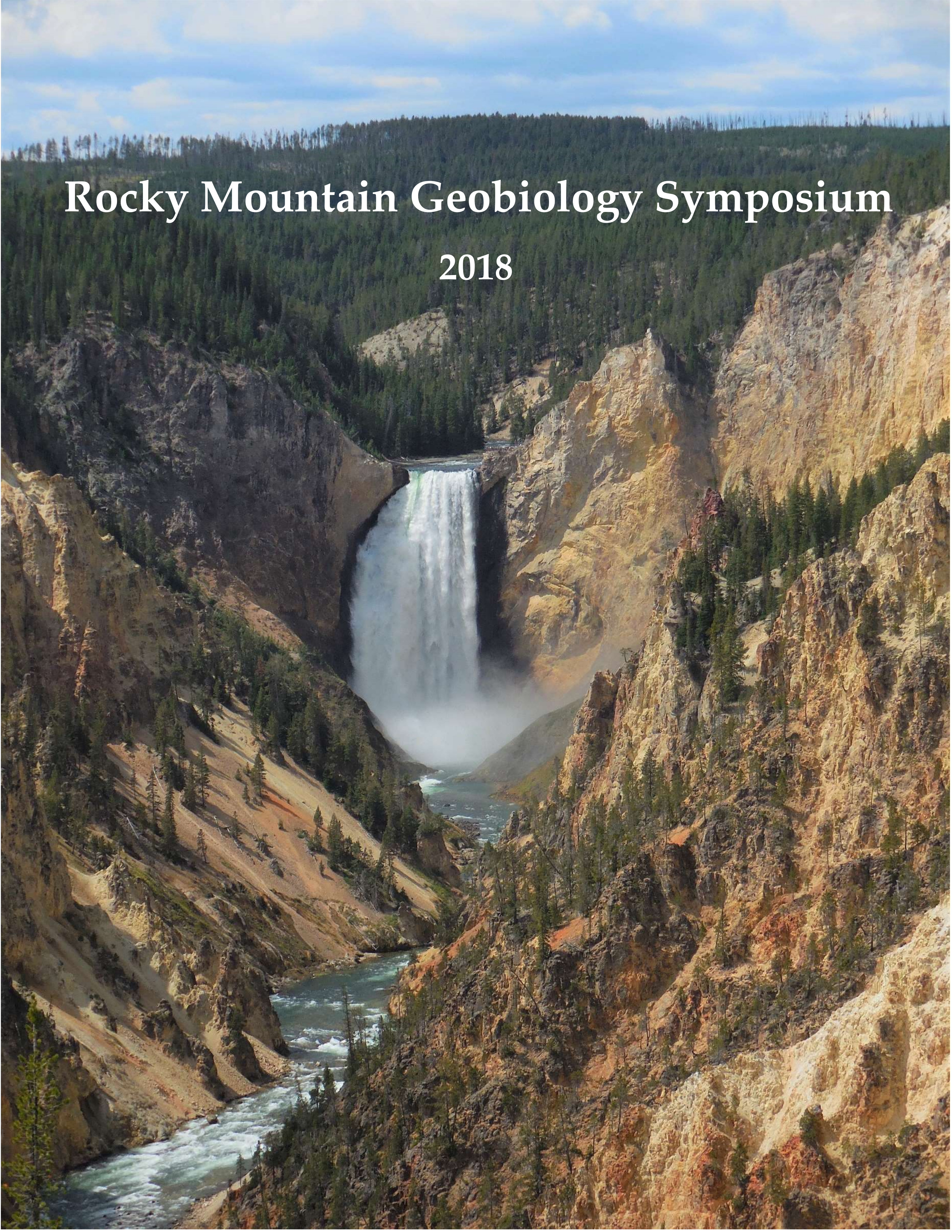

Rocky Mountain Geobiology Symposium

2018

Hosted by:
Emily Kraus, Dr. Blake Stamps, and Dr. John Spear
Colorado School of Mines

Logo design:
Kalen Rasmussen

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Time	Event
8:15 am	**Registration opens, coffee and bagels provided**
8:45	**Opening remarks – *Dr. John Spear, Dr. Blake Stamps, Emily Kraus***
9:00	**Benjamin W. Johnson -** Insights into Paleoarchean ocean conditions: inverse modeling from the Panorama District, Australia
9:30	**Michael P. Manzella -** Seeing the trees for the forest of microbial community biomass stoichiometry
10:00	**Aaron Meilijson -** Life in a pinch of salt: organic proxies for the ecology and oceanographic environment of the deep Mediterranean Messinian salinity crisis
10:30	**Julia McGonigle -** Diversity and Metabolic Characterization of the Bonneville Salt Flats
11:00	**Karen Wawrousek -** Microbial communities in produced waters from the Greater Green River Basin in Wyoming
11:30	**Lunch**
12 – 1:30pm	**Poster session – Coolbaugh Atrium**
1:30	**Donna L. Drogos** - Biogeochemistry of Produced Water from Unconventional Wells in the Powder River Basin, Wyoming
2:00	**Annika Mosier** - Unexpected versatility in the metabolism and ecophysiology of nitrite-oxidizing *Nitrotoga* sp. in freshwater habitats
2:30	**Jesse Colangelo-Lillis -** Syntrophy-driven evolutionary adaptation of a sulfate reducing bacterium diminishes its non-syntrophic growth physiology and alters its isotope phenotype in an unexpected direction
3:00	**Victoria Petryshyn, Invited Talk -** Everything I thought I knew was wrong: lessons from the stromatolites of Walker Lake
3:30	**Keynote Address – *Dr. Annette Summers Engel***
4:45	**Closing remarks – *Dr. John Spear, Dr. Blake Stamps, Emily Kraus***
6:30 pm	**Reception – New Terrain Brewing with dinner & drinks provided.**

Oral Presentations:

Insights into Paleoarchean ocean conditions: inverse modeling from the Panorama District, Australia

Benjamin W. Johnson, Boswell Wing.
University of Colorado Boulder

The Paleoarchean represents a key transition in Earth history. The appearance of a continuous, recognizable rock record and the appearance of key metabolisms (e.g., nitrogen fixation) indicate both the biosphere and geosphere are evolving in novel ways. Determining surface conditions such as temperature, nutrient availability, and ocean depth would greatly assist interpretation of the geologic record. We use existing O-isotope data from the Panorama District in the Pilbara of Australia to determine seawater composition and temperature.

