PURDUE APPLIED MICROBIOME SCIENCES

# Midwest Microbiome Symposium: 2024

## Resilience & Microbial Communities

*A Symposium at Purdue University*

PURDUE.AG/MICROBIOME

**May 13-15, 2024**

*Beck Agricultural Center*
*West Lafayette, Indiana*

PURDUE UNIVERSITY

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## Organizing Committee

### Symposium Organizing Committee

Chair: Caitlin Proctor, Agricultural and Biological Engineering & Environmental and Ecological Engineering
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Leopold Green, Department of Biomedical Engineering
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## Career Panel

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*Biomedit*

Tim Johnson
*Associate Professor*
*Purdue Universiy*

## Trainees Social

### Student & Postdoc Mixer

The Graduate Student & Postdoc Mixer will be hosted at ***Lafayette Brewing Company***, on Monday, May 13 and begins at 7 pm.

Food will be provided. Drinks are available for purchase.

Lafayette Brewing Company
622 Main St
Lafayette, IN 47901

## Symposium Schedule

| MONDAY, MAY 13 | |
|---|---|
| **TIME** | **AGENDA** |
| 12:00 – 12:45 pm | **Registration**<br>*Beck Agricultural Center, West Lafayette, IN* |
| 12:45 – 1:00 pm | **Welcome Remarks**<br>*Karen Plaut, Caitlin Proctor, Purdue University* |
| **SESSION 1** | **Modeling/Measuring Resilience of Microbiomes** Convened by: *Leo Green* |
| 1:00 – 1:25 pm | Ilya Slizovskiy, Purdue University<br>*Advancements in metagenomics for microbiome-wide profiling of genes and genomes of public health importance* |
| 1:25 – 1:45 pm | Annabel Biruete, Purdue University<br>*Dietary fiber, the gut microbiome, and chronic kidney disease-mineral and bone disorder* |
| 1:45 – 2:00 pm | Margaret Thairu, University of Wisconsin-Madison<br>*Behavior, Biology, and Well-being: Investigating changes in the gut microbiome in response to app-based well-being training* |
| 2:00 – 2:15 pm | Yonaida Valentine, The Ohio State University<br>*Circulating inflammation may be central to gut microbiota-mediated neuroinflammation and low emotionality after chemotherapy.* |
| 2:15 – 2:30 pm | Rwivoo Baruah, Purdue University<br>*Understanding the role of molecular weight in dietary fiber utilization by human gut microbiota* |
| 2:30 – 3:00 pm | Coffee & Tea Break |
| 3:00 – 4:00 pm | **KEYNOTE SPEAKER**<br>Paul Jensen, University of Michigan<br>*Exploring phenotypes and genotypes with a robot scientist* |
| 4:00 – 4:15 pm | Suzanne Alvernaz, University of Illinois<br>*Creation and Validation of LIMÓN - Longitudinal Individual Microbial Omics Networks* |
| 4:15 – 4:30 pm | Mariana Guzmán Sánchez, Purdue University<br>*Insights into Gut Microbial Ecology: Modeling Interactions in Inulin Fermentation Communities* |
| 4:30 – 6:00 pm | **Poster Session 1 and Reception** |
| 7:00 pm | Trainee Social<br>*Lafayette Brewing Company, 622 Main Street* |

## Symposium Schedule *continued*

| TUESDAY, MAY 14 | |
|---|---|
| **TIME** | **AGENDA** |
| 8:45-9:00 am | **Welcome Remarks -** *Bernard Engel, Purdue University* |
| **SESSION 2** | **Ecosystem Resilience** Convened by: *Laramy Enders* |
| 9:00-10:00 am | **KEYNOTE SPEAKER -** Adina Howe, Iowa State<br>*Midwest Microbiomes: From Rump to Runoff* |
| 10:00-10:30 am | Esther Ngumbi, University of Illinois, Urbanna-Champaign<br>*Submerged in Stressors: How flooding, herbivory, and their interaction alter soil microbial communities and plant chemistry* |
| 10:30-11:00 am | Coffee & Tea Break |
| 11:00-11:15 am | Jacob Adler, Purdue University<br>*Rain Garden Soil Microbiome Biodiversity at Purdue University* |
| 11:15-11:30 am | James Riddell, The Ohio State University<br>*Methane-suppressing treatments greatly alter viral community activity in a thawing permafrost soil bioreactor* |
| 11:30-11:45 am | Daniel Raudabaugh, Purdue University<br>*Employment of a novel protocol to understand the influence of earlier colonizers on the fungal community composition and structure of rock faces* |
| 11:45-12:00 pm | Bridget Hegarty, Case Western Reserve University<br>*A Snapshot of Drinking Water Distribution System Phage Communities* |
| 12:00-1:30 pm | **LUNCH** (Provided) |
| **SESSION 3** | **Resilience in One Health** Convened by: *Patricia Wolf* |
| 1:30-2:30 pm | **KEYNOTE SPEAKER -** Purna Kashyap, Mayo Clinic<br>*Hiding in plain sight: Biofilms as a mechanism by which pathogens persist in the gut* |
| 2:30-2:45 pm | Tingting Ju, Purdue University<br>*Missing microbes: Characterizing gut microbial communities in production animals* |
| 2:45-3:00 pm | Kris Martens, The Ohio State University<br>*Metagenomic sequencing detects injury-specific changes to the microbiome 30 days following traumatic brain injury in rodents* |
| 3:00 – 3:30 pm | Coffee & Tea Break |
| 3:30-4:00 pm | Maria Elisa Caetano-Silva, University of Illinois<br>*Psychological stress-induced disruptions to intestinal mucosal compartments and the microbiome: Insights into stress pathways* |
| 4:00-4:15 pm | Robert Glowacki, Cleveland Clinic<br>*Identification of genes and physiological factors that mediate strain-level variation in biofilm formation among Bacteroides thetaiotaomicron isolates* |
| 4:15-4:30 pm | Pallavi Singh, Northern Illinois University<br>*One-health approach to unravel the gastrointestinal microbial ecology and enteric pathogen shedding in bison* |
| 4:30 - 6:00 pm | **Poster Session 2 and Reception** |

## Symposium Schedule *continued*

| WEDNESDAY, MAY 15 | |
|---|---|
| **TIME** | **AGENDA** |
| 8:45-9:00 am | **Welcome Remarks** |
| **SESSION 4** | **Engineering Microbiomes** Convened by: *Caitlin Proctor* |
| 9:00-9:30 am | Karen Dannemiller, The Ohio State University<br>*Utilizing microbial resilience in indoor spaces for improved detection of markers associated with human health outcomes* |
| 9:30-9:45 am | Maysa Niazy, University of Guelph, Canada<br>*Towards high-throughput microbial diversity analysis: Application of the latent variable modeling approach to investigate vaginal microbiota communities in sows* |
| 9:45-10:00 am | Anna Clapp Organski, Purdue University<br>*Oral contraceptive usage impacts exercise-induced changes in the gut microbiota* |
| 10:00-10:15 am | Xinran Zhang, University of Michigan<br>*Medium chain carboxylic acid recovery from acid whey using anaerobic dynamic membrane bioreactor* |
| 10:15-10:30 am | Zachary Liechty, Air Force Research Lab<br>*Shigellosis results in persistent changes to the gut microbiome* |
| 10:30-11:00 am | Coffee & Tea Break |
| 11:00-12:00 am | **KEYNOTE SPEAKER**<br>Ameet Pinto, Georgia Institute of Technology<br>*Towards microbiome inclusive drinking water management* |
| 12:00-1:30 pm | **LUNCH** (provided) & trainee career panel |
| 1:30-2:00 pm | **Poster Highlights**<br>*Short highlights of standout posters, to be selected* |
| 2:00-2:15 pm | **Poster Awards Ceremony and Closing Remarks**<br>*Steve Lindemann* |

## KEYNOTES:

### Paul Jensen

Paul Jensen combines artificial intelligence, laboratory automation, and high-throughput genomics to built robot scientists that answer biological questions. He leads a research group at the University of Michigan that has performed over one million autonomous experiments in synthetic biology and oral microbiology. Paul trained as an engineer and microbiologist at the University of Minnesota, the University of Virginia, and Boston College. He is an ASQ Certified Quality Engineer and co-founder of the biotech company Cerillo, Inc.

Our lab website is: http://jensenlab.net

Title: *Exploring phenotypes and genotypes with a robot scientist*

### Adina Howe

Dr. Howe leads the *Genomics and Environmental Research in Microbial Systems* (GERMS) Laboratory. Her research goals are to help understand and manage the impacts of microbiology as we continuously change the environment that we live in. The GERMS team research projects include the production, resilience, and safety of food, energy, and water resources; the impacts of land management strategies; the connection of environmental and animal microbiomes; and the large-scale detection of biomarkers for environmental health. Dr. Howe is in the Department of Agricultural and Biosystems Engineering at Iowa State University and is also a part of Iowa State University's Environmental Science Program, Interdepartmental Microbiology Program, and Bioinformatics and Computational Biology Program. The GERMS Lab is currently supported by the USDA National Institute of Food and Agriculture; the National Science Foundation; and the U.S. Department of Energy, Office of Biological and Environmental Research. In her free time, she enjoys playing bottom-tier city volleyball and board games and hiking with her family including two goofy dogs.

Website: https://www.germslab.org/

Title: *Midwest Microbiomes: From Rump to Runoff*

### Ameet J. Pinto

Dr Ameet Pinto is an Environmental Engineer and Carlton S Wilder Associate Professor in Civil and Environmental Engineering at Georgia Institute of Technology (Georgia Tech). Ameet is a Chemical Engineer from Institute of Chemical Technology (University of Mumbai) with post-graduate degrees in Environmental Engineering from the University of Alaska (2005) and Virginia Tech, USA (2009). Prior to joining Georgia Tech in 2021, Ameet was an Assistant Professor at Northeastern University and Lecturer/Senior Lecturer at the University of Glasgow.

## KEYNOTES:

*Ameet J. Pinto, continued*

Their research has received support through prestigious grants and awards like EPSRC's Bright IDEAS Award in 2015, the NSF CAREER and ISME/IWA Rising Star Awards in 2018, 2019 Paul L Busch Award for Innovation in Applied Water Quality Research and the IWA MEWE Mid-Career Award in 2023. Their research focuses on the development and application of state-of-the-art molecular and modelling tools to monitor and manage the microbiology of drinking water systems to improve the sustainability of treatment processes and enhance the safety and security of drinking water.

Website: www.pintolab.com

Title: *Towards Microbiome Inclusive Drinking Water Management*

### Purna C Kashyap

Dr. Purna Kashyap is practicing gastroenterologist and Professor of Medicine and Physiology, the Bernard and Edith Waterman Director of the Microbiome program, and Director of the germ-free mouse facility in the Center for Individualized Medicine at Mayo Clinic, Rochester, MN. The NIH funded Gut Microbiome laboratory led by Dr. Kashyap is focused on delineating the complex interactions between diet, gut microbiome, and host gastrointestinal physiology. The laboratory uses germ-free mouse models in conjunction with measures of gastrointestinal physiology in vitro and in vivo to investigate effects of gut microbial products on host gastrointestinal function. In parallel, they use a systems approach incorporating multi-omics, patient metadata, and physiologic tissue responses in human studies, to aid in discovery of novel microbial drivers of disease. The overall goal of the program is to develop novel microbiota-targeted therapies. Dr. Kashyap has published nearly 100 peer reviewed articles including journals like Cell, Cell Host Microbe, Science Translational Medicine, Nature Communications, and Gastroenterology. He was inducted to American Society of Clinical Investigation in 2021. He has previously served on the scientific advisory board of American Gastroenterology Association Gut Microbiome Center, and on the council of American Neurogastroenterology and Motility Society. He now serves on the council and the research committee of AGA, in an editorial role for Gut Microbes and as an ad hoc reviewer on NIH study sections.

Title: *Hiding in plain sight: Biofilms as a mechanism by which pathogens persist in the gut*

## Other Titles:

### *Advancements in metagenomics for microbiome-wide profiling of genes and genomes of public health importance*

Ilya Slizovskiy *
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Metagenomic DNA sequencing enables the culture-free profiling of bacteria and their genes within microbiomes. However, implementation of metagenomic workflows for detection and characterization of antimicrobial resistance genes (i.e. resistomes) and pathogens in regulatory surveillance systems is limited, owing to the low sequencing sensitivity for clinically meaningful metagenomic detail relative to the background off-target microbiota and host DNA. Targeted metagenomic sequencing methods are gaining popularity for their advantages in per-base cost relative to the increased public health and context-specific information gain. We introduce and review advantages, limitations, and opportunities of novel targeted metagenomic sequencing methods, including: **t**arget-**e**nriched **l**ong-read **seq**uencing (*TELSeq*) and **m**etagenomic **t**arget **a**mplified **g**enome sequencing (*mTAG-seq*). These novel metagenomic workflows will be discussed in relation to their human, animal, environmental, and food safety applications. It is anticipated that scalable library construction, rapid runtime, and information-rich data afforded by further improvements in these sequencing modalities extends the application of metagenomics to the public health landscape.

### *Dietary fiber, the gut microbiome, and chronic kidney disease-mineral and bone disorder*

Annabel Biruete*
Department of Nutrition Science, Purdue
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Chronic kidney disease (CKD) affects 15% of the United States population. As the kidneys are the master regulators of homeostasis, in advanced CKD, virtually every organ and system is affected. CKD-mineral and bone disorder (CKD-MBD) is a highly prevalent systemic disorder characterized by biochemical abnormalities, bone abnormalities, and extraskeletal calcifications. Current treatments have focused on limiting dietary phosphorus intake, intestinal phosphate binders, and therapies that lower parathyroid hormone (PTH). Despite these treatments, high levels of circulating phosphorus and PTH remain prevalent in people with kidney failure; thus, innovative therapies are of interest. Dietary fiber may impact the three hallmarks of CKD-MBD, via mechanisms dependent and independent of the gut microbiome. However, the effects of dietary fiber may depend upon the fiber's physicochemical and functional properties, including viscosity and fermentability. We will discuss the beneficial effects of supplementing the prebiotic fiber inulin in a model of slowly progressive CKD-MBD and preliminary data on the impact of four dietary fibers based on fermentability and viscosity. Ultimately, dietary fiber may represent a low-cost intervention to improve CKD-MBD.

## Other Titles: *continued*

### *Behavior, Biology, and Well-being: Investigating changes in the gut microbiome in response to app-based well-being training*

Margaret Thairu*, Kris Sankaran, Simon Goldberg, Richie Davidson, Jo Handelsman, Wisconsin Institute of Discovery, University of Wisconsin-Madison, Madison, WI.
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In recent years there has been an increase of people suffering from various mental health disorders and a decreased sense of well-being exacerbated by the COVID-19 pandemic. Depression is leading the way (with an anxiety comorbidity rate of ~68%) with a prediction to be the leading cause of disability by 2030. Current treatments and interventions available to help manage mental health disorders, such as depression and anxiety, fail to provide benefit to a large proportion of the population indicating the need for novel interventions to treat these mental health conditions. Studies have found that mindfulness-based interventions can be as effective as pharmacological and/or psychological interventions in reducing symptoms of various physical and psychological disorders such as anxiety, depression, stress, and chronic pain. Mindfulness training has the added benefit of being highly accessible. However, the biological mechanisms by which mindfulness helps increase overall well-being are not well understood. Neuropsychiatric disorders such as anxiety and depression are associated with changes in the gut microbiome, highlighting the need to disentangle mechanisms that mediate interactions between our gut and brain. To begin unraveling the mechanisms that drive the interactions between gut microbiome and brain we embarked on the Behavior, Biology, and Well-being (BeWell) study, a fully remote randomized controlled trial, focused on testing the effects of a meditation-based smartphone app (Healthy Minds Program) on depression symptoms and the microbiome. Participants recircuited from across the country with elevated symptoms of depression received the Healthy Minds Program (HMP) app with or without meditation practice included (i.e., active control) or to a usual care control condition. Stool samples were collected at baseline and 3-month follow-up. Though currently ongoing, preliminary results have found that full HMP app and the active control influences microbiome composition and they improve depression symptoms. These results suggest there is a dynamic interplay between mental training, behaviors, and the gut microbiome, that can lead to improved health phenotypes. Based on these preliminary results we plan to expand our study to interrogate the mechanistic microbial drivers of improved well-being that arise as a result of mindfulness practice. If we can dissect the factors that mediate the microbiome and mood, then we can find ways to create synergistic treatments for psychiatric disorders.

### *Circulating inflammation may be central to gut microbiota-mediated neuroinflammation and low emotionality after chemotherapy*

Yonaida Valentine*, Audrey Duff, Lindsay Strehle, Lauren Otto, Melina Seng, Michael Bailey, Leah M Pyter.
Institute for Behavioral Medicine Research, The Ohio State University, Columbus, OH.
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Nearly 500,000 U.S. cancer patients receive chemotherapy treatment yearly. In the U.S., up to 40% of breast cancer survivors report increased depressive symptoms and up to 70% of breast cancer patients report increased anxiety symptoms. Our lab has previously demonstrated that chemotherapy administration in mice induces gut dysbiosis, inflammation, and behavioral deficits.

## Other Titles: *continued*

We have also shown that the chemotherapy-induced changes to the gut microbiota alone, causes anxiety-like behavior and neuroinflammation. However, the mechanisms by which the chemotherapy-transformed gut microbiota, mediates changes in the brain and behavior are not well understood and are rarely studied in a clinically-relevant manner. Here, we used neurodevelopmentally and immunologically healthy conventional mice to test the hypothesis that gut microbiota transplanted from chemotherapy-treated mice humorally activate microglia to induce neuroinflammation, and anxiety- and depressive-like behaviors in recipients. First, female C57/Bl6J mice received an antibiotic pre-treatment to knockdown commensal gut bacteria before receiving intra-gastric transplants of gut contents from mice that were directly injected with paclitaxel chemotherapy (30mg/kg i.p. for 6 doses every other day) or vehicle treatment (Veh-GMT or Chemo-GMT). Mice were then assessed for several chemotherapy-induced behavioral deficits or microglia were collected. In separate cohorts, brain tissues were collected to assess region-specific neuroimmune morphological and gene expression changes. Fecal samples were collected throughout for 16S rRNA sequencing of the gut microbiome. Preliminary data indicate our GMT paradigm effectively manipulated the gut microbiome. Chemo-GMT increased expression of inflammatory mediators in the blood (CCL2, TNFa); increased neuroinflammation in the hippocampus (Tnfa, Nlrp3); and increased microglia expression of Tnfa. These effects coincided with increased depressive-like behaviors compared to Veh-GMT controls. Analysis of the morphological difference in microglia induced by Chemo-GMT are ongoing. Together, these results suggest that the chemotherapy-transformed gut microbiota may mediate depressive-like behavior through increased circulating inflammation and an altered microglia profile. Thus, microbiota-targeted interventions for chemotherapy-associated behavioral side effects should target these markers in the blood and brain.

### *Understanding the role of molecular weight in dietary fiber utilization by human gut microbiota*

Rwivoo Baruah*, Tianming Yao and Stephen R. Lindemann
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Dietary fibers are widely regarded to modulate the human gut microbiome. The ability of dietary fibers to reshape the gut microbiome is influenced by several factors such as concentration, monosaccharide composition, branching type/ratio and molecular weight. The microbial members most commonly observed in otherwise-identical fermentations of polysaccharides varying in molecular weight differ due to involvement of different carbohydrate-active enzymes. To study this phenomenon, we used the microbial alpha-glucan (dextran) produced by the lactic acid bacterium Weissella cibaria as a model dietary fiber. Dextran was produced in vitro using sucrose as a substrate and dextransucrase purified from W. cibaria DSM 14295. The molecular weight of purified dextran was in the range of 1 x 10^7 Da with ~96% (1-6) and ~4% (1-3) glucose linkages. To obtain dextran of different molecular weights, enzymatic and acid hydrolysis methods were employed. Use of enzyme dextranase on dextran from Weissella cibaria DSM 14295 in optimized conditions yielded lower molecular weight dextran of ~2.4 x 10^3 Da, and acid hydrolysis of dextran yielded lower molecular weight dextran in the range of ~1 x10^4- 1 x 10^5 Da. We fermented these dextrans of varying molecular weights in sequential fecal fermentation for up to 7 passages, allowing us to understand the restructuring of the gut microbial community on the basis of fiber molecular weight and its influences on production of key metabolites, short-chain fatty acids (SCFAs).

