



# 2019 MIDWEST GEOBIOLOGY

## Washington University in St. Louis

8<sup>th</sup> Annual

Midwest Geobiology Symposium

20–21 September 2019

~ at ~

Washington University in St. Louis

Department of Earth and Planetary Sciences



AGOURON  
INSTITUTE



*Funding from Washington University in St. Louis College of Arts & Sciences provided jointly by the Department of Earth and Planetary Sciences and the McDonnell Center for Space Sciences.*



# Welcome to MWGB 2019!

We are excited to host the eighth annual iteration of the Midwest Geobiology Symposium and for the meeting's return to Washington University in St. Louis for the first time since its inception in 2012. This year, we have about 70 participants representing more than 16 institutions from the Midwest and beyond, with 30 presenters delivering talks and sharing posters.

Each year, the Midwest Geobiology Symposium brings together students and early career scientists in geobiology and affiliated fields throughout the Midwest to share and discuss research, connect and network with each other, and delight in shared interests, insights, and experiences. We look forward to seeing those of you who have been to past meetings and welcome all of the newcomers. We hope this won't be your last MWGB!

We would like to thank the Agouon Institute, the McDonnell Center for Space Sciences, and the Departmental of Earth and Planetary Sciences at WashU for supporting the meeting.

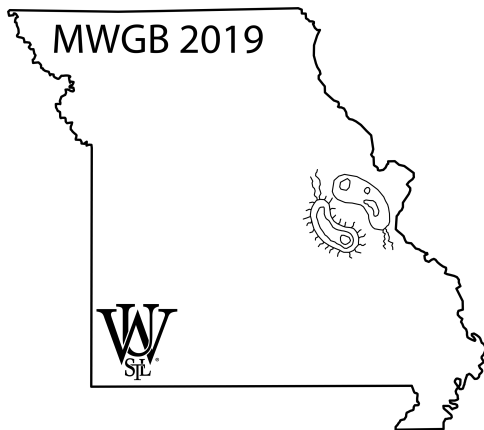
Sincerely,  
The MWGB 2019 Organizing Committee

Alex S. Bradley  
David A. Fike  
Jake Ogata Bernstein  
Robert Kupper  
Nadia Sae-lim  
R. Seth Wood

# Logo Design Contest

We solicited designs from members of the department and loved all of them! To honor the work each contributor put into their design, we decided to include the design submissions here.

Nadia Sae-lim and Greg Ledingham, two second-year students, designed the logo that we chose to represent MWGB 2019, which appears on the front cover and on the meeting website. Congratulations, Nadia and Greg!



# Table of Contents

Orientation information.....	1
Schedule of events.....	2
Speakers.....	3
Posters.....	5
Abstracts ( <i>alphabetical by presenting author</i> ).....	8
Conference attendees.....	28
Author index.....	32
Space for notes.....	36

# Orientation information

The weekend will begin with an icebreaker reception at Urban Chestnut, a local brewery/restaurant in The Grove. Come join us Friday 20 September at 6:00pm at 4465 Manchester Avenue, St. Louis, MO 63110 for food, drink, and general merriment!

On Saturday, we will be in Whitaker Hall on the East end of the WashU campus. The meeting will be split between the Whitaker Atrium for meals, coffee breaks, and an afternoon poster session and Whitaker 100 for general remarks and oral presentations.

See the map below for the location of Whitaker Hall and a parking garage (free on weekends). The East end of campus is also a short (~5-min.) walk from the Skinker MetroLink stop and several bus lines.



# Schedule of Events

## FRIDAY (9/20)

6:00 – 9:00      Icebreaker reception  
*at Urban Chestnut Grove Brewery and Biergarten*  
4465 Manchester Avenue, St. Louis, MO 63110

## SATURDAY (9/21)

8:00 – 9:15      Breakfast & presentation set-up

9:20 – 9:30      Opening remarks

9:30 – 10:45      Oral session 1

10:45 – 11:00      Morning coffee break

11:00 – 12:15      Oral session 2

12:15 – 1:30      Lunch

1:45 – 2:35      Oral session 3

2:35 – 2:50      Afternoon coffee break

2:50 – 3:40      Oral session 3, cont.

3:45 – 3:55      Closing remarks

4:00 – 6:00      Poster session  
*with snacks and drinks*

# Speakers

## Oral Session 1

- 9:30 – 9:50 **Anna Bennett**\* Senthil Murugapiran, Jeff Havig, and Trinity Hamilton  
“Phototrophic community distribution and morphology along a geothermal stream: Insights into both modern and ancient microbial ecology”  
*University of Minnesota*
- 9:55 – 10:15 **Jared Zavorski**\* and Ziming Yang  
“Mercury biogeochemical processes in Lake Michigan sand dune ecosystem”  
*Oakland University*
- 10:20 – 10:40 **Jarunetr (Nadia) Sae-lim**\* Bronwen Konecky, Neil Michelutti, Chris Grooms, Jack Hutchings, Isla S. Castañeda, Jeff Salacup, and John Smol  
“Investigation of climate, terrestrial ecosystems, and aquatic productivity over the last millennium from the Lake Sibinacocha Watershed, Peruvian Andes”  
*Washington University in St. Louis*

~ Morning Coffee Break ~

## Oral Session 2

- 11:00 – 11:20 **Leah Trutschel**\* Buz Barstow, and Annette Rowe  
“Investigating extracellular electron uptake in the model mineral-reducing microbe *Shewanella oneidensis* MR-1”  
*University of Cincinnati*
- 11:25 – 11:45 **R. Seth Wood**\* Aivo Lepland, Jennifer Houghton, Clive Jones, Marta Torres, and David A. Fike  
“Geochemical evolution of methane seeps off the Lofoten-Vesterålen continental margin, Norway”  
*Washington University in St. Louis*
- 11:50 – 12:10 **Matthew J. Selensky**\* Magdalena R. Osburn, and Jennifer G. Blank  
“Organic and isotopic biosignatures of lava tubes”  
*Northwestern University*

~ Lunch ~



### Oral Session 3

- 1:45 – 2:05 **Petra Vaiglova**\*  
“Of cattle and feasts: Using multiple isotopes to understand Neolithic management of animals at Makriyalos, northern Greece”  
*Washington University in St. Louis*
- 2:10 – 2:30 **M. Joseph Pasterski**\* Raveendra Wickramasinghe, Luke Hanley, and Fabien Kenig  
“Femtosecond-Laser Desorption Laser Postionization-Mass Spectrometry of organic-rich and organic-lean geologic samples”  
*University of Illinois at Chicago*

~ Afternoon Coffee Break ~

### Oral Session 3, cont.

- 2:50 – 3:10 **Mingfei Chen**\* Jessica L. Conroy, Robert A. Sanford, Joanne C. Chee-Sanford, and Lynn M. Connor  
“Interpreting lacustrine bulk sediment  $\delta^{15}\text{N}$  values using metagenomics: A case study from a hypersaline lake system”  
*University of Illinois at Urbana-Champaign*
- 3:15 – 3:35 **Matthew S. Smart**\* Gabriel M. Filippelli, William Gilhooly, and Jessica H. Whiteside  
“Decoding Devonian mass extinctions: New evidence linking land plant expansion to marine anoxia”  
*Indiana University—Purdue University Indianapolis*

# Posters

**Azam Baharlouei**\* and Scott D. Hamilton-Brehm

“Enrichment of microbes from benthic and subsurface ecosystems for the conversion of waste biomass into value-added molecules”

*Southern Illinois University Carbondale*

**Roger N. Bryant**,\* Ryan Ogiore, Maya L. Gomes, Jill D. Pasteris, and David A. Fike

“Marcasite in mid-Cretaceous marine sediments reflects ocean acidification”

*The University of Chicago*

**Caitlin Casar**\* and Magdalena Osburn

“Big data in geobiology: Applications to DeMMO”

*Northwestern University*

**Kelsey E. Doiron**,\* Arndt Schimmelmann, Hường Nguyễn-Văn, Dương Nguyễn-Thùy, and Simon C. Brassell

“Sedimentary biomarkers in Lake Biển Hồ, Vietnam, record temporal changes in phytoplankton dynamics”

*Indiana University Bloomington*

**Fotios Fouskas**,\* William P. Gilhooly, III, Molly D. O’Beirne, Josef P. Werne, and Alice Bosco-Santos

“S-C-Fe dynamics for the competition of sulfurization pathways within the long-term history of Mahoney Lake, Canada”

*Indiana University—Purdue University Indianapolis*

R. Agustin Mors, **Fernando J. Gomez**,\* Cecilia Mlewski, and Ricardo A. Astini

“Spheroidal carbonate particles within travertine and tufa deposits in the Argentine Puna plateau: Exploring the physical, chemical, and biological controls on textures and microfabrics”

*Centro de Investigaciones en Ciencias de la Tierra*

**Jennifer Houghton**,\* David A. Fike, and Daniele Scarponi

“Pyrite  $\delta^{34}\text{S}$  records changes in environment of deposition across glacial-interglacial cycles in the Valle di Manche, Italy”

*Washington University in St. Louis*

**Chase S. Howard**\* and Gregory K. Druschel

“Short-term temporal variations in the physiochemical conditions of a eutrophic freshwater lake”

*Indiana University—Purdue University Indianapolis*

**Raisa Islam**,\* Elizabeth D. Swanner, Chad A. Wittkop, and Gabrielle Ledesma

“Microscale resolution on how sedimentary pyrite forms”

