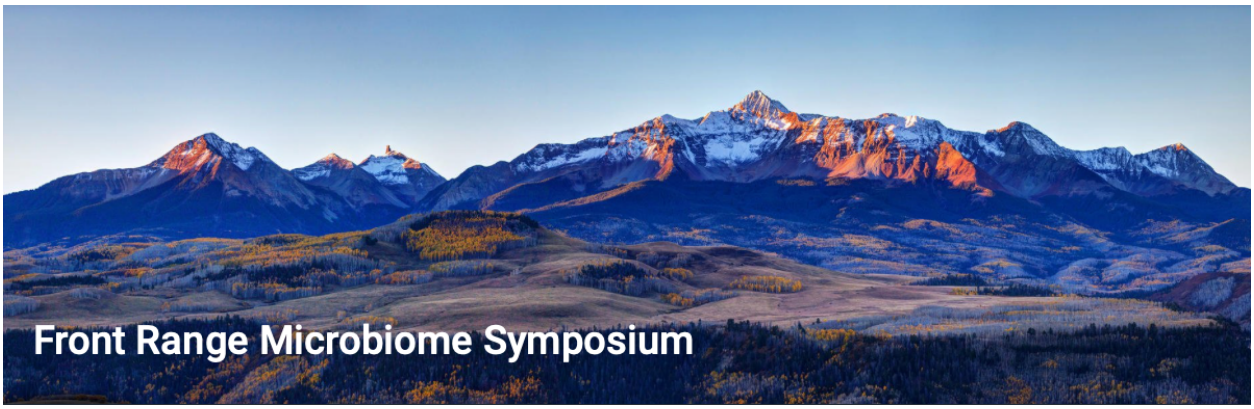

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VICE PRESIDENT FOR RESEARCH
COLORADO STATE UNIVERSITY

April 18th-19th, 2024

April 18th, 5-8 pm: Pre-symposium Mixer: at Gilded Goat Brewing Co., midtown location (3500 College Ave., Fort Collins, CO)

April 19th, 8 am – 5 pm: Alumni Center, Canvas Stadium, Colorado State University, 701 W Pitkin St, Fort Collins, CO 80523 from 8 am – 5 pm

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Alumni Center, Canvas Stadium, Colorado State University, 701 W Pitkin St, Fort Collins, CO 80523 from 8 am – 5 pm

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Organizing Committee

Dr. Tiffany Weir, Professor and Graduate Program Director, Food Science and Human Nutrition, Colorado State University

Dr. Weir is an Associate Professor of Food Science and Human Nutrition. She studies the impact of diet on the human gut microbiota and the interaction between these microbes and the downstream development of cardiometabolic diseases. She also teaches Fermentation Microbiology and courses for the Nutrition and Food Science graduate program. Dr. Weir studied Microbiology at Penn State University before completing the Cell and Molecular Biology PhD program at Colorado State University. Prior to becoming a professor at CSU, she spent time study microbial ecology in the Peruvian rainforest as a visiting scientist at the Pontifical Catholic University of Peru.

Dr. Susan De Long, Associate Professor, Department of Civil and Environmental Engineering, Colorado State University

Dr. De Long is an Associate Professor in the Department of Civil and Environmental Engineering at Colorado State University. She earned a M.S. and Ph.D. at University of Texas-Austin. Dr. De Long's research is focused on developing environmentally sustainable biotechnologies. Her research group applies molecular biology tools to investigate microbial communities involved in successful treatment processes and leverages this knowledge to improve treatment process design. Additionally, Dr. De Long's group is conducting research to discover genes and enzymes involved in degradation of recalcitrant pollutants; this knowledge will support development of molecular biology assays to track key microbes in natural or engineered systems to guide development of the next generation of treatment technologies.

Steering Committee

Dr. Cresten Mansfeldt – University of Colorado, Boulder

Dr. Karen Wawrousek – University of Wyoming

Dr. Joshua Chan – Colorado State University

Dr. Pankaj Trivedi – Colorado State University

Dr. Vienna Brunt – University of Colorado, Anschutz

Dr. Christopher Stamper – Department of Veterans Affairs, Rocky Mountain Regional VAMC

Dr. Alexander Pak – Colorado School of Mines

Dr. Antisar Afkairin – Colorado State University

Dr. Toru Ishii – University of Colorado, Anschutz

Program

Thursday 18th, April 2024

5:00 pm – 8:00 pm: Pre-symposium mixer at Gilded Goat Brewing Company 3500 College Avenue, Fort Collins, CO 80525 (Midtown Fort Collins location)

Friday 19th, April 2024

8:00 am – 8:45 am: Doors open and breakfast snacks

8:45 am – 9:00 am: FRMS Organizing Committee – opening remarks

9:00 am – 10:00 am: Keynote: Serita Frey, *Going underground: Unearthing the Role of Microbial Traits on Social Carbon Dynamics*

10:00 am – 10:30 am: Invited Talk – Mohit Verma, *Field-deployable Biosensors for One Health Microbiomes*

10:30 am – 11:30 am: Poster Session 1

11:30 am – 12:15 pm: Contributed Talk (15 min) Reed Woyda, *DRAM2: Empowering Microbiome Exploration and Advanced Multi-Omics Analytics for Ecosystem-Specific Profiling*

Contributed Talk (15 min) Jennifer Fouquier, *EXPLANA: A user-friendly workflow for EXPLoratory ANAlysis and feature selection in cross-sectional and longitudinal microbiome studies*

Contributed Talk (15 min) Tom Lennard Stach, *Full-factorial Mesocosm Experiments Reveal Stressor Effects on Stream Microbiomes*

12:15 pm – 1:00 pm: Lunch

1:00 pm – 2:00 pm: Poster Session 2

2:00 pm – 2:50 pm: Lightning Talk (10 min) Chris Stamper, *Major Findings and Lessons Learned in a Longitudinal Veteran Microbiome Study: The Unites-States Veteran Microbiome Project (US-VMP) Study*

Lightning Talk (10 min) Ashley Scadden, *Altered Gut Microbiota Diversity Metrics and Composition Over Time in Participants from the Daily caloric Restriction versus Intermittent Fasting Trial (DRIFT)*

2:00 pm – 2:50 pm: Lightning Talk (10 min) Xingfeng Huang, *A Coal-derived Soil Amendment Promotes the Plant Growth of Sweet Pepper (Capsicum annuum) and Changed the Rhizosphere Microbial Communities*

Lightning Talk (10 min) Hannah Rodgers, *Plant Breeding Alters the Rhizosphere Environment of a Novel Grain Crop*

Lightning Talk (10 min) Marina Nieto-Caballero, *Airborne Microbes from Thawing Permafrost Landscapes in the Arctic*

2:50 pm – 3:00 pm: Break

3:00 pm – 3:30 pm: Invited Talk – Matt Olm, *Applying Metagenomic Sequencing to Decipher the Human Gut Infant Microbiome*

3:30 pm – 4:00 pm: Invited Talk – Boahemaa Adu-Oppong, *My Life among Microbes: A Casual Chat about a Career in Science*

4:00 pm – 4:10 pm: Closing Remarks & Awards

4:10 pm – 5:00 pm: Wine and Cheese Social Hour

Contributed Talks

DRAM2: Empowering Microbiome Exploration with Advanced Multi-Omics Analytics for Ecosystem-Specific Profiling

Keywords: multi-omics, computational biology, metagenomics, metabolism, microbiome

Reed Woyda, Bridget B. McGivern, Kaela Amundson, Jared Ellenbogen, Chris Chorpenning, Laura Mason, Laura Moore, Laura Schaerer, Lady Grant, Katherine Kokkinias, Valerie Steitz, Sophie Jurgensen, Rory Flynn, Janaka N Edirisinghe, Sabina Leanti La Rosa, Christopher S Miller, Jessica Prenni, Phil B. Pope, Christopher S Henry, Kelly C. Wrighton, and Mikayla A. Borton

Microbiome sequencing generates a deluge of genomic information for bacteria and archaea, demanding robust computational methods to rapidly profile the functional potential of genomes across ecosystems. To meet this need, we expanded the capabilities of DRAM (Distilled and Refined Annotation of Metabolism) to build DRAM2, a multi-omics data analytics platform for rapid, ecosystem-specific microbiome reporting. At its core, DRAM2 annotates and organizes volumes of genomic data, enabling users to discern physiologically relevant information from assembled microbial community sequencing information. New features in DRAM2 include the ability to i) integrate microbial data from multiple biological modalities, ii) visualize microbial data in an interactive web interface, and iii) curate annotations using non-homology-based methods. Collectively, these upgraded features enabled rapid classification of microbial genomes into traits, providing users with a per-genome list of metabolic traits (e.g. sulfate reducer or denitrifier) and assignment to biogeochemical cycles (e.g. sulfur or nitrogen). Integration of several new databases in combination with these ecosystem rulesets allow users to summarize annotations for specific ecosystems, including agricultural soil, engineered systems, the human gut, and permafrost applications. For improved accessibility, we have implemented DRAM2 with Nextflow, making it freely available on GitHub and within KBase. The latter is a point-and-click interface, alleviating the need for computational resources and enabling integration of DRAM annotations into other KBase tools, such as genome scale metabolic models. We demonstrate the immediate value of DRAM2's capabilities and highlight improvements that are critical to deciphering mechanisms underpinning microbiome function.

EXPLANA: A user-friendly workflow for EXPLoratory ANAlysis and feature selection in cross-sectional and longitudinal microbiome studies

Keywords: Bioinformatics, exploratory analysis, software tools, longitudinal, feature selection

Jennifer Fouquier, Maggie Stanislwaski, John O'Connor, Catherine Lozupone

The potential for disease treatment through gut microbiome modification has contributed to an increase in longitudinal microbiome studies (LMS). Gut microbiome modification can occur through factors such as diet, probiotics, or fecal transplants. Scientific data often motivates researchers to perform exploratory analyses to identify features that relate to a response. LMS are cumbersome to analyze though, often leading to lost information and research barriers. Some LMS analytic challenges include data integration, compositionality, dimensionality reduction, and the need for mixed-effects models for non-independent data. Additionally, LMS can be observational (i.e., measurements made over time, related to natural fluctuations in symptoms/measurements), with no expected change compared to baseline, making baseline values less informative. LMS can also be interventional, with expected changes compared to baseline, making baseline values more informative. Thus, calculating feature changes for each subject over time is dependent on different reference values. To address these challenges, we developed EXPLANA, a data-driven feature-selection workflow that supports numerical and categorical data. We implemented machine-learning models for repeated measures, feature-selection methods, and visualizers for explaining how selected features relate to the response. With one script, analysts can build models to select important features and obtain an analytic report that textually and graphically summarizes results. EXPLANA had good performance using twenty simulated data models yielding an average AUC of 0.91 (range: 0.91-1.0) and improvements compared to an existing tool (AUC: 0.95 and 0.56; precision: 0.82, and 0.14, respectively). EXPLANA can identify unique features dependent on different contexts of change, including novel order-dependent categorical features.

Full-factorial mesocosm experiments reveal stressor effects on stream microbiomes

Keywords: River, Microbiome, Stress, Mesocosm, Full-factorial

Tom Lennard Stach, Aman Deep, Iris Madge Pimentel, Dominik Buchner, Mikayla A. Borton, André Soares, Jörn Starke, Till L.V. Bornemann, Kelly C. Wrighton, Daniela Beisser, Florian Leese and Alexander J. Probst

Stream ecosystems represent an integral link between terrestrial, marine, and freshwater ecosystems. While it is widely accepted that stream microbiomes play an essential part in ecosystem services, their response to anthropogenic stressors and their ability to recover after stressor release is heavily understudied. Here, we show the effect of salinity (0.5 mS/cm increase), temperature (3.5 C increase), flow velocity (14.2 vs 3.5 cm/s), and combinations thereof on microbial populations in sediments using genome-resolved metagenomics and metatranscriptomics. Using an outdoor mesocosm system with 64 experimental units, we investigated structural and functional changes in the microbiome after two weeks of stressor exposure and after a subsequent two-week recovery phase. Based on marker gene (ribosomal protein S3 gene) analysis we demonstrate that flow velocity is a key factor for stream microbiomes. Reduced flow velocity outcompeted the influence of the other stressors resulting in significantly restructured microbial communities and for example lower abundance of diatoms. With increasing temperature, one metagenome-assembled genome (MAG), annotated Bacteroidetes, increased in relative abundance, and its encoded genes were significantly higher expressed. While microbiomes changed in species composition, their encoded functions did not substantially change across the experiment. However, we identified short-term adaptations based on differential gene expression depending on the individual stressors or their combinations. After two weeks of recovery, stream microbiome composition between the different treatments reconverged. This study demonstrates the great sensitivity of stream microbiomes in their response to anthropogenic stressors and their functional resilience.

Lightning Talks

Major Findings and Lessons Learned in a Longitudinal Veteran Microbiome Study: The United States-Veteran Microbiome Project (US-VMP) Study

Keywords: Microbiome, Longitudinal Sampling, Gut, Mental Health, Veterans

Christopher E. Stamper, Andrew J. Hoisington, Kelly A. Stearns-Yoder, Teodor T. Postolache, Christopher A. Lowry, Lisa A. Brenner

Our understanding of the human microbiome and its potential for affecting human physiology and behavior has experienced unprecedented growth. Researchers have shown that the human gut microbiome is impacted by external factors (e.g., diet, gender, age, environmental exposures, etc.) and has been implicated in many physical and mental health conditions, including anxiety, mood, and trauma- and stressor-related disorders such as posttraumatic stress disorder. United States (US) military Veterans are a unique population in that their military-related exposures can have consequences for both physical and mental health, but the microbiome of this population has been understudied. To rectify that knowledge gap, the US Veteran Microbiome Project (US-VMP) was started with a model to serially collect microbiome and health-related data from those seeking care within the Veterans Health Administration. The ongoing research effort has collected over 6,000 fecal, oral, and skin microbiome samples from 484 participants. Using this study, we have identified important factors associated with primarily the fecal microbiome to include traumatic brain injury, alcohol use disorder, posttraumatic stress disorder, diet, social equity, homelessness, and medication usage. The long-term sampling effort has also enabled within-person analysis before and after physical illnesses (e.g., COVID-19, sleep apnea) and mental health outcomes (e.g., depression, anxiety). In addition, the US-VMP study has led to quality control improvements from sample stabilization methods, technical variation in sequencing centers, use of positive and negative controls, and impact of low biomass on sequencing. This presentation summarizes the major findings from US-VMP and our lessons learned during the six-year study.

