Argonne NATIONAL LABORATORY

ANLSoilMetagenomics Workshop2011

3rd Annual Argonne National Laboratory Soil Metagenomics Workshop

OCTOBER 5th - 7th 2011

Hilton Chicago Indian Lakes Resort
Bloomingdale, IL

microbial function in a changing world

ORGANIZING COMMITTEE

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We would like to thank our sponsors for their generous support

October 5, 2011

Welcome to the 3rd Annual Argonne Soil Metagenomics Workshop!

Soil microbes play a critical role in terrestrial biogeochemical cycles that drive global climate. Relatively little is known, however, about microbial community dynamics in space and time owing to the overwhelming structural and functional complexity of such communities in soil. The 3rd Annual Argonne Soil Metagenomics Workshop brings together a multidisciplinary group of scientists to tackle some of the challenges associated with linking microbial function to soil and ecosystem processes that impact Earth's changing climate.

Recent advances in sequencing technology have enabled access to staggering volumes of information about soil microbial communities. The challenge now lies in interpreting such data to develop a conceptual model of how microbes shape larger-scale processes. A primary aim of this workshop is to develop strategies for applying sequence data to ecological questions spanning from soil microsites to whole ecosystems.

The needs of this interdisciplinary community are ongoing, and we hope this workshop will boost necessary communication among soil scientists, ecologists, computational scientists, and molecular microbiologists. We strive to make such interdisciplinary interaction a hallmark of the Argonne Soil Metagenomics Workshops to promote continued expansion of the boundaries of soil metagenomics research. We hope that the agenda's combination of session introductions and facilitated discussions, invited and contributed talks, forum discussions, and poster sessions provide a unique venue and ample opportunity for interaction among the diverse group of scientists who are pushing the boundaries of soil metagenomics research.

Sincerely,

The Organizing Committee

AGENDA

Wednesday, October 5

3:00 pm **Welcome**
Location: Conference Center, Trillium 3

Forum **Nuts and Bolts of Soil Metagenomics**

3:15 *Introduction*
Dionysios Antonopoulos, Argonne National Laboratory

3:30 *Selecting appropriate sequencing strategies*
Leaders, Dionysios Antonopoulos & Jack Gilbert, Argonne National Laboratory
Featuring Suzanne Kennedy, Director of Research and Development at MoBio

4:10 *Analysis and visualization of community structure (16S) data*
Leader, Rob Knight, University of Colorado

4:50 *Analysis and visualization of shotgun metagenomic data*
Leader, Folker Meyer, Argonne National Laboratory

5:30 *RCN: TerraGenome—The Soil Metagenome Network*
David Myrold

5:35 *Break*

6:00 pm ***Buffet dinner***
Location: Conference Center, Trillium 4

7:00 pm **Keynote talk**
What can microbial ecology do for soil metagenomics?
James Prosser, University of Aberdeen

8:00–9:30 pm **Poster Session I**

AGENDA

Thursday, October 6

8:00 am ***Continental breakfast***

Location: Conference Center, Trillium 3

Session 1: Edaphic Drivers of Microbial Function
Chair & invited speaker, Mark Waldrop, US Geological Survey

8:30 am *The Mold in the Machine: Metagenomics, Models, and Moore's Law*
M. Waldrop, USGS

9:00 am *Invited talk: Relationship of Edaphic Factors to 16S and 18S rRNA Gene Diversity*
C. Lauber, University of Colorado

9:20 am *The relative abundance of ammonia oxidizing archaea is related to land use management at three contrasting experimental sites*
P. Dorr de Quadros, K. Zhalnina*, A. Davis-Richardson, K. Gano, J. Fagen, C. Brown, A. Giongo, S. Daroub, I. Clark, S. McGrath, P. Hirsch, E. Triplett

9:40 am *Metagenomic Exploration of a Prairie Soil*
T. Delmont, E. Prestat, K. Keegan, M. Faubladier, P. Robe, I. Clark, E. Pelletier, P. Hirsch, F. Meyer, J. Gilbert, D. Paslier, P. Simonet, T. Vogel*

10:00 am Discussion

10:10 am *Break*

Session 2: Climate-microbe Feedbacks
Chair & invited speaker, John Crawford, University of Sydney

10:30 am *GxE: Inferring the Microbial Environment*
J. Crawford

11:00 am *Invited talk: Function and evolution of decomposer fungi in a changing climate*
L. van Diepen*, E. Morrison, A. Pringle, C. Sthultz, S. Frey

11:20 am *Antarctic-MAPs: Predicting the population structure of Antarctic soil microbial communities as a function of environmental conditions*
P. Larsen*, J. Gilbert

11:40 am *Large-scale manipulated field experiments to describe the responses of biological soil crust microbial communities to long-term elevated CO_2*
B. Steven*, J. Belnap, R. Evans, L. Gallegos-Graves, C. Kuske

12:00 pm Discussion

12:10 pm ***Lunch (provided)***

AGENDA

Session 3: Spatial structure of microbial communities across scales
Chair & invited speaker, Mark Bailey, Center for Hydrology and Ecology, Oxford, UK

1:30 pm *Biogeography of microbes in soils?*
M. Bailey*, R. I. Griffiths, B. Thompson, C. van Der Gast & A. Whiteley

2:00 pm *Invited talk: The spatial, phylogenetic and abundance dimensions of environmental and historical filters on bacterial community structure*
D. Nemergut

2:20 pm *Ecology of Diatomaceous Biological Soil Crust Assemblages in Yellowstone Geothermal Soils*
J. Meadow*, C. Zabinski

2:40 pm *Using the RDP classifier to predict novelty and reduce the search space for finding novel organisms*
G. Rosen*, Y. Lan, Q. Wang, J. Cole

3:00 pm Discussion

3:10 pm *Break*

Session 4: Temporal Dynamics
Chair & invited speaker, Allan Konopka, Pacific Northwest National Laboratory

3:30 pm *Nature's pulsing paradigm (with apologies to the Odum family)*
A. Konopka

4:00 pm *Invited talk: Distinct resuscitation patterns of soil microbes following early-season wet-up*
S. Placella, E.L. Brodie*, D.D. Ackerly, M.K. Firestone

4:20 pm *Microbial ecology of switchgrass decomposition across a tropical forest rainfall gradient*
K. DeAngelis*, T. Hazen, S. Borglin, D. Chivian, J. Fortney, B. Simmons, W. Silver

4:40 pm *Does long-term drought alter the response of soil microbial communities to moisture?*
S. Evans*, M. Wallenstein

5:00 pm Discussion

5:10 pm *Break*

5:30–7:00 pm Poster session II

Dinner on your own

Friday, October 7

8:00 am ***Continental breakfast***

Session 5: **Integrating Ecological Theory in Metagenomics Projects**
Chair & invited speaker, Jay Lennon, W. K. Kellogg Biological Station, Michigan State University

8:30 am *An ecological perspective on soil metagenomics*
J. Lennon

9:00 am *Invited talk: Permafrost Metagenomics*
J. Jansson

9:20 am *Molecular Ecological Networks In Response to Elevated CO_2*
J. Zhou*, Y. Deng, F. Luo, Z. He

9:40 am *Perturbing complex microbial communities for metagenomic discoveries: one 'designed' evenness at a time*
T. Delmont*, E. Prestat, E. Pelletier, D. LePaslier, P. Simonet, T. Vogel

10:00 am Discussion

10:10 am *Break*

Session 6: **Plant-Microbe Interactions**
Chair & invited speaker, Mary Firestone, University of California, Berkeley

10:30 am *Isotopic and molecular characterization of a soil microbial community present in an AMF hyphosphere*
M. Firestone*, E. Nuccio, D. Herman, J. Pett-Ridge, A. Hodge

11:00 am *Invited talk: Interactions between plant litter decomposition and microbial communities: Aboveground and belowground effects*
M. Strickland

11:20 am *Metagenomics of the Rhizobium leguminosarum-plant host symbiosis*
J. Imperial*, B. Jorrin, G. Laguerre

11:40 am *Deep sequencing of soil microbial communities of bioenergy crops*
A. Howe, E. Cardenas, A. Garoutte, J. Guo, P. Chain, J. Fish, Q. Wang, J. Cole, J. Tiedje

12:00 pm Discussion

12:10 pm ***Lunch (provided)***

Session 7: **Lowering the Barriers to Implementing Metagenomics in Soils: Forum Report**
Chair, Dionysios Antonopoulos, Argonne National Laboratory

1:30 pm Overview
Dionysios Antonopoulos

AGENDA

1:40 pm *Selecting appropriate sequencing strategies*
J. Gilbert
2:00 pm *Analysis and visualization of community structure (16S) data*
G. Caporaso
2:20 pm *Analysis and visualization of shotgun metagenomic data*
F. Meyer

2:40 pm Discussion

3:00 pm ADJOURN

POSTERS

Poster session I: Even-numbered titles

Poster session II: Odd-numbered titles

Edaphic drivers of microbial function

1. *North American soil metagenomes cluster by biome and edaphic factors*
   Dionysios A. Antonopoulos[1,2], Sarah L. O'Brien[1], Kevin P. Keegan[1], Areej Ammar[1], Brandon S. Bates[1], Jennifer M. Brulc[1], J. Gregory Caporaso[4], Marc H. Domanus[1], Mark D'Souza[5], Rob Edwards[3,6], Tifani Eshoo[1], Rachel E. Gallery[7], Aaron Garoutte[1], Carol S. Giometti[2], Elizabeth M. Glass[3], Julie D. Jastrow[2], Rebecca H. Kao[7], Kenneth M. Kemner[2], Rob Knight[4,8], Kelly A. Skinner[2], Rick Stevens[3,5], Andreas Wilke[5], Jared R. Wilkening[1], R. Michael Miller[2], Folker Meyer[1,3,5]

2. *Characterizing Cellulolytic Enzymes from Soil Using Proteomics: Do differences in enzyme diversity drive home-field advantage for litter decomposition?*
   Barbara Fricks, Ken Reardon, Matthew Wallenstein

3. *Coffee from the ground up: rustic coffee plantations provide better habitats for soil fungal communities than sun coffee plantations*
   Krista L. McGuire, Stacy M. Philpott, Rosie Gradoville, Caitlyn Gillikin

4. *A comparative analysis of different fertility practices in an arable Indiana Soil*
   Matt A. Rudisill, Lori A. Hoagland

5. *Identification of genes involved in iron reduction in anoxic peat soils*
   Archana J. Srinivas, Ling Han, J. Matthew Haggerty, David A. Lipson, Elizabeth Dinsdale

6. *Transcriptional response of Ralstonia pickettii to high copper concentrations*
   Fan Yang, Thomas C. Voice, David T. Long, Terence L. Marsh

Spatial structure of microbial communities across scales

7. *The First Use of Biofilm Microbial Community Structure as an Indicator of the Impact of Two Dams on the Elwha River (Washington)*
   W.D. Eaton, D. Barry

8. *Diversity and Activity of Fungal Laccase across an Ecosystem Gradient*
   Dara B. Goodheart, Jen Kerekes, Benita Ho, Tom Bruns, Mary K. Firestone

9. *Discriminating Native Subsurface Microbes from Drilling Mud Contaminants at Depths of 1.0-2.2 KM in the Illinois Basin Using 454 Pyrotag Sequencing*
   Yiran Dong, Isaac Cann, Roderick Mackie, Nathan Price, Theodore M. Flynn, Robert A. Sanford, Philip Miller, Nicolas Chia, Charu Gupta Kumar, Pan-Jun Kim, Mayandi Sivaguru, Bruce Fouke

10. *Geochemical Controls Over the Spatial Distribution of Biota in the McMurdo Dry Valleys, Antarctica*
    J.E. Barrett

Climate-microbe feedbacks

11. *Soil nutrient legacies surpass the effects of CO_2 and O_3 concentration on mycorrhizal communities*
    Carrie Andrew, Erik Lilleskov

12. *Elevated Atmospheric Carbon Dioxide Alters the Composition of Soil Microbial Communities*
    John J. Kelly, Emily Peterson, Jon Winkelman, Catherine Putonti, Steven T. Rier, and Nancy C. Tuchman

13. *Exploring the Functional Role of Soil Microorganisms under Climate Change Conditions in a Temperate Forest*
    Carley Kratz, Erik Lilleskov, Andrew J. Burton

14. *The Effects of Climate and Land-Use Change on Soil Microbial Diversity in the Mongolian Steppe*
    Aurora MacRae-Crerar, Brenda Casper, Peter Petraitis, Bazartseren Boldgiv

15. *Aspects of Adaptation by Same Bacterial Genus to Diverse Environments*
    S. Geetha Saarunya, Tatiana Vishnivetskaya, Karuna Chourey, Alice C. Layton, Susan M. Pfiffner

16. *Expanded diversity of bacterial nosZ genes and corresponding functional activity beyond denitrifiers revealed in different agricultural soils*
    Robert A. Sanford, Joanne C. Chee-Sanford, Lynn M. Connor, Frank E. Löffler

17. *Metagenomics Sequencing Analysis of Grassland Soil Microbial Community in Response to Long Term Elevated CO_2*
    Qichao Tu, Zhili He, Liyou Wu, Patrick Chen, Gary Xie, Jizhong Zhou

18. *Soil Metabolism and Microcalorimetry; A Unique combination*
    Hameed Ullah, Jose A. Simoni, Cláudio Airoldi, Ali Riaz

Temporal dynamics

19. *Microbial seasonal succession and soil biogeochemistry during spring thaw in arctic tundra*
    Kate M. Buckeridge, Samiran Banerjee, Steven D. Siciliano, Paul Grogan

20. *Microbial Resuscitation Strategies Following Soil Wet-Up Are Phylogenetically Clustered*
    Sarah A. Placella, Eoin L. Brodie, Mary K. Firestone

21. *Metagenomic-based analysis of fungal and bacterial communities in isothiocyanate-amended soil*
    Ping Hu, Anil Somenahally, Frank M. Hons, Terry J. Gentry

Integrating ecological theory in metagenomics projects

22. *Characterization of Fungal Communities associated with Mesofauna in Low pH Forest Soils*
    Anzilni Fathia Amasya, Rida Oktorida Khastini, Kazuhiko Narisawa, Makiko Watanabe

23. *Increased fire incidence in California annual grasslands can alter soil microbial responses to multi-factor global change*
    K. Docherty, J. Gutknecht

24. *Microbial communities in restored wetland sediments*
    Shaomei He, Mark Waldrop, Lisamarie Windham-Myers, Tanja Woyke, Susannah G. Tringe

25. *Functional metagenomics of plant litter decomposition*
    Mari Nyyssönen, Claudia Weihe, Michael L. Goulden, Kathleen K. Treseder, Jennifer B.H. Martiny, Adam C. Martiny, Steven D. Allison, Eoin L. Brodie