*Iowa State University*

**Joshua E. Kuether**,\* Trinity L. Hamilton, and Jeff R. Havig

“Hydrothermal hypoliths: A comparison of extremophilic rock-dwelling microorganisms with other hydrothermal photo- and chemotrophic communities across acidic and alkaline pH ranges”

*University of Minnesota*

**Robert J. Kupper**\* Nanqing Zhou, Clara Chan, Aaron Thompson, and Jeffrey G. Catalano  
“Abiotic Fe(II) oxidation behavior in trioctahedral smectites: Rates, extents, and products”  
*Washington University in St. Louis*

**Judy Malas**\* Sarah Khoury, Michael Tanzillo, Gracie A. Fischer, Ian D. Patete, Jean E. Bogner, and D’Arcy Meyer-Dombard  
“Trash or treasure? Biogeochemical cycling in landfill ecosystems”  
*University of Illinois at Chicago*

**Jocelyn A. Richardson**\* Samuel M. Webb, Ritimukta Sarangi, Britt Hedman, Keith O. Hodgson, David A. Fike, Sascha Roest-Ellis, and Nicholas J. Tosca  
“The SMB Resource at SSRL: Geological, biological, and environmental applications using X-ray spectroscopy and imaging”  
*SLAC National Accelerator Laboratory*

**Niloufar L. Sarvian**\* Matthew T. Hurtgen, Andrew D. Jacobson, Adam C. Maloof, and Magdalena R. Osburn  
“A stable strontium isotope record of pre-Sturtian carbonates spanning a large  $\delta^{13}\text{C}$  anomaly”  
*Northwestern University*

**Christopher J. Schuler**\* Peter K. Kang, Jill M. McDermott, Dean M. Peterson, Cody Sheik, William Dowd, WoongHee Lee, Cara M. Santelli, and Brandy M. Toner  
“Mineralogical influences on the habitability of a banded iron formation”  
*University of Minnesota*

**Derek A. Smith**\* Brian A. Lerch, Karen C. Abbott, and Sarah C. Bagby  
“A co-limited approach to the black queen”  
*Case Western Reserve University*

**Melanie K. Suess**\* Clive Jones, David A. Fike, and Alexander S. Bradley  
“Single-cell analysis of microbial metals content by SIMS”  
*Washington University in St. Louis*

**Linda Vu**\* and Amy Cheng Vollmer  
“*Pseudomonas aeruginosa* biofilm formation after cultivation in simulated microgravity”  
*University of Cincinnati*

**Jacob R. Waldbauer**\* Maureen L. Coleman, Amy E. Zimmerman, and Lichun Zhang  
“Proteomic tracking of microbial nitrogen sourcing and utilization”  
*The University of Chicago*

**Jinshu Yan**\* Neha Sharma, Elaine D. Flynn, Daniel E. Giammar, and Jeffrey G. Catalano  
“Controls on trace metal speciation and availability in wetland soils”  
*Washington University in St. Louis*



# **ABSTRACTS**

*alphabetical by presenting author*

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## Enrichment of microbes from benthic and subsurface ecosystems for the conversion of waste biomass into value-added molecules

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AZAM BAHARLOUEI<sup>1</sup> AND SCOTT D. HAMILTON-BREHM<sup>1</sup>

<sup>1</sup>Southern Illinois University Carbondale, Carbondale, IL, USA

There is an urgency to find sustainable sources of biofuels and value-added chemicals. Waste biomass such as plant-derived lignocellulose is an abundant renewable carbon resource. This research is specifically aimed at screening environmental microbes from benthic and subsurface ecosystems that can derive the energy from common sugars to reduce lignin, furfuryl alcohol, and furfural into high-value products or biofuels.

Environmental samples were obtained from three sites: 1) a subsurface water sample from Amargosa Valley, California, 2) a benthic sediment sample from Southern Illinois University Carbondale Campus Lake, and 3) a benthic sediment sample from Fly Geyser in Northern Nevada. These sites are chronically exposed to complex carbon structures, remain anaerobic, and contain a high diversity of microorganisms.

The organic products produced by stable cultures of anaerobic bacterial enrichments were screened using High Performance Liquid Chromatography (HPLC) and Nuclear Magnetic Resonance (NMR) Spectroscopy. Microbial communities identified by Next Generation sequencing targeting the V4 region of 16S rRNA gene resulted in a total of 2.8 million DNA sequences, representing 7,746 operational taxonomic units. The most abundant microorganisms identified in the enrichments were from the phyla Firmicutes and Proteobacteria. In lignin containing enrichments, the cultures were found to be dominated by the genera *Candidatus Caldatri bacterium*, *Clostridium*, and *Hypnocyclicus*. In furfural containing enrichments, the cultures were found to be dominated by *Clostridium* and *Aeromonas*. In presence of furfural, cultures were dominated by *Clostridium*, *Morella*, *Lachnoclostridium*, and *Helicobacterium*. Here we demonstrate successful screening of anaerobic bacteria not previously identified as having the potential to produce renewable value-added molecules.

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## Phototrophic community distribution and morphology along a geothermal stream: Insights into both modern and ancient microbial ecology

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ANNA BENNETT,<sup>1</sup> SENTHIL MURUGAPIRAN,<sup>1</sup> JEFF HAVIG,<sup>2</sup> AND TRINITY HAMILTON<sup>1</sup>

<sup>1</sup>Department of Plant and Microbial Biology, University of Minnesota, St. Paul, MN, USA

<sup>2</sup>Department of Earth Science, University of Minnesota, Minneapolis, MN, USA

Modern geothermal systems are often studied in conjunction with the rock record to determine ancient microbial ecology. Filaments, microbial mats and stromatolites have been recovered from rocks dating back to the Archean, but evidence for the origin of these structures is often contradictory. Phototrophic bacteria form filaments, mats and stromatolites in a variety of environments given the ubiquity of light. However, the relationship between phototrophic morphology and geochemistry in geothermal systems is poorly constrained.

Rabbit Creek (RC), a circum-neutral geothermal stream in the Midway Geyser Basin of Yellowstone National Park, provides a natural temperature gradient ranging from a near boiling source to ~45°C 250 meters downstream. Near the source, the creek is host to yellow and orange phototrophic mats at the upper temperature limit of photosynthesis (72°C). In contrast, green and orange filamentous phototrophs are more common downstream in the cooler water where they form long ropes. In addition to sulfide and temperature, other features of the stream change with distance from the source. For example, oxygen increases with distance from the source, pH increases as CO<sub>2</sub> outgasses, and the width and depth of the stream bed change leading to variable flow. Thus, RC is a suitable environment to glean the adaptive methods to changing environmental conditions and community shift distribution of ancient and modern bacterial phototrophs.

Based on reports of abundant Cyanobacteria in phototrophic mats in circum-neutral YNP hot springs, we expected RC mats and filaments to be comprised largely of Cyanobacteria. Further, we expected community composition to change over the geochemical gradients observed in RC along with the changes observed morphology. 16s rRNA data revealed a relatively consistent phototrophic community structure at the phylum level (3.8:1 ratio of Chloroflexi:Cyanobacteria) whereas alpha diversity increases with decreasing temperature. At higher taxonomic resolution, there is a 1:1 ratio of *Roseiflexaceae* (Chloroflexi) to *Leptococcaceae* (Cyanobacteria) across sites. This is indicative of a transcriptional shift in the community population, not necessarily a shift in community structure, suggesting that the microbial community is adapting morphologically.

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## Marcasite in mid-Cretaceous marine sediments reflects ocean acidification

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ROGER N. BRYANT,<sup>1,2</sup> RYAN OGLIORE,<sup>3</sup> MAYA L. GOMES,<sup>4</sup> JILL D. PASTERIS,<sup>1</sup> AND DAVID A. FIKE<sup>1</sup>

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Marcasite, the rarer FeS<sub>2</sub> mineral, is notable for its presence in marine black shales. Laboratory experiments suggest that marcasite should only form in very acidic conditions (pH < 6) that are absent in modern marine sediment pore waters. One proposed explanation for sedimentary marcasite formation is that the partial oxidation of abundant pyrite in black shales produced the necessary low pH conditions. A major problem with the pyrite oxidation hypothesis is that it requires oxygenation of sediments thought to have been deposited under severely deoxygenated water columns, e.g., during oceanic anoxic events (OAEs). To further investigate this problem, we selected sediments of various lithologies deposited over a range of water depths during the mid-Cretaceous 'OAE-2'. We used laser Raman microprobe analysis (LRM) and secondary electron microprobe energy dispersive X-ray spectroscopy (SEM-EDX) to characterize and determine the relative abundances of acid-insoluble sulfur-bearing minerals across OAE-2 at the different sites. Marcasite is present at all sites and is neither exclusive to the traditional OAE-2 interval nor the black shale units. In addition to isolated euhedra, marcasite is also present as overgrowths on framboidal pyrite and as coarse cements in polyframboidal pyrite aggregates, suggesting that the marcasite postdates pyrite. We therefore suggest that the chemistry of the water column (i.e., low pH due to high atmospheric *p*CO<sub>2</sub>) played a role in producing acidic pore waters, and the high organic matter content of sediments further lowered the pH of sediment pore waters within and close to the OAE-2 interval through the generation of organic acids, facilitating the formation of early diagenetic marcasite.