Altered gut microbiota diversity metrics and composition over time in participants from the Daily caloric Restriction versus Intermittent Fasting Trial (DRIFT)

Keywords: microbiota, obesity, weight loss, intermittent fasting

Ashley W. Scadden, Jennifer Fouquier, Cathy Lozupone, Danielle M. Ostendorf, Paul S. MacLean, Edward L. Melanson, Daniel H. Bessesen, Victoria A. Catenacci, and Maggie A. Stanislawski

Alterations in gut microbiota have been associated with obesity and may influence weight loss. Here we analyzed 16S rRNA gene sequencing and clinical data from N=150 individuals with overweight or obesity enrolled in the Daily caloric Restriction versus Intermittent Fasting Trial (DRIFT), a one-year weight loss intervention comparing the efficacy of two dietary approaches: daily caloric restriction (DCR) and intermittent fasting (IMF). Participants were predominantly white (86.7%) females (72.7%) averaging 42.1 years of age (SD: 8.9). Overall, participants exhibited significant improvements in clinical outcomes, including reduced weight ($p<0.05$), which was more pronounced in the IMF group. We examined diversity metrics and genus-level gut microbiota composition using regression-based methods. Alpha diversity, including Shannon diversity index, richness, and evenness, increased significantly ($p<0.05$). We assessed longitudinal changes in gut microbiota from baseline to 12 months and identified 40 genera that changed significantly (false discovery rate, $q<0.05$), many of which are consistent with prior dietary intervention research, e.g., decreased abundance of Dialister and Faecalibacterium. We observed changes in overall microbiota composition over time (beta diversity based on the Aitchison distance metric; $p=0.0002$). Eight taxa showed differential change by intervention group over time ($q<0.05$), e.g., Butyricimonas, a butyrate producing-taxon, which increased more in the IMF group. Lastly, we will report results obtained from a new machine-learning based software tool, EXPLANA (EXPLoratory ANAlysis) to identify important features predictive of clinical outcomes. Together, our results elucidate the impact of dietary interventions on gut microbiota and associations with clinical outcomes, potentially informing future personalized weight loss interventions.

A Coal-derived Soil Amendment Promotes the Plant Growth of Sweet Pepper (Capsicum annuum) and Changed the Rhizosphere Microbial Communities

Keywords: Coal-derived Soil Amendment, Sweet Pepper (Capsicum annuum), Rhizosphere Microbial Communities

Xing-Feng Huang, Paul H. Fallgren, Song Jin, Kenneth F. Reardon

Previous studies have shown that a coal-based soil amendment could improve soil organic matter content and promote plant growth. To better understand the effects of coal-based amendment on plant growth and crop productivity, comprehensive analyses have been carried out to investigate the effects of coal-based amendment on the growth of sweet pepper (Capsicum annuum), pepper fruit production, soil properties, and rhizosphere microbial communities. Applying coal-based amendment did not change the soil pH but significantly increased soil organic matter. Moreover, the application of coal-based amendment not only promoted pepper growth but also pepper fruit production resulting in significantly higher pepper plant height, plant weight, and root dry weight than the reference. The effects of coal-based amendment on rhizosphere microbial communities have been analyzed by 16S rRNA and ITS gene sequencing analysis. The application of coal-based amendment increased the richness and diversity of the rhizosphere bacterial communities. The PCoA clustering patterns indicated the different treatments affected the pepper rhizosphere microbial communities differently. The abundance of Proteobacteria decreased by 33% and Chloroflexi increased by 34% compared to the reference at 12 weeks. LEfSe analysis has identified biomarkers which showed significant differences in different treatments. Vicinamibacteraceae and Mycobacteriaceae were identified in coal-based amendment treatments respectively at both 5-week and 12-week. Soil fungal communities also changed; the amendment led to decreases in the relative abundances of Basidiomycota and increases in Ascomycota. These findings showed that applying the coal-derived soil amendment can be used as a soil amendment to improve soil properties and promote crop production.

Plant breeding alters the rhizosphere environment of a novel grain crop

Keywords: plant breeding, rhizosphere, sustainable agriculture

Hannah Rodgers, Linda van Diepen, Urszula Norton

Kernza, the first developed perennial grain crop, promises to create climate-resilient agroecosystems in part by supporting a soil microbiome similar to perennial prairie. Kernza presents a unique opportunity to study the effects of plant breeding on the rhizosphere, since it was recently bred from intermediate wheatgrass (Thinopyrum intermedium). To investigate this, we characterized the rhizosphere microbiome of Kernza from nine breeding cycles, starting from wild-type intermediate wheatgrass. We measured a variety of organic matter pools, enzyme activities, phospholipid fatty acids, and both bacterial and fungal diversity using amplicon sequencing. Plant breeding significantly reduced arbuscular mycorrhizal fungal and total microbial biomass, as well as dissolved organic carbon & nitrogen and extracellular enzyme activities. Bacterial and fungal alpha-diversity were not affected by breeding cycle, but specific microbial functional groups were. Plant breeding for grain yield created plants with less labile organic matter and microbial biomass and activity in the rhizosphere, suggesting a trade-off between yield and root exudates. A better understanding of the agricultural rhizosphere can help researchers and plant breeders collaborate to create sustainable, resilient agroecosystems, and guide future work into the possibility of breeding crops for enhanced positive microbial associations in the rhizosphere.

Airborne microbes from thawing permafrost landscapes in the Arctic

Keywords: Bioaerosol, permafrost, ice nucleating particles

Marina Nieto-Caballero, Thomas C. J. Hill, Kevin R. Barry, Christina S. McCluskey, Thomas A. Douglas, Paul J. DeMott, Sonia M. Kreidenweis, Jessie M. Creamean

The rapid warming of Arctic regions, outpacing global rates by up to four times, is leading to significant changes in these fragile cryospheric landscapes. This study focuses on Northern Alaska, where the thawing of permafrost, some preserved for up to 2 million years, is reintroducing ancient microorganisms to the environment. These microbes, previously preserved in frozen tundra soils, are spreading to thermokarst lakes, rivers, and the Arctic Ocean through erosion and groundwater drainage, altering the composition of these water systems. Permafrost microbes can be exposed and introduced to the atmosphere via various known mechanisms. Here, we explore the local microbial sources of bioaerosols in Northern Alaska, assessing a broad range of environmental samples (i.e., terrestrial, water bodies, and vegetation). Although seawater and brackish water are the predominant bioaerosol sources in the region, we present evidence for the first time, to the best of our knowledge, for the signatures of permafrost-originating microbes and their corresponding contribution to bioaerosols in Northern Alaska. This finding forewarns the importance of permafrost thawing for future atmospheric impacts, as it is known to be enriched with ice nucleating particles, rare aerosols that play a critical role in altering cloud properties, precipitation patterns, and the overall radiative budget despite their low concentrations. Our findings emphasize the complex interactions between terrestrial changes and cloud processes in the Arctic region, highlighting the need to research these underappreciated implications of permafrost thawing under the influence of a warming Arctic.

Poster Abstracts (Alphabetic Order by Presenter Last Name)

Poster #43: The Microbiome And Chronobiology Of Dairy Cattle Milk

Keywords: Milk, Microbiome, Chronobiology

Khalid Al Lakhen - Metcalf lab

Human milk is one of the first sources of nutrients & immunoregulatory compounds an infant receives. It's also one of the first sources of microbes that will colonize the infant's gut. Therefore, it's essential to understand any trends in these milk components and how they may impact the infant's health from an early age. Some bioactive compounds in milk, such as melatonin, are known to cycle over the course of a day and are essential for transferring chronobiological information to infants to establish their circadian rhythm. Although the microbiota of human milk has been studied previously, it is unclear whether it is among the milk components that vary diurnally and longitudinally. A significant variation could point toward the importance of the timing for breastfeeding in shaping an infant’s gut microbiome and gut health. The chronobiology of human milk and its impact on infant development and health is poorly understood because intensive diurnal and longitudinal milk sampling is difficult to do in humans, especially in very early lactation, when the most rapid and likely impactful changes occur. To overcome this obstacle, we will use dairy cattle as a model to intensively characterize dairy milk's diurnal and longitudinal chronobiology. We will analyze milk levels of macronutrients, IgA, lactoferrin, cortisol, and IgG of the dairy milk samples collected across the 34 days with each collection day having a morning and night sample collected (12 hours apart). In addition, 16s rRNA amplicon sequencing was performed to analyze the microbial composition of milk.

Poster #41: Leveraging genomic insights for a biogeographical understanding of fractured shale microbiome function

Keywords: metagenomics, database, terrestrial subsurface

Kaela K. Amundson, Mikayla A. Borton, Rebecca A. Daly, Daniel S. Alessi, Julian Damashek, Casey Hubert, Anna M. Martini, Sophie L. Nixon, Gabrielle Sheffer, Karen E. Wawrousek, David W. Hoyt, Elizabeth K. Eder, Allison Wong, Kelly C. Wrighton, Michael J. Wilkins

Subsurface shales underly much of North America and are economically important for the recovery of oil & natural gas. However, many microorganisms that persist in shale wells contribute to common production challenges such as bioclogging, corrosion, souring, and scaling. Given that shale formation temperature and salinity of produced waters can vary considerably between geographically distinct basins, there is need to understand and predict spatial and temporal patterns of microbial functional potential in these ecosystems. To investigate this, we performed metagenomic sequencing on 209 water samples collected from 36 hydraulically fractured wells across nine shale basins in North America, as well as two international basins (UK & China). Many of these samples were from 19 wells with timeseries data (n=161), and a majority paired with NMR metabolomic data (n=128), which together revealed significant differences in concentrations of detectable metabolites, taxonomic profiles, and the complete absence of a core microbiome across basins. From these samples we built a comprehensive genomic database for fractured shales containing 978 unique medium- and high-quality MAGs and over 7 million unique genes. We leveraged a genome-resolved approach to uncover the differences in key metabolisms, such as fermentation and sulfate and thiosulfate reduction, as well as key traits, such as osmoprotection strategies, across the microbiomes of geographically distinct basins. Together, this approach provides new insights into spatial and temporal patterns of microbial functional potential and demonstrates how -omics tools can be applied to make informed decision for reservoir management in the terrestrial subsurface.

Poster #38: Investigating Desiccation-Tolerant Environmental Cyanobacteria

Keywords: Cyanobacteria, EPS, Soil, Desiccation-Tolerance

Camille Angeles, Dr. James Henriksen, Dr. Christie Peebles

Cyanobacteria are photosynthetic microorganisms found in most aquatic and terrestrial environments. Some species of cyanobacteria are capable of surviving desiccation. The mechanisms behind their desiccation tolerance are not well understood or widely studied, but their exopolysaccharides (EPS) are thought to play a large role. To investigate the EPS of desiccation-tolerant cyanobacteria, we collected and enriched cyanobacterial cultures from various environmental sources and selected for desiccation tolerance and EPS production. We obtained 12 desiccation-tolerant cyanobacteria species and are currently experimenting with EPS production conditions and investigating the contents of the produced EPS.

Poster #47: The Effect of Dietary Components on C. difficile Infection and the Gut Microbiome

Keywords: Fat, fiber, gut microbiome, C. difficile infection

Madison Apgar, Casey Martin, Elena Wall, Keith Hazleton, Catherine Lozupone

Consumption of high-fat/low-fiber diets has been shown to increase mortality in a murine model of antibiotic-associated Clostridioides difficile infection. Increased mortality was linked with greater antibiotic-induced disturbance of the microbiome compared to chow-fed mice. High-fiber diets are hypothesized to prevent mortality in antibiotic-associated C. difficile infection by protecting the gut microbiome from disturbance and facilitating the production of beneficial metabolites that promote a gut microbial ecosystem not conducive to C. difficile growth, including butyrate and secondary bile acids. Here, we tested whether supplementation of high and low-fat purified diets with a cocktail of soluble fibers improved survival by maintaining production of these metabolites. Indeed, dietary supplementation with soluble fibers increased survival. However, sustained secondary bile acid and butyrate production and an associated dampening of inflammation or C. difficile toxin production, was not observed. Surprisingly, mice fed purified diets supplemented with soluble fibers had higher cecal and colonic inflammation that was not correlated with C. difficile toxin levels. Furthermore, blooms in suspected opportunistic pathogens following challenge with antibiotics and C. difficile were observed in mice fed the low fiber purified diets and not with soluble fiber supplementation. Taken together, our results suggest that dietary supplementation with soluble fibers may protect against mortality from C. difficile in this murine model through inducing inflammatory responses that can protect against secondary infections. More work needs to be done to elucidate the mechanism underlying increased inflammation with soluble fiber supplementation.

Poster #15: Electro-enhanced anaerobic digestion

Keywords: anaerobic digestion, bioelectrochemistry

Claire Bailey, D. Bartholet, P. Zaytseva, C. Bailey, K. F. Reardon

In standard anaerobic digestion (AD) of municipal wastewater, diverse microbial communities transform sewage biomass into carbon dioxide and methane, which can be used as a renewable energy source. However, these known greenhouse gases are less valuable, less energy dense, and less environmentally friendly than the more reduced volatile fatty acid (VFA) and solvent metabolites produced as intermediates in the AD process. We hypothesize that we can introduce metabolic shifts away from methanogenesis and towards these reduced chemicals by supplying additional electrons to the AD system — a process we call electro-enhanced anaerobic digestion (electro-AD). During AD, some electroactive microbes exchange electrons to balance their intracellular NAD+/NADH ratio, obtaining energy via direct intracellular electron exchange (DIET). The ability to harness these electrons makes them sensitive to changes in oxidation-reduction potential (ORP) of the system, which can be manipulated with the application of an electron-donating potential from a working electrode. We have shown that electro-AD systems establish electroactive biofilm communities on the working electrode and, under certain conditions, increase yields of VFAs. By combining electrochemical, taxonomic, and metabolomic analyses to small-scale electro-AD reactors, we can begin to understand the complex interactions between microbes and electrodes that lead to increased production of desired metabolites.

Poster #49: Metabolic interactions and core taxa underpinning high methane fluxes across terrestrial freshwater wetlands

Keywords: methanogenesis, wetlands, redox

Emily Bechtold, Danhui Xin, Maricia Pacheco, Anthony Sigman-Lowery, Brandy Toner, Yu-Ping Chin, William Arnold, Mike Wilkins

The Prairie Pothole Region (PPR) of North America contains millions of small depressional wetlands with some of the highest methane fluxes ever reported in terrestrial ecosystems. In wetland saturated soils, two conventional paradigms are (i) methanogenesis is the final step in the redox ladder, occurring only after more thermodynamically favorable electron acceptors (e.g., sulfate) are reduced, and (ii) methane is primarily produced by acetoclastic and hydrogenotrophic pathways. However, previous work in PPR wetlands observed co-occurrence of sulfate-reduction and methanogenesis and the presence of diverse methanogenic substrates likely generated through abiotic processes. Through field and laboratory studies, we investigated the contribution of different methanogenic pathways to methane generation in PPR wetland sediments. Field studies coupled microbial community compositional analyses (i.e., 16S rRNA) with electrochemistry measurements, revealing dominance of methylotrophic methanogens in specific wetlands with high sulfate concentrations. Complementary laboratory mesocosm metatranscriptomic experiments assessed the importance of added field relevant substrates (formate, acetate, and methanol) to fueling methanogenesis in surface sediments. These experiments supported field observations, highlighting the transcriptional activity of methylotrophic methanogens in response to methanol amendment. This resulted in the highest methane production across the experiment, suggesting a greater role for methylotrophic methanogenesis in wetland methane production than previously understood. The use of non-competitive substrates by many methylotrophic methanogens allows these metabolisms to bypass thermodynamic constraints, and can explain co-occurrence patterns of sulfate-reduction and methanogenesis. This study demonstrates that the current models of methanogenesis in wetland ecosystems insufficiently represent methane cycling in some of the highest methane emitting environments.

