

## FRMS 2022 Poster Abstracts

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Amundson, Kaela

Colorado State University

Poster 1

Session 1

Title: Predators of the Deep: virus-host dynamics in the terrestrial subsurface

Viruses are ubiquitous in every ecosystem studied to date, including the deep subsurface. Even so, the impact of viruses on bacterial and archaeal hosts, and the associated effects on biogeochemical cycling, remains challenging to detangle. Given the dependence of viruses on their hosts for replication, investigations into viral ecology and impacts on host metabolism require the identification of host-viral linkages, which is a challenging step in many ecosystems. However, lower microbial diversity in many subsurface shale environments presents a unique opportunity to investigate virus-host dynamics at the genome-level. Here, we temporally sampled six hydraulically fractured oil & gas wells in the Niobrara shale formation and recovered 202 unique metagenome assembled genomes (MAGs) and 2,176 unique viral MAGs (vMAGs) from 78 metagenomes. Using protospacers from the >60% of MAGs with CRISPR-Cas arrays, we achieved viral linkages with approximately 45% of all MAGs, including a remarkable >70% of dominant MAGs. Leveraging these abundant host-viral linkages across time series data, we investigated how diverse host populations respond to viral predation. Most MAGs recovered from this ecosystem were likely undergoing viral predation and could generally be grouped by differing responses to viral predation. Further, we report evidence for an ongoing virus-host 'arms race' in this ecosystem, where continuous viral predation is driven by shifting viral populations that target individual taxa. Our genome-centric results in a relative low diversity system expand our knowledge of host-viral interactions in deep fractured shales and provide greater understanding of the role viral infection may play in other natural ecosystems.

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Flynn, Rory

Colorado State University

Poster 2

Session 2

Title: Distillation and Refinement of Metabolism Annotations Enables Rapid Understanding of Microbial and Viral Functional Potential

Metagenomic sequencing generates a deluge of genomic information for microbial life. This wealth of information is squandered without software to annotate the functional potential encoded in genomic data. We created DRAM (Distillation and Refinement of Annotations of Metabolism), a tool that analyzes and curates genome annotations from multiple databases, summarizing the functions encoded by genome with speed and reproducibility. DRAM's viral component, DRAM-v, uses virus specific databases and methods to triage the potential auxiliary metabolic genes, using proven metrics. Here, we present DRAM2.0, an update that rapidly expedites processing of data and provides powerful new options. In addition to its own predetermined set, DRAM2.0 can now assimilate user-provided databases and selected ecosystem-specific content (e.g., phytohormones for agricultural soils), for more customized genome metabolism summaries. We improved the descriptive power of DRAM with more

modules, allowing more detailed refinement of transporters, sporulation, and antimicrobial metabolisms. We developed a synthesis feature that provides a list of functional traits for each genome, enabling users to quickly assign genomes a range of adjectives covering distinct metabolic roles (e.g., methanogenic, respiratory, and photosynthetic). We overcome well known challenges in homology-based annotation precision by including phylogenetic tree placement. Moreover, DRAM and DRAM-v are now freely accessible in KBase, a graphical interface, removing dependence on computational resources and enabling integration of DRAM annotations into other KBase tools, such as genome scale metabolic models. We demonstrate the immediate value of DRAM's current capabilities and highlight improvements that are critical to deciphering mechanisms underpinning microbiome function.

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Kokkinias, Katherine

Colorado State University

Poster 3

Session 1

Title: Weathering the inflammatory storm: A multi-omics analysis of the microbiome in response to Salmonella infection and diet-induced inflammation over time

Inflammation caused by pathogens and diet alters the chemical resources available in the gut and the composition of the intestinal microbiome. While there are some diet-induced metagenomic studies, few studies use a multi-omics perspective to explore the intersection of diet and pathogen susceptibility, knowledge that can identify key metabolic pathways during intestinal inflammation. Using a CBA/J mouse model, we show a high fat diet (HFD) resulted in decreased fecal microbial diversity and increased inflammation relative to a high fiber diet (chow). We also show mice fed HFD have enhanced susceptibility when challenged with *Salmonella enterica* serovar Typhimurium, a model pathogen which uses inflammation to colonize the gut. We created a genome-resolved database of metagenomes derived from mice treated with and without *Salmonella* under two dietary regimes resulting in 124 dereplicated microbial genomes to be used for metatranscriptome recruitment. We discovered taxa that responded positively (*Enterococcus*) and negatively (*Clostridia*) to *Salmonella* regardless of diet, while some had diet-specific *Salmonella* responses (*Lactobacillus*). Consistent with theory from pure-culture experiments, our metatranscriptome data validated that *Salmonella* respire nitrate and oxygen to outcompete commensal fermentative microorganisms, with greater gene expression of respiratory genes observed in inflamed HFD relative to chow. Ongoing efforts are using genome-resolved gene expression networks between *Salmonella* and the commensal microbiome to better understand metabolic networks impacting *Salmonella* colonization and infection in the gut. Our findings have identified metabolic pathways of commensal microbes that can weather *Salmonella* infection under diet-induced inflammation which, upon further testing, might offer the basis for therapeutic intervention.

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Leleiwi, Ikaia

Colorado State University

Poster 4

Session 2

Title: Creation of a Gut Microbial and Viral Genome Catalog Exposes Gene Content and Taxonomic Variation in Inflamed and Non-Inflamed Mice

Mouse models are increasingly used to inform how the gut microbiome modulates human disease. Of these mouse models, CBA/J mice are widely used for immunology, metabolism, and enteric inflammation studies, yet their microbiome remains comparatively understudied. Here we utilized deep metagenomic sequencing (> 30 Gbp/mouse) combined with custom assembly methods to re-construct the first microbial genomes from the feces of CBA/J mice. We generated a genomic catalog that included 2,281 bacterial and 114 viral metagenome assembled genomes and sampled over 139,000 unique microbial genes. Genome refinement and dereplication, based on completion and contamination criteria, resulted in 504 medium or high-quality MAGs and 113 unique microbial genomes. Querying previously reported 16S rRNA genes from fecal CBA/J communities revealed the database inventoried the most prevalent and abundant microbial genera in non-inflamed and inflamed mouse guts. These dereplicated genomes spanned 7 phyla with over 86% (n=98) representing novel microbial species that lacked prior genomic sampling. This resource is also the first genomic investigation of the inflamed mouse gut, where inflammation reduced overall microbial community diversity by 40%, but enhanced the recovery of novel, inflammation-specific, genomes that were low abundant members in the uninflamed gut. Significant increases in respiratory metabolic capabilities and propionate production occurred in the inflamed communities, concomitant with decreases in relative butyrate and acetate production potential. Importantly, the CBA/J database contains microbial members previously not sampled in existing microbial genome catalogs from other mice, demonstrating the value of host-specific microbiota resources for enabling precision microbiome manipulations in the future.

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Arnolds, Kathleen

National Renewal Energy Laboratory

Poster 5

Session 1

Title: IMAGINE Biosecurity. Mesocosm based methods to evaluate biocontainment strategies and impact of industrial microbes upon native ecosystems.

Industrial production microbes and their associated bioproducts have emerged as an integral component of a sustainable bioeconomy. However, the rapid development of these innovative technologies does raise some biosecurity concerns, namely, the risk of environmental escape. Thus, the realization of a bioeconomy hinges not only on the development and deployment microbial production hosts, but also on novel biocontainment designs that can be rigorously assessed via a robust testing pipeline that allows for detection of rare escapees, effect of associated bio-products, and impact on native ecologies. To date, most biocontainment strategies have been largely evaluated in the lab, often in monocultures and under tightly regulated conditions, thus their escape potential has never been thoroughly evaluated under conditions that even begin to recapitulate an environmental escape, where they would face complex microbial communities, a diverse array of resources and metabolites that may influence escape frequency, as well as increased probability of horizontal gene transfer. Here, we are developing methods that utilize soil mesocosms to evaluate the efficacy of novel biocontainment strategies and to assess the impact of production systems upon terrestrial soil microbiome dynamics. This methodological pipeline will allow us to screen a broad range of biocontainment modules in diverse microbes from different trophic regimes via a down-selection strategy. To our knowledge

these efforts represent a first in-kind testing pipeline of this rigor and will allow NREL to set a national standard for the assessment of the safety of industrial microbes and their associated bioproducts, accelerating the realization of a secure bioeconomy.

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Bazany, Kathryn  
Colorado State University  
Poster 6  
Session 2

Title: Drought affects interkingdom microbial connections in plant rhizosphere

We examined the influence of water deficit stress on bacteria, fungi, and protists associated with corn and sugar beet rhizospheres. We found that the impact of water deficit stress on rhizosphere alpha and beta diversity varied by microbial kingdom. Bacteria and protists were more strongly influenced by water deficit conditions than fungi. Inter-kingdom interactions were influenced by the environmental condition as well. Positive interactions between kingdoms increased in the water deficit condition, which could indicate that the water deficit stress was exerting a top-down control that had a greater influence than predation and competition in determining community composition. Within each of these kingdoms we identified phyla that were most influenced by the water deficit condition, indicating various levels of drought tolerance or sensitivity. Insights into these complex interactions will advance our understanding of the ecological processes that govern microbiome assembly and will be crucial in the development of targeted and effective microbial amendments that can improve crop fitness and productivity under drought. These findings additionally make a compelling case for including protists in future soil microbiome research in order to further understanding of multi-kingdom interactions.

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Boot, Claudia  
Colorado State University  
Poster 7  
Session 1

Title: The shadow metabolism of L-sugars and their potential for carbon sequestration

Life is thought to operate on the principle of homochirality, where one three-dimensional form of an organic compound dominates the molecular operations of life: L-amino acids in proteins, D-sugars in carbohydrates, L-nucleosides in DNA. The non-dominant, mirror image form of these molecules, e.g. L-sugars, are thought to be extremely rare or nonexistent in nature, however, we have observed a widespread shadow metabolism for L-sugars in soils from across the US. Our interest in biotic processing of these sugars is two-fold, first to understand their biochemical underpinning and determine the ubiquity of microbial capability for L-sugar synthesis and consumption, and second to explore the potential of L-sugars as a mechanism for carbon sequestration in soils. We will present an overview of our understanding of the known and predicted biochemistry of L-sugars and preliminary results for their soil carbon sequestration potential. We will also introduce the Keck Metabolism Collective, a nascent working group dedicated to understanding the myriad molecules, modes of energy transformation and environments for microbial metabolism across the globe.

