oxidation, such as the activity of iron-oxidizing microbes or the presence of chemical oxidants, can trigger the precipitation of iron-silicates, particularly at lower pHs ~7 to 7.5. The activity of an Fe(II)-oxidizing phototroph, *Rhodospseudomonas palustris* (*R. pal*), produced an Fe(II-III) intermediate phase which further oxidized into an Fe(III) oxide similar to goethite. A small pulse of oxygen (~10<sup>-6</sup> atm) resulted in an initial goethite-like precipitate that then transformed to a mixed Fe(II-III) phase after aging in ferrous iron and silica. However, both Fe(II-III) phases were considerably more oxidized than the iron-silicate nanoparticles. Thus, our current work is focused on exploring the effects of low-level iron oxidation by *R.pal* in a Fe(II)-buffered and high-silica system as we continue to constrain the conditions necessary to produce low-Fe(III) greenalite.

## **Megafire microbial ecology: Impact on soils immediately after variable intensity wildfire (Poster #4)**

**Alexander S. Honeyman**, John R. Spear  
Colorado School of Mines

Wildfires are a historically natural occurrence in the American West. Nonetheless, recent fire seasons were the most intense and destructive in recorded American history. Fires are burning hotter, longer, and more frequently than years past, devastating financial reserves of suppression operations and obliterating entire cities. Large tracts of land, too, are left scarred with uncanny burn intensity—annually evoking photographs of moonscapes hardly reminiscent of the biome once present. In June, 2018, the ‘416 Fire’ ignited in Durango, Colorado and ultimately consumed 54,129 acres of forest and watershed. In an effort to characterize the effects of various burn severities on soils, we conducted a spatial microbiome study in conjunction with collation of satellite remote sensing data describing spectral fingerprints of burned lands. As a fusion study, we explore the interdependencies of burn severity and microbial community structure immediately after wildfire. Collectively, we aim to understand how relationships between microbiomes / genetic banks inform remote sensing measures and, inversely, how the ecological recovery trajectory of lands can be estimated by imaging from space.

## **Biological methane cycling in subsurface serpentinization-impacted waters** (Oral presentation)

**Emily A. Kraus**, Blake W. Stamps, Kaitlin R. Rempfert, Daniel B. Nothaft, Eric S. Boyd, Juerg M. Matter, Alexis S. Templeton, John R. Spear  
Colorado School of Mines

The subterranean biosphere hosts novel microbial life, the understanding of which has broad implications for early evolution on Earth and the emergence of life on other rocky planets. Cellular metabolism in the absence of surface-derived organic carbon must rely on chemical energy generated from subsurface water-rock reactions. One such water-rock interaction that can generate chemical energy for chemolithoautotrophic life is serpentinization, a process wherein mantle rock is hydrated resulting in the formation of molecular hydrogen that can be utilized by microorganisms as an electron donor. Methanogenic archaea use  $H_2$  and  $CO_2$  to produce methane in these conditions, but abiotic methane production is also thermodynamically possible, leaving the sources of methane in terrestrial serpentinizing environments undefined. In the Samail ophiolite of Oman, the microbial contribution to methane cycling is currently in debate with unusual  $\delta^{13}C_{CH_4}$  values fall outside typical abiotic/biotic values. To resolve the contribution of biology to the unusual observed  $\delta^{13}C_{CH_4}$  values in the Samail, we examined the composition, functional potential, and inferred functionality of microbial life in the ophiolite waters. Biomass for SSU rRNA, metagenomic, and metatranscriptomic sequencing was recovered from seven wells intersecting a range of rock and fluid types. Methanogenic, methanotrophic, and methylo-trophic activity was detected in hyperalkaline fluids and is thus likely impacting the isotopic composition of  $CH_4$  in the Samail ophiolite. These results support the relevance of biology in methane cycling in the deep subsurface of the Earth and indicate a possible need to reconceptualize biosignature ranges for  $\delta^{13}C_{CH_4}$ .

