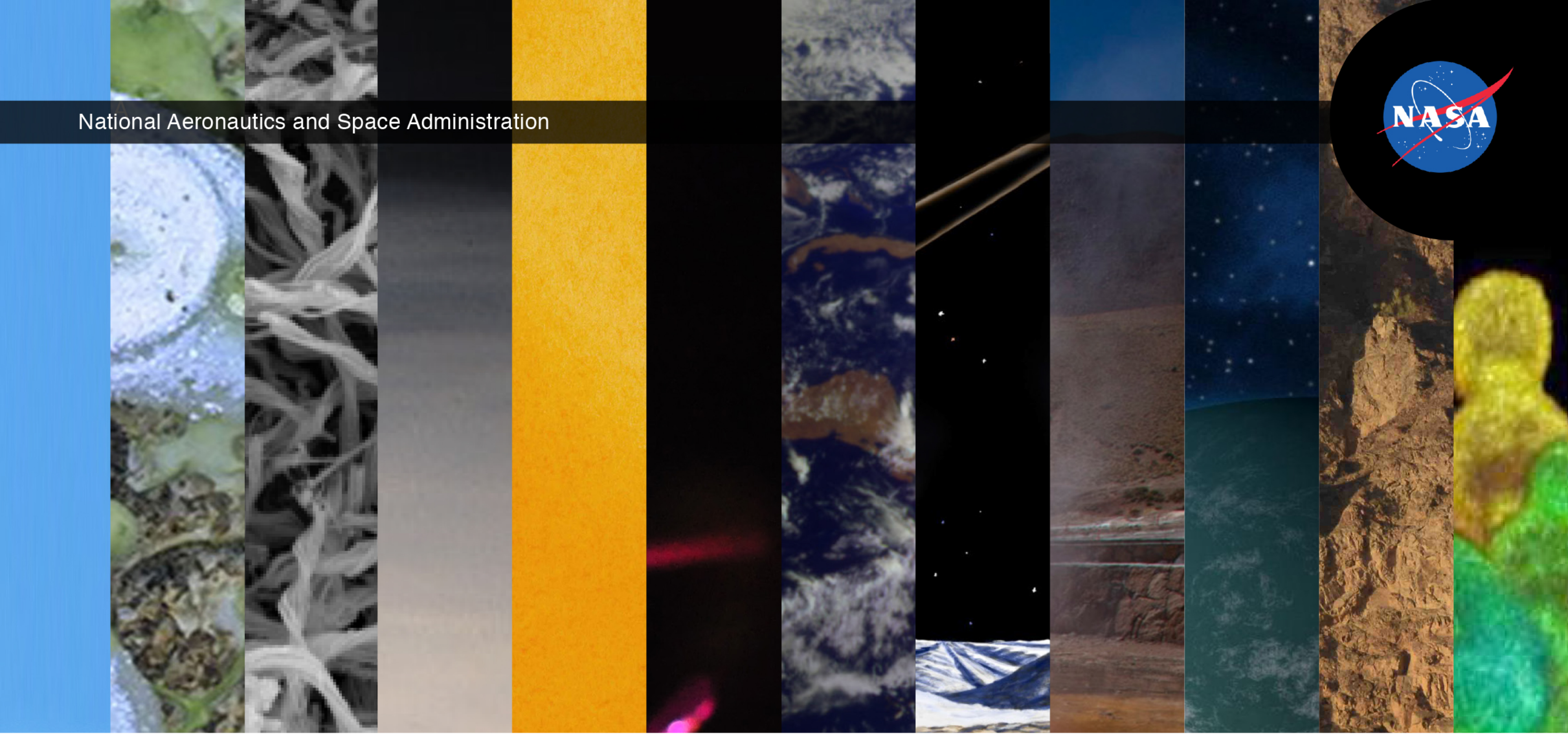


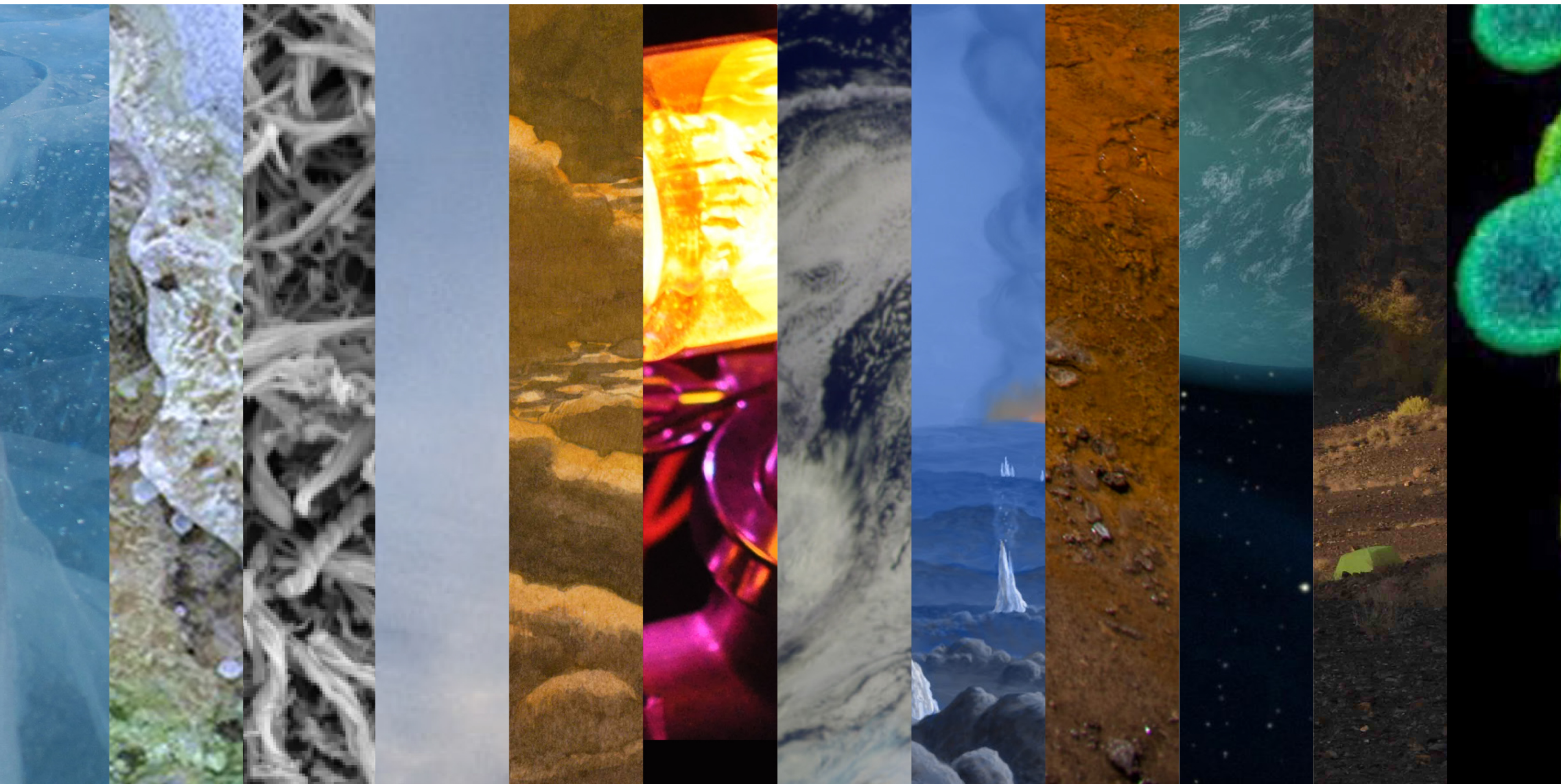
National Aeronautics and Space Administration

NASA ASTROBIOLOGY INSTITUTE

2017 Annual Science Report

Life Underground

University of Southern California



Life Underground

Lead Institution:
University of Southern California

Team Overview

Principal Investigator:
Jan Amend

The Life Underground (LU) team developed and employed field, experimental, analytical, and modeling approaches aimed at detecting and characterizing microbial life in the subsurface—the intraterrestrials—and their host environments. We posited that if life exists, or ever existed, on Mars or other planetary bodies in our Solar System, evidence thereof would most likely be found in the subsurface. Our team took advantage of unique opportunities to explore the subsurface ecosystems on Earth through boreholes, mine shafts, sediment cores, marine vents and seeps, and deeply-sourced springs. Access to the subsurface—both continental and marine—and broad characterization of the rocks, minerals, fluids, and microbial inhabitants were central to this study. Our four major research themes were:

- Access to the subsurface
- *In-situ* life detection and characterization
- Guided cultivation of intraterrestrials
- Energy flow and metabolic modeling

Team Website: https://dornsife.usc.edu/life-underground

2017 Executive Summary

The Life Underground team continued its research into the continental and marine deep subsurface biosphere on Earth, with an eye towards potentially habitable environments on other planetary bodies. In 2017, we had major accomplishments in all four of our research themes—access to the subsurface, in