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Bouley, Clara

University of Wyoming

Poster 8

Session 2

Title: Source Tracking via Molecular Characterization of FRNA Coliphages

Recreational activity in surface water contributes to a significant number of annual cases of illness per year. Importantly, fecal contamination of these waters presents a major hazard of exposure to pathogenic microorganisms and subsequent illness. There is a pressing need for accurate source attribution. Microbial source tracking (MST) is a methodology that can aid in elucidating the sources of fecal contamination and inform remediation efforts to mitigate pathogen exposure risk. We made use of male-specific (F+) Coliphage, a bacteriophage which is strongly associated with fecal material, to act as our marker for the MST approach. This group of bacteriophage is an apt marker for gauging fecal contamination presence and source through their abundance in feces and known associations with specific hosts types. This RNA virus comprises four genogroups, wherein GII and GIII are associated with mainly human and domestic sewage, GIV with animals and livestock, and GI with both human and animal feces. Environmental water samples from the Jackson, Wyoming area were tested for these four genogroups with digital PCR following concentration with an anion exchange resin concentration method and RNA extraction directly from the resin. Digital PCR allowed for absolute quantification for each of the four coliphage genogroups, and yielded detection mainly in genogroups I and III among the 22 pooled samples tested. As both of these genogroups have association with human fecal material, it is suggested to human sewage may have been a contributor to the elevated levels of coliforms, and potentially pathogens, in these waters.

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Broeckling, Corey

Colorado State University

Poster 9

Session 1

Title: Enabling cutting edge metaproteome analysis at Colorado State University: Introducing the NSF-funded Orbitrap Eclipse Mass Spectrometer for proteome analysis of microbial communities.

Mass spectrometry-based proteomics is a powerful tool for large-scale protein identification. However, typical data dependent acquisitions can have a limited dynamic range and insufficient sensitivity for thorough characterization of high-complexity samples. Metaproteomics extends proteomics approaches to analysis of the collective proteome of microbial communities, providing a qualitative and quantitative description of the proteins in microbiome samples. The complexity of metaproteomes demands novel analytical technology to maximize the depth of coverage. In this presentation we describe the Thermo Orbitrap Eclipse with Field Asymmetric Waveform (FAIMS) interface, a high-end instrument acquired through funding from the National Science Foundation: Major Research Instrumentation program. This instrument will enable development and implementation of cutting-edge metaproteomic applications at Colorado State University. We demonstrate the functionality of this instrument by through analysis of two commercially available microbial community standards (ZymoBIOMICS Microbial Community – 10 species, Cat. D6300 and Gut Microbiome, Cat. D6331 – 21 species). The Eclipse + FAIMS was able to identify proteins from every species in these samples, with as many as 911 proteins

identified from a single species in the 21 species mixture. This instrumentation and approach will enable deep and broad descriptions of the biochemical activity in microbiome samples and facilitate a deeper understanding of the dynamics of the proteome of individual species as they exist within the larger microbiome.

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Burcham, Zachary

Colorado State University

Poster 10

Session 2

Title: Geographical Variation of Microbial Dynamics during Human Decomposition

Decomposition is one of the most important global processes. During decomposition, animal remains are broken down by chemical processes and scavengers which together help to prevent the buildup of organic waste and drive global ecosystem processes. Microorganisms break down the bulk of vertebrate organic matter into simpler compounds, but our understanding of these microbial dynamics is limited even though they have interdisciplinary value for both forensics and ecology. Here, 36 corpses were allowed to decompose in terrestrial environments at three willied-body donation facilities across the United States. Corpse skin and associated gravesoil were sampled over 21 days of decomposition. We performed amplicon sequencing to estimate the archaeal, bacterial, and microbial eukaryotic decomposition communities. Shotgun metagenomic sequencing was utilized to characterize microbial functional dynamics in the corpse adjacent gravesoil. Metabolomic profiling from both the skin and gravesoil was used to characterize metabolic intermediates associated with decomposition. This study used multi-omic datasets to build machine learning models with the goal of using 'omic data feature abundance and geographic location as predictors of time since death and successfully created accurate models from multiple data types. The microbes driving these models resemble a complex assemblage from multiple sources such as human, soil, and invertebrates. While decomposition progresses, the microbial communities undergo phylogenetic turnover and alter their nutrient cross-feeding and competitive interactions. Further, we find that lipid build up occurred in our semi-arid environment suggesting microbial activity may be hindered and that microbial processes of decomposition likely do not behave the same across geographic locations.

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Caro, Tristan

University of Colorado-Boulder

Poster 11

Session 1

Title: Quantifying the growth rates of microbes in soil with stable isotope probing lipidomics

The rate at which organisms grow and reproduce is fundamental to our understanding of ecosystems. While soil microbiologists routinely quantify soil microbial biomass levels and the growth rates of individual taxa in culture, we have a limited understanding of how quickly microbes actually grow in soil. For this work, we posed the simple question: What is the typical generation time of soil microorganisms in soil? We answered this question by implementing a new taxa-agnostic approach to quantify microbial growth: lipidomic hydrogen stable isotope probing. For this method, heavy water ( $2\text{H}_2\text{O}$ ) is added directly to soil as a tracer of membrane lipid biosynthesis. By measuring the  $2\text{H}$  enrichment of intact polar lipids through compound-

specific gas chromatography isotope ratio mass spectrometry, we were able to quantify the apparent growth rates of both bulk soil microbial communities and specific community subsets. We find that mean apparent generation times in soil are exceptionally slow (from ~3 to 13 weeks), but with a wide range in inferred generation times within a given community. We note that the abundance of membrane components does not correlate with their specific turnover rate, suggesting that the more abundant community members are not necessarily faster growing. More generally, we demonstrate the utility of lipidomic hydrogen stable isotope probing for measuring microbial growth rates in soil and highlight the importance of measuring growth rates to complement more standard analyses of soil microbial communities.

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de Melo Ferreira, Djennyfer Karolaime

Colorado State University

Poster 12

Session 2

Title: Microbial and geochemical contributions to methane fluxes in saline, coastal wetlands

Despite their relatively small land coverage, wetlands represent the largest natural source of atmospheric methane (CH<sub>4</sub>), a potent greenhouse gas. Coastal wetlands experience dynamic freshwater or saltwater intrusion events, and CH<sub>4</sub> emissions are poorly quantified. Although salinity is thought to suppress methanogenesis in saltwater wetlands, the mechanism and tipping points of this effect are not well understood. As such more data on the microbiome responses to a range of salinities is needed, especially studies that account for variation in above ground land cover (ecosites) and below ground soil texture and geochemistry. To address the microbial contributions to CH<sub>4</sub> fluxes, we sampled soil cores and surface water from three ecosites (Spartina spp., Juncus spp., and unvegetated open water) of the Barataria Bay coastal saline marsh, in Louisiana, USA. Methane fluxes to the atmosphere were measured with chamber measurements, while porewater concentrations of methane were measured at cm-depth intervals with in-situ peepers. In the overlying water and from three depths in the soil column (0-10, 20-30 and 40-50cm) geochemical measurements (e.g. dissolved oxygen, temperature, redox potential and Fe<sup>2+</sup> concentrations) were paired to microbial community membership analyzed by 16S rRNA amplicon sequencing. Together these findings will elucidate how changes in wetland characteristics affect methanogen, methanotroph, and overall community membership and structure in a model coastal wetland.

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DiLegge, Michael

Impello Biosciences

Poster 13

Session 1

Title: Drought-induced host recruitment of a “thirst-quenching” rhizosphere microbial community

Plant associated microbial communities can play a strong role in plant developmental performance through the efforts of nutrient acquisition and tolerance to both biotic and abiotic stressors. Plants can reshape the soil and resulting rhizosphere microbiota over repeated cultivation of the same host species in one agroecosystem. These communities can be influenced by the host plant in response to an outside stimulus such as high salinity, drought stress, or soil

nutrient availability. Due to the already present agricultural issue of limited water availability, one method of alleviation is to identify microbial communities that assist crops growing under drought conditions. We adapted the conceptual framework of host-associated microbiome conditioning in a system with limited water availability. Selected host plants in this study were hemp (*Cannabis sativa* cv. Janie's Cherry) and tomato (cv. Roma) to observe plant-mediated reshaping of microbial communities under two generations of drought stress conditions. Interestingly, both plant types under limited water conditions in the second generation showed increased biomass when cultivated with microbial communities conditioned by the previous generation, compared to the control plants under severe drought with no applied microbes. Rhizosphere soils of all plant replicates in both generations, as well as the starting soil inoculum in the absence of plants, is undergoing 16S Illumina MiSeq sequencing to identify the resulting bacterial communities that likely played a role in alleviating drought stress in generation two.

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Fouquier, Jennifer

University of Colorado Anschutz Medical Campus

Poster 14

Session 2

Title: A workflow for variable selection in longitudinal microbiome studies

Gut microbiome imbalances are linked to diseases including HIV, autism, and obesity. Longitudinal microbiome studies (LMS) are increasingly common for understanding how microbiome changes relate to factors they may influence such as metabolites, immune markers, and disease symptoms. Integration of microbiome data with other datasets is performed to identify features that relate to a response of interest. There are many analytical challenges with LMS including sampling of individuals requiring mixed-effects models to handle non-independent data. Additionally, LMS can be observational (i.e., measurements made over time, related to natural fluctuations in symptoms) or involve interventions, such as diet modifications. In observational studies, the lack of an intervention often reduces the importance of baseline reference values, while interventional studies emphasize them. Thus, it is necessary to understand feature changes in the context of difference reference points. To aid exploratory analysis, we are developing a feature selection workflow for integration, feature reduction and calculation of feature differences for each subject, and between timepoints, using different reference points for varying LMS designs. Next, Mixed Effects Random Forests identify variables, then post-hoc statistics are performed. This workflow is being developed and validated using simulated data engineered to contain important relationships, notably categorical deltas which are identified using this tool. When applied to a non-simulated agrarian diet intervention aimed to improve health in HIV+ individuals, 49% of the variation in metabolic disease score was explained with variables including microbiome, demographic, sexual behavior, and diet. This workflow streamlines the complex process of feature selection in LMS with the goal of generating new hypothesis to advance microbiome research.