Poster #37: Integrating BROADN research teams through an undergrad summer research program

Keywords: REU, BROADN, Aerobiome

Brad Borlee, PhD; Beth Hayes, PhD; BROADN REU Students; BROADN REU Mentoring Team

BROADN is an NSF supported discovery network that is an acronym for BII Regional OneHealth Aerobiome Discovery Network. The goal of BROADN is to support a diverse team of researchers to make fundamental discoveries about the microbiome of the air (the 'aerobiome'). The BROADN Research Experience for Undergraduates (REU) program located at Colorado State University in Fort Collins offers a ten-week summer program for undergraduate students. Under the mentorship of a diverse team of scientists within interdisciplinary laboratories, the BROADN REU provides students with a unique opportunity to engage in hands-on research. Through collaborative efforts, participants contribute to fundamental discoveries about the aerobiome and its response to environmental challenges. Our goal is to train undergraduate researchers, equipping them with essential skills to navigate the complexities of the aerobiome within the context of BROADN's research goals. Students are engaged in answering fundamental questions regarding the aerobiome. These questions include: What microbes are in the air? Where do they come from, where do they go, and how do they survive while they are in the air? This authentic research experience is part of the broader mission of BROADN. The program not only emphasizes research but also aims to unite BROADN research teams, promoting synergy and collaboration between interdisciplinary research programs to focus on answering questions about the aerobiome that are not possible to answer within a single discipline or laboratory. BROADN is committed to building a collaborative environment that enhances scientific exploration and contributes to the advancement of knowledge in aerobiome research.

Poster #19: Imaging Metabolism: Luminescent Nanosensors to Image Oxygen Metabolism in Microbial Communities

Keywords: metabolism, biofilms, oxygen, imaging

Kevin J. Cash, Samuel C. Saccomano, Megan P. Jewell, Anne A. Galyean, Alexa A. David, Tony Tien, Pilar A. Martin, Cassandra C. Soeldner, Natalie E. Mudd, J. Kirk Harris, Edith T. Zemanick

Studying oxygen is essential in understanding microbial life. We developed oxygen-sensitive luminescent nanosensors for spatiotemporal imaging of oxygen concentration in microbial communities (example systems: bacterial biofilms and brewing yeast cultures).

Bacterial biofilms are the cause of chronic infections of wounds, medical implants, and immunocompromised patients such as those with cystic fibrosis (CF). These biofilms often exhibit antibiotic resistance that makes them medically difficult to treat. Unfortunately, current antibiotic susceptibility testing (AST) approaches are not clinically useful for CF. Being able to monitor target analytes within the structure of the biofilm is critical for understanding biofilm disease biology and its response to treatment. Current methods of oxygen monitoring such as microelectrodes limit monitoring to one dimension and cannot adequately capture dynamics. We used our nanosensors to measure oxygen concentrations in Pseudomonas aeruginosa biofilms with minimal disruption to the biofilm structure. We can use this oxygen data as a proxy for metabolic response in antibiotic treatment, and with our assay determine antibiotic susceptibility from both sensitive and resistant strains.

We also used a similar approach to monitor brewing yeast. Understanding brewing yeast is traditionally performed on volume scales used in brewing, with 1-liter sizes being considered very small. This precludes high throughput approaches common in other microbiological investigations. We demonstrated the ability of nanosensor-based oxygen monitoring to track beer fermentation in microwell plates, enabling higher throughput testing. We compared fermentation properties and the differing antimicrobial response of a common brewing strain (a Kolsch style yeast) and a Norwegian Kviek style yeast.

Poster #10: Evaluation of the chimeric peptide on Pierce's disease control and its impact on the grapevine microbiome

Keywords: Pierce's disease, Xylella fastidiosa, Antibacterial peptide, Grape Microbiome

Jeongyun Choi, Goutam Gupta, Supratim Basu, Narattam Sikdar, Pankaj Trivedi

Pierce's disease (PD), caused by the xylem-limited bacterium Xylella fastidiosa (Xf), poses a significant threat to the global wine industry. Our group developed a chimeric peptide by combining two peptides belonging to the plant immune system. While antimicrobial peptides (AMPs) have been reported to reduce pathogen titers in plants, their impact on the plant microbiome, crucial the plant growth and productivity, remains poorly understood. In this study, we applied the peptide solution to the grapevine canopy twice, one month apart, and monitored Xf titers and microbiome structure in leaves and bark. Our result revealed that Xf predominantly colonizes symptomatic leaves rather than bark. Also, the peptide treatment significantly reduced Xf titers in the leaves. Microbiome structure changes were observed in infected leaves, independent of peptide treatment, with no significant changes in the bark. Importantly, these microbiome alterations were driven by pathogen infection rather than the peptide treatment. Our findings suggest the chimeric antimicrobial peptide as a promising solution for the Pierce's disease demonstrating efficacy in reducing Xf titers in leaves with minimal impact on the plant microbiome.

Poster #54: Complementary soil quality and microbiome metrics enable in-depth analysis of soil health and function.

Keywords: soil health, 16S, agriculture, SMAF, bioindicators

Christopher Chorpenning, Oliver Hoffman, Jim Ippolito, Kelly Wrighton

Soil health is the basis of sustainable crop production with the soil microbiome being an integral component of the soil ecosystem. Currently, biological aspects of soil health are estimated using phospholipid fatty acid testing, enzyme assays, and microbial biomass and diversity metrics. However, it is increasingly recognized that these microbial measurements are difficult to interpret and fail to provide credible inferences of soil health. To investigate how microbiome data may be used in assessing soil health, we paired 16s/ ITS rRNA gene analysis -uncovering respective prokaryotic and fungal community metrics and co-occurrence networks- with the Soil Management Assessment Framework (SMAF) for measures of soil health. Soil samples were taken from 3 sites in southern Minnesota, with each site including an active conventional farm (high-disturbance; HD) and acreage of established native tall grass prairie (low-disturbance; LD). SMAF scores were statistically higher in LD across sites for overall soil health, biological, and physical scores with the most significant category being the biological indicators: microbial biomass carbon, soil organic carbon, beta-glucosidase assay, and potentially mineralizable nitrogen (n.s.). Microbiome data may reveal key indicator taxa that correlate with soil health or network behaviors that are conserved to LD fields or fields with higher SMAF scores, suggesting microbiome indicators of soil health and function. This study introduces and meshes established tools in microbiome analysis to conventional methods in soil health measurement, allowing for further insight into indicators of soil's capacity to provide ecosystem services.

Poster #13: Effects of Mycobacterium aurum DSM 33539 on biological signatures of stress-induced neuroinflammation, stress resilient behaviors, and gut microbiome in adult male rats

Keywords: Mycobacterium Aurum DSM 33539, neuroinflammation, stress-resilience

Clifton AB, Sago SA, Kessler LR, Sterrett JD, Zambrano CA, Loupy KM, Marquart BM, D'Angelo HM, Fonken LK, Frank MG, Maier SF, Lowry CA.

Stress-related psychiatric disorders, such as anxiety disorders, mood disorders, and trauma and stressor-related disorders such as posttraumatic stress disorder (PTSD) are increasing in prevalence. Emerging evidence suggests that upregulation of inflammatory markers is a risk factor for such disorders. In contrast, immunization with whole-cell, heat-killed preparations of Mycobacterium vaccae NCTC 11659 (recently reclassified as M. kyogaense sp. nov. NCTC 11659) or M. vaccae ATCC 15483, bacterial strains with anti-inflammatory and immunoregulatory properties, have been shown to stabilize the gut microbiome, prevent stress-induced exaggeration of peripheral inflammation, neuroinflammation, and anxiety-like defensive behavioral responses following exposure to chronic psychosocial stress. The extent to which the anti-inflammatory and stress resilience effects of M. vaccae strains generalize to other mycobacterial strains is not known. We tested the hypothesis that Mycobacterium aurum DSM 33539, a mycobacterial strain that is believed to be phylogenetically related to M. vaccae 15483 [Dai et al., 2011, J Clin Microbiol, 49(6): 2296-2303], has the same stress resilience effects as M. vaccae. Here we show that, when assessed 24 h following inescapable stress (IS) exposure, immunization with M. aurum DSM 33539 reduces IS-induced changes in the alpha and beta diversity of the gut microbiome. These data are consistent with the hypothesis that M. aurum DSM 33539 has similar stress resilience-promoting properties to M. vaccae NCTC 11659.

Poster #7: Differential effects of antiretroviral treatment on immunity and gut microbiome composition in people living with HIV in rural versus urban Zimbabwe

Keywords: HIV, Intestinal Microbiome, ART Response

Angela Sofia Burkhart Colorado, Alessandro Lazzaro, Charles Preston Neff, Nichole Nusbacher, Kathryn Boyd, Suzanne Fiorillo, Casey Martin, Janet C. Siebert, Thomas B. Campbell, Margaret Borok, Brent E. Palmer, Catherine Lozupone

Background: The widespread availability of antiretroviral therapy (ART) has dramatically reduced mortality and improved life expectancy for people living with HIV (PLWH). However, even with HIV-1 suppression, chronic immune activation and elevated inflammation persist and have been linked to a pro-inflammatory gut microbiome composition and compromised intestinal barrier integrity. PLWH in urban versus rural areas of sub-Saharan Africa experience differences in environmental factors that may impact the gut microbiome and immune system, in response to ART, yet this has not previously been investigated in these groups. To address this, we measured T cell activation/exhaustion/trafficking markers, plasma inflammatory markers, and fecal microbiome composition in PLWH and healthy participants recruited from an urban clinic in the city of Harare, Zimbabwe, and a district hospital that services surrounding rural villages. PLWH were either ART naïve at baseline and sampled again after 24 weeks of first-line ART and the antibiotic cotrimoxazole or were ART-experienced at both timepoints. Results: Although expected reductions in the inflammatory marker IL-6, T-cell activation, and exhaustion were observed with ART-induced viral suppression, these changes were much more pronounced in the urban versus the rural area. Gut microbiome composition was the most highly altered from healthy controls in ART experienced PLWH, and characterized by both reduced alpha diversity and altered composition. However, gut microbiome composition showed a pronounced relationship with T cell activation and exhaustion in ART-naïve PLWH, suggesting a particularly significant role for the gut microbiome in disease progression in uncontrolled infection. Elevated immune exhaustion after 24 weeks of ART did correlate with both living in the rural location and a more Prevotella-rich/Bacteroides-poor microbiome type, suggesting a potential role for rural-associated microbiome differences or their co-variates in the muted improvements in immune exhaustion in the rural area. Conclusion: Successful ART was less effective at reducing gut microbiome-associated inflammation and T cell activation in PLWH in rural versus urban Zimbabwe, suggesting that individuals on ART in rural areas of Zimbabwe may be more vulnerable to co-morbidity related to sustained immune dysfunction in treated infection.

Poster #36: Microbial Source Tracking for Fecal Indicator Bacteria in Boulder Creek

Keywords: Microbial Source Tracking, Fecal Indicator

Allison Cook, Cresten Mansfeldt

The City of Boulder, CO devotes resources to maintain the Escherichia coli concentration below regulated waterway level thresholds. An annual pipe cleaning in conjunction with the University of Colorado Boulder serves as a one method to remove storm sewer systems as potential point sources. However, the origin of E. coli within and the effectiveness of the cleaning of storm sewers remain undemonstrated. Therefore, to explore the impact of pipe-cleaning on managing the resident storm sewer microbiome, three key objectives are explored: (1) determine whether background E. coli result in false positives, (2) identify the host-origin of E. coli signals, and (3) and quantify the effectiveness of pipe cleaning. Supporting these objectives, nine sampling campaigns were conducted between June-October 2023, withdrawing runoff from three sites within Boulder Creek and the adjoining stormwater network both before and after cleaning. Specific concentrations of fecal indicators were measured following an Environmental Protection Agency quantitative polymerase chain reaction qPCR method capable of distinguishing likely human, ruminant, canine, or avian contamination. Overall, the results are suggestive of a human origin of the E. coli signal within the stormwater network. Combining this qPCR data with additional E. coli plate counts, geospatial information, and precipitation will assist in further understanding key factors driving these levels within the stormwater sewer system and Boulder Creek.

Poster #2: Examining trade-offs between sugarcane aphid resistant and susceptible sorghum lines.

Keywords: Sorghum, Resistant, Susceptible, Trade-offs, Rhizosphere

Stephanie Cromwell, Paul Ayayee, Timothy Dickson, Joe Lious

Sorghum, Sorghum bicolor (L.) Moench, is a staple crop worldwide and a high-carbohydrate climate resilience crop critical to global food production and security, feeding billions of people. Although Sorghum can adapt to various environmental conditions and tolerate abiotic stressors such as drought and extreme temperatures, recent studies show that Sorghum production has decreased in the United States and around the world over the years due to climate change and sugarcane aphid (SCA) damage, a major sorghum phloem-feeding pest. In this study, we investigated the effects of drought and salinity on Sorghum variety crops, the SCA-resistant sorghum line (SC265) and the SCA-susceptible sorghum line (SC1345), productivity, as well as their impact on their rhizosphere microbial diversity. The experiment consisted of the two sorghum plant lines (n= 10) subjected to five treatments: control (without salinity and drought stress), low and high drought and salinity stresses (for 30 days once they reached the 5 leaf -vegetation state). Our results revealed that drought had a more significant effect on plant growth, productivity, and rhizosphere composition than salinity in both plant lines. The most significant differences were found in the high drought compared to all other treatments. Investigation of the bulk soil and rhizosphere microbial community in the experimental treatments are ongoing to examine recruitment from bulk soil into rhizosphere between sorghum lines varying in insect susceptibility. Alpha diversity metrics and beta diversity compositional analyses were used to examine the differences in rhizosphere microbial community composition.

Poster #26: Identification of Microbial Functions Important to Soil Health Indicators using Conditional Random Forests

Keywords: soil health, microbiome, machine learning, microbial function

Heather L. Deel, Daniel K. Manter, Jennifer M. Moore

Soil health and the indicators used to measure it are widely used, but the importance of microbial functions to these soil health indicators is understudied. Identifying microbial functions that influence indicators including active carbon, aggregate stability, autoclaved-citrate extractable protein, respiration, soil organic matter, water capacity, and nutrient content could provide new targets for improving soil health through altering function of the microbial community. Over 600 samples were collected from 2016-2018 from 26 states across the U.S. from cropland and rangeland systems. The microbial community was characterized through 16S ribosomal RNA sequencing, and the function of the microbial community was predicted using PICRUSt2. Additionally, samples were submitted for the Comprehensive Assessment of Soil Health at Cornell University Soil Health Lab for soil health indicator measurements. Conditional random forest machine learning and the microbial enzyme data were used to predict soil health indicators to determine which microbial functions influence indicators the most. For many indicators, enzymes involved in nitrogen and carbon cycling appeared to be the most important. For other indicators, enzymes with unknown function in the soil were most important. This study provides new targets for better understanding the mechanisms by which microbes can contribute to healthy soils.