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## Big data in geobiology: Applications to DeMMO

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CAITLIN CASAR<sup>1</sup> AND MAGDALENA OSBURN<sup>1</sup>

<sup>1</sup>Northwestern University, Evanston, IL, USA

As the field of geobiology expands and high-throughput DNA sequencing technology advances, it becomes increasingly important to maximize our ability to handle big data. Big data is defined as datasets that are so large that typical software tools cannot process such data in a reasonable amount of time. A variety of tools for efficient data analysis, pipeline standardization, and documentation are available across a range of programming languages that are underutilized in the geobiology community. Further, big datasets contain a large amount of information that must be distilled and conveyed to the community in a straightforward, meaningful way. Thus, it is equally important for the geobiology community to make big data accessible to others by making use of available tools and platforms for effective communication and collaboration. Here we describe our approach to handling and presenting a large metagenomic and geochemical dataset generated through long-term research at the Deep Mine Microbial Observatory (DeMMO) in Lead, SD.

**Data management:** We tackle data management using SQL, a language designed to build and query large databases, and R packages RPostgres and Tidyverse that interface with SQL databases.

**Getting more from your data:** We standardize and optimize our data processing pipeline using a custom-built R package, DeMMOgorgon, that encompasses the data cleaning, microbiome analysis, and figure-making tools required for our desired output. We use machine learning approaches, i.e. predictive modelling, to query patterns in our data to gain insight into the controls on metabolic function and biomass distribution in the deep subsurface.

**Public Access:** We make our data accessible to the public through interactive web applications built using R Shiny, hosted on our website [deepminemicrobialobservatory.com](http://deepminemicrobialobservatory.com). Additionally, we make our code available to the community to promote method transparency at [github.com/deepsurfer](https://github.com/deepsurfer) and provide detailed documentation with embedded code chunks on our website. Some of these features are currently disabled but will become available after data publication.

Our efforts have allowed us to optimize our efficiency and get the most out of our data while fostering communication with the public and other members in the geobiology community.

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## Interpreting lacustrine bulk sediment $\delta^{15}\text{N}$ values using metagenomics: A case study from a hypersaline lake system

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MINGFEI CHEN,<sup>1</sup> JESSICA L. CONROY,<sup>1,2</sup> ROBERT A. SANFORD,<sup>1</sup> JOANNE C. CHEE-SANFORD,<sup>3,4</sup> AND LYNN M. CONNOR<sup>3,4</sup>

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<sup>2</sup>Department of Plant Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA

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Nitrogen (N) is a limiting nutrient in lacustrine systems, and bulk organic matter (OM) stable isotope ratios of N ( $\delta^{15}\text{N}$ ) are widely used in lake sediment studies to interpret N source inputs and lake trophic status. However, the traditional interpretations of lacustrine sedimentary  $\delta^{15}\text{N}$  often yield conflicting results with lacustrine fossil records. Furthermore, many aspects of the microbial N-cycle have substantial isotopic fractionation factors and likely wield an enormous influence on bulk sedimentary  $\delta^{15}\text{N}$ . Yet few studies apart from N-fixation have attempted to link diverse microbial, N-related activity to sedimentary  $\delta^{15}\text{N}$  in lake sediment records. Therefore, we proposed to link the geochemical data of a lake sediment core to the microbiome profiles analyzed from



extracted sediment DNA using metagenomics to reveal insight into the specific drivers of lacustrine sedimentary  $\delta^{15}\text{N}$  variability. As a case study, we focused on a ~1600 year-long sediment record from hypersaline 'Lake 1', located on Kiritimati Island, Republic of Kiribati (2°N, 157°W). We observed a robust  $\delta^{15}\text{N}$  trend through time, with a higher  $\delta^{15}\text{N}$  value (5.72‰) in the surface mat and a lower  $\delta^{15}\text{N}$  value (-0.75‰) in a buried mat. The metagenomics profile showed significant differences in abundant taxa between the surface- vs. buried sediment mats. In the surface mat, *Proteobacteria*, *Bacteroidetes*, and *Cyanobacteria* were the most abundant bacterial phyla comprising dominant species that are known to harbor genes for complete denitrification. Species from phyla candidate division *Zixibacteria* and *Chloroflexi*, known to contain nitrification genes (*hao* and *nxr*), were the dominant bacterial taxa in the buried mat. Functional gene annotations using the KEGG database and SEED subsystem indicated genes comprising an incomplete denitrification pathway that were less abundant in the buried mat compared with that in the surface mat. In contrast to the surface mat, annotated *nifH* genes in the buried mat were affiliated with methanogens, suggesting the possible coupling of N-fixation and methanogenesis in the older sediment. We conclude that these spatial-temporal  $\delta^{15}\text{N}$  values are controlled by the relative abundance of N-related microbial taxa as well as nitrification/denitrification genes. We further plan to retrieve metagenome-assembled genomes (MAGs) to construct the functional potential of N cycling processes in the sediment core.

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## Sedimentary biomarkers in Lake Biền Hồ, Vietnam, record temporal changes in phytoplankton dynamics

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KELSEY E. DOIRON,<sup>1</sup> ARNDT SCHIMMELMANN,<sup>2</sup> HƯƠNG NGUYỄN-VÂN,<sup>2</sup> DƯƠNG NGUYỄN-THÙY,<sup>2</sup> AND SIMON C. BRASSELL<sup>1</sup>

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<sup>2</sup>VNU University of Science, Hanoi, Vietnam

The Pleiku volcanic field of the Central Highlands of Vietnam features maar lakes ranging in age from 2.4–0.2 Ma. Sediment archives from these lakes provide opportunities to elucidate environmental and climatic change linked to variations in the strength of the S. Asian monsoon. This investigation focuses on biomarker records in sediments from Lake Biền Hồ seeking evidence for temporal variations in phytoplankton and inputs from terrestrial vegetation.

Prominent components in samples from 1 to 23 cm sediment depth in Lake Biền Hồ comprise: (i) C<sub>19</sub>–C<sub>33</sub> *n*-alkanes & *n*-alk-1-enes, (ii) C<sub>34</sub>–C<sub>37</sub> mono- & bicyclic botryococcenes, (iii) hopanes, hopenes, & 2β-methylhopanes, and (iv) des-A-lupane & des-A-arbor-9(11)-ene. The depth differences reflect changes in the Lake Biền Hồ environment and its phytoplankton community, which today includes green & golden algae, diatoms, dinoflagellates, euglenoids, and cyanobacteria. The plant wax *n*-alkanes attest to terrestrial inputs whereas the predominance of C<sub>25</sub> at 1 & 6 cm depth reflects emergent macrophytes. *n*-Alk-1-enes suggest that contributions from chlorophytes increase below 11 cm. The abundance of *n*-alkenes, des-A-lupane & des-A-arbor-9(11)-ene resembles sediments from Lake Chala, also a maar lake. The dominance of cyclic botryococcenes at 6, 11 & 16 cm contrasts with their minor amounts at other depths and echoes their prevalence in maar lakes and other tropical settings. Depth variations in hop-22(29)-ene and ββ-hopane abundances attest to temporal changes in bacterial inputs, whereas the occurrence of 2β-methylhopanes at 20 & 23 cm reflects cyanobacteria.

Biomarker compositions of sediments from Lake Biền Hồ resemble those of other maar lakes and their depth profiles reflect a lake setting sensitive to environmental change, characterized by: (i) anoxia, coupled with inputs from terrestrial plant waxes, (ii) a succession of phytoplankton populations, including *Botryococcus* algae, likely responding to nutrients, and (iii) enhanced contributions from emergent macrophytes. Future work will focus on analysis a suite of longer (~15 m) <sup>14</sup>C-dated sediment cores providing a 30 kyr record of the history of Lake Biền Hồ, including productivity and monsoonal changes through the last glacial cycle.

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## S-C-Fe dynamics for the competition of sulfurization pathways within the long-term history of Mahoney Lake, Canada

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FOTIOS FOUSKAS,<sup>1</sup> WILLIAM P. GILHOOLY III,<sup>1</sup> MOLLY D. O'BEIRNE,<sup>2</sup> JOSEF P. WERNE,<sup>2</sup> AND ALICE BOSCO-SANTOS<sup>1,3</sup>

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<sup>3</sup>Geology and Natural Resources, University of Campinas, Campinas, São Paulo, Brazil

Reduced sulfur (S) undergoes inorganic and organic transformations within water column and sediments. Studies have focused on modern euxinic lakes where S isotopes can potentially differentiate the two competitive sulfurization pathways that lead to pyrite and organo-S compounds (OSCs) formation. The large community of phototrophic purple S bacteria, sulfate reducers, and the high sulfate/sulfide levels within Mahoney Lake (~400/35 mM) make this lake an end-member of extreme euxinia. Despite the high sulfate content, the  $\Delta^{34}\text{S}_{\text{SO}_4\text{-H}_2\text{S}}$  is similar to isotopic offsets observed in other euxinic sites (~50‰). A 3.6 m core (age-dated to 7700 B.P.) was recovered from the deep basin of the lake. The concentration of total organic S (TOS; ~2 wt.%) is significant relative to the inorganic S phases (pyrite and AVS; <0.5 wt.%) within the sediment. The  $\text{S}_{\text{org}}:\text{C}_{\text{org}}$  molar ratio increases in the top of the core (last 500 yrs), while it remains stable within the rest of the core, suggesting there are no diagenetic effects over the long history of the lake. The  $\delta^{34}\text{S}$  values of pyrite are highly uniform (~ -23 ‰) and decreased to 33 ‰ in the bottom of the core. The  $\delta^{34}\text{S}$  value of TOS also progressively decreased with depth. The isotope offset between pyrite and TOS changed downcore from -15‰ (last 500 yrs where pyritization is preferential over OM sulfurization consuming the mostly available  $\text{H}_2\text{S}$ ) to nearly 0‰ (high competition of both sulfurization pathways). Thus, we suggest that the sedimentary system of Mahoney Lake is relatively “open” and that fast sulfurization reactions of abundant  $\text{H}_2\text{S}$  and OM lead to large amounts of TOS with low  $\delta^{34}\text{S}$  values. In addition, Fe speciation varies over depth and this variability contributes to the relative dominance of each sulfurization pathway. Overall the variability in the Fe pools, together with the changes of  $\text{S}_{\text{org}}:\text{C}_{\text{org}}$  ratio within Mahoney Lake influence the partitioning of reduced S in pyrite and TOS, and therefore the competition of both sulfurization pathways.