## **Needle litter decomposition induces soil biogeochemical shifts and selective pressures** (Poster #10)

**Laura Leonard**, Kristin Mikkelson, Zhao Hao, Eoin Brodie, Kenneth Williams, Jonathan Sharp  
Colorado School of Mines

A resurgence in Spruce Beetle infestation is occurring in Southwest Colorado causing concern for the broader impacts of bark-beetle infestation in mountainous forests. A unique aspect of bark-beetle tree mortality is a pulsed release of pine needles to the forest floor during tree death, which contributes additional organic matter to the surface. This increased supply of carbon and nutrients can impact terrestrial nutrient and carbon cycling with implications for ecosystem recovery and watershed function. The goal of this project is to isolate the relative biogeochemical contributions of different types of needle decay from rhizospheric processes to better understand the selective pressures of needle decay. To answer this, pine needles were harvested as a function of beetle-impacted spruce, non-impacted spruce, and non-impacted lodgepole pine and transported to open meadow plots where they were exposed to natural weathering processes over the course of two years. The chemistry of the needles changed over time with significant decreases in the C:N ratios of both types of spruce needles, and an increase in lodgepole needle C:N. The needle decay induced increases in soil respiration with enhanced  $CO_2$  production in contrast to needle-free controls. Respiration under lodgepole needles revealed the largest magnitudes over time, with values about twice that of needle-free controls during summer high moisture events. Modest increases in  $CH_4$  consumption under needle decay were also observed along with clear seasonal responses of increased  $CO_2$  production and  $CH_4$  consumption during high moisture events. These trends were analogous in the second year, but the magnitudes were lower due to drier soil conditions. Porewater was collected, and trends suggest higher averages of dissolved organic carbon under lodgepole and spruce needle decay; however, no trends were observed in association with dissolved nitrogen. These geochemical results reveal mechanistic shifts in soil carbon cycling over time that are largely driven by needle decay, while also responding to soil moisture. Microbial community analyses revealed clustering of bacterial and fungal communities that differentiated as a function of needle chemistry. Significant temporal shifts were also apparent with clustering as a function of time, with increased relative abundances of nitrogen fixers and heterotrophic microorganisms in response to increased needle decay. These results implicate the potential for biogeochemical shifts in soils as a function of litter chemistry and soil moisture that can aid in the prediction of functional ecosystem shifts within montane watersheds experiencing large-scale tree mortality.

## **Predicting compositions of fluids and geochemical energies for microbes in the deep continental serpentinizing subsurface (Poster #3)**

**James Leong**, Alta Howells, Kirtland Robinson, and Everett Shock  
Arizona State University

The serpentinization of ultramafic rocks generates some of the most reduced and alkaline fluids on Earth. These fluids provide energy for many microorganisms and thus have profound implications for the habitability of the deep subsurface. Subsurface geochemical energies that can be exploited by microorganisms can be quantified through analysis of deep-seated fluids sampled through wells or surface expressions (springs). However, analysis of several high-pH fluids from both wells and springs reveals ample oxidized dissolved species that could be introduced by atmospheric and/or shallow groundwater infiltration. In this work, we calculate the composition of serpentinization-generated fluids devoid of atmospheric and shallow groundwater influences using constraints from fluid-mineral equilibria. From these predicted compositions, we conclude that most hyperalkaline fluids have some minor extent (<5%) of surface/shallow subsurface influence. Some samples have less influence (<1%) and could be interpreted as the most pristine representatives of the deep subsurface. Available energy supplies through various redox reactions are also quantified using these predicted compositions, focusing on two microbial processes: hydrogenotrophic methanogenesis and hydrogen oxidation. Limited but available energy (0.1-6.0 joule/kg fluid) can be exploited by methanogens, permitting the possibility of deep communities in serpentinizing aquifers. More energy is available for methanogenesis (0.1-20 joule/kg fluid) and hydrogen oxidation (0-70 joule/kg fluid) when upwelling deep-seated fluids mix with shallow groundwater. Available energy for both reactions varies with mixing extent where methanogenesis is favored in fluids characterized by no to low extents of mixing while hydrogen oxidation is favored in systems characterized by higher extents of mixing.

## **Marine bivalve aspect ratio, shell size, and their relationship to a latitudinal gradient (Poster #2)**

**Sarah Leventhal**  
University of Colorado Boulder

In this study, I investigated the relationship that the morphological diversity and the species diversity of extant marine bivalves have with latitude. It is known that bivalve species diversity is latitudinally sensitive, with species richness peaking in the tropics and steadily declining towards the poles. The goals of this investigation were to determine whether the morphological diversity of bivalves is similarly impacted by latitude, and to assess the impact that variation in species diversity may have on the variation in morphological diversity. Shell aspect ratio and size were considered as morphological characters; these metrics were assessed across 3,831 extant marine bivalve species from every major ocean and spanning from the tropics to the poles. Additionally, three families of bivalves (the Veneridae, the Cardiidae, and the Philobryidae) were subject to further analysis to determine their individual morphological and species diversity patterns, as well as how much variance there is within different groups of bivalve taxa. The results indicate that bivalve aspect ratio and size are invariant with latitude on a global scale, but that some specific taxonomic groups of bivalves exhibit deviation from this large-scale occurrence.

