Pacific Northwest
NATIONAL LABORATORY

Multi-omics for Microbiomes Conference

2019 Report

September 2019

1Janet K Jansson
2Monica G Moffett
3Deborah Moles
4Evelyn Callister

U.S. DEPARTMENT OF ENERGY

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Multi-omics for Microbiomes Conference

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Conference report authors:
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Pacific Northwest National Laboratory
Richland, Washington 99354

About

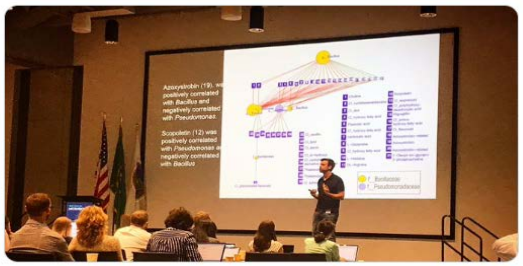
This conference brings together a cross-disciplinary group of scientists who apply multi-omics approaches to understand complex microbial communities, who develop tools to analyze and integrate omics data, and who develop omics technologies to increase our knowledge and understanding of the microbiome.

The importance of microbiome research is increasingly being recognized because of the critical roles that microbial communities play in the environment and human health. However, we are currently in the “discovery phase” of microbiome science and lack a mechanistic understanding of the roles that individual microbes and communities of microbes play in different environments and the impacts of perturbations on microbial community functions. The time is therefore ripe for us to exchange knowledge and expertise, and this conference is a significant step in doing so.

Janet Jansson
Conference Chair
Earth and Biological Sciences Directorate
Pacific Northwest National Laboratory
https://pnnl.cvent.com/multiomics

2019 Tweet & Photo Highlights

Science at PNNL @sciencePNNL · Jul 24
"Bacteria are everywhere. You cannot control your exposure." - @gilbertjacka, our **#MultiMicro2019** keynote speaker, this morning on human and environmental microbial health.
#Microbiomes #bacteria @PNNLab #pnnlab



5 13

Laura Tipton @lauraomics · Jul 26
Thank you to @JanetJansson @MonchGrace and all of the @PNNLab for a great **#MultiMicro2019** conference!

2 2 11

Monica Moffett @MonchGrace · Jul 24
Huge shout out to our sponsors and exhibitors. We couldn't have done it without you! **#MultiMicro2019** @thermofisher @ElsevierConnect @Metabolon @Biocrates_ @illumina @EMSLscience @PhaseGenomics @DOEKBase @ISME_microbes @MB_Insights

2 10

Kuesel Lab @kuesel_lab · Jul 26
Today Kirsten is giving a talk at the **#MultiMicro2019** conference @PNNLab presenting our work in the @CRC_AquaDiva and the #BalanceOfTheMicroverse #ExcellenceCluster
Thank you @sawi_mpi for providing the pictures.

4 20

D. Scott Merrell (Lab) @scottymerrell1 · Jul 24
Nothing is sterile! Human milk microbiome **#MultiMicro2019**

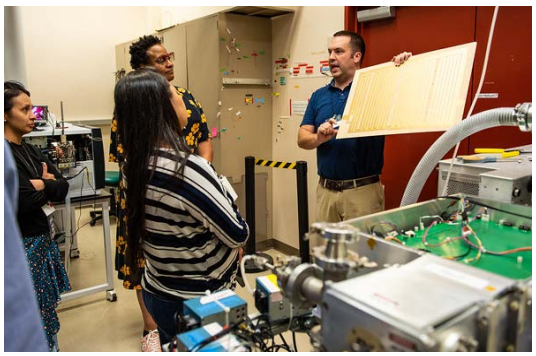
Science at PNNL @sciencePNNL · Jul 25
The **#MultiMicro2019** crew enjoying a beautiful evening at @REACHmuseum after a long, awesome day of presentations. Tomorrow: Day 3, including tours of @EMSLscience.
#science #scientificconference #microbiome #Biology

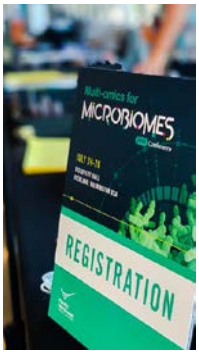
Erick Cardenas @erickcardenasp · Jul 26
@JanetJansson closes the conference **#MultiMicro2019**. Really good talks and posters. I learned a lot about the microbiome of humans (and also their milk), soils, fungi, groundwater, and the built environment. 🧬🧪🍄🌲💩🏙️.

3

Elisha Wood-Charlson @ElishaMariePhD · Jul 25
Great review of ocean viral economics work by Matt @Lab_Sullivan. Fun to see how far we've come. Now, to get soils on board - no small feat (punny, right? 😉)
#MultiMicro2019 #virusluv

2 2 15


Microbially-Mediated Plant
Friesen et al. 2011 AR

Multi-omics for
MICROBIOMES
REGISTRATION

PNNL





Multi-omics for
MICROBIOMES
2019 Conference



Multi-omics for
MICROBIOMES

1.0 Conference Overview

The 2019 Multi-Omics for Microbiomes Conference was a huge success and covered a range of topics of interest to the field of microbial ecology. For example, the two keynote speakers were selected to highlight recent advances in disparate areas of microbial ecology. Prof. Jack Gilbert from UCSD, San Diego, CA, gave the opening keynote address and provided an overview of microbiome research ranging from the human gut microbiome, to the built environment. Prof. Kirsten Kusel, Friedrich Schiller University, Jena, Germany gave the second keynote address, focusing on understanding mechanisms underlying development of the groundwater microbiome. In addition, there were 22 plenary talks that covered a range of topics. The session themes included environmental and human microbial ecology, advances in multi-omics technologies, the soil microbiome and the microbiome & defense health. Twelve shorter contributed talks were selected from the abstracts. Two of these were ISME award recipients as described in more detail below.

The general session was held in a common auditorium where attendees listened to 28 speakers over the course of 2.5 days. The general session was followed by a half day of tours through several labs in the Environmental Molecular Sciences Laboratory and a DOE KBase Workshop.

Attendees shared more than 200 tweets using **#MultiMicro2019**. To see the full recap of social media posts, check out the Twitter moment we created here.

This event was supported by the Microbiomes in Transition Initiative LDRD Program at the Pacific Northwest National Laboratory, a multi-program national laboratory operated by Battelle for the DOE under Contract DE-AC06-76RL01830.

2.0 Sponsor Acknowledgements

The sponsors were acknowledged in several ways; for example, in the program book as follows: "My thanks to all of you who are attending, and especially to our sponsors: ISME, Microbiome Insights, Thermo Fisher Scientific, Illumina, Phase Genomics, Metabolon, Biocrates, and Elsevier."

In addition, the sponsor logos were displayed throughout the conference on the speaker podium and on slides and flat screens.

3.0 ISME Support

As part of our marketing strategy, we promoted ISME in several different ways. We included ISME logos and ISME18 announcements in all of the intermission slides as well as on the slides that were able to be viewed in all hallways. We also included the ISME logo on the website and promoted the society via several social media posts. You can see some examples in the photo/tweet highlights at the beginning of this report.

This year we used ISME funds to support two early career scientists via the ISME Young Investigator Grant. This grant provided a $1,000 travel stipend to each winner. Recipients were selected from the abstract submissions based on the quality of their science and validation that they were early career scientists. The award recipients were Dr. Kristin Meyer (Baylor College of Medicine) and Dr. Thibault Stalder (University of Idaho). Each of the ISME Awardees presented an oral contributed talk at the conference. Full abstracts and authorship listing can be found in the program (see Appendix A). Their presentation titles are shown below:

1. **Gut Microbiome Composition and Mold Exposure are Associated with Allergic Symptoms After Hurricane Harvey**

   Kristen Meyer[1], Kristi Hoffman, Abi Oluyomi, Xiangjun Gu, Jesus Sotelo, Dan Na Luo, Georgina Armstrong, Joe Petrosino, Cheryl Walker, and Melissa Bondy
   [1]Baylor College of Medicine

2. **Finding the Hosts of the Resistome and Plasmidome**

   Thibault Stalder[1], Maximilian Press[2], Shawn Sullivan[2], Ivan Liachko[2], and Eva Top[1]
   [1]University of Idaho, and [2]Phase Genomics

In addition to the items listed above, we also noted in the program that ISME Sponsored the conference poster session. Of the 193 attendees', there were a total of 66 posters submitted to the conference. Appendix B contains a list of authors and poster titles.

4.0 Participant Metrics

The multi-omics for microbiomes conference hosted 193 attendees from various institutions and backgrounds.

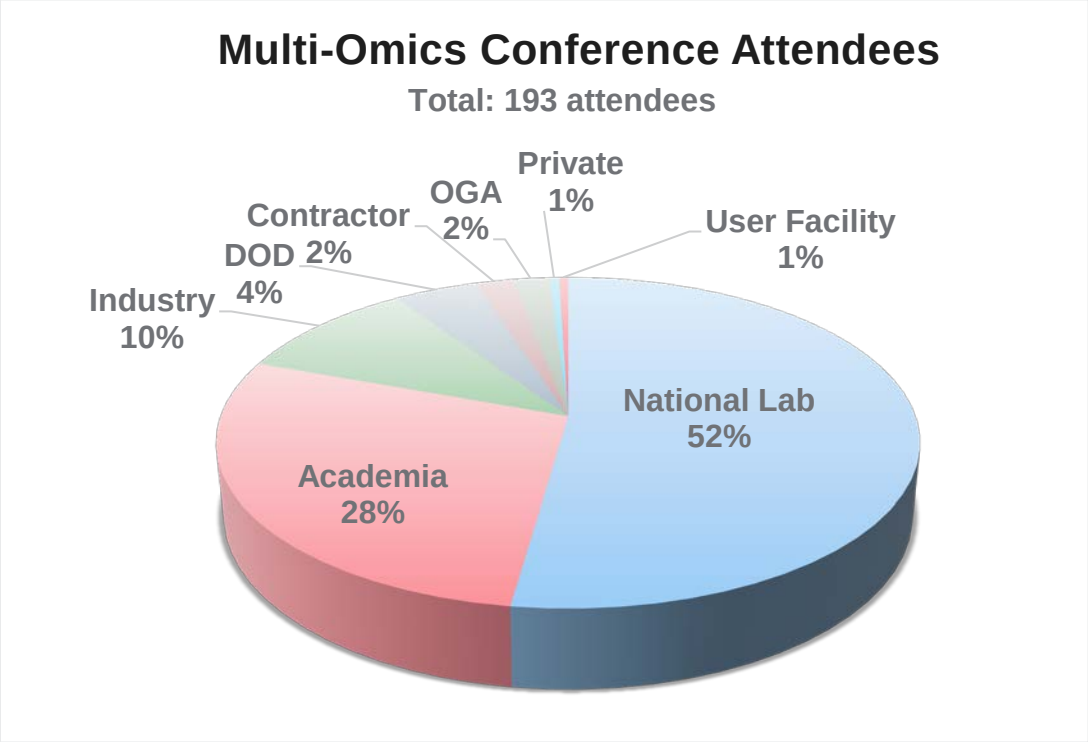


Figure 1 For a full breakdown of who attended, please view Appendix C.

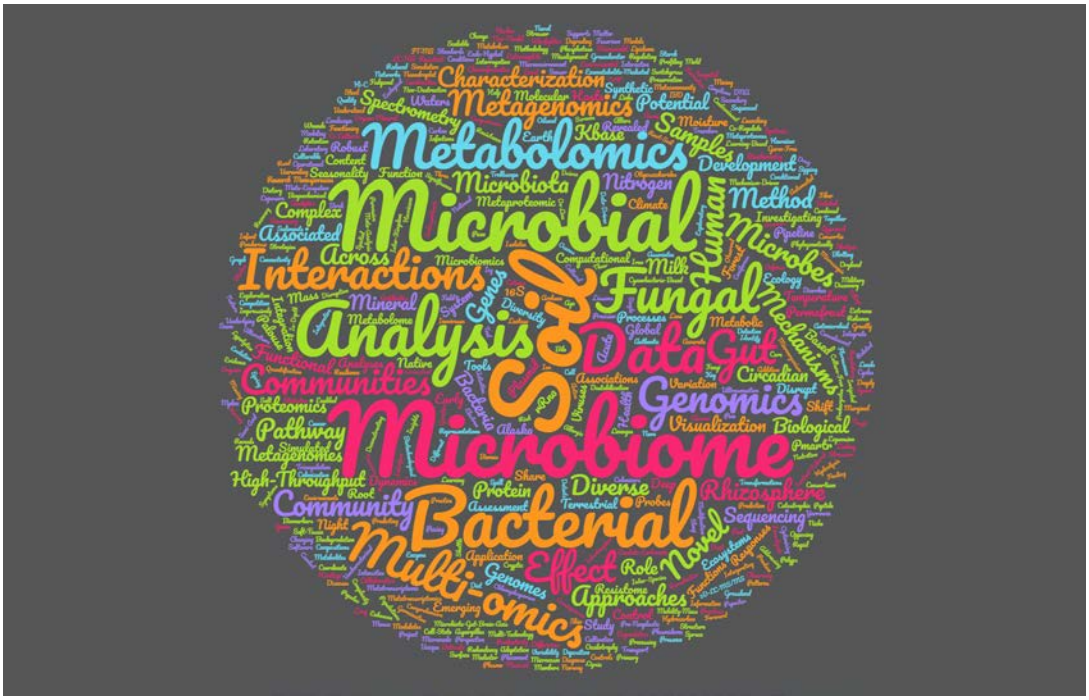
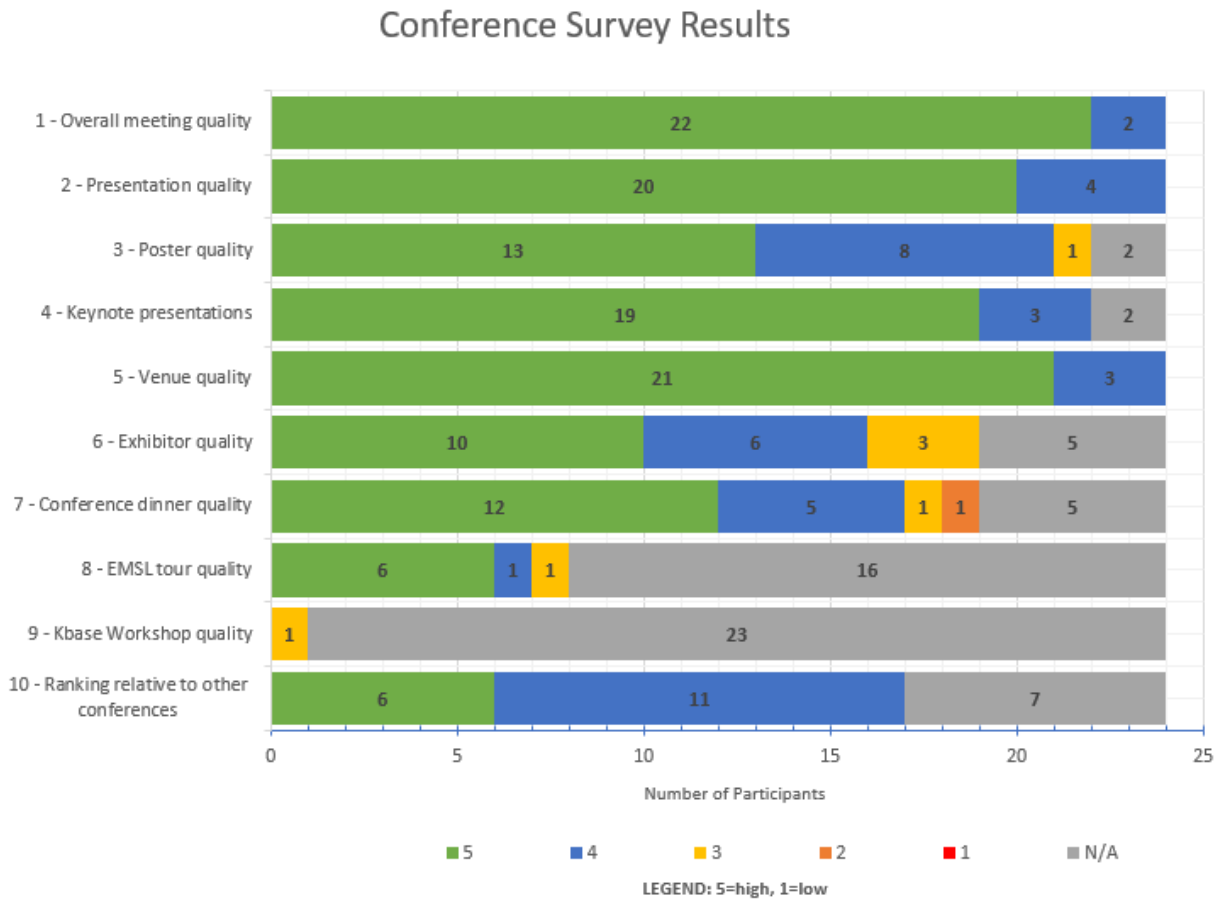


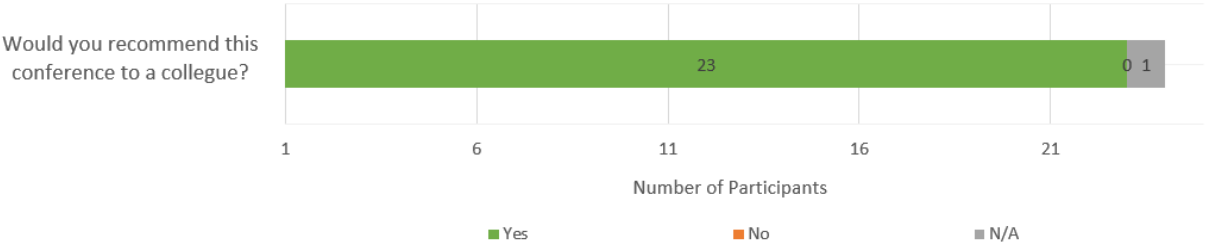
Figure 2 Wordle of all poster/talk titles at Multi-omics Conference.

Figure 2. Participant Affiliation Logo Cloud

5.0 Feedback

A survey was sent out to participants to gauge the usefulness and impact of the conference. While we didn't receive a response from everyone, we were extremely encouraged by the responses we did receive.





Appendix A – Scientific Program

Tuesday, July 23	
5:00 – 7:00 pm	**Early Registration** *Discovery Hall, 650 Horn Rapids Road, Richland, WA 99354*
WEDNESDAY, July 24	
7:00 – 8:15 am	**Registration and Check-in** *Morning Refreshments available*
8:15 – 8:30 am	**Conference Welcome & Overview** *Janet Jansson, Pacific Northwest National Laboratory*
8:30 – 9:30 am	**Opening Keynote: Human and Environmental Microbial Health: A Global Perspective** *Jack Gilbert, University of California, San Diego*
9:30 - 11:45 pm	**Microbiomes Across Different Ecosystems** *Chair: Vanessa Bailey, Pacific Northwest National Laboratory*
9:30 – 10:00 am	**Ecological Dynamics of Synthetic Microbial Communities** *Ashley Shade, Michigan State University*
10:00 – 10:30 am	**Multi-omic Triangulation of Bacterial Lignin Modification Pathways** *Bill Mohn, University of British Columbia*
10:30 – 10:45 am	**Break**
10:45 – 11:00 am	**Contributed Talk: Launching the National Microbiome Data Collaborative** *Elisha Wood-Charlson, Lawrence Berkeley National Laboratory*
11:00 – 11:30 am	**Biotechnological Potential of Extreme Soils: Integrative Multi-omics and Cultivation Assessment Approaches** *Alexandre Soares Rosado, University of California Davis*
11:30 - 11:45	**ISME Young Investigator Awardee: Gut Microbiome Compositions and Mold Exposure are Associated with Allergic Symptoms after Hurricane Harvey** *Kristen Meyer, Baylor College of Medicine*
11:45 – 1:00 pm	**Break for Lunch** *(on your own)* *See list of lunch vendors in full program* *Expo & Poster Viewing*
1:00 - 5:00 pm	**Microbiomes Across Different Ecosystems** *Chair: Ryan McClure, Pacific Northwest National Laboratory*
1:00 – 1:30 pm	**SIPping the Icy Waters of Alaska: Multi-omics Approaches to Emerging Contaminant Biodegradation Research** *Mary Beth Leigh, University of Alaska Fairbanks*
1:30 – 2:00 pm	**The Mucosal and Microbial Landscape of Pre-Neoplastic Polyps** *William DePaolo, University of Washington*

WEDNESDAY, July 24 (continued)	
2:00 – 2:15 pm	**Contributed Talk: Cryptic Inoviruses Revealed as Pervasive in Bacteria and Archaea across Earth's Biomes** *Simon Roux, Joint Genome Institute*
2:15 – 2:45 pm	**Got Microbes? An Update on the Human Milk Microbiome** *Michelle "Shelley" McGuire, University of Idaho*
2:45 - 3:00 pm	**Session Q&A**
3:00 – 3:15 pm	**Break**
3:15 – 3:45 pm	**High-Throughput Genetics in Non-Model Bacteria** *Adam Deutschbauer, University of California – Berkeley*
3:45 – 4:00 pm	**Contributed Talk: Using Genomic Data to Identify Bacterial Associates of Fungi** *Geoffrey House, Los Alamos National Laboratory*
4:00 – 4:30 pm	**The Switchgrass Rhizosphere Microbiome and Nitrogen Transformations on Marginal Lands** *Maren Friesen, Washington State University*
4:30 – 4:45 pm	**ISME Youg Investigator Awardee: Finding the Hosts of the Resistome and Plasmidome** *Thibault Stalder, University of Idaho*
4:45 – 7:00 pm	**Poster Session & Expo** *Sponsored by International Society for Microbial Ecology* *Heavy Appetizers and Refreshments provided* **Posters Numbered odd in program will present 5:00pm – 5:40pm** **Posters Numbered even in program will present 5:40pm – 6:20pm**
THURSDAY, July 25	
7:30 – 8:00 am	**Registration and Check-in** *Morning Refreshments available*
8:00 – 11:30 am	**Thematic Session: Exploration of the Terrestrial Microbiome** *Chair: Janet Jansson, Pacific Northwest National Laboratory*
8:00 – 8:30 am	**Simulated Climate Change and Organic Matter Stoichiometry Co-regulate Carbon Destabilization in Active Peat** *Emily Graham, Pacific Northwest National Laboratory*
8:30 – 9:00 am	**Investigating Bacterial-Fungal Interactions** *Patrick Chain, Los Alamos National Laboratory*
9:00 – 9:15 am	**Contributed Talk: Transport of Mineral Cations by Fusarium Chlamydosporum in a Mineral Doped Soil Micromodel System** *Arunima Bhattacharjee, Pacific Northwest National Laboratory*
9:15 – 9:30 am	**Session Q&A**
9:30 – 9:45 am	**Break**
9:45 – 10:15 am	**Interkingdom Interactions of the Soil Microbiome** *Kirsten Hofmockel, Pacific Northwest National Laboratory*
10:15 – 10:30 am	**Contributed Talk: Soil Mineral Alterations by a Bacterial Electron Shuttle Produce Opposing Effects Upon Wheat Iron Nutrition Under Dryland and Irrigated Conditions** *Melissa LeTourneau, US Department of Agriculture – Agricultural Research Service*
10:30 – 11:00 am	**Multi-Omics Enabled Quantification of Microbial Controls on Biogeochemical Cycles in Permafrost Ecosystems** *Neslihan Taş, Lawrence Berkeley National Laboratory*

THURSDAY, July 25 (continued)	
11:00 – 11:30 am	**Viruses in Nature: Lessons From the Oceans, Soils and Humans** *Matthew Sullivan, Ohio State University*
11:30 – 1:30 pm	**Break for Lunch** *(on your own)* *See list of lunch vendors in full program* *Expo & Poster Viewing*
1:30 – 4:45 pm	**Thematic Session: Human Microbiome and Defense Health** *Chair: Justin Teeguarden, Pacific Northwest National Laboratory*
1:30 – 2:00 pm	**Skin and Soft-Tissue Infections in the Age of Genomics: Analyses in Military Trainees at Fort Benning, GA** *D. Scott Merrell, Uniformed Services University of the Health Sciences*
2:00 – 2:30 pm	**Data-Driven Methods for Optimizing Sensor Placement in Microbial Genomes to Diagnose Cell-State** *Enoch Yeung, University of California – Santa Barbara*
2:30 – 2:45 pm	**Contributed Talk: Functional Gene Analysis of the Gut Microbiota During Acute and Convalescent Travelers' Diarrhea** *Ryan Johnson, Uniformed Services University*
2:45 – 3:00 pm	**Contributed Talk: Acute Diet Stressor Alters Inter-Species Competition for Resistant Starch in the Gut Microbiota** *Ida Pantoja, US Army Natick*
3:00 – 3:15 pm	**Break**
3:15 – 3:45 pm	**Long Read Tools for Precision Genomics and Metagenomics** *Chris Bradburne, Johns Hopkins University*
3:45 – 4:15 pm	**Effects of Sleep and Circadian Disruption on the Microbiota-Gut-Brain-Axis** *Kenneth P. Wright, University of Colorado*
4:15 – 4:45 pm	**The Gut Microbiome as a Potential Mediator of Warfighter Responses to Operational Stressors** *J. Phillip Karl, US Army Research Institute of Environmental Medicine*
5:00 pm	**Bus departs PNNL for conference hotels**
5:45 pm	**Bus departs Hotels for The REACH** – *See full schedule for exact pick-up times*
6:00 – 8:00 pm	**Dinner and Tour - The REACH Museum** *Transportation provided - see shuttle schedule in full program.*
FRIDAY, July 26	
7:30 – 8:00 am	**Registration and Check-in** *Morning Refreshments available*
8:00 – 9:00 am	**Keynote: Formation of a Groundwater Microbiome: From Patterns to Mechanisms and Functions** *Kirsten Küsel, Friedrich Schiller University Jena*
9:00 am – 12:00 pm	**Thematic Session: Metabolomics & Cheminformatics** *Chair: Tom Metz, Pacific Northwest National Laboratory*
9:00 – 9:30 am	**From Microbes to Metabolites: Tools to Help Integrate Metabolomics with Microbiomics** *David Wishart, University of Alberta*
9:30 – 10:00 am	**Metabolomics with Microbiomics** *Elaine Holmes, Health Futures Institute, Murdoch University*
10:00 – 10:15 am	**Contributed Talk: Metabolomic Profiling of Diverse Microbial Communities for the Earth Microbiome Project (EMP500)** *Sneha Couvillion, Pacific Northwest National Laboratory*

FRIDAY, July 26 (continued)	
10:15 – 10:30 am	**Break**
10:30 – 11:00 am	**Computational Metabolomics: Decreasing Our Reliance on Authentic Standards** *Jamie Nuñez, Pacific Northwest National Laboratory*
11:00 – 11:15 am	**Contributed Talk: Learning Accurate Representations of Microbe-Metabolite Interactions** *James Morton, University of California, San Diego*
11:15 – 11:45 am	**Multi-omics Integration in KBase to Understand Ecology in Diverse Microbiomes** *Chris Henry, Argonne National Laboratory*
11:45 – 12:00 pm	**Closing Remarks** *Janet Jansson, Pacific Northwest National Laboratory*
12:00 pm	**Adjourned**
1:00 – 2:30 pm	**OPTIONAL - Environmental Molecular Sciences Laboratory (EMSL) Tour** *Participants: You must pre-register. Check-in at Registration Desk by 12:30 pm. Close-toed shoes are required.*
1:00 pm	**Tour 1: Hi-Res Imaging Lab – Scott Lea** **Tour 2: Cell Isolation and Systems Analysis (CISA) Lab – Will Chrisler** **Tour 3: Nuclear Magnetic Resonance (NMR) Lab – Robert Young & Chaevien Clendinen** **Tour 4: Mass Spectrometry Lab – Danny Orton** **Tour 5: My EMSL - Super Computer Lab – Lee Ann McCue**
2:30 – 4:30 pm	**OPTIONAL – KBase Workshop** *Participants: You must pre-register for this workshop! Questions should be directed to engage@kbase.us.*