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## Spheroidal carbonate particles within travertine and tufa deposits in the Argentine Puna plateau: Exploring the physical, chemical, and biological controls on textures and microfabrics

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R. AGUSTIN MORS,<sup>1</sup> FERNANDO J. GOMEZ,<sup>1,2</sup> CECILIA MLEWSKI,<sup>1</sup> AND RICARDO A. ASTINI<sup>1</sup>

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The Terma Los Hornos is an active travertine-tufa system developed in the high-altitude volcanic plateau of the Puna region of Catamarca, Argentina. Here, by using classic petrography, SEM, confocal microscopy and microbial diversity techniques, we study mm- to cm- scale spheroidal carbonate particles to determine the role microorganisms and related physical-chemical processes may have played in their formation. We recognize different carbonate particles including a) *spheroids* (0.1-1.6 cm in size) with rough outer surfaces and an internal microstructure composed of dendriform micrite-rich clumps intimately associated with a microbial community; and b) *pisoids* (0.3-3.9 cm in size) with smooth exteriors and concentrically laminated interiors. Spheroids are typically associated with Cyanobacteria (e.g. *Rivularia*) followed by Proteobacteria (mostly alphaproteobacteria) and Bacteroidetes among others. On the other hand, pisoids do not show a microbial community associated with the carbonate microtextures. This suggests that textural differences can be partially associated with the presence of an active microbial community.

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# Pyrite $\delta^{13}\text{C}$ records changes in environment of deposition across glacial-interglacial cycles in the Valle di Manche, Italy

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The sulfur isotopic composition ( $\delta^{34}\text{S}$ ) of pyrite in the sedimentary record is thought to reflect the porewater sulfide  $\delta^{34}\text{S}$  at the time of deposition resulting primarily from the activity of sulfate reducing bacteria. Recent studies have highlighted the influence of the environment of deposition, specifically the feedback between water depth, sedimentation rate, and connectivity of sediment porewater with the overlying water column, on the resulting  $\delta^{34}\text{S}_{\text{pyrite}}$ . In this study, we present a new  $\delta^{34}\text{S}_{\text{pyrite}}$  record for the Valle di Manche section, Crotona Basin, southern Italy that spans the Early-Middle Pleistocene boundary, including two complete glacial-interglacial cycles (MIS 18 – 22). The sequence stratigraphy within the Crotona Basin is well characterized and provides an improved reconstruction of the history of deposition during a period of tectonically driven increasing accommodation, allowing continuous deposition even during terminal interglacial high-stands. Extensive companion datasets exist for this section that independently constrain the paleoclimate and paleoenvironment, including detailed records of  $\delta^{18}\text{O}$  from benthic *U. peregrina*, pollen, planktonic nannofossils, and benthic macrofauna.

The  $\delta^{34}\text{S}_{\text{pyrite}}$  record presented here broadly confirms previous patterns of stable, isotopically depleted values during interglacial periods and highly variable, isotopically enriched values during glacial periods observed in MIS 1-7 sedimentary sequences from the Gulf of Lion, northwestern Mediterranean. However, in combination with the existing companion paleoenvironmental datasets, this study can evaluate several variables that may influence microbial sulfate reduction and thus the  $\delta^{34}\text{S}_{\text{pyrite}}$  record, including sedimentation rate, oxygen levels, type of substrate, storm events, and organic carbon supply.

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## Short-term temporal variations in the physiochemical conditions of a eutrophic freshwater lake

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Eagle Creek Reservoir (ECR), Indiana is the site of an on-going project to research and better understand the effects of Harmful Algal Blooms (HABs) and the processes governing their formation/proliferation. Sampling has been conducted on surface waters via boat across fourteen sampling sites, mostly concentrated on the northern portion of the reservoir (which is more dynamic and thought to be the origin of most ECR HABs.) Data collected in 2018 demonstrated significant spatial variations on a scale of just thousands or hundreds of feet with significant temporal variations also being noted on several days to week-long timescales. In order to assess temporal changes on a shorter timescale replicate measurements were performed a total of five times with roughly 15 minute intervals between each at one location. Water samples were collected using a Van Dorn sampler at ~1m depth and sub-sectioned into unfiltered and filtered (0.22  $\mu\text{m}$  filter) aliquots before being stored on ice. Additionally, organic material was collected on a Sterivex filter for genetic analysis then frozen, and unfiltered subsamples taken for T&O compound analysis (through GC/MS) and cell counts. Measured variables included SRP, total P, total dissolved P, dissolved nitrate, TSS, Chl-A, PC, metals (Ca, Mg, K, Na, Fe, Mn,) cell counts (cyanophytes/chlorophytes primarily,) MIB, Geosmin, and MIB/Geosmin synthase concentrations.

The results indicate certain variables show significant variation over this timescale while others do not. Those parameters associated with overall water conditions and nutrient levels (such as SRP, metals) appeared to have statistically insignificant temporal variations and remained largely static. Meanwhile, key variables for monitoring the presence and extent of HABs (such as cell counts, Chl-A, PC) showed significant differences over a short time period. These results will inform ongoing work on how to couple drone- and satellite-based remote sensing information with water chemistry.

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## Microscale resolution on how sedimentary pyrite forms

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Sedimentary pyrite is one of the most abundant minerals on the earth's surface [1], but the chemical mechanisms that are involved in its creation at low temperatures from primary minerals during early sedimentary diagenesis are diverse, and constrained primarily from experiments rather than natural systems. The change in oxidation state from  $S^{2-}$  to  $S^0$  to form pyrite during sedimentary diagenesis is enigmatic [2], as pyrite forms in reducing sediments. The oxidation state in pyrite indicates that  $S$  needs to be oxidized, and either  $Fe$  removed or  $S$  added from a putative primary  $FeS$  precipitate to ultimately form pyrite ( $FeS_2$ ) [3]. Lab-based experiments have documented several mechanisms of pyrite formation, but past field-based studies on pyrite formation pathways relied on bulk extractions with operationally defined  $S$  phases (i.e. acid vs. chromium volatile sulfide) rather than direct detection of sulfur-bearing phases.

To address this, we studied sediments and water-column particulates forming in a ferruginous lake with bulk and microscale techniques. Brownie Lake in Minneapolis, MN is a meromictic lake with bottom water  $Fe^{2+}$  exceeding 1000  $\mu\text{M}$ , and maximum sulfate concentrations of 80-100  $\mu\text{M}$ . Bulk X-ray fluorescence (XRF) indicates 0.5%  $S$  in Brownie sediments, while secondary electron microscopy (SEM)-coupled energy dispersive spectroscopy (EDS) indicates the presence of both  $Fe$  and  $S$  in particulates

from the anoxic water column. Microscale imaging of embedded particulates and sediments by synchrotron-based XRF documents co-localization of Fe and S, consistent with iron sulfide formation, Mössbauer spectroscopy of particulates indicates a highly reactive FeS phase, while microscale synchrotron-based X-ray absorption spectroscopy (XAS) indicates very reduced S in particulates, with more oxidized sulfur in sediments. We will discuss how these results can inform the chemical mechanisms by which sedimentary pyrite forms in ferruginous sedimentary environments.

[1] Rickard D, Luther III GW. 2007. Chemistry of Iron Sulfides. *Chemical Reviews* 107: 514-62. [2] Picard A, Gartman A, Girguis PR. 2016. What Do We Really Know about the Role of Microorganisms in Iron Sulfide Mineral Formation? *Frontiers in Earth Science* 4. [3] Goldhaber MB, Kaplan IR. 1974. The sulfur cycle. In *The Sea*, ed. ED Goldberg, pp. 569-655. New York, N.Y.: Wiley-Interscience

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## Hydrothermal hypoliths: A comparison of extremophilic rock-dwelling microorganisms with other hydrothermal photo- and chemotrophic communities across acidic and alkaline pH ranges

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Modern extremophiles are ideal microbial communities to study and compare with Earth's early harsh environments. Recent evidence suggests that terrestrial hot springs were present 3.5 billion years ago, which necessitates the study and characterization of modern hydrothermal microbial communities further. Much of the current focus is on microbial communities within the hydrothermal pools, however, this study investigates modern hypolithic microbial communities living below siliceous rock at the soil-air interface around the edges of hot springs in Yellowstone National Park. Hypolithic communities are understood to utilize their siliceous covering as UV protection within extreme environments. The hypolithic productivities are compared with the productivities of subaerial phototrophs, subaqueous phototrophs, and subaqueous chemotrophs across acidic and alkaline pH ranges. This study compares the microbial community composition across all of the microbial categories and pH ranges. Overall carbon uptake ( $\mu\text{g C uptake/g biomass/hr}$ ) ranged from 5-1000 (n=16) for hypoliths, 15-2750 (n=8) for subaerial phototrophs, 5-10000 (n=10) for subaqueous phototrophs, and 0-1400 (n=7) for subaqueous chemotrophs. Hydrothermal hypolithic productivity and composition are overall similar to the other microbial communities. Notable variations for the hypolithic communities are seen when comparing the acidic and alkaline microbial compositions.