situ life detection and characterization, guided cultivation of intraterrestrials, and energy flow and metabolic modeling—that led to numerous high impact publications. Participation in and, in many cases, leadership of these projects significantly advanced the training and opportunities for our undergraduate and graduate students, post-doctoral scholars, and junior investigators.

We obtained unique access to the continental subsurface via legacy boreholes at the Sanford Underground Research Facility (SURF) in South Dakota, the BLM-1 well in Nevada, and serpentinizing springs in California. The marine subsurface was sampled with a remotely operated vehicle in the North Atlantic, as part of an IODP expedition off the coast of Japan, and on cruises to the Monterey Canyon in California and the Gulf of California in Mexican waters.

We continued to develop and refine a spectral imaging pipeline that correlates mineral, organic, and biologic analyses across wide spatial scales, down to the sub-cellular level. Natural and incubated field samples, including marine carbonates, humic substances, and biofilms were put through this pipeline, yielding robust data on various spatially resolved microbe-mineral interactions and microbial formation of hydrous carbonates on dolomite surfaces in very high pH fluids. Working with model organisms, we showed that deep UV Raman spectra could differentiate cells in the lag, exponential, and stationary growth phases, and when combined with stable isotope probing, metabolic activity could be assessed even more directly. Part of this analysis suite provides essential ground-truthing for SHERLOC, a Mars 2020 mission flight instrument.