Appendix B – Poster List

NO	First Name	Last Name	Organization	Title
1	Caylon	Yates	Penn State University	Niche expansion by bacterial isolates conditioned to novel soils
2	Cheryl	Chow	Second Genome, Inc	Mechanism-driven, multi-omics approach using multi-technology meta-analysis for novel microbiome-derived drug discovery in IBD
3	Christopher	Anderton	Pacific Northwest National Laboratory	Soil moisture modulates inter-kingdom interactions as observed using a simulated soil core
4	Huaihai	Chen	Pacific Northwest National Laboratory	Global soil metagenomic evidence supports functional redundancy in soil microbes
5	Jane	Lucas	University of Idaho	Antibiotics and temperature disrupt native Palouse soil communities and their function
6	Karl	Weitz	Pacific Northwest National Laboratory	Unraveling the Molecular Mechanisms of the Birch Effect in Soils
7	Katherine	Naasko	Washington State University	Soil Enzyme Activities Vary Greatly Across Tillage Intensities in Semi-Arid Palouse Soils
8	Kehau	Hagiwara	National Institute of Standards and Technology	Addressing Sample Preservation Strategies for Metabolomics Best Practices within Microbiome Multiomics Studies
9	Kristin E.	Burnum-Johnson	Pacific Northwest National Laboratory	Metaproteomic approaches for in-depth characterization of complex soil microbial communities
10	Laura	Tipton	University of Hawaii at Manoa	Metagenomic Community Dynamics in Hawaiian Fishpond Sediments
11	Mahantesh	Halappanavar	Pacific Northwest National Laboratory	Scalable Graph Analytics for Predicting Protein Functions in Metagenomes
12	Nicholas	Be	Lawrence Livermore National Laboratory	Metagenomic interrogation of the microbial microenvironment in combat wounds
13	Ondrej	Uhlik	University of Chemistry and Technology, Prague	Phylogenetically novel prokaryotes in Czech spring waters of cultural heritage significance
14	Ryan	Pace	University of Idaho	Variation in human milk oligosaccharides, protein, and lactose are related to variation in milk and infant stool microbiota
15	Stephen	Eacker	Phase Genomics	Linking the Resistome to the Microbiome: A Culture-Free Method Links Plasmid, Virus, and Antimicrobial Resistance Genes to their Hosts in Complex Microbial Populations
16	Ying	Zhu	Pacific Northwest National Laboratory	High-throughput single cell proteomics based on nanodroplet sample processing and ultrasensitive LC-MS
17	Yi-Syuan	Guo	University of Connecticut	Microbe-mediated Soil Moisture Retention and its Variability
18	Adriana	Torres-Ballesteros	Rothamsted Research	Microbiome connectivity drives productivity in Oilseed Rape Crops
19	Alison	Thurston	Cold Regions Research and Engineering Laboratory - ERDC	Microorganism Communities Associated with Dust Deposition on Snow
20	Allison	Thompson	Pacific Northwest National Laboratory	Exploratory data analysis and interactive visualization of FT-MS data
21	Amy	Zheng	Vanderbilt University	Development of metabolomics approaches to study 2D and 3D bacterial-fungal co-cultures
22	Christopher	Whidbey	Pacific Northwest National Laboratory	Multi-omic characterization of dietary fiber degradation in the gut microbiome
23	Dan	Naylor	Pacific Northwest National Laboratory	A Novel Methodology for Deconstructing the Complex Soil Microbiome
24	Eric	Johnston	Oak Ridge National Lab	Effects of nitrogen addition on Populus rhizosphere microbiome and early root colonization
25	Fabio	Palmieri	University of Neuchatel	Biological control of Aspergillus niger through bacterial oxalotrophy
26	Fadi	Abdi	Biocrates Life Sciences	Assessment of the Microbiota Metabolome and Its Role in Chronic Systemic Diseases
27	John	Chodkowski	Michigan State University	Consequences of diversity on microbial responses to exometabolite-mediated interactions in a synthetic microbial community

28	Julia	Kelliher	Los Alamos National Laboratory	Investigating the endo-hyphal fungal microbiome through comprehensive genomic screens
29	Komi	Messan	Cold Regions Research and Engineering Laboratory	The role of a rapid changing temperature in microbial metabolic processes during permafrost thaw
30	Laura	Kaminsky	Penn State University	Characterization of consistent early microbial colonizers of soils
31	Lee	Bergstrand	University of Waterloo	Micromeda: a pathway prediction pipeline and web visualization tool for functional comparisons of microbial genomes and metagenomes
32	Pilar	Junier	University of Neuchatel	Mining genomic data to investigate the evolution of key mechanisms regulating bacterial-fungal interactions
33	Ruonan	Wu	Pacific Northwest National Laboratory	Piecing together DNA viromes from deeply sequenced soil metagenomes
34	Ryan	Trexler	Penn State University	Metatranscriptomic and 16S rRNA gene analysis of a Cyanobacteria-based soil surface consortium with and without a diverse underlying soil microbiome
35	Salvador	Castaneda Barba	University of Idaho	Hi-C Method Detects Plasmid-Host Associations in Soil
36	Saskia	Bindschedler	University of Neuchâtel	The role of bacterial-fungal interactions in the functioning of the oxalate-carbonate pathway in soils
37	Shobhan	Gaddameedhi	Washington State University	Circadian biomarkers of cancer risk associated with night shift in humans
38	Simone	Lupini	University of Houston	Soil bacterial and fungal communities interactions in a microcosm column
39	Tomas	Vetrovsky	Institute of Microbiology of the CAS	Seasonality of microbial processes at the root-soil interface in a coniferous forest as revealed by the combination of metatranscriptomics, metagenomics and metabolomics
40	Vivian	Lin	Pacific Northwest National Laboratory	Root blotting method for non-destructive spatial analysis of phosphatase activity in the rhizosphere
41	Yuqian	Gao	Pacific Northwest National Laboratory	The Combined Application of MPLEx, on-line 2D-LC-MS/MS, and Automated Data Analysis Pipeline Enhances the Deep Analysis of Soil Metaproteomes
42	Zander	Human	Microbiology Institute of the CAS	The effects of seasonality and nitrogen content on the Norway spruce (Picea abies) forest soil microbiome
43	Gherman	Uritskiy	John Hopkins	Resilience and adaptation mechanisms of an extremophile community after a catastrophic climate event
44	Aaron	Robinson	Los Alamos National Laboratory	Genomic analysis of diverse members of the fungal genus Monosporascus reveals novel lineages, unique genome content and the potential to harbor bacterial endosymbionts
45	Agne	Nixon	Pacific Northwest National Laboratory	A robust florescence assay for detection of primary and secondary bile salt hydrolysis in the gut microbiome
46	Aivett	Bilbao	Pacific Northwest National Laboratory	Ion mobility-mass spectrometry-based proteomics of soil microbiome using context-tailored pathway information and targeted data
47	Alice	Dohnalkova	Pacific Northwest National Laboratory	Genomic, Molecular and Microscopic Insights into the Organo-Mineral Associations in a Ponderosa Pine Rhizosphere
48	Colin	Brislawn	Pacific Northwest National Laboratory	Share Impressively with DataHub
49	David	Degnan	Pacific Northwest National Laboratory	PSpecteR: A proteomics data analysis application in R
50	Demosthenes	Morales	Los Alamos National Laboratory	Observing the effect of bacterial presence on fungal spore germination.
51	Jennifer	Kyle	Pacific Northwest National Laboratory	Circadian misalignment through night shift simulation disrupts plasma lipidome
52	JOON-YONG	LEE	Pacific Northwest National Laboratory	Metaproteomic data analysis with deep learning-based de novo peptide sequencing
53	Kelly	Stratton	Pacific Northwest National Laboratory	pmartR: Software for Quality Control and Statistics Robust to Missing Data for Mass Spectrometry-based Biological Data

54	Kristoffer	Brandvold	Pacific Northwest National Laboratory	Function-based characterization of gut microbiome metabolism using activity-based probes and germ-free mouse models
55	Michal	Strejcek	University of Chemistry and Technology, Prague	Differences in community structure analysis based on 16S rRNA gene using high-throughput amplicon and whole-metagenome shotgun sequencing of samples with vastly uncharacterized microbiomes
56	Michelle	Davison	Pacific Northwest National Laboratory	Recovery of culturable bacteria from soil
57	Neeraj	Kumar	Pacific Northwest National Laboratory	Computational Modeling of Metabolic and Regulatory Networks of Yarrowia lypolytica
58	Rachel	Richardson	Pacific Northwest National Laboratory	Visualization of Large Biological Mass Spectrometry Datasets via Integration of pmartR and trelliscopejs
59	Robert	Cottingham	Argonne National Laboratory	KBase Orgs: How to coordinate and share data & analyses in KBase across a laboratory, project or any community of practice
60	Robert	Danzak	Pacific Northwest National Laboratory	Meta-ecosystem metabolomics: Interpreting metabolomes through the lens of metacommunity ecology
61	Robert	Jones	United States Army Corps of Engineers Engineering Resource and Development	Terrestrial Hydrocarbon Degrading Bacterial Diversity and Development: a Three Year Remediation Case Study in Utqiaġvik, Alaska
62	Sophie	Colston	US Naval Research Laboratory	Field forward sequencing in naval environments
63	Yuliya	Farris	Pacific Northwest National Laboratory	Development and Analysis of Reduced Complexity Microbial Consortia Emerging from Native Grassland Soil Systems
64	Heather	Brewer	Pacific Northwest National Laboratory	Getting Tough Fungal Samples to Spill Their Proteins for MS Analysis

Appendix C – Attendee Affiliation

Row Labels	Count of Company
Academia	**55**
Baylor College of Medicine	1
Friedrich-Schiller-University, Jena	1
Institute of Microbiology of the CAS, v. v. i., Prague	2
Johns Hopkins University	2
Michigan State University (MSU)	2
Murdoch University	1
Ohio State University	1
Oregon Health & Science University (OHSU)	2
Oregon State University (OSU)	2
Pennsylvania State University	3
Rothamsted	1
Seattle University	1
University of Waterloo	1
University of Alaska, Fairbanks	1
University of Alberta	1
University of British Columbia	1
University of California Davis	1
University of California San Diego School of Medicine	1
University of Chemistry and Technology, Prague	2
University of Colorado Boulder	2
University of Connecticut	1
University of Hawaii at Manoa	2
University of Houston	1
University of Idaho	5
University of Neuchâtel	3
University of Pennsylvania	1
University of Washington	1
Vanderbilt University	1
Vanderbilt University, School of Engineering	1
Washington State University (WSU)	9
Weill Cornell Medicine	1
Contractor	**4**
Oak Ridge Associated Universities (ORAU)	3
Simons Foundation	1
DOD	**7**
Air Force/TSGT	1
Cold Regions Research and Engineering Laboratory (ERDC)	3
US Army	1
US Army MEDCOM	1
US Naval Research Laboratory	1
	20

Industry	
Biocrates Life Sciences	2
CosmosID	1
Elsevier	1
Illumina	3
Metabolon INC.	2
Microbiome Insights Inc.	3
Phase Genomics, Inc.	4
Second Genome	1
Thermo Fisher Scientific	2
USANA Health Sciences	1
National Lab	**101**
Argonne National Laboratory	3
Armed Forces Radiobiology Research Institute, Uniformed Services University (AFRRI)	1
DOE Systems Biology Knowledgebase (KBase)	1
Lawrence Berkeley National Laboratory (LBNL)	3
Lawrence Livermore National Lab	1
Los Alamos National Laboratory	7
Oak Ridge National Laboratory	3
Pacific Northwest National Laboratory (PNNL)	82
OGA	**4**
National Institute of Standards and Technology (NIST)	1
Uniformed Services University	2
USDA-ARS, Wheat Health, Genetics and Quality Unit	1
Private	**1**
Private	1
User Facility	**1**
Department of Energy Joint Genome Institute	1
Grand Total	**193**

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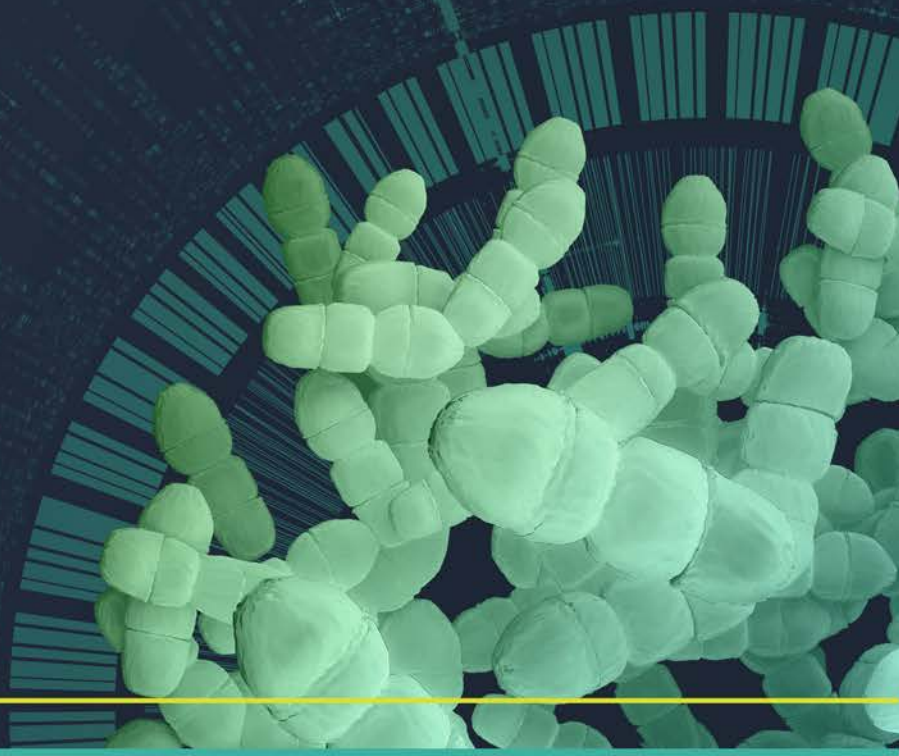
Multi-omics for

MICROBIOMES

2019 Conference

CONFERENCE PROGRAM



July 24-26

Discovery Hall

Richland, Washington U.S.A.

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NATIONAL LABORATORY

SPECIAL THANKS TO THE COMMITTEE WHO PUT THIS CONFERENCE TOGETHER:

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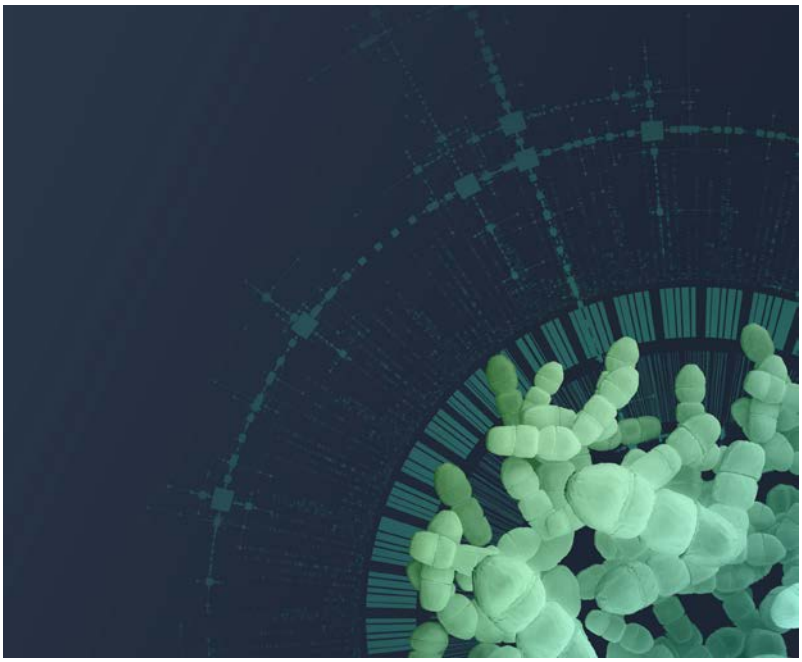

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WELCOME!

Thank you for joining us at ***the 3rd Biennial conference on Multi-omics for Microbiomes***. This conference brings together a cross-disciplinary group of scientists who apply multi-omics approaches to understand complex microbial communities, who develop tools to analyze and integrate omics data, and who develop omics technologies to increase our knowledge and understanding of the microbiome.

The importance of microbiome research is increasingly being recognized because of the critical roles that microbial communities play in the environment and human health. However, we are currently in the "discovery phase" of microbiome science and lack a mechanistic understanding of the roles that individual microbes and communities of microbes play in different environments and the impacts of perturbations on microbial community functions. The time is therefore ripe for us to exchange knowledge and expertise, and this conference is a significant step in doing so.

My thanks to all of you who are attending, and especially to our sponsors: ISME, Microbiome Insights, Thermo Fisher Scientific, Illumina, Phase Genomics, Metabolon, Biocrates, and Elsevier.

Janet Jansson
Conference Chair
Earth and Biological Sciences Directorate
Pacific Northwest National Laboratory

Tuesday, July 23	
5:00 – 7:00 pm	**Early Registration** *Discovery Hall, 650 Horn Rapids Road, Richland, WA 99354*
WEDNESDAY, July 24	
7:00 – 8:15 am	**Registration and Check-in** *Morning Refreshments available*
8:15 – 8:30 am	**Conference Welcome & Overview** *Janet Jansson, Pacific Northwest National Laboratory*
8:30 – 9:30 am	**Opening Keynote: Human and Environmental Microbial Health: A Global Perspective** *Jack Gilbert, University of California, San Diego*
9:30 - 11:45 pm	**Microbiomes Across Different Ecosystems** *Chair: Vanessa Bailey, Pacific Northwest National Laboratory*
9:30 – 10:00 am	**Ecological Dynamics of Synthetic Microbial Communities** *Ashley Shade, Michigan State University*
10:00 – 10:30 am	**Multi-omic Triangulation of Bacterial Lignin Modification Pathways** *Bill Mohn, University of British Columbia*
10:30 – 10:45 am	**Break**
10:45 – 11:00 am	**Contributed Talk: Launching the National Microbiome Data Collaborative** *Elisha Wood-Charlson, Lawrence Berkeley National Laboratory*
11:00 – 11:30 am	**Biotechnological Potential of Extreme Soils: Integrative Multi-omics and Cultivation Assessment Approaches** *Alexandre Soares Rosado, University of California Davis*
11:30 - 11:45	**ISME Young Investigator Awardee: Gut Microbiome Compositions and Mold Exposure are Associated with Allergic Symptoms after Hurricane Harvey** *Kristen Meyer, Baylor College of Medicine*
11:45 – 1:00 pm	**Break for Lunch** *(on your own)* *See list of lunch vendors in full program* *Expo & Poster Viewing*
1:00 - 5:00 pm	**Microbiomes Across Different Ecosystems** *Chair: Ryan McClure, Pacific Northwest National Laboratory*
1:00 – 1:30 pm	**SIPping the Icy Waters of Alaska: Multi-omics Approaches to Emerging Contaminant Biodegradation Research** *Mary Beth Leigh, University of Alaska Fairbanks*
1:30 – 2:00 pm	**The Mucosal and Microbial Landscape of Pre-Neoplastic Polyps** *William DePaolo, University of Washington*

WEDNESDAY, July 24 (continued)	
2:00 – 2:15 pm	**Contributed Talk: Cryptic Inoviruses Revealed as Pervasive in Bacteria and Archaea across Earth's Biomes** *Simon Roux, Joint Genome Institute*
2:15 – 2:45 pm	**Got Microbes? An Update on the Human Milk Microbiome** *Michelle "Shelley" McGuire, University of Idaho*
2:45 - 3:00 pm	**Session Q&A**
3:00 – 3:15 pm	**Break**
3:15 – 3:45 pm	**High-Throughput Genetics in Non-Model Bacteria** *Adam Deutschbauer, University of California – Berkeley*
3:45 – 4:00 pm	**Contributed Talk: Using Genomic Data to Identify Bacterial Associates of Fungi** *Geoffrey House, Los Alamos National Laboratory*
4:00 – 4:30 pm	**The Switchgrass Rhizosphere Microbiome and Nitrogen Transformations on Marginal Lands** *Maren Friesen, Washington State University*
4:30 – 4:45 pm	**ISME Youg Investigator Awardee: Finding the Hosts of the Resistome and Plasmidome** *Thibault Stalder, University of Idaho*
4:45 – 7:00 pm	**Poster Session & Expo** *Sponsored by International Society for Microbial Ecology* *Heavy Appetizers and Refreshments provided* **Posters Numbered odd in program will present 5:00pm – 5:40pm** **Posters Numbered even in program will present 5:40pm – 6:20pm**
THURSDAY, July 25	
7:30 – 8:00 am	**Registration and Check-in** *Morning Refreshments available*
8:00 – 11:30 am	**Thematic Session: Exploration of the Terrestrial Microbiome** *Chair: Janet Jansson, Pacific Northwest National Laboratory*
8:00 – 8:30 am	**Simulated Climate Change and Organic Matter Stoichiometry Co-regulate Carbon Destabilization in Active Peat** *Emily Graham, Pacific Northwest National Laboratory*
8:30 – 9:00 am	**Investigating Bacterial-Fungal Interactions** *Patrick Chain, Los Alamos National Laboratory*
9:00 – 9:15 am	**Contributed Talk: Transport of Mineral Cations by Fusarium Chlamydosporum in a Mineral Doped Soil Micromodel System** *Arunima Bhattacharjee, Pacific Northwest National Laboratory*
9:15 – 9:30 am	**Session Q&A**
9:30 – 9:45 am	**Break**
9:45 – 10:15 am	**Interkingdom Interactions of the Soil Microbiome** *Kirsten Hofmockel, Pacific Northwest National Laboratory*
10:15 – 10:30 am	**Contributed Talk: Soil Mineral Alterations by a Bacterial Electron Shuttle Produce Opposing Effects Upon Wheat Iron Nutrition Under Dryland and Irrigated Conditions** *Melissa LeTourneau, US Department of Agriculture – Agricultural Research Service*
10:30 – 11:00 am	**Multi-Omics Enabled Quantification of Microbial Controls on Biogeochemical Cycles in Permafrost Ecosystems** *Neslihan Taş, Lawrence Berkeley National Laboratory*

THURSDAY, July 25 (continued)	
11:00 – 11:30 am	**Viruses in Nature: Lessons From the Oceans, Soils and Humans** *Matthew Sullivan, Ohio State University*
11:30 – 1:30 pm	**Break for Lunch** *(on your own)* *See list of lunch vendors in full program* *Expo & Poster Viewing*
1:30 – 4:45 pm	**Thematic Session: Human Microbiome and Defense Health** *Chair: Justin Teeguarden, Pacific Northwest National Laboratory*
1:30 – 2:00 pm	**Skin and Soft-Tissue Infections in the Age of Genomics: Analyses in Military Trainees at Fort Benning, GA** *D. Scott Merrell, Uniformed Services University of the Health Sciences*
2:00 – 2:30 pm	**Data-Driven Methods for Optimizing Sensor Placement in Microbial Genomes to Diagnose Cell-State** *Enoch Yeung, University of California – Santa Barbara*
2:30 – 2:45 pm	**Contributed Talk: Functional Gene Analysis of the Gut Microbiota During Acute and Convalescent Travelers' Diarrhea** *Ryan Johnson, Uniformed Services University*
2:45 – 3:00 pm	**Contributed Talk: Acute Diet Stressor Alters Inter-Species Competition for Resistant Starch in the Gut Microbiota** *Ida Pantoja, US Army Natick*
3:00 – 3:15 pm	**Break**
3:15 – 3:45 pm	**Long Read Tools for Precision Genomics and Metagenomics** *Chris Bradburne, Johns Hopkins University*
3:45 – 4:15 pm	**Effects of Sleep and Circadian Disruption on the Microbiota-Gut-Brain-Axis** *Kenneth P. Wright, University of Colorado*
4:15 – 4:45 pm	**The Gut Microbiome as a Potential Mediator of Warfighter Responses to Operational Stressors** *J. Phillip Karl, US Army Research Institute of Environmental Medicine*
5:00 pm	**Bus departs PNNL for conference hotels**
5:45 pm	**Bus departs Hotels for The REACH –** *See full schedule for exact pick-up times*
6:00 – 8:00 pm	**Dinner and Tour - The REACH Museum** *Transportation provided - see shuttle schedule in full program.*
FRIDAY, July 26	
7:30 – 8:00 am	**Registration and Check-in** *Morning Refreshments available*
8:00 – 9:00 am	**Keynote: Formation of a Groundwater Microbiome: From Patterns to Mechanisms and Functions** *Kirsten Küsel, Friedrich Schiller University Jena*
9:00 am – 12:00 pm	**Thematic Session: Metabolomics & Cheminformatics** *Chair: Tom Metz, Pacific Northwest National Laboratory*
9:00 – 9:30 am	**From Microbes to Metabolites: Tools to Help Integrate Metabolomics with Microbiomics** *David Wishart, University of Alberta*
9:30 – 10:00 am	**Metabolomics with Microbiomics** *Elaine Holmes, Health Futures Institute, Murdoch University*
10:00 – 10:15 am	**Contributed Talk: Metabolomic Profiling of Diverse Microbial Communities for the Earth Microbiome Project (EMP500)** *Sneha Couvillion, Pacific Northwest National Laboratory*

FRIDAY, July 26 (continued)	
10:15 – 10:30 am	**Break**
10:30 – 11:00 am	**Computational Metabolomics: Decreasing Our Reliance on Authentic Standards** *Jamie Nuñez, Pacific Northwest National Laboratory*
11:00 – 11:15 am	**Contributed Talk: Learning Accurate Representations of Microbe-Metabolite Interactions** *James Morton, University of California, San Diego*
11:15 – 11:45 am	**Multi-omics Integration in KBase to Understand Ecology in Diverse Microbiomes** *Chris Henry, Argonne National Laboratory*
11:45 – 12:00 pm	**Closing Remarks** *Janet Jansson, Pacific Northwest National Laboratory*
12:00 pm	**Adjourned**
1:00 – 2:30 pm	**OPTIONAL - Environmental Molecular Sciences Laboratory (EMSL) Tour** *Participants: You must pre-register. Check-in at Registration Desk by 12:30 pm. Close-toed shoes are required.*
1:00 pm	**Tour 1: Hi-Res Imaging Lab – Scott Lea** **Tour 2: Cell Isolation and Systems Analysis (CISA) Lab – Will Chrisler** **Tour 3: Nuclear Magnetic Resonance (NMR) Lab – Robert Young & Chaevien Clendinen** **Tour 4: Mass Spectrometry Lab – Danny Orton** **Tour 5: My EMSL - Super Computer Lab – Lee Ann McCue**
2:30 – 4:30 pm	**OPTIONAL – KBase Workshop** *Participants: You must pre-register for this workshop! Questions should be directed to* **engage@kbase.us**.

NOTES

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INVITED SPEAKERS

Opening Keynote: Human and Environmental Microbial Health: A Global Perspective

Jack Gilbert

University of California, San Diego

Understanding the microbial ecosystem dynamics of our planet is central to our role as custodians of the planet. The Earth Microbiome Project aimed to characterize the microbial diversity of the diverse ecosystems across our world, which we have used to model the ecosystem dynamics of these environments. Integrating these models with agricultural policy provides a framework on which to determine how climate change and shifting policy will influence the microbial metabolic dynamics, which will affect our ability to modulate system scale outcomes. The human microbiome is quickly being recognized as a dynamic part of the human ecosystem, and research is starting to demonstrate that using ecology to understand this ecosystem has profound benefits for patient wellness. The immune system controls our interaction with the microbial world, and yet the microbial communities in our bodies are central to modulating the immune response. Changes in the human microbiome have substantial influence on atopy, neurological disorders, metabolic disorders, and a range of complex conditions and disease states. We will discuss evidence of these mechanisms of interaction and how we have started to disturb the delicate balance of the immune-microbe equilibrium, impacting the development and function of our immune systems. Central to this disturbance is the distance we have placed between our children and the microbial world, which has been demonstrated to have a substantial influence on their physiological, immunological, neurological and even endocrinological development. Applying new strategies to identify how the microbial ecosystem correlates with diseases states and treatment efficacy through Microbiome-Wide Association Studies (MWAS) is altering the trajectory of precision medicine, and providing a new framework for facilitating patient care.