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## Abiotic Fe(II) oxidation behavior in trioctahedral smectites: Rates, extents, and products

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Fe(II)-bearing trioctahedral smectites form during anoxic weathering of basaltic rock and are predicted to have been widespread on the early Earth. They are observed in the oceanic subsurface on the modern Earth but degrade in the oxygenated environment at the surface. Due to difficulty in sampling and studying these minerals, their potential as electron donors for microorganisms remains poorly constrained. In this study we synthesized trioctahedral Fe(II)-bearing smectites in order to first study the rate, extent, and products of their abiotic oxidation by dissolved O<sub>2</sub> and nitrite, a reactive intermediary produced during denitrification.

Smectites were synthesized using a sol-gel method with hydrothermal aging performed under anoxic conditions. Structural formulae were derived from bulk elemental abundances measured by ICP-OES. Oxidation kinetics were measured on suspensions in a freshwater media and exposed to 5 mM sodium nitrite, a continuous flow of filtered air (21% O<sub>2</sub>), or a microaerophilic gas mixture (78% N<sub>2</sub>, 20% CO<sub>2</sub>, 2% O<sub>2</sub>). Oxidation extent was monitored throughout the 30-day experiments via colorimetry on a subsample of smectite by dissolved by ammonium hydrogen fluoride.

Oxidation by nitrite was minimal over the timeframe of the experiments. Dissolved O<sub>2</sub> caused partial oxidation of Fe(II) in the smectites with an unreactive portion remaining. Oxidation was more rapid in media equilibrated with the air relative to those under microaerophilic conditions, and proceeded to a greater extent. This behavior is consistent with the incomplete oxidation seen in natural samples and reduced dioctahedral smectites and may be caused by non-Nernstian redox behavior of iron, likely controlled by the local environment of the iron within the structure. Mössbauer and XRD analyses of synthesized smectites and oxidized products will also be presented and indicate that iron remains in the clay structure following oxidation. These analyses provide comparative data for interpreting future biotic studies and identify potential biosignatures of microbial iron oxidation.

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## Trash or treasure? Biogeochemical cycling in landfill ecosystems

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Increased understanding of the microbial processes driving landfill degradation is vital not only for waste management, but also for understanding biogeochemical cycling. Landfilled MSW (municipal solid waste) can reach depths of >100 m and, in many respects, can be characterized as an “extreme” environment (deep, dark, hotter than ambient). Like many natural ecosystems, landfills are inherently heterogeneous and rich with organic and inorganic materials that fuel a diverse array of microbial metabolism. Decomposition of MSW components (dominated by cellulose from paper, garden, and food waste) occurs under primarily anaerobic conditions via a series of hydrolytic, fermentative, acidogenic, acetogenic, and methanogenic microbial reactions. Additional microbial and abiotic reactions occur in landfills including cycling of nitrate, sulfate, and iron reduction. Also, like natural ecosystems, landfills are susceptible to disruptions that can hinder normal landfill function and waste degradation. For example, while landfill ecosystems are typically “mesophilic”, ranging from 23-45 °C, internal landfill temperatures have been observed at up to 100 °C. Thermophilic conditions in these “hot” landfills may

suppresses rates for the terminal step in anaerobic degradation, methanogenesis. Additionally, observed methane production from landfills is often less than the theoretical expected values. Here we investigate microbial responses at mesophilic conditions to the addition of non-carbon substrates in the landfill ecosystem, toward the understanding of potential causes for disruption to waste degradation. We present geochemical results from experimental landfill microcosms in response to the addition of antibiotics, sulfur, iron, and microplastics. These results have implications for both landfill management and broader biogeochemical cycling in “extreme” environments. Understanding microbial ecosystem responses at mesophilic conditions will also help inform future investigation into thermophilic landfill conditions.

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## Femtosecond-Laser Desorption Laser Postionization-Mass Spectrometry of organic-rich and organic-lean geologic samples

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We use the MS-imaging technique, femtosecond-laser desorption post ionization-MS (fs-LDPI-MS), to determine the spatial distribution of organic compounds within geologic material (Hanley *et al.*, 2019). We analyzed two sample sets: (1) a -164 Ma organic rich mudstone (14.2 wt. % total organic carbon, TOC); and (2) a -93 Ma organic-lean burrowed carbonate (1.0 - 2.1 wt. % TOC). Prior to this study, all geologic units had been analyzed via GC-MS to determine the lipid biomarker composition.

In both sample sets, organic-S fragment ions dominated the organic portion of the low ionization energy components of the samples and were heterogeneously distributed as micron scale particles throughout the samples. These results indicate that MS-imaging of organics is possible in both organic-rich and organic-lean geologic samples.

This work is supported by the NASA Exobiology Program.

**References:** Hanley L., *et al.* (2019) *Annual Review of Analytical Chemistry*, 12, 225-245 and refs. therein.

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## The SMB resource at SSRL: Geological, biological, and environmental applications using X-ray spectroscopy and imaging

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The Stanford Synchrotron Radiation Lightsource (SSRL) Structural Molecular Biology (SMB) facility develops, operates and supports synchrotron-based tools for research in biological, environmental, geological and biomedical science. These techniques include macromolecular crystallography, small angle x-ray scattering, multiple x-ray spectroscopy methods (XAS) and x-ray fluorescence (XRF) imaging. The SMB resource aims to increase and sustain research within the scope of the DOE Office of Biological and Environmental Research (BER) mission. The SMB resource has several  $\mu$ -X-ray Fluorescence ( $\mu$ -XRF) imaging and X-ray Absorption Near Edge Structure (XANES) spectroscopy experiments covering a wide range of x-ray spot sizes (0.5 to 250 microns), energies (2 to 23 keV), and sample size (up to 300 x 600 mm). Integrated beam line scheduling allows proposals to map multiple elements, from phosphorus to technetium (K-edge) as well as information through the actinide series (L-edge), across different spatial scales. Ambient analytical conditions allows for versatile samples and *in situ* experiments. Our capabilities include combining imaging with spectroscopy to generate individual images of the speciation of the element(s) of interest. The applications of these techniques to the environmental and biological sciences are varied spanning research areas from subsurface remediation, biogeochemistry and cycling of nutrients, to the chemistry of metal uptake and regulation by organisms. We present examples of the role and speciation of sulfur in biological marine carbonate and in a variety of species of plant roots from different ecosystems, the expression of transition metal transporters in seedling phenotypes and plant leaves, and phosphorus speciation and abundance on early Earth.

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## Investigation of climate, terrestrial ecosystems, and aquatic productivity over the last millennium from the Lake Sibinacocha Watershed, Peruvian Andes

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Historical hydroclimate data from high elevations in the Andes, South America are spatially inconsistent and most temperature observations are either short or nonexistent. Here we present a record from the central high Andes in order to understand long-term interactions among alpine ecosystems, climate changes, and human influences during the past millennium. We present initial biomarker data from sediment cores collected from the watershed of Lake Sibinacocha (13.86 S, 71.02 W; 4860 m a.s.l.), ~20 km northwest of the Quelccaya Ice Cap. We analyzed long-chain *n*-alkanes and *n*-alkanoic acids (leaf waxes) to reconstruct terrestrial vegetation community and precipitation, branched glycerol dialkyl glycerol tetraethers (brGDGTs) to reconstruct temperature, and bulk and isotope carbon and nitrogen to reconstruct past aquatic productivity. Preliminary results based on the MBT<sup>5</sup>Me calibration, which yields brGDGT temperatures similar to present-day lake temperatures, reveal that the Sibinacocha watershed warmed by ~2°C in the last ~200 years. The average chain length of long-chain *n*-alkanes suggests a stable regional vegetation community, with possible long-range transport of waxes from lowland ecosystems. This particular presentation aims to examine and evaluate the interpretation of biomarker data in order to better understand relationships between the high Peruvian Andean



environment and climate change due to both natural forcing and human influence. This will provide a foundation for future paleo-ecological research and water resource management in the central Andes.

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## A stable strontium isotope record of pre-Sturtian carbonates spanning a large $\delta^{13}\text{C}$ anomaly

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The marine carbon isotope record ( $\delta^{13}\text{C}$ ) tracks Neoproterozoic glacial events with large negative anomalies ( $\sim -8\%$ ) that interrupt sustained intervals of positive values. The mechanisms responsible for these  $\delta^{13}\text{C}$  variations remain enigmatic. To evaluate the relationship between carbon isotopes and global-scale glaciation, we used a high-precision TIMS method [1] to measure both radiogenic ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) and stable ( $\delta^{88/86}\text{Sr}$ ) strontium isotope compositions of marine carbonate rocks from the Copper Cap Formation (Mackenzie Mountains, Canada), which were deposited just before the Sturtian snowball Earth event (dated  $\sim 717$  Ma) [4].