26. *Predicting bacterial growth efficiency with ecological theory and metagenomic data*
    Ben R. Roller, Thomas M. Schmidt

Plant-microbe interactions

27. *Soil inoculation as a tool to manipulate plant-soil feedbacks and improve management of exotic knapweed invasions.*
    Thomas A. Grant III, Mark W. Paschke

28. *Inhibitory effects of Bromelia pinguin (Bromeliaceae) on soil ecosystems in primary forests of Costa Rica*
    C. Looby, W.D. Eaton

29. *Effects of Land Management and Habitat Type on Organic Carbon (C) and Nitrogen (N) Content and Biomass, and Abundance and Diversity of Soil Fungal and Non-Fungal Eukarya in a Tropical Lowland Region of Costa Rica.*
    K.M. McGee, W.D. Eaton

30. *Nitrogen cycling and root-zone microbial communities in rangeland restoration plots*
    Jeanette M. Norton, Xu Li, John M. Stark

31. *Consequences of oak tree loss on soil microbial community composition in eastern temperate forests of North America*
    Ramya Rangamannar, Ika Djukic, Krista L. McGuire, Bill Schuster, Kevin Griffin

32. *Mechanisms of plant root impacts on litter decomposition in soil*
    Shengjing Shi, Donald Herman, Trent Northen, Eoin Brodie, Mary Firestone

Informatics and wet-lab technique development

33. *Public Domain Functional Metagenomics*
    Trevor Charles, Katja Engel, Jiujun Cheng, Andre Masella, Gabriel Moreno-Hagelsieb, David Rose, Josh Neufeld

34. *Screening for novel metabolic genes from soil metagenomic libraries using a Sinorhizobium meliloti megaplasmid deletion strain*
    Jiujun Cheng, Shirley Wong, Tanya Romantsov, David Rose, Josh Neufeld, Trevor Charles

35. *Demonstration of a metagenomics analysis pipeline on Everglades Histosol*
    Austin Davis-Richardson, Christopher T. Brown, Kateryna Zhalnina, Kelsey A. Gano, Jennie Fagen, Adriana Giongo, Samira H. Daroub, Eric W. Triplett

AGENDA

36. *Gene-Targeted Metagenomics Assembly*
    Jordan A. Fish, Qiong Wang, Yanni Sun, C. Titus Brown, James M. Tiedje, James R. Cole

37. *The Earth Microbiome Project: A new paradigm in geospatial and temporal studies of microbial ecology*
    Jack A Gilbert, Janet Jansson, Rob Knight, Sarah Owens, Ella Rakowski

38. *A Methodology Study for Metagenomics using Next Generation Sequencing*
    Deborah Grove, Sushmita Singh, Istvan Albert, Doug Bintzler, Kip L. Bodi, Mary Ann Bruns, Ken Dewar, Greg Gloor, Timothy J. Johnson, Jan Kieleczawa, Robert H. Lyons, Thomas A. Neubert, Anoja G. Perera, Robert Steen, Michael Zianni

39. *Validation and application of Illumina sequencing to comparative soil metagenomics*
    Chengwei Luo, Konstantinos T. Konstantinidis

40. *Functional screening of soil metagenomic libraries for bacterial conjugation functions*
    Cveta Manassieva, Kathy Lam, Jiujun Cheng, Trevor Charles

41. *Evaluation of SCODA as a novel electrophoretic technology for DNA purification from soil and other environmental samples*
    Jason Maydan, Hau-Ling Poon, Joel Pel, Katja Engel, Josh Neufeld, Andre Marziali

42. *RCN: TerraGenome—The Soil Metagenome Network*
    David D. Myrold, Janet K. Jansson, Folker Meyer, James M. Tiedje, Eric W. Triplett

ABSTRACTS

Characterization of fungal communities associated with mesofauna in low pH forest soils

Anzilni Fathia Amasya[1], Rida Oktorida Khastini[2], Kazuhiko Narisawa[2], Makiko Watanabe[1]

[1]*Graduate School of Urban Environmental Science, Tokyo Metropolitan University;* [2]*College of Agriculture Ibaraki University*

This study aimed to characterize the fungal communities associated with mesofauna collected from low pH forest soils in Japan. Nine repeats of soil samples were collected from *Fagus* forest in Nagano prefecture, central Japan, during July – August 2011. Litter and humic layers within a 20x20 cm plot were weighed in the field while A horizons for mesofauna collection and soil analysis were collected using a cylinder with the volume of approximately 400 cm^3 and 800 cm^3 , respectively. Soils in A horizons were collected within 10 cm in depth. Soil mesofauna were collected using modified Tullgren method for 7x24 hours under a 5 watt light bulb with an average of 14.4 cm distance from the soil surface, kept in a constant 24^0C room temperature. Apart from the soil mesofauna, fungal sclerotia – which are resting bodies of mainly ectomycorrhizal species – were also found. Nematodes showed the highest relative abundance followed by Protura and Collembola. Sclerotia were spherical black with an approximate diameter range of 0.5–1.5mm, and an average density of 1.5 g kg^{-1} soil. T-RFLP (terminal restriction fragment length polymorphism) profiling and clone libraries with the internal transcribed spacer for fungi (primers ITSf1 and ITSf5) were used to analyze the fungal communities consumed by soil mesofauna. Soil mesofauna fungal communities were analyzed separately between Nematodes, Protura, Collembola, Acari and Araneae. The fungal communities found in mesofauna bodies were expected to correlate with the relative abundance of sclerotia-forming fungi.

Soil nutrient legacies surpass the effects of CO_2 and O_3 concentration on mycorrhizal communities

Carrie Andrew[1,2], Erik Lilleskov[3]

[1] The Field Museum, Chicago, Illinois; [2] Northeastern Illinois University, Chicago, Illinois; [3] Northern Research Station, Houghton, Michigan

Here we present a comprehensive overview of ectomycorrhizal fungal response to elevated CO_2 and O_3 across time within a FACE field site context. Ectomycorrhizal fungal communities do respond to CO_2 and O_3 levels, but the response is dependent upon the tissue (mushrooms or mycorrhizas). Aboveground,

the response is straightforward: increased photosynthesized carbon leads to increased sporocarp production, with stabilizing effects on communities over time. Belowground, however, the story is contingent on soil nutrient legacy effects, which more strongly affect belowground communities than do CO_2 or O_3 levels. This has strong implications for understanding mycorrhizal community dynamics within the context of continual climatic change.

North American soil metagenomes cluster by biome and edaphic factors

Dionysios A. Antonopoulos[1,2], Sarah L. O'Brien[1], Kevin P. Keegan[1], Areej Ammar[1], Brandon S. Bates[1], Jennifer M. Brulc[1], J. Gregory Caporaso[4], Marc H. Domanus[1], Mark D'Souza[5], Rob Edwards[3,6], Tifani Eshoo[1], Rachel E. Gallery[7], Aaron Garoutte[1], Carol S. Giometti[2], Elizabeth M. Glass[3], Julie D. Jastrow[2], Rebecca H. Kao[7], Kenneth M. Kemner[2], Rob Knight[4,8], Kelly A. Skinner[2], Rick Stevens[3,5], Andreas Wilke[5], Jared R. Wilkening[1], R. Michael Miller[2], Folker Meyer[1,3,5]

[1]Institute for Genomics and Systems Biology, [2]Biosciences Division, [3]Mathematics and Computer Science Division, Argonne National Laboratory, Argonne, IL 60439, [4]Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309, [5]Computation Institute, The University of Chicago, Chicago, IL 60637, [6]College of Sciences, San Diego State University, San Diego CA 92182, [7]NEON, Inc., Boulder, CO 80301, [8]Howard Hughes Medical Institute, Boulder, CO 80309

Soil microbial communities are critical components of terrestrial ecosystems, but their astounding complexity has hampered development of an integrated understanding of their functional capabilities. A metagenomics approach that combines large-scale sequencing and computing to access the collective gene content of these communities can confront the challenge of linking microbial diversity and function to climate change-relevant biogeochemistry by relating annotated gene sets to important environmental variables. Here we show that soil microbial communities across North America have distinct functional gene repertoires that clearly segregate by biome (forest vs. grassland) and are driven by local edaphic conditions (nitrogen and pH) regardless of climate or geography. Furthermore, we present the first work to identify sets of genes that ostensibly underlie previously enigmatic relationships between soil microbial community functions and discrete edaphic properties. These findings demonstrate that metagenomics can improve the foundation for uncoupling integrated environmental drivers of microbial community function across ecosystems.

Biogeography of microbes in soils?

Mark J. Bailey, R. I. Griffiths, B. Thompson, C. van Der Gast & A. Whiteley

Molecular Microbial Ecology Group, Centre for Ecology & Hydrology, Crowmarsh Gifford, Wallingford, Oxfordshire, UK, OX10 8BB

Soils are the substrate that sustain terrestrial ecosystems, they underpin pant productivity and are a critical sink and important source of Carbon and GHG. Plant derived organic matter inputs also contribute significantly to soil bacterial community composition and associated soil processes. Advances in metagenomics and high throughput methods provide the tools to determine the factors that influence and regulate the relative abundance and activity of microbial populations locally or across geographical space. In my overview I will comment on a number of recent approaches to map the local and regional variation in microbial (and specific gene) abundance and distribution. These investigations reveal a strong correlation in vegetation type, soil quality, nutrient (C / N / P), pH etc. with bacterial diversity, community structure and functioning. AMF as a vital component of terrestrial ecosystems form mutually beneficial relationship with plant roots that directly influence above ground plant diversity and productivity. At both local and regional scales AMF community composition reflects strain adaptation to the prevailing soil conditions including a clear response to agricultural manipulation. These and other studies demonstrate marked and reproducible patterns in the biogeographic distribution of microbial communities, and reveal patterns consistent with land habitat classes. Furthermore soil disturbance in the farmed environment appears to dampens the signature of any near local differences in community composition. These large scale field-based studies add to a growing body of evidence of the impact of soil nutrient status and aboveground vegetation on the relative abundances of dominant soil microbial taxa. Understanding these interactions and impacts are key to developing soil management practices that maintain soil quality and productivity for the provisioning of ecosystem services.

Geochemical controls over the spatial distribution of biota in the McMurdo Dry Valleys, Antarctica

J. E. Barrett[1]

[1]Department of Biological Sciences, Virginia Tech, Blacksburg, VA, USA 24061.

Soil microbial communities are influenced by a combination of historical and contemporary processes operating over millennial to daily time scales. For example, geological substrate constrains plant and soil communities over pedogenic time scales, while spatial and temporal variation in subsurface hydrology may influence microbial communities over days to seasons. Distinguishing among historic and contemporary influences over soils can provide insight into assembly of microbial communities in heterogeneous environments. Here I present research investigating the influence of geochemical gradients on the spatial distribution of soil biota in an Antarctic polar desert. The McMurdo Dry Valleys comprise the largest ice-free area of the Antarctic continent and host a variety of glacial,

lacustrine, and fluvial landscapes dominated by arid mineral soils developing on unconsolidated tills and poorly weathered bedrock. No vascular plants or vertebrates inhabit the dry valleys, and foodwebs are comprised of algae, bacteria, fungi, protozoa, and very few taxa of metazoan invertebrates. Antarctic soil ecosystems are useful models for investigating controls over microbial communities because the remoteness of Antarctica limits dispersal of cosmopolitan organisms, and the combination of extreme conditions (*i.e.*, cold, dry, saline, alkaline) and fine-scale spatial heterogeneity create a diverse mosaic of habitats that select for distinct biological communities. Soil microbial communities were investigated across a sequence of tills within Taylor Valley spanning a well-characterized range of physical and geochemical properties and surface exposure ages. Microsites with evidence of active subsurface hydrology were co-located with dry sites within each till and community structure and diversity was assessed using a T-RFLP community profiling approach. Variance in microbial communities was associated with differences among the various tills. Influences of active hydrology on soil communities were most evident in the oldest and geochemically most extreme tills suggesting that the influences of contemporary soil processes are constrained by landscape history.

Microbial seasonal succession and soil biogeochemistry during spring thaw in arctic tundra

Kate M. Buckeridge[1,2], Samiran Banerjee[3], Steven D. Siciliano[3], and Paul Grogan[1]

[1]Queen's University, Canada; [3]University of California, Santa Barbara; [3]University of Saskatchewan, Canada

Microbial community seasonal succession may have large impacts on soil biogeochemical dynamics, through the release of nutrients stored in the microbial cytoplasm or through changes to microbial functional groups during spring thaw. Our objective was to characterize seasonal soil biogeochemistry and microbial community functional group succession during a year in arctic tundra, with emphasis on the thaw period. We predicted that winter snow depth would be a strong control on the microbial community and soil biogeochemistry.

We sampled soil biogeochemistry (inorganic and organic C, N and P) and soil microbial biomass and community structure (using chloroform-fumigation-extraction, epiflourescent microscopy, PLFA and qPCR (16S/18S)), from late winter until fall 2007, in a Canadian low arctic birch hummock tundra (Daring Lake, NWT). To investigate the impact of deepened snow on these factors, we sampled under ambient (0.3 m) and experimentally deepened (1 m) snow.

Microbial community structure and soil biogeochemistry were most dynamic during spring thaw. During thaw two important soil temperature periods were associated with elevated soil biogeochemical pools, of approximately 3 weeks each when all soils hovered near -5°C, and then near 0°C. All of our microbial biomass and microbial community analytical methods agreed that soil bacteria and fungal

biomass peaked in these two spring periods; that these soils are dominated by fungi; and that fungi declined at the end of the 2nd period more than bacteria, and later under deeper snow. Our results support two broad conclusions: (1) that snow depth is an important control on spatial variability in soil and microbial nutrient pools, and thus possibly vegetation, across the tundra and (2) that microbial seasonal succession is an important mechanism contributing to temporal variability in tundra biogeochemistry.

Public domain functional metagenomics

Trevor Charles[1], Katja Engel[1], Jiujun Cheng[1], Andre Masella[1], Gabriel Moreno-Hagelsieb[2], David Rose[1], Josh Neufeld[1]

[1]University of Waterloo; [2]Wilfrid Laurier University

A limitation of functional metagenomics as it is currently practiced is that constructed libraries are typically project-specific and maintained in isolation. In an effort to enable the sharing of genetic material from environmental samples for the benefit of the scientific community, we have established the Canadian MetaMicroBiome Library (CM^{2}BL; http://cm2bl.org). This repository contains metagenomic libraries originating from composite soil samples that have been characterized by extensive SSU gene and whole-genome sequencing, generating taxonomic profiles and metabolic pathway information for each sample. To facilitate the identification of libraries of interest, sample information can be retrieved by environmental context and DNA sequence data. High quality large insert libraries are constructed in custom broad host range cosmid Gateway® entry vectors, allowing for phenotypic screening in a wide range of microbial surrogate hosts. The screening host range can be further extended by transfer of the cloned fragments to desired Gateway® destination vectors. Phenotypic screening of these metagenomic libraries provides access to truly novel functions that would otherwise be missed by sequence-based surveys or host-limited screening. While CM^{2}BL is being initiated with soil samples collected from across Canada, there are several functional metagenomics efforts in existence throughout the world. We propose that this effort be extended to an international network of linked public domain metagenomic library resources.

