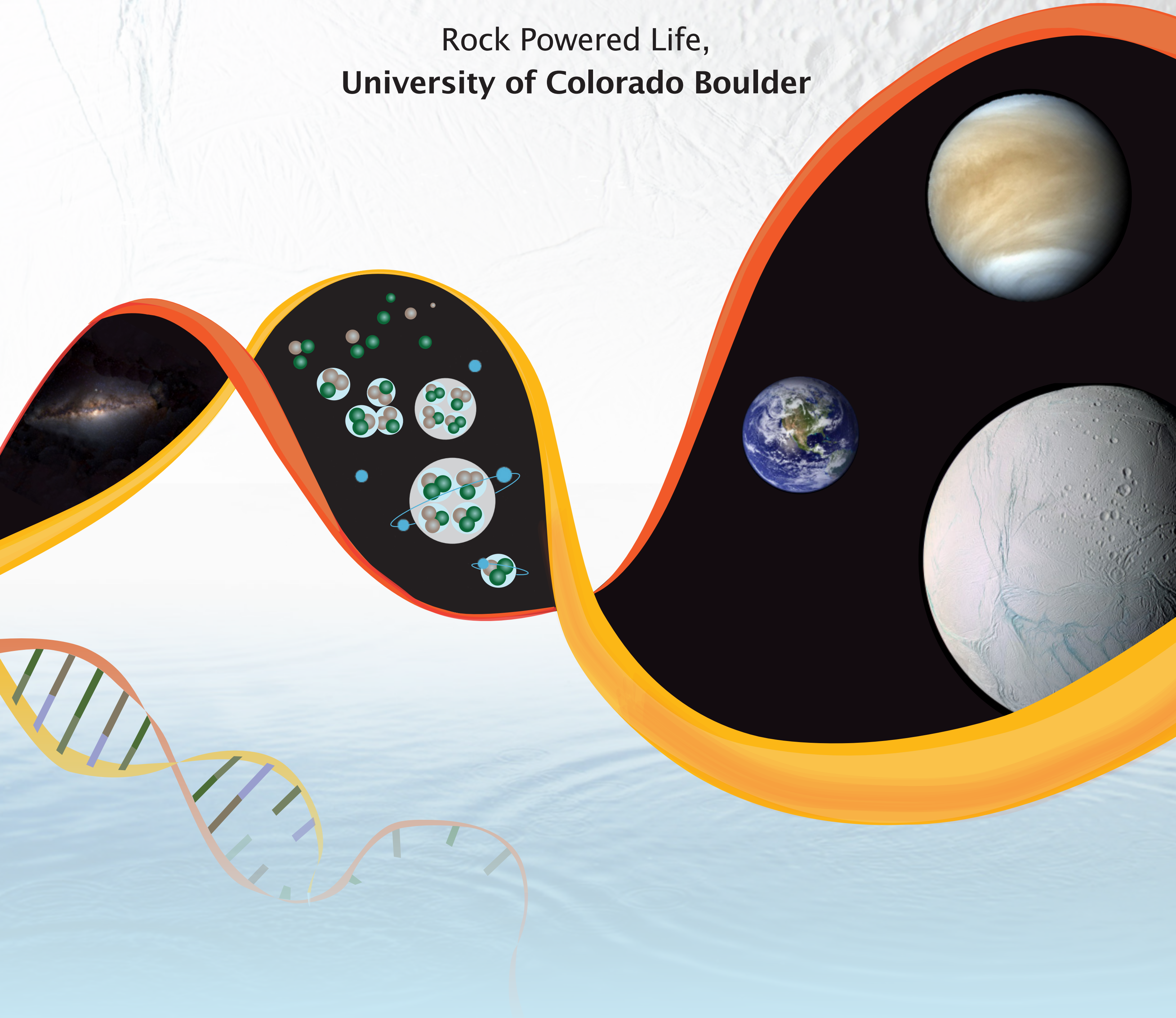
National Aeronautics and Space Administration

NASA Astrobiology Institute 2016 Annual Science Report

Team Report:

Rock Powered Life,

University of Colorado Boulder



RPL
ROCK POWERED LIFE

Lead Institution:
University of Colorado Boulder

Team Overview

Principal Investigator:
Alexis Templeton

The goal of the Rock Powered Life Team is to reveal how energy flows from the lithosphere to the biosphere. Rocks store chemical energy that can power living systems when released through extensive interaction with water. However, there is a need to mechanistically define how, when, and where geological systems can directly sustain biological activity, particularly within the vast realm of environmental conditions where rocks and water react across the shallow subsurface of rocky planets and moons.

RPL identifies controls upon the production and consumption of energy sources such as hydrogen, methane and carbon monoxide in "Serpentinizing Systems", which are environments where ultramafic rocks react with water. Establishing how chemical and biological processes are coupled together during Serpentinization will have important implications for developing strategies for detecting extraterrestrial life beyond Earth, such as in the shallow crust of Mars and icy satellites (Europa, Enceladus), and improving hypotheses concerning primordial ecosystems on Earth.

RPL efforts in 2016 led to significant accomplishments within four main project areas:

- Hydrogeochemistry of serpentinizing systems
- Microbial community composition and function in rock-hosted ecosystems
- Mineralogical evolution in serpentinizing systems
- Experimental, theoretical, and modeling constraints on pathways of low-temperature water/rock interaction

Team Website: http://www.colorado.edu/lab/rockpoweredlife

2016 Executive Summary

The Rock Powered Life Team identifies how ecosystems are supported by energy sources released during water/rock interaction. We focus on the process of "Serpentinization", which occurs whenever ultramafic rocks come into contact with water. Although we can identify active and ancient serpentinization processes throughout our Solar System, we need to determine how the chemistry, mineralogy, fluid dynamics and microbial metabolism couple together to sustain life activity. Thus RPL investigations can feed into the development and implementation of upcoming missions; several Co-Is have been actively engaged in landing-site discussions and the search for rock-hosted life for Mars2020, as well as planning efforts for the Europa Multiple Fly-By mission and the Europa Lander Science definition team.