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Gilfillan, Darby

Colorado State University

Poster 15

Session 1



## Title: An Enteric Enquiry: Rotavirus Vaccination using a Recombinant Probiotic and the Host-Microbiome Response

Rotavirus causes 250,000 annual deaths among children under five.<sup>1</sup> Lower-middle income countries have reduced protective immune responses to the two attenuated-live oral vaccines available; therefore, an alternative vaccine is necessary.<sup>2</sup> We engineered the probiotic bacterium *Lactobacillus acidophilus* as a recombinant, orally delivered vaccine platform (rLA) expressing the viral capsid protein VP8 and two adjuvants (FimH/FliC). Here we evaluate specific immune responses induced by probiotic immunization and interactions between host and our rLA. Hypothesis: rLA vaccination against rotavirus will induce protective immune responses. Systems-level understandings of mechanisms regulating vaccine-induced protection will inform future vaccine strategies. Summary/Results: We vaccinated mice with our rLA on two immunization schedules (three/five doses) with subsequent rotavirus challenge. We evaluated anti-VP8 immune responses generated by the rLA using ELISpot and protection via fecal shedding of the rotavirus antigen. Results indicate a delay in viral shedding and increased antigen-specific B-cells in the five-dose vaccine group compared to controls and the three-dose group. To assess the host immune system/microbiome/vaccine interaction, we orally immunized mice with the same rLA strain and collected ileum containing a Peyer's patch 24-hours after dosing. We applied metagenomic (structure), metatranscriptomic (function), and metabolomic (metabolites) analyses. Preliminary results successfully detected both adjuvants expressed in our vaccinated group and upregulation of immune-system related genes. Future studies will further examine the host and microbiome during immunization programs. Conclusions: Our research indicates rLA can induce a protective immune response against rotavirus while capturing some of the immediate changes occurring within the host/microbiome/immune system environment due to rLA vaccination.

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Gonzales Diaz, Andie  
Colorado State University

Poster 16

Session 2

Title: Climate Change Influence Microbial Community Assembly in the Rhizosphere of Boreal Tree Species

The temperate-to-boreal forest ecotone is a critical habitat which is predicted to experience overall increase in the future climate patterns including higher global temperature and longer periods of extreme drought than lower latitude biomes. Significant functional changes have already been documented, like forest range contraction or migration. The ability of plants to face environmental stresses is partly mitigated by the microbiome inhabiting the soil, rhizosphere, roots, and other plants tissues. However, we have limited understanding on how multiple climate change drivers individually or interactively shape the microbial community structure of plants growing in temperate-to-boreal forest ecotone. Here, we collected rhizosphere soil samples from plants growing under ambient, warming, drought, and combined warming and drought treatments from Boreal Forest Warming in an Ecotone in Danger (B4WarmED) experiment being conducted at two sites in Minnesota. We profiled the microbial community structure by amplicon sequencing. Our results demonstrate that the climate change drivers significantly impact the structure of rhizosphere associated bacterial and fungal community. We are now

exploring the microbial assembly processes that are impacted by climate change drivers. Understanding the dynamic relationships between microbiome and plants during future climate change scenarios is essential for predicting and potentially managing plant-microbiome interactions to increase plant resilience to abiotic stresses.

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Grant, Lady

Colorado State University

Poster 17

Session 1

Title: Probing the response of soil microbiomes to decadal differences in tillage

Tillage and soil management practices influence the physical, chemical, and microbial structures of soils, which in turn alter soil biogeochemical cycling critical to soil health. While management practices that promote an effective soil microbiome are of great interest as microbial processes can impact crop productivity and maintain sustainable agroecosystems, it remains unclear how specific tillage practices affect soil microbial communities in semi-arid soils. In this study we used 3 widely used tillage treatments that spanned a gradient of tillage severity from less (strip tillage, ST) to intermediate (minimum tillage, MT) to most intense (conservation tillage, CT). Soil tillage treatments were maintained for the past ten years at the Kerbel field site, part of Colorado State University's Agricultural Research, Development and Education Center (ARDEC) in Fort Collins, CO. Here we sampled the bulk and rhizosphere soils for bacterial and fungal community composition along the tillage treatments with samples collected temporally across three key growth stages of maize production (emergence, vegetative, reproductive). We posited that soil fungal and bacterial communities would statistically differ across the three tillage treatments, with bulk, surface soils from the conventional tillage having the most distinct membership and lowest microbial diversity. To test this hypothesis, we collected over 311 spatially and temporally resolved samples that included 16S rRNA gene and ITS amplicon sequencing data paired to geochemical measurements and soil health indices. Our study will begin to resolve how tillage management practices shape the soil microbiomes and the biogeochemical processes they drive.

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Hey, Maya

Colorado State University

Poster 18

Session 2

Title: Case studies on precision fermentation in alternative food/protein industries: a rhetorical approach

This poster analyzes three case studies that utilize precision fermentation to bypass macro-organisms in the production of proteins destined for human consumption. Rather than derive these proteins from natural pathways, these ventures use micro-organisms as bioreactors and engineer them to optimize the production of compounds of interest. By studying three instances of bypassing—dairy without the cow, egg protein without the chickens, breast milk without the human—this poster compares the rhetorical framing of the food product, its messaging, and the organisms/technologies these companies use to actualize their intervention. The poster concludes with three provocations meant to engage with microbiome researchers: (1) what are other

instances of using one organism as a tool to bypass another? (2) what goes into the calculation of which organisms to expend and which to bypass? (3) what, if any, ramifications does precision fermentation have on skin, gut, and oral microbiomes when consuming these food products?

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Hill, Jessica

Colorado State University

Poster 19

Session 1

Title: *Caenorhabditis Elegans*: a Model for Genetic Interaction between the Gut Microbiota and Intestinal Epithelial Cells

The human gastrointestinal tract is home to trillions of microorganisms that dynamically comprise our gut microbiome. Alterations in our gut microbial community are associated with numerous diseases. At the forefront of these host-microbe interactions are intestinal cells, which help establish and maintain the beneficial symbiotic relationships we develop with our gut microbiota. Several fundamental processes underlying these host-microbe interactions however remain unclear. How do intestinal cells differentiate between commensals versus pathogens? Which intestinal cell responses promote commensal selection in the gut? Addressing these questions with current models has been challenging. Thus, we leverage the tractability and simplicity of *Caenorhabditis elegans*. We hypothesize that intestinal cells display unique innate immune activity due to their spatial sub-functionalization in the gut, and that these cell-specific immune functions allow for distinct interactions with gut bacteria and are required for commensal vs pathogen recognition. Organization of bacteria- and community-specific immune responses within the intestine are evaluated by determining the transcriptional response of intestinal cells to commensal and pathogenic gut bacteria. We also hypothesize that distinct intestinal cell innate immune receptors are important for recognizing commensal bacteria and promoting their colonization in the gut. Regulation of commensal bacterial colonization in the gut by intestinal cells is tested by determining the role of C-type lectin (CLEC) innate immune receptors in selecting for and maintaining commensal gut bacteria. These investigations clarify how innate immunity is regulated on a transcriptional level throughout the intestine and how it contributes to the selection and maintenance of host-microbe relationships in the gut.

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Holzhausen, Elizabeth

University of Colorado-Boulder

Poster 20

Session 2

Title: The association between breast milk miRNA and the infant gut microbiota: a potential mechanism for developmental programming of the infant gut microbiota

Breastmilk contains non-coding microRNAs, which regulate post-transcriptional gene expression and can be contained in extracellular vesicles (EV-miRNAs). While the exact role of breastmilk EV-miRNAs remains uncertain, they can survive digestion and may be taken up by infant cells. Thus, EV-miRNAs may alter infant gut microbiota, via epigenetic programming or as a nutritional component. Participants included 110 mother-infant dyads from the Southern California Mother's Milk Study. EV-miRNAs were measured 1-month postpartum, and infant gut microbiota were assessed at 1- and 6-months. EV-miRNAs examined were present in  $\geq 70\%$

of samples and were quantified as counts/million. We used principle components analysis to test whether EV-miRNA profile was associated with maternal/infant characteristics and negative binominal models to analyze genus counts, with an offset of total reads and adjustment for proportion of rRNA reads, date of EV extraction, and volume of skim milk. We adjusted for multiple testing using the Benjamini-Hochberg procedure, with a threshold of  $P < 0.05$ . Maternal and infant characteristics were not associated with EV-miRNAs. Abundance of 17 EV-miRNAs (e.g., miR-92b, miR-150, miR-30b; associated with infant immune function) were associated with *Streptococcus*, *Veillonella*, or an unidentified genus from family Enterobacteriaceae at 1-month (effect estimate range 0.86-1.1). *Veillonella* is common in the microbiome of breastfed infants and high levels of *Streptococcus* have been linked with adiposity. 5 positive associations (distinct from associations at 1 month) between EV-miRNAs and *Prevotella* and the unidentified genus from family Enterobacteriaceae were observed at 6 months. These results provide preliminary evidence that breastmilk EV-miRNAs may impact the infant gut microbiota.