Seeing the trees for the forest of microbial community biomass stoichiometry

Michael P. Manzella, E.K. Hall.
Colorado State University

In community ecology it is often possible to examine multiple phenotypic aspects, traits, for each member of the community. For example, if examining a forest, it is easy to measure the height and diameter at breast height of individual trees. However, in microbial community ecology, it is difficult to measure traits for individual microorganisms in situ due to technical constraints. Thus, microbial traits are commonly measured at the community level, masking the individual phenotypes that contribute to the community average. Single-cell techniques, however, are able to unmask this hidden diversity. At Colorado State University, we employed a technique (energy dispersive spectroscopy (EDS)) capable of measuring carbon (C), nitrogen (N), and phosphorus (P) within single bacterial cells with femtomolar detection limits. Using EDS, we can determine the elemental makeup, stoichiometry, of single bacterial cells within populations and communities. We show that the mean biomass C:N:P generated by EDS had similar values to those obtained by established bulk methods. However, the application of EDS also allowed us to assess the phenotypic plasticity and diversity of biomass stoichiometry within the population at different timepoints during a bacterial growth curve. Finally, we examined biomass C:N:P within individual bacteria in naturally-occurring microbial communities from three Colorado lakes. We show that clonal populations generated substantial phenotypic diversity in response to changing environmental conditions, and that this response differed among bacterial taxa. However, the distribution of these phenotypes was normally distributed around a local maximum in a probability density function. Within the natural bacterial communities, we show that both N:P and C:P contained a number of local maxima, indicating the presence of different stoichiometric guilds within each community. The reasons for these stoichiometrically-divergent populations is unclear, but are likely related to metabolic state, phylogeny, or the existence of microenvironments where conditions differ. Divergence in the stoichiometric distribution between these two experiments points to the importance of single-cell determinations of microbial stoichiometry to uncover phenotypic diversity in microorganisms.

Life in a pinch of salt: organic proxies for the ecology and oceanographic environment of the deep Mediterranean Messinian salinity crisis

Aaron Meilijson, Yizhaq Makovsky, Josh Steinberg, Frits Hilgen, Julio Sepúlveda.
INSTAAR, University of Colorado Boulder

The Mediterranean Messinian salinity crisis (MSC) is perceived as an environmental crisis governed by climatic and tectonic controls, which affected the salinity of the global ocean and influenced the biogeochemistry of the Mediterranean Sea. Ongoing research of the MSC in marginal and intermediate sections is coupled with new offshore seismic data and previously inaccessible wells from the Levant Platform. This new material allowed us to produce a stratigraphic model and test hypotheses about the distal occurrence of the MSC. High-resolution sedimentological, faunal and geochemical data from the entire massive Messinian evaporite section of the deep Mediterranean basin allows us to constrain that the deposition of the salt occurred during the complete 640 kyr-long event rather than limited to the 50 kyr 'MSC acme' as previously thought. Moreover, the deep-basin of the Levant was barren of eukaryotic life throughout most of this duration. Thus, brine formation, salt precipitation, and faunal extinction seems to have taken place in a non-desiccated basin with a restricted but often open connection with the Atlantic Ocean, which provides a new understanding of the mechanisms governing salt deposition during the MSC, as well as the geologic record by analogy. This presentation will focus on the results of a detailed organic-geochemical and geobiological characterization of these deposits. Our goal is to characterize the microbial life and the physical-chemical properties (e.g., sea surface temperature, salinity, and redox conditions) that dominated this extreme environment through the analysis of lipid biomarkers.

Diversity and Metabolic Characterization of the Bonneville Salt Flats

Julia McGonigle, William Brazelton.
University of Utah

An 18 square mile salt flat on Mars has recently been speculated to be a remnant of the last large lake which may have potentially hosted life on the red planet. Similar large scale salt deposits exist on Earth, such as the Bonneville Salt Flats in Utah. These salt flats are a remnant of a massive lake that stretched further than the Great Salt Lake in the Pleistocene. Although these salt flats on Earth currently undergo ephemeral wet/dry cycles the salt flats on Mars no longer experience, they nonetheless represent a Mars analog and provide the opportunity to learn more about extreme ecosystems that support microbial communities on Earth.Comprehensive microbial studies have been conducted on salt flats in other locations, but to date all studies done at the Bonneville Salt Flats have been limited to culture-based approaches. We are using culture-independent approaches for the first time to investigate the microbial community of these salt flats. Sequencing of the universal taxonomic marker, the 16S rRNA gene, was performed on DNA extracted from 8 locations. Diversity and species composition was compared between pits. Metagenomic sequencing was also performed to determine microbial role in nitrogen and sulfur cycling and hydrocarbon degradation. In addition to sequencing efforts, incubation experiments using media enriched with 13C- glucose, acetate, and bicarbonate were done to investigate activity levels of the microbial community.

Microbial communities in produced waters from the Greater Green River Basin in Wyoming

Karen Wawrousek, Donna Drogos, Mike Urynowicz, Charles Nye, Scott Quillinan.
University of Wyoming

Despite the prevalence of unconventional natural gas production, little is understood about the downhole microbial ecosystems and how these microbes affect biogeochemical reactions. To better understand this naturally occurring ecosystem, Illumina MiSeq 16S rDNA sequencing was used to identify microbes brought to the surface with the produced waters of five hydraulically fractured wells. Samples were collected at the water-gas separator of each well, and well depths ranged from approximately 9,500ft to 11,500ft. Correlations between pH, salinity, and metals naturally present in the groundwater, as well as biocides used during fracturing and production, were made when analyzing differences in microbial communities. Preliminary results identify several bacterial and archaeal families including: Clostridiales, Thermoanaerobacterales, Synergistales, Alteromonadales, and Thermotogales. Of the 5 sampled wells in the Green River Basin, 16 microbes were identified in all samples. These microbes common to all 5 samples include Anaerobaculum, Thermovirga, and an unclassified Clostridiaceae. Unclassified 16S sequences representing unique unidentified microbes are analyzed for their presence in multiple samples, and microbial populations across wells are analyzed to better understand the microbial communities present in these exotic environments.

