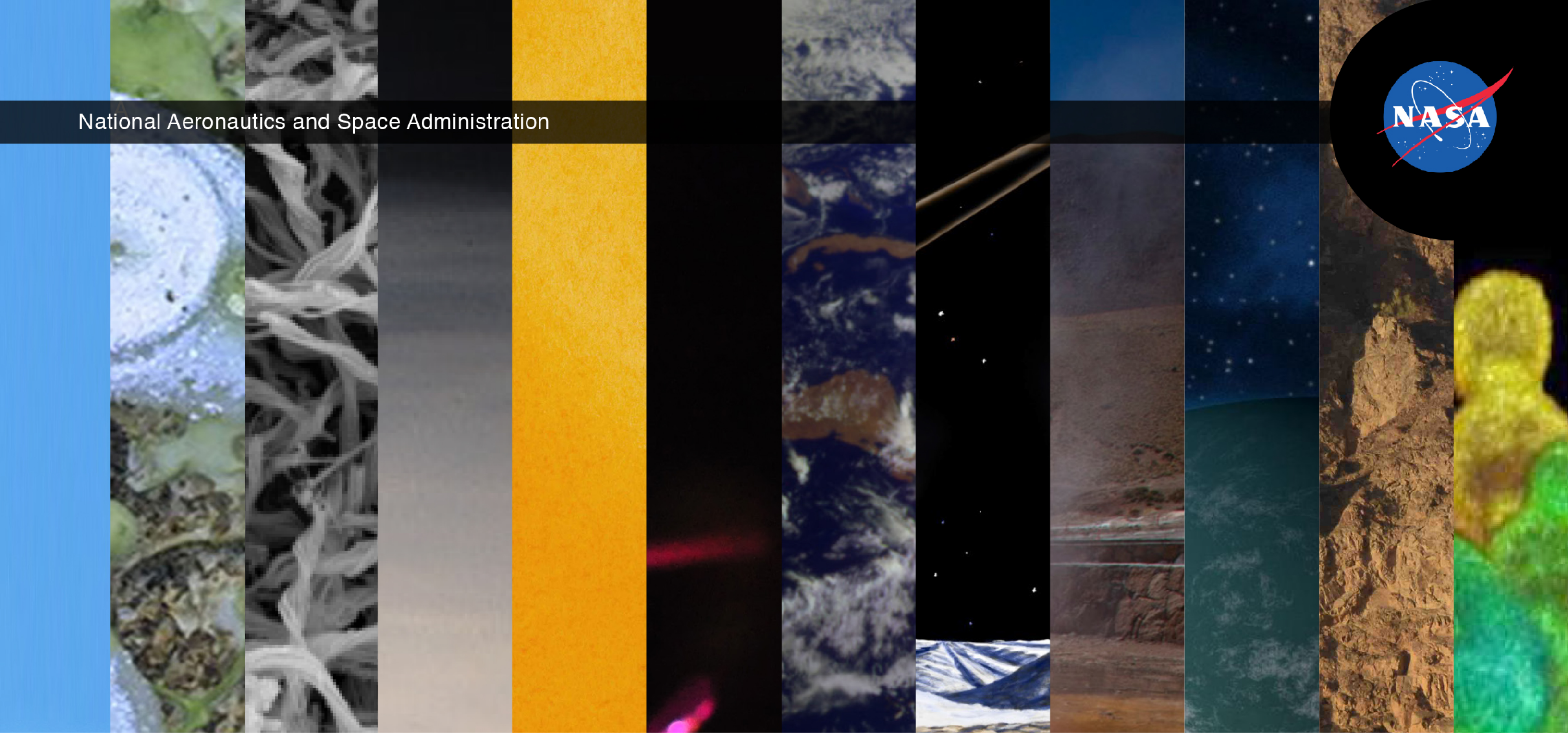
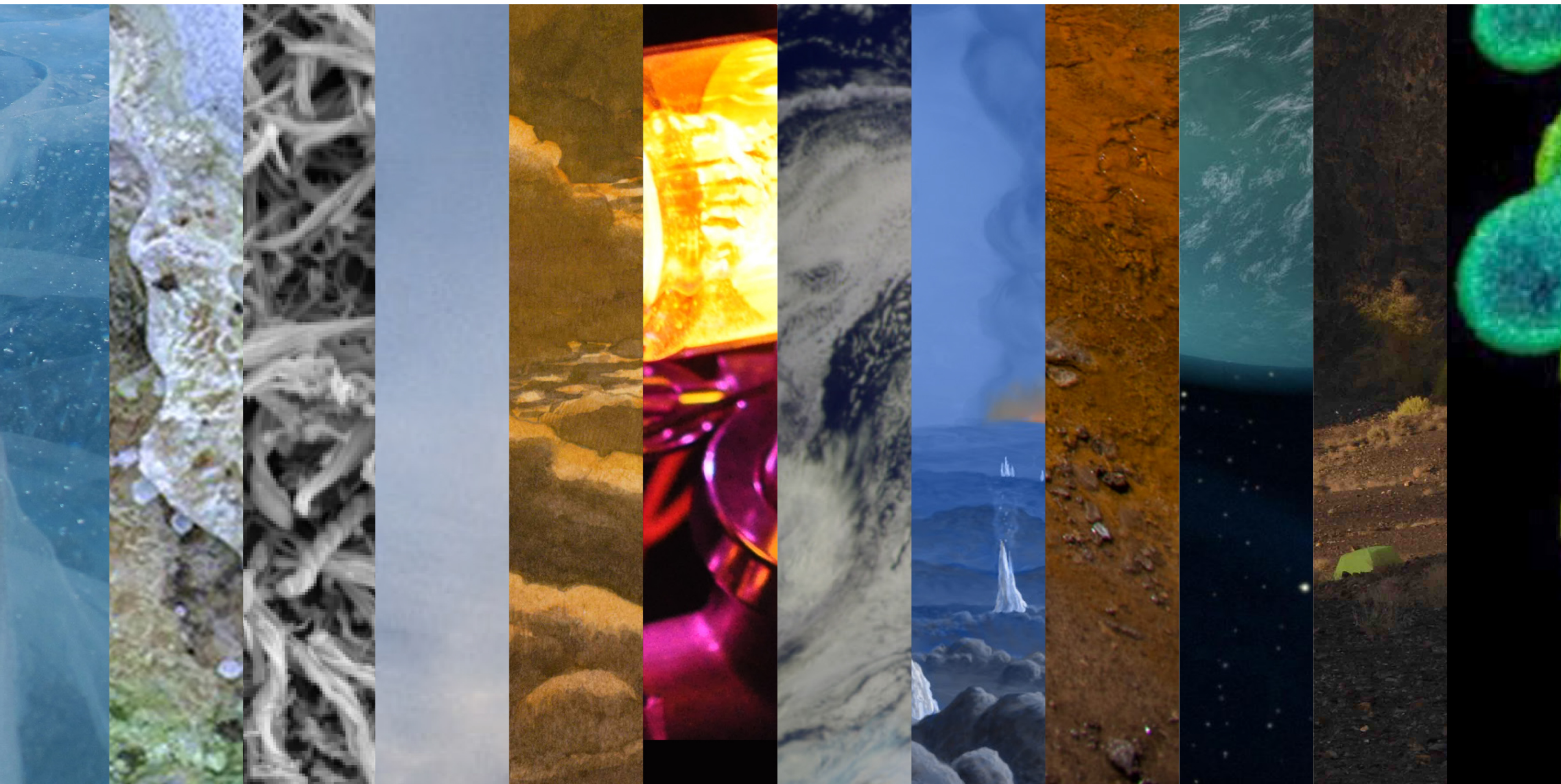