The application of electrochemical techniques, both *in situ* and in the laboratory, greatly advanced our cultivation and characterization of intraterrestrials. We developed and employed a new reactor at SURF, demonstrating via cyclic voltammetry both cathodic and anodic current flow, indicative of microbially mediated sulfur reduction and oxidation. In a laboratory study, we showed that a pure methanogen culture could operate multiple metabolic mechanisms, relying either on cell-secreted hydrogenases or on direct cell-to-surface contact. Lastly, electrochemistry was also used to further characterize the physiology of two novel bacterial strains, previously isolated from SURF by members of the LU team.

Fig. 1. Caitlin Casar harvesting flow-through cultivation experiments at the SURF DeMMO 3 site. Photo credit: Brittany Kruger.

Fig. 2. Rose Jones setting up electrode colonization experiments on the North Pond cruise. Photo credit: Jacqueline Goordial.

Fig. 3. A) Magdalena Osburn, (B) Moh El-Naggar (left) with Victoria Orphan, (C) Annette Rowe, (D) Elizabeth Trembath-Reichert

Within our theme on energy flow and metabolic modeling, we identified an array of aqueous organic compounds in ocean basement fluids, evaluated the potential energy from several dozen fermentation reactions, and modeled the rates of sedimentary organic matter degradation by microorganisms. We also demonstrated that dead biomass likely does not support more than a tiny fraction of microbial metabolism in oligotrophic marine sediments, and that in hydrothermal systems a wide range of redox processes can provide the requisite energy to vast microbial communities.

Mentoring our early career scientists was an essential component of our LU project. Their successes, including awards, invited lectures, professional appointments, and post-doctoral and graduate fellowships, ensure a strong future in astrobiology research at NASA and elsewhere. Here, we highlight just a few examples from 2017: Magdalena Osburn received a David and Lucile Packard Fellowship, Moh El-Naggar was a finalist for the Blavatnik National Awards in life sciences, post-doc Annette Rowe accepted a tenure-track faculty position at the University of Cincinnati, and post-doc Elizabeth Trembath-Reichert's PNAS paper was selected for the Cozzarelli Prize.

Team Members

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William Abbey
Abigail Allwood
Rohit Bhartia
Lina Bird
Sean Bouchard
Caitlin Casar
David Case
Gray Chadwick
Allison Comrie
Bethany Ehlmann
Moh El-Naggar
Evan Eshelman
Jayme Feyhl-Buska
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Yuri Gorby
Scott Hamilton-Brehm
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Yamini Jangir
Rose Jones
Amruta Karbelkar
Chris Kempes
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Bonita Lam
Doug LaRowe
Kyle Metcalfe
Alice Michel
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Tullis C. Onstott
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Magdalena Osburn
Sahand Pirbadian
Brandi Reese
Nerissa Rivera-Laux
Alberto Robador
Annette Rowe
Joshua Sackett
Everett Salas
Cecilia Sanders
Haley Sapers
Pratixa Savalia
Barbara Sherwood-Lollar
Daan Speth
Elizabeth Trembath-Reichert
Greg Wanger
Josh West
Holly Willis
Hank Yu

Project Reports

Access to the Subsurface

The LU team carried out fieldwork at a number of continental and marine subsurface locations, including the SURF in South Dakota, BLM-1 in Death Valley, the Cedars spring system in Northern California, a deep-sea sediment pond (North Pond) near the Mid-Atlantic Ridge, Monterey Canyon in Northern California, and sediments off the coast of Muroto (Japan).

In February, May, July, September, October, and December, a group led by Maggie Osburn visited the network of six legacy boreholes that constitute the Deep Mine Microbial Observatory (DeMMO) at SURF (Fig. 4). These visits add to an established long-term geochemical and microbiological monitoring program that began more than 2 years ago, and also facilitated the installation of experiments and collection of data by several LU lab groups. BLM-1 was visited twice by LU members from DRI, JPL, and Caltech to test a newly designed and custom built *in situ* snap sampler (Niskin-like device for collecting borehole water, Fig. 5), and to deploy and retrieve long-term mineral incubation experiments. Ken Nealson's group led several sampling trips to the serpentinizing springs at the Cedars.

Several LU members participated in expeditions to various marine subsurface sites. In October, Beth Orcutt led a cruise with the remotely operated vehicle (ROV) Jason to the oceanic crust borehole observatory network at North Pond. We recovered pristine crustal fluids and hundreds of microbial rock colonization experiments that were deployed in 2011 as part of IODP Expedition 336 (Fig. 6). Experiments are on-going with these crustal fluids to enrich for iron- and

Fig. 4. Borehole packer and flow through colonization reactors at DeMMO (SURF, South Dakota).

Fig. 5. Custom Snap Sampler being deployed at BLM-1 (Death Valley).