Screening for novel metabolic genes from soil metagenomic libraries using a *Sinorhizobium meliloti* megaplasmid deletion strain.

Jiujun Cheng, Shirley Wong, Tanya Romantsov, David Rose, Josh Neufeld, Trevor Charles

University of Waterloo

We are interested in identifying and studying novel genes for catabolic pathways in soil bacteria. Many of these genes might not be expressed in *E. coli*, which is the most common host used for metagenomic screening. We have developed efficient screening methods using members of the α–proteobacteria, an important soil taxon. The RmF726 strain of the alfalfa symbiont *Sinorhizobium meliloti* harbors a large deletion in megaplasmid pSymB that removes a number of genes, including those involved in α-galactoside, β-galactoside and β-ketoadipate catabolic pathways, and dicarboxylic acid transport. As a result, the strain is unable to grow using sole carbon sources whose catabolism requires the functions of these pathways, such as lactose, melibiose, protocatechuate and succinate. Growth on other carbon sources is unaffected. In an effort to identify novel genes from soil bacteria for these pathways we are performing functional complementation of this deletion strain using the Canadian MetaMicroBiome Library (CM^2BL) broad host range metagenomic cosmid libraries. This complementation is carried out by introducing the pooled library clones, maintained in *E. coli*, by *en masse* conjugation into the deletion mutant strain, followed by selection for growth on defined media with the indicated carbon source. Isolated clones are then confirmed by conjugal transfer back into *E. coli*, and from there back into the original deletion mutant. DNA sequence analysis of the complementing cosmid clones provides the basis for further biochemical characterization. The use of phenotypic selection of pooled metagenomic libraries greatly simplifies the isolation of complementing clones.

GxE: Inferring the microbial environment

John Crawford

University of Sydney

The metagenome of the soil is an important beginning to our understanding of the origin and maintenance of the soil processes that sustain our environment. However, the genome on it's own does not provide a full picture and somehow, and in parallel, we need to consider how we factor in the enormous diversity of microenvironments in the soil at the scale of the microbial habitat that influence gene expression. I will discuss the role of modelling in making the invisible observable, the concept of emergent parsimony, and how important the GxE interaction will be for a full understanding of soil.

Demonstration of a metagenomics analysis pipeline on Everglades Histosol

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Agricultural treatment of soil using fertilizers, agrochemicals and tillage modify the chemical, physical and biological characteristics of soil. These alterations affect the carbon and nitrogen cycles, pH, oxygen content, moisture and the composition and activity of the microbial community. Common side-effects of consistent agriculture include nitrogen leaching and reduction of soil fertility. To investigate the effect of agriculture on the functional characteristics of agricultural soil, a sample of organically-rich Histosol muck was taken from the Everglades Agricultural Area (Histosol - Florida, USA). A shotgun metagenome was generated from this sample using 454 pyrosequencing and is being analyzed with a pipeline implemented in Python. Here, this pipeline is presented. Metagenomic reads are trimmed based on quality scores. Velvet is used to perform de novo assembly of trimmed reads. Open Reading Frames (ORFs) are predicted from contigs using Prodigal in metagenome mode. Raw reads are reference assembled to ORFs to obtain coverage using CLC Assembly Cell, resulting in quantification of each identified function. Using the hierarchical functional annotation contained in SEED, coverage at different levels of function are calculated, allowing the investigator to look at functional characteristics at broad or narrow perspectives. Proteins identified in the SEED database can then used to generate visual pathway maps using KEGG. Any reads that are not identified as proteins are compared to the Ribosomal Database Project 16S rRNA database to obtain taxonomic information. The constituents of the pipeline are free and open source except for CLC Assembly Cell which is preferred for its performance. However, comparable Open Source reference assemblers should suffice. The source for the pipeline will be made available on GitHub upon publication.

Microbial ecology of switchgrass decomposition across a tropical forest rainfall gradient

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Tropical soils are capable of quickly and completely deconstructing plant materials to basic components under low and fluctuating redox conditions. To better understand decomposition under fluctuating redox potentials, we buried litterbags filled with switchgrass in four sites along a rainfall gradient in the Luquillo Experimental Forest in Puerto Rico, ranging from drier, rapidly fluctuating redox to wetter, low and slowly fluctuating redox. In all four sites we followed decomposition over two years; at 1, 4, 13, 30, 60 and 96 weeks, litter and adjacent soil samples

were collected and analyzed for litter chemistry and microbial community activity and composition. The driest site had the least mass lost and the lowest C:N ratio after two years. While the highest soil enzyme activities belonged to the wettest site, one of the intermediate, fluctuating redox sites had the highest litter enzyme activity. Cellulase activities increased steadily from 1 to 30 weeks then leveled off, while phenol oxidase and peroxidase activities peaked much earlier. Small subunit ribosomal RNA genes were pyrosequenced with universal primers specific for bacteria, archaea and eukaryotes, yielding over 40,000 unique taxa after quality filtering, dereplication and clustering at 97% identity. We detected higher richness in litter than soil for the driest sites, but higher richness in soil than litter for the wettest sites. Community profiles calculated using phylogenetic distance (via FastUnifrac) showed that during early decomposition (weeks 1-13), soil and litter bacterial communities were distinct, while the fungal soil and litter communities were indistinguishable; during later decomposition (weeks 30-96), bacterial soil and litter communities were indistinguishable while fungal communities were distinct. A relatively small set of taxa was highly abundant during early-stage decomposition, including Beta- and Gammaproteobacteria, Firmicutes and Stramenopiles. These data reveal a succession of fungi and bacteria that conspire to accomplish decomposition under low and fluctuating redox conditions.

Perturbing complex microbial communities for metagenomic discoveries: one "designed" evenness at a time.

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One of the biggest challenges in metagenomics is the reconstruction of genomes directly sequenced from complex environments. To stimulate assembly efficiency without applying whole genome amplification, the evenness of microorganisms from a grassland soil was highly modified under microcosm conditions prior deep sequencing efforts. Soil communities were incubated either at 37°C, under anaerobic condition or under aerobic conditions with specific enrichments (different concentrations of salt, mercury, heavy metals or diesel) for four months. The relative functional and taxonomical reproducibility observed among replicates emphasizes the non random aspect of the communities' responses to the various environmental modifications. Under most of the conditions, several taxa and functions increased significantly in relative distribution in comparison to natural communities, controls, and other conditions. Results provide valuable information about how complex communities respond during controlled

environmental modifications (*e.g.*, unusual distribution of resistance genes and specific mobile genetic elements). Interestingly, large genetic fragments (hundreds of kilo bases) were reconstructed under different conditions and could represent entire genomes in some cases. These fragments provide information about the genetic environment of genes and genetic elements of interest. Thus, we were able to reconstruct indigenous soil genomes selected by relatively harsh or specific conditions.

Metagenomic exploration of a prairie soil

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The soil ecosystem is critical for human health, affecting aspects of the environment from key agricultural and edaphic parameters to critical influence on climate change. Soil has more unknown biodiversity than any other ecosystem. We have applied diverse DNA extraction methods coupled with high throughput pyrosequencing to explore 4.88 billion base pairs of metagenomic sequence data from the longest continually studied soil environment (Park Grass experiment at Rothamsted Research in the UK). Results emphasize important DNA extraction biases and unexpectedly low seasonal and vertical soil metagenomic functional class variations. Clustering-based subsystems (CBSS) and carbohydrate metabolism had the largest quantity of annotated reads assigned although less than 50 % of reads were assigned at an E value cutoff of 10^{-5}. In addition, with the more detailed subsystems, cAMP signaling in bacteria (3.24 +/- 0.27 % of the annotated reads) and the Ton and Tol transport systems (1.69 +/- 0.11%) were relatively highly represented. The most highly represented genome from the database was that for *Bradyrhizobium japonicum*. The metagenomic variance created by integrating natural and methodological fluctuations represents a global picture of the Rothamsted soil metagenome that can be used for specific questions and future inter environmental metagenomic comparisons. However, only 1% of annotated sequences correspond to already sequenced genomes at 96% similarity and E values of less than 10-5, thus, considerable genomic reconstructions efforts still

have to be performed. Attempts at assembling contigs helped understand possible genetic associations as well as the future effort needed to sequence all soil microbial genomes.

Function and evolution of decomposer fungi in a changing climate

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Fungi are ubiquitous in terrestrial ecosystems and play an important role in biogeochemical cycling because of their function as litter decomposers. It has been demonstrated that increased nitrogen (N) deposition decreases fungal biomass, reduces their diversity and changes relative abundance of particular groups and species. In addition increased N can slow litter decomposition and reduce lignolytic enzyme activity. To understand the relationship between fungal community dynamics and changes in litter decay in response to climate change we made use of an existing long-term study in which a northern hardwood forest has been exposed to simulated increased N deposition for over 20 years (Chronic N Addition plots, Harvard Forest, Petersham, MA). The Chronic N Addition Experiment consists of three treatments (ambient, 5 and 15 g N m^{-2} y^{-1}).

We performed 454 sequencing of fungal DNA in the forest floor, and extracted RNA for metatranscriptomic analysis. Furthermore we isolated fungi from litter collected at each of the treatments to test fungal growth rate and decomposition ability under three different N levels. Preliminary data from 454 sequencing of the forest floor indicated a decrease in Shannon diversity and changes in relative abundance of dominant fungal taxa with N fertilization. *Hypocrea/Trichoderma sp.*, an Ascomycete, was present in all three N treatments. However, *Hypocrea/Trichoderma sp.* isolates from the low N treatment had a slower growth and decomposition rate compared to isolates from control and high N treatment. The observed changes in growth rate and decomposition rate indicate that even if fungal species persist in a higher N environment, their functioning might be affected, which in turn can change the functioning of the ecosystem they inhabit.

Increased fire incidence in California annual grasslands can alter soil microbial responses to multi-factor global change

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Wildfire in California annual grasslands is an important ecological disturbance and ecosystem control. Regional and global climate changes that affect aboveground biomass will alter fire-related nutrient loading and promote increased frequency and severity of fire in these systems. This can have long-term impacts on soil microbial dynamics and nutrient cycling, particularly in N-limited systems such as annual grasslands. We examined the effects of a low-severity fire on microbial biomass and specific microbial lipid biomarkers over three years following a fire at the Jasper Ridge Global Change Experiment. We also examined the impact of fire on the abundance of ammonia-oxidizing bacteria (AOB), specifically *Nitrosospira* Cluster 3a ammonia-oxidizers, and nitrification rates 9 months post-fire. Finally, we examined the interactive effects of fire and three other global change factors (N-deposition, precipitation and CO_2) on plant biomass and soil microbial communities for three growing seasons after fire. Our results indicate that a low-severity fire is associated with earlier season primary productivity and higher soil-NH_4^+ concentrations in the first growing season following fire. Belowground productivity and total microbial biomass were not influenced by fire. Diagnostic microbial lipid biomarkers, including those for Gram-positive bacteria and Gram-negative bacteria, were reduced by fire 9-months and 21-months post-fire, respectively. All effects of fire were indiscernible by 33-months post-fire, suggesting that above and belowground responses to fire do not persist in the long-term and that these grassland communities are resistant to fire disturbance. While N-deposition increased soil NH_4^+, and thus available NH_3, AOB abundance, nitrification rates and Cluster 3a AOB, similar increases in NH_3 in the fire plots did not affect AOB or nitrification. We hypothesize that this difference in response to N-addition involves a mediation of P-limitation and increased soil water content as a result of fire, possibly enhanced by increased plant competition and arbuscular mycorrhizal fungi-plant associations after fire.

Discriminating native subsurface microbes from drilling mud contaminants at depths of 1.0-2.2 KM in the Illinois Basin using 454 pyrotag sequencing

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Knowledge of composition, structure and activity of microbial communities in deep subsurface is of fundamental importance for practical management of groundwater resources, hydrocarbon extraction and carbon sequestration. However, technical challenges of collecting deep subsurface samples free of contamination have left the identity and function of these populations a mystery. To overcome this issue, the newly developed Schlumberger Quicksilver MDT probe (MDT) and conventional drill stem test (DST) were deployed to collect formation water from different horizons of a 2.2 km deep well in the Illinois Basin. IL. Microbial communities of these samples were compared with that of the drilling fluid to differentiate indigenous species from contamination.

Five water samples contaminated with drilling mud were collected at 1 km depth using the DST method, and 3 formation water samples were obtained using the Quicksilver MDT probe at the horizons ranging from 1.8 to 2.2 km. Geochemistry and microbial communities inhabiting in these sampling horizons were evaluated with a suite of geochemical, microscopic, and molecular phylogenetic analyses (e.g., T-RFLP, clone libraries and 454 pyrosequencing). In addition to distinct geochemical conditions observed, significantly different microbial communities among horizons were also suggested by molecular analyses. Pyrosequencing results indicated the MDT samples to be much less similar to the drilling mud (< 2 % shared) than the mud-contaminated DST samples (11 – 27% of sequences shared). Moreover, comparison of the microbial communities from which potential contaminating populations had been removed indicated heterogeneous distribution of indigenous microbial composition in the Illinois Basin, even within the same stratigraphic geological formation.

Our work suggests that pyrosequencing is a sensitive tool to determine indigenous subsurface bacterial communities. The diversity of indigenous microbial communities inhabiting in different horizons may result from the geochemical gradients (e.g. total dissolved salts (46 – 158 g/L), pH (5.9 – 8) or T (28.7 – 52.1 ˚C)) in the formations.