Biogeochemistry of Produced Water from Unconventional Wells in the Powder River Basin, Wyoming

Donna L. Drogos, Karen E. Wawrousek, Michael A. Urynowicz, Charles W. Nye, Scott A. Quillinan.
University of Wyoming

Microbial activity in waters associated with unconventional oil and gas reservoirs can profoundly affect options for treatment, storage, disposal, and reuse of produced water (PW); frac fluid composition; biocide use; near borehole reservoir properties; and microbially induced gas production. Improved identification of microbial communities is required to develop targeted solutions for detrimental microbial activity such as biofouling and to exploit favorable activity such as microbial induced gas production. We quantified the microbial communities and inorganic chemistry in PW samples from six unconventional oil and gas wells in the Powder River Basin in northeast Wyoming. The wells are horizontal completions in the Frontier, Niobrara, Shannon, and Turner formations at depths of 10,000 to 12,000 feet, with PW temperatures ranging from 93OF to 130OF. Biocides utilized in frac fluids primarily included glutaraldehyde and Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) with first production occurring in 2013. Geochemical results for PW are: pH 6.5 to 6.9; alkalinity (as CaCO3) 219 to 519 ppm; salinity 13,200 to 22,300 ppm; and TDS 39,364 to 62,725 ppm. Illumina MiSeq 16S rRNA sequencing identified the majority of communities in PW are related to anaerobic, thermophilic, halophilic, chemoheterotrophic, and chemoorganotrophic bacteria, including Thermotoga, Clostridiaceae, Thermoanaerobacter, Petrotoga, Anaerobaculum, Clostridiales, Desulfomicrobium, and Halanaerobiaceae. These findings are important for identification of biogeochemical reactions that affect the organic-inorganic-microbial interactions among reservoir rocks, formation waters, and frac fluids. These biogeochemical reactions have implications for the handling and reuse and of frac fluids; determining biocide application; discouraging detrimental effects such as biofouling; and identifying the potential for biogenic gas production.

Unexpected versatility in the metabolism and ecophysiology of nitrite-oxidizing *Nitrotoga* sp. in freshwater habitats

Annika C. Mosier, Andrew M. Boddicker.
University of Colorado Denver

Nitrite-oxidizing bacteria (NOB) play a critical role in the removal of nitrogen pollution from freshwater systems by metabolizing nitrite to nitrate, which is then removed via assimilation, denitrification, or anammox. Recent studies revealed that NOB are phylogenetically and metabolically diverse, and yet most of our knowledge of NOB comes from only a small number of cultured representatives from marine and engineered systems. Using enrichment methods and genomic sequencing, we identified four novel *Candidatus* Nitrotoga NOB species from freshwater sediments and water column samples in Colorado, USA. Genome assembly revealed highly conserved 16S rRNA gene sequences, but a surprisingly broad diversity in metabolic potential including genes for nitrogen, sulfur, hydrogen, and organic carbon metabolism. Genomic predictions suggest that *Nitrotoga* can metabolize in low oxygen or anaerobic conditions, which may support a previously unrecognized environmental distribution of these organisms. An array of antibiotic and metal resistance genes likely allows *Nitrotoga* to withstand environmental pressures in impacted systems. Phylogenetic analyses revealed a deeply divergent nitrite oxidoreductase subunit A gene (*nxrA*) not represented in any other NOB, suggesting a novel evolutionary trajectory for *Nitrotoga*. *Nitrotoga*-like 16S rRNA gene sequences were prevalent in riverine sediments and water columns. This work considerably expands our knowledge of the *Candidatus* Nitrotoga genus and improves our understanding of their role in the global nitrogen cycle.

Syntrophy-driven evolutionary adaptation of a sulfate reducing bacterium diminishes its non-syntrophic growth physiology and alters its isotope phenotype in an unexpected direction

Jesse Colangelo-Lillis, N. Stopnisek, D. Stahl, B. Wing.
University of Colorado Boulder

Sulfate-reducing microbes (SRM) fractionate sulfur isotopes to an extent dictated by substrate and product concentrations and the kinetics of the proteins employed in transport and metabolism: this fractionation potential is their isotope phenotype. Through this phenotypic lens, analyses of sulfur isotopes in the geological record are utilized to place constraints on metabolic innovations and environmental shifts throughout Earth's history. In this work we test the assumption that isotope phenotypes have not been altered by SRM evolutionary adaptation since the time of signal preservation, allowing contemporary fractionation behaviors to serve as models for ancient ones. We employ an experimental evolution system in which a sulfate reducing bacterium (Desulfovibiro vulgaris Hildenborough, DvH) was forced into syntrophy with a methanogen (Methanococcus maripaludis, Mm) for 1000 generations. Clonal populations derived from evolved lineages of DvH display overlapping but unique mutation sets that frequently include loss of functional genes essential to sulfate respiration. Evolved lines that retained sulfate respiration capacity had diminished functionality, exhibited by reduced growth rate and cell-specific sulfate reduction rate. Relative to the ancestral DvH, we found that diminished sulfur isotope fractionation by evolved descendants accompanied this diminished functionality (up to 4 ‰). This difference contrasts with the canonical physiological relationship between respiration rate and sulfur isotope fractionation, where the magnitude of fractionation decreases with increasing respiration rate. We will discuss these results in the context of quantitative models of sulfur isotope fractionation by DvH, which we will use to compare and contrast the isotopic consequences of enhanced and diminished functionality in the sulfate reduction pathway. While variation in observed fractionation after only 1000 generations of adaptation suggests that contemporary isotope phenotypes may not serve as accurate proxies for ancient ones, it also potentially offers a roadmap for disentangling ecological changes from evolutionary ones in the geological sulfur isotope record.

Invited Talk:

Everything I thought I knew was wrong: lessons from the stromatolites of Walker Lake

Victoria Petryshyn
University of Southern California

Stromatolites - laminated structures commonly attributed to the activity of microbial mats, constitute some of the oldest putative fossils on Earth, with a record going back at least 3.5 billion years. However, the processes that control the different aspects of stromatolite growth are poorly understood. Because of this,

determining whether microbes were directly or indirectly involved in the stromatolite formation can be difficult—and this endeavor becomes more difficult as geologic time passes and organic signals degrade. Still, as layered, accretionary structures that form subaqueously—and therefore record chemical information about their formation environment—stromatolites are potentially useful tools for fine-timescale environmental and biological reconstructions, if they can be properly interpreted. In order to highlight the potential of stromatolites as biologic and climatic indicators, I will present evidence from stromatolites found on the shores of Walker Lake, an alkaline lake in western Nevada. These Holocene-age forms display the mm-scale lamination and domed growth pattern frequently found in Archean and Proterozoic stromatolites, yet are young and unaltered, allowing for analyses that cannot be performed on ancient samples. Detailed studies of growth rate, growth direction, texture, morphology, biotic community structure, and organic matter incorporation reveal that the conventional stromatolite growth model is in need of revision.