Ecological Dynamics of Synthetic Microbial Communities

Ashley Shade

Michigan State University

Synthetic microbial communities provide a controlled way to manipulate and understand the drivers and consequences of diversity and function. We have designed a simple and transferable synthetic community approach to interrogate the interactions of community members that are mediated by extracellular metabolites (exometabolites). We use this approach to ask how interactions among community members change over time, or in response to experimental treatment. Our aim is to use the system understand the consequences of those pairwise member interactions for system-level community outcomes, like resilience and functionality. The synthetic community system offers the potential to collect and integrate rich multi-omic datasets, including transcriptomics to track member gene regulation, mass spectral analysis of exometabolites to track chemical signals and community goods, and flow cytometry to track member success using cell counts or activity staining. The talk will share our latest results using our synthetic community approach, which asks how member interactions change over stationary phase when community resources are limited.

Multi-omic Triangulation of Bacterial Lignin Modification Pathways

Bill Mohn
University of British Columbia, Vancouver, BC

Biotechnological Potential of Extreme Soils: Integrative Multi-Omics and Cultivation Assessment Approaches

Alexandre Soares Rosado
University of California, Davis

How can extremophiles help us find new paths to environmental biotechnology?

Extreme environments and extremophiles are characterized by being a great source of novel biomolecules with unique properties. There is a growing interest in industries for biomolecules, with low toxicity, higher activity and stable under extreme conditions, which are often imposed during industrial processes. Therefore, it is necessary to access underexploited areas with high biotechnology importance.

Among the extreme environments, Antarctica is emblematic and is suffering serious environmental threats. For example, territorial exploration through centers of monitoring, scientific research and tourism lead to contamination, mainly by crude oil and its derivatives. Some places such as the King George Islands and Deception Island (a volcanic island with several geothermal sites with temperatures varying from 0 to 110°C) are examples of ecosystems that have been affected by the contamination by hydrocarbons and are true natural laboratories and rich in microbial diversity of various extreme life spectra.

Our research group has summarized different beneficial roles of microbiomes and proposed new approaches to potentially improve ecosystem resilience. Optimizing and increasing the rate of contaminant degradation, carbon sequestration and nitrogen fixation are important in the modern world. Major advancements are expected in the near future, especially regarding studies on new metabolic pathways from extremophilic microorganisms, by exploring new culture methods and synthetic biology. Here I will present our recent data on novel bacterial groups from hot or cold extreme soils (High salinity and high solvent contamination, Antarctic cold and hot soils) and explain how we are manipulating these microbiomes to protect ecosystems against different threats and also providing potentially sustainable biotechnological applications in Bioremediation, agriculture and energy sector.

SIPping the Icy Waters of Alaska: Multi-omics Approaches to Emerging Contaminant Biodegradation Research

Mary Beth Leigh[1], Christopher P. Kasanke[1,2], and R. Eric Collins[3]

[1]Institute of Arctic Biology, University of Alaska Fairbanks, USA; [2]Current affiliation: Pacific Northwest National Lab, USA; [3]College of Fisheries and Ocean Sciences, University of Alaska Fairbanks, USA

Sulfolane is an industrial solvent an emerging contaminant that is associated with one of the largest contaminated groundwater plumes in the state of Alaska as well as numerous sites globally. Despite its widespread use, little is known about the microbes capable of biodegrading sulfolane or the biodegradation pathways involved. We combined DNA-based stable isotope probing (SIP) with genome-resolved metagenomics to identify microorganisms and putative pathways associated with sulfolane biodegradation in a sulfolane-contaminated aquifer in subarctic Alaska. We identified the primary sulfolane degrader, which comprised ~85% of the labeled community in the amplicon sequencing dataset, as closely related to *Rhodoferax ferrireducens* strain T118. We obtained a 99.8%-complete metagenome-assembled genome for this strain, allowing us to identify putative pathways of sulfolane biodegradation. Although the 4S dibenzothiophene desulfurization pathway has been proposed as an analog for sulfolane biodegradation, we found only a subset of the required genes, suggesting a novel pathway specific to sulfolane may be involved. DszA, the enzyme likely responsible for opening the sulfolane ring structure, was encoded on both the chromosome and a plasmid. Based on our SIP findings, we assessed the distribution of sulfolane-degrading bacteria throughout the 6.4 km-by-4 km-wide contaminated plume, which revealed that sulfolane biodegradation potential is widespread throughout the aquifer, yet is likely inactive under normal conditions. However, the sulfolane-metabolizing *Rhodoferax* sp. was the most dominant microbe in an effective experimental air-sparge system, suggesting that injecting air into the aquifer may stimulate sulfolane biodegradation in situ. This study demonstrates the power of integrating DNA-SIP with metagenomics to characterize emerging organic contaminant degraders and putative pathways and shows how multi-omics data can be applied toward larger-scale environmental process questions.

The Mucosal and Microbial Landscape of Pre-Neoplastic Polyps

William DePaolo

University of Washington

Colorectal cancer (CRC) is the third most common cancer and fourth most common cause of cancer-related death worldwide. Increasing evidence implicates gut bacteria as critical players in CRC. Despite this evidence, the potential role of the microbiome in the development of early colonic neoplasia and whether it can influence the transition from polyp to carcinoma are not known. Since CRC arises along different molecular pathways, and from specific lesions at specific sites, it is possible that distinct commensal bacteria could be involved in each pathway, lesion type, and location. Yet no studies have characterized the gut microbiota of colorectal polyps according to these features. Another intriguing possibility is that changes in the tissue microenvironment due to malignant transformation or local inflammation may provide selective pressure for commensals with certain genes, behaviors and metabolic needs. Therefore, the microbes associated with and around these lesions likely play a more important role than their luminal counterparts. Using a multi-disciplinary approach that pairs next generation sequencing with traditional microbiological techniques, we have begun to study the biogeography associated with pre-cancerous polyps and establish putative genotype-phenotype associations between bacteria and specific polyp sub-types.

Got Microbes? An Update on the Human Milk Microbiome

Michelle "Shelley" McGuire

University of Idaho

Human milk provides sole-source nutrition for exclusively breastfed infants. In addition to the traditional nutrients, milk contains myriad biologically active substances such as hormones, immune factors and cells, nucleic acids, and enzymes. Although long believed sterile, milk is also a rich source of microbes. Substantial research is needed to understand the importance of these microbes to maternal and infant health, but experts postulate that they may be involved in maintaining breast health and colonizing the infant's gastrointestinal (GI) and respiratory tracts with specific taxa that confer early-life and life-long health and wellbeing (McGuire 2017), including optimization of the immune system (LeDoare 2018; Moossavi 2018; Ojo-Okunola 2018).

The origin of the microbial communities in milk is still controversial but thought to be multifactorial including via transport from the mother's GI tract (enteromammary pathway), retrograde inoculation from the infant's mouth during suckling, and the breast skin. Indeed, elegant studies with women suffering from mastitis have shown that maternal consumption of probiotics can result in the appearance of the same bacterial species in the milk (Arroyo 2010; Jiménez 2008), supporting an enteromammary pathway. In a recent report from the CHILD study, Azad and colleagues provided convincing evidence that whether a mother exclusively feeds her infant at the breast or uses a pump to express her milk is related to variation in the microbiome of her milk. Biagi (2018) also found that the milk microbiome changes as women transition from exclusively pumping to feeding their infants at the breast. In addition, we and others have found strong correlations among variations in milk, infant oral, and maternal fecal microbiomes (Pannaraj 2017; Williams 2019), suggesting that these microbial communities are linked and likely interact with each other. Importantly, even women who have never breastfed have a rich microbial community in their mammary glands (e.g., Urbaniak 2016), illustrating that these microbes are reaching the breast via routes not related to suckling.

Factors influencing variation in the human milk microbiome are likely extensive and may include maternal health, nutritional status and dietary patterns, antibiotic use, time postpartum, genetics, and cultural practices. However, substantial inconsistencies exist in the literature regarding these relationships, and this may be due to a plethora of mediating factors. For instance, data from the INSPIRE study which involved 412 relatively healthy mothers and infants living in 8 countries provide rigorous evidence that the milk microbiome varies around the world (Lackey 2019). We have also shown previously that an infant's social environment is associated with the microbial diversity of his/her mother's milk (Meehan 2018). As such, studies relating maternal and environmental factors to the milk microbiome should take into consideration culture, geography, and behaviors. The potential impact of maternal diet is one such factor. Little is known about whether a mother's acute and chronic dietary patterns influence the microbial community structure of her milk, but evidence from our research group and several others suggests that this is a distinct possibility (Williams 2017). Specifically, women consuming more amino acids appear to produce milk with higher levels of Proteobacteria. There are also likely relationships between micronutrient intakes and the milk microbiome. Carefully conducted, controlled intervention studies are needed to investigate and understand better these possibilities.

Arroyo. Clin Infect Dis. 2010;50:1551-8; Biagi. Front Microbiol. 2018;9:2512; Jiménez. Appl Environ Microbiol. 2008;74:4650-5; LeDoare. Front Immunol 2018;9:361; Lackey. Front Nutr. 6:45. doi:10.3389/fnut.2019;00045; McGuire. Curr Opin Biotechnol. 2017;44:63-6; Meehan. Am J Hum Biol. 2018;30:e23131; Moossavi. Front Pediatr. 2018;6:197; Ojo-Okunola. Nutrients. 2018;10: pii:E1643. doi:10.3390/nu10111643; Pannaraj. JAMA Pediatr. 2017;171:647-54; Urbaniak. Appl Environ Microbiol. 2016;82:5039-48; Williams. J Nutr. 2017;147:1739-48; Williams. J Nutr. 2019;149:902-14.

High-Throughput Genetics in Non-Model Bacteria

Adam Deutschbauer

University of California, Berkeley

The Switchgrass Rhizosphere Microbiome and Nitrogen Transformations on Marginal Lands

Maren L. Friesen[1], Richard Allen III White, Renee Petipas, Brett Younginger, Chandra N. Jack, Emily McLachlan, Alan W. Bowsher, Darian Smercina, Lisa K. Tiemann, and Sarah E. Evans

[1]*Washington State University*

Switchgrass (*Panicum virgatum*) is model bioenergy crop with the potential for growth on marginal lands to expand US yield and production. A major challenge for utilizing marginal lands is that they are highly limited in nitrogen, yet switchgrass often shows little growth enhancement under fertilization. We used high-throughput metagenomic sequencing of switchgrass rhizosphere two-weeks apart pre- and post-nitrogen fertilizer application, then elucidated the community structure, bulk metabolic potential, and resolved 28 individual bacteria genomes via metagenomic *de novo* assembly. The overall community structure and diversity did not significantly change over the treatment; however, the bulk metabolic potential carbohydrate-active enzymes were depleted under fertilizer treatment. We resolved genomes from the 'most wanted' soil taxa, and identified a novel putative diazotroph. We also present 3 years of field data collections to characterize switchgrass, soil biogeochemistry, and the rhizosphere microbiome under contrasting nitrogen regimes. A temporal dataset finds contrasting seasonal fluctuations at two sites, while a single-timepoint data collection over six sites spanning Michigan and Wisconsin documents variability over space. A greenhouse experiment identifies selection on belowground functional traits and documents relationships between these traits and members of the microbiome, particularly *Micromonospora* and *Devosia*. Finally, sterile growth systems have been developed to analyze root exudation as well as target specific interactions between switchgrass and nitrogen-fixing bacteria.

Simulated Climate Change and Organic Matter Stoichiometry Co-Regulate Carbon Destabilization in Active Peat

Emily B. Graham, Montana Smith, Sheryl Bell, Allison Thompson, David Hoyt, Malak Tfaily, and Kirsten S. Hofmockel

Pacific Northwest National Laboratory

Peatlands sequester up to 30% of global carbon (C) and are vulnerable to losses in C storage due to climate change. Biogeochemical activity in peat is vertically-stratified, and recent work has highlighted that poorly-understood intermediate peat harbors enhanced respiratory processes. Despite knowledge that active zones are migrating downward due to climate-driven water table suppression, a spatially-explicit understanding of the mechanisms governing organic matter cycling in peat remains elusive. To improve our ability to predict future peatland C destabilization, we investigate peat active layers using multi-omic characterization and novel analytical approaches that elucidate molecular processes associated with C cycling under simulated climate change at Spruce and Peatland Response Under Changing Environments (SPRUCE). We found that (1) warming response of microbial C cycling was distinct in peat mesotlem (30-40 cm) as compared to the more frequently-studied acrotelm (0-10 cm) and (2) the mechanisms governing C metabolism were intertwined with organic nitrogen (N), sulfur (S), and phosphorous (P) cycling. Temperature effects were more pronounced in the mesotlem where changes in water table height have a direct effect on redox status. In the mesotelm, we show decomposition of more chemically-complex organic matter with strong connectivity to organic N in contrast to the rapid cycling of highly bioavailable organic compounds in the acrotelm where organic P- and S- are linked to C cycling. Additionally, specific microbial

taxa were linked to biologically-connected metabolite modules that differentially responded to maximum temperature and CO2 perturbation at each layer. The mesotelm showed an association of anaerobic and nitrogen cycling organisms with metabolite transformations, with a shift towards more salt tolerant microorganisms under simulated climate change. In the acrotelm, climate change-enriched metabolite transformations were associated with methane oxidizing microorganisms in contrast to more generic heterotrophic organisms and extracellular enzyme activity at ambient conditions. We therefore advance that changes in the predominate zone of biogeochemical investigation – the acrotelm – are not reflective of activity in deeper peat. In light of predicted climate-driven changes in the vertical distribution of biogeochemical activity, we determine that interconnectivity between C biochemistry and other elemental cycles are key to improving C cycling predictions in newly active peat. In total, our work demonstrates that environmental attributes that are poorly-represented in models—including organic matter biochemistry and interconnectivity across elemental cycles—are critical considerations for accurately predicting losses in peatland C storage due to climate change.

Investing Bacterial-Fungal Interactions

Patrick Chain

Los Alamos National Laboratory

Fungi and bacteria form different types of associations that are central to numerous environmental processes. This project's goals are to investigate bacterial interactions with fungi at a fundamental level, to establish the types of interactions, their breadth both phylogenetically and functionally, and later, to characterize these interactions at the molecular level. Prior work suggested that bacteria and fungi exploit each other both on the surfaces of fungal mycelial networks, as well as within mycelia. We begin by exploring the diverse nature of these associations by screening both genomic databases as well as culture collections. Available fungal genome projects are screened for the presence of bacterial genomic signatures, while the fungal collections are screened using an approached based on sequencing of the 16S rRNA gene combined with microscopic confirmation of the presence of endohyphal bacteria. For some isolates, we explore some of the interactions phenotypically, by monitoring growth during confrontation assays. We have used proven devices called 'fungal highway' columns, to isolate bacteria capable of utilizing mycelia as a dispersal mechanism, and are testing a 3D fabrication of this type of device for the routine exploration of such interactions. Growth phenotypes of some of these bacterial and fungal isolates, as well as known interacting bacterial-fungal pairs, are examined. Collectively, these studies begin to shed light into the diversity, and range of interactions that occur among these dominant microbial players. https://genomicscience.energy.gov/research/sfas/lanlbfi.shtml

This study was supported by a U.S. Department of Energy Biological and Environmental Research Science Focus Area grant (grant no. DE-AC52-06NA25396).

Interkingdom Interactions of the Soil Microbiome

Kirsten S. Hofmockel[1] and Janet K. Jansson[2]

[1]Environmental Molecular Sciences Division; [2]Biological Sciences Division, Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory, Richland, WA

As climate changes, the mesic grassland ecosystems of the continental United States are predicted to experience increasing periods of drought. The influence of extended drought on functions carried out by interacting members of microbial communities across trophic scales is largely unknown but vital for understanding and predicting outcomes of future climate regimes on soil health and biofuel feedstock sustainability. We used a multi-scale approach to identify the molecular mechanisms underpinning interactions between soil microorganisms in an effort to define how water availability affects interkingdom interactions and the decomposition and cycling of chitin, an abundant molecule in soil. To understand how water availability influences decomposition dynamics among bacteria, archaea and fungi, we conducted

coordinated experiments on field-derived soil communities ranging from model organisms to model soil consortia and soil incubations. By coupling these empirical efforts with individual-based models, we developed new understanding of how soil moisture influences the way enzymes, metabolites, and microbial community members interact to decompose organic carbon. Our results demonstrate how the phenotypes of bacteria and fungi vary depending on the ecological context, including the presence of other organisms, moisture or resource availability, and spatial structure of the microbial habitat. Through the development of reproducible and tractable model soil consortia our research revealed that the taxonomic richness and structure of the soil habitat significantly influences the stability of our soil consortia. Spatial structuring of the community also affected interspecies interactions in space and time, as demonstrated through simulations of individual-based microbial models. Inter-species interactions (including mutualism, competition, antagonism, commensalism, and amensalism) developed unique patterns of spatial organization as communities evolving in time that may influence soil microbiome decomposition dynamics under field conditions. Using understanding derived from simplified systems and modeling experiments, we examined how CO2 respiration by the soil microbiome responds to shifting water regimes. Real-time mass spectrometry coupled with isotopic tracers provided quantitative evidence for the rapid response of soil microbes to changes in soil moisture and the metabolic changes accompanying the elevated respiration. Together these results illustrate the importance of interkingdom interactions and spatial structuring of soil microbiomes in regulating decomposition and CO2 release from soil ecosystems under changing soil moisture conditions.

Multi-Omics Enabled Quantification of Microbial Controls on Biogeochemical Cycles in Permafrost Ecosystems

Neslihan Taş

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Arctic soils store large amounts of biomass and water from warmer periods in the history of the Earth that became preserved in permafrost during cooling and glaciation events. Permafrost soils contain a broad diversity of cold-adapted microbes, whose metabolic activity depends on environmental factors such as temperature changes that cause cycles of freezing and thawing in the soil. Microbial metabolism leads to decomposition of soil organic matter and can substantially affect the cycling of nutrients. However, the relationship between permafrost microbial properties and biogeochemical cycles is poorly understood. Emerging metagenomic studies are uncovering the extent and depth of the permafrost genetic reservoir. In this talk will address how multi-omic approaches aid deciphering the functional and phylogenetic evolution of permafrost microbial communities during thaw across multiple arctic locations.

Viruses in Nature: Lessons from the Oceans, Soils, and Humans

Matthew Sullivan

Ohio State University

Microbes are recently recognized as driving the energy and nutrient transformations that fuel Earth's ecosystems in soils, oceans and humans. Where studied, viruses appear to modulate these microbial impacts in ways ranging from mortality and nutrient recycling to extensive metabolic reprogramming during infection. As environmental virology strives to get a handle on the global virosphere (the diversity of viruses in nature), we face challenges to organize this 'sequence space' (create a sequence-based viral taxonomy), link these viruses to their natural hosts (who infects whom), and establish how virus populations are structured (ecological drivers) and impact natural ecosystems (their impacts). Here I will share current thinking on how to study viruses in complex communities and how these efforts are revealing new biology in the oceans, soils and the clinic that will help enable a new generation of ecosystems biology and medical treatments.

Skin and Soft-Tissue Infections in the Age of Genomics: Analyses in Military Trainees at Fort Benning, GA

D. Scott Merrell

Uniformed Services University of the Health Sciences

Skin and soft-tissue infections (SSTIs) are often caused by *Staphylococcus aureus* and remain one of the most predominant bacterial infections seen in the hospital today. Moreover, certain populations, such as congregate military personnel, are at increased risk for *S. aureus* colonization and SSTI. This is true of Infantry soldiers undergoing One Station Unit Training (OSUT) at Fort Benning, GA, where as many as 1 in 10 of these individuals will develop an SSTI. In an effort to understand the role of the microbiota in development of SSTI, we have completed a number of studies that have investigated the following: 1) differences in nasal microbiota in healthy individuals as compared to those suffering from SSTI, 2) multi-body site (nasal, oropharyngeal, inguinal, and perianal) microbiota differences in healthy individuals as compared to those suffering from SSTI, and 3) microbiota differences seen in purulent abscesses as compared to non-purulent cellulitis samples. Current data indicate that the nasal microbiota differs significantly in individuals that have SSTI, that colonization with S. aureus results in significant changes in the resident microbiota, that the inguinal region may be an unappreciated source of *S. aureus* spread, and that while abscesses are predominated by *S. aureus*, cellulitis samples contain atypical bacteria such as *Rhodanobacter terrae*. Current work includes a longitudinal study that followed trainees upon arrival at Ft. Benning; microbiota swabs were collected from multiple body sites regularly throughout the course of the three-month training exercise. Ongoing analyses of these samples will allow us to watch the individual- as well as group-level evolution of the microbiota in a congregate setting and thus, allow us to identify any correlates of subsequent SSTI risk. Completed and ongoing studies at Ft. Benning can serve as a model for comparable studies to be conducted at other study locations and within additional branches of the US military.

The Gut Microbiome as a Potential Mediator of Warfighter Responses to Operational Stressors

J. Philip Karl

US Army Research Institute of Environmental Medicine

The gut microbiome and its human host coexist in a dynamic, bidirectional relationship that is largely mutually beneficial, but can be perturbed by exposures that directly impact the host, the gut microbiome or both. Recent evidence implicates the gut microbiome and its metabolites as modulators of host responses to environmental, physical, and psychological stress, and there is growing recognition that supporting a healthy and resilient gut microbiome may contribute to health and performance optimization of Warfighters. However, the extent to which military stress impacts the Warfighter gut microbiome, and the implications for Warfighter health and performance are just now beginning to be explored. This talk will consider the potential role of the gut microbiome in Warfighter health and performance, and discuss findings of recently completed studies conducted within the U.S. Army Research Institute of Environmental Medicine (USARIEM) examining relationships between dietary and environmental exposures, the gut microbiome, and host responses during military relevant stress.

Disclaimer: The views expressed in this abstract are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government.

Long Read Tools for Precision Genomics and Metagenomics

Christopher Bradburne

John Hopkins University, Engineering for Professionals

Genome and metagenomic projects are poised to benefit from the emergence of long read sequencing and informatics technologies. More difficult, is deciding when to employ them over traditional short read, high-quality platforms for genome projects and metagenomic applications. We have developed tools such as 'META' to predict and demonstrate performance of different approaches to metagenomic analyses and genome assembly projects in the emerging era of long-read sequencers. META combines several metagenomic analyses into one, allowing a user to select the best performing tool, or combine them in a system-style output. Our analysis approaches also allow differentiation between genomic regions with very small differences and rare variants, while structural variant discovery tools are enabling new insights into actionable phenotypes for mammalian disease and performance.

Effects of Sleep and Circadian Disruption on the Microbiota-Gut-Brain-Axis

Kenneth P. Wright

University of Colorado

Data-Driven Methods for Optimizing Sensor Placement in Microbial Genomes to Diagnose Cell-State

Enoch Yeung

University of California – Santa Barbara

Optimal sensor placement is an important yet unsolved problem in systems and synthetic biology. In this talk we make use of the Koopman observability gramian to develop an algorithm for optimal sensor placement from time-series biological measurements. The Koopman operator lifts nonlinear dynamics to a higher dimensional space where the dynamics evolve approximately linearly in time. Data in biology are often sampled sparsely in time, therefore a method is developed to compute a temporally fine-grained Koopman operator from the temporally coarse-grained Koopman operator. In the case of noisy data, a closed form expression for the error in the coarse-grained Koopman operator is derived. A novel algorithm for optimal sensor placement is developed for the case of a fixed initial condition. The method is finally demonstrated on a simulation example of a biological network. https://arxiv.org/pdf/1906.00944.pdf

Final Day Keynote Speaker: Formation of a Groundwater Microbiome: From Patterns to Mechanisms and Functions

Kirsten Küsel

Friedrich Schiller University, University of Jena

The terrestrial deep biosphere hosts the majority of the earth's microbial biomass. Whereas life in deep saline waters is suggested to be sustained mainly by H2 and CO2, microbes in near-surface groundwater still receive input from recently fixed organic carbon. Groundwater are important drinking water reservoirs, and especially karst aquifers account for about 25% of the human global groundwater supply. Within the research center AquaDiva, we aim to understand the links between the surface and subsurface biogeosphere, especially how organisms inhabiting the subsurface reflect and influence their environment, and affect water and matter transiting the subsurface. To achieve this, we have constructed a novel infrastructure, the Hainich Critical Zone Exploratory (CZE), which provides an excellent platform for studying the formation of groundwater microbiomes and following changes in microbial community structures and their functions in space and time. Our data demonstrate that both surface inputs and the local hydrogeological setting are crucial to influencing groundwater microbiomes. The proximity to the recharge area gave prominence to high bacterial diversity in the groundwater. But environmental selection played an increasingly important role in shaping pelagic microbiomes along the flow paths. Despite the presence of young organic carbon, the microbes assimilated also 14C-free organic carbon to varying degrees. The amount incorporated depended on local biogeochemistry, which was linked to physiological strategies of the core community species. Furthermore, we could show the existence of complex food webs with several levels along the trophic cascade up to metazoan top predators. High genetic potentials for chemolithoautotrophy were correlated with more complex food webs independent of the local environmental conditions, suggesting a substantial input of carbon to the subsurface food web via light-independent CO2-fixation.

From Microbes to Metabolites: Tools To Help Integrate Metabolomics with Microbiomics

David Wishart

University of Alberta

In this presentation I will describe some of the software and databases that my lab has developed over the past few years to facilitate the integration of metabolomics with microbiome studies. First I will describe the Human Metabolome Database (HMDB) and highlight the content it has with regard to microbial metabolites and microbial metabolism. This brief overview will also describe a recently updated sub-database covering the human fecal metabolome. This database is also being used to help define a fecal reference standard for NIST. Next I will describe a new pathway database, called PathBank, which is being designed to facilitate the development of model organism pathway databases. In particular I will highlight PathBank's capabilities to create comprehensive microbial metabolic pathways for almost any microbe. Finally I will discuss BioTransformer, a new tool for predicting microbial metabolism (as well as liver metabolism) of both endogenous and exogenous compounds. This tool could be particularly helpful in the characterization of novel microbial metabolites arising from (gut) microbial transformation of xenobiotic compounds.

Metabolic Phenotyping of Microbial Signatures in Biofluids

Elaine Holmes; Nicholson JK, Turnbaugh P, Elliott P, and Li JV

Health Futures Institute, Murdoch University, Perth, Western Australia

The complexity and metabolic regulation of the human ecosystem is partially controlled by the gut microbiome, which interacts with the mammalian system at the level of genes, proteins and metabolism. Dysbiosis is associated with a wide range of diseases including inflammatory bowel disease, metabolic syndrome, certain cancers and neurodevelopmental conditions such as autism. The metabolic phenotype can provide a window onto dynamic biochemical responses to physiological and pathological stimuli and also contains information relating to the metabolic activity and function of the gut microbiome. Metabolic profiling strategies for analyzing biosamples, encompassing high-resolution spectroscopic methods (NMR spectroscopy, LC-MS, GC-MS, REIMS etc) in combination with multivariate statistical modeling tools, have been shown to be well-suited to generating metabolic signatures reflecting gene-environment interactions and has been used to generate diagnostic signatures of disease [1].

Examples of urinary or faecal metabolites that are products of the microbiota, or microbiota-host interactions include phenols, indoles, bile acids, short chain fatty acids and choline derivatives, all of which can be quantitatively profiled using spectroscopic technology.

Landmark studies in both animal models and humans have shown that obese, lean and insulin resistant individuals carry a different gut microbial composition [2]. Clear differences in microbially-derived metabolites have been shown in urinary, fecal and plasma profiles from obese individuals with metabolites such as hippurate and phenylacetylglutamine being associated with leaner phenotypes [3]. Similarly, bariatric surgery induces a shift in microbial metabolites, which is mirrored in the metagenome [4] and which may have trans-generational impact. By correlating events across multiple biological levels (metagenome, metabolome, plasma miRNAs), we have gained information on the mechanisms by which weight loss is achieved and also shown the influence of bariatric surgery on neonatal outcomes.

The influence of the microbiome on metabolic phenotype is evident in neonates and early life colonization of the gut relates to later life disease. Preterm infants have increased risk of metabolic syndrome and cardiovascular disease in adulthood. We have shown that preterm birth imposes a persistent metabolic signature, observable in adults and characterized by human-microbial co-produced metabolites such as methylamines, hippurate and bile acids. This lecture will give an overview of the microbial influence over human phenotypes.