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Honeyman, Alexander  
Colorado School of Mines

Poster 21

Session 1

Title: Statistical learning for hybrid microbiome-biogeochemical predictions in post-wildfire soils

Soil biogeochemical processes regulate plant growth and nutrient losses that affect water quality, yet the temporal response of soil after variable intensity wildfire is difficult to explain and predict. To address this issue, we examined two wildfires in Colorado, USA across the first and second post-fire years and leveraged Statistical Learning (SL) to predict and explain biogeochemical responses. We found that SL predicts biogeochemical responses in soil after wildfire with surprising accuracy. SL models trained on a hybrid feature space of biogeochemistry and microbiome data typically gave the most accurate predictions. Sometimes, microbiome data alone were more predictive than biogeochemical measurements—and these predictions depended on complex covariances amongst features that were learned by statistical models. A data science approach to the assessment of post-fire soils may enable practical tools for monitoring recovery.

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Huang, Xingfeng  
Colorado State University

Poster 22

Session 2

Title: Coal-derived Soil Amendment Promotes Plant Growth and Shapes Rhizosphere Microbial Communities of Lettuce (*Lactuca sativa*)

Recent studies showed that application of coal-based amendments could improve soil physicochemical properties and thus increase crop yield. However, there is still a lack of comprehensive analysis how coal-based amendments influenced soil physicochemical properties and microbial communities. In the present study, it was found the application of coal-based amendment promoted the plant growth of lettuce. The plant fresh weight of the coal-based amendment treatment was approximately twice that of the reference while the raw coal treatment

had similar fresh weight to the reference. The addition of coal-based amendment improved the soil organic matter content in the soils but did not significantly increase the content of phosphate and potassium while the organic fertilizer treatments showed higher phosphate and potassium in comparison to the reference. Furthermore, lettuce rhizosphere microbial communities of both bacteria and fungi have been investigated by 16S rRNA and ITS gene sequencing using a 2 × 250 MiSeq flow cell. The PCoA clustering patterns suggest the additions of coal-based amendment, organic fertilizer, and raw coal affected the lettuce rhizosphere microbial communities differently. In comparison to the reference, the coal-based amendment treatments both at low and high dosages decreased the relative abundances of Actinobacteria and Proteobacteria and increased Actinobacteria and Chloroflexi significantly in the bacterial community and decreased the relative abundances of Basidiomycota and increased Ascomycota significantly in the fungal community. The ability of enrich or attract more beneficial microbes in the rhizosphere may be one of the reasons to promote plant growth by coal-based amendment.

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Jech, Sierra

University of Colorado-Boulder

Poster 23

Session 1

Title: Field cultivation of climate-adapted cyanobacteria for dryland biocrust restoration

Biological soil crusts (biocrust) are photosynthetic communities of moss, lichen, and cyanobacteria that thrive in dryland systems and influence ecosystem functions like soil stability, fertility, and hydrology. Biocrusts are sensitive to mechanical disturbance (e.g., development) which can result in land degradation. Efforts to restore biocrust include cultivating moss, lichen, and cyanobacteria in the laboratory, greenhouse, or field and then inoculating soils where biocrust has been lost. Biocrust restoration success can be highly variable and dependent on climate. It is prudent to consider future climate in restoration strategies, so that restoration efforts have the best chance of long-term success. One option for improving biocrust restoration success is to include hot-adapted cyanobacteria (e.g., *Microcoleus steenstrupii*) in cold-desert biocrust inocula. To test the hypothesis that *M. steenstrupii* could survive cultivation in cold-desert conditions, biocrust sourced from the Colorado Plateau, Sonoran Desert, and Mojave Desert were field cultivated on the Colorado Plateau for one year. We used 16S rRNA sequencing and qPCR to identify and quantify the microbial communities of each biocrust inoculum as compared to intact biocrust at each desert source. We identified compositional differences (e.g. cyanobacterial communities) for each biocrust source and for the resulting cultivated inoculum. This work informs future research on dryland restoration with biocrust and the practice of restoration under changing climate.

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Jones, Zackary

Aggrego Data

Poster 24

Session 2

Title: Community Science: The Vermi-microbiome Project

The goal of the Vermi-Microbiome Project is to educate vermicomposters about modern microbial ecology through community based data analysis. The value of vermicompost lays

mainly in its rich microbial community. Samples were collected nationally from 25 different vermi-composters to better understand the broad ecology of different vermicompost types. Metadata about each producer and vermicompost in the form of questionnaires and traditional STA certified compost physical and chemical properties, respectively. A broad range of composting inputs, methods, and chemical characteristics were overserved. Despite this variability, a core microbiome analysis revealed a common set of organisms across nearly all vermicompost samples. The number one most abundant and prevalent organisms were members of the genus *Chryseolinea*. This genus was seen in nearly every vermicompost sample with at least 1% relative abundance, and about half of the vermicompost samples have at least 3.5% of *Chryseolinea*. These organisms are also known endophytes which might help explain the wide plant benefits seen from vermicompost of all types.

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Kimmell, Louisa

Colorado State University

Poster 25

Session 1

Title: Comparing soil microbial communities across degraded, intact, and restored dryland ecosystems

Escalating soil degradation and ecosystem service losses across drylands highlight a clear need for strategic approaches to use active restoration to achieve management goals. In recent decades, there has been growing interest in using soil microbe inoculation and/or “transplants” from intact reference sites to promote dryland restoration success. Yet, use of biotic soil restoration treatments is often not guided by a preliminary understanding of soil community structure and what organisms and/or functions are lacking in degraded communities. Consequentially, significant gaps remain in our understanding of how best to incorporate biotic soil and plant restoration treatments into dryland restoration planning and decision-making. This study explores how the composition and diversity of microbial communities differs across degraded, intact, and restored sites in dryland environments. Microbial communities were analyzed using soil samples from sites managed under RestoreNet, an existing restoration network that systematically tests the effectiveness of plant-based restoration strategies in western US drylands. I am predicting that there will be greater microbial diversity in intact and restored sites, but a greater relative abundance of stress-tolerant microbes in degraded sites. The findings from this study will help land managers and restoration practitioners select the proper microbial treatments for restoration in drylands, as well as decide whether or not to use plant-based treatments for recovery of soil microbial communities.

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Laurita, Nicole

University of Colorado-Denver

Poster 26

Session 2

Title: Response of freshwater ammonia-oxidizing bacteria to environmental pressures based on genomic predictions and physiology

In Colorado streams, microbes perform vital steps in the nitrogen cycle, which entails cycling between gaseous dinitrogen and its bioavailable forms. While every cell needs nitrogen, too

much bioavailable nitrogen leads to eutrophication and its devastating effects on the environment. Current anthropogenic pollution is overloading these nitrogen cycling microbes and threatening the health of Colorado freshwater ecosystems. This study examines how ammonia-oxidizing bacteria (AOB that convert ammonia into nitrite) respond to environmental stress in Colorado rivers with a particular focus on metal pollution. We report the enrichment and near-complete genome sequences of three *Nitrosomonas oligotropha* AOB representatives from the South Platte, Cherry Creek, and Cache la Poudre rivers. A global biogeography analysis (based on a query of over 270,000 SRA submissions from 16S rRNA gene amplicon studies) showed *N. oligotropha*-like sequences were commonly found in freshwater and wastewater environments across the globe and were largely absent from soil or marine settings. Our Colorado *N. oligotropha* genomes contained a wide range of metal resistance genes. Except for some lead and mercury resistance proteins missing in one of the genomes (CP45), all three riverine *N. oligotropha* genomes encode the same metal resistance proteins: iron, manganese, zinc, nickel, copper, silver, chromium, tellurium, and selenium. The presence of these predicted proteins may be due to environmental pressures of metal pollution in the South Platte River basin from naturally occurring and human induced processes (e.g., weathering, mining, wastewater effluent). Future efforts will extend these studies to include transcriptomics to measure expression of different genes under metal stress (e.g., changes in stress response genes, carbon cycling, energetics). By studying the degree of metal tolerance in AOB, we aim to identify Colorado streams where AOB will be negatively impacted by elevated dissolved metal concentrations in order to guide remediation efforts.

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Lucero, Hannah

Colorado State University

Poster 27

Session 1

Title: Genome Guided Cultivation of Synthetic Microbial Consortia Probes the Interactions underpinning *Salmonella enterica* Colonization of the Gut

*Salmonella enterica* is one of the most significant food-borne pathogens, resulting in 94 million cases of gastroenteritis annually world-wide. It is widely recognized that gut colonization of this pathogen depends on it outcompeting commensal microorganisms. Microbial multi-omic data collected by our team during *Salmonella* infection revealed specific microbial interactions and changes in nutrient usage over the course of pathogen infection. Mining this data provides a road map of the *Salmonella* most-wanted microorganisms, a list of bacteria that are most susceptible or resistant to *Salmonella* infection, and a description of their relative abundance and metabolic performance changes over time. Our goal is to obtain pure cultures of these strains to enable synthetic consortia for interrogating the metabolic dependencies between *Salmonella* and other gut inhabitants, potentially revealing new avenues for therapeutics. Currently we have isolated seven of the top ten most wanted microorganisms in pure culture. As an example of the power of this approach, we demonstrate that a *Lactobacillus johnsonii* strain we isolated shares 100% 16S rRNA gene identity to a bacterium consistently co-occurring with *Salmonella* during peak infection. In vitro experiments revealed this strain not only has genes to resist *Salmonella* inflammation, but also secretes a soluble compound inhibiting *Salmonella* growth. Preliminary annotation suggests this compound is likely a bacteriocin, with ongoing proteomic and metabolic efforts confirming this identification. Future work using mouse models will test the efficacy of

harnessing competition from these most-wanted microorganisms or their antimicrobial agents as biotherapeutics during *Salmonella* infection.