## Effect of growth rate on the carbon and hydrogen isotopic composition of biomass and lipids in *Saccharomyces cerevisiae* (Poster #16)

Ashley E Maloney, Sebastian H Kopf, Xinning Zhang  
Princeton University

Cells have the fascinating ability to alter the quantity, form, and isotope composition of their biomolecules in response to changes in the external environment or to internal metabolic processes. Lipids are particularly useful geochemical biomarkers because they are sensitive, long-lived, and often source-specific. The hydrogen and carbon isotope ratios of lipids have been primarily used to reconstruct past abiotic parameters, but the striking response of hydrogen and carbon isotope ratios to metabolic shifts suggests that lipid isotopes can provide important information about energy metabolism. Metabolically flexible microbes are opportune organisms for testing the effects of metabolism on important geochemical markers.

Previous studies show that lipid hydrogen and carbon isotopes in prokaryotes are sensitive to different metabolisms, but how do heterotrophic eukaryotes respond to metabolic variability? Baker's yeast (*Saccharomyces cerevisiae*) is an ideal model organism since it is capable of overflow metabolism, a seemingly wasteful preference for aerobic fermentation when consuming glucose (instead of respiration when fed glycerol). In this study we grew ammonium-limited chemostat cultures of *S. cerevisiae* to assess the impact of substrate and growth rate on lipid hydrogen and carbon isotopes. Fatty acids from cultures grown at slower growth rates (1 d<sup>-1</sup>) were up to 20‰ <sup>3</sup>H-enriched compared to faster growing cultures (5 d<sup>-1</sup>), indicating decreases in lipid-water fractionations with slower growth. Similarly for carbon isotopes, biomass and fatty acids from slowly growing cultures were enriched (up to 1‰ for biomass and up to 8‰ for fatty acids) relative to faster growing samples, in this case representing increased sample-substrate fractionation at slower growth rates. No significant difference was detected between cultures fed glucose or galactose. The effect of chemostat-controlled growth rate on hydrogen isotope fractionation in heterotrophic eukaryotic microbes has not been previously reported, but the trend echoes that found in chemostat cultures of a eukaryotic phytoplankton and batch cultures of a heterotrophic prokaryote. This study highlights the sensitivity of lipid isotopes from eukaryotic microbes to fundamental metabolic properties.

## Origin and cycling of methane in the Samail Ophiolite, Oman (Oral presentation)

Daniel Nothaft, Hannah Miller, Jeemin H. Rhim, David T. Wang, Shuhei Ono, Sebastian Kopf, Juerg Matter, Mark Conrad, Eric Boyd, Alexis Templeton  
University of Colorado Boulder

The reaction of water and dissolved inorganic carbon (C) with peridotite can produce hydrogen (H<sub>2</sub>) and reduced C compounds capable of sustaining microbial life. Where peridotite hydration occurs, methane (CH<sub>4</sub>) is often the most abundant form of C in ultra-basic (pH>10) reacted waters, reaching mM concentrations. However, the mechanism(s) of CH<sub>4</sub> formation at low temperature and the role of microbes in the subsurface production and consumption of CH<sub>4</sub> in peridotites are yet to be established. Here, we report geochemical, isotopic, and molecular microbiological data from subsurface waters in the Samail Ophiolite, Oman, a site of present-day peridotite weathering at temperatures <60°C. Ultra-basic waters with high [CH<sub>4</sub>] (>100µM) exhibit intra-molecular isotopic disequilibrium and elevated C<sub>1</sub>/(C<sub>2</sub>+C<sub>3</sub>) ratios (~10<sup>-2</sup>-10<sup>-5</sup>) suggesting that a considerable proportion of CH<sub>4</sub> is microbially produced. We find that 16S rRNA gene sequences associated with CH<sub>4</sub>-cycling taxa are ubiquitous in the aquifer. Waters with the highest proportion of methanogenic 16S rRNA gene sequences in our sample set (~20% of total reads) also hosted CH<sub>4</sub> with radiocarbon dates of 9.6-13.3 ka. This suggests that methanogens are metabolizing C compounds that were relatively recently derived from atmospheric CO<sub>2</sub>. Elsewhere in the ophiolite, CH<sub>4</sub> radiocarbon dates are indistinguishable from blanks, indicating that CH<sub>4</sub> is derived from C that was last in contact with the atmosphere >50ka. The diversity of hydrogeologic regimes in our study allow us to interrogate how microbial methanogenesis functions at high pH conditions where CO<sub>2</sub> is scarce. Specifically, δ<sup>13</sup>C values of CH<sub>4</sub> sampled from boreholes on opposite sides of a geologic contact range from -73±13‰ VPDB (3-year mean ±1 sd) to +4±3‰ VPDB (4-year mean ±1 sd) in the gabbro- and peridotite-hosted contact boreholes, respectively. This drastic variation may be explained by groundwater mixing resulting in hydrogenotrophic methanogenesis under a

