

7

th Annual

Argonne National Laboratory Soil Metagenomics Meeting

OCTOBER 21st- 23rd, 2015

Lisle/Naperville Hilton Hotel
Lisle, IL



Acknowledgements



illumina®

We would like to thank our sponsors for
their generous support

ArgonneSoilMetagenomicsMeeting2015

October 21, 2015

Welcome to the 7th Annual Argonne Soil Metagenomics Meeting!

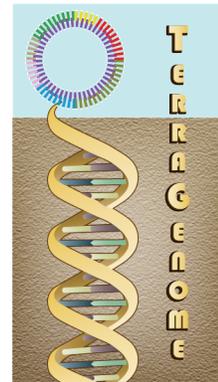
The aim of this series of meetings has been to incite discussion on the challenges and opportunities afforded by next-generation DNA sequencing and associated 'omics approaches to understand the complex nature of soil microbial communities. This meeting aims to serve the research community by providing a venue for interaction among ecologists, microbiologists, bioinformaticists, and 'omics enthusiasts — all focused on the wonders of life in soil. Through oral presentations, posters, discussion sessions, and participation by industry sponsors, the meeting will cover any aspect of soil metagenomics research, from technical development to data analysis to science breakthroughs.

We hope you share your research, meet some new folks, learn something new, and enjoy the meeting!

Sincerely,

The Organizing Committee

Sarah O'Brien, Chair
Sarah Owens
Darlyn Mishur
Dionysios Antonopoulos
Folker Meyer
Will Trimble
David Myrold



AGENDA

Wednesday, October 21

7:30 **Registration**
Continental breakfast

8:15 **Introduction & announcements**

Session I: The Underexplored Biosphere

8:30 *Ecological and evolutionary insight into the persistence of soil bacteria*
Jay T. Lennon, Stuart E. Jones

8:50 *Phylogenetic distribution of resuscitation genes in soil microbes*
Fan Yang, Stuart E. Jones, Jay T. Lennon, Adina Howe

9:10 *Molecular hybrid synthetic long reads reconstruct full genomes from the rare biosphere with functional potential elucidated by high resolution mass spectrometry*
Richard Allen White III, Eric M. Bottos, Taniya Roy Chowdhury, Carrie D. Nicora, Kristin Burnum-Johnson, Lee Ann McCue, Erin Baker, Janet K. Jansson

9:30 *Uncovering Earth's virome*
David Paez-Espino, Alexander D. Thomas, Emiley Eloie-Fadrosh, Marcel Huntemann, Amrita Pati, Edward Rubin, Natalia N. Ivanova, Nikos C. Kyrpides

9:50 *Moldy dark matter: Inferring the taxonomic and functional diversity of soil fungi through a combined meta-'omic strategy*
J.R. Herr

10:10 *Soil, lateral gene transfer, and hybrid genomes—Invited*
Robert G. Beiko

10:40 Break

Session II: Breakthroughs in Informatics

11:00 *Accurate detection and quantification of target genes in shotgun metagenomes: Method development and application to the nitrogen cycle genes*
Luis H. Orellana, Luis M. Rodriguez-R, Kostas T. Konstantinidis

11:20 *Hunting down frame shifts: Processing amplicon data of biphenyl and benzoate dioxygenase genes*
Michal Strejcek, Tomas Macek, Ondrej Uhlik

11:40 *RefSoil: Continuous progress in reference soil database*
Jinlyung Choi, Erick Cardenas, Ryan Williams, Adina Howe

12:00 Discussion

12:15 **Lunch**

AGENDA

1:30 **Posters I**

Session III: Frozen(ish) Soils

3:00 *Soil microbial communities as sentinels of environmental change—
Invited*

Martin Hartmann

3:30 *Control of boreal forest soil decomposition processes by plant secondary
compounds*

Mary-Cathrine Leewis, Mary Beth Leigh

3:50 *Serving as a glimpse into the past, Alaskan paleosols harbor unique
microbial diversity*

Robyn A. Barbato, Natalia Vinas, Robert Jones, Karen Foley, Thomas
Douglas, Julie Esdale, Edward Perkins

4:10 *The genetic potential for siderophore-mediated dissimilatory iron
reduction in arctic soil microbial communities*

Archana J. Srinivas, Elizabeth A. Dinsdale, David A. Lipson

4:30 *Frozen in time? Microbial strategies for survival and carbon metabolism
over geologic time in a Pleistocene permafrost chronosequence*

Rachel Mackelprang, Thomas A. Douglas, Mark P. Waldrop

4:50 Discussion

Group photo

5:00 **Mixer – cash bar**

6:30 **Plenary session – Keynote talk and buffet dinner**

What do 'omics do best?

David D. Myrold

Thursday, October 22

7:30–8:30 **Continental breakfast**

Session IV: Microbial members of the ecosystem

8:30 *Identifying factors controlling methane emissions across freshwater
wetland gradients—Invited*

Kelly Wrighton

9:00 *Composition of bacterial communities tracks salinity and flooding
gradients*

Pamela Weisenhorn, Jack A. Gilbert, Loretta Battaglia

AGENDA

- 9:20 *Soil bacterial and fungal community dynamics during conversion of Conservation Reserve Program (CRP) fields to cropping systems*
Mamatha Kakarla, Jennifer Moore-Kucera, Lisa Fultz, Chenhui Li, Veronica Acosta-Martinez, Jon Zak, Kameswara Rao Kottapalli
- 9:40 *Diverse carbon utilization from field collected rhizosphere soils using paired metagenomic and metatranscriptomic approaches*
Aaron Garoutte, Jim Tiedje

10:00 Break

- 10:20 *Using critical zone observatories to understand how microbial community structure changes with elevation*
Chelsea Carey, Stephen C. Hart, Clifford Riebe, Sarah Aciego, Molly Blakowski, Emma Aronson

10:40 *The importance of dispersal in microbial communities—Invited*
Kabir G. Peay

11:10 Break

Session V: If a tree falls in the forest, do the microbes respond?

- 11:30 *Forest harvesting affects the soil microbial diversity of nitrogen- and carbon-cycling genes: A study of five ecozones across North America*
Erick Cardenas, Kendra Maas, Luis Orellana, Kostas T. Konstantinidis, William W. Mohn

11:50 *Structure and activity of denitrifier communities in biochar-amended soil and their impact on N₂O emissions*
Johannes Harter, Ivan Guzman Bustamante, Stefanie Kühfuß, Reiner Ruser, Reinhard Well, Oliver Spott, Mohamed El-Hadidi, Daniel Huson, Andreas Kappler, Sebastian Behrens

12:10 *Stable isotope probing reveals long-term impacts of timber harvesting on cellulolytic soil community*
Roland Wilhelm, Erick Cardenas, Hilary Leung, András Szeitz, Lionel D. Jensen, William W. Mohn

12:30 *Linking ecosystem processes to individual microbes in forest soils*
Petr Badrian, Salvador Lladó-Fernández, Rubén López-Mondéjar, Tomás Vetrovsky, Adina Chuang, Howe, Katharina Riedel, Lucia Zifcáková

12:50 Discussion

1:00 **Lunch**

2:00 **Posters II**

AGENDA

Session VI: Microbes in a changing world

- 3:30 *Soil microbial community responses to warming as revealed by comparative metagenomics—Invited*
Eric Johnston, Chengwei Luo, Luis M. Rodriguez-R, Liyou Wu, Shi Zhou, Kai Xue, Zhili He, Mengting Maggie Yuan, Yiqi Luo, Edward A.G. Schuur, James R. Cole, James M. Tiedje, Jizhong Zhou, Kostas Konstantinidis
- 4:00 *Increased soil C storage under anthropogenic N deposition: lignocellulolytic bacterial ascendance in a fungal world*
Zachary B. Freedman, Rima A. Upchurch, Donald R. Zak, Lauren C. Cline
- 4:20 *Changes in microbial community structure and function in response to long-term N addition as revealed by soil metaproteomics—Invited*
Katharina M. Keiblinger, Stefan J. Forstner, Stefanie Kloss, Stephan Fuchs, Kathrin Riedel, Martin H. Gerzabek, Sophie Zechmeister-Boltenstern
- 4:50 *Methylophony and archaeal heterotrophy dominate processing of sub-root soil carbon*
Cristina N. Butterfield, Zhou Li, Peter Andeer, Susan Spaulding, Trent Northen, Brian C. Thomas, Chongle Pan, Robert Hettich, K. Blake Suttle, Susannah Tringe, Jillian F. Banfield
- 5:10 *Why is it so difficult to incorporate metagenomics into traditional soil models? —Invited*
Kathe Todd-Brown
- 5:40 Discussion
- 6:00 *Dinner on your own*

Friday, October 23

7:30–8:30 **Continental breakfast**

Session VII: Breakthroughs at the bench

- 8:30 *Single cell genomics: From science fiction to mainstream microbiology—Invited*
Ramunas Stepanauskas
- 9:00 *BacEx segregates microbial DNA from eukaryotic DNA “clutter” allowing for more efficient microbiome sequencing*
Robert T. Yamamoto, Allyn Forsyth
- 9:20 *Deconstructed PCR: Improved library preparation tools for high throughput amplicon sequencing*
Stefan J. Green, Ankur Naqib

AGENDA

9:40 *Advanced omic approaches to soil microbe characterization—Invited*
Mary S. Lipton, Carrie D. Nicora, Thomas O Metz, Erin Baker, Richard
A. White III, Taniya Roy Chowdhury, Eric M. Bottos, Jennifer E. Kyle,
Colin J. Brislawn, Kristin E. Burnum-Johnson, Samuel Payne, Chris
Whidbey, Natalie C. Sadler, Janet K. Jansson

10:10 Discussion

10:20 Break

Session VIII: Co-occurrence and interactions among microbes

10:40 *Microbial soil community responses to extreme disturbance: Insights
from the ongoing subterranean Centralia coal mine fire—Invited*
Ashley Shade

11:10 *Investigating microbial co-occurrence patterns based on metagenomic
compositional data*

Yuguang Ban, Lingling An, Hongmei Jiang

11:30 *Elucidating microbial interactions that drive decomposition through a
co-occurrence framework*

Ryan J. Williams, Kirsten S. Hofmockel, Adina Howe

11:50 *Modeling the Pseudomonas fluorescens sulfur regulome*

Sarah Zerbs, Peter Korajczyk, Frank R. Collart, Philippe Noirot, Peter
E. Larsen

12:10 *Molecular mechanisms of a plant-fungus-bacterial community
interaction*

Shalaka Shinde, Sarah Zerbs, Peter E. Larsen, Jonathan R. Cumming,
Frank R. Collart, Steve J. Callister, Philippe Noirot

12:30 *Rhizosphere versus endosphere bacterial microbiomes: How much do
they overlap?*

Marketa Polivkova, Lucie Musilova, Michal Strejcek, Jachym Suman,
Tomas Macek, Ondrej Uhlik

12:50 Discussion

1:15 **Adjourn**

Lunch

AGENDA

Posters

Session I: Titles with odd numbers

Session II: Titles with even numbers

- 1. High resolution DNA stable isotope probing reveals that root exudate addition to soil changes the identity of the microbes that degrade cellulose but not the rate of degradation**
Ashley N. Campbell, Charles Pepe-Ranney, Anh Vinh T. Nguyen, and Daniel H. Buckley
- 2. Capturing the spatial variability of microbial communities within agricultural soils**
Sarah Castle, Jessica Gutknecht, Linda Kinkel, Carl Rosen, Michael Sadowsky, Deborah Samac
- 3. Plant-microbe interactions: The *Populus* atlas**
Melissa Cregger, Miranda Crouch, Zamin Yang, Dale Pelletier, Rytas Vilgalys, Chris Schadt
- 4. Effect of a changing in the aboveground plant community on the soil physicochemical properties and microbial community**
Marie Duhamel, Kabir Peay
- 5. Multi-year functional soil metagenomic characterization of cellulosic bioenergy feedstock production systems**
DS Duncan, JM Tiejde, GP Robertson, RD Jackson
- 6. Microbial community arsenic tolerance and the fate of arsenic in soil**
Taylor K Dunivin, John Chodkowski, Jackson Sorensen, Ashley Shade
- 7. Niche and neutral community dynamics in parallelized aerobic, single carbon-source enrichment cultures**
Theodore M. Flynn, Jason C. Koval, Kenneth M. Kemner, Dionysios A. Antonopoulos
- 8. Salinity effects in soil microbial communities, enzyme activity and carbon biogeochemistry in tidal freshwater wetlands**
Georgios Giannopoulos, Dong Yoon Lee, Olivia De Meo, Scott Neubauer, Bonnie Brown, Rima Franklin
- 9. Metagenomics and physiology based analysis of bacterial communities from differently managed agricultural soils**
Timothy Gsell and Craig Sweet

AGENDA

- 10. Study of seasonal variation of desert keratinophilic fungi**
P. Hamm, R. C. Mueller, J. Belnap, C. R. Kuske, A. Porras-Alfaro
- 11. Biogeography and metabolism of dominant bacterial populations in tundra soils**
Eric R. Johnston, Luis M. Rodriguez-R, Liyou Wu, Zhili He, Edward A.G. Schuur, Yiqi Luo, James M. Tiedje, Jizhong Zhou, and Konstantinos T. Konstantinidis¹
- 12. Manipulating soil temperature and moisture to uncover microbial community trends for modelling purposes**
Robert M Jones, Karen L Foley, Charles M Reynolds, Robyn A Barbato
- 13. Meta- and isolate- genomic analyses of grassland soil microbial communities**
Ulas Karaoz, Heejung Cho, Kateryna Zhalnina, Mary Firestone, Eoin Brodie
- 14. Understanding microbial community responses and limitations to the SPRUCE deep peat heat experiment**
Laurel Kluber, Samantha Allen, J. Nicholas Hendershot, Paul Hanson, Christopher Schadt
- 15. Plant response to various phosphorus sources under nutrient limitation**
Peter Korajczyk, Shalaka Shinde, Sarah Zerbs, Frank R. Collart, Philippe Noirod
- 16. The influences of thorny bamboo growth on the bacterial community in badland soils**
Yu-Te Lin and Chih-Yu Chiu
- 17. Metagenomic and comparative analysis in agricultural and mining soils in Guanajuato, Mexico**
María Elena López-Pérez, Gabriela Ana Zanol, María Cristina del Rincón-Castro
- 18. Soil bacterial community structure and function shift along a successional series of tidal flats in the Yellow River Delta**
Xiaofei Lyu, Junbao Yu, Bin Ma, Scott Chang
- 19. Microbial ecology of restored floodplains**
Christopher W Marshall, Sarah L O'Brien, Kenneth M Kemner, Edward J O'Loughlin, Neil R Gottel, Silvia Alvarez Clare, Aaron A Best, Theodore M Flynn, and Jack A Gilbert
- 20. Spatio-temporal dynamics of Phymatotrichopsis root rot disease in alfalfa hay production fields**
Chakradhar Mattupalli, Casey Curtsinger, Corey A. Moffet, James K. Rogers, Carolyn A. Young

AGENDA

- 21. Conversion of Amazon rainforest to agriculture alters community traits of methane-cycling organisms**
Kyle Meyer, Ann Klein, Jorge Rodrigues, Klaus Nüsslein, James M. Tiedje, Titus Brown and Brendan J.M. Bohannon
- 22. Proteomics of soil and sediment: Protein identification by *de novo* sequencing of mass spectra complements traditional database searching**
Samuel Miller, Adriana Rizzo, Jacob Waldbauer
- 23. BAC Sudoku sequencing strategy for *in silico* screening of large-insert soil metagenomic libraries**
Scott Monsma, Jinglie Zhou, Alinne Pereira, Blaine Pfeifer, Timothy Bugni, Scott R. Santos, Megan Niebauer, Erin Ferguson, Ron Godiska, ChengCang Wu, David Mead, and Mark R. Liles
- 24. Bacterial and archaeal ammonia oxidizers are reduced by increasing timber harvest intensity in surface and subsurface soils of the western Gulf Coastal Plain**
Ryan M. Mushinski, Thomas W. Boutton, and Terry J. Gentry
- 25. Distribution of fungi in arid microenvironments and their potential role on plant growth**
Cedric Ndinga Muniania, Katrina Sandona, Jayne Belnap, Cheryl R. Kuske, Andrea Porras-Alfaro
- 26. Agricultural nitrogen management affects response to ammonium and ammonia oxidizer communities**
Jeanette Norton, Yan Ouyang, John Stark, Mussie Habteselassie
- 27. Imaging soil bacteria *in situ***
Sarah L. O'Brien, Matthew D. Whiteside, Deirdre Sholto-Douglas, Alice Dohnalkova, Daniel Durall, Doga Gursoy, Melanie D. Jones, Libor Kovarik, Barry Lai, Christian Roehrig, Shane Sullivan, Stefan Vogt, Kenneth Kemner
- 28. Effects of soluble electron shuttles on microbial Fe(III) reduction and methanogenesis in wetland sediments**
Edward J. O'Loughlin, Margaret F. Sladek, Dionysios A. Antonopoulos, Theodore M. Flynn, Jason C. Koval, Christopher W. Marshall, and Kenneth M. Kemner
- 29. Exploring Verrucomicrobia populations in grassland soil using Molecu**
Sarah M. Owens, William L. Trimble, Sarah L. O'Brien, Stephanie M. Greenwald, Dionysios A. Antonopoulos, and Folker Meyer

AGENDA

- 30. The role of environmental and genetic factors in shaping the microbiome of a highly olfactory bird species**
Douglas S. Pearce, Brian Hoover, Gabrielle A. Nevitt and Kathryn Docherty
- 31. Comparative analysis of metatranscriptomes provide insights of microbial communities under warming conditions**
William Rodriguez, Lauren Alteio, Grace Pold, Linda van Diepen, Serita Frey, Jerry Melillo, Kristen DeAngelis, Jeffrey Blanchard
- 32. *Nicotiana* roots recruit rare rhizosphere taxa as major root-inhabiting microbes**
Muhammad Saleem, Audrey D. Law, Luke A. Moe
- 33. Fungal community diversity in long-term biochar amended forest soils**
Jessica Sarauer, Amy Ross-Davis, Mark Coleman
- 34. Discovering thermophilic diversity in temperate soils affected by a subterranean coal fire**
Jackson Sorensen, Sang-Hoon Lee, Ashley Shade
- 35. The effects farming practices on fungal communities associated with *Glycine max* (soybeans)**
Terri Tobias, Sara Dean, Winthrop Phippen, Joel Gruver, Andrea Porras-Alfaro
- 36. Heavy metal tolerant fungal community analysis from temperate pine forest soil using Illumina sequencing**
Terry Torres Cruz, Cedar Hesse, Cheryl Kuske, Andrea Porras-Alfaro
- 37. The diversity of metagenomes in MG-RAST**
William L. Trimble, Travis Harrison, Jared Bischof, Kevin P. Keegan, Folker Meyer
- 38. Who's on first? Bacterial and fungal colonization of fresh soil minerals**
Thea Whitman, Rachel Neurath, Ping Zhang, Tong Yuan, Joe Zhou, Peter Weber, Jennifer Pett-Ridge, Mary Firestone
- 39. Soil microbial community composition affects the weed suppression potential of green manures**
Anthony Yannerell, Yi Lou
- 40. How does microbial community composition and function change in ageing primary boreal forest ecosystems?**
Stephanie A. Yarwood, Tamara Walsky, Mona N. Högberg

AGENDA

41. Identifying *Pseudomonas fluorescens* genetic resources for metabolism of diverse sulfur nutrients

Sarah Zerbs, Peter Korajczyk, Frank R. Collart, Philippe Noirot, and Peter E. Larsen

42. Active, Near-Surface Methanogens Contribute to Greater Methane Emissions in Exposed Mud Flats in a Freshwater Wetland

J. C. Angle, A. B. Narrowe, M. A. Jackson, G. J. Smith, M. D. Johnston, K. C. Stefanik, D. N. Marcus, R.A. Daly, R. T. McVeety, M. J. Wilkins, C. S. Miller, and K.C. Wrighton

43. Peatland microbial community responses to plant functional group, water table and peat depth

L.J. Lamit, J.T. Lennon, E.A. Lilleskov

44. The Effect of Root Exudate 7,4'-dihydroxyflavone and Naringenin on Soil Bacterial Community Structure

Márton Szoboszlay, Alison White-Monsant, Luke A. Moe

ABSTRACTS

Active, near-surface methanogens contribute to greater methane emissions in exposed mud flats in a freshwater wetland

J. C. Angle, A. B. Narrowe², M. A. Jackson¹, G. J. Smith¹, M. D. Johnston¹, K. C. Stefanik¹, D. N. Marcus¹, R.A. Daly¹, R. T. McVeety¹, M. J. Wilkins¹, C. S. Miller², and K.C. Wrighton¹

¹Department of Microbiology, The Ohio State University, Columbus, Ohio 43210

²Department of Biology, University of Colorado Denver, Denver, Colorado 80204

Concentrations of atmospheric methane, a potent greenhouse gas with climate forcing 26 times stronger than carbon dioxide, are increasing in response to climate change. Despite temperate terrestrial wetlands representing the largest source of methane to the atmosphere, we know little about the microbial factors that control methane release. At a temperate wetland field site on Lake Erie, we observed the 16S rRNA gene dominance of a single *Methanosaeta* OTU in shallow surface exposed mudflats. The detection of near-surface methanogens motivated us to investigate methane production potential activity across three horizontal microsites and two vertical depths across the wetland. On average, exposed mudflats emitted six times and two times more methane than plant covered and open water microsites respectively over a two-year period. The greatest *in situ* sediment pore-water methane concentration was observed in mudflat surface samples, which was statistically greater than other shallow samples from plant and open water microsites and also greater than deeper (>23 cm) samples from all microsites. Consistent with these findings, 40-day laboratory methane production enrichments revealed the greatest methane production from surface mudflats and surface open water samples. These values were statistically greater than those from plant surface samples, and all deeper locations across the sampling transect, which were uniformly exhausted at 5.2 $\mu\text{g}\pm 0.0$ CH₄-C per gram sediment. We hypothesize that elevated pore water sulfate concentrations in plant surface sediments competitively inhibit methanogenesis, while methanogenesis at depth may be constrained by reduced substrate availability. Ongoing analysis of microbial 16S rRNA and *mcrA* transcripts will characterize the distribution and activity of methanogens across these spatial gradients. Together these results demonstrate that methanogenic populations are present and active in the shallow fraction (first 5 cm) of exposed mudflats, which may significantly contribute to methane release from these microsites across wetland ecosystems.

Linking ecosystem processes to individual microbes in forest soils

Petr Baldrian¹, Salvador Lladó-Fernández¹, Rubén López-Mondéjar¹, Tomáš Větrovský¹, Adina Chuang Howe², Katharina Riedel³, Lucia Žifčáková¹

¹Laboratory of Environmental Microbiology, Institute of Microbiology of the ASCR, Prague, Czech Republic; ²Iowa State University, Ames, USA; ³Ernst-Moritz-Arndt Universität Greifswald, Germany

Microbes are important drivers of soil processes in forest soils that mediate decomposition as well as nutrient transfer from primary producers into soil. Metatranscriptomics,

metaproteomics or enzyme activity assays can give us a fair picture about the functioning of whole microbial communities, but they are per se not able to identify individual microbial species, participating in the soil processes. The aim of this work was to address the functional potential of dominant microbial species in forest topsoil and to track their real activity in the ecosystem by the combination of shotgun metatranscriptomics, strain isolation and characterization and proteomics of their cultures. This helped us to define the roles of dominant microbes in forest topsoil and to analyze whether their transcription *in situ* differs among horizons and seasons with tree photosynthetic activity and no activity. Isolation has yielded 20 bacterial strains representing some of the dominant molecular OTU from the ecosystem, some of them likely represent novel taxa at the genus level. Among dominant bacteria, interestingly, strains belonging to Acidobacteria showed the highest production of decomposition-related enzymes and the highest counts of glycosyl hydrolases in genomes, in contrast to Bacteroidetes and Proteobacteria. When metatranscriptomic reads were mapped to the genome sequences obtained, it was recorded that their transcript profiles *in situ* differ among horizons and among seasons. The same was noted for fungi from the same ecosystem. Furthermore, culture-based studies followed by proteomics of bacteria showed that by far not all predicted genes involved in decomposition are expressed in the presence of their substrates and that their decomposition systems are variable and complex, and may contain components that were previously not noticed. The results show that a combination of contemporary methods is able to answer the question about the role of individual taxa in their environment.