Poster #20: Structure & Interactions of the Airborne Microbial Communities

Keywords: Aerobiome, Microbiome, Global Warming, Climate Change

Avinash Dhar, Yuan Jing, Marina Nieto-Caballero, Beth Hayes, Kristen Otto, Thomas Hill, Jessica Metcalf, Paul DeMott, Lily Jones, Claudia Mignani, Ashley Milller, Jane E Stewart, Brad Borlee, Mark T Hernandez, Sue VandeWoude, Sonia.Kreidenweis, Jan E. Leach, Pankaj Trivedi

Microbial communities sustain fundamental ecosystem services, including nutrient cycling, primary production, energy transformation, and climate regulation. However, characterizing these communities within the aerobiome is complex due to low biotic concentrations and the need for detailed sampling strategies. Addressing these challenges, our research provides a detailed census of the aerobiome's taxonomic composition and functional potential. We demonstrate that airborne microbial communities are not only diverse and dynamic but also intricately complex, paralleling terrestrial and aquatic biomes. These communities' intricate networks are shaped by multiple environmental factors, leaving them susceptible to disturbance. Our findings elucidate the principles underlying the assembly, dynamics, stability, and vulnerability of the aerobiome. The insights gained are crucial for a deeper understanding of aerobiome dynamics and for predicting how these aerial microbial networks interact with and influence environmental processes.

Poster #1: EcoGenoRisk: Computational Biology for Synthetic Biological Risk

Keywords: Computational, Biology, Sythetic

John Docter, Cresten Mansfeldt

Synthetic biological (synbio) organisms pose a unique challenge for effective risk assessment and mitigation. With self-replication, synbio organisms have the potential to greatly impact environmental and human health. EcoGenoRisk is a bioinformatic ecological risk assessment software tool that quantifies the likelihood and damage of synthetic microbial cell release into the environment. EnvCen, the current focus of development, utilizes computational biology to identify impacted habitats, characterize community function shift, calculate community repair through microorganism random immigration, and report community function loss. Each one of these operations contributes to a risk probability score that will build upon the tool's other analyses, giving the user a data backed insight into a synbio organism's potential for harm. Ultimately, as synthetic biology becomes adopted across economic sectors, EcoGenoRisk will give both public and private actors the necessary ability to understand synthetic biology risk and protect human and environmental health.

Poster #14: Differences in microbial community abundance, diversity, and functional attributes on regenerative vs. conventional farms

Keywords: soil microbiome, regenerative agriculture, soil carbon, no-till, cover crops

Elizabeth Ellis, Laura Mason, Keith Paustian, Kelly Wrighton

Regenerative agriculture is an approach to farming that uses soil conservation to regenerate agroecosystem function and improve the provisioning of ecosystem services. Practices that fall under the umbrella of regenerative agriculture include no-till farming, use of cover crops, diversified rotations, and integrating animals into cropping systems. Regenerative agriculture improves soil and ecosystem health by rebuilding soil organic matter (SOM) and soil organic carbon (SOC) lost through years of conventional practices (i.e., heavy tillage, fallow, over application of inputs, etc.). Although the importance of the soil microbial community in SOC cycling is well known, the ways that regenerative agriculture changes the structure and function of the microbial community require further investigation. The objective of this study is to elucidate differences in microbial community abundance, diversity, and functional attributes through an observational study of farms employing regenerative and conventional practices. We sampled soils from eight commercial farms throughout Iowa using one of two management systems: 1) no-till + cover crops (regenerative), or 2) annual tillage + winter fallow (conventional). We obtained samples for four depth increments (0-15, 15-30, 30-60, and 60-100 cm) allowing us a rare glimpse into the microbial world of agricultural subsoils. Through 16S and ITS DNA amplification and sequencing, we will characterize the microbial community structure between the study farms and elucidate potential differences in soil carbon stocks to a depth of one meter.

Poster #55: Methods and Software for the Analysis of Longitudinal Compositional Microbiome Data

Keywords: zero-inflation, software, Bayesian, longitudinal , high-dimensional

Brody Erlandson, Ander Wilson, Matt Koslovsky

A major aim in human microbiome studies is investigating the feasibility of designing personalized interventions that modulate and maintain the composition of the microbiome to diagnose and treat microbiome-associated diseases. Microbiome studies frequently follow individuals to monitor how changes in their microbiome composition may be related to an exposure or treatment, such as dietary intake, over time. Analysis of longitudinal microbiome studies is challenging due to the correlation between repeated measures for each individual, as well as the high-dimensional, compositional structure of microbiome data and potential zero-inflation. We propose and illustrate a new model for compositional microbiome data collected in longitudinal studies. Specifically, our method extends the recently developed zero-inflated Dirichlet-multinomial model to repeated measures microbial count data. Our method includes random effects to account for correlation between repeated measures of exposures, time-varying coefficients to investigate how a covariate's relation with relative abundances may vary over time, and induces sparsity to scale to high-dimensional covariate and compositional spaces. As such, our method is able to recover the complex relationships between covariates and microbial composition, ultimately helping the design and evaluation of personalized treatment strategies for human health and disease.

Poster #25: Increased Levels of Glucocorticoids is Associated with Increased Abundance of Colonic Helicobacter in a Model of Psychosocial Stress

Keywords: stress, microbiome, helicobacter, endocrinology

Espino MA, Reber SO, Siebler PH, Donner NC, Morton JT, Smith DG, Kopelman JM, Lowe KR, Wheeler KJ, Fox JH, Hassell JE Jr, Greenwood BN, Jansch C, Lechner A, Schmidt D, Uschold-Schmidt N, Füchsl AM, Langgartner D, Walker FR, Hale MW, Lopez Perez G, Van Treuren W, González A, Halweg-Edwards AL, Fleshner M, Raison CL, Rook GA, Peddada SD, Knight R, Lowry CA

Unhealthy lifestyle factors such as poor diet, lack of exercise, and an inability to cope with stress contribute to the development of inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease. Colonic species of Helicobacter are implicated in the development of gastrointestinal symptoms in UC. In this secondary analysis of a published study [Reber et al., 2016, PNAS, 113 (22), E3130-E3139], the relationship between basal measures of hypothalamic-pituitary-adrenal axis (HPA axis) activity and the relative abundance of different strains of Helicobacter in the gut microbiome was established. In a previous experiment, mice were exposed to a model of repeated psychosocial stress and either immunized with a series of heat-killed preparations of Mycobacterium vaccae NCTC 11659 or vehicle injections. Microbiome analysis revealed that stress exposure increased the relative abundance of Helicobacter. Although immunization with M. vaccae did not prevent stress-induced increases in the relative abundance of Helicobacter, it ameliorated many stress-related symptoms including spontaneous colitis. We hypothesized that basal HPA-axis activity, such as plasma concentrations of adrenocorticotropic hormone (ACTH) and corticosterone, is positively associated with increases in the relative abundance of Helicobacter in the gut microbiome. The correlation between plasma concentrations of ACTH/corticosterone and the relative abundance of Helicobacter was analyzed. Plasma concentrations of corticosterone, but not ACTH, were positively correlated with increases in the relative abundance of Helicobacter. Future studies will investigate the mechanisms through which the presence of Helicobacter leads to exaggeration of stress-induced pathology, and how M. vaccae prevents these effects.

Poster #5: Genome-scale Metabolic Modeling of Aspergillus oryzae

Keywords: Aspergillus, fungi, fermentation, MATLAB, FBA

Emmanuel Ezika, S.H.J. Chan

Aspergillus oryzae is a filamentous fungus used extensively in the fermentation industry to produce healthy fermented sauces such as soy sauce, miso, and sake, and the production of industrial enzymes such as amylase, lipase, and protease. This research aims to have an improved reconstructed metabolic network that can accurately predict increased growth and biomass yield of A. oryzae on different carbon sources. The COBRA Toolbox in MATLAB was used for the network reconstruction of a recently improved genome-scale metabolic A. oryzae RIB40 model from the model paper. Information for the reconstruction was collected from biochemical pathways like MetaCyc, online protein databases like UniProt, data platforms like KBase, and literature. The reconstructed metabolic network was transformed into a mathematical framework for simulation-based Flux Balance Analysis (FBA) to predict the fungi growth rate with minimal media on different carbon sources (glucose, xylose, glycerol, and maltose). The flux balance analysis result of the original model (O) from the model paper, my reconstructed model (R), and the result from the literature (L) showed that R had a higher growth rate on glucose and xylose, the same growth rate as O but higher than L on glycerol, and a higher growth rate than O but a little bit lower than L on maltose. My reconstructed model is an improved metabolic network of the A. oryzae RIB40 model and thus is a relevant resource to gain more insight into our understanding of A. oryzae physiology to engineer an increase in amino acid synthesis during fermentation.

Poster #9: Bristol Stool Form is Associated with Anxiety and Depressive Symptoms in Stressed Adults

Keywords: Stool form, mental health, anxiety, depression, microbiome-gut-brain axis

Lauren Finkelstein, Christopher Lowry, Joanna Arch

Background: Stool form is associated with gut microbiota richness, which in turn is associated with mental health outcomes via the microbiota-gut-brain axis. This study evaluates the direct relationship between self-reported stool form and psychological symptoms, with the goal of improving our understanding of associations among these symptoms using an easy-to-administer screening tool. Methods: Data were collected as part of a randomized trial of stressed adults in Colorado (N=108) who received a probiotic supplement or placebo for 46 days. They completed the Bristol Stool Form Scale and measures of stress, anxiety, and depression at baseline and again on the final day of supplementation. Data were analyzed using multi-level models with random intercepts for participant. Results: In models that account for person-level random intercepts, compared to participants with normal stool, participants with soft stool reported higher levels of anxiety ($p=0.015$) and depression ($p=0.014$), but not stress ($p=0.125$). These differences are such that the average participant with normal stool reported mild anxiety symptoms while the average participant with soft stool reported moderate anxiety symptoms. Similarly, the average participant with normal stool reported depression symptoms 24.30% under the clinical cutoff for major depression, compared to participants with soft stool who were 1.23% below this threshold. We observed no differences in mental health outcomes for participants with hard compared to normal stool (p's>0.689). Conclusion: These findings indicate that soft stool form is associated with higher levels of anxiety and depressive symptoms, but not stress levels, in a population of stressed adults.

Poster #18: Disentangling the interacting disturbances of mountain pine beetle (MPB) kill and salvage logging on microbiome-mediated vegetation recovery

Keywords: Mountain pine beetle, salvage harvesting, ectomycorrhizal fungi, greenhouse bioassay, amplicon sequencing

Julie A. Fowler, Kya M. Sparks, Timothy S. Fegel, Sarah J. Hart, David M. Barnard, Charles C. Rhoades, Michael J. Wilkins

Large scale outbreaks of mountain pine beetles (MPB) in Colorado over the past few decades have led to the loss of millions of acres of ponderosa and lodgepole pine trees and are often followed by salvage harvesting of dead stands. However, the impacts of the interaction between MPB outbreaks and the time until salvage harvesting on the soil microbiome and associated vegetation recovery are unknown. To elucidate these interactions, we conducted a vegetation and soil sampling campaign in State Forest, Colorado on sites within six treatment classes. These treatment classes represent undisturbed forests, those that were clear-cut before an MPB outbreak, and those that suffered MPB-related mortality and were cut down either close to the time of stand death or decades later. Microbiome profiling through 16S rRNA gene and ITS amplicon sequencing revealed that host tree death prior to clear-cutting leads to decreased soil ectomycorrhizal fungi populations, which are necessary for conifer tree establishment and persistence. To support this observation, ongoing greenhouse bioassay experiments are investigating ectomycorrhizal colonization of lodgepole pine seeds using soils from all six treatment classes and two moisture regimes. Bioassay analyses will include quantifying ectomycorrhizal colonization of the pine roots, amplicon sequencing of rhizosphere soils, and ecophysiological assessment of the pine seedlings. Coupled with extensive vegetation characterization from the field sites, these data will reveal long-term impacts of MPB outbreaks and management operations on belowground soil microbiomes and vegetation recovery.

Poster #21: Functional Resistances of Aerobiome Bacteria That Might Promote Survival in the Atmosphere

Keywords: Aerobiome, Bacteria, Ultraviolet light, Reactive Oxygen Species (ROS), Co-resistance

Adam Gillison, Pankaj Trivedi, Paul Demott, Thomas Hill, and Amaya Garcia Costas

Research in the human and soil microbiomes has shown the importance of interactions between complex organisms and microbes in those environments. The aerobiome, microbes in the atmosphere, is an additional microbial environment that has been understudied, due to its low biomass density and a lack of reliable sampling tools. Yet, thanks to recent advancements in the development of modern analytical techniques, we are discovering that microorganisms in the aerobiome have roles in ecosystem diversity and health, disease outbreaks, and potentially the Earth's hydrologic cycle. At the same time, the known functional properties and physiological traits of the atmospheric microorganisms themselves are limited. Due to the constant exposure to ultraviolet (UV) light in the atmosphere, it is predicted that a higher resistance to this environmental stressor is one trait shared by aerobiome microorganisms. To determine this the UV-B tolerance of aerobiome bacteria was examined. Bacteria with the highest UV-B tolerance were identified and characterized, including possible UV-resistance mechanisms and co-resistance. This research provides a framework to evaluate the functional properties of atmospheric microorganisms identified in community composition studies.

Poster #50: Effects of Cannabidiol (CBD) on Gut microbiome of Manduca sexta.

Keywords: Gut microbiome, CBD, insect

Shannon Isenhart, Dr. Amaya Garcia Costas

Tobacco hornworms are a common pest found throughout the continental United States and in neighboring continents and islands. They feed on nightshade family plants, decimating common crops. Due to their short life cycle and ease of care, tobacco hornworms are model organisms for caterpillar and moth studies, especially in agricultural-related research. CBD is a secondary metabolite found in Cannabis sativa commonly known as the hemp plant. Being one of over 100 secondary metabolites, it is believed to be part of the plant's defense against pests. Previous research has shown that after being exposed to CBD, tobacco hornworms have a high failure rate shedding their exuvia, causing them to die. In addition, CBD exposure caused significant changes to gene expression in these hornworms. We hypothesize that Manduca sexta's gut bacteria might play a role in detoxification or changed physiology during this CBD exposure by either metabolizing and altering CBD or changing their own metabolism. To investigate these potential roles of the gut microbiome we intend to examine bacterial community composition and functionality changes in the gut of tobacco hornworms. Briefly, these are reared with either CBD and MCT oil, MCT oil, or nothing mixed into their artificial food starting at the 3rd instar. At the 5th instar tobacco hornworms are euthanized, their gut dissected, and DNA extracted. 16S rRNA sequencing will be used to identify the bacteria present in the gut of each caterpillar during the different conditions. Future studies will examine the changes in functional properties of the gut microbiome.