In RPL's efforts to detect and characterize signatures of rock-powered life, we found that "false biosignatures" can be produced when gases such as hydrogen sulfide react with dissolved organic carbon. These reactions initiate the self-assembly of sulfur minerals encapsulated in carbon within forms that strikingly resemble microbial cells and extracellular structures (Cosmidis and Templeton, 2016).

The RPL Team significantly expanded the projects being conducted in subsurface peridotite rocks that have been variably serpentinized. Intensive groundwater sampling and biological experimentation occurred at the NAI Coast Range Ophiolite Microbial Observatory (CROMO) and Oman. The RPL Team members in the Science Party of International Ocean Discovery Program (IODP) Expedition 357 to the Atlantis Massif also had the opportunity to preserve and analyze cores recovered from the shallow subsurface at core repositories in Bremen, Germany and Kochi, Japan.

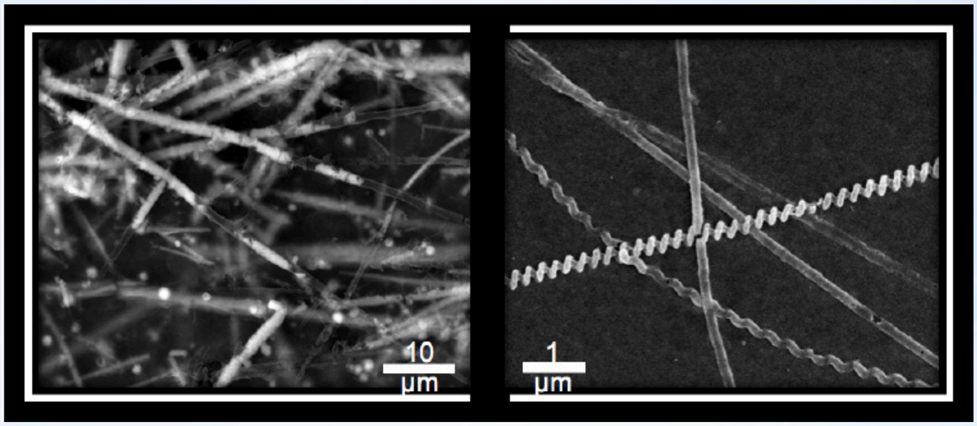


Fig. 1. Scanning electron micrographs of self-assembled carbon tubes loaded with elemental sulfur. These microstructures form abiotically but can be easily confused with modern and ancient microfossils (Cosmidis and Templeton, 2016).

Fig. 2. RPL graduate student Leong (ASU) characterizing the geochemistry of fluids detected at hyperalkaline seeps in the Samail ophiolite, Oman.

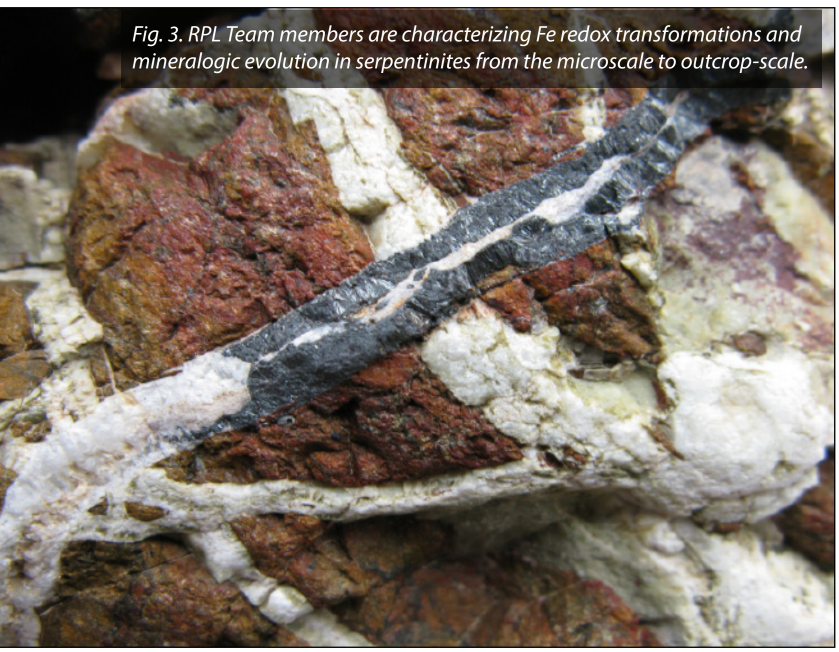


Fig. 3. RPL Team members are characterizing Fe redox transformations and mineralogic evolution in serpentinites from the microscale to outcrop-scale.

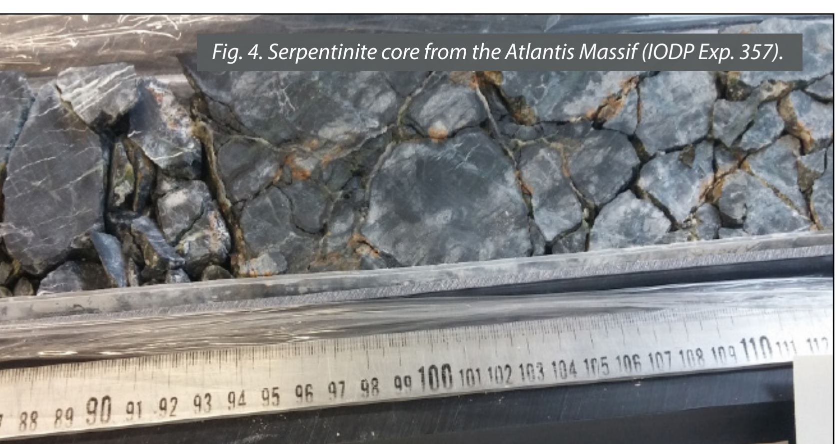


Fig. 4. Serpentinite core from the Atlantis Massif (IODP Exp. 357).

