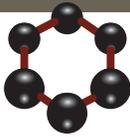


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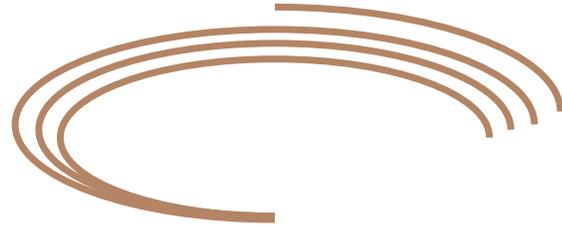
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We are grateful for the artistic contributions of Big Car Collaborative (cover logo, MWGB program), Ted Flynn (Chicago logo, MWGB 2014 pint glasses), and Kristin Woycheese (Sue logo, MWGB 2014 pint glasses).

Welcome to the third annual Midwest Geobiology Symposium!

The Midwest Geobiology symposium is an opportunity for undergraduate students, graduate students, and postdocs to share their research with the regional geobiology community. This annual event brings together students, faculty, and researchers from Midwestern colleges, universities, and research institutions.

Geobiology is an inherently interdisciplinary research field that focuses on the coevolution of Life and the Earth. It encompasses diverse fields such as geology, geochemistry, biology, microbiology, chemistry, oceanography, climate science, and engineering. The Midwest Geobiology symposium provides a venue for the regional geobiology community to discover the recent advances occurring in each other's laboratories, to provide critical feedback to ongoing research, and foster collaboration and new research directions. We continue to encourage the participation of researchers at all levels, from undergraduates to senior scientists and faculty.

This year's symposium is being held at the renowned Field Museum of Natural History in the heart of downtown Chicago. This venue, home to one of the nation's finest collections of fossils, minerals, and meteorites provides a unique opportunity for university and national laboratory-based geobiologists to interact with Field Museum scientists, many of whom are experts in closely-related fields such as mineralogy, isotope geochemistry, and paleontology. By bringing together scientists from these diverse and interconnected fields in such a vibrant setting, we hope that this year's symposium will strengthen ties formed at previous meetings and forge new opportunities for geobiological research.

Sincerely,

The Midwest Geobiology 2014 Organizing Committee

Theodore Flynn (Argonne National Laboratory)
D'Arcy Meyer-Dombard (University of Illinois at Chicago)
Magdalena Osburn (Northwestern University)
Jacob Waldbauer (The University of Chicago)

Program of Events – Saturday, September 27th, 2014

8:30 am **Arrival and Breakfast**

Oral Session 1 – Molecular Geomicrobiology

9:00 am **Welcoming Remarks**

9:10 am **Wesley Swingley**, Northern Illinois University.

Microbial community analyses of the Nachusa Grasslands (Illinois) prairie restoration chronosequence

9:30 am **Sarah Cadieux**, Indiana University.

Phototrophic purple sulfur bacteria in a dilute dimictic Arctic lake under ice cover

9:50 am **Yiran Dong**, University of Illinois at Urbana-Champaign

Phylogenetic and metabolic activity of an Aquificales-dominated microbial community inhabiting the apron and channel facies of mammoth hot springs, Yellowstone National Park, USA

10:10 am **Kristin Woycheese**, University of Illinois at Chicago

Deep subsurface microbes in terrestrial serpentinizing seeps

10:30 am **Coffee Break**

Oral Session 2 – Geochemistry in Geobiology

11:00 am **Qiang Yu**, University of Notre Dame

Sulfhydryl binding sites within bacterial extracellular polymeric substances (EPS)

11:20 am **Sora Kim**, University of Chicago

The ecological dynamics of sharks based on stable isotope analysis

11:40 am **Steven Chemtob**, Washington University in St. Louis

Mechanisms and Si isotope signatures of iron-silica aqueous interaction in the Precambrian ocean

12:00pm **Lunch**

Oral Session 3 – Environmental Microbiology and Bioremediation

1:30 pm **Carol Beaver**, Western Michigan University

Elevated magnetic susceptibility in crude oil contaminated sediments points to the presence of hydrocarbon-degrading microbial communities

1:50 pm **Xikun Liu**, The University of Iowa

Diversity of the epoxyalkane:coenzyme M transferase gene (etnE) at different vinyl chloride contaminated sites

2:10 pm **Linduo Zhao**, Miami University – Ohio

Investigation of effects of microbial iron redox cycles on clay mineral properties and its potential application in nitrate removal

2:30 pm **Nicolas Clercin**, Indiana University – Purdue University Indianapolis

Identification of taste-and-odor producing and degrading bacteria in a freshwater reservoir, central Indiana

2:50 pm **Closing Remarks**

3:00 pm **Poster Session**

5:00 pm **Meeting End**

Reconstruction of ancient microbial phylogenetic diversity from fossil travertine

ABIGAIL E. ASANGBA, ROBERT A. SANFORD, AND
BRUCE W. FOUKE

Department of Geology, University of Illinois at Urbana-Champaign

Hot-spring environments preserve biomarkers representative of the microbial life that existed in the paleo-environments in which they formed. This makes them an important target in the search for microbial fossils that formed on the early earth and potentially other planets. The study of carbonate (travertine) precipitating springs is especially relevant due to the recent evidence of carbonate deposits on Mars. Extraction and characterization of these biomarkers should result in a better understanding of ancient ecosystems in extreme environments, evolution of microbial communities over geological time, and long-term preservation of biomarkers. Biomarker preservation depends on trapping of microbes during travertine precipitation, as well as on all post-depositional water-travertine interactions (diagenesis). This study therefore seeks to systematically study preservation potential of DNA biomarkers during precipitation, fossilization and diagenesis of modern through ancient travertine. This information is being used to reconstruct the phylogenetic and metabolic diversities of the ancient meso- and thermophilic microbes and their native hot-spring environments. The Mammoth Hot-Springs corridor of Yellowstone National Park (YNP) contains thermal springs (73°C at the vent) that are actively and rapidly (≤ 5 mm/day) precipitating travertine, as well as a complete time-series of travertine deposits and Mammoth and in nearby Gardiner, Montana, that extend back to the Pleistocene (~33 ka). Samples for comparative study were obtained from the Cakmak quarry (~700-900 ka) in the Denizli Basin of Turkey. Genomic DNA was extracted from modern through ancient travertine bulk rock samples from the apron and channel, the pond and the proximal slope travertine depositional facies. Community profiles generated from T-RFLP analysis were used to compare phylogenetic diversity of the microbial communities present in the various depositional facies of the modern and diagenetically altered fossil travertine. Multivariate statistics was used to quantify differences among the T-RFLP profiles from each sample. Results from 16S rRNA gene sequencing in combination with petrographic analysis of fossil travertine will be used to investigate the effect of diagenetic contamination and alteration on microbial community composition and metabolism.

Use of stable carbon isotopes to assess anaerobic and aerobic methane oxidation in hypersaline ponds

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Methane oxidation (MO) is known to play a significant role in reducing methane (CH₄) concentrations in sediments and water columns throughout a variety of aqueous environments. In marine systems, for example, it is thought that more than 80% of CH₄ produced is oxidized before reaching the atmosphere. However, under hypersaline conditions, little work has been done. Of the few hypersaline studies undertaken, it is unclear to what extent MO occurs, although cells of anaerobic methanotrophs have been identified in salinities up to halite saturation. The focus of this study was to investigate anaerobic and aerobic methane oxidation in organic rich microbial mats and endoevaporite crusts of hypersaline ponds. The two main study areas were the Atacama Desert in Chile, and Guerrero Negro in Mexico. To track microbial consumption of CH₄ to carbon dioxide (CO₂), ¹³C-labeled CH₄ was added to the headspace of incubation vials containing mat and evaporite slurries. After a known incubation period, a portion of the biologically produced gaseous headspace was analyzed for $\delta^{13}\text{C}_{\text{CO}_2}$. If MO were occurring, the measured $\delta^{13}\text{C}_{\text{CO}_2}$ values would be more enriched in ¹³C compared to control incubations where no ¹³CH₄ was added. In addition to ¹³CH₄ treatments, inhibitors (molybdate, picolinic acid, and BES) were also added to determine the effect different substrate conditions would have on MO. The largest difference between $\delta^{13}\text{C}_{\text{CO}_2}$ values of ¹³CH₄-containing incubations and corresponding controls was approximately +5 to +7‰ in anaerobic treatments of microbial mat and evaporite crusts from Salar de Llamara in Chile. The $\delta^{13}\text{C}_{\text{CO}_2}$ values for the majority of ¹³CH₄ treated incubations, including ¹³CH₄ treatments with added inhibitors, were within ~1‰ of respective controls. Based on the low amount of ¹³C-enrichment in $\delta^{13}\text{C}_{\text{CO}_2}$ values, it appears that little, if any, MO is occurring in these hypersaline systems.

Elevated magnetic susceptibility in crude oil contaminated sediments points to the presence of hydrocarbon-degrading microbial communities

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²Oklahoma State University, Stillwater, OK 74078

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Traditionally, the monitoring of hydrocarbon bioremediation has been done by geochemical and microbiological methods, not geophysical ones. One geophysical method, magnetic susceptibility (MS), is a possible alternative for the monitoring of oil biodegradation, because iron-reducing bacteria may precipitate magnetite as they oxidize hydrocarbons. The presence of magnetite will increase the MS of the sediment. In order to evaluate this method, we measured the MS in wells and of cores from a hydrocarbon-contaminated aquifer in Bemidji, MN. We used a 16S rRNA gene high throughput sequencing approach to identify the populations of the microbial community in areas of the sediments with high and low MS. We found that wells and cores from the contaminated sediments had higher MS than uncontaminated sediments, with the highest peak occurring in the methanogenic plume undergoing oil degradation. In the hydrocarbon smear zone above the water table, iron-reducing and hydrocarbon-degrading microbes similar to *Albidiferax* were present. In the oil plume in the aquifer, the hydrocarbon-degrading methanogenic consortium consisted of *Methanoregula* and the syntrophic *Smithella*. A conceptual model will be presented showing the interrelatedness of iron reducers, methanogens, and elevated MS, and how this method can guide microbiological sampling during the monitoring of oil spill bioremediation.

Molecular insights into the Calescamantes dark matter phylum

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¹Northern Illinois University, DeKalb, IL

²University of Nevada Las Vegas, Las Vegas, NV

The vast majority of microbial life remains uncatalogued due to the inability to cultivate these organisms in the laboratory. This microbial “dark matter” represents a substantial portion of the tree of life and of species that control chemical cycling in most ecosystems. While many of these keystone species are at concentrations too low to construct robust assemblies from community genomes (metagenomes), single-cell genomics allows for the targeting and sequencing of individual cells, without the limitations of metagenomics or culture bias. In this work we coordinated single-cell isolation and sequencing with metagenomics in order to construct a near-complete genome from a dark matter phylum. The Great Basin is home to many high temperature hot springs that harbor thermophilic microbes that are of interest for understanding how life originally evolved on Earth. Great Boiling Spring (GBS) has become a model system for Great Basin studies, with multiple ecological gradients targeted for dark matter studies. Here we present the genome analyses of Calescamantes, a bacterial dark matter phylum. Calescamantes make up ~5% of the GBS community, and a similar proportion of communities in springs at Yellowstone National Park (Bison Pool and Octopus Spring) and Tengchong County, Yunnan Province, China (Gongxiaoshe Spring); insufficient for complete assembly using metagenomics alone. Using a co-assembled single-cell genome as an anchor, metagenomic reads were clustered at each spring by nucleotide frequency using machine learning algorithms, allowing robust assembly without the risk of chimerism. Metabolic reconstruction completed apparent metabolic gaps in the single-cell genome, and suggests that Calescamantes strains at GBS and each of the other springs are facultative anaerobic respirers that can putatively utilize nitrite and/or nitrous oxide (but not nitric oxide) as electron acceptors when oxygen is limited. Draft genomes and metabolomes will be leveraged for further efforts at tailored media construction for the cultivation of these elusive organisms. This work represents an important step toward the genetic analysis of microbial dark matter phyla through the coordinated analysis of traditional community genomes and targeted single-cell sequencing.