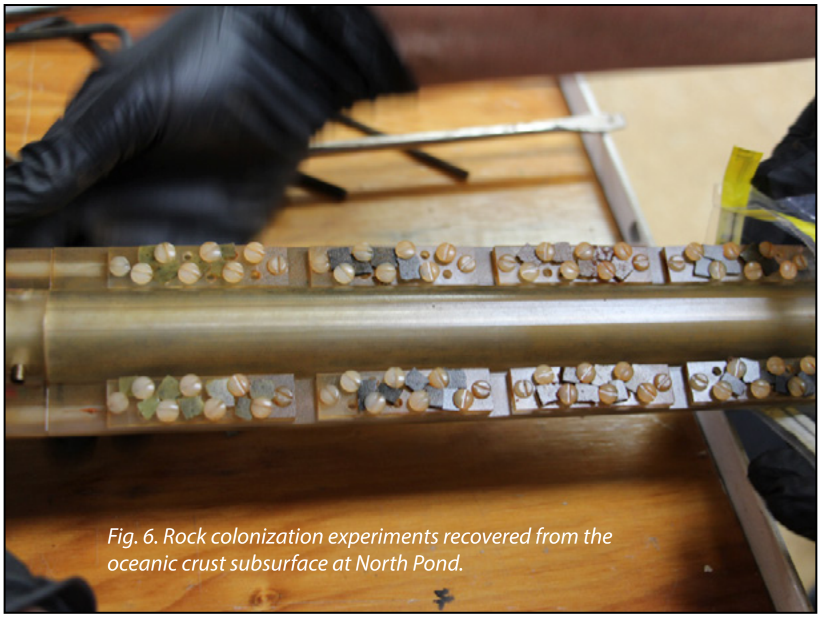

Fig. 6. Rock colonization experiments recovered from the oceanic crust subsurface at North Pond.

sulfur-oxidisiny members of the microbial community. New fluorescence approaches for sorting out individual active cells are also underway. Victoria Orphan's group participated in IODP Expedition 370 (Temperature Limit of the Deep Biosphere off Muroto) and in two cruises to Monterey Canyon on the R/V Western Flyer to test ROV-deployed *in situ* stable isotope probing techniques in sediments at ~1000 m water depth. They also returned to hydrothermal vents in the Pescadero Basin, Gulf of California to follow up on samples collected in 2015.

In situ Life Detection and Characterization

Rohit Bhartia and Victoria Orphan developed a correlated spectral imaging pipeline that fuses mineral, organic, and biologic analyses to bulk analytical methods (genetic diversity/organic inventory) on laboratory standards and at multiple field subsurface environments. This includes *in situ* experiments for assessing mineral-specific microbial colonization and metabolic activity that spans the macro to microscale. Field and laboratory incubations of natural and defined minerals in deep subsurface environments including BLM-1, SURF, subseafloor coal and shale beds, and methane seep carbonates have been used for optimization of various components of the pipeline.

The spectral pipeline includes coarse-scale localization of mineral associated biomass (MOSAIC); bulk mineralogical characterization; 10's of µm scale UV and green Raman analysis; single cell-resolved imaging; and chemical and isotopic mapping (e.g., SEM-EDX, nanoSIMS). This has led to the incarnation of a biological, organic, and mineral imaging instrument (TORUS), a bench top version of the Mars2020 SHERLOC deep UV Raman/fluorescence instrument. The LU team also demonstrated fluorescence correlation to biomass, UV Raman analysis of microbial physiological states correlated to variation in nucleic acids and aromatic amino acids content, and mineralogical control of microbial colonization and authigenic biomineralization (Fig. 7). Lastly, in addition to spectral collection, we developed a Multi-INstrument-Database (MIND) and a Raman/fluorescence spectral processing pipeline that will also support SHERLOC/Mars 2020 spectral processing and instrument testing efforts.

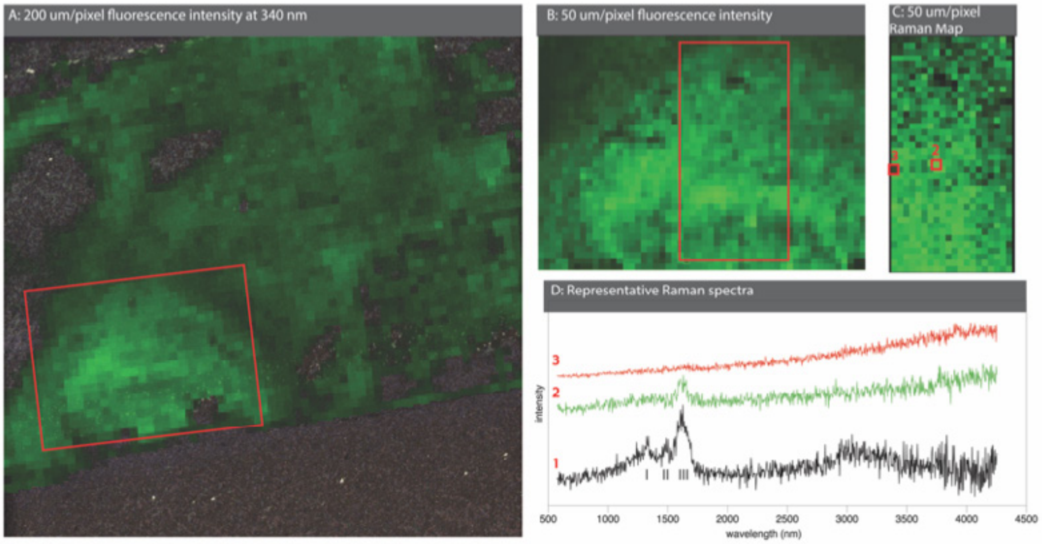

Fig. 7. DUV spectroscopy was used to localize biomass on a rock coupon incubated for 3 months at SURF. (A) DUV fluorescence scan (340 nm intensity) at 200 µm/pixel overlain on SEM image of one coupon; (B) fluorescence at ~570-4250 nm intensity at a resolution of 50 µm/pixel; (C) deep UV Raman spectra of red insert in (B) at 50 µm/pixel; (D) representative spectra of (1) Raman shifts associated with aromatic molecules, including vibrations attributed to adenine (I), guanine (II), and the amino acids tryptophan, tyrosine, and phenylalanine (III); (2) a bright pixel in (C) ascribed to a greater proportion of a biological component than (3) a dark pixel.