Investigating microbial co-occurrence patterns based on metagenomic compositional data

Yuguang Ban¹, Lingling An^{2,3}, Hongmei Jiang¹

¹Department of Statistics, Northwestern University, Evanston, IL 60208, USA;

²Interdisciplinary Program in Statistics, University of Arizona, Tucson AZ 85721, USA;

³Department of Agricultural and Biosystems Engineering, University of Arizona, Tucson AZ 85721, USA

The high-throughput sequencing technologies have provided a powerful tool to study the microbial organisms living in various environments. Characterizing microbial interactions can give us insights into how they live and work together as a community. Metagenomic data are usually summarized in a compositional fashion due to varying sampling/sequencing depths from one sample to another. We study the interaction patterns of microbial organisms using their relative abundance information. Analyzing compositional data using conventional correlation methods has been shown prone to bias that leads to artifactual correlations. We propose a novel method, REBACCA, to identify significant co-occurrence patterns by finding sparse solutions to a system with a deficient rank. To be specific, we construct the system using log ratios of count data and solve the system using the l_1 -norm shrinkage method. Our comprehensive simulation studies show that REBACCA achieves higher accuracy in general when a sparse condition is satisfied and runs considerably faster than the existing comparable method. The proposed method is also applied to several real metagenomic datasets.

Serving as a glimpse into the past, Alaskan paleosols harbor unique microbial diversity

Robyn A. Barbato¹, Natalia Vinas², Robert Jones¹, Karen Foley¹, Thomas Douglas³, Julie Esdale⁴, Edward Perkins²

¹US Army Cold Regions Research and Engineering Laboratory, Hanover, NH

²US Army Environmental Laboratory, Vicksburg, MS

³US Army Cold Regions Research and Engineering Laboratory, Fairbanks, AK

⁴ US Army Alaska, Fairbanks, AK

Climate projections for the 21st century indicate the potential for a pronounced warming in the Arctic and sub-Arctic regions. In particular, interior Alaska is expected to warm approximately 5°C by 2100, changing the landscape significantly. The warmer temperatures and altered precipitation regime are expected to initiate changes to soil biogeochemical attributes in addition to the biodiversity of plants and animals in these ecosystems. Since paleosols formed long periods ago, they may offer insight into the microbial diversity of the past, similar to syngenetic permafrost sediment and ice features that have trapped biochemical evidence of an environment at that time. We hypothesized that the microbial communities in the paleosols were significantly different than nearby soils, mainly due to the unique system inputs at the time the feature formed. Our objective was to investigate microbial diversity from soils collected around and within an exposed bluff in interior Alaska. Of particular interest was the krotovina, an ancient rodent nest, near the bottom of the bluff, which would offer unique carbon substrates available to nearby microbes. Following extraction of genomic DNA, the 16S rRNA gene was sequenced and analyzed using the QIIME pipeline. Our results revealed that while there were common phyla present in these cold-adapted soils, the microbial communities in the paleosols and the krotovina were significantly different than those from nearby surface soils. In fact, the microbial communities in the krotovina were most discrete when compared to the other soils. A high abundance of sequences related to Actinobacteria in these older soils suggested the potential for more bioactive metabolites such as antibacterials and antifungals in that time frame. We suspect that local carbon substrates and micronutrients, rather than weather-related conditions influenced community composition in these soils. Metagenomic analysis of these soils would offer a more informed view of past environmental conditions in interior Alaska.

Soil, lateral gene transfer, and hybrid genomes.

Robert G. Beiko,

Faculty of Computer Science, Dalhousie University, Halifax, Nova Scotia, Canada

Lateral gene transfer (LGT) is the phenomenon whereby genetic material is transferred from a donor organism to a recipient. Successful LGT events require opportunity (for example, physical proximity), mechanisms such as plasmid conjugation, and recombination and persistence in the genome. LGT can dramatically reshape the biochemical capacities of an organism; recent work suggests a central role for LGT in extremophile evolution, and very recent events that have affected critical pathways in synthetic communities.

Earlier results suggested that gene transfer rates in soil acidophiles are also very high. In particular, the phylogenetic affinities of genes in *Acidithiobacillus* were so different that it could not be reliably assigned to any proteobacterial class. Dozens of other organisms, and notably other sulphur-oxidizing acidophiles previously classified in the same genus as *Acidithiobacillus*, were implicated in a wide range of gene-sharing relationships. However, the organisms considered were not necessarily sampled from the same location, and the question of LGT among co-occurring organisms remains.

To address this question in a different manner, I examined the diversity of 16S ribosomal RNA gene sequences from a single sample of acidic soil, and identified reference genomes with very similar 16S sequences to serve as proxies for the inhabitants of the soil. Comparative genomics and phylogenetics suggested a high degree of gene sharing amongst the proxy organisms, including the two most abundant genera, *Acidobacterium* and *Terriglobus*. These results suggest that LGT contributes to an “ecology of genes” that links members of a microbial community. Precise answers about the role of LGT will require the recovery of genomes from the microorganisms in a given sample, but mosaic genomes will present unique challenges to read classification and assembly.

Methylotrophy and archaeal heterotrophy dominate processing of sub-root soil carbon

Cristina N. Butterfield¹, Zhou Li², Peter Andeer³, Susan Spaulding¹, Trent Northen³, Brian C. Thomas¹, Chongle Pan², Robert Hettich², K. Blake Suttle⁴, Susannah Tringe⁵, Jillian F. Banfield¹

¹Department of Earth and Planetary Science, University of California Berkeley, Berkeley, California; ²Oak Ridge National Laboratory, Oak Ridge, Tennessee; ³Lawrence Berkeley National Laboratory, Berkeley, California; ⁴Department of Ecology and Evolutionary Biology, University of California Santa Cruz, Santa Cruz, California; ⁵DOE Joint Genome Institute, Walnut Creek, California

Half of all photosynthesized carbon on Earth is deposited into soil. Much of this organic carbon is respired by microorganisms but little is known about the controls on carbon export from the root zone. We studied the microbial response to a carbon pulse mediated by the first fall rain event in a 40 cm deep soil in grassland located in a Mediterranean climate in coastal Northern California. We collected data from ten samples from two regions below the root zone (10 – 20 cm and 30 – 40 cm) before the rain and several days after and used a genome-resolved approach to link active metabolic pathways (proteomics and metabolomics) to specific bacteria and archaea. All of the bacterial and archaeal phylogenetic trees displayed diversity patterns resembling dandelion seed heads radiating from the tips of every branch. These microbes respond positively to the extractable sugars and amino acids pulsed in by the rain as evidenced in the near-complete disappearance of metabolites at the deeper depth and the omnipresence of sugar and amino acid transporters in the proteomics data. The first rain event briefly disrupted the shallow community (the Verrucomicrobia and Actinobacteria decreased and the Archaea increased in abundance before returning to pre-rain abundance levels) while the deeper community was not similarly perturbed. In fact, heterotrophic Thermoplasmatales dominated all other microbial groups, and along with the rest of the Archaea, composed about 20% of the microbial community at the aerobic deeper soil depth. Notably, methylotrophic proteins from little-studied (but abundant) Gemmatimonadetes and Candidate Phylum Rokubacteria

top every proteomics abundance list, demonstrating that methyl containing compounds are a significant substrate in carbon biogeochemistry at these depths. These results link novel, diverse organisms to robust metabolic pathways and carbon compound dynamics in order to describe the fate of carbon exported out of this critical transition zone.

High resolution DNA stable isotope probing reveals that root exudate addition to soil changes the identity of the microbes that degrade cellulose but not the rate of degradation

Ashley N. Campbell^{1,2}, Charles Pepe-Ranney², Anh Vinh T. Nguyen², and Daniel H. Buckley²

¹Chemical Sciences Division, Lawrence Livermore National Laboratory, Livermore, California, USA; ²School of Integrative Plant Sciences, Cornell University, New York, USA

Plant roots release compounds, such as root exudates, which can alter soil organic matter (SOM) decomposition and have large impacts on soil carbon (C) retention. The changes in SOM turnover resulting from the addition of organic and/or inorganic substrates are termed 'priming effects'. In this study we examine the effects of root exudates on the priming of cellulose added as particulate organic matter. We amended soil microcosms with ¹³C-cellulose in the presence or absence of artificial root exudate additions and incubated for 45 days. Soils receiving the root exudate (RE) were given either one large dose or multiple, small doses of RE. We tracked operational taxonomic units (OTUs) assimilating ¹³C from cellulose (herein, 'responder') over time using DNA stable isotope probing coupled with next generation sequencing. All treatments exhibited the same pattern for the rate of cellulose-¹³C respiration over time, characterized by three phases, (I) increase in respiration rate between days 8-19, (II) decrease in rate between days 20-33, and (III) a plateau from days 34-47. However, cellulose responders were different depending on treatment and time of sampling (days 14, 28 and 45). We identified a total of 10,361 OTUs, of which there were 369 cellulose responders in the cellulose only treatment, 273 in the repeated, small dose RE treatment, and 358 in the RE single, large dose treatment. Most of the cellulose responders found in all treatments belonged to phyla *Bacteroidetes*, *Planctomycetes*, *Proteobacteria*, *Verrucomicrobia*, and *Chloroflexi*. The response time of phyla varies; for instance, more OTUs in *Bacteroidetes* were observed on day 14 and diminish with each subsequent sampling time. On the other hand, OTUs in *Verrucomicrobia* increased in response over time. Our study shows no priming effect resulting from the addition of root exudates, although the identity of the microbial mediators of cellulose decomposition varies in each treatment.

Abstract preparation by A.N.C. was performed under the auspices of the U.S. Department of Energy by LLNL under Contract DE-AC52-07NA27344.

Forest harvesting affects the soil microbial diversity of nitrogen- and carbon-cycling genes: A study of five ecozones across North America

Erick Cardenas¹, Kendra Maas¹, Luis Orellana², Kostas T. Konstantinidis², and William W. Mohn¹

¹University of British Columbia, ²Georgia Institute of Technology

Soil microbes are important mediators of nutrient cycling in forests, thus processes that affect them can potentially affect the health of the trees aboveground and vice versa. Since forests are slow to regenerate, long term studies are needed (and relevant) to understand the effect of harvesting on tree productivity, and on the phylogenetic and functional diversity of the soil microbial communities. We studied the effect of forest harvesting in five different ecozones from five sites of the Long Term soil productivity study of North America (LTSP). Samples (n=107) from both mineral and organic layers and from four different organic matter removal regimes were taken at each ecozone ten years after harvesting and replanting and their microbial community were analyzed using shotgun metagenomics. Metagenomes were used to specifically study genes involved in carbon and nitrogen cycle by comparing high-quality sequence reads against the Carbohydrate Active Enzyme (CAZy) database and a custom database of nitrogen cycling genes which included genes involved in nitrogen fixation, denitrification, ammonia oxidation, and dissimilatory nitrate reduction to ammonia.

Diversity analysis showed that harvesting tended to decrease the abundance of CAZy genes while increasing the abundance and diversity (Shannon index) of nitrogen cycling genes although the effects were not consistent across ecozones. Permutational multivariate analysis of variation attributed the variation in CAZy and nitrogen cycling gene profiles mostly to site and soil layer differences, and in few cases to differences in harvesting regimes. Harvesting predictors were not consistent across ecozones although eleven CAZy and nine nitrogen cycling genes were predictors of soil layer across the five ecozones. This study shows that harvesting can have long term effects that can potentially influence the soil productivity through nutrient cycling changes.

Using Critical Zone Observatories to understand how microbial community structure changes with elevation

Chelsea Carey^{1,2}, Stephen C. Hart^{3,4}, Clifford Riebe⁵, Sarah Aciego⁶, Molly Blakowski⁵, and Emma Aronson¹

¹ Department of Plant Pathology and Microbiology, University of California, Riverside, Riverside, CA, USA; ² Environmental Systems Program, University of California, Merced, Merced, CA, USA; ³ Life and Environmental Sciences, University of California, Merced, Merced, CA, USA; ⁴ Sierra Nevada Research Institute, University of California, Merced, Merced, CA, USA; ⁵ Department of Earth and Environmental Sciences, University of Michigan, Ann Arbor, MI, USA; ⁶ Department of Geology and Geophysics, University of Wyoming, Laramie, WY, USA

Biogeographic patterns and community responses to climate change can be assessed using elevational gradients, which function as space-for-time substitutions. While surveys across multiple elevations have frequently been used to determine climate and other

environmental controls on aboveground (e.g., plant) communities, only recently have microbial communities been investigated in the same way. As a result, our ability to generalize and predict trends in microbial structure (composition and diversity) across elevational gradients - and to infer the mechanisms that shape elevational biogeographic patterns over time - remains limited. In order to determine spatial and temporal patterns of soil microbial community structure in the Sierra Nevada, California, we sampled soil from four elevations (400 m to 2700 m) along the Southern Sierra Critical Zone Observatory. Ten replicate soil cores (0-10 cm depth) were taken from each site in April, June, July, and August 2014. Soil microbial DNA was extracted, amplified for the 16S rRNA gene (V3 - V4 region), and sequenced on an Illumina MiSeq. OTU richness, both within and among phyla, decreased non-linearly with elevation. In addition, PCoA of the UniFrac metric illustrated clustering of soil communities by elevation. Within a given site, however, both richness and community composition remained relatively stable over time, indicating that elevation was a stronger determinant of microbial community structure than sampling date. Approximately 150 to 250 OTUs persisted within a site across time (the “core” microbiome), while the number of shared OTUs across sites within a given date was much more variable (approximately 400 shared OTUs across sites in April and only 50 in June). Continuing research to investigate the consistency, potential driving mechanisms, and functional importance of these trends across multiple elevational gradients within the Critical Zone Observatory network, will help advance our knowledge of soil microbial structure in montane landscapes.

Capturing the spatial variability of microbial communities within agricultural soils

Sarah Castle¹, Jessica Gutknecht², Linda Kinkel¹, Carl Rosen², Michael Sadowsky^{2,3}, Deborah Samac^{1,4}

¹University of Minnesota, Dept. of Plant Pathology; ²University of Minnesota, Dept. of Soil, Water, and Climate; ³University of Minnesota, Biotechnology Institute; ⁴USDA-ARS, Plant Science Research Unit

Understanding patterns in spatial variability of soil microbial communities can provide important insights into the mechanisms that control ecosystem function. The level of replication required to adequately characterize the variability of soil communities across both small and large geographic and edaphic gradients, however, is not well understood. Here we asked: How extensive does sampling need to be in order to capture variability across different spatial scales? Furthermore, how much information do we lose by decreasing sampling effort?

To address this, we utilized a recently established agricultural research network in three geographically and edaphically distinct sites across Minnesota: Grand Rapids, Waseca, and Lamberton. At each site, we identified five 24 x 24 meter plots and collected surface soil from six individual locations per plot. Illumina sequencing of 16S rRNA genes (V4 region) was used to characterize sensitivity of community data to sample pooling (physical or computational). We rarefied individual samples and physical composites to a depth of 20,000 sequences per sample, and computational composites were created by first summing OTU occurrences across individual samples and then rarefying to this same depth.

Community richness and diversity were greater in computationally-pooled soils than either individual or physically-pooled soils for two of the three sites (Grand Rapids and Waseca). By contrast, community richness and diversity did not differ between the two methods of compositing for Lamberton and a single physical composite provided a sufficient assessment of both richness and diversity. The effort and expense associated with extracting and sequencing replicate soil samples is often a motivation for physically pooling spatially explicit samples. We recommend that researchers consider that the effectiveness of pooling may vary by soil type.

RefSoil: Continuous progress in reference soil database

Jinlyung Choi¹, Erick Cardenas², Ryan William¹, Adina Howe¹

¹Iowa State University, ²University of British Columbia

Microbes directly shape the abiotic and biotic soil environment. Consequently, research on soil microbes improve our ability to sustainably manage this important natural resource, including our understanding of nutrient cycling management, agricultural yields, and protection against disease and pathogens. Sequencing technologies have transformed our ability to study soil microbes, offering access to these populations without the challenges and biases of traditional cultivation approaches. Using sequencing strategies, the structure and functions of soil microbial communities is becoming increasingly accessible. A key challenge to leveraging soil sequencing datasets to understand soil health and function is the availability of high quality soil reference genomes. As the number of soil sequencing project increase, manually-curated reference genomes would provide a foundation for interpretation of this data.

In this effort, we have created a soil reference database, named RefSoil, that includes manual curation of high-quality, completed microbial genomes that have their origins from soil isolates. This project was inspired by the Human Microbiome Project (HMP) Data Analysis and Coordination Center (DACC), which used a similar effort to guide metagenomic analysis and future genome sequencing targets for microbiome studies. We have identified genomes from 1000 soil organisms and have investigated their distribution in the context of isolate genomes available in NCBI (RefSeq) and known microbial domains of biology. Additionally, we have identified the distribution of these genomes in environmental sequencing datasets, including datasets in the Earth Microbiome Project, creating a global soil isolate resource. We will present the status of the RefSoil database, including its phylogenetic and functional composition, and discuss challenges and opportunities going forward based on its ability to capture soil biodiversity.

Plant-microbe interactions: The *Populus* atlas

Melissa Cregger¹, Miranda Crouch¹, Zamin Yang¹, Dale Pelletier¹, Rytas Vilgalys², Chris Schadt¹

¹BioSciences Division, Oak Ridge National Laboratory, ²Duke University, Durham NC

Plants and microorganisms are intricately linked. Understanding the interactions between these organisms can result in novel uses of microorganisms to aid in plant health, productivity, and alterations in ecosystem functions like carbon sequestration. Using next generation amplicon sequencing, we assessed the bacterial communities associated with *Populus deltoides* and *Populus trichocarpa/deltoides* hybrids from approximately 30 unique environments spanning the leaves down to the roots. We found that bacterial communities clustered by tree environment ($p < 0.01$), and within some of the tree environments, by tree species ($p < 0.01$). Interestingly, we saw distinct bacterial community members across the different plant environments, which may denote differences in bacterial community function across these environments. This atlas of bacterial communities associated with *Populus* will aid in our understanding of how bacterial communities may function across this plant host.

Microbial communities associated with the biotransformation potential of insensitive explosives in surface soils

Fiona Crocker¹, Carina Jung¹, Karl Indest¹, Dawn Hancock¹, Alon Blakeney², and Jed Eberly¹
¹USACE Engineer Research and Development Center, Vicksburg, MS ² Bennett Aerospace, Vicksburg, MS

New explosive formulations that are less sensitive to external stimuli are being incorporated into current munitions. However, very little is known about whether these new insensitive explosives could pose ecological or human risks, especially since many DoD lands are located in critical ecosystems. The objective of this project was to determine if molecular ecology approaches could be used to assess microbial community diversity and function as an early indicator of disturbance in ecosystems and the microbial populations associated with biotransformation. We evaluated the effects and biotransformation potential of the explosives 2,4-dinitroanisole (DNAN), 3-nitro-1,2,4-triazole-5-one (NTO), and the new IMX-104 formulation on soil microbial communities. High throughput sequencing was used to determine changes in community diversity and to infer which phylotypes were correlated with biotransformation of the explosives. Aerobic and anaerobic biotransformation of the explosives was observed in soil microcosms with and without supplemental carbon and nitrogen. With IMX-104, significant hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) degradation did not occur until DNAN and 50% of the NTO had been degraded. Principal Coordinates Analysis (PCoA) of the weighted UniFrac distances showed that the presence or absence of carbon explained 47.33% of the variation in microbial community diversity, indicating that carbon rather than IMX104 had the greatest effect on community diversity. Four families, Sphingomonadaceae, Rhizobiaceae, Comamonadaceae, and Bradyrhizobiaceae, exhibited a statistically significant increase in relative abundance in IMX-104 plus nitrogen supplemented microcosms compared to microcosms without IMX-104. Metagenomic analysis of soil communities is providing valuable information on the types of microorganisms associated with biotransformation

of explosives which can be used to inform best management practices that sustain ecosystems on military lands.

Effect of a changing in the aboveground plant community on the soil physicochemical properties and microbial community.

Marie Duhamel¹, Kabir Peay¹

¹Stanford University, Department of Biology

Brutal perturbations can lead to important shifts in ecological state potentially impacting ecosystem functioning. The Vision Fire that occurred in 1995 in Point Reyes (CA) led to a severe rearrangement of the monospecific vegetation stands from this site, with an important increase of *Ceanothus thyrsiflorus* abundance. After the fire, this system did not return to its original state. In this context, we looked at the effect of the establishment of these new plant species on the soil properties and microbial communities.

Samplings were performed in sites colonized by *Baccharis pilularis* before and after the fire, and sites that switched from *B. pilularis* to *Pinus muricata* and from *B. pilularis* to *C. thyrsiflorus* hypothesizing that as these plants display distinct pools of root symbionts (ie. *P. muricata* colonized by ectomycorrhiza, *B. pilularis* by arbuscular mycorrhiza, *C. thyrsiflorus* by nitrogen fixing bacteria), the soil microbial communities from these stands should be specific. The shift in the plant community is also likely to impact the soil physicochemical properties of the different stands as they import different litter in addition to the various functions performed by their symbionts.

To test the hypotheses, soil, litter and leaves were sampled for carbon, nitrogen, pH, and decomposition rate measurements. Metabarcoding was used to assess bacterial and fungal identity in soil samples.

C. thyrsiflorus stands displayed the highest nitrogen content in litter, leaves and soil. The decomposition rate was the highest for *B. pilularis* and the lowest for *P. muricata*. The metabarcoding data show significant differences in the fungal but not in the bacterial species richness. Both the bacterial and the fungal community compositions differ significantly. These changes are most likely linked; this implies a modification of the ecological processes taking place in these vegetation stands and potentially an evidence for a microbial alternative stable state

Microbial community arsenic tolerance and the fate of arsenic in soil

Taylor K Dunivin¹, John Chodkowski¹, Jackson Sorensen¹, Ashley Shade¹

¹Department of Microbiology and Molecular Genetics Michigan State University, East Lansing, MI USA

The One Health initiative recognizes that human health is linked to the health of animals and the environment. Arsenic contamination in ground water is an important environmental health concern that can impact wildlife, livestock, and humans. Arsenic's toxicity and bioavailability depends largely on its oxidation state: the pentavalent arsenate (As^{5+}) is less soluble and less toxic compared to the trivalent arsenite (As^{3+}). The oxidation state of arsenic depends partially on microbial transformation. We anticipate that an

improved understanding of the microbial ecology of arsenic tolerance will help to understand arsenic speciation in the environment and improve risk assessment. The Centralia, Pennsylvania coal seam fire has been burning since 1962, and the town has been abandoned. As the coal burns, heavy metals, including arsenic, are released and deposited in the soil where the microbial community must cope. Centralia provides an interesting case study for microbial responses to long-term arsenic pollution. We examined the diversity and functions of arsenic tolerant microbes in an active soil vent using culture dependent and -independent methods. We isolated arsenic tolerant microorganisms belonging to several genera, including *Acinetobacter*, *Bacillus*, *Enterobacter*, *Laceyella*, *Paenibacillus*, *Pseudomonas*, and *Stenotrophomonas*. We characterized several genes conferring tolerance to arsenic and investigated the minimum inhibitory concentrations of arsenate. With metagenome sequencing of the soil, we performed targeted assembly an arsenite efflux pump, *arsB*, and discovered a diverse group of potentially arsenic tolerant microorganisms not represented among our isolates. In future works, we will characterize arsenic transformation capabilities of our isolates to further reveal microbial relationships with arsenic and expand our metagenome analysis to include other genes conferring microbial transformation of arsenic. Our study will offer a microbial community perspective on the fate and toxicity of arsenic contamination in soils. This perspective may improve prediction of groundwater contamination and, ultimately, contribute to reducing arsenic poisoning.