spectrum of C-limited to H<sub>2</sub>-limited conditions. This study provides new insights into the CH<sub>4</sub> dynamics and fate of C in ultramafic rocks undergoing low-temperature hydration and carbonation.

**Evaluating hydrothermal microbial communities and associated insights into the rock record** (Poster #5)

**K. Rasmussen**, B.W. Stamps, S.M. Ulrich and J.R. Spear  
Colorado School of Mines

Hydrothermal ecosystems have been posed as potential environments for the origin of life. Additionally, hydrothermal sites excel at lithifying the cooler surrounding environment, leading to the entrainment of surrounding biota (macro- and micro-) into the rock record. Silicification of microorganisms is a mechanism by which microbiota may be lithified and preserved as resilient microfossils, making silicified environments ideal for the study of biosignatures from early Earth and possibly other planets. However, there is much debate regarding the microbial role in silicification, and gaps in knowledge are widespread regarding the entirety of microbial lineages involved and possible ecosystems where silicification can occur. Furthermore, the silicified rock record is dominated by both filamentous and coccoidal cells, indicating certain morphologies silicify more readily than others. When drawing conclusions from the rock record, researchers are faced with the stark reality that entire lineages of microorganisms and environmental insights may be unaccounted for due to observed preferential silicification. The focus of this work is to advance our understanding of the microbial response to silica precipitation and biomineralization at an actively silicifying hot spring. This study may reveal novel information regarding life on early Earth and serve as an analog for similar dormant environments on Mars. This project encompasses biological and water chemistry sampling at Steep Cone Geyser (Yellowstone National Park), evaluation of potential biogeochemical cycles, and 16S rRNA gene microbial community analysis of the microbial mats over both spatial and temporal transects along the outflow channels. Microfossil samples along a time series rockfall have been examined using an FE-SEM. Lastly, morphological inferences were made from the 16S data and compared to the FE-SEM results to evaluate possible discrepancies between the rock record and living microbial community.

**Salinity tolerance in cyanobacteria** (Oral presentation)

**Jennifer Reeve**, Boswell Wing  
University of Colorado Boulder

Oxidation of the Earth's atmosphere occurred at the Great Oxidation Event (GOE) approximately 2.3 billion years ago, however evidence for localized oxic conditions exists in the geologic record dating back to 3.0 billion years ago. Cyanobacteria are believed to have been the driving force behind the oxygenation of Earth's atmosphere, and there are two basic hypotheses about how they effected this major geobiological transition. One hypothesis states that once Cyanobacteria arose, the oxygenation of Earth's atmosphere occurred rapidly and immediately as the energetic potential of oxygenic photosynthesis is significantly higher than most other autotrophic metabolisms. A second, 'ecological' hypothesis states that Cyanobacteria were restrained to freshwater benthic habitats such that oxidation was limited to localized events until they evolved the ability to thrive in marine environments, thereby explaining the delayed oxygenation of Earth's atmosphere. One key aspect of this hypothesis is that reconstructions of the ancestral habitats of early Cyanobacteria imply a strong intolerance to seawater salinity levels. However, these ancestral reconstructions of salinity tolerance in Cyanobacteria have been based on the habitats of isolation of modern Cyanobacteria and a consideration of salinity tolerance as a discrete, rather than continuous, cyanobacterial trait. In this presentation I will evaluate these assumptions through a meta-analysis of literature data as well as targeted laboratory experiments with key strains of modern Cyanobacteria. My preliminary results suggest we may need to rethink the current characterization of ancestral Cyanobacteria as isolated to terrestrial environments.