Guided Cultivation of 'Intraterrestrials'

At SURF-DeMMO 4, postdoctoral scholar Annette Rowe and undergraduate researcher Karla Abuyen installed a new *in situ* enrichment reactor incorporating both minerals and electrodes (Fig. 3.1). Based on geochemical analyses, we targeted sulfur oxidizing metabolisms. In two experimental incubations (March-May and August-October), microbial electrochemical activity (i.e., current) was monitored, with an observed high cathodic current indicative of active electron uptake. Interestingly, periodic anodic currents were also observed. Cyclic voltammetry suggested that the observed electron flow was mediated or catalyzed by a biological reaction. This suggests the potential for cycling redox states in the environment between sulfur reducing and sulfur oxidizing conditions. We are now comparing the enriched microbial communities and attempting to cultivate the relevant electrochemically active sulfur oxidizing and reducing populations.

One of the LU team's aims is to better understand microbial electron uptake, with a focus on the limits and astrobiological relevance of this metabolic strategy. Annette Rowe completed a series of experiments demonstrating the potential for cathodic current generation via pure cultures of Methanosarcina barkeri, a model methanogen from the lineage with cytochromes (Fig. 3.2). She observed two mechanisms, one that relies on cell-secreted hydrogenases that catalyze H2 production on external surfaces, and one, previously undescribed, that appears to involve direct cell interaction with surfaces. This latter mechanism likely has important implications to energy conservation in this astrobiologically important group of organisms.

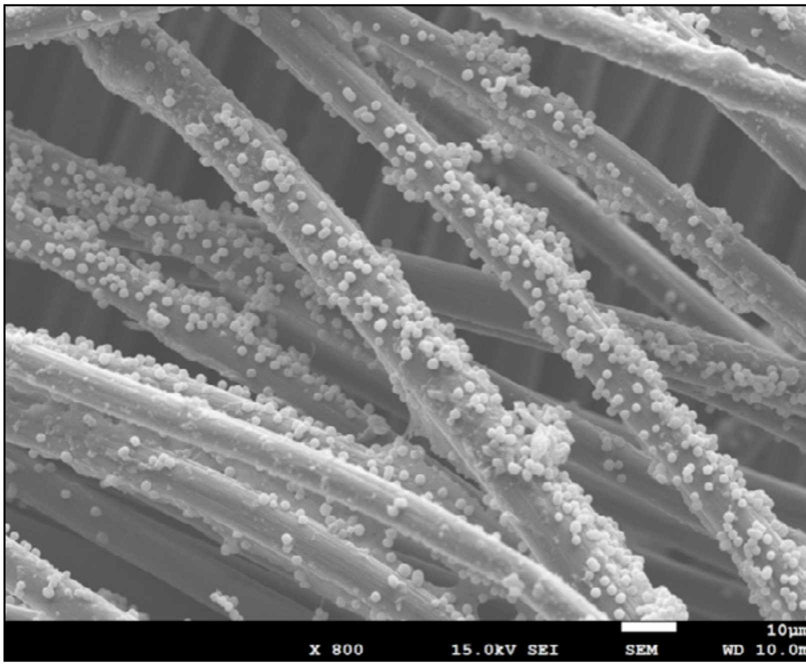

Fig. 8. Scanning electron micrograph of wild type M. barkeri cells attached to a carbon cloth electrode. Electrode incubated for five days at -500 mV SHE.

USC graduate students Amruta Karbelkar and Nicole Beedle completed the electrochemical characterization of two new Bacillus and Comamonas strains isolated from SURF using an *in situ* electrochemical colonization reactor. The Bacillus strain is capable of using an electrode as a respiratory electron acceptor, while the Comamonas strain performs the reverse process of electron uptake from surfaces. Both strain characterizations are examples of our subsurface-to-lab workflow for electrode-based capture, enrichment, isolation, and mechanistic evaluation of electrochemically active microbes.

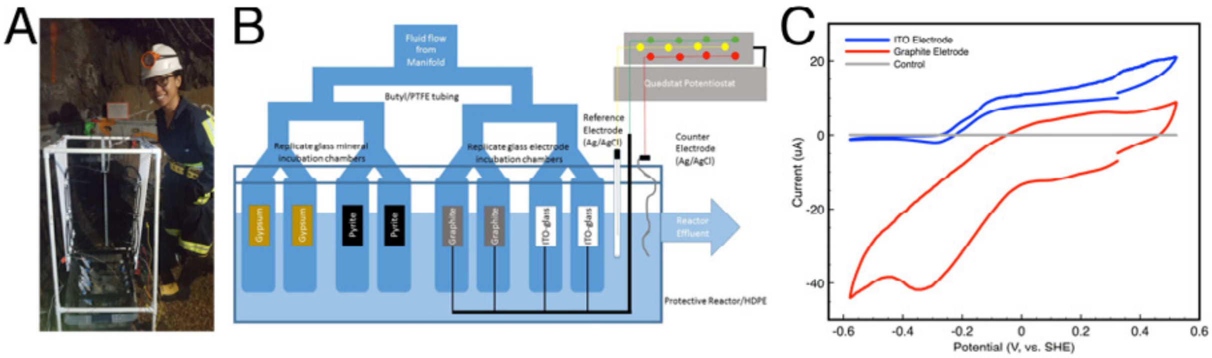

Fig. 9. (A) LU undergraduate researcher Karla Abuyen installing incubation reactor at SURF; (B) reactor schematic showing utilization of two electrode materials (poised at -200 mV SHE) and two redox active minerals; (C) cyclic voltammetry data suggesting electrochemical activity, with the graphite electrode generating negative (cathodic) current and the ITO electrode generating positive (anodic) current.