## Other Titles: *continued*

### *Creation and Validation of LIMÓN - Longitudinal Individual Microbial Omics Networks*

Suzanne Alvernaz*, Beatriz Peñalver Bernabé
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Microbial communities continually adapt to their surrounding environment. Such communities play pivotal roles in countless ecosystems from environmental to human health. Perturbations of these communities have been linked to a myriad of diseases, including mental health and neurological disorders, Chron's disease and cancer. The function of each individual microorganism depends on their interactions with other members of their community. Furthermore, these multispecies ecosystems are dynamic making it essential to have robust methods to understand how alterations in their environment effect interactions among community members. Existing methods for identifying temporal microbial adaptations have been focused on abundance variations in individual taxa, leaving a crucial underexplored area of how microbial interactions evolve overtime and how dynamic microbial networks could be specific to each of the entities from which repeated measurements are taken from (e.g., different mice or individuals, distinct spatial locations). Doing so would require novel statistical approaches to handle the complicated nature of compositional repeated count data. To address this gap in knowledge, we have developed a pipeline, LIMÓN – Longitudinal Individual Microbial Omics Networks. This novel platform includes methods to (1) handle dynamic temporal data, (2) account for sample covariates, and (3) identify sample-specific network characteristics from the overall aggregate network across time. In LIMÓN, longitudinal count data compositionality is removed using a zero-inflated negative binomial linear mixed model. Our approach allows users to perform sample covariate correction at this step to remove confounding effects, such as subject age or weight which is often necessary in human studies. Covariate corrected microbial values are extracted and undergo centered-log ratio normalization and network inference with Sparse inverse covariance Estimation for Ecological Association Inference (SPIEC-EASI) for each specified time point. Finally, individual networks from the overall aggregate network at each time point are estimated using Linear Interpolation to Obtain Network Estimates for Single Samples (LIONNESS). This provides sample specific networks that users can leverage to evaluate how individual network topology proprieties change over time (e.g., number of nodes, edges, communities, centrality characteristics). Thus, LIMON provides a unique platform to reveal the heterogenous evolving relationships between microbial network properties and sample features of interest overtime.

### *Insights into Gut Microbial Ecology: Modeling Interactions in Inulin Fermentation Communities*

Mariana Guzmán Sánchez*, Stephen Lindemann, Rubesh Raja
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The gut microbiome, a complex ecosystem, undergoes structural and functional changes in response to environmental variations. However, the empirical complexity of microbial communities poses challenges that traditional in vivo and in vitro approaches struggle to address alone. In this study, we aimed to mathematically model the degradation of inulin by gut microbial communities, focusing on a community comprising *Klebsiella pneumoniae*, *Escherichia coli*, and *Bifidobacterium*

## Other Titles: *continued*

*dentium*. We propose a cybernetic modeling approach, which considers overall regulation and control mechanisms within the ecosystem. Batch monocultures under anaerobic conditions were conducted using fortified buffer supplemented with one of the following carbon sources -glucose, fructose, sucrose, kestose- at 0.4% concentrations. Cultures were incubated for 8 to 12 hours at 37ºC, with samples collected at 1-hour intervals. Bacterial growth was assessed by measuring optical density at 600 nm, while microbial biomass was determined by dry weight through traditional oven drying. Substrate concentration was analyzed using High-Performance Liquid Chromatography (HPLC). These experiments provided growth parameters for each strain, subsequently incorporated into the model to account for biomass and substrate concentrations. The model demonstrated accurate fits with experimental data, enabling simulations of microbial interactions within the community. These findings offer valuable insights into the dynamics of gut microbial communities and have implications for understanding their ecological functions and responses to environmental stimuli.

### *Submerged in Stressors: How flooding, herbivory, and their interaction alter soil microbial communities and plant chemistry*

Esther Ngumbi, University of Illinois, Urbanna-Champaign
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Record-breaking flooding events have increased across North America with detrimental impacts on agricultural plants, crop productivity, and environmental sustainability. In nature, flooding can happen concurrently with other stressors. To date, our understanding of how flooding impacts plant defense and stress mitigation strategies remains limited. Further, we lack a clear understanding of how soil management history shapes flooding-driven impacts. Motivated by forecasts that project more frequent and severe flooding events in the future, this study was conducted to examine how flooding, insect herbivory, and their combination impact soil microbial communities that underpin plant health and volatile organic compounds that mediate ecological interactions among plant associating communities. We characterized tomato plant headspace volatile emissions and the dynamic changes in soil microbial communities in flooded and non-flooded plants with and without herbivory by Manduca sexta. Our results revealed that belowground soil microbial communities and aboveground plant emitted VOCs were altered by flooding and the stress combination of flooding and insect herbivory. Soil management history was the dominant driver dictating microbial response to flooding. Notably, flooding decreased the abundance of beneficial and keystone microbial taxa group. Additionally, flooding increased the abundance of pathogenic fungal communities. Taken together, our findings suggest that the simultaneous exposure of tomato plants to flooding and insect herbivory profoundly changes below and aboveground plant defense and stress mitigation strategies with potential consequences for microbe facilitated and chemically mediated plant-insect interactions.

## Other Titles: *continued*

### *Rain Garden Soil Microbiome Biodiversity at Purdue University*

Jacob Adler*
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Diverse garden soil microbiomes are vital for cycling nutrients which improve plant health and production yield. Further, specific bacteria are beneficial for hydrocarbon pollutant removal and to assist with water quality. Also, the diverse bacterial species within the soil microbiome can help reduce common pollutants in combined sewer overflow events. Purdue University and the West Lafayette, IN community has been especially interested in reducing common pollutants and these overflow events. To that effect, Purdue University has increased the number and diversity of types of rain gardens on campus over the past 20 years. This project studied the impact the various rain garden developments at Purdue University has had on the soil's microbiome. Soil samples for various sites were collected from Purdue University in January 2024. The geolocation of the sites was obtained as well as the age and stage of the sites. We examined the soil's functional biodiversity using carbon source utilization analysis (EcoPlate). Results were used to calculate Shannon Diversity Index, Evenness, and Richness values. Further, genetic biodiversity of the soil's bacteria species was also determined by genomic DNA extraction followed by 16S ribosomal RNA sequencing. Sequences were processed and analyzed with Nephele DADA2 and QIIME2.0 pipelines. Finally, the soil moisture content and pH were evaluated, as they have been shown to impact soil microbial biodiversity. Here we report data on various stages of rain garden development. It is important to continue to monitor the effects of green landscape design and rain gardens so that we can discover if there are possible benefits of providing these spaces for soil microbiomes and water quality in our community.

### *Methane-suppressing treatments greatly alter viral community activity in a thawing permafrost soil bioreactor*

James Riddell*, Bridget McGivern, John Bouranis, Sophie Jurgensen, Kayla Borton, Ami Fofana, Laura Schaerer, Jared Ellenbogen, Matthew Sullivan, Kelly Wrighton, Malak Tfaily, Microbiology, The Ohio State University, Columbus, OH,
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As human activities warm Earth, microbes are metabolizing increasingly available organic carbon in near-surface thawing permafrost soils into carbon dioxide (CO2) and methane, further contributing to planetary warming. We tested inhibitors of methanogenesis in a bioreactor model system derived from thawed permafrost soil samples and found catechin, a flavonoid subunit of tannin, reduced methane emissions by 80%, as well as the transcription of key microbes related to methanogenesis. Microbial community metabolism is modulated by viruses, where they influence their hosts through mortality and metabolic reprogramming. Here, I will determine the impact of catechin treatment on the transcriptional abundance of bioreactor viruses.

We recovered 5,500 species-level viral populations by mining bioreactor metagenomic assemblies. 3,700 of these viruses were detected in at least one metatranscriptomic sample out of the 45 samples taken over the 35-day experiment. 55% of these viruses were differentially abundant in the catechin treatment compared to the control, suggesting catechin has a significant impact on the bioreactor viral community activity. Genus-level host predictions could be assigned to 64%

## Other Titles: *continued*

of the transcriptionally detected viruses using machine-learning based virus-host prediction algorithms. 38 viruses were predicted to infect five methanogen hosts. Many of these viruses had significantly lower transcriptional abundances in the catechin and condensed tannin treatments, but we also saw instances where viruses were enriched in catechin and condensed tannin treatments, and observed trends in viral transcriptional abundance that significantly differed from their predicted hosts. Lastly, ecological statistics revealed viral community composition clustered by treatment group, and not by sampling time point.

This ecogenomics study investigates how a model bioreactor viral community changes in response to a methane-suppressing compound. As we begin exploring geoengineering methods to reduce methane emissions from thawing permafrost, we need to evaluate unintended consequences to the permafrost microbiome, and viruses are a critical group that needs to be studied because they greatly influence microbial community composition and metabolism.

### *Employment of a novel protocol to understand the influence of earlier colonizers on the fungal community composition and structure of rock faces*

Daniel B. Raudabaugh *, M. Catherine Aime
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To characterize the community composition and structure of microbiomes, researchers use high throughput sequencing technology, which generates short sequences. These short sequences are then compared against large databases to determine the identity of those sequences. Unfortunately, the short sequences are insufficient to identify many species, resulting in a large percentage of unclassified sequences. Therefore, we have designed a new protocol to increase the sequence length, reduce the number of unclassified sequences, and increase confidence in taxonomic determination. To test this new protocol, we chose to examine the mycobiome of earlier colonizers of rock faces, which consisted of lichens, mosses, and rock ferns. For this study, we hypothesized that the fungal community composition and structure of rock faces are influenced more by the community assemblage of the early rock colonizers than by environmental abiotic factors. Therefore, we collected moss (*Dicranum* sp.), lichen (*Cladonia* sp.), and rock ferns (*Polypodium virginianum*) from two adjacent rock faces in West Virginia. Both rock faces contained the same species, but one was more recently colonized compared to the other, based on the maturity and size of the earlier colonizers. Samples were divided into moss-underlying substrate, fern root-underlying substrate, fern leaf, and lichen-underlying substrate, resulting in three technical replicates per sample or a total of 24 samples, plus one control. DNA was extracted from 0.5 g of each sample using the Omega E. Z.N.A. soil DNA kit, followed by PCR and library preparation. Sequencing was performed on an Illumina Miseq with 2x 250 bp paired-end sequencing, and the resulting files were processed using DADA2 and clustered into OTUs at 97% sequence similarity using Decipher. Taxonomy was assigned based on sequence similarity against the NCBI database. In total, 2986 OTUs were identified, of which 1071 were fungi. The top five most abundant species were two *Taphrina* spp., a *Mortierella* sp., and two Chytridiomycota species. The presence of fungal species was statistically significant ($p = 0.001$, $r2 = 0.38$) based on colonization age; host/tissue type was also statistically significant ($p = 0.001$, $r2 = 0.51$); and host tissue type combined with site age was statistically significant ($p = 0.001$, $r2 = 0.9525$). These results suggest that the composition of the earlier colonizers was more important than abiotic factors

## Other Titles: *continued*

in determining community composition. Interestingly, the dominant fungal genera appeared to be unique to this environment, prompting future exploration of additional sites. In addition, the new protocol was successful in reducing the number of unclassified sequences by 50% overall. Consequently, there were only 20 fungal sequences that could not be identified to the phylum level, resulting in less than 2% unclassified sequences for this group. Therefore, the utility of this protocol will be further evaluated and offers researchers another valuable tool in studying fungal microbiomes.

### *A Snapshot of Drinking Water Distribution System Phage Communities*

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In drinking water distribution systems, the impacts of viruses on drinking water quality and human health are increasingly recognized. Beyond the direct impacts of viral pathogens, bacteriophages' ("phages", or viruses that infect bacteria) may impact water quality in other less direct ways by influencing the proliferation of bacteria that can lead to human disease, nitrification, and corrosion. In this presentation, I will present my work investigating the effects of phages on drinking water quality. I will begin by sharing recent work of mine that provided the first glimpses of how phage diversity varies in drinking water distribution systems, demonstrating that distribution system water chemistry influences drinking water richness and evenness. Distribution systems with residual disinfectant had fewer viral populations (p-value = 3.0*10-6) and less even viral community structures (p-value=0.001) than distribution systems without residual disinfectant. I further found that drinking water phages carry genes relevant for surviving the specific stresses of drinking water distribution systems (e.g., oxidative stress and nitrogen limitation). However, this work focused on genomes mined from publicly available metagenomes from studies querying the bacterial community. Therefore, the sequenced viruses may be biased towards particle associated viruses, those actively infecting their host, and free viruses trapped by the filter. To more comprehensively study the viral community, it is necessary to design new studies using filters small enough to collect viral particles.

In the second half of the presentation, I will share the next steps of this work: namely, the results from a recent sampling campaign specifically designed to address this gap in previous studies. The efficacy of drinking water concentration was assessed with virus spike-and-recovery experiments. After comparison, it was determined that filter choice significantly influenced viral recovery of the spike, with the highest recovery found using the InnovaPrep ultrafilter concentrating pipettes. Samples were also taken using the Sterivex 0.2 µm pressure filters commonly used for studying drinking water bacterial communities, allowing for comparison of the viral communities revealed through previous studies to our improved method. All samples were metagenomically sequenced using a combined long and short read approach, allowing for the creation of MAGs and identification of viral populations. These sequences were then run through a new viral identification tool, VHIP, to predict viral-host interaction networks. In this presentation, we will present these networks with a particular focus on Mycobacteria and other opportunistic pathogens.

## Other Titles: *continued*

In sum, this research demonstrates that viral assemblages are diverse across drinking water systems and shaped by treatment choices, expands our understanding of phage-host interactions, and highlights the need for further research efforts to characterize the influence viruses have on drinking water distribution system microbiology.

### *Missing microbes: Characterizing gut microbial communities in production animals*

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The gut microbiota is involved in various vital physiological processes for the host, with its composition heavily influenced by factors like diet and environment. Understanding the dynamics and patterns of the gut microbiota and the underlying factors shaping microbial communities in production animals holds promise for targeted interventions aimed at manipulating the gut microbiome to achieve beneficial health outcomes. We hypothesized that the practice used in the intensive livestock production system can potentially minimize the exposure of animals to beneficial commensal bacteria. Employing 16S rRNA gene amplicon sequencing, we characterized the gut microbiota of broiler chickens raised in different production environments. Additionally, we investigated the difference in the gut microbiota between wild and domestic pigs to provide further insights into the effects of domestication. We observed notable distinctions in the gut microbiota of broiler chickens between those raised in intensive versus extensive production systems. Specifically, we identified a collection of bacterial taxa prevalent in extensively raised birds that were either absent or diminished in birds from the intensive production settings. Notably, taxa such as Bacteroides contributed to improved microbial functionalities in breaking down complex polysaccharides and producing short-chain fatty acids. Conversely, bacterial taxa such as Enterobacteriaceae and Campylobacter jejuni were found to be significantly more abundant in birds from the intensive production environment. Interestingly, contrasting patterns were observed when comparing gut microbial community structures between domestic and wild pigs with domestic pigs exhibited higher alpha-diversity indices in their gut microbiota. However, wild pigs consistently harbored more bacterial genera known to degrade fibers, while the gut microbiota of domestic pigs exhibited elevated levels of potentially pathogenic genera such as Campylobacter, Fusobacterium, and Escherichia-Shigella. These disparities in the gut microbiota suggest an impact of current rearing practices on the establishment and evolution of gut microbial communities in production animals. Furthermore, we investigated potential management strategies, including barn sanitation methods, using a poultry model to explore effective approaches for inhibiting the colonization of zoonotic pathogens such as C. jejuni in the gut.

### *Metagenomic sequencing detects injury-specific changes to the microbiome 30 days following traumatic brain injury in rodents*

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Traumatic brain injury (TBI) causes cognitive impairment, increases risk for psychiatric disease, and exacerbates related symptoms such as risky decision-making and impulsivity. Impaired monoamine neurotransmission is a likely contributor to such symptoms, with serotonin signaling contributing to anxiety and mood-related disorders, impulsive dysfunction, and impaired decision-making. Despite

## Other Titles: *continued*

this knowledge, precisely why these systems are vulnerable to TBI is unknown. However, emerging data indicate a role for the gut microbiome. Gut dysbiosis occurs rapidly after TBI and may persist for years in patients.

In a previous study, our lab manipulated the microbiome of rodents using antibiotic dysbiosis. We then assessed function on the Rodent Gambling Task, a clinically relevant assessment of impulsivity and decision-making. The findings from the study showed a delay in the onset of TBI symptoms in the antibiotic cocktail group pointing to a potential causal role for the gut microbiome in psychiatric disease following TBI. 16S amplicon-based sequencing identified broad changes in the microbiome but could not identify species-level information and injury-specific differences resolved by 14 days post injury. To better understand the mechanisms at play, we performed metagenomic shotgun sequencing. From these data, we were able to construct bacterial metagenome-assembled genomes (MAGs) to determine changes occurring at key time points post injury and at the species level. The results of this study showed that TBI and antibiotics differentially affected the prevalence of multiple MAGs. These differences persisted 30 days following injury. Of particular interest to our lab, sequencing identified several MAGs associated with behavioral performance even after accounting for manipulations of TBI and antibiotics identifying potential therapeutic targets in the gut.

### *Psychological stress-induced disruptions to intestinal mucosal compartments and the microbiome: Insights into stress pathways*

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Inflammatory bowel disease (IBD) is a chronic inflammation of the gastrointestinal tract that poses a significant challenge due to the complex nature of its etiology and the limited understanding of the role of environmental factors, such as psychological stress, in its development. While genetic factors in IBD have been extensively studied, the mechanisms underlying the impact of stress on the disease remain elusive. Interactions between gut microbiota and abnormal immune responses is thought to be central, yet the specific pathways involved are not fully understood. This talk will explore how psychological stress modifies gut epithelial-microbiome interactions at the mucosal interface and the underlying signaling pathways. Our study, using a murine social disruption stress (SDR) model, reveals significant changes in the transcriptomic profile of intestinal epithelial cells (IECs) upon stress, particularly upregulation of ROS/RNS signaling. Interestingly, blockade of β-adrenergic receptor (AR) prevented these changes, suggesting a key role for β-AR in mediating stress effects, despite IECs not expressing β-AR. The transcriptomic profile of the gut immune cells, i.e. intraepithelial lymphocytes (IELs), also changed dramatically with stress but returned to baseline upon stress + β-AR blockade. Furthermore, stress affected host-microbiome interactions, with β-AR blockade rescuing the stress-induced decrease in alpha diversity and relative abundance of stress-sensitive bacterial taxa. These findings suggest that psychological stress disrupts epithelial physiology, and key gut immune cells, expressing β-AR, directly interface with IECs and the gut microbiota. Understanding how stress-induced modification of IECs, IELs, and

## Other Titles: *continued*

the gut microbiota leads to mucosal disruption and IBD predisposition is crucial for developing targeted interventions.

Keywords: Psychological stress, ROS signaling, host-microbiome interactions, inflammatory bowel disease, intestinal epithelial cells, immune response.

Supported by NIH R01DK131133-01.

### *Identification of genes and physiological factors that mediate strain-level variation in biofilm formation among Bacteroides thetaiotaomicron isolates.*

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The gut microbiome is subject to myriad insults that threaten their fitness in the intestine, prominent among them being inflammation. Despite this, using a gnotobiotic model of inflammatory bowel disease we found that commensals like Bacteroides thetaiotaomicron retained fitness in the inflamed intestine, suggesting an evolution of strategies potentiating their resilience to inflammatory stressors. However, the mechanisms responsible have remained ill-defined. Using a panel of 24 different Bt isolates, we have tested resistance to reactive oxygen species and biofilm-forming capacity, strategies that provide resistance to inflammatory mediators. Our data reveal the existence of significant strain-level variation in these responses, with select strains displaying potent natural biofilm formation, and greater resistance to ROS, contrasting with the well-studied type strain, Bt-VPI-5482. To uncover the molecular pathways underlying these features, we performed a transposon-mutagenesis based screen in one of these isolates, strain Bt-5951, and identified genes that control natural biofilm formation. Although purified bile promotes biofilms in Bt-VPI-5482, it had little impact on biofilm formation in Bt-5951. By contrast, the microbiota-transformed secondary bile acid, lithocholate, significantly expanded the biofilm-forming capacity of Bt-5951. Moreover, those genes required for natural biofilm formation in Bt-5951 were also important for bile-induced biofilm formation in Bt-VPI-5482. Thus, our data suggest a common biofilm-formation pathway downstream of strain-selective molecular cues that elicit the biofilm response. Collectively our work sheds light on novel mechanisms through which microbiome members mediate their survival in the face of inflammatory insults and provides insights into strain-specific strategies that facilitate stable niche colonization.