Multi-year functional soil metagenomic characterization of cellulosic bioenergy feedstock production systems

DS Duncan¹, JM Tiejde², GP Robertson³, RD Jackson¹

¹ Department of Agronomy & DOE-Great Lakes Bioenergy Research Center, University of Wisconsin-Madison; ² Center for Microbial Ecology & DOE-Great Lakes Bioenergy Research Center, Michigan State University; ³W.K. Kellogg Biological Station & DOE-Great Lakes Bioenergy Research Center, Michigan State University

The soil microbiome of an agroecosystem greatly influences its productivity, nutrient cycling, carbon footprint, and other management-relevant properties. Components of a cropping system, including management activity and crop species, in turn shape soil microbial communities with potentially long-term effects. We studied soil microbial communities in a bioenergy feedstock cropping systems trial three to five years after its establishment. The trial included both monocultural (continuous no-till corn, switchgrass, miscanthus, and hybrid poplar) and polycultural (early successional community, mixed native grasses, and prairie species assemblages) agroecosystems. We characterized soil microbial communities using functional gene abundance based on shotgun metagenomic sequencing, profiling the functional genetic potential of the entire community. Preliminary results indicate overall gene abundances are largely similar among all systems. Despite this, we observed significant system-level differences. Within-system interannual variability was relatively high, particularly in continuous corn. Further analyses will focus on changes in the abundance of denitrification pathway genes (*nirK/S*, *norB*, *nosZ*), as well as on the effects of nitrogen fertilizer in the switchgrass and prairie systems.

Niche and neutral community dynamics in parallelized aerobic, single carbon-source enrichment cultures

Theodore M. Flynn^{1,2}, Jason C. Koval², Kenneth M. Kemner¹, and Dionysios A. Antonopoulos^{1,2}

¹Biosciences Division, Argonne National Laboratory

²Institute for Genomics and Systems Biology, Argonne National Laboratory

A primary challenge in microbial ecology is understanding the environmental forces that shape the structure and function of microbial communities. While niche adaptation plays a strong role in determining what organisms are active in a given environment, stochastic factors such as dispersal limitation are significant factors as well. This poses a significant challenge to creating phylogenetically-resolved predictive models of microbial activity, particularly in light of the breathtaking complexity of microbial communities in terrestrial environments such as soil, water, and sediment. In this study, we utilize parallel bioreactors to examine the effect of nutrient amendment on various microbial communities taken from six disparate environments: soil from a temperate prairie and forest, tropical forest soil, subalpine forest soil, and surface water and sediment from a palustrine emergent wetland. Dilute subsamples of material from each environment were used to inoculate 96-well microtiter plates containing triplicate wells amended with one of 31 carbon sources from 6 different classes of organic compound (phenols, polymers, carbohydrates, carboxylic acids, amines, amino acids). After incubating each well aerobically in the dark for 72 hours, we analyzed the amount of microbial activity as evidence by an NADH-reactive indicator dye and took subsamples for microbial community analysis. Amplicons of the 16S rRNA gene were sequenced on the Illumina MiSeq platform for both the microtiter enrichments and the initial inocula and analyzed using a combination of QIIME and the R statistical package "phyloseq." We found most enrichments were dominated by one or several OTUs from the Proteobacteria (e.g. *Pseudomonas*, *Agrobacterium*, and *Ralstonia*) or Bacteroidetes (e.g. *Chryseobacterium*). Conversely, organisms from the phyla Verrucomicrobia, Actinobacteria, and Acidobacteria were much less abundant in the enrichments compared to the inocula. Overall, the 10 most abundant genera in the enrichments, which comprise >80% of all sequences there, represent only 1-8% of all sequences in the original inoculum. That the initial abundance of an organism does not determine the extent to which it became enriched provides evidence for niche adaptation within the enrichments by organisms better adapted to the nutrient-rich conditions within the microtiter plate. Yet across all of the microtiter plate enrichments, we found that the environmental source used to inoculate the well, not the carbon source it was amended with, was the strongest factor influencing the overall structure of the community in each enrichment. This suggests neutral, stochastic factors (the original composition of the source community), will also shape how the composition of the community as a whole responds to changing environmental conditions.

Increased soil C storage under anthropogenic N deposition: lignocellulolytic bacterial ascendance in a fungal world

Zachary B. Freedman¹, Rima A. Upchurch¹, Donald R. Zak^{1,2}, and Lauren C. Cline¹

¹School of Natural Resources & Environment and ²Department of Ecology and Evolution, University of Michigan, Ann Arbor, Michigan, USA 48109

Future rates of atmospheric nitrogen (N) deposition reduce organic matter decay by repressing activities of saprotrophic soil microorganisms, which may result in greater terrestrial carbon (C) storage. Thus, understanding how microbial communities respond to future rates of atmospheric N deposition has global implications for the cycling and storage of C in soil. For two decades, we have experimentally increased NO₃ deposition in replicate stands of northern hardwood forest in the Great Lakes region of North America, resulting in reduced soil respiration and increased soil C storage. Here, we paired extracellular enzyme assays with shotgun metagenomes to determine: i) if changes in microbial exo-enzyme activity has occurred alongside changes in the functional potential of the saprotrophic microbial community, and ii) if bacterial and fungal genes mediating litter decay respond similarly to experimental N deposition.

Two decades of experimental N deposition reduced the activity of microbial exo-enzymes mediating the decay of plant detritus. This reduction in enzyme activity occurred concomitantly with greater relative abundance and altered composition of bacterial functional pathways mediating the metabolism of carbohydrates, aromatic compounds, as well as respiration. Furthermore, 50 of 60 significant bacterial gene pathways increased in relative abundance under experimental N deposition. Conversely, fungal genes mediating the metabolism of common litter components (*i.e.*, cellulose, hemicellulose, lignin, pectin, and xylan) were not affected by chronic N deposition. Our results indicate that the functional potential of the saprotrophic fungal community was resilient to chronic N deposition, but saprotrophic soil bacteria, which degrade organic matter less efficiently than their fungal counterparts, were favored by this agent of environmental change. Greater abundance of bacterial gene pathways in the forest floor suggests lignolytic bacteria play an important function in litter decay as rates of N deposition increase in the future.

Diverse carbon utilization from field collected rhizosphere soils using paired metagenomic and metatranscriptomic approaches

Aaron Garoutte¹ and Jim Tiedje¹

¹Michigan State University

Carbon cycling in soils plays an important role the global carbon cycle as either a source or sink of greenhouse gases CO₂ and CH₄. By pairing metagenomics and metatranscriptomics community potential for carbon compound utilization can be ascertained as well as providing a snapshot of which genes are transcribed. We collected rhizosphere soil from a *Panicum virgatum* (switchgrass) plot and bulk soil from a nearby root free soil, in which continuous *Zea mays* (corn) was grown. Samples were collected from moist soil in late July near maximum plant growth from the Kellogg Biological Station in southwest Michigan. JGI provided 2.18 billion base of metagenome and 1.98 billion base of metatranscriptome.

Assembled contigs were annotated using the MG-RAST SEED Subsystems and the Carbohydrate Active Enzyme (CAZy) databases. Differential abundance analysis of Subsystems annotations of bulk and rhizosphere metagenomes showed the rhizosphere enriched for metabolism of putative root exudate compounds such as di- and oligosaccharides, organic acids and monosaccharides, while the bulk soil was enriched for use of polysaccharides and monosaccharides potentially related to root decomposition of the annual corn crop. Differential expression analysis of the metatranscriptomes showed potential use of a wider variety of carbon compounds in addition to the compounds found to be differentially abundant in the rhizosphere metagenomes. Analysis of CAZy annotations of rhizosphere metatranscriptomes showed a greater diversity of glycoside hydrolases than did the bulk soil. Taken together these data suggest that the rhizosphere is utilizing a wider variety of carbon compounds than suggested by their companion metagenomes while the bulk soil metatranscriptome mostly reflects activities of the metagenome.

Salinity effects in soil microbial communities, enzyme activity and carbon biogeochemistry in tidal freshwater wetlands

Georgios Giannopoulos^{1*}, Dong Yoon Lee², Olivia De Meo², Scott Neubauer², Bonnie Brown³, Rima Franklin¹

¹ Microbial Ecology; ² Wetland Biogeochemistry; ³ Environmental Genetics, Department of Biology, Virginia Commonwealth University, 1000 W Cary St. Richmond, VA 23284.

*presenting

One of the more certain effects of global climate change is the continued rise in sea level, which can lead to more frequent flooding and saltwater intrusion into tidal freshwater wetlands; even modest changes can alter plant community composition, water chemistry, and soil redox status. Most prior studies of these phenomena have focused on biogeochemistry and ecosystem function, while relatively little research has considered the soil microbial communities that govern these responses. The goal of our research is to link a metagenomics-based characterization of soil microbial communities with process-level measurements of important ecosystem carbon transformations, and to examine their collective responses to environmental change. This research primarily focuses on an on-going in situ field manipulation at a pristine freshwater wetland in the Chesapeake Bay (Virginia, USA). Throughout the field manipulation, we monitored soil biogeochemistry, enzyme process rates, ecosystem gas exchange and microbial community structure. Preliminary results are consistent with prior studies that demonstrate an effect of salinity on the activity of several hydrolytic and oxidative enzymes associated with organic matter breakdown. These differences, combined with changes in ecosystem-scale CH₄ and CO₂ fluxes, suggest that the contribution of different microbial processes to carbon transformations changed over both temporal (field manipulation) and spatial (transect survey) scales. Analyses of gene abundance and expression associated with key microbial functional groups (esp. methanogens and iron- and sulfate-reducing bacteria) will help us link ecosystem responses to microbial community composition. Metagenomic and metatranscriptomic analyses will help us further disentangle the complex interactions between vegetation, microbial communities, biogeochemical transformations, and ecosystem processes.

Deconstructed PCR: Improved library preparation tools for high throughput amplicon sequencing

Stefan J. Green^{1*} and Ankur Naqib^{1,2}

¹ DNA Services Facility, Research Resources Center, University of Illinois at Chicago, Chicago, IL; ² Dept. of Bioengineering, University of Illinois at Chicago, Chicago, Illinois, USA

The polymerase chain reaction is sensitive to mismatches between primer and template, and mismatches can lead to inefficient amplification of targeted regions of DNA template. In amplification reactions where many targets are intended, such as bacterial small subunit ribosomal RNA gene amplification from mixed communities, primers are frequently heavily degenerate. Inefficiencies due to different melting temperatures between different primers within a degenerate primer pool, in addition to mismatches between primer binding sites and primers, can distort the relative abundance of targets in the original DNA pool. We have developed a two-step, two primer set PCR strategy to reduce the distortion associated with primer mismatch and primer degeneracy based on a fundamental deconstruction of the PCR reaction. This method separates primer-template annealing from exponential amplification, allowing for exponential amplification to be performed with non-degenerate primers, thereby reducing bias during PCR. The utility of this method is shown in mock constructed communities and from environmental genomic DNA. The utility of this strategy touches many areas of research, including PCR with degenerate primers, PCR with primers potentially containing mismatches (including single nucleotide polymorphisms, SNPs) to known and unknown templates, multiplex PCR for target capture, and quantitative PCR with degenerate primers. The method is simple to perform and is limited to PCR mixes and a single exonuclease step which can be performed without reaction cleanup. An improved version of the protocol has been developed, providing for greatly increased sample throughput and a mechanism to reduce chimera formation together with reduction in amplification bias.

Metagenomics and physiology based analysis of bacterial communities from differently managed agricultural soils

Timothy Gsell¹ and Craig Sweet¹

¹Governors State University, Chemistry and Biological Science Division, College of Arts and Sciences

Management of soil usually has a profound effect on the bacterial communities residing within them. In many cases the supplemented nitrogen and phosphorus from fertilizer into some managed soils has dramatically impacted microbial diversity, causing species shifts in soil that crop plants rely on for health and growth. In this study the bacterial communities of 3 historically differently managed soils were tested with metagenomics and physiology based approaches. These two analyses were supported by data collected for total bacterial counts and diversity indices data comparison among them. The three different soil practices included organically farmed, conventional farmed and human derived biosolid waste application. Prairie restoration soil was also collected and composited for analysis and represented another management practice in non-agriculture land use. All management

practice based field sites were located on the Governors State University campus. Metagenomics data includes an Illumina 16s analysis of all bacterial from phyla to species found in composite field soil sample DNA. Relative abundances of the major representative types are described. Biolog Eco-plates determined community level physiological changes in bacterial carbon usage patterns by resident cells in each sample type. Kruskal–Wallis test results showed significance between sites for both total bacteria and diversity ($p < .05$). Post Dunn analysis revealed the organic plot had significantly high diversity but also significantly lower total bacteria compared to that of the biosolid plot. PCA was run to determine similarity between Biolog Ecoplate data and showed differences between soil types from the managed soil sites. Molecular analysis suggests some uniformity between major soil bacterial types in all samples at the class and order levels, with more substantial distinctions being made at the genus/species level. Differences in bacterial phyla representation between all different managed soils were surprisingly similar to the prairie restoration soil samples.

Study of seasonal variation of desert keratinophilic fungi

P. Hamm¹, R. C. Mueller³, J. Belnap², C. R. Kuske³, A. Porras-Alfaro¹

¹Western Illinois University, Biological Sciences, 1 University Circle, 61455, Macomb, IL, USA.

²US Geological Survey, Climate Variability and Change, 2290 S. West Resource Blvd., 84532, Moab, UT, USA

³Los Alamos National Lab, Environmental Microbiology, P.O. Box 1663, 87545, Los Alamos, NM, USA

Soil fungi in desert ecosystems present adaptations to very harsh conditions such as high soil surface temperatures and limited organic matter and water. The diversity and abundance of fungi in these systems, including those that can degrade keratin, are poorly known. The objective of this project was to document keratinophilic fungi from different biological soil crusts and rhizosphere soils collected in an arid grassland using culturing techniques and Illumina sequencing. Soil samples were collected near Castle Valley, UT. Keratinophilic fungi were isolated using different baits and isolated in Sabouraud Dextrose Agar and Malt Extract Agar. Fungi were identified using ITS and LSU rRNA sequences. One hundred-eighteen fungi were isolated representing a total of 33 Operational Taxonomic Units at 97% similarity. The culture collection was dominated by the orders Pleosporales, Eurotiales, Mortierellales, and Hypocreales and dominant taxa were represented by *Alternaria*, *Aspergillus*, *Mortierella*, and *Fusarium*, respectively. *In vitro* bioassays were conducted to confirm the capacity of these fungi to degrade keratin. Next generation sequencing was congruent with the culture collection findings that *Alternaria* represents the most common taxa representing >40% of the samples for both rhizosphere and biocrust soils. *Fusarium* was the next most abundant keratinophile followed by two *Chaetomium* species, *Phoma*, *Preussia*, *Aspergillus*, a Pezizomycete, and *Monosporascus*. Lichen biocrust showed the highest seasonal variation for OTU 1 (*Alternaria*) with lower abundance (40%) in April and June and a dramatic increase (>80%) during the months of August to November. OTUs 44 and 74, both *Chaetomium* spp., showed the highest abundance in the rhizosphere and below biocrust samples. OTU 59 (*Fusarium*) showed the highest variability for the biocrust samples with a large increase from March to July. Illumina data combine

with culture dependent studies could be used as a reliable tool that could provide specific information on poorly study communities such as keratinophiles in desert ecosystems.

Structure and activity of denitrifier communities in biochar-amended soil and their impact on N₂O emissions

Johannes Harter¹, Ivan Guzman Bustamante², Stefanie Kühfuß², Reiner Ruser², Reinhard Well³, Oliver Spott⁴, Mohamed El-Hadidi⁵, Daniel Huson⁵, Andreas Kappler¹ and Sebastian Behrens^{1,6}

¹ Geomicrobiology and Microbial Ecology, Center for Applied Geosciences, University of Tuebingen, Tuebingen, Germany;

² Fertilisation and Soil Matter Dynamics, Institute of Crop Science, University of Hohenheim, Stuttgart, Germany;

³ Institute of Climate-Smart Agriculture, Johann Heinrich von Thünen-Institut, Braunschweig, Germany;

⁴ Department Soil Physics, Helmholtz Centre for Environmental Research – UFZ, Halle/Saale, Germany;

⁵ Algorithms in Bioinformatics, Center for Bioinformatics, University of Tuebingen, Tuebingen, Germany;

⁶ Department of Civil, Environmental, and Geo-Engineering, University of Minnesota, MN, USA.

Nitrous oxide is a strong greenhouse gas with an almost 300 times higher global warming potential than CO₂. The main sources of N₂O are microbial-mediated nitrogen transformation reactions in soils. Denitrification, the microbial reduction of NO₃⁻ to N₂ occurs frequently in oxygen-limited zones and represents one of the major N₂O-producing pathways. As some denitrifiers lack the genetic potential for N₂O reduction and this step is highly oxygen and pH sensitive, complete denitrification is often impaired, what can result in elevated N₂O release from soils. Soil amended with biochar has been demonstrated to reduce N₂O emissions in laboratory microcosms and in the field. Although N₂O emission mitigation due to soil biochar amendment has frequently been reported for different soils and biochars it remains unclear how biochar affects the structure and activity of the denitrifying microbial community in soils.

We setup soil microcosms containing 5% (w/w) wood-derived biochar, adjusted the water-filled pore space to 90%, and added NH₄NO₃ at field applications rates. We quantified geochemical parameters (e.g. pH, NO₃⁻, NO₂⁻, NH₄⁺) and N₂O emission rates. Genes and transcripts of functional marker genes for microbial denitrification (e.g. *nirK*, typical and atypical *nosZ*) were quantified by real-time PCR and analyzed by Illumina sequencing. Soil biochar amendment decreased N₂O emission rates and promoted the expression of crucial functional marker genes for complete microbial denitrification. Sequence analyses of the N-cycling marker genes and their transcripts revealed biochar induced community shifts. While 16S rRNA gene diversity was only slightly affect by biochar amendment during microcosm incubation, functional gene abundance and transcription levels varied among

taxonomic groups over time suggesting the contribution of rare but active taxa to soil N₂O emissions.

Our findings further the mechanistic understanding of the complex coupling between nitrogen pools, nitrogen-transforming microorganisms and nitrogen gas fluxes in biochar-amended soils.

Soil microbial communities as sentinels of environmental change

Martin Hartmann

Swiss Federal Research Institute for Forest Snow and Landscape Research WSL,
Birmensdorf, Switzerland

Soils are complex and dynamic biological systems containing diverse organisms that span all three domains of life. Interdependent constituents of this vast microbiota play essential roles in virtually every biogeochemical process on earth, making them ideal sentinels of environmental change. Methodological constraints, however, have long limited our capability to survey microbial diversity at a throughput, coverage and phylogenetic resolution required to draw adequate ecological conclusions. In this light, novel high-throughput DNA sequencing technologies are about to revolutionize our understanding of the key factors driving soil microbial diversity.

Climate change and land use are global key forces altering soil microbial communities with potentially significant impact on ecosystem functioning. I will demonstrate how we can use targeted high-throughput sequencing of ribosomal markers coupled to integrative visualization techniques to evaluate the impact of these forces on soil microbial diversity. For example, soil microbial diversity is central to the productivity and viability of agricultural and forest ecosystems, but we still have a rather poor understanding of sustainable management strategies and associated disturbance thresholds. Some of our recent studies have shed more light on the impact of logging and agricultural management on soil microbial diversity and associated ecosystem functions. Beyond these management strategies, rapid global warming has potentially huge effects on the soil microbiome locked up in the cryosphere, eventually releasing a largely unknown genetic resource into the environmental cycle. In this context, I will show first results from a pilot project where we aim at unravelling microbial diversity hidden in alpine permafrost soils of central Europe. These examples demonstrate the potential of using microbial communities as sentinels of environmental change. Such knowhow is important to better understand microbial diversity on our planet and the environmental drivers that change these communities with potential impact on ecosystem functioning at regional and global scales.

Moldy dark matter: Inferring the taxonomic and functional diversity of soil fungi through a combined meta-'omic' strategy

J. R. Herr

Department of Plant Pathology & The Center for Plant Innovation Science, University of Nebraska, NE 68588

It has long been understood that fungi contribute to many key ecosystem processes. This is particularly important in soils, where fungi facilitate nutrient mobilization and are the main drivers of organic matter decomposition. Fungi forming symbiotic associations with plants are responsible for nutrient uptake and thereby directly affect plant growth and fitness. Additionally, fungi in soils contribute to carbon sequestration, shape seedling establishment, and evidence suggests that mycorrhizal fungi may contribute to the distribution of carbohydrates from one plant to another, directly regulating the survival of nurse seedlings. The soil matrix also provides a habitat for the growth and survival of plant pathogenic fungi. Despite their important ecological roles, there is a paucity of information regarding taxonomic and functional diversity, especially in soils. This is due largely to the fact that most fungi are unculturable, lack known sexual structures, and are known only by nucleotide identification. Next-generation sequencing technologies have revolutionized the ability to use sequence data to address ecological and physiological questions, and fungi have not been immune to these advances. Using publically available amplicon, metatranscriptomic, and metagenomic datasets and a metagenome assembly strategy, I will provide a framework for taxonomic and functional fungal diversity in soil. Reconstructing whole genomes of unculturable soil fungi is not possible, but capturing large portions of the fungal genomic component certainly is possible. I will focus particularly on the genomic diversity of arbuscular mycorrhizal fungi in grassland ecosystems and will discuss the potential for monitoring plant pathogen outbreaks in crop systems using a monitoring strategy with metagenomic sequencing.

Biogeography and metabolism of dominant bacterial populations in tundra soils

Eric R. Johnston¹, Luis M. Rodriguez-R¹, Liyou Wu², Zhili He², Edward A.G. Schuur³, Yiqi Luo², James M. Tiedje⁴, Jizhong Zhou², and Konstantinos T. Konstantinidis¹

¹Georgia Institute of Technology, Atlanta, GA 30332, USA; ²University of Oklahoma, Norman, OK 73019, USA; ³Northern Arizona University, Flagstaff, AZ, 86011, USA; ⁴Michigan State University, East Lansing, MI 48824, USA.

The degree to which soil microbial communities differ between and within soil ecosystems remains poorly understood; yet, is essential for research efforts endeavoring to holistically understand soil microbial ecology in the context of ecosystem functioning, and how environmental changes affect microbial activities and services. Under a multi-institutional DOE-supported project, we have begun investigations on microbial communities from Alaskan tundra permafrost (AK), which has been experimentally warmed 2-4°C above ambient temperature *in-situ*. Our shotgun-metagenomic datasets obtained early in the experimental phase to describe these communities allowed for near-complete coverage of the community by sequencing (~92% breadth), including the recovery of twenty-seven high quality draft genomes of novel taxa. Based on well-replicated samples, we show that all of

these taxa represent sequence-discrete, abundant populations and that, collectively, they comprise up to 9% of the entire soil microbial community. These genomes represent diverse taxonomic groups and metabolic lifestyles tuned toward sulfur cycling, methanotrophy, and plant-derived carbon oxidation. Several of the assembled populations were also detectable in geographically distant tundra habitats (up to 99.7% nucleotide identity and full representation of the genome sequence), apparently playing important roles in regional ecosystem functioning. Their ubiquity across both small and large scales, from single meter to several hundred-kilometer distances, allowed for a unique assessment of soil population biogeography across spatial and environmental gradients, as well as areas of recent climate change-relevant disturbances. Thus, these assembled genomes provide potential model organisms for future *in-situ* experimental manipulations; for instance, through the design of population-specific PCR for assessing gene transcript (activity) level, allowing potential linking of methane, nitrogen, SOM, etc. turnover to individual populations.