Energy Flow and Metabolic Modeling

Jan Amend guided several studies on the geochemical energy available to microorganisms in continental and marine subsurface environments. For example, a team led by Doug LaRowe used Fourier transform ion cyclotron resonance mass spectrometry to determine the identity of organic compounds in ocean basement fluids that are available for heterotrophic microbial communities, calculated the energy yields from 47 fermentation reactions at a range of subsurface environmental conditions, modeled the 3-D distribution of temperature in marine sediments, and applied a reaction transport model to quantify the rates at which microbes consume energy from particulate organic carbon degradation in marine sediments globally (Fig. 10). Postdoctoral scholar James Bradley showed that, counter to claims in the literature, the oxidation of dead microbial biomass (necromass) likely provides only a small fraction of the maintenance power demands of subseafloor microbial communities in oligotrophic sediments. He then incorporated this into a model that predicts energetic controls on microbial physiology in marine sediments. Focusing on hundreds of hydrothermal systems globally, graduate student Guang-Sin Lu computed the energy yields from more than 700 potential chemolithotrophic metabolisms, and in parallel, a team of LU scientists specifically targeted the energy yields from nitrogen cycling at the Loihi Seamount (Hawaii) hydrothermal vent field and in a deep-sea sediment pond (North Pond) near the Mid-Atlantic ridge.

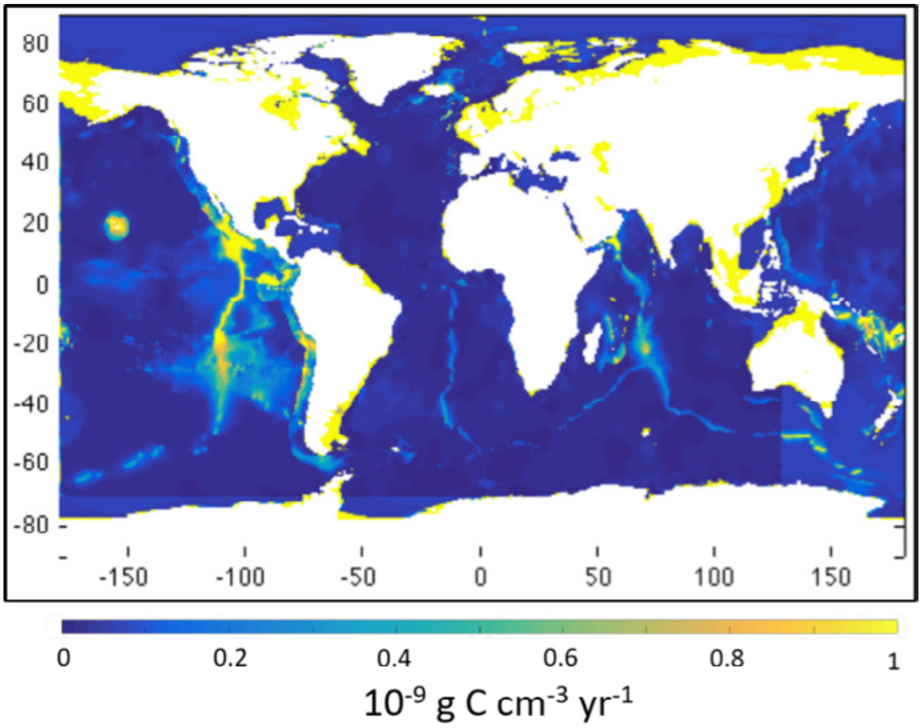

Fig. 10. Global rates of particulate organic carbon (POC) degradation in sediments that were deposited at the beginning of the Pleistocene (~2.6 Ma). Despite orders-of-magnitude differences in POC deposition fluxes and highly variable initial organic matter reactivity levels, global rates of microbial heterotrophy are calculated to converge at about 1 nmol of carbon per cubic centimeter per year in sediments that are 2.6 Ma.

Field Work

Sanford Underground Research Facility, Lead, South Dakota, USA

This former Homestake gold mine in the Black Hills of South Dakota hosts a major underground research laboratory, the Sanford Underground Research Facility (SURF). While SURF activities are focused on high-energy physics, it is now also a key site in deep subsurface biosphere research including the Deep Mine Microbial Observatory (DeMMO). The laboratory accesses about ~170 km of mine workings, with a subset currently maintained and ventilated, to a depth of ~1.5 km. Black Hills geology is complex and this site provides subsurface access to key Precambrian metamorphic rocks, ore bodies, and basalts.