Computational Metabolomics: Decreasing Our Reliance on Authentic Standards

Jamie R. Nuñez, Sean M. Colby, Thomas O. Metz, Justin G. Teeguarden, Ryan S. Renslow

Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory (PNNL), Richland, WA, USA

Identification of small molecules in complex biological samples will be essential to many future precision medicine-based breakthroughs. For example, the ability to identify all compounds in a sample of human blood could guide studies focused on the effect of being exposed to different conditions (e.g., day shift vs. night shift) and enable detection of disease long before patient's typical treatment regiments. Non-targeted metabolomics is an emerging field focused on meeting these needs, but there are major roadblocks for these analyses that must be overcome. First, the gold standard for unambiguous identification of small molecules is based on comparing two or more orthogonal properties of data from analysis of reference materials to experimental data acquired in the same laboratory with the same analytical methods. This represents a significant limitation since <1% of known molecules are available in pure form, and it is

estimated that we know far less than a hundredth of all natural molecules on Earth. Second, most data of complex samples is collected on a mass spectrometer and most experimental features remain unannotated, leaving a large portion of potentially useful data completely unused. To address these needs, we are (i) advancing chemical property predictions (e.g. ion mobility collision cross section, NMR, and IR spectra prediction) through the creation and utilization of a large-scale computational chemistry platform, the in silico chemical library engine (ISiCLE), (ii) developing a deep-learning network (DarkChem) to generate novel structures that represent candidate molecules that may be present in complex samples, as well as providing their chemical property calculations within milliseconds, and (iii) establishing algorithms and scoring guidelines to assist in chemical identification when multiple types of data are available. Here, I will discuss each of the tools we have developed, several published applications, and the future of metabolomics at PNNL.

Integrating Multi-Omics Data in the KBase Platform to Validate Models, Discover New Pathways, and Improve Ecological Understanding

Chris Henry

Argonne National Laboratory

The Department of Energy Systems Biology Knowledgebase (KBase) is a knowledge creation and discovery environment designed for both biologists and bioinformaticians. KBase integrates a large variety of data and analysis tools, from DOE and other public services, into an easy-to-use platform that leverages scalable computing infrastructure to perform sophisticated systems biology analyses. Recent advances in the KBase platform have enabled integration of metabolomics and transcriptomics data into models derived from genomic data. In one example, we integrate exometabolite data from the Web of Microbes to fill in missing pathways in six isolate genomes, further elucidating the specific genes and pathways responsible for consuming or producing each exometabolite. Second, using the JCVI minimal genome strain, JCVI-syn3.0, we combined cheminformatics and modeling tools in KBase to integrate metabolomics data to discover likely new pathways in metabolite damage in this genome. As a final example, we integrated transcriptomes and metabolomes from the DSM strain of *B. subtilis* to predict the active metabolic pathways in multiple knockout strains across multiple growth conditions. All of these examples demonstrate how the tools and pipelines in KBase can enable the extraction of new biological insights and experimentally testable predictions from multi-omics data. We will also introduce new functionalities in the KBase platform to facilitate sharing and rapid discovery of new multi-omics datasets available within the platform. This includes the ability for users to create their own organization pages, with which they can associate all analyses and datasets performed for that organization within KBase. This also includes tools to rapidly identify nearby genomes and their associated omics datasets within the KBase platform.

CONTRIBUTED TALKS

Launching the National Microbiome Data Collaborative

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The National Microbiome Data Collaborative (NMDC) is ready to start developing resources and building functionality that will empower the research community to more effectively harness microbiome data through a collaborative, open, and integrative data science ecosystem. The NMDC seeks to address fundamental roadblocks in microbiome data science, including 1) the implementation of FAIR data principles (making data findable, accessible, interoperable and reusable), 2) connecting data resources and compute, and 3) developing a framework for community engagement that supports open science and shared ownership.

This will be one opportunity (of many) for microbiome researchers to engage in a discussion around the newly drafted NMDC roadmap, which outlines the initial 2-year development effort and pilot work funded by the Department of Energy. As this effort is community-driven, a short presentation will be followed by time to share ideas and ask questions.

Cryptic Inoviruses Revealed as Pervasive in Bacteria and Archaea Across Earth's Biomes

Simon Roux

DOE Joint Genome Institute

Filamentous single-stranded DNA viruses from the Inoviridae family (inoviruses) exhibit unique morphological and genetic features. While the vast majority of known bacteriophages carry double-stranded DNA genomes encapsidated into icosahedral capsids, inoviruses are instead characterized by rod-shaped or filamentous virions which carry a circular single-stranded DNA genomes of ~5-20kb. Inoviruses are also uniquely able to establish a chronic infection, enabling them to propagate with minimal negative impacts while still modulating host cell physiology and pathogenicity. Owing to their peculiar morphology and simple genome amenable to genetic engineering, several inoviruses are widely used for biotechnological applications, including for phage display or as drug delivery nanocarriers. Yet despite these remarkable properties, few inoviruses have been characterized, and these are known to infect only a limited range of bacterial hosts. Here we show that the current 56 members of the Inoviridae family represent a minute fraction of a highly diverse group of inoviruses. Using a new approach to specifically detect inovirus sequences through custom HMM profiles and a machine learning classifier, we identified 10,295 inovirus-like genomes from publicly available microbial genomes and metagenomes. Collectively, these were estimated to represent six distinct proposed inovirus families infecting both bacteria and archaea across virtually every ecosystem, calling for a complete re-evaluation of the diversity and role of inoviruses in nature. While small, inovirus genomes as a whole encode an expansive functional diversity including toxin-antitoxin systems and other gene expression modulation systems, which distribution is shaped by frequent gene exchange with unrelated groups of viruses, plasmids and transposable elements. Finally, we uncovered evidence of both synergistic (CRISPR evasion) and antagonistic (superinfection exclusion) interactions with co-infecting dsDNA viruses, which were confirmed experimentally in a Pseudomonas model. Capturing this previously obscured component of the global virosphere in an expanded and restructured genome catalog thus provides a renewed framework for further investigation of the different impacts inoviruses have on microbial ecosystems, and for exploration of their extraordinary potential for novel biotechnological applications and manipulation of microbial cells.

Using Genomic Data to Identify Bacterial Associates of Fungi

Geoffrey House[1], Aaron Robinson[1], Andrea Lohberger[2], Fabio Palmieri[2], LaVerne Gallegos-Graves[1], Julia Keliher[1], Demosthenes Morales[1], Armand Dichosa[1], Debora Rodrigues[3], Hang Nhuyen[3], Saskia Bindschedler[2], Jean Challacombe[4], Jamey Young[5], Pilar junier[2], and Patrick Chain[1]

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The amount of publicly available fungal genome sequencing data is increasing quickly. This is due in large part to the 1000 Fungal Genomes Project through the Joint Genome Institute (JGI), with data from over 1300 fungal isolates represented in JGI's Mycocosm database. However, because many fungi have bacteria and viruses associated with them, these DNA sequence datasets from fungi could also provide information about the associated microbiome of these fungal isolates. Furthermore, because the 1000 Fungal Genomes Project seeks to span the full range of known fungal diversity, this presents a unique opportunity to use these DNA sequences to start understanding the diversity of bacteria that may form associations with a wide range of fungi. To this end, we have developed a bioinformatics pipeline that consists of commonly used tools and custom scripts to identify signals of bacteria that co-occur with hundreds of different fungal isolates. We begin the analysis with raw DNA sequencing reads from fungal genome projects and then remove all identifiable fungal DNA sequencing reads in order to reduce spurious similarities to bacteria. Next, we assemble the remaining reads into longer contigs that contain more information to assist in taxonomic classification, and utilize these contigs for discovery of bacteria. We then use the presence of single-copy marker genes to group contigs into bins putatively representing single bacterial genomes. Finally, we use a taxonomy classifier with a custom reference database to identify contigs within these bins with specific bacterial attributions.

We illustrate the utility of this approach using sequence data from 10 *Monosporascus* fungal isolates. Based on observations from the original genome assembly process, three of these isolates are expected to have associated *Ralstonia pickettii* bacteria, and the other seven isolates are not. Using this bioinformatics pipeline, we correctly identified two of the three isolates expected to have associated *Ralstonia* bacteria and confirmed the identification of *Ralstonia pickettii*. These two identified isolates were the ones expected to have the highest amount of *Ralstonia* endobacteria, and for one fungal isolate an estimated 88% of the *Ralstonia* genome was assembled from the fungal sequencing data. The pipeline correctly did not identify any of the other seven fungal isolates as having an associated bacterial signal. We are evaluating how sensitively this pipeline identifies bacterial signals over a range of bacterial abundances. Because determining whether the identified bacterial signals represent true fungal associates or simply sample contaminants remains a challenge, we will continue validating these bacterial signals using fluorescence in situ hybridization (FISH) visualization of bacteria either on or within the *Monosporascus* hyphae. This pipeline is effective in identifying the presence of strong bacterial genomic signals in fungal sequencing data while producing few false positive results in controlled tests. In the future, this workflow can be applied to a wider variety of metagenome samples, including complex soil metagenomes, to identify potential bacterial associates of fungi from a range of environments and to begin understanding the mechanisms that underpin these bacterial and fungal interactions.

Transport of Mineral Cations by *Fusarium Chlamydosporum* in a Mineral Doped Soil Micromodel System

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Fungal species are foundational members of soil microbiomes, where their contributions are key for community resilience under environmental stresses. This is in part due to the ability of fungi to access resources through their ability to form extended and exploratory mycelial networks in adverse environments. For example, in low nutrient environments, some soil fungi can extract essential elements such as K, Na, Fe, etc. by degrading soil minerals using organic acids exuded from their hyphal tips. Thus, fungal mycelial webs can contribute towards weathering and translocation of soil minerals and facilitate plant nutrient uptake from soil. However, the molecular mechanisms underlying mineral transport by fungal hyphae through soil remain poorly understood. To address this knowledge gap, we created a new soil chip platform to study nutrient transport by fungal hyphal networks under different environmental conditions. The soil chips were doped with soil minerals and were manufactured to emulate soil pore characteristics. We applied the soil chips to determine how mineral elements are taken up and translocated by Fusarium chlamydosporum under low nutrient and drought conditions. We observed an increased hyphal density and fungal mechanosensory response (thigmotropism) around obstacles and through pore spaces (~12 µm) in mineral doped microfluidic channels when compared to control conditions without minerals. Time-of-flight secondary ion mass spectrometry (TOF SIMS) analysis of fungal hyphae grown in mineral doped channels demonstrated that the fungi were able to translocate K and Na ions. In addition, we applied proteomics to the fungal biomass extracted from the soil chips to determine what proteins are expressed to enable fungal species to access the soil micronutrients. Essential mineral element sensing and transport by fungi, as demonstrated in this work, provides fungal communities with significant survival advantages over other species by bypassing competition in resource limited soil ecosystems. This study provides the first direct proof of hyphal translocation of mineral elements from a mineral surface under nutrient limiting conditions. Together, these findings provide new knowledge about signaling pathways that enable fungal sensing and weathering of soil mineral nutrients.

Soil Mineral Alterations by a Bacterial Electron Shuttle Produce Opposing Effects Upon Wheat Iron Nutrition Under Dryland and Irrigated Conditions

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Phenazine-1-carboxylic acid (PCA) is a natural antibiotic that is produced in high concentrations by Pseudomonas spp. in dryland wheat rhizospheres in the low-precipitation zone of the Columbia Plateau in central Washington and Oregon. PCA is thought to support anaerobic respiration via dissimilatory reduction of iron- and manganese-bearing minerals, but this respiratory strategy has seldom been studied in oxygen-rich environments such as semi-arid agro-ecosystems where the bioavailability of Fe is often limited due to its presence in oxidized, insoluble forms. We therefore compared Fe concentrations in rhizosphere soil extracts and plant tissues that had been grown with controlled soil moisture in the presence of the PCA-producing strain ***Pseudomonas synxantha* 2-79** or its PCA-deficient mutant in order to test the hypothesis that PCA-producing rhizobacteria enhance the bioavailability of Fe to wheat. Under both dryland and irrigated soil moisture regimes, the PCA-producing strain dramatically altered the crystallinity of soil Fe minerals and replenished the supply of poorly crystalline Fe in the root-depletion zone relative to PCA-free controls. However, the impact upon Fe uptake and translocation by wheat differed between the two soil moisture regimes. Multi-scale X-ray absorption spectroscopy further indicated that the Fe redox

status and identity of Fe-bearing minerals were largely un-affected under dryland conditions despite the significant changes in crystallinity. We attribute these differences between the dryland and irrigated treatments to differences in both soil moisture and in PCA-mediated biofilm development as described in prior work. These findings suggest that the potential efficacy of microbial ecological engineering to promote bio-fertilization or other processes relating to crop and soil health is strongly conditions-dependent – especially given the well-established ability of microbial communities to engineer micro-environments via biofilm development in a conditions-dependent manner.

Functional Gene Analysis of the Gut Microbiota during Acute and Convalescent Travelers' Diarrhea

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Travelers' diarrhea (TD) is a major detriment to job performance and operational readiness among deployed military personnel to tropical and sub-tropical regions. Although antibiotics are effective for reducing TD symptoms and duration, prolonged treatment regimens are often distracting and can negatively impact the resident microflora. Indeed, microbial dysbiosis in the gut is associated with increased susceptibility to gastrointestinal infections, often by antibiotic resistant enteric pathogens. To date, most microbiome analyses have studied microbial structure/composition, while the functional capabilities of the gut microbiota remain largely unstudied. Thus, we set out to understand how the gut microflora are functionally impacted by TD and subsequent antibiotic treatment. To address these knowledge gaps, we collected fecal samples from deployed USA & UK military service members that presented with acute non-inflammatory diarrhea. A sample was collected at acute presentation (day 1, prior to antibiotics, n = 125), as well as 7 (n = 64) and/or 21 (n = 135) days following a single-dose of antibiotics (azithromycin (500 mg), levofloxacin (500 mg), or rifaximin (1650 mg) all in combination with loperamide). Each stool sample underwent culture and TaqMan RT-PCR analyses for pathogen detection. Purified DNA from each sample was analyzed using the HuMiChip v2.0 microarray which contains over 133,000 gene-specific probes involved in various microbial-host processes including antibiotic resistance, virulence, stress responses, and nutrient metabolism. Ordination analysis revealed that subjects with severe TD symptoms had distinct functional profiles from subjects with mild or moderate symptoms. We also noted functional differences associated with geographic location (Kenya, Djibouti, and Honduras). However, we did not observe overt functional differences between pre- and post-antibiotic treatment samples. Furthermore, the data showed no evidence supporting increased acquisition of antibiotic resistance determinants post treatment. Together, these results indicate that single-dose antibiotic regimens may not drastically alter the functional profile of the host gut microflora and should inform future treatment options and antibiotic stewardship in TD patients.

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Acute Diet Stressor Alters Inter-Species Competition for Resistant Starch in the Gut Microbiota

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Gut microbiome homeostasis in vivo is due to complex microbial interactions which can be perturbed by acute stress-induced changes in the gastrointestinal environment, potentially leading to dysbiosis. Diet in particular, can greatly influence the dynamics of gut microbiome composition and metabolism. Here, the influence of a sudden change in diet, namely 21 days sole sustenance on the Meal, Ready-to-Eat (MRE) U.S. military combat ration relative to volunteers on habitual diet (HAB), on population dynamics within the gut microbiome was examined using resistant starch (RS2) as a model fermentable fiber substrate. Fecal samples collected from 10 individuals before and after consuming their habitual diet or only MREs for 21 days underwent 24hr in vitro fermentation in nutrient-rich media supplemented with RS2 under ascending colon domain-specific conditions. 16S rRNA amplicon and Whole Genome Sequencing (WGS) were used to measure community composition and functional potential. Principal Coordinates Analysis (PCoA) showed clustering by diet group and fermentation time point (0hr-24hr), but no apparent clustering by study day within the MRE group. This suggested that consuming a MRE diet for 21 days did not have major effects on community structure in response to RS2. However, more subtle effects on changes in the relative abundances of individual taxa were detected. These effects included a greater increase in *Dorea spp.* and smaller decrease in ***Akkermansia muciniphila*** relative abundances in the presence of RS2 in the MRE group at day 21 relative to day 0 and HAB diet 0-21 days. Conversely, the relative abundances of potentially harmful microbes both from the Proteobacteria Phylum (***Desulfovibrio spp.*** and ***Haemophilus parainfluenzae***) decreased to a greater extent in the presence of RS2 in the MRE group at day 21 relative to day 0 and HAB diet 0-21 days. WGS analysis revealed that starch degradation pathways were more abundant in MRE samples on day 0 vs. day 21 whereas no differences were observed in HAB samples. ***Dorea longicatena*** and ***Ruminococcus torques*** were identified among the top 10 bacterial species contributing to this pathway. These findings suggest that consuming an MRE diet for 21 days minimally effects changes in gut microbiota community structure in response to RS2. Additionally, this study demonstrates how in vitro fermentation can be applied to extend findings derived from human studies aiming to understand effects of stress on competitive nutrient:microbiome interactions.

Metabolomic Profiling of Diverse Microbial Communities for The Earth Microbiome Project (EMP500)

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There is increasing evidence that microbial communities are responsible for a wide range of processes critical to the health of the ecosystems they inhabit and that they impact them in ways which we are yet to fully understand. Sequencing technologies have enormously advanced our knowledge of microbial diversity and composition and have paved the way for functional omics approaches to obtain a molecular and mechanistic understanding of these complex systems. The Earth Microbiome Project (EMP) is an unprecedented effort to map microbial life on the planet, which was made possible by crowd-sourced sample collection and standardized protocols. The first phase of the project focused primarily on cataloguing microbial diversity by generating 16S rRNA amplicon sequencing data from over 27000 samples originating from 7 continents and 43 countries and involved more than 500 researchers. In order to gain functional insights in addition to community composition, the second phase of the project (EMP500) includes metagenomic sequencing and metabolomics analysis of approximately five hundred samples. In this talk I will describe the GC-MS based metabolomics analysis of the EMP500 samples that was carried out at PNNL. We optimized standardized extraction protocols and analysis pipelines, which was challenging given the diverse nature of samples, including soils, feces and sediments, and limited sample amounts. Comparative statistical analyses across samples using PCA and PLS-DA scores plots show clustering based on the environment the sample was collected from. For example, captive terrestrial mammal fecal samples clustered together with captive reptile and amphibian fecal samples, and there was clustering between Palmyra algal samples, ocean sediments, postglacial pond sediments and playa lake samples, which were all derived from aquatic environments, both saline and non-saline. We also identified interesting metabolites within individual study samples. In whale fecal samples, we detected elevated amounts of L-ornithine, a non-proteinogenic amino acid that is an intermediate produced in the urea cycle. In soil samples from Centralia, Pennsylvania, where a coal mine fire burns uncontrolled, we detected fluoranthene and pyrene, polycyclic aromatic hydrocarbons that forms on incomplete combustion of organic compounds. Collectively, our findings demonstrate that metabolomics can provide a mechanistic understanding of functions carried out by microbiomes and also inform us about the environmental factors or stresses that shape the resident microbiota.

Learning Accurate Representations of Microbe-Metabolite Interactions

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The use of multi-omics has seemingly unlimited potential to transform biology. With multiple omics datasets, it may be possible to vastly improve diagnostics, automate drug discovery and possibly identify culturing conditions to grow unknown microbes. However, there are several conceptual challenges for developing techniques to combine microbiome and metabolomics datasets. First, multiple high-dimensional multiomics datasets must be first integrated into a single coherent analysis framework in order to create an intuitive global perspective the underlying biological system. Tools that perform this task such as Canonical correspondence analysis often suffer from complex model interpretation, complicating the process of identifying individual microbe-metabolite interactions. Second, both microbiome and metabolite datasets are both compositional, meaning that total microbe/metabolite counts cannot be inferred from these measurements. This latter aspect severely limits the utility of conventional correlation techniques such as Pearson and Spearman, yielding unreliable correlation values that can approach upwards of 100% false discovery rate (***Weiss et al.*** 2016); (***Friedman*** 2012). Similar problems have been encountered in natural language processing and have been resolved by estimating conditional probabilities of words using neural networks, a notable example being word2vec (Mikolov 2013), a type of compositional matrix factorization of the conditional probability matrix. Here we propose to extend this approach to handle two omics datasets to identify microbial-metabolite interactions in an accurate and interpretable manner. This approach aims to estimate the conditional probabilities of observing a metabolite given the presence of a given microbe. The estimated conditional probability vectors are scale-invariant, negating the need to measure total microbial biomass as suggested by (***Vandeputte et al*** 2017). We demonstrate this method's improvement over Pearson and Spearman correlations in simulations generated from partial differential equation modeling of microbe-metabolite interactions in a biofilm. Furthermore, the proposed method has not only validated known P. aeruginosa-produced metabolites in the context of cystic fibrosis, but also resolved contradictory cyanobacteria-metabolite interactions in previously published studies in context of soils wetting.

ISME YOUNG INVESTIGATOR AWARDEES

Finding the Hosts of the Resistome and Plasmidome

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The rapid spread of antibiotic resistance among bacterial pathogens is a serious human health threat. A wide range of environments have been pointed out as reservoirs of the antibiotic resistance genes (ARGs) we find in today's pathogens. ARGs often travel across populations of diverse microbes on mobile genetic elements such as plasmids and integrons. However, limited information is available about the bacterial hosts of the ARGs and the replicons that carry them in these ecosystems. The main bottleneck is our inability to track mobile genetic elements without having to isolate individual organisms or cells, leaving a substantial gap in our understanding of the ecology of antimicrobial resistance. Proximity-ligation methods such as Hi-C have been introduced as methods to detect interactions between DNA molecules originating in the same cell within microbial communities. Here we hypothesize that by linking ARGs, plasmids, and integrons to their hosts using Hi-C, we can determine the natural reservoirs of ARGs and mobile genetic elements promoting their spread.

We applied the in vivo proximity ligation method Hi-C to a real microbial community known to be a reservoir for ARG and plasmids, i.e., municipal wastewater. A sample of raw wastewater from the municipal wastewater treatment plant of Moscow, Idaho (USA), was used to prepare the Hi-C and shotgun libraries and sequenced. ProxiMeta Hi-C deconvolution yielded >1 000 clusters of contigs and led to the reconstruction of several novel metagenome-assembled genomes (MAGs).

To show that proximity ligation can reconstructs a plasmid-host association from a wastewater community, we first validated the ability of Hi-C to assemble the genome of a completely sequenced plasmid-bearing bacterium from the wastewater metagenome. To a fraction of a wastewater sample, we added the E. coli K12::gfp containing the multi-drug resistance plasmid pB10::rfp so it represented approximately 10% of the total bacterial community. Deconvolution produced a >97% complete E. coli genome, and Hi-C linkage between pB10::rfp and its host was extremely strong relative to other clusters. This confirmed that Hi-C can accurately ascertain plasmid-host relationships within a natural diverse microbial community.

Next, we identified the natural reservoir of ARGs, plasmids, and integrons. By physically linking the ARGs, plasmids, and integrons to their hosts in wastewater we could identify their in situ host range. The IncQ plasmids and class 1 integrons had the broadest host range in this wastewater. Bacteria belonging to Moraxellaceae, Bacteroides and Prevotella, and especially Aeromonadaceae were the most likely reservoirs of ARGs in this community. We detected both previously known and novel associations between ARGs, mobile elements and host genomes, thus validating this method. While several questions about the accuracy and sensitivity of this approach are under investigation by our team and others, we show that in vivo proximity ligation can help identify the natural carriers and the in situ host range of ARGs, plasmids, and integrons in a microbial community.

Gut Microbiome Composition and Mold Exposure are Associated with Allergic Symptoms After Hurricane Harvey

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In August 2017, Hurricane Harvey dropped over 50 inches of rain on Houston, Texas leading to record-breaking catastrophic flooding across the city. Over 100,000 homes were flooded, displacing thousands of Houstonians and causing an estimated $125 billion in damage. After the immediate threat of floodwaters, long-term health concerns remained including chemical exposures to toxins released into the floodwaters from Superfund sites and biological exposures from mold and other pathogens in flooded homes. As a part of the NIH Disaster Research Response (DR2) efforts, an interinstitutional collaborative study was rapidly launched to assess the impact of these environmental exposures. Within one month of the hurricane, 208 subjects were enrolled from four study locations within Harris County, followed by enrollment of 266 subjects at 12-months post-Harvey, including 125 longitudinal subjects. Questionnaires were administered to detail subjects' flooding exposures and health outcomes, with a focus on allergic symptoms associated with mold exposure (sinus, throat and eye irritation, headache, skin rash, shortness of breath, wheezing, chest pain, and cough). Microbiome samples were collected (oral, nasal, stool, and household swabs) and were profiled with 16S rRNA gene sequencing (all sample types) and ITS2 sequencing (nasal and household swabs) to characterize the bacterial and fungal microbiota, respectively. Our analysis revealed that subject-reported exposure to visible mold (inside or outside the home) was significantly associated with increased risk of sinus and eye irritation at 1-month post-Harvey, while the presence of mold in the home was associated with increased risk of these symptoms at 12-months post-Harvey (OR >2.5, $q < 0.10$). Beta diversity analysis (Binary Jaccard) of microbiome samples revealed that the composition of the nasal and stool microbiome varied across Houston neighborhoods and was associated with demographic factors including race/ethnicity and education (EnvFit, $q < 0.05$). After controlling for demographic variables, subject-reported exposure to visible mold was significantly associated with nasal fungal microbiota composition (PCoA, $p = 0.002$), suggesting that nasal microbiome sampling may be an effective method to monitor individual mold exposures. However, no association was detected between nasal mycobiome composition and allergic health outcomes (EnvFit, $q \geq 0.05$). Surprisingly, we found that the stool microbiome at 1-month post-Harvey was significantly associated with allergic health outcomes: the stool microbiome (bacteria) of subjects reporting at least one allergic symptom within 1-month of Hurricane Harvey was distinct compared to subjects that did not experience any allergic symptoms (EnvFit, $q < 0.05$). Intriguingly, this association was strongest for the stool microbiome (bacteria), whereas no association was seen with the fungal or bacterial microbiota at any other sample site tested (nasal, saliva, household swabs; EnvFit, $q \geq 0.05$). Together, these data reveal a previously unappreciated association between mold exposure and the composition of gut microbiota with allergic symptoms after a flooding disaster. These novel findings generated two interesting hypotheses: (1) do allergic symptoms drive changes in gut microbiota composition; or (2) do changes in gut microbiota play a role in systemic allergic responses, potentially via modulation of the immune system's effector vs tolerant responses to environmental antigens such as mold spores.

POSTERS

1. Niche Expansion by Bacterial Isolates Conditioned to Novel Soils

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Introducing isolated microorganisms into diverse soil environments, in an attempt to confer beneficial functions (e.g. N2 fixation), has been a popular agricultural concept for over a century. However, poor consistency in isolate establishment and function remain the primary hurdles for this potential management approach. Most isolated bacteria are likely to be well-adapted to the abiotic conditions (e.g. pH, available carbon sources) of the soil they were collected from, but we hypothesize that conditioning bacterial isolates to novel soil environments will expand their niche breadth, to reflect conditions in the new soils. To examine this, we introduced four taxonomically distinct bacterial isolates (collected from the same soil), as well as a consortium composed of all four, to four different "away" soils, and allowed them to grow in these new soils for varying lengths of time. We are assaying changes in bacterial growth in each new soil environment through respiration assays and colony counts. We are also monitoring for changes in growth in the "home" soil, which could reflect tradeoffs resulting from adaptation. Developing microbial isolates that are specialized to particular soil types could lead to microbial products that are more responsive to the heterogeneous conditions found both between, and within, agricultural systems.

2. Mechanism-Driven, Multi-Omics Approach Using Multi-Technology Meta-Analysis for Novel Microbiome-Derived Drug Discovery in IBD

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Microbiome-driven drug discovery holds great promise for therapeutic development. However, microbiome and host interactions are difficult to disentangle, owing largely to the complexity of a diverse microbiome and the myriad possible direct or indirect interactions through which a microbial product can influence its host. In particular, inflammatory bowel disease (IBD) is an indication for which microbiome-based therapeutics have been proposed but not yet validated. The difficulty in drug discovery for IBD, whether from the microbiome or not, is related to the disease heterogeneity, multiple etiologies and wide variation in treatment responses. To focus drug discovery on key biological targets for targeted patient populations with IBD, we have developed an approach that utilizes paired host gene expression data (RNAseq) in concert with microbiome data (16S NGS, 16S PhyloChip) to identify bacteria of interest from multiple clinical cohorts. We focused our analyses on inflammatory responses associated with the IL-23 pathway, which is a clinically validated target for IBD. By segmenting patients based on their gene expression profile, we hypothesized that the specificity of therapeutics for IBD could be improved and better targeted in a move towards precision medicine. This multi-omics approach leveraged strain-level annotations and multi-technology, meta-analysis (MTMA) to identify bacterial strains that were consistently associated

with a specific host gene expression profile. We identified many strains that were associated with a pro-inflammatory or anti-inflammatory state according to IL-23-related gene expression data from biopsy samples. We tested a subset of the 13 strains associated with the reduced inflammatory state (aka reduced IL-23-related gene expression) in vitro using a primary cell assay to confirm biological activity. Two of the three tested strains demonstrated anti-inflammatory activity and were found to suppress IL-17-secretion. By segmenting the patient cohort according to gene expression profiles, we found strains of interest that differ from those previously observed with traditional comparative analyses according to clinical diagnoses (e.g. ulcerative colitis, Crohn's disease, and control), and we successfully confirmed the predicted in vitro activity of some of these strains. Thus, these results suggest that paired multi-omics analysis in conjunction with multi-technology meta-analysis can improve specificity and increase the probability of discovering novel microbiome-derived therapeutics for heterogeneous diseases such as IBD.