Soil microbial community responses to warming as revealed by comparative metagenomics.

Eric Johnston¹, Chengwei Luo¹, Luis M. Rodriguez-R¹, Liyou Wu², Shi Zhou², Kai Xue², Zhili He², Mengting Maggie Yuan², Yiqi Luo², Edward A.G. Schuur³, James R. Cole⁴, James M. Tiedje⁴, Jizhong Zhou², and Kostas Konstantinidis¹.

Georgia Institute of Technology, Atlanta, GA 30332, USA ¹; University of Oklahoma, Norman, OK 73019, USA ²; Northern Arizona University, Flagstaff, AZ, 86011, USA ³; Michigan State University, East Lansing, MI 48824, USA ⁴.

How complex soil microbial communities respond to natural or human-induced fluctuations, including major perturbations such as global climate change, remains poorly understood, severely limiting our predictive ability for soil ecosystem functioning and resilience. Under a multi-institutional DOE-supported project, we have begun investigations on microbial communities from Alaskan tundra permafrost (AK) and Oklahoma temperate grassland (OK) soils, both of which have been experimentally warmed 2 to 4 °C for five years above ambient temperature *in-situ*. Our results showed small but significant shifts in community composition, gene expression, and functional metabolic potential compared to control (un-warmed) adjacent communities. Greater taxonomic composition differences were observed at the OK site relative to AK, presumably resulting from longer generation times due to the less optimal conditions for growth at permafrost soils. The most pronounced bacterial taxon shifts observed at OK site, which were somewhat also observed at the AK site, were an increase in abundance of *Actinobacteria* and decrease in *Planctomycetes*, both representing major phyla in soils. In terms of functions, the communities of AK warmed plots were enriched in metabolic pathways related to labile carbon mobilization and oxidation whereas fewer of these patterns were observed in the OK communities, indicating that soil C is more vulnerable to microbial respiration at AK. Collectively, our findings suggest that microbial communities of grassland soils play important roles in mediating feedback responses of the soil ecosystem to climate change and that even short periods of warming induce significant changes in microbial community function and composition. To enable this research, we have developed several bioinformatics tools that addressed practical limitations during the comparative analysis of the soil metagenomes such as how to assess the fraction of the community captured by a

metagenomic dataset. These tools are available for online analysis through <http://enve-omics.gatech.edu/>

Manipulating soil temperature and moisture to uncover microbial community trends for modelling purposes.

Robert M Jones¹, Karen L Foley¹, Charles M Reynolds¹, Robyn A Barbato¹

¹USACE ERDC Cold Regions Research and Engineering Lab

Models capable of estimating soil microbial community trends lack the knowledge of which environmental data are most relevant and how and to what degree their fluxes would impact the system. Given that soil temperature and moisture are well established factors that influence microbial activity, our research investigated the degree to which these factors alter the principle communities. We developed an initial model of soil activity by measuring soil respiration and community composition from four soils subjected to varying physiochemical attributes, a range of temperatures and moistures (5 to 30°C and -23,121 kPa to -7 kPa respectively), in quadruplicate. Once the soils reached stable respiration rates, they were destructively sampled to extract DNA, sequence the 16S rRNA gene, and visualize community shifts. Our results showed that communities changed significantly from one temperature and moisture point to the next. The magnitude of separation; however, ranged from broad to minor depending on the soil. For example, the soil with the highest organic matter (OM) content contained a distinctive 30°C community. Furthermore, within the 30°C incubation, the differing moistures showed significant separation; however, the lower three temperatures (5, 15, and 25°C) displayed no significant separation based on temperature. Oppositely, the soil with the lowest OM harbored distinctive communities based on both temperature and moisture. Overall, community differences according to changes in moisture and temperature were most significant when transitioning from low to middling levels. At higher levels, the differences were slight, suggesting minor shifts in dominant microbes. Therefore, temperature and moisture may be helpful in uncovering principle community shifts though the clarity of the shifts becomes less distinguishable at higher temperatures and moistures. For minor community shifts, other factors may be more useful in modelling soil activity. Our future work will look into manipulating other soil properties to further develop community trend lines.

Soil bacterial and fungal community dynamics during conversion of Conservation Reserve Program (CRP) fields to cropping systems

Mamatha Kakarla¹, Jennifer Moore-Kucera¹, Lisa Fultz², Chenhui Li¹, Veronica Acosta-Martinez³, Jon Zak⁴, Kameswara Rao Kottapalli⁵

¹Plant & Soil Sciences, Texas Tech University, Lubbock, TX; ² School of Plant, Environmental and Soil Sciences, Louisiana State University AgCenter, LA; ³USDA-ARS, Lubbock, TX; ⁴ Biological Sciences, Texas Tech University, Lubbock, TX; ⁵ Center for Biotechnology & Genomics, Texas Tech University, Lubbock, TX.

We investigated changes in soil bacterial and fungal communities during conversion of Conservation Reserve Program (CRP) land to cropland. Soil samples (0-10cm and 10-30cm)

were collected in December 2012, 2013, and 2014 from three long-term (>20 y) CRP fields and three fields recently converted (within 1-2 y in 2012) from CRP (>20 y) to dryland crop production. Genomic DNA was isolated from the soil samples and the V3-V4 (16S rRNA) and ITS1 regions were amplified for bacterial and fungal community analysis, respectively using the Illumina MiSeq platform. Soil bacterial diversity and richness estimates were higher in converted fields compared to CRP fields. These increased levels of diversity in converted systems were likely the result of incorporation and redistribution of soil organic material into the lower depth during conversion of CRP to cropping fields. Distinct soil bacterial communities were present between the two systems. The CRP fields were dominated by relative abundance of oligotrophic taxa such as *Acidobacteria*, *Verrucomicrobia*, *Chloroflexi*, *Rubrobacteria*, and *Cyanobacteria*. Converted fields were dominated by copiotrophic taxa such as *Bacteroidetes*, *Firmicutes*, α -*proteobacteria*, and β -*proteobacteria*. Converted fields also were characterized by *Gemmatimonadetes* that respond to N fertilization. Hence, conversion of management systems resulted in a shift of bacterial communities from low C turnover oligotrophic species to high C turnover copiotrophic species. Fungal diversity and richness were not affected by management conversion but, community composition changed as CRP fields were converted to cropping systems. The distinct fungal communities in CRP fields include members of *Agaricomycetes*, *Onygenales*, *Chaetothyriales*, *Glomerales*, *Boletales*, *Russulales*, and *Botryosphaeriales*. Converted fields were dominated by members of *Sordariomycetes*, *Mortierellales*, *Xylariales*, *Hypocreales*, *Pleoporales*, and *Sordariales*. Our results show that conversion of CRP to cropping systems result in rapid shift of microbial communities that might have major implications on potential C storage in these ecosystems.

Meta- and isolate- genomic analyses of grassland soil microbial communities.

Ulas Karaoz¹, Heejung Cho^{1,2}, Kateryna Zhalnina^{1,2}, Mary Firestone^{1,2}, Eoin Brodie^{1,2}

1 Earth and Environmental Sciences, Lawrence Berkeley National Laboratory, Berkeley, CA, United States.

2 University of California Berkeley, Berkeley, CA, United States.

As part of a large effort whose overarching goal is to determine the impact of the interactions between plant roots and soil microbial communities on organic C decomposition and stabilization processes in soil, we are using isolate genomics-assisted metagenomics. To begin to define the functional roles of microbial community members, soil samples were collected from a Northern California annual grassland towards the end of the summer dry season and during peak plant productivity. Ultradeep shotgun sequencing was performed, yielding a total of nearly 0.77Tb of high quality sequence in addition to sequence data from samples taken during rhizosphere succession. From the same soils, we built a repository of 290 bacterial isolates obtained from multiple dilute media formulations incubated over a period of 2.5 months. Thirty-nine isolates have been characterized physiologically and sequenced yielding draft assemblies. Strain-level heterogeneity of relatives of our sequenced isolates is being assessed and their response to root growth evaluated. Time series metagenomes are being used to refine metagenome binning.

(1) An assembly driven approach was taken to obtain draft genomes, leading to dozens of partial to near complete genomes, that were binned based on tetranucleotide profiles and coverage across the various metagenomes. Soil was dominated by Bacteria (*Actinobacteria* and *Alphaproteobacteria*), with a more minor contribution of Archaea and Eukarya.

Actinobacterial bins were primarily from Actinomycetales and Solirubrobacterales while Proteobacterial bins were primarily from Rhizobiales. Although present in all samples, several Rhizobiales and Micrococcales genome bins were more abundant in rhizosphere. Current work is focused on the identification of C metabolism pathways in genomic bins based on the preliminary annotations. (2) High-stringency (99-100% sequence identity) mapping of metagenomic reads to the isolate genomes suggest that although the isolates only represent a small fraction of the total microbial community, several Actinobacterial and Alphaproteobacterial isolates are highly represented strains.

Changes in microbial community structure and function in response to long-term N addition as revealed by soil metaproteomics

Katharina M. Keiblinger¹, Stefan J. Forstner¹, Stefanie Kloss¹, Stephan Fuchs², Kathrin Riedel², Martin H. Gerzabek¹ and Sophie Zechmeister-Boltenstern¹

¹ Institute of Soil Research, University of Natural Resources and Life Sciences, Peter-Jordan-Str. 82, A-1190 Vienna, Austria

² Institute of Microbiology, University of Greifswald, Friedrich-Ludwig-Jahnstrasse 15, 17489 Greifswald, Germany

Since the mid-19th century, terrestrial ecosystems have been receiving ever-increasing amounts of nitrogen (N) via atmospheric deposition. In many systems N is regarded the limiting nutrient for plants and microbes, however, the effects of high N on structure and function of soil microbial communities are poorly understood.

To elucidate the effects of enhanced N-input on the soil microbial communities and their effects on C-sequestration we sampled a long-term forest fertilization experiment (Klosterhede, Denmark). The dominant plant is Norway spruce (*Picea abies*) the soil is a haplic podzol. N was added at a rate of 35 kg N ha⁻¹ y⁻¹ for >20 years on top of 25 kg N ha⁻¹ y⁻¹ of background deposition. In spring 2014, we took samples from one organic and one mineral soil horizon in control and N-treatment plots. The soil metaproteome was extracted with SDS-phenol and NaOH. The combination of these two complementary extraction protocols aims on an efficient and comprehensive metaproteome profiling. Proteins were separated by SDS-PAGE, digested and analysed by LC-MS/MS.

Identified proteins ranged from 913 to 1288 per sample across treatments and extraction buffers. N-fertilization resulted in less proteins extracted from organic horizons. Although microbial biomass in total was not affected by long-term forest fertilization, metaproteomics indicated a shift in microbial communities and their depth distribution. In the organic horizon, N addition resulted in a relative decrease of eukaryotic proteins and a concomitant increase of bacterial proteins, regardless of extraction method. In the mineral horizon, the pattern was the exact opposite, i.e. eukaryotic proteins increased at the expense of bacterial protein. Within the eukaryotes, relative abundances of ascomycotal and basidiomycotal proteins generally decrease with N addition in both horizons.

Metaproteomics enables us to investigate these changes with respect to possible effects on soil C storage at even finer taxonomic resolution.

Understanding microbial community responses and limitations to the SPRUCE deep peat heat experiment.

Laurel Kluber¹, Samantha Allen¹, J. Nicholas Hendershot¹, Paul Hanson^{2,3}, Christopher Schadt^{1,3}

¹Biosciences Division, ²Environmental Sciences Division, and ³Climate Change Science Institute, Oak Ridge National Laboratory,

The Spruce and Peatland Responses Under Climatic and Environmental Change (SPRUCE) experiment is a large-scale ecosystem manipulation experiment designed to examine how peatland ecosystems respond to increased temperature and CO₂ levels. This experiment is expected to lead to various changes in ecosystem processes, including microbially mediated biogeochemical cycling, thus ultimately altering the overall C balance of these ecosystems. The initial phase of this experiment began over the summer of Summer 2014 by heating deep subsurface peat to +2.25, +4.5, +6.75 and +9.0 °C above ambient plots with a target heating zone of 1.5-2 meters depth. Peat cores were collected in September 2014 and June 2015 and microbial communities were examined at twelve discrete depths across the entire peat profile to a depth of 200 cm. In an effort to identify potentially limiting factors on decomposition and microbial community change, we conducted a 70 day microcosm incubation of deep peat (150-200 cm depth) at 6 and 15 °C to mimic ambient and +9 °C SPRUCE conditions. To determine whether microbial activity was limited by factors other than temperature, additional treatments included elevated pH and the addition of N and P. Incubation microcosms were monitored for CO₂ and CH₄ production, and microbial community dynamics were assessed using qPCR and amplicon sequencing. Increasing temperature had positive effects on both CO₂ and CH₄ production while elevated pH only resulted in greater CH₄ production. The effects of elevating temperature and pH in combination with N, P or N+P additions were more variable. Although temperature had little effect on the overall microbial community structure, there was a shift in the size of bacterial and archaeal populations. In contrast, elevated pH and N additions seemed to have the largest influence on community structure.

Plant response to various phosphorus sources under nutrient limitation

Peter Korajczyk¹, Shalaka Shinde¹, Sarah Zerbs¹, Frank R. Collart¹, Philippe Noiroi¹

¹Biosciences Division, Argonne National Laboratory

Phosphorus is a major nutrient element essential for plant health. In natural ecosystems phosphorus is present as inorganic phosphate, organic forms, and insoluble complexes with calcium or iron. Only inorganic phosphate (P_i) can be taken up by plants but it is usually present at very low concentrations. Most work has examined plant response to inorganic phosphate limitation without consideration of alternative P sources. To more closely model natural soil conditions, we have analyzed P accessibility by providing Aspen seedlings with different P forms as inorganic phosphate, organic P phytate (P_o) or insoluble P tricalcium phosphate (TCP) sources. *Populus tremuloides* seedlings were grown on agar plates utilizing one of these sole P sources at 5, 15, 25, 50, or 100 μM total phosphorus. After 5 weeks of growth, shoot and root fresh and dry weights were measured. Aspen seedling showed significant changes in biomass due to different P doses and P sources. Decreased biomass

was prominently observed on reduced P_i media; to a lesser extent in plants grown on P_o also showed concentration-dependent decreases in biomass. In TCP treatment we observed decreases in biomass with increasing nutrient concentration, however, plant phenotypes indicated that the plants experience more stress at lower TCP concentrations. Further biochemical parameters are being developed to identify metrics besides biomass indicative of P stress on different P sources and describe specific plant strategies to address P limitation when alternative P nutrients are present. This experiment demonstrates that two poorly available P sources support different levels of growth and that both P concentration and speciation affects the plant productivity.

Peatland microbial community responses to plant functional group, water table and peat depth

L.J. Lamit,¹ J.T. Lennon,² E.A. Lilleskov^{1,3}

¹School of Forest Resources and Environmental Science, Michigan Technological University, 1400 Townsend Dr., Houghton, MI 49931. ²Department of Biology, Indiana University, Bloomington, Indiana, USA. ³USDA Forest Service, Northern Research Station, Forestry Sciences Laboratory, 410 MacInnes Drive, Houghton, MI 49931

Peatlands represent a globally important pool of soil carbon, the stability of which is vulnerable to drought and disturbance. Microorganisms play pivotal roles in carbon cycling, and it is key to understand factors that influence peatland microbial communities. Although standard approaches utilize DNA to characterize microbial communities, taxa represented in DNA pools may not all be transcribing, suggesting that RNA-based approaches reveal a clearer picture of active microbial community structure. We used prokaryote 16S rRNA gene sequencing (bacteria, archaea) and quantitative PCR (qPCR; bacteria only) of DNA and RNA to understand how peatland microbes are influenced by water table (average, lowered), plant functional group (ericaceous shrubs, sedges) and peat depth (10-20cm, 30-40cm) in a mesocosm experiment. We hypothesized that these factors will influence microbial communities, and that RNA will more strongly reflect their influence than DNA. Quantitative PCR supported our hypotheses: 1) bacterial DNA decreased with depth and was unaffected by other factors. In contrast, 2) bacterial RNA increased with depth, but this increase was modulated by a 3-way interaction with plant functional group and water table. Sequencing provided mixed support for our hypotheses: 3) there were distinct differences in composition between DNA and RNA for both domains, and 4) their compositions were both influenced by depth, plant functional group and water table. However, 5) DNA and RNA revealed similar patterns of community response to the three factors. We show that peatland microbial communities are structured by moisture and vegetation, two factors likely to be influenced by climate change and disturbance. However, the effects of moisture and vegetation occur within the context of the much stronger effect of depth. Importantly, results from DNA can sometimes run in contrast to those from RNA, indicating that the use of both DNA and RNA is important for characterizing microbial communities.

Control of boreal forest soil decomposition processes by plant secondary compounds

Mary-Cathrine Leewis^{1*}, Mary Beth Leigh¹

¹University of Alaska Fairbanks, Institute of Arctic Biology, Fairbanks, Alaska, U.S.

*mcleewis@alaska.edu

Hundreds of thousands of different secondary plant metabolites (SPMEs) are produced by plants, however the quantity and variety of compounds vary enormously between plant species/progenies and shift in response to environmental conditions. We hypothesize that SPMEs released through litterfall and root turnover in the boreal forest control ecosystem carbon cycling by inhibiting microbial decomposition processes, which are overcome partially by increased aromatic biodegradation of microbial communities that also fortuitously prime soils for accelerated biodegradation of contaminants. This study aims to reveal how SPMEs, released through litter deposition, exert control on cycling of complex organic matter (carbon and nutrients) in soil, and how these chemical controls are overcome by shifts in microbial communities that also affect resilience to contaminants in the boreal forest. All sampling was conducted at the long-term common tree gardens located in the Kevo Subarctic Field Research Institute. Soils and litter (stems, roots, senescing leaves) were collected from 3 different birch progenies from Iceland, Finland, and Siberia that have been reported to contain different SPME content (low, medium, high, respectively). Soils and surface litter from beneath each progeny were also collected. We characterized the SPME content of these plant progenies and used a variety of traditional microbiological techniques (i.e. enzyme assays & functional incubation assays) and advanced molecular techniques (i.e. gene-targeted metagenomics) to address the hypothesis that different levels of SPMEs will result in different microbial community structure and function. Results indicate that microbial populations associated with trees of different SPME content significantly differ in their enzymatic potential to breakdown cellulose and degrade diesel-range organics. Results also indicated that microbial communities (bacterial and fungal) significantly vary in composition in accordance with dominant tree species present. This study offers novel, fundamental information about phytochemical controls on ecosystem processes, resilience to contaminants, and microbial decomposition processes.

Ecological and evolutionary insight into the persistence of soil bacteria

Jay T. Lennon¹, Stuart E. Jones²

¹Indiana University; ²University of Notre Dame

Microorganisms typically experience conditions that are suboptimal for growth and reproduction. Despite this, many populations of bacteria survive by entering a reversible state of reduced metabolic activity, or dormancy. Our previous work has demonstrated that persistence is an important trait that contributes to the maintenance of microbial diversity. However, a variety of persistence strategies are thought to have evolved among bacteria, which might have different costs and trade-offs. In this presentation, first, we will outline the theoretical frameworks and expectations for bacterial persistence under energy limitation (i.e., starvation). Second, we will describe results from a long-term experiment that quantified persistence for a phylogenetically diverse collection of soil bacteria. Results

from these multi-year experiments suggest there is upwards of four-orders-of-magnitude of the variation in bacterial death rate under starvation. Some strains persisted with almost no loss of viability while others rapidly succumbed to energy limitation. Interestingly, for the majority of our strains, the decay rate of viability significantly deviated from first-order expectations suggesting that bacterial death rates declined over time. Simulation models and follow-up experiments including genomic analyses indicate that this type of functional response may arise from cannibalism, evolution, or possibly both. The findings have implications for understanding ecological and evolutionary of persistence in natural and managed ecosystems.

The influences of thorny bamboo growth on the bacterial community in badland soils

Yu-Te Lin¹ and Chih-Yu Chiu¹

¹Biodiversity Research Center, Academia Sinica, Nankang, Taipei, Taiwan

The badland soils originated from mudstone was hard for plant growth because of its high salinity and poor chemical and physical properties. Thorny bamboo (*Bambusa stenostachya*) is one of the few plant species can survive in this area. To investigate the respond of bacterial community in this badland soils to the vegetation cover, soils from thorny bamboo plantation and bare area were sampled and analyzed with barcoded pyrosequencing technique. The results revealed that *Actinobacteria* predominated in the bare soil communities, but *Acidobacteria*, *Actinobacteria* and *Proteobacteria* were the most abundant group in the thorny bamboo soils. Non-metric multidimensional scaling analysis with the distribution of abundant OTUs also revealed the different clusters between thorn bamboo and bare area communities. The bacterial community diversity in the thorny bamboo plantation was higher than that in the bare soils. The soil properties, including pH, soluble organic carbon and nitrogen and electrical conductivity were related to the bacterial structure and/or diversity. Further analyses also indicated these factors affected the distribution of *Acidobacteria*, *Actinobacteria*, α - and γ -*Proteobacteria*. The growth of thorny bamboo in the badland soil could change soil properties and in turn directly and/or indirectly affect soil bacterial structure and diversity.

Advanced omic approaches to soil microbe characterization.

Mary S. Lipton, Carrie D. Nicora, Thomas O Metz, Erin Baker, Richard A. White III, Taniya Roy Chowdhury, Eric M. Bottos, Jennifer E. Kyle, Colin J. Brislawn, Kristin E. Burnum-Johnson, Samuel Payne, Chris Whidbey, Natalie C. Sadler, Janet K. Jansson

Pacific Northwest National Laboratory

Microbes in soil do not live in isolation, and as such, understanding the function of microbes within the context of their community is critical to the characterization of the community as a whole. Macromolecule expression profiles reveal the actual functional potential of any biological system, but obtaining this information from the biological matter in soil can be challenging. Chemical heterogeneity, biological diversity resident within soils, along with microbial strain variation complicates omic based experiments and data analysis. To this end, we have developed a suite of omics based approaches for the characterization of

macromolecules from soil. Effective protein extraction represents a large challenge in soil proteomic analyses and we evaluated a series of protein extraction methods for a particular type of soil soils that effectively allow for the characterization the functional potential of the resident microbes in the soil including a multi-omics extraction (MEO) allows the isolation of proteins, lipids and metabolites concurrently To address the challenges of throughput and high amounts of humic acids and minerals in the protein extracted from soil, ion mobility spectrometry measurements were integrated between liquid chromatography (LC) and MS analyses. Using this approach IMS separated the humic acids from the peptides of interest prior to MS detection due to their vastly different structures. MS analyses with either gas chromatography or LC-IMS front separations were also performed on the lipids that were extracted using the MEO approach. In the lipidomic studies, novel lipids, such as highly unsaturated triglycerides with three very long carbon chains that have never been observed at PNNL previously, were detected. Hundreds of metabolites ranging from amino acids, nucleic acids and sugars were also detected in soil samples from the aqueous fraction of the MEO preparations from soil.