Astrobiologists are participating in a number of projects aimed at detecting and characterizing microbial life in the subsurface. The DeMMO is a 3D microbial observatory established using select legacy boreholes from the surface to the 4850 level (~1.5 km) that tap into deep subsurface fluids. Monitoring of the geochemistry and microbial inhabitants of these sites is of key astrobiological interest. Additionally, targeted experiments at DeMMO sites include *in situ* electrode-assisted cultivation of subsurface microbes and colonization experiments on rock and mineral chips to learn about biofilm formation.

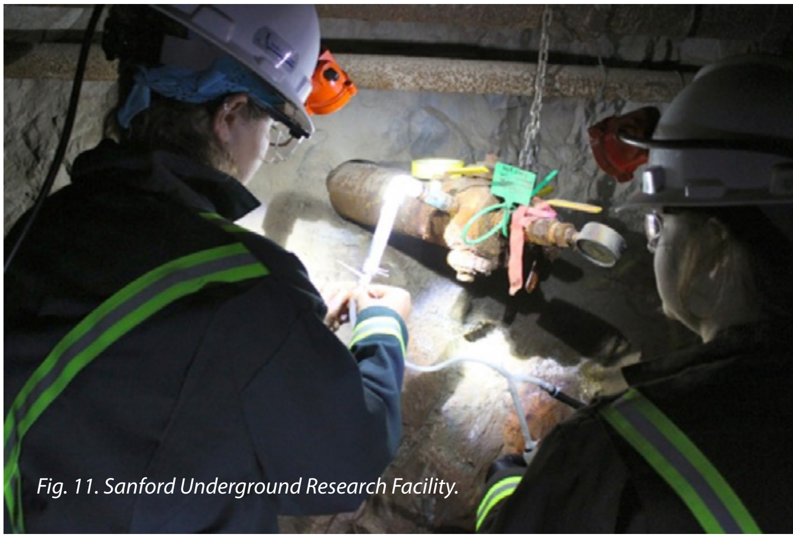

Fig. 11. Sanford Underground Research Facility.

Amargosa Valley (BLM-1)

The Death Valley Flow system consists of a highly fractured regional groundwater system covering hundreds of square kilometers of Nevada and California. The BLM-1 borehole is located just inside California on the border with Nevada in the Amargosa Valley. Situated just east of Death Valley, BLM-1 was drilled >700 m deep to monitor the ground waters slowly migrating from Nevada into California.

Determining the rate of colonization of minerals, the distribution of those biosignatures on minerals, and the activity of individual populations in the deep subsurface helps astrobiologists understand the extent of life in these extremely low biomass systems. To guide the search for potential subsurface life elsewhere in the solar system, astrobiologist must first be able to identify it here on Earth.

For the last two years experiments have been lowered to the bottom of BLM-1 and incubated *in situ* for months at a time. These long-term experiments show the rate of colonization of freshly exposed minerals, the major players in the microbial community, and whether the community is stable over time. Once samples are collected from the field the minerals are examined with Deep-UV spectroscopic tools to characterize the distribution of organics and biosignatures there upon.

Fig. 12. BLM-1 is a ~700 m deep monitoring well drilled into a carbonate aquifer on the edge of Death Valley National Park.

The Cedars

The Cedars, located in Sonoma County, California, hosts groundwater-fed minerals springs discharging from serpentinizing peridodite. Serpentinization, the hydration of olivine and pyroxene minerals at moderate temperatures (100 to 300°C), generates heat, hydrogen, and other materials relevant to supporting autotrophic life, and is an analog environment relevant to where life is thought to have emerged. Olivine and pyroxene make up >50% of the Earth's mantle. Additionally, these are minerals that are present on other solar system bodies (planetary moons, Mars, meteorites, etc.), suggesting the potential for these life-fueling processes elsewhere in the solar system. Localized within a serpentine mountain range, The Cedars hosts mineral springs, travertine

Fig. 13. The Cedars Grotto Spring Pool 1 (GPS1).

Fig. 14. The Cedars Barnes Spring Complex.

deposits, and minerology indicative of serpentinization, as well as chromium ore. Serpentinized fluids from this site are thought to originate from two sources including a shallow meteoric water sources, as well as fluids contacting a deeper marine sediment layer (the Franciscan Subduction Complex). These sources vary in age, ionic strength, and the endemic microbial communities present.

Research at The Cedars has centered on understanding the metabolic potential of microbes that persist in this environment, as well as the adaptations for life under extreme high pH conditions. Microbial community analysis and metagenomics studies have provided insight in to the potential metabolisms present in these systems, including chemolithoautotrophs and photoautotrophs. This work is supported by the isolation of the first autotrophs from a serpentinizing spring, Serpentinamonas, a hydrogen consuming aerobe that is commonly found in terrestrial serpentinizing systems, and its tolerance and adaptations to high pH environments is currently being investigated.

Other research at The Cedars has focused on understanding microbe mineral interactions in this analog environment. The first successful enrichment and isolation of a high pH mineral reducing microbe from The Cedars has provided insight into the potential of solid phase minerals as terminal electron acceptors under anaerobic conditions. The microbe isolated is capable of reducing magnetite—a common end-product of serpentinization. This site is also being used to field test instruments selected for the Mars 2020 Rover. Microbe-mineral interactions were assessed for a variety of environmentally relevant minerals incubated in spring waters using correlative spectroscopic approaches, combining DUV fluorescence and Raman mapping with molecular microbiological approaches (i.e., 16S rRNA gene characterization). Pre and post mineral mapping is providing insight into the selective attachment of microbes to various minerals, and in some cases the altered mineralogy (both formation and mineral weathering) corresponding with microbial attachment.