## **More than meets the eye: Microbial communities and their viral predators govern carbon and nitrogen transformations in the hyporheic zone (Poster #12)**

**Josué Rodríguez-Ramos**, Mikayla Borton, Garrett Smith, Lindsey M. Solden, Rebecca Daly, Jorge Villa, Emily B. Graham, Samuel O. Purvine, Evan Arntzen, Hyun-Seob Song, William C. Nelson, Mary Lipton, Gil Bohrer, James Stegen, Kelly C. Wrighton  
Colorado State University

Microorganisms are known catalysts for carbon and nitrogen biogeochemical cycles in hyporheic zones, yet the microbial metabolisms underlying these processes are largely unknown. Moreover, the viral ecology of river sediments is to a great extent unexplored. Here, we performed metagenomic and metaproteomic analyses on 6 sediment cores, with samples collected at 10 cm intervals from the surface to 60 cm in depth. We reconstructed 67 near-complete genomes from the 5 most abundant and active microbial lineages including members of Nitrospirae, Thaumarchaeota, Firmicutes, Proteobacteria, as well as members from 6 other phyla. Genome metabolic predictions indicated that ammonium is a central metabolite connecting all organisms in this system. Expression data confirmed that organic nitrogen degradation (e.g. peptides and amino acids) by Proteobacterial and Firmicutes members provides a source of ammonium to the system. One of the most dominant and active members in this system, the Thaumarchaeota (now called Crenarchaeota), oxidize ammonium into nitrite. Subsequent nitrite oxidation to nitrate by members of the Nitrospiraceae fuel denitrification yielding nitrous oxide and other nitrogen gasses. Evidence for carbon transformations include autotrophic carbon dioxide utilization or heterotrophic sources utilizing phenolics, which could provide simple sugars for respiration. Consistent with our proteomic data, porewater and chamber measurements demonstrated the greatest nitrous oxide concentrations near the sediment surface, with nitrous oxide and carbon dioxide fluxes governed by river stage. Our viromics analyses recovered 412 unique viral populations, several of which may contribute to carbon and nitrogen cycling in this system. In silico predictions linked a 19 kb viral genome to 3 active Thaumarchaeota genomes, demonstrating viral predation of nitrogen cycling microorganisms. Viruses also encoded glycoside hydrolase and amino acid genes, indicating viral influence of carbon degradation and ammonium formation in this system. Together these findings uncover microbial and viral roles that impact carbon and nitrogen fluxes from river systems.

## **Rare earth element interactions with wastewater treatment microbial communities (Poster #14)**

**Olivia Salmon**, Gary Vanzin, Sarena Nguyen, Junko Munakata-Marr, Yoshiko Fujita  
Colorado School of Mines

As industrial and medical uses of rare earth elements (REEs) become more common, the presence of REEs in waste streams discharging to wastewater treatment facilities is expected to increase. To date, little is understood about interactions of REEs with the microbial communities that are at the heart of wastewater treatment systems.

To investigate these relationships, fed-batch, bench-scale bioreactors were developed to mimic aerobic activated sludge and anaerobic sludge digester conditions in wastewater treatment systems. Aerobic and anaerobic test reactors are treated with high-priority REEs as salts and chelated compounds. Wastewater quality parameters such as chemical oxygen demand are tested weekly in aerobic reactors and bimonthly in anaerobic reactors. No significant impacts on wastewater treatment effectiveness in either bioreactor system have been observed from lower REE treatments ranging from 0.09 to 20 ppm. Preliminary results indicate potential inhibition of nitrification processes in aerobic bioreactors treated with 50 ppm of gadolinium and yttrium salts.

In tandem with these efforts, we prepared culture plates amended with treatments of high-priority REEs and inoculated with mixed wastewater cultures. Early results indicate complete inhibition of colony growth on media treated with yttrium at concentrations that exceed 260 ppm and on media treated with neodymium at concentrations of 400 ppm. Partial inhibition has been noted on media treated with 400 ppm gadolinium, lanthanum, or europium. Culture plate results will inform additional REE treatments in bench-scale bioreactors and be used to further explore the relationships between priority REEs and the microbial communities of wastewater treatment systems. Ultimately, this research will shed light on potentially harmful interactions of REEs with wastewater treatment systems and, if needed, inform future preventative and remedial strategies to address these impacts.