Poster Presentations:

Uncovering the role and isotopic impact of membrane nitrate transport in the nitrogen cycle

Ciara Asamoto, Sebastian Kopf.
University of Colorado Boulder

Nitrogen is a critical nutrient for all life on Earth and yet balancing our global nitrogen budget remains elusive. Microbes are key in mediating each aspect of the nitrogen cycle, including the fixation of dinitrogen gas into bioavailable nitrogen and the re-release of bioavailable nitrogen back to the atmosphere through denitrification, and anaerobic oxidation of ammonium. Denitrification in particular leaves a significant isotopic signal in the environment and is thus useful as a biosignature for nitrogen cycling processes. However, variation in this signal has led researchers to believe additional factors other than denitrification may affect this isotopic signal. A potential source of variation is the relative rate of nitrate transport across the cell membrane. Understanding the physiology of the organisms involved is thus an important prerequisite for researchers attempting to fully constrain global nitrogen cycling. Here we present the results of physiological experiments measuring nitrate uptake rates of Paracoccus denitrificans, a model denitrifier. This is the first step towards understanding the relationship between transport rates and resulting nitrogen isotope fractionation found in the environment. Subsequent work will utilize gene knockouts of transporters and reductases in the nitrate reduction pathway to more concisely analyze any isotopic effects associated with transport rates.

Understanding translation through experimental evolution of Chlamydomonas reinhardtii

Amanda L.D. Bender, Boswell A. Wing.
University of Colorado Boulder

Ribosomes are crucial to life as we understand it. Ribosomes are the sites of protein synthesis (translation) within all living cells, and proteins perform a vast array of functions within organisms. When contemplating the origin of life, it is crucial to consider how the ribosome evolved to its current state. Interestingly, ribosomal function and structure are largely conserved across extant biology. In order to better understand the evolution of translation, we seek to understand: Can ribosomal function be re-evolved? Our goal is to test for adaptability in ribosomal function by subjecting model organisms with dysfunctional chloroplast ribosomes to selective photosynthetic pressure. We use mutant strains of a green algae (Chlamydomonas reinhardtii) with poorly functioning chloroplast ribosomes. C. reinhardtii is an ideal model organism for this approach because it can be grown under photoautotrophic, mixotrophic, and heterotrophic conditions. We are working with experimental strains that have varied chloroplast ribosome mutations: some are deficient in chloroplast ribosomes and require acetate for growth, some synthesize limited chloroplast ribosomal proteins, and others have mutations that affect peptide progression through a distorted exit tunnel. We will gradually subject these mutant strains to increased photoselective pressure. All strains will initially be grown under heterotrophic conditions (i.e., with 13C-enriched acetate) in the dark. After multiple generations, mixotrophic conditions will be introduced (i.e., light added to growth system). Then photoautotrophic conditions will be introduced (i.e., growth media without acetate), intended to select for improved photosynthetic capacity and chloroplast ribosome function. For comparison, wild type C. reinhardtii will be grown alongside the mutant strains, and the selection experiment will be repeated without photoselective pressure. Carbon isotopic analysis of the resulting biomass will allow us to quantify the relative contribution of (13C-enriched) heterotrophic growth versus photoautotrophic carbon assimilation in the evolved mutant strains. This isotopic assay will provide evidence of adaptive improvements in translation by chloroplast ribosomes. We will present experimental design and preliminary results.

The role of urease in carbonate precipitation in the Great Salt Lake

Ryan Clay, Dezmond Swain.
Weber State University

Microbialites are sedimentary structures that are formed through the binding and trapping of grains or precipitation of minerals by members of the microbial communities associated with the microbialite. Expansive microbialite deposits composed mostly of carbonate minerals exist in the Great Salt Lake (GSL), but the exact mechanism of their formation is not known. We hypothesize that one mechanism could be microbial urease activity. The enzyme urease breaks down urea into ammonia and carbonate (CO32-), which in turn can react to form carbonate minerals. Bacteria cultured from the microbialite and lakewater were found to be positive for urease activity using a urease broth test. We are isolating urease-positive organisms to determine if they are representative of the microbialite microbial community as a whole by comparing isolates' 16S gene sequences to microbialite metagenomes. Finally, we will perform precipitation tests by spiking cultures with excess Ca2+ and watching for carbonate precipitation. Precipitation will be measured quantitatively via atomic absorption spectroscopy and analyzed for structure using a scanning electron microscope. The results will allow us to assess whether GSL microbialites formed as a result of microbial urease activity.

Microscale mineralogical characterization of actively serpentinizing peridotite, Oman

Eric T. Ellison, Lisa E. Mayhew, Kaitlin R. Rempfert, Alexis S. Templeton, The Oman Drilling Project Phase 1 Science Party.
University of Colorado Boulder

Ultramafic rocks are far from equilibrium with fluids circulating near the surface of the Earth, leading to multiple stages of alteration and oxidation and the production of energy-rich gases such as hydrogen. Phase 1 of the Oman Drilling Project in early 2017 saw the completion of exploratory rotary drilling through 400m of hydrating rock at the active serpentinization site BA-1A in the Samail ophiolite. We collected fresh drill cuttings every 10 m and packaged them anaerobically to preserve the in-situ Fe redox state as a function of depth. The drill cuttings contained variably serpentinized peridotite, together with frequent rodingite (gabbro altered by the calcium-rich fluids derived from the serpentinization of the host peridotite). Optical microscopy, Raman spectroscopy, electron microprobe analysis, and synchrotron-based microscale X-ray absorption fluorescence spectroscopy were applied to characterize the mineralogy and Fe speciation. Fe(II) was abundant in relict olivine, Fe sulfide, ferroan brucite, and magnetite, while Fe(III) was abundant in magnetite and andradite garnet. Fe substitution into serpentine minerals was dominated by Fe(III) near the surface (~ 85% Fe(III)/∑Fe), but Fe(II) accounted for a greater proportion with increasing depth (~45% Fe(III)/∑Fe at 390 m). The mineral association clinopyroxene + garnet ± clinochlore ± xonotlite ± talc ± actinolite was interpreted as resulting from rodingitization/Ca-metasomatism of gabbroic veins or dikes. One of the most surprising observations was the presence of metal sulfides in the serpentinized peridotite, generally associated with brucite in mesh cores. While sulfide was present in drill cuttings from all examined intervals, it appeared to be most abundant in the upper 100 m. These sulfide minerals may be a direct result of in-situ sulfate reduction, and may offer an exciting potential fingerprint of subsurface "rock-powered" microbial activity. The abundant olivine, pyroxene, and brucite indicates that these rocks may indeed be undergoing modern active serpentinization. There is substantial reducing power remaining in the rock in the form of reactive Fe(II)-bearing minerals, with the potential to fuel the production of biologically labile, energy-rich electron donors including hydrogen at low temperatures.