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Luna, Emily

Colorado State University

Poster 28

Session 2

Title: Microbiomes in Honeydew of Russian Wheat Aphids Increase Aphid Virulence to Plants

Phenotypic responses of plants to biotic stresses are often studied as interactions between two species; however, in the phytobiome, these responses frequently result from complex interactions involving several organisms. We previously demonstrated that variation in chlorosis on wheat caused by feeding of Russian wheat aphid (*Diuraphis noxia*) is determined, in part, by aphid-associated bacteria. Although saliva from aphids contains culturable bacteria, using scanning electron microscopy (SEM), bacteria were not detected in high numbers in the salivary glands or foregut of the aphids. Culturing studies show that bacteria in the same genera of those identified in aphid saliva are also present in aphid honeydew, including bacteria in the families Enterobacteriaceae and Erwiniaceae. One bacterium detected in honeydew, *Erwinia iniecta*, has the genetic capacity to interact with both the plant and the aphid. Currently, studies are underway to determine the role of honeydew-associated bacteria in initiation of plant defenses in plants. Preliminary data support the hypothesis that honeydew-associated bacteria contribute to aphid virulence by suppressing the plants' insect defense mechanisms, allowing the aphid to feed freely

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Lutsiv, Tymofiy

Colorado State University

Poster 29

Session 1

Title: Relandscaping the Gut Microbiota with a Whole Food: dose-response effects of Common Bean

Underconsumption of dietary fiber is an underappreciated concern associated with the increasing global burden of chronic diseases. This is attributed predominantly to the gut microbiota, composition and function of which depend on the amount and quality of fiber in the diet. Common bean, *Phaseolus vulgaris* L., is a fiber-rich food whose potency is overlooked in the realm of diet-microbiota-host interactions. To elucidate bean effects on the gut microbiota, a model for dietary induced obesity was investigated. Diet formulations containing increasing doses of cooked bean (0 to 70% dietary protein from bean) were fed for 16 weeks to male and female C57BL/6J mice. At study termination, 16S rRNA sequencing was used to assess cecal microbial communities and the mass of adipose depots was determined. Bean-containing diets significantly re-landscaped the gut microbial ecosystem and dose-dependently reduced mesenteric and subcutaneous fat mass. Dietary bean reduced the Firmicutes:Bacteroidetes ratio, decreased  $\alpha$ -diversity, and increased  $\beta$  diversity—effects that were more pronounced in female versus male mice. Bean consumption suppressed bacterial taxa associated with disease states and increased the prevalence of taxa linked to obesity prevention. Overall, these data demonstrate dose-responsive and sex-dependent changes to the gut microbial community in a widely studied model of dietary induced obesity.



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Mansfeldt, Cresten  
University of Colorado-Boulder  
Poster 30  
Session 2  
Title: Database-driven microbial risk and benefit analyses

The rapid expansion of sequencing archives increasingly supports in silico microbial risk/benefit assessments. These databases house a wide range of information, from the genomic structure of individual species to the community composition of complex environments. One aspect of either risk or benefit is analyzing the prevalence of an antagonist (e.g., known human or ecological health hazards) or protagonist (e.g., plastic degrading microbiota) throughout diverse microbiomes. Prevalence analyses advance knowledge by focusing on the known-knowns within databases. However, the size and detail of modern databases enable the exploration of known-unknowns such as the likelihood of invasion of a synthetic biology organism within a specific environment or the emergence of a new functional trait or pathogen of concern. These examples provide the basis for two distinct pipelines currently under development: EcoGenoRisk and PrarieMiner. The focus of EcoGenoRisk is the comparative assessment of the predicted function of an invading organism through its genome annotation to the functional profile of the host environment to estimate the likelihood of invasion and establishment. In comparison, PrarieMiner investigates between metagenomes of similar type to investigate those environments with the most evolutionary “chatter” within functions of interest, such as bioremediation or virulence. These types of analyses provide a database-focused summative assessment of microbial risk housed within the twenty-five years of information collected during the genomics era, developing flexible and updating tools that can incorporate the incredible wealth of information anticipated to be gained over the next twenty-five years.

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May, Alyssa  
Colorado State University  
Poster 31  
Session 1  
Title: Rapid identification of microbes using MALDI-TOF Biotyper

Microbiology laboratories have revolutionized their microbial identification process in recent years through the use of matrix-assisted laser desorption ionization methods coupled with time-of-flight mass spectrometry (MALDI-TOF MS). MALDI-TOF MS is particularly favorable for clinical patients that require rapid diagnoses for effective treatment of bacterial infections or researchers that require rapid and cost-effective identification of thousands of bacterial isolates. At the Mass Spectrometry facility in the Analytical Resources Core Materials and Molecular Analysis Center (ARC-MMA) on the CSU campus, we utilize “Biotyper” technology from Bruker Daltonics to accomplish this. The technique requires minimal sample prep and can identify each microbial species in a matter of seconds. Microbial samples are identified via reference libraries or through creation of custom libraries. This talk will discuss the technology behind the MALDI Biotyper method and present a case study for accelerating the development of monoclonal antibody treatments targeted at treating colorectal cancer in collaboration with the University of Florida.

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Mohrlang, Amanda  
Colorado State University  
Poster 32  
Session 2  
Title: Electro-Enhanced Anaerobic Digestion

Anaerobic digestion (AD) of wet organic wastes, such as food wastes and municipal wastewater, is an important microbiome-driven industrial process. Although AD currently focuses on methane as its primary product, the release of carbon dioxide as a byproduct and the low economic value of methane are major shortcomings. In electro-enhanced AD, anaerobic digestions are supplemented with inexpensive electrons from renewable energy with the hypothesis that these will be internalized as additional reducing equivalents that lead to increased production of more valuable products such as volatile fatty acids (VFAs). The detailed effects of implementing electro-enhanced AD on the microbial community structure and microbial metabolism are not known. The goal of this project is to investigate the effect of applied electrical potentials on VFA production, microbial community structure, and the electron transfer behavior of bacteria involved in electro-enhanced AD. By combining these electrochemical, genomic, and metabolomic data, the mechanisms of electro-enhanced AD can be elucidated, and this information can be applied to improve AD processes. Initial experiments demonstrate that electron supplementation significantly increases VFA production. Electron transfer behavior of electrode biofilms is also highly dependent on electron supplementation, suggesting that applied potentials affect the colonization of bacteria on the electrode surface.

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Moore, Laura  
Colorado State University  
Poster 33  
Session 1  
Title: Contributions of soil microbes and their life history strategies to mineral-associated organic matter (MAOM) formation

Bacteria and fungi play an essential role in soil carbon (C) cycling and storage as microbial decomposition products lead to the formation of mineral-associated organic matter (MAOM). Given that microbial diversity is often positively associated with decomposition rates, it is likely that differences in microbial diversity influence the formation of MAOM, especially under varying degrees of labile substrate availability. This project aims to investigate linkages between microbial diversity, labile substrate availability, and MAOM formation through an incubation experiment. We will fractionate Central Plains Experimental Range (CPER) soil by size into MAOM and particulate OM (POM), and then recombine it in three different ratios of MAOM and POM (75% MAOM/25% POM; 50%/50%; 25%/75%) with the goal of manipulating our 'start' microbial communities. We will apply 'low,' 'medium,' and 'high' amounts of dissolved <sup>13</sup>C-labeled inputs representative of rhizodeposition at CPER for four months. After harvesting, we will link community composition and diversity (measured with 16S and ITS amplicon sequencing) and soil respiration to new MAOM formation. We hypothesize that i) high microbial diversity will contribute to lower MAOM formation as more C will be released through mineralization, and ii) this relationship strengthens with increasing substrate availability. As this

experiment is still in the early phases of set-up, this framework and expected results will be presented. Ultimately, this study will help us to develop a better understanding of the relationships between microbial community dynamics and C storage and help define microbial traits that would be most useful to include in global change models.

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Natter, Nicole

Colorado State University

Poster 34

Session 2

Title: Longitudinal SARS CoV-2 identification in human stool and associated gut microbiota

Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) has caused the deaths of millions and infected over 200 million since its emergence in December of 2019. Diagnostic tests for SARS-CoV-2 typically involve nasopharyngeal and saliva biospecimens, whereas little is known regarding the persistence of SARS-CoV-2 detection following acute infection in human stool. A cohort of adults with confirmed SARS-CoV-2 infection were asked to submit stool samples over the course of six months to one year as part of the Northern Colorado COVID-19 Biorepository (NCT04603677). Of 139 participants enrolled, 109 adults provided at least one stool sample. From the 63 females and 46 males analyzed so far, 39.4% tested positive at least once in the stool and 14% had persistent positive stool detection by either real-time PCR or ddPCR. Additionally, a relationship between BMI and microbiota composition was identified in a subset of participants by 16S sequencing that merit investigation alongside differences in persistent stool virus detection. This pilot analysis supports a higher SARS-CoV-2 PCR detection rate in adults who are overweight and obese when compared to normal weight. Moreover, a higher number of females with positive SARS-CoV-2 PCR in stool suggests potential for sex differences. We conclude that the intestinal colonization of virus merits further investigations with respect to changes to microbiota and persistent immune activation, as well as development of post-acute sequelae, namely long-COVID symptoms. Future studies are also needed to evaluate differences between variants for gastrointestinal involvement during initial disease progression and in cases of re-infection during the pandemic.

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Pinedo, Santiago

Colorado State University

Poster 35

Session 1

Title: Biotypic Differences in Bacteria of Russian Wheat Aphid Honeydew and Their Effects on Barley

Wheat and barley make up an enormous portion of human diets globally and are vital in feeding a growing population. The Russian wheat aphid (RWA) feeds on these crops, and infestations lead to severe yield loss. To prevent this, resistance genes were incorporated into plants. One gene prevented RWA1 (biotype 1) from feeding, but eventually, a new aphid biotype, RWA2 (biotype 2), evolved that is able to feed on the plants. The biotypes are genetically identical, leaving the question “what allows RWA2 to feed on previously resistant plants?”. We hypothesize that bacteria in aphid honeydew (HD, excrement) may help RWA overcome plant resistance. To test this, bacteria were isolated from collected HD of both biotypes (RWA1: 27

cfu/HD droplet, RWA2: 30 cfu/HD droplet). Colony PCR was performed to amplify DNA in the 16s rRNA region of the isolated bacteria, and the amplicons were sequenced. Of the HD-associated bacteria sequenced, 94% of the isolates grouped closely to members in the genus *Staphylococcus*. We are currently introducing each of the HD-associated bacteria into barley plants to determine their effect on plant phenotype. Future studies will determine the role of HD-associated bacteria in plant defense against aphid feeding. Characterizing the microbial communities in RWA1 and RWA2 will inform us on how interactions with bacteria may affect aphid virulence, and ultimately lead to more sustainable methods to prevent aphid infestation of crops, a step towards improving the stability of global food sources.