## **Precipitation of carbonate by Great Salt Lake microbialite communities: Geochemical modeling and laboratory experiments (Poster #18)**

**Danielle Saxer**

Weber State University

Great Salt Lake (GSL) is home to extensive beds of microbialites, rocks assumed to have been formed by microorganisms found on and within the structure. Microbialites are among the best pieces of evidence for the early emergence of life on Earth and they record information about past environments. Understanding how and under what conditions they form will help us understand ancient life and environments. The GSL offers a living laboratory for understanding how microbialites form in terrestrial environments, what they record in terms of environmental parameters, and how microbes influence their growth and chemistry. One outstanding question is which organisms (if any) are responsible for building the GSL microbialites. This study explores the potential of several organisms found in the GSL microbialites to influence carbonate saturation and drive the precipitation of calcium carbonate and formation of the microbialites. PHREEQC was used to model carbonate saturation in waters surrounding a microbialite reef at Ladyfinger Point on Antelope Island State Park. The effect on carbonate saturation of microbial metabolisms and shifts in environmental parameters were also modeled. In addition, we conducted preliminary laboratory experiments to monitor precipitation of carbonate under lake-mimicking conditions under the influence of several different microbial communities, some of which facilitated carbonate precipitation, while others (including photosynthesis by cyanobacteria) did not. Here we present preliminary results of ongoing long-term precipitation experiments.

## **Does increased nutrient availability allow microbes to avoid water limitation in extreme environments? (Oral presentation)**

**Solon, Adam J., Vimercati, L., Darcy, J.L., Gendron, E.M.S., and Schmidt, S.K.**

University of Colorado Boulder

As essential building blocks of cellular structures, nitrogen (N) and phosphorus (P) are required components of all known life. The search for life beyond Earth may require consideration of where and when nitrogen and phosphorus, along with liquid water, are available for biological use. In cold, dry, and nutrient-limited ecosystems, resource pulses of water, nitrogen, and phosphorus may be necessary for the persistence of multi-trophic microbial communities and can inform how life might survive in extraterrestrial locations. Our recent research in a glacial forefield in the Peruvian Andes demonstrated that the addition of P to field soils allows the development of complex microbial communities in sites that were previously thought to be limited by cold and low water availability. These findings challenged our understanding of the cold-dry limits for life on Earth and perhaps elsewhere in the Universe. In this talk I will briefly review the physiological explanation for these findings from Peru and discuss my ongoing research to test this nutrient-limitation/pulse-growth hypothesis in even more extreme environments such as the high volcanoes of the Atacama Desert and the Dry Valleys of Antarctica and the Himalayas.

## **Carbon chemistry of intact versus chronically drained peatlands in the southeastern USA (Poster #21)**

**Craig A. Stricker**, Judith Z. Drexler, Kevin A. Thorn, Jamie A. Duberstein, and Sam Rossman  
United States Geological Survey

The Great Dismal Swamp (GDS) is a large temperate swamp in Virginia/North Carolina with peat soils historically resistant to microbial decomposition. However, this peatland has been subject to ~200 years of disturbance during which extensive drainage, fire suppression, and wide-spread logging have increased decomposition and dramatically decreased the distribution of Atlantic white cedar (AWC). The purpose of this study was to determine the impact of long-term drainage and AWC loss on the carbon chemistry of GDS peats. Peat cores were collected from three drained GDS vegetation communities (pocosin, AWC, and red maple-black gum) and compared to cores collected from an intact, undrained AWC peatland at the Alligator River National Wildlife Refuge (AR) in North Carolina, USA. The AR peats had higher lignin content in the deeper peat intervals, and lignin content and % organic carbon were largely invariant with depth compared to the GDS peats. The concentrations of syringyl group phenols were greater in the surface layers of GDS peats, likely reflecting the selective removal of AWC and transition from gymnosperms to angiosperms. Acid to aldehyde ratios for vanillyl and syringyl group phenols indicated that the GDS peats were more decomposed, particularly at depth, and that this occurred under aerobic conditions. Moreover, solid state  $^{13}\text{C}$  NMR confirmed a coincident loss of carbohydrates and increase in recalcitrant byproducts of carbohydrate degradation with depth. These data indicate that long-term drainage has accelerated the decomposition of peat at the GDS, reducing the capacity and stability of the carbon sink.