The combination of  $^{87}\text{Sr}/^{86}\text{Sr}$  and  $\delta^{88/86}\text{Sr}$  measurements can uniquely resolve signals of global weathering *and* carbonate burial rates. The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of seawater represents a balance between radiogenic inputs from continental weathering and non-radiogenic inputs from hydrothermal alteration of the oceanic crust [2], constraining Sr input fluxes, but not the Sr output flux, which primarily occurs via marine carbonate burial. Modern marine carbonates (both biotic and abiotic) preferentially incorporate lighter Sr isotopes relative to seawater [3]. Therefore, the  $\delta^{88/86}\text{Sr}$  value of seawater is sensitive to global carbonate burial rates. Here we present the first  $\delta^{88/86}\text{Sr}$  record prior to the Sturtian snowball Earth event.  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios (0.706-0.714) show a weak positive correlation to  $\delta^{13}\text{C}$  values and weak negative correlation with  $\delta^{88/86}\text{Sr}$  values (0.30‰-0.51‰). Notably, many of the  $\delta^{88/86}\text{Sr}$  values are high relative to typical Phanerozoic seawater values (0.386‰), and several samples downsection have elevated  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios. Interpretation of these data requires consideration of both primary and secondary drivers of isotopic variation. To this end, we are investigating trace element compositions, microdrilling for phase-specific isotope measurements, and performing petrographic analyses. Primary observations suggest relatively good preservation of many lithological phases, including those with unusually high radiogenic Sr isotope ratios.

[1] Andrews et al. (2016) [2] Palmer and Edmond (1992) [3] Pearce et al. (2015) [4] Rooney et al. (2014)

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## Mineralogical influences on the habitability of a banded iron formation

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The deep continental biosphere is separated spatially and temporally from photosynthetically derived energy sources and supported by chemolithoautotrophic primary producers. To explore how mineralogy affects the habitability of a fractured rock aquifer, we analyzed the aqueous chemistry of four boreholes, as well as the fracture distribution and mineralogy of rock cores from the Soudan Mine Underground State Park. The mine allows access to the Vermillion Range banded iron formation of the Canadian Shield. The boreholes begin at a depth of 714 m below ground surface and range from 100-140 m in total length. Boreholes and resulting cores intersect fractures containing a highly concentrated calcium chloride brine, with total dissolved solids reaching concentrations more than two times that of seawater. Archived cores show rock units composed of alternating zones of chlorite schist, banded jasper, and iron ore. X-ray computed tomography of core segments was used to identify areas of interest for thin sectioning. We will use micro-probe X-ray and electron characterization techniques to describe alteration reactions within fractures zones that supply substrates and nutrients to the microbes living in the deep biosphere.

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## Organic and isotopic biosignatures of lava tubes

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Lava tubes host diverse microbial communities on Earth and are analogs to potentially habitable environments on Mars. The basalt walls of these shallow caves can provide mineral energy sources for *in situ* lithoautotrophic metabolisms. However, organic carbon from the surface likely influences these environments, which may result in the outcompetition of basalt-hosted autotrophs. Therefore, constraining the balance between heterotrophy and autotrophy is critical for studying lava tubes as Mars analogs. The lava tubes of Lava Beds National Monument, CA (LBNM) are situated in a low-productivity, semi-arid surface environment, thereby mimicking the presumably low-productivity conditions of the Martian surface. Thus, lava tube microbial communities from LBNM are theoretically more likely to harbor autotrophic metabolisms compared to their tropical counterparts in Hawaiian or Azorean lava tubes.

Intact polar lipids (IPLs) are the membrane lipids of extant microbial communities. IPL profiling is a non nucleic acid-based approach to estimate both taxonomy and biomass in an environment. Stable carbon isotope abundances ( $\delta^{13}\text{C}$ ) of individual IPLs can be compared to bulk organic  $\delta^{13}\text{C}$  ( $\delta^{13}\text{C}_{\text{org}}$ ). This proffers a glimpse into the metabolic pathways expressed by the microorganisms that produce each IPL. Here, we present IPL profiles from a variety of LBNM lava tube features, with IPL-specific  $\delta^{13}\text{C}$  values when available.

Biosignatures were analyzed from a suite of lava tube sample types, encompassing biological and mineral features.  $\delta^{13}\text{C}_{\text{org}}$  was measured on an elemental analyzer coupled to an isotope ratio mass spectrometer

(IRMS). Extracted IPLs were derivatized into fatty acid methyl esters (FAMES), which were characterized and quantified via GC-MS analysis. FAMES were then run on a GC-IRMS for IPL-specific  $\delta^{13}\text{C}$ .

LBNM lava tube microbial communities are dominated by branched, rather than straight-chain, IPLs. Lava tube biofilms tend to exhibit more depleted  $\delta^{13}\text{C}_{\text{Org}}$  compared to overlying surface soil and mineral-like cave features. Branched and cyclopropyl IPLs tend to be more depleted in  $\delta^{13}\text{C}$  compared to straight-chain isomers. This indicates either the presence of non-Calvin cycle carbon metabolisms or repeated cycles of mineralization and C fixation in LBNM cave communities. Future stable isotope probing work will attempt to distinguish between these two possibilities.

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## Decoding Devonian mass extinctions: New evidence linking land plant expansion to marine anoxia

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The evolutionary advancement of fully developed root structures by land plants in the later Devonian revolutionized the Earth's surface, constituting the emergence of the first Critical Zone. This innovation within the terrestrial biosphere initiated a pseudo-modern process of soil formation dynamics, and in turn significantly impacted nutrient and carbon cycling within the global oceans. It has been theorized that this transformation upon Earth's surface increased nutrient flux to paleo oceans, resulting in widespread anoxia and potentially driving multiple marine mass extinctions. To provide evidence linking these events, land-based measures of P weathering and mobilization during this time were developed to provide a dynamic and quantitative basis for assessing the global role of plant and soil evolution. Here we present results from multiple Devonian lacustrine sequences from both Euramerica and Gondwana which show evidence of a net loss of P during root development coincident with the appearance of the first trees. Critically, two separate Mid Devonian study sites reveal a near identical net loss of P, providing critical support to the hypothesis that the expansion and diversification of land plants released unprecedented levels of nutrients driving global eutrophication and anoxia.

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## A co-limited approach to the Black Queen

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Microbial competition is believed to be a primary driver of community structure, dynamics, and evolution. Bioinformatic investigations of natural populations suggest that microbes derive a fitness benefit from genome streamlining (Giovannoni, et al., 2014). Recently, the Black Queen Hypothesis postulated that even essential activities might be lost in genome streamlining, so long as the products of those activities are supplied as public goods by other organisms (Morris, et al., 2012). Under this hypothesis, purely selfish loss-of-function “beneficiaries” rely on the continued production of public goods by a non- streamlined lineage (“helpers”). Helpers bear the metabolic cost of public good production, with the benefit that they become indispensable to the community, potentially leading to the evolution of stable mutualisms. The original Black Queen model considered the case of iron limitation and reactive oxygen species (ROS) detoxification, with ROS- detoxifying enzymes as the public goods. Here, we take a theoretical modelling approach to examine Black Queen dynamics under iron and nitrogen co-limitation (after Saito, et al., 2008). We explore how mutants that have lost nitrogen fixation and extracellular siderophore (iron- scavenging molecules) production may invade and overtake resident populations. Our initial exploration of the model suggests that, under co-limitation, the extent of public good accessibility to the entire community impacts the development of stable helper/beneficiary mutualisms. We aim to further develop this model in conjunction with empirical laboratory experimental approaches to directly investigate the processes shaping microbial community evolution in different environments.

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## Single-cell analysis of microbial metals content by SIMS

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Secondary Ion Mass Spectrometry (SIMS) has an established history of use in biological imaging to determine the elemental or isotopic content of cells at cellular and subcellular resolutions. We tested the imaging capacities of a Cameca 7f-GEO SIMS that has been upgraded with a new source and detector. A high-brightness RF plasma nanoprobe O<sup>-</sup> source allows more sensitive detection of positive secondary ions such as those generated from metals. A Resistive Anode Encoder (RAE), installed as a detector, allows stigmatic ion imaging. In concert, the new equipment allows sensitive detection of positive secondary ions and co-registration of positive and negative ion maps over an area of approximately 10,000 μm<sup>2</sup>. This area is sufficient to obtain distributions of elements of isotopes in a statistically meaningful population of single cells. We analysed metals content (manganese, iron, cobalt, copper, zinc, and molybdenum) in wild type strains of *Escherichia coli* and *Methylobacterium extorquens* and in evolved isolates derived from the *M. extorquens* ancestor.

We will present results that detail the imaging capabilities of the upgraded 7f-GEO, and report the heterogeneity in metals contents among individual cells within each population under various growth conditions.

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## Investigating extracellular electron uptake in the model mineral-reducing microbe *Shewanella oneidensis* MR-1

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*Shewanella oneidensis* MR-1 is an important model organism for the study of extracellular electron transfer (EET)—the ability of an organism to either deposit and/or take up electrons from outside the cell, often from solid phase minerals or electrodes. The Mtr respiration pathway is the result of the characterization of the mineral “breathing” phenomenon in MR-1. However, it was found that this pathway could be reversed when MR-1 was grown on an electrode. This electron uptake process appears to be affected by more than just genes in the Mtr pathway though. To investigate this -100 transposon generated knockout mutants were screened for redox activity. 24 of interest were then further screened on a cathode to determine if they had a deficient phenotype. Of those 24, 6 contained interrupted genes of an unknown function and are now being screened again as clean knockouts.