The relative abundance of ammonia oxidizing archaea is related to land use management at three contrasting experimental sites

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Agricultural land use management including fertilization and tillage affects the chemical and physical properties of soil including pH, organic matter content, porosity, pH, moisture levels, and microbial community diversity. Increased

nitrification rates in agricultural soils lead to nitrate leaching. The objective of this study was to detect changes in the relative abundance and diversity of ammonia oxidizing Archaea (AOA) in non-agricultural and agricultural soils from three different sites including the Broadbalk Experiment at Rothamsted Research (Alfisol soil in Harpenden, UK), the Everglades Agricultural Area (Histosol in Florida, USA) and the Kellogg Biological Station (Alfisol soil in Michigan, USA) using 16S rRNA barcoded Illumina sequencing. Shotgun metagenomic sequencing of the Everglades soil was also done. Soil pH, N-NO_3 and N-NH_4 content, total nitrogen, organic carbon and moisture content were measured to determine whether they correlate with the relative abundance Archaea at these sites. All the three sites, AOA relative abundance was significantly higher in agricultural soils, indicating that changes in soil caused by agricultural management favors ammonia oxidation by Archaea. The AOA:AOB ratios for Broadbalk, Everglades and Kellogg were 11:1, 198:1 and 12:1, respectively, for the agricultural plots and 6:1, 97:1 and 13:1, respectively, for the non-agricultural plots. The relative abundance of AOA ranged between 0.4 to 8.2 % of total reads. *Candidatus* Nitrososphaera within Thaumarchaeota was the most abundant archaeal genus at all sites. Moreover, successional plots at the Kellogg Biological Station was showed that the relative abundance of Archaea increased with time since conversion of plots to agriculture. In the agricultural plots, pH was higher and moisture content and the organic carbon were lower compared to the non-agriculture plots. For all three sites, pH was positively correlated with archaeal relative abundance and can be considered one of the strongest soil factors that drives archaeal communities in agriculture.

The first use of biofilm microbial community structure as an indicator of the impact of two dams on the Elwha River (Washington)

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Two dams constructed in the early 1900's on the Elwha River stopped all upstream movement of anadromous salmonids and their subsidy of marine-derived nutrients (MDN) beyond river mile mile 4.9, and greatly reduced all downstream movement of organic materials and large woody debris (LWD). Prior to dam removal and river restoration beginning in fiscal 2011/12, we studied the effects the dams had on the riverine biofilm microbial community, and assessed if variations in this community could be used as indicators of change for monitoring the effectiveness of the Elwha River Restoration Project. Biofilm samples were collected from the upper (EU; above the dams), the lower (EL; below the dams), and the middle (EM; between the dams) reaches of the river, and from the unrestricted Quinault River (QU). We determined the DNA sequence diversity of 168 bacterial 16s rRNA and 106 cyanobacterial internal transcribed space (ITS) PCR product-

generated clones from these different samples. This diversity analysis separated out all four locations by microbial community structure. It also showed that the microbial populations in EU and QU regions were closely related, the EL somewhat related to these regions, and the EM region not closely related to the other three regions. These relationships paralleled the amount of LWD in the sites, as the QU and EU have the most LWD, the EM the least, with the EL having intermediate levels of LWD. Moreover, only the EL and QU sites contain salmon—raising the question discussed by riparian community scientists "are salmon and their MDN or are the LWD the key ecosystem determinants in Pacific Northwest rivers?"—our results suggest the latter. We showed this suite of tools should be used as part of river monitoring and assessment plans to assess river conditions and effects of restoration activities.

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Soil microbial communities to moisture: does drought history matter?

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Drought is likely to become more frequent and more severe under future climate regimes. Despite microorganisms' important role in ecosystem function and our extensive knowledge of community responses to disturbance in plant systems, we do not know how microbial communities will respond to extreme moisture events, and whether this response may depend on ecological history. In this study, we tested whether drought history alters the response of active microbial communities to moisture. We sampled soils from 11-year drought manipulations at the shortgrass steppe LTER and subject soils to a range of moisture levels in the lab to investigate changes in microbial community composition and function. We isolated the active community by using BrDU-incorporation and examined composition by pyrosequencing the immunocaptured DNA, and measured decomposition rate by measuring CO_2 efflux from 1-year incubations. Drought history strongly affected which bacteria species were active in response to moisture. Samples from the same drought treatment in the field were more similar than samples subject to the same lab moisture, although moisture had a stronger influence on community structure at the wettest and driest levels. Although specific taxonomic groups showed different responses to both drought and moisture exposure in the lab, we did not find a strong relationship between phylogeny and drought-response. Respiration rates were significantly different among moisture levels, but drought history did not have a strong effect on the respiration response over a range of moistures. This suggests that drought does select for more tolerant microbial species, and the proportion of the community active under certain conditions is influenced by microbial ecological history. However, our results

suggest that these community changes may not affect broad ecosystem-level processes, such as respiration, and community changes may not be predictable based on phylogenetic relations.

Isotopic and molecular characterization of a soil microbial community present in an AMF hyphosphere

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We investigated the interactions of an arbuscular mycorrhizal fungi (AMF), *Glomus hoi*, with a soil microbial community involved in litter decomposition. Two-compartment microcosm units were used; in half of the microcosms, AM hyphae accessed ^{13}C,^{15}N- labeled *Plantago lanceolata* root litter in soil; in half, AM hyphae were excluded.

Succession of the soil community mediating decomposition was assessed by PLFA and ^{13}C-PLFA analysis. In AMF-invaded root litter, Gram-negative, Gram-positive, and 16:1w5*c* markers became less ^{13}C enriched relative to their counterparts in non AMF-invaded microcosms during active decomposition. These patterns of ^{13}C:^{12}C enrichment may result from AMF supply of ^{12}C from the plant to the decomposing soil microbial community. High density microarray analysis (Phylochip) of the bacterial and archaeal communities, indicated that about 10% of the 3700 taxa detected in the soil either increased or decreased in response to the presence of AM hyphae. Communities responding to the AMF, exhibited strong phylogenetic clustering. The mechanisms through which the AM hyphae alter the prokaryotic community are hypothesized based on the stable isotopic evidence.

Gene-targeted metagenomics assembly

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Soil and other very large metagenomes tax the abilities of current-generation short-read assemblers. In addition to space and time complexity issues, most assemblers are not designed to correctly treat reads from closely related populations of organisms. We are developing a gene-targeted approach for

metagenome assembly. In this approach, information about specific genes is used to guide assembly and gene annotation occurs concomitantly with assembly. This approach combines a space-efficient De Brujin graphical representation of the reads with a protein profile Hidden Markov Model for the gene(s) of interest. To limit the search, we use a heuristic to first identify nucleotide k-mers that translate to peptides found in a set of representatives of the target protein family. These k-mers, along with the positions of the peptides in the HMM representation define a set of search start points. Contigs are then assembled by applying graph path-finding algorithms in both directions on the combined De Bruijn-HMM graph structure. Using this technique we have been able to extract complete nifH protein coding regions from a 1G subset of a soil metagenome. Interestingly, simulation experiments indicate that paths optimized on HMM score often incorporate sequencing artifacts. Future work will focus on improving search efficiency, extending the method to extract multiple high-scoring paths, and separating sequencing artifacts from low-coverage rare populations.

Characterizing cellulolytic enzymes from soil using proteomics: Do differences in enzyme diversity drive home-field advantage for litter decomposition?

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Enzymes drive microbial cycling of nutrients in all ecosystems. yet a full understanding of specific enzymes involved remains unclear. Current enzyme assays allow us to understand some of the functions present in a system but do not indicate the diversity of iso-enzymes involved in degrading specific substrates. Recent studies using metabolomics indicate that different communities may decompose a common leaf litter using different enzymes, which can confer a home-field advantage when they are able to degrade litter from their native site more quickly than the rates achieved by naïve communities. Our objective in this study was to examine whether microbial communities with different environmental histories utilize different enzymes to degrade a common leaf litter.

We created an inocula of soil microorganisms from grassland and forest soils by adding switchgrass (a potential biofuel) litter to each of the soils; incubated the soils for 1 week at 25oC; and extracted proteins and enzymes using DI water. We added 0.5 mL of inoculum to 19.5 mL Hoagland's solution and 0.050 g switchgrass, and measured respiration rates over a 3-week incubation. After 1, 7, and 21 days, we extracted proteins and measured enzymatic activity for 1,4 glucosidase (AG); 1,4 ß-glucosidase (BG); cellobiohydrolase (CB); and ß- cylosidase (XYL). Using zymograms we can characterize differences in molecular weights of proteins and comparative quantity of cellulolytic enzymes to activity. We are also developing an

approach to identify enzymes involved in litter decomposition using mass-spectrometry based proteomics. However, extracting proteins from soil remains a challenge and our protocol is still being refined.

The results of our study will provide new insights into the functional differences between microbial communities that differ in composition. It will also provide new detail on the enzymes involved in litter decomposition, which could be applied to developing more efficient production of biofuels from switchgrass.

The Earth Microbiome Project: A new paradigm in geospatial and temporal studies of microbial ecology

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For three decades we have had the capability to take a molecular peak at microbial life on our planet. However, it is only within the last few years that it has become possible to explore the microbial earth with any semblance of depth. With these exciting new technologies comes great responsibility; not least of these is the necessity to appropriately record information about each study. Here we present the Earth Microbiome Project (www.earthmicrobiome.org), an international effort to redefine our exploration of this frontier. Here we will outline the project aims and goals, and explain why and how the paradigm shift must occur. There are more bacteria in the oceans alone than stars in the known universe, and hence we need a systematic and defined approach to survey this biosphere. Importantly, a survey is only as good as the analysis of the data. Here we will employ revolutionary new algorithms to explore the synergy between existing data and new data to explore overlap and identify gaps in knowledge. Additionally, through metagenomic sequencing we will create a metabolic map of microbial life on this planet, which will shape and define our perspective of how life responds and adapts to changes in the environment. The preliminary EMP projects will be discussed in terms of their immediately findings and how much they tell us about the task ahead. O'Dors Law of Biology states that "Physics and chemistry have laws, while biology only has lawyers looking for loopholes". It is essential that we better define these biological laws and help to reduce the knowledge gap that prevents us from managing the resources of this planet effectively.

Diversity and activity of fungal laccase across an ecosystem gradient

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Fungal laccases are the primary ligninolytic enzymes in the environment and are critical in decomposition of plant matter and hence the global carbon cycle. The activity and structure of laccases have been studied, however, the diversity of laccases within and across different ecosystems has not been well characterized. The aims of this study are to determine the diversity of fungal laccases across different ecosystems and assess if the diversity of fungal laccases affects laccase activity in these ecosystems. To address these goals, the diversity and activity of fungal laccases was determined in soil along a latitudinal transect that includes boreal (Alaska: Black spruce forest and Tussock grassland), temperate (California forests: SF Bay Area, Coastal Redwoods, and Sierra Conifer and California grasslands: SF Bay Area and Northern CA) and tropical ecosystems (Wet tropical forests: Puerto Rico and Panama, Tropical Cloud forest: Costa Rica and Dry tropical forest: El Salvador).

Fungal ITS and fungal laccase diversity was investigated by pyrosequencing using Qiime and Mothur tools. The abundance of fungal laccases in each ecosystem site was determined using relative quantitative PCR. Laccase activity across the different ecosystems was assessed using a phenol oxidase assay. These analyses suggest that 1) fungal ITS diversity is greater in grassland ecosystems, 2) fungal laccase diversity varies across this ecosystem gradient and that 3) differences in fungal laccase diversity affects laccase activity in these ecosystems.

Soil inoculation as a tool to manipulate plant-soil feedbacks and improve management of exotic knapweed invasions

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The interactions of plants with soil fauna and flora has recently received a great deal of attention due to the potential for plant-soil feedbacks to influence plant community composition, successional dynamics, and the spread of invasive species. To augment our understanding of plant-soil feedbacks, we used soil from native and invasive plant communities to study the impacts of inoculation on two invasive knapweed species. A field based restoration experiment was conducted in four US states and followed up with similar greenhouse experiments. Our goal was to determine the potential of soil inoculation as a tool to aid in the management of exotic knapweed invasions, improve the restoration of native vegetation, and to increase our understanding of plant-soil feedbacks that can be utilized for ecologically-based management. Field experiments analyzed vegetation, soil nutrients, soil microbial biomass (C and N), and soil microbial composition using the Fatty Acid Methyl Ester (FAME) method. Our studies show that soil inoculation can

affect the vegetation, soil nutrients, and microbial communities, although the results of inoculation were variable and highly species and site specific. Inoculation with soil of native plant communities decreased cover and density of *Centaurea stoebe* (spotted knapweed), but had little effect on *Acroptilon repens* (Russian knapweed). Multi-variate analysis of vegetation, microbes, and soil nutrients using Gradient Boosting Machines identified very different nutrient and microbial functional groups as strong predictors of knapweed or native vegetation. Inoculation treatments were weak predictors of vegetation. Restoration and management of knapweed infestation may be improved through inoculation and modification of microorganisms or nutrients, although additional research is necessary to understand the feedbacks between plants, soil, and microbes. An improved understanding of plant-soil feedbacks may make it possible manage invasive plant populations through the subtle modification of ecosystem processes, such as: nutrient cycling, litter decomposition, pathogen accumulation, or competition.

A methodology study for metagenomics using next generation sequencing

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Metagenomics is one of several genomics applications which has benefited immensely from the high throughput and cost efficacy of Next Generation sequencers. Although hundreds of studies on metagenome analysis have been published over the past few years, the methodology for conducting them is still very much evolving. The DNA Sequencing Research Group (DSRG) of The Association of Biomolecular Research Facilities (ABRF) is conducting a study to evaluate the influence of various sample preparation methods, specifically DNA extraction and amplification approaches, on data output along with a comparative analysis of Next Generation sequencing platforms. We will study the effect of these different experimental and technical strategies on determination of sample biodiversity. ABRF is an international society dedicated to promoting communication and cooperation among core laboratories. ABRF Research Groups advance core biomolecular technologies by sponsoring research studies that help researchers and facilities evaluate analytical techniques and methodologies, as well as help establish good laboratory practices. More information is available at http://www.abrf.org/

ABSTRACTS

Microbial communities in restored wetland sediments

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Restoration of wetlands has great potential to reverse land subsidence on peat islands previously drained for agriculture, thereby reducing risk of levee failure. In addition, the high primary production and slow decomposition rates found in restored wetlands may result in a net atmospheric CO_2 sequestration. However, one major concern is the microbial emission of CH_4 that could potentially offset the benefits of carbon fixation by wetland vegetation. Thus in wetland ecosystems, microorganisms play key roles in governing greenhouse gas flux. In this preliminary study, we collected belowground samples from a restored wetland largely vegetated with cattail and tule from a pilot-scale restoration project. We selected three sites that exhibited gradients in physicochemical conditions and peat accretion rates. From each site, we collected samples from the bulk decomposed material, cattail rhizomes and tule rhizomes. Pyrosequencing of amplified V8 regions of 16S rRNA genes was used to generate microbial community profiles. Our data indicated that wetland community composition was primarily governed by sampling sites, and secondarily by sample types. Particularly, wetland communities from these three sites transited in a direction consistent with the gradients along these sites. Some OTUs (operational taxonomic units) show high 16S rRNA gene sequence identity to characterized microorganisms that perform lignocellulose decomposition, fermentation, denitrification, sulfate reduction, methanogenesis and methane oxidation. These provided a glimpse of likely processes that these microbes mediate. In addition, incubation experiments were conducted to evaluate CH_4 and CO_2 flux, which varied considerably among sample sites and types. Several methanogenic archaeal OTUs were associated with the high CH_4 flux samples, likely contributing to the observed methane emission. Further, metagenomic analyses are being conducted to reveal the microbial functions important for long-term carbon sequestration and nutrient cycling by the restored wetland microbial community.