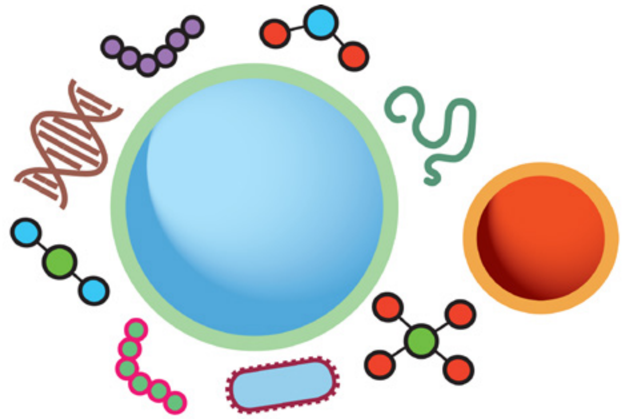
NASA Astrobiology Institute

2017 Annual Science Report

Habitability, Life Detection, and the Signatures of Life on a Terrestrial Planet

University of Wisconsin-Madison





Habitability, Life Detection, And The Signatures Of Life On The Terrestrial Planets

Lead Institution:
University of Wisconsin-Madison

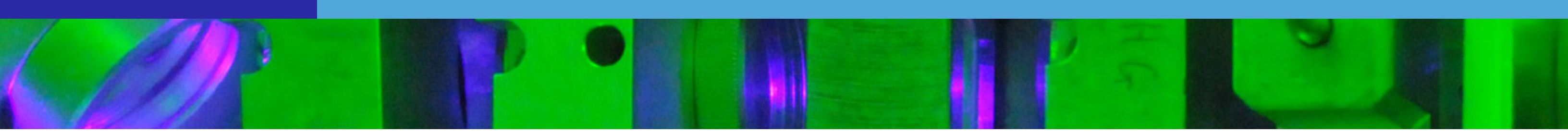
Principal Investigator:
Clark Johnson

Team Overview

The Wisconsin Astrobiology Research Consortium pursues research and education on habitability, life detection, and the signatures of life on the terrestrial planets, with a focus on Earth and Mars. This effort is fundamentally built around a broad interpretation of Life Detection, which includes not only detection of the organic signatures of life in modern and ancient environments, on Earth or other planetary bodies, but also the inorganic signatures of life, which may have the greatest fidelity over billion-year timescales and complex geologic histories. Biosignatures developed from laboratory experiments are field-tested in modern and ancient environments on Earth, which in turn inform new experimental studies, producing an iterative process of testing and evaluation. The goal is to ultimately develop the interpretive context needed to evaluate the potential for life on other planetary bodies, as well as to understand the evolution of life on Earth.

The three research components of our program are:

- Developing methods for life detection on Mars and in Mars analog environments
- Biosignatures: developing the tools for detection of ancient life and determining paleoenvironments
- Life detection in the ancient terrestrial rock record



Team Website: http://geoscience.wisc.edu/astrobiology

2017 Executive Summary

The research portfolio in the last year (Year 5 of CAN-6) included 26 projects that spanned the team's three research themes on life detection, biosignature development, and the ancient terrestrial rock record. This research effort involved 14 lead investigators from eight institutions, and major collaborations with seven other current and former NAI Teams, as well as astrobiologists in the U.S., Europe, Israel, Japan, China, New Zealand, Australia, and South Africa. The results of these efforts were published in 39 peer-reviewed publications in Year 5. 2017 concludes the team's NAI membership, which included CAN-4 and CAN-6. *In toto*, the team's decade of research and EPO activities involved 17 co-investigators at nine institutions, trained 29 post-doctoral fellows and 32 graduate students, and published 223 papers in the peer-reviewed literature.

Our efforts on Mars and Mars-analog environments produced new discoveries on the longevity of Mars geologic activity; explored the inter-relations of electron transport, isotopic exchange, and energy utilization in microbial Fe cycling in both acidic and neutral-pH environments; and studied the preservation of biomarkers in extreme conditions, in preparation for new and on-going missions. The major focus on microbial Fe cycling provides fundamental insights into modern terrestrial systems, as well as providing the context for understanding new discoveries on Mars.

A major focus has been on development of new biosignatures, as well as the new analytical approaches needed to detect them. This included experimental studies on new stable isotope systems, including K and Si isotopes, which provide insight into habitability and microbial element cycling; understanding the formation conditions for carbonates, including temperature and fluid composition; and the isotopic signals recorded by coupled Fe-S microbial processes. Biosignature analysis in ancient and complex terrestrial rocks, as well as samples returned from Mars, requires an *in situ* approach, with focus on features at the micron scale, and major advances were made in capillary absorption spectrometry, Raman spectroscopy, confocal laser scanning microscopy, secondary ion mass spectrometry, and femto second laser ablation.

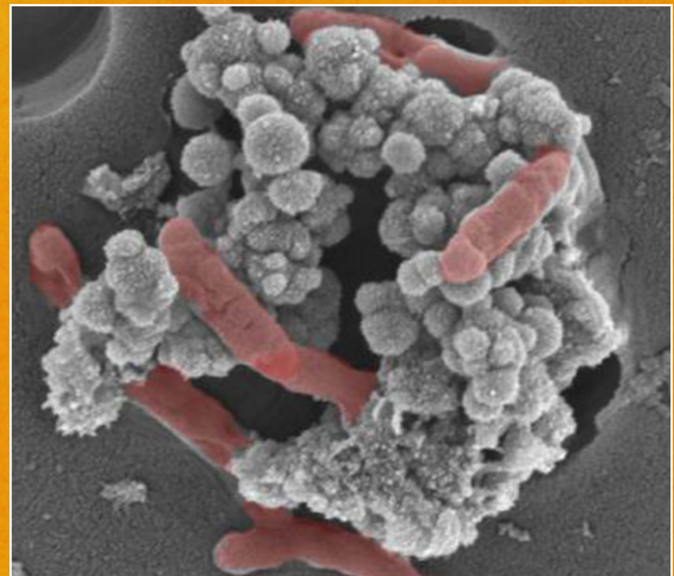


Fig. 1. Cryo-SEM image demonstrating the intimate cell-mineral association of an aerobic pyrite-oxidizing enrichment culture after ca. 45 days of growth. Cells are shown in false orange color. Micron-size pyrite particles are aggregates of numerous small framboidal crystallites. Image featured on the cover of Geobiology, September 2017 issue (Percak-Dennett et al., 2017). Image credit: Deborah Powell, University of Delaware Bioimaging Center.