To screen them, the MR-1 mutants are grown in biological reactors containing a 3-electrode system. The working electrode, which is poised at -600 mv, acts as an electron donor and the current can be measured over time (Chronamperometry) and across voltages (Cyclic Voltammetry). These are then compared to the WT, where deficient strains have been found to have a marked reduction in electron uptake (40-50%). The deficient strains represent potential novel components of the electron uptake pathway and may elucidate how/if MR-1 can “eat” solid substrates *in situ*.

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## Of cattle and feasts: Using multiple isotopes to understand Neolithic management of animals at Makriyalos, northern Greece

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The Late Neolithic site of Makriyalos presents evidence for large-scale consumption of domestic animals during communal feasting activities; a scale that is unparalleled in other Neolithic contexts. The aim of this study is to explore how these animals were obtained and managed in preparation for the feasts using a series of stable isotopic measurements. Bulk bone collagen  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of humans and animals are used to assess the long-term diets of the animals and their contribution to human subsistence. Sequential tooth enamel carbonate  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values are used to investigate the animals' short-term seasonal dietary and mobility patterns.  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios from cattle tooth enamel carbonate are used to test the possibility that some of the animals were born outside of the local landscape and brought to the site to be slaughtered at the ritual feasts. The results suggest that the Neolithic farmers managed their domestic herbivores in different ways, making use of distinct zones of the local coastal landscape for animal pasture. Some animals may have been brought to the site from the nearby Pieria mountains.

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## ***Pseudomonas aeruginosa* biofilm formation after cultivation in simulated microgravity**

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*Pseudomonas aeruginosa* can attach to surfaces and form aggregates known as biofilms. It has been previously found that *P. aeruginosa* cultivated in space form thicker and structurally different biofilms than those grown in Earth gravity. The purpose of our study was to investigate how microgravity, simulated in a laboratory setting, would influence the biofilm formation abilities of *P. aeruginosa* PA14 wild type strain as well as mutants  $\Delta flgK$  and  $\Delta pelA$ . While  $\Delta flgK$  is defective in the initialization of biofilm formation,  $\Delta pelA$  is hindered in biofilm growth and maintenance. The bacteria were cultivated in a High Aspect Ratio Vessel (HARV) on a Rotary Cell Culture System (RCCS) that was used to simulate microgravity. For the Earth gravity control cultures, the RCCS was oriented horizontally and cultures were rotated in HARVs around a vertical axis. Incubation time was six days, and, in contrast to studies done by others, the bacteria were allowed to grow into stationary phase without replenishment of culture medium. At the end of the incubation time, the bacteria were extracted and cultured in a 24-well plate under identical conditions in Earth gravity. After 24 hours, the robustness of biofilm formation was compared by removing the soluble culture from the wells, staining with crystal violet, solubilizing the remaining biofilm, and quantifying spectrophotometrically. Additionally, the concentration of pyocyanin produced during cultivation was determined through extraction and spectrophotometry. Cultures grown under simulated microgravity had a lower biofilm formation ability as well as lower pyocyanin production compared to those grown under Earth gravity. This raises the possibility that *P. aeruginosa* experiencing nutritional starvation under long-term simulated microgravity may become less virulent.

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## **Proteomic tracking of microbial nitrogen sourcing and utilization**

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Proteins are one of the main biomolecular constituents of microbial cells, often comprising more than half of cell biomass and most of a cell's nitrogen quota. As a result, nitrogen is a limiting nutrient in many microbial ecosystems, including much of the surface ocean and many carbon-rich high-latitude soils. We have developed novel proteomics techniques to track the incorporation of isotopically labeled N substrates into individual microbial proteins, affording a view of the physiology of microbial nitrogen utilization with unprecedented molecular depth and specificity. An initial application of these techniques to sourcing of N for protein synthesis during viral infection of marine picocyanobacteria (Waldbauer & Coleman *et al.*, 2019) revealed that bacteriophages can acquire more than 40% of their protein N from the environment after infection begins, and that the balance of host cell-derived versus medium-derived N shifts over the course of infection. Protein-specific N incorporation data also showed that a small set of host-encoded proteins continue to be produced during infection; many of these have functions believed to enhance the productivity of phage infection and thus may represent a form of viral remodeling of host gene expression and metabolism. We are currently expanding the application of these proteomic techniques to investigate the utilization of various organic and inorganic N sources by microbial communities in oligotrophic ocean surface waters.

Waldbauer J.R.\*, Coleman M.L.\*, Rizzo A.I., Campbell K.L., Lotus J.M., and Zhang, L.. (2019) Nitrogen sourcing during viral infection of marine cyanobacteria. *Proceedings of the National Academy of Sciences*. 116: 15590-15595 (\*co-first/corresponding authors)

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## Geochemical evolution of methane seeps off the Lofoten-Vesterålen continental margin, Norway

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Methane seeps are a critical component of the global carbon cycle and climate. In marine environments, sulfate-coupled anaerobic oxidation of methane (AOM-SR) modulates the release of methane into the overlying water column. In this study, barite crusts precipitated near the sediment-water interface off the Lofoten-Vesterålen continental margin, Norway, are used to elucidate the geochemical evolution of two recently discovered cold seeps. Specifically, scanning electron microscope (SEM) images are used to identify generational growth structures and dissolution textures in barite, and secondary ion mass spectrometry (SIMS) is used to measure the  $\delta^{34}\text{S}$  composition of individual barite grains.  $\delta^{34}\text{S}$  values span more 50‰ in individual samples ( $\sigma = 1$ ), and  $\delta^{34}\text{S}$  values  $> 100$ ‰ (VCDT) are observed. Paired petrographic and isotopic assessments suggest these barites precipitated in temporally and spatially variable microenvironments near the sulfate-methane transition zone (SMTZ) associated with extreme sulfate depletion and distillation. Changes in methane flux induce variable rates of AOM-SR relative to sulfate diffusion and permit dynamic migration of the sulfate-methane transition zone (SMTZ). Variations in SMTZ depth are recorded by successive increases and decreases in  $\delta^{34}\text{S}$  values along barite growth axes and episodic dissolution due to sulfate depletion. These findings are supported by pore water analyses,  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  measurements of carbonates, and  $\delta^{34}\text{S}$  measurements of bulk sediments. Additionally, these barite crusts are compared to the isotopic composition and petrographic textures of other diagenetic and cold seep barite.

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## Controls on trace metal speciation and availability in wetland soils

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Trace metals are essential for biogeochemical processes such as denitrification and methanogenesis. Trace metals are retained in soils via multiple mechanisms, including adsorption on minerals, coprecipitation in minerals, and complexation by organic matter. Previous laboratory studies suggest that low trace metal availability inhibits microbial processes, but few investigations have evaluated whether trace metal limitations occur in natural systems. If present, such limitations are likely controlled by the speciation of trace metals and the resulting impact on their mobility in natural environments.

Our work investigates the conditions in natural aquatic systems that produce trace metal limitations on biogeochemical processes, seeking to study the controls on trace metal speciation and availability in natural wetland soils. Soils and sediments were collected from a marsh wetland, a riparian wetland, and the base of a stream. The mineralogy of these samples was determined by powder XRD, which shows that the soils are composed primarily of quartz but also contain clay minerals. Aqueous and solid-phase metal concentrations were determined by ICP-MS. Variations in trace metal contents suggest that their availability varies among field sites. Sequential extractions suggest that Cu and Ni, metal essential to denitrification and methanogenesis, differ in their bioavailability. XAFS spectra of the native trace metals were analyzed by PCA, revealing that 4 to 5 distinct species of Cu and Ni are present. Linear combination fitting shows that Cu appears bound primarily to sulfide minerals and organic matter while Ni occurs bound to reduced sulfur, carboxyl groups on organic matter, and mineral surfaces. CuS and NiS abundance weakly correlated with sulfur content. Despite having low sulfur contents (0.02 to 0.24 wt.%), this constituent of the soils appears to exert a dominant control on Cu and Ni speciation.

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# Mercury biogeochemical processes in Lake Michigan sand dune ecosystem

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Lake Michigan hosts the largest freshwater sand dune system in the world. The shorelines of Lake Michigan support many species of rare and endemic wildlife, and are economically important to industry and tourism. However, due to industrial pollution and atmospheric deposition, toxic levels of methylmercury (MeHg) in Lake Michigan fish have been reported since the 1970's, but little is known what biogeochemical parameters control the MeHg formation in the Lake Michigan sand dune ecosystem. Here, we conducted anaerobic incubation experiments with beach sand collected from Ludington, Michigan, and we examined the effects of organic carbon, inorganic nitrogen, and iron oxide minerals, on mercury methylation. Despite nutrient poor, low-organic carbon conditions, our results show a high microbial activity in carbon degradation and mercury methylation in the Lake Michigan sediments. After adding acetate as a carbon substrate, we observed a rapid acetate consumption in the first 20 days of incubation, while the MeHg level increased from 2 to 300 pg/g soil. Ammonium addition had little effect on carbon degradation or mercury methylation, suggesting these processes are not nitrogen limited. In iron oxide-enriched sands (~1% in total dry weight), however, we observed an inhibition of MeHg formation by up to 90%, suggesting a strong negative effect of iron oxide minerals on methylation. This study highlights a few key biogeochemical factors that control mercury methylation in the Lake Michigan sand dune, which could provide new insights into understanding carbon and mercury cycling in the Great Lakes ecosystems.