Poster #11: Interrogating how Clostridia gut metabolites influence peripheral inflammatory response in Autism Spectrum Disorder

Keywords: autism, dysbiosis, inflammation, metabolite, macrophage

Sydney Johnson, Emily Perkins, Quinn Pogge, Julie Moreno, Katriana Popichak

Autism Spectrum Disorder (ASD) encompasses a group of developmental disorders associated with repetitive interests and impacted social behaviors. With the rising diagnosis rates of ASD, and limited therapeutic intervention, there is a growing emphasis on researching its underlying causes. Surprisingly, children with ASD exhibit dysbiosis, an imbalance in gut microbial composition; presenting with a higher concentration of Clostridium histolyticum than those not diagnosed with ASD. Upon treatment with a Clostridium-targeting antibiotic, ASD-associated psychological symptoms decrease, suggesting that ASD may emanate from the gut. C. histolyticum metabolizes phenylalanine into m-tyrosine (m-tyr), a known analog for the dopamine precursor, L-DOPA, further metabolized by bacteria to form 3-hydroxyphenylpropionic acid. When 3-hydroxyphenylpropionic acid undergoes beta-oxidation, it becomes 3-(3-hydroxyphenyl)-3-propionic acid (HPHPA). Additionally, it's shown that HPHPA levels are elevated in urine samples from children with ASD, further suggesting that the cause of ASD not only originates from the gut, but that this particular bacterial species plays a substantial role in disease progression Thus, we hypothesize that the C. histolyticum metabolites, m-tyr and HPHPA, lead to peripheral inflammatory response mediated by innate immune cells, macrophages, that exacerbates neuronal dysfunction in ASD. . To test this hypothesis, we exposed a macrophage, murine cell line, RAW 264.7, to both m-tyr and HPHPA and s measured viability and pro-inflammatory gene expression. Additionally, we measured neuronal viability upon treatment with macrophage conditioned media to determine whether HPHPA/m-tyr exposed macrophages release neurotoxic mediators. Taken together, these data demonstrate novel targets for more effectively treating ASD.

Poster #35: Can Aquatic Microbes Close the Solubility Gap of Pyrogenic Carbon?

Keywords: Wildfire, aquatic, sequencing

William Johnson, Professor Cresten Mansfeldt

During wildfire, biogenic carbon in ecosystems is transformed. While a majority of burned carbon is oxidized to form CO2, the remainder is converted into pyrogenic organic material (PyOM), which has been found to be abundant in soil, inland waters, and oceans through the quantification of condensed aromatic carbon. The ubiquity of pyrogenic carbon in aquatic environments is complicated by the apparent insolubility of condensed and high-temperature pyrogenic species in laboratory and modeling studies. The ability of PyOM to enter the dissolved phase may be explained in part by microbial oxidation of particulate PyOM. This research seeks to characterize the microbial transformation products of PyOM in water-sediment biodegradation tests using spectroscopy. In order to determine which community members may be adapted to interfacing with PyOM, 16S rDNA sequencing data are used to evaluate short term microbial succession at the water-sediment interface. As PyOM is associated with the toxic, but generally hydrophobic class of compounds known as polycyclic aromatic hydrocarbons, analysis of these microbial data seeks to inform questions of both contaminant mobilization and aquatic ecosystem response. This research suggests the importance of aquatic microbes for understanding the ultimate fate of pyrogenic carbon, as well as the chemical identity of its transformation products in water.

Poster #61: Getting into the MUCC reveals microbial carbon cycling dynamics in freshwater wetlands

Keywords:

Sophie K. Jurgensen, Aaron Gondran, Emily Bechtold, Angela Oliverio, Christopher E. Chorpenning, Alexandra M. Wettengel, Jared Ellenbogen, Reed Woyda, Christopher Henry, William Riley, Yu-Ping Chin, Gil Bohrer, Eric Ward, Sheel Bansal, Mike Wilkins, Mikayla Borton, Jorge Villa, Kelly Wrighton

Despite a small aerial coverage, wetland soils store nearly one third of the soil organic carbon and represent the largest natural source of atmospheric methane (CH4) on this planet. Constraints on microbial metabolism in these soils dictate the balance between the soil carbon sequestered and released as greenhouse gases, yet these controllers are poorly defined across diverse wetland systems, limiting transferability to modeling frameworks. Here we employed a coordinated, standardized sampling effort to collect paired microbiome, geochemical, and carbon greenhouse gas production and flux measurements from wetlands in the U.S. Department of Energy's Ameriflux network. These 10 wetlands (5 marshes, 2 bogs, and 3 fens) range in geographic location, mean annual soil temperature (4-23 ⍰C), precipitation (833-1616 mm), and CH4 emissions (1-24.6 g m2). Data from this effort contributes to the collaboratively built Multi-omics to Understand Climate Change (MUCC) genomic resource, a microbial catalog of these climatically important soils. Currently the MUCC database contains 17,333 metagenome-assembled genomes representing 2,502 unique genomes, with 57% lacking prior characterization. Our first investigation from the highest CH4 methane emitting wetland (US-OWC) revealed coordinated gene expression networks explained the greatest variability in CH4 and CO2 emissions. This was complemented by a subsequent network analysis incorporating 5 other wetlands which revealed microbial membership to be a better predictor of CH4 fluxes than abiotic factors alone. By leveraging paired geochemical, metabolomic, and GHG measurements with multi-omics data, we provide new insights into the unifying patterns and drivers of methane flux across freshwater wetlands.

Poster #34: Clustering zero-inflated multivariate compositional count data

Keywords: compositional, clustering, semiparametric, Bayesian, diet

Kevin Korsurat, Matt Koslovsky

Individuals who share similar microbial composition may respond similarly to treatment for chronic diseases such as cancer. Thus, the identification of subgroups with similar microbial compositions can help researchers design and evaluate personalized intervention and/or treatment protocols. Existing methods for clustering microbiome data are limited as they do not accommodate the high-dimensional, zero-inflated, and compositional structure of the data. Further, they may require specifying the number of clusters a priori or tend to overestimate the number of clusters in the data. We propose a novel Bayesian semiparametric mixture model that addresses these limitations by assuming the observed data and latent clusters labels follow a zero-inflated Dirichlet-Multinomial distribution. In simulation, we demonstrate the method's improved clustering and estimation performance compared to existing nonparametric and model-based clustering algorithms for microbiome data. We then apply the proposed method to data collected in a dietary intervention study investigating the effects of nutrient dense, fiber-rich foods on the gut microbiomes of infants in Nicaragua and Mali. Our approach identifies distinct clusters of microbial composition at 6-, 8-, and 12-month measurements, largely differentiated by infants' nationality.

Poster #39: Modulation of Culicoides sonorensis Microbiome in Response to Veterinary Pharmaceuticals

Keywords: midge, Culicoides, blue tongue virus

Colin Korte, Jonathan Rodriguez, Tyler Sherman, Phillip Shults, Christie Mayo, Jessica Metcalf, Bradley Borlee, Grace Borlee

Culicoides sonorensis is an ecologically relevant species of biting midge and a prominent vector of livestock and wildlife diseases, such as blue tongue virus, African horse sickness virus, and epizootic hemorrhagic disease virus. Although none of these viruses can be transmitted to humans, potential methods of controlling and repressing transmission are emerging areas of research. The bacterial composition of the midge midgut may influence its ability to transmit disease. Manipulating the gut microbiome of this arthropod vector may reduce the frequency at which these diseases are transmitted. To determine how the microbiome of the midgut can be altered by exposure to drugs or antibiotics commonly used in livestock veterinary medicine, lab-reared C. sonorensis midges were fed bloodmeals treated with either flunixin (NSAID), fenbendazole (antihelminth), dexamethasone (corticosteroid), tetracycline (antibiotic), or ceftiofur (anti-infective). Midges were surface sterilized in 70% ethanol and the bacterial composition of the midge was determined via 16S rRNA gene sequencing. Overall trends of microbial diversity between treatments were similar, except for the ceftiofur treatment, which induced decreased diversity. Midges that had ingested bloodmeal with ceftiofur had a higher relative abundance of Aeromonas spp. and Morganella spp. as compared to the other treatments. The ceftiofur bloodmeal-treated midges had a decreased relative abundance of other notable bacteria such as Acinetobacter. Other drug or antibiotic treatments resulted in less dramatic changes in diversity with the most fluctuations of relative abundance being in the Acinetobacter spp. Future studies will investigate how these changes in the microbiome alter the dynamics of blue tongue virus replication and transmission.

Poster #24: Increased toxin copy number promotes containment efficacy in both lab and industrial strains of Saccharomyces cerevisiae

Keywords: biocontainment, whole genome sequencing, Saccharomyces cerevisiae, genetic engineering, industrial microbes

Natalie Lamb, Kimberly Rosenbach, Thomas Musselwhite, Katie Arnolds, Riley Higgins, Gabriella Li, Jeff Linger, Michael Guarnieri

The emerging bioeconomy is dependent on a multitude of bioproducts made by engineered and modified microorganisms. While these genetically modified organisms (GMOs) are integral to a sustainable bioeconomy, there are also concerns about the impact GMOs could have on the surrounding environments if they were to escape from lab or industrial conditions. To understand genetic components that impact effective biocontainment, we have paired the CamOff switch adapted from Pseudomonas putida with the bacterial toxin relE in Saccharomyces cerevisiae. The CamOff-relE system was integrated in both a lab strain (BY) and an industrial strain (PE-2) of yeast. We have evaluated the impact of increasing copy number of the toxin relE and found that biocontainment is most effective with two or four copies of relE, added in a homozygous fashion to diploid yeast. High throughput screening of biocontained strains for 72 hours in liquid culture revealed rare escape events and the time of escape varied by genetic isolate. To understand mechanisms that may enable escape, we performed whole genome sequencing on seven distinct escapees. Four out of seven escapees had nonsynonymous mutations in either CamOff or relE. The other three escapees had no mutations in the containment system, but instead gained mutations in genes involved in DNA replication and repair, along with mannose biosynthesis. We hypothesize that the combination of multiple mutations in these pathways promotes escape in strains of CamOff-relE yeast. This work provides a suite of containment strains for future assessment of the impact of GMO introduction to distinct environments.

Poster #45: Colorado Riverine Nitrosomonas oligotropha and Metal Resistance

Keywords: Nitrogen; Metal; Stream; Pollution; Ammonia-Oxidizing Bacteria

Nicole Laurita; Annika Mosier; Andrew Boddicker

Microbes play pivotal roles cycling nitrogen between gaseous dinitrogen and its bioavailable forms. While every cell needs nitrogen, excessive nutrient loading leads to an overburdened nitrogen cycle and eutrophication in freshwater ecosystems. Metal toxicity can stress or kill these microbes, further exacerbating eutrophication. To protect these vital microbes, it is important to understand their habitat as well as their mechanisms of metal resistance or lack thereof. This study summarizes historic stream concentrations of ammonia and bioavailable metals in the South Platte River Basin and explores potential non-point source contributors within the subwatersheds. This study also examines ammonia-oxidizing bacteria (AOB), or microorganisms that convert ammonia into nitrite, collected from streams within the South Platte River Basin. We report the enrichment and near-complete genome sequences of three Nitrosomonas oligotropha AOB representatives from the South Platte River, Cherry Creek, and Cache la Poudre River. To assess the potential for metal resistance, we examine the genomes for metal resistance genes, alongside operon analysis, with comparative genomics with other Nitrosomonas. By examining the conditions of the riverine bacteria as well as their metal resistance genes, we aim to understand their environmental pressures while gauging their potential susceptibility or resiliency to metal toxicity.

Poster #8: From Dirt to Data: Comparing Liquid Chromatography-Mass Spectrometry Methods for Soil Metaproteomics

Keywords: metaproteomics, microbiome, mass spectrometry, soil proteomics

Dorathea Lee, Gustavo Diaz, Mikayla A Borton, Meagan E Schipanski, Valerie A Seitz, Jessica E Prenni, Kelly C Wrighton, Corey D Broeckling

Recently, metaproteomics has emerged as a powerful microbiome phenotyping platform, providing a more dynamic picture of symbiotic interactions and biochemical functions to complement metagenomic and metatranscriptomic approaches. Soil metaproteomics provides functional characterization of microbial communities imperative to agricultural and environmental monitoring. Studies are limited by highly complex samples, concomitant challenges posed by analysis of myriad proteomes. While Data Dependent Acquisition (DDA) has been the standard for many years, fractionation and strategies like Data Independent Acquisition (DIA) could provide better characterization but have not been explored fully. In this work, we evaluate the performance of DIA, DDA, and DDA coupled with High-Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS) gas-fractionation for low-biomass bottom-up metaproteomics analysis. Samples were 0.5g aliquots of soil from a CSU agricultural field and ZymoBIOMICS Microbial Community Standard (Zymo Research). Samples were run on a Vanquish Neo coupled with an Orbitrap Eclipse (Thermo Scientific). Preliminary DDA testing of soil samples identified 3866 proteins and 7020 peptides assigned to 92 different taxa. This demonstrates that standard DDA can achieve high-quality data and a reasonably high number of IDs within the scope of low-biomass metaproteomics, but improvements are possible. We will compare these DDA results to DIA and FAIMS for both samples, including number of peptide IDs, protein IDs, and sequence coverage. We will also discuss reproducibility, variation in taxa/species representation, and respective merits/demerits of each method. If possible, we will develop recommendations as to which method may be better for certain sample types or experiments to guide future metaproteomics research.

Poster #33: Microbiomes in Agroecosystems and Production Soils (MAPS): hundreds of metagenome-assembled genomes from agricultural soils across Colorado as a reference to surveying soil health.

Keywords: Soil health; Soil microbiome; Metagenomics; Metatranscriptomics; databases

Laura Mason, Christopher Chorpenning, Bridget McGivern, Lady Grant, Tad Trimarco, Valerie Seitz, Agustin Nuñez, Lisa Eash, Peter Olayemi, Steve Fonte, Troy Bauder, Erik Wardle, Jessica Prenni, Jim Ippolito, Meagan Schipanski, Reza Keshavarz Afshar, Gene Kelly, Mikayla Borton, Kelly Wrighton.

Soil health is the basis of sustainable crop production with the soil microbiome being an integral component of the soil ecosystem. Currently, biological aspects of soil health are estimated via PLFA, enzyme assays, and microbial biomass and diversity metrics. However, it is increasingly recognized that these microbial measurements are difficult to interpret and fail to provide credible inferences of soil health. To interrogate existing and emerging microbiome diagnostics of soil health relevant to semi-arid agroecosystems, we constructed a genomics-enabled database and software framework. Our first major hurdle was overcoming the lack of comprehensive bacterial genomic databases for agricultural soil microbiomes, which prohibits the development of more sensitive measurements of microbial activity relevant to soil health. To build this resource, we obtained 108 agricultural soil metagenomes across Colorado, spanning seasons, geography, crops, and management practices. We created the Microbiomes in Agroecosystems and Production Soils (MAPS) database, a genome-resolved catalog of 610 metagenome-assembled genomes (MAGs) representing 22 bacterial and archaeal phyla. Of the MAGs recovered, 15% were from novel, illustrating the untapped microbiology harbored in agricultural soils. We next developed annotation software, DRAM-Ag, to classify this genomic content, revealing microbiota contributions to nutrient cycling and C storage. Currently, we are leveraging this genomic annotation system to analyze a multi-year dataset with paired traditional soil health metrics and metatranscript data, enabling machine learning approaches for the discovery of microbial activity-based determinants of soil health. This research contributes to emerging microbiome metrics for soil health with utility for producers and researchers alike.