Deep sequencing of soil microbial communities of bioenergy crops

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Plant-associated microbes play an important role in plant health and production, and thus have a potential role on the productivity and sustainability of crops used for biofuels. We studied the plant-microbe dynamic by surveying the microbial communities of two biofuel crops, switchgrass and Miscanthus, in two US locations, Michigan and Wisconsin, using whole shotgun sequencing, and targeted pyrotags of 16S rRNA genes.

Bacterial community membership was analyzed using 16S rRNA gene sequences obtained both from amplification as pyrotags and by extraction of their fragments from the metagenome shotgun reads. 16S rRNA gene short reads (Illumina) were identified by comparison to well known 16S rRNA genes in the RDP database using BLAST, HMMER, and INFERNAL. These short reads aligned to the region targeted by our pyrotag data shared high sequence similarity, and at the phylum-level, community profiles were similar for pyrotag and shotgun data. The similarities between shotgun and pyrotag 16S rRNA sequences suggest that sequencing bias is minimal and supports the use of shotgun metagenomic sequencing for detecting 16S rRNA genes.

Significant grouping of metagenome assemblies and pyrotag sequences by associated plant type were observed. A significant perMANOVA result is observed when samples are grouped according to sampling location (Michigan vs Wisconsin). Ordination of the samples by principal components supports the perMANOVA results. At the genus and OTU level, samples separate by plant and location while at the gene function level (COGs), samples separate only at the plant level. These results suggest that although there is variation in species composition between the Wisconsin and Michigan soils, the functions carried out by the soil communities are conserved.

This work will allow us to further explore genes involved in plant growth promotion; carbon, nitrogen, and phosphorus cycling and soil type responsive traits which, combined with site-specific environmental metadata, can be used to explore the effects of gene suite and habitat on plant-microbe-soil relationships.

Metagenomics-based analysis of fungal and bacterial communities in isothiocyanate-amended soil

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Isothiocyanates (ITCs), such as those released from the biomass of many *Brassicaceae* spp., may play an important role in organic strategies for controlling pathogens and weeds; however, the impacts of ITCs on non-target soil microbial communities are not well understood. We investigated microbial community responses to 4 types of ITCs (allyl, benzyl, phenyl, and butyl) in a microcosm study using Weswood loam soil. The microcosms were amended with 50 μg g^{-1} of an individual ITC and incubated at 25°C for 28 days. Community qPCR assays were used to evaluate relative abundances of soil microbial populations after 2, 7, 14, 21,

and 28 days. Soil microbial community composition after 2, 7, and 28 days was determined through tag-pyrosequencing using 454 GS FLX titanium technology, targeting ITS and 16S regions for fungal and bacterial communities respectively. Our results showed that the application of ITCs suppressed either soil fungal or bacterial abundance, depending upon the ITC applied, and altered soil microbial community composition. The allyl ITC greatly decreased soil fungal abundance by >6 times compared with the unamended control and significantly changed both fungal and bacterial community composition. In contrast, butyl ITC had a larger impact on bacterial rather than fungal abundance and composition. Our findings suggest that incorporation of different ITCs could differentially impact non-target soil microbial populations and composition.

Metagenomics of the *Rhizobium leguminosarum*-plant host symbiosis

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Grain legumes of the *Pisum, Vicia, Lens*, and *Lathyrus* genera establish a nitrogen-fixing, root nodule symbiosis with *Rhizobium leguminosarum* bv. viciae soil bacteria. Rhizobia are minor soil inhabitants, and their ability to establish an endosymbiosis with legumes has been interpreted as a mechanism to maintain and increase their populations in soil. Classic biodiversity studies using host plants as traps suggest that soil rhizobial populations are highly diverse and that, even within the same cross-inoculation group, different plant hosts select different rhizobial genotypes among those present in a given soil. This diversity, at least in part, can be explained by the presence of numerous (3-6) large plasmids in *Rhizobium*, accounting for up to 30% of the genome.

We have applied a genomic / metagenomic approach to the study of the host-plant differential selection of soil rhizobial genotypes. *Pisum sativum*, *Lens culinaris*, *Vicia sativa* and *V. faba* plants have been used to trap symbiotic rhizobia from a well characterized farm soil. Isolates from one-hundred nodules have been pooled, their DNA extracted and sequenced with Illumina technology (500 bp paired-end libraries, 100 bp reads, 40 million reads / sample). Apart from plant-associated read groups, available reference genomes (*R. leguminosarum* bvs. viciae and trifolii, and *R. etli*) have been used for binning reads into additional groups. Since any attempt at read assembly is prone to generate chimaeras, we restricted reference-guided assembly to specific ORFs of interest. These include well-conserved, single-copy housekeeping genes (in order to estimate the extent of genetic polymorphism in the populations), *repABC* plasmid replication genes, symbiotic genes (*nod*, *fix* and *nif*), and the identified plant host-specific reads. The ongoing analysis will allow a definition of the extent and nature of the host-plant selection of rhizobial genotypes, and will provide targets for future functional studies.

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Permafrost metagenomics

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Permafrost contains vast reservoirs of organic carbon that may become accessible to microbial degradation as it thaws in a warming climate. However, the microbial identities and functions responsible for carbon turnover and greenhouse gas emission are currently unknown. Therefore, we took a metagenomics approach to determine the identities and functions of microbial communities in different Alaskan permafrost, seasonally thawed active layer, and thermokarst bog samples, before and after thaw. During the incubations, some of the samples were incubated with unlabeled and 13C-labeled substrates to monitor microbial respiration. There were significant shifts in the microbial community compositions during thaw as well as shifts in several functional genes involved in cycling of methane and carbon. Comparative metagenomics between samples revealed the greatest differences in the bog sample compared to the other samples. Also, we found that the pathways represented in the active layer were different from permafrost layer samples. The sequence data was sufficient for obtaining a draft genome of a novel methanogen, and this was linked to observed evolution of methane from the samples during thaw. We validated the phylogenetic data obtained from the metagenome data by pyrotag sequencing and qPCR of specific taxa. Together these data confirmed that actinobacteria populations increased after thaw. However, many shifts we observed at the phylogenetic level were sample specific, thus emphasizing the need for replicate metagenomes. We also found a cosmopolitan OTU representative of an uncultivated Chloroflexi in all samples that is now the target for single cell sequencing. In summary, our studies have revealed a rapid response of frozen permafrost communities to thaw and suggest that the methane cycle and nitrogen cycle are immediately impacted.

Elevated atmospheric carbon dioxide alters the composition of soil microbial communities

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The concentration of CO_2 in the atmosphere has been increasing for the last 150 years, from 270 ppm prior to the industrial revolution to the current level of approximately 388 ppm, and is projected to exceed 700 ppm by the end of the century. This ongoing increase in atmospheric CO_2 is believed to be due to the extensive use of fossil fuels and changes in land use patterns. Although elevated

atmospheric CO_2 is unlikely to have direct effects on soil microbial communities, we hypothesized that elevated CO_2 would impact the soil ecosystem indirectly through CO2-induced plant responses that influence below-ground processes. To test this hypothesis, cloned trembling aspen (*Populus tremuloides*) seedlings were grown individually in outdoor, open-top chambers under either elevated (720 ppm, ELEV) or ambient CO_2 (360 ppm, AMB). After 5 years of treatment, soil cores (20 cm depth) were collected from the chambers. Soils from the ELEV chambers showed significantly lower levels of both soil nitrate and total soil nitrogen compared to the AMB soils. T-RFLP analysis of bacterial 16S genes demonstrated that ELEV soils had significantly different bacterial community composition than AMB soils. Q-PCR revealed that there were significantly more denitrifying bacteria (based on *nirK*) and significantly fewer bacterial ammonia oxidizers (based on bacterial *amoA*) in the ELEV soils, while there was no difference in the numbers of archaeal ammonia oxidizers (based on archaeal *amoA*). Clone library sequencing of these functional genes indicated lower *nirK* diversity with ELEV treatment, but no change in the diversity of bacterial or archaeal *amoA* genes. Tag pyrosequencing of 16S genes also demonstrated shifts in bacterial community composition, and metagenomic sequencing is currently being used to assess shifts in community functional potential. In summary, these data indicate that growth of plants under elevated atmospheric CO_2 can shift microbial community composition, which could have significant implications for soil ecosystem function.

Nature's pulsing paradigm (with apologies to the Odum family)

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"Steady state" is likely to be rare in natural ecosystems, particularly with respect to biological community composition. The forces that might lead to temporally stable self-organization of microbial communities are opposed by external physical and chemical pulses as well as by biological interactions. The temporal scales at which these variabilities operate can range over 6 orders of magnitude, and affect the rhythms of biological processes from intracellular biochemistry to ecosystem functionality. However, the periodicities of external forcing factors will be ecosystem-specific and sampling strategies should reflect the temporal scale of microbial community variability and relate these to (at least) important physical or chemical pulses. Studies with these design features remain rare in microbial ecology, and may be particularly challenging in soil ecosystems that also contain extensive spatial heterogeneity.

Exploring the functional role of soil microorganisms under climate change conditions in a temperate forest

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There are many potential feedbacks between the climate system and the function of the soil microbial community. Microorganisms drive a wide variety of biogeochemical processes; however the ways in which microorganisms respond to changing temperature and moisture conditions is not well understood. This study was conducted at an experimental site in a northern Michigan temperate forest with both temperature (+4 °C) and moisture manipulations (+30%), which mimic future climate change scenarios in the region. One goal of this study is to link the biogeochemical cycling of nutrients to the activity of microorganisms in the soil under potential climate change conditions. This will ultimately allow us to gain a broader understanding of feedback mechanisms between the climate system and soil microorganisms. Enzyme assays were used to partition microbial metabolism into groups ranging from the decomposition of labile organic compounds, such as glucose, to more recalcitrant compounds, such as polyphenols. The assays were conducted at two different temperatures to mimic soil temperatures in the field. These analyses have shown that after three months of warming microbial metabolism remains elevated at higher temperatures, regardless which experimental treatment the soil came from. Soil respiration data also indicate that microbial metabolism has not acclimated to the higher temperatures in the warmed plots. This indicates a short-term positive feedback between the climate system and soil microbial activity in which warming triggers higher rates of metabolism, which cause more CO_2 to be released into the atmosphere, leading to further warming. It is probable that rates of microbial metabolism will acclimate to the climate change manipulations in the long-term. Future research will include conducting quantitative PCR on mRNA extracts to determine differences in activity of functional genes related to carbon and nutrient cycling and examining the metagenome and metatranscriptome of the soil microbial community.

Antarctic-MAPs: Predicting the population structure of Antarctic soil microbial communities as a function of environmental conditions

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Antarctic ecosystems are predicted to experience unequalled impacts due to climate change. Data from previous Antarctic studies ranging in latitude from the Falkland Islands (51° S) to the base of the Antarctic Peninsula (72° S) and from vegetated and fell-field locations demonstrated that bacterial diversity was mostly organized by latitude-dependent factors such as mean temperature, soil moisture, and vegetation cover. As temperatures rise and patterns of rainfall are altered in response to global warming, these changes will have direct impacts on the bacterial community structure of the Antarctic soils, and hence it is necessary to explore predictive models to determine the extrapolative impacts of such change on the wider ecosystem. To achieve this we have created Microbial Assemblage Prediction (MAP), a tool for generating predictive models of microbial population structure as a function of environmental conditions. MAP uses bacterial population and environmental parameter data to generate an ecological interaction network. This network predicts the relationships between specific environmental parameters and bacterial taxa, and between bacterial taxa with other bacterial taxa. This interaction network is expressed as a set of mathematical equations such that the relative abundances of bacterial taxa are expressed as functions of environmental conditions. The MAP model is validated by correctly reproducing previously observed community structure, and then is used to extrapolate community structure for those ecological conditions predicted to occur due to global warming. Methods used here are generally applicable and can be used to model microbial soil or marine communities from a variety of ecological locations and make predictions as to how those populations may be altered in response to climate change.

Relationship of edaphic factors to 16S and 18S rRNA gene diversity

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Soil bacterial communities are known to be highly diverse and are distributed across landscapes based on the prevailing edaphic conditions. However, it is unknown if soil fungi and eukaryotes have a similar relationship to edaphic factors as many studies have focused on these organisms only at smaller geographic distances. Here we used barcoded pyrosequencing of 16S and 18S rRNA genes to examine the distribution of these communities and their relationship to edaphic factors in soils collected from four continents. 16S rRNA genes were sequenced on the Illumina HiSeq platform to characterize bacterial communities while 454 pyrosequencing was used to assess the distribution and diversity of fungal and

eukaryotes from the same collection of soils. We found Illumina sequencing of the 16S rRNA gene yielded similar results to previous surveys indicating soil pH drives bacterial community structure and composition. Analysis of 18S rRNA genes showed the composition of fungal and eukaryotic communities were largely driven by differences between biomes and were linked to temperature, precipitation and latitude. Our results indicate forces shaping biomes are also important for structuring fungal and eukaryotic populations in soil, while bacteria appear to be affected more by soil chemistry.

An ecological perspective on soil metagenomics

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A major goal of ecology is to identify the mechanisms that maintain biodiversity. In turn, we often want to understand how this biodiversity influences ecosystem processes. Over the past few decades, advances in molecular tools have allowed us to catalog the diversity of taxa and genes in wide range of habitats. In particular, metagenomics offers a unique opportunity to test existing ecological theory regarding the biogeography and coexistence of microorganisms. In addition, metagenomics may generate information that will spur new ecological theory. In this talk, I will highlight case studies where metagenomics has been used to test ecological principles. I will follow this with some work from my laboratory that focuses on the mechanisms and implications of dormancy for microbial seed banks and the maintenance of biodiversity.