Basin Evolution Analysis of Newark Canyon Formation, Nevada: A reconstruction of palustrine and lacustrine carbonate depositional environments

Anne C. Fetrow, Kathryn E. Snell, Russell Di Fiori, Sean P. Long, Josh W. Bonde
University of Colorado Boulder

The hinterland of the Cretaceous to Paleogene Sevier fold-thrust belt, the "Nevadaplano", is a hypothesized high elevation orogenic plateau like the modern Andean Altiplano-Puna Plateau, yet the timing of uplift and maximum elevation are subject to debate. The Lower to mid-Cretaceous Newark Canyon formation (KNC) in Nevada records the deposition of palustrine carbonates interbedded with fluvial siliciclastics, poorly-developed paleosols, and fossiliferous lacustrine carbonates, and is associated with early thrust faulting and folding in the hinterland of the Sevier fold-thrust belt. This period of geologic history was also marked by global temperatures ~14°C warmer and had at least eight major ocean anoxic events (OAEs). These events have been well documented in positive δ13C excursions in the marine sediment record, but it is still unknown if signals from OAEs were truly 'global' perturbations as they have not yet been documented in terrestrial environments. The KNC is a rare example of a preserved terrestrial environment from the Early Cretaceous and thus presents an opportunity to examine how a terrestrial system responds climatically and environmentally to considerable global climate variation and potential regional elevation change. This study aims to understand the deposition of the distinct palustrine and lacustrine carbonate facies, and to provide sedimentological and biogeochemical context for future paleoclimate and paleoaltimetry studies. Here we present preliminary clumped isotope (Δ47), δ13C, and δ18O stable isotope data that suggest an evolution from a variable wetland and channelized fluvial setting to a low energy, shallow lacustrine system. We also present stratigraphy (0.5m scale) and facies characterization of the KNC from analysis of thin sections using optical and cathodoluminescence microscopy. Preliminary facies description and stratigraphic analysis suggest heterogeneous deposition along distal portions of a fan delta system that deposited locally-sourced material from the proximal highland. Variability in color, grain sorting, and other features (e.g. fossil assemblages, root traces) suggest that these environments had fluctuating water saturation levels, duration of subaerial desiccation, localized pH levels, and inconsistent fluvial energy levels.

Assessing the Biogenicity of Great Salt Lake Microbialites using Geochemical Measurements of Lake and Groundwater

Jess Gann, Kisten Mayfield.
Weber State University

Microbialites are defined as structures built by microbial communities through trapping and binding, or precipitation. However, the interplay between biotic and abiotic formation mechanisms is not well understood. Microbialites are key to understanding early Earth biology and surface environments due to the rich record of microbialites in periods dating back as far as 3.5 billion years. Calcium carbonate in the form of calcite, aragonite, or dolomite is the core molecule in many of these formations including those in the Great Salt Lake. We hypothesized that the microbialites in the Great Salt Lake formed at an intersection of briney lake water and fresh spring water without microbiological involvement. To test this hypothesis, measurements were taken on site to provide a snapshot of the aqueous chemistry surrounding the microbialite structures. Through the use of the analytical tool Geochemist Workbench, we uncovered strong evidence suggesting abiotic precipitation of carbonates is not possible using the conditions currently existing in The Great Salt Lake. This means the presence of microbialite structures is either due to microbial involvement in the past or present, or the chemical environment has changed from the point when abiotic precipitation was possible.

Fresh Snowfall Microbiology and Chemistry are Driven by Geography in Storm-Tracked Events

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

Snowfall is a global phenomenon highly integrated with hydrology and ecology. Forays into studying bioaerosols and their dependence on aeolian movement are largely constrained to either precipitation-independent analyses or *in-silico* models. Though snowpack and glacial microbiological studies have been conducted, little is known about the biological component of meteoric snow. Through culture-independent phylogenetic and geochemical analyses, we show that the geographical location at which snow precipitates determines snowfall's composition. Storm-tracking, furthermore, can be used as a valuable environmental indicator to trace down what factors are influencing bioaerosols. We estimate annual deposits of up to ~10 kg of bacterial / archaeal biomass per hectare along our study area of the eastern Front Range in Colorado. The dominant kinds of microbiota captured in an analysis of seven snow events at two different locations, one urban, one rural, across the winter of 2016/2017 included phyla *Proteobacteria*, *Bacteroidetes*, *Firmicutes* and *Acidobacteria*, though a multitude of different kinds of organisms were found in both. Taxonomically, *Bacteroidetes* were more abundant in Golden snow while *Proteobacteria* were more common in Sunshine samples. Chemically, Golden snowfall was positively correlated with some metals and ions. The work also hints at better informing the 'everything is everywhere' hypotheses of the microbial world and that atmospheric transport of microbiota is not only common, but is capable of disseminating microbiota of different physiologies and genetics that then affect ecosystems globally. Snowfall, we conclude, is a significant repository of microbiological material with strong implications for both ecosystem genetic flux and general bio-aerosol theory.