Poster #12: Soil respiration after irrigation retirement influenced more by changes in substrate than moisture availability

Keywords: soil respiration, heterotrophic respiration, carbon, agroecosystem, soil moisture

Violeta Mendoza Martinez, Agustin Nuñez, Veronica Acosta-Martinez, Meagan Schipanski

Over half of the world's fresh water is used in crop production and use far exceeds local water availability and recharge rates. With climate change, agriculture will be pressured into reducing its water consumption and large areas of currently irrigated farmland across the Western U.S. will likely transition into dryland agriculture. The effects this will have on global soil C dynamics, however, remain unclear. In 2016, a transitional study was established at CSU's ARDEC in Northern Colorado to understand how stopping irrigation will affect soil C turnover in a no-till, continuous maize agroecosystem. Earlier results showed limited responses of the soil microbial community to the adoption of the new watering regime, but two years of accumulated plant residues brought differences in soil heterotrophic respiration (Rh) rates suggesting a possible co-limitation of water and available C to the microbial community. With the present study, we further explored the relationship between soil moisture and C inputs in shaping the soil microbial community under the new watering regimes. Two seasons of data collection showed decreases in available soil water, bacteria, fungi, protozoa and actinomycetes FAME biomarkers, activities of four extracellular enzymes and soil autotrophic respiration in response to both reductions in irrigation and plant inputs, with strong interactive effects between the two factors. However, plots under dryland conditions had higher concentrations of DOC and muted differences in soil Rh when compared to their irrigated counterparts; differences in Rh between fallow treatments were more pronounced. Correlations of soil respiration with moisture were weak or even negative, thus suggesting soil moisture was not a strong direct driver of Rh. In contrast, monthly soil moisture measurements had a stronger, direct effect on Rh than substrate availability as estimated by water-extractable DOC.

Poster #22: Soil bacteriomes of competing plants; bacterial taxa that are individually tailored to a plant and bacterial taxa that are shared.

Keywords: Bacteriome, plants, bulk soil, rhizosphere

Derek Newberger, Ioannis Minas, Daniel Manter, Jorge Vivanco

Plant inter-/intraspecific competition and facilitation has been repeatedly studied, however, there is little empirical information about the shared bacteriome of multiple plants. Here, the bulk soil and rhizosphere of individual plants was analyzed in a microcosm study containing different combinations and densities of cover crops: Medicago sativa, Brassica sp., and Fescue sp. In the bulk soil, bacterial alpha diversity did not increase with either plant diversity or density. Alternatively, there was a trade-off, where each plant combination enriched different beneficial bacteria. Interestingly, Azospirillum spp. which was enriched in alfalfa and brassica monocultures, were not enriched in the bulk soil of alfalfa-brassica mixtures. Alternatively, phytohormone producer Pseudarthrobacter phenanthrenivorans was enriched. Furthermore, mixtures of all three plants showed an increase in abundance of Planctomyces sp. SH-PL14 and Sandaracinus amylolyticus which were not represented in the monocultures. Rhizobacterial beta diversity was promoted by increasing plant diversity around the target plant species. In contrast, rhizobacterial beta diversity was reduced by increasing plant density for each plant mixture. When intraspecific competitors increased, a few rhizobacteria were consistently enriched for each plant species. A few bacterial taxa were shown to have conditional associations such as being enriched within only the highest plant densities. These bacterial taxa may alleviate plant competition within these spaces. Lastly, there is evidence of bacterial sharing of nitrogen fixers from alfalfa to fescue. Overall, rhizobacterial recruitment is plant species specific, with intraspecific competitors helping to establish a particular bacteriome and interspecific competitors shifting the target plant's bacteriome.

Poster #52: Effects of Indoor versus Outdoor Cadaver Decomposition on the Microbiome

Keywords: Decomposition, indoor environment, post-mortem interval (PMI), machine learning

Victoria Nieciecki, Zachary M. Burcham, Kristen Otto, Aaron Lynne, Sibyl Bucheli, and Jessica Metcalf

Estimating the postmortem interval (PMI) is a critical step in many crime scene investigations. Current methods that rely on visual assessments of decomposition stage or entomology are dependent on dynamic environmental factors that may decrease PMI accuracy. Previous studies have shown that a distinct microbial network assembles at the site of vertebrate remains to break down organic matter in a predictable manner regardless of climate and geography. Recent work has shown these key decomposers are valuable predictors in novel microbial-based PMI estimation techniques. While these studies provide valuable forensic tools for estimating PMI, they exclusively rely on data collected from controlled, outdoor forensic research facilities. Several factors such as temperature, humidity, and exposure to insects may alter the fate of human remains decomposing indoors, impacting the accuracy of PMI estimates. In this study, we investigate the effects of shelter on microbial succession during human decomposition across 2 seasons and provide important considerations for PMI modeling. Human cadavers were left to naturally decompose in sheltered enclosures along with paired outdoor controls for 21 days. We found that outdoor skin samples were colonized by a more diverse microbiome that was distinct from communities recovered from indoor skin samples, giving rise to different microbial community trajectories over time. Finally, a machine learning model that was trained on outdoor bodies more accurately predicted the PMI of outdoor cadavers compared to those placed indoor. Together, our findings suggest that incorporating indoor cadaver data into PMI models may improve predication capabilities across most decomposition environments.

Poster #16: High-Fiber/Low-Fat Diet Modulates Immune and Metabolic Phenotypes in HIV Independent of the Gut Microbiome Composition

Keywords: HIV, Diet, Metabolic Disease, Inflammation

John O'Connor, Jennifer Fouquier, Charles P Neff, Tyson Marden, Suzanne Fiorillo, Janet C. Siebert, Nichole Nusbacher, Janine Higgens, Thomas Campbell, Brent Palmer, Catherine Lozupone

Background: Human immunodeficiency virus (HIV) remains a global health threat affecting the lives of millions living with the virus. HIV microbiomes, particularly in men who have sex with men (MSM), are characterized by Prevotella rich Bacteroides poor (PRBP) enterotypes. PRBP enterotypes correlate with increased metabolic disease and chronic inflammation but are observed in agrarian populations consuming high-fiber/low-fat diets. Additionally, western high-fat diets exacerbate simian immunodeficiency virus disease, providing motivation to assess the the effect of diet-microbiome matching with high-fiber/low-fat diets on inflammatory/metabolic-disease in HIV. Methods: In this study, a 4-week dietary intervention was conducted. Participants, enrolled at the University of Colorado, were randomized to a high-fiber/low-fat agrarian diet (AD) or a low-fiber/high-fat western diet (WD). Fecal and mucosal microbiomes were characterized using 16S ribosomal RNA gene sequencing. Inflammatory and metabolic markers were measured in blood. Cytometry by Time of Flight was used to quantify Immune cell populations in blood and colonic biopsy. Results: Eighty-two participants, 36 HIV(+)MSM, 21 HIV(-)MSM, and 25 HIV(-)Non-MSM, were enrolled. AD was associated with decreased high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) as well as reduced interleukin-6 (IL-6) in HIV(+)MSM. Without notable changes in the fecal or mucosal microbiome, AD conferred anti-inflammatory immune cell phenotypes in HIV(+)MSM. Conclusions: In this study, we showed that high-fiber/low-fat diets can reduce acute inflammation and pro-inflammatory immune cell phenotypes in HIV(+)MSM. This, combined with alterations in blood cholesterols, underscore the potential for diet to impact negative clinical outcomes in chronic HIV infection.

Poster #59: Decoding Nanobody-Induced Depolymerization of Surface-Layer Protein Lattices Using Molecular Simulations and Machine Learning

Keywords:

Adam J Cecil, Mukund Gurumurthi, Ethan V Halingstad, Alexander J Pak*
* presenting author

Surface-layer proteins (SLPs) are multi-domain proteins that self-assemble into a nanoporous lattice, the so-called S-layer, on the exterior of many bacteria and archaea. The S-layer protects and aids signaling between cells and has been identified as a virulence factor in pathogens. Recently, a subset of camelid nanobodies targeting the B. anthracis SLP were found to disarm the pathogen by disassembling the lattice; infections in murine models were cleared within 6 days, suggesting that nanobodies may be used as therapeutics to prevent pathogenesis. However, several nanobodies bound to the same site of the SLP did not cause lattice disassembly, suggesting that binding affinity alone cannot account for the functional outcome of all nanobody therapeutics. We hypothesize that inhibitory nanobodies enact strain across the lattice to a greater degree than noninhibitory nanobodies, causing lattice disassembly. To test our hypothesis, we combine insights from atomistic molecular dynamics (MD), coarse-grained (CG) MD, and machine learning (ML) classification. Using systematically derived CG models, we discuss how nanobodies bind to and restrict collective motions throughout the assembled S-layer. We also discuss the use of ML models to classify non-binding, binding-noninhibitory, and binding-inhibitory nanobodies based only on the dynamics of the SLP binding region. Using explainable artificial intelligence methods, these ML models will be inspected in conjunction with dynamical insights from CGMD to uncover the specific biophysical mechanisms that drive lattice depolymerization and direct identification of motifs that lead to inhibitory action in binding nanobodies. In the future, we will leverage these insights to design nanobodies with enhanced inhibitory behavior to target S-layer virulence factors in pathogenic bacteria.

Poster #56: Tracking rice paddy methanogen community dynamics and interactions across a growing season

Keywords: Methane, Rice

Raegan Paul, Emily K. Bechtold, Michael J Wilkins

Rice is a staple crop for more than half of the global population, with its production covering roughly 1.7 million km2 globally. Importantly, through the activity of methanogenic archaea, rice cultivation accounts for 12% of global anthropogenic methane emissions. Despite these significant contributions to global greenhouse gas production, the seasonal dynamics and interactions within methane cycling communities in rice paddies are poorly understood. Here, I investigated the abundance and diversity of methanogens over a growing season within Arkansas rice fields. Depth-resolved soil samples were retrieved from 8 fields before and after flooding, allowing us to test the hypothesis that prolonged flooding increases reducing conditions resulting in higher methanogen abundances. Pre-flooding 16S rRNA gene analyses revealed initial high relative abundances of methanogens (~2% of the community) that decreased significantly upon initial flooding, likely due to the growth of other community members in response to soil wetting. Later in the growing season, methanogen relative abundances rebounded to approximately 3% of the microbial community, and included enrichment of methylotrophic methanogens. Quantitative PCR analyses measuring the absolute abundance of methanogens (via the mcrA gene) supported the patterns observed in the relative abundance data. Within the methanogen community, archaea affiliated with the family Methanomassilicoccaceae and the genus Methanocella, Methanobacterium, Methanosarcina and Methanothrix were found within at least 60% of all soils, reflecting a core methanogen community in these samples. Importantly, similar methanogen community composition has been observed in many other rice growing regions, highlighting the presence of consistent methanogen membership with rice paddy soils.

Poster #17: Dietary resistant starch supplementation increases gut luminal deoxycholic acid abundance in mice

Keywords: Resistant starch; 7-α-dehydroxylation; bile acid; gut microbiome; DCA; metagenomics

Melanie A. Reuter, Madelynn Tucker, Zara Marfori, Rahaf Shishani, Jessica Miranda Bustamante, Rosalinda Moreno, Michael L. Goodson, Allison Ehrlich, Ameer Y. Taha, Pamela J. Lein, Nikhil Joshi, Ilana Brito, Blythe Durbin-Johnson, Renu Nandakumar, and Bethany P. Cummings

Bile acids (BA) are among the most abundant metabolites produced by the gut microbiome. Primary BAs produced in the liver are converted by gut bacterial 7-α-dehydroxylation into secondary BAs, which can differentially regulate host health via signaling based on their varying affinity for BA receptors. Despite the importance of secondary BAs in host health, the regulation of 7-α-dehydroxylation and the role of diet in modulating this process is incompletely defined. Understanding this process could lead to dietary guidelines that beneficially shift BA metabolism. Dietary fiber regulates gut microbial composition and metabolite production. We tested the hypothesis that feeding mice a diet rich in a fermentable dietary fiber, resistant starch (RS), would alter gut bacterial BA metabolism. Male and female wild-type mice were fed a diet supplemented with RS or an isocaloric control diet (IC). Metabolic parameters were similar between groups. RS supplementation increased gut luminal deoxycholic acid (DCA) abundance. However, gut luminal cholic acid (CA) abundance, the substrate for 7-α-dehydroxylation in DCA production, was unaltered by RS. Further, RS supplementation did not change the mRNA expression of hepatic BA producing enzymes or ileal BA transporters. Metagenomic assessment of gut bacterial composition revealed no change in the relative abundance of bacteria known to perform 7-α-dehydroxylation. P. ginsenosidimutans and P. multiformis were positively correlated with gut luminal DCA abundance and increased in response to RS supplementation. These data demonstrate that RS supplementation enriches gut luminal DCA abundance without increasing the relative abundance of bacteria known to perform 7-α-dehydroxylation.

Poster #58: Iodoform suppress methane and rewires food anaerobic digestion pathways towards longer short and medium chain FAs

Keywords:

Jorge L. Rico, Rebecca Daly, Reed Woyda, Parsa Ghadermazi, Joshua Chan, Kelly Wrighton, Kenneth Reardon, Susan De Long

Advanced anaerobic digestion (AD) technologies can convert organic residues into added-value chemicals and fuel precursors. This rewired AD (RWAD) approach requires directing feed carbon selectively into FAs (FA) instead of methane. However, there is lack of understanding of how iodoform impacts microbiomes in RWAD more broadly. This study investigated the effects of iodoform on microbiomes in RWAD of food waste using anaerobic wastewater sludge as a microbial inoculum. Significant differences were observed in the production of gases and in the FA product spectrum. As expected, methane was produced in controls but not in reactors with iodoform. Interestingly, acetic, and propionic acid production was highest in controls, while butyric and hexanoic acids were highest in iodoform treated reactors. Comparative microbiome analysis revealed differences in the microbiome structure and in the metagenomes. Metagenomic analysis at the gene level revealed significant effects across metabolic pathways in energy conservation, carbon fixation, and assimilatory sulfate reduction, among others. These metabolic shifts likely promoted redirection of the central intermediate acetyl-CoA towards chain elongation reactions under methanogenesis inhibition with iodoform.

Poster #40: Credibility and Expectancies of Probiotic Supplementation as a Means of Supporting Healthy Stress Regulation

Keywords: Probiotics, Stress, Human Health

Ethan Robinson, BA, Lauren Finkelstein, MA Joanna Arch, PhD University of Colorado, Department of Psychology and Neuroscience

Background: Treatment credibility and outcome expectancies predict treatment response for traditional psychiatric medications. Despite recent interest in using probiotics to support mental health outcomes, little is known about the credibility of this approach in treatment-seeking populations. The aim of this study is to describe treatment credibility and expectancies, with important implications for treatment uptake and efficacy. Methods: Data were collected as part of a randomized trial where Colorado adults (N=103, ages 18-45) reporting elevated stress were randomized to receive a probiotic or placebo for 46 days. At baseline, after being presented the study rationale, participants completed the Credibility/Expectancy Questionnaire (CEQ). Data were analyzed descriptively and inferentially, using multivariate regression to examine key predictors. Results: On average, participants thought that the probiotic supplement offered seemed between “somewhat” and “very” logical, and they predicted that their stress levels would improve approximately 37% at the end of supplementation. The probiotic treatment was rated as slightly more than “somewhat” credible (mean=18.05, SD=3.86, possible range 3-27) and the average treatment outcome expectation score was slightly less than “somewhat” (mean=13.01, SD=4.54). Older participants reported higher treatment credibility ($p = 0.021$) and expectancies ($p = 0.040$), and participants with a bachelor’s degree reported higher credibility compared to those with an advanced degree ($p = 0.046$). We observed no differences based on gender identity or baseline stress level. Conclusion: Among participants in a trial of probiotics for stress regulation, the average participant reported moderate to high treatment credibility and outcome expectancies, suggesting significant trust in this approach.