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Nieciecki, Victoria

Colorado State University

Poster 36

Session 2

Title: Microbial Community Response to Vertebrate Remains is Soil Depth Dependent

During vertebrate decomposition, nutrient-rich fluids enter the surrounding soil producing a localized resource hotspot. While microbial community composition has been shown to predictably change in response to these decomposition fluids at the soil surface, few studies have characterized subsurface responses. In this study, we longitudinally sampled forest soils below decomposing human cadavers and from adjacent control sites in 2 cm increments to a depth of 10 cm. Soil samples were collected over three seasons at Sam Houston State University's Southeast Texas Applied Forensic Science Facility and corresponded with active decay, advanced decay, and the dry stage of decomposition. We detected microbial community changes in the top 2-4 cm of soils that had been exposed to decomposition fluids during the spring and summer months. Interestingly, we observed microbial community changes extending into the lower 8-10 cm of soil only during the spring season. These preliminary findings indicate that subsurface microbial communities respond to decomposition nutrients and the maximum depth of detection is likely dependent on season. We aim to investigate microbial responses at deeper soil depths and incorporate soil characteristic measurements into future experiments. In addition to improving our understanding of decomposition ecology, we anticipate that incorporation of these findings into forensic investigations could help law enforcement locate clandestine graves.

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Rico, Jorge

Colorado State University

Poster 37

Session 1

Title: Microbiome Analysis of Iodoform Effects on Advanced Anaerobic Digestion Processes for Production of Fatty Acids

Advanced anaerobic digestion (AD) technologies can transform organic residues into added-value chemicals and fuel precursors. This rewired AD (RWAD) approach requires selectively directing feed carbon into fatty acids instead of methane. For such an approach, studies in RWAD have used methanogen inhibitors, like iodoform, and demonstrated success in arresting methanogenesis while producing fatty acids. However, there is a lack of understanding of how iodoform is associated with the microbiome in RWAD. This knowledge gap is one of the

limitations in the field for optimizing process performance. This study investigated the effects of iodoform addition in RWAD of food waste using anaerobic wastewater sludge as a microbial inoculum. Microbiome reactors were acclimated to operating parameters for one month, followed by a five-day experimental run with five reactor replicates per treatment (with and without iodoform addition). Significant differences were observed in the production of gases and the fatty acid product spectrum. As expected, methane was produced in control reactors but not in the iodoform treated. 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 the metagenome. These differences were driven by *Prevotella* (gen) and *Peptostreptococcacea* (fam), which dominated iodoform reactors; and *Alcaligenes* (gen) and *Rombustia* (gen), which dominated in controls.

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Risk, Briana

Colorado State University

Poster 38

Session 2

Title: *Bacillus Subtilis* DE111 Improves Endothelial Dependent Vascular Dilation in Mice Fed a Western Diet

Vascular dysfunction often precedes the development of cardiovascular disease in which data suggests a role of the gut microbiome in the development of this defect. In a pilot study of healthy humans, we observed the spore-based probiotic, *Bacillus Subtilis* DE111 (DE111), improved reactive hyperemia index (RHI) scores, indicating improved vascular dilation. The current study examined the efficacy of DE111 in a diet-induced obesity model in mice. Male C57/BJ mice were fed a standard diet (SD; n=24) or Western diet (WD; n=24) for 8 weeks to induce vascular dysfunction, then further divided such that (n=12) half of the animals on each diet received 1 billion CFU/d of DE111. WD and WD+DE111 mice exhibited similar increases in body weight (SD: 31.2±2.3 vs WD: 47.4±3.3 and WD+DE111: 45.7±4.0) and impairments in glucose tolerance compared to SD mice (SD: 237.1±68.0 vs WD: 410±26.9 and WD+DE111: 460±116.8, p<0.05). A 28% decrease in vessel dilation was observed with mice on a WD as compared to mice on a SD after addition of the endothelial-dependent dilator acetylcholine (AUC, 918.3±49.25, p<0.05). However, the addition of DE111 improved endothelial mediated vessel dilation in mice fed a WD+DE111 (AUC, 991.7±47.9, p<0.05). This increase was observed independent of body weight changes and glucose tolerance. Further analyses are underway to establish the mechanisms of this gut-vasculature crosstalk, but our animal and human studies both suggest that DE111 may support vascular health.

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Rodriguez, Josue

Colorado State University

Poster 39

Session 1

Title: A finely scaled temporal analysis of microbial and viral communities in the Erpe river

While it is known that microbial and viral communities play key roles in biogeochemical cycles, most microbiome studies focus on single time points or are temporally resolved to scales that

may not be appropriate for microbial and viral community life cycles. As a result, the extent at which viral and bacterial communities turn over or remain stable are poorly understood in natural systems. To assess how viral and microbial communities change and influence biogeochemical cycling processes over time in a fluvial system, we sampled surface waters and sediments from the Erpe River in Germany every 3 hours over a 48-hour period. This resulted in 32 metagenomes that were paired to geochemical measurements and FTICR-MS data, providing unprecedented insights into the dynamics and processes of these two river compartments. We reconstructed 125 microbial and 1250 viral metagenome assembled genomes, and found, consistent with our geochemistry, distinct microbial and viral communities were associated with each compartment. Our analyses demonstrated that some viral community members remained stable across 70% of the time series, hinting at the possibility of continuous predation roles. Using computational approaches, we linked 29 viral genomes to microbial genomic hosts, elucidating possible virus-host relationships that could influence carbon, nitrogen, and sulfur cycling in river ecosystems. Together, these findings bolster support for the idea that microbial and viral communities can exist as taxonomically, and metabolically stable communities along a flowing river corridor; and provide insights that could affect ecosystem models attempting to constrain the underpinning dynamics of biogeochemical cycles in rivers.

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Sachdeva, Neha

Colorado State University

Poster 40

Session 2

Title: Investigating the role of prokaryotic CYP450 in TOrCs biodegradation using multi-omics tools: A proof of concept study

The increasing use of organic compounds has led to trace organic contaminants (TOrCs) water pollution. The conventional wastewater treatment processes fail to completely remove TOrCs from the treated wastewater streams. Tapping the TOrC-degrading potential of wastewater microbiota could provide a cost-effective solution to TOrCs pollution. Cytochrome-P450 enzymes (CYP450) metabolize pharmaceuticals in humans. While several studies have indicated the role of the human gut microbiome in human CYP450 synthesis, few studies have investigated the role of prokaryotic CYP450 in TOrC biodegradation. The dearth of molecular tools and functional gene biomarker assays has limited these investigations to just the monitoring of TOrC degradation rates, mostly for pure cultures cultivated with single TOrCs.

Therefore, we are using a multi-omics approach to investigate the role of prokaryotic CYP450 enzymes (wastewater microbiome) in simultaneous biodegradation of twelve TOrCs. The microbiota, retrieved from activated sludge and sediments historically receiving wastewater effluent, were acclimated to high concentrations (50 ug/L) of selected TOrCs (as cocktail) for 8 weeks. The metagenomic data were used to design rhAmp-PCR primers, which were used to screen for functional CYP450 genes/biomarkers using a novel Functional gene Amplicon Sequencing Test (FASt) approach. A proof-of-concept study was conducted, wherein these acclimated cultures were cultivated with the TOrCs cocktail, with and without CYP450 inhibitor. FASt biomarker assays and rhAmp-primers will be used to screen for the presence/absence of CYP450 genes in these samples and investigate whether the TOrC biodegradation (metabolomics) is linked to specific prokaryotic CYP450 enzymes. Metaproteomics will be used to investigate enzyme expression.

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Santangelo, Brook

University of Colorado Anschutz Medical Campus

Poster 41

Session 1

Title: Using microbiome knowledge graphs to gain mechanistic insight of disease

The gut microbiome is known to play an integral role in human health. Sequencing technologies have enabled the profiling of thousands of individual gut microbiome samples, from which we have begun to discern correlations between microorganisms and disease. However, it is challenging to characterize these correlations mechanistically utilizing the vast amount of microbiome knowledge that exists. Here we propose to bridge this divide by incorporating microbial concepts into a large biomedical knowledge graph for further functional interpretation of the results of microbiome studies. Over 3000 relationships defining the metabolism and gene regulation of microbes were added to the PheKnowLator knowledge graph (Callahan, T., bioRxiv, (2020)) using the W3C Web Ontology Language (OWL) scheme. Vector embeddings of each entity were generated from the knowledge graph using Node2Vec. Cosine distance between a microbe and a disease, cellular process or metabolic pathway is used as a metric for relatedness. This process identified a potential contributing microbe in inflammatory bowel disease, *Bifidobacterium animalis*, which metabolizes chlorogenic acid into caffeic acid, a metabolite that modulates intestinal disease. The representation of additional microbiome-relevant entities in the knowledge graph allowed us to use vector embeddings to hypothesize connections between microbes and disease and then gain mechanistic insight through analysis of the graph. Future work will expand this knowledge base using text mining approaches and ultimately enable hypotheses surrounding the molecular mechanisms of diseases influenced by the microbiome.

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Schopflin, LeAundra

Colorado State University

Poster 42

Session 2

Title: Ecological processes of the dryland soil microbiome shift across a systemic breakdown phase aridity threshold

Although the soil microbiome of dryland systems is vital to ecosystem functioning, we know relatively little about how its members will respond to the increasing pressures of climate change. The drylands that host these microbiomes comprise 41% of the earth's surface and rank among the most susceptible environments to climate disturbance. Under the increasing pressure of climate driven stress, dryland ecosystems can cross aridity thresholds that instigate rapid change in both plants and microbial communities. To study these joint effects, we conducted a global metagenomic study of 54 dryland sites across six continents. We aimed to understand the effect of a systemic breakdown phase aridity threshold (AI = 0.8) on the soil microbiome and its associated ecological functions. Our metagenomic analysis recovered 94 significantly correlated gene groups of which 38 were correlated with semi-arid plots (below AI 0.8) and 56 were correlated with arid plots (above AI 0.8). Within the semi-arid group, steroid hormone biosynthesis and sphingolipid and histidine metabolisms were associated with hyphae formation,

cell membrane synthesis and repair, and transcriptional regulation. Within the arid group, ribosome biogenesis, DNA replication proteins, and the calcium signaling pathway were associated with cell regulation, DNA repair, motility, and antibiotic synthesis. Our data reveal a distinct change in soil microbiome processes associated with crossing the 0.8 AI aridity threshold. In the context of ongoing climate change, understanding this aridity threshold and its long-term effects will be vital for mitigating sudden climate change caused ecological disruptions in the already tenuous dryland soil microbiome.