Microbial degradation of organic matter and community response in coastal marshes

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²Department of Earth and Planetary Sciences, Washington University in St. Louis, St. Louis, Missouri

Marshes are important environments for global biogeochemical cycles due to their high productivity and ability to gather and store considerable amounts of organic carbon. Much of the short term organic carbon turnover in coastal marshes is controlled by soil microbial communities and changes in the structure of these communities will impact their carbon-cycling capacity. The structure of these microbial communities is largely controlled by local environmental conditions that vary due to both natural and anthropogenic phenomena. The rates of organic carbon degradation and associated shifts in microbial diversity were experimentally assessed in response to changes in depth, distance to water, and the possible impact of the introduction of oil from the *Deepwater Horizon* spill in April 2010. This was done using a series of batch microcosms of soil or sediment and sterilized seawater gathered from Louisiana marshes dominated by the cordgrass *Spartina alterniflora* with differing environmental conditions. Native microbial communities were all dominated by Proteobacteria and unclassified bacteria, but deviated markedly at lower taxonomic levels. *S. alterniflora* leachate was added as a source of labile dissolved organic matter (DOM), and the rate of humification, determined from fluorescence measurements, was used as a proxy for its microbial degradation. Degradation rates were similar for all sites except for the inland sediment of the more heavily oiled sites, which were an order of magnitude greater. Changes in degradation rates were associated with a shift in microbial community structure. Although Proteobacteria and unclassified bacteria were still dominant discernable changes were observed within proteobacterial classes and Clostridia groups. These results imply that observed shifts in microbial community structure and increases in the rates of microbial degradation of DOM at more heavily oiled marshes may be caused by a priming effect due to the introduction of oil into the system. These results may have impacts on carbon cycling and help to explain changes within marshes affected by the spill.

Investigating the genetic basis of a paleohydrologic biomarker using *Solanum pennellii*, a desert relative of domestic tomato

AMANDA L. D. BENDER¹, MELANIE SUESS¹, DANIEL H. CHITWOOD², AND ALEXANDER S. BRADLEY¹

¹Washington University in St. Louis

²Donald Danforth Plant Science Center

The relative abundances of D/H and ¹³C/¹²C in leaf waxes incorporate information about the plant's growth environment and physiology, and are genetically controlled. Due to their recalcitrance in sediments, these molecules can be used as an informative paleoclimate proxy. Measurements of leaf wax δ D are used as a proxy for meteoric water, which is strongly controlled by hydrology and temperature. Values of δ^{13} C from leaf material relate to carbon fixation and water use efficiency – the ratio of carbon assimilation to transpirational water loss.

However, use of leaf waxes as a biomarker is hindered by a limited understanding of the influence of plant physiology on leaf wax δ D values. We present a biological approach to understand the biological controls on leaf wax δ D values. This approach leverages different hydrological adaptations in two closely related tomato species: *Solanum lycopersicum* (domestic tomato) and its “wild” desert relative, *Solanum pennellii*.

Preliminary analyses have identified quantitative trait differences between these two lines of tomato. We have detected a significantly higher abundance of branched alkanes in the leaf wax profiles of the “wild” *S. pennellii* plants than in the leaf wax profiles of domestic *S. lycopersicum* plants. The unbranched alkanes might create a more tortuous diffusion path for water in the leaf cuticle of *S. pennellii*, which would decrease permeability and possibly conserve water in its native desert habitat. We have also detected differences in the δ^{13} C values of leaf wax alkanes between the two strains.

To determine whether these differences can be associated with quantitative trait loci (QTLs), we will examine traits of introgression lines (ILs) of *S. pennellii* into *S. lycopersicum* – a population containing chromosome fragments from the “wild” *S. pennellii* introgressed in the genetic background of *S. lycopersicum*. By screening the ILs, we hope to determine QTLs associated with differences in leaf wax abundance, structure, and isotopic composition (δ D and δ^{13} C).

Evaluating the role of transhydrogenases in lipid D/H ratios

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We investigate the importance of transhydrogenases in determining lipid D/H ratios using the model organism *Methylobacterium extorquens*.

Transhydrogenases are a class of oxidoreducto enzymes that are responsible for transferring reducing power between intracellular pools of pyrimidine nucleotides. At least three classes of transhydrogenases have been described: i) the proton translocating transhydrogenase PntAB, which is known to carry an extremely large isotope effect; ii) the soluble energy-independent transhydrogenase UdhA, which is present in many proteobacteria; iii) the electron-bifurcating transhydrogenase NfnAB, present in many anaerobic bacteria and archaea.

In wild-type *Methylobacterium* grown on C1 compounds such as methanol or methylamine, NADPH is produced by methylene tetrahydromethanopterin (H_4 MPT) dehydrogenase during the cytoplasmic oxidation of formaldehyde to formate. Lipids produced under these conditions are enriched in deuterium relative to media water by ~80 permil. We compare this to an engineered mutant (EM) in which the H_4 MPT pathway has been inactivated and replaced by the non-orthologous glutathione-dependent pathway from *Paracoccus denitrificans* that produces NADH but not NADPH. In the EM strain, *pntAB* is upregulated and transhydrogenase activity is increased. Lipids in this strain are depleted in deuterium relative to media water by ~200 permil. In several evolved strains derived from EM, transhydrogenase expression is higher than in EM, correlating with restoration of NAD(H) and NADP(H) pools towards the wild type, and lipids in these strains are enriched in deuterium relative to the EM.

We compare these results to a *pntAB* deletion mutant which has been complemented with *pntAB* on an inducible promoter, allowing a direct comparison between transhydrogenase expression levels, NADPH/NADP ratios, and lipid D/H ratios.

Disentangling deposited from endemic microbes in a terrestrial hot spring

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Hot spring ecosystems are considered windows into the deep biosphere; however, they are also influenced by surface processes. To understand microbial dynamics that occur in these hot springs, endemic microbes need to be disentangled from microbes deposited from the surrounding environment. To this end, daily samples of water and sediment were taken from two hot springs in Tengchong, China (Gumingquan and Diretiyan). In addition, soil was collected from near the two springs. Gumingquan was a larger pool with no visible conduit for overland water flow into the spring. The microbial community and geochemical parameters did not change for Gumingquan and remained distinct from the surrounding soils. In contrast, Diretiyan was a smaller spring situated under a cliff where water could be seen flowing into the spring during rain events and the temperature fluctuated from 48-66°C depending on the amount of water flow into the spring. When the lowest temperature of the spring was recorded the sediment and water community was more similar to the surrounding soil than the community that existed at the maximum temperature. The soil samples were dominated by Actinobacteria, Acidobacteria, and Proteobacteria. The hot spring was dominated by Aquificales and Crenarchaeota when it was not raining. During heavy rain the hot spring was dominated by Proteobacteria and Cyanobacteria. The original hot spring community recovered within 24hrs of the rain stopping. These results indicate that rain events can wash microbes into the hot spring, which can alter the community structure and influence our views of the deep biosphere.

The mineralogy of the Beck Spring Dolomite: Implications for the breakup of a Supercontinent and the diversification of microbial life

ROGER N. BRYANT

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The Neoproterozoic Beck Spring Dolomite in Death Valley, CA, was deposited in shallow water on a carbonate platform on the Western margin of Laurentia. Overall marine regression was periodically offset by syn-sedimentary normal faulting, associated with the rifting of Rodinia. The interbedded shales and carbonate of the Upper Cherty member of the Beck Spring include a distinctive oncolite-bearing bed, just above a layer from which early diagenetic sedimentary talc was sampled. These pieces of evidence are all indicative of a major drowning event. A negative $\delta^{13}\text{C}$ anomaly coinciding with the Upper Cherty member at the top of the Beck Spring Dolomite correlates globally with the pre-Sturtian Islay anomaly. This study found sedimentary talc to be far less prevalent in the Beck Spring than in analogous continental margin deposits, where it could be argued that the pore fluid pH-increasing influence of anaerobic Fe-respiring microorganisms more frequently outweighed the pore fluid pH-decreasing influence of evaporation. This may have resulted from healthier microbial populations than in the Beck Spring, potentially as a result of lower disruption of the populations by tectonic activity. Higher levels of Al^{3+} in the Beck Spring relative to other Laurentian margin deposits resulted from greater siliciclastic influx (likely as a result of the more frequent syn-depositional tectonic activity than the Beck Spring experienced) – this would also have prohibited talc formation. Mineralogical evidence for heightened Al^{3+} comes from the presence of several low temperature Mg-silicates in the absence of talc: sepiolite, palygorskite, corrensite and clinochlore. The lower Crystal Spring–upper Crystal Spring unconformity in the lower Pahrump Group records large-scale regional tilting and a major hiatus that could be argued to represent the period of uplift and associated initial lack of sedimentation that might have resulted from the greater geothermal heating of W. Laurentia relative to E. Laurentia by the same superplume responsible for initiating the breakup of Rodinia. The resulting subsidence would have exacerbated syn-sedimentary faulting in the Beck Spring, potentially placing pressure on marine microbial ecosystems.

Phototrophic purple sulfur bacteria in a dilute dimictic Arctic lake under ice cover

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Oxidation of sulfide under anoxic conditions is one of the most important ecological properties of phototrophic purple sulfur bacteria (PSB). Dense populations of PSB are typically associated with sulfate-sulfide gradients occurring within the photic zone of meromictic, saline lakes. However, in two consecutive years we identified a dense PSB population under ice in a shallow, dilute, dimictic Greenlandic lake (Potentilla lake). Potentilla lake is located 5 km from the terminal moraine of the Russell Glacier, on the ice-free margin of western Greenland. This lake occurs within a series of lakes in a narrow valley overlying a structural shear zone. In winter of 2013, directly below a sharp redox gradient at 6 m, a horizon of purple-to-pink colloidal particles was observed. Water from this interval was filtered and DNA of retained solids extracted. Amplicon sequencing of 16S rRNA genes on a MiSeq Illumina platform identified the family *Chromatiaceae* as the most abundant phylotype at 39%. *Lamprocystis*, an obligate phototroph, was the dominant genus (30%). In winter of 2014, a population of PSB was again found at this interval, where the concentration of dissolved sulfide ranged from 0.5 to 6.8 μM . Detailed microbial community composition and water column chemistry in winter 2014 will be explored in order to better understand the unique occurrence of PSB in a dilute, dimictic low-sulfide Arctic lake.

Microbially-influenced Fe-Cycling within high pH serpentinizing springs of the Zambales Ophiolite, Philippines

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The Zambales ophiolite region in the Philippines contains high pH springs associated with serpentinization. At the surface where calcium-saturated fluids mix with air, fluid becomes aerobic and diffusion of carbon dioxide occurs. At depth, there are low concentrations of dissolved inorganic carbon and oxygen, and high concentrations of methane and hydrogen. Redox potential of iron in the fluids is largely dependent on pH. Ferrous iron is unstable at a high pH, and spontaneously reacts with atmospheric oxygen to ferric iron, which is then hydrolysed to ferrihydrite. The reaction kinetics may be too rapid for microbes to harness energy for growth; however, cells have been documented to act as nucleation sites for ferrihydrite precipitation in natural environments. Precipitates that sink to the subsurface act as substrates for microbes where they may carry out ferric iron reduction in the presence of hydrogen. Predictions made about Gibbs energy of reaction for iron metabolisms in serpentinizing systems show that ferric iron reduction in the subsurface is energetically favorable (Cardace, et al., 2013).

Spring fluid and rock samples from the Zambales region were collected in September 2013. Time series microcosms including sample rock, spring fluid, and gas simulating the spring surface and subsurface were set up in June 2014 to investigate microbial growth rates and microbial reaction products over one year. Pulverized rock within the microcosms provides surfaces for microbial attachment, and polished rock chips facilitate observation of microbe-mineral associations. In order to simulate the surface and subsurface, gas headspaces are comprised of CO₂:Air and CH₄:H₂, respectively. Sterilized microcosms serve as abiotic controls. Microcosms are being monitored for cell counts via fluorescence microscopy, SEM, and XRD to examine cell growth rates, microbial action on mineral surfaces, minerals forming around cells, and changes in mineralogy at intervals of three, six, nine, and twelve months. After one year, microbial community structure and iron metabolizers will be identified via DNA sequencing. Results thus far as well as expected future results will be discussed.

Mechanisms and Si isotope signatures of iron-silica aqueous interaction in the Precambrian ocean

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Banded iron formations (BIFs), Fe- and Si-rich chemical sediments common in Precambrian successions, are a critical tool for understanding the compositional and biological evolution of the Precambrian ocean [1]. A poorly understood aspect of BIF genesis is the origin of Si and its association with the primary precipitates that formed these units. Si could have been transported to BIF deposition sites by adsorption on hydrous iron oxide surfaces [2]; alternatively, Fe and Si may have coprecipitated as a single phase [3]. Stable Si isotope ratios may provide key constraints on BIF genesis, but the fractionation factors (ϵ) for each mechanism are poorly constrained. Here we explore variations in ϵ with Fe-Si interaction mechanism (adsorption vs. coprecipitation).

In adsorption experiments, sodium silicate solutions (125-2000 μ M, pH 8.1) were reacted with hematite particles of various morphologies for 24 hours. Adsorption curves were consistent with monomeric Si adsorption until surface site saturation (\sim 145 μ mol Si/g hematite) and polymeric adsorption at higher [Si]. Solutions spanned $\delta^{29}\text{Si} = +0.15\text{‰}$ to 2.07 ‰ and are consistent with a fractionation factor $\epsilon_{29} = -1.72 \pm 0.22\text{‰}$ (2σ) over the entire range of [Si], larger than previously reported by [4] (-0.54 to -0.81 ‰).