Inhibitory effects of *Bromelia pinguin* (Bromeliaceae) on soil ecosystems in primary forests of Costa Rica

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Few studies have been performed on the ecology of *Bromelia pinguin* (Bromeliaceae), a bromeliad commonly found in large patches within primary forests of Central America and the Caribbean. Previously, Camacho-Hernández et al. (2001) found that the fruit pulp of this bromeliad possessed antifungal activity, and Looby et al. (2011) found decreased fungal DNA and differences in C and N nutrient levels in bromeliad soils in comparison to adjacent primary forest soils. The current study showed that, although the standing pools of Total N and Dissolved Organic C

did not differ between the bromeliad and primary forest soils, the bromeliad soils had lower rates of N and C biomass development, less laccase activity (suggesting less basidiomycete activity), and much less fungal 18s rRNA and basidiomycete ITS region DNA than found in the adjacent primary forest soils. The results of T-RFLP analyses conducted also showed that the community diversity and abundance of basidiomycete and the fungal pathogen *Mycena* sp. DNA were less in the bromeliad forest. This has interesting implications for research on the potential control of the fungus *Mycena citricolor*, which causes American Leaf Spot disease, known as ojo de gallo, in Costa Rica, and millions of dollars in losses to the coffee agriculture industry per year. Metagenomic studies of the soil community DNA also showed clear differences in the populations of the Basidiomycota (Tremellales, Agricales, Thelephorales, Boletales representatives), the Ascomycota (Helotiales representatives), the Zygomycota (Mortierellales representatives) and the Glomeromycota (Glomerales representatives) between the bromeliad and primary forest soils.

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Validation and application of Illumina sequencing to comparative soil metagenomics

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High-throughput next generation sequencing (NGS) technologies are increasingly used for the analysis of soil communities but several important challenges remain. Most importantly, it is essential to evaluate the quality of short read assemblies from such complex communities, especially in comparison with that of longer reads (e.g., Roche 454, ~500 bp). Furthermore, assembling individual genomes from complex metagenomic data remains a challenge.

In this study, we compared the two most frequently used sequencing platforms, the Illumina and Roche 454 technologies on the same community DNA sample. We found that the platforms provided a comparable view of the community sampled, e.g., the derived assemblies overlapped in ~90% of their total sequences, and that Illumina provided equivalent, if not better, assemblies with 454. Further, we evaluated whether an individual genotype or species can be robustly assembled from complex metagenomes and found that at least 20X coverage is required for this purpose and that contig length does not increase significantly with higher than 30X coverage.

Based on these and other findings, we compared metagenomic datasets originating from two soils; one that was undergoing infrared heating by 2°C over ten

years, simulating the effect of predicted global warming, and the other set was control. We report the comparison on the two metagenomes and offer insights on soil microbial communities' response to warming perturbation.

The effects of climate and land-use change on soil microbial diversity in the Mongolian Steppe

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How the microbial ecology of the Mongolian Steppe shifts with climate change is a central question under investigation in the five-year PIRE Mongolia Project (Partnerships in International Research and Education, http://mongolia.bio.upenn.edu/). Through this project, plant ecologists, biogeochemists, soil scientists, and climate modelers, are empirically documenting the effects of climate and land-use change on this arid ecosystem. Though many global change studies strive to link ecosystem shifts to microbial mechanisms, few have integrated edaphic, floristic and climatic data with microbial community data produced by Next-Generation Sequencing techniques, as is being done in the PIRE Mongolia Project. Moreover, the experimental design is comparable to numerous other climate change studies, allowing for the production of globally relevant data. Presently, Mothur software (http://www.mothur.org/wiki/Main_Page) is being used to analyze 16S pyrosequencing data from the soil microbial community of this well replicated, multi-factorial experiment, which includes warming, watering, grazing and topography treatments. The average annual temperature at the Lake Hövsgöl Long-Term Ecological Research (LTER) site has increased by 1.7ºC since 1963, and some of the greatest projected increases in temperature are associated with northern Mongolia. Hence, this site is therefore ideally suited for studying ecological shifts driven by climate change. One of the initial hypotheses in this moisture-limited system is that microbial diversity will decrease with warming and increase with watering. By the end of this summer, 16S data will be integrated with spatial and temporal data that includes information on carbon and nitrogen fluxes, changes in plant phenology and shifts in climate.

Functional screening of soil metagenomic libraries for bacterial conjugation functions

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Bacterial conjugation is considered to be an important factor in horizontal gene transfer, facilitating the distribution of genetic material throughout genomes within a given community. Conjugation allows for the transfer of DNA elements, such as plasmids, from donor to recipient, resulting in transconjugants carrying characteristics of both parents. Transfer systems required for conjugation are encoded by *tra* genes. In an effort to discover previously unidentified types of conjugal transfer systems, a corn-field soil metagenomic library in the tetracycline resistant cosmid pJC8 was screened for potential *tra* genes by using a mating assay. A culture of the pooled library, harbored in *E. coli* DH5α, was mixed with a culture of a rifampicin resistant derivative of DH5α, and incubated together for several hours on agar plates in the absence of antibiotics. The mating spot was suspended and plated out on agar containing rifampicin and tetracycline. Colonies that arose were putative transconjugants made possible by the presence of *tra* genes on the DNA of the clone that was transferred. The conjugation ability and efficiency were confirmed by mating individual clones with the streptomycin resistant *E. coli* strain HB101. Several clones with unique restriction enzyme banding patterns were identified for further DNA sequence analysis coupled with subcloning experiments designed to provide information about the nature of these *tra* genes. We have thus identified conjugation systems on metagenomic clones using a simple mating assay.

Evaluation of SCODA as a novel electrophoretic technology for DNA purification from soil and other environmental samples

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Purification of microbial DNA from soil for metagenomic studies is complicated by co-extraction of humic acids and phenolic compounds that inhibit cloning, amplification and sequencing. These contaminants must be removed for successful metagenomic library construction and DNA sequencing. Boreal Genomics has commercialized the Synchronous Coefficient of Drag Alteration (SCODA) technology as a novel electrophoretic method of DNA purification that provides superior purity, greater yield, and higher molecular weight DNA than obtained by traditional silica and ion exchange columns and phenol:chloroform-based purification approaches.

Here we present results of quantitative PCR (qPCR) experiments using universal 16S rDNA primers on DNA prepared from a wide range of soils and environmental samples including agricultural soil, arctic tundra, temperate rainforest soil, silty clay-rich loam, bitumen and oilsands, and hydrocarbon-rich seabed sediments. Data from dilution series show DNA samples purified by SCODA exhibit less PCR inhibition than samples prepared by other methods; therefore, more template DNA can be used in PCR without overwhelming the reaction with

inhibitors. Our results demonstrate that higher yields and better PCR amplification are achieved from DNA purified by SCODA than from DNA prepared with other methods including commercial columns and traditional phenol:chloroform extraction.

Boreal's protocols deliver DNA up to 10 kb, 50 kb or even 1 Mb in length depending on the downstream requirements and the lysis procedures that are used. The ability to recover high molecular weight DNA facilitates large-insert library construction and reliable identification of novel species and intact functional pathways. The combination of superior inhibitor removal and highly efficient DNA recovery from low abundance or low biomass samples enables isolation of rare DNA sequences from challenging environmental samples, promising a more complete representation of microbial diversity in metagenomic studies.

Effects of land management and habitat type on organic carbon (C) and nitrogen (N) content and biomass, and abundance and diversity of soil fungal and non-fungal eukarya in a tropical lowland region of Costa Rica.

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Poor land management practices have caused extensive habitat damage to the lowland forests of Costa Rica over 40 years. To fully understand the effects of such practices, it is critical to determine how these practices impact the soil ecosystems, yet too few studies have examined this, especially in the tropics. We studied soils from four unique habitats within the Maquenque National Wildlife Reserve of Costa Rica: 1) previously unharvested primary forests; 2) large patches in these forests dominated by the bromeliad *Bromelia pinguin* (Bromeliaceae) that has antifungal properties; 3) pasture cleared of primary forest in the early 1980's; and 4) secondary forest allowed to regenerate following clearing in the early 1980's. We measured the differences in the amount and rate of production of dissolved organic C (DOC), total N, and microbial C and N biomass levels in soils, and the abundance and diversity of fungal and non-fungal eukaryote clones developed from soil DNA. The rate of DOC production was greatest in the primary and secondary forest soils; the amount and rate of production of C biomass was least in the bromeliad soils; the amount and rate of production of total N and N biomass was greatest in primary forest, and intermediate in secondary forest soils. These differences were compared with differences found in the composition of the representatives of the non-fungal soil eukaryotic groups Myriapoda, Arachnida, Hemiptera, Coleoptera, Amphipoda, Diptera, Polychaeta, Oligochaeta, Collembola, Trichoptera, Isoptera, Lepidoptera, and Hymenoptera; representatives from the water mold protists Oomycetes; representatives from the Basidiomycota fungal groups Tremellomycetes and Agaricomycetes; representatives from the Ascomycota

fungal groups Leotiomycetes, Saccaaromycetes, and Sordariomycetes; and representatives from the fungal group Glomeromycota. All results were analyzed in the aggregate to look for indicators of soil ecosystem condition and the effects that these non-bacterial groups might have on the biomass development.

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Coffee from the ground up: rustic coffee plantations provide better habitats for soil fungal communities than sun coffee plantations

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The intensification of coffee production in tropical agroecosystems has resulted in a decrease in the diversity and abundance of many forest species; however the impact of management techniques on soil microbial communities has rarely been evaluated. Here, we compared soil fungal communities in a shade coffee plantation, a sun coffee plantation, and a nearby rain forest. We tested the hypotheses that 1) increasing intensification of coffee management alters fungal community composition and decreases extracellular enzyme activity; 2) shade coffee plantations harbor fungal communities that are more similar to unmanaged, forest soil communities; and 3) soil fungal communities in sun coffee have more distinct fungal communities from rain forest soils than do rustic coffee plantations. To test these hypotheses, we collected ten composite soil cores (0-20 cm) from six plots in each site and sequenced fungal DNA using 454 pyrosequencing. From the same soil samples, extracellular enzyme assays were conducted for enzymes involved in the cycling of cellulose, hemicellulose, chitin, and amino acids. Results from extracellular enzyme assays were variable and there was no ubiquitous trend connecting management intensity to microbial function for the six enzymes analyzed in this study. However, as hypothesized, we found that increasing intensification of coffee production altered fungal community composition, as fungal communities in the sun coffee plantation were distinct from rain forest soil communities (ANOSIM $P < 0.05$). Fungal communities from the rustic coffee plantation, by contrast, were not significantly different from either the rain forest soil or the sun coffee plantation communities, indicating that rustic farms may be more able than sun coffee farms to support soil fungal diversity found in intact rain forest. This study provides some of the first evidence that soil microbial responses to coffee management intensity are similar to that of higher taxa, although the implications of these results on ecosystem function need further study.

Ecology of diatomaceous biological soil crust assemblages in Yellowstone Geothermal soils

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High-throughput sequencing technologies have recently allowed for ecological research at unprecedented detail in microbial communities in many previously understudied environments. As a result, microbial communities are being increasingly utilized to address long-standing ecological questions that are suddenly more tractable in these compact assemblages. This field, however, is still somewhat unsettled in several aspects of methodology. For instance, it is unclear at present what scale (soil core vs. single particle) best represents a more relevant look at soil microbial communities and their inherent heterogeneity, and how to appropriately apply existing ecological analyses to these complex data. Here, we present a metagenomic look at a previously unstudied alkaline-chloride diatomaceous biological soil crust assemblage in geothermal soils of Yellowstone National Park as an effort to address some of these outstanding issues. In this study, we analyzed microbial assemblages from single particle scale to soil core scale for both prokaryotic and eukaryotic microbial consortia, with an emphasis on vertical distribution of taxa. We also suggest multivariate analysis methods that adequately describe microbial datasets on different scales. This soil system is unique in that striking visual heterogeneity is apparent in color and texture on several spatial scales, and this allows for a comparison of visual differences with actual community compositions as well as with soil chemical and physical characteristics. Our results support the idea that some heterogeneity in soil systems is lost with a 'California in a blender' approach to soil microbial community sampling, and that the study of microbial community composition and structure at the soil particle scale might yield important insight into microbial ecology. Additionally, depth of sequence-sampling has different consequences on prokaryotic and eukaryotic communities, and the choice of sampling scale strongly depends on underlying gradients and questions being addressed.

RCN: TerraGenome—The Soil Metagenome Network

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The National Science Foundation recently funded a Research Coordination Network (RCN) to facilitate soil metagenomics studies. This presentation introduces the TerraGenome RCN to the soil metagenomics community and solicits its input

about the activities of the network. We anticipate that TerraGenome will accomplish its purpose by holding periodic meetings to plan strategies and share information, coordinating sequencing and bioinformatics activities, hosting workshops to train students and scientists in metagenomic analysis, and generally enhancing communication and information sharing through a website and list-server. The most fundamental impact of TerraGenome will be to build and support a diverse community of researchers in the U.S. and around the world who have an interest in uncovering the taxonomic and functional diversity of soil microorganisms. Bringing together this community, with scientists representing several disciplines, will help to train students and postdoctoral associates to tackle an inherently interdisciplinary question. This is most clearly demonstrated from the workshops it will sponsor. The TerraGenome website will broadly disseminate the products of our Network activities, with a special effort to provide web-based tools for use by K-12 educators and students. Through its activities TerraGenome will help to provide the genetic foundation to better understand the diversity of microbial processes upon which the health and functioning of the planet is based. For example, the processes involved in cycling carbon, nitrogen, sulfur, and other elements are key determinants of plant and ecosystem productivity. Practically, this translates into producing food and fiber for humanity. Furthermore, the huge diversity of soil organisms represents a relatively untapped reservoir of potentially useful microbial enzymes and products, such as enzymes for cellulosic energy production, and antibiotics and other pharmaceuticals.

The spatial, phylogenetic and abundance dimensions of environmental and historical filters on bacterial community structure

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Microbes are the most abundant and diverse organisms on the planet, and are important for industry, medicine and public health as well as for understanding and predicting ecosystem responses to global climate change. Yet, we lack a synthetic understanding of microbial biogeography. Although the relative contribution of environmental and historical filters may vary across different systems, evidence is mounting that both can be important factors in determining community structure. In this talk, I will highlight the roles of the spatial scales of environmental filters on soil microbial community composition. Specifically, I will examine the relationship between soil chemistry and different phylogenetic and abundance fractions of the soil bacterial community. Additionally, I will show mesocosm data that suggest that the colonization order of different low abundance organisms leads to distinct successional trajectories that may have affects on overall microbial community structure. The goal of this work is to provide new insights into the spatial, phylogenetic and abundance dimensions through which environmental and historical filters operate.