3. Soil Moisture Modulates Inter-Kingdom Interactions as Observed Using a Simulated Soil Core

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Understanding the basic biology that underpins soil microbiome interactions is required in order to predict the metaphenomic response to environmental shifts, such as changing moisture content. A significant knowledge gap is how such changes will affect microbial community structure and its metabolic landscape. Here, we visualized the metabolome of interacting organisms within the soil habitat by obtaining high resolution multidimensional maps of the compositional and functional state of soil microbial communities. Using a custom-built simulated soil core system, called the SoilBox, we demonstrate changes in microbial community growth and composition in different soil environments, contingent upon access to reservoirs of nutrient sources. The SoilBox, designed to press functionalized-glass slides against the soil surface at different depths from the top of the soil surface, emulates the probing depth of a common soil core. This enabled determining both the spatial organization of the microbial communities that form on the slides and their metabolites by using confocal microscopy in combination with matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI), respectively. We found that increased adhesion of soil microbial biomass occurred on slides functionalized with chitin islands, with the seeding attachment event occurring as early as 2 h. The MALDI-MSI data showed a high abundance of bacterial-related lipid families on the chitin islands and low abundance on areas without chitin. Confocal microscopy measurements of these samples confirmed the increased growth of microbial biomass and consumption of chitin. The microbial growth and community composition were also sampled at different moisture regimes (i.e., 14%, 24%, and 34% water to total soil weight). Fungal hyphal networks bridging different chitin islands over distances of 17 mm were observed only in the driest of conditions, indicating that such bridges could potentially act as fungal highways during drought conditions. In all, through the use of correlative imaging platforms, these results illustrate how the SoilBox system can provide unprecedented spatial information about interactions within soil microbial communities as a function of changing environments. We anticipate that further use of our SoilBox approach will be invaluable in probing specific intra- and inter-kingdom metabolic networks arising from a gradient of environmental stresses.

4. Global Soil Metagenomic Evidence Supports Functional Redundancy in Soil Microbes

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Understanding the relationship between soil microbial functional profiles and taxonomic compositions is essential to predict ecosystem functions under various environmental disturbances. It is often presumed that although microbial communities are sensitive to disturbance, ecosystem functions remain relatively stable, as soil microbes are likely to be functionally redundant. Moreover, it is found that microbial diversity reduction in natural soils would not affect "broad" ecosystem processes carried out by a wide range of soil microbes, such as substrate decomposition, but may affect "narrow" processes specialized by certain microorganisms. However, the degree of microbial functional redundancy in soil is still controversial, and thus a comprehensive study to analyze both microbial functional and taxonomic variations based on soil metagenomics on global scales is urgently needed. Here, we show a worldwide comparison of 845 soil metagenomes annotated in functional database of SEED Subsystems and taxonomic database of RefSeq in MG-RAST server from 51 publications and 56 MG-RAST studies. We aim to evaluate to what extent soil microbial functional and taxonomic diversities respond to multiple types of perturbations and whether soil microbes involved in "broad" processes are more functional redundant than "narrow" functions, such as soil nutrient cycling. Our results showed that despite a significant linear correlation between taxonomies and functions, taxonomic compositions of soil microbes had lower pairwise similarity, larger global variations, and were more affected by climate variation and soil properties than functional profiles. Even though functional similarity between "broad" and "narrow" functions remain similar, soil microbes carrying out "broad" ecosystem processes had higher similarity of microbial taxonomic composition than "narrow" processes. The "narrow" functions had more significant correlation between functional and taxonomic compositions than "broad" processes. For beta diversity, classification of 17 climate zones resulted in greater separation in taxonomic compositions than functional profiles. Microbial taxonomic composition of "broad" and "narrow" functions were also significantly different, in which the "broad" processes shared more dominant microbial groups and the "narrow" functions were more abundant in Proteobacteria. Microbial taxonomies formed a larger and more complex co-occurrence network with more module structures than functions. Functional network was closely inter-related among different categories, whereas taxonomic network was more cooperatively interactive in the same phylum/class. The taxonomies of "broad" processes also formed a larger and more complex co-occurrence network than the "narrow" functions, in which the interactions in the "broad" processes were mainly positive but the "narrow" functions only had negative links. Therefore, our results suggest that microbial taxonomic compositions vary to a larger extent than functional profiles in terrestrial ecosystems across the globe, supporting that soil microbes are more functionally rather than taxonomically redundant with a higher level of functional redundancy in the "broad" processes than the "narrow" functions.

5. Antibiotics and Temperature Disrupt Native Palouse Soil Communities and Their Function

Janet Lucas, and Michael Strickland

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Antibiotic compounds are frequently used to promote the health and growth of livestock. As with many medicines, the un-metabolized and active compounds are eliminated through animal waste products (up to 80% of the total dosage) and introduced to the surrounding environment. Additionally, with rising temperatures, livestock are predicted to produce lower yields, increasing our demand for growth-promoting antibiotics. How these introduced, active compounds interact with temperature to shift soil food webs and their function is poorly resolved. To better understand the interactive effects of antibiotics and temperature on soil ecosystems, we conducted a mesocosm experiment. We introduced high or low

doses of the broad-spectrum and commonly administered livestock antibiotic, Monesin, to environments at 15, 20 and 30C. Using multi-omic techniques, we examined microbial community composition and antibiotic resistant gene prevalence, as well as soil functions through a range of assays. We found that antibiotic addition interacts with temperature to disrupt microbial communities and change antibiotic resistant gene prevalence. We also found that antibiotics and temperature shifted microbial community function, but these effects were often not interactive. For example, microbial biomass was decreased in antibiotic treatments but not temperature dependent, while CO2 production was highest in mesocosms at 30C but was unaffected by antibiotic additions. Combined these results underscore the varied but dramatic influence antibiotics and temperature can have on soil ecosystems. The result that antibiotic additions are equal to or greater than the response to temperature highlights the need to monitor anthropogenic introductions of antibiotics, as these can have strong effects on soil community structure and their function.

6. Unraveling the Molecular Mechanisms of the Birch Effect in Soils

Mary Lipton, Montana Smith, Karl Weitz (Presenter)**, and Kirsten Hofmockel**

Pacific Northwest National Laboratory

Microbes in soils control how carbon and other vital nutrients are cycled. However, there are still limitations in many of the analyses we perform to understand this heterogeneous system, including microbial respiration. In many experiments, studying microbial respiration is limited, with no ability to analyze in real time. Often an infrared gas analyzer is used to capture single point CO2 measurements, taken at discrete time intervals and analyzed as a cumulative function We report here on the utility of direct atmospheric monitoring Real-Time High Definition Mass Spectrometry (RTHD-MS) to assess the activity of regenerated living microbial communities through rehydration of the dormant microorganisms in soil.

Electron impact (EI) data acquisition periods of up to 24hrs were collected and revealed new information on the effects of hydration on dormant microbial communities. Water was added to soils and respiration was monitored for 24 hours. Initial CO2 bursts were observed seconds after exposure to water and soil respiration slowly increased until reaching a point of stasis after approximately 90 minutes. This response would be missed with single point measurements. When adding a glucose solution, we observed a biphasic response where the first phase was equivalent to the initial burst of CO2 after hydration followed by a second larger burst of CO2 that could be due to the metabolism of the glucose. We also observed responses that are consistent with glycolysis, including an increase in water in the atmosphere and an uptake of oxygen. To further elucidate this phenomena we added 13C labeled glucose, and we observed the same response, with 12CO2 in the initial burst, and 13C observed much later. This gives insight to the Birch effect, showing that upon hydration microbes are utilizing carbon from osmolytes before they access outside carbon. While these results have been strongly hypothesized, it has not been previously shown. This system allows for detailed monitoring of soil response to substrate addition and can be extended to many other applications."

7. Soil Enzyme Activities Vary Greatly Across Tillage Intensities in Semi-Arid Palouse Soils

Katherine Naasko

Washington State University

Enzymes are key biological indicators of soil health, as they provide insight into metabolic processes, microbial activity and biogeochemical productivity of soil. Specialized enzymes drive decomposition processes, carbon cycling and nutrient cycles of nitrogen, sulfur and phosphorus, and therefore influence availability of these nutrients for uptake by plant roots and other living soil organisms. Enzymes facilitate important ecosystem services that allow for agricultural sustainability and optimal management in terms of nutrient and water management. We evaluated enzyme activity as one of many soil health metrics in the semi-arid, Palouse region of Eastern Washington and Northwestern Idaho. The study encompassed

soil samples from recently harvested, winter wheat sites in pairs of conventional tillage (CT) and no-till (NT) management. We studied p-nitrophenol release associated with six enzymes: beta-glucosidase, beta-glucosaminidase, arylsulfatase, phosphodiesterase, acid phosphatase and alkaline phosphatase. Activities of beta-glucosaminidase and arylsulfatase enzymes were higher in CT sites compared to NT. Beta-glucoside enzyme activity was correlated with hot-water extractable carbon. Phosphatase enzyme activities were correlated with soil pH and permanganate oxidizable carbon. Enzyme activity, in terms of key relationships with important soil carbon and nitrogen pools, show the region's biogeochemical productivity and discuss potential impacts on soil health of the Palouse soil series.

8. Addressing Sample Preservation Strategies for Metabolomics Best Practices within Microbiome Multiomics Studies

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Multiomics studies have largely been focused on genomic, transcriptomic, and proteomic analyses because of the sample amenability to complementary fields. However, metabolomic analyses require additional communications and modifications, particularly in regards to sample collection and sample handling. The largest concern arises from processing biological samples in a manner that ensures the observed metabolomic results are representative of the system perturbation of interest. As multiomics studies integrate metabolomic analyses, it is important to address experimental design modifications to avoid compromising or biasing observed metabolomes. Because of the time-sensitive nature of the metabolome, metabolomics studies require a high level of rigor and consistency across the entire workflow. Numerous publications have discussed the importance of aspects like sample collection, metabolic quenching, extraction methods, stability of the extracted metabolome, measurement design, and data handling as it pertains to metabolomics analyses. The accepted best practices within the metabolomics community aim to reduce operator-sourced or workflow-based biases and increase robustness, reproducibility, and validity of research outcomes.

In particular, microbial (microbiome) samples intended for metabolomic analyses within a multiomics study require an acute awareness of an additional step: sample preservation/stabilization using solutions (fixatives). These solutions are routinely employed in genomic and transcriptomic studies and may not be amenable to metabolomic analyses. This study investigates the impact of two common sample preservation solutions, RNAlater™ and 95% ethanol, on microbial metabolomic profiles relative to lyophilization. Aliquots of lyophilized bacterial cells were incubated with either RNAlater™, 95% ethanol, or untreated (lyophilized) for 48 hours at -80°C. Polar and non-polar metabolomes were extracted from treated cells using a Bligh and Dyer extraction protocol. NMR-based metabolomic analyses allowed for direct and unambiguous comparison of these common preservation methods. The results from this systematic approach to sample storage solution impacts on metabolomic profiles outline important considerations for multiomics researchers. This work will better inform best practices for the experimental design of and sample handling for microbial multiomics studies that incorporate metabolomic analyses.

9. Metaproteomic Approaches for in-Depth Characterization of Complex Soil Microbial Communities

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Mass spectrometry (MS)-based metaproteomic approaches are transforming our ability to understand and characterize microbial communities in environmental and biological systems. These measurements are even enabling enhanced analyses of complex soil microbial communities, which are the most complex microbial systems known to date. At PNNL we have improved sample processing steps (Fig. 1), implemented improved on-line liquid chromatography (LC) and gas phase separations prior to MS detection (Fig. 2), and advanced both MS instrumentation and data processing methods to provide comprehensive metaproteomic characterization of soil communities. We have applied these advanced methods and instrumentation to study Kansas prairie soil samples to gain a better understanding of the members and functions of this microbial community, while evaluating the changes taking place upon biological and environmental perturbations.

10. Metagenomic Community Dynamics in Hawaiian Fishpond Sediments

Laura Tipton, Kiana Frank, Rosanna Alegado, and Nicole Hynson

University of Hawaii at Manoa

Hawaiian fishponds (loko i´a) and their associated aquaculture have both great potential to feed future generations and a deep history in Hawaiian culture. Aquaculture production levels are influenced by many environmental factors, including the local microbes. To examine how microbes interact with and influence environmental factors that ultimately influence fishpond production, we started with the community that resides in the sediments of the He`eia Fishpond. In particular, He`eia Fishpond is capable of producing 0.4 metric tons of fish annually, mirroring their historical production. It is an 88-acre shallow coastal estuary located on the windward coast of O`ahu that is between 600 and 800 years old. While the structure of the He`eia Fishpond is being actively restored, shifts in land-use upstream have resulted in erosion and deposition of a 20 cm deep sediment layer on top of the original carbonate benthos.

Near-shore marine sediments, including those in He`eia Fishpond, are known to exhibit diel (day-to-night) shifts in both geochemical composition and bacterial and archaeal community structure. Taxonomic, geochemical, and functional gene qPCR data suggest these patterns in redox and community structure are driven by complex, cryptic metabolic interactions. Metagenomic approaches are required to examine community scale metabolic pathways and discover gene and gene-family level associations with diel associated redox shifts. By integrating 16S rRNA gene amplicon based analyses with metagenomics, in the context of the geochemical environment, we are able to examine a more comprehensive view of the taxonomic diversity and the functional potential of organisms across different environmental regimes.

In addition to bacterial taxonomic data (from targeted amplicons of the 16S rRNA gene) and geochemistry measurements, we produced metagenomic data from both an Illumina MiSeq and an Oxford Nanopore MinION sequencer. Metagenomic reads confirmed the presence of non-bacterial members of the fishpond community including protists, algea, and importantly, fish. Using reads from two sequencing platforms has allowed us to combine accurate short reads from the MiSeq with reads that are long enough to cover whole genes from the MinION. Our long reads average ten times the length of our short reads, but have a loss in accuracy of 9.5 percentage points (a decrease in Q-score of 27.2). Therefore, it is in combining these metagenomic reads before integrating them with taxonomic and geochemistry measurements, that we are able to get the most information about community scale metabolic pathway potential.

11. Scalable Graph Analytics for Predicting Protein Functions in Metagenomes

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Currently the known protein 'universe' is large and rapidly expanding due to advancements in sequencing technology. The ability to represent protein sequence similarity as graphs and operate on these graphs to extract meaningful biological knowledge is fundamentally limited by issues of data size and computational tools. The explosion in the number and diversity of sequences is a considerable computational challenge but represents an unparalleled and largely untapped opportunity for scientific advancement.

On the other hand, the push for exascale systems has resulted in an unprecedented scale of computing power with tens of billions of computing units (hardware threads). However, the complex and hierarchical nature of these computer architectures pose significant challenges for efficient software development and use. The current and forthcoming Department of Energy leadership class facilities represent an unparalleled and largely untapped opportunity for scientific advancement.

Using a subset of data consisting of 24.8 million sequences stored in over 127,000 files from a database known as PATRIC (https://docs.patricbrc.org/), we present a computational pipeline and preliminary results from building a protein similarity graph by computing over 309 trillion pairwise comparisons resulting in over 37 billion pairs (edges) and 2 Tera Bytes of disk storage. We present initial results from graph analytics on this network showing that we can efficiently annotate proteins using this method and that new insights can be gained coupling this approach with machine learning to extend functional annotations. We will also present scalable tools for graph clustering (community detection), influence maximization, b-matching and graph coloring with numerous applications in computational biology of interest to the community."

12. Metagenomic Interrogation of the Microbial Microenvironment in Combat Wounds

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The increasing incidence of severe, survivable combat injuries in recent conflicts necessitates new approaches to managing wounds from the battlefield. More accurate prognostic guidance is needed, including metrics that assess microbial colonization and infection. Metagenomics-based approaches have the potential to fill this need, and, in combination with statistical modeling, could provide critical guidance for wound infection care. To examine the utility of these tools, wound tissue and effluent samples were examined from 78 wounds in combat-injured patients, including wounds with successful or failed healing. Over 380 samples underwent whole metagenome and targeted antimicrobial resistance gene sequencing. The combined assessment of microbial population and targeted sequencing of 800+ resistance genes yielded a comprehensive portrait of bioburden content in combat wounds. Distinctions were observed across separate wounds at comparable progression points, as well as observation of shifting bioburden content and function across time within single wounds. Amongst resistance gene families associated with resistance to 13 antimicrobial categories, macrolide, aminoglycoside, and beta-lactam resistance genes were the most prevalent at initial wound samplings (49%, 34%, 31% respectively). Statistical modeling identified categories of microbial genomic variables, including resistance, with utility for predicting outcome, and revealed that the predictive value may shift over time. In a mixed modeling

approach, macrolide-lincosamide-streptogramin (MLS) resistance was among the top categories where a large negative effect was fit at the final wound sampling, indicating association with healing failure. Predictive systems such as hidden Markov models are being applied to follow injury samplings in sequence, creating temporally-informed microbial profiles reflective of distinct clinical outcomes. These analyses could also generate synthetic data to simulate diagnostic scenarios. Such insight on content and progression of wound bioburden could predict the trajectory and outcome of infection in combat wounds, facilitating more accurate therapeutic guidance. Adjustment of corresponding treatment at an individual level could reduce morbidity, aid recovery, and improve long-term outcomes.

13. Phylogenetically Novel Prokaryotes in Czech Spring Waters of Cultural Heritage Significance

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There is a diverse range of deep springs in the Czech Republic, each of which, whether hot, radon or brine, is characterized by an extreme, yet stable and unique, environment that has enabled its indigenous microorganisms to evolve for centuries. Although all the Czech deep spring waters are justifiably considered to be a national treasure given their therapeutic benefits, not much research on their microbial life has been carried out. A classic example of a unique subsurface water environment is the hot springs in the spa town of Carlsbad (Karlovy Vary) of cultural heritage significance, whose waters are 18 thousand years old. The structure of the Carlsbad springs results from complex geological formations and intense tectonic activity. Another source of hot spring water can be geothermal activity combined with the decay of radioactive elements. Natural groundwater containing radioactive isotopes is located in a limited number of locations around the world, including the Czech spa town of Jáchymov in the Ore Mountains. Its radon-saturated waters are approximately 13 thousand years old. We hypothesized that prokaryotes found in these ancient waters would be unique from many points of view – phylogenetically with respect to as-yet-undetected taxa, metabolically with respect to the production of biologically active compounds, and ecologically with respect to unusual life strategies and interactions. In order to test our hypothesis, we sampled 4 water springs from both Carlsbad and Jáchymov and analyzed microbial communities therein using cultivation-based approaches, 16S rRNA gene amplicon sequencing and shotgun metagenomics. Cultivation using oligotrophic media resulted in the isolation of only a few bacterial taxa which clustered mostly with Proteobacteria, Actinobacteria and Firmicutes. Despite that, some novel species of bacteria have been isolated. Analysis of 16S rRNA gene amplicons revealed that both yet-to-be cultured Bacteria and Archaea are very abundant in the spring waters in addition to common chemolithotrophs. Shotgun metagenomics allowed us to retrieve tens of high-quality metagenome-assembled genomes (MAGs) and gain deeper insight into the phylogeny and metabolic potential of the prokaryotes inhabiting these ancient waters.

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14. Variation in Human Milk Oligosaccharides, Protein, and Lactose are Related to Variation in Milk and Infant Stool Microbiota

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Background: Along with providing direct nourishment, milk also contains microbes and metabolic substrates (e.g., human milk oligosaccharides, HMO) used by host-associated microbes residing within the infant gastrointestinal tract. Prior data from our INSPIRE consortium have independently shown that profiles of HMO and human milk/infant stool microbiomes in healthy women and infants varies across populations. However, the relationship between milk composition (e.g., HMO) and milk and infant stool microbiomes has not been assessed. Here we examined the association between HMO, lactose, and protein concentrations in milk with paired human milk and infant stool microbial communities collected as part of the INSPIRE study. We hypothesized that (1) variation in milk and infant stool microbiomes is associated with variation in HMO, lactose, and protein concentrations in milk and (2) population differences in milk microbiome composition are predictive of bacterial membership within infant stool samples.

Methods: Milk and infant stool (n=357 maternal-infant dyads) were collected from eleven geographically and culturally diverse sites located across eight countries (Ethiopia, The Gambia, Ghana, Kenya, Peru, Spain, Sweden, and the United States). DNA was extracted from samples and the V1V3 region of the bacterial 16S rRNA gene amplified and sequenced. DNA extraction blanks processed alongside milk samples were utilized as negative controls (n=23) for filtering of putative contaminants with decontam. Sequence data were processed using DADA2. Dirichlet multinomial mixture modeling was used to cluster samples into community state types (CST). SourceTracker was used to predict the likely origin of infant stool microbes. HMO, lactose, and protein concentrations were generated from high-performance liquid chromatography and spectrophotometric assays.

Results: Population of origin was strongly associated with variation in milk and stool microbiomes; however, analysis of CST revealed both milk and stool microbiomes contained fewer discrete clusters than number of populations (PERMANOVA $p<0.001$). We identified four milk clusters (lactotypes) and two infant stool clusters (enterotypes). Examination of milk lactose/protein concentrations revealed significant differences by population and CST. As expected, differences in HMO profiles were driven by 2'-fucosyllactose (2'FL); in concordance, lactotypes varied in the proportion of secretors. Several significant associations between HMO and the structure of milk and infant stool microbiomes were identified. For example, 2'FL was a significant predictor of the community structure of both milk and infant stool. Interestingly, lactose was correlated with the milk microbiome, but not infant stool. Finally, we found that the proportion of infant stool taxa predicted to arise from milk varied across populations, with non-western populations containing an increased proportion of predicted milk taxa.

Conclusions: Data indicate that variation in milk HMO and lactose/protein profiles are associated with variation in the structure of milk and infant stool microbiomes, and that the proportion of infant stool microbes of predicted milk origin vary across populations. Given our results, as well as prior data on the influence of other environmental variables (e.g., pumped versus direct breastfeeding), additional longitudinal studies are needed to better understand the stability of this complex network of host-microbe interactions with respect to environmental factors, as well as its impact on postnatal maternal-infant health.

15. Linking the Resistome to the Microbiome: A Culture-Free Method Links Plasmid, Virus, and Antimicrobial Resistance Genes to their Hosts in Complex Microbial Populations

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Background: The rapid spread of antibiotic resistance is a global health threat. A range of environments have been identified as reservoirs of the antibiotic resistance genes (ARGs) found in pathogens, but we lack understanding of the origins of these ARGs and their spread from environment to clinic. This is partly due to an inability to identify the bacterial hosts of ARGs and the mobile genetic elements that mediate horizontal gene transfer due to the loss of intra-cellular contiguity upon DNA extraction.

Methods: In two recent studies we describe the application of proximity-ligation methods for the determination of the in situ host range of numerous ARGs, viruses, plasmids, and integrons within complex microbiome samples. This method forms physical junctions between sequences present within the same cell prior to DNA extraction. Subsequent sequencing generates a dataset that robustly connects mobile elements to their hosts and can assemble de novo genomes from mixed communities.

Results and Conclusions: Our application of this technology to complex wastewater and rumen samples yielded hundreds of novel ARG-, virus-, and plasmid-host interactions, as well as over a thousand new microbial genomes. These studies highlight the power of the proximity-ligation approach to deconvolving microbiome samples and foreshadow the development of rapid culture-free strategies for tracking and managing the spread of antimicrobial resistance.

16. High-Throughput Single Cell Proteomics Based on Nanodroplet Sample Processing and Ultrasensitive LC-MS

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Biological tissues contain a variety of cell types and subtypes with distinct functions, and understanding heterogeneity at the single cell level is of great interest for biomedical research. Although MS-based proteomic analyses are capable of quantifying thousands of proteins, the extension to single cell studies has been largely ineffective. However, this is not due to the insufficient sensitivity of current LC-MS systems, rather it is largely the result of inefficient single cell isolation and large sample losses during sample preparation. To address these challenges, we developed the nanoPOTS (Nanodroplet Processing in One-pot for Trace Samples) to efficiently process and analyze single mammalian cells containing <0.2 ng total proteins. A microfabricated glass chip with photolithographically-patterned hydrophilic wells was used as reaction vessels for multiple-step proteomic sample preparation. Cultured murine cells or primary human cells were directly sorted into the microchip by flow cytometry (FACS). Only viable cells were selected by pre-labeling cells with a fluorescence dye. A home-made robotic platform was employed to dispense nanoliter reagents into nanowells for proteomic processing. The total processing volume was below 200 nL. Both label-free and isobaric labelling (TMT 10-plex)-based protein quantification were evaluated. An ultralow flow nanoLC (30-μm i.d.) and a Lumos Orbitrap MS were used to maximize the sensitivity. Generated data were analyzed using MS-GF+, Maxquant, and an R-based biostatistics pipeline. The coupling of viability staining and FACS can efficiently isolate single cells into nanowells at high throughput and almost 100% successful rate, while background contamination was significantly minimized. Using HeLa cells as a model sample, the label-free workflow resulted in an average protein identification of 670 protein groups and 332 proteins were quantifiable among the tested single cells. We evaluated the feasibility of our label-free single

cell proteomics platform to differentiate human cell types from a clinical specimen. As a demonstration, primary lung epithelial and mesenchymal cells from a 12-month old female donor were analyzed and 485 proteins identified across the single cells. Principal component analysis (PCA) revealed the single-cell proteome clustered by cell type. Statistical analysis revealed a panel of proteins that were enriched for each cell type, including known epithelial-specific and mesenchymal-specific marker proteins. To improve the analysis throughput, single cells were labeled by isobaric labelling (TMT 10-plex) approach, where at most 10 single cells can be quantified in single shot LC-MS. Pairwise correlations of TMT ion intensities for single-cell-sized digest (0.2 ng peptide in each channel) were from 0.97 to 0.99, indicating excellent quantification reproducibility. Using three cultured murine cell populations (C10, SVEC and Raw cells) labeled with TMT tags, we were able to identify over 1,600 proteins with over 1200 quantified among total "72 single cells. The three cell populations partitioned clearly based on their single cell proteomes alone utilizing various unsupervised methods (PCA, tSNE, hierarchical clustering). Using the TMT 10-plex-based workflow, we were able to quantify >56 single cells in a single day. Further improvement in throughput is expected after optimizing LC gradient time and MS parameters.

17. Microbe-Mediated Soil Moisture Retention and its Variability

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The rhizosphere is the selected region of soil that have dynamic interactions between plant roots and microorganisms. For example, soil bacteria living near plant roots secrete extracellular polymeric substances (EPS), a carbohydrate polymer that can alter soil structure, retain soil moisture and further improve plant growth. In turn, soil structure and water content modulate microbial access to aqueous and gaseous substrates, and thereby control microbial activity. EPS promote soil moisture via three ways: (i) by serving as hydrogel, swelling during wet conditions and remaining hydrated during dry conditions, (ii) by altering soil surface properties through creating water repellent surfaces, and (iii) by promoting the formation of soil particle aggregates.