In coprecipitation experiments, solutions of Na-silicate and Fe(II) chloride (1-10 mM) were prepared anaerobically, then air-oxidized for 3 days to induce precipitation. At low [Si], magnetite formed; near silica saturation, lepidocrocite and ferrihydrite formed. A fraction of aqueous Si (40-90%) was incorporated into the oxidative precipitates. The Si isotope fractionation behavior was distinct from adsorption ($\epsilon_{29} = -1.17 \pm 0.52\text{‰}$, 2σ). Thus, the mechanism of Fe-Si aqueous interaction affects ϵ ; reconstruction of paleo-ocean $\delta^{29}\text{Si}$ may require additional constraints on which mechanism dominated. These isotopic analyses will be paired with Si K-edge X-ray absorption spectra of the precipitates to determine how Si coordination affects isotopic fractionation.

[1] Klein (2005) *Am. Min.*, **90**, 1473-1499. [2] Fischer and Knoll (2009) *GSA Bull.*, **121**, 222-235. [3] Rasmussen et al. (2014) *GSA Bull.*, doi:10.1130/B30944.1. [4] Delstanche et al. (2009) *Geochim. Cosmochim. Acta*, **73**, 923-934.

Identification of taste-and-odor producing and degrading bacteria in a freshwater reservoir, central Indiana

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Freshwater Actinobacteria (Actinomycetes) and Cyanobacteria (blue-green algae) cause a multitude of water-quality concerns by producing taste-and-odor (T&O) compounds. These algal metabolites may have a significant economic impact and are of particular interest in lakes, reservoirs, and rivers that are used for drinking-water supply, recreation, or aquaculture. These odorous compounds (*e.g.*, 2-methylisoborneol (MIB), trans-1,10-dimethyl-trans-9-decalol (geosmin)) that frequently impair the water treatment process and the production of drinking water are produced intracellularly and released into the water naturally when organisms die or chemically when an algaecide treatment is applied to the water body. Once released, metabolites can either be found in the dissolved fraction or bound to particles that settle to the bottom sediments. These organic compounds are readily available as a food source for other bacteria that have the ability to degrade them.

Investigations focused on Eagle Creek Reservoir (Central Indiana) that frequently witnesses seasonal episodes of MIB and geosmin. Water samples were collected biweekly, from May to October, near the dam of the reservoir to characterize the seasonal variability and, at four discrete depths (0, 3, 6 and 10 meters) to characterize the spatial distribution of the bacterioplankton. After an odorous event during the spring 2013, the reservoir received a copper-based algaecide treatment in order to terminate the internal production of taste-and-odor compounds by eliminating the growing producers. To understand the relationship between the microbial communities, habitats and production/degradation of odorous metabolites, a metagenomic technique using the 16S rRNA gene was conducted. Results indicated that Cyanobacteria, Proteobacteria, Bacteroidetes and Actinobacteria were the most abundant taxa of the bacterioplankton. Statistical analysis determined that MIB occurrences were correlated to the presence of Actinomycetes belonging to the genus of *Streptomyces* ($p < 0.01$), whereas occurrences of geosmin were linked to some cyanobacterial taxa such as *Planktothrix* ($p < 0.001$) and *Phormidium* ($p < 0.01$). After application of the algaecide, degradation of released metabolites showed a positive correlation with the growth of some bacteria, represented by Sphingomonadales (α -Proteobacteria), Burkholderiales (β -Proteobacteria), Pseudomonadales (γ -Proteobacteria) and Flavobacteriales (Bacteroidetes). MIB degradation was mainly carried out by α -Proteobacteria (*Sphingomonas*, *Sphingobium*; both $p < 0.001$), γ -

Proteobacteria (*Pseudomonas*; $p < 0.001$) and Flavobacteriales (*Flavobacterium*; $p < 0.001$). The degradation of geosmin appeared to be closely related to Flavobacteriales (*Chryseobacterium*; $p < 0.01$). A Canonical Component Analysis (CCA) explaining 72.3% of the total variance of the dataset revealed four different clusters. These clusters describe habitats where bacterial groups can be found within the water column under different reservoir's hydrodynamical conditions. Actinobacteria were mostly found in the upper layers of the water column by the end of the spring in association with elevated nitrate levels whereas cyanobacterial growth was favored by more stable conditions and higher water temperatures during the summer stratification. Bacterial degraders were identified in the anoxic bottom waters of the hypolimnion.

Did iron-oxidizing chemolithotrophic bacteria play a role in the formation of early phosphorites?

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Phosphorites, marine sedimentary units of high phosphate content, first appear in the Paleoproterozoic in the wake of the initial rise of atmospheric oxygen. Rising oxygen levels are attributed to microbial oxygenic phototrophy which would have induced locally steep redox gradients – energy-rich niches likely to have stimulated the evolution of microbial metabolisms. At the same time however, diurnal fluctuations would have also required a strategy for dealing with alternating oxic-anoxic redox regimes. Today, the utilization of high-energy bonds in polyphosphates (polyP) is recognized as one such strategy. Under oxic conditions phosphate is taken up and stored intracellularly as polyphosphates, while under anoxic conditions the stored bond energy is accessed through the cleaving off of phosphate ions from the polyphosphates chain. Here we present microfossil evidence of filamentous bacteria preserved within Paleoproterozoic stromatolites. The distinct morphology of the microfossils resembles that of the extant iron-oxidizing and stalk-forming *Mariprofundus ferrooxydans* PV-1 recently found to encode genes for the uptake and storage of polyphosphates. Expulsion of phosphate into a confining stromatolitic matrix may have driven phosphate concentrations to supersaturation with respect to apatite and contributed to the formation of these unusually phosphate-rich stromatolites. These findings suggest that the early evolution of polyphosphate utilization may have been a strategy for dealing with locally fluctuating redox conditions as the Earth begins to become oxygenated, and may also offer insight into the occurrence of the earliest pulse of Paleoproterozoic phosphorites.

Geobiological interpretation of a travertine core from Yellowstone National Park

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Mammoth Hot Springs in Yellowstone National Park is the second-largest travertine-depositing hot spring system in the world. The spring system has been actively depositing calcium carbonate travertine for ~8000 years, and has built up to 75 m of travertine across its ~4 km² area. In 1967, the United States Geological Survey undertook a drilling initiative in the Mammoth Hot Springs area in order to better understand the thickness and subsurface conditions of the travertine complex. This study presents a new stratigraphic analysis of the USGS Y-10 core from Mammoth Hot Springs that places the Y-10 core in a geobiological facies context, based on the facies model defined for the modern spring system by Fouke et. al (2000). Previous stratigraphic analysis of the core has focused on understanding the variation in petrographic characteristics in the travertine, but has not emphasized the geobiological context of the deposit. Using a geobiological facies framework for stratigraphic analysis of the Mammoth Hot Springs travertine lends insight for better interpretation of the ancient biological, physical, and chemical conditions of this dynamic spring system. Careful analysis of the core shows that the same geobiological characteristics that were used to define the modern facies model are observed in the Y-10 core, indicating that the general spring biology, chemistry, and hydrology have remained similar over time. Patterns of progradation and regression, observed in the modern as a response to spring flow, can also be observed in the Y-10 core. An overall shift towards more proximal facies indicates that the spring outlet likely moved over time due to changes in flow rates.

Phylogenetic and Metabolic Activity of an Aquificales-dominated Microbial Community Inhabiting the Apron and Channel Facies of Mammoth Hot Springs, Yellowstone National Park, USA

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Filamentous ‘streamer’ microbial communities dominated by Aquificales (e.g., *Sulfurihydrogenibium spp.*) are found in neutral pH terrestrial springs at geographically diverse hydrothermal sites. These thermophilic bacteria interact with the indigenous geochemical and hydrologic factors and plays important role in carbon, nitrogen, sulfur and/or hydrogen cycling, which end up enhancing the formation of morphologically distinct CaCO₃ travertine mineral precipitants. To understand the ecophysiology of *Sulfurihydrogenibium spp.*, metagenomic, metatranscriptomic and metaproteomic analyses were conducted on a model microbial community from Narrow Gauge hot spring in the Mammoth Hot Spring complex of Yellowstone National Park. The Apron and Channel travertine depositional facies is an environment of shallow turbulent sheet flow at temperatures of 70-72°C, pH of 6.2-6.5, flow rates of 0.3-10 cm/s) in which distinct filamentous travertine deposits are precipitated. Illumina pyrosequencing targeting V3-V5 hypervariable region of the 16S rRNA genes, metagenome and metatranscriptome yielded 0.19, 60.8 and 55.6 million paired-end reads. Meanwhile, 244 proteins extracted from the bio-mat of the hot spring were assigned to Order Aquificales. All of these data libraries consistently indicated dominance of *Sulfurihydrogenibium spp.*, with the relative abundance 89.3, 97.8, 94.8 and 97.3 % for the 16S rRNA genes

identified by pyrosequencing, metagenome and the open reading frames (ORFs) identified from the metagenome and metatranscriptome, respectively. Parallel comparative assignment of metagenomic and metatranscriptomic ORFs into Cluster of Orthologous Groups (COG) categories suggested active transcription of the ones assigned to the COG categories related to energy production and convention, translation and biogenesis as well as posttranslational modification. Functional characterization of the highly expressed genes and protein analyses suggested the metabolic strategies employed by the *Sulfurihydrogenibium spp.*, such as carbon-, nitrogen-, sulfur-cycling, membrane synthesis, heat tolerance and flagella synthesis, allow them to dominate these turbulent flow environments.

Lower-Middle Ordovician sulfur isotope stratigraphy at Shingle Pass, Nevada, USA: implications for marine redox conditions and the Great Ordovician Biodiversification Event (GOBE)

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Here we report sulfur isotopic data ($\delta^{34}\text{S}$) from the Great Basin region of North America measured from the Lower-Middle Ordovician carbonate strata of the Pogonip Group at Shingle Pass, NV, USA. Paired sulfur isotope analyses were measured from carbonate-associated sulfate (CAS) and disseminated sedimentary pyrite from bulk carbonate lime mudstones and wackestones. Parallel positive isotope excursions in both $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ (2‰ and 11‰, respectively) in the uppermost *Rossodus manitouensis* conodont Zone (Tremadocian Stage) are interpreted to record a short-lived period (~0.2 myr) of the upwelling of ocean anoxia with evidence for elevated burial rates of organic matter and pyrite. Modeling results indicate that the size of the Ordovician sulfate reservoir (6×10^{18} mol SO_4 ; 4.6 mM) was similar to estimates drawn from fluid inclusion data and other modeling studies and that this event could have released up to 3×10^{18} mol of oxygen to the atmosphere-ocean system. This perturbation appears to represent one of the last major isotope excursions in the early Paleozoic where changes to the carbon and sulfur cycles were coupled, suggesting that this interval marks an important transition in marine redox conditions. This oxygenation event occurred prior to and during some of the earliest pulses of biodiversification that record the onset of a major radiation of animal life known as the Great Ordovician Biodiversification Event. Oxygenation also occurred prior to an increasing trend in regional biodiversity based on biodiversity data compiled from published occurrence data of most animal groups from measured sections in the nearby Ibex area, central Utah.

Rethinking secular $\delta^{34}\text{S}$ records: Stratigraphic trends and biogeochemical interpretations

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Sulfur isotope ratio data ($\delta^{34}\text{S}$) provide a framework for reconstructing both global redox budgets and microbial metabolic activity over Earth history. However, as the record of ancient oceanic conditions becomes better resolved, reports of coeval but divergent isotopic proxies are becoming increasingly common. These sulfur isotope records are characterized not just by divergent $\delta^{34}\text{S}$ values, but also by differences in the spatial signature and magnitude of isotopic variability. Such discordant data suggest that we do not fully understand how isotopic signatures are incorporated and eventually preserved in the rock record. Here we examine the spatial signature and magnitude of isotopic variability in modern marine systems as a function of depositional environment and differential microbial metabolic activity. Varying depositional conditions, particularly sedimentary reworking, are seen to play a major role in generating and modifying the isotopic signatures of sulfur phases in modern environments. These observations can be extrapolated to investigate records of sulfur cycling in ancient strata. The results suggest that many apparent secular $\delta^{34}\text{S}$ trends may be related to changing depositional environment rather than changes in the global sulfur cycle. Further, this environmental dependence can also help explain coeval but discordant $\delta^{34}\text{S}$ data from within and between sedimentary basins. Together, these observations provide new insights that enable us to reflect on and refine our interpretations of chemostratigraphic $\delta^{34}\text{S}$ data that have the potential to constrain the behavior of the sulfur cycle over geological timescales.