Nitrogen cycling and root-zone microbial communities in rangeland restoration plots

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In the Intermountain West (USA), cheatgrass (*Bromus tectorum*) has infested over 40 million ha of native sagebrush – bunchgrass communities and these communities are quite resistant to re-establishment of desirable native perennials. We hypothesize that belowground feedbacks from shifts in nutrient cycling and microbial communities are responsible for the observed persistence of cheatgrass and ask whether intrinsic characteristics of the soil or plant history are more influential on these shifts. We examined microbial and nutrient dynamics in soils collected from replicated restoration plots established 27 years ago. At the time of establishment disturbed plots were fumigated with methyl bromide before planting. We assessed ammonia–oxidizing bacteria (AOB) and archaea (AOA), soil bacteria, soil fungi and arbuscular mycorrhizal fungi (AMF) in response to contrasting soil history and plant species dominance. The AO community was examined by real-time PCR and clone libraries of *amoA.* The ratio of AOA /AOB *amoA* copies was higher (17) in the undisturbed native soil than in either the fumigated cheatgrass dominated (2.1) or the fumigated climax plant community soil (1.0). Bacterial and fungal community structure was assessed by amplified ribosomal spacer analysis (ARISA) and by tagged pyrosequencing. ARISA results suggested only minor shifts in the overall microbial community structure with the contrasting plant communities. Analysis of pyrosequencing data is ongoing. The survey of AMF was accomplished by DNA extraction from plant roots (4 plant species) followed by PCR amplification targeting AMF, cloning, and RFLP of the 18S rRNA gene. Our results showed that plant species could affect shifts in the AMF community and that these differences were more pronounced in fumigated soils than in the native plant control soils suggesting a soil history effect. Further pyrosequencing work on targeted functional genes is in progress.

Functional metagenomics of plant litter decomposition

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Understanding the relationship between community composition and functionality is essential to predicting how ecosystems respond to global change. In this study, our aim is to link microbial taxa with functional traits involved in plant litter decomposition, a model process for carbon cycling, and use this information to investigate how community response to altered rainfall and nitrogen availability impacts decomposition rates.

To identify hydrolytic activities related to plant litter decomposition high molecular weight DNA and fungal messenger RNA is isolated from decomposing plant litter undergoing rainfall and nitrogen manipulation and cloned to fosmid and plasmid libraries, respectively. Both types of libraries are screened for activities involved in cellulose, hemicellulose, chitin, lignin and starch degradation as well as peptide breakdown and mineralization of organic phosphate using novel functional assays. These automated high throughput assays are based on colorimetric and fluorescent substrates and have been optimized to allow simultaneous screening of endolytic and exolytic hydrolysis activities required during different stages of degradation. In addition, the *Escherichia coli* strain used as expression host in the functional screens has been modified with gene-specific knockouts to reduce the wild-type activities that can reduce the sensitivity of screening assays.

Clones expressing the targeted activities are being sequenced and the identified hydrolytic genes will be related to phylogenetic context, enzyme kinetics, biogeochemical measurements and microbial community dynamics through mathematical models to predict how litter decomposition rates change as microbial communities respond to environmental change. This presentation focuses on the development and screening of functional metagenomic libraries.

Distinct resuscitation patterns of soil microbes following early-season wet-up

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Soil microbes in arid and semi-arid ecosystems experience an acute water potential upshock following the first rainfall after extended drought periods. Despite this severe physiological stress, these rainfall events are associated with large pulses of greenhouse gases, demonstrating rapid metabolic recovery of the microbial community. Is this reactivation a random process or do specific microbial groups employ distinct resuscitation strategies? In one example of ongoing work we followed the responses of soil microbes across two California annual grasslands to a wet-up event following a typical summer drought. Using high-density microarray (PhyloChip) analysis of 16S rRNA we detected four primary response strategies following wet-up. Phylogenetically related groups of taxa were either continually-present, responded within 1 hour of wet-up, within one day of wet-up, or after one day. Thus it appears that the large carbon dioxide pulse following wet-up is the sum

of activities of a succession of microbial groups with distinct resuscitation strategies. Ongoing work is focused on identifying the succession of metabolic processes contributing to soil carbon mineralization during these pulsed-activity events.

Microbial resuscitation strategies following soil wet-up are phylogenetically clustered

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The first rainfall following a long dry period in arid and semi-arid ecosystems provides an acute water potential change that can be both a severe physiological stress and a defined stimulus for the revival of soil microbial communities rendered inactive by low-water conditions. We followed the responses of indigenous microbial communities to the addition of water to soils taken from two California annual grasslands following a typically dry Mediterranean summer. Differential resuscitation was evident within 1 hour of wet-up using high-density microarray (PhyloChip) analysis of *16S rRNA*. We identified three response strategies based on when taxa had the highest relative ribosomal quantity: rapid-responders (within 1 hour of wet-up), intermediate-responders (between 3 and 24 hours following wet-up), and delayed-responders (24 to 72 hours post wet-up). These data suggest that the large carbon dioxide pulse produced concurrently with wet-up results from the activity of different groups of metabolically active microorganisms over time. Relative ribosomal quantity of rapid-responders was as high in the prewet dry soils as at any other time, suggesting that some organisms may be poised to respond to the wet-up event in that they preserve their capacity to synthesize proteins rapidly. Microbial response patterns clustered phylogenetically, and were primarily conserved at the phylum level, with the clustering of responses largely consistent across the two soils. Microbial resuscitation strategy following wet-up of dry soil appears to be a phylogenetically conserved ecological trait.

What can microbial ecology do for soil metagenomics?

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For the past 20 years, 'first-generation' molecular techniques have been used to characterise soil microbial communities. These techniques have demonstrated, and continue to demonstrate high diversity in these communities and the existence

of previously unsuspected, high-level, taxonomic groups. Early descriptive studies were soon complemented by those aimed at identification of links between community composition, soil characteristics and environmental factors and determination of mechanisms driving soil microbial community composition. Attempts were also made to link community composition to ecosystem function, and these increased with the development of quantitative PCR methods for estimation of abundance of both 16S rRNA and functional genes and methods designed to estimate activity of specific groups, e.g. stable isotope probing and analysis of transcriptional activity. Again, these new techniques provided useful information, but studies often followed techniques rather than determining critically which techniques were required for specific ecological questions. More positively, they activated interest in broader ecological questions and their relevance to soil microbial diversity, activity and ecosystem function, and awakened long-standing debates, such as those surrounding bacterial and archaeal species concepts. High-throughput sequencing techniques are now significantly advancing, and increasingly replacing first-generation molecular techniques and should significantly improve our ability to address these questions. Metagenomics, metatranscriptomics and, inevitably and inexorably, soil metaproteomics and metabolomics, introduce something different and something new. They provide an holistic view of, e.g. gene or transcript content, independent of soil structure or even cellular structure. While current emphasis is placed on technical and bioinformatics challenges, the greatest challenges are arguably conceptual, in determining which, if any, fundamental ecological questions can be addressed using metagenomics; whether new concepts are required to direct meaningful experimental studies; and the extent to which knowledge of microbial ecology gained during the past 130 years can inform such conceptual challenges.

Consequences of oak tree loss on soil microbial community composition in eastern temperate forests of North America

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In the Eastern North America forests, genus *Quercus* (Oak) represents one of the foundational tree taxa. However, the future of oak forests is uncertain as forests are impacted by events such as insect herbivory, pathogen introduction and human disturbance; hence, the feedback to nutrient cycling will in part be dependent on changes in the associated microbial communities which in turn may dramatically impact ecosystem services. The main objective of this study was to evaluate the

consequences of oak loss on soil microbial communities by experimentally mimicking pathogen-induced mortality. In 2008 a tree-girdling experiment was established in Black Rock Forest, New York (USA) with the following treatments: all oak trees were girdled, 50% of oak trees were girdled, all non-oak trees were girdled, and no trees were girdled (control). Each treatment was replicated three times in 20m X 20m plots. From each plot, soil (0-20cm) and litter (O_{i-e}) was collected in the summer of 2010. Using phospholipid fatty acid (PLFA) analysis and 454 pyrosequencing, samples were analyzed for microbial biomass and fungal community composition. Principal component analysis (PCA) of PLFA patterns revealed that the microbial communities were compositionally distinct among different treatments, and that increased bacterial dominance resulted in the treatments where all oak trees were girdled. Results from PLFA analyses were corroborated by 454 sequence analysis. Shifts in fungal communities were more pronounced when all oaks were girdled, indicating that a loss of oak tree species from eastern temperate forests could have significant implications for fungal diversity and function.

Predicting bacterial growth efficiency with ecological theory and metagenomic data

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Growth efficiency relates the amount of carbon entering microbial biomass to the total carbon utilized by microbes, including carbon respired to CO_2. It has been traditionally thought that CO_2 flux at the ecosystem level was robust to variation in microbial community composition, and thus growth efficiency differences between species, because CO_2 is the product of many different organisms and processes. Microbial species are not all equivalent, but have varying physiological capabilities which are often used to group microbes into distinct ecological strategies. Many classification schemes have been used to distinguish ecological strategies of bacteria, such as the r/K theory of MacArthur and Wilson, Winogradsky's allochthonous/autochthonous dichotomy, and Poindexter's copiotrophic/oligotrophic organisms. Bacteria can also be grouped into ecological strategies with genomic data, using *rrn* operon copy number. The ability to use genomic data to predict the ecological strategy of a bacterial community could have large implications if the physiological adaptations relevant to ecosystem processes, such as growth efficiency, varied along with ecological strategy.

In a phylogenetically diverse collection of bacteria, there is a significant negative relationship between *rrn* operon copy number and growth efficiency, providing a testable mechanism for relating microbial community composition and physiology to CO_2 flux. We hypothesize that microbial communities dominated by low *rrn* operon copy number bacteria will use carbon more efficiently than

communities with a greater abundance of high *rrn* operon copy number bacteria. Soil metagenomes from two different land use treatments at Kellogg Biological Station have been analyzed for differences in *rrn* operon copy number per average genome. Future work is planned to measure bacterial community BGE in environments with significantly different *rrn* operon copy number per average genome. The impact of this work will be an increased understanding of how microbial community composition and physiology can alter CO_2 flux in terrestrial ecosystems.

Using the RDP classifier to predict novelty and reduce the search space for finding novel organisms

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Currently, the naïve Bayesian classifier provided by the Ribosomal Database Project (RDP) is one of the most widely used tools to classify 16S rRNA sequences, mainly collected from environmental samples. We show that RDP has 97+% assignment accuracy and is fast for 250bp and longer reads when the read originates from taxon known to the database. Because most environmental samples will contain organisms from taxa whose 16S rRNA genes have not been previously sequenced, we aim to benchmark how well RDP and other competing methods can discriminate these novel from known taxa. Because each fragment is assigned a score (such as boostrap in RDP), we "train" a threshold to discriminate between novel and known organisms using this threshold, and observe its performance on a test set. The threshold that we determine tends to be conservative (low sensitivity) for naïve Bayesian methods, leaving room for improvement. Nonetheless, RDP performs better than other methods tested, measured by the f-measure and the area-under-the-curve on the receiver operating characteristic of the test set. By constraining the database to well-represented genera, sensitivity improves 3-15%. Finally, we show that the detector is a good predictor to determine novel abundant taxa (especially for finer levels of taxonomy where novelty is likely). We conclude that selecting a read-length appropriate RDP bootstrap score can significantly reduce the search space for identifying novel genera and higher-levels in taxonomy. In addition, having a well-represented database significantly improves performance while having genera that are "highly" similar does not make a significant improvement. On a real dataset from an Amazon Terra Preta soil sample, we show that the detector can predict (correlates to) whether novel sequences will be assigned to new taxa when the RDP database "doubles" in the future.

A comparative analysis of different fertility practices in an arable Indiana soil

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The use of alternative fertility practices is ever-growing in agriculture. Increased awareness of the negative environmental consequences (e.g. denitrification) of conventional production coupled with increases in both consumer demands for organically grown vegetables and cost of mineral fertilizers have prompted growers to use biologically based soil amendments. A variety of biologically based amendments are commercially available, and application may reduce nutrient loss in comparison to conventional fertilizers. However, using these amendments to effectively meet crop nutritional needs can be challenging due to their chemical properties and less characterized release rates of plant-available Nitrogen under conditions in Indiana. This research will seek to determine how fertility amendments are impacting nutrient losses, crop productivity, and microbial ecology in an arable Indiana soil. The following amendments will be evaluated in both a high tunnel and open field setting: unamended control, chicken manure compost, green manure, and urea. Nutrient losses via denitrification will be quantified by periodically collecting and analyzing gas samples to determine flux rates of the harmful greenhouse gas nitrous oxide. Impacts on production will be determined through total yield and fruit quality of two vegetable crops: Swiss chard (*Beta vulgaris*) and bell peppers (*Capsicum annuum*). Microbial community composition will be monitored throughout the growing season and estimated using T-RFLP of 16S rRNA. Gene frequencies of relevant Nitrogen cycling genes (*amoA*, *nosZ*) will be quantified using qPCR and diversity calculated using T-RFLP. Combined, these approaches will lead to a better understanding of how different fertility practices impact the sustainability, productivity, and microbial ecology of an arable Indiana soil.

Aspects of adaptation by same bacterial genus to diverse environments

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Microorganisms are considered to be the oldest forms of life and are known to occupy all the major environments including air, water, and soil. Extreme environmental conditions such as the hot or cold temperatures may affect the genomic evolution of these organisms. The genetic alterations which occur within a taxonomic group would enlarge the concept of bacterial species and their adaptation to diverse environments. One example is the genus *Exiguobacterium*, which thrives from subfreezing to thermal environs.

The ability of *Exiguobacterium* genus members to survive and proliferate in both low and high thermal regimes could be attributed to the core genome containing essential cold and heat stress responsive genes which have evolved through genomic rearrangements and or horizontal gene transfer.

The sequences of *Exiguobacterium sibiricum* 255-15 (GenBank no. CP001022) and *Exiguobacterium* sp. AT1b *(*GenBank no CP001615) were downloaded from NCBI. These bacteria belong to different species, as evidenced from the average nucleotide identity between these strains. It was calculated using the Jspecies software (http://www.imedea.uib.es/jspecies/) and was found to be 69.2%; which is below the 94% cut off expected for *in silico* species delineation. Out of 81 stress-responsive genes studied, nine genes were common in both genomes (1). MAUVE multiple genome alignment software was used to identify regions of genomic rearrangements (2). Probabilistic analysis of horizontal gene transfer was performed on the nine genes based on the criteria specified by Koonin et al. (3).

The results from this study can be further used to explore the adaptation mechanisms of a microorganism for extreme environments.