Microbial overprinting of original stable isotopic signatures in lacustrine carbonate

Miquela Ingalls, Katie Snell.
University of Colorado Boulder

Chemical lake sediments record paleoenvironmental and paleoclimatic information from ancient terrestrial environments. The abundance and distribution of carbon and oxygen isotopes within a carbonate mineral can be related to the environment in which it formed (i.e. temperature, aridity, altitude, vegetation). However, we hypothesize that interactions between microbes and carbonate sediments prior to lithification can directly alter the original isotopic composition of carbonate (e.g. by metabolism) or indirectly alter the sediment pore water DIC pool (e.g. by respiration) from which authigenic carbonate precipitates. To test this hypothesis, we track down-core changes in carbon, oxygen, and clumped isotopic composition of carbonate sediments, organic matter, and pore water DIC. When there is a strong microbial influence in the pore water-carbonate system, we expect an anti-correlated down-core trend in d13C values of organic matter and carbonate, i.e. a paired negative shift in d13Ccarbonate with a positive shift in d13Corg due to mixing of carbon pools (DIC and DOC). In addition, carbonates associated with respiration of organic matter typically have lower d18O values6, and thus d18O can be used as a secondary indicator of microbial influence on carbonate stable isotopes. Here we present preliminary stable isotope data from lake water, sediment cores, and pore water from Mono Lake and Great Salt Lake. We find an anti-correlation between d13Corg and d13Ccarb with depth and suggest that this trend may indicate carbon cycling between the inorganic and organic carbon pools by microbial respiration within sediment pore space. We chose modern lakes with relatively well known recent climate in order to be able to compare d18Ocarb and Δ47-derived temperature reconstructions with historical temperature and water level data and robust proxy records of relatively recent climate, and to assess the use of d18Ocarb as a paleo-aridity proxy. This study demonstrates that in situ microbial processes in lake sediments can overprint primary isotopic values via mixing of C isotopes. "Unmixing" in the geologic record using standard isotope mixing models may require knowledge of the paleo-microbial community and their associated activities in the ancient sediments to make necessary assumptions about the magnitude of alteration and mixing.

Needle induced biogeochemical shifts and the influences of climatic variables

Laura Leonard, Kristin Mikkelson, Jonathan O. Sharp.
Colorado School of Mines

Forests within North America have been experiencing large-scale insect infestation for several years in addition to more recent climate-related factors that are collectively expected to magnify the infestation. To better understand how forest ecosystems respond to disruptions, we are investigating how seasonal fluctuations such as temperature and snow melt timing influence needle decay. Past research in our group has revealed shifts in soil edaphic properties and microbial ecological signatures under beetle-impacted lodgepole trees when compared to healthy trees. To better understand these observed shifts, pine needles were harvested from the forest floor as a function of beetle-impacted spruce, non-impacted spruce, and non-impacted lodgepole pine and transported to Crested Butte. Plots were established at three distinct elevations to analyze various effects on carbon and nitrogen export into the atmosphere and hydrosphere. Key findings over one year suggest that needle chemistry and seasonality influence gas flux. All three needle types had more pronounced shifts in gaseous flux from the needle-free controls with lodgepole needles displaying the most significant shifts. Seasonality has also altered the subsurface gas flux with an observed increase in CO_2 production and CH_4 consumption during high moisture events. Porewater samples collected after snowmelt indicate increased concentrations of DOC, TN, and SUVA in the accelerated snowmelt plot in contrast to a natural snowmelt plot at the same elevation. Initial microbial analysis suggests potential differences between the needle-free control upper organic horizon in contrast to the horizons beneath needle decay. Ongoing work will focus on the decoupling of climatic variables and edaphic changes from microbial shifts. Overall, these observations suggest multiple biogeochemical shifts could occur due to increased needle decay and seasonal moisture events. This project has implications for forest recovery and nutrient export that could aid in the prediction of functional ecosystem shifts within montane watersheds experiencing further stressors from climate change.

Variations in Fe and mineralogy in subsurface serpentinites from the Atlantis Massif

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The Atlantis Massif (AM) is an oceanic core complex wherein (ultra)mafic mantle rocks uplifted through fault activity are exposed to seawater. The AM underlies Lost City, a hydro-thermal vent field exuding warm temperature (~40-90°C), high pH fluids with H_2 and dis-solved C-species [1]. Water/rock reactions alter the mineralogy and Fe undergoes dynamic redox transformations that control the composition of these energy-rich fluids, thus influencing the habitability and activity of any associated subsurface biosphere. Hard rock drilling conducted by IODP Exp. 357 recovered a complex suite of highly serpentinized, variably oxidized peridotites [2]. For a detailed understanding of rock geochemical transformations, we employed bulk and microscale quantitative, integrated mineralogic, elemental, and Fe oxidation state analyses of this unique sample set. Bulk analyses reveal a wide variation in Fe redox state between the different samples and a variety of secondary mineral phases that always include serpentine and sometimes include the more Si-rich phases talc-chlorite-amphibole. Raman microscale imaging revealed multiple generations of serpentine with unique distributions within the sample. Quantitative elemental images (EPMA-WDS) show variation in the Fe content of serpentine phases. Iron re-dox state maps were generated from synchrotron radiation μXRF (x-ray fluorescence) image data collected at multiple energies within the Fe K-pre-edge region, which is quantitatively sensitive to the oxidation state of Fe. Comparison to standards with known Fe redox and co-ordination states enabled assessment of $Fe^{3+/}$FeTotal at every pixel. Combining these complementary microscale techniques makes it possible to observe variation in Fe redox between different minerals within a sample (such as different generations of serpentine), but also be-tween different samples. Revealing these mineralogic and chemical signatures of serpentinites recovered at different depths and across borehole transects may aid in unraveling the many episodes of reaction and chemical overprinting and the possible interplay of biotic and abiotic processes at work in this water/rock reaction system. [1] Kelley et al. 2005. Science, 307:1428-1434. [2]http://publications.iodp.org/proceedings/357/357title.html