Community level physiological profiling of diverse environments reveals functional and taxonomic diversity within aerobic, single carbon-source enrichments

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Microbial communities in complex, heterogeneous environments can be comprised of thousands of different taxa. Characterizing these communities using 16S rRNA gene-based assays provides only a coarse profile of the functional capabilities of the microbes present. Community level physiological profiling (CLPP) provides a different, functional view of microbial communities by measuring a community's ability to respire various carbon substrates by using a redox indicator dye that turns a vivid purple in the presence of actively respiring microbial cells. The broad functional diversity of a particular community can therefore be inferred from the pattern of purple (dye+) wells on a 96 well microtiter plate. The extent to which the active organisms in dye+ wells represent populations that are actively respiring *in situ*, however, is unknown. In this project, we combine CLPP and molecular microbial ecology to better understand the relationship between the composition and function of microbial communities and biogeochemical processes in nature. CLPP experiments were inoculated using soil suspensions or natural waters from five disparate terrestrial and aquatic environments. We then sequenced 16S rRNA gene amplicons from each well as well as the original material using the Illumina MiSeq platform. We found that CLPP creates similar but not identical clustering patterns as phylogenetic profiles based on the 16S rRNA gene. Furthermore, 16S rRNA-based analysis of the enriched communities within each well cluster in the same manner as the phylogenetic analysis of the inocula. Many of the most highly enriched populations, however, are not the most abundant organisms in the original environment. These results suggest that while the identity of the most "active" members of a community is determined in part by the original environment, functional assays that are based on microbial growth only partially recapitulate the true functional diversity of a microbial community. Still, CLPP can provide valuable insight into active microbial processes by identifying sub-communities that respire specific carbon compounds.

In vitro and *in situ* analysis of microbial diversity at Chocolate Pots hot springs, Yellowstone National Park

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Previous research into the microbial community at Chocolate pots hot spring (CP), Yellowstone National Park (YNP) has identified an abundance of organisms supported by dissimilatory iron reduction (DIR). Previous most probable number (MPN) enumerations and enrichment culture studies confirmed the presence of endogenous microbial communities that reduced native CP Fe(III) oxides. Enrichment cultures demonstrated sustained DIR coupled to the oxidation of acetate and lactate through repeated transfers over ca. 450 days. Bacterial sulfate reduction to sulfide, and abiotic oxidation of sulfide by ferric oxide, was determined to not contribute extensively to the observed level of iron reduction. Genomic DNA was extracted from enrichment cultures for 16S rRNA gene pyrosequencing in order to identify putative populations involved in DIR. Results indicated that dominant OTUs from the enrichment cultures were closely affiliated with the well known Fe(III) reducer *Geobacter metallireducens*. Additional taxa included relatives of sulfate reducing bacterial genera *Desulfohalobium* and *Thermodesulfobivrio*. A metagenomic analysis of enrichment cultures is underway in anticipation of identifying genes involved in DIR and thermotolerance. Current studies are aimed at interrogating the *in situ* microbial community at CP. Core samples were collected along the flow path at CP and subdivided into 1 cm depth intervals. A parallel study investigated *in vitro* microbial DIR in sediments collected from three of the coring sites. DNA was extracted from samples from both studies for 16S and metagenomic sequencing in order to obtain a more detailed understanding of the vertical and longitudinal distribution of microbial taxa throughout CP than was previously undertaken. These studies will provide insight into the coupled geochemical-microbial system, demonstrating how genomic properties change with depth and distance in a Fe-rich, neutral pH geothermal environment.

Capture and cultivation of microorganisms using magnetic, lipid-bound antibodies

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Despite advancing techniques in microbiology, an estimated 98% of all microbial species on Earth have yet to be isolated in pure culture. Natural samples, once transferred to the lab, are commonly overgrown by “weed” species whose metabolic advantages enable them to monopolize available resources. Developing new methods for the isolation of thus-far uncultivable microorganisms would allow us to better understand their ecology, physiology and genetic potential. Physically separating target organisms from a mixed community is one approach that may allow enrichment and growth of the desired strain. Here we report on a novel method that uses known physiological variations between taxa, in this case membrane lipids, to segregate the desired organisms while keeping them alive and viable for reproduction. Magnetic antibodies bound to the molecule squalene, which is found in the cell membranes of certain archaea, but not bacteria, enable separation of archaea from bacteria in mixed samples. Viability of cells was tested by growing the separated fractions in batch culture. Efficacy and optimization of the antibody separation technique are being evaluated using qPCR and cell counts. Future work will apply this new separation technique to natural samples.

Sulfur and oxygen isotope insights into sulfur cycling in shallow-sea hydrothermal vents, Milos, Greece

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Shallow-sea (5m depth) hydrothermal venting off Milos Island provides an ideal opportunity to target transitions between igneous abiogenic sulfide inputs and biogenic sulfide production during microbial sulfate reduction. Seafloor vent features include large white patches containing hydrothermal minerals (elemental sulfur and orange/yellow patches of arsenic-sulfides) and cells of sulfur oxidizing and reducing microorganisms. Despite visual evidence for the close association of vent organisms and hydrothermalism, the sulfur and oxygen isotope composition of pore fluids did not permit delineation of a biotic signal separate from an abiotic signal. Hydrogen sulfide in the free gas had uniform $\delta^{34}\text{S}$ values ($2.5 \pm 0.28\text{‰}$, $n = 4$) that were nearly identical to pore water H_2S ($2.7 \pm 0.36\text{‰}$, $n = 21$). In pore water sulfate, there were no paired increases in $\delta^{34}\text{S}_{\text{SO}_4}$ and $\delta^{18}\text{O}_{\text{SO}_4}$ as expected of microbial sulfate reduction. Instead, pore water $\delta^{34}\text{S}_{\text{SO}_4}$ values decreased (from 21‰ to 17‰) as temperature increased across each hydrothermal feature. We interpret the inverse relationship between temperature and $\delta^{34}\text{S}_{\text{SO}_4}$ as a mixing process between oxic seawater and ^{34}S -depleted hydrothermal inputs that are oxidized during seawater entrainment. An isotope mass balance model suggests secondary sulfate from sulfide oxidation provides at least 15% of the bulk sulfate pool. Coincident with this trend in $\delta^{34}\text{S}_{\text{SO}_4}$, the oxygen isotope composition of sulfate tended to be ^{18}O -enriched in low pH (<5), high temperature (>75°C) pore waters. The shift toward high $\delta^{18}\text{O}_{\text{SO}_4}$ is consistent with equilibrium isotope exchange under acidic and high temperature conditions. The source of H_2S contained in hydrothermal fluids could not be determined with the present dataset; however, the end-member $\delta^{34}\text{S}$ value of H_2S discharged to the seafloor is consistent with equilibrium isotope exchange with subsurface anhydrite veins at a temperature of $\sim 300^\circ\text{C}$. Any biological sulfur cycling within these hydrothermal systems is masked by abiotic chemical reactions driven by mixing between low-sulfate, H_2S -rich hydrothermal fluids and oxic, sulfate-rich seawater.

A combined molecular and isotopic study of anoxygenic photosynthesis in meromictic lakes of the northwestern United States

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The sulfur isotope composition of dissolved sulfate and sulfide within the chemocline of anoxic lakes can be an indication of green and purple sulfur photosynthetic activity. This signal is, however, small and variable, with fractionations on the order of -3 to +5‰ [1]. It is therefore advantageous to investigate the environmental and ecological effects on this signal so that these influences can be taken into account when estimating the contribution of anoxygenic phototrophs to the sulfur cycle in aquatic environments.

This project aims to investigate the ways in which anoxygenic phototroph community structure and lake water geochemistry impact the sulfur isotope fractionation expressed during anoxygenic photosynthesis in meromictic lakes. During the summer of 2013, water column profile analysis of six lakes in the Pacific Northwest (located in eastern Washington and western Montana) were conducted to assess photosynthetically available radiation, salinity, pH, temperature, dissolved solids, and specific conductivity. Water column samples were obtained to determine the sulfur isotopic composition of dissolved sulfate and sulfide, major ion and sulfide concentrations. Microbial samples were also collected for genetic sequencing. Initial results found green (*Chlorobiaceae* sp.) and purple (*Lamprocystis purpurea*) bacteria at the same depth in one of the study lakes. These data, in addition to the same suite of samples collected in the summer of 2014, provide insight into relationships between the isotopic composition of sulfur (in H₂S, S⁰, and SO₄), lake water chemistry, and the presence or absence of green and purple sulfur bacteria.

[1] Zerkle et al. (2009) *Geochim. Cosmochim. Acta*, **73**, 291-306

Environmental controls on elemental sulfur production by *Thiomicrospira thermophila* using the Sox multi-enzyme pathway

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Elemental sulfur, thiosulfate, and polythionates, can be biogeochemically important in marine sediment environments even when their steady-state concentrations are low (<μM) due to rapid, hidden (or ‘cryptic’) recycling. The Sox pathway is a versatile multienzyme pathway responsible for oxidizing thiosulfate (S₂O₃) that has been demonstrated in physiologically diverse organisms, including green sulfur bacteria, purple sulfur bacteria, and colorless sulfur bacteria [1]. *T. thermophila* uses the Sox enzymes for sulfur catabolism and can completely oxidize thiosulfate to sulfate or partially oxidize it to elemental sulfur, rapidly switch from one mechanism to another as a function of pH. The inhibition of the enzyme SoxCD combined with the activation of SoxL is shown here to be essential for elemental sulfur formation via polysulfide. Both the rate of sulfate production and growth rate are tied to the activity of SoxCD in batch growth. At low pH, an additional enzyme (thiosulfate dehydrogenase) is activated that oxidizes thiosulfate to tetrathionate (S₄O₆). Both S₂O₃ consumption and SO₄ production rates are significantly decreased once S₄O₆ production begins. The formation of elemental sulfur via a modified Sox pathway such as this may be common among marine sulfur oxidizing autotrophs and mixotrophs.

[1] Friedrich et al. (2001) *Appl. Environ. Microbiol.*, **67**, 2873-2882.

Behavior of antimony(V) under sulfate-reducing conditions.

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Introduction

Antimony (Sb) is a metalloid that is used in a variety of industrial manufacturing settings. While antimony has an assortment of useful applications, it has a similar toxicity to other Group 15 elements. As the mining and use of antimony continues to increase, environmental concerns involving the element grow. Antimony(V) and (III) are the two most abundant oxidation states for the element. Little is known about the redox transitions between the two species in a contaminated environmental setting. In order to better understand the behavior antimony in anoxic environments we have set up systems under sulfate-reducing conditions.

Experimental Methodology

The behavior of Sb(V) under sulfate-reducing conditions was examined in aqueous suspensions containing 2 mM KSb(OH)_6 , 50 mM Fe(III) (as ferrihydrite), 10 mM sulfate, and 10 mM lactate, that were inoculated with sediment from a wetland on the campus of Argonne National Laboratory in Argonne, Illinois. Samples were collected over time to track changes in the concentrations of Sb, Fe(II), sulfate, and lactate. Samples were also collected for microbial community analysis. X-ray absorption fine-structure (XAFS) spectroscopy was used to determine Sb valence state and chemical speciation.

Discussion of Results

Lactate was rapidly fermented to acetate and propionate, with the latter serving as the primary electron donor for dissimilatory sulfate reduction (DSR). Sb X-Ray absorption near structure (XANES) analysis showed complete reduction of Sb(V) to Sb(III) within 4 weeks, concurrent with DSR and formation of FeS. Sb extended X-ray absorption fine structure (EXAFS) spectroscopy indicated that the reduced phase a mixture of S- and O-coordinated Sb(III). These results suggest that reduction of Sb(V) is likely in sulfidogenic environments.

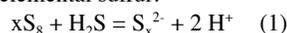
Reactivity of elemental sulfur nanoparticles

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Sulfur biogeochemical cycling involves a vast network of chemical reactions including electron exchange, polymerization (S-S bonding), and aggregation that leads to α -S₈, via the step of forming nanoparticulate elemental sulfur or S_{8(nano)}.

Elemental sulphur participates in nucleophilic and hydrolysis reactions to form sulfur intermediates, such as polysulfide ions. The nucleophilic reaction with sulfide (as H₂S or HS⁻, reaction 1), is a key process in the dissolution of elemental sulfur:



This reaction can occur in its forward (chain elongation reactions of polysulfide to form S₈ rings) or reverse (coarsening process of S₈-rings to form S_{8(nano)} and α -S₈) direction. Its overall direction is dictated by the amount of polysulfide present, however the surface area and surface character of elemental sulfur are tested on how they influence the kinetics of the reaction.

Here we present some preliminary data on the kinetics of elemental sulfur consumption (reaction 1) that have been tested in various sizes and surface properties of the sulfur nanoparticles. The surface area and presence (or absence) of surfactant molecules influences the kinetics of polysulfide formation. The cycling of that reaction may play a significant role to the element's bioavailability to microorganisms, which incorporate the nanoparticulate (rather than the bulk α -S₈) form of elemental sulfur or polysulfides in their specific metabolisms [1].