Poster #62: Microbiome keeps the multifunctionality of rude ram ecosystems highly resistant to climate change

Keywords:

Rocío Rodríguez, Antonio Gallardo, Luis Villagarcia, Guiyao Zhou, Tadeo Sáez-Sandino, Samuel Castejón, Ana López, Jesús G.P. Rodríguez, Felipe Bastida, Manuel Delgado-Baquerizo, Pankaj Trivedi

The global-scale abandonment of rural areas is resulting in a mosaic of disturbed ecosystems dominated by ruderal vegetation. Yet, the impacts of climate change on the functioning of ruderal ecosystems remain virtually unknown. Here, we conducted a 7-year field experiment to evaluate, for the first time, the long-term impacts of warming (~3 °C increase), rainfall exclusion (33% reduction), as well as their combination, on the capacity of a ruderal Mediterranean ecosystem to maintain multiple ecosystem services. We found that, in general, ruderal ecosystems are highly resistant to climate change with little effects of warming and rainfall exclusion on plant biodiversity and multiple ecosystem services. In fact, we detected some small but positive impacts of climate change on individual services with warming increasing plant biomass, soil carbon stock, and soil organic matter decomposition, and rainfall exclusion increasing soil carbon stocks. Our results highlighted the complexity of climate change interactions in explaining the capacity of ruderal ecosystems to support multiple ecosystem services, and further highlight the overall resistance of these already disturbed ecosystems to climate change.

Poster #28: Mechanisms of bacterial degradation of 1,4-dioxane at the Lowry Landfill Superfund Site

Keywords: monooxygenase, wastewater, biodegradation, metagenomics, bacteria

Jessie Romero, Timberley Roane, Chris Miller, Annika Mosier

The chemical 1,4-dioxane (dioxane) is an emerging contaminant affecting numerous drinking water sources across the US. Due to its potential carcinogenic effects in humans, efforts are underway to remove dioxane from the environment. A promising strategy to remediate contaminated groundwater is biodegradation, which is mediated by certain soluble di-iron monooxygenase enzymes (SDIMOs) in bacteria and fungi. The Lowry Landfill (Aurora, Colorado) was designated as a Superfund Site in 1984 due to disposal practices that contaminated the surrounding environment but has since reduced a nearby dioxane plume using a bacterial pump-and-treat system. This study aimed to characterize the bacterial community composition of moving bed bioreactors (bioreactors) within the treatment system, to identify SDIMOs potentially contributing to dioxane biodegradation, and to search the gene neighborhoods of putative SDIMOs to elucidate potential dioxane biodegradation mechanisms. Support media from one of three bioreactors were collected over three years. 16S rRNA gene sequencing revealed bacterial communities consisting of Nitrospiraceae, Nitrososphaeraceae, Nitrosomonadaceae, Pseudonocardiaceae, Hyphomicrobiaceae, and other families. Metagenomic shotgun sequencing showed a high abundance of Group V SDIMOs, namely dioxane monooxygenase (Dxm)-like proteins. These proteins were phylogenetically related to directly metabolic and co-metabolic SDIMOs. Gene neighborhoods of Dxm-like proteins showed enzymes potentially involved in the degradation of dioxane intermediates and other chemicals and genes possibly conferring horizontal gene transfer. Metagenome-assembled-genomes (MAGs) associated with these proteins were classified as Pseudonocardia, Mycobacterium, Hyphomicrobiaceae, Baekduia, and genera of the Chloroflexota phylum. Further elucidation of dioxane biodegradation at the Lowry Landfill can support future remediation efforts at other dioxane-impacted sites.

Poster #30: Leveraging integrated resources to predict mechanisms of the host-associated microbiome in disease

Keywords: microbiome, knowledge graphs, mechanistic inference

Brook Santangelo, Marcin Joachimiak, Catherine Lozupone, Lawrence Hunter

Increasingly widespread coverage of sequencing gut microbiomes have enabled studies which emphasize the importance of the host-associated microbiome in disease. A growing catalog of resources that capture host and microbe metabolic function can serve as the basis for understanding host-microbe interactions and uncovering novel hypotheses. Integrating these resources to take advantage of their content is challenging due to variable formatting and incomplete mappings between concepts. We developed a method to interrogate this knowledge space by representing it as a structured network of microbes, enzymes, metabolites, pathways, and diseases. We introduce a knowledge graph (KG) which includes over 800,000 microbes and their relationships with other concept types, including all annotated microbial enzymes from the Universal Protein Resource (UniProt), serving as one of the most comprehensive microbe-disease resources to date. We performed embedding-based and path finding analyses to evaluate plausible mechanistic paths describing the role of microbes in disease, particularly for correlative findings of microbial involvement in disease from the Disbiome database. We also apply such methodologies to known causal relationships between microbes and disease to evaluate their accuracy. We see this KG as an important advancement in using automated methods to uncover mechanistic explanations for microbe-disease associations.

Poster #4: Arctic Food Webs: Deciphering Microbial Methane Metabolism in Over-Winter Carbon Flux

Keywords: metagenomics, permafrost, winter, metabolism, SIP

Laura G. Schaerer, Jared B. Ellenbogen, Mikayla A. Borto, Reed Woyda, Bridget B. McGivern, Jeffrey A. Kimbrel, Richard A. White, Neslihan Tas, Eugenie S. Euskirchen, Jack McFarland, Janet K. Jansson, Mark P. Waldrop, Steven J. Blazewicz, Kelly C. Wrighton

New knowledge gleaned from multiomics has shown that microorganisms in frozen soils can produce greenhouse gasses including methane and carbon dioxide. This leaves open the possibility that winter greenhouse gas production may be an underappreciated aspect of the annual carbon cycle. At present, the metabolic pathways responsible for producing methane and carbon dioxide in permafrost under frozen conditions are unknown. Here we employed heavy water stable isotope probing paired with metagenomic sequencing to identify active microorganisms and metabolic pathways in Alaskan peat soils after six months and one year of laboratory simulated winter conditions under anaerobic conditions. As evidence for microbial activity, methane and carbon dioxide steadily accumulated during the incubation period. After 370 days, samples incubated at –1°C had 47-fold more methane and 25-fold more carbon dioxide than the control samples maintained at –20°C. The most abundant taxa in the heaviest (most active) fractions were from the phyla Bacteroidota and Firmicutes which support active soil carbon oxidation resulting in carbon dioxide emission. Further, members of the Methanomicrobiales, Methanomasiliicoccales, and Methanosarcinales were identified in the heaviest fraction, highlighting the diversity of methanogenesis (hydrogenotrophic, methylotrophic, and acetoclastic respectively) pathways present in these soils. These results point to a complex metabolic web mediating winter methane release. Results support that the soil microbial community residing in permafrost is diverse, active, and produces greenhouse gasses even when experiencing freezing conditions. This investigation highlights commonly overlooked winter greenhouse gas production and deepens insights into winter carbon cycling within environmentally sensitive habitats susceptible to climate change.

Poster #6: Cover Crop Root Exudates Differentially Influence Soil Microbial Function and Phytohormone Production in Agricultural Soil Microbiomes

Keywords: metagenomics, metabolomics, soil microbiome, MAGs

Valerie A. Seitz, Bridget B. McGivern, Mikayla A. Borton, Jacqueline M. Chaparro, Meagan E. Schipanski, Jessica E. Prenni, Kelly C. Wrighton

Cover cropping is an agricultural practice that uses secondary crops to support the growth of a primary crop. Cover crops may elicit ecosystem services through chemical interactions with the soil microbiome via root exudation, or the release of plant metabolites, such as phytohormones, from roots. Phytohormones exuded by plants can activate the rhizosphere microbiome, yet managing this chemical interaction remains an untapped mechanism for optimizing plant-soil microbiome interactions. Here, we link variability in cover crop phytohormone root exudate composition to changes in soil microbiome functionality. Exudate profiles from 4 cover crop species were used as the chemical inputs to decipher microbial responses. These distinct exudate profiles, along with a no exudate control, were amended to agricultural soil microcosms with microbial responses tracked over time using metabolomes and genome-resolved metatranscriptomes. Our findings illustrated microbial metabolic patterns were unique in response to cover crop exudate inputs over time. In particular, sorghum and cereal rye amended microcosms stimulated novel microbial members who produced indole-3-acetic acid and gibberellic acid 4, two important phytohormones, over time. We also identify broad changes in microbial nitrogen cycling in response chemical inputs. Finally, we constructed a soil microbial genomic catalog of microorganisms responding to cover crop inputs, a public resource for agriculturally-relevant microbes. Our findings emphasize the tractability of high-resolution multi-omics approaches to investigate processes relevant for agricultural soils, opening the possibility of targeting specific soil biogeochemical outcomes through precision agricultural practices that use cover crops and the microbiome as levers for enhanced crop production.

Poster #32: Examination of Microbial DNA and Chemical Characteristics in Two Rivers Fed by Produced Water from Oil and Gas Extraction

Keywords: Produced Water, Microbiome, QIIME2, Taxonomy

Shad Sellers, Beyhaad Nemati

Natural microbial communities differ greatly depending on the ambient nutrients available to the microbes. Microbial communities in streams are greatly affected by both the nutrients available in the stream as well as physical properties, such as temperature and redox potential. Produced water is the water brought to the surface through oil and gas production and serves as a primary source for two streams in Wyoming. This water is often low in oxygen content and high in salinity. Water samples were collected from several sites along each stream. To analyze microbial communities present in streams fed by produced water, prokaryotic 16S rDNA and eukaryotic ITS sequences were sequenced and analyzed. Each was then classified according to taxonomy using QIIME2. Collected water samples were analyzed for major and minor anions and cations, pH, and redox potential. Water chemistry analysis shows that each stream has a very different level of oxygenation, a significant difference in the amount of sodium and chloride ions, and a range of pH levels. The 16S (prokaryotic) and ITS (eukaryotic) taxonomies are being analyzed and compared with water chemistry to more fully understand the microbiomes created by produced water contamination in streams.

Poster #3: Maternal weight loss restores the gut microbiome and cardiometabolic disease in BPH/5 female offspring, a genetic mouse model of hypertension

Keywords: microbiome, pregnancy, hypertension, obesity, lactobacillus

Jenny L. Sones, Jon L. Lacour, Kalie F. Beckers, Chin-Chi Liu

Blood pressure high (BPH)/5 are a spontaneous mouse model of hypertension. Adult females have obesity due to hyperphagia. In pregnancy, they recapitulate the cardinal signs of preeclampsia (PE), late gestational rise in maternal blood pressure and proteinuria. BPH/5 placentae have abnormal development with increased markers of inflammation, tumor necrosis factor (TNF)-α and interleukin (IL)-6. BPH/5 offspring have fetal growth restriction (FGR) and adult cardiometabolic disease. Because BPH/5 phenocopy key features of PE observed in women, they have been used to study adverse maternal and fetal outcomes. Women with obesity have a 30% increased risk for PE. To further explore this risk factor, monitored food intake was employed in BPH/5 females beginning at conception (pair-feeding). This resulted in attenuation of the late-gestational maternal hypertension and FGR. Therefore, we hypothesized that BPH/5 offspring born to pair-fed vs. ad libitum fed dams have decreased evidence of adult cardiometabolic disease. BPH/5 male and female adult offspring born to pair-fed dams had increased relative abundance of Lactobacillus sp. in their gut microbiome (3-fold), decreased blood pressure, and cardiomegaly, but only females had decreased body weight, fat mass, and circulating leptin. Adult BPH/5 females given oral Lactobacillus sp. had a significant reduction in body weight without changing food intake and decreased inflammation at the pregnant maternal-fetal interface (TNF-α, IL-6). These findings support the importance of maternal obesity and gut dysbiosis in adverse pregnancy outcomes. Further studies are needed to understand the role of Lactobacillus sp. in the prevention of PE programming of cardiometabolic disease in offspring.

Poster #60: Quality Assessment and Quantifying Variation Across 16S rRNA Sequence Data – From Sample Collection to Sequencing in Human Microbiome Studies.

Keywords:

Christopher E. Stamper, Andrew J. Hoisington, Joseph C. Ellis, Lisa A. Brenner

The rapid shift in sequencing technologies over the last decade revealed a myriad of connections between the microbiome and human health. During this rapid growth, knowledge on quality assessment was not a primary focus impacting reproducibility. Since 2016, the Rocky Mountain MIRECC has sequenced ~5000 fecal, oral, and skin 16S rRNA microbiome participant samples plus over 100 technical replicates (e.g. commercial mock community and positive control samples). Here we present a survey of sequencing results to examine variation in the following: sampling collection techniques, shipping at ambient conditions, DNA concentration, sequencing runs. A direct comparison between stabilized fecal samples and unstabilized swab samples from the same participants revealed sampling methods significantly influenced microbial community results. These differences persisted even if swabs samples were frozen within 36 hours of collection. Shipping swabs at ambient conditions resulted in increases in Gammaproteobacteria and Bacilli that correlated with transit days. Technical replicate data from 18 sequencing runs revealed limitations in accuracy while precision was robust for mock community and human positive control samples. The sample type with the highest technical replication also had the highest DNA concentrations. Importantly, technical variation in the microbial community due to sequencing run was significantly less than biological variation. Overall, this research highlights the importance of the continued pursuit of standardization in microbiome research in order to limit technical noise in microbiome data and increase the reliability of metanalyses combining multiple studies.

Poster #46: Host-microbiome dual transcriptome analysis with HoMi profiles host-microbe crosstalk at mucosal surfaces

Keywords: metatranscriptomics, transcriptomics, host-microbe crosstalk, symbiosis, software

John Sterrett, Anne Brauweiler, Nichole Nusbacher, Katherine Littlefield, Brent Palmer, Catherine Lozupone

Interactions between a microbiota and its host occur most frequently at mucosal surfaces, such as the colonic mucosa in the case of the gut microbiome. Largely, previous work studying the transcriptional activity of the gut microbiota has evaluated the fecal metatranscriptome. However, the fecal metatranscriptome does not assess the activity of mucosa-associated microbes or the host; therefore, it cannot be used to assess host-microbiome crosstalk. To resolve this, we have developed a protocol for host-microbiome dual transcriptomics from mucosal biopsies containing both host and microbial matter. Other research groups have used dual transcriptome methods to assess interactions between a single pathogen and its host, but dual transcriptomics has not yet been applied to the full microbiome scale. We optimized nucleic acid extraction techniques to extract both host and microbial material from a single sample, from which samples can either be split into polyadenylated (host mRNA-dominated) and non-polyadenylated (microbe-enriched) fractions or sequenced as a bulk library. Additionally, we have developed a software package for processing and statistical analysis of dual transcriptomic data, which manages software environments and parallel processing of data on a high-performance compute cluster. Our package contains statistical tools for metatranscriptome differential expression analysis and associations between host and microbial transcripts using both zero-inflated beta regression and linear regression methods. The processing and statistics modules have been benchmarked against in silico synthetic communities and communities assembled from real datasets.