Here, we employed emulated soil micromodels to systematically investigate the physical, chemical, and biological factors that contribute to soil moisture retention at the pore sale. Emulated soil micromodels are microfluidic devices with a physical structure that closely resembles sandy loam soil. EPS was collected from stationary-phase Sinorhizobium meliloti cultures and suspended at different concentrations in growth media salts or in artificial groundwater. Drying experiments using emulated soil micromodels and EPS solutions of different compositions showed that EPS acts with micro-scale pore structures to strongly retain moisture and alter the spatial distribution of hydrated spaces. Our drying experiment results showed that 0.0015% (w/w) and 0.006% (w/w) EPS solutions dried 8 and 16 times respectively slower than deionized water, respectively. These same EPS solutions maintained 6 ± 1% and 35 ± 21% of residual saturation at steady state, respectively, compared with 0% of residual saturation maintained by deionized water. Drying experiments using other physical geometries confirmed that the water-retaining properties of EPS act only in the inter-aggregate micropore regime (ca., <20 µm diameter), and NOT in intra-aggregate macropores (ca., 1mm diameter). Dilute solutions of EPS decrease the rate of water loss and also amplifies the variability of moisture content in micropores, potentially contributing to functional variability and resiliency or rhizosphere systems. Longer term, we anticipate that the impact of this work will be to aid in the development of sustainable agriculture biotechnology for increased production of biomass on marginal lands.

18. Microbiome Connectivity Drives Productivity in Oilseed Rape Crops

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A well-balanced microbiome at the plant–soil interface is vital for crop production as microbial communities are responsible to soil functions and ecosystem sustainability. This balance is vulnerable to agricultural management practices, including crop rotation, fertilizer regime, pesticide use, irrigation, and continuous cropping. As a result of human population growth and the limited arable lands, continuous cropping has become trendy in agriculture. This practice has been associated with poor plant growth, low yield, and soil-borne diseases. We tested if yield crops of Oilseed rape depends on microbial taxa interactions at the plant–soil interface. The main goal of this study was (1) to explore the links between bacterial and fungal communities at 3 stages in Oilseed Rape crops, and (2) identify microbial communities' connections that lead to yield decline incontinuous cropping systems.

We used field experiments at 2 locations (Warwick Crop Centre and Rothamsted) with two rotations systems: Continuous and Virgin. Sampling of each plot was done at 3 crop stages; 8 leaf stage, at green bud and post flowering. We collected three samples types Bulk soil, Rhizosphere and Roots at 4 randomly positions within the plot. For microbial community analysis, DNA was extracted and marker genes (16S rRNA, 18 rRNA and ITS) were amplified and sequenced. We performed diversity and differential analysis with Phyloseq, Vegan and Deseq2. Microbial networks were constructed with iGraph and the Sparse Correlations for Compositional data algorithm (SparCC). We used visual analytics to explore patterns associated to crop yield.

In root samples, we detect a stronger effect of continuous cropping, measured in differences of microbial composition across rotation systems. We identified taxonomic groups linked to nitrogen and carbon cycling in soil as the most susceptible to continuous cropping. Our results suggest that Nitrogen fixing bacteria (classified with the genera Devosia and Nitrospira) could be used as an indicator for a "healthy soil" for Oilseed Rape crops. At the same time bacteria involved in the breakdown of organic carbon in the soil (from the genera Chthoniobacter) has been found as one of the potential drivers of soil productivity. As expected microorganism that Increase Carbon and Nitrogen in soil promote overall soil health and plant growth.

Microbial taxa are more connected in continuous rotations when compared with virgin crops and connectivity increases along sampling stages. Co-occurrence networks also revealed candidate taxa that may be important to the maintenance of structure and function of soil microbiome as Streptomyces and Flavobacterium. However microbial taxa identified as hubs in "healthy environments" are isolated nodes in continuous crops. This could lead to the establishment and growth of other taxa with potential pathogen activity. Visual analytics indicate that connectivity of keystone microbial taxa in each rotation is related to yield in Oilseed Rape crops. This is the first indication, at taxonomic level, of the dependency of microbial networks in Oilseed Rape crop productivity.

19. Microorganism Communities Associated with Dust Deposition on Snow

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Dust is transported onto snow covered regions either via wind redistribution (dry deposition) or from the atmosphere during a snowfall event (wet deposition). Dust particles can carry microbial organisms from the dust source region to the deposition region. As some microorganisms are able to survive in extreme environments, including the coldest regions of the earth, cell proliferation can occur when growth needs are met. Microorganisms become incorporated into, and can greatly alter, snowpack physical properties including snow structure, pore structure and resulting radiative and mechanical properties. These processes affect the surrounding hydrology on a macro-scale. In this interdisciplinary study, we examine the microbial communities deposited through dust transport and the effects this deposition has on the snow matrix. We examined the microbial communities deposited on snow by dust events in snow samples from the Rocky Mountains in Colorado, USA and the Phoenix Ice Runway near the U.S. McMurdo Station in Antarctica. Microbial communities in the snow samples were examined by genomic and culturing approaches. We found differences in community make up based on global location and between dust events at a single location. Further understanding of microbial associated dust dependent melt affects can aid in snow melt predictions and provide information for snow based infrastructure.

20. Exploratory Data Analysis and Interactive Visualization of FT-MS Data

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The high resolution and mass accuracy of Fourier transform mass spectrometry (FT-MS) has made it an increasingly popular technique for analyzing complex mixtures. Thus, there is a growing demand for informatics tools to analyze FT-MS data to investigate the composition and fundamental chemical properties of complex organic matter. To date, most researchers have utilized custom dataset-specific scripts and pipelines for the downstream processing and visualization of FT-MS data, and the availability of open-source tools has been limited to specific visualizations (Kew, et al., 2017).

We present FREDA (https://msc-viz.emsl.pnnl.gov/FREDA/), a user-friendly web interface providing users with data formatting and processing, filtering, visualization, and sample and group comparison functionalities. FREDA is based on an open-source R package, ftmsRanalysis (http://github.com/EMSL-Computing/ftmsRanalysis), which provides an extensive collection of plotting methods and enables expedient, flexible, and interactive visualization of complex datasets.

FREDA and ftmsRanalysis provide the ability to calculate chemical properties based on the molecular formulae of observed compounds, filter based on characteristics of the data, and create various plots designed to explore the dataset. Data are uploaded as .csv files containing the mass values, peak intensities, and elemental composition information for the experimental samples. FREDA allows a user to summarize their data and calculate many chemical properties of the observed compounds, including the Nominal Oxidation State of Carbon (NOSC), Double Bond Equivalent (DBE), and various elemental ratios (O:C, H:C, etc), along with tabular and graphical summaries of these calculated values. FREDA also provides mechanisms to filter data based on mass, the number of times a compound is observed across the entire dataset, formula assignments, or any other chemical property. The visualizations available in FREDA include: 1) van

Krevelen plots, 2) Kendrick mass defect plots, 3) density plots of calculated values, 4) custom scatter plots, and 5) principal coordinates analysis plots. Users may plot data for single samples, groups of samples, a comparison of two samples, or comparisons of two groups of samples (i.e., treatment groups). Comparisons enable users to identify compounds that are unique to a group of samples, defined either by presence/absence thresholds or a qualitative statistical test. Finally, FREDA supports downloads of the filtered and processed data, with all calculated values, and any figures of interest. We present an example analysis of FTICR-MS data from a soil microbiology study to demonstrate FREDA's core functionality and highlight the interactive visualizations.

21. Development of Metabolomics Approaches to Study 2D and 3D Bacterial-Fungal Co-Cultures

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The goal of this project is to understand the complex metabolic interactions between bacterial and fungal partners in mixed culture environments that mimic natural soil microbiomes. Our team is developing metabolomics and stable isotope labeling approaches to assess the meta-metabolism of both 2D and 3D co-culture systems.

First, we are developing methods for spatial profiling of extracellular metabolites in 2D cultures using Desorption Electrospray Ionization – Imaging Mass Spectrometry (DESI-IMS). The DESI-IMS uses an electrospray mechanism to ionize metabolites from surfaces under ambient conditions. These methods are being applied to study the interactions of the fungus Aspergilllus niger (A. niger) with the bacteria Pseudomonas putida (P. putida) and Cupriavidus oxalaticus (C. oxalaticus). Our hypothesis is that the interactions between fungal:bacterial pairs is dependent on spatial distribution of oxalic acid. A. niger produces oxalic acid for a better growth environment, which lowers the pH of the medium. C. oxalaticus metabolizes oxalic acid and thereby antagonizes A. niger growth by raising the local pH in the vicinity of the bacterial colonies. In contrast, P. putida coexists with A. niger without altering the pH of the medium. We have developed and are currently testing a DESI-IMS strategy to spatially profile the concentration of oxalic acid and other metabolites by growing 2D co-cultures on semi-permeable membranes placed atop agar plates. Using this approach, we expect to investigate nature of the metabolic interactions between these species.

Second, we are developing a novel 3D culture system to isolate and study bacterial:fungal pairs from soil microbiomes. The 3D culture design allows us to mimic conditions in soil using a column filled with glass beads. We have validated our ability to detect a range of metabolites extracted from bacterial and fungal species using GC-MS, and we are currently optimizing our approach to directly extract metabolites from cells grown in this 3D culture system. Once these methods are fully developed, our next steps will be to enrich both axenic cultures and defined co-cultures with 13C-labeled nutrients followed by analysis of isotope enrichment over a series of sample time points. Mathematical models will be developed to aid in the interpretation of the isotope labeling data and the estimation of intracellular metabolic fluxes and extracellular exchange rates. These studies will provide a more detailed understanding of the metabolic interactions that occur in 3D soil microcosms.

With this two-pronged approach, we expect to better understand the microbial metabolic processes that contribute to nutrient assimilation and carbon/nitrogen-cycling in the soil.

22. Multi-Omic Characterization of Dietary Fiber Degradation in the Gut Microbiome

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Pacific Northwest National Laboratory

Despite the importance of the gut microbiota to mammalian health and physiology, there are limited tools available to study biochemical function within the gut. While metagenomic and metatranscriptomic studies can help to identify the genes present and expressed respectively, they are not able to identify which enzymes and organisms are truly responsible for a given activity in vivo. To overcome this limitation, we have developed an approach that applies activity-based protein profiling (ABPP) to gut microbiome samples. ABPP uses small-molecule chemical probes to covalently label enzymes with a given activity, allowing enrichment and protein identification using mass spectrometry. Here, we demonstrate the potential of ABPP in gut microbiome research, by utilizing a chemical probe to detect active glycoside hydrolases in mice given diets that were rich or poor in dietary fiber. We identified 18 different carbohydrate-active enzymes (CAZys) belonging to 16 different CAZy families. We can further correlate protein activity measured by ABPP with metagenomic abundance and metabolomics data to develop a multi-omic characterization of fiber degradation. This demonstrates the capacity for ABPP to reveal novel, key insights into true biochemical function within the microbiome. Because probes for ABPP have already been developed for a variety of enzymatic activities such as proteases and ATPases, this approach can be readily applied wide-range of functions of interest.

23. A Novel Methodology for Deconstructing the Complex Soil Microbiome

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The soil microbiome represents one of the most complex types of microbial communities, encompassing thousands of taxa and numerous metabolic pathways, complicating easy analysis of this system as a whole. This study was conducted to establish an alternative methodology for studying the soil microbiome. Specifically, our goal was to 'break down' the complex microbiome into component parts based on carbon metabolism patterns, then studying each part in higher resolution than would be afforded using a holistic approach, before subsequent reconstruction of the broader soil microbiome.

Selective enrichments with defined growth conditions and carbon substrates were used to obtain metabolically distinct subsets ('functional modules') of the soil microbiome. We hypothesized that i) reproducible, predictable functional modules exist as a function of enrichment conditions, and that ii) in combination, these modules will encompass a large extent of the diversity inherent in the soil microbiome. Initial functional modules were designed around metabolism of common carbon intermediates, before being expanded to include breakdown of complex polysaccharides, resistance to antibiotics, and alternative redox states under anaerobic conditions. Functional modules were first evaluated through 16S amplicon sequencing to obtain estimates of community composition. Less permissive metabolic niches (anaerobic condition or use of complex polysaccharides as a sole carbon source) induced a more distinct community profile with higher richness and more variability between replicates, whereas simpler carbon substrates were dominated by opportunistic Pseudomonas species. To further expand the diversity of our functional modules, a subset of functional modules were combined with environmentally relevant abiotic stresses, in order to evaluate the efficacy of alternative growing conditions on capturing a wider extent of soil diversity. Finally, we performed transcriptomic analysis for five functional modules to see the extent of functional diversity captured through our approach.

24. Effects of Nitrogen Addition on Populus Rhizosphere Microbiome and Early Root Colonization

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Plants are colonized by numerous microorganisms serving important symbiotic functions that are vital to plant growth and success. In many terrestrial ecosystems, plant growth is primarily limited by nitrogen (N) availability; thus, understanding microbial taxa involved in N cycling and plant uptake of N is important for agricultural production and sustainability. In this study, we sought to better understand how nitrogen fertilization alters soil rhizosphere and root endosphere microbial communities during early colonization for two cottonwood species, Populus deltoides and P. trichocarpa. For this, eighty plants representing either species were grown in a greenhouse for a period of 2-3 months with and without added nitrogen using soils from Oregon and West Virginia, which had distinct chemical properties and initial soil microbial communities. Root and rhizosphere microbiomes of select plants were investigated using both shotgun metagenomics and 16S rRNA gene and ITS2 PCR amplicon sequencing. Nitrogen fertilization was found to have pronounced effects on several plant growth factors, e.g., chlorophyll density, plant N allocation, root vs. aboveground biomass allocation, and also corresponded to differences in root and rhizosphere microbiomes. However, these effects were unique to the different soils surveyed as well as the two different Populus species evaluated in this study, thus demonstrating the importance of differences between host species as well as initial soil communities.

25. Biological Control of Aspergillus Niger Through Bacterial Oxalotrophy

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The worldwide emergence of (multi)drug-resistant pathogenic fungi in the last decades is a major problem for human health, agriculture and food security. There is an urgent need to find more sustainable therapeutic approaches to mitigate the rise of antifungal drug resistances. Some phytopathogenic fungi such as Sclerotinia sclerotiorum or Botrytis cinerea are known to use oxalic acid as a pathogenicity factor, but no such link between oxalic acid production and pathogenicity has yet been made in the case of human and animal pathogens. However, oxalate crystals have been reported in the case of aspergillosis, which is one of the fungal-related diseases with increased prevalence in the past few years. Some Aspergillus species, including A. niger, have been associated to a variety of health issues ranging from mild allergies to severe invasive pulmonary infections. For some of these diseases, oxalic acid might play a role in pathogenesis. Oxalic acid is commonly produced by soil fungi and usually occurs in the form of the mineral calcium oxalate. In soils the sink to this mineral is its consumption by oxalotrophic bacteria. The aim of this study is to assess the biocontrol potential of oxalotrophic bacteria to control the growth of A. niger in-vitro and ex-vivo.

Co-cultures confronting A. niger with Pseudomonas putida (non-oxalotrophic control) and Cupriavidus oxalaticus (oxalotrophic bacterium) were performed in-vitro in media with differing nutrient composition (including liquid lung cells medium). The same confrontations will be also conducted in a tissue-engineered lung model. Oxalic acid concentration, as well as different lung epithelial cell inflammatory parameters, will be measured, and cell injury will be assessed.

C. oxalaticus controls fungal growth, but this depends on the media composition. Also, the oxalotrophic bacterium controls oxalic acid production, spore germination and filamentous growth when co-cultured with A. niger in liquid lung cells medium.

With this study, new insights into the biocontrol potential of oxalotrophic bacteria on A. niger are highlighted. This offers a new venue for an alternative and more sustainable therapeutic approach that reduce the risk of resistance selection.

26. Assessment of the Microbiota Metabolome and Its Role in Chronic Systemic Diseases

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Biocrates Life Sciences

Summary: Causal links between the microbiota and diseases are still lacking to a large extent. Metabolomics allows the investigation of microbial metabolic activities and is thus the ideal tool for assessing functional host-microbiome interaction. Human plasma and fecal samples were analyzed by using the newly developed standardized MxP® Quant 500 targeted metabolomics kit allowing for the quantification of 500+ host and gut bacteria-derived metabolites by mass spectrometry. Future applications will further demonstrate the importance of metabolic phenotyping of the holobiont for the investigation of functional microbiome-host interactions, and its causal link pathophysiological processes and chronic systemic diseases, including microbiota-related drug interaction.

Introduction: The microbiome research has reshaped our understanding of human biology and how microbes impact our pathophysiological processes. The role of the microbiome and its symbiotic relationship with the host have shed light on understanding many chronic systemic diseases. Metabolomics is known to provide a snapshot of the phenotype of the holobiont and help investigate its metabolic activities. Here, we discuss the application of newly developed standardized, quantitative targeted assay for multiplexed analysis of host and gut bacteria-derived metabolites by mass spectrometry.

Methods: Human plasma and fecal samples were analyzed by using the MxP® Quant 500 targeted metabolomics kit (Biocrates) allowing for multiplexed analysis of 600+ metabolites by mass spectrometry. 10 µL sample volume were pipetted on a 96 well-plate, preloaded with internal standards. After derivatization and extraction, LC-MS/MS and FIA-MS/MS analyses were performed (Agilent 1290 Infinity UHPLC –SCIEX QTRAP® 5500). MetIDQ™ software was used for the entire automated workflow, from sample registration to quality-controlled results.

Results: In total, over 600 metabolites from 14 small molecule and 12 lipid classes including metabolites produced and/or biochemically modified by gut microbiota were analyzed by using the MxP® Quant 500 kit. The LC-MS analysis provided quantitative results of small molecules covering bile acids, indole derivatives, amino acids and related compounds, amongst other classes. Acylcarnitines, lipids, and monosaccharides were analyzed by FIA-MS. Metabolites were quantified with a high precision in both plasma and fecal samples. To a large extent, the metabolites quantified in feces overlap with those in plasma covering numerous gut microbiota-derived metabolites. A higher number of lipids, especially phosphatidylcholines and triglycerides, are quantified in human plasma compared to fecal samples.

Conclusions & Discussion: The functional microbiome-host interaction is a key aspect leading to new insights and understanding commonalities in chronic systemic diseases such as diabetes, non-alcoholic fatty liver disease, cancer, neurodegenerative diseases, autoimmune diseases, and cardiovascular diseases. In addition to host metabolites, a multitude of metabolites produced or modified by gut microbiota could be quantified in human plasma and fecal samples by using a newly developed standardized, quantitative targeted metabolomics kit solution. This includes metabolites of the choline metabolism, the tryptophan metabolism, bile acids, and branched-chain amino acids. Future applications will further demonstrate the importance of metabolic phenotyping specifically assessing functional gut microbiota-host crosstalk, and its causal link to pathophysiological processes and chronic systemic diseases, including microbiota-related drug interaction.

27. Consequences of Diversity on Microbial Responses to Exometabolite-Mediated Interactions in a Synthetic Microbial Community

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Interactions among microbial populations are expected to have consequences for microbial community function, but it is challenging to observe microbial interactions in the environment. Exometabolites are one method of microbial interaction via excreted chemical signals, and they can be used to cue or inhibit neighboring populations. We developed a synthetic microbial community approach using a transwell system. In this system, discrete member populations are physically separated but share a media reservoir of community goods, including resources and exometabolites. Our system allows for the investigation of microbial interactions facilitated by exometabolites. Here, we used the transwell system to ask how diversity (number of members) and time over stationary growth influence community exometabolites and member gene transcription. Community members included Burkholderia thailandensis, Chromobacterium violaceum, and Pseudomonas syringae. These common environmental bacterial strains were chosen because of their established genomic resources and exometabolite production, and reports of interactions among them. We quantified and compared the dynamics of exometabolite production and transcriptional responses across 7 community conditions- monoculture (3), pairwise (3), full (1). We found that B. thailandensis had a strong influence on its neighbors' transcriptional responses and hypothesized that competitive strategies, like antimicrobial production, were responsible. Indeed, B. thailandensis had the greatest number of up-regulated biosynthetic gene clusters in pair and full community conditions. Interspecies co-expression networks revealed that B. thailandensis was enriched for up-regulated antibiotics, and mass spectrometry analysis confirmed the production of some of these antibiotics. To explore how increased diversity may result in non-additive and potentially unexpected community outcomes, we also report unique transcriptional and exometabolite outcomes observed only in the full community. We expect that this work will contribute to building a systems-level understanding of the impacts of microbial interactions for community outcomes.

28. Investigating the Endo-Hyphal Fungal Microbiome Through Comprehensive Genomic Screens

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The endo-hyphal fungal microbiome contains many uncharacterized inhabitants and interactions. This fungal microbiome, coupled with an extensive network of extracellular interactions with bacteria and plants within the soil, contribute to the complex ecosystem services facilitated by fungi. We sought to characterize the members of the intracellular fungal microbiome as a way to better understand the roles of fungi and their associated endosymbionts. Based on a screening established from sequencing of the 16S rRNA gene of four distinct fungal collections from different geographic locations, we have identified taxonomic signatures of many bacteria not previously known to be associated with fungi. One of these signatures that was found across all culture collections, is a recurrent signal of sequences that are identical to various plant chloroplasts. Several techniques will be utilized in trying to validate the potential associations between fungi and the bacteria represented by these signatures, including FISH staining, isolation, and additional targeted bioinformatic screens using whole genome sequence data. Additional analysis of the data from these culture collections will aid in our understanding of the fungal microbiome and its components.

29. The Role of a Rapid Changing Temperature in Microbial Metabolic Processes During Permafrost Thaw

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Approximately one fourth of the Earth's terrestrial surface is ground that has been continuously frozen for at least two years, called permafrost. Given the numerous studies pointing to an evidence of the acceleration of a warming climate, the fate of permafrost is of great concern. The extent of permafrost thaw as the global temperatures rise has prompted numerous questions related to the release of greenhouse gases (e.g. CO2, CH4, etc.) and overall impact on key ecosystem processes. Here, we sought to understand biochemical processes as permafrost thaws. To do so, we analyzed metabolites from microbial cells originating in frozen permafrost and compared them to cells from thawed permafrost. Through multivariate and univariate statistical methodologies, our study revealed a high similarity in the thawed permafrost metabolome at different sites using both untargeted and targeted metabolomics. We however observed an overall shift in the permafrost metabolic processes as the system thawed. These findings provide an important outlook on the biogeochemical response to permafrost thaw, a knowledge that will become crucial as the Arctic and Antarctic landscapes continue to change due to climate warming.

30. Characterization of Consistent Early Microbial Colonizers of Soils

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A fundamental issue in soil microbiome research is that our ability to sequence soil microorganisms has far outstripped our ability to describe their ecological niche. This is due to the resistance of most microorganisms to culturing. We have developed a soil mesocosm colonization approach to segregate microbes that are consistent early colonizers of sterile soils, thereby providing new ecological information about this subset without direct cultivation. Sterile recipient soil mesocosms were put in contact with unsterilized soil acting as a microbial source. Bacterial colonizers of the recipient soil were characterized at two and eight weeks. We describe the taxonomic and functional overlap of microbial colonizers from source soil microbiomes with different abiotic soils conditions (pH, nutrient profile) and land use histories (agricultural or forested). We also describe how recipient soil nutrient conditions and salinity shape the successful colonizer pool, indicating potential abiotic optima of different bacterial colonizers. This information is of practical use in understanding soil functional development following agricultural practices disrupting soil microbiomes (e.g. soil fumigation, anaerobic soil disinfestation).

31. Micromeda: A Pathway Prediction Pipeline and Web Visualization Tool for Functional Comparisons of Microbial Genomes and Metagenomes

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Technological advances and democratization of genome sequencing over the past decade have facilitated the acquisition of microbial genomes, environmental metagenomes, and metagenome-assembled genomes (MAGs) to the point where gene-based pathway prediction is now a commonly used bioinformatics approach for many microbiology research groups. With thousands of functions potentially encoded by each new microbial genome or metagenome, an important challenge remains to identify effective computational methods for data reduction and comparative visualization of those functions. In order to address these challenges, we developed Micromeda as a combined pathway prediction and web visualization tool. Micromeda allows researchers to generate interactive web-based heat maps for comparing the predicted presence and absence of biochemical pathways and other biological properties across multiple genomes. The web visualization components of Micromeda allow users to search and filter for pathways of interest, revealing pathways that are found in some microbial genomes but not in others. Micromeda generates these visualizations from pathway annotations produced by a custom bioinformatics pipeline based on InterProScan and the Genome Properties database. To support the development of Micromeda, we produced the Pygenprop library, which carries out pathway prediction and allows for programmatic comparison of pathways across multiple organisms. For the more coding inclined researcher, Pygenprop facilitates Jupyter Notebooks-based analyses, including the generation of multivariate statistical ordinations of thousands of genomes based on predicted functional profiles. Additionally, Pygenprop is compatible with scikit-learn, allowing for machine learning-based phenotype prediction from pathway data. Micromeda and Pygenprop are being used to develop a plugin for the newly developed Automatic Tool for Local Assembly Structures (ATLAS) pipeline for metagenome assembly and analysis. Overall, Micromeda and Pygenprop will streamline multi-sample omic analysis workloads and facilitate rapid comparisons of complex functional pathways in genomic and metagenomic data.

32. Mining Genomic Data to Investigate the Evolution of Key Mechanisms Regulating Bacterial-Fungal Interactions

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The availability of an increasing number of genomes encompassing fungal phylogenetic diversity offers a unique opportunity to generate hypotheses regarding the evolution of specific molecular pathways regulating the interactions of fungi with bacterial partners. Genomic screening was applied to investigate i) innate mechanisms of fungal immunity in response to extracellular bacterial partners; ii) the role of low molecular weight organic acids for recognition and metabolic exchange between partners; and iii) potential mechanisms of intra-colony communication and signal transmission. To address each one of these points, specific protein domains of key proteins involved in the synthesis of melanin, oxalic acid and ionic channels were analyzed. An interesting pattern of distribution is starting to emerge separating mycorrhizal fungi and other fungal groups known to host endobacteria from other soil fungi.

33. Piecing Together DNA Viromes from Deeply Sequenced Soil Metagenomes

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The diversity of soil viromes and their potential influences on the host communities, i.e. population control, metabolic add-ons and co-evolution have been understudied in soil. This is due to several challenges including: 1) low sequencing depth, 2) lack of comprehensive soil viral reference databases and 3) lack of computational approaches to access viral diversity and auxiliary metabolic genes (AMGs). To address these challenges, we designed an optimized workflow, 'VirFunnel' for more confident mining of viral sequences from soil metagenomes. VirFunnel ranks searches from multiple databases and integrates results from existing bioinformatics tools. Then the sequences are categorized according to different levels of confidence. We applied VirFunnel to screen for soil viral sequences in three deeply sequenced soil metagenomes (~1TBp for each) from Washington, Kansas and Iowa. To gain more insights into viral diversity and taxonomy, the soil viral sequences were first clustered with reference viral genomes based on the protein sharing network using vConTACT (v2-0.9.10). Tetranucleotide frequency analysis was then used to group related but un-clustered viral fragments to complement the vConTACT results. The resulting annotated AMGs with KEGG orthology (KO) numbers from the three metagenomes were grouped into 515 KEGG pathways and 54 modules. KO coverage of a certain pathway was calculated as an index of viral contribution to a particular metabolic module. The 3D structures of the predicted proteins were obtained from the translated AMGs by searching against the Protein Data Bank (PDB). By integrating the viral information into our current microbial network and metabolic modeling platforms we will further extend our understanding of soil metaphenomes and interkingdom dynamics in response to environmental perturbations.

34. Metatranscriptomic and 16S Rrna Gene Analysis of a Cyanobacteria-Based Soil Surface Consortium with and Without a Diverse Underlying Soil Microbiome

Ryan Trexler, Xin Peng, Mary Ann Bruns, and Terrence Bell

Pennsylvania State University

Cyanobacteria-based soil surface consortia (SSC) are currently being developed as potential agricultural inoculants for adding fixed carbon and nitrogen to soils and to improve soil structure. Similar to other microbial inoculants, little is known about how already established microbes, and the resulting interactions, influence inoculant establishment, persistence, and function after addition. Cyanobacteria-based SSCs provide a tractable model for examining these interactions as their abundant surface growth allows for direct observation and sampling. Here we added a Cyanobacteria-based SSC (DG1) to soil microcosms differing in the presence or absence of a diverse, established microbial community (low / high diversity) and in nitrogen status (added urea / no added urea). The presence of a diverse underlying soil microbiome did not affect Cyanobacterial relative abundance, though non-cyanobacterial members of the consortium decreased in relative abundance. In contrast, the relative abundance of Cyanobacteria decreased in response to urea addition. Both the presence of a diverse underlying microbiome and the addition of urea reduced the abundance of transcripts assigned to Cyanobacteria. Results demonstrate the utility of this system to examine interactions between SSC inoculants and established soil microbiomes. Future work will specifically focus on determining SSC gene expression as a factor of underlying microbiome diversity and soil nitrogen status, and whether these factors affect functions related to inter-microbial competition.