In Oman, RPL labs conducted comparative geochemical analyses of hyperalkaline surface seeps vs. deep subsurface fluids to determine which mineral/fluid equilibria control fluid chemistry and the distribution of oxidants and reductants. This work created new insights into the reactions that give rise to abiotic vs. biotic sources of dissolved organic carbon (Robinson et al., in prep). RPL also identified high concentrations of methane in deep subsurface fluids that may have been partially produced and/or consumed by microbial activity (Miller et al., 2016; Miller et al. 2017). Further insight into the subsurface microbial communities in the ophiolite was developed through a comprehensive multivariate statistical analysis of the deep subsurface fluid chemistry and 16S rRNA data sets (Rempfert et al., 2017).

RPL Team members made significant advances in understanding the mineralogical characteristics of low-temperature serpentinizing systems, by developing a technique coupling synchrotron-radiation based x-ray spectroscopic data with Raman data to quantitatively assess the Fe oxidation state of variably serpentinized rocks from Oman and the Atlantis Massif. RPL efforts revealed key metastable mineral phases such as Fe-bearing brucite react to produce hydrogen during progressive stages of water/rock interaction (Miller et al., 2016; Mayhew et al., in review; Ellison et al., in prep). RPL Team members also initiated geophysical surveys of the CROMO landscape to develop 3D models of the subsurface changes in the magnetic properties of the serpentinites.

Deciphering pathways of methane production and consumption is a theme that bridges across RPL laboratories, with several labs investigating unconventional metabolic pathways expressed during microbial methanogenesis and their signatures to identify mechanisms of methane formation (Miller et al., in prep; Rhim et al., in prep; Beuter et al. in prep). Abiotic water/rock experiments by RPL Team members have also been critical to identify first-order controls on H_2 production and CH_4 production during low temperature serpentinization (McCollom and Donaldson, 2016; Miller et al., in revision).

Hydrogeochemistry of Serpentinizing Systems

Fluids that circulate through mafic and ultramafic rocks are extensively modified by a combination of water/rock reaction and *in-situ* microbial activity. In order to identify habitable rock-hosted environments, it is critical to understand first order controls on the aqueous geochemistry of the fluid system, which directly affects the energy landscape and the fluxes of energy sources and oxidants.

In Oman, graduate students Rempfert, Miller, and Nothaft, PI Templeton and collaborators Kelemen and Matter completed an intensive survey of deep fluids hosted in peridotite and gabbro rocks within the Samail Ophiolite. Four distinct fluid chemistries were identified: moderately alkaline fluids circulating in the shallow subsurface, highly reducing, hyperalkaline fluids rich in dissolved H_2 and CH_4 stored at depth within the peridotite, oxidizing fluids hosted in gabbros, and variably "mixed" fluids detected where peridotites are hydrologically connected to gabbro rocks. The RPL Team detected a surprising abundance of oxidants, particularly nitrate and sulfate, which strongly modulates the potential to support microbial activity.

Graduate students Leong, Robinson, Canovas and Co-I Shock are conducting comparative geochemical analyses of fluid chemistry in hyperalkaline surface seeps vs. deep subsurface fluids to determine which mineral/fluid equilibria control fluid chemistry and the distribution of oxidants and reductants. This work has created new insights into the reactions that give rise to abiotic vs. biotic sources of dissolved organic carbon in serpentinizing systems. Robinson used thermodynamic calculations to model organic acid formation during specific low-temperature mineral hydration reactions, and incorporated the products of these reactions into anaerobic metabolic models that consider carbonate, small molecular weight organic acids, methane, carbon monoxide, hydrogen, sulfate and nitrate (Robinson et al., in prep).

RPL also uses the Coast Range Ophiolite Microbial Observatory (CROMO) as a key field-site for unraveling the coupled hydrology, geochemistry and microbiology of serpentinizing systems. Several dimensions were added to groundwater monitoring efforts by graduate student Williams and Co-I Shrenk to support geochemical and biological modeling of the serpentinization-influenced system at CROMO.