### *One-Health Approach to Unravel the Gastrointestinal Microbial Ecology and Enteric Pathogen Shedding in Bison*

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Bison, as keystone species in tallgrass prairies, profoundly impact ecosystem restoration and biodiversity, and more importantly as farm-raised for their meat. Foodborne zoonotic pathogens affect food safety and are an important concern in the United States. A multitude of factors may affect bison health including diet, water, pathogen status, and seasonal variation. Depending on their environment, they may encounter agricultural runoffs and other animals, providing a platform

## Other Titles: *continued*

for pathogen transmission in shared agroecosystems. Bison, therefore, may serve as a reservoir for pathogens and transfer them from farm to fork. However, the dynamics of pathogen transmission and the role of intestinal microbes in bison are unknown. This project assessed the intestinal microbial population of bison longitudinally, its function, and the variation that influences pathogen colonization and shedding. For these analyses, amplicon sequencing targeting16S rRNA (V4-V5) gene sequencing, microscopy for enteric parasites, and culture-based detection for bacterial pathogens were performed from wild and farm-raised bison, longitudinally. Bison intestinal microbiome was found to be affected by variation in diet and seasonal impact (p<0.0001). Various groups of enteric parasites that may impact bison health were detected changing with seasonal variation except for Eimeria which was found to be in 90% of the animals all year round. Bacterial pathogens that are important for human health were also detected including shiga toxin-producing E. coli, Salmonella, and Clostridium. Future studies will involve identifying key microbial patterns that are important for bison health and that contribute to pathogen colonization. This systematic and longitudinal study will provide data-driven insights for on-the-ground outcomes to improve bison health and prairie restoration efforts by identifying key management strategies and improving food safety.

### *Utilizing microbial resilience in indoor spaces for improved detection of markers associated with human health outcomes*

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Any occupied space is home to a rich and diverse microbial community. These microbes are resilient to low moisture conditions in indoor spaces and originate from people, outdoor air, pets, items in the space, and other sources. This talk will highlight two examples of ways in which resilience of these microbes are associated with human health. First, exposure of asthmatics to mold in housing costs an estimated $22.4 billion per year in the US alone. This harmful growth is generally limited by moisture availability. Improved understanding of fungal function upon exposure to moisture in indoor spaces can lead to the development of improved indicators of harmful mold growth in homes. Second, the COVID-19 pandemic has led to over 1 million deaths in the US and impacted all of our lives. Environmental tracking of SARS-CoV-2 and other viruses is necessary to understand disease prevalence and identification of variants in the population. We utilize the persistence of viral nucleic acids deposited in indoor spaces to develop a novel environmental monitoring strategy for respiratory illnesses at the building scale using vacuumed dust. Together, these projects demonstrate how improved understanding of different aspects of microbial resilience can lead to development of novel tools to improve human health.

## Other Titles: *continued*

### *Towards High-Throughput Microbial Diversity Analysis: Application of the latent variable modeling approach to investigate vaginal microbiota communities in sows*

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Sow performance is a key component of commercial pig farms' productivity. Reproductive loss can be caused by stillborn piglets, high oestrus returns, and vaginal discharges. The consequences of a healthy vaginal microbiome may include a fast recovery post-farrowing, resulting in a lower likelihood of urogenital infection, vaginal discharge, or prolapse. Adverse health outcomes, like vaginosis and premature birth, have been linked with diverse vaginal microbial communities in females. However, in sows, little is currently known about the vaginal microbiome's transition from late gestation to post-farrowing, as well as the differences in microbiome between older sows and gilts. Here, we aimed to determine what changes in the vaginal microbiome as a result of farrowing, whether the vaginal microbiome of the sow post-farrowing influences subsequent sow reproductive status, and whether an abnormal vaginal microbiome is associated with vaginal discharge or other health issues post-farrowing. A total of 50 primiparous (0 parity) and 58 multiparous (1-6 parity) were chosen for the study. Vaginal swabs were collected at three separate times (the last three weeks of gestation, the farrowing week, and two weeks post-farrowing). The reproductive performance of the following gestation was obtained, including the number of piglets weaned per mated sow per year (PWPMS), stillbirth occurrences, wean-to-estrus interval, and vaginal discharge (a scale of 0-1 where 0 indicates no discharge; a discharge of 1 is mild; and a discharge of 2 is severe). Microbiome composition was assessed by sequencing the V4 region of the 16S rRNA gene.

The vaginal microbiotas of sows demonstrated noticeable variations over gestational periods for all parities, and decreased bacterial diversity was identified in the multiparous group compared to the primiparous. During the gestational period, there was an observed inverse correlation, with Firmicutes bacteria increasing while Proteobacteria decreased in most animals. Based on the Bray-Curtis dissimilarity index, there was no correlation between vaginal microbiota and reproductive success; however, within the primiparous group, there were clear shifts in the microbiome of gilts with severe vaginal discharges and a high stillbirth rate compared to gilts with no discharges and a lower stillbirth rate. Significant differences were also identified in bacterial relative abundances between gilts with no discharges and those with two score discharges. Gilts with no discharges or stillbirths had lower diversity compared to those with less reproductive performance, severe discharges, and a higher stillbirth rate, as indicated by Shannon diversity (P < 0.012, P < 0.022, respectively). We confirmed that vaginal microbiota was altered in gilts with severe vaginal discharges, stillbirths, and delay-oestrus returns, resulting in lower reproductive performance using the Latent Dirichlet Allocation model.

This study identifies the dynamics of the vaginal microbiota in sows during the gestational period, and the vaginal shifts observed point to potential directions for modifying these communities to improve the sows' performance and overall health. Our analyses using latent class models allow for

## Other Titles: *continued*

the exploration of heterogeneous populations and reveal substructures in vaginal ecosystems with potential clinical and biological associations. These findings will also serve as a reference for future studies focusing on the microbiome.

### *Oral contraceptive usage impacts exercise-induced changes in the gut microbiota*

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Oral contraceptives (OC) are the second most common contraceptive approach in the US, but their usage has been linked with increased cardiometabolic incidents. We recently showed that OC decreased physical activity and energy expenditure in mice, and this perturbation was lessened by the introduction of exercise. Exercise provides metabolic protection, partly through the modulation of the gut microbiota and energy metabolism. However, the role of the gut microbiota in exercise-related protection on OC-induced metabolic dysfunction is not known. OBJECTIVE: We aim to characterize the shifts in gut microbiota in response to exercise and OC use, and relate these microbial changes with changes in energy expenditure. METHODS: Female C57BL/6J mice were provided with or without voluntary wheel running (VWR vs. Sedentary) and fed a high-fat diet with vehicle or OC (Veh vs. OC) for twelve weeks, resulting in a 2x2 factorial design. Cecal microbiota was assessed using 16S rRNA gene sequencing. RESULTS: While the cecal microbiota of Sedentary-OC group clustered differently (p<0.05) than the Sedentary-Veh, substantial OC-driven taxonomic differences were not detected. To our surprise, exercise elicits a stronger impact on the gut microbiota at the community level than OC after 12 weeks of usage. Differential abundance analysis of bacterial taxa at the class level showed that VWR-Veh group had significantly (p<0.05) greater Clostridia and significantly lower Bacilli compared to Sedentary-Veh and Sedentary-OC. At the genus level, VWR-Veh group had significantly (p<0.05) greater Turicibacter and an uncultured genus from Lachnospiraceae compared to Sedentary-Veh and Sedentary-OC. Interestingly, VWR-OC treatment shifted these taxa to be more similar to the VWR-Veh group proportionally. Clostridia was positively associated with non-resting energy expenditure ($\rho$=0.37, p=0.014), total energy expenditure ($\rho$=0.3, p=0.042), and activity-based energy expenditure ($\rho$=0.38, p=0.011). CONCLUSIONS: Our data demonstrated that 12 weeks of OC use did not drastically impact the gut microbiota. However, a significant interaction between exercise and OC on the gut microbiota was present whereby exercise-induced changes in microbial taxa associated with energy expenditure may be diminished with the use of OC.

### *Medium Chain Carboxylic Acid Recovery from Acid Whey using Anaerobic Dynamic Membrane Bioreactor*

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Lactate-based chain elongation (LCE) is a promising anaerobic biotechnology used to recover medium chain carboxylic acids (MCCAs) from organic wastes such as acid whey (AW). MCCAs are straight-chain mono-carboxylic acids with six to 12 carbons with many industrial and agricultural

## Other Titles: *continued*

applications. Barriers to the commercialization of AW LCE lie in the need to minimize competitive microbial metabolisms and improve LCE bioreactor systems. AW is an attractive waste for LCE due to its high production rate and high concentration of MCCA precursors (lactate and short chain carboxylic acids). However, the presence of lactose and glucose in AW presents challenges in effectively managing microbial pathways, consequently yielding lower MCCA productivity in previous LCE studies. Conventional LCE bioreactor systems require costly external filtration steps for downstream MCCA extraction and recovery. Anaerobic dynamic membrane bioreactors (AnDMBRs) utilize a mesh supported in-situ cake layer (a dynamic membrane biofilm) for effective solids-liquid separation and can produce clear effluent, therefore removing the external filtration steps required by conventional CE bioreactors. Motivated by these challenges and advancements, the objectives of this study were to 1) determine the impact of AW composition on LCE efficiency and the LCE microbiome and 2) demonstrate continuous MCCA production using an AnDMBR and simulated AW.

Batch experiments, conducted in triplicate at pH 5.5 and 37°C, explored the impact of AW composition on LCE performance by supplementing locally collected AW with lactate and lactose. Various lactate:lactose molar ratios (1, 1.5, 2, 2.5, 3, and 4) were investigated. Applying the AW composition determined from these experiments, a simulated AW was used to feed a continuous-flow AnDMBR system. The average hydraulic retention time was 3.2 ± 0.6 days, and the organic loading rate was 10.6 g COD/L/d. Samples were collected thrice weekly from the batch experiment and the AnDMBR to monitor carboxylic acid, lactic acid, and lactose concentrations. Biomass samples were collected weekly for 16S rRNA and 16S rRNA gene sequencing.

In the batch experiment, a maximum caproate (C6) production (36.25 ± 0.65 mM C) occurred for the lactate:lactose molar ratio of 4, indicating improved LCE efficiency with lactate-rich AW. Additionally, lactate-rich AW increased the relative abundance and activity of microbial populations belonging to the Megasphaera genus (putative LCE organisms). A simulated AW with a lactate:lactose molar ratio of 4 was used to feed the AnDMBR. The system produced high levels of MCCAs and yielded high-quality permeate with a total suspended solids (TSS) of 0.58 ± 0.01 g TSS/L. The dynamic membrane biofilm contributed to chain elongation performance, evidenced by greater caproate (C6) and heptanoate (C7) concentrations in the permeate than in the mixed liquor (caproate increased from 227 ± 7.0 to 235.05 ± 5.9 mM C and C7 increased from 181.36 ± 6.2 to 183.77 ± 5.7 mM C, across the dynamic membrane).

This work identified the optimal AW composition for LCE, enriching a microbial community with active chain-elongating microbial populations. The AnDMBR system demonstrated efficient filtration as well as MCCA production from simulated AW. Future work will focus on characterizing AnDMBR suspended biomass and dynamic membrane biofilm, aiming to sustain MCCA production and high LCE efficiency using real acid whey and fermented food waste as substrates.

## Other Titles: *continued*

### *Shigellosis results in persistent changes to the gut microbiome*

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Shigella is a significant cause of dysentery predominantly affecting children in middle- and low-income countries, as well as international travelers. While the primary symptoms caused by Shigella or other diarrhea-inducing pathogens resolve within a few days, persistent molecular and physiological changes can occur in infected individuals. Infection can lead to a reduced gut permeability, increased likelihood of developing irritable bowel syndrome, and increased prevalence of antimicrobial resistance genes. While some reports have demonstrated pathogen-induced diarrhea can alter the gut microbiome, no studies have examined the effects of Shigella inoculation on microbiome composition in a controlled infection setting. Here, we profiled the fecal microbiomes of 45 individuals infected with Shigella sonnei 53G before infection, during infection, and after antibiotic treatment. This model allowed for a detailed exploration of microbiome temporal dynamics during infection, as well as a comparative analysis between those who did and did not experience severe symptoms (shigellosis). We found that alpha diversity decreased to a greater degree in individuals with shigellosis and that decreases in alpha diversity significantly correlated with the relative abundance of Shigella. Furthermore, perturbations in microbial composition during infection compared to the pre-infection state were significantly larger in individuals diagnosed with shigellosis than those who were not. After recovery (28 days after infection), those with shigellosis still had persistent changes to their microbiomes while those without shigellosis recovered to a composition resembling their pre-infection microbiome. Shigellosis-induced changes include an increased abundance of multiple ASVs classified as Lachnospiraceae and Veillonella, and decreased abundance in ASVs classified as Alistipes, Faecalibacterium, and Oscillospiraceae. Correlation analysis also identified various ASVs that increased in abundance alongside Shigella, including Strepotcoccus ssp., Veillonella ssp., and Hemophilus parainfluenzae. This study elucidated the dynamics of the gut microbiome under Shigella infection, and identifies an altered state of the microbiome after recovery that could lead to insights about long-term issues arising from Shigella infection and potential interventions to lessen its burden.

## Poster Agenda

| Poster Number | Presenter |
|---|---|
| 1 | Simerdeep Kaur, Purdue University<br>*Nucleic acid detection of live pathogens* |
| 2 | Laurie Spencer, Northern Illinois University<br>*Molecular Characterization of Intestinal Microbiome in Wild Bison* |
| 3 | Taylor Beck, The Ohio State University<br>*Investigating the impact of a combined blueberry and galacto-oligosaccharide dieton gut microbiome composition in a sickness-induced rodent model* |
| 4 | Apollo Stacy, Cleveland Clinic<br>*Impact of the metabolite nitrate on oral health and disease* |
| 5 | Wily Sic, Purdue University<br>*Fungal microbiome associated with tar spot of corn (Phyllachora maydis) over time in Indiana.* |
| 6 | Yuxin Wang, Purdue University<br>*The interaction between microbiome metabolites and the colon epithelial barrier in vitro* |
| 7 | Tessa Cannon (Wilde), The Ohio State University<br>*Insights into SIV from Sooty Mangabey Saliva and Gut Microbes* |
| 8 | Benjamin Levine, University of Illinois at Urbana-Champaign<br>*Prebiotic dietary fiber consumption confers resilience to psychological stress and intestinal dysmotility* |
| 9 | Anthony Yannarell, University of Illinois at Urbana-Champaign<br>*Engines for rapid adaptation: symbiont evolution facilitates host tolerance to stress* |
| 10 | Zachary Lewis, The Ohio State University<br>*Optimizing DNA extraction and host depletion for shotgun metagenomics of high-host low-microbial biomass urine samples.* |
| 11 | Thaisa Cantu-Jungles, Purdue University<br>*Synergistic potential of fiber mixtures to support gut microbiota structure and short-chain fatty acid production* |
| 12 | Blake Williams, University of Illinois Chicago<br>*The Role of Sex in the Gut Microbiota-Fetal Axis during Pregnancy* |
| 13 | Adam Quinn, Purdue University<br>*Effects of Cereal Brans on Gut Microbiome Structure and Function* |
| 14 | Emily Spicuzza, Purdue University<br>*Development of Biofilm in a Hydroponics System* |
| 15 | Apekshya Chhetri, Purdue University<br>*Diet quality impacts circulating tryptophan metabolites and the diversity of tryptophanase-harboring bacteria* |

## Poster Agenda

| Poster Number | Presenter |
|---|---|
| 16 | Frederico Barros, Purdue University<br>*Influence of Sorghum Phenolic Compounds and Dietary Fibers on Short-Chain Fatty Acid Production* |
| 17 | Ashley Kayabasi, Purdue University<br>*Rapid Field Biosensor for Detecting Fecal Contamination on Fresh Produce Farms* |
| 18 | Sulemana Issifu, Purdue University<br>*Pairwise Combinations of Biological Nitrification Inhibitors Reveal Antagonism and Synergism Against Nitrifiers and Soil Nitrification* |
| 19 | Dielson Vieira, Purdue University<br>*Analysis of the cecum microbiome of the guinea pig: from species to fasting* |
| 20 | Cindy Mayorga, Purdue University<br>*Use of polystyrene nanoparticles as particle models for Raman spectroscopy* |
| 21 | Seyedeh Nooshan Mirmohammadali, Purdue University<br>*The Impact of Autoclaved versus Non-autoclaved Diet on Cecum and Colon Gut Microbiome Composition of Rats with Chronic Kidney Disease and Normal Kidney Function* |
| 22 | Hannah Toth, Purdue University<br>*Using metagenomics to investigate the pathogen landscape and diversity of soft rot and blackleg infected potatoes* |
| 23 | Mohamed Kamel, Purdue University<br>*Rapid Detection of Bovine Respiratory Disease Pathogens and Associated ARGs in Cattle Using Paper-Based Loop-Mediated Isothermal Amplification (LAMP)* |
| 24 | Noelle Toong, Benjamin David, Vasumitra Rao, Univerisity of Michigan Ann Arbor<br>*Quality Control for Automated Microbial Growth Experiments* |
| 25 | Nafisa Rafiq, Purdue University<br>*A Field Deployable Heating System for Microbial Detection Using Loop-Mediated Isothermal Amplification (LAMP) Assay* |
| 26 | Alfonso Carrillo, The Ohio State University<br>*Sub-daily virus sampling reveals population, but not community level differences at the Bermuda Atlantic Time Series* |
| 27 | Laura Bran, Purdue University<br>*Development of biosensor for the detection of inflammatory blood biomarker in cattle* |
| 28 | Jordan Mason, The Ohio State University<br>*Fecal and Urine Microbiota of Pet Dogs Vary with Breed and Sex* |
| 29 | Sophia Stokes, The Ohio State University<br>*BefA Protein Induces Increased Growth in Germ-Free American Cockroaches* |

## Poster Agenda

| Poster Number | Presenter |
|---|---|
| 30 | Barsha Bhattarai, Northern Illinois University<br>*Mapping Antibiotic Resistant Genes (ARGs) and Bacteria (ARBs) in Diverse Environmental Niches* |
| 31 | Bibek Raut, Purdue University<br>*Quantitative Analysis of Nucleic Acids on Paper Sensors* |
| 32 | Jessica Li, Univerisity of Michigan Ann Arbor<br>*A novel ultra-high-throughput microfluidic approach to grow and manipulate biofilms* |
| 33 | Shreya Milind Athalye, Purdue University<br>*Viral Characterization and Real-Time Monitoring Using Raman Spectroscopy* |
| 34 | Ethan Hillman, Univerisity of Michigan Ann Arbor<br>*Bifidoacteria Leverage Type IVA Pili to Capture Resistant Starch and Enhance the Metabolism of an Isoluble Substrate* |
| 35 | Melanie Kessler-Mathieu,<br>*Analysis of fungal microbiome composition of wheat subjected to different moisture and CO2 simulated storage conditions.* |
| 36 | Kyungyeon Ra, Purdue University<br>*Lyophilize Loop-Mediate Isothermal Amplification method* |
| 37 | Hisako Masuda, Indiana University<br>*Interactions between gut microbiota, medicinal herbs and a short-term plant-based diet* |
| 38 | Iqra Nazir, Purdue University<br>*Impact of bacteriophage therapy against Salmonella Gallinarum on chicken gut microbiota* |
| 39 | Anubhav Basu, University of Illinois at Urbana-Champaign<br>*Raffinose Family Oligosaccharide utilization by Bacteroides* |
| 40 | Audrey Ellis, Purdue University<br>*Establishing a Gnotobiotic Mouse Model for Studying Swine-Microbiome Interactions* |
| 41 | Mirian Aparecida de Campos Costa, Purdue University<br>*Impacts of Regular Black Tea Kombucha Consumption on the Gut Microbiota of Individuals With or Without Obesity* |
| 42 | Zainab Alzoubi, University of Illinois at Urbana-Champaign<br>*Do nutrition interventions improve gastrointestinal symptoms during cancer treatment? A systematic review and meta-analysis.* |
| 43 | Pei-Ru Jin, Purdue University<br>*Gut Bacterial Metabolite 3-Phenylpropionic Acid Alleviates Acetaminophen-induced Hepatotoxicity by Inhibiting Cytochrome P450 2E1 Expression* |
| 44 | Kyoungjae Won, Purdue University<br>*Fusobacterium nucleatum Metabolites Promote Colorectal Cancer Cell Proliferation through Activating Aryl Hydrocarbon Receptor* |