## **Growth characteristics of planktonic and particle-associated states by sulfate-reducing bacteria (Poster #20)**

**Kasdi Sujono**, Jesse Colangelo, Boswell Wing  
University of Colorado Boulder

The distribution of sulfur isotopes in geologic materials reveals information about Earth history and biogeochemical processes, and largely attributed to microbial processes in the sediment, specifically by sulfate-reducing bacteria (SRB). The interplay between growth conditions, microbial growth, and isotopic fractionation have been investigated in vitro and modeled in silico, but only in homogenous, suspended cultures, (planktonic state), rather than in environments more representative of contemporary environments, where SRB are sediment associated (particle-associated state). In this study, we investigate the growth characteristics and sulfur isotopic fractionation by the sulfate-reducing bacterium, *Desulfovibrio vulgaris* strain Hildenborough (DvH) in planktonic and particle-associated states. Assessment on the growth characteristics of DvH showed that the cell-specific sulfate-reduction rate (csSRR) in the planktonic state (PLK-PLK) = 293 fmol  $\text{SO}_4^{2-}$ /cell/day, and the particle-associated component the particle-associated state (PA-PA) = 228 fmol  $\text{SO}_4^{2-}$ /cell/day were comparably similar, with the differences of  $\pm 65$  fmol  $\text{SO}_4^{2-}$ /cell/day. As for the planktonic component of the particle-associated state (PLK-PA) showed a very low csSRR, at 0.26 fmol  $\text{SO}_4^{2-}$ /cell/day. These similarities between PLK-PLK and PA-PA cultures showed SRB metabolized similarly regardless the environment. However, within a mixed condition (between PA-PA and PLK-PA), SRB seems to favor to metabolize while associated with the surrounding particles. The results of our research indicate that current experiments designed to study the sulfate-reducing bacteria are appropriate to be performed without considering the actual physical environment representative of the one in which SRB are actually reducing sulfate.

## **Temporal insights into subsurface microbial ecology and rock-equilibrated fluid geochemistry at Edgar Experimental Mine (Poster #13)**

**Patrick Thieringer**, Alex Honeyman, Matthew Schreiner, John Spear  
Colorado School of Mines

Microbial communities inhabiting subsurface ecosystems are taking precedence in providing insights into early life on Earth and possible analogs for planetary studies. These subsurface ecosystems harbor unique metabolic pathways allowing studies to investigate the microbial adaptations necessary to thrive in otherwise unfavorable conditions. Following groundwork conducted by a previous monitoring project, this study aims to reveal temporal relationships alongside geochemical and microbiological analyses of microbial communities present at Edgar Experimental Mine. Previously drilled boreholes were fitted with a packer to collect leaking groundwater and were periodically extracted. Samples retrieved from extractions occurred beginning after an approximately 2 year dormant period from the initial study, and then collected every 3-4 weeks for the following 3 months. Fluid extractions were syringe microfiltered for DNA extraction and 16s rRNA gene sequencing while the collected fluid samples were processed through IC/ICP for geochemistry. Extracting microbial communities over both short and long intervals offers the opportunity to examine not only how geochemistry fluctuates over varying periods of recharge, but also how phylogeny functions over dormant versus rapid incubation periods. This will provide deeper insight into the dominant microbial members unveiling more into the potential metabolic processes important to subsurface communities of this mine. By investigating the temporal effects, more can be understood about how fluid-rock interactions affect the fluid geochemistry over periods of fluid build-up, as well as how hydrological microbial communities can grow and adapt.

## **Is the IPCC perhaps underestimating Earth's climate sensitivity? Insights from clumped isotopes in pedogenic siderites from the PETM (Oral Presentation)**

**Joep van Dijk**  
University of Colorado Boulder

## **Nitrogen cycle dynamics during the Cretaceous Oceanic Anoxic Event 2 (OAE2) in the Western Interior Seaway (Poster #7)**

**Jonn van Oosten-Edl**, F. Garrett Boudinot, Brett Davidheiser-Kroll, Kathryn Snell, Julio Sepúlveda  
University of Colorado Boulder