[1] Boyd and Druschel (2013) *Appl. Environ. Microbiol.*, **79**, 2061-2068

Using leaf wax biomarkers to constrain land-use changes associated with Mississippian settlements

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We attempt to better understand the context of environmental and land-use changes near the Cahokia (Mississippian) settlement over the past two millennia by examination of environmental records from lake sediments. Several sediment cores were taken from Horseshoe Lake (Madison County, IL), about a mile away from the Cahokia mound settlement site. We detected abundant leaf wax lipids throughout the core. In conjunction with pollen analysis, leaf waxes have the ability to preserve information about the history of vegetation in the area surrounding the lake. In particular, we aim to address two questions: i) can we place age constraints on the onset and disappearance of extensive maize agriculture in the area surrounding Horseshoe Lake? ii) can we identify any significant changes in paleohydrology in this region over the course of the last two millennia?

To address agricultural changes, we take advantage of the fact that unlike most plants native to the Mississippi valley, maize uses C4 carbon fixation, which typically results in an enrichment of ^{13}C in its biomass relative to other vegetation. By examining the ^{13}C content of leaf waxes through the sediment core, we may be able to detect shifts coincident with the onset and decline of agricultural land use by the Cahokian settlement. Leaf waxes also contain paleohydrological information, manifested in their deuterium to hydrogen (D/H) ratio, which derives from that of environmental water [1]. Observations of excursions in leaf wax D/H could be indicative of hydrological stress, such as associated with a drought.

Analysis of the 2011 Horseshoe Lake core revealed that waxes were abundant enough to continue with isotopic analysis on the 2012 Horseshoe Lake core. ^{13}C values from the 2012 Horseshoe Lake core suggest maize agriculture peaked in the area ~900 CE. Further study will involve detailed measurement of D/H leaf waxes in the 2012 core from Horseshoe Lake.

[1] Sachse, D. et al. (2012) *Ann. Rev. Earth Planet. Sci.*, **40**, 221-249.

The influence of wastewater effluent on microbial communities in an urbanized river system

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Communities of microorganisms in highly urbanized aquatic ecosystems are regularly acclimating to the changing flux of nutrients caused by environmental conditions and anthropogenic inputs. Wastewater treatment plants have the potential to significantly impact the microbial communities when the effluent is discharged directly into these environments. The Chicago River is a system of channels and canals that flows through a highly urbanized area with water inputs mainly from domestic pumpage and stormwater runoff. It includes the Terrence J. O'Brien Water Reclamation Plant on the North Shore Channel that presumably contributes to chemical and biological fluxes downstream of the facility through its release of 0.787 million m^3 per day on average of treated but non-disinfected wastewater effluent. Disinfection of the effluent is scheduled to begin in 2015. Using a metagenomic approach, we investigated the bacterial composition and genetic potential in sites upstream and downstream of the effluent discharge to determine the impacts of wastewater effluent inputs on the riverine microbial communities. Concurrent with a significant increase in nitrogen downstream of the effluent input, we evaluated shifts in the microbial communities that could be linked to the organisms and chemical composition of the effluent. These results suggest that the treatment facility significantly affects microbial community dynamics. This effort will help gauge how the implementation of disinfection might alter downstream microbial community composition and metabolism.

Geoscience Education: Engaging in field-based scientific inquiry in Yellowstone National Park, an example of how science REALLY works

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Outreach and education programming emphasizing field-based learning and scientific inquiry, has exposed k-100 learners to the wonders of Yellowstone and the unique interface of geology and biology at Mammoth Hot Springs, in Yellowstone National Park.

This hot-spring system has been the focus of three distinct programs, all grounded in a scientific inquiry model, including Expedition Yellowstone!, a residential program for 4th to 8th grade students, Biocomplexity, a semester-long course for college honors undergraduates at the University of Illinois Urbana-Champaign, and a field-based course for adult learners hosted through the Yellowstone Association Institute, Mammoth Microbes and Global Connections.

While each program focuses on a specific audience, field-based immersion and scientific process skills are targeted using a model of scientific inquiry that emphasizes the dynamic nature of science. Our suggested scientific inquiry model provides an alternative framework to the typical way the Scientific Method is presented in most classrooms and understood by the general public. The standard model for performing scientific investigations is portrayed in a very linear, step-wise fashion. This often-taught directional model fails to accurately portray how real science works.

Using our model of scientific inquiry, students of all ages experience the creative and authentic scientific process in a geobiological context through three unique education initiatives in Yellowstone National Park.

The ecological dynamics of sharks based on stable isotope analysis

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The ecological roles of sharks vary as meso- and apex predators in estuarine and pelagic environments. However, the diet, environment, and behaviour of modern and ancient sharks is difficult to study using traditional ecological and paleontological methods. Stable isotope analysis is a biogeochemical tool often used to study the ecology of mammals, birds, and teleosts but this technique was only recently introduced to shark ecology. Here, I use stable isotope analysis to determine ecological dynamics in the following modern and ancient shark systems:

1) White sharks are often depicted as apex predators that forage on marine mammals. However, a comparison of isotope niche width within vs. between individuals revealed a distribution of specialists and generalists within the California population.

2) Fossil shark teeth from Peru record a transition from *Cosmopolitodus hastalis* and an unnamed *Carcharodon* species to the modern *C. carcharias* during the mid-Miocene to early Pliocene. Although this transition is attributed to increased upwelling, oxygen isotope values suggest that environmental conditions did not dramatically change.

3) Modern sand tiger sharks live in marine habitats at tropical to temperate latitudes. However, there are fossil sand tiger shark teeth in Eocene sediments in the Arctic and Antarctic. A paleosalinity estimate based on the isotopic analysis of Eocene Arctic shark teeth portrays a freshwater dominated Eocene Arctic Ocean.

Acid extraction overestimates total Fe(II) in the presence of Fe(III) oxide and Fe(II) sulfide minerals

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Aquatic and terrestrial environments are dynamic systems where coupled microbiological, geochemical, and hydrological processes define the complex interactions that drive the biogeochemical cycling of major and minor elements. Fe(III) is ubiquitous in these environments and is typically present as various minerals, including Fe(III) oxides such as ferrihydrite, lepidocrocite (γ -FeOOH), goethite (α -FeOOH), and hematite (α -Fe₂O₃). Ferrous [Fe(II)] sulfide minerals [e.g., mackinawite (FeS), greigite (Fe₃S₄), pyrrhotite (FeS_{1+x}), and pyrite (FeS₂)] are also common in soils and sediments, particularly under anoxic conditions. Accurate determination of Fe(II) concentrations and distribution is key to understanding iron redox processes involved in the biogeochemical cycling of Fe in environmental and engineered systems and acid extraction followed by a colorimetric assay is a widely used approach for determination of Fe(II) in soil and sediment samples. Under sulfidogenic conditions, these samples can contain both metal sulfides and Fe(III) oxides. We compared the Fe(II) concentrations determined by acid extraction followed by colorimetric analysis to those determined by extended X-ray absorption fine structure (EXAFS) spectroscopy for samples containing Fe(III) oxide (as ferrihydrite, lepidocrocite, or goethite) and mackinawite (FeS). Our measurements of Fe(II) concentrations in these systems showed that concentrations determined by acid extraction were 1.5 to 3 times higher than those determined by EXAFS analysis depending on the Fe(III) oxide, due to reduction of Fe(III) to Fe(II) by sulfide following acidification (which causes dissolution of FeS and release of sulfide into solution) of the samples. These results illustrate the need for caution when using acid extraction with colorimetric analysis to determine Fe(II) concentrations in samples containing Fe(III) oxides and ferrous sulfide minerals.

The ecophysiology of sulfur isotope fractionation by sulfate reducing bacteria in response to variable environmental conditions

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Microbial sulfate reducers (MSR) drive the Earth's biogeochemical sulfur cycle. At the heart of this energy metabolism is a cascade of redox transformations coupling organic carbon and/or hydrogen oxidation to the dissimilatory reduction of sulfate to sulfide. The sulfide produced is depleted in the heavier isotopes of sulfur relative to sulfate. The magnitude of discrimination (fractionation) depends on: *i*) the cell-specific sulfate reduction rate (csSRR, Kaplan & Rittenberg (1964) *Can. J. Microbio.*; Chambers et al. (1975) *Can. J. Microbio.*; Sim et al. (2011) *GCA*; Leavitt et al. (2013) *PNAS*), *ii*) the ambient sulfate concentration (Harrison & Thode (1958) *Research*; Habicht et al. (2002) *Science*; Bradley et al. *in review*), *iii*) both sulfate and electron donor availability, or *iv*) an intrinsic physiological limitation (e.g. cellular division rate). When neither sulfate nor electron donor limits csSRR a more complex function relates the magnitude of isotope fractionation to cell physiology and environmental conditions. In recent and on-going work we have examined the importance of enzyme-specific fractionation factors, as well as the influence of electron donor or electron acceptor availability under carefully controlled culture conditions (e.g. Leavitt et al. (2013) *PNAS*). In light of recent advances in MSR genetics and biochemistry we utilize well-characterized mutant strains, along with a continuous-culture methodology (Leavitt et al. (2013) *PNAS*) to further probe the fractionation capacity of this metabolism under controlled physiological conditions. We present our latest findings on the magnitude of S and D/H isotope fractionation in both wild type and mutant strains. We will discuss these in light of recent theoretical advances (Wing & Halevy (2014) *PNAS*), examining the mode and relevance of MSR isotope fractionation in the laboratory to modern and ancient environmental settings, particularly anoxic marine sediments.

Diversity of the epoxyalkane:coenzyme M transferase gene (*etnE*) at different vinyl chloride contaminated sites

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Vinyl chloride (VC) is a known human carcinogen and a common groundwater contaminant. It is usually generated via anaerobic reductive dechlorination at chlorinated solvents (usually chloroethenes) contaminated sites. VC can be aerobically oxidized by bacteria in aerobic zones. An important enzyme that participates in the process of VC oxidation is Epoxyalkane: Coenzyme M transferase, encoded by the gene *etnE*. It is currently used as a biomarker for VC oxidizers at contaminated sites. However, knowledge about the diversity and distribution of *etnE* in the environment is limited because *etnE* has not yet been recovered from the environment directly. In order to expand our current understanding of *etnE* diversity, six sites with different geological and contamination conditions were surveyed: Carver, MA, Naval Air Station (NAS) Oceana, VA and Soldotna, AK are VC-contaminated groundwater sites (aquifer sampled); Fairbanks, AK and Kotzebue, AK are groundwater site without VC (aquifer sampled); Altona (a balsalt site), Australia is also VC contaminated (vadose zone sampled). New touch-down PCR and nested PCR methods were developed to successfully amplify *etnE* from environmental samples with low *etnE* abundance (~60-70 gene copies/ μ L of DNA sample). PCR products were cloned and Sanger-sequenced. Finally 150 partial *etnE* sequences were recovered from all the environmental samples. Three major groups of *etnE* were found using phylogenetic analysis. The majority of *etnE* from VC-contaminated sites grouped in Clade I, the largest *etnE* group with *etnE* previously identified from *Mycobacterium*, *Pseudomonas*, and *Ochrobactrum* strains. Several sequences retrieved from VC-contaminated soil in Australia and from the relative pristine site in Kotzebue, AK group with the recently discovered *etnE* found in *Mycobacterium* strain NBB4 (isolated in Australia) in Clade I. Some of the NAS Oceana sequences grouped with Clade II, which includes *etnE* from *Nocardioides* and *Gordonia* strains. A new group (Clade III) emerged, with some of the Soldotna sequences, *Xanthobacter* Py2 EaCoMT gene and the putative *etnE* from a marine ethene-assimilating *Hailea* strain recently isolated. Most of the *etnE* recovered from Kotzebue and Fairbanks have either internal stop codons or a 7-bp deletion. Our *etnE* survey significantly expanded our knowledge of the functional gene diversity in the environment and *etnE* dataset, which will help develop new probes for qPCR and better diagnose VC degradation at contaminated sites.

Investigating isotope fractionation by ATP sulfurylase

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The oxygen isotope ratio of marine sulfate and of sulfate in the geologic record is determined in part by isotope fractionation that occurs during microbial dissimilatory sulfate reduction (DSR). In putative models of DSR, the first step in the process is the activation of environmental SO_4^{2-} to adenosine phosphosulfate (APS) by the enzyme ATP sulfurylase. In this work, our goal is to determine the oxygen isotope fractionation imposed by ATP sulfurylase. We have performed the reaction catalyzed by ATP sulfurylase [$\text{SO}_4^{2-} + \text{ATP} \rightarrow \text{APS} + \text{pyrophosphate}$] *in vitro*, which yielded significant APS product and confirmed that this is a practicable method to produce samples for isotopic analysis. In preparation for isotope analyses, we are developing a technique for the isolation of SO_4^{2-} and APS from the reaction mixture by a combination of selective sulfate precipitation with barium ions and anion-exchange chromatography. In constraining the fractionation associated with this enzymatic step, we will complement models of DSR pathways with enzyme-specific fractionation constraints, which will provide an improved understanding of microbial sulfate reduction and the global sulfur cycle.