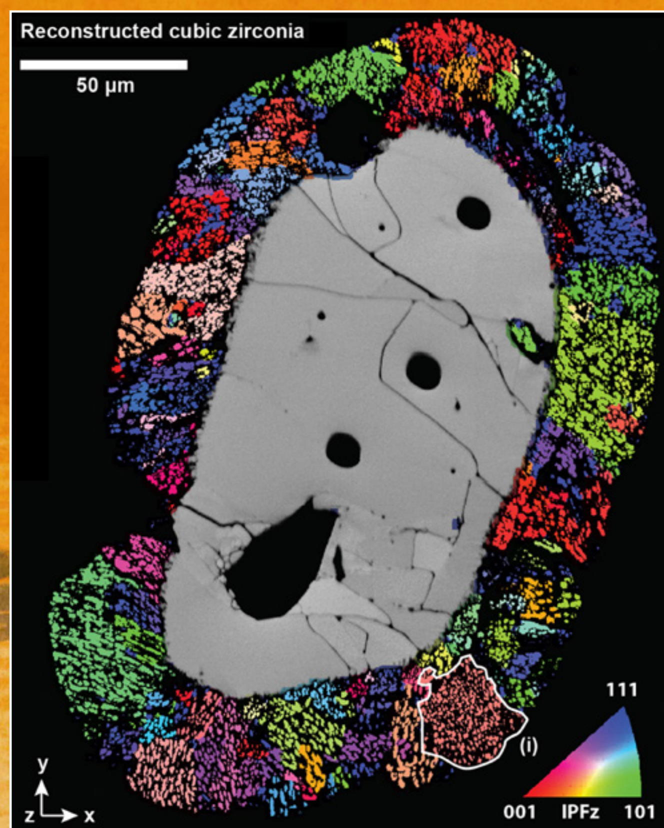


Fig. 2. Electron backscatter diffraction of zircon is shown in grayscale based on EBSD pattern quality (band contrast), with colored map showing crystallographic orientations of reconstructed parent cubic zirconia grains. Inverse pole figure (IPF) orientation color scheme. Documentation of cubic zirconia overgrowths document an impact melt >2370 oC. See Timms et al. (2017). Image credit: Aaron Cavosie.

Background Image: Artist conception of the Earth at ~4Ga, where continental crust is minor and the atmosphere is an organic haze. Credit: Hannah Bonner.

The team's efforts on the ancient terrestrial rock record studied habitability issues and searched for direct evidence of ancient life on Earth via microfossils. Work addressed the temperatures of the ancient oceans directly through studies of the isotopic compositions of Archean cherts, and indirectly through analysis of reconstructed enzyme stabilities. The delivery of nutrients from the continental crust, required for an early biosphere, was studied using Archean marine carbonates. The effects of planetary impacts on the early Earth was investigated using detrital zircons, including a reconstruction of the pressure and temperature effects of large impacts. Finally, early Archean microfossils were studied to constrain the metabolic diversity of the early biosphere, which new work shows was remarkably complex.

Education and Public Outreach (EPO) activities included those aimed at the general public, as well as at the university level. Two astrobiology summer camps were led for kids 6-11 years old, where participants thought about definitions of life, built their own rovers and designed Mars bases. Two other EPO projects were in full swing for Year 5, working with very different audiences. "Astrobiology Afterschool" leverages a network of Madison community centers and schools (38 in total) that provide after school and evening programming for audiences under-represented in the sciences. Integration of astrobiology into this learning network was a primary goal, which exposed many children and their families to concepts and careers previously unknown to them. "Holding Space" is a hands-on astrobiology program led in assisted-living facilities for seniors. The goal is that this programming will be a gateway to cultivate relationships with residents that blossom into recording their memories, when they were young, of NASA's space program. Finally, university-based instruction in astrobiology involved approximately 300 students, including freshman non-science majors, undergraduate science majors, and graduate students.

Fig. 3. EPO lead Brooke Norsted tending the WInogradsky Garden - every few months Geology Museum personnel top off the water on the 3-foot high Winogradsky column that is a highlight of the NAI-supported astrobiology exhibit. Credit: Geology Museum.

Background Image: Artist conception of the Earth at ~3Ga, where, by this time, extensive continental crust exists and traces of oxygenic photosynthesis is starting to influence the atmosphere. Credit: Hannah Bonner.

Project Reports

Developing Methods for Life Detection on Mars and in Mars Analog Environments

Our work on Mars and Mars analog environments ranges from studies of Martian meteorites to terrestrial hydrothermal systems, and include connections to space missions. To better evaluate early Martian environmental conditions, Brian Beard and colleagues have used geochronological methods to determine Martian meteorite ages. They discovered that ejection age measurements for all the depleted shergottites are the same, implying that they represent magmas erupted from the same area for two billion years; these results indicate surprisingly long-lived, plume-driven magmatic activity on Mars.

Turning to Mars analog environments, Eric Boyd and Eric Roden studied energy utilization of microorganisms in Dragon Spring, Yellowstone (Fig. 4), in an attempt to determine how organisms "decide" on which substrates to use for maximum energy yield. Through the application of physiological studies and thermodynamic calculations, they showed that the energetic costs of assembling the biochemical machinery required to process growth substrates factors into the preference of substrate usage by microorganisms. These results help to explain why microorganisms do not always utilize growth substrates that maximize energy yield.

Eric Roden led three interrelated lines of research aimed at understanding Fe-based chemolithotrophic microbial metabolism. First, the role of extracellular electron transfer (EET) in neutral-pH Fe(II)-oxidizing bacterial (FeOB) metabolism was investigated using the genomes of 73 neutral-pH FeOB, and these results greatly extended our understanding of bacterial EET and provide candidate genes for future research. Second, the microbiological and genomic characteristics of a subsurface Fe(II)-silicate weathering front were studied as a terrestrial analog for potential Fe-based chemolithotrophic microbial communities on