Metagenomic and comparative analysis in agricultural and mining soils in Guanajuato, Mexico

María Elena López-Pérez¹, Gabriela Ana Zanor¹, María Cristina del Rincón-Castro^{1†}

¹División de Ciencias de la Vida. Universidad de Guanajuato. Ex Hacienda El Copal Km. 9 Carretera Irapuato-Silao; A.P. 311; C.P. 36500, México. †Corresponding author (cdelelrincon@ugto.mx)

Two samples of soil were analyze, agricultural soil (MASE) and mining soil (SMI), from Guanajuato, México. The analysis included physical-chemical properties, trace elements, total DNA extraction and 454 GS FLX sequencing. MASE showed high contents of active phases of soil (clay: 61.21% and 8.27% organic matter), 6.44 pH, and was very high in nitrogen (0.38 %), indicating fertility conditions with a good exchange of nutrients. SMI presented loam texture, 8.10 pH, and high content in organic matter (7.40%) and nitrogen (0.17%). MASE showed below of the total reference trace elements concentration (CR_T). However, SMI showed high concentrations of As, Zn, Cu and Pb. Total number of reads from MASE and SMI soils were 175,240 with an average in length sequences of 411 bases. The 16S rRNA sequences aligned to SILVA database were 3,716 to MASE and 3,425 to SMI, while for the 18S rRNA sequences obtained were 17,299 and 16,760 respectively. The Phylum with the highest proportion of 16S rRNA gene corresponds to Proteobacteria (38% overall). The Acidobacteria Phylum was the second most representative with 24.2% for the sample SMI and 11.6% for MASE. MASE soil showed in a greater proportion the Actinobacteria Phylum (19.0%) when compared with SMI soil (14.2%). MASE soil showed the highest proportion of 18S rRNA gene, (63.1%) corresponding to the unidentified Fungi, followed by other organisms unassigned to SMI (49.8%). However, the Ascomycota Phylum was found in both samples. The Glomeromycota Phylum from 18S rRNA was found in SMI, but not in MASE. The OTUs estimated from the 16S rRNA were 254 to MASE and 206 to SMI. For the 18S rRNA, was obtain 98 and 110 OTUs, respectively. This study could help to understand and explain the correlation between two different soils, their properties, and the presence of certain soil microorganisms.

Key words: metagenomics, DNA, 16 S rRNA, 18S rRNA, physicochemical soil properties

Soil bacterial community structure and function shift along a successional series of tidal flats in the Yellow River Delta

Xiaofei Lyu^{1,2,3}, Junbao Yu^{1*}, Bin Ma¹, Scott Chang³

¹ Key Laboratory of Coastal Environmental Processes and Ecological Remediation, Yantai Institute of Coastal Research (YIC), Chinese Academy of Sciences (CAS); Shandong Provincial Key Laboratory of Coastal Environmental Processes, YICCAS, Yantai, Shandong, China

² University of Chinese Academy of Sciences, Beijing, China

³ Department of Renewable Resources, University of Alberta, Edmonton, Alberta, Canada

* Correspondence author: Pr. Junbao Yu

Tidal flats are critical components of coastal ecosystems and are characterized by high primary productivity and diversity. The bacteria in tidal flat sediments drive various biogeochemical processes. However, the structure and function of bacterial community in tidal flats are poorly understood. Successional tidal flat ecosystems form natural environmental gradients, which may influence bacterial community. Microbial communities also face large seasonal variations in temperature in temperate coastal regions. This study aimed to reveal how bacterial communities respond to the seasonal variation along a successional series of tidal flats (subtidal, intertidal and supratidal flats). Bacterial community composition and diversity from tidal flats were analyzed over four seasons by 16S rRNA genes using the Ion Torrent PGM platform. The relative abundances of Acidobacteria, Gemmatimonadetes, and Nitrospirae increased, while Chloroflexi, Actinobacteria, and Firmicutes decreased from subtidal to supratidal flat. Bacterial phylogenetic diversity increased, and phylogenetic turnover decreased, from subtidal to supratidal flat. Moreover, the bacterial community structure differed significantly among seasons. Temperature, Na⁺, SO₄²⁻, and nitrite and clay contents were the main drivers and explained 14.4% of the variability. Despite major compositional shifts, functional capacity predicted with PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) revealed high similarity in most of level-3 KEGG (Kyoto Encyclopedia of Genes and Genomes) Orthology groups. The predicted soil bacterial metagenomes in winter had lower relative abundances of functional genes associated with biogeochemical processes, such as nitrogen metabolism, methane metabolism, vitamins metabolism and energy metabolism. Taken together, our study indicates that the bacterial community structure and function in tidal flats shifted along the tidal flat gradient. This research provides new insights on the impact of a tidal gradient on bacterial community in coastal ecosystems.

Frozen in time? Microbial strategies for survival and carbon metabolism over geologic time in a Pleistocene permafrost chronosequence

Rachel Mackelprang¹, Thomas A. Douglas², Mark P. Waldrop³

¹California State University Northridge, Northridge, CA

²Cold Regions Research and Engineering Laboratory, Fairbanks, AK

³United States Geological Survey, Menlo Park, CA

Permafrost is gaining interest as a model for exobiology. Since six of the other eight planets in our solar system, as well as their moons, asteroids, and comets are permanently frozen, life on these celestial bodies is most likely to be found in a subzero environment. On Earth, life can exist in permafrost for millennia and may act as an analogue reflecting potential inhabitants on extraterrestrial cryogenic bodies. Active microbial life exists in even the most ancient permafrost, but we know little about the strategies utilized by permafrost microbes that enable survival over geologic time. Here we describe a 16S rRNA gene and shotgun metagenomic study targeting a chronosequence (12kyr – 35kyr) of Pleistocene aged permafrost. 16S rRNA sequencing and analysis showed decreasing microbial diversity and higher abundance of endospore-forming Firmicutes in increasingly older samples. 16S rRNA gene and metagenomic data showed significant age-based clustering. The youngest samples were enriched in genes involved in the degradation of complex polysaccharides whereas older samples had significantly greater abundance of genes involved in lipid and amino acid degradation. These data suggest increasing reliance on scavenging of detrital biomass in older permafrost. A gene common in older samples is involved in cadaverine production, which could explain the putrefied smell of Pleistocene permafrost. A significantly greater abundance of chemotaxis genes in the oldest samples suggests that thermotaxis and chemotaxis may be modulated by overlapping systems. We binned 30 draft genomes from metagenomic assemblies including several members of Thaumarchaeota, which the 16S rRNA gene data showed to be the most abundant archaeal group across all ages. Together, these data increase understanding of how permafrost microbes transform organic matter over geologic time and identify biochemical adaptations enabling long-term survival with no influx of new energy or materials.

Microbial ecology of restored floodplains

Christopher W Marshall, Sarah L O'Brien, Kenneth M Kemner, Edward J O'Loughlin, Neil R Gottel, Silvia Alvarez Clare, Aaron A Best, Theodore M Flynn, and Jack A Gilbert

Argonne National Laboratory, Argonne, IL; North Central College, Naperville, IL; Hope College, Holland, MI, University of Chicago, Chicago, IL

Freshwater flooding of terrestrial soils occurs naturally, but residential and commercial land use can exacerbate flooding and sedimentation of rivers and lakes, causing environmental and economic damage. Restoring private and public property to natural floodplains can help alleviate the damage due to flooding events, but little is known about the effect that periodically waterlogged soils have on microbial activity and the resultant greenhouse gas emission to the atmosphere. Because flooding these soil systems generates an anaerobic state due to heterotrophic oxygen consumption, the resulting change in microbial community structure and metabolism leads to CH₄ flux to the atmosphere.

Substantial carbon (C) release from waterlogged soils to the atmosphere impacts atmospheric concentrations of carbon dioxide (CO₂) and methane (CH₄) (which has ~25x the greenhouse capacity of CO₂, and has steadily increased in the atmosphere for the last 200 years). Hence, we must quantify these interactions to understand their net effect on atmospheric radiative forcing of methane. The present study utilizes extensive 16S rRNA amplicon sequencing data to correlate floodplain sites at different ages since restoration (<5 years, 5-10 years, >10 years) to the microbial ecology particular to that site. Additionally, moisture, depth, terminal electron acceptor concentrations, pH, and methane flux are all examined to ascertain the complexities of microbial activity in response to land-use change. Results from this study indicate that the variability of the microbial communities could largely be explained by site location, soil depth, C:N ratio, and pH. Microbial diversity was higher at older floodplains and decreased with deeper sampling depths. The latter correlated with a decrease in moisture, total carbon, and total nitrogen with depth. Additionally, Deltaproteobacteria OTUs were significantly enriched at lower depths compared to Verrucomicrobia and Bacteroidetes at higher soil depths. Furthermore, each site had certain taxa that were significantly enriched, laying the foundation for building site-specific microbial signatures. We propose that these signatures could eventually be used for determining floodplain health and predictive modeling of greenhouse gas emissions from waterlogged soils.

Spatio-temporal dynamics of *Phymatotrichopsis* root rot disease in alfalfa hay production fields

Chakradhar Mattupalli¹, Casey Curtsinger², Corey A. Moffet³, James K. Rogers², Carolyn A. Young¹

¹Forage Improvement Division, The Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73401, USA

²Agricultural Division, The Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73401, USA

³USDA-ARS, Southern Plains Range Research Station, 2000 18th St, Woodward, OK 73801, USA, (formerly the Agricultural Division, The Samuel Roberts Noble Foundation)

Phymatotrichopsis Root Rot (PRR) is a devastating disease caused by the soil-borne fungus, *Phymatotrichopsis omnivora* that affects many dicots including alfalfa. Alfalfa stands in the southern Oklahoma region can be heavily infested by *P. omnivora* and affected fields have shortened stand life. Little information is available as to how PRR spreads in alfalfa hay production fields. Understanding the spatio-temporal dynamics of PRR is essential for epidemiology and disease management. Hence in 2015, we established an experimental site by selecting five diseased sites from a 120-acre alfalfa hay production field at the Noble Foundation's Red River Farm, Burneyville, OK. Plant samples will be collected from June 2015 through October 2015 representing alfalfa's active growth phase. First and second samplings were performed in June and August respectively from ten quadrats along a transect spanning unaffected and diseased sectors that included some surviving plants. Three plants collected from each quadrat were assessed for shoot and root characteristics. DNA was extracted from root samples and end-point PCR was performed to detect *P. omnivora* using ITS primers specific for the pathogen. During the first sampling period, *P. omnivora* was detected in roots of healthy plants that were 3.5 meters away from the disease front in the unaffected sector indicating the pathogen's progression prior to visible

symptomology. Within 35 days, the disease has progressed into the unaffected sector by a distance of 0.5 to 0.7 meters across the disease sites. In addition, a substantial decrease in biomass of plants from the diseased sector was noted between first and second sampling periods suggesting interplay of host, environment, and microbial community factors. Field data from this study will be used to generate hypotheses for conducting rhizosphere metagenomic studies that may unravel the microbial community fluctuations influencing growth and survival of *P. omnivora*.

Conversion of Amazon rainforest to agriculture alters community traits of methane-cycling organisms

Kyle Meyer^{1**}, Ann Klein^{1**}, Jorge Rodrigues², Klaus Nüsslein³, James M. Tiedje⁴, Titus Brown⁵ and Brendan J.M. Bohannan^{1*}

¹Institute of Ecology and Evolution, Department of Biology, University of Oregon, Eugene, OR

²Department of Land, Air and Water Resources, University of California, Davis, CA

³Department of Microbiology, University of Massachusetts, Amherst, MA

⁴Department of Plant, Soil, and Microbial Sciences, Michigan State University, East Lansing, MI

⁵Department of Population Health and Reproduction, University of California, Davis, CA

* Corresponding author (bohannan@uoregon.edu)

**These authors contributed equally to this work.

Land use change is one of the greatest environmental threats worldwide, especially to tropical forests. The Amazon rainforest has been subject to particularly high rates of land use change, primarily to cattle pasture. Land use change in the Amazon has been shown to result in significant effects on ecosystem functions, including changes to the rates and controls of biogeochemical cycles. A commonly observed response to cattle pasture establishment in the Amazon is the conversion of soil from a methane sink in rainforest, to a methane source in pasture. However it is not known how the organisms that mediate methane flux are altered by land use change. Here we show markers for methanotrophs (methane-consuming microorganisms) differ between forest and pasture in the Amazon, while fewer markers for methanogens (methane-producing microorganisms) appear to differ. Pasture soils have a significantly lower methanotroph-to-methogen ratio. Type II (Alphaproteobacteria) methanotrophs are disproportionately affected, showing a decrease in relative abundance as well as a shift in taxonomic composition, diversity, and evenness. This is of concern because Type II methanotrophs play a significant role in the consumption of atmospheric methane. In addition, we observed that the abundance of genes from the particulate methane monooxygenase (pMMO) pathway significantly declined following conversion, while genes from the soluble methane monooxygenase (sMMO) pathway did not. Cells containing pMMO have a higher affinity for methane than sMMO containing cells, and thus may play a larger role in the consumption of atmospheric methane. Our observations suggest that methane-consuming organisms are more vulnerable to land use change in Amazon soils than methane-producing organisms, and this vulnerability may underlie the response of methane flux to land use change in these soils.

Proteomics of soil and sediment: Protein identification by *de novo* sequencing of mass spectra complements traditional database searching

Samuel Miller, Adriana Rizzo, Jacob Waldbauer

Department of Geophysical Sciences, University of Chicago

Proteomics has the potential to elucidate the metabolic pathways and taxa responsible for *in situ* biogeochemical transformations. However, low rates of protein identification from high resolution mass spectra have been a barrier to the development of proteomics in complex environmental samples. Much of the difficulty lies in the computational challenge of linking mass spectra to their corresponding proteins. Traditional database search methods for matching peptide sequences to mass spectra are often inadequate due to the complexity of environmental proteomes and the large database search space, as we demonstrate with soil and sediment proteomes generated via a range of extraction methods.

One alternative to traditional database searching is *de novo* sequencing, which identifies peptide sequences without the need for a database. BLAST can then be used to match *de novo* sequences to similar genetic sequences. Assigning confidence to putative identifications has been one hurdle for the implementation of *de novo* sequencing. We found that accurate *de novo* sequences can be screened by the quality score and length of the sequences and the complementary use of multiple *de novo* sequencing algorithms. Screening criteria are verified by comparing the results of *de novo* sequencing and traditional database searching for well-characterized proteomes from simple biological systems. The BLAST hits of screened sequences are interrogated for taxonomic and functional information. The behavior of BLAST searches for short peptide sequences with small numbers of possible amino acid errors guides the interpretation of these results.

We applied *de novo* sequencing to organic topsoil and marine sediment proteomes. Peak-rich proteomes, which can result from various extraction techniques, yield thousands of high-confidence protein identifications, an improvement over previous proteomic studies of soil and sediment.

BAC Sudoku sequencing strategy for *in silico* screening of large-insert soil metagenomic libraries

Scott Monsma¹, Jinglie Zhou², Alinne Pereira², Blaine Pfeifer³, Timothy Bugni⁴, Scott R. Santos², Megan Niebauer¹, Erin Ferguson¹, Ron Godiska¹, ChengCang Wu⁵, David Mead¹, and Mark R. Liles²

¹Lucigen Corporation, 2905 Parmenter St., Middleton WI, USA; ²Department of Biological Sciences, Room 101 Rouse Life Sciences Building, 120 West Samford Avenue, Auburn University, Auburn AL, 36849 USA; ³Department of Chemical and Biological Engineering, 904 Furnas Hall, State University of New York at Buffalo, Buffalo NY 14260; ⁴School of Pharmacy & Department of Chemistry, 777 Highland Ave, University of Wisconsin-Madison, Madison WI 53705-2222; ⁵Intact Genomics Inc., 1100 Corporate Square Dr., Suite 257, St. Louis, MO 63132

Soil microorganisms express diverse bioactive natural products; however, the majority of soil microbes are recalcitrant to cultivation. We are using a metagenomic approach to bypass cultivation and directly capture the DNA from diverse microbial genomes in natural environments such as soils. A metagenomic library from an agricultural soil (Cullars Rotation, Auburn, AL) was constructed in a broad host-range BAC vector that contained 19,200 clones with an average insert size of 110kb. Screening was accomplished using strategy termed BAC Sudoku sequencing, wherein a pooling strategy is used to multiplex the clones for sequencing, while still providing the ID of individual clones. BAC clones were sequenced in pools (row, column, and plate) using indexed primers and paired end reads on an Illumina HiSeq. Contigs were assembled for each pool and screened for secondary metabolite gene clusters using antiSMASH, resulting in identification of >1000 novel PKS/NRPS pathway-containing clones. The cloned pathways are very divergent from known pathways, with the GC content varying from 41 to 76% and the amino acid identity of the KS domains ranging from 32 to 83% to the best matching BLAST hit. Expression of these PKS pathway-containing clones in *E. coli* strain BTRA engineered for polyketide expression has resulted in multiple clones with evidence for heterologous expression of a cloned PKS pathway. These results indicate a high degree of unique sequence space has been recovered from large-insert metagenomic clones and a subset of these clones are capable of being heterologously expressed to produce secondary metabolites, thereby expanding our available resources for natural product discovery.

Bacterial and archaeal ammonia oxidizers are reduced by increasing timber harvest intensity in surface and subsurface soils of the western Gulf Coastal Plain

Ryan M. Mushinski¹, Thomas W. Boutton¹, and Terry J. Gentry²

¹Dept. Ecosystem Science & Management, Texas A&M University; ²Dept. Soil & Crop Sciences, Texas A&M University

Disturbances such as timber harvesting have the potential to diminish forest productivity by removing limiting nutrients in the harvested biomass, increasing the potential for nutrient losses from soil, and altering the structure and function of soil microbial communities. The Long-Term Soil Productivity (LTSP) experiment was initiated to address concerns over possible losses of soil productivity due to disturbance associated with forest management. We determined the effect of forest harvest intensity (i.e., no-harvest, merchantable bole/stem-only harvest, and whole-tree harvest + forest floor removal) on bacterial and archaeal *amoA* copy number, soil microbial biomass carbon (MBC) and nitrogen (MBN), $\text{NH}_3/\text{NH}_4^+$ and NO_3^- pool sizes, and other soil physicochemical properties in acidic surface and subsurface soil of a *Pinus taeda* L. forest, 18-yrs post-harvest, at the Groveton LTSP site in eastern Texas. Soils were sampled (0-10, 10-30, 30-60, and 60-100 cm) seasonally during 2014-2015. We quantified *amoA* with DNA-based qPCR, microbial biomass by chloroform fumigation/extraction, and $\text{NH}_3/\text{NH}_4^+$ and NO_3^- with colorimetric assays. The abundances of both bacterial and archaeal *amoA* were influenced by harvest method, soil depth, and time. Archaeal *amoA* was significantly more abundant than bacterial *amoA*, at all depths. MBC and MBN were generally unaffected by harvest method; however, differences were found with respect to soil depth and time. $\text{NH}_3/\text{NH}_4^+$ and NO_3^- concentrations were influenced significantly by harvest treatment, soil depth, and time. Results indicate that harvest methods that minimize organic matter removal will favor a

higher number of ammonia oxidizing bacteria and archaea, increase soil N availability, and promote the sustainability of forest productivity.

What do 'omics do best?

David D. Myrold,

Oregon State University

The use of next generation sequencing technology has been applied to studies of soil microbial communities for about a decade, initially with amplicon-based techniques. Shotgun metagenomics of soil soon followed, as did application of transcriptomic and proteomic methods, resulting in an initial pulse of omics studies reported in the soils literature. Although technical challenges persist and will need to be solved before the application of omics to studying soil systems becomes standard, significant progress has been made in addressing many of these challenges. Thus, it is appropriate that we step back from the excitement of being able to make the novel measurements of soil microbial communities that omics have enabled and ask ourselves: What do omics do best?

Distribution of fungi in arid microenvironments and their potential role on plant growth

Cedric Ndinga Muniania¹, Katrina Sandona¹, Jayne Belnap³, Cheryl R. Kuske², Andrea Porrás-Alfaro¹

1. Western Illinois University, 2. Los Alamos National Laboratory, 3. US Geological Survey

In arid ecosystems, fungi form complex microbial communities with plants and other photosynthetic organisms. Many of these fungi are likely to contribute to plant survival, soil protection, and nutrient enrichment. However the role and diversity of these fungi are not well known, especially their potential pathogenicity or growth promoting properties that could impact nearby plants. We collected soil and isolated fungi from different microenvironments in an arid grassland near Moab, UT. The biocrust (BSC) fungi were isolated from lichen, moss and cyanobacteria, and rhizosphere soils were collected from two plants, *Bromus tectorum* and *Hilaria jamesii*. Fungi were isolated using a serial dilution technique and identified using ITS rRNA sequencing. Abundance and seasonal variation of the fungi in the different microenvironments will be determined using Illumina sequencing. From the 906 fungi isolated, 806 have been sequenced and Ascomycota was the dominant phylum. Pleosporales was the dominant order in the BSC and Eurotiales was the dominant order in the rhizosphere. The most dominant genera included *Aspergillus*, *Coniochaeta*, *Alternaria*, *Preussia*, *Cladosporium*, *Chaetomium* and *Penicillium*. Seed germination experiments using dominant taxa were conducted in corn and soybean to determine potential roles of these fungi on plant growth. Dark septate fungi, in particular *Cladosporium* and *Alternaria*, promote plant growth by stimulating root production and stem elongation. In addition of dark septate fungal adaptation to arid ecosystems, this growth promoting ability could be an important factor to help plants to cop with heat and drought conditions.

Agricultural nitrogen management affects response to ammonium and ammonia oxidizer communities

Jeanette Norton¹, Yang Ouyang¹, John Stark¹, Mussie Habteselassie²

¹Utah State University, ²University of Georgia

Nitrification provides a link between ammonium (product of mineralization and major fertilizer) and denitrification. Understanding nitrification is therefore central to our ability to predict and manage soil N losses and nitrous oxide production. A multi-year experiment was conducted in Utah and Georgia USA to examine N-source effects on nitrification in agricultural systems. N-sources include low and high levels of ammonium sulfate fertilizer (100 and 200 kg_N/ha) and manure composts. Real-time quantitative PCR targeting *amoA* was used to follow changes in bacterial (AOB) and archaeal ammonia oxidizer (AOA) populations. Amplicon pyrosequencing of *amoA* characterized community and selective inhibition differentiated ammonia oxidation mediated by AOB versus AOA. Changes in population size for the AO in response to N treatments were found in the first year based on potentials and by the second season effects were significant for gene abundance. Differential inhibition and quantitative PCR revealed that while AOA gene counts were higher, AOB populations were more dynamic and responsible for an equal or greater fraction of the ammonium oxidized. The combination of kinetic and metagenomic approaches has brought us closer to the goal of linking the capable organisms to the process rate and extent in the environment.

Identifying microbial habitats in soil using quantum dots and x-ray fluorescence microtomography

Sarah L. O'Brien¹, Matthew D. Whiteside², Deirdre Sholto-Douglas¹, Alice Dohnalkova³, Daniel Durall⁴, Doga Gursoy¹, Melanie D. Jones⁴, Libor Kovarik³, Barry Lai¹, Christian Roehrig¹, Shane Sullivan¹, Stefan Vogt¹, Kenneth Kemner¹

¹Argonne National Laboratory

²Vrije Universiteit Amsterdam

³Pacific Northwest National Laboratory

⁴University of British Columbia, Okanagan

The metabolic activities of soil microbes are the primary drivers of biogeochemical processes controlling the terrestrial carbon cycle, nutrient availability to plants, contaminant remediation, water quality, and other ecosystem services. However, we have a limited understanding of microbial metabolic processes such as nutrient uptake rates, substrate preferences, or how microbes and microbial metabolism are distributed throughout the three-dimensional pore network of the soil. Here we use a novel combination of imaging techniques with quantum dots (QDs, engineered semiconductor nanoparticles that produce size or composition-dependent fluorescence) to locate bacteria in the three-dimensional pore network of a soil aggregate. First, we show using confocal and aberration-corrected transmission electron microscopies that bacteria (*Bacillus subtilis*, *Pseudomonas fluorescens*, and *Pseudomonas protogens*) actively take up and internalize CdSe/ZnS core/shell QDs conjugated to biologically relevant substrates. Next, we show that cells bearing QDs can be identified using fluorescence imaging with hard x-rays at 2ID-D at

the Advanced Photon Source (APS). Finally, we demonstrate that the Se constituent to the QDs can be used to label bacteria in three-dimensional tomographic reconstructions of natural soil at 0.5 nm spatial resolution using hard x-rays at 2ID-E at the APS. This is the first time soil bacteria have been imaged in the intact soil matrix at such high resolution. These results offer a new way to experimentally investigate basic bacterial ecology in situ, revealing constraints on microbial function in soil that will help improve connections between pore-scale and ecosystem-scale processes in models.