Shimokita Peninsula (Japan)

The Shimokita Peninsula coal beds house a microbial assemblage that has been buried for millions of years, yet is similar in composition to a modern, terrestrial swamp community. This system was accessed by IODP Expedition 337, which recovered cores from the deepest hole ever explored

Fig. 15. Off-shore of the Shimokita Peninsula, Japan, are deeply buried coal beds (2 km below seafloor) that house a microbial community that has utilized the same organic carbon source for 25 million years.

by marine scientific ocean drilling. The site interrogates depth and pressure limits of life and found the lowest cell abundances of any IODP mission. The coal beds, however, appear to be a "hot bed" of microbial abundance and activity. This suggests that if ample organic carbon is available, microbes can persist in this harsh, low-energy environment and may extend to planetary interiors being a potential safe haven for future astrobiological targets.

Samples collected from this site were utilized for a range of scientific purposes, though microbiology was a core focus. In addition to *in situ* microbial characterization, both stable and radioisotope probing experiments were conducted to determine the range of microbial metabolisms and rates of metabolism surrounding and within the coal beds. Through the novel application of deuterated water as a passive tracer of microbial activity, followed by single-cell NanoSIMS analysis, microbial biomass is estimated to regenerate on the order of years to hundreds of years in these incubations. These rates are much faster than other deep biosphere estimates for even shallower systems, suggesting this more sensitive technique may provide new insight into deep biosphere, and low biomass/energy astrobiological targets in general.

Atlantis Massif, North Atlantic

The Atlantis Massif is an ocean core complex on the western flank of the Mid-Atlantic Ridge, where mantle crust has been uplifted to the surface. Mantle crust rich in the mineral peridotite undergoes serpentinization fluid-rock reactions when exposed to seawater, leading to the abiotic development of hydrothermal fluids enriched in alkalinity, hydrogen and small carbon compounds. For example, the characteristic Lost City Hydrothermal Vent field is located on the Atlantis Massif, where high pH fluids mix with seawater to form expansive carbonate chimneys. Such subsurface serpentinization fluids and processes may support chemosynthetic life that utilizes the hydrogen and small carbon compounds, although life may be challenged by the high pH conditions. Studying life in this marine subsurface serpentinizing system can help astrobiologists to understand life's potential to exist in serpentinizing systems on other worlds such as Enceladeus and Europa.

The Atlantis Massif provides an opportunity to study subseafloor microbial communities associated with serpentinization. Here, astrobiologists study life's adaptation to extremes of pressure and high pH, and also how microbial communities may take advantage of the chemicals generated from fluid-rock serpentinization processes. These studies also focus on determining chemical fingerprints and biosignatures of life supported by serpentinization, which may be used for identifying serpentinization-supported life on other worlds. For these studies, astrobiologists are participants in Integrated Ocean Drilling Program (IODP) expedition 357, which cored into the Atlantis Massif in 2015 to collect rock and fluid samples. Observatories were also installed in some of the boreholes, to enable collection of pristine serpentization fluids during future missions.

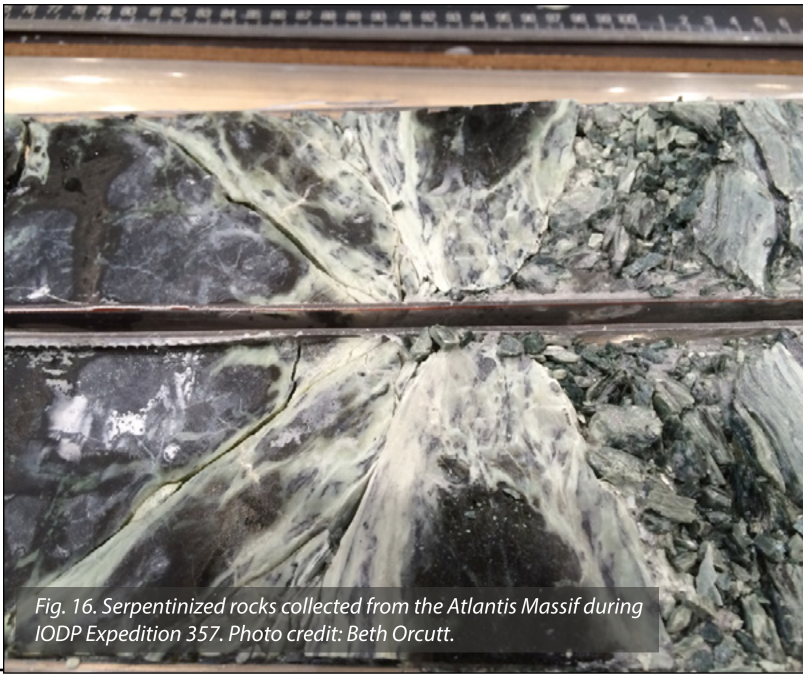

Fig. 16. Serpentinized rocks collected from the Atlantis Massif during IODP Expedition 357. Photo credit: Beth Orcutt.

Life Underground: 2017 Publications

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