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R. Seth Wood, *Graduate student*

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Christopher Yen, *Ph.D. student*

# Author index

*alphabetical by surname*

<u>Name</u>	<u>Affiliation(s)</u>	<u>Abstract(s)</u>
Karen C. Abbott	Case Western Reserve University	Smith et al.
Ricardo A. Astini	Centro de Investigaciones en Ciencias de la Tierra	Mors et al. (Gomez*)
Sarah C. Bagby	Case Western Reserve University	Smith et al.
Azam Baharlouei	Southern Illinois University Carbondale	Baharlouei and Hamilton-Brehm
Buz Barstow	Cornell University	Trutschel et al.
Anna Bennett	University of Minnesota	Bennett et al.
Jennifer G. Blank	Blue Marble Space Institute of Science	Selensky et al.
Jean E. Bogner	University of Illinois at Chicago	Malas et al.
Alice Bosco-Santos	Indiana University—Purdue University Indianapolis University of Campinas	Fouskas et al.
Alexander S. Bradley	Washington University in St. Louis	Suess et al.
Simon C. Brassell	Indiana University Bloomington	Doiron et al.
Roger N. Bryant	The University of Chicago Washington University in St. Louis	Bryant et al.
Caitlin Casar	Northwestern University	Casar and Osburn
Isla S. Castañeda	University of Massachusetts Amherst	Sae-lim et al.
Jeffrey G. Catalano	Washington University in St. Louis	Kupper et al. Yan et al.
Clara Chan	University of Delaware	Kupper et al.
Joanne C. Chee-Sanford	University of Illinois at Urbana-Champaign	Chen et al.
Mingfei Chen	University of Illinois at Urbana-Champaign	Chen et al.
Maureen L. Coleman	The University of Chicago	Waldbauer et al.
Lynn M. Connor	University of Illinois at Urbana-Champaign	Chen et al.
Jessica L. Conroy	University of Illinois at Urbana-Champaign	Chen et al.
Kelsey E. Doiron	Indiana University Bloomington	Doiron et al.
William Dowd	Lehigh University	Schuler et al.
Gregory K. Druschel	Indiana University—Purdue University Indianapolis	Howard and Druschel
David A. Fike	Washington University in St. Louis	Bryant et al. Houghton et al. Richardson et al. Suess et al. Wood et al.
Gabriel M. Filippelli	Indiana University—Purdue University Indianapolis	Smart et al.
Gracie A. Fischer	University of Illinois at Chicago	Malas et al.

Elaine D. Flynn	Washington University in St. Louis	Yan et al.
Fotios Fouskas	Indiana University—Purdue University Indianapolis	Fouskas et al.
Daniel E. Giammar	Washington University in St. Louis	Yan et al.
William P. Gilhooly, III	Indiana University—Purdue University Indianapolis	Fouskas et al. Smart et al.
Maya L. Gomes	Johns Hopkins University	Bryant et al.
Fernando Gomez	Washington University in St. Louis Centro de Investigaciones en Ciencias de la Tierra	Mors et al. (Gomez*)
Chris Grooms	Queen's University	Sae-lim et al.
Trinity Hamilton	University of Minnesota	Bennett et al. Kuether et al.
Scott D. Hamilton-Brehm	Southern Illinois University Carbondale	Baharlouei and Hamilton-Brehm
Luke Hanley	University of Illinois at Chicago	Pasterski et al.
Jeff Havig	University of Minnesota	Bennett et al. Kuether et al.
Britt Hedman	SLAC National Accelerator Laboratory	Richardson et al.
Keith O. Hodgson	SLAC National Accelerator Laboratory	Richardson et al.
Jennifer Houghton	Washington University in St. Louis	Houghton et al. Wood et al.
Chase S. Howard	Indiana University—Purdue University Indianapolis	Howard and Druschel
Matthew T. Hurtgen	Northwestern University	Sarvian et al.
Jack Hutchings	Washington University in St. Louis	Sae-lim et al.
Raisa Islam	Iowa State University	Islam et al.
Andrew D. Jacobson	Northwestern University	Sarvian et al.
Clive Jones	Washington University in St. Louis	Suess et al. Wood et al.
Peter K. Kang	University of Minnesota	Schuler et al.
Fabien Kenig	University of Illinois at Chicago	Pasterski et al.
Sarah Khoury	University of Illinois at Chicago	Malas et al.
Bronwen Konecky	Washington University in St. Louis	Sae-lim et al.
Joshua E. Kuether	University of Minnesota	Kuether et al.
Robert J. Kupper	Washington University in St. Louis	Kupper et al.
Gabrielle Ledesma	Iowa State University	Islam et al.
WoongHee Lee	University of Minnesota	Schuler et al.
Aivo Lepland	Geological Survey of Norway	Wood et al.
Brian A. Lerch	University of North Carolina at Chapel Hill	Smith et al.
Judy Malas	University of Illinois at Chicago	Malas et al.
Adam C. Maloof	Princeton University	Sarvian et al.

D’Arcy Meyer-Dombard	University of Illinois at Chicago	Malas et al.
Jill M. McDermott	Lehigh University	Schuler et al.
Neil Michelutti	Queen’s University	Sae-lim et al.
Cecilia Mlewski	Centro de Investigaciones en Ciencias de la Tierra	Mors et al. (Gomez*)
R. Agustin Mors	Centro de Investigaciones en Ciencias de la Tierra	Mors et al. (Gomez*)
Senthil Murugapiran	University of Minnesota	Bennett et al.
Đường Nguyễn-Thùy	VNU University of Science	Doiron et al.
Hướng Nguyễn-Văn	VNU University of Science	Doiron et al.
Molly D. O’Beirne	University of Pittsburgh	Fouskas et al.
Ryan Ogliore	Washington University in St. Louis	Bryant et al.
Magdalena R. Osburn	Northwestern University	Casar and Osburn Sarvian et al. Selensky et al.
Jill D. Pasteris	Washington University in St. Louis	Bryant et al.
M. Joseph Pasterski	University of Illinois at Chicago	Pasterski et al.
Ian D. Patete	University of Illinois at Chicago	Malas et al.
Dean M. Peterson	University of Minnesota Duluth	Schuler et al.
Jocelyn A. Richardson	SLAC National Accelerator Laboratory	Richardson et al.
Sascha Roest-Ellis	University of Oxford	Richardson et al.
Annette Rowe	University of Cincinnati	Trutschel et al.
Jarunetr (Nadia) Sae-lim	Washington University in St. Louis	Sae-lim et al.
Jeff Salacup	University of Massachusetts Amherst	Sae-lim et al.
Robert A. Sanford	University of Illinois at Urbana-Champaign	Chen et al.
Cara M. Santelli	University of Minnesota	Schuler et al.
Ritimukta Sarangi	SLAC National Accelerator Laboratory	Richardson et al.
Niloufar L. Sarvian	Northwestern University	Sarvian et al.
Daniele Scarponi	University of Bologna	Houghton et al.
Arndt Schimmelmänn	VNU University of Science	Doiron et al.
Christopher J. Schuler	University of Minnesota	Schuler et al.
Matthew J. Selensky	Northwestern University	Selensky et al.
Neha Sharma	Washington University in St. Louis	Yan et al.
Cody Sheik	University of Minnesota Duluth	Schuler et al.
Matthew S. Smart	Indiana University—Purdue University Indianapolis	Smart et al.
Derek A. Smith	Case Western Reserve University	Smith et al.
John Smol	Queen’s University	Sae-lim et al.
Melanie K. Suess	Washington University in St. Louis	Suess et al.
Elizabeth D. Swanner	Iowa State University	Islam et al.

Michael Tanzillo	University of Illinois at Chicago	Malas et al.
Aaron Thompson	University of Georgia	Kupper et al.
Brandy M. Toner	University of Minnesota	Schuler et al.
Marta Torres	Oregon State University	Wood et al.
Nicholas J. Tosca	University of Oxford	Richardson et al.
Leah Trutschel	University of Cincinnati	Trutschel et al.
Petra Vaiglova	Washington University in St. Louis	Vaiglova
Amy Cheng Vollmer	Swarthmore College	Vu and Vollmer
Linda Vu	University of Cincinnati	Vu and Vollmer
Jacob R. Waldbauer	The University of Chicago	Waldbauer et al.
Samuel M. Webb	SLAC National Accelerator Laboratory	Richardson et al.
Josef P. Werne	University of Pittsburgh	Fouskas et al.
Jessica H. Whiteside	University of Southampton	Smart et al.
Raveendra Wickramasinghe	University of Illinois at Chicago	Pasterski et al.
Chad A. Wittkop	University of Minnesota	Islam et al.
R. Seth Wood	Washington University in St. Louis	Wood et al.
Jinshu Yan	Washington University in St. Louis	Yan et al.
Ziming Yang	Oakland University	Zaporski and Yang
Jared Zaporski	Oakland University	Zaporski and Yang
Lichun Zhang	The University of Chicago	Waldbauer et al.
Nanqing Zhou	University of Delaware	Kupper et al.
Amy E. Zimmerman	The University of Chicago	Waldbauer et al.



# Notes