Molecular features of dissolved organic matter produced by picophytoplankton

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Compounds derived from picophytoplankton through exudation, grazing and viral lysis contribute a large portion of labile DOM to the ocean. This labile DOM is rapidly turned over by and exchanged among microbial communities. However, identifying labile DOM compounds and tracking their sources and sinks in ocean ecosystems is complicated by the presence of non-labile DOM which has a significantly larger reservoir size and longer residence time. This study focuses on investigating labile DOM produced by single-strain cyanobacteria isolates via different modes of release and varied nutrient conditions. DOM compounds are analyzed by high-resolution mass spectrometry. Putative formula assignments to ions present in *Synechococcus* WH7803 exudate span a range of compositional space overlapping several classes of biochemicals, such as lipids, pigments, protein, nucleotides and carbohydrates. Incubation experiments using combined whole seawater and diluent of grazer-free or viral-free water at the BATS time-series station in Sargasso Sea will yield complimentary data. The compositional features of each type of DOM could serve as future proxies for different modes of DOM production in the oceans.

Psychrophilic adaptations in cytochromes from marine hydrocarbonoclastic bacteria

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Marine microorganisms such as *Colwellia psychrerythraea* and *Marinobacter hydrocarbonoclasticus* are important both for general global biogeochemical cycling and for specific roles in hydrocarbon degradation. These organisms encounter a range of temperatures in the marine environment, including many regions (the deep oceans and the poles) at low temperatures. We are working to elucidate molecular mechanisms of cold adaptation in these microorganisms through study of cytochrome c_{552} , a small electron-transfer protein. Using UV-visible absorption, circular dichroism, and NMR spectroscopies, X-ray crystallography, and electrochemistry, we are investigating the structure, dynamics, and energetics of homologous cytochromes from *Colwellia* and *Marinobacter*. We report that overall protein stability and protein dynamics (flexibility) are not coupled simply in this protein. Our structural and electrochemical analyses suggest specific functional adaptations for life in the cryosphere.

Microbial communities catalyzing commodity chemical and fuel production

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Increasing concerns over the effect of global warming have inspired increased attention on renewable energy and chemical production technologies. Despite this, the US remains the world's largest petroleum consumer, with consumption attributed primarily to transportation (71% of total US consumption) and industry (23%), including chemical production. Microbial electrosynthesis is a sustainable process capable of generating transportation fuels and commodity chemicals while reducing greenhouse gas emissions and minimizing dependence on fossil fuels. Microbial electrosynthesis systems (MESs) are fuel cell-type devices that utilize autotrophic microorganisms to catalyze electron transfer from an electrode to a desired product with carbon dioxide as the only carbon source. To date, mixed microbial communities (microbiomes) confer the highest chemical production rates and the most versatile production profile compared to other biocatalysts in MESs. Highly productive microbiomes originating from brewery wastewater were selected on graphite cathodes poised at -590mV vs SHE in MESs. Acetate, methane, hydrogen, formate, propionate, and butyrate were produced at varying rates depending upon the microbial community and reactor conditions. A time course analysis over 300 days of multiple electrosynthetic reactors indicated a relatively stable community dominated by *Acetobacterium*, *Methanobacterium*, and *Sulfurospirillum* in addition to a more variable community of less abundant OTUs. Metagenomic reconstruction of the community indicates that the Wood-Werkman pathway is the central metabolic scaffold key to driving microbial electrosynthesis from CO₂. This study contributes to the understanding of electrode-associated community dynamics in response to a negatively poised electrode potential and has broad implications for application of microbiome catalysts. Given that the global commodity chemical market is greater than \$2 trillion, MES technology has immense potential for the renewable chemical and fuel production industry. Additionally, MESs provide a unique tool to understand the fundamental processes behind microbial extracellular electron transfer.

Microbial ecology of terrestrial serpentinizing springs

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Subsurface serpentinization produces highly reduced, high pH waters that provide microbial habitat, with characteristics including appreciable dissolved hydrogen and methane, little inorganic carbon, and limited electron acceptors. As these waters approach the oxygenated surface environment, microbial biomes shift to include members that can metabolize oxygen. As such, serpentinizing springs represent ecotones where these two distinct environments meet. Here, we characterize several springs strongly influenced by serpentinization in the Zambales ophiolite (the Philippines), and an ephemeral spring in the Tekarova ophiolite (Turkey). Field and laboratory measurements of fluids allow us to define the geochemical environments that embody these ecosystems.

Serpentinizing fluids in the Zambales ophiolite range from pH 9-11, and have low dissolved oxygen (0.06-2 mg/L) and ORP in the source pools. Springs produce extensive travertine terraces, and low flow systems produce calcite rafts/flakes. Variable flow rates indicate variable subsurface-surface mixing capacity. Measurements of dissolved gases reveal concentrations of H₂ and CH₄ up to 10⁻⁴ M, CO₂ up to 10⁻³ M, and trace amounts of CO. These sites provide potential for differing degrees of mixing/reaction with local meteoric water, local hydrothermal fluids, and adjacent sedimentary rock unit.

The Tekarova ophiolite has been venting gas for thousands of years. Previous work (e.g., Hosgormez et al., 2008) has reported that the vents are releasing gas of >85% CH₄ and up to 11% H₂ by volume. We have discovered a gas vent with a small flowing spring source. Preserved travertine terraces indicate that the source has been present for some time, but is likely ephemeral. SEM and XRD indicate that biofilms are calcite-based, and host microbial communities, but that calcite precipitation is unlikely biologically driven. Total carbon content in biofilms increases downstream, but organic carbon content decreases. Based on the geochemistry, all springs should support iron and sulfate reduction as well as methanotrophy and methanogenesis. Heterotrophic processes may be significant. Sequencing of 16S rRNA genes support these hypotheses, based on nearest neighbor metabolic functions. Results will be compared with other known serpentinizing fluids to place these microbial habitats in context of global biogeochemistry.

Protein-based functional analysis of tundra soil microbial communities

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Permafrost-affected soils contain approx. 1,500 Pg of organic carbon in the upper 3 m, accounting for over half of the total soil C_{org} pool. The fate of this C in a rapidly warming Arctic is uncertain. The conversion of a fraction of this C to GHGs with increasing microbial activity could add significantly to the 800 Pg C currently in the atmosphere, and a similar feedback may have caused Paleocene-Eocene hyperthermals (DeConto et al., 2012, *Nature*).

Long-term warming and fertilization experiments at Toolik Field Station on the North Slope of Alaska provide one means of understanding climate-induced changes in soil biogeochemical cycles. These experiments confirm pan-Arctic observations that deeply rooting woody shrubs are expanding at the expense of native tussock sedges and non-vascular vegetation. Floral succession increases C storage, but this may be a transient effect dwarfed by changes in the much larger soil C pool. Long-term measurements of soil C storage have high variance due to the heterogeneity of permafrost soils. Data on changes in microbial community function would complement measurements of C stocks and fluxes. Protein expression profiles from “metaproteomics” experiments are a proxy for microbial community function. Metaproteomics experiments are facilitated by an expanding number of soil metagenomic surveys of permafrost-affected soils.

I collected 2¼” diameter soil cores to the permafrost table from different environments in the vicinity of Toolik Field Station, including geomorphologically similar areas with either abundant tussocks or shrubs. Cores were frozen at -80°C within 6 hours of collection to capture *in situ* microbial activity. In the laboratory, different techniques for protein extraction and purification are being tested on soils to optimize the yield of biogeochemically relevant peptides in mass spectra.

This poster focuses on the environmental variables taken into account in the comparison of soil cores and plans for the comparison of experimentally manipulated plots. It also focuses on the comparison of protein extraction and purification techniques for metaproteomics in complex sediment samples.

Physiological response of *O. annularis* to pollution gradients

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Orbicella annularis is an abundant framework-building Scleractinian coral throughout the Caribbean Sea. The *O. annularis* holobiont (biotic and abiotic components of the coral) is weakened by increased exposure to sewage and ship bilge pollution. This is evidenced by altered tissue cellular composition, and skeletal structure. Exposure to pollution is characterized by decreased skeletal growth, as well as decreased zooxanthellae and chromatophore tissue cell densities. Healthy colonies have been studied at five sites on the leeward coast of Curacao, along a systematically decreasing pollution concentration, and were sampled from the back-reef depositional facies of a protected fringing reef tract. A unidirectional current flows to the NW past the city of Willemstad, a large point source of human sewage and ship bilge. This setting creates an ideal natural laboratory for *in situ* experimentation to quantitatively track the impact to coral physiology along a gradient from pristine to polluted seawater. Our lab has established laser scanning microscopy for three-dimensional (3D) quantification of zooxanthellae, and chromatophore cellular tissue density. X-ray computed tomography (BioCT) was used for analysis of skeletal density. Initial results suggest that zooxanthellae density decreases as seawater pollution concentration increases. Chromatophore density exhibits no significant correlation with pollution concentration, yet varies dramatically within each site. This suggests zooxanthellae density is impacted by the environment while chromatophore density is more genetically controlled. Skeletal density shows no significant relationship with pollution concentration. However, different *O. annularis* colonies at the same reef site show significant variation in skeletal density banding patterns. This implies that skeletal density may be more influenced by genetics than previous thought.

Linking gross N-transformation rates to microbial gene expression in an agricultural soil

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Agricultural soils have long been identified as a source of the potent greenhouse gas nitrous oxide (N₂O) owing to the application of nitrogenous fertilizers and microbial activity, including denitrification. Less commonly investigated is the role of dissimilatory nitrate reduction to ammonia (DNRA), an alternate pathway for the reduction of nitrate (NO₃⁻). While also a potential source of N₂O, DNRA largely results in N retention. In order to determine the relative importance of DNRA in an agricultural soil, and monitor microbial activity, ¹⁵N labelled NH₄Cl and KNO₃ were applied to intact soil cores (0-10 cm and 11-30 cm depth) incubated under a summer diurnal temperature regime reflective of natural temperature profiles observed at specified depths. At nine time points, replicate cores were sampled for soil chemistry, isotopic analysis and nucleic acid extraction. Soil extracts will be analysed for ¹⁵N-NO₃⁻ and ¹⁵N-NH₄⁺ using mass spectrometry. Microbial communities will be monitored using 16S rDNA based T-RFLP analysis and expression levels of relevant N-cycling genes (e.g. *nrfA*) will be determined using qRT-PCR. Successful RNA extraction and *nrfA* cDNA generation in test extracts indicates a population capable of DNRA in this soil environment. Resultant data will allow for the linking of quantitative gene expression to gross N-transformation rates, providing novel insight into N-cycling in agricultural soils.

A novel nanoparticle approach for imaging soil bacteria

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Soil microbes are the primary drivers of biogeochemical processes and many other ecosystem services. However, we have a limited understanding of microbial metabolic processes such as substrate uptake rates and preferences. Here we use a novel imaging technique with quantum dots (QDs, engineered semiconductor nanoparticles that produce size or composition-dependent fluorescence) to measure bacterial uptake of substrates of varying complexity. We found that uptake of QDs conjugated to organic substrates varied depending on growth conditions and substrate, suggesting that they are a useful indicator of bacterial ecology. On average, QD assimilation was six times greater when nitrogen or phosphorus was limiting, and phosphoserine uptake was higher compared to other substrates, likely because it was the only compound providing both nitrogen and phosphorus. X-ray fluorescence images collected at the Advanced Photon Source were consistent with those produced with conventional microscopy, indicating that the XRF can detect bacterial uptake of CdSe-core QDs. These findings offer a new way to experimentally investigate basic bacterial ecology such as metabolic activity and biofilm development and function.