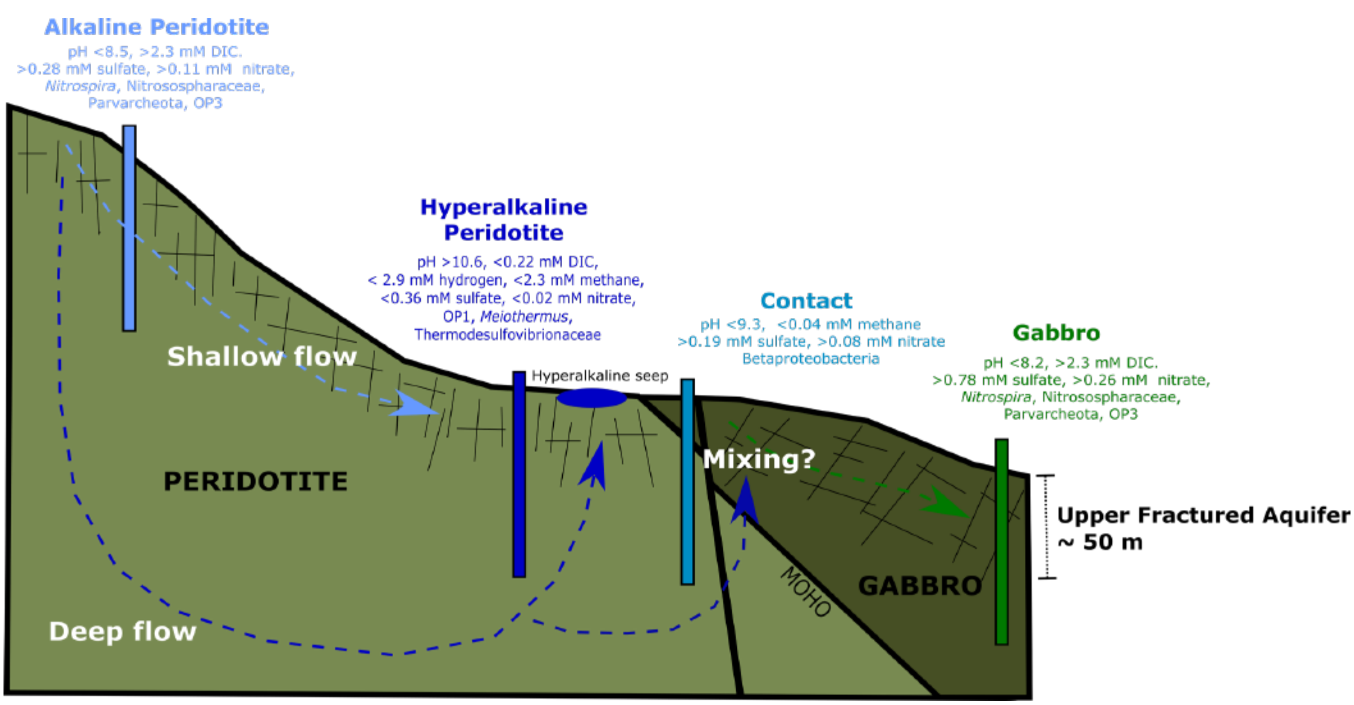


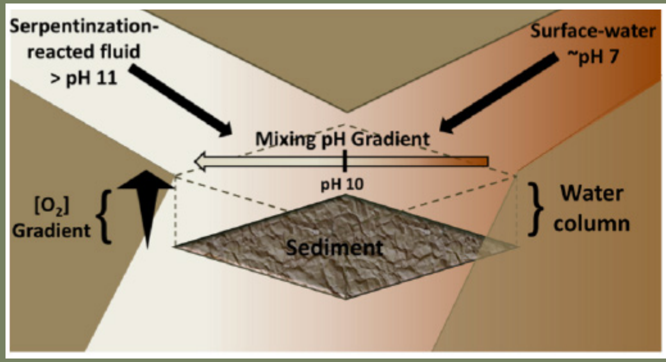
Fig. 4. Schematic cross-section of the Samail ophiolite, Oman, showing different fluid chemistry and flow regimes. Information about distinct differences in the dominant microbial organisms detected in each fluid type (see Project #2) is also shown in this figure adapted from Rempfert et al. (2017).

Microbial Community Composition and Function in Serpentinizing Systems

The RPL Team is focused on detecting and characterizing the microbial life hosted within serpentinizing systems. Large 16S ribosomal RNA gene data sets were generated for hyperalkaline seeps (Shock, Howells) and deep subsurface fluids (Rempfert, Miller, Nothaft, Templeton) in Oman. Multivariate statistical methods were used to evaluate how community composition is shaped by the distinct geological and hydrological settings (Rempfert et al, 2017; Howells et al., in prep). Metagenomic data sets are undergoing assembly and functional analysis by graduate student Kraus, Co-I Spear and Co-I Boyd.

Co-I Brazelton and Postdoc Twing optimized methods of DNA extraction and purification from low-biomass subsurface serpentinite samples from the Atlantis Massif collected during IODP Exp. 357. Now it will be possible to build genomic data sets and closely integrate this data with mineralogical and geochemical data. Co-I Schrenk is also generating a "map" of substrates used by sulfate reducing bacteria in these same AM core samples.

The RPL Team is developing protocols for intensive field-based measurements of the rates of production and consumption of C, H and S species during microbial metabolism in serpentinites. An overarching area of research involves identifying organisms sustained by transformations of CO_2, CO, formate, and methane. Graduate student Bueter and Co-I Boyd developed protocols to quantify rates of formate and CO metabolism in serpentinite-hosted



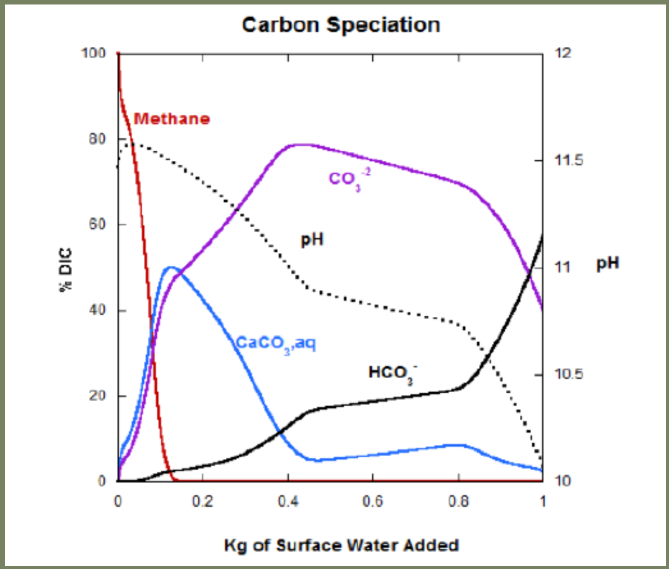


Fig. 6. Mixing between serpentinization-reacted water and surrounding surface water engenders a range of habitats where carbon speciation takes different forms. 16S rRNA gene analysis was done on DNA extracts from sediment and planktonic samples to evaluate the influence of carbon speciation across the established mixing gradient on microbial community composition, diversity and function.

Fig. 7. Co-I Brazelton and graduate student Thornton participated in a sampling party at the Kochi Core Center in February 2016 to process preserved Atlantis Massif Expedition 357 samples in a consistent, standardized manner to enable better cross-comparisons among labs conducting microbiological analyses. The Brazelton lab is currently optimizing methods for extraction and purification of DNA from these samples.

