



2016 NASA Astrobiology Early Career Collaboration Award Report:
**Investigations of Methane, Sulfur, and Iron in the Serpentinite Subsurface using
Depth-Resolved Biogeochemical Analyses, Stable Isotope Geochemistry, and
Microcosm Approaches**

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Image shows the Core Shed Well locations at CROMO. CSW 1,1 is the largest diameter well located in the shaded area (second well on the left).

Summary:

With the funding from the NAI Early Career Collaboration award, a detailed investigation of biological methane oxidation within the serpentinite groundwater at the Coast Range Ophiolite Microbial Observatory (CROMO) was conducted to constrain how microbial community composition and activity is linked to methane, sulfur, and iron availability with depth.

Field Work:

During a trip to CROMO at the beginning of June 2016, depth profiles of methane, methane stable isotopes, oxygen, iron, sulfur, pH, and microbial community composition (16S rRNA analysis) were determined in the water column within a central monitoring well (CSW1,1). With the support of Mike Kubo, samples were collected at depths where dissolved oxygen concentrations were 100%, 50%, 15%, and 0% of air saturation. Members of the Schrenk Lab (Lauren Seyler and Lindsay Williams) extracted



DNA and RNA from Sterivex filters taken at each sampling point for sequencing analysis. Dawn Cardace (University of Rhode Island) took detailed field notes throughout this extensive process, which provided an accurate record of how much water was drawn from the well and timing for when water samples and measurements were taken. With the endless assistance of Tori Hoehler and Mike Kubo, microcosm incubations were conducted to quantify rates of methane consumption. Groundwater was pumped from the 15% O₂ air saturation point and the anoxic well bottom directly into bottles for laboratory incubation. The Schrenk, Cardace, Hoehler, and Ono lab members present were essential in this process.

Microcosm Work:

All microcosms were inoculated with ¹³CH₄ gas at Ames, with parallel experiments run following the addition of an iron or sulfur compound, respectively. Changes in fluid chemical composition were monitored at multiple time points by colorimetric methods (sulfide, ferrous and total iron), ICP-MS (total sulfur, iron), and gas chromatography (methane). The evolution of ¹³C labeled CO₂ -- the presumable product of methane oxidation -- was quantified using membrane-inlet mass spectrometry (MIMS) and, as an independent control, isotope ratio mass spectrometry (IRMS). To understand how cell densities change over time, samples were preserved at each time point for microscopic cell counts, and will be analyzed at Michigan State University this fall. At the experiment-end, samples for 16S rRNA will be extracted to define the change in microbial communities from the *in situ* well populations. The results of these in-progress microcosms and geochemical analyses will be presented at the Serpentine Days conference in Sète, France this September. A follow-up trip to Ames will be made in December 2016 to continue monitoring the microcosms.



Microcosm experiment created from water from CSW 1,1. Oxic bottles (green tape) contain water from the 15% oxygen air saturation level. Anoxic bottles (teal tape) have been inoculated with water from the anoxic well bottom. Each set was run in triplicate, with three 0.22 µM-filtered controls for each amendment added (methane only, sulfur, or iron respectively).



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