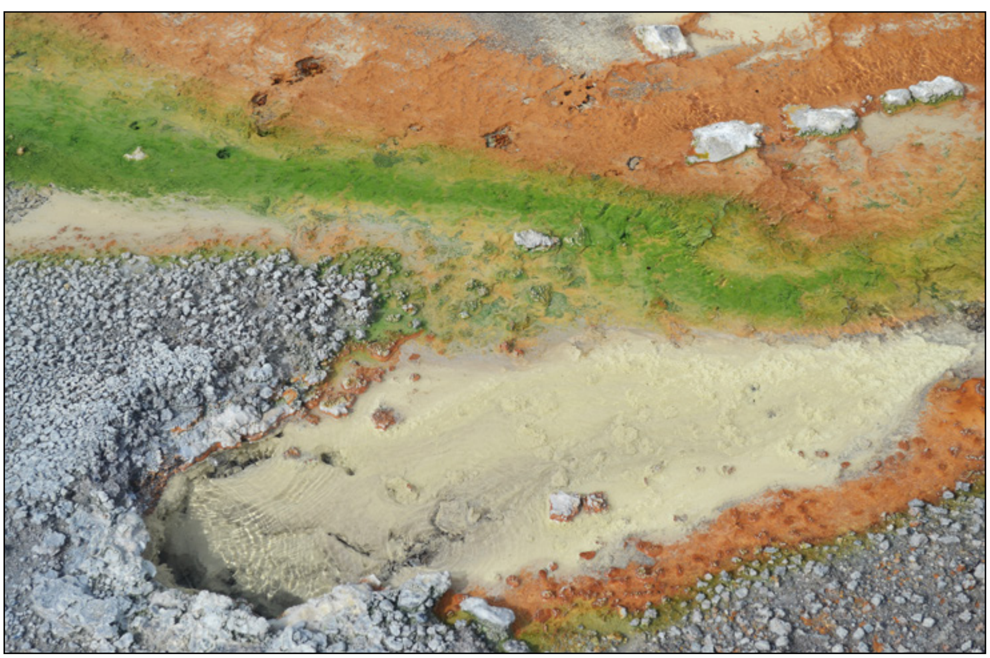
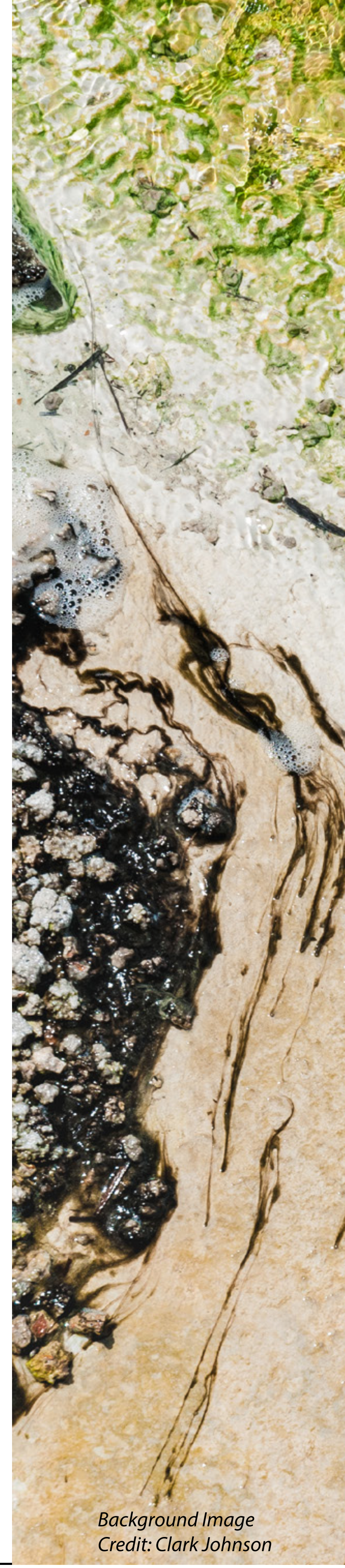


Fig. 4. Although microbes are expected to prefer substrates that have the highest energy yield, the study of Amenbar et al. (2017), featured on the cover of Nature Geoscience *(August 2017 issue) shows that a metabolically flexible archaeon exhibits preference for lower energy substrates. Photo shows overlapping gradients in mineral substrates capable of supporting microbial metabolism in Dragon Spring, Yellowstone National Park. Image credit: Eric Boyd.*



Background Image Credit: Clark Johnson

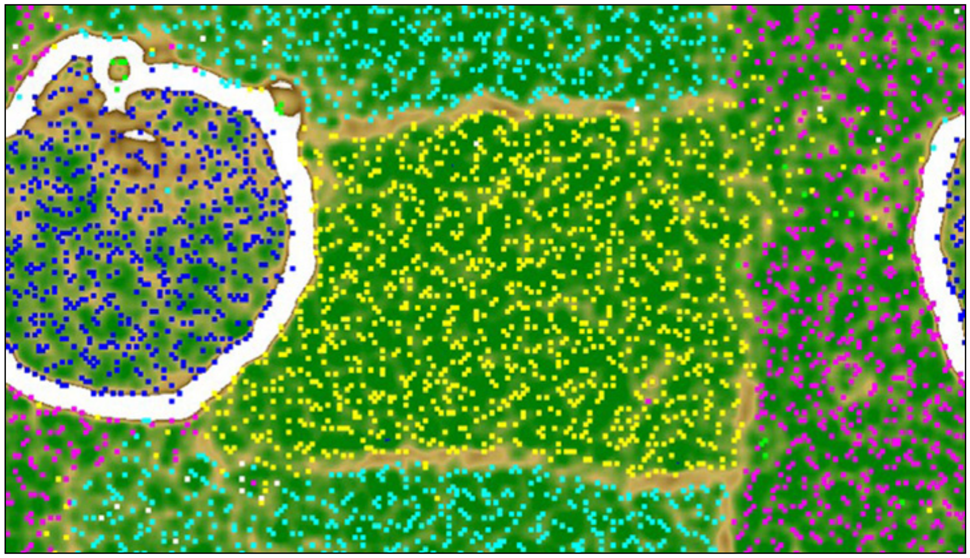


Fig. 5. Microbial oxidation of pyrite, under neutral-pH and aerobic conditions, is a potentially significant source of chemical energy in both terrestrial and Martian rocks. Metagenomic analysis documents the presence of Fe and S oxidation pathways. Image shows an Emergent Self-Organizing Map (ESOM) of genomic sequence fragments from the aerobic microbial pyrite-oxidizing culture. See Percak-Dennett et al. (2017). Image credit: Eric Roden.

Mars or other rocky planets. Metagenomic sequencing revealed the presence of homologs to several of the EET systems described in the first project. Third, microbial mediation of neutral-pH aerobic oxidation of pyrite was studied in the laboratory, a significant source of chemical energy in both terrestrial and Martian rocks. Metagenomic analysis (Fig. 5) indicated the presence of Fe and S oxidation pathways in several dominant organisms in the cultures. These results demonstrate the ability of aerobic microbial activity to accelerate pyrite oxidation (see also Fig. 1 in Executive Summary).

Eric Boyd, Clark Johnson, and Brian Beard took a new look at dissimilatory iron reduction (DIR), which, at neutral pH, suggests that Fe isotope fractionations are driven by electron exchange between $Fe(II)_{aq}$ and the reactive component of the iron oxide at the mineral surface. In the new work, to test if DIR under acidic conditions produces Fe isotope fractionation, they studied cultures of the thermoacidophile *Acidianus* strain DS80 grown with two solid-phase iron minerals as electron acceptors, H_2 as the electron donor, and CO_2 as the carbon source. Surprisingly, the results show that, despite the low pH, which should preclude sorbed Fe(II), extensive Fe isotope exchange occurred. This work has the potential to revolutionize the use of Fe isotopes as a biosignature in acidic systems.