35. Hi-C Method Detects Plasmid-Host Associations in Soil

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Plasmids play a significant role in the spread of antibiotic resistance genes in the environment and have been shown to emerge from farm or feedlot settings. From farms, these plasmids and their antibiotic resistance genes can spread further through agricultural application of manure as fertilizer. The limitations of current methods have prevented us from gaining further insight into the fate of antibiotic resistance plasmids in manure enriched soil. To address this knowledge gap, we are using the Hi-C method to study the fate of plasmids in soil. Hi-C uses proximity ligation to physically link DNA molecules that are present in the same cell, such as plasmids and the chromosome of their host, within microbial communities. Our first objective was to test the limits of Hi-C to detect plasmid transfer in soil. We set up mock communities in soil with decreasing numbers of Pseudomonas putida carrying promiscuous multi-drug resistance plasmid pB10, hereby referred to as transconjugants. The Hi-C method was applied to each of these communities and the resulting Hi-C libraries were sequenced. In our sample with the lowest amount of transconjugants tested so far, 0.1% of the total bacterial community, we still detected links between pB10 and its host. Our results show that the Hi-C method is capable of detecting links between plasmids and their hosts in soil, but given low plasmid transfer frequencies in soil, this limit is not low enough to detect natural plasmid transfer. Future work will include a target capture approach aimed at enriching pB10-containing Hi-C reads. As a culture independent approach, the Hi-C method has the potential to significantly improve our understanding of the range and rate of spread of antibiotic resistance genes that are introduced into soil. This can lead to important insights into the main players in the antibiotic resistance gene dissemination network in soil.

36. The Role of Bacterial-Fungal Interactions in the Functioning of the Oxalate-Carbonate Pathway in Soils

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Fungi and bacteria are both essential actors of biogeochemical cycling in soils, a crucial aspect of ecosystem functioning. While their individual roles have been under intensifying study, far less is known regarding how bacterial-fungal interactions (BFI) affect soil functioning. Both organisms have shared the same environment for millions of years resulting in a myriad of interactions from mutualism to antagonism. Describing such interactions in complex systems such as soils is challenging, as their regulation depends on trophic, biotic, and abiotic factors. Here, we focused on the oxalate-carbonate pathway (OCP), a natural process in which both fungi and bacteria play a crucial role by transforming the poorly soluble calcium-oxalate (Ca-ox) into CO2, and eventually into carbonate. The OCP has a major impact on soil functioning by triggering alkalinisation in acidic soils, which in turn has effects at the ecosystem scale: it positively impacts soil nutrient content and it may lead to the formation of a C sink under a mineral form. As a result, the OCP is a pertinent model to study the impact of microorganisms on both, soil and ecosystem functioning. Using a soil microcosm study with two physically-separated compartments, one with bacteria and/or fungi and one with a Ca-ox source, we were able to highlight that BFI are essential for the functioning of the OCP in soil. Indeed, a correlation between fungal-driven bacterial dispersal, oxalate consumption, and pH changes was observed. Interestingly, fungal and bacterial communities responded differently to the presence or absence of Ca-ox. The two same fungal families (Nectriaceae and Mortierellaceae) dominated the communities, followed by different, less abundant fungi. On the other hand, the contribution of four dominant bacterial families (Burkholderiaceae, Chitinophagaceae, Alcaligenaceae, Flavobacteriaceae) differed in presence or absence of Ca-ox in the soil. Therefore, mycelial networks effectively increased Caox bioaccessibility to bacteria and thus promoted their oxalotrophic activity, but the specificity between fungi and bacteria strongly depended on the presence of Ca-ox. By using a relevant and natural biogeochemical process such as the OCP, we highlight the overlooked role of BFI in biogeochemical processes. As a consequence, future studies aiming at unravelling the factors that drive soil functioning should definitely benefit from integrating BFI.

37. Circadian Biomarkers of Cancer Risk Associated with Night Shift in Humans

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Circadian misalignment has been identified as a risk factor for declining human health associated with modern living conditions such as night shift work. Although epidemiological studies suggest increased risk of various cancers with night shift work, biomarkers and mechanisms are yet to be elucidated. Investigation of the circadian transcriptome and its association with canonical cancer pathways may offer new insights. Here we assessed the transcriptome of 778 genes linked to ten hallmark cancer pathways along

with 18 circadian clock genes using NanoString nCounter analysis method, in lymphocytes collected at 3-hour intervals during a 24-hour constant routine protocol following three day of simulated night shift or day shift in healthy young adults (n=7 in each condition). We found that 16.3% of the genes exhibited significant circadian rhythmicity after day shift as compared to 12.6% after night shift. Of these, 6.6% genes showed circadian rhythmicity in both conditions, with 11 of these genes demonstrating a significant timing (i.e., circadian phase) difference between the two condition. Functional analysis revealed that circadian regulation of a key canonical cancer pathway, genomic stability, was significantly impacted by simulated night shift. These results suggest that loss of circadian regulation of genes involved with canonical cancer pathways may precipitate increased cancer risk in night shift workers.

38. Soil Bacterial and Fungal Communities Interactions in a Microcosm Column

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Fungi and bacteria are cosmopolitan microorganisms that play a key role in ecology, agriculture, forestry and human health. The interactions between those two microorganisms in soil ecosystem are complex and various (from antagonism to mutualism) and can be affected by many environmental factors (Montoya, Pimm and Sole 2006).

This project is focused on the mechanism of interaction between two of the major groups of microbes in the soil community: fungi and bacteria. Understanding the complexity of such environment could give us a better understanding of how the communication between these two microorganisms could affect ecosystem functions.

Our group focused on the collection of fungi and bacteria communities from soil using an in-house designed microcosm column. Similar device has been described as a novel method for the isolation of the bacteria associated with the fungal mycelium in the so-called "fungal highway" (Simon, et al. 2015). For this study four different plant-related media, namely cornmeal, oatmeal, sorghum grain and potato carrot, were used for the isolation of the communities from six different locations. The locations were chosen based on the different plant coverage. The sterile microcosms were placed on the designed area and after one week of contact with the soil, the agar media contained on the top of the column cap was transferred to a new agar plate without antibiotic or fungicide to enrich the total bacterial and fungal community grown in the column.

The DNA of the enriched community was sequenced using ITS and 16S rRNA primers for fungi and bacteria, respectively, in an Illumina Miseq instrument. The obtained data was processed in order to obtain the relative abundance of each community using the QIIME 2 analysis package. A phylogenetic tree was built to better understand the distribution and genomic distance of the different microbes obtained. Further analysis has been conducted to correlate the diversity within the samples and the characteristics of the soil using a Principal Component Analysis.

From the different communities, 45 fungal isolates and 53 exo-bacteria associated were subsequently isolated and tested to determine the type of interaction between two microorganisms obtained from the same community. Two fungi, one belonging to the Didymellaceae family and the other identified as Fusarium oxysporum were selected for further investigation due to their interactions with their associated-bacteria (Microvirga, Paenibacillus and two Bacillus spp.).

Further investigations to comprehend this complex ecosystem will involve the identification of the fungal isolates and the metabolites involved in this microbial interaction.

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39. Seasonality of Microbial Processes at the Root-Soil Interface in a Coniferous Forest as Revealed by the Combination of Metatranscriptomics, Metagenomics and Metabolomics

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Bacteria and fungi are important drivers of ecosystem processes in forest soils, mediating both decomposition and nutrient transfer from and to trees, the main primary producers. It was demonstrated recently, that microbial activity varies among seasons. Here we have explored the contribution of bacteria and fungi to soil processes in a coniferous forest in the context of the seasonal differences among metabolomes. The combination of metabolomics, metagenomics, metatranscriptomics and microbial community sequencing was used to assess the seasonality of nutrient availability at the root/soil interface and its effects on the composition of the microbiomes and their contribution to C and N cycling in early and late vegetation season and early and late winter. Profound differences in metabolome profiles were found between periods of tree activity during the vegetation season and in summer. While the communities of bacteria and fungi were similar across all seasons, their activity differed seasonally. The rhizosphere and root compartments were the most dynamic, but roots, comprising ectomycorrhizal symbionts of tree roots showed higher activity of fungi, especially in summer. The C-cycle processes were dominated by bacteria in soil, but by fungi in litter and the composition of the seasonal metabolomes had further effects on the intensity of utilization of individual C compounds across seasons. In all compartments, seasons of vegetation activity were associated with microbial growth and turnover of cell wall components while in winter, reserve compounds including trehalose, glycogen and mannitol represented important C resources. Our results show that the understanding of soil microbiome functioning is impossible without considering plant activity, demonstrated by the seasonality of metabolomes of soil microhabitats. Forest soils represent highly complex systems where bacteria and fungi locally dominate and contribute together to the C and N cycling in the ecosystem. This work was supported by the FICUS project "The Impacts of Nitrogen Availability and Seasonal Dynamics on Plant-Microbial Interactions Affecting C and N Cycling in Coniferous Forest Soils".

40. Root Blotting Method for Non-Destructive Spatial Analysis of Phosphatase Activity in the Rhizosphere

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Phosphorus (P) is an essential nutrient for plant growth, although much of the phosphorus in soils may not be bioavailable depending on pH and geochemical environment. Understanding how plants access P from their environment is essential to evaluating the growth potential and productivity of plants, particularly in nutrient poor systems. Organic P sources, such as P contained within organic matter, can act as a major source of P in some soil systems. Phosphatases, which act on organic P to liberate inorganic phosphate, are produced by both plants and microbes and are considered one of the most active classes of enzymes in soil. We have developed a root blotting method to image phosphatase activity in the rhizosphere in a spatial manner. Proteins from the rhizosphere can be transferred to a nitrocellulose membrane with retention of enzymatic activity and spatial distribution. Subsequent application of a fluorogenic phosphatase indicator, DDAO phosphate, enables visualization of areas with high phosphatase activity. The proteins can then be fixed to the membrane, and treatment with a fluorescent total protein stain (SYPRO Ruby blot stain) allows for visualization of total protein distribution. Taken together, the images of root phosphatase activity and total protein localization can be mapped back to the root architecture and provide insight into factors affecting the spatial distribution of enzymatic activity and protein accumulation in the rhizosphere. Notably, this method can be applied to plants growing in rhizoboxes containing sand or soil and can be performed multiple times in a non-destructive manner. We anticipate that this fluorescent indicator imaging technique on root blots can be used in diverse plant-microbe-soil systems to better understand the role of phosphatases in P acquisition and soil P cycling.

41. The Combined Application of MPLEx, on-line 2D-LC-MS/MS, and Automated Data Analysis Pipeline Enhances the Deep Analysis of Soil Metaproteomes

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Metaproteomics is a promising technique to study microbial activity in environmental samples and to obtain an in-depth characterization of microbial interactions. However, due to the complexity of soil samples, it is very challenging to extract and analyze soil metaproteomes. Here we utilize the combination of a simultaneous metabolite, protein, and lipid extraction (MPLEx) method, an on-line two-dimensional chromatographic separation approach (2D LC-MS/MS), and a Python based automated data analysis pipeline to enhance our ability in deep analysis of soil samples. The MPLEx extraction protocol helps extract protein from soils with more identification of peptides compared to well-known soil extraction techniques, at the same time it provides opportunities for simultaneous analysis of both metabolites and lipids of the same soil samples. Additionally, the on-line 2D LC-MS/MS fractionates the digested soil proteome in 2 dimensions while coupled directly to the MS instrument. It greatly outperformed the 1D LC-MS/MS in providing significantly more number of peptides identified and proteins observed from soils and has great advantage in dealing with limited biomass compared to conventional off-line 2D LC-MS/MS. Lastly, we developed a Python based automated data analysis pipeline to ensure fast and accurate analysis of spectra counts and intensities from huge databases. As a demonstration, we applied the combination of MPLEx, on-line 2D-LC-MS/MS and automated data analysis pipeline to characterize Kansas native prairie soil metaproteomes in great depth.

42. The Effects of Seasonality and Nitrogen Content on the Norway Spruce (Picea Abies) Forest Soil Microbiome

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The world's forests forms a major component of the global carbon cycle and the microorganisms inhabiting these forest soils play several important roles in the functioning of these ecosystems. Forest soil microbiomes are directly and indirectly affected by the surrounding environmental conditions such as temperature, moisture and organic and inorganic nutrient fluctuations. For example, carbon (C) and nitrogen (N) availability is known to have important consequences on the composition and functioning of soil microbiomes. By following seasonal changes in forest soil microbiomes, we can elucidate the effects of C availability on microbial community composition because the photosynthetic carbon input into soil by coniferous trees is largely absent during cold winters. Here, we characterise the bacterial and fungal communities in Picea abies forests in four seasons in sites in the Czech Republic (high N) and in Norway (low N) by sequencing the 16S rRNA and ITS2 amplicons on the Illumina Miseq platform. This community data was then combined with metabolomic profiles obtained using GC-MS to determine whether different soil compartments could be separated among seasons and N content. By separately sampling P. abies roots, rhizosphere soil, bulk soil and litter, we attempted to identify at which soil horizons the greatest seasonal differences in microbial community composition occurs. Bacterial and fungal communities were different in high and low N sites, and were dominated by completely different bacterial and fungal taxa. Metabolite profiles were correlated with community data and separated the different soil compartments and samples from high and low N sites. Furthermore, bacterial communities differed across seasons in both high and low N sites, with large effect sizes appearing in all soil compartments. The composition of fungal communities was not significantly affected by season, although the relative abundance of some of the dominant fungal taxa decreased in colder seasons. However, ergosterol measurements revealed that fungal biomass does change significantly across seasons. We suggest that more sensitive methods such as community profiling based on RNA-based templates are required. On the contrary, bacterial communities seem to respond faster to seasonal differences, and changes in their composition could be detected in all of the horizons sampled.

43. Resilience and Adaptation Mechanisms of an Extremophile Community After a Catastrophic Climate Event

Gherman Uritskiy, Adam Munn, Samantha Getsin, Diego Gelsinger, James Taylor, and Jocelyne DiRuggiero

John Hopkins University

Microbial communities play a predominant role in the biosphere and, as such, understanding the mechanisms underlying their resistance and resilience is essential to predict the impact of climate change on Earth's ecosystems. However, knowledge of the temporal dynamics in response to acute disturbances is lacking in natural microbial communities. To address this knowledge gap, we used a multi-omics approach to investigate the composition, functional potential, and transcriptional landscape of an endolithic (inside rock) desert microbial community following a rare and catastrophic rainfall event. Our results suggest that the rapid change in osmotic pressure during the rain resulted in a mass death event, leading to a major restructuring of the community taxonomic structure, functional potential, and proteome composition. While the community functioning recovered over the course of a year, the recovered microbiome was comprised of an entirely new set of organisms. The two shifts in community composition – the initial response and the recovery – resulted in a similar degree of functional change but it was achieved by different mechanisms. These two types responses, or modes, allowed for inference of a general microbiome

adaptation model, which can be potentially applied to explain and predict the taxonomic and functional flux in other ecosystems following major environmental changes. We also found that the convergence of the functional potentials of these segregated communities was starkly contrasted by their unique transcriptional landscapes, which challenges some pre-existing assumptions regarding the relationship between genomic and transcriptional elements of microbiomes.

44. Genomic Analysis of Diverse Members of the Fungal Genus Monosporascus Reveals Novel Lineages, Unique Genome Content and the Potential to Harbor Bacterial Endosymbionts

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The genus Monosporascus represents an enigmatic group of fungi important in agriculture and widely distributed in natural arid ecosystems. Of the eight described species of Monosporascus, two (M. cannonballus and M. eutypoides) are important pathogens on the roots of members of the Cucurbitaceae in agricultural settings. The remaining six species are capable of colonizing roots from a diverse host range without causing obvious symptoms of disease. Recent molecular and culture studies have shown that members of the genus are nearly ubiquitous as root endophytes in arid environments of the Southwestern United States. Isolates have been obtained from apparently healthy roots of grasses, shrubs and herbaceous plants located in central New Mexico and other regions of the Southwest. These isolates displayed a wide range of morphological phenotypes in culture and neither asexual nor sexual spore production has been observed in these Southwestern isolates.

Phylogenetic and genomic analyses reveal substantial diversity among these isolates. The New Mexico isolates include close relatives of M. cannonballus and M. ibericus, as well as isolates that represent previously unrecognized lineages. To explore evolutionary relationships within the genus and gain insights into potential ecological functions, we sequenced and assembled the genomes of three M. cannonballus isolates, one M. ibericus isolate and six diverse New Mexico isolates. The assembled genomes were significantly larger than what is typical for the Sordariomycetes despite having predicted gene numbers similar to other members of the class. Variation in the M. cannonballus genomes indicated substantial diversity in genome size and gene content within the species.

While regions of the Monosporascus genomes exhibit synteny with genomes of diverse members of the Xylariales, certain regions do not. Genomic regions lacking clear synteny with other members of the Xylariales are in general AT-rich and are in some cases enriched for genes involved in secondary metabolism. Comparisons of predicted carbohydrate-active enzymes and enzymes involved in pathogenicity suggest that endophytic Monosporascus isolates possess higher numbers of genes for both groups of enzymes than do pathogenic lineages. Several Monosporascus isolates appear to harbor bacterial endosymbionts from the genus Ralstonia.

45. A Robust Florescence Assay for Detection of Primary and Secondary Bile Salt Hydrolysis in the Gut Microbiome

Agne Nixon, Kristoffer Brandvold, and Aaron Wright

Pacific Northwest National Laboratory

The composition of mammalian gut microbiome shapes the host's physiology in many ways, including through modification of endobiotic metabolites, such as bile acids. These acids and their derivatives facilitate absorption of hydrophobic nutrients, and additionally regulate host signaling pathways through binding host receptors. The host in-part determines the composition of the bile salt pool through production of the primary bile salts from cholesterol in the liver. The microbiome can also change the composition of this pool through a variety of metabolic transformations. An important microbial modification of bile salts is hydrolysis of a conjugated amino acid moiety, which can subsequently be used by microorganisms as a nutrient source. Bile salt deconjugation, which is catalyzed by bile salt hydrolase (BSH), is a key modification because it is required for all further microbial metabolism, which can include reduction and oxidation of the sterol core. Increasing evidence suggests a correlation between the bile acid composition and various diseases, such as colon cancer, obesity, and Alzheimer's disease. The therapeutic relevance of the BSH enzyme warrants a need for a sensitive and simple assay for continuous monitoring of BSH activity. Thus, the aim our research was to develop a continuous fluorescence assay that allows for characterization of BSH activity with purified protein, cell lysates, and whole cells with both primary and secondary bile salts. To achieve this goal, we created a suite of synthetic substrates, which yield a fluorescent product upon BSH-dependent turnover. With this assay, we report for the first time an in vivo characterization of BSH activity on all primary and some of the most relevant secondary bile salts that are commonly associated with various human pathologies.

46. Ion Mobility-Mass Spectrometry-Based Proteomics of Soil Microbiome Using Context-Tailored Pathway Information and Targeted Data

Aivett Bilbao, Joon-Yong Lee, Yuqian Gao, Lisa Bramer, Ruonan Wu, Erin S. Baker, Janet Jansson, Kristin E. Burnum-Johnson,

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Soil is one of the most important global carbon reservoirs which supports the terrestrial biosphere through complex microbial ecosystems. The information for modelling and understanding the collective physiological responses of the soil microbial community at the phenotype level is provided by protein expression. However, the complexity of thousands of different species in the soil metaproteome challenges the analytical and computational strategies maturely applied in bottom-up mass spectrometry (MS) analyses for protein samples with fewer organisms.

Here we present a computational approach to perform accurate quantitation and capture regulation of proteomic signatures in soil. Our method relies in two key aspects. First, biological context-tailored constrains are added in an informed manner by selecting specific signaling pathways of interest based on kinetic modelling of consortium level metabolic pathways. Second, the increased sensitivity and dynamic range afforded by ion-mobility is exploited by extracting the signals of in-silico generated protein targets, in a directed fashion from the raw data.

This method is developed to compare soil samples under glycine nutrient treatment against untreated controls where the treatment increased proliferation and enriched for specific microbial species. Samples from three locations were collected in triplicate at the Konza Long-Term Ecological Research field station in Kansas. Protein extracts were digested into peptides and analyzed by mass spectrometry combined with liquid chromatography (LC) and ion mobility (IM) separations. A list of 1279 proteins related to pathways of interest was generated from novel modeling approaches to identify these treatment-enriched microbial species. Our approach allows us to probe proteomics signals in data generated with ion mobility to gain insight into the microbial biochemical mechanisms in soil.

47. Genomic, Molecular and Microscopic Insights into the Organo-Mineral Associations in a Ponderosa Pine Rhizosphere

Alice Dohnalkova[1], Colin Brislawn[1], Malak Tfaily[2], Rosalie K. Chu[1] and Peyton Smith[3]

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Micro-scale organo-mineral associations in soils are the preferred habitat for ubiquitous microbial communities, where complex processes including mineral aggregate formation, microbial mineral weathering, and soil organic matter (SOM) stabilization all occur in a narrow zone of biogeochemical gradients. This study aimed to investigate these processes in a pine forest rhizosphere experiment. We placed in-growth mesh bags containing biotite in the ponderosa pine rhizosphere and after 9 months analyzed their contents for bacterial and fungal microbiomes, highlighting the influence of bacterial and fungal composition and the importance of Betaproteobacteria in mineral weathering processes. The molecular-level identification of newly-formed organic compounds was obtained by Fourier-transform ion cyclotron resonance mass spectrometry. We also used high-resolution electron microscopy to examine the nature of organo-mineral associations, aggregation, and mineral weathering processes.

Significant phylogenetic differences in alpha- and beta-diversity were observed among the mineral-associated bacteria as compared to the source microbiome in the bulk soil, with the bacterial microbiome differing more than the fungal. These results are in agreement with research [1, 2] showing that the chemical composition of soil minerals drives the composition of mineral-associated microbial communities. We showed linkages amongst organo-mineral associations and organic carbon processes that were driven by newly formed organic matter produced by microbial activity in mesh bags with biotite.

We conclude that the dynamics of microbial colonization of mineral surfaces in soil are foundational to the stabilization of SOM and to the soil organic carbon cycling, and that multiple approaches must be combined to gain insight into soil processes on larger scales.

[1] Colin, Y., et al., Mineral Types and Tree Species Determine the Functional and Taxonomic Structures of Forest Soil Bacterial Communities. Applied and Environmental Microbiology, 2017. 85(4).

[2] Ahmed, E., et al., Mineral Type Structures Soil Microbial Communities. Geomicrobiology Journal, 2017. 34(6): p. 538-545.

48. Share Impressively with DataHub

Colin Brislawn, Jason McDermott, Ian Smith, Dave Millard, and Mark Borkum

Pacific Northwest National Laboratory

We all strive to make our research data Findable, Accessible, Interoperable, and Reusable, but that's easier said than done. Our team has been leveraging DataHub to organize, publish, and promote our multi-omics data and share our science with the world. From automatic data collection to DOI minting and custom web pages, learn how you can make DataHub work for you.

49. PSpecteR: A Proteomics Data Analysis Application in R

David Degnan, Lee Ann McCue, Lisa Bramer, and Aivett Bilbao

Pacific Northwest National Laboratory

Proteomics research aims to understand cellular processes and gene functions at the phenotypic level by analyzing the whole set of expressed proteins within biological systems. The preferred method to identify and quantify proteins is liquid chromatography coupled to mass spectrometry (LC-MS), where either intact proteins (top-down) or proteins digested into peptides (bottom-up), are eluted from an LC column, ionized, and analyzed with MS. The resulting LC-MS data files are large and compressed in binary formats (.raw), requiring specialized analysis software. This complicates quality control (QC) and visualization of LC-MS data, specifically the extraction of raw spectra and metadata, evaluation of fragmentation pattern reproducibility, and assessment of spectrum quality. We used extant packages in R to develop an open source graphical user interface (GUI) to interact with proteomic LC-MS .raw files, PSpecteR. Our app allows users to import .raw or XML-formatted MS data and either call a protein identification algorithm to match the spectra to proteins (e.g., MSPathFinder or MSGF+) or provide the identified protein IDs and FASTA database files. Using peptide-spectrum matches, in-silico fragment ions of peptides are generated and raw MS/MS spectra are automatically annotated with multiple ion types that the user can interactively chose and change. PSpecteR also offers visualization of protein fragmentation patterns with ppm error heatmaps, testing of various post-translational modifications, mapping diagrams of the identified sequences, plotting features across spectra metadata, extracted ion chromatograms (XICs), and the ability to export all generated plots, tables, and spectra. PSpecteR is a user- and coder-friendly GUI which demonstrates that, like code, GUIs can be flexible and open source.

50. Observing the Effect of Bacterial Presence on Fungal Spore Germination.

Demosthenes Morales[1,2], Julia Kelliher[2], Geoffrey House[2], Aaron Robinson[2], Laverne Gallegos-Graves[2], James Werner[1], Patrick Chain[2]

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Linking bacterial and fungal co-occurrence in soil to functional interactions is a present challenge in understanding complex microbial networks. Next-generation sequencing (NGS) offers a broad view of these interactions but lacks the resolution to catalogue behaviors as either mutualistic or antagonistic, for example. Here, we are characterizing bacteria and fungi at the single cell level to elucidate the influence of spatial and temporal dependent interactions. In our first case, we investigate the influence bacteria have on the germination of the fungal biocontrol agent, Trichoderma harzianum, and other Trichoderma species. Using brightfield microscopy, we determine the rate of germination for a population of spores in the presence of bacterial partners relative to independent spores. This visual phenotypic result will motivate further molecular investigations to determine the contribution by microbial partners in influencing fungal growth. We find that bacterial presence is linked to either acceleration or retardation of spore germination and may have implications in promoting efficient growth of beneficial fungal strains especially in stressed environments, which is of interest for biotechnological applications.

51. Circadian Misalignment Through Night Shift Simulation Disrupts Plasma Lipidome

Jennifer Kyle[1], Lisa Bramer[1], Daniel Claborne[1], Kelly Stratton[1], Kent Bloodsworth[1], Justin Teeguarden[1], Thomas Metz[1], Shobhan Gaddameedhi[2], and Hans Van Dongen[2]

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The circadian system coordinates daily rhythms in lipid metabolism including lipid storage and utilization. Disruptions to internal circadian rhythmicity due to altered sleep/wake schedules, such as in night shift work, has been implicated in increased risk of metabolic disorders, including obesity, type 2 diabetes, and cardiovascular disease. To determine the impact of night shift work on the blood plasma lipidome, an in-laboratory simulated shift work study was conducted in the Sleep and Performance Research Center at Washington State University Spokane. Fourteen healthy adults (aged 22–34, 4 females, 10 males) were randomized to 3 days on simulated night shift (n=7; scheduled wakefulness 18:00–10:00) or simulated day shift (n=7; scheduled wakefulness 06:00–22:00). This was followed by 24 hours of constant routine with hourly isocaloric food intake, during which subjects were kept awake with constant semirecumbent posture under fixed laboratory conditions. During the constant routine, blood samples were collected at 3-hour intervals through an intravenous catheter. Lipids were extracted from subjects' blood plasma samples assayed with untargeted liquid chromatography-tandem mass-spectrometry (LC-MS/MS). Over 400 discrete lipid molecular species were identified and quantified across 374 LC-MS peaks covering 5 lipid categories (sphingolipids, glycerolipids, glycerophospholipids, sterols, and fatty acyls) and 21 subclasses (e.g., ceramide, diacylglycerophosphocholines (PCs), and triglycerides (TGs)). Depending on the prior shift schedule, 24-hour mean levels were altered for 14% of lipids. Specifically, mean levels reduced after the night shift schedule were mostly phospholipids and statistically enriched (EASE score, $p<0.05$) lipids containing the fatty acid 18:2, specifically PC lipids, phospholipids containing 18:1, and TGs containing 12:0. Following the simulated night shift schedule, 33% of lipids underwent a statistical (p-value <0.05) phase shift. These lipids were dominantly monoacylglycerophosphocholines (LPCs), including all LPCs with C18 and C20 fatty acids, and TGs. As these lipid changes were measured under constant routine, they were endogenous and not an artifact of masking by behavioral schedules. In conclusion, circulating plasma lipids were highly altered after simulated night shift work, with preliminary evidence of dysregulation of cholesterol and triglyceride metabolism.