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| Poster Number | Presenter |
|---|---|
| 45 | Erica Long, Purdue University<br>*A prediction model of tulathromycin antibiotic treatment success in pre-weaned dairy calves with bovine respiratory disease* |
| 46 | Anurag Pujari, Purdue University<br>*Functional characterization of a GH43 enzyme from Bacteroides in Sorghum arabinoxylan cleavage* |
| 47 | Andrei Badilla-Aguilar, University of Notre Dame<br>*Transport and fate of manure-borne antibiotic resistance genes in flowing water* |
| 48 | Devin North, University of Notre Dame<br>*Analysis of Temporal and Geographic Variation in Human Pathogens for Wastewater-Based Epidemiology (WBE) Applications* |
| 49 | Daisy Ugalde, University of Illinois at Urbana-Champaign<br>*Intestinal and hepatic histology is altered by soluble fiber consumption in an FXR/SHP-dependent manner* |
| 50 | Kien Vu, University of Notre Dame<br>*Application of Dye/Enzyme treatment for removing free nucleic acids in wastewater* |
| 51 | Jeremy Beales, The Ohio State University<br>*Baseline gut microbiome alpha diversity predicts gastrointestinal symptoms in breast cancer patients receiving chemotherapy* |
| 52 | Bilal Ahmed, Purdue University<br>*Development of a LAMP-based Paper-pad Biosensor for Genetic Trait Detection of Corn (Zea mays) and Soy (Glycine max)* |
| 53 | Maria Luisa Savo Sardaro, Northwestern University<br>*How the gut microbiome can be shaped by the use of complex dietary fibers in food* |
| 54 | Boyu Jiang, Purdue University<br>*Predicting host-microbiome interactions: integrating iColonEpithelium and microbial community-scale metabolic models* |
| 55 | Jordan Rindels, University of Illinois at Urbana-Champaign<br>*Psychological stress and dietary fiber regulate markers of inflammation and anxiety* |
| 56 | Anjali Vanamala, Purdue University<br>*The Impact of Anorexia Nervosa on the Gut Microbiota* |
| 57 | Angela del Aguila, The Ohio State University<br>*Exploring the role of intraperitoneal immune cells in the communication between the gut microbiome and the brain* |
| 58 | Angela Kent, University of Illinois at Urbana-Champaign<br>*Harnessing Microbiome Associated Phenotypes for Sustainable Nutrient Retention in Agroecosystems* |

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|---|---|
| 59 | Emily Kelleher, Northwestern University<br>*Characterization of the Gut Microbiome in Sri Lankan Asian Elephants (Elephas maximus): A Comparative Study* |
| 60 | Brandon Schemerhorn, Purdue University<br>*Shotgun Metagenomic Analysis of Schizaphis microbiome in response to Biotype, Carried CYDV and Time* |
| 61 | Miguel Alvarez Gonzales, Purdue University<br>*In vitro degradation of wheat bran phenolic compounds by human gut microbiota* |
| 62 | Noah Brown, University of Illinois at Urbana-Champaign<br>*Prairie plant species differ in their resilience to fungicide-induced alterations of foliar endophytic mycobiomes* |
| 63 | Marcelo Guerrero, Purdue University<br>*Experimental evolution of gut microbes on inulins varying in degree of polymerization* |
| 64 | Sadia Marjia Ferdous, Case Western Reserve University<br>*Predicting Phage Cocktail Efficacy: A Step towards Phage-based Bacterial Control for Water Security* |
| 65 | Rajsri Raghunath, Purdue University<br>*Cereal fiber type and bran particle size differentially selects for gut microbial community composition and function under high dilution pressure* |
| 66 | Ritesh Ray, Northern Illinois University<br>*Comparative Genomics of Shiga toxin producing E. coli (STEC) harbored in bison and its impact on intestinal microbiome* |
| 67 | Ainsley Tran, University of Illinois Chicago<br>*Determining the causal role of the gut microbiota in perinatal depression: A pilot study* |
| 68 | Andrew Fleck, Purdue University<br>*HealthyHerd: A Web-Based Platform to surveil the spread of SARS-CoV-2 variants in Animal Populations* |
| 69 | Arval Viji Elango, Purdue University<br>*Understanding the microbiome of Controlled Environment Agriculture towards the development of a microbial control* |
| 70 | Clay Swackhamer, Purdue University<br>*Three patterns emerge in the gut microbial community during in vitro fecal fermentation of dietary fibers varying in their level of compexity* |
| 71 | Cristian Salinas , Purdue University<br>*Microbial Community Analysis on Coffee Roots under Legume Intercropping Systems as an Alternative To Nitrogen Supply in Colombia* |

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| 72 | Danyang Duan, University of Illinois at Urbana-Champaign<br>*Ecological Memory of Nitrogen Fertilizers on Soil Microbiome Structure Becomes Evident at Peak Growth of Miscanthus* |
| 73 | Edward Moncada, Purdue University<br>*Deciphering Fiber Tolerance: Impact of Gut Bacterial Dysbiosis and Fiber Physicochemical Properties* |
| 74 | Gopal Palla, Purdue University<br>*Field-deployable paper-based colorimetric LAMP biosensor for the detection of antimicrobial-resistant genes of Bovine respiratory disease* |
| 75 | Henrique Petry Feiler, Purdue University<br>*Unveiling the Impact of Nanoplastics on Plant-Rhizosphere Dynamics: A Study of Tomato and Lettuce Microbiomes* |
| 76 | Jose Haro-Reyes, Purdue University<br>*Anti-colitic activity of phlobaphenes and anthocyanins in IL-10 -/- mice is dependent on the human donor microbiota* |
| 77 | Josiah Davidson, Purdue University<br>*Development of Nucleic Acid-based Diagnostics for the Detection of Viral Respiratory Pathogens in Humans and Animals on Paper* |
| 78 | Muhtashim Rafiq Chowdhury, Case Western Reserve University<br>*Understanding the influence of houseplants on the indoor microbiome* |
| 79 | Paul Oladele, Purdue University<br>*Evaluating the effect of lyoprotectants in preserving community structure and bacterial viability in the lyophilized fecal community of pigs.* |
| 80 | Victoria Gutierrez, Purdue University<br>*Biofilm formation of Bifidobacterium spp. in sorghum arabinoxylan fermentation* |
| 81 | Trever Thurgood, Purdue University<br>*Carbon Source-Specific Regulation of Nitrogen Fixation in Paraburkholderia xenovorans* |
| 82 | Lauren Sofia Yepes Fernandez & Maria Alejandra Osorio Marulanda, Purdue University<br>*Whole Food Fibers For Support Of Key Gut Bacteria For Human Health* |
| 83 | Natalie Duvanenko, Purdue University<br>*A research proposal: root microbiomes and aphid resistance* |
| 84 | Aliya Ehde, Purdue University<br>*The Molecular Ecology of Drinking Water in Building Plumbing as a Function of Stagnation* |

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## *1: Nucleic acid detection of live pathogens*

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Herein, we propose a point-of-care biosensor for detecting live pathogens contaminating beef products. Biosensing of live pathogens is based on isothermal nucleic acid amplification on a paper-based device. A colorimetric dye is employed to indicate the amplification product for visual results. The assay incorporates a compound propidium monoazide (PMA) that makes the DNA from dead cells inaccessible for amplification. This approach is especially applicable for pathogens that can enter a viable but non-culturable state (VBNC)

## *2: Molecular Characterization of Intestinal Microbiome in Wild Bison*

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American bison (Bison bison) are large ruminants native to North American grasslands that were hunted to near extinction in the late 1800s. Bison are a keystone species; herds are being reintroduced into nature preserves to act as ecosystem engineers aiding in prairie restorations. Wild bison were reintroduced at Nachusa Grasslands preserve in 2014 as part of The Nature Conservancy's native tallgrass prairie restoration strategy. Intestinal microbiome compositional analysis can be used to indicate host health. Studies of domestic cattle have shown microbiome fluctuations in direct response to shifts in dietary composition and quality. The quantity and quality of wild bison forage may be affected by variations in environmental conditions (i.e., temperature and precipitation). Only a few studies have explored the intestinal microbiome of free-ranging bison, with sample sizes and study lengths relatively small, and no studies have included bison herds from the Midwestern United States. Our study aims include conducting a longitudinal assessment of bison intestinal microbiome composition, across multiple years and seasons. Our specific research questions are: (1) What is the intestinal bacterial composition and abundance of free-ranging bison at Nachusa Grasslands?; and (2) What are the factors that contribute to community shifts across years (2018 – 2021) and seasons (winter, spring, summer, and fall)? To answer these questions, we performed 16S rRNA (V4-V5) gene sequencing utilizing the Illumina NovaSeq SP 2x250. Fecal samples (N=148) and rectal swab samples (N=46) were collected using aseptic techniques and stored at -80°C until further processing. Community DNA was isolated, sequencing libraries were prepared in-house and shipped to Rush University Medical Center Microbiome Core Facility (Chicago, IL). The resultant 30,543 sequence reads were analyzed for taxonomic and compositional characterization using QIIME2. We performed alpha-diversity (Shannon) with Kruskal-Wallis pairwise tests, and beta-diversity (Bray-Curtis and Jaccard) with PERMANOVA pairwise tests, across sampling years and seasons. These diversity tests revealed statistically significant differences between years (2018-2021) and seasons (winter, spring, summer, fall). Further analyses will investigate the relative abundances of bacterial taxa, and correlations between the composition shifts and temperature and precipitation fluctuations. Overall, these data will highlight environmental influences on the intestinal microbiome of the animals, which in turn are imperative to understand how this may affect bison health.

## *3: Investigating the impact of a combined blueberry and galacto-oligosaccharide dieton gut microbiome composition in a sickness-induced rodent model*

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The human diet aids in modulating the gut-brain axis, a two-way signaling system between the nervous system and microorganisms in the gut. Cognitive function is influenced by this relationship and has been shown to decline over time due to age-related structural and functional changes. Development of a functional food product for clinical studies to serve as an intervention for improved cognitive function via the gut-brain axis was achieved by creating a confection containing blueberry anthocyanins (ACNs, a polyphenol) and galacto-oligosaccharides (GOS, a prebiotic), which have both been previously studied to reduce systemic inflammation and promote healthy bacterial populations in the gut, respectively. It is hypothesized that consumption of blueberry ACNs and GOS will increase the diversity of the gut microbiome and mitigate effects of cognitive decline. A sickness induced rat model was used to evaluate behavioral and physiological parameters when fed a control and a treatment diet containing similar relative amounts of ACNs and GOS as the confection. Fecal samples were collected before and after inducing systemic inflammation via lipopolysaccharide (LPS, an endotoxin) injection at multiple time points to mimic cognitive decline in humans. Sickness scores were measured starting at 16 hours post LPS injection for five days and standard cognitive functions tests (Elevated Plus Maze, Forced Swim Test, and Morris Water Maze) were performed starting three days after LPS injection. The treatment diet given to rats pre-LPS injection showed a significant effect on the gut microbiome compared to the control diet. Further, effects of the diet post-LPS injection were maintained over fourteen days. These findings indicate that the intervention diet effectively modifies the gut microbiome and future studies will compare its composition as it relates to behavioral and physiological outcomes after induced sickness.

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## 4: Impact of the metabolite nitrate on oral health and disease

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Commensals that respire the metabolite nitrate are abundant across the body, particularly the oral cavity, where dietary nitrate (e.g., from leafy greens) is concentrated to millimolar levels within saliva. In response to nitrate, oral Neisseria can generate nitric oxide (NO), a compound associated with systemic health benefits. However, nitrate can also be generated as a byproduct of inflammation, particularly inducible NO synthase (iNOS) activity, which is heightened in periodontal (gum) disease, one of the most prevalent inflammatory conditions worldwide. The source of nitrate may therefore play a critical role in determining the relationship between commensals, pathogens, and the host. We hypothesize that diet-derived nitrate promotes health, while inflammation-derived nitrate promotes disease. To test this hypothesis, we have developed a synthetic community comprising the commensal Neisseria mucosa (Nm) and the periodontal pathogen Fusobacterium nucleatum (Fn). To model diet-derived nitrate, we cultured the community as biofilms on media ± nitrate. To model inflammation-derived nitrate, we injected the community into mice to create microbially defined abscesses. In the biofilm model, nitrate addition to mono-cultures promoted Nm, while not affecting Fn. In contrast, nitrate addition to co-cultures drove Nm to inhibit Fn, specifically via the antimicrobial nitrite. Strikingly, in the infection model, Fn was not recoverable from abscess mono-infections, but highly abundant in co-infection, which also increased iNOS expression, suggesting a role for inflammation-derived nitrate. Our results show that depending on the source of nitrate, nitrate-respiring commensals may drive disease or promote health, which in the future could be harnessed as a microbiota-mediated therapy.

## 5: Fungal microbiome associated with tar spot of corn (Phyllachora maydis) over time in Indiana.

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Tar spot is an emergent disease spreading in the Midwest since 2015 and is already present in some states in the Northeast and Southeast of the United States. From 2018 to 2020, estimated yield losses due to tar spot across the Midwestern states totaled 241 million bushels. This disease is caused by Phyllachora maydis and is associated with Monographella maydis and Coniothyrium phyllachorae in Mexico, but not in the United States. M. maydis was initially considered to cause fish-eye lesions, however, microbiome studies showed that it is not required for fish-eye development. C. phyllachorae was described as a mycoparasite of P. maydis, however its presence and role in tar spot disease are still unclear.

The identification of fungal species associated with tar spot can provide insight into the management of this disease since potential biological control agents can be identified. The association of Coniothyrium with P. maydis is of particular interest since this genus has been reported and used as a biological control for fungal pathogens such as Sclerotinia sclerotiorum. Coniothyrium was reported in Mexico by morphological descriptions and by next-generation sequencing in the United States. Paraphaeosphaeria spp. and Fusarium spp. have also been reported in the United States in high abundance. The presence and abundance over time of Coniothyrium in tar spot lesions can provide insight into its role and association with P. maydis. Therefore, we are using next-generation sequencing to identify the abundance of Coniothyrium over time in tar spot lesions in Indiana.

Infected corn leaves were collected in Indiana, during the early, middle, and late disease development stages. DNA was extracted from tar spot lesions and the Internal Transcribed Spacer 2 region (ITS2) was used for metagenomic sequencing. We found similar fungal abundances over time with fewer taxa at late-stage disease development within samples and between disease stages. Coniothyrium was the second-most-abundant taxon just below fungi assigned as incertae sedis, which we suspect to be P. maydis. The abundance of Coniothyrium was similar over the three disease stages. Other genera present in high abundance over time are Bullera and Tilletiopsis, which also have been reported as potential biological control agents. The abundance of these pathogens is similar over time and P. maydis is the only species showing differential abundance at late stages compared to early disease stages.

## 6: The interaction between microbiome metabolites and the colon epithelial barrier in vitro

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Objectives: To investigate microbial metabolites' impact, mechanism, and transport on the epithelial monolayer.

Methods: We established a model system by seeding 1×10^5 cells/cm^2 Caco2 and HT29 cells (9:1) onto the apical chamber in a 12-well transwell plate in DMEM with 10% FBS and 1% penicillin-streptomycin at 37°C with 5% CO^2. Transepithelial electrical resistance was monitored. Upon monolayer formation, the cells were treated with 1-10 mM SCFA sodium in the apical part in PBS, with PBS-only and DMEM-only controls. SCFA and glucose levels were analyzed using gas chromatography and GOPOD. Cell viability was assessed using a cell counting kit, propidium iodide staining, and fluorescence microscopy.

Results: After 5 mM SCFA sodium treatment for 7 days, TEER values (Ω • cm^2) were: DMEM (283.73 ±11.57), PBS (283.73 ± 23.13), butyrate (328.53 ± 29.78, P = 0.006 vs DMEM and P = 0.016 vs PBS), butyrate with low glucose DMEM basolateral (840 ± 54.41, p < 0.0001 vs DMEM, PBS, and the butyrate with high glucose DMEM basolateral), Acetate and propionate increased TEER a little.

The propionate (mM) in the apical chamber was 1.25 ± 0.14, basolateral was 2.49 ± 0.17 (P = 0.0006) with 0.66 ± 0.09 g/L glucose left; High-glucose DMEM basolaterally with butyrate (mM) in the apical chamber was 0.52 ± 0.03, basolateral was 0.93 ± 0.11 (P = 0.003) with 1.22 ± 0.16 g/L glucose left (P = 0.004 vs

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PBS, P < 0.0001 vs acetate and propionate); Low-glucose DMEM basolaterally, apical butyrate was 0.38 ± 0.01, basolateral was 1.11 ± 0.05 (P < 0.0001), with 0.02 ± 0.01 g/L glucose left ( P = 0.0003 vs PBS). No acetate transportation.

Sodium butyrate decreased cell viability to 67.48 ± 4.20 % (P = 0.0008 vs DMEM, and P = 0.008 vs PBS), while sodium propionate and sodium acetate did not have an effect compared to PBS. PI staining revealed no difference in nuclei staining between DMEM and different sodium butyrate concentrations (1, 2, 5, 10 mM). The CFSE staining showed that 5 mM sodium butyrate didn't affect the cell viability.

Conclusions: Our study reveals that a basolaterally fed co-cultured epithelial monolayer facilitates microbiome-epithelium interaction studies. The cells transport butyrate and propionate from the apical to the basolateral side and utilize butyrate alongside glucose. Sodium butyrate decreased cell viability mainly through inhibition of cell proliferation yet significantly enhanced TEER. These findings establish a relevant model for investigating microbiome-epithelial metabolic interactions, elucidating the complex interplay between microbiome metabolites and intestinal epithelium.

### *7: Insights into SIV from Sooty Mangabey Saliva and Gut Microbes*

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The microbiome plays a critical role in primate health and is shaped by many environmental factors. In Human Immunodeficiency Virus (HIV) and Simian Immunodeficiency Virus (SIV), gut epithelial damage, and microbial translocation from the gut to systemic circulation are key determinants of disease progression and are also associated with increased viral loads and CD4+ T-cell depletion. However, in natural SIV host species such as sooty mangabeys (Cercocebus atys), the gut barrier remains intact, and microbial translocation does not occur despite high levels of viral replication. Moreover, disease progression to AIDS is not commonly observed. This phenomenon has promoted interest in the microbial communities that play a role in maintaining gut homeostasis in SIV. Here we examine gut and oral microbiota of sooty mangabeys from Emory National Primate Research Center (NPRC) and their free-ranging counterparts living in Ivory Coast's Taï Forest – completing the first characterization of SIV in the saliva of wild sooty mangabeys. Our results indicate that saliva sampling is more accurate than fecal sampling for noninvasively detecting SIV in sooty mangabeys. SIV viral load in saliva was found to be significantly higher in mangabeys housed at Emory NPRC than those living in the Tai Forest. We observed differences in alpha and beta diversity metrics of the salivary microbiota based on SIV status. We establish correlations between the gut and oral microbiota, viral load, and several social and ecological variables. These results highlight the importance of a wholistic approach in examining microbiome-disease interactions in wild primate populations. Further, if diet or the microbiome can be modulated to improve gut barrier integrity, decrease microbial translocation and the associated immune activation, and ultimately prevent increased viral titers and progression to AIDS, this could have major implications on HIV / SIV treatment and AIDS prevention.