Richard Quinn continued work on the recovery of mineral-associated biomolecules to inform correlated studies on the stability and detection of biomarkers in space environments. Using Fe seeps in Yellowstone National Park as an analog for ancient Mars, the preservation of lipid biomarkers has been measured using gas chromatograph-mass spectroscopy (GC-MS). These results have been characterized in the context of anticipated future mission data using a European Space Agency (ESA) ExoMars Mission Raman flight prototype (Centro de Astrobiología). The results are also being used to inform ongoing selection and preparation of samples for real-time, *in situ* study of the effects of the space environment in astrobiologically relevant materials using an external platform on the International Space Station. These space environment studies are being performed in collaboration with the Organic Exposure in Orbit (OREOcube) and ExoCube missons which have advanced to phase A/B under ESA funding.

Biosignatures: Developing the Tools for Detection of Ancient Life and Determining Paleoenvironments

Development of new biosignatures, as well as new analytical methods, is key to advancing our understanding of how life evolved on Earth or other planets, as well as to prepare for future sample return missions. The stable K isotope system is being developed by Brian Beard's group as a proxy for weathering, which bears on planetary habitability. New experiments and theoretical calculations constrain stable K isotope fractionation factors, which is a critical aspect for developing this new weathering proxy. Clark Johnson led two efforts aimed at isotopic proxies, the first being completion of a large experimental effort on stable Si isotopes; these results now explain the contrast in Si isotope compositions of cherts in iron formations relative to common Fe-free cherts, and highlights that unusual Si isotope ratios likely record biological cycling of both Fe and Si. In a second project, "clumped" (^{13}C-^{18}O) isotope fractionation was used to understand the thermal history of the Neoarchean Campbellrand carbonate platform (South Africa), the results of which match inferences obtained using organic geochemistry by the MIT NAI team.

Several fundamental studies of carbonates, oxides, and fluids were pursued by the team. Max Coleman investigated paleomicrobial biosignatures in diagenetic carbonates using trace sulfate S and O isotopes in siderite nodules that formed by both sulfate-reducing (SR) and iron-reducing bacteria (FeR). Both unexpectedly showed little sulfate processing, suggesting recycling. When FeR and SR were cultured together, they worked symbiotically, where FeR activity was stimulated by SR, a finding not predicted by thermodynamics. Huifang Xu studied the effects of solution chemistry and biopolymers on Ca-Mg-carbonate mineral textures and compositions in sedimentary environments. Chris Romanek completed experiments to determine the physicochemical controls on the incorporation of magnesium in calcite. The results suggest that aqueous Mg/Ca and temperature primarily control the Mg-content of calcite, while precipitation rate, PCO_2 and solution ionic strength exert only minor influences, a discovery that permits the use of Mg contents in calcite as a proxy of temperature and fluid chemistry. Huifang Xu led two projects on advancing fundamental understanding of aqueous and mineral properties. The first was aimed at determining natural indices for the chemical "hardness/softness" of metal cations and ligands in aqueous systems. The second project focused on a new mineral, ε-Fe_2O_3 (luogufengite), and its magnetic coercivity, which potentially bears on magnetism on Mars (Fig. 6).

A major effort was made across the team at developing new analytical methods to be used in future biosignature research. Max Coleman continued development of the new ultrasensitive analytical technique for stable isotope analysis, Capillary Absorption Spectrometry, and his team was been able to get a very good spectrum from samples <1 picomole, leading to the next step of validating isotopic compositions. Bill Schopf continued to push the limits of Raman spectroscopy and confocal laser scanning microscopy, where his group established the composition, preservation mode, and biogenicity of 760 Ma fossil protozoans in sediments from Brazil; because both techniques are non-intrusive and non-destructive, they are ideal for analyses of fossil-like objects in Mars rocks. John Valley's group completed a five-year project to create standards and calibrate *in situ* Secondary

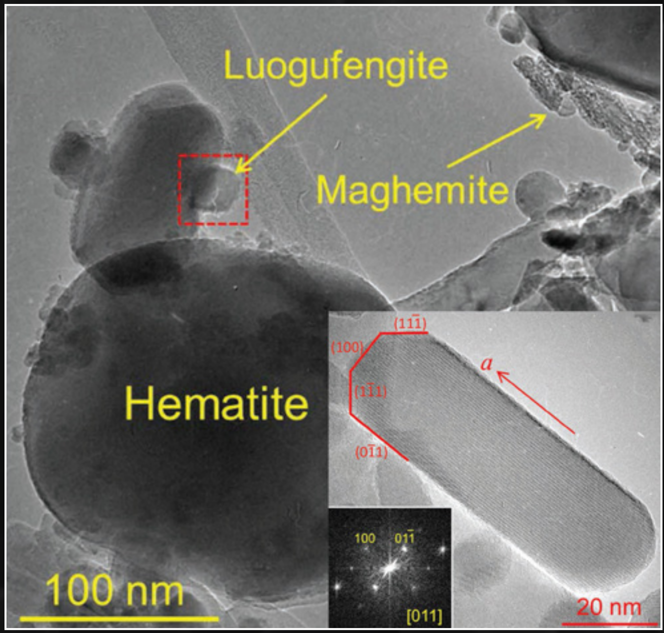


Fig. 6. Luogufengite is a newly discovered nano-mineral that has high coercivity and strong remanent magnetization that may explain magnetic features observed on Mars. Bright-field TEM image shows that hematite crystals are generally larger than luogufengite, whereas maghemite crystals are generally smaller than luogufengite. Inset shows HRTEM image of the rod-shape of luogufengite elongated along the a-axis. See Xu et al. (2017). Image credit: Huifang Xu.

Background Image Credit: Clark Johnson

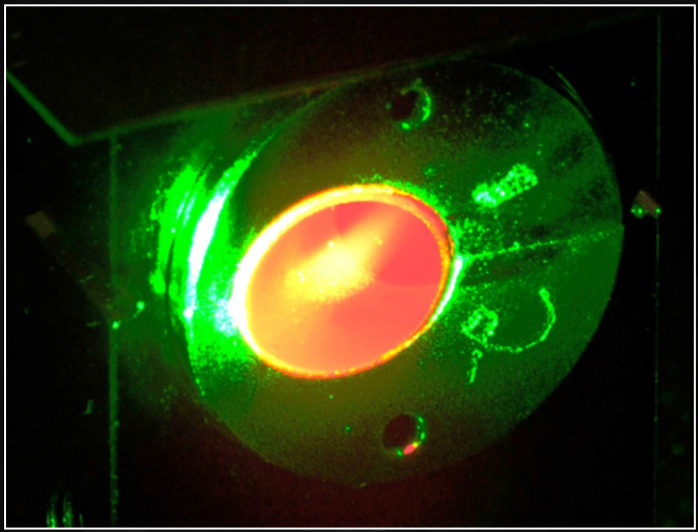


Fig. 7. Ultra-fast (femtosecond, or 10^{-15} s) laser ablation is a new in situ analysis method that is well suited to the very small samples that will be returned from Mars. Here, a red Ti sapphire crystal is pumped with a green 532nm Nd:Yag laser to produce the 800nm reddish femtosecond laser pulse. The intense bright areas in the center is where the pump and femtosecond seed laser are focused onto a Ti sapphire crystal to amplify the pulse energy of the femtosecond laser. Image credit: Brian Beard.