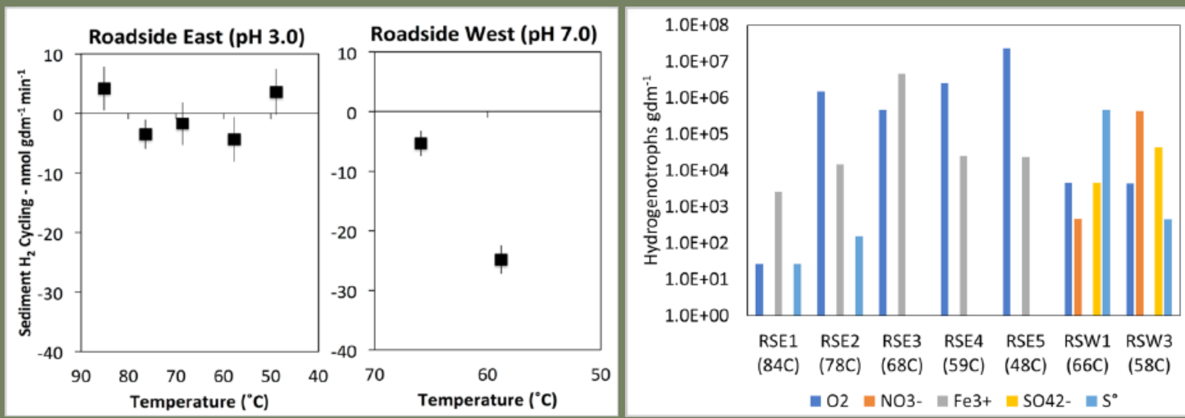


Fig. 8. (A) Rates of net H_2 transformation in sediment-associated microbial communities in selected sites along Roadside East (RSE) and West (RSW) hot springs (YNP). (B) Abundance of autotrophic hydrogenotrophs in sediments from RSE and RSW that are capable of coupling with specified oxidants.

ecosystems using ^{13}C labeled substrates. In addition, Postdoc Seyler developed approaches for metabolite analysis of hyperalkaline fluids, and graduate student Sabuda was hosted at NASA Ames by Hoehler and Som to trace methane oxidation pathways.

RPL is quantifying rates of H_2 production and consumption in hot springs in Yellowstone National Park. Graduate student Lindsay, Co-I Boyd, and Co-I Hoehler analyzed rates of H_2 transformation in the context of community level productivity, RNA-based analyses, the abundance of hydrogenotrophs and geochemical data (Lindsay et al., in prep.). These data reveal a strong correspondence between oxidant availability, the modes and kinetics of H_2 transformation, and 16S rRNA gene transcripts affiliated with known H_2 metabolizing taxa (Fig. 8AB). Specifically, the Team noted a relationship between the availability of oxidants, which is controlled largely by subsurface geophysical processes, and the use of those oxidants by resident taxa. Due to the decreasing solubility of the preferred oxidant, oxygen, for thermoacidophilic hydrogenotrophs in Roadside East spring, rates of net H_2 transformation were lower than in the lower temperature, circumneutral Roadside West spring.

To further identify the populations involved in H_2 transformation in rock hosted ecosystems, graduate students Lindsay and Dunham, RPL postdoc Daniel Colman, and Co-I Boyd have developed a metagenomics guided RNA based approach to quantify and sequence transcripts of hydrogenases encoded in hydrogenotrophic taxa. These approaches are currently being applied to rock hosted ecosystems in hot springs in Yellowstone, basaltic subglacial sediments in Iceland, and biofilm and planktonic communities in Oman.

Team Members

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Emily Kraus
Mike Kubo
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Julia McGonigle
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Shuhei Ono
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Julio Sepulveda
Lauren Seyler
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Sanjoy Som
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John Spear
Christopher Thornton
Masako Tominaga
Chris Trivedi
Katrina Twing
Lindsay Williams
Kristin Woycheese

Mineralogical Evolution in Serpentinizing Systems

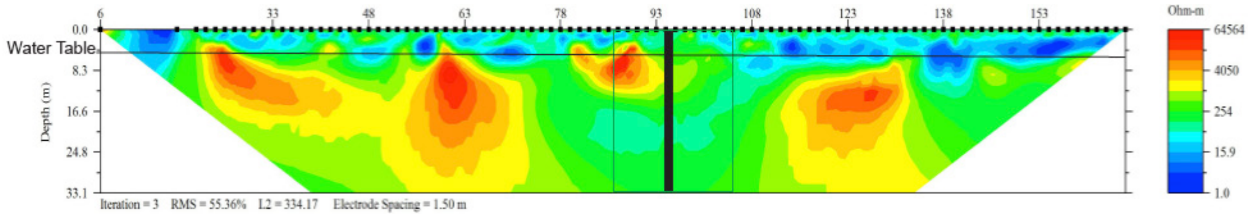

Fig. 9. Resistivity measurements around the Quarry Valley well at CROMO.

Significant advances were made in identifying mineralogical evolution in serpentinizing systems, from the microscale to the outcrop scale. Templeton, Mayhew, Ellison and Miller focused on detailed mineralogical characterization of Oman peridotites that have undergone variable extents of water/rock reaction and Fe-oxidation (Miller et al. 2016; Miller et al., in revision; Mayhew et al. in review; Ellison et al. in prep). A key finding is that brucite ($Mg,Fe(OH)_2$) is a metastable mineral that is distributed throughout partially-altered serpentinites. Brucite traps Fe(II) into the mineral structure, particularly at low temperatures, creating a reservoir of Fe(II). When brucite is destabilized by further water/rock reaction, significant Fe release and oxidation can occur. Such reactions produce H_2 and formate, which serve as electron donors that may sustain biological activity in serpentinizing systems.

In these efforts, Mayhew and Ellison developed an approach utilizing synchrotron-radiation based x-ray fluorescence multiple energy mapping within the pre-edge of the Fe K-edge XANES for quantitative assessment of the Fe oxidation state of variably serpentinized rocks from surface outcrop and from subsurface drilling at Atlantis Massif (AM) and Oman. Data are co-registered with Raman spectroscopy data to determine the oxidation state of specific mineral phases.