Effects of soluble electron shuttles on microbial Fe(III) reduction and methanogenesis in wetland sediments

Edward J. O'Loughlin^{1*}, Margaret F. Sladek¹, Dionysios A. Antonopoulos¹, Theodore M. Flynn¹, Jason C. Koval¹, Christopher W. Marshall¹, and Kenneth M. Kemner¹

¹ Biosciences Division, Argonne National Laboratory, Lemont, IL 60439

Iron redox cycling by microorganisms is a significant component of C cycling and energy flux in many aquatic and terrestrial environments. Dissimilatory iron-reducing bacteria (DIRB) are phylogenetically diverse microorganisms that obtain energy by coupling oxidation of organic compounds or H₂ to reduction of Fe(III) to Fe(II). Because of the relative insolubility of most Fe(III)-bearing minerals, many DIRB use soluble electron shuttles (e.g., quinones, flavins, phenazines, and reduced sulfur species) to transfer electrons from the cell to external electron acceptors. Studies investigating effects of electron shuttles on microbial Fe(III) reduction have typically been conducted under axenic conditions. To better understand how electron shuttles influences microbial Fe(III) reduction in the presence of a diverse microbial community, we examined the effects of different electron shuttles (9,10-anthraquinone-2,6-disulfonate acid (AQDS), 9,10-anthraquinone-2-carboxylic acid (AQC), and 5-hydroxy-1,4-naphthoquinone (lawsone, NQL)) on the bioreduction of Fe(III) oxide and methanogenesis in microcosms inoculated with wetland sediment. Our results showed no significant enhancement of Fe(III) reduction in the presence of NQL or AQC relative to the no shuttle (NS) control; however both the rate and extent of Fe(II) production were enhanced in the presence of AQDS. The onset of methanogenesis was earlier in the presence of AQDS compared to NQL and NS, but in each case methane production was not evident until Fe(II) production ceased. Methanogenesis was completely inhibited in the presence of AQC, highlighting the potential for electron shuttles to influence microbial processes not involving microbial respiration using insoluble electron acceptors. Systems amended with AQC were dominated by microorganisms classified in the family *Pelobacteraceae* (avg. 45.4% total abundance), while *Geobacteraceae* dominated in microcosms amended with AQDS (30-48%), NQL (51.9%), or NS (37%). *Geobacteraceae* sequences were of much lower abundance in the AQC enrichments, accounting for only 8% of the total abundance on average and only 3-4% in two of the three replicates. While closely related to the *Geobacteraceae*, organisms in *Pelobacteraceae* lack c-type cytochromes and are unable to transfer electrons directly to ferric iron. This suggests AQC may inhibit direct reduction of ferric iron by organisms such as *Geobacter*, allowing *Pelobacter* spp., which would otherwise be outcompeted, to dominate.

Accurate detection and quantification of target genes in shotgun metagenomes: method development and application to the nitrogen cycle genes

Luis H Orellana¹, Luis M Rodriguez-R² and Konstantinos T Konstantinidis^{1,2}

School of Civil and Environmental Engineering¹ and School of Biology², Georgia Institute of Technology, Atlanta, Georgia, USA.

Metagenomics can elucidate the diversity, abundance, and dynamics of microbial genes and pathways participating in biogeochemical transformations of nutrients in a variety of ecosystems. However, thresholds that accurately discriminate between true and false positive matches during sequence similarity searches of metagenomes are rarely evaluated. To overcome these limitations, we developed a methodology aimed to identify position-specific, most-discriminant bitscore thresholds for target genes. Our methodology employs the receiver operating characteristic (ROC) curve to analyze simulated shotgun metagenomic reads that map onto well-curated reference protein sequences, and a sliding window across the length of the reference sequences to deal with non-discriminative domains shared between different proteins. Using popular search algorithms such as BLASTx, our strategy showed an improved false discovery rate, up to 38-fold, when compared to the common practice of using a fixed e-value. We coded our approach into an automated pipeline, called ROcker, and used it to investigate the abundance and diversity of nitrogen cycle genes in Illumina metagenomes, such as nitrous oxide reductase (*nosZ*), mediating the reduction of the potent greenhouse gas, N₂O, to N₂. Atypical *nosZ* genes were 2 times more abundant, on average, than their typical counterparts in most soils. Further, our comparative assessment of N-cycle genes indicated a higher reduction potential of N₂O in marine sediments versus terrestrial soils. We also quantified nitrification genes in the same soil metagenomes, such as the ammonia monooxygenase genes (*amoA*), using a ROcker model that accurately discriminates short-gene fragments from their archaeal counterpart and methane monooxygenase (*pmoA*) genes. Our study provides a bioinformatic strategy for reliable detection of target genes in metagenomes and expands the known sequence diversity of key nitrogen cycle genes.

Exploring Verrucomicrobia populations in grassland soil using Moleculo

Sarah M. Owens¹, William L. Trimble², Sarah L. O'Brien¹, Stephanie M. Greenwald¹, Dionysios A. Antonopoulos¹, and Folker Meyer¹

Biosciences Division, Argonne National Laboratory¹; Computation Institute, University of Chicago²

We investigated soil bacterial community structure in a switchgrass stand at high spatial resolution to determine whether biogeographic trends occurred at the centimeter scale (O'Brien et al. 2015; in review). Bacterial communities were highly heterogeneous, with abrupt changes in relative abundance of bacterial phyla from samples collected only centimeters apart. Such heterogeneity did not obscure larger-scale trends; however, at the ecosystem scale bacterial community composition and structure were subtly, but significantly, altered by fertilization, with higher alpha diversity in fertilized plots. We also identified a single Verrucomicrobia-derived OTU that dominated many of our small-volume soil cores (<2 g each). Here, we use complementary sequencing approaches to determine

whether this OTU is taxonomically coherent or is comprised of multiple species/strains. We hypothesize that if it is many species/strains, they may have conserved elements (core genome) and variable regions that suggest variations in ecology or function, potentially defining a pan-genome for the Verrucomicrobia in grasslands (Fierer et al. 2012, 2013; Bergmann et al 2011). In order to test our hypothesis, we selected 4 samples rich in Verrucomicrobia for Moleculo long-read sequencing and shotgun sequencing, and generated shotgun metagenomic sequences (Illumina Hiseq2000) for an additional set of 12 soil samples. Samples from fertilized and unfertilized soils were sequenced to determine whether this field treatment impacts the gene content of the species/strains of Verrucomicrobia observed. While the Long-read Moleculo data assembled well, and confidently provided 10kb contigs, those contigs could not be assembled further to get a better picture of the Verrucomicrobia genomes present. This limitation resulted from a lack of redundancy in the reads because of the incredible diversity of the soil microbiota. While the long-reads did not provide whole genome assemblies, we were able to get better bacterial and protein annotations than with short read data. In addition, a comparison of raw shotgun reads with assemblies of shotgun reads and synthetic long reads will provide an assessment of the accuracy of current methods and will shed light on any potential biases in using high-throughput and long-read sequencing to understand the biochemistry of microbes in soil and elsewhere.

Uncovering Earth's virome

David Paez-Espino¹, Alexander D. Thomas¹, Emiley Eloie-Fadrosch¹, Marcel Huntemann¹, Amrita Pati¹, Edward Rubin¹, Natalia N. Ivanova¹, Nikos C. Kyrpides¹

¹Department of Energy, Microbial Genome and Metagenome Program, Joint Genome Institute, Walnut Creek, USA.

Viruses are the most abundant biological entities on Earth. They play an essential role in all biogeochemical cycles, and impact microbial populations and diversity. Paradoxically, we know very little about viruses due to the complexity in the isolation of the host where they rely on and the lack of large culture collections, which extremely restricts the number of available viral sequences. To address these limitations, we combined a variety of computational methods to interrogate cal. 5 Tb of sequence data from over 4,000 diverse metagenomic samples for the presence of viruses. We discovered over 133 thousand viral scaffolds (cal. 2.25 Gb of viral sequences), representing more than a twenty-fold increase in the amount of sequence data derived from isolated double-stranded DNA viruses. We developed a coherent framework for clustering viral sequences, and revealed that 96.1% of all clustered viral groups were novel. Further, 10% of the identified viral groups were connected to their host organism(s) by using a combination of (a) known host-virus interactions from isolate viral genomes, (b) CRISPR-spacer matches from a newly developed 3.5 million spacer database, and (c) a novel viral tRNA encoding approach specifically linked to host tRNA sequence. Remarkably, these approaches revealed numerous host-viral associations across hundreds of previously unrecognized genera and doubled the number of bacterial phyla putatively infected by viruses. In addition, we determined viral diversity and distribution across distinct habitats revealing a strong endemism for the vast majority of the viral groups within the same ecosystem category. The paucity of information for soil-derived viruses led us to further investigate viral groups across soil samples. Our results

highlight the expansive viral diversity found globally, and provides insight into habitat distribution and previously unrecognized host-viral interactions.

The role of environmental and genetic factors in shaping the microbiome of a highly olfactory bird species

Douglas S. Pearce¹, Brian Hoover², Gabrielle A. Nevitt² and Kathryn Docherty¹

(1)Biological Sciences, Western Michigan University, Kalamazoo, MI, (2)Neurobiology, Physiology and Behavior, University of California- Davis, Davis, CA

Background/Question/Methods

Many animal species rely heavily on olfaction for self-recognition, mate selection, foraging and predation. The microbiome is hypothesized to play a role in an animal's odor signature by fermenting compounds found in secretions and producing odorous metabolites. Individual microbiomes can be affected by habitat, but individual animal genetics can also shape specific microbial communities. While microbial communities have been shown to influence the social interactions several mammalian studies, no studies have demonstrated this in birds. *Procellariiformes* are highly olfactory seabirds that rely on odors to locate food, relocate nests, and identify conspecifics and potential mates. In this study, we collected skin swab samples from dorsal and ventral locations from twenty pairs of Leach's storm petrels from a well-established colony at Bon Portage Island in Nova Scotia, Canada. We purified microbial DNA from body swabs and surrounding burrow soil for next-generation sequencing. We hypothesized that birds in mated pairs harbor significantly different microbial communities and potentially exploit these differences to avoid inbreeding and spread of microbial pathogens.

Results/Conclusions

We processed paired-end sequences using mothur v.1.34.4 and visualized multivariate microbial community data using nonmetric multidimensional scaling (NMDS), analysis of similarity and permutational multivariate analysis of variance. Dorsal and ventral samples collected from individual birds clustered separately. Results indicate that petrels in mated pairs do not carry different microbial communities from their mates. Additionally, there was no effect of sex on microbial community composition, suggesting that the microbiome is not sex-specific in these birds, but may be related to other genetic traits. Additionally, burrow location did not impact bird-associated microbial communities, providing evidence that bird-associated microbial assemblages are influenced more by the individual bird and its mate than by habitat. This study provides the first robust examination of factors that shape the microbiome of a highly olfactory avian species.

The importance of dispersal in microbial communities

Kabir G. Peay¹

¹Department of Biology, Stanford University, Stanford CA 94305-5020

Dispersal plays a central role in most ecological and evolutionary theories. While microbial ecology has mostly moved beyond the Baas-Becking debate, an alternative framework for understanding the effects of dispersal on microbial community dynamics is needed. Using

experiments and observational studies of fungi, I demonstrate a number of ways in which dispersal affects the composition and richness of fungal communities. First, while many fungi are capable of long-range dispersal, I show that dispersal limitation can occur at even very local scales due to the influence of spore quantity and arrival time on fungal competition. Spore trap data confirm that spore deposition is highly spatially and temporally heterogeneous, even within a homogeneous habitat type. Second, dispersal ability is an important functional trait. Fungi within a community exhibit great differences in overall spore production and longevity, both of which influence the predictability of community assembly. Finally, while dispersal is difficult to measure across hundreds or thousands of kilometers, observations from population genomics and continental studies of community composition provide evidence that long-range dispersal is not frequent enough to homogenize the geographic structure of populations or communities. Based on these results, I argue that dispersal is one of the central principles necessary to understand the ecology of microbial communities.

Rhizosphere versus endosphere bacterial microbiomes: how much do they overlap?

Marketa Polivkova¹, Lucie Musilova¹, Michal Strejcek¹, Jachym Suman¹, Tomas Macek¹, Ondrej Uhlík¹

¹University of Chemistry and Technology, Prague, Faculty of Food and Biochemical Technology, Department of Biochemistry and Microbiology, Technická 3, 166 28 Prague 6, Czech Republic

Many environmental microorganisms are associated with terrestrial plants. Areas with extensive plant-microbial interactions include primarily rhizosphere and endosphere. The beneficial plant-microbe interactions are essential to nutrient acquisition, disease suppression, or growth promotion and survival of plants in unfavorable conditions. Whereas rhizosphere diversity have been a subject of many studies, endosphere microbiomes are still mostly unrevealed. Consequently, little is known about how similar in terms of microbial diversity the rhizosphere and endosphere are. Since the rhizosphere is a known entry point for endophytic bacteria, we hypothesized that endophytes would be mostly a subset of rhizosphere bacteria. Additionally, since variable soil conditions influence the diversity of rhizosphere bacteria, we also hypothesized that similar changes would occur in the endosphere. Therefore, we studied bacterial diversity in the rhizosphere and endosphere of *Tamarix parviflora* plants cultured under different environmental conditions, including different soil type, increased soil salinity, or bioaugmentation with allochthonous bacteria. Furthermore, we analyzed the community structure differences in ten selected plant species growing naturally under the same environmental conditions.

Our results show that endophytes are in general less diverse than rhizosphere bacteria but are not necessarily a subset of the rhizosphere microbiota. We also demonstrate that endophytes, unlike the rhizosphere bacteria, may be stable despite the conditions under which the plant is cultivated – all investigated *T. parviflora* plants were dominated by *Candidatus Uzinura diaspidicola* of the *Bacteroidetes*, which was previously described as an endosymbiont of armored scale insects. In the rhizosphere, this bacterium was detected only in minute quantities. Our results also indicate that there are differences in the endophytic communities in roots and stems, both of which differ from the structure of

rhizosphere communities. Overall, our study brings new insight into the diversity of plant-associated bacteria and how their diversity is shaped.

Acknowledgement: Funding is acknowledged of the Czech Science Foundation projects no. 13-20414P and 13-28283S.

Comparative analysis of metatranscriptomes provide insights of microbial communities under warming conditions

William Rodriguez¹, Lauren Alteio¹, Grace Pold¹, Linda van Diepen⁴, Serita Frey⁴, Jerry Melillo⁵, Kristen DeAngelis³, Jeffrey Blanchard²

¹Graduate program in Organismic and Evolutionary Biology, University of Massachusetts – Amherst; ²Biology Department, University of Massachusetts – Amherst; ³Microbiology Department, University of Massachusetts – Amherst; ⁴Department of Natural Resources and Environment, University of New Hampshire; ⁵Marine Biological Laboratories

Terrestrial ecosystems serve as a carbon sink, but increasing temperatures enhance soil respiration beyond the rates of carbon dioxide fixation by plants creating positive feedbacks in biogeochemical cycles. In the soil the organic layer decreases under experimental warming. We hypothesize that if labile carbon or easier to utilize carbon stocks decrease after long-term warming conditions, then microbes will adapt and evolve to digest more recalcitrant carbon still present in the organic layer. Our experimental sites consist of 3 ongoing warming experiments SWaN (2006), Barre Woods (2003), and Prospect Hill (1991) at the Harvard Forest in Petersham, MA. Each experimental site has buried cables increasing the temperature in soil +5°C relative to ambient (control plots). To determine changes in microbial physiology, 48 samples were collected from the plots for community RNA sequencing (metatranscriptomics). Analyses were performed using SEED and KEGG databases to assess the treatment effect. Interestingly, more significant changes by treatment occur at the older plots (Barre Woods and Prospect Hill than SWaN). Increasing the soil temperature affect genes related with pathways and complexes including ribosome, starch and sucrose metabolism, ABC transporter, and oxidative phosphorylation. This could be related to microbial mechanisms for adapting to temperature changes.

***Nicotiana* roots recruit rare rhizosphere taxa as major root-inhabiting microbes**

Muhammad Saleem¹, Audrey D. Law¹ and Luke A. Moe^{1*}

¹Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY, USA, 40546-0312

Muhammad Saleem: m.saleem@uky.edu; Audrey Law: audrey.law@uky.edu; Luke A. Moe: luke.moe@uky.edu

Root-associated microbes have a profound impact on plant health, yet little is known about the distribution of root-associated microbes among different root morphologies or between rhizosphere and root environments. We explore these issues here with two commercial varieties of burley tobacco (*Nicotiana tabacum*) using 16S rRNA gene amplicon sequencing from rhizosphere soil, as well as from primary, secondary, and fine roots. While rhizosphere

soils exhibited a fairly rich and even distribution, root samples were dominated by *Proteobacteria*. A comparison of abundant OTUs between rhizosphere and root samples indicated that *Nicotiana* roots select for rare taxa (predominantly *Proteobacteria*, *Verrucomicrobia*, *Actinobacteria*, *Bacteroidetes* and *Acidobacteria*) from their corresponding rhizosphere environments. The majority of root-inhabiting OTUs (~80 percent) exhibited habitat generalism across the different root morphological habitats, although habitat specialists were noted. These results suggest a specific process whereby roots select rare taxa from a larger community.

Fungal community diversity in long-term biochar amended forest soils

Jessica Sarauer¹, Amy Ross-Davis², Mark Coleman¹

¹University of Idaho, ²USDA Forest Service, Rocky Mountain Research Station

Roughly 42 million acres of US forests have been impacted by bark beetles since 1996, resulting in wood that is a bioenergy resource. Biochar and biofuels are co-products of thermochemical conversion of forest biomass. Biochar has the potential to sequester carbon and improve soil quality when used as a soil amendment. We are investigating the effects of biochar as a soil amendment on microbial communities. One objective of our research is to determine if microbial community diversity increases with the addition of biochar, given that biochar can serve as a refuge to microorganisms and cause physical and chemical changes in soil. DNA was extracted from 43 soil samples collected from three long-term biochar research sites in the northwestern USA. Soils across the selected sites reflect a continuum of volcanic tephra input: 1) deep pumice, (Umpqua) 2) fine-textured Andisol, (Purdue Creek) and 3) coarse grained granitic soil absent any significant tephra influence (Swift Creek). Double-barcoded LSU (Large Subunit) amplicons were sequenced on a paired-end 300bp Illumina MiSeq platform. Resulting sequences were analyzed using the *dbc Amplicons* package (<https://github.com/msettles/dbcAmplicons>) at the University of Idaho IBEST facility. Sequencing data represent 6 phyla of Fungi, 30 classes, 99 orders, 255 families, and 702 genera, with the most common genus being *Umbelopsis*, a ubiquitous soil zygomycete. Biochar did not have an effect on number of sequences or OTU's (operational taxonomic unit) generated, richness (Chao), or diversity (Shannon-Weaver Index) at any location, except OTU richness at Purdue Creek where richness was significantly higher among biochar samples compared to the control. Although there were no treatment effects at any location, there were overall differences among sites. Swift Creek had significantly lower numbers of sequences and OTU's than both Umpqua and Purdue Creek and less diversity than Umpqua, which could be due to differences in soil characteristics.

Microbial soil community responses to extreme disturbance: Insights from the ongoing subterranean Centralia coal mine fire

Ashley Shade

Michigan State University Department of Microbiology and Molecular Genetics

The Centralia, Pennsylvania coal seam fire has been burning near-surface since 1962. It has created an extreme environment that supports coal fire-adapted microbial life. Heat, steam and combustion products (CO, CO₂, SO_x and NO_x) vent upward from the fire through the overlying soils, increasing soil surface temperatures to over 80°C. Soil chemistry is altered by both spontaneous and microbially-mediated chemical reactions. As the fire expands into new areas, it also retreats from some affected sites, which can then recover. This unusual habitat provides an opportunity to investigate the selective pressures and community processes that promote microbial community stability in the face of extreme, ongoing disturbance. In 2014, we collected soils in Centralia along a chronosequence of historical and current fire activity. From these soils, we used a combination of culture-dependent and -independent approaches to understand the community's response to and recovery from the fire. Our results show that complementary structural and functional responses among microbial community members contribute to a community's stability in the face of extreme disturbance, and suggest that typically rare taxa that can survive the disturbance as community intermediates may play an important role in driving community resilience.

Molecular mechanisms of a plant-fungus-bacterial community interaction

Shalaka Shinde¹, Sarah Zerbs¹, Peter E. Larsen¹, Jonathan. R. Cumming², Frank R. Collart¹, Steve J. Callister³ and Philippe Noirot¹

¹Biosciences Division, Argonne National Laboratory, Lemont, IL, ²Biological Science Division, West Virginia University, Morgantown. ³Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA

Plants form close, symbiotic relationships with common fungi and bacteria found in soils. This plant-microbial community plays a crucial role in forest productivity and the resiliency of the ecosystem to climatic stress. Although nutrient cycling and exchange of mineral nutrients are key features of this interaction, very little is known about the molecular mechanisms that underpin this process or the role of these processes in the formation of community structure. To address this research gap, we have developed a unique tripartite system comprising of *Populus trichocarpa* tree seedlings, *Laccaria bicolor* and *Paxillus involutus* ectomycorrhizal fungi, and *Pseudomonas fluorescens* mycorrhizal helper bacterial strains. Our goal is to assess the impact of soil community structure on plant biomass under conditions of nutrient stress and to identify the specific molecular mechanisms of community interaction that arise as a consequence of nutrient limitation. We investigate the phenotypic, biochemical, and physiological responses of plants in the context of transcriptomic, metabolomics and proteomic analyses. We have explored the molecular mechanisms underlying these interactions and have constructed models for the interaction of plants during association with the ectomycorrhizal fungi and/or mycorrhizal helper bacteria. Transporters related to inositol, proteins and magnesium are up-regulated during bacterial colonization, which can be affiliated to changes in the root growth and morphology

and carbon-nutrient exchange at the interface. Transporters such as those associated with bicarbonate, nodulin EamA-like and potassium are up-regulated during fungal colonization, which can be connected to structural changes of roots due to mycorrhizal fungi and cation exchange capacity in the rhizosphere. The combined experimental and computational approach will facilitate identification of molecular pathways that are key for interactions with plants and achieve a genome-based, dynamic systems-level understanding of organism and community function.

Discovering thermophilic diversity in temperate soils affected by a subterranean coal fire

Jackson Sorensen¹, Sang-Hoon Lee^{1,2}, Ashley Shade¹

¹Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI USA; ²School of Civil, Environmental, and Architectural Engineering, Korea University, Seoul, South Korea

Centralia, PA is the site of a long burning coalmine fire. The coalmine caught fire in 1962 and continues to spread at a rate of approximately 2 meters per year along the coal seams. As the fire spreads, the overlying soils can reach temperatures upwards of 80 degrees Celsius for prolonged periods, until the fire recedes and allows for thermal recovery. Utilizing the known history and other characteristics of this fire, we collected a chronosequence of soils from Centralia that represent pre-, current, and post- disturbance states. We probed the thermophilic diversity of microbes along this chronosequence through 16S rRNA amplicon and shotgun metagenome sequencing. We identified taxa (97% sequence identity OTUs) and functional genes (COG categories) that exhibit positive temperature responses, and compared these findings with the JGI Integrated Microbial Genome's collection of sequence (hyper)thermophilic genomes. Furthermore, we deeply sequenced (45Gbp) the direct metagenome of the hottest sample in this chronosequence as well the metagenome of a pool of its enriched thermophilic taxa cultured on TSA50 agar plates at 60 degrees Celsius. From these extensive sequencing efforts we assembled several large contigs representing partial genomes from thermophilic taxa. Our next steps are to use thermophilic taxa in temperate soils as models for studying the contributions of typically rare taxa to microbial community resistance to and resilience from disturbances.