Ion Mass Spectrometry (SIMS) analysis of Ca-Mg-Fe-Mn carbonates. This SIMS approach will provide the most accurate high-resolution C and O isotope data for interrogating signs of life in samples returned to Earth by Mars 2020 and subsequent missions. In addition, a new analytical technique was developed that permits simultaneous *in situ* SIMS analysis of C and N isotopes in organic matter. Finally, Brian Beard led an effort at pushing the state-of-the-art of femto second laser ablation (fs-LA) for *in situ* isotopic analysis, an approach that complements SIMS methods, depending upon sample specifics. This research has centered on how fs-LA (Fig. 7) is different from "conventional" lasers, which use longer (nanosecond) pulse widths, and how fs-LA can be used for isotopic analysis without matrix matching.

Life Detection in the Ancient Terrestrial Rock Record

The Earth remains the only known example of life's origin and evolution, and study of the early Earth provides an interpretive context for the search for life on other planets. A key component to such efforts is better understanding the habitability of the early Earth. The temperature of the Archean oceans, for example, continues to be debated. John Valley led an effort in using O isotopes in cherts to infer ocean temperatures, which included a detailed petrographic and *in situ* (SIMS) O isotope study of stromatolite-bearing units of the Strelley Pool Chert (SPC), Australia. They showed that bulk measurements of $\delta^{18}O$ for chert are complex mixtures representing multiple events and are not a faithful record of ancient seawater. There are multiple generations of quartz in the SPC, and the youngest quartz is higher in $\delta^{18}O$ than previously published secular trends of $\delta^{18}O$ vs. age for cherts that have been misinterpreted to record temperature evolution of the oceans. The continued ambiguity in the temperature of the Archean oceans is important to resolve - Bill Schopf continued his work on analysis of modern photosynthetic prokaryotes and eukaryotes and their thermostabilities, which suggest that temperatures of Earth's surface environment decreased from approximately 75°C in the Archean to approximately 35°C in the Devonian. Another component related to habitability was led by Clark Johnson on the evolution of the continental crust, which has become increasingly recognized as the primary source in the early Earth for nutrient delivery to the oceans. This effort used Sr isotopes in Archean marine carbonates, and identified a continual increase in seawater $^{87}Sr/^{86}Sr$ ratios from ~3.2 to ~2.8 Ga that must reflect an increasing extent of evolved (granitic) continental crust exposed to weathering (Fig. 8). This in turn indicates a very early origin of plate tectonics (>3.2 Ga), and raises the possibility that the presence of emergent continental crust, and its delivery of nutrients (especially P), was the key variable in permitting a rapidly evolving and expanding early Archean biosphere.

One of the challenges to an early Earth biosphere is the potentially sterilizing effect of bolide impacts. Aaron Cavosie's group used micro- to nano-scale methods to investigate shock effects in accessory minerals (zircon, monazite, others), and high-pressure phases (reidite). Studies of shocked minerals from South Africa and elsewhere provide texturally-constrained U-Pb data that accurately date impacts. The results bode well for searching for evidence of early Earth impacts in

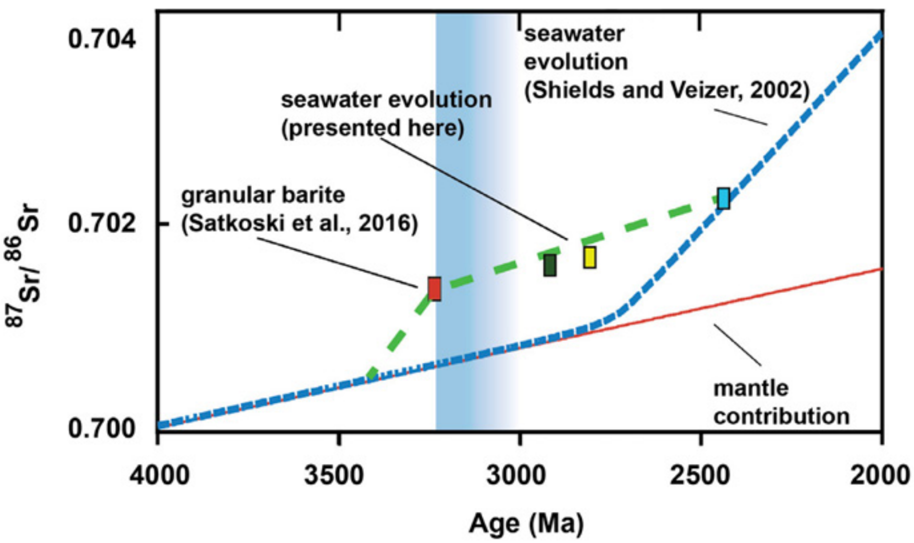


Fig. 8. Erosion of the continents provides a first-order constraint on nutrient delivery (e.g., P), which in turn controls the size of the biosphere. New work on the Sr isotope compositions of the Archean oceans shows a much larger continent delivery than previously thought. Here, age versus $^{87}Sr/^{86}Sr$ ratio is shown, where higher $^{87}Sr/^{86}Sr$ indicates enhanced delivery of continental nutrients, shown by the dashed green curve, relative to previous estimates (dashed blue curve). The green (Red Lake) and yellow (Steep Rock) colored data are the best estimates of the seawater $^{87}Sr/^{86}Sr$ ratios at 2.94 and 2.80 Ga. The teal colored datum is from the Campbellrand carbonate at the end of the Archean. The first enhanced continental input occurs at 3.2 Ga (red box). See Satkoski et al. (2017). Image credit: Clark Johnson.

populations of detrital grains in Archean siliciclastic sediments. Cavosie created the first pressure-temperature diagrams that relate zircon, silica, zirconia, and all known polymorphs under the extreme conditions of a planetary impact. The P-T diagrams provide new insights into conditions experienced by crustal rocks during meteorite impacts, and also insights into the fate of zircon under mantle conditions. This effort led to the discovery of the hottest crust yet documented on Earth (at >2370 °C), as identified by dissociation of zircon to cubic zirconia in the impact melt from the Mistastin impact structure in Canada (see also Fig. 2 in Executive Summary).