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Expanded diversity of bacterial *nos*Z genes and corresponding functional acitvity beyond denitrifiers revealed in different agricultural soils

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Decades of study on bacterial reduction of N_2O have relied largely on a database of knowledge primarily related to known denitrifiers. Recently, *nos*Z, the gene encoding nitrous oxide reductase, was found to be functional in several strains of the non-denitrifying ammonifier *Anaeromyxobacter dehalogenans*, and the gene sequence was distinct from *nos*Z genes of denitrifiers. Agricultural soils from two very different drainage classes were collected in Illinois to evaluate the distribution of these unique *nosZ* genes. Using newly designed PCR-primers we revealed at least two new clusters of *nos*Z present in agricultural soils that would have remained undetected using previously published *nos*Z primer sets. Two of the new classes

were most similar to *nosZ* genes found in *Anaeromyxobacter* and *Opititus terrae* populations. To further investigate the extent of *nos*Z diversity, soil-derived enrichments were actively maintained under a range of acetate:nitrate or nitrite ratios. DNA from these cultures, representing carbon-rich or carbon-limiting conditions, were analyzed using a our suite of new *nosZ* primers as well as ones that target denitrifiers.

Carbon-rich enrichment cultures were dominated by *nos*Z genes with closest similarity to *Dechloromonas aromatica* (82-83% identical). Typical denitrifier *nos*Z genes were not detected. With carbon-limiting conditions, both denitrifier- and new *nos*Z homologs were detected, the latter group include sequences related to *Anaeromyxobacter* (88%) and *D. aromatica* (85-86%). Enrichment cultures with acetate alone and no N-oxides showed only an increased detection of *nos*Z related to *Anaeromyxobacter*, consistent with the hypothesis that these metabolically diverse populations may play multiple ecological roles in electron-accepting processes and are underinvestigated for their role in N-cycling. The results suggest that there is much higher diversity of *nos*Z in soil beyond denitrifiers and corresponding populations harboring these genes are active and underinvestigated in current N-budgets. New methods must be reevaluated to investigate nitrous oxide reduction in natural environments.

Mechanisms of plant root impacts on litter decomposition in soil

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Soil organic carbon (C) is the largest component of the terrestrial C cycle, with fluxes through the C pool mediated by soil microorganisms and their interactions with plant roots and their exudates. In this study, we examined the effects of living *Avena barbata*, common in California grasslands, on the decomposition of ^{13}C-labeled *A. barbata* root litter in a California annual grassland soil. The decomposition rates of labeled root litter in the presence of *A. barbata* were determined and compared with the decomposition rates in a no-plant treatment by measuring total CO_2 and $^{13}CO_2$ fluxes. Factors which may influence the decomposition process by microorganisms, such as N availability, were also investigated. In addition, the metabolic profiles of *A. barbata* root exudates collected in sterile hydroponic systems were characterized. These data, in addition to decomposition rates measured in the presence and absence of living plant roots will guide future investigations of the microbial metabolic pathways responsible for rhizosphere C processing. This work will be extended to distinguish the C-cycling transcriptome of rhizosphere microbial communities utilizing ^{13}C-labeled exudates to provide a mechanistic basis for organic matter priming in the rhizosphere.

ABSTRACTS

Identification of genes involved in iron reduction in anoxic peat soils

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Iron reduction is an important metabolic pathway in anaerobic environments. In arctic peat soils, it is the major pathway for respiration. This pathway has implications for understanding the factors which act as controls over CO_2 and CH_4 fluxes from the arctic soil ecosystem. We investigated four metagenomes which were obtained from soils acquired from four different depths of the research site on the Arctic Coastal plain near Barrow (71°17′44″N 156°45′59″W). Four soil samples from depths of upto 40 cm were obtained from on the Arctic Coastal plain near Barrow. Once the DNA was extracted from the soil samples, sequenced and assembled, the four microbial metagenomes were uploaded in MG – RAST server (the Metagenome - Rapid Annotation using Subsystems Technology) and annotated. The results from the four metagenomes were subjected to a phylogenetic profile comparison to see what phyla, order and genera were represented in each metagenome. A metabolic profile comparison was conducted between the four metagenomes to identify the major metabolic pathways represented in each metagenome. Identifying sequences coding for the enzymes which are involved directly or indirectly with the iron reduction metabolic pathway was of high priority. Approximately 95% of all four metagenomes were made up of bacterial sequences from phyla such as Proteobacteria and Actinobacteria. Bacteroidetes was the most abundant group within Proteobacteria. Interesting correlations between cytochrome and siderophore levels were seen at certain soil depths (10-20 cm) showing that these molecules are involved in the complex respiratory pathway which the bacteria in these soils utilize .These preliminary results are suggest that iron reduction is an important metabolic pathway in these soils and appear to involve the activity of extracellular biomolecules such as cytochromes(involved in electron transport) and siderophores(involved in iron acquisition) where anaerobic conditions prevail.

Large-scale manipulated field experiments to describe the responses of biological soil crust microbial communities to long-term elevated CO_2

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Biologic soil crusts (biocrusts) consist of cyanobacteria and other phototrophic bacteria, algae, mosses and their associated heterotrophic fungi and

bacteria. These communities are important components of arid ecosystems where they colonize the plant interspaces. Our studies have been conducted at the Nevada Desert Research Center Free Air CO_2 Enrichment (FACE) facility to describe the effects of ten years of elevated atmospheric CO_2 on these sensitive microbial communities. Using a combination of environmental measurements and shotgun metagenome sequencing we are describing the effects of this environmental manipulation on the structure and function of the biocrust microbial communities.

Within the shotgun metagenomes, several microbial taxa were found to respond to the CO_2 treatment through shifts in relative abundance. However, in some cases these responses were contradictory to results from 16S rRNA pyrotag sequencing. Taxonomic assignments of shotgun metagenome reads did not identify known *Cyanobacteria* species that inhabit the biocrusts such as *Microcoleus vaginatus*, suggesting that the taxonomic bins may not have accurately reflected the known microbial community. However, the shotgun metagenomes detected differences in the functional potential of the microbial communities due to the CO_2 treatment. In the ambient CO_2 treatment, processes such as the anaerobic synthesis of chlorophyll and electron transport proteins were enriched compared to elevated CO_2 conditions. In contrast, genes for photosynthetic oxidative phosphorylation and fatty acid biosynthesis were enriched in the elevated CO_2 treatment. Taken together these results suggest that the elevated atmospheric CO_2 conditions resulted in taxonomic, functional, and physiological changes in the biocrust microbial communities.

Interactions between plant litter decomposition and microbial communities: Aboveground and belowground effects

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Soil microbial and plant communities are intimately linked. In fact the bulk of ecosystem processes and biogeochemical transformations are driven by these two communities of organisms. However, the specific relationship between plants and microbes and how this relationship regulates processes and community dynamics is far from understood. Here, I present the results from research that seeks to examine how plant and soil microbial communities are related and how compositional change in the microbial community affects its functional role in the environment. This will be divided into three sections with the first examining how change in both microbial community composition and litter decomposition rates are related to a shared history of litter inputs. Second, I demonstrate how a change in root exudates, particularly small additions of low molecular weight carbon compounds, induce shifts in microbial communities. Finally, I examine how shifts in plant community composition induced by changes in aboveground trophic structure (presence/absence of herbivores and presence/absence of predators) influence soil microbial communities. Together this compilation of research demonstrates how

changes in plant derived inputs and plant community composition influence soil microbial communities and how metagenomic approaches can be paired with experimental designs aimed at understanding the interaction between plants and microbes. Furthermore, this research suggests that our understanding of the linkages between both plant and microbial communities must also consider other interactions within ecosystems.

Metagenomics sequencing analysis of grassland soil microbial community in response to long term elevated CO_2

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Understanding the responses of biological communities to elevated CO_2 (eCO_2) is a central issue in ecology, but little is known about the impact of eCO_2 on the structure and function of the below ground microbial community due to the extremely diverse and uncultivable nature of most soil microorganisms. In this study, 24 soil samples (12 from ambient CO_2 (aCO_2), 12 from eCO_2, and all with 16 plant species and ambient nitrogen) were collected from BioCON experiment site located in the Cedar Creek Area in University of Minnesota. Both 454 and Illumina-based metagenomics sequencing were performed for each sample, resulting in a total of ~ 120 GB sequence data. To achieve a best sequence assembly of the soil metagenomics, various strategies and approaches were used for assembling Illumina and 454 data from 24 samples. Reads-based analysis for Illumina data was also performed by mapping short Illumina reads to genes predicted from 454 reads and assembled contigs. A core microbial gene and taxonomy catalogue, mainly dominated by Actinobacteria and Proteobacteria, was also identified. Many novel functional genes were detected. Although not much difference of the proportions of taxonomy and function processes can be detected at high level classification (such as phylum level and COG function processes), noticeable differences between aCO_2 and eCO_2 samples can be observed by detrended correspondence analysis at lower levels of taxonomy and functions (such as OTU level of functional genes). Microbial ecological networks were also constructed and analyzed, providing novel insights into potential activities and feedbacks of microbial community in response to eCO_2.

Soil metabolism and microcalorimetry: A unique combination

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All physical, chemical and biological processes are accompanied by changes in energy which means, heat. This makes calorimetry one of the most suitable techniques to study all these process[1]. Soil is a typical example of this, mainly due to the fact that its behavior is related to all these processes. Soil microorganism, microbial biomass and organic matter are the most important parameter of soil quality[2] and they could be evaluated, at the same time, using Calorimetry, more generally by ITC [3]. In this study we have evaluated the effects of seasonal sampling as well as the influence of the experimental condition on the glucose degradation by Brazilian soil, using Calorimetry. The results show a decrease in the metabolism of the soil microorganisms upon storage from one month ($\Delta Hmet$= -5.80 J g^{-1}) to three months ($\Delta Hmet$= -2.73 J g^{-1}) and so on. On the other hand the oxygen atmosphere seems to decrease the metabolism ($\Delta Hmet$= -5.87 J g^{-1}) in comparison with the CO_2 admission ($\Delta Hmet$ = -10.4 J g^{-1}). This results show the increase of the microorganism stress provoked by the adverse condition of the carbon dioxide addition. It is an important factor in order to study the influence of the green house effect on the soil metabolism, and vice versa. Other results showed the increase of the metabolic energy with the increase of the As^{3+} addition, from ($\Delta Hmet$= -9.99 J g^{-1}) for 50 ppm of As^{3+} to ($\Delta Hmet$ = -11.9 J g^{-1}) for 1,000 ppm and a decrease of the metabolism concomitant with the herbicide (glyphosate, 0.13M) addition ($\Delta Hmet$= -2.44 J g^{-1}).

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The mold in the machine: Metagenomics, models, and Moore's Law

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Linking molecular scale microbial community data to ecosystem models requires that microbiologists 1) have a comprehensive understanding of the environmental factors that structure microbial communities 2) understand how shifts in the composition of the microbial community affects ecosystem function, and 3) are measuring the correct variables at the correct temporal and spatial scales that models require. Metagenomics presents us with a vast (and ever increasing) account of the molecular composition of soil (mostly prokaryotic) microbial community, but this high degree of detailed information is arguably anathema to the structure of ecosystem models, which often work best with a limited set of driving variables and relationships, but with high temporal and spatial resolution. In this presentation, I will address the state of knowledge regarding controls on microbial community composition at ecosystem and continental scales and highlight cases in which microbial community information can help explain ecosystem processes and

be utilized in ecosystem models. However, many of these approaches are outside the realm of metagenomics and include food web dynamics, symbiotic relationships, C stabilization processes, and physiological assays. For metagenomic analysis to be utilized by current ecosystem models, data will have to be binned into functional genes or groups that directly link to an ecosystem function, be quantitative and spatially and temporally resolute, and be conducted in conjunction with other soil microbial techniques, including biomass and rate parameters.

Transcriptional response of *Ralstonia pickettii* to high copper concentrations

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We have previously described and studied copper resistant bacteria *Ralstonia pickettii* strains 12D and 12J that were isolated from heavy metal (Cu, Cd, Zn, and Ni) contaminated lake sediment. Our results revealed that *R. pickettii* strains (12D and 12J) were resistant to high concentrations of copper and sequestered up to 27.44 and 38.19 mg copper per gram dry weight of cells, respectively. We have investigated the transcriptional response of *R. pickettii* to copper by Illumina sequencing of extracted RNA. With multiplex sequencing, we obtained 13-18 million reads (55 bp/read) per sample and 78-91% of the sequences from sample were mapped to *R. pickettii* 12D or 12J reference sequences by Bowtie. While the majority of the reads were aligned to ribosomal RNA sequences, 500 thousand to 1 million sequences from copper supplemented cells were mapped to annotated mRNA genes and 50 to 70 thousand reads were mapped to intergenic regions in *R. pickettii* 12D and 12J grown in copper. The amount of mRNA transcripts obtained from cells grown in the absence of copper were substantially less, however, with 19 (12D) and 61 (12J) thousand mapped mRNA reads and 6 (12D) and 65 (12J) thousand mapped intergenic reads only. Despite the differences in the abundance of transcripts, the alignments showed that transcripts encoding for copper resistance, heavy metal efflux pumps and heavy metal translocating P-type ATPase were abundant in both copper grown *R. pickettii* 12D and 12J cells, as expected. We also observed that citrate synthase encoding transcripts were substantially more abundant in copper exposed cells, suggesting that citrate may be used by the cell to sequester copper. In addition, sequences aligned to extracellular binding receptors and signal proteins may further help us in understanding the copper binding mechanisms in *R. pickettii* 12D and 12J.

Molecular ecological networks in response to elevated CO_2

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Understanding the interactions among different species and their responses to environmental changes is a central goal in ecology. Although the network interactions and their responses to environmental changes have been intensively studied in macroecology, very little is known in microbial ecology because defining the network structure in a microbial community is of a grand challenge. Here, a novel random matrix theory (RMT)-based conceptual framework is developed for identifying phylogenetic molecular ecological networks using metagenomics sequencing data of 16S rRNA genes from soil microbial communities in a long-term grassland FACE (Free Air CO_2 Enrichment) experiment. Our results demonstrated that RMT-based network approach is powerful in delineating phylogenetic molecular ecological networks in microbial communities. Also, the structure of the identified networks under ambient and elevated CO_2 was substantially different in terms of overall network topology, network composition, node overlap, module preservation, module-based higher order organization (meta-modules), topological roles of individual nodes, and network hubs. These results suggested that elevated CO_2 dramatically altered the network interactions among different phylogenetic groups/populations. In addition, the changes in network structure were significantly correlated with soil carbon and nitrogen content, indicating the potential importance of network interactions in ecosystem functioning. Elucidating network interactions in microbial communities and their responses to environmental changes is fundamentally important for research in microbial ecology, systems microbiology, and global change.

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