In separate efforts, Co-I Shock and graduate student Leong have initiated work to conduct comparative mineralogical and Fe-redox assessments between peridotites and gabbros from Oman and the SW Indian Ridge. In addition, Co-I Cardace and graduate students Johnson and Sousa are developing microFTIR methods to map serpentinite mineralogy on polished rock

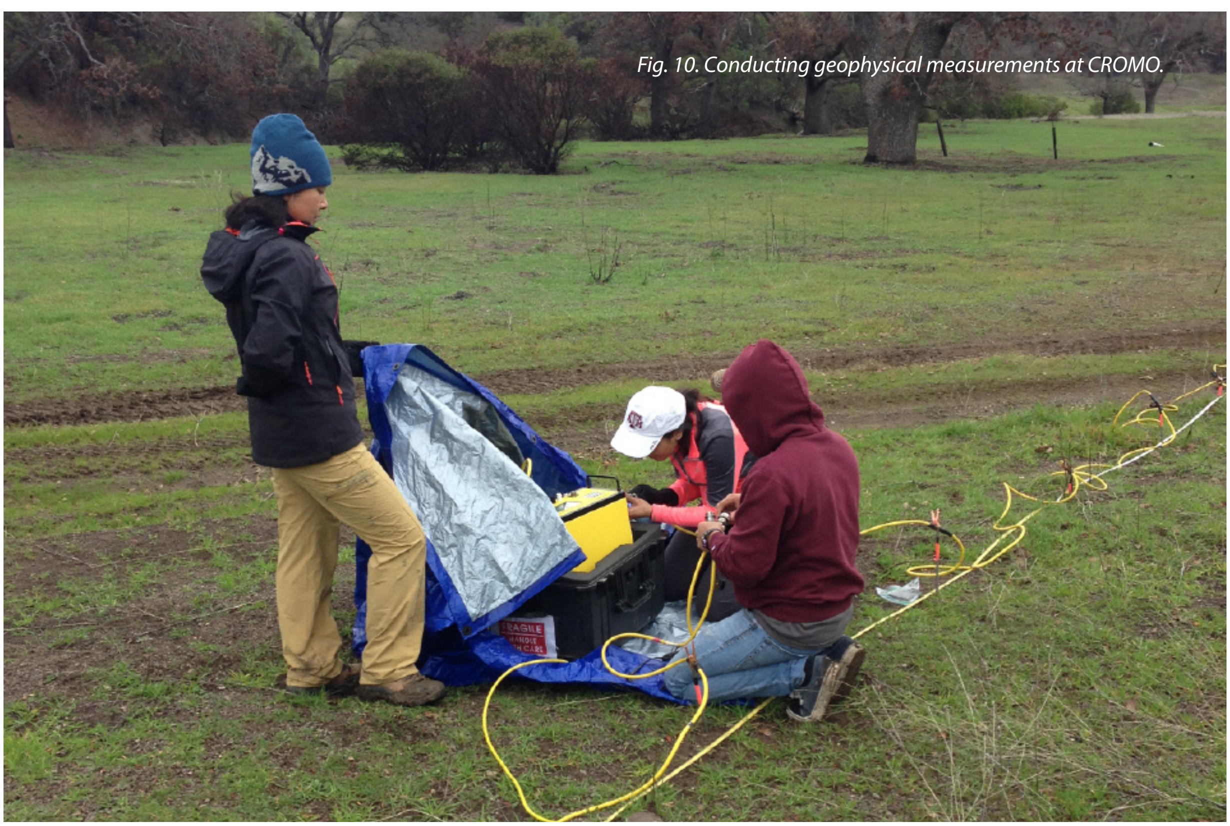

Fig. 10. Conducting geophysical measurements at CROMO.

chips to track changes in chemistry and mineralogy of samples from CROMO and to monitor biofilm formation in the subsurface environment. Cardace also supported Co-I Tominaga and graduate student Ortiz in initiating geophysical surveys at CROMO to map subsurface changes in the hydrological and magnetic properties of the serpentinites. Electrical resistivity tomography surveys and photogrammetry will be used to make a high resolution 3D model and detect zones of ongoing water/rock interaction, particularly zones where hydrogen production is connected to increased magnetic character of the serpentinites.

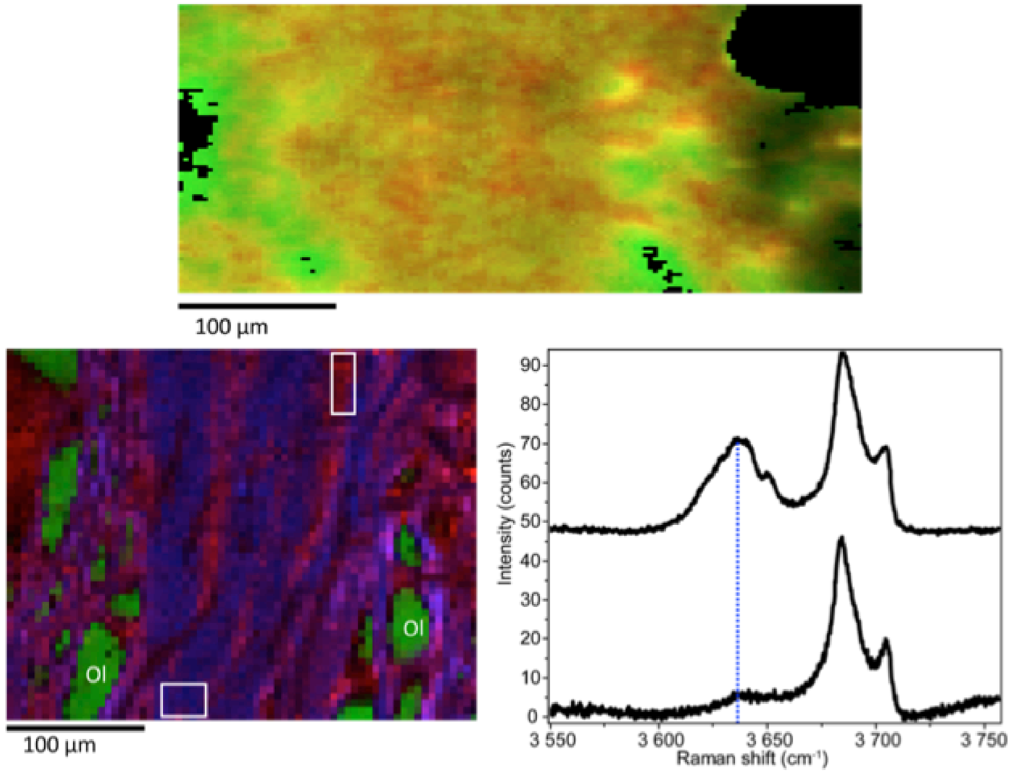

Fig. 11. Microscale characterization of a partially serpentinized Oman peridotite. Top: Iron redox map showing variation in Fe(III)/∑Fe ratio from ~0% (green) in olivine to ~65% in serpentine (red). Left: Raman map illustrating the heterogeneous distribution of lizardite-brucite mixtures (blue) and lizardite without significant brucite (red) in an overlapping area. This heterogeneity contributes to some of the redox variation observed in serpentine. Right: Raman spectra from the blue and red areas indicated by rectangles in the Raman map. The upper spectrum is from the blue area and contains a major brucite peak at the position indicated by the vertical dashed line. The lower spectrum (red area) has no significant brucite peak.