Microbial diversity of the highly-alkaline Calumet Wetlands in south Chicago, Illinois

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The Calumet Wetlands located in south Chicago, Illinois, USA is a historical steel waste dumping site with very high alkalinity (pH>13.2) in a non-saline environment. Anthropogenic alkaline sites are distinct from naturally-occurring alkaline systems both in their extreme pH as well as their high heavy metal concentrations, and many of the worst of these waste sites are in close contact with low-income neighborhoods. In this study we sought to characterize microbial diversity and metabolism at the Calumet Wetlands through the synergistic analysis of environmental physicochemical and sequence data. Environmental 16S rRNA amplicons and whole sequence data (metagenomes) were sequenced from numerous Calumet locations, as well as across horizontal and vertical gradients within the most extreme alkaline site. Analyses of taxonomic abundance using both 16S amplicons and metagenome assemblies identified a large fraction of Betaproteobacteria, including species related to the hydrogen-oxidizing genus *Hydrogenophaga*, in terrestrial environments most closely associated with the alkaline water (but not as prevalent in the water itself). Deep, anaerobic samples transitioned from the dominant Betaproteobacteria to members of phylum Actinobacteria and the Firmicute genus *Exiguobacterium*. Cultivation of heterotrophic and lithotrophic microorganisms was informed by environmental conditions in order to isolate novel alkaliphilic taxa. While sequence analysis revealed a number of undescribed phyla within the sample locations, culturing revealed a bias toward easily-grown genera within the Firmicutes and Actinobacteria. Most notable among these is the genus *Exiguobacterium*, which not only composed nearly half of cultured isolates, but was also highly abundant in deep, anaerobic sequence datasets. The integration of geochemical, bioinformatic, and microbial analyses at Calumet provides a strong foundation for further study of this hyperalkaline, heavy metal-contaminated environment. Insights gleaned from Calumet may help illuminate the possible risk/benefit of microbes in these toxic environments, and how to appropriately treat similar industrial waste sites around the world.

Isotopic composition of sulfate assemblages in an arctic gossan

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West et al. (2009) reported an arctic gossan overlying Archean massive, volcanogenic sulfide deposits at High Lake area in Nunavut, Canada. The gossan mineral assemblage contains jarosite, gypsum, iron-oxides, and iron-sulfates and serves as a natural laboratory for examining ancient Martian surficial processes. To investigate proportions of oxygen isotopes from water and atmospheric O₂ in different sulfate minerals, water-soluble sulfate (WSS), acid-soluble sulfate (ASS), DTPA solution-soluble sulfate (DSS), chromium reducible sulfur (CRS) are sequentially extracted from 23 samples originally analyzed by West et al. (2009). Oxygen isotopic compositions of WSS, ASS, and DSS were analyzed as well as sulfur isotope of WSS, ASS, DSS, and CRS. The δ³⁴S values of WSS, ASS, DSS, and CRS range from -0.7‰ to +3.3‰ (average= +1.1‰), -0.8 to +3.3 ‰ (average= +1.3‰), -1.1‰ to +2.5 ‰ (average= +0.6‰), -0.5‰ to +8.3 ‰ (average= +2.9‰), respectively. The δ¹⁸O values of WSS, ASS, and DSS range from -13.2‰ to -3.5‰ (average= -9.2‰), -14.1 to -4.2 ‰ (average= -9.8‰), and -16.1 to -5.2‰ (average= -13.8‰), respectively. The sulfate concentration of WSS, ASS, DSS, and CRS range from 132 to 48565 ppm (average= 4806 ppm), 386 to 18660 ppm (average= 10414 ppm), 511 ppm to 17679 ppm (average= 7700 ppm), 36 ppm to 12570 ppm (average= 1181 ppm), respectively. There are strong correlations among δ³⁴S values, δ¹⁸O values, and sulfate concentration for WSS, ASS, and DSS but no apparent correlation between δ³⁴S values and sulfur concentration for diverse sulfate and CRS. The values for δ³⁴S and δ¹⁸O DSS are generally lower than corresponding WSS and ASS. There is no a significantly difference between δ³⁴S and δ¹⁸O values for WSS and ASS. Our data suggest that the three kinds of sulfate resulted from sulfide oxidation in local precipitation water (δ¹⁸O_{H₂O} ≈ -23‰). DSS probably precipitates from solution followed by WSS and ASS during seasonally recurrent events of dissolution and precipitation. It appears that the DSS records the primary water-isotopic signal. WSS is likely gypsum mineral and ASS is likely combination of jarosite and Fe-sulfate minerals, but the mineral source of DSS is unknown. Further mineral examination by XRD and EDS is needed. Detail study mechanism of mineral assemblages and corresponding isotopic composition of gossans under cold climates in the active layer above permafrost where seasonal water saturation occurs could shed light on our understanding of ancient surficial geochemical process on Mars.

The Neoproterozoic Carbon Cycle: Insights from the 2.7 Ga Campbellrand carbonate platform, South Africa

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Prior to the rise of atmospheric oxygen (Great Oxidation Event; GOE) the operation of the carbon (C) cycle, and its influence on atmospheric chemistry, remains poorly understood. From a proprietary drill core (BH1-Sacha), recovered from the Griqualand West structural basin in the Northern Cape Province, South Africa, C-isotope data from carbonates ($\delta^{13}\text{C}_{\text{carb}}$) and organic matter ($\delta^{13}\text{C}_{\text{org}}$) is presented. These data offer new insights into the Neoproterozoic (2.65–2.5 Ga) C-cycle. These data ($\delta^{13}\text{C}_{\text{carb}}$ and $\delta^{13}\text{C}_{\text{org}}$) are observed to co-vary, revealing periodically synchronous negative excursions, with $\delta^{13}\text{C}_{\text{carb}}$ reaching anomalously light values (-11 ‰). With the exception of $\delta^{13}\text{C}_{\text{carb}} < -6$ ‰, the C-isotope records of BH1-Sacha are deemed to be primary signals, reflective of the original paleoenvironment via intra-basinal correlation and synchronicity with sulphur-isotope records reflecting wider atmospheric processes. Development of an Archean C-cycle model results in a large dissolved inorganic carbon reservoir that efficiently buffers $\delta^{13}\text{C}_{\text{carb}}$ against simple perturbations. Model simulations suggest that, in order to generate $\delta^{13}\text{C}_{\text{carb}}$ and $\delta^{13}\text{C}_{\text{org}}$ signals equivalent to that observed in the empirical BH1-Sacha dataset, simultaneous increases in photosynthesis and remineralisation is the most likely cause of the signals observed in the chemostratigraphy of BH1-Sacha. These processes are hypothesized to initiate and promote a productivity driven positive feedback loop. Such a feedback loop is an interpretation that is consistent with the hypothesized periodic existence of a bi-stable organic-rich atmosphere caused by elevated methanogen productivity. These coupled data-modeling inferences strengthen the proposed Neoproterozoic bi-stable atmosphere and confirm the sensitive nature of the atmosphere to biogenic influxes during the juvenile state of the early Earth.

Biosiliceous “glass ramps” as alternative states of marine ecological systems in deep time

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The investigative tools, nomenclature, and research queries applied to marine sedimentary rocks differ substantially between siliciclastic and carbonate systems. Bio-siliceous “glass ramps” represent a third sedimentary regime that remains enigmatic despite its importance to both paleontology and economic resource development. The dynamism in metazoan influence on these ecosystem shifts is unknown.

A glass ramp model based on new field work in Jurassic rocks of the Central Peruvian Andes is compared to models published on other prominent glass ramps of the Phanerozoic: the Mississippian of southern Kansas, the Permian of North America, and the Eocene of western Australia. Each example was a widespread shallow shelf habitat dominated ecologically by siliceous demosponges. The temporal, paleogeographic, climate, and marine chemistry conditions that prevailed during formation of each interval are compared. Ecology and paleogeography of the sponges and affiliated faunas is also compared, with emphasis on initiation and termination conditions. Glass ramps are an alternative ecosystem state expressed at the local and continental scale, and in deep time they appear to respond to both long term geochemical revolution and brief environmental upheaval.

Experimental apatite precipitation associated with Liesegang bands and microbial EPS

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Multiple lines of evidence indicate microbial influence on the precipitation of marine apatite. We have studied apatite precipitation in a double diffusion setup in which gelatin separates a cation solution from an anion solution, allowing for observation of a slower rate of precipitation of the apatite minerals similar to the rates of *in situ* precipitation. Liesegang bands (L-bands) are a precipitation feature observed in such systems due to the interactions of diffusing ion fronts. To test the effects of both L-banding and microbial activity on the precipitation of apatite, we established two setups using ion solutions of the stoichiometry of fluorapatite, one with a string of microbial extrapolymeric substance, EPS, running the length of the gelatin plug and one without. The first site of observed precipitation in both setups was an L-band offset to the anion side within the gelatin. A second L-band formed to the cation side of the first within 24 hours. In the EPS setup, precipitates were noted on the EPS outside the L-band areas after one week. After 13 days we harvested the L-bands and the EPS, then analyzed mineralogy by XRD, morphology by ESEM, and chemistry by EDS. Among the results: 1) all precipitates were identified as fluorapatite, 2) precipitation of apatite directly on/within the EPS – whether at the site of the L-band or away from it – was more uniform in morphology and size than precipitation within either of the L-bands but not in proximity with EPS; 3) precipitation on EPS at the location of L-banding was more densely packed and exhibited slight structural differences compared to precipitates on EPS away from the L-banding; 4) though precipitation in the first L-band of both the EPS+ and EPS– setups exhibited the same mineralogy, chemistry, and morphology, a comparison of the second L-bands of the two setups showed a difference in their calcium to phosphorus ratios and the morphological consistency and maturity of the precipitates. We conclude that: 1) L-banding has a slight impact on the density of, and possibly the structural morphology of, apatites precipitated on EPS; and 2) that the presence of EPS and/or organisms within the EPS may impact ion concentrations of the moving ion front, leading to differences in the precipitates of the second L-band.

Microbial community analyses of the Nachusa Grasslands (Illinois) prairie restoration chronosequence

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The natural prairie ecosystem, once found across much of the Midwestern United States, has been reduced to a fraction of its original extent, mainly due to human agricultural practices and the removal of native animal taxa. To reduce the negative consequences of replacing species-rich prairies with monoculture croplands, The Nature Conservancy (among others) has established a long-term prairie restoration project at the Nachusa Grasslands in Northern Illinois. In this study we sought to answer the question of whether prairie restoration had a significant impact on microbial population, and, if so, on what time scale do microbial populations begin to reflect those in native prairie soil (i.e. do they parallel the return of plant, insect, and animal populations to the restored prairies). Microbial diversity was analyzed across the 27-year old chronosequence via environmental 16S rRNA amplicon sequencing from samples taken at the end of last year's growing season (October 2013) and the beginning of this year's (April-June 2014). Our results show that microbial communities are most similar between prairie restoration sites of similar age, with a strong separation between the newest restoration sites (< 5-years old) and the oldest/remnant sites. Increasing restoration age is strongly tied to an increase in Verrucomicrobia, a phylum known to be prevalent in prairie soils, contributing to the statistical similarities between the oldest restorations and the remnant prairies. Conversely, chaotic/inconsistent microbial populations in the most recently planted field highlight the rapid changes under way in the earliest stages of restoration, a process which appears to take ~5 years to settle toward a stable community. This research confirms the success of the Nachusa Grasslands at reestablishing diverse microbial communities seen in native, undisturbed Illinois prairies, and suggests potential targets for improving restoration efforts using "native prairie" microbial inoculations.

[Co(III)-EDTA]⁻ reduction by thermophilic methanogen *Methanothermobacter thermautotrophicus*

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Methanothermobacter thermautotrophicus, an obligate thermophilic methanogen was used to reduce Co(III) in aqueous solution. Experiments were conducted in a growth medium with H₂/CO₂ as substrate with initial Co(III) concentrations of 1, 2, 4, 7 and 10 mM in the form of [Co(III)-EDTA]⁻. Spectrophotometric measurement of aqueous Co(III) concentration over time showed a complete reduction of the 1, 2, and 4 mM Co(III) within 14, 108 and 156 hours, respectively. However, reduction efficiency was significantly reduced at higher concentrations (7 and 10 mM) with reduction extents of only about 70% in both cases associated with its toxic behavior to cells which was also supported by the observed inhibition in methanogenesis and a decrease in total cellular protein over the period of bioreduction. Time course spectra of X-ray absorption near-edge structure (XANES) and electron paramagnetic resonance (EPR) discovered a progressive reduction of Co(III) to Co(II). Our results demonstrated the ability of *M. thermautotrophicus* to reduce Co(III) to Co(II) and its potential implications for predicting the fate of ⁶⁰Co in the high temperature subsurface radioactive disposal sites.