52. Metaproteomic Data Analysis with Deep Learning-Based De Novo Peptide Sequencing

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Meta-omics data is a crucial component to understand the function and composition of the microbial communities in soil environments. Especially, metaproteome data can better address the microbial activities well correlated with protein abundances, while metagenome and metatranscriptomic data may not directly describe cell functions and conditions. Bioinformatic challenges such as data complexity, lack of protein databases for environmental samples, and the following inaccurate estimation of false discovery rates (FDRs), however, hinder metaproteomic data analysis. In this study, we present a novel framework to analyze the metaproteomic data from soil samples to identify the peptides and proteins by incorporating the deep learning-based de novo peptide sequencing. Kaiko is a deep learning model trained for database-free peptide identification. The Kaiko model was trained by about 5.76 million spectra obtained by high resolution mass spectrometry representing 1 million unique peptides using rigorous quality controls.

Comparing with the conventional tools, Kaiko showed better performance in predicting peptide sequences from unseen MS/MS spectra with the de novo approach. Soil samples of three sites were collected at the Konza Prairie Biological Station (KPBS), a Long–Term Ecological Research (LTER) site representative of native tallgrass prairie in the Flint Hills of eastern Kansas. In a metaproteomic workflow, we introduced the de novo peptide sequencing with Kaiko to extract the most probable peptide sequences for each spectrum. Based on the Kaiko predictions, highest scoring taxa were identified by performing alignment search against the reference protein sequences. After building a database containing protein sequences of these taxa from the reference sequences, we performed MS-GF+ against this database and identified the peptides and proteins by estimated FDRs using the target decoy approach. We compared the proposed pipeline with the typical one based on the database construction from metagenome sequences. We will present that de novo sequencing leads to more and complementary identifications for soil metaproteomic data.

53. pmartR: Software for Quality Control and Statistics Robust to Missing Data for Mass Spectrometry-based Biological Data

Kelly Stratton, Lisa Bramer, Lee Ann McCue, Bobbie-Jo Webb-Robinson, Bryan Stanfill, Daniel Claborne, Allison Thompson, and Iobani Godinez

Pacific Northwest National Laboratory

Prior to statistical analysis of mass spectrometry (MS) data, quality control (QC) of the identified biomolecule peak intensities is imperative to reduce process-based sources of variation and extreme biological outliers. Without this step, statistical results can be biased. Additionally, liquid-chromatography-MS proteomics data presents inherent challenges due to large amounts of missing data that require special consideration during statistical analysis. We present pmartR, an open-source R package, for proteomics data at the peptide- or protein-level (either unlabeled or labeled with an isobaric tag), as well as for metabolomics and lipidomics data. The pmartR package provides a single software tool for QC (filtering, normalization and outlier identification), exploratory data analysis (EDA), visualization, and statistical analyses robust to missing data. This is accomplished using simple function calls on data objects which are automatically updated with information about the analysis as it is performed in order to streamline the data exploration, QC, and statistical analysis process for the user. Additionally, pmartR provides a unique capability to create an interactive user interface (UI) for exploring the data and statistical results. With a few simple function calls, a user can create the UI of visualizations of the data for each biomolecule and data can be filtered and sorted according to quantities (e.g. p-values from statistical results) or characteristics of the biomolecule (e.g. number of peptides per protein). The open source pmartR package is available for download at http://github.com/pmartR/pmartR. We demonstrate pmartR analysis capabilities using proteomics data from mice exposed to smoke to demonstrate the core functionality of the package and to highlight the capabilities for handling missing data.

54. Function-Based Characterization of Gut Microbiome Metabolism Using Activity-Based Probes and Germ-Free Mouse Models

Kristoffer Brandvold, Regan Volk, Whitney Garcia, Agne Nixon, Bryan Killinger, Teresa Gibbins, Kimberly Tyrrell, and Aaron Wright

Pacific Northwest National Laboratory

The animal gut houses a complex community of microbes that greatly expands the scope of metabolic transformations that can occur within the host organism. Our work is focused on how the chemistry of the microbiome affects the host, with a particular emphasis on the liver. We seek to gain a mechanistic understanding of how microbiome function affects host physiology on the molecular level. The cornerstone feature of our approach is determining which microbes are engaging in a particular activity, rather than solely defining community composition by conventional sequencing methods that are agnostic to

function. We determine functionally active microbes by using activity-based probes that selectively label active enzymes, which allows us to determine identity by either proteomics or fluorescence-based analyses. We are currently studying how the microbiome modifies both endobiotics (bile salts) and xenobiotics (environmental pollutants) using our function-based approach. The authors will discuss development of novel activity-based probes for application in gut microbiome research, and how these can be combined with germ-free mouse models to provide a mechanistic understanding of gut microbiome metabolism.

55. Differences in Community Structure Analysis Based on 16S Rrna Gene Using High-Throughput Amplicon and Whole-Metagenome Shotgun Sequencing of Samples with Vastly Uncharacterized Microbiomes

Michal Strejček, Gabriela Nováková, Tereza Šmrhová, Ondřej Uhlík

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Geothermal and mineral waters have drawn attention mainly due to the possibility of discovering phylogenetically novel and unique taxa, potentially producing novel biologically active molecules. Water of many such springs comes from a depth of several hundred meters to kilometers. Although originally rainwater, having spent up to thousands of years in the geological bedrock, it cannot be contaminated by human activities. Microbial life in these waters very often remains largely unexplored.

In this study, we collected metagenomic DNA from four radon-containing subsurface water springs from Jáchymov, Czech Republic, which were analyzed by high-throughput amplicon sequencing (HTAS) with universal prokaryotic primers targeting the V4-V5 areas of the 16S rRNA gene, as well as the whole-metagenome shotgun sequencing (WMSS). We attempted to specifically target and reconstruct full-length 16S rRNA genes from the short reads, calculated their coverage and compared them to the HTAS-obtained sequences. We hypothesized that the genomes of the most abundant taxa detected by HTAS would be preferentially sequenced using shotgun sequencing and aimed to verify whether HTAS of 16S rRNA gene can be used to predict how many sequences need to be obtained from WMSS to describe dominant taxa in the community. Thus, we further attempted to recover metagenome-assembled genomes (MAGs). The 16S rRNA gene sequences extracted from the retrieved high-quality MAGs represented 30% and 15% of sequences that were derived from WMSS and HTAS, respectively.

Overall, both methods – HTAS and WMSS – showed comparable ability to describe the most abundant taxa of bacterial community. Interestingly, in the case of Archaea the HTAS data captured five times more taxa even though the universal prokaryotic primers were designed to maximize bacterial coverage, not the archaeal. However, the calculated coverages of WMSS derived from full length 16S rRNA genes and abundances of HTAS 16S rRNA fragments did not correlate. Furthermore, taxa with high abundance in HTAS data were not consistently recovered as high-quality MAGs.

While HTAS of 16S rRNA gene represents an important technique in microbial ecology, and is often used for differential abundance testing (designed experiments), our results show that these data cannot be used to predict how much sequencing effort is required to sufficiently recover dominant taxa.

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56. Recovery of Culturable Bacteria from Soil

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Pacific Northwest National Laboratory

Rapid recovery of culturable bacteria from environmental samples is a critical component to enable detection and further characterization of potential pathogens. We are developing a method for fast, flexible, small-scale recovery of cells from soil using a Warden Silt Loam model. Three model species were used to test our pipeline: GFP-tagged soil bacteria Pseudomonas fluorescens and Arthrobacter chlorophenolicus, and an mNEON-expressing E. coli Nissle. Recovered cell culturability was assessed with CFU plating and cell counting on a Novocyte. Isolation buffer composition, and method of disruption were optimized to achieve recovery rates greater than 50%.

57. Computational Modeling of Metabolic and Regulatory Networks of Yarrowia lypolytica

Neeraj Kumar, Lucas Naville, Samuel Britton, Erin Bredeweg, and Scott Baker

Pacific Northwest National Laboratory

Modifying genetic and metabolic pathways in microbes to design systems with the desired function remains challenging. Our inability to anticipate how the host organism will respond is due to the fact that many fundamental gaps exist between a molecular-level analysis and a cellular-level understanding of metabolic modeling essential to uncover interactions and dynamics of complex microbial systems. New computational tools are needed for quantitative modeling of cell that predict metabolite levels in high throughput manner, characterize thermodynamics and kinetics of individual reactions and energetics requirement of most likely metabolic pathways. We applied our newly developed ODE-based optimization approach based on statistical thermodynamics for developing a central metabolic model of Yarrowia lipolytica for the efficient production of itaconic acid. We used maximum entropy production principle to derive fluxes through a central metabolic network. The predicted metabolite concentrations produced from maximum entropy production rate solution is then be compared to those typically expected from the experiment using a loss function from which post-translational regulation of enzymes was inferred. We then re-optimized the system with the inferred regulation and determine optimal rate constants for the metabolic network from the metabolite concentrations and reaction fluxes.

58. Visualization of Large Biological Mass Spectrometry Datasets Via Integration of pmartr and Trelliscopejs

Rachel Richardson[1], Lisa Bramer[1], Ryan Hafen[2], and Lee Ann McCue[1]

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Biological datasets require visualization strategies for efficient interpretation and communication of underlying trends. As high-throughput measurement techniques progress and massive biological datasets become accessible, data visualization methods beyond summary visualizations (e.g. principal component analysis) prove to be unwieldly or impractical. In particular, mass spectrometry (MS) datasets can yield thousands of biomolecule abundance measurements across multiple samples in a single experiment. Maintaining resolution of statistical tests and data trends therefore requires new data visualization strategies. *Trelliscopejs* is an R package with the ability to sort and filter subsets of complex data visualizations

on-the-fly. It allows researchers to visualize and assess the entirety of any dataset in great detail. We demonstrate the benefits of integrating *trelliscopejs* with the MS quality control and statistics package, *pmartR*. We show the benefits of this workflow on an MS-generated lipid abundance dataset, highlighting a subset of the number of visualizations that are available and demonstrating how *trelliscopejs* can be used to depict trends in relative abundance of lipids. Researchers can thereafter select and evaluate significantly different biomolecules for further investigation.

59. Kbase Orgs: How to Coordinate and Share Data & Analyses in Kbase Across a Laboratory, Project or any Community of Practice

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http://kbase.us

Project Goals: The Department of Energy Systems Biology Knowledgebase (KBase) is a knowledge creation and discovery environment designed for both biologists and bioinformaticians. KBase integrates a large variety of data and analysis tools, from DOE and other public services, into an easy-to-use platform that leverages scalable computing infrastructure to perform sophisticated systems biology analyses. KBase is a freely available and developer extensible platform that enables scientists to analyze their own data within the context of public data and share their findings across the system.

KBase Orgs (short for Organizations) is the newest way to coordinate your research in KBase. Members of your team, laboratory or project can now connect with each other and share Narratives (data and analyses) via a KBase Org. In science, researchers are members of labs, teams, groups, collaborations, and projects that work together with shared data and analyses. In KBase, Orgs are a way for teams of scientists to share their data and associated analyses that are in the Narratives they create with each other as a group.

Org members can see information about the team and a list of the Narratives associated with the organization to which they can request access. View-only access is granted upon request while all other access levels to the Narrative (e.g., copy, read/write) are granted by the Narrative owner. KBase users can be members of more than one Org, and Narratives with the associated data might also be added to more than one Org.

Why use Orgs?:

- Allow your collaborators to quickly and easily view each other's Narratives
- Centralize data & shared analyses for a given project, laboratory, or institution
- Create a public Org to share both public and private Narratives (members can request access to private Narratives)
- Join multiple Orgs to keep your Narratives & data organized and shared across you collaborators
- Connect with researchers of similar interests & share your work with the KBase community at large

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60. Meta-Ecosystem Metabolomics: Interpreting Metabolomes through the Lens of Metacommunity Ecology

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Environmental metabolomics has the potential to provide insight into biogeochemical cycles by elucidating the metabolic processes occurring within an ecosystem. By using ultrahigh-resolution mass spectrometry, we can obtain a broad, untargeted characterization of environmental metabolomes. Recent work has revealed pronounced variation in metabolomes through space and time, suggesting that knowledge about the ecological processes underlying metabolome variability is necessary to develop transitive principles and enhance predictive capabilities. We propose integrating concepts and tools developed in metacommunity ecology with environmental metabolomics. In order to accomplish this, we generated relational dendrograms that incorporate information derived from molecular characteristics of each metabolite, akin to phylogenetic trees in microbial ecology. We applied a null model frequently used to understand the drivers of biological species distributions to samples obtained from global riverine ecosystems to determine the ecological processes acting upon metabolomes. Our results indicate that metabolomes from these rivers are structured by different combinations of deterministic and stochastic assembly processes. We further show that specific groups of metabolites contributed disproportionately to the observed ecological patterns by developing a new metric we term 'feature-level β–nearest taxon index (βNTIfeature)', indicating that certain metabolite groups occur more frequently than others. Metabolites containing CHO (e.g., lignins, tannins) drove many similarities among samples while metabolites containing functional groups with a variety of elements (e.g., CHONSP) contributed to divergences between samples. By analyzing metabolomes as ecological assemblages, we demonstrated that metabolomes experience differential structuring processes across riverine ecosystems and that certain subsets of metabolites disproportionately drive observed patterns.

61. Terrestrial Hydrocarbon Degrading Bacterial Diversity and Development: a Three Year Remediation Case Study in Utqiaġvik, Alaska

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We are still at the cusp of understanding the Arctic microbiome and how their functional capabilities could accelerate the cleanup of pollutants. Degradation at sites in the High Arctic is particularly important because of the extreme weather conditions and limited growing season. Several large terrestrial oil spills occurred at the former Naval Arctic Research Laboratory (NARL) site in Utqiagvik, Alaska, which persisted in the soil for over 50 years. Over a three-year period we implemented a bioremediation study utilizing a mixture of cold-tolerant plants (two grasses and a clover) along with a nutrient amendment, to understand if the presence of additional nutrients and plant exudates could stimulate hydrocarbon degradation and the impact it would have on the microbial community. To study this impact we performed various hydrocarbon analyses along with 16S rRNA sequencing on the soil samples from the four treatments; control, nutrient amended plant amended, nutrient and plant amended which were applied across five sites. We hypothesized that given the soil microbiota's historic and long-term exposure to high levels of hydrocarbon contaminants, the addition of nutrients and plants would preferentially promote hydrocarbon degrading genera as they are the best suited for survival, are primed for degradation by plant

exudates, and would have access to previously limited nutrients allowing for complex reactions. The petroleum hydrocarbon tests indicated that the abundance of contaminants was decreasing overtime, most effectively in the plant and nutrient and plant amended plots. Additionally, we found that the types of hydrocarbon degrading bacteria and their relative abundances were not homogenous across sites or by treatment. Interestingly, there were a higher abundance of known hydrocarbon degrading bacteria like Arthrobacter, Flavobacterium, and Pseudomonas in the plant and nutrient and plant treatments clearly demonstrating that they were enriched due to the addition of plants. Also, the prevalence of hydrocarbon degrading bacteria was consistently highest in the plant and nutrient and plant amended test plots mirroring the higher loss of hydrocarbons in these plots. Through this research, we have observed the abundance of hydrocarbon degrading bacteria in a high Arctic soil and the potential ability of the community to shift in response to contamination with the addition of nutrient and plant amendments suggesting flexible bioremediation potential.

62. Field Forward Sequencing in Naval Environments

Sophie M. Colston, NRC Postdoctoral Fellow; Nathanael Reynolds, Code 6900; Judson Hervey, Code 6910; Jeffrey S. Erickson Code, 6930; Chadwick Y. Yasuda, Code 6900; Dustin J. Harrison, Naval Medical Research Center; Gary J. Vora, Code 6910

The U.S. Navy operates within diverse environments that spans both maritime operating areas and shore installations. The U.S. Naval Research Laboratory (NRL) supports a number of research initiatives within these sites, including the analysis of genomic/metagenomic and transcriptomic data to characterize discrete microorganisms or mixed microbial communities. NRL also coordinates in-house capabilities to conduct parallel proteomic and metabolomic analyses when possible. In an effort to complement and expand current technologies and capabilities, NRL has implemented the use of Oxford Nanopore's sequencing device, the MinION, leveraging its portability and real-time data acquisition to enable DNA/RNA sequencing in austere, non-traditional lab settings and/or in scenarios that require rapid responses. As an accompaniment to the MinION, NRL has also developed a portable laboratory that can be immediately deployed and can support a complete workflow from sample collection and processing, PCR amplification, library preparation, sequencing, and data analysis. This kit has been used in a pilot study to investigate the microbial diversity of interior surfaces onboard a naval vessel with the eventual objective to track the subsequent changes to the communities of this built environment during various stages of a deployment cycle. The capability has also been demonstrated in the Navy's expeditionary mobile laboratory, with the primary aim to augment current assays for pathogen detection from various source matrices. In addition to targeted assays, there are ongoing efforts to improve unbiased sequencing protocols in the field to characterize complex environmental and clinical samples such as water filters, biofilms, urine, and soil, especially in situations with limitations on sample storage and transport. Onsite sample processing and sequencing methods allow for a rapid assessment of a sample's genetic cache, facilitating timely taxonomic classification and the identification of potential determinants associated with disease or antimicrobial resistance that may impact human health in a naval operational environment.

Acknowledgements

We would like to thank the Naval Facilities Engineering Command for supporting this research and Argonne National Laboratory genomic center for making the sequencing possible.

63. Development and Analysis of Reduced Complexity Microbial Consortia Emerging from Native Grassland Soil Systems

Ryan McClure, Dan Naylor, Colin Brislawn, Yuliya Farris (presenter), Sarah Fansler, Kirsten S. Hofmockel and Janet K. Jansson

Pacific Northwest National Laboratory

Soil microbial communities are critical to the overall carbon cycle and to the decomposition of complex biopolymers such as chitin and cellulose. Despite the critical nature of these microbiomes, a detailed understanding of how the interactions between members lead to emergence of community functions is lacking. This is due, in part, to the complex nature of the soil microbiome with thousands of species across several kingdoms contributing to the overall response of soil. In order to gain a more detailed view of the soil environment we took an approach based on developing and analyzing reduced complexity microbial consortia that contain fewer species than the native soil but are still representative of this site and are more experimentally tractable. Data collected from these reduced complexity communities and resulting knowledge and hypotheses can then be applied to the more complex native side to increase our understanding of microbiome functions in soil. To generate these consortia, we collected samples from our native field site containing a grassland silt loam soil. This native soil was then diluted to various levels, and cultured in irradiated sterile native soil with N-acetyl-glucosamine (NAG). We confirmed that initial dilutions decreased the richness and complexity of our native soil and found that culturing these dilutions in soil lead to stable microbial consortia containing both bacteria and fungi. In addition to cultivation in soil, we also developed consortia on plates using chitin itself as a major source of carbon and nitrogen. These plate communities represented microbiomes that were even further reduced in complexity (containing between 20-70 OTUs) while again containing a diverse community of several different phyla. We also isolated several constituent microbial species from these communities and examined their growth under several different carbon and nitrogen sources finding that each species had specific nutrient sources that it preferred. This series of reduced complexity consortia are powerful tools that can be used by the soil community at large to interrogate the response of soil microbiomes to a number of perturbations and to confirm critical interactions between microbial species, particularly inter-kingdom interactions that characterize the emergent behavior of soil microbiomes. Further knowledge of these interactions will help us better understand the overall metaphenome of soil systems, especially as they respond to critical perturbations including drought.

64. Getting Tough Fungal Samples to Spill Their Proteins for MS Analysis

Heather Brewer[1], Candice Swift[1], Samuel Purvine[1], Michelle O'Malley[1]

[1]Pacific Northwest National Laboratory; [2]University of California, Santa Barbara

Understanding fungi and how they break down lignins and cellulose are on the rise with the advent of biofuels, however fungal samples are notorious for being difficult to process for proteomic analyses. Since the cell walls are made of cellulose and chitin, fungal lysis isn't as simple as the process for bacterial or even mammalian tissue samples. We have often utilized the MPLEx method (Nakayasu, et. al. 2016, mSystems, 1 (3) (2016), pp. e00043-16) to extract protein, metabolite and lipid analytes from the same sample. Modifying the lysis method by combining the chemicals utilized with mechanical disruption enabled us to extract proteins previously unobserved in fungal samples. We were able to additionally observe a greater number of peptides when compared to previous lysis methods using bead beating as the mechanical disruption process. This modified method not only yields better global proteomic coverage for fungal samples, but it can also supply lipid and metabolite data simultaneously for a greater understanding of metabolic pathways and the biology of these organisms.


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LAMB VINDALOO - $10

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Seasonal vegetables in a creamy tomato, onion, cashew, coconut milk sauce with a mild blend of Indian herbs and spices. Served with basmati rice.

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Raita – $1

DRINKS

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Menu:

Hot and Spicy Honey Chicken $9
Crispy chicken chunks stir fry in a hot and spicy honey sauce with broccoli, carrots, bamboo shoots, snow peas and water chestnuts and chili pods. *Comes with a side of rice and salad.*

Orange chicken $9
Crispy chicken chunks stir fry in a tangy, orange sauce with broccoli and orange peels. *Comes with a side of rice and salad*

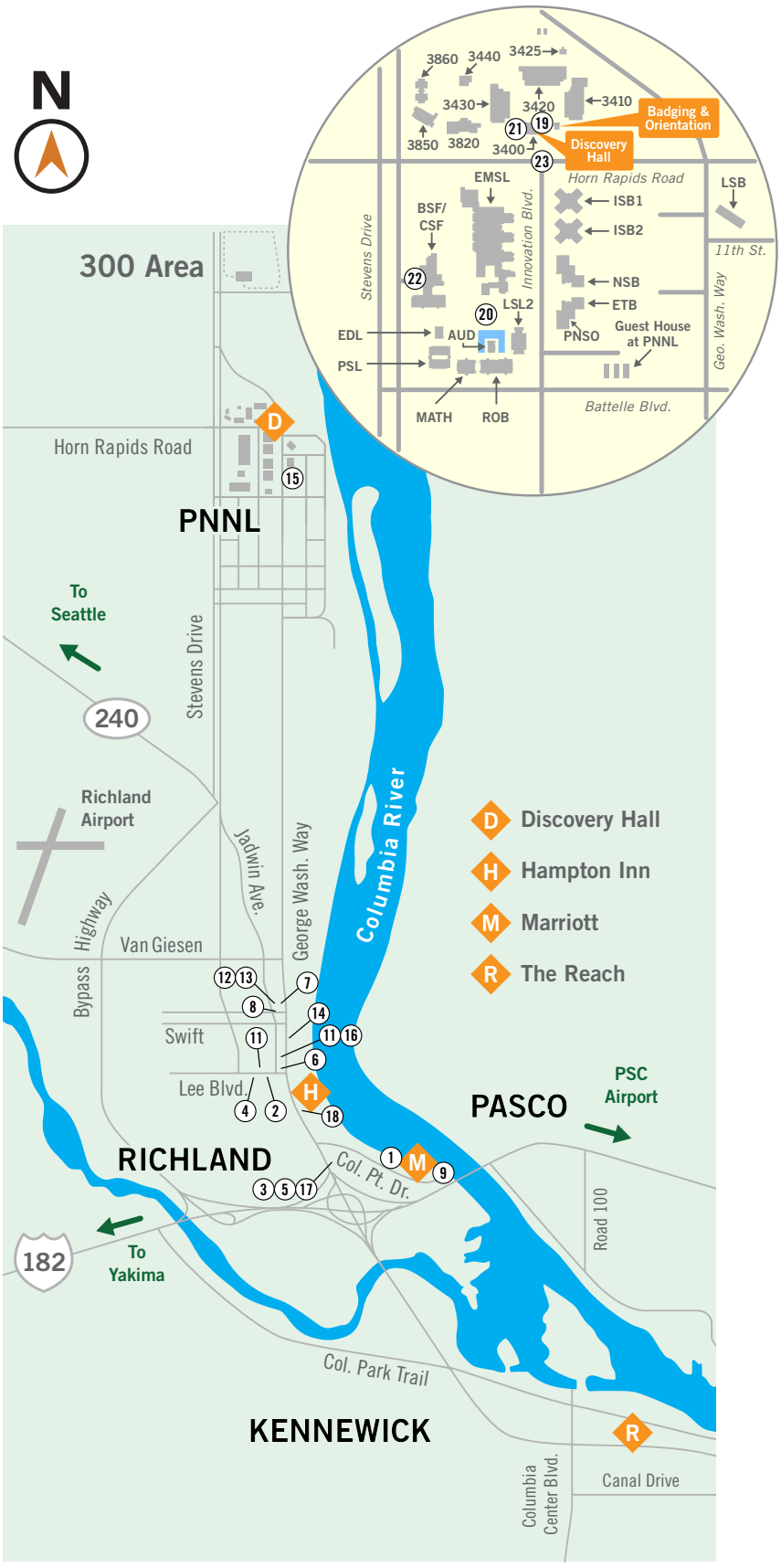
Kindra Lunchable: 2 spring rolls, 2 egg rolls and four chicken potstickers $10

Chicken pad Thai (gluten free) $8
Sautéed rice noodles with eggs, bean sprouts, fresh green onions, and in-house tamarind sauce, served with crushed peanuts and a slice of lime.

Veggie pad Thai (gluten free) $8

Complimentary water for attendees with a conference badge!

AREA MAP


N
300 Area
3860
3440
3425
3430
3420
3410
3850
3820
3400
Badging & Orientation
Discovery Hall
Horn Rapids Road
EMSL
BSF/ CSF
Stevens Drive
Innovation Blvd.
ISB1
ISB2
LSB
11th St.
NSB
ETB
LSL2
EDL
AUD
PNSO
Guest House at PNNL
Geo. Wash. Way
PSL
MATH
ROB
Battelle Blvd.
Horn Rapids Road
PNNL
To Seattle
240
Stevens Drive
Richland Airport
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George Wash. Way
Columbia River
Highway
Van Giesen
Bypass
Swift
Lee Blvd.
PASCO
PSC Airport
RICHLAND
Col. Pt. Dr.
Road 100
182
To Yakima
Col. Park Trail
KENNEWICK
Columbia Center Blvd.
Canal Drive
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DINING

1 Anthony's
2 Apollo
3 Applebee's
4 Atomic Ale House
5 Bento Teriyaki
6 Casa Mia
7 Denny's
8 Fat Olives
9 Lu Lu
10 Montorosso's
11 Porters
12 Some Bagels
13 Spudnut Shop
14 Sterlings
15 Venezia

Coffee Shops:

16 Kagan Coffee & Crepes
17 Starbucks
18 Roasters

On Campus:

19 Hot Tamale (Wed. & Thurs. Only)
20 Fast & Curryous (Thurs. only)
21 Kagen Deli
22 Kindra's Cafe
23 Kindra's Wok 'n Roll (Wed. only)

BUS SCHEDULE

Wednesday, July 24th

AM Service

7:10 am Depart Courtyard by Marriott, Richland for Discovery Hall
7:15 am Depart Hampton Inn, Richland for Discovery Hall

PM Service

5:30 pm - Depart Discovery Hall for Courtyard by Marriott & Hampton Inn, Richland
6:00 pm - Depart Discovery Hall for Courtyard by Marriott & Hampton Inn, Richland
6:30 pm - Depart Discovery Hall for Courtyard by Marriott & Hampton Inn, Richland
7:00 pm - Depart Discovery Hall for Courtyard by Marriott & Hampton Inn, Richland

Thursday, July 25th

AM Service

7:10 am - Depart Courtyard by Marriott, Richland for Discovery Hall
7:15 am - Depart Hampton Inn, Richland for Discovery Hall

PM Service

5:00 pm - Depart Discovery Hall for Courtyard by Marriott & Hampton Inn, Richland
5:45 pm - Depart Marriott & Hampton Inn Richland hotels for The REACH Museum
7:30 pm - Depart The REACH Museum for hotels
8:00 pm - Depart The REACH Museum for hotels
8:30 pm - Depart The REACH Museum for hotels
9:00 pm - Depart The REACH Museum for hotels (last bus)

Friday, July 26th

AM Service

7:10 am - Depart Courtyard by Marriott, Richland for Discovery Hall
7:15 am - Depart Hampton Inn, Richland for Discovery Hall

PM Service

12:15 pm - Depart Discovery Hall for Pasco Airport and hotels
2:30 pm - Depart EMSL for Pasco Airport and hotels
4:45 pm - Depart EMSL for Pasco Airport


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