Experimental, Theoretical, and Modeling Constraints on Pathways of Water/Rock Interaction in Presence and Absence of Biological Processes

Experimentally deciphering pathways of hydrogen and methane production and consumption bridges across RPL laboratories. Co-I McCollom conducts theoretical and experimental studies of serpentinization of ultramafic rocks under abiotic conditions to identify first-order controls on H_2 production. In recent water/rock reaction experiments where olivine and harzburgite were hydrated at 90ºC, no appreciable H_2 or CH_4 production occurred (McCollom and Donaldson, 2016). However, ongoing experimental work by McCollom has demonstrated that H_2 production can be enhanced at high pH when this was tested at 200ºC. In contrast, the Templeton lab demonstrated production of notably high concentrations of H_2 and formate when partially serpentinized rocks were reacted at 100ºC at alkaline and hyperalkaline pH (Miller et al., in review). Thus, RPL will continue efforts to critically evaluate which factors control the production of H_2 and reduced C compounds during water/rock reaction.

The pathways of methane formation are particularly enigmatic and not easily inferred from gas chemistry, isotope composition or source characteristics. After investigating the detection of high concentrations of methane with a notably enriched $\delta^{13}C$ isotopic signature in Oman (Miller et al., 2016; Miller et al. 2017), Miller and Templeton initiated a study that examines

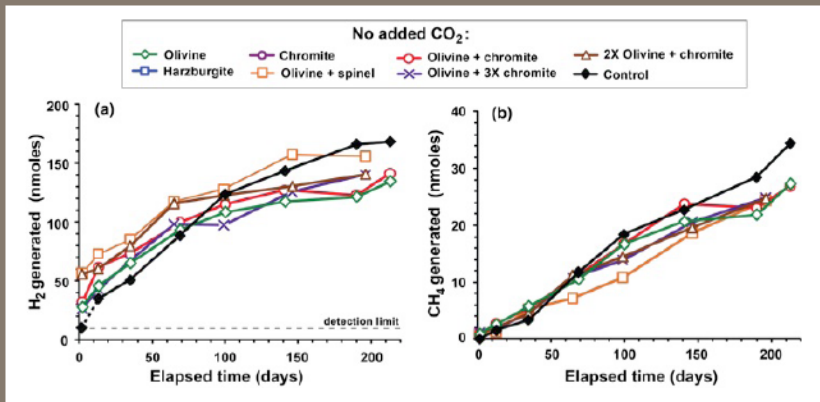

Fig. 12. Production of (a) H_2 and (b) CH_4 during incubation of ultramafic rocks and minerals with water at 90°C in laboratory experiments (McCollom and Donaldson, 2016). In all cases, the experiments produce less H_2 and CH_4 than mineral-free controls (black diamonds), indicating that reactions involving the minerals did not produce detectable amounts of these compounds during the experiment.

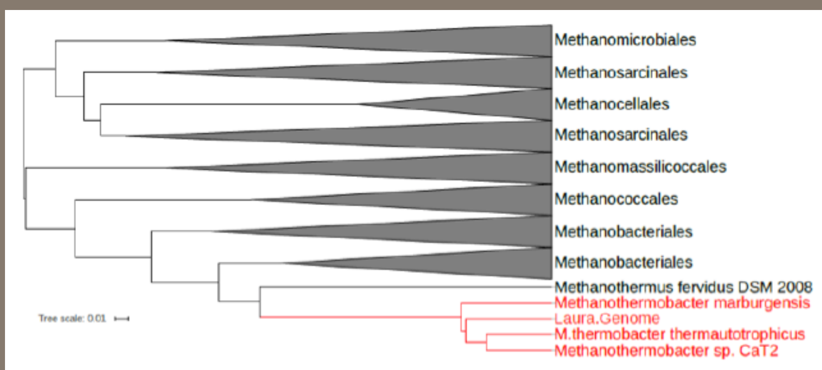

Fig. 13. Phylogenomic reconstruction of a novel methanothermobacter strain, in combination with additional informatics analysis, reveals key similarities and differences with existing strains. The genome also serves the important role as a scaffold for mapping RNAseq reads obtained when the strain is grown under Fe reducing and non-reducing conditions.

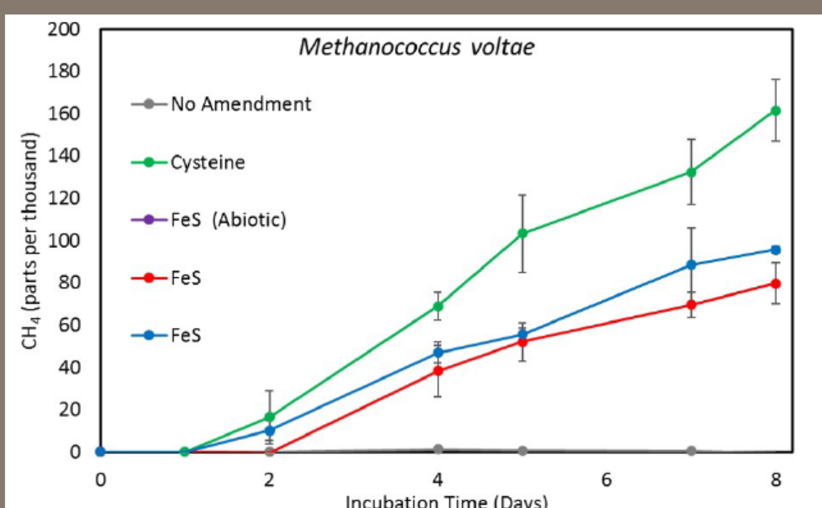

Fig. 14. Methane production by Methanococcus voltae during growth in medium amended with varying forms of reduced sulfur compounds, including pyrite (FeS_2) and mackinawite (FeS) minerals.