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Seitz, Valerie

Colorado State University

Poster 43

Session 1

Title: Variation in root exudate composition influences soil microbiome membership and function

Interaction between plants and soil microorganisms are predominantly mediated by root exudates, which are low molecular weight compounds (e.g. sugars, organic acids, amino acids, phenolics, and secondary metabolites) released by the plant roots into the soil environment. Root exudation patterns can vary depending on plant developmental stages, species and even within genotypes, which can lead to different microbial associations. Root exudates primarily influence the rhizosphere and can mediate biological processes including influencing resistance to pathogens, plant signaling, and recruitment of beneficial microorganisms. While previous studies have demonstrated a clear link between root exudates and the composition of the rhizosphere microbiome there remains a gap in our understanding of how these interactions translate to soil function. Here, we used a laboratory scale soil reactor system coupled with exometabolomics, 16S rRNA and metagenomics analysis to characterize the functional changes in the soil microbiome under two divergent root exudate conditions. Our results demonstrate an acute remodeling of the microbial community upon exudate addition followed by a long term (20 day) convergence to a similar community structure. Exometabolite data supports the bacterial production of multiple organic and phenolic acids as well as the plant growth promoting auxin (Indole-3-acetic acid; IAA). Metagenomic assembled genomes enabled identification of specific genes enriched in the microbial communities that support the metabolite response to root exudate composition. The results of this study represent an important first step in our understanding of the functional outcome of these plant-microbe interactions and how this response may vary based on root exudate composition.

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Stanislawski, Maggie

University of Colorado Anschutz Medical Campus

Poster 44

Session 2

Title: Host genetics and gut microbiota in asthma among US Hispanics/Latinos: the Hispanic Community Study/Study of Latinos

Asthma is a heterogeneous condition influenced by genetic, demographic, and environmental factors. Gut microbiota may also play a role in asthma. In this study, we used shotgun metagenomic stool microbiota data from the Hispanic Community Health Study/Study of



Latinos to investigate how stool microbiota characteristics relate to asthma among US Hispanic/Latinos, while evaluating and accounting for the impacts of obesity and demographic/environmental factors. We used regression-based models that were sequentially adjusted for covariates to assess the associations of alpha diversity, beta diversity, and microbiota taxa and functional pathways with asthma among N=2404 participants (N=191 with current asthma, N=2213 with no history of asthma), predominantly of Cuban, Mexican, Puerto Rican and Dominican heritage. In fully adjusted models, we observed that increased risk of asthma was associated with lower taxonomic evenness (OR=0.85; 95% CI: 0.73, 0.99; p-value=0.038), significant differences in community-level taxonomic (p<0.001) but not functional structure (p-value=0.133), and differential abundance of numerous taxa and functional KEGG modules, such as decreased *Oxalobacter* abundance and reduction in functional modules related to carbohydrate metabolism. However, these relationships were subject to substantial confounding by factors related to socioeconomic status and immigration history, and they varied significantly with obesity status and ethnic background, with stronger microbiota associations among Mexicans and Dominicans. We also examined microbial associations with an asthma polygenic risk score (results pending). These results support that asthma is associated with differences in the stool microbiota and illustrate the importance of considering the influence of confounding and effect modification when evaluating this relationship.

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Sterrett, John

University of Colorado-Boulder

Poster 45

Session 1

Title: Gut microbiome and short chain fatty acid profiles of individuals with human immunodeficiency virus in rural Appalachia

Human Immunodeficiency Virus (HIV) is associated with altered gut microbiome composition and an increased risk of metabolic syndrome. We analyzed liver steatosis and fibrosis, gut microbiomes, and short chain fatty acid (SCFA) profiles of 19 HIV+ individuals vs 20 HIV- (body mass index- and age-matched) control participants from rural Appalachia. Stool samples were sequenced using 16S rRNA metabarcoding to generate 2x300 base pair reads, and SCFA concentrations were quantified via gas chromatography with flame ionization. Amplicon sequence variants were generated using DADA2, a phylogenetic tree was created using saté-enabled phylogenetic placement, and taxonomy was assigned using a naïve-Bayes classifier trained on the SILVA database. Samples were rarefied to 52,495 reads, Faith's phylogenetic diversity was used to assess alpha diversity, and unweighted UniFrac was used for beta diversity. We found increased liver steatosis (Welch's  $t(37.7)=2.59$ ,  $p=0.013$ ) and fibrosis (Welch's  $t(28.9)=2.02$ ,  $p=0.052$ ) in HIV+ individuals. A Kruskal-Wallis test revealed no difference in alpha diversity by HIV status, but a PERMANOVA revealed a difference in microbiome composition (pseudo-F = 1.91,  $p = 0.003$ ). Additionally, we found lower fecal concentrations of acetate ( $H(1)=7.94$ ,  $p=0.005$ ) and butyrate ( $H(1) = 8.23$ ,  $p = 0.004$ ) in HIV+ individuals. Differential abundance testing (Gneiss, ANCOM) and co-occurrence network module analysis (SCNIC + ANCOM) revealed increased relative abundances of the phylum Bacteroidota and the genus *Prevotella*, as well as a decreased relative abundance of an uncultured group of *Erysipelatoclostridiaceae* in HIV+ individuals. Overall, this study showed a difference in liver markers, microbiome composition, and SCFA profiles in HIV+ individuals.

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Stevens, Bo  
USDA ARS  
Poster 46  
Session 2

Title: MinION reduces costs while producing comparable results to MiSeq

Illumina MiSeq is the current standard for characterizing microbial communities in soil. The Oxford Nanopore Technologies MinION sequencer is quickly gaining popularity because of the low initial cost and longer reads. However the accuracy of MinION, per base, is much lower than MiSeq (90% versus 99.9%). The effects of this difference in base-calling accuracy on taxonomic and diversity estimates remains unclear. We compared the output of short reads of MiSeq to short and long reads of MinION using similar bioinformatic methods with a mock community and agricultural soil samples. For all three methods, we found that taxonomic assignments of the mock community at genus and species level matched expectations with minimal deviation from our mock community (74% to 85% Bray-Curtis similarity). Beta diversity of each method produced unique communities, clustered by method. Communities characterized with short reads were more similar to each other than to the MinION long read community. Results were not always consistent across methods when comparing differences between sites. We compare various OTU filtering thresholds to accurately estimate alpha diversity, which was overestimated by all methods. Estimates of alpha diversity and differential abundance were improved with an OTU filtering threshold of 0.07% of sample abundance for MiSeq and MinION data. Compared to established methods, MinION produces output similar to that of MiSeq, suggesting that these techniques are interchangeable.

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Thieringer, Patrick  
Colorado School of Mines  
Poster 47  
Session 1

Title: Metagenomic and pangenomic investigation into microbial diversity and pressures for adaptation in a subsurface serpentinizing environment of Oman

Serpentinization – the hydration and oxidation of ultramafic olivine-rich rock connected to the reduction of water – leaves fluids that are geochemically reduced at hyperalkaline pH with appreciable concentrations of H<sub>2</sub> and CH<sub>4</sub> that can readily serve as electron donors for microbial metabolisms. The Samail Ophiolite in Oman serves as an ideal location for investigating microbial communities at a terrestrial site of low-temperature serpentinization. The spatial resolution of microbial communities has yet to be fully investigated at the Samail Ophiolite, as well as determining what geochemical pressures drive alpha diversity. This study leverages subsurface fluid samples collected from seven previously drilled boreholes ranging from neutral (7.3) to hyperalkaline (11.43) pH. Discrete depth profiles (1m to >200m) were assessed by employing a packer system to confine isolated depth intervals, a submersible pump for open borehole fluid extraction, and an air-tight gas sampler to collect from strict regions within boreholes. Samples were processed for 16S rRNA gene and metagenomic sequencing. We used an oligotyping approach to deconstruct what geochemical pressures drive alpha diversity amongst distinct microbial populations. We employed metagenomes to make use of pangenomic

investigation to evaluate sub-population diversity and how specific organisms adapt to unique conditions in each well to carve out distinct niches. Our study helps to improve understanding the patterns of environmental distribution and genomic diversity of microbial populations within a deep subsurface serpentinizing environment.

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Vega, Michael

Colorado School of Mines

Poster 48

Session 2

Title: Methanotrophs support denitrification over dissimilatory nitrate reduction to ammonia (DNRA) and anammox in a benthic wetland biomat

Competition among denitrifying, DNRA, and anammox bacteria often dictates whether aqueous nitrogen is exported from (denitrification or anammox), or retained within (DNRA), a natural or engineered system. Here, we report how methanotrophs impact the contributions of these processes within a benthic biomat community that colonizes open-water engineered wetlands. In-situ  $^{15}\text{NO}_2^-$  column tracer incubations and genome-resolved metatranscriptomics determined that denitrifiers outcompete DNRA and anammox bacteria, with stable isotopes suggesting denitrification was driven by gross  $\text{NO}_2^-$  regeneration supplied from nitrate reduction or ammonia oxidation. Estimates of nitrate loss suggest that ammonia-derived  $\text{NO}_2^-$  may have comprised as much as 79% of gross  $\text{NO}_2^-$  regeneration. Interestingly, amplicon sequencing and metagenomics identified limited evidence of ammonia-oxidizing bacteria and archaea, and all mapped transcripts encoding for ammonia or methane monooxygenase were assigned to a novel methanotrophic metagenome assembled genome (MAG) of the genus *Methylotetracoccus*, potentially implicating methanotroph co-metabolism – rather than traditional nitrification – as a significant source of ammonia-derived  $\text{NO}_2^-$  regeneration. We observed that this MAG expresses genes encoding for nitrogen assimilation and nitric oxide respiration and hypothesize that the latter is a detoxification mechanism following hydroxylamine oxidation during methanotroph nitrification. Based on metatranscriptomic data, this *Methylotetracoccus* MAG is also the dominant producer of methanol, cross-feeding C1 compounds to methylotrophic denitrifiers, and to a lesser extent, DNRA bacteria. Other methane and nitrogen rich ecosystems, such as natural wetlands, may contain similar niches where methanotrophic bacteria directly cycle nitrogen while cross-feeding reactive nitrogen species and C1 compounds to methylotrophic denitrifiers, ultimately favoring denitrification over DNRA and anammox.