Poster #42: Practical Characterization and Modeling of Higher Flow in a Water Condensation Growth Tube Bioaerosol Sampler

Keywords: bioaerosol, instrumentation, aerosol, sampling

Braden Stump, Dominick Heskett, Patricia Keady, Gregory S. Lewis, Arantzazu Eiguren-Fernandez

The BioSpot-VIVAS™ bioaerosol sampler currently has a default flow rate of 8LPM. During ambient bioaerosol sampling, higher volumetric flowrate is highly desired. Higher flow rates can reduce sampling time required to achieve limit of detection and can provide better statistical significance in experimental results. It was recently hypothesized based off work previously completed at Aerosol Dynamics that the BioSpot-VIVAS™ sampler's growth tube could be operated with the same efficiency of aerosol capture (in certain environments) above 8LPM, up to 15LPM. This hypothesis was tested via two methods. Uranine collection efficiency testing and modeling was performed to confirm activation of aerosol, and to confirm and quantify the performance of the BioSpot-VIVAS bioaerosol sampler at higher flow rates. Computational Fluid Dynamics modeling was also completed on the inlet of the instrument to confirm large aerosol 2.5 um – 10 um are efficiently transported through the inlet of the instrument into the growth tubes. These experiments aim to confirm that the BioSpot-VIVAS™ bioaerosol sampler can be operated at flowrates up to 15 LPM without compromising scientific specifications.

Poster #31: Sourdough Starter Culture Microbiomes Influence Functional and Nutritional Characteristics of Wheat Bread

Keywords: lactic acid bacteria, fermentation, food systems

Charlene Van Buiten, Caitlin Clark, Ashley Ohstrom, Eva Keohane, Molly Smith, Benjamin E. Wolfe, Jessica Prenni, Josephine Wee, Charlene Van Buiten

Sourdough fermentation is an ancient food processing technology wherein wild bacteria and yeasts leaven dough in baked goods like breads. Known as “starter cultures”, the communities of bacteria and yeast used to inoculate dough can be generated de novo from the growth of microorganisms endogenous to the flour and water used to create dough, then re-used continuously in food production. This leads to unique microbial profiles that have been shown to be influenced by factors such as origin, age and storage conditions. While all sourdough starter cultures produce the same core metabolic outputs (i.e., carbon dioxide, organic acids), the diversity of the microbial ecologies in sourdough fermentations lead to differences in functional characteristics of their resulting bread products including (1) nutritional profile such as mineral content and starch digestibility, (2) quality as it pertains to organoleptic properties and shelf life, and (3) immunological parameters including inflammation and gluten immunogenicity for individuals with celiac disease and gluten intolerances. The overarching objective of this effort is to characterize relationships between microbial populations in sourdough starter cultures and these functional outputs, based on the hypothesis that unique microbial community structures are associated with differences in physical and chemical outputs of fermentation, which cause functional differences in bread products as they relate to quality and healthfulness. Our findings suggest that microbial diversity drives differences in key characteristics of sourdough breads including organic acid profiles, texture and metabolomic profiles, while other characteristics, such as mineral profile as established by ionomics, appear consistent between doughs created with a controlled recipe. With regard to health and safety, we have established differences in protein degradation based on sourdough microbiomes which affect gluten-mediated inflammation in an in vitro model of celiac disease. These findings provide insight towards the use of targeted processing approaches to produce more functional and sustainable bread products that meet the needs and demands of modern consumers.

Poster #44: Investigating functional microbial determinants of disease in early rheumatoid arthritis

Keywords: rheumatoid arthritis; microbiome; metabolism; enzymes

Alexandra Vita, Marie L. Feser, Kevin D. Deane, Tiffany Weir

Background/Significance: Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation and deterioration of tissue within the joints, often associated with distinct gut microbial profiles. Modifiable lifestyle factors, such as diet, are being investigated for their utility in rebalancing the gut microbiota and supporting RA disease management. However, the extent to which diet and specific diet-derived compounds exert local and systemic effects may be directly affected by the functional capacity of an individual's gut microbiome. Purpose: The purpose of this study is to gather preliminary data to investigate functional microbial determinants of disease in adults with early RA. These data will inform microbial biomarker optimization for future, more targeted data collection on microbial enzyme genes and correlation with dietary data. Methods: Fecal samples of adults with early RA (n=10) and healthy controls (n=10) were provided by the University of Colorado (UC) Denver Population and Data Science Core (P30-AR079369) and underwent metagenomic shotgun sequencing at the UC-Denver Cancer Center Genomics Core (P30-CA046934). Assembled genomes were annotated taxonomically and functionally. Fecal donor health characteristics were also described, with disease activity scores of RA donors being used in analysis. Analyses: Planned analyses include (1) evaluating functional differences (e.g., enzyme genes, pathogenicity, metabolic pathways) of gut microbiota between early RA and healthy controls; and (2) correlating functional measures to disease activity scores of early RA samples. Discussion: This is an essential stepping-stone toward future data collection investigating whether microbial metabolism of specific diet-derived phytocompounds (e.g., polyphenols) predicts RA disease outcomes.

Poster #53: Using Ecological Succession in the Intestine for Microbiome Restoration and Prevention of Clostridioides difficile Infection

Keywords: Succession, Aerotolerance, Gut

Elena S. Wall, Dr. Casey Martin, Dr. Keith Hazleton, Dr. Catherine Lozupone

Ecological succession of the intestinal microbiota describes the predictable turnover in species during their establishment and development within a host. Pioneer species are foundational members in this process that prepare the environment for colonization by late successional species. The healthy adult microbiome is a low oxygen environment typically dominated by strict anaerobes. A disruption in the composition and distribution of host-associated resident microbes can alter the intestinal environment, reducing microbial diversity and leading to increased oxygen levels in the gut. We aim to characterize a group of bacteria that we refer to as butyrate-producing pioneer species (BPPSs). Based on in vitro experimentation and genomic analysis, these bacteria are resistant to higher levels of oxidative stress than late successional species and have the potential to produce butyrate from simple substrates. We hypothesize that these qualities are adaptations of BPPSs that allow them to colonize a more oxygen-rich niche during early succession of the gut, in infancy or after disturbance events. We also predict that this colonization can facilitate the return to a climax community in disturbed guts with low microbial diversity and prevent opportunistic infections. Our work highlights the traits of BPPSs and their potential to impact succession in a mouse model.

Poster #48: Ultra-high-throughput single-microbe sequencing enabled by semi-permeable capsules

Keywords: Single-cell sequencing, microbial genomics, microbial ecology, high-throughput single-microbe analysis

Andrew Watson Vaidotas Kiseliovas, Vaida Kurmauskaite, Simonas Jocys, Lorea Campos, Rapolas Zilionis

Whole-genome sequencing opens a window to understanding the diversity and function of unculturable microorganisms. Metagenomic sequencing is attractive for its straightforward sequencing library preparation from bulk environmental samples but only offers limited resolution into individual species. On the other hand, single-microbe sequencing offers true single-clone resolution but can only meaningfully address the high biological diversity expected in environmental samples if performed on thousands of individual cells in parallel. To satisfy this throughput requirement, well- and droplet-based approaches keep evolving in parallel. However, these approaches suffer from a fundamental trade-off between throughput and versatility. Our Semi-Permeable Capsule (SPC) technology combines the throughput of droplets with the versatility of wells and enables a virtually unlimited number of processing steps on genetic material from thousands of individual microbes in parallel. This study aimed to demonstrate the use of SPCs for barcoding 10,000 individual microbial genomes to obtain single-microbe whole genome sequencing data of unprecedented quality. For a proof-of-concept demonstration, we processed E. coli and B. subtilis bacteria. Sequencing results showed >90% genome recovery from individual cells. Next, we applied the method for environmental samples. We encapsulated microbial cells from soil, pond water and saliva samples, lysed cells at alkaline conditions, amplified their genomes, and employed a split-pool approach to add unique cellular barcodes. We then sequenced an aliquot of barcoded cells and evaluated technical metrics related to de novo assembly of SAGs. We conclude that the compartmentalization of microbial cells into SPCs allows the generation of high-quality whole-genome data at scale.

Poster #27: CHOP chemotherapy in canine cancer patients increases gut microbiota dysbiosis index

Keywords: Dysbiosis, Gut microbiome, chemotherapy

Annika M. Weber, Juan Aragon, Nora Jean Nealon, Madison Tipton, Jennifer Thomsen, Hend Ibrahim, Kristen Weishaar, Sangeeta Rao, Jan Schudolski, Jonathan Stockman, and Elizabeth P. Ryan

Chemotherapy can cause adverse gastrointestinal (GI) side effects in dogs and people. The overall objective of this study was to assess the impact of chemotherapy treatment with cyclophosphamide, hydroxydaunorubicin, oncovin, and prednisone (CHOP) on GI dysbiosis in dogs with cancer. We hypothesized that CHOP chemotherapy produces compositional and functional shifts in the canine fecal microbiota that are associated with increased dysbiosis. Fecal samples from canine cancer patients were compared for microbiota and metabolites before and after the first cycle of chemotherapy. Dogs undergoing chemotherapy for cancer (n= 26) were consented by client owners to provide fecal and blood for targeted metabolite and microbiota analysis including, but not limited to phytosterols, bile acids, and commensal bacterial populations. A dysbiosis index from fecal testing was calculated for each dog and revealed that the dysbiosis index significantly increased following chemotherapy ($p = 0.012$). There was a significant increase in fecal E. coli levels noticed post chemotherapy ($p= 0.04$), and a significant decrease in fecal Clostridium hiranonis levels post chemotherapy ($p=0.0083$). Furthermore, there were phylum shifts whereby Proteobacteria increased, and Fusobacteria decreased post chemotherapy. There were significantly decreased levels arachidonic acid ($p=0.0251$) and cholestanol ($p=0.0278$) after chemotherapy timepoint when compared to the pre chemotherapy timepoint. While preliminary, these findings suggest that the GI symptoms following CHOP may be indicated by shifts in gut microbiota structure and function. Addressing gut dysbiosis with dietary treatment that supply key nutrient and fiber rich foods may prove to be beneficial in dogs with cancer.

Poster #51: Gut microbiome dysbiosis in an equine model of spontaneous osteoarthritis

Keywords: Dysbiosis, osteoarthritis, equine, translational medicine

Zoë J. Williams, Lyndah Chow, Gabriella Piquini, Ashana Patel, Meagan Rockow, Dean Hendrickson, Luke Bass, Steven Dow, Lynn Pezzanite

Osteoarthritis (OA) is a painful and debilitating condition impacting 33 million people in the US. Spontaneous OA is prevalent in horses (80% of horses >15 years) and the equine preclinical model of OA is translationally relevant for human orthopedic disease due to greater similarities in cartilage thickness, articular loading forces and joint volume compared to other laboratory species. Understanding of the pathogenesis is evolving, but OA is increasingly thought to be a multifactorial disease in which the innate immune system and dysregulation of the gut microbiome play essential roles to drive OA. This study aimed to evaluate this further in the equine model, comparing microbial populations in feces and circulating peripheral blood mononuclear cells (PBMC) between horses with and without OA, and to correlate these findings with cytokine levels in plasma and synovial fluid (SF). Horses (n=18) were enrolled (n=12 healthy, n=6 OA). DNA was extracted from feces and PBMC for 16S sequencing using library preparation from the Earth Microbiome protocol with 515F and 806R primers. Cytokine levels (n=23) were analyzed via equine multiplex immunoassay. Findings revealed differences in taxonomic composition between feces (P=0.003) and PBMC (P=0.01) in horses with and without OA. Horses with OA had elevated inflammatory biomarkers in SF (IL-1ß (p=0.02), IL-6 (p=0.005), G-CSF (P=0.02)) and plasma (IFN-γ (P=0.004), IL-18 (P=0.0007), IL-6 (P=0.02), IP-10 (P=0.02)) in OA. These findings support further evaluation of microbial dysbiosis in OA progression and horses as a relevant preclinical model to investigate the gut-joint-axis of OA across species.

Poster #23: Comparing the effects of probiotic Bacillus subtilis DE111 administration alone to combined bacteriophage and probiotic administration on metabolic markers and gut microbiota

Keywords: probiotic, gut microbiota, Bacillus subtilis

Natasha Williams, Tiffany Weir

The gut microbiota is an important modulator of human health; making probiotic interventions an attractive option for supporting gastrointestinal (GI), cardiovascular, and immune system health. Bacillus subtilis is a spore-based probiotic that has shown great potential in improving mild-to-moderate gastrointestinal distress and reducing systemic inflammation. We hypothesized that the co-administration of B. subtilis DE111 with a commercial cocktail of Escherichia coli-targeting bacteriophages would exhibit enhanced beneficial effects over the probiotic administered alone. In a double-blinded, placebo-controlled parallel-arm clinical trial, healthy adults consumed (1) B. subtilis, (2) B. subtilis + bacteriophages, or (3) placebo for 6 weeks. Stool samples were used to examine gut microbiota and gut metabolites (SCFA, fecal secretory IgA, and lipocalin). Blood samples were analyzed for metabolic markers of inflammation (CRP, lipid profile, HbA1c, zonulin, and cytokines). Questionnaires and stool charts were used to examine changes in bowel habits and perceived GI symptoms. There were no significant changes in alpha and beta diversity parameters for the microbiota across the study. Some changes in metabolic markers possibly could have clinical relevance but didn't reach statistical significance. B. subtilis DE111 administered alone seems to have a greater influence on GI symptoms than administered in combination with a bacteriophage cocktail. Additional research is warranted to identify the population sector that can potentially benefit from the consumption of probiotic B. subtilis DE111.

Poster #29: Supporting microbiome research with mass spectrometry-based analytical resources

Keywords: bile acids, untargeted, metabolomics, uremic toxins, short-chain fatty acids

Linxing Yao and Corey Broeckling

The Analytical Resource Core (ARC) at Colorado State University houses seventeen mass spectrometers, including both high-resolution and quadruple instruments, coupled to ultra-performance liquid chromatography systems and gas chromatography systems, enabling extensive services for metabolomics, proteomics, and ionomics. These mass spectrometry-based analytical approaches have been utilized to support various microbiome research. The metabolomics involves the detection, identification, and quantification of small molecules produced by the microbiome. For hypothesis-generating discovery studies, the comprehensive profiling of known and unknown metabolites in each sample determines differences among treatment groups. For hypothesis-driving study, well-defined groups of metabolites such as bile acids, short-chain fatty acids, and uremic toxins, are quantified using customized methods. Both targeted and untargeted approaches are well established at ARC. Method development for the metabolites that are not covered by our routine analyses is also available. The general workflow of full service analysis at ARC starts with sample organization and randomization, metabolite extraction and clean-up as needed, data acquisition with quality control, data processing and interpretation. Examples of routine metabolomics assays offered at ARC and how the data were used in microbiome research are presented.