the rates and isotopic compositions of microbial methanogenesis at high pH (Miller et al., in prep). Co-I Ono and graduate student Rhim complemented analysis of subsurface CH_4 samples by measuring the isotopologues of methane from Oman, and they are also measuring the isotopologues of methane produced under highly controlled laboratory conditions in bioreactor experiments. Co-I Boyd and graduate student Beuter are also examining the physiology of model methanogens. They have examined modes of anaerobic methane consumption with alternative electron acceptors (e.g. sulfate, Fe(III)-oxides), and have most recently demonstrated that at least three methanogen lineages can reductively dissolve pyrite (FeS_2), and assimilate FeS, which would be a novel source of Fe and S for this metabolism and provides new perspectives on FeS_2 mobilization in highly reduced serpentinite environments (Beuter et al., in prep).

In order to interpret the chemical and biological dynamics observed at our field sites at CROMO and in Oman, collaborator Som is using RPL data sets to design and constrain cell-scale reactive transport models to predict the evolution of fluid chemistry during serpentinization across a broad range of environmentally feasible temperatures, water/rock ratios and reaction extents. These models feed into analysis of the potential to sustain methanogenesis across chemical gradients we have observed in our field systems.

Rock Powered Life: 2016 Publications

Colman, D.R., J. Feyhl-Buska, K.J. Robinson, K.M. Fecteau, H. Xu, E.L. Shock, and E.S. Boyd. (2016). Ecological differentiation in planktonic and sediment-associated chemotrophic microbial communities in Yellowstone hot springs. *FEMS Microbiology Ecology* 92(9): fiw137. DOI: 10.1093/femsec/fiw137

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Feyhl-Buska, J., Y. Chen, C. Jia, J.-X. Wang, C.L. Zhang, and E.S. Boyd. (2016). Influence of growth phase, pH, and temperature on the abundance and composition of tetraether lipids in the thermoacidophile Picrophilus torridus. *Frontiers in Microbiology*. 7:1323. DOI: 10.3389/fmicb.2016.01323

Fortney, N.W., B.J. Converse, S. He, B.L. Beard, C.M. Johnson, E.S. Boyd, and E.E. Roden. (2016). Microbial Fe(III) oxide reduction potential in Chocolate Pots, Yellowstone National Park. *Geobiology* 14(3):255-75. DOI: 10.1111/gbi.12173

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Harrold, Z., M.L. Skidmore, T.L. Hamilton, L. Desch, K. Amada, W. van Gelder, K. Glover, E.E. Roden, and E.S. Boyd. (2016). Aerobic and anaerobic thiosulfate oxidation by a coldadapted, subglacial chemoautotroph. *Applied and Environmental Microbiology* 82: 1486-1495. DOI: 10.1128/AEM.03398-15. 3

Hindshaw, R.S., T.H.E. Hinton, E.S. Boyd, M.R. Lindsay, and E.T. Tipper. (2016). Influence of glaciation on mechanisms of mineral weathering in two high Arctic catchments. *Chemical Geology* 420: 37-50. DOI: 10.1016/j.chemgeo.2015.11.004

Hindshaw, R. S., S. Q. Lang, S. M. Bernasconi, T. H. E. Heaton, M. R. Lindsay, and E. S. Boyd. (2016). Origin and temporal variability of unusually low δ13C-DOC values in two High Arctic catchments. *J. Geophys. Res. Biogeosci*. 121, 1073–1085, DOI: 10.1002/2015JG003303

Hochstein, R., M.J. Amenabar, J. Munson-McGee, E.S. Boyd, and M. Young. (2016). Acidianus tailed spindle virus: a new large tailed spindle virus discovered by culture-independent methods. *Journal of Virology* 13: 3458-6810.1128. DOI: 10.1128/JVI.03098-15

McCollom, T.M. and Donaldson, C. (2016). Generation of Hydrogen and Methane during Experimental Low-Temperature Reaction of Ultramafic Rocks with Water. *Astrobiology*. DOI: 10.1089/ast.2015.1382

Miller, H.M., Matter, J.M., Kelemen, P., Ellison, E.T., Conrad, M.E., Fierer, N., Ruchala, T., Tominaga, M., and Templeton, A.S. (2016). Modern water/rock reactions in Oman hyperalkaline peridotite aquifers and implications for microbial habitability. *Geochimica et Cosmochimica Acta*, v. 179, p. 217–241, DOI: 10.1016/j.gca.2016.01.033

Miller, H.M., Matter, J.M., Kelemen, P., Ellison, E.T., Conrad, M., Fierer, N., Ruchala, T., Tominaga, M., Templeton, A.S. (2016). Reply to "Methane origin in the Samail ophiolite: Comment on 'Modern water/rock reactions in Oman hyperalkaline peridotite aquifers and implications for microbial habitability. *Geochimicaet Cosmochimica Acta*. DOI: 10.1016/j.gca.2016.11.011

Parker, S.R., West, R.F., Boyd, E.S., Feyhl-Buska, J., Gammons, C.H., Johnston, T.B., Williams, G.P., Poulson, S.R. (2016). Biogeochemical and microbial seasonal dynamics between water column and sediment processes in a productive mountain lake: Georgetown Lake, MT, USA. *Journal of Geophysical Research - Biogeosciences* 121: 2064–2081. DOI: 10.1002/2015JG003309

Urschel, M.R., Hamilton, T.L., Roden, E. E. and Boyd, E.S. (2016). Substrate preference, uptake kinetics, and energetics in a facultatively autotrophic, thermoacidiphilic crenarchaeote. *FEMS Microbiology Ecology* 92. fiw069. DOI: 10.1093/femsec/fiw069