The Cretaceous Ocean Anoxic Event 2 (OAE2, ~94 Ma) was a time of high sea-level and warm greenhouse climate, and was characterized by enhanced marine productivity and carbon burial. These conditions resulted in the widespread deposition of black shales and a global perturbation of the carbon cycle. While decades of research have disentangled some of the complex biogeochemical dynamics during OAE2, a detailed understanding of the nitrogen cycle during this event (i.e., nitrogen transformations through redox-controlled microbial processes), remains elusive. We present a high-resolution (centennial-to-millennial time scales) bulk nitrogen stable isotope record from an expanded sedimentary section of OAE2 in the Smokey Hollow #1 core (SH1, southern Utah) that provides a record of changes in marine ecology and biogeochemistry from a marginal setting in the Western Interior Seaway (WIS). By comparing our record to data from planktonic ecology (e.g., foraminifera, nanoplankton, algal biomarkers) from the same site, as well as other nitrogen isotope records during this event, we propose a schematic model for nitrogen cycling that invokes changes in marine productivity, ecological successions, water circulation, and redox conditions across OAE2. A new astronomical timescale is used to estimate rates of change and temporal relationships between nitrogen cycling and other biogeochemical and climatic feedbacks. We will discuss potential implications of nitrogen cycling during OAE2 for the study of global change in the Anthropocene.

**Influence of diatom photosynthesis on denitrification activity within a benthic, open water wetland biomat** (Poster #9)

**Michael Vega**, Adam Brady, Evelyn Lundeen, Kristin Mikkelson, Jonathan Sharp  
Colorado School of Mines

Constructed wetlands offer a unique opportunity to investigate fundamental biogeochemical processes in a managed, engineered environment. Located in southern California, the Prado Wetlands contain open water cells characterized by a shallow water column, geotextile lined bottom, and absence of macrophytes, selecting for a benthic microbial community that alleviates nitrogen loading into the Santa Ana river. Phylogenetic inquiry revealed this thick (5–15cm), photosynthetic ‘biomat’ assemblage to contain diatoms complemented by a diverse array of heterotrophic bacteria. Previous research characterizing nitrogen loss pathways within the biomat demonstrated compelling rates of denitrification with contributions from anammox, yet the effect of photosynthesis and respiration on nitrogen cycling remains uncertain. The purpose of this study was to explore how photosynthesis driven redox shifts influence the spatial and temporal distribution of denitrification activity within the biomat. We hypothesized that denitrification occurs just beneath the zone of photosynthesis and is most pronounced at night, when the distance to the oxic/anoxic interface is minimized. To this end, biomat porewater was collected using a modified minipoint sampling technique and cores were flash frozen and subsectioned at depths complementary to porewater for genomic and geochemical inquiry. Current results suggest that redox shifts associated with photosynthesis and respiration influence denitrification functionality, with activity being concentrated in the upper  $\approx 2$ cm and most prevalent at night. This work aims to enhance our understanding of diel nutrient cycling in wetlands and sheds light on how benthic photosynthesis can influence denitrification in both engineered and natural systems.

**Soil microbial communities and seedling success** (Poster #15)

**Corinne M Walsh**, Noah Fierer  
University of Colorado Boulder

Interactions between plants and soil microbes are critical to ecosystem functioning and the maintenance of plant productivity in both unmanaged and agricultural ecosystems. There is considerable academic and commercial interest in understanding the identities and effects of soil microbes surrounding and inside plants, but despite the ubiquity of these relationships, the identities, dynamics, and controls of many plant-associated microbial communities remain poorly resolved. While some plant growth promoting interactions are mediated by soil-dwelling microbes, many bacteria and fungi can reside in plant tissues and function as endophytes. Here I investigate symbiotic and potential “probiotic” microbes in wheat (*Triticum aestivum*), a crop plant that accounts for  $\sim 30\%$  of all the cereal grain consumed in the world today.

With a collection of over 200 natural and agricultural soils, I have established a laboratory-based platform to study aspects of the plant-microbe relationship in the context of naturally derived soil microbial communities. I examine the effects and dynamics of native soil microbial communities on the early life of wheat using a culture-independent approach (via 16S and ITS high-throughput sequencing) combined with an in-vitro seedling germination assay. This research strategy is designed to answer the following questions: Q1: To what extent do native soil microbial communities influence wheat seed germination success? Q2: Across a large range of soil types with distinct soil microbial communities, what are the specific microbial taxa, or combinations of taxa, that promote germination success? Q3: Across this same range of soil microbial communities, are there specific soil-derived lineages that are consistently recruited into the host plant tissue and become endophytes? By combining a large number of diverse source microbial communities with a controlled experimental design, this work will provide important insight into the effects of soil-dwelling microbes for an agriculturally important plant.