### *8: Prebiotic dietary fiber consumption confers resilience to psychological stress and intestinal dysmotility*

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Introduction: Irritable bowel syndrome (IBS) is a common yet poorly understood condition characterized by severe intestinal dysmotility. Psychological stress is an environmental exposure that enhances the frequency and intensity of intestinal dysmotility and reduces the abundance of microbial genera that confer stress resilience and synthesize γ-aminobutyric acid (GABA) - a neurotransmitter that modulates intestinal motility via enteric nervous system activity. In direct contrast, prebiotic-rich diets reliably enhance the abundance of microbes that confer stress resilience and increase luminal GABA concentrations. Hence, the objective of this work was to characterize the effect of prebiotic dietary fiber consumption on the host stress response and intestinal motility. Our central hypothesis is that prebiotic consumption will reduce stress resilience and reduce intestinal dysmotility.

Methods: Male and female C57BL/6 mice (age 6-8 weeks, n=6, N=96) were randomized into sixteen groups with factors of sex (male/female), two weeks of diet (1. Fiber-free diet [FFD] (base diet), 2. FFD + 20% cellulose [CELL] [non-prebiotic fiber control], 3. FFD + 10% CELL + 10% short-chain fructooligosaccharide [scFOS] [short-chain prebiotic fiber], or 4. FFD + 10% CELL + 10% inulin [INU] [long-chain prebiotic fiber]), and two hours of daily restraint stress exposure during the second week on diet (stressed vs non-stressed). On day one of restraint, blood was collected at 0, 30, 60, 90, 120 min, and one-hour post-stress (180 min) to quantify corticosterone via ELISA. Adrenal mass (a marker of stress reactivity) was measured as a percentage of total body mass. Colonic motility was measured ex-vivo via an organ bath and force transducer system with LabChart analysis software. Colon tissue was exposed to a physiologically relevant concentration of GABA or a control buffer. Subsequently, it was chemically stimulated with acetylcholine to elicit a contractile response. Corticosterone, adrenal mass, and motility data were tested via three-way ANOVA (sex, stress, and diet factors) with post-hoc LSD.

Results: Females had higher adrenal mass vs males (p<0.00001). Male-INU had lower adrenal mass vs Male-FFD (p=0.01). Stressed tended to have higher adrenal mass in males (p=0.1). During stress, corticosterone increased across time (p<0.00001) with a diet-by-time interaction (p<0.001). At 120 min, Stressed-CELL, Stressed-scFOS, and Stressed-INU had lower corticosterone vs Stressed-FFD (all p<0.05). The total corticosterone response to stress was lower in CELL (p=0.01) and scFOS (p=0.007), but not INU (p=0.35) vs FFD. CELL and INU reduced contraction force in both stressed (CELL p=0.03, INU p=0.007)

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and non-stressed (CELL p=0.01, INU p=0.007) conditions. All fiber diets (p<0.05) reduced contraction duration in the non-stressed condition, but only INU reduced contraction with stress (p=0.01). With stress, GABA incubation reduced contraction force (p=0.01), and duration (p=0.01) compared to controls.

Conclusions: Overall, these data indicate that dietary fiber consumption confers resilience to stress-induced glucocorticoid secretion and colonic dysmotility – suggesting that nutritional therapies may be a feasible strategy to mitigate the stress-associated symptomatic burden in IBS. Ongoing experiments are investigating whether these data are related to changes in gut microbiome and enteric nervous system structure.

### *9: Engines for rapid adaptation: symbiont evolution facilitates host tolerance to stress*

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Mutualistic symbiosis can fuel rapid adaptation to environmental stress. This may be especially likely when one of the partners is a microbe that can quickly evolve because of short generation times and horizontal gene transfer. However, environmental stresses could also threaten the stability of symbioses by imposing selective pressures on the partners that are contrary to the selective forces that maintain the partnership. We sought to determine the impact of multiple stresses on a mutualistic symbiosis using a model legume-bacterial symbiosis involving white clover (Triofolium repens) and Rhizobium leguminosarum. We hypothesized that the existing nitrogen-based partnership between clover and R. leguminosarum would help this symbiosis more quickly adapt to an additional source of environmental stress. To test this hypothesis, we conducted a multi-generation experiment in the greenhouse. We created an inoculum of 28 well-characterized R. leguminosarum strains that differed in their net fitness effects on clover. We then combined this inoculum with clover plants in a fully-factorial experiment crossing water stress (well-watered vs. droughted conditions) with nitrogen stress (low vs. high soil nitrogen content). We also included an additional set of treatments that included only soil inoculated with the R. leguminosarum mix (i.e. no plants) in order to distinguish between adaptation due to the symbiosis from adaptation of the bacteria alone. At the end of each clover generation, we passaged soil (with clover roots and root nodules for treatments containing plants) to another round of selection for a total of four clover generations. At the end of the fourth generation, we crossed each of our 8 historical treatments into well-watered and droughted conditions, and we planted clover plants into each in order to assess the fitness impact of the evolved R. leguminosarum on clover. We found evidence of environmental matching: clover plants under well-watered conditions grew best when paired with historically well-watered R. leguminosarum, and droughted clover plants grew best when paired with historically droughted R. leguminosarum. Crucially, this environmental matching was only apparent for R. leguminosarum strains that had a plant partner during the adaptation phases of the experiment. We interpret this result as indicating that the symbiosis was necessary to facilitate adaptation to the watering environment. Furthermore, because environmental matching was more apparent under historical low-nitrogen conditions than under high-nitrogen conditions, and because high-nitrogen is known to degrade the clover-rhizobium mutualism, we interpret this as further evidence that maintenance of the symbiosis during the water-selection phase facilitates rapid adaptation. We conclude that symbiosis can lead to rapid adaptation in the face of novel environmental stresses.

### *10: Optimizing DNA extraction and host depletion for shotgun metagenomics of high-host low-microbial biomass urine samples.*

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Recent studies highlight the role of microbiota in cancer development and therapeutic response. However, efforts to characterize the urobiome (microbiota of the urinary tract) and their role in bladder cancer (BC) have been limited due to technical challenges including low microbial biomass and high host cell shedding in urine. While some studies have identified links between the urobiome and BC, additional mechanistic studies with standardized methods are needed. Few studies have attempted shotgun metagenomic sequencing in urine, and none have attempted to assemble microbial genomes from metagenomes. Here we evaluate differing methods for host cell removal and their effects on urobiome profiles in healthy dogs. We collected urine from seven dogs and fractionated samples into 3.0 mL aliquots. Aliquots were spiked with a standard concentration of canine cells, modelling a biologically relevant host-cell burden. Samples then underwent DNA extraction followed by shotgun metagenomic sequencing. We tested six different methods of DNA extraction, five of which included host cell removal steps: QIAamp Bacteremia (no host removal), QIAamp DNA Microbiome, Molzym MolYsis, NEBNext Microbiome DNA Enrichment, Zymo HostZERO, and Propidium Monoazide. Sequences were analyzed using MetaPhlAn4 or standard metagenome assembled genome (MAG) pipelines, and statistics were performed in R. Dog (p=0.001) but not extraction method (p=0.9) drove overall differences in microbial composition. DNA Microbiome, Molzym, and Zymo HostZERO reduced the burden of host DNA compared to samples extracted with Bacteremia. DNA Microbiome also maximized the number of unique microbial species observed (p=0.01) and overall microbial diversity (p=0.002, Shannon entropy). Finally, DNA Microbiome produced the greatest number of MAGs, though overall recovery of MAGs was low. DNA Microbiome was the most effective extraction method for urobiome profiling in samples with relatively high host-cell burdens. We also demonstrate that shotgun metagenomic profiling is feasible at both the gene- and genome-resolved level in urine samples.

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## *11: Synergistic potential of fiber mixtures to support gut microbiota structure and short-chain fatty acid production*

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Prebiotic dietary fibers are a recognized approach for modulating the gut microbial community, but most prebiotics are made from single fiber components. This study hypothesized that a mechanistic approach to blending fibers could enhance the growth of bacterial groups beneficial to health. Initially, pooled in vitro fecal fermentations were used to create dietary fiber blends that support complementary microbial groups associated with health. Subsequent experiments compared microbial responses to these blends against single fiber components using fecal samples from 10 healthy individuals. Results demonstrated that the designed fiber blends were superior in promoting diverse bacterial taxa, resulting in higher alpha diversity and unexpectedly greater production of short-chain fatty acids (SCFAs). Additionally, fiber blends led to unique community structure shifts and specific taxa changes not seen with single fibers, indicating a synergistic effect when fibers are combined. Designed mixtures also showed more consistency to boost several taxa, particularly butyrate-producing bacteria from the Clostridium cluster XIVa, in gut microbiota communities from different donors. These outcomes underscore the potential of strategically designed fiber blends to support a varied microbial population, enhance SCFA production, and achieve more uniform responses to dietary fibers among people. Ultimately, leveraging the synergistic capabilities of designed fiber blends offers a potentially superior strategy for future prebiotic fiber development.

## *12: The Role of Sex in the Gut Microbiota-Fetal Axis during Pregnancy*

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Introduction: Sex differences have been identified in many biological processes, including immune response and disease prevalence. Recent research has shown that the microorganisms that reside in the human gastrointestinal system are distinct in XX and XY individuals and can modify sex hormones, such as estrogens. XX fetus produce higher levels of estrogens than XY fetus, however, no research has explored the role of fetal sex in the maternal gut microbiota. Studies have shown that individuals pregnant with XY fetus are more likely to experience perinatal depression (PND) and that the pathogenesis of PND may be linked to the gut microbiome through the gut-brain axis (GBA), highlighting the importance of better understanding the gut microbiota-fetal axis during pregnancy. This study aims to fill in this current gap in knowledge by exploring the associations between fetal sex and the maternal gut microbiota composition and its metabolic functions.

Methods: We used publicly available 16S rRNA sequencing data from fecal samples from 485 healthy pregnant individuals recruited from a hospital in Guangzhou (China), with 49% carrying an XX fetus. We identified bacterial taxa present in those samples using DADA2 and predicted metabolic potential with PICRUSt2. To identify differences in enzyme abundance based on fetal sex, we used Linear Models in MaAsLin2 correcting for age, gestational age, BMI before pregnancy, residency status, and parity. Models were corrected by multiple comparisons. To identify differences in bacterial taxa abundance based on fetal sex, we used Zero-Inflated Gaussian Models in metagenomeSeq correcting for the covariates listed above.

Results: We found pregnant individuals that carry XX fetuses had higher predicted levels of beta-glucuronidase (FDR-corrected p-value=0.17), a bacterial enzyme that deconjugates estrone-3 and estradiol-17-glucuronides into unbound estrone and estradiol which can then be circulated through the bloodstream. These circulating estrogens may be transferred back to the growing fetus, potentially acting as a positive feedback loop toward female sex differentiation and reproductive organ development.

Conclusion: Differences in fetus-produced hormone levels may affect and be affected by the composition of the gut microbiota of pregnant individuals in a sex-specific manner.

## *13: Effects of Cereal Brans on Gut Microbiome Structure and Function*

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Dietary fiber consumption plays a major role in human gut microbiome structure and function. Gut microbiota are responsive to subtle chemical and structural differences in fibers during in vitro fermentations resulting in the promotion of different genera and different rates of metabolite production. What is not as well understood is the stability and resilience of microbiome communities during highly targeted dietary interventions with fibers. This project sought to test whether structure and function of gut microbiomes could be altered by feeding human subjects wheat and sorghum bran and whether these changes were fiber specific. Both brans were separately fed to three subjects for two weeks separated by a washout period. Stool samples were collected at baseline and post-feeding to determine changes to microbiome community structure using 16S rRNA gene sequencing. Stool samples were also used as inocula for in vitro fermentations with both brans, and resulting fermentation rates were evaluated by measuring pH, gas, and short-chain fatty-acid production. Consumption

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of either bran led to increases in many Bacteroidetes genera in subject 2. Bran specific effects also occured with greater proportions of Bifidobacterium and Massiliprevotella after wheat bran feeding and Ruminococcus and Faecalibacterium for sorghum bran. Bran feeding broadly impacted in vitro fermentation rates by increasing rates of initial pH decline. After subjects consumed wheat bran, in vitro production of butyrate increased only in wheat bran fermentations. These results support the hypothesis that gut microbiome composition and capability can be impacted in targeted ways by habitual consumption of different brans.

## *14: Development of Biofilm in a Hydroponics System*

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Hydroponics is a system where plants are grown in a circulating nutrient solution, often indoors, to stimulate faster growth and higher yields compared to traditional soil-based practices. However, pathogens can circulate freely in hydroponic systems if the microbiome is not kept under close observation. Although pathogens are unwanted microbial growth, beneficial biofilms in controlled environment agriculture systems can aid plant growth, increase nutrient uptake, and build pathogen resistance. This study aims to design efficient biofilm collection and monitoring techniques to enhance the productivity of these systems. Experiments were conducted to explore differences in microbial growth with 4 unique biomass collection methods: coupons, swabs, nutrient solution (planktonic), and rockwool substrate throughout the system. Based on previous testing with Kratky jars, we hypothesize that coupons have the most biofilm and diversity due to ideal growth conditions on the coupons. The microbiome was quantified (DNA yields) and will be characterized in each phase of the system under multiple iterations to track where microbes are coming from and the best methods to measure them. This study included measured biomass in a basil nutrient film hydroponics system with (a) no plants, (b) poor growing conditions, and (c) better growing conditions. Each system was quantified with flow cytometry (planktonic), and DNA was extracted from biofilm (swab, coupons, rockwool) and planktonic samples. Microbiome characterization is ongoing. Plant measurements were also recorded in all systems. Coupons had higher DNA yields (0-0.135 ng/mm2) compared to swabs (0-0.00289 ng/mm2) in all systems. Total cell counts were similar for both systems with plants, but were lower in the system before plants were introduced. These results will give insight into methods needed to sustain a healthy microbiome. Overall, developing strategies for microbiome monitoring and control will promote plant growth and prevent the spread of harmful pathogens throughout the system. Expanding this field of research will have significant implications for sustainable agriculture. The findings from this study can set a foundation for more uniform research of this critical microbiome. Further research will be directed towards the application of these methods in large-scale hydroponic setups and their potential impact on crop yield and quality.

## *15: Diet quality impacts circulating tryptophan metabolites and the diversity of tryptophanase-harboring bacteria*

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Objective: Tryptophan is an essential amino acid that is decreased with lower dietary quality. Tryptophan metabolism by host and microbial enzymes forms protective (indole) and deleterious (5-hydroxytryptamine—5-HT) metabolites that play critical roles in colorectal cancer (CRC). Indole is produced by tryptophanase (tnaA) harboring bacteria (THB). Our previous work revealed THB to be significantly associated with CRC in African American/Black individuals (AA/Bs). Thus, we aimed to determine if dietary quality impacts the gut microbiome and circulating tryptophan metabolites in human participants.

Methods: Stool and fasting (~12 hr) venous blood were collected from 138 participants between 45-75 years of age living in Chicago. Plasma tryptophan and 5-HT were quantified using UPLC-MS. Microbial genomes were shotgun sequenced and differences between microbial features were assessed. Dietary quality was calculated from two 24-hour recalls using the USDA HEI-2015 with HEI<51 set as a cut-point for poor dietary quality. Using NCBI's open-source database, tnaA amino acid sequences were obtained and compared against the bacterial groups for relative sequence similarities. Relative changes in metabolites among the two HEI groups were validated using non-parametric (Wilcoxon) t-test with a p-value of 0.05.

Results:

Participants were 52% AA/B and 57% female with a mean age of 59.6 (±6.2). African American/Blacks had poor dietary quality, higher circulating 5-HT, and lower circulating tryptophan (p<.001). Using HEI-2015, 77 and 76 genera were associated with HEI <51 and HEI ≥ 51, respectively. Analysis of associated genera revealed the THB Porphyromonas, Akkermansia, and Lachnospiraceae to be associated with HEI ≥ 51 and Escherichia, Fusobacterium, Odoribacter, and Lachnospiracae to be associated with HEI <51. Homology analysis of tnaA sequences from representative species within associated genera ranged from 25% - 87%.

Conclusion: AA/Bs have lower serum tryptophan, higher serum 5-HT, and lower dietary quality as calculated by HEI-2015. The low sequence homology among THB demonstrates a need to understand THB activity among diverse bacteria that can be impacted by dietary quality.

Funding Sources: LTH, PG, EM, JR, HRH: R01CA204808, U54MD012523; MLO: T32CA057699; AC: RSG-23-1154989-01-CSCT.

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## 16: Influence of Sorghum Phenolic Compounds and Dietary Fibers on Short-Chain Fatty Acid Production

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Sorghum, a cereal of growing interest for its potential health benefits, is rich in a combination of phenolic compounds and dietary fibers. This study aimed to assess how fibers and phenolic compounds from toasted sorghum flours influence the production of short-chain fatty acids (SCFA). Two sorghum genotypes (BRS 501 white, without tannins, and BRS 305 tannin sorghum) underwent direct dry heat toasting. Subsequently, the flours were subjected to an in vitro gastrointestinal phase followed by an in vitro fecal fermentation model, and SCFA were quantified using GC-MS. The results revealed a consistent increase in SCFA concentrations from time 0 to 24 hours. Regarding to acetate, the highest concentration was observed in samples white sorghum phenolic extracts (WSE) and tannin sorghum phenolic extracts (TSE) with fructooligosaccharides (FOS) (WSE + FOS = 37.55 mM ± 4.03; TSE + FOS = 38.02 mM ± 0.66). This can be attributed to the presence of FOS, with a slight enhancement by phenolic compounds. This enhancement was particularly evident in propionate, where the highest concentration was found in WSE + FOS (29.59 mM ± 1.53), followed by TSE + FOS (19.89 mM ± 0.61), compared to FOS alone (17.33 mM ± 0.76). When comparing the results from white and tannin sorghums flours with no supplementation (WSD and TSD, respectively), no significant difference in the concentration of all SCFAs was observed. Additionally, for both WSD and TSD alone, when compared to supplementation with their phenolic extracts, an increase in the concentration of acetate and propionate was observed in the WSD + WSE sample, and no significant difference was noted in TSD + TSE. These findings suggest that, despite tannin sorghum flour having a higher dietary fiber content than white sorghum flour, this factor did not influence SCFA production as much as supplementation with phenolic extracts. Additionally, the data indicate that the phenolic composition in each extract can play a crucial role in influencing the production of SCFA and indicating a health potential.

## 17: Rapid Field Biosensor for Detecting Fecal Contamination on Fresh Produce Farms

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The demand for paper-based point-of-care (POC) laboratory-based nucleic acid amplification tests (NAATs) has surged, specifically in clinical settings. However, there is an increasing need for POC NAATs tailored for on-farm applications that provide feasibility, accuracy, and rapid results. Thus, our aim was to devise an accurate, quick, and easily reproducible method for identifying Bacteroidales as a fecal contamination biomarker for fresh produce farms by employing loop-mediated isothermal amplification (LAMP). We developed a field-deployable heating and imaging system compatible with a user-friendly, paper-based biosensor, ensuring uniform heating and temperature control at 65 degrees Celsius. The heater is compact, efficiently powered, and capable of real-time heat generation. Samples from a commercial lettuce farm underwent preparation for a colorimetric LAMP assay and were placed inside the integrated device. After an hour, positive samples for Bacteroidales exhibited a color change from red to yellow, while negative samples remained unchanged. Our device displayed 100% accuracy in comparison with lab-based tests, indicating its potential for detecting fecal contamination for on-farm settings. This study highlights the ability to utilize this device as a tool within the fresh produce industry, offering on-farm deployment capabilities for rapid results. Leveraging these on-farm results can enable the development of effective strategies, particularly in the cultivation and harvesting of produce, ultimately enhancing food safety standards.