Development of reservoir rock GeoBioCell: A microfluidic flowcell for subsurface microbe-water-rock interaction experimentation

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A better understanding of subsurface microbe-water-rock interaction in the Earth's outer crust is of critical importance because it strongly influences the basic petro-physical properties of sedimentary rock. Microfluidic flowcell prototypes of subsurface reservoir systems, called micromodels/GeoBioCells, have been used to replace traditional column experiments, which contain mostly inert pore structure and therefore lack biogeochemical heterogeneities in actual subsurface rock reservoirs. In this study, we developed a next-generation microfluidic experimental test bed, herein called the Reservoir Rock GeoBioCell (RRGBC), in which an actual piece of subsurface reservoir rock is mounted within a microfluidic flowcell to overcome the GeoBioCells limitations. Custom petrographic rock sections (0.5 mm thick) were prepared from siliciclastic sandstone cores using SuperGlue® adhesive for impregnation. The SuperGlue from the pores was removed with acetone. The thin section was placed in a custom made PDMS mold between inlet and outlet microfluidic channels. Another PDMS layer was placed to sandwich the rock core and pressure seal was applied to form test bed flow cell. Multi-photon laser confocal microscopy of the RRGBC showed pore connectivity to an imaging depth of ~400 μm within the thin section. The geochemical reactive sites were characterized using Raman Microscopy, confirming the presence of reactive quartz. A fluorescent tracer test was conducted to identify micro-flow paths and solute breakthrough within the thin section. In a multiphase flow experiment an aerobic mixed-culture oil-degrading bacteria was grown on light oil injected in pores. Biofilm growth was tracked with an oxygen sensitive fluorophore. The RRGBC developed in this study is useful for quantitatively testing and monitoring the physical, chemical and biological factors that affect subsurface carbonate diagenesis and other biogeochemical reactions.

Abundance and diversity in hot springs of Tengchong County, China

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Early life potentially originated in extreme environments that are similar to present day hot springs. Due to many geochemically unique hot springs, Tengchong County in Yunnan Province, China could serve as an analogue to early life conditions. Water and sediment samples from 36 sites were analysed by qPCR and 16S rRNA gene sequencing. Temperature of the investigated samples ranged from 40°C to 93°C and pH from 2.3 to 9.7. Highly acidic sites (pH 2.3–3.5) had an average bacterial 16S rRNA gene copy number of E+06 whereas, the alkaline sites (pH 8–9.07) had an average of E+04. Illumina MiSeq sequencing produced a total of 3424971 reads that clustered into 53851 OTUs at the 97% identity level. Overall, the sediment samples had a higher abundance and diversity than the water samples from the same site. The bacterial phylum Proteobacteria dominated in lower temperature sites (40–70°C), while archaeal genus *Pyrobaculum* was dominant in high temperature sites (75°C–90°C). A large amount of unclassified Bacteria were also detected through all sites. Thus, the presence of diverse microbial communities in these sites demonstrate their ability to thrive in extreme conditions and further genetic analysis could help in understanding the origin of microbial life on primitive Earth.

Culturing cellulolytic bacteria from high pH serpentinizing springs

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Cellulose is the single most abundant organic compound on the planet, accounting for nearly half of the total terrestrial biomass. Due to its sturdy, crystalline structure it resists degradation to a large extent, making the cellulolytic activity of many bacterial groups a significant part of the global carbon cycle. Currently, there is no information available about bacterial cellulose degradation in high pH serpentinizing systems. Serpentinization is the oxidative, hydrologic alteration of ultramafic mantle rock, thereby resulting in highly reduced fluids associated with H₂ and CH₄ gases. These deep subsurface fluids breach the surface in a number of seeps around the world. Our field sites include a number of serpentinizing seeps on the main island of Luzon, Philippines. Large quantities of dead and decaying vegetation are observed to have fallen into the springs from the surroundings, driving the hypothesis that microbial cellulose degradation is occurring in these springs at the surface. The pH values at these sites range from ~8 – 12, and temperatures range from ~30 - 35 °C. The goal of this study is to culture and isolate cellulolytic bacteria from these high pH springs. A cellulose-based medium adjusted to pH 10.4 was inoculated with 6 different environmental samples obtained from various springs in the Philippines, and incubated at 35 °C in both anaerobic and aerobic conditions. After 3 weeks, growth was observed in 2 of the 6 experiments, both aerobic and anaerobic, as evidenced by a distinct turbidity in the experimental tubes. DAPI microscopy was used to verify presence of bacterial cells, and showed abundant cells of varying morphologies. Future work to commence in this study will be identification of cultured bacteria through use of 16S gene sequencing, as well as identification of cellulase genes present in environmental samples. Discovery of potentially novel bacterial strains and cellulase genes may be achieved through studying these specific extreme environments that have heretofore not been adequately studied for degradation of cellulose.

Passalid beetles harbor different microbial communities specialized in cellulose breakdown

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Herbivores play an important role in food chains and carbon cycle. However, the microorganisms that inhabit their guts are the ones responsible for the cellulose breakdown and consequent release of greenhouse gases. Here we studied the Passalid beetle from Costa Rica *Veturius* sp., this beetle only feed on decay wood and presents a subsocial behavior that may lead to the acquisition and sharing of microbial symbionts for efficient cellulose degradation. We sampled 5 *Veturius* sp family groups from 5 different logs and analyzed the gut microbiome of larvae and adults as well as the woody gallery material in which they resided. DNA samples were sent to the JGI for 16S rRNA pyrotag sequencing and community metagenomic sequencing. We processed the amplicon sequences using MOTHUR, sequences were clustered into OTUs at 97% similarity. Metagenomes were assembled and functionally annotated using Pfam database. Our results showed that adult, larvae and gallery material harbor significantly different communities, sharing less than 3% of total OTUs (11 712). Also, we found that Firmicutes and Tenericutes were enriched in both larvae and adults of the Passalid beetles. Furthermore, larvae microbial community showed an important component of archaea. We found 71 different glycosyl hydrolases in the metagenomic contigs based on Pfam search. Methanogenesis functions were only found in the larvae metagenomes, suggesting that those performed the final steps of cellulose decomposition. Finally, we compared the protein profiles of several microbiomes showed that Passalid larvae gut microbiomes cluster with the cow rumen and separate from other insect microbiomes suggesting a possible functional convergence of these systems.

Nitrogen fixation genes in oil-contaminated sediments

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Oil contamination of sediments has become a widespread environmental problem. Microorganisms are able to effectively degrade hydrocarbons and reduce levels of contamination. The many types of hydrocarbons in crude oil provide an abundant amount of carbon source to the microenvironment. Previously, soil samples from an oil pipeline rupture in Bemidji, Minnesota, were obtained and a 16S rRNA gene clone library was constructed to observe the variety of microorganisms present. This revealed 16S rRNA gene sequences similar to microorganisms known to be able to break down hydrocarbons and produce methane. However, how petroleum degrading microbes attain nitrogen for biosynthesis has been largely unexplored. The site in Bemidji contains low amounts of nitrate and ammonium; therefore, one method of obtaining nitrogen from the environment is through nitrogen fixation. Thus we constructed a clone library based on the *nifH* gene. The *nifH* gene encodes the nitrogenase enzyme, which converts atmospheric nitrogen to ammonia. The vadose zone in the contaminated sediment showed *nifH* genes belonging to organisms similar to the genera *Azorhizobium*, *Mesorhizobium*, and *Methanofollis*. The production of more clone libraries from the free phase and dissolved phase contaminated sediments will enable us to characterize the microorganisms that provide fixed nitrogen in the different soil environments.

Deep subsurface microbes in terrestrial serpentinizing seeps

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Our understanding of the biogeochemistry of deep subsurface ecosystems is growing, but is constrained by the general inaccessibility of the deep subsurface. Terrestrial serpentinizing seeps provide ready access to deep subsurface microbiota. The habitability of the deep subsurface, like any other biome, is dependent on the presence of electron donors and acceptors. Serpentinization (the hydrous alteration of ultramafic rock) may provide the foundation of an H₂-based microbial trophic web. Samples were collected for fluid geochemistry and microbial community characterization at terrestrial serpentinizing seeps in Antalya, Turkey (Yanartaş) and Zambales ophiolite range, the Philippines (Manleluag and Poon Bato). Environmental DNA was extracted and submitted for high-throughput sequence analysis on the Illumina MiSeq platform. Chemolithotrophic and heterotrophic microbial communities were detected in sediments collected at the seep source; these sediments were continuously exposed to highly reducing, alkaline, fluids that exhibited low levels of dissolved inorganic carbon.

The most commonly encountered archaeal taxa affiliated with the genus *Methanobacterium*, while Proteobacteria (particularly Comamonadaceae), Bacteroidetes (mainly Bacteroidales and Chitinophagaceae) and Firmicutes (predominantly Bacillaceae and Clostridiaceae) were the most abundant affiliated bacterial taxa. Transect sampling of seeps suggested that the deep subsurface “signature” was retained in the environmental genomic DNA tens of meters downstream. Moreover, abiotic precipitation of calcium carbonate (triggered by serpentinizing fluid exposure to atmospheric carbon dioxide) appears to promote cellular entombment via provision of a nucleation surface. These results suggest that terrestrial serpentinizing seeps serve as excellent analogs to study the potential for the long-term preservation of microfossils, and may be particularly relevant to serpentinization-based astrobiology queries.

Sulfhydryl binding sites within bacterial extracellular polymeric substances (EPS)

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Metal-sulfhydryl binding represents a dominant mechanism responsible for metal adsorption onto bacterial cell envelopes under low metal loading conditions, and therefore can play an important role in the transport, bioavailability and transformation of metals in natural environments. Although sulfhydryl binding sites within bacterial cell envelopes have been identified and characterized in previous studies, it is unknown whether these sulfhydryl binding sites also exist within bacterial extracellular polymeric substances (EPS). In the present study, the sulfhydryl concentrations within bacterial biomass samples before and after removing the EPS were measured by coupling selective site-blocking, potentiometric titration and surface complexation modeling in order to determine the sulfhydryl concentrations of the EPS. The experiments involved the Gram-negative species *Shewanella oneidensis* as well as the prolific EPS-producing species *Pseudomonas putida*, and three different approaches were employed for EPS removal, including cation exchange, enzyme degradation and EDTA treatment. UV-vis and Live/Dead staining results demonstrated that no significant cell lysis occurred during the removal of EPS using any of the techniques, and SEM images confirmed that the EPS that extensively coated the bacterial cells prior to treatment was effectively removed by each of the three EPS removal approaches. The measured sulfhydryl concentration within *P. putida* samples prior to EPS-removal was 34.9±9.5 μmol/g, and no sulfhydryl sites were detected for cells after EPS removal by any of the three methods used, indicating that virtually all of their sulfhydryl sites are located on the EPS molecules. In contrast, the sulfhydryl site concentration within the *S. oneidensis* samples increased from 32.6±3.6 to 51.9±7.2 μmol/g after the removal of EPS, indicating that the EPS from the *S. oneidensis* biomass contained fewer sulfhydryl sites than were present within the cell envelopes. The results from this study suggest that the sulfhydryl concentrations on bacterial EPS molecules are independent of the EPS extraction protocols used, but that the sulfhydryl concentrations within the EPS vary significantly from one bacterial species to another. This study is the first to document sulfhydryl sites within EPS. It is crucial to quantify the distribution and concentration of these sites on other species in order to determine the effect of bacterial cells and EPS molecules on metal cycling in the environment.

Investigation of effects of microbial iron redox cycles on clay mineral properties and its potential application in nitrate removal

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Phyllosilicates are ubiquitous in nitrate-contaminated aquifers and have high Fe contents that can serve as an iron source for microbial respiration. The objective of this research is to determine the ability of the phyllosilicate nontronite (NAu-2) to remove nitrate by undergoing repeated microbial-driven redox cycles by the dissimilatory Fe(III)-reducing bacteria, *Shewanella putrefaciens* CN32, and nitrate-dependent Fe(II)-oxidizing bacteria, *Pseudogulbenkiania* sp. Strain 2002. Three redox cycles were conducted in bicarbonate- and PIPES-buffered medium. The extents of Fe(III) reduction, Fe(II) oxidation, nitrate reduction, and its various intermediate products were measured by wet chemical methods. For each cycle, Electron Energy Loss Spectroscopy confirmed Fe oxidation state. Mineralogical changes were identified by using X-ray diffraction (XRD), ^{57}Fe -Mössbauer spectroscopy, and infrared absorption spectroscopy. For all three cycles, nitrate was completely reduced to nitrogen gas under both bicarbonate- and PIPES- buffered conditions. As redox cycle increased, bio-reduction extents of Fe(III) in NAu-2 decreased by 33% and 48% in PIPES- and bicarbonate-buffered medium, respectively; however, bio-oxidation extents increased by 66% and 55% in the same medium, respectively. Despite the change of OH-stretching vibration band and OH-bending vibration bands in NAu-2 structure along Fe redox cycles, XRD data showed interlayer spacing of NAu-2 to be constant along the same Fe redox cycle. ^{57}Fe -Mössbauer spectrum of bio-reduced NAu-2 sample from the 2nd cycle displayed both Fe(III)-Fe(II) and Fe(II)-Fe(II) pairs in clay mineral structure. After the bio-oxidation of the 2nd cycle, most Fe(II)-Fe(III) pairs were oxidized. The result of this study shows that Fe in biogenically reduced or oxidized NAu-2 could serve as a renewable iron source for multiple biogenic redox cycles. It also provides a prospect of applying N-Fe redox cycles to remove nitrate contaminant in soils and groundwater systems.

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