Origin and Cycling of Short Chain Alkanes in the Samail Ophiolite, Oman

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The aqueous alteration of peridotite supports subsurface microbial ecosystems and links the geosphere, hydrosphere, and atmosphere via the production of reduced gases and the mineralization of dissolved CO_2. CH_4 is the most abundant reduced C compound in reacted fluids, often reaching mM concentrations, but the formation mechanisms and the role of biology in the production/consumption of CH_4 and other hydrocarbons in peridotites, especially at low temperatures, is unclear. I use isotope geochemical approaches to evaluate mechanisms of C reduction and microbial alkane oxidation in the Samail ophiolite of Oman, which is undergoing serpentinization <60˚C. Using submersible pumps in boreholes that tap into geochemical regions of interest including hyperalkaline reducing fluids and fluid mixing zones, I have sampled water and dissolved gases in the Samail Ophiolite. Additionally, I have sampled at multiple isolated depth intervals in a single peridotite borehole using a packer system to contrast deep, reducing fluids with more oxic upper aquifer fluids in order to assess chemical and biological processes occurring across this redox transition. I will present concentrations and C and H isotopic compositions of C1-C6 alkanes to compare against trends characteristic of abiotic polymerization and/or microbial oxidation. Concentrations and $\delta^{13}C$ of dissolved inorganic carbon ($\sum CO_2 = CO_2 + HCO_3^- + CO_3^{2-}$) will be reported to further assess potential microbial alkane oxidation. High $\delta^{13}C$ of CH_4 (up to +4 ‰ vpdb) and Δ13CH3D values reflecting unreasonably high apparent temperatures of formation (>500˚C) do not support a thermogenic origin for CH_4, but rather microbial methanogenesis under kinetic influence (e.g., high H_2 and/or CO_2 limitation) and subsequent methanotrophy.

Succession of a founding microbiome after seeding in a multiple-compartment anaerobic bioreactor for domestic wastewater treatment

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Engineered wastewater treatment systems are useful environments for studying microbial ecology phenomena. Mixed-culture activated sludge systems are the most commonly used form of wastewater treatment today; however, activated sludge systems are energy-intensive and require substantial oxygenation to promote microbial growth. Anaerobic wastewater treatment systems have gained increasing attention due to their generation of useful end products, such as methane-rich biogas for onsite energy generation. Many of the most promising anaerobic reactor configurations, such as the anaerobic baffled reactor (ABR), are the subject of current pilot-scale research and require further study prior to widespread full-scale implementation. While the degradation of complex organics found in domestic wastewater (i.e., carbohydrates, fats, and proteins) to CH_4 and CO_2 has been studied in reactor systems such as the ABR, the spatiotemporal development of microbial communities in each reactor compartment after inoculation has not been well studied. We employ 16s rRNA gene sequencing, bioinformatic approaches, and statistical analyses to examine microbial community succession and persistence of the founding microbiome - concepts commonly explored in plant ecology and island biogeography. Our results suggest that the microbial communities in the ABR followed two successional trajectories after inoculation. The first trajectory was driven by unmanaged performance variations (i.e., pulse disturbances to the sludge blanket) and low wastewater temperatures. When disturbances ceased and wastewater temperatures increased, Euryarchaeota increased in relative abundance and caused the communities in each reactor compartment to become more similar. Interestingly, members of the founding microbiome (i.e., the seed sludge) decreased substantially during the first successional trajectory, but increased in relative abundance during the second successional trajectory.

Evaluation of the Beer Microbiota

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Microorganisms are a key component in beer production. Without them, fermentation would not occur, ethanol would not be produced, and the social culture surrounding beer would be forever changed. Historically, yeast has been utilized as the main microorganism in beer production, with *Saccharomyces cerevisiae* being the most commonly used species. However, with the emergence of craft beer and the numerous new styles of beer being brewed, different strains and species of yeast are being used. Additionally, during the brewing process, the wort may be inoculated by microorganism from the surrounding environment. These contaminant microbes may then grow and impact the final flavor profile of the beer. The focus of this project was to examine the microbial communities of beer, with a focus on sour style beers. Beer samples were obtained from breweries across the US and filtered. DNA was extracted from the filtrate and the 16S rRNA gene was amplified. The amplicons were then sequenced using next generation sequencing technology, and the results were analyzed using various bioinformatics tools.

Salinity tolerance of cyanobacteria Synechococcus sp PCC 7002

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Recent phylogenetic and geochemical analyses have placed the origin of cyanobacteria and oxygenic photosynthesis in a freshwater environment at least 2.2 billion years ago. However, these results rely on several assumptions about the stability and conservation of traits across billions of years. Here we present the results from a series of experiments testing the tolerance of the unicellular cyanobacteria Synechococcus sp. PCC 7002 to varying levels of salinity in its growth media. These results provide a basis for beginning an evolution scale experiment on cyanobacterial salinity preference with the aim of testing the underlying assumptions in our understanding of cyanobacterial evolution.

Subsurface Biogeochemical Cycling of Nitrogen in an Ultramafic Aquifer

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Despite nitrogen being a key nutrient for life, little effort has been made to characterize sources of nitrogen to the deep subsurface, especially in rock-hosted ecosystems. Nitrogen may also be a significant energy source to the deep biosphere, with reduced forms of nitrogen such as ammonium used as an electron donor for metabolism, or oxidized forms such as nitrate used as an electron acceptor to "breathe" in the absence of dissolved oxygen. Accordingly, determining the origin and form of nitrogen present to the subsurface biosphere is critical for understanding how nitrogen availability may influence and limit autotrophic microbial abundance in the deep subsurface. N and O isotopic analyses of nitrogen species detected in fluids sampled from boreholes (>300 m depth) drilled in peridotite in the Samail Ophiolite of Oman were applied to trace the source and subsequent cycling of nitrogen in a deep, ultramafic aquifer ecosystem. To evaluate possible geologic sources of nitrogen, bulk N isotopic measurements of rock chips obtained from rotary drilling during Phase 1 of the Oman Drilling Project at hole BA-1A were also conducted. High concentrations of nitrate (>100 uM) with a meteoric isotopic signature were detected in shallowly sourced fluids, suggesting rainwater may be an important source of nitrogen to the subsurface aquifer. Deeper fluids were conversely enriched in ammonium and depleted in dissolved dinitrogen gas. Concurrently, the potential for microbial participation in the cycling of nitrogen at depth was assessed based on the presence of genes for nitrogen metabolism in borehole fluid metagenomes. The combined isotopic and functional gene analyses suggest a transformation of nitrogen speciation within the subsurface with at least some biological involvement through active nitrification and dissimilatory nitrate reduction. The disparate functional gene composition between shallow and deep subsurface fluids also indicates that the ecology of the subsurface microbial community may be influenced by the availability of oxidized nitrogen species in this peridotite-hosted environment.

Metabolic investigation of microbial communities found in a sulfur-dominated glacial spring system in the Canadian High Arctic.