The genetic potential for siderophore-mediated dissimilatory iron reduction in arctic soil microbial communities

Archana J. Srinivas¹, Elizabeth A. Dinsdale¹, David A. Lipson¹

¹San Diego State University

Dissimilatory iron reduction (DIR), a respiratory pathway in which ferric iron (Fe³⁺) is reduced to ferrous iron (Fe²⁺), contributes to carbon dioxide (CO₂) efflux from soils of the wet sedge tundra in the Arctic coastal plain (ACP), Alaska. DIR can competitively inhibit the production of methane, a stronger greenhouse gas than CO₂, from Arctic soils. Siderophores are microbial metabolites secreted to dissolve Fe³⁺ from soil minerals in iron deficient systems, making Fe³⁺ available for micronutrient uptake. DIR depends on Fe³⁺ availability in

soil; however, as the ACP is not iron deficient, siderophores present in Arctic soils may play a role in anaerobic respiration by solubilizing more Fe^{3+} for DIR.

Soils from which DNA was extracted were sampled at different depths from drained thaw lake basins (DTLB), a dominant feature in the Arctic tundra. DTLBs form a chronosequence of soil development, and are classified by age as young, medium, old and ancient. Of the twelve metagenomes used in this study; four were sequenced from DNA from soils from 4 different depths from the medium basin; and eight metagenomes were sequenced from soils from 2 different depths of the 4 basin types.

Siderophore biosynthesis, regulation, uptake, and membrane transport genes were identified in all metagenomes. Several bacterial families were involved in siderophore production in Arctic soils, including some DIR bacteria. Siderophore transport and uptake genes found in genomes of bacteria belonging to Acidobacteriaceae imply an opportunistic relationship between Acidobacteria and siderophore synthesizers.

The presence of siderophore associated genes in genomes of DIR bacteria suggest that Arctic soil microbes use siderophore-mediated dissolution of iron minerals to maintain a pool of dissolved Fe^{3+} in soils for DIR. This study provides insight into the mechanisms of DIR in this ecosystem, and has relevance for understanding Arctic soil respiration in the context of climate change.

Single cell genomics: from science fiction to mainstream microbiology

Ramunas Stepanauskas

Bigelow Laboratory for Ocean Sciences, East Boothbay, Maine

In less than a decade, single cell genomics evolved from science fiction to a high-throughput technology for accessing hereditary information at the most basic level of biological organization. It is increasingly utilized to decipher the metabolic potential, evolutionary histories and in situ interactions of environmental microorganisms. The establishment of the Bigelow Laboratory Single Cell Genomics Center has enabled a broad access to this technology by environmental microbiologists. In my presentation, I will provide examples of various types of microbial single cell genomics applications. I will also discuss several recent technology developments, which improve genomic data recovery from individual cells and allow its integration with cell's phenotype properties.

Hunting down frame shifts: Processing amplicon data of biphenyl and benzoate dioxygenase genes

Michal Strejcek¹, Tomas Macek¹, Ondrej Uhlík¹

¹*Department of Biochemistry and Microbiology, Faculty of Food and Biochemical Technology, University of Chemistry and Technology, Prague, Czech Republic*

Functional gene ecological analyses using amplicon sequencing can be challenging as translated sequences are often burdened with shifted reading frames. The aim of this work

was to evaluate several bioinformatics tools designed to correct errors which arise during sequencing therefore reducing the effect of those errors on frame-shifts (FS). Genes encoding for alpha subunits of biphenyl (*bphA*) and benzoate (*benA*) dioxygenases were used as model sequences. FrameBot, a FS correction tool, was able to reduce the number of detected FS to zero. However, up to 43.1% of sequences were discarded by FrameBot as non-specific targets. Therefore, we proposed a *de novo* mode of FrameBot for FS correction, which works on a similar basis as common chimera identifying platforms and is not dependent on reference sequences. By nature of FrameBot *de novo* design, it is crucial to provide it with data as error free as possible. We tested the ability of several publicly available correction tools to decrease the number of errors in the data sets, namely: AmpliconNoise, Maximum Expected Error (MEE) filtering and Single Linkage Pre-clustering (SLP). The combination of MEE filtering and SLP proved the most efficient read procession. Applying FrameBot *de novo* on the processed data enabled analysis of BphA sequences with minimal losses of potentially functional sequences not related to those previously known.

The effect of root exudate 7,4'-dihydroxyflavone and naringenin on soil bacterial community structure

Márton Szoboszlay¹, Alison White-Monsant², Luke A. Moe¹

¹ Department of Plant and Soil Sciences, University of Kentucky, Lexington, Kentucky, USA

² Department of Animal, Plant and Soil Science, Centre for AgriBioscience, La Trobe University, Melbourne, Australia

Our goal was to investigate how root exudate flavonoids influence the soil bacterial community structure and to identify members of the community that change their relative abundance in response to flavonoid exudation. Using a model system that approximates flavonoid exudation of *Medicago sativa* roots, we treated a soil with 7,4'-dihydroxyflavone and naringenin in two separate experiments using three different rates: medium (equivalent to the exudation rate of 7,4'-dihydroxyflavone from *M. sativa* seedlings), high (10× the medium rate), and low (0.1× the medium rate). Controls received no flavonoid. Soil samples were subjected to ATP assays and 16S rRNA gene amplicon sequencing. The flavonoid treatments caused no significant change in the soil ATP content. With the high 7,4'-dihydroxyflavone treatment rate, operational taxonomic units (OTUs) classified as *Acidobacteria* subdivision 4 increased in relative abundance compared with the control samples, whereas OTUs classified as *Gaiellales*, *Nocardoidaceae*, and *Thermomonosporaceae* were more prevalent in the control. The naringenin treatments did not cause significant changes in the soil bacterial community structure. Our results suggest that the root exudate flavonoid 7,4'-dihydroxyflavone can interact with a diverse range of soil bacteria and may have other functions in the rhizosphere in addition to *nod* gene induction in legume-rhizobia symbiosis.

The effects farming practices on fungal communities associated with *Glycine max* (soybeans).

Terri Tobias¹, Sara Dean², Winthrop Phippen³, Joel Gruver³, Andrea Porras-Alfaro¹

¹ Western Illinois University, Biological Sciences, Macomb, IL. ²University of New Mexico, Biological Sciences, Albuquerque, NM. ³Western Illinois University, School of Agriculture, Macomb, IL.

Both *Glycine max* (soybean) and *Thlaspi arvense* (pennycress) are important oilseed crops. These species are valuable agricultural plants and potential resources for renewable biofuels and industrial products. Pennycress is a member of the Brassicaceae plant family and can be grown over the winter months in corn stubble fields as a potential cover crop helping prevent erosion. In addition, pennycress does not compete with food production making it a viable option for biofuels. The objectives of this study were to compare the fungal communities associated with soybean roots grown under organic and conventional farming practices and to determine the potential impact of pennycress on microbial communities when used as a cover crop prior to soybean planting. Roots were collected in the summer of 2013 and 2014 from three treatment plots (conventional soybeans, organic soybeans, and soybeans following pennycress) at the Western Illinois University experimental farms. Roots were sequenced with fungal primers using 454 pyrosequencing and a total of 227,505 sequences were obtained. The most dominant fungal phylum in all plot treatments was Ascomycota. Results suggest that host (8% of variation), practice (8%) and year (21%) all had highly significant impacts on fungal community composition. In addition, there were also significant host x year and practice x year interactions. Soybean roots in all treatments were dominated by the order Hypocreales with the most abundant species being *Fusarium*. Pennycress roots were dominated by the fungal order Eurotiales with the most abundant species being *Penicillium citreonigrum*. Microbial diversity is a key component of soil health and soil microorganisms provide a wide variety of services to plant communities. Understanding these interactions and the potential effects on agricultural practices could provide important insights on plant health and management.

Why is it so difficult to incorporate metagenomics into traditional soil models?

Kathe Todd-Brown

Pacific Northwest National Laboratory, Richland, WA, USA

Soil biogeochemistry is a mix of abiotic and biotic mechanisms however processes of interest, such as carbon cycling, are frequently biologically mediated. There are several challenges and potential ways forward to incorporate genomic information into traditional first-order linear carbon decomposition models.

Traditional models split soil carbon into several pools. Carbon leaves these pools at a rate proportional to the total carbon in that pool. Some of this carbon is diverted to other pools; the rest leaves the system as carbon dioxide. These carbon pools are conceptual combinations of physical availability and chemical recalcitrance of the organic carbon with an implied microbial community associated with each pool.

Measuring the substrate pools directly via chemical and physical characterization has proven frustrating due to the challenges posed by soil structure and chemical heterogeneity. If, as is assumed in traditional models, there exists a group of microbes that are physiologically adapted to a particular substrate pool, then the change in relative abundance of that microbial community should be proportional to the change in substrate pool over time. By quantifying that relative microbial abundance via quantifiable microbial markers, we can hopefully track the relative change in the associated substrate pool. Functional gene quantification techniques like GeoChip could provide one way to do this that can inform the decay rates of the substrate pools.

There are several obvious shortcomings to this approach, for example; life history may lead microbes to not behave 'optimally' and substrate heterogeneity within modeled pools could lead to successional behavior. However, this provides a testable hypothesis to further develop traditional models. Biogeochemistry promises to be a rich line of microbial, ecological, and computational research for years to come.

Heavy metal tolerant fungal community analysis from temperate pine forest soil using Illumina sequencing

Terry Torres Cruz¹, Cedar Hesse², Cheryl Kuske², Andrea Porras-Alfaro¹

¹Western Illinois University; ²Los Alamos National Laboratory

The release of heavy metals to the environment has increased continuously due to technological and industrial activities. Heavy metal tolerant fungi have been described in contaminated soils and water but it is unknown how abundant and diverse they are in natural ecosystems. The objective of this project is to isolate and identify heavy metal tolerant fungi from Duke Forest soil samples and determine their abundance using Illumina sequencing. Samples collected from Duke Forest in North Carolina were serially diluted and inoculated on MEA supplemented with antibiotics and metal concentrations between 100 ppm and 1000 ppm of FeSO₄, ZnSO₄, CuSO₄, Al₂(SO₄)₃, Pb(NO₃)₂, Cr(NO₃)₃, NiCl₂, and CdCl₂ at 25°C. Fungal isolates were sequenced for the ITS and LSU regions, and analyzed using BLAST and UNITE databases for identification. A total of 439 isolates were obtained, from which the majority were isolated using Pb supplemented media. The most common and diverse genera isolated are *Penicillium* and *Trichoderma*. Phylogenetic analysis was also performed for these two genera, showing that Duke Forest soils contain potential novel species for both of them. Genera isolated in this study have not been reported or tested as heavy metal tolerant, including *Umbelopsis*, *Pochonia*, *Geomyces*, *Trichocladium*, *Bionectria* and *Ilyonectria*. The distribution and abundance of metal resistant taxa was determined using Illumina databases obtained from the same soils. The most common genera isolated in cultures were present in all soil horizons and corresponded to the most frequent taxa in the Illumina data; and samples that were non detectable by the next generation sequencing correspond to OTUs with low isolation rates. This research supports the fact that non-contaminated soils contain a great diversity of microorganisms with potential for bioremediation.

Composition of bacterial communities tracks salinity and flooding gradients

Pamela Weisenhorn^{1,2}, Jack Gilbert^{1,2,3,4}, Loretta Battaglia⁵

¹Argonne National Laboratory; ²Department of Ecology and Evolution, University of Chicago; ³Marine Biological Laboratory; ⁴Field Museum; ⁵Southern Illinois University Carbondale

Sea level rise is threatening coastal wetlands, which serve a crucial role in storm protection and as critical fisheries habitat. Thus, there have been many efforts to mitigate existing damage through restoration and more recently, assisted migration efforts that anticipate future community shifts. Bacteria play a key role in mediating ecosystem level response to a rising sea, including decomposition, marsh accretion, and nutrient availability to maintain plant health. Further, specific bacterial taxa, such as plant growth promoting bacteria or bacteria inhibitory to plant pathogens, can have strong effects on primary productivity. However, bacterial communities are not considered in plans to restore wetland habitats, due in part to the 'field of dreams' assumption that bacterial community structure and function will return with plant restoration. To evaluate the potential for successful anticipatory restoration through assisted migration, we examined differences in bacterial community composition across 4 vegetation zones (pine forest, brackish marsh, fresh marsh, and salt marsh) of 4 replicate marsh-pine island complexes along the Mississippi Gulf Coast that are currently experiencing sea level rise. We found marked taxonomic and PICRUSt-predicted functional differences in bacterial community composition across this short 100 cm elevation gradient. Thus, through assisted migration efforts, migrant plants will be interacting with novel bacterial communities. Ongoing work will address how these interactions may influence both plant establishment and bacterial community dynamics, as well as tease apart effects of vegetation from edaphic factors in shaping wetland soil bacterial communities.

Moleclo hybrid synthetic long reads reconstruct full genomes from the rare biosphere with functional potential elucidated by high resolution mass spectrometry

Richard Allen White III¹, Eric M. Bottos¹, Taniya Roy Chowdhury¹, Carrie D Nicora¹, Kristin Burnum-Johnson¹, Lee Ann McCue¹, Erin Baker¹, Janet K Jansson¹

¹Biological Sciences Division, Pacific Northwest National Laboratory, Richland, Washington 99352, USA

Hybrid synthetic long reads from the high-throughput sequencing Illumina platform (e.g Moleclo) provide >8 kb read lengths with 99.99% accuracy. Moleclo has enabled resolution of complex repeats in eukaryotic genomes including *C. elegans* and *Botryllus schlosseri*; however, metagenomic applications of this technology remain largely unexplored. Kansas native prairie soil presents the greatest challenge for *de novo* assembly from metagenomic shotgun reads due to its high microbial diversity (e.g >1,000 OTUs g⁻¹) and high microbial cell density (>10⁷ g⁻¹). Approximately 300 million reads from the HiSeq 2500 rapid mode (e.g 250 bp paired reads) using hybrid synthetic long reads Moleclo yielded 16.9 k reads >9 kbp and 6.2 k reads >10 kbp. By comparison, assembly attempts from similar prairie ecosystems with >400 Gbs provided only 9 contigs (>10 kbp). Moleclo synthetic long reads data resulted in >100 genomic bins, 20 partial genomes, and 3

complete genomes from the rare biosphere (e.g TM7, Zixi, OD1). Using this approach, a novel candidate phyla was detected using Molecuola in the Kansas native prairie soil by binning with using self-organizing maps (ESOM). Amongst the completed genomes in this analysis, the *Candidatus Zixibacterium* strain JKJ-1 in Kansas soil has the potential for hemicellulose degradation (e.g xylan metabolism) and has two copies of arylsulfatase that are linked to soil desiccation and environmental warming. High resolution mass spectra from a Q Exactive mass spectrometer were mapped to Molecuola synthetic long reads resulting in over 20,000 peptide identifications. Upon protein roll-up of the identified peptides, ~100 proteins were found to correlate with members of the rare biosphere (e.g TM7, Zixi, OD1) with functions relating to digesting recalcitrant carbon sources (e.g lignin and xylan). These results demonstrate the synergistic potential of integrating Molecuola hybrid synthetic long reads in systems biology approaches to establish a functional link between metagenomics and metaproteomics.

Who's on first? Bacterial and fungal colonization of fresh soil minerals

Thea Whitman¹, Rachel Neurath¹, Ping Zhang², Tong Yuan², Joe Zhou², Peter Weber³, Jennifer Pett-Ridge⁴, Mary Firestone¹

¹Department of Environmental Science Policy and Management, University of California-Berkeley; ²Institute for Environmental Genomics, University of Oklahoma; ³Nuclear and Chemical Sciences Division, Lawrence Berkeley National Laboratory; ⁴Physical Biosciences Division, Lawrence Berkeley National Laboratory

Soil organic matter (SOM) stabilization by soil minerals is an important mechanism influencing soil C cycling. Microbes make up only a few percent of total SOM, but have a disproportionate impact on SOM cycling. Their direct interactions with soil minerals, however, are not well characterized. We studied colonization of fresh minerals by soil microbes in an *Avena barbata* (wild oat) California grassland soil microcosm. Examining quartz, ferrihydrite, kaolinite, and the heavy fraction of the native soil, we asked: (1) Do different minerals select for different communities, or do random processes drive the colonization of fresh minerals? (2) What factors influence which taxa colonize fresh minerals? After incubating mesh bags (<18 μm) of minerals buried next to actively growing plant roots for 2.5 months, we used high-throughput sequencing of 16S and ITS2 genes to characterize the microbial communities colonizing the minerals. We found significant differences between the microbial community composition associated with different minerals and soil for both bacteria and fungi. We found a higher relative abundance of arbuscular mycorrhizal fungi with ferrihydrite and quartz, and nanoscale secondary ion mass spectrometry (NanoSIMS) imaging of these minerals suggests that some fungal hyphae are moving C directly from roots to mineral surfaces. Additionally, we found significant enrichment of groups of microbes with potential characteristics of predation, swarming, N-fixing, faunal symbiosis or parasitism, or fast growth in minerals. Our findings suggest that: (1) Microbial colonization of fresh minerals is not a fully passive or neutral process (2) Motility, predation, vector transport, alternative metabolisms and potential for fast growth may all facilitate colonization of soil minerals.

Stable isotope probing reveals long-term impacts of timber harvesting on cellulolytic soil community

Roland Wilhelm¹, Erick Cardenas¹, Hilary Leung¹, András Szeitz², Lionel D. Jensen³ and William Mohn¹

¹Department of Microbiology & Immunology, University of British Columbia, Vancouver, BC, V6T 1Z3, Canada.

²Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, V6T 1Z3, Canada.

³Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, AB, T6G 2E1, Canada.

Forest soils form from decaying plant matter comprised largely of plant cell wall (lignocellulose) which is gradually decomposed by the activity of microorganisms. The process of decomposition has major influences on the biological and physicochemical characteristics of soil. We examined whether different timber harvesting had long-term effects on the cellulose-degrading community and whether the potential rate of cellulose decomposition was affected. We measured the rate of cellulose decomposition and identified the organisms responsible using artificially labeled ¹³C-cellulose in a method known as stable isotope probing. With harvesting, we observed substantial decreases in the abundance of fungal cellulose degraders, particularly *Sordariomycetes*, accompanied by increases in bacterial cellulose degraders, particularly *Actinobacteria*. We found no indication of the disappearance of any cellulose-degrading organisms as a result of harvesting, but observed a consistent decline in abundance of *Verrucomicrobia* with increasing levels of organic matter removal. Bacterial cellulose degraders were diverse, while there were only a few dominant fungi. Combining stable isotope probing with metagenomics proved to be an effective method for reducing the complexity of metagenomes, while targeting a particular functional group within communities. Assemblies of these metagenomes included 17-25% of total reads, in contrast to <1% for the total metagenomes. As a result, we recovered draft quality genomes of five putatively cellulolytic bacteria (*Kitasatospora sp.*, *Opiritaceae spp.*, *Herbaspirillum sp.* and *Caulobacteraceae spp.*) and one fungus (*Myceliophthora thermophile*). Better assembly enabled the examination of genomic architecture relating to the carbohydrate-active enzymes in these organisms. This study joins a growing body of research elucidating long-term effects of harvesting on forest soil decomposer communities, while expanding our knowledge of cellulolytic activity in the uncultured community of organic and mineral soils.

Elucidating microbial interactions that drive decomposition through a co-occurrence framework

Ryan J. Williams(1), Kirsten S. Hofmockel(2), Adina Howe(1)

1 Agricultural and Biosystems Engineering, Iowa State University

2 Ecology, Evolution, and Organismal Biology, Iowa State University

The fundamental problem with determining links between microbes, carbon (C) cycling, and climate system feedbacks is that soil microorganisms are functionally and phylogenetically diverse due to the variety of soil C-substrates. Traditionally, microbial C-

cycling has been summarized by extracellular enzymes, which are proximal indicators of decomposition. Next-generation sequencing technologies are also now providing massive amounts of data characterizing what microorganisms are present (marker gene sequencing) and what traits they harbor (metagenomes). Integrating these datasets, we have asked the questions: Which microorganisms coexist and potentially interact to drive C-cycles, what C-cycling traits are involved, and are these related to our biogeochemical indicators of decomposition? Using a Bayesian co-occurrence framework, we combined datasets to answer these questions in two different ecosystems: soil aggregates isolated from agricultural systems and decomposing logs in forests. When analyzing co-occurrence networks, we identified fungi that may ultimately be responsible for producing the extracellular enzymes measured commonly in laboratory assays. Several fungal taxa including members of *Claviceps*, Orbiliaceae, and Phaeosphaeriaceae increased linearly in abundance with sequences associated with enzyme families including cellulases and cellobiohydrolase. Independent analyses of genomes representing these fungal taxa confirmed the presence of cellobiohydrolase genes (e.g. *cbhA*), confirming their potential role in soil C-cycling. When applying these analyses to microbial data collected from decomposing logs, we have found co-occurrence relationships between fungal taxa and extracellular enzymes were most common among later stages of decomposition and were specific to the log species. Several fungal taxa co-occurring with greater cellobiohydrolase activity belong to the saprobic order, Helotiales. Representative genomes from this group were also identified as containing cellobiohydrolase genes (*cbhB*) as well. Overall, these results suggest that co-occurrence analyses can identify putative relationships between microbes and enzymatic potential while identifying potential drivers of decomposition and C-cycling.

Identifying factors controlling methane emissions across freshwater wetland gradients

K.C. Wrighton*[#], G.S. Smith, J.C. Angle, A.B. Narrowe, M. Jackson, M. Johnston, K.S. Stefanik, R.A. Daly, M.J. Wilkins, D. Hoyt, P.J. Mouser, M. Tfaily, L. Pasa-Tolic, C.S. Miller[#]

* presenting

[#] corresponding

Freshwater temperate wetlands represent the largest natural source of methane emitted to the atmosphere, yet we know little about the factors impacting emission in these habitats. Here, we identify the biogeochemical and microbial genomic determinants impacting methane cycling, and the scale at which they operate along temperate freshwater wetland gradients. Mud ecosites lacking above-ground vegetation or standing water produced significantly more methane in colder seasons, while summer plant primary productivity had the greatest methane emission. Consistently, the greatest *in situ* methane and methanogenic potential was associated with surface samples with high dissolved oxygen concentrations, and not the deeper anoxic samples. 16S rRNA gene analyses and shotgun metagenomics revealed *Methanosaeta* spp. were dominant in high methane emitting surface samples. Two reconstructed surface *Methanosaeta* genomes encode multiple, different oxygen tolerance mechanisms. We posit that recalcitrant carbon constrains methanogenic activity, and we observed statistically different high-molecular-weight carbon profiles and a greater concentration of methanogenic substrates in methane-rich pore waters from surface samples. Although methanotrophs were broadly distributed across ecosites and depths, the highest relative abundances were detected in surface samples, and thus strongly correlated

to *in situ* methane and oxygen concentrations. Near-complete reconstructed *Methylobacter* genomes revealed redox tolerance as an explanation for site-wide prevalence, with the potential for methane oxidation coupled to oxygen and nitrate. Despite the high richness and broad phylogenetic diversity of wetland soils, the majority of methane cycling across time, season, and depth was limited to a handful of taxa active primarily in shallow, rather than deep, samples. Together, our findings show that carbon quality and the abundance of specific microbial genera, not depth or dissolved oxygen concentrations, are key predictors of ecosystem-scale methane emissions.