A combined effort by Schopf's and Valley's teams on Archean microfossils and *in situ* C isotopes targeted the famous 3.4-3.5 Ga microfossils from Australia. Studies of the Strelley Pool Chert documented microfossils that represent a shallow water microbial consortium composed of anaerobic H_2S-producing sulfuretums and H_2S-consuming phototrophs, which constrains speculations about the time of origin of O_2-producing photosynthesis. These results, which suggest an anoxic phototrophic biosphere in the early Archean, agree well with earlier work by Johnson and Beard using U-Th-Pb and Fe isotopes on similar-age units. In a second study, the biogenicity of microfossils in ~3,465 Ma Apex Chert was confirmed by *in situ* SIMS analysis of $\delta^{13}C$ values of numerous individual fossils, the oldest diverse assemblage of cellular microfossils now known in the geological record. The morphologies and range in isotopic compositions indicate the presence of photosynthetic bacteria, methanogens, and methanotrophs, all anaerobic members of near-basal lineages of the phylogenic tree of life (Fig. 9).

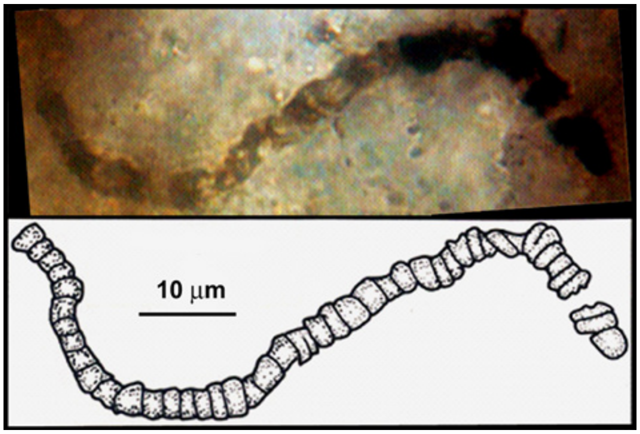


Fig. 9. A key component to understanding the earliest life on Earth is documentation of the oldest microfossils, as well as determining what metabolic processes were used by ancient life. This image shows a cellular filamentous microbe (Primaevifilum amoenum) from the ~3,465 Ma Apex Chert of Western Australia interpreted, on the basis of SIMS $\delta^{13}C$ isotope measurements, to be a methane-consuming γ-Proteobacterium. See Schopf et al. (2017). Image credit: William Schopf.

Field Work

Habitability, Life Detection, and the Signatures of Life on the Terrestrial Planets

Field work in 2017 was led by Co-I's Max Coleman, Richard Quinn, and Eric Roden that touched on a variety of projects. As part of Coleman's work on the O and S isotope compositions of carbonate-associated sulfate, which holds promise for understanding the origin of seawater sulfate back to the Paleoproterozoic Great Oxidation event, Coleman is "ground-truthing" such work on much younger rock sequences where independent data exist for seawater compositions. This includes work on the Miocene Monterey Formation (Fig. 10). Richard Quinn's field work focused on modern iron springs in Yellowstone National Park, where his group is interested in lipid compositions of flocculent biofilms containing chemolithoautotrophs such as *Leptothrix* and *Gallionella* at Chocolate Pots Hot Springs in Yellowstone National Park (Fig. 11). This field-based work explores the nature of organic degradation processes in Fe(II)-rich groundwater springs— environmental conditions that have been identified as highly relevant for Mars exploration. Understanding the potential of sedimentary environments to capture and preserve fossil biosignatures is of vital importance in the selection of the best landing sites for future astrobiological missions to Mars. Finally, Eric Roden led field studies aimed at integrating field and laboratory results on the microbial role in pyrite oxidation. A field project at the Shale Hills Critical Zone Observatory in Pennsylvania (Fig. 12) is examining the role of chemolithotrophic FeOB in a terrestrial subsurface pyrite weathering system. Material from the colonized minerals is being used to establish enrichment cultures in the laboratory with specimen pyrite serving as the sole energy source. Preliminary studies have demonstrated the presence of such organisms in Shale Hills groundwater.

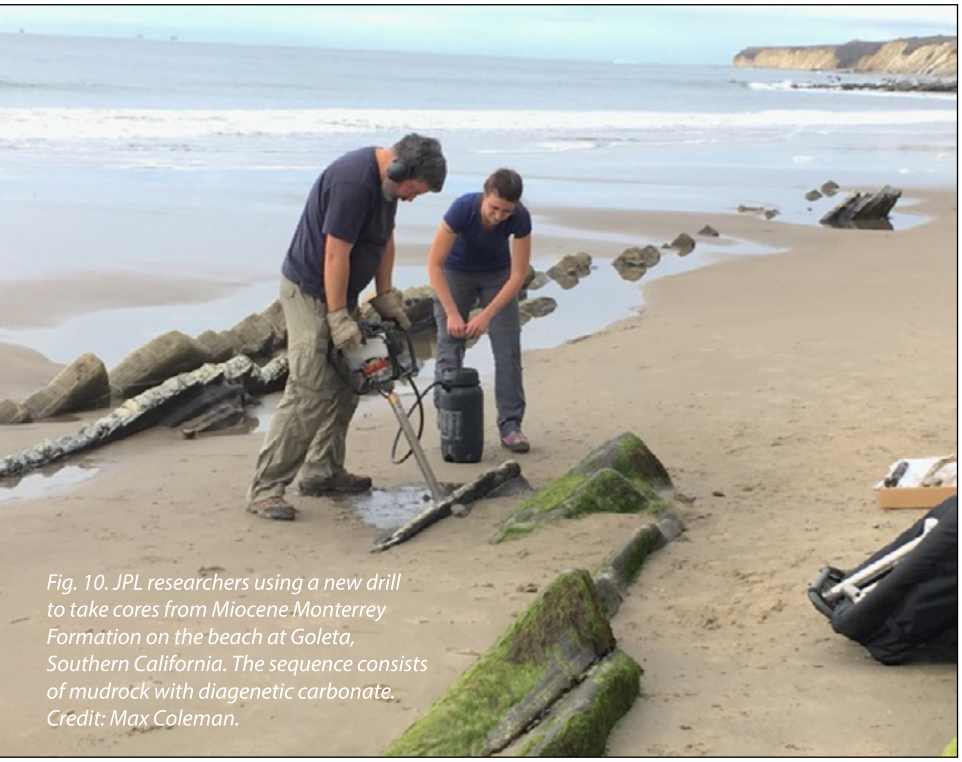


Fig. 10. JPL researchers using a new drill to take cores from Miocene Monterrey Formation on the beach at Goleta, Southern California. The sequence consists of mudrock with diagenetic carbonate. Credit: Max Coleman.