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Vilonen, Leena

Colorado State University

Poster 49

Session 1

Title: Resilience of bacterial communities and limited legacies of fungal communities after a four-year drought in a mesic grassland

The soil microbiome remains largely unstudied, particularly in regards to microbiome response to global change drivers. One such driver, drought, is increasing in intensity and frequency and is expected to intensify with worsening climate change. Further, legacy effects or impacts of drought after drought has subsided could have lasting impacts after drought. Thus, our study

aimed to understand how the soil microbiome responds after drought and whether legacy effects persist post-drought. We measured soil microbial community response post-drought for two years after a four-year experimental drought in a mesic grassland. Our two treatments in the experimental drought either chronically reduced rainfall by 66% or intensely reduced rainfall by completely eliminating rainfall until 45% of annual rainfall was achieved. The bacterial community had no legacies in the first season but showed small positive legacies in the second growing season. In the first and second growing seasons, we found small differences in beta diversity between the control and intense treatment for fungal communities. Further, we found that the two main phyla of fungi Ascomycota and Basidiomycota showed reduced relative abundance post-drought. Both bacteria and fungi showed small legacies, which is likely attributed to the drought resistant nature of grassland microbial communities and the importance of paleoclimate on microbial communities vs. current climate. Overall, these results indicate that microbial community composition shows few legacies to drought post a four-year experimentally-induced drought.

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Von Eggers, Jordan

University of Wyoming

Poster 50

Session 2

Title: Controls on biogeography and assembly of lake sedimentary microbial communities

Microbial communities play a key role in lake ecosystems by performing critical functions including nutrient cycling of terrestrial and aquatic organic matter. Yet, little is known about what controls microbial community assembly in lake sediments, given that they are continually buried into deeper sediment layers, or how vertical organization varies across large spatial scales. Here, we cataloged archaeal and bacterial communities with 16S DNA metabarcoding along the transition from organic, oxic surficial sediments into the low-energy, anoxic zone. We analyzed 46 sediment cores, on average 22 cm deep, from 36 subalpine lakes in Wyoming across a temperature, depth, elevational, and latitudinal gradient. The composition of microbial assemblages varied consistently along a sediment depth gradient, and along a gradient from shallow, warm lakes to deeper, cooler lakes. Geographic location did not appear to contribute to microbial community composition, suggesting dispersal is not limiting community assembly. Preliminary analyses suggest a degree of environmental filtering on microbial communities related to lake temperature in a predictable fashion, which dissipates as the microbes are incorporated into deeper sediments. During the transition to deeper sediments, we observed a successional trend at the phylum-level. There was a rapid loss of Cyanobacteria, Verrucomicrobia, and Bacteroides, followed by a secondary decline in Proteobacteria, along with an increase of Chloroflexi, Planctomycetes, and Miscellaneous Crenarchaeota Group abundances. Understanding the processes that structure sediment communities across space and time may also shed light on past climate conditions, whose signatures may be retained as indicators of environmental change over longer time scales.

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Walecka, Devin

University of Colorado-Denver

Poster 51

Session 1

Title: Characterizing the gut microbiome of golden retrievers using highly-replicated 16S rRNA sequencing

Microbes in the human gastrointestinal tract have shown strong associations with health and disease; however, less is known about the role of the gut microbiome in companion dogs. Golden retrievers are ideally suited as study subjects because they are one of the most common dog breeds in America and have higher rates of obesity compared to other dog breeds. Using 394 fecal samples provided by the Morris Animal Foundation's Golden Retriever Lifetime Study, we characterized the gut microbiome of 224 purebred golden retrievers. To our knowledge, this is one of the largest single-breed cohort in canine microbiome research to date. By leveraging a Course-based Undergraduate Research Experience (CURE), our study also demonstrates the utility of a novel, highly-replicated sampling approach designed to quantify the technical variability inherent in our 16S rRNA sequencing protocol, filtering out low-quality samples. Consistent with other mammals, the canine gut microbiome is predominantly inhabited by phyla Firmicutes and Bacteroidetes (80%). However, the abundances of these two phyla vary greatly from dog to dog, despite the lower genetic diversity of a single-breed cohort. Although constrained by limited variability in cohort body condition score, our high-confidence, replicated community profiling does not support previously reported associations between body condition score and the Firmicutes/Bacteroidetes ratio or body condition score and alpha diversity. This project establishes the range of normal biological variation in gut microbial community across a large cohort of golden retrievers, which is central to future studies examining the link between microbial community, environment, and health and disease.

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Walsh, Corinne

University of Colorado-Boulder

Poster 52

Session 2

Title: Does dirt do the work? Investigating the influence of soil microbes on seed properties

Could the flavor of plant-based foods be influenced by the microbiome of the soil in which those plants are grown? The chemical content and flavor profile of crops is known to be affected by numerous factors including plant variety, harvest time, climate, and soil type. The role of the soil microbiome in determining crop flavor is less well understood. To test the role soil microbes play in determining flavor chemistry, we grew mustard plants in a greenhouse study and manipulated only the microbes applied to the soil. We then characterized the chemical composition of mustard seeds produced to test our hypothesis that plants grown with different soil microbiomes will produce seeds that have distinct chemical profiles. We focused our chemical analysis glucosinolates, a family of spicy-tasting compounds that the mustard plant concentrates in its seeds as defense against insects and microbes. In addition to characterizing the chemical composition of the mustard seeds, we explored how the soil microbiome impacts the seed microbiome through effects on the parent plant. While it is well understood that the soil microbiome plays a large role in determining the rhizosphere and root microbiomes, less is known about the impact of soil microbes on the seed microbiome. To tease apart drivers of the seed microbiome, we explored the relationships between the soil, rhizosphere, root, and seed microbiomes. We hypothesize that the soil microbiome experienced by the parent plant may

contribute to the structure of the seed microbiome, leaving an inheritable legacy of the soil community.

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Weber, Annika

Colorado State University

Poster 53

Session 1

Title: Rice bran in ready-to-use therapeutic foods (RUTFs) for microbiota-targeted treatment of childhood malnutrition

Severe acute malnutrition (SAM) affects more than 14 million children around the world and causes nearly half of all child deaths under the age of five. SAM treatment involves provisions of ready-to-use therapeutic foods (RUTF), which have greatly lowered mortality rates. However, recovered children still have a more immature and dysbiotic gut microbiome than their regionally matched healthy counterparts. Such findings may be related to the recovered child's continued susceptibility to infection and SAM relapse. Emerging attention is placed on repairing the child gut microbiota via consumption of prebiotics. Rice bran in particular is a prebiotic that is nutrient rich and has demonstrated capability to modulate gut microbiota. This notion is now being applied in a clinical trial in Indonesia, where currently more than 2 million children under age five are affected by SAM. Here, 400 children with SAM will be enrolled and treated with a locally produced RUTF, half of which with the addition of 5%-rice bran. We will then characterize the gut microbial and metabolite changes related to this gut targeted RUTF (RUTF + rice bran) in the improvement of SAM treatment. We predict to see microbial changes in align with published literature on the healthy Indonesian child's gut microbiome, such as increased genus level *Prevotella* and *Faecalibacterium*. To explore this objective, we will 1) determine the gut microbiome changes in bacterial diversity/taxonomic shifts and 2) examine shifts in the blood and stool metabolome. This study will yield crucial findings on improved SAM treatment options for sustained recovery.

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Woyda, Reed

Colorado State University

Poster 54

Session 2

Title: Occurrence and persistence of food-borne pathogens in peanut hull-based litter under antibiotic-free broiler chicken production

We conducted an on-farm longitudinal study to evaluate the occurrence and persistence of *Campylobacter* species under a "No Antibiotic Ever (NAE)" production system. The broiler farm was comprised of four broiler houses (> 22, 000 birds/house) and used peanut hulls for bedding material. While many studies have quantified *Campylobacter* presence in poultry litter, no studies have been performed using peanut hulls as a bedding material with litter reuse. Broiler litter samples (n = 288) were collected at the beginning (7 to 10 days after bird placement) and at the end (4-6 weeks after broiler placement) of each grow-out cycle (49 days), for three consecutive flocks. The study resulted in successful isolation of *Campylobacter* species over 3 grow-out cycles with an unequal presence across 4 co-located grow houses on a single farm. Our results indicated that *Campylobacter* species were most prevalent during the first grow-out cycle,

during the late grow-out phase ( $P < 0.001$ ) and within specific grow houses ( $P < 0.05$ ). Additionally, occurrence was more likely at low ( $\leq 79^{\circ}\text{F}$ ,  $P < 0.0001$ ) temperatures, high pH ( $> 7$ ,  $P < 0.05$ ) and at high litter moisture ( $> 25\%$ ,  $P < 0.001$ ). Here we further characterize the genetic diversity of *Campylobacter* species isolated over multiple grow-out cycles and make comparisons of *Campylobacter* occurrence to other commonly used bedding materials. *Campylobacter* abundances were quantified and subjected to antibiotic susceptibility testing and whole genome sequencing. Our results suggest that use of peanut hull-based litter has similar *Campylobacter* occurrence as pine shavings and that gene content is correlated with grow-out cycles.