Presentation Title: BacEx segregates microbial DNA from eukaryotic DNA “clutter” allowing for more efficient microbiome sequencing—*Cancelled*

Robert T. Yamamoto¹, Allyn Forsyth²

¹Zova Systems, LLC, San Diego, CA, USA, Presenting author; ²San Diego State University, San Diego, CA, USA

To improve and streamline costs of the metagenomic analysis of complex microbiomes, we have repurposed restriction endonucleases as methyl specific DNA binding proteins. We call these reagents “BacEx” and have developed a simple, fast technique for specific binding and extraction of microbial genomic DNA away from eukaryotic DNA clutter. Using genomic DNA mixtures, we demonstrate 80% recovery of *Escherichia coli* genomic DNA even when only femtogram quantities are spiked into 10 µg of human DNA background. Binding is very specific with less than 0.5% of human DNA bound. Next Generation Sequencing of input and enriched synthetic mixtures shows over 100-fold enrichment of target genomes relative to human and plant DNA with little apparent bias. Comparable enrichment is seen when sequencing complex microbiomes such as those from creek water and human sample types. Data will be presented on these and other metagenomics sample types.

Robert.yamamoto@zovasystems.com, 760-271-7760

Soil microbial community composition affects the weed suppression potential of green manures

Anthony Yannarell¹ and Yi Lou¹

¹Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign

The use of cover crops as green manures can suppress the emergence of early season weeds, but this effect depends on interactions between the green manures and the soil microbial community. Soil microbes may enhance weed suppression (increased plant pathogen populations) or diminish it (degradation of allelochemicals). We conducted weed seed germination assays using live and sterilized soils from a variety of farm systems. We then partitioned the total weed suppression in these treatments into components representing the unique contribution of soil microorganisms, the unique contribution of green manures, and their interaction. We used high-throughput DNA sequencing to understand how microbial suppression and the microbe-green manure interaction depend on soil microbial community composition. We found that green manures have a high, but

temporary, suppressive effect on weed seed germination. Live soil microbial communities also have an inherent capacity to suppress weed germination, and the magnitude of microbial suppression depended on microbial community composition. Microbial weed suppression was highest in organic systems and lowest in no-till systems, indicating that different management techniques can be used to foster weed suppressive microbial communities. In general, we found live microbial communities diminished the effectiveness of green manures, leading to a negative interaction between green manures and microbes. However, the strength of this negative interaction varied along with temporal and system-based differences in microbial community composition. We conclude that understanding this microbial variation, in the context of management decisions that directly affect soil microbial communities and their activities, can help farmers optimize cover cropping and green manure strategies for maximal weed suppression.

Phylogenetic distribution of resuscitation genes in soil microbes

Fan Yang¹, Stuart E. Jones², Jay T. Lennon³, Adina Howe¹

¹Department of Agricultural and Biosystems Engineering, Iowa State University; ²Department of Biological Sciences, University of Notre Dame; ³Department of Biology, Indiana University

Soil microbes are associated with a wide range of growth factors, influencing their metabolic activities and ability to replicate. Growth of bacteria in the environment is crucial for maintaining ecosystem health as well as predicting the potential health hazard. One such category among these growth factors is resuscitation-promoting factors (Rpf) that controls bacterial cell growth and resuscitation from starvation induced dormancy. Historically, Rpf-associated genes have been identified mainly in members of Actinobacteria and hypothesized to be unique among this phylum. Recent comparative genomic studies have revealed that members of Firmicutes also contain growth factors similar to Rpf and that this Rpf function may be more broadly distributed than previously predicted. We investigated the phylogenetic distribution of *rpf* genes in soil microbial genomes and found that genes sharing similarity to bacterial resuscitation-promoting factors are broadly present in microbes that have been isolated and sequenced from both aquatic and soil environments. Our results revealed that two clades of *rpf* genes (Actinobacteria clade and Firmicutes clade) were identified in 16 phyla, including Actinobacteria, Proteobacteria, and Firmicutes. As expected, the Actinobacteria clade *rpf* genes were observed in majority of the known free-living Actinobacteria (over 93%) and 2% Actinobacteria also had *rpf* genes resembling those found in the Firmicutes clade, suggesting that members of Actinobacteria respond to Actinobacteria Rpf mainly. In contrast, the Firmicutes clade *rpf* genes were identified in 69% and 74% of known free-living Proteobacteria and Firmicutes, respectively, while 41% and 62% of the respective phyla had *rpf* genes similar to those of the Actinobacteria clade. This suggests that members of Proteobacteria and Firmicutes may be more responsive to different resuscitation-promoting factors. Overall, our results indicate that Rpf are common among free-living bacteria and bacteria from different phyla may have evolved different strategies in responding to these factors to promote cell growth.

How does microbial community composition and function change in ageing primary boreal forest ecosystems?

Stephanie A. Yarwood¹, Tamara Walsky¹, Mona N. Högborg²

¹Department of Environmental Science and Technology, University of Maryland, College Park, Maryland, USA

²Department of Forest Ecology and Management, Swedish University of Agricultural Science, Umeå, Sweden

Along the Fennoscandia coastline, glacial rebound results in ~8 mm of land uplift annually. As land is uplifted, mineral weathering, and plant colonization are visual signs of ecosystem ageing. Previous studies in Sweden and Finland have shown that under such circumstances microbial community composition changes, but those results have largely been based on techniques that characterize dominant species. By applying amplicon Illumina sequencing of the 16S rRNA gene, we investigate the recruitment of new species during ecosystem development and the extent to which bacterial and archaeal species are correlated to each other at different ecosystem ages between 25 and 560 years. We focus more than the previous studies on the early, rapid changes microbial community composition and ecosystem age. Using the bioinformatics program, PiCrust, and Q-PCR we also investigate the difference in N cycling function across the ecosystem.

Identifying *Pseudomonas fluorescens* genetic resources for metabolism of diverse sulfur nutrients

Sarah Zerbs^{1*}, Peter Korajczyk¹, Frank R. Collart¹, Philippe Noirot¹, and Peter E. Larsen¹

¹Biosciences Division, Argonne National Laboratory

Sulfur can become a limiting nutrient in soil communities with sufficient nitrogen, phosphorus and carbon. While inorganic sulfate is the preferred sulfur source for plants and microbes this form composes only about 5% of the total sulfur present in soil. The remaining sulfur is a dynamic and structurally diverse mix of other inorganic sulfur complexes and organosulfur compounds. Increased knowledge of the mechanisms for transport and utilization of potentially bioavailable sulfur by bacteria is needed to understand microbial community structure and nutrient mobilization in soil. We examined the sulfur utilization capabilities of *Pseudomonas fluorescens*, a soil bacterium which is able to metabolize many recalcitrant small molecules including organosulfur compounds. However, in *P. fluorescens* metabolism of alternative sulfur sources only occurs under sulfur limitation stress, which makes identification of these responses challenging.

A combination of comparative genomics and transcriptomics data from bacterial monocultures supplemented with multiple organosulfur sources was used to identify genes that participate in sulfur limitation responses. Analysis of the *P. fluorescens* SBW25 genome indicated an abundance of genes with predicted sulfur/organosulfur transport, metabolism, and regulation of sulfur metabolism functions. The percentage of cys/met residues in protein sequences was used to predict gene expression under sulfur limited conditions. Differentially expressed genes in sulfate-stressed transcriptomics data sets correlated well with reduced cys/met content. Transcript expression patterns under varying nutrient conditions indicated that some genes with annotations related to sulfur metabolism were

not induced by sulfur limitation. Additional genes with hypothetical or poor annotations were also differentially expressed, indicating that they may function in the sulfur limitation response. Ongoing characterization of nutrient turnover activity in this bacterial species will be used to identify genes or families of genes present in a microbial community that contribute to necessary community nutrient acquisition functions.

Modeling the *Pseudomonas fluorescens* sulfur regulome

Sarah Zerbs, Peter Korajczyk, Frank R. Collart, Philippe Noirot, and Peter E. Larsen*
Biosciences Division, Argonne National Laboratory

Sulfur can become a limiting nutrient in soil communities with sufficient nitrogen, phosphorus and carbon. While the preferred sulfur source in soils is inorganic sulfate, this form of sulfur composes only about 5% of the total sulfur present in soil. The remaining sulfur in soils is a dynamic and structurally diverse mix of other inorganic sulfur species and organosulfur compounds. Soil bacteria must be able to utilize a diverse blend of bioavailable sulfur sources in order to optimize survival in a constantly changing, competitive environment.

To understand how bacteria interact with their nutrient environment, we modeled the sulfur regulome of representative soil bacteria, *Pseudomonas fluorescens*, as an Artificial Neural Network (ANN). The bacterial regulome integrates information collected from multiple transmembrane sensors to drive patterns of gene regulation and behavior. To better mimic how bacteria receive information about their environment, sulfur nutrients were considered, not as specific molecular compounds, but as vectors of quantitative chemical characteristics. The corresponding computational model links networks of transmembrane sensors, enzymes, and transcription factors to predict the gene expression patterns and relative growth of *P. fluorescens* on a variety of sulfur-containing media. The *P. fluorescens* transcriptome and biomass when cultured on nine sulfur-containing media types were accurately modeled using ANNs. Use of ANN models also provided the opportunity to make additional predictions for *P. fluorescens* growth of different sulfur sources. The ANN extrapolates gene expression patterns and growth on a variety of possible sulfur sources beyond the set of nutrients used to train the ANN model. Also, the ANN predicts the effects of gene knock outs on growth. Results indicate that modeling the regulome as an ANN provides useful insights into how soil bacteria interact with their environment and proposes testable hypotheses for specific molecular biology experiments.

PARTICIPANTS

Jordan Angle, The Ohio State University
angle.42@buckeyemail.osu.edu

Dionysios Antonopoulos, Argonne National Laboratory
dion@anl.gov

Petr Baldrian, Institute of Microbiology of the ASCR
baldrian@biomed.cas.cz

Robyn A. Barbato, US Army CRREL
robyn.a.barbato@erdc.dren.mil

Robert Beiko, Dalhousie University
rbeiko@dal.ca

Lori Biederman, Iowa State University
lbied@iastate.edu

Jeff Blanchard, University of Massachusetts Amherst
jeffb@bio.umass.edu

Anaïs Boyd, Carleton College
boyda@carleton.edu

Cristina Butterfield, UC Berkeley
cristina.butterfield@gmail.com

Ashley Campbell, Lawrence Livermore National Laboratory
campbell87@llnl.gov

Erick Cardenas, University of British Columbia
carden24@mail.ubc.ca

Chelsea Carey, University of California Riverside
chelsea.carey@ucr.edu

Sarah Castle, University of Minnesota
sccastle@umn.edu

Michelle Catania, The Morton Arboretum
mcatania@mortonarb.org

Benli Chai, Michigan State University
chaibenl@msu.edu

Zhongqiang(John) Chen, DuPont
zq.john.chen@gmail.com

Charles Chih-Yu Chiu, Academia Sinica
bochiu@sinica.edu.tw

Hee jung Cho, UC Berkeley/Lawrence Berkeley National Laboratory
beeologai@berkeley.edu

Jin Choi, Iowa State University
jinchoi@iastate.edu

Todd Ciche, Monsanto Co.
todd.ciche@monsanto.com

Jim Cole, Michigan State University
colej@msu.edu

PARTICIPANTS

Phil Colgan, Iowa State University
colganph@gmail.com

Melissa Cregger, Oak Ridge National Laboratory
creggerma@ornl.gov

Emelia DeForce, MO BIO Laboratories, Inc.
edeforce@mobio.com

Kathryn Docherty, Western Michigan University
kathryn.docherty@wmich.edu

Marie Duhamel, Stanford University
mduhame2@stanford.edu

David Duncan, UW-Madison
dsduncan@wisc.edu

Taylor Dunivin, Michigan State University
dunivint@msu.edu

Jared Flater, Iowa State University
jflater@gmail.com

Ted Flynn, Argonne National Laboratory
tflynn@anl.gov

Josh Franken, MO BIO Laboratories, Inc.
jfranken@mobio.com

Zac Freedman, University of Michigan
zacf@umich.edu

Aaron Garoutte, Michigan State University
garoutte@msu.edu

Terry Gentry, Texas A&M University
tgentry@ag.tamu.edu

Georgios Giannopoulos, Virginia Commonwealth University
george.z.giannopoulos@gmail.com

Jack Gilbert, University of Chicago
gilbertjack@anl.gov

Eric Goldman, MO BIO Laboratories, Inc.
egoldman@mobio.com

Barry Goldman, Monsanto
barry.s.goldman@monsanto.com

Vicente Gomez-Alvarez, US EPA
Gomez-Alvarez.Vicente@epa.gov

Maylin Gonzalez, Instituto Alexander Von Humboldt
magonzalez@humboldt.org.co

Neil Gottel, Argonne National Laboratory
ngottel2@gmail.com

Robin Graham, Argonne National Laboratory
grahamrl@anl.gov

PARTICIPANTS

Stefan J. Green, University of Illinois at Chicago
GreenDNA@uic.edu

Stephanie Greenwald, Argonne National Laboratory
smoormann@mcs.anl.gov

Tim Gsell, Governors State University
tgsell@govst.edu

Santosh Gunturu, Michigan State University
gunturus@msu.edu

Paris Hamm, Western Illinois University
ps-hamm@wiu.edu

Miranda Hart, UBC Okanagan
miranda.hart@ubc.ca

Johannes Harter, University of Tuebingen
johannes.harter@uni-tuebingen.de

Martin Hartmann, Swiss Federal Institute for Forest, Snow and Landscape Research WSL
martin.hartmann@wsl.ch

Josh Herr, University of Nebraska - Lincoln
joshua.r.herr@gmail.com

Mindy Hong, Northwestern University Department of Statistics
mindyhong2019@u.northwestern.edu

Adina Howe, Iowa State University
adina@iastate.edu

Hongmei Jiang, Northwestern University
hongmei@northwestern.edu

Eric R. Johnston, Georgia Institute of Technology
eric.johnston65@gmail.com

Robert M Jones, Cold Regions Research and Engineering
Robert.M.Jones@erdc.dren.mil

Mamatha Kakarla, Texas Tech University
mamatha.kakarla@ttu.edu

Ulas Karaoz, Lawrence Berkeley National Laboratory
ukaraoz@lbl.gov

Mehdi Keddache, Illumina
mkeddache@illumina.com

Katharina M. Keiblinger, University of Natural Resources and Life Sciences Vienna, Institute of
Soil Research
katharina.keiblinger@boku.ac.at

Ken Kemner, Argonne National Laboratory
kemner@anl.gov

Frédéric Kendirgi, Agrinos Inc
frederic.kendirgi@agrinos.com

Angela Kent, University of Illinois at Urbana-Champaign

PARTICIPANTS

akent@illinois.edu

Md A.W. Khan, University of Texas
mdakhan@mavs.uta.edu

Helena Avila-Arias, Purdue University
wisemr@purdue.edu

Ann M. Klein, University of Oregon
annmaureenklein@gmail.com

Kostas Konstantinidis, Georgia Institute of Technology
kostas@ce.gatech.edu

Peter Korajczyk, Argonne National Laboratory
pkorajczyk@anl.gov

Jason C Koval, Argonne National Laboratory
ckoval2@gmail.com

Jamie Lamit, Michigan Tech. University
ljlamit@mtu.edu

Peter Larsen, Argonne National Laboratory
plarsen@anl.gov

Mary-Cathrine Leewis, University of Alaska Fairbanks
mc.leewis@gmail.com

Jay T. Lennon, Indiana University
lennonj@indiana.edue

Di Liang, Michigan State University
100liangdi@gmail.com

Mary S. Lipton, Pacific Northwest National Laboratory
mary.lipton@pnnl.gov

Alex Liu, Agrinos
alex.liu@agrinos.com

Maria Elena Lopez Perez, UNIVERSIDAD DE GUANAJUATO
marinli2110@gmail.com

Xiaofei Lyu, Yantai Institute of Coastal Zone Research Chinese Academy of Sciences
xiaofeilv01@gmail.com

Rachel Mackelprang, California State University Northridge
rachel.mackelprang@csun.edu

Chris Marshall, Argonne National Laboratory
cmarshall@anl.gov

Hisako Masuda, Indiana University Kokomo
masudah@iuk.edu

Chakradhar Mattupalli, The Samuel Roberts Noble Foundation
cmattupalli@noble.org

Morgan McPherson, University of Nebraska - Lincoln
morgan.r.mcpherson@gmail.com

PARTICIPANTS

Kyle M Meyer, University of Oregon
kmeyer@uoregon.edu

Folker Meyer, Argonne National Laboratory
folker@anl.gov

Meghan Midgley, The Morton Arboretum
mmidgley@mortonarb.org

Samuel Miller, University of Chicago
samuelmiller10@gmail.com

Darlyn Mishur, Argonne National Laboratory
mishur@mcs.anl.gov

Keithanne Mockaitis, Dow AgroSciences, LLC
kmockaitis@dow.com

Sara Molinari, Rice University
sm85@rice.edu

Scott Monsma, Lucigen Corp.
smonsma@lucigen.com

Ryan M. Mushinski, Texas A&M University
rm1463@tamu.edu

Mehmet Burcin Mutlu
mbmutlu@anadolu.edu.tr

Dave Myrold, Oregon State University
david.myrold@oregonstate.edu

Ankur Naqib, University of Illinois at Chicago
anaqib2@uic.edu

Cedric Ndinga Muniania, Western Illinois University
c-ndingamuniania@wiu.edu

Philippe Noirot, Argonne National Laboratory
pnoirot@anl.gov

Jeanette Norton, Utah State University
jeanette.norton@usu.edu

Sarah O'Brien, Argonne National Laboratory
slobrien1@gmail.com

Edward O'Loughlin, Argonne National Laboratory
oloughlin@anl.gov

Luis (Coto) Orellana, Georgia Institute of Technology
lhorellana@gatech.edu

Sarah Owens, Argonne National Laboratory
sarah.owens@anl.gov

David Paez-Espino, DOE-JGI
adpaezespino@lbl.gov

Doug Pearce, Western Michigan University
douglas.s.pearce@wmich.edu

PARTICIPANTS

Kabir Peay, Stanford University
kpeay@stanford.edu

Peter Pellitier, University of Michigan
ptpell@umich.edu

Jennifer Pett-Ridge, Lawrence Livermore National Laboratory
pettridge2@llnl.gov

Andrea Porras-Alfaro, Western Illinois University
a-porras-alfaro@wiu.edu

SR Prabhu, TerraBioGen Technologies Inc.
prabhu@terrabiogen.com

Josh Rehberger, Agro BioSciences
josh.rehberger@agro-biosciences.com

William G. Rodriguez, University of Massachusetts Amherst
wrodriguez@acad.umass.edu

Muhammad Saleem, University of Kentucky
m.saleem@uky.edu

Rob Sanford, University of Illinois at Urbana-Champaign
rsanford@illinois.edu

Jessica Sarauer, University of Idaho
sara8172@vandals.uidaho.edu

Chris Schadt, Oak Ridge National Laboratory
schadtcw@ornl.gov

Drew A. Scott, Southern Illinois University
drews2222@gmail.com

Ashley Shade, Michigan State University
shade.ashley@gmail.com

Shalaka Shinde, Argonne National Laboratory
sdesai@anl.gov

Garrett Smith, The Ohio State University
smith.10284@osu.edu

Jackson Sorensen, Michigan State University
jacksonwsorensen@gmail.com

Archana J. Srinivas, San Diego State University
archieniv@gmail.com

Ramunas Stepanauskas, Bigelow Laboratory for Ocean Sciences
rstepanauskas@bigelow.org

Michal Strejcek, University of Chemistry and Technology,
Prague strejcem@vscht.cz

Marton Szoboszlai, University of Kentucky
marton.szoboszlai@uky.edu

Clotilde Teiling, Illumina

PARTICIPANTS

cteling@illumina.com

Terri Tobias, Western Illinois University
tl-tobias@wiu.edu

Kathe Todd-Brown, Pacific Northwest National Laboratory
ktoddbrown@gmail.com

Terry Torres Cruz, Western Illinois University
tj-torrescruz@wiu.edu

Will Trimble, Argonne National Laboratory
trimble@anl.gov

Keith Turner, Monsanto
keith.turner@monsanto.com

Ondrej Uhlik, University of Chemistry and Technology, Prague
ondrej.uhlik@vscht.cz

Pan Wang, Northwestern University, Statistics Department
panwang2012@u.northwestern.edu

PENG WANG, University of Nebraska - Lincoln
pwang16@unl.edu

Qiong Wang, DuPont Pioneer
wangqion@gmail.com

Kim Wegener, Monsanto
kimberly.m.wegener@monsanto.com

Pamela Weisenhorn, Argonne National Laboratory
pweisenhorn@anl.gov

Seth Wenner, Agro BioSciences
seth.wenner@agro-biosciences.com

Richard Allen White III, Pacific Northwest National Laboratory
richard.white@pnnl.com

Thea Whitman, UC Berkeley
whitman@berkeley.edu

Roland Wilhelm, University of British Columbia
rwilhelm@mail.ubc.ca

Andreas Wilke, Argonne National Laboratory
wilke@mcs.anl.gov

Ryan Williams, Agricultural and Biosystems Engineering, Iowa State University
ryanjwtx@gmail.com

Kelly Wrighton, The Ohio State University
kwrighton@gmail.com

Fan Yang, Iowa State University
fyang@iastate.edu

Tony Yannarell, University of Illinois
acyann@illinois.edu

PARTICIPANTS

Stephanie Yarwood, University of Maryland
syarwood@umd.edu

Sarah Zerbs, Argonne National Laboratory
szerbs@anl.gov



PowerMag[®] Soil DNA Isolation Kit

Magnetize your Research with ClearMag[®] Technology

Automated Isolation of high quality DNA from soil, environmental samples and stool samples.

www.mobio.com

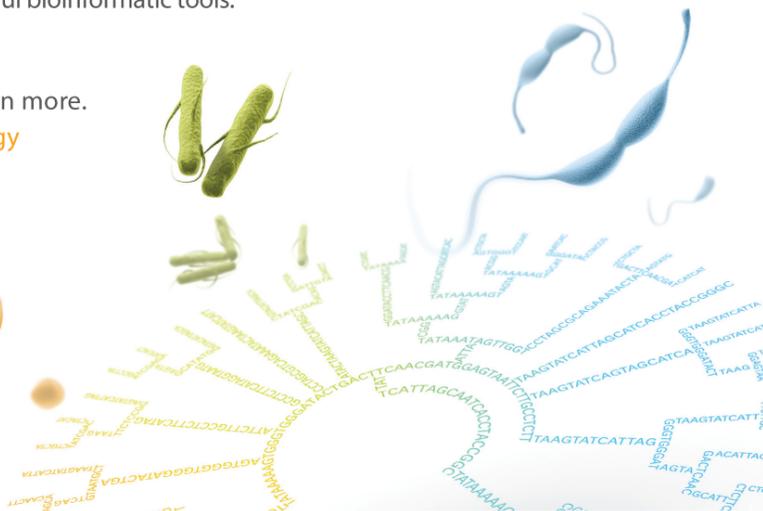


Accelerate genomic breakthroughs in microbiology.

Gain deeper insights with powerful bioinformatic tools.

Visit the Illumina exhibit to learn more.

www.illumina.com/microbiology



© 2015 Illumina, Inc. All rights reserved. Illumina and the pumpkin orange color are trademarks of Illumina, Inc. and/or its affiliates in the US and/or other countries.