## 18: Pairwise Combinations of Biological Nitrification Inhibitors Reveal Antagonism and Synergism Against Nitrifiers and Soil Nitrification

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Nitrification is the microbially-mediated oxidation of reduced nitrogen (NH4+) to NO3–, a process that poses ecological risks and economic losses to farmers. While undesirable synthetic nitrification inhibitors have been largely used to control nitrification, biological nitrification inhibitors(BNI), bioactive metabolites released by plants to restrict nitrification favoured by NH4+ conditions, has been reported as a nature-based antidote and have been found in many Gramineae crops. We investigated the single as well as the interactive/pairwise effects of prominent BNIs (caffeic acid, vanillic acid, vanillin, and phenylalanine) which were co-exuded favoured by NH4+ nutrition in the rhizosphere of the perennial wheatgrass, Thinopyrum intermedium (Kernza®) against multiple strains of ammonia-oxidisers (Nitrosomonas europaea, Nitrosospira multiformis, Nitrosospira briensis, and Nitrososphaera viennensis) and soil nitrification. CA, VA, PHE, and VAN tested at a concentration of 500 µM individually inhibited microbial growth of the nitrifiers up to VAN (29%), VA (38%), PHE (100%), and CA (100%), depending on the strain. Pairwise combinations (CA+VA, CA+VAN, VA+PHE, and VA+VAN) test at 200 µM per metabolite, resulted in microbial inhibitions of up to CA+VA (100%), CA+VAN (100%), VA+PHE (100%), and VA+VAN (16%). Only, VA+VAN (9.60) and CA+VA (q = 14) interacted synergistically against N. briensis, while their interactions were antagonistic against all other test strains. VA+PHE resulted in synergism against N. briensis (q = 42.54) and N. tenuis (q = 3.12). While soil nitrification was reduced by the addition of each of the metabolites (up to 50%) and the pairwise combinations (up to 43%) of them, we found antagonistic interactions between them. Our findings confirm the coexistence of multiple nitrification inhibitors in the rhizosphere of T. BNI-positive crops and suggest that both synergistic and antagonistic interaction may exist between coexisting metabolites in the rhizosphere.

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## *19: Analysis of the cecum microbiome of the guinea pig: from species to fasting*

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Guinea pigs have historically served as a dietary staple in Peru and other regions, alongside being a vital model for studying human intestinal functions. The National Institute for Agricultural Innovation (INIA) of Peru introduced three guinea pig breeds—Peru, Inti, and Andina—in 2004, 2005, and 2013, respectively. Despite the importance of these breeds, limited research has explored the cecum microbiota, which could shed light on bacterial influences relevant to meat production and disease modeling. Fasting, the temporary cessation of food intake, is known to alter the microbiota in various animal species. However, its effects on guinea pig cecum microbiomes and potential breed differences remain unexplored. This study aimed to investigate the impact of fasting on the cecum microbiome across three guinea pig breeds—Andina, Inti, and Peru—and to assess the influence of host genetics on microbiota structure. Using sequencing and analysis of the V4 hypervariable region of the 16S rRNA gene, we characterized bacterial communities in cecum mucosa samples. For the species project fifteen guinea pigs were distributed into three groups according to their breed: Andina (5), Inti (5), and Peru (5). For our fasting approach, we established two treatment groups (fasting and fed), for each of the three guinea pig breeds: Andina, Inti, and Peru. The study involved twenty-eight guinea pigs, which were divided into the following groups: Andina-fed (five), Andina-fasting (five), Inti-fed (four), Inti-fasting (five), Peru-fed (five), and Peru-fasting (four). Based on the comparison between the species, we discovered that four main phyla were shared between the three breeds: Bacteroidota, Firmicutes, Spirochaetota, and Synergistota. . When we analized the groups with and without fasting, the results indicated a significant difference in beta diversity between the treatment groups for the Peru breed (P-value = 0.049), but not for the treatment groups of the Andina and Inti breeds. The dominant phyla across all groups were Firmicutes and Bacteroidetes. We observed variations in the abundance of different taxa in the cecum microbiota when comparing the treatment groups for each breed. Additionally, there was a higher number of unique taxa observed in the fasting groups compared to the fed groups. We discovered that the genus Victivallis was the only one present in all fasting groups across all breeds. However, the resilience of the gut microbiome remained largely intact across all breeds, suggesting potential adaptive mechanisms. Nevertheless, the Peru breed exhibited distinct responses to fasting, indicating a greater susceptibility to dietary changes compared to Andina and Inti breeds. Further analysis revealed unique genera associated with fermentation capacity in each breed, suggesting potential functional relationships between microbiota composition and industrial profiles. These findings underscore the importance of considering host genetics and dietary interventions in studying guinea pig microbiomes and their implications for health and production.

## *20: Use of polystyrene nanoparticles as particle models for Raman spectroscopy*

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Raman Spectroscopy is an optical technique that analyses qualitatively and quantitatively by measuring the inelastic scattering of light due to molecular vibrations. It is a useful analytical tool for identifying various materials, particles, molecules, and microorganisms. Raman spectroscopy provides i) fast data acquisition, ii) reliability, iii) minimal sample preparation, and iv) applicability to organic and inorganic materials. The advantages of Raman spectroscopy make it an analytical tool that is useful for studying microorganisms in pharmaceutical, biomedical, or environmental applications. In this work, we use polystyrene nanoparticles as model particles to help understand parameters such as size, concentration, and composition that can affect Raman scattering signals—at the same time, providing an understanding of the fundamentals of Raman spectroscopy and optimizing experimental parameters for specific applications. This study employed a range of polystyrene nanoparticles, varying in size below 1000 nm and at different concentrations. Raman spectra of polystyrene nanoparticle suspensions were collected using 96-well plates as a platform at an excitation laser of 785 nm and 100% laser power. The characteristic peak of polystyrene was detected at 1001 cm-1 with the Raman setup used in this study, while it was absent in the 96-well plate and water. When exposed to a 785 nm excitation laser with 100% laser power, polystyrene nanoparticles were unaffected in size. As particle size decreases and concentration decreases, the Raman intensity at the 1001 cm-1 characteristic peak decreases. Polystyrene nanoparticle spectra at different sizes could be collected in liquid media without implementing concentration techniques. Thorough data processing and analysis can enhance the accuracy of quantifying polystyrene nanoparticle signals within prominent peaks. By using polystyrene nanoparticles as particle models, we can analyze different parameters of Raman spectroscopy, such as wavelength, laser power, or objective lens, as well as parameters in nanoparticles, such as size or concentration in the acquisition of Raman spectra. A comprehensive understanding of these parameters and their optimization is useful when studying similar particle sizes, such as microbes or small molecules. We expect that this study can provide additional insights into the interaction of nanoparticles in the size range of other particles similar in size and how they can affect the performance in Raman spectra collection. In future work, we aim to characterize and detect bacteria and viruses with small sizes using Raman spectroscopy.

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## *21: The Impact of Autoclaved versus Non-autoclaved Diet on Cecum and Colon Gut Microbiome Composition of Rats with Chronic Kidney Disease and Normal Kidney Function*

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Background:
Dietary factors influence the gut microbiome. Our group previously showed that an autoclaved diet increased advanced-glycation end products (AGEs), and the consumption of this diet led to a faster decline in kidney function and some complications derived from kidney disease. AGEs, formed through glycation, exacerbate oxidative stress and may worsen CKD progression, leading to dysbiosis. The objective of this project was to examine if autoclaving a grain-based diet impact the gut microbiota composition of rats with chronic kidney disease (CKD) compared to normal littermates.

Methods:
We assigned 32 male CKD rats (Cy/+) and 32 normal littermates (NL) to one of two diet groups: grain-based non-autoclaved diet (NAC; Envigo Teklad Global 2018 without autoclaving) or an autoclaved diet (AC; autoclaved Envigo Teklad Global 2018) and the. Rats consumed the diet from birth until euthanasia at 32 weeks of age. We collected fecal and cecal samples from each rat, extracted DNA, and sequenced the V4 region of the 16S rRNA gene.

Results:
There were no significant differences between alpha and beta diversity based on AC vs. NAC diet. There was significant difference between the samples between cecal and fecal samples. There was no difference between the relative abundance of amplicon sequence variants (ASVs) between samples based on the diet. However, unclassified Ruminococcaceae and Bilophila had a higher relative abundance in colon samples fed with NAC diet in comparison to AC diet.

Conclusion:
Low amount of AGEs may not have an impact on the gut microbiome composition of rats with CKD and their littermates

## *22: Using metagenomics to investigate the pathogen landscape and diversity of soft rot and blackleg infected potatoes*

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Diagnostics are essential for effective plant disease management. Molecular methods, such as PCR, are frequently used to precisely identify known pathogens, but these methods can fail to identify unknown, quickly evolving, or emerging plant pathogens. In this study, we conducted a microbiome analysis to identify the potato soft-rot and blackleg pathogens, Pectobacterium and Dickeya, respectively, which threaten global seed production. As these two pathogens cause similar symptoms, co-infect, and have genetic variability, it is challenging to use typical diagnostic tools. Further these pathogens undergo frequent genomic evolution and speciation. Samples from the Wisconsin Seed Potato Certification Tissue Culture Laboratory originally tested with qPCR were inconclusive even though they displayed typical soft rot and blackleg symptoms. Therefore, we used metagenomic sequencing (MGS) as a tool to characterize the pathogen landscape of soft-rot and blackleg infected potatoes collected from Wisconsin over a 4-year period (2017-2020). Using short-read sequencing, metagenomics sequencing we confidently identified disease-causing agents for the inconclusive qPCR samples and uncovered diversity within samples. We discovered pathovars of Pectobacterium that were not tested with qPCR were present in many samples. Additionally, metrics were developed to establish clear guidelines on dictating genome completeness, utilizing the N50 and BUSCO score values as parameters. Our results provide compelling evidence to support the use of MGS for potato soft rot and blackleg diagnostics.

## 2*3: Rapid Detection of Bovine Respiratory Disease Pathogens and Associated ARGs in Cattle Using Paper-Based Loop-Mediated Isothermal Amplification (LAMP)*

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Bovine Respiratory Disease (BRD) is a major health issue in livestock, leading to significant economic losses worldwide. Prompt and precise identification of BRD pathogens and their antimicrobial resistance patterns is crucial for effective treatment and management. We introduce a novel, field-deployable diagnostic tool utilizing paper-based loop-mediated isothermal amplification (LAMP). This method enables the rapid, on-site detection of both viral and bacterial pathogens responsible for BRD from clinical samples without the need for prior DNA extraction. Our approach not only identifies the causative agents within 60 minutes but also discerns their antimicrobial resistance genes. This paper-based method's simplicity, cost-effectiveness, and accuracy make it an ideal solution for veterinary applications, ensuring timely and targeted therapeutic interventions. This advancement represents a significant leap toward improving animal health and biosecurity at the farm level.

Keywords: Bovine Respiratory Disease, LAMP, field-deployable diagnostics, antimicrobial resistance, viral and bacterial pathogens

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## 24: Quality Control for Automated Microbial Growth Experiments

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Most species of bacteria are understudied. Automated growth profiling with laboratory robots increases the pace of research on uncharacterized microbes; however, laboratory automation can make experimental errors difficult to detect. We developed three tools to improve the quality and analysis of automated microbial growth experiments. First, we developed a simple dye-based assay to monitor liquid handler performance. The assay detects changes in dispense volumes using only an absorbance plate reader and two inexpensive chemical dyes. Second, we designed an algorithm for placing positive and negative controls in microplate experiments. The algorithm searches for plate configurations that are optimal for detecting errors. Third, we built Gaussian Process regression models to correct for error due to spatial variation in microplate growth experiments. These tools address challenges that arise when experiments are scaled up with laboratory automation and provide a foundation for implementing quality control practices in biological research.

## 25: A Field Deployable Heating System for Microbial Detection Using Loop-Mediated Isothermal Amplification (LAMP) Assay

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Nucleic acid testing has become a prominent method for rapid microbial detection. Unlike polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP) is a simple method of nucleic acid amplification where the reaction can be performed at a constant temperature and the output provided in a colorimetric format. A transparent water bath is a desirable instrument to perform the heating and observe the visual results. However, existing methods of heating water are not convenient for loading and unloading the test samples. Here, we developed a field-deployable water bath—an isothermal heater called IsoHeat for short–which is solely dedicated to performing LAMP reactions and can heat the water up to 85°C. Using 3D-printing and laser-cutting technology, we fabricated different parts and mechanically assembled the parts to develop the entire device. Users can commence the heating by pressing the start button on the screen after entering the target temperature. Subsequently, the device heats up the water bath and maintains the target temperature through a PID algorithm-based control system. We demonstrate that IsoHeat can operate in environmental temperatures ranging from 5-33 °C and it can conduct LAMP reactions in liquid format as well as in paper-based devices. IsoHeat is more efficient and user-friendly compared to a commercially available immersion-heating device, which is often used to perform LAMP reactions. This newly developed device would be helpful to detect pathogens conveniently in the field (e.g., at point-of-care for human applications, on farms for plant and animal applications, and in production facilities for food safety applications).

## 26: Sub-daily virus sampling reveals population, but not community level differences at the Bermuda Atlantic Time Series

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The oceans cover two-thirds of the planet and play key roles in global biogeochemical cycling, including buffering against human-accelerated climate change. Oligotrophic oceans are nutrient-limited and thermally stratified waters that represents the majority of ocean surface area and carbon cycling in these regions need to be understood as thermal stratification and ocean nutrient loss are predicted to intensify under future climate scenarios. The Bermuda Atlantic Time Series (BATS) acts as a model oligotrophic ocean ecosystem studied via monthly sampling since 1988. Microbes in these oceans contribute to biogeochemical cycling and ecosystem function but do so under constraints imposed by viruses whose dynamics are particularly understudied. Here we complement ocean time series studies by establishing a high-resolution time series dataset (every 4 or 12 hours, respectively for surface versus deep chlorophyll maximum waters, for 112 hours) at the BATS station during 2019 late summer-stratified conditions to establish a baseline understanding of virus community dynamics using de novo viromes generated through 39 large-scale virus concentrates. Aggregated community diversity metrics such as Inverse Simpsons and bray-curtis dissimilarity were consistent at each depth over time, but distinct between depths. Abundances of particular viral populations did significantly change temporally. Viral population abundance patterns inferred from metagenomic sequencing either lacked any diel periodicity (87.01%), peaked in abundance during the daytime (11.02%), or peaked in abundance during the nighttime (1.97%). Host predictions revealed viruses with 24-hour cycles in abundance tended to have predicted hosts with day/night differences in surface metagenomic abundances. Host predictions within diel cycling viruses in the DCM were also found to be different to those in the non-diel viruses. Surprisingly, virus-encoded metabolic genes were relatively invariant between the diel and non-diel viruses, suggesting that other genes drive niche differentiation in these populations (selection analyses are ongoing). Together these findings provide baseline observations critical for incorporating viruses into predictive models of the oligotrophic oceans.

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## 27: Development of biosensor for the detection of inflammatory blood biomarker in cattle

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Haptoglobin, a glycoprotein binding free hemoglobin, serves as a biomarker for inflammation in cattle as in this species Hp gives a particularly strong response to infection, with the concentration increasing in the circulation over 100 times [1]. It plays a crucial role in diagnosing and monitoring conditions like infection and trauma. Identifying elevated haptoglobin levels enables timely treatment, preventing disease complications. Developing biosensors for on-site testing promises enhanced efficiency and timely interventions, revolutionizing disease management in remote areas.

## 28: Fecal and Urine Microbiota of Pet Dogs Vary with Breed and Sex

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Recent studies, across host species, have demonstrated a role for host-associated microbiota in cancers, metabolic disorders, urinary tract diseases, and response to therapy. In dogs, notable differences in disease incidence occur across breed; for example, Scottish Terriers have a twenty-fold higher risk of developing urothelial carcinoma compared to mixed-breed dogs. Earlier studies in dogs suggest that the fecal microbiota vary according to breed and age, but additional research is necessary to establish stronger links between breed, microbiota, and disease. Importantly, healthy urine microbiota (or the urobiome) remain generally understudied. Here, we sought to characterize the impacts of age, sex, and breed on the fecal and urine microbiota in healthy dogs. We collected mid-stream free catch urine and fecal samples from 59 dogs: Thirty-two Scottish Terriers and 27 dogs of "other" breeds (12 different breeds represented including 6 mixed breed dogs). Twenty-seven dogs were male (eight intact) and 32 were female (7 intact). DNA was extracted, and 16S rRNA amplicon sequencing was performed. Sequence processing, diversity analyses, and statistical testing were performed using QIIME2 and Prism. The fecal microbiota differed according to sex: males had significantly lower microbial diversity than females ($p=0.0017$). The fecal microbiota of Scottish Terriers were significantly more diverse than those of other breeds ($p=0.0068$). The urine microbiota differed significantly in composition by sex ($p=0.015$). Additionally, the urine microbial composition of Scottish Terriers was significantly different from those of other breeds ($p=0.019$). No differences by age were found in urine or fecal microbial diversity or composition. Our results demonstrate that sex impacts the microbiota in companion dogs. We also report that Scottish Terriers differ from other breeds in the diversity and composition of urine and fecal microbiota, warranting further investigation into the links between breed-related disease and the host - associated microbiota.

## 29: BefA Protein Induces Increased Growth in Germ-Free American Cockroaches

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The symbiotic relationship between organisms and their associated microbiota has been shown to play a vital role in host health and fitness. American cockroaches (Periplaneta americana) have diverse gut microbiomes that resemble the levels of complexity seen in human microbiomes. When reared without their normal gut microflora, these organisms exhibit notable growth and developmental issues. A previously uncharacterized protein, named BefA (Beta Cell Expansion Factor A), has been shown to restore some wild-type phenotypes in germ-free hosts. This experiment investigates whether the BefA protein can rescue several growth defects seen in germ-free P. americana. By inoculating germ-free cockroach nymphs with Escherichia coli genetically modified to either produce or not produce BefA, we found that the presence of the protein is correlated with increased growth and development. The nymphs inoculated with BefA-producing bacteria expressed larger body sizes, more consistent sizes between individuals, and faster molting than the group without BefA. These data suggest that the BefA protein plays a functional role in the symbiotic relationship between the host and its microflora.

## 30: Mapping Antibiotic Resistant Genes (ARGs) and Bacteria (ARBs) in Diverse Environmental Niches.

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An emerging concern in contemporary times—antimicrobial resistance (AMR) in livestock and humans—may lead to an era devoid of effective antibiotics. By 2050, infections caused by antibiotic-resistant bacteria (ARB) are projected to contribute to approximately 10 million deaths globally. While human activities such as misuse and overuse of antibiotics are the primary causes of antibiotic resistance, the environment also plays a crucial role in the spread of ARBs and AMR genes. Within the environmental domain, AMR can undergo several mechanisms of development and dissemination. Therefore, the goal of this study is to detect the prevalence of antimicrobial-resistant genes (ARGs) and ARBs within the environmental domain. This investigation encompasses urban, rural, and agricultural regions along the river, including the wastewater treatment plant, facilitating systematic sample collection across various seasons. We

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employ traditional culture methods targeting carbapenem and cephalosporin-resistant bacteria, complemented by molecular techniques to detect multiple AMR genes. Our findings reveal the abundance of carbapenem (~6.5 x $10^4$/L) and cephalosporin (~8.2 x $10^4$/L)-resistant bacteria across all samples, including those from rural water sites. Furthermore, multiple resistance genes such as blaSHV, blaTEM, ermB, tetM, qnrS, qnrB and aadA were identified in almost all samples, suggesting that the environment serves as a reservoir for ARBs and ARGs. Future studies will evaluate microbial community analysis to unravel the community role in this transmission. This study provides valuable insights into AMR in environmental settings for informed epidemiological and quantitative risk assessment modeling and developing effective strategies to combat AMR.