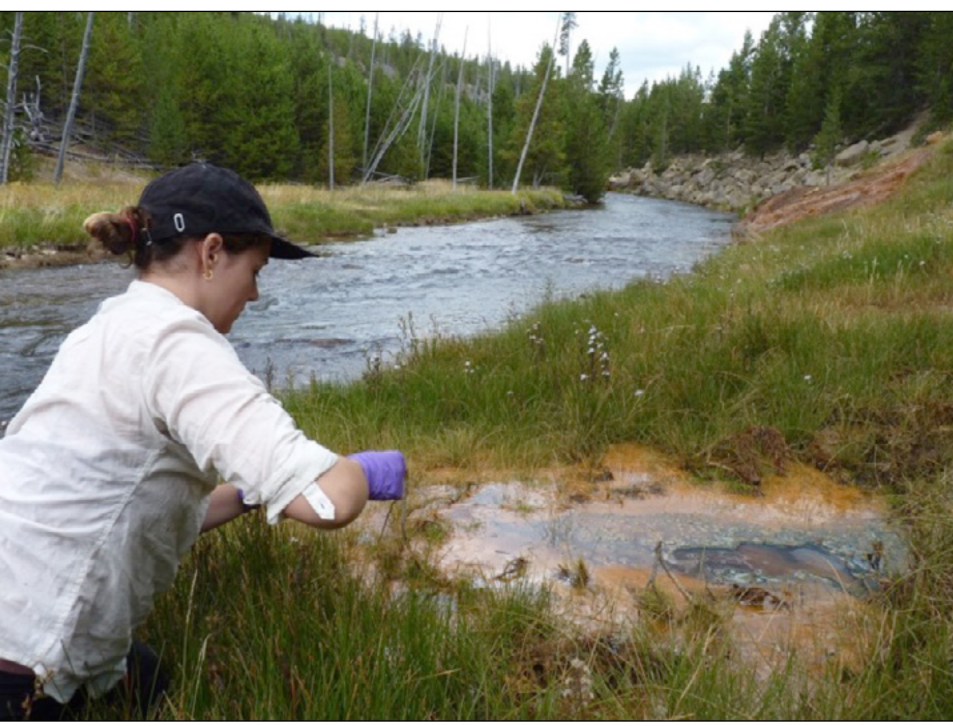
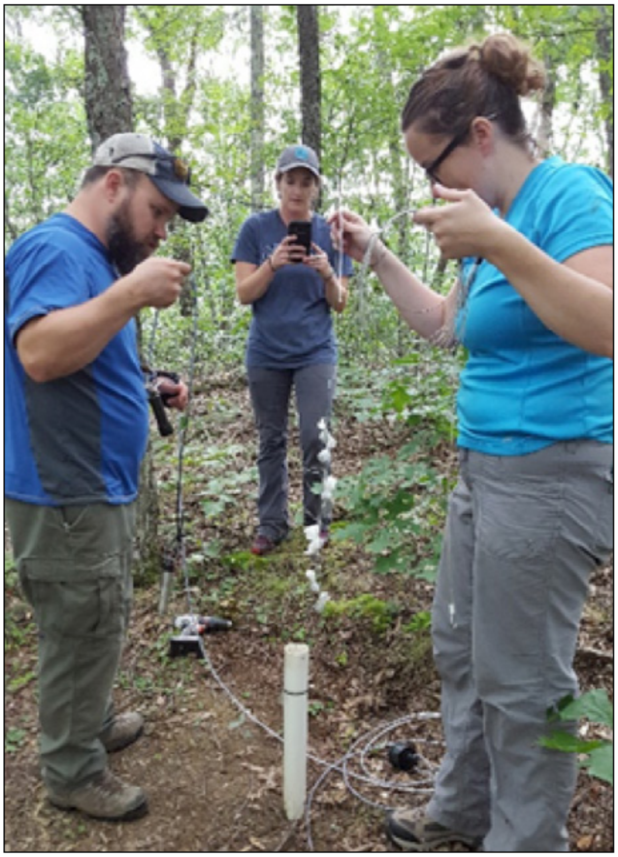


Fig. 11. (Left) NAI researcher Taylor Kelly (SETI Institute) collecting samples at the Chocolate Pots Hot Springs in Yellowstone National Park. Field research conducted under Yellow Research Permit YELL-2017-SCI-1549. Credit: Richard Quinn.



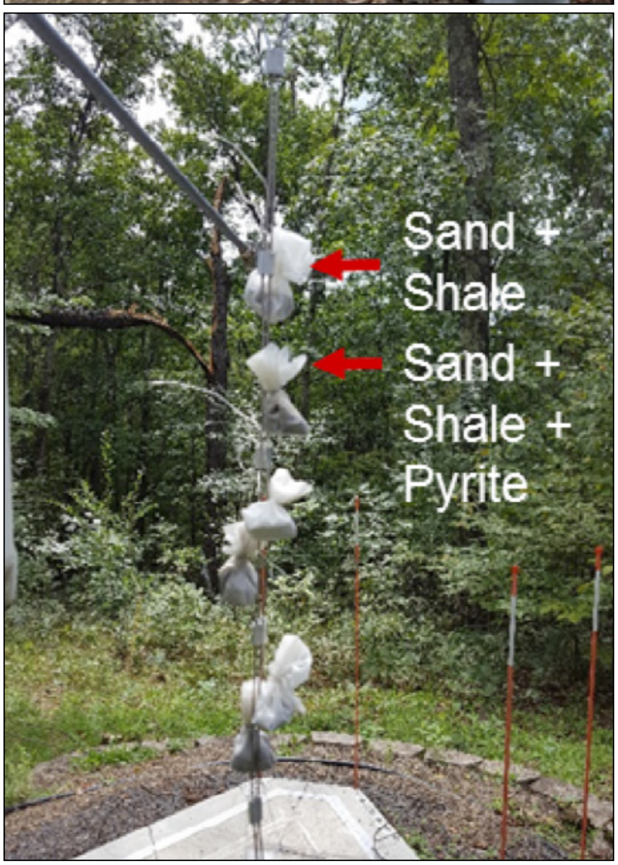


Fig. 12. University of Wisconsin students deploying mineral "bug traps" in groundwater wells at the Shale Hills Critical Zone Observatory in Pennsylvania, in search of chemolithotrophic pyrite-oxidizing microorganims. Credit: Eric Roden.

Team Members

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Georgio Casaburi
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Jennifer Mobberley
Stephanie Montalvo
Luana Morais
Des Moser
Stephanie Napieralski
Alexander Nemchin
Mason Neuman
Noah Nhleko
Brooke Norsted
Shuhei Ono
Ian Orland
Jeffrey Osterhout
Niki Parenteau
Mark Pearce
Alberto Perez-Huerta
John Peters
Pascal Philippot
Noah Planavsky
Ty Prosa
Richard Quinn
Steve Reddy
Mark Reed
Pam Reid
David Reinhard
Antonio Ricco
William Rickard
Minako Righter
Eric Roden
Alejandro Rodriguez Navarro
Christopher Romanek
Guilherme Romero
Isaac Rudnitzki
Farid Salama
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Juergen Schieber
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Habitability, Life Detection, and the Signatures of Life on the Terrestrial Planets: 2017 Publications

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