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The Galilean moon Europa is a unique environment that makes it an ideal target for astrobiological investigation. Specifically, the presence of sulfur constituents on the icy surface supports the hypothesis that sulfur-based metabolisms could be fueled by water-rock interactions within the subsurface coupled with the delivery of oxidants via cycling of surface ice. It is impossible to currently study the potential for microorganisms on Europa, however, proxies on Earth do exist. Borup Fiord Pass (BFP) is a sulfur-dominated glacial spring system found on Ellesmere Island, Nunavut, Canada. BFP hosts one of the few cold-temperature sulfur springs that have been found in the Arctic. Along with being a candidate site to study low-temperature sulfur cycling, it has also been implicated as one of the best Earth-based analogs to study in preparation for a Europan lander. Early research at BFP has revealed preliminary data about spring geochemistry, microbiology, biomineralization, and putative sulfur metabolisms. BFP is visually striking as large accumulations of sulfur-like material on top of regional ices can be seen to cover an area of tens to thousands of square meters. These accumulations on top of the ice are accompanied by large proglacial icings (aufeis), which form when spring flow is frozen down-valley during winter months. Investigations into the microbial communities at BFP that utilize sulfur constituents for metabolic processes have revealed the presence of sulfur-oxidizing organisms from the class Epsilonproteobacteria as well as an abundance of the genus Flavobacterium. Preliminary work examining 16S rRNA gene sequencing data has shown the presence of some of these organisms over the course of years and through various sample types. How they are metabolically active is not yet fully understood and is the next step in our research at BFP. Through the use of metagenomics and metatranscriptomics we will further evaluate the growth of these microorganisms in such an extreme environment. Initial gene clusters, or operons, of interest, include sox, sor, sqr, apr, psr, ttr, and dsr as well as other metabolic, photosynthetic, and carbon fixation gene pathways. Our work will help to further guide the search for potential astrobiological targets on Europa, specifically at water/ice interfaces.

Metagenomic analysis of an energy-positive anaerobic hybrid reactor system for treatment of domestic wastewater under psychrophilic conditions

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Worldwide, microorganisms are the catalysts for cleaning domestic wastewater. While aerobic microbial treatment is efficient at meeting regulatory wastewater discharge requirements, three to four percent of all domestic energy generated powers this process. Our research centers on transforming this aerobic treatment into an energy-positive anaerobic process. We established a pilot scale, three-compartment anaerobic baffled reactor (ABR) being continuously supplied domestic wastewater from a 250-unit housing complex at the Colorado School of Mines. To better understand the membership and function of the microbial communities that develop in the ABR we performed metagenomics on 18 reactor DNA samples. Here we present initial findings of this metagenomics study, including a temporal-spatial analysis, and the prevalence of antibiotic-resistance genes in reactor communities.

Assessing the role of diurnal cycling on nitrogen biogeochemistry in a wetland biomat

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Treatment wetlands offer a unique opportunity to query fundamental biogeochemical processes in a controlled and engineered environment. This study investigates nitrogen cycling in an open water wetland characterized by its shallow (<30 cm) water column, absence of macrophytes, and geotextile lined bottom. As a result, a diffuse biomat (up to 15 cm thick) reproducibly colonizes the wetland bottom containing a microbial assemblage dominated by diatoms and proteobacteria. The wetland effectively removes nitrate from secondary wastewater effluent with treatment being driven by anaerobic microbial processes (denitrification with potential contributions from anammox). A shallow water column permits photosynthesis near the biomat/water interface during the day, resulting in oxygen concentrations that exceed 20 mg/L. At night, microbial respiration consumes oxygen and the water column becomes undersaturated (~5 mg/L). Preliminary observations of oxygen profiles within the biomat suggest that it is scavenged abruptly (within ~1 cm of the biomat/water interface) during the day, yet it is unknown how this changes through diurnal cycles. In addition to oxygen, photosynthesis is known to produce organic carbon exudates that can fuel heterotrophic metabolisms and alter bacterial community structure in stratified sediments. Collectively, oscillating light and dark cycles may influence oxygen diffusion and carbon availability in benthic sediments containing microalgae. Because denitrification and anammox are (i) sensitive to oxygen and (ii) reliant on different forms of carbon, it is important to understand how the distribution of these nutrients is controlled by photosynthesis and respiration. This project therefore explores the role of diurnal cycling in regulating the microbial functionality and associated nitrogen

removal pathways in a benthic biomat responsible for nitrogen attenuation in a demonstration scale treatment wetland.

Microbial Tortoise and Hare: Deciphering the proteome of slow growing and fast-growing microorganisms

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Microorganisms tune cellular resource allocation and enzyme production in response to different growth conditions and environmental constraints. The fundamental trade-off between maximal growth speed and maximal energy efficiency has been observed to dictate protein allocation in fast-growing microbial communities. However, the resulting dynamics of proteomic shifts at environmentally relevant, slower growth rates are less well understood. This is crucial to advance our understanding of microbial systems in soils & oceans, chronic infections in medicine, as well as optimization of many industrial microbial processes. This present work investigates the proteomic investment strategies of the unicellular bacterial model organism Escherichia coli in a growth-rate limiting environment. E. coli were grown in carbon-limited chemically and physiologically static continuous culture (chemostat) conditions at precisely controlled nutrient supply rates equivalent to generation times of 2 hours and 23 hours, respectively. After maintaining steady-state for at least 4-6 generations, the organism was exposed to heavy nitrogen (15N) and the incorporation rate of the isotope into specific proteins was quantified for over 600 protein fragments by LC-MS/MS and spectral fitting. Between the two growth-rate conditions, we detected shifts in proteomic allocation in terms of both proteins present, and in terms of cellular investment in peptide synthesis and degradation (protein turnover). Unsurprisingly, a majority of the proteome remained consistent across growth rates due to the identical nutritional environment, but a substantial subset (20%) of the proteins detected were unique to each growth condition. As growth rate decreased, we identified a relative increase in cellular investment into proteins associated with carbon assimilation, and a decrease in cellular investment into proteins associated with amino acid and nucleotide synthesis. Further, in the slow growing condition we observed a significant increase in turnover of proteins involved in metabolic pathway functions. This work demonstrates dynamic stress response strategies in both proteomic remodeling and proteomic reprioritizing. These results scratch the surface of what is possible for investigating the macroeconomics of the proteome and provide support for an experimental avenue for studying life in "slow motion".

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