### *31: Quantitative Analysis of Nucleic Acids on Paper Sensors*

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Loop-mediated isothermal amplification (LAMP)-based nucleic acid tests on paper facilitate on-site diagnostics by enabling prompt visual interpretation of results through the use of colorimetric reporters. However, the interpretation of color gradients with the naked eye becomes challenging in the presence of complex samples or low copy numbers, often due to slow or incomplete reactions. To address these challenges, we have developed automatic color analysis software and an easy-to-assemble isothermal heater that maintains a constant temperature of 65°C. This heater is integrated with a flat-bed scanner, which captures time-series images of the colorimetric paper sensor, ensuring precise temperature control essential for LAMP reactions and high-quality image capture necessary for effective image processing. We tested our system's performance using a range of bacterial and viral genes, obtaining an accuracy exceeding 95% in interpreting results and generating a calibration curve for the quantitative estimation of target nucleic acids. Moreover, our high-throughput cartridge, comparable in size to a standard microtiter plate and equipped with a large imaging window from the scanner, enabled simultaneous LAMP reactions on up to 150 paper sensor pads, significantly enhancing throughput. The robustness and adaptability of the instruments have been demonstrated through successful testing in both laboratory settings and animal farms. This system's demonstrated ability to deliver rapid, digitized, and reliable LAMP analysis confirms its potential to revolutionize diagnostics both in the lab and in the field, establishing it as an essential tool for the further development and testing of molecular diagnostic methods.

### *32: A novel ultra-high-throughput microfluidic approach to grow and manipulate biofilms*

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Biofilms are an important form of microbial growth consisting of a single, or more commonly multiple, species in a structured 3D extracellular matrix. The spatial organization and physical attributes of the matrix confer important emergent properties on biofilms that are not present in planktonic cultures – for instance, increased resistance to physical removal and antibiotics. While interest has increased in studying biofilms due to these special properties, studies on multispecies biofilm communities remain limited. This is due in part to the combinatorial complexity of communities; the number of possible interspecific interactions increases exponentially with the number of community members. We thus aim to develop novel approaches to grow and analyze biofilms in an ultra-high-throughput manner to probe interactions in combinatorial subcommunities. By utilizing nanoliter-scale water-in-oil microfluidic droplets that serve as parallel miniaturized bioreactors, this "divide-and-conquer" conceptual and technological framework can be employed to rapidly screen multispecies biofilms and characterize interspecies interactions in a comprehensive manner or to identify subcommunities with enhanced properties of interest. As an initial step in developing this novel framework, we successfully co-encapsulated plastic microspheres in microdroplets with Pseudomonas putida, a model biofilm former, and/or Sphingopyxis panaciterrae, a robust biofilm-forming isolate from the Ann Arbor drinking water system. We observed surface-associated microbial growth whose growth dynamics mirrored those seen in milliliter-scale cultures in 96-well polystyrene plates, demonstrating the capability of this platform to support ultra-high throughput growth of parallel biofilms. Future work will focus on integrating existing tools to extract and analyze genetic materials from these microsphere-associated cells. We will then utilize these tools to grow biofilms derived from drinking water biofilms and, in combination with single-droplet barcoding, screen them for resistance to invasion by Pseudomonas aeruginosa, an opportunistic waterborne pathogen.

### *33: Viral Characterization and Real-Time Monitoring Using Raman Spectroscopy*

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Raman Spectroscopy is a technique that can analyze molecules qualitatively and quantitatively without damaging the sample. It can be used on aqueous samples, making it a preferred choice for biological samples. This technique requires minimal sample preparation and is nondestructive, which makes it an excellent Process analytical technology (PAT) tool. Previously, we demonstrated the use of Raman spectroscopy in qualitative and quantitative measurements of biologics, highlighting its ability to distinguish between several different microbes and mammalian cells.1 To further explore Raman Spectroscopy, we developed a tool to monitor the quality and quantity of viral particles in a continuous flow setup. Such a tool can accelerate applications of continuous

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manufacturing for the vaccine industry and alleviate public health by supporting continuous supply. We characterized the attenuated human cytomegalovirus (CMV) under various concentrations and flow rates. Our results demonstrate the limit of detection (LoD) (2.36 x 10^10 particles/mL) for CMV particles in continuous flow (via the Raman spectroscopy method) by addressing the effect of flow rate, concentration, and integrity of samples. Our future work focuses on improving the sensitivity of Raman spectroscopy by advancing in-line probes and integrating acoustic devices and machine-learning tools.

(1) Maruthamuthu, M. K.; Raffiee, A. H.; De Oliveira, D. M.; Ardekani, A. M.; Verma, M. S. Raman Spectra-Based Deep Learning: A Tool to Identify Microbial Contamination. MicrobiologyOpen 2020, 9 (11), e1122. https://doi.org/10.1002/mbo3.1122.

### *34: Bifidoacteria Leverage Type IVA Pili to Capture Resistant Starch and Enhance the Metabolism of an Isoluble Substrate*

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The composition and activity of the gut microbiome are largely influenced by dietary fibers, including Resistant Starch (RS) – a plant polysaccharide that evades degradation in the small intestine but can be metabolized by specialized bacteria in the colon. However, our understanding of how bacteria in the turbulent gut environment capture insoluble substrates like RS remains limited. In our study of 140 healthy participants who supplemented their diets with RS from potatoes, Bifidobacteria – specifically Bifidobacterium adolescentis and Bifidobacterium pseudocatenulatum - were found to be the most responsive bacteria, constituting up to 24% of the relative abundance. To further investigate these organisms, we isolated Bifidobacteria from fecal samples to assess their degradation of RS. We observed that cultivars of Bifidobacteria flocculate starch granules due to their attachment to RS granules via a pilus filament. When grown on RS or maltodextrin – a subunit of RS – B. pseudocatenulatum 851 induced the expression of a cluster of genes corresponding to a Type IVa sortase-dependent pilus. When pilus-expressing B. pseudocatenulatum 851 cells are exposed to resistant starch, we found that they adhered to the RS granules and more rapidly metabolized the resistant starch compared to cells that were not expressing the pilus. Similar Type IVa pilus gene clusters associated with multiple species of Bifidobacterium were found in metagenomes from the interventional dietary study. Additionally, related pilus gene clusters were identified in published metagenomes from people worldwide, suggesting that these pili are often involved in interactions between diet and the human gut microbiome.

### *35: Analysis of fungal microbiome composition of wheat subjected to different moisture and CO2 simulated storage conditions.*

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Wheat grains are naturally colonized in the field by complex microbial communities that potentially affect the quality of seeds when the crop is stored. Spoilage of the grain during storage due to fungal growth and mycotoxin contamination are associated with significant food safety risks. Though, there are only a few studies on how stored wheat grain microbial communities change in different storage conditions. In this study, we evaluated the effect of different moisture levels (12%, 15%, 18% and 21%) and open or sealed storage over a period of 0 to 50 days on the microbial communities and mycotoxin concentrations (ochratoxin and fumonisins).

Samples were collected at T0, T28 and T50 days and mycotoxins were quantified by ELISA. The level of lipid peroxidation was measured using the thiobarbituric acid reactive substances (TBARS) method. Amplicon sequence analysis using high throughput sequencing was performed to characterize the changes/shifting occurring on the fungal communities according storage conditions and time.

The results showed an increase over time in lipid peroxidation level according to moisture content. Samples at T0 were measured at 20 µM of malondialdehyde (MDA)/mg while samples at 22% moisture, under sealed storage and located at the bottom of the storage barrel after 50 days were measured at 70 µM of MDA/mg. For ochratoxin and fumonisins, the concentrations were below 1 µg/Kg at T0 for all samples. Small variations were observed over time. However, for treatment with sealed storage and 22% moisture, samples located at the bottom of the storage container after 50 days reached 50 µg/Kg of ochratoxin and 12 µg/Kg of fumonisins.

Metagenomics analysis findings were that Alternaria, Penicillium, Gibberella/Fusarium, Cortinarius and Aspergillus were the dominant core fungal taxa, which are common pathogens associated with wheat and common sources of mycotoxins. The fungal communities were found to shift according to storage conditions: time and moisture levels. Alternaria, Gibberella/Fusarium relative abundances decreased over time, whereas Aspergillus and Penicillium, responsible for most of the damage in stored grain increased in most samples. During simulated storage of wheat, fungi that are commonly associated with grains in the field were gradually replaced with those more typically associated with storage. There may be value in these results for developing superior control strategies to prevent mold infestations and mycotoxin contamination during grain storage.

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## 36: Lyophilize Loop-Mediate Isothermal Amplification method

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Loop-mediated isothermal amplification (LAMP) assay has been used to detect several different pathogens in fresh produce fields where nearby animal facilities can be a source of contamination. In the recent fieldwork trip, we found a shelf-life of paper-based LAMP assay for its best performance was only up to 3 days when paper pads were stored at 4C. It may have a longer shelf-life with lower temperatures, but not all users may have access to the freezer in the field in reality. Because of a short shelf-life, we had to prepare the assay in the lab and ship overnight to the field worrying about if we will receive them on time. This lyophilizing method was part of the big project to make a user-friendly portable system that can be used in the field for low cost and fast test results. Lyophilizing LAMP assay can save hours of reaction preparation time when running an assay for lots of samples. We also found that the shelf-life of lyophilized colorimetric LAMP assay was up to 60 days. No longer than 60 days of experiment was done, but with this result, we will be able to handle a larger scale assay in the field without worrying about assay performance due to shelf-life.

## 37: Interactions between gut microbiota, medicinal herbs and a short-term plant-based diet

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While the positive health impacts of medicinal plants have been well-documented, the exact mechanisms of actions have not been thoroughly studied. For example, their impacts on gut microbiome and interactions with different types of food remain largely unknown. Ginger and curcumin both exert anti-oxidative and anti-inflammatory effects on the human body. As a part of an evidence-based community health improvement program through promoting a healthy plant-based diet, we investigate the impacts of ginger and curcumin on participant's health metrics and gut microbiota. Healthy adult participants between the ages of 18 and 45 were recruited to partake in ginger and curcumin supplements daily for a period of one week. The gut microbial composition was analyzed by shotgun metagenomic sequencing of fecal samples. We will report current progress of studying the supplements' impacts on gut microbiota and whether these supplements work synergistically with a plant-based diet and improve a variety of health metrics. Our preliminary data identified several bacterial taxa whose sequence abundance specifically increased or decreased after the diet period with supplements (p<0.05). Interestingly, supplements seem to exert opposing impacts on the abundance of four Bacteroides species that have a known link to human immune response.

## 38: Impact of bacteriophage therapy against Salmonella Gallinarum on chicken gut microbiota

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Fowl typhoid is among the most significant poultry bacterial diseases worldwide. Its causative agent, Salmonella Gallinarum, is endemic in many developing countries with infections leading to significant bird and economic losses. Antibiotics are often used to prevent or control fowl typhoid; however, such practices contribute to the continually global challenge of antibiotic resistance. As such, the use of bacteriophages to control bacterial infections in food animals has gained significant attention. Previously, we developed a polyphage prototype that significantly reduced Salmonella Gallinarum in experimentally challenged chickens. Here, we evaluated the impact of treatment with this polyphage prototype on microbial communities that surround the targeted bacteria. Day old broiler chickens were distributed randomly into five pens according to the treatment (n=34 chickens/pen); 1) chickens receiving no Salmonella Gallinarum challenge and no phage treatment; 2) chickens receiving a Salmonella Gallinarum challenge but no phage treatment; 3) chickens receiving Salmonella Gallinarum challenge and unprotected phages (500 µL at 1010 PFU/mL; oral gavage); 4) chickens receiving Salmonella Gallinarum challenge and phages protected by microencapsulation; and 5) chickens receiving Salmonella Gallinarum challenge and a mixture of protected and unprotected phages. At 7 and 21 days of age, five chickens from each treatment group were euthanized and the microbial communities in cecal contents were characterized by 16SrRNA sequencing.

The statistical analysis revealed there was no significant differences in microbial communities of treatments according to alpha diversity metrices (Observed features, Faith's PD) while beta diversity metrices (weighted and unweighted UniFrac) represented dissimilarities in microbial composition at different age through pairwise PERMANOVA analysis (p < 0.05). Additionally, taxonomy analysis using reference sequence classifier highlighted that age was a determinant of microbiome development. Furthermore, differentially abundant taxa in treatment groups were analyzed through DESeq2, and Edge R revealing that treatment with mixture of protected and unprotected phages significantly enhanced the proliferation of genera thought to be beneficial for chicken such as Fecalibacterium, Blautia, and Lactobacillus. While chickens challenged with Salmonella and received no treatment over-represented Lactobacillus fermentum and Streptococcus reflect intestinal inflammation and antibody production. These results indicate that beyond reducing targeted bacteria in the chicken, phage treatment may play a beneficial role in modulation of chicken gut microbiota. Treatment with phage cocktail targeting Salmonella Gallinarum specifically may enhance the growth of beneficial gut microbiota in chicken and inhibit growth of potential opportunistic pathogens. Taken together, this research opens the possibilities for using bacteriophages as an effective means to control fowl typhoid without causing increases in antibiotic resistance.

Keywords: Salmonella Gallinarum, microbiota, polyphage prototype

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## 39: Raffinose Family Oligosaccharide utilization by Bacteroides

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The gut microbiome plays an important role in health and disease. Bacteroides species are successful colonizers of the human gut and can utilize a wide variety of complex polysaccharides and oligosaccharides that are indigestible by the host. For this, they use enzymes encoded in gene clusters called Polysaccharide Utilization Loci (PULs). While recent work has uncovered the PULs required for use of many dietary and host-associated polysaccharides, the specificity of many PULs remains unclear. In particular, the genes required for use of simpler oligosaccharides, including the Raffinose Family Oligosaccharides (RFOs), is unknown. In this report, we show that a novel duplication in an α-galactosidase gene BT1871, which resides in PUL24, confers an in-vitro growth advantage on RFOs to Bacteroides thetaiotaomicron. Further, mutations in the anti-sigma gene controlling the expression of this α-galactosidase also confers growth advantage to B. thetaiotaomicron. We then show that complete breakdown of RFOs requires hydrolases from another PUL working in concert with BT1871. We also determine that the master regulator of carbohydrate utilization BT4338 plays a role in controlling RFO utilization in B. thetaiotaomicron. Finally, we show that homologs of BT1871 are widespread among Bacteroides and contribute to melibiose utilization. Our findings shed light on how Bacteroides species utilize an important dietary oligosaccharide.

## 40: Establishing a Gnotobiotic Mouse Model for Studying Swine-Microbiome Interactions

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Previous human health studies have shown germ-free mice to be a useful model to determine the role of the gut microbiome on human phenotypes, like obesity. To determine the feasibility of similar research regarding the swine gut microbiome, we performed fecal microbiota transplants (FMT) from sows (n=3) and 12-week old piglets (n=3) to germ-free mice (n=24). Mice at 4-6 weeks of age were given an oral gavage containing a fecal sample from a swine donor. Cecal and fecal samples were collected from the mice approximately two weeks after receiving FMT treatment. These samples were then sequenced and analyzed for community composition. The mice utilized in the study exhibited normal behavior and growth following FMT, with no instances of mortality or morbidity observed. Recipient mice also exhibited different microbiota compositions, similar to the differences between donor sows and piglets. Sow donors and their recipients exhibited higher alpha diversity compared to their piglet counterparts. Beta-diversity measurements, such as Bray-Curtis, further revealed dissimilarities between sow and piglet donors and recipients. These beta-diversity measurements also indicated that within piglet and sow recipients, replicate mice sharing the same donor exhibited similar community compositions compared to replicate mice with a different individual donor. Bacteria from families such as Lachnospiraceae, Ruminococcaceae, and Prevotellaceae were identified in both donors and their recipients, suggesting successful colonization following FMT, with relative abundance differing based on donor type. Other bacterial families such as Lactobacillaceae were present in donors but not recipients. The success observed in this study suggests that germ-free mice could serve as a valuable model for investigating the interactions between swine and their gut microbiome, thus providing insights into the impact of the microbiome on swine health phenotypes.

## 41: Impacts of Regular Black Tea Kombucha Consumption on the Gut Microbiota of Individuals With or Without Obesity

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Objectives
To evaluate the regular consumption of black tea kombucha on the gut microbiota of individuals with and without obesity.

Methods
This is a clinical, chronic study, lasting eight consecutive weeks. Subjects were allocated into two groups, according to their features: normal weight + black tea kombucha; obese + black tea kombucha. The participants were instructed to drink 200 mL of black tea kombucha per day and maintain their usual diet and physical activity pattern throughout the study to avoid any biases. Stool samples were collected at baseline and the 8th week of treatment and stored in an ultra-freezer at -80°C until analysis. After extracting the DNA from fecal samples, the sequencing was performed considering the bacterial regions V3 and V4 of the 16S rRNA gene (primers 341F/806R) and the internal spacers transcribed from the nuclear ribosomal DNA (ITS1 and ITS2) of the fungal rRNA region (primers ITS1F and ITS2R).

Results
Thirty-seven participants completed the study: 21 from the normal weight group and 16 from the obese group. After eight weeks of intervention, kombucha favored microorganisms such as Bacteroidota, Akkermanciaceae, and Prevotellaceae and reduced the abundance of microorganisms associated with obesity, such as Ruminococcus and Dorea, especially in the obese group. There was an increase in fungal diversity, greater abundance of Saccharomyces, and a decrease in Exophiala and Rhodotorula. Pichia and Dekkera, two of the main microorganisms found in kombucha and SCOBYs, were identified as biomarkers after the intervention.

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Conclusions
Our study is the first clinical trial investigating the impacts of regular kombucha consumption on the gut microbiota. The results suggest that regular kombucha consumption could modulate the gut microbiota of individuals with or without obesity, and the results were even more promising in the last group. However, we emphasize the importance of a balanced diet on the overall health.

## *42: Do nutrition interventions improve gastrointestinal symptoms during cancer treatment? A systematic review and meta-analysis.*

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Cancer therapy is associated with various gastrointestinal (GI) symptoms. The most common symptoms: nausea, diarrhea, vomiting, and constipation can be dose-limiting, potentially leading to treatment failure and reduce overall patient quality of life. Preventing weight loss and malnutrition are common targets of nutrition therapy in these patients, but less attention has been given to their ability to reduce GI symptoms. More specifically, recent findings have been leaning towards targeting the gut microbiome using prebiotics, probiotics, or synbiotics. The microbiome has been an emerging field for disease progression/recession, metabolite production, and immune function. During cancer therapy, the microbiome is modified eventually influencing the intensity of these symptoms. Therefore, the objective of this study was to assess the ability of nutrition therapy to reduce GI side-effects of cancer treatment via systematic review and meta-analysis of the literature.

A systematic keyword search was conducted in Scopus and PubMed databases. The search algorithm included all possible combinations of terms from the following 3 groups: (1) GI Symptoms; (2) Cancer Treatment; and (3) Nutrition. Potentially relevant articles were screened by title and abstract, then full text against the study inclusion criteria. A meta-analysis was performed on articles meeting inclusion criteria to estimate the pooled effect size on GI symptoms, divided by nutrition intervention type (oral intake, oral nutrition supplement [ONS], or dietary counseling). Subgroup analyses were further conducted based on cancer therapy, and specific nutrient intervention. All statistical analyses were performed in Stata using 2-sided tests with p < 0.05 as the threshold for statistical significance. 16,013 articles were captured by the search algorithm, and 144 studies met inclusion criteria for meta-analysis. Articles reported 13 different GI symptoms, resulting in 238 total meta-analyses by symptom, cancer treatment, and nutrition intervention subtype. Meta analyses indicated that probiotic supplementation had one of the strongest effects on reducing diarrhea (p < 0.001), nausea (p = 0.01), and vomiting (p = 0.02) incidences regardless of cancer or treatment type. Fiber supplementation strongly reduced mucositis incidence (p < 0.001) but had no effect on diarrhea incidence (p > 0.05). Synbiotic supplementation reduced diarrhea incidence (p=0.04) but had no effect on both mucositis and constipation incidence (p > 0.05).

This meta-analysis supports the use of specific nutrition therapies in treatment of cancer therapy induced GI symptoms and identifies those that require additional investigation. As the microbiome may be a potential target for controlling GI symptoms, it differs from person to person and may be influenced by cancer location and treatment. Future studies should explore personalized nutrition strategies to combat these symptoms.

## *43: Gut Bacterial Metabolite 3-Phenylpropionic Acid Alleviates Acetaminophen-induced Hepatotoxicity by Inhibiting Cytochrome P450 2E1 Expression*

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Acetaminophen (APAP)-induced hepatotoxicity has been a major cause of acute liver failure worldwide and is caused by APAP bioactivation into a toxic metabolite by hepatic drug-metabolizing enzymes. The gut microbiota has emerged as a modulator of the hepatic expression of drug-metabolizing enzymes and may contribute to inter-individual variability in APAP-induced hepatotoxicity. Our group previously reported that a gut bacterial metabolite 3-phenylpropionic acid (PPA) alleviates APAP-induced hepatotoxicity in mice. This was accompanied by decreased hepatic protein (but not mRNA) levels of cytochrome P450 2E1 (CYP2E1), the major enzyme responsible for APAP bioactivation. To elucidate the mechanisms of PPA action, PPA effects on APAP toxicity and CYP2E1 expression were investigated in primary mouse hepatocyte culture and AML-12 mouse hepatocyte cell line. PPA, at physiological concentrations, decreased APAP-induced cytotoxicity (determined by the lactic dehydrogenase, LDH, release) in both primary mouse hepatocytes and AML-12 cells. Furthermore, we observed a decreased CYP2E1 protein expression in the PPA-treated hepatocytes, suggesting that the previous in vivo findings in PPA-supplemented mice are attributable to the direct effects of PPA on CYP2E1 expression. Unlike in mice, however, PPA treatment decreased the mRNA levels of CYP2E1 in primary mouse hepatocytes. Blocking protein synthesis through co-treatment with cycloheximide minimally impacted the decrease in CYP2E1 protein levels induced by PPA, supporting the minimal effects of PPA on the degradation rate of CYP2E1 protein. These results suggest the role of PPA in the transcriptional regulation of CYP2E1 in primary mouse hepatocytes. Elucidation of detailed molecular mechanisms underlying PPA action in primary mouse hepatocytes and AML-12 cells is underway. Overall, revealing the detailed mechanisms of PPA lays the foundation for a better understanding of how the gut microbiota impacts drug-induced liver toxicity.

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## *44: Fusobacterium nucleatum Metabolites Promote Colorectal Cancer Cell Proliferation through Activating Aryl Hydrocarbon Receptor*

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Fusobacterium nucleatum (Fn) has gained attention due to its role in the progression of colorectal cancer (CRC). However, the identities and biological role of Fn-derived metabolites in colorectal cancer remain largely unknown. Aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that binds to a broad spectrum of chemicals. Dysregulation of AhR is implicated in various diseases, including CRC. We hypothesized that AhR could detect previously unidentified metabolites produced by Fn. To validate this hypothesis, we extracted the culture supernatants of Fn with ethyl acetate, dried, reconstituted them in DMSO, and tested for AhR activation. Organic extracts, ranging from the log phase to the stationary phase, activated AhR in HepG2 cells, suggesting the presence of AhR-activating metabolites in Fn culture. Using activity-guided fractionation and various spectroscopic methods (high-resolution MS and 1D & 2D-NMR), we identified three metabolites (Fusotrisindoline; FTIN, Trisindoline; TIN, Streptindole; STIN) as AhR agonists. Among the three metabolites, Fn predominantly produces FTIN under our culture condition, which also serves as the most potent activator of AhR. Cell-based ligand competition assay demonstrated that these metabolites interact directly with AhR. In vitro cell proliferation assay showed that FTIN promotes the proliferation of CRC cells (H508, SNU-C4). This effect was abolished when AhR was suppressed either through siRNA-mediated knockdown or pharmacological inhibition. Considering the enrichment of Fn in CRC tissues, we explored whether Fn could promote CRC progression by activating AhR. Fn enhanced the cell proliferation of CRC cells. However, tnaA deletion mutant of Fn, lacking the ability to produce FTIN, showed weaker enhancement in CRC cell proliferation. in vivo mouse model using SNU-C4 xenograft showed Fn enhanced tumor growth, while tnaA deletion mutant did not show any enhancement. We measured FTIN levels in both human CRC and corresponding adjacent normal colon tissues and observed a significant elevation of FTIN levels in the cancerous tissues when compared to their normal counterparts. In summary, we have discovered new AhR-activating Fn metabolites that contribute to the advancement of CRC. These findings establish a solid basis for a more comprehensive understanding of Fn's role in the CRC progression.

## *45: A prediction model of tulathromycin antibiotic treatment success in pre-weaned dairy calves with bovine respiratory disease*

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Bovine respiratory disease (BRD) is a bacterial disease which causes respiratory illness particularly common in beef and dairy calves in which microbes infect the lung cavity, leading to morbidity and mortality. BRD has been identified as the leading cause of death in pre-weaned dairy calves, leading to significant economic consequences for producers because of animal death, reduced feed efficiency, and treatment cost. BRD is typically diagnosed on-site based on visual signs including coughing, nasal discharge, fever, labored breathing, or decreased appetite or water intake. Due to the lack of specificity in determining the bacterial cause of BRD, it can be difficult to determine which antibiotic treatment would be most successful.

Many farms regularly collect animal data to document events such as birth weight, serum protein levels, movement records, feed intake, and pneumonia treatments. The goal of this experiment is to develop a machine-learning algorithm based on animal metadata which could predict antibiotic treatment success. A large five-year data-centric dataset from a local Indiana dairy was cleaned and translated into an animal-centric format. A random forest model was employed to determine correlation between factors and treatment successes, which will then be applied to use in farm settings as a rapid antibiotic determinant. It was discovered that milk intake, feeder approximation, and date of treatment had the highest influence on treatment success of the antibiotic tulathromycin. Lowering the treatment decision time will increase likelihood of treatment success and therefore decrease economic impact from morbidity and mortality.

## *46: Functional characterization of a GH43 enzyme from Bacteroides in Sorghum arabinoxylan cleavage*

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Arabinoxylan, a complex plant polysaccharide, serves as a carbon source for diverse microbial communities. This study investigates the functional diversity of glycoside hydrolase family 43 (GH43) enzymes within such a community enriched on white sorghum arabinoxylan. Metagenomic analysis revealed a remarkable abundance and diversity of GH43 family genes, suggesting a potential for distinct enzymatic functionalities despite shared ancestry. We hypothesize that these enzymes target specific sites on the arabinoxylan molecule, contributing to its efficient degradation by the microbial consortium.

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To unravel this functional diversity, we employ a molecular biology approach. Individual GH43 genes will be isolated from the enriched microbial community through targeted gene cloning and expressed in a suitable host organism. This will allow for the isolation and characterization of each enzyme's specific activity and substrate specificity. By analyzing the enzymatic properties of these GH43 isolates, we aim to:

Identify distinct functionalities: Determine the enzymatic activities of each GH43 isolate, focusing on their ability to cleave different glycosidic linkages within the arabinoxylan structure.

Characterize substrate specificity: Define the preferred target sites on the arabinoxylan molecule for each enzyme, elucidating their roles in the overall degradation process.

Uncover synergistic interactions: Investigate potential synergistic interactions between different GH43 enzymes within the consortium, leading to a more comprehensive understanding of the cooperative degradation mechanism.

This project will provide valuable insights into the functional repertoire of GH43 enzymes within a microbial consortium degrading arabinoxylan. By elucidating the specific contributions of individual enzymes, we aim to gain a deeper understanding of the intricate mechanisms underlying efficient polysaccharide breakdown by microbial communities. This knowledge can contribute to the development of novel strategies for bioconversion of plant biomass into valuable products.

### *47: Transport and fate of manure-borne antibiotic resistance genes in flowing water*

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Antibiotic use in animal husbandry represents the primary source of antibiotic consumption worldwide. In 2020, farm animals were administered more than 99,000 metric tons of antimicrobials. Antibiotic Resistance Genes (ARGs) are commonly found in cattle manure, frequently employed as a soil amendment, and capable of contaminating nearby ditches. Despite ARGs being frequently detected in water ecosystems, their transport and fate in streams and rivers remain poorly understood.

To address this knowledge gap, we conducted experiments in an artificial stream to estimate removal rates from the water column (k; m-1) for three ARGs (mefA, tetQ, and tetW) and a ruminant fecal marker (bacR). We then compared the intra-target and across-target k variability utilizing ANCOVAS. We then optimized a Stochastic Mobile Immobile Model (SMIM) to disentangle the overall removal into two components: rs and rh, which refer to temporal immobilization rates (sec-1) in the surface (i.e., water column) and the subsurface (i.e., streambed), respectively. Finally, we applied the SMIM framework across four streams to theoretically predict the downstream travel distance of ARGs and bacR.

Our results demonstrated measurable removal for all the targets in all experimental replicates (n=3). Our results suggest that bacR might be valuable for inferring contamination of ARGs like mefA and tetQ from cattle manure, but it could overestimate tetW concentration in freshwater streams. We found that rh values were orders of magnitude larger than rs for both ARGs and bacR (t-test; p < 0.05). These findings suggest that ARGs and bacR are being removed from the water column through immobilization reactions occurring in the streambed. We also predicted that the 90% removal (or T90) of targets occurs within the first 500 m in all streams except in a slow-flow pastoral stream where ARGs can travel for up to 1400 m downstream.

Our model does not differentiate between the individual processes that contribute to the removal of targets from the stream. Instead, it lumps all storage zone processes into a single rate represented by rh. We thus propose that these removal reactions might include sorption events (e.g., adsorption and absorption); biochemical breakdown of DNA; assimilatory uptake mediated by biofilms; predation by protozoa; and natural decay of bacteria. We believe that most of these reactions occur in the benthic biofilms at the sediment-water interface, which might be driving the removal of ARGs and bacR from the water column.

Our study establishes a baseline for comprehending the transportation of antibiotic-resistant genes found in manure through flowing surface waters. To our knowledge, this is the first study that robustly characterizes the transport characteristics of ARGs by employing manure injection in a stream and a stochastic modeling tool (i.e., the SMIM) to understand the influence of the subsurface in the removal of targets. We show that ARG removal is dominated by reactions occurring in the subsurface, including on benthic surfaces and in the hyporheic zone. Application of the model to different types of streams allowed us to analytically predict a T90 that theoretically the distance at which 90% removal of ARGs and bacR would occur in a specific stream scenario. Our results suggest a need for a holistic water monitoring strategy for manure-impacted streams, including analyzing both water and sediments. removal on the ecology of streams and rivers.

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## 48: Analysis of Temporal and Geographic Variation in Human Pathogens for Wastewater-Based Epidemiology (WBE) Applications

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Wastewater-based epidemiology (WBE) has become a primary method of tracking human pathogenic viruses since the onset of the COVID-19 pandemic. WBE allows for the surveillance of many pathogens from a singular source without having to collect clinical samples and can inform community-scale public health decisions. Many methods have been published to concentrate and quantify viruses from wastewater in WBE. Yet, many still need to consider the specificities of viral targets, small-scale temporal shifts in concentration, or variations of the sewage matrix. Here, we demonstrate the need for a pathogen-specific WBE sampling and sample processing approach. Samples of primary influent were collected from a Northern Indiana wastewater treatment plant over 21 days to demonstrate daily shifts in pathogen presence and concentrations, and physiochemical tests were performed. Samples were also collected from various wastewater treatment plants across the continental United States to show variation by geographic location. PCR was performed on all samples for common viral indicators and pathogens. Samples were also sequenced using the QIAseq xHYB Adventitious Agent Panel, which enriched sequences for 132 pathogens of interest. PCR results indicate consistent signals from viral indicator targets, measured with CrAssphage and PMMoV, but 1-2 log10 day-to-day spikes occurred for all assayed human pathogens. The sequencing data also demonstrates a significant daily change in abundance for some pathogens, while others had a consistent signal across the entire sampling period. Thus, WBE sampling frequency needs to be designed to fit the pathogen of interest, as sampling once weekly will fail to detect the presence or fluctuation of some diseases. We also report significant variations in alpha diversity based on geographic location, demonstrating the need for meticulous experiment design in WBE.

## 49: Intestinal and hepatic histology is altered by soluble fiber consumption in an FXR/SHP-dependent manner

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Intro: Farnesoid X recepteor (FXR) is a nuclear bile acid (BA) receptor with multiple physiological functions across disparate organ systems. Such functions include BA synthesis, BA trafficking, and regulation of cellular proliferation, which highly influences function of organs such as the intestine and liver. However, BA composition varies across the gut-liver axis, thus influencing FXR activation and function. Furthermore, dietary modification, particularly fiber consumption, alters host and microbial BA metabolism. The objective of this study was to understand how soluble fiber alters enterohepatic histology in an FXR-dependent manner.

Methods: A total of (N=46) 9–10-month-old mice were utilized in a 2x2x2 study design (diet by genotype by sex). Diets were a fiber-free diet (FFD) vs a 10% beta-glucan, 10% cellulose diet (BG). Genotypes included wild type (WT) vs FXR-SHP double knock out mice (DKO). Hematoxylin and eosin staining was used to identify crypt depth and villus height morphology along the gastrointestinal tract. Liver histology was examined using Halls bile and trichrome staining.

Results: Bile staining indicated more hepatic bile accumulation in WT-FFD vs all other groups (all p < 0.05). Trichrome staining demonstrated less collagen formation in WT-BG compared to all other groups (all p < 0.05). Ileum villus height and crypt depth were higher in DKO vs WT (p=0.004 and p=0.003 respectively), and females compared to males (p=0.03 and p=0.007 respectively). Cecum crypt depth was lower in WT-FFD vs all other groups (all p < 0.05). Distal colon crypt depth was lower in WT-BG vs all other groups (all p < 0.05).

Discussion: Both hepatic and intestinal histology were impacted by soluble fiber consumption in both segment and FXR-dependent manners. This highlights the importance of FXR signaling on the structure and function of the enterohepatic axis. Ongoing studies are investigating expression of genes regulated by FXR related to gut-liver axis physiology.

## 50: Application of Dye/Enzyme treatment for removing free nucleic acids in wastewater

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Wastewater plays a crucial role in monitoring the presence of pathogens and genetic material, such as DNA and RNA, which can pose risks to human and environmental health. Molecular methods, such as PCR and metagenomic sequencing, are powerful tools to determine pathogen presence and abundance but are fundamentally challenged by their ability to discriminate nucleic acids from viable versus non-viable microorganisms. Multiple methods exist to exclude free nucleic acids, including intercalating dyes and nuclease enzymes; however, all current methods have well-recognized limitations that have challenged their broad adoption. This study compared multiple representative free nucleic acid removal methodologies in wastewater, including propidium monoazide (PMA), RNase, DNase, and combinations. In particular, the removal of representative native viral fecal indicators, cross-assembly phage (crAssphage) and pepper mild mottle virus (PMMoV) was evaluated. The results demonstrated that combining PMA and enzyme-based pretreatment approaches effectively

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removed free nucleic acids from wastewater samples. Specifically, 21% crAssphage and 35% PMMoV were removed from the wastewater samples. Sequencing outcomes revealed the high removal efficiency of this approach in removing Alphapapillomavirus, Mamastrovirus, Roseolovirus. However, some double-stranded DNA genomes, such as Adenovirus, Betapolyomavirus, and Dependoparvovirus, resisted these pretreatment methods. The developed protocol presents a specific, sensitive, and reliable approach to wastewater analysis, enabling the detection and quantification of viable pathogens. The findings highlight the importance of implementing pretreatment strategies to assess and quantify viable genetic material in wastewater samples accurately.

### *51: Baseline gut microbiome alpha diversity predicts gastrointestinal symptoms in breast cancer patients receiving chemotherapy*

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Chemotherapy treatment frequently causes debilitating gastrointestinal symptoms (diarrhea, nausea, vomiting), which are inadequately managed by current treatments. Recent research indicates that the gut microbiome plays a significant role in the pathogenesis of these side effects. The aim of the current study was to identify pre-chemotherapy gut microbiome markers that predict gastrointestinal symptom severity after chemotherapy in patients with breast cancer. Participant factors that modulated the predictive microbiome markers were also examined, as well as potential underlying mechanisms of the gastrointestinal side effects (microbiome disruption, intestinal inflammation or permeability). Fecal samples, blood, and gastrointestinal symptom scores were collected from 59 breast cancer patients before, during, and after chemotherapy treatment. Lower pre-chemotherapy microbiome alpha diversity and the abundance of specific microbes (e.g., Faecalibacterium, Lachnospiraceae FC020 group, and Lachnospira) predicted greater chemotherapy-induced diarrhea and nausea/vomiting symptoms. Participant factors associated with lower pre-chemotherapy microbiome alpha diversity were human epidermal growth factor receptor 2 (HER2) positive tumor status, an intact primary tumor (neoadjuvant status), and a greater proportion of dietary calories from carbohydrates. Lower baseline alpha diversity also predicted higher microbiome disruption with chemotherapy, which was positively associated with concurrent diarrhea symptoms. The results indicate that certain types of cancer patients are more likely to have lower microbiome diversity before chemotherapy treatment, which is predictive of greater gastrointestinal symptoms and a less resilient microbiome during chemotherapy. These patients may be strong candidates for microbiome-directed preventative interventions (e.g., diet change) prior to chemotherapy.

### *52: Development of a LAMP-based Paper-pad Biosensor for Genetic Trait Detection of Corn (Zea mays) and Soy (Glycine max)*

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Field-based genotyping emerges as a pivotal tool for monitoring seed production quality and facilitating prompt remediation actions if necessary. This approach also allows for the confirmation of trait identity thereby ensuring stringent quality control measures. In our study, we investigated the efficacy of paper-based Loop-Mediated Isothermal Amplification (LAMP) of nucleic acid (DNA) as a viable method for field genotyping for corn and soy. We validated DNA-LAMP assays tailored for corn and soy traits. The optimization of LAMP assays and leaf sample extractions for integration into paper-based test cartridges was meticulously carried out. This innovative approach promises to deliver on the crucial requirements of being easy, rapid, robust, cost-effective, and featuring a straightforward readout, thus offering a practical solution for field-based genotyping needs.

### *53: How the gut microbiome can be shaped by the use of complex dietary fibers in food*

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Lifestyle changes over the past century contribute to microbiome disruption and loss of beneficial microbes. The Western diet, low in fiber and high in fat and sugar, contributes to the bacterial depletion and chronic inflammatory diseases. These conditions can be prevented with the increase of fiber intake, by promoting their use in the food industry. However, knowledge of which dietary fibers promote which microbial taxa is still lacking. To address this gap, we carried out a pilot fermentation study, incubating fecal samples from two individuals with three dietary fibers (inulin, pectin, and dextran (Weissella cibaria)) with different levels of complexity (i.e. polysaccharide length, branching). We hypothesized that the level of complexity would influence the fermentability and the bacteria able to use them. We predicted that less complex fibers would be more accessible to bacterial species with higher growth rates. We sampled incubations at 0-4-8-24-32-48h to assess the long-term impacts of fiber consumption on the microbiome. Through 16S rRNA sequencing, we found a similar microbial shift across fiber treatments during the first 8 hr, but changes became fiber-specific after 24 hours. These results showed how it is important to use long incubation experiments to characterize fiber's prebiotic properties and that more complex fibers have a more beneficial impact on the GM.

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## 54: Predicting host-microbiome interactions: integrating iColonEpithelium and microbial community-scale metabolic models

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The gut microbiota plays a predominant role in dietary nutrient metabolism and absorption in the colon. Host-microbiome interactions are complex, and we need tools for assessing the biochemical exchanges between these systems. Genome-scale metabolic models (GEMs) are powerful tools for exploring exchanges between host-microbiome systems. To understand the underlying mechanisms involved in host-microbiome interactions, our present work focuses on modeling colonocytes, the dominant human colonic epithelial cell type. We used a combination of four methods (iMAT, tINIT, CORDA, and pymCADRE) and the generic human reconstruction, Recon3D, to generate the first-ever human colonocyte GEM, which we call iColonEpithelium. We tested the specificity of our consensus reconstruction using the information from 47 reported metabolic functions known to be essential for human colonocytes. In order to facilitate the exchange of metabolites between iColonEpithelium and a microbial community-scale metabolic model (MCMM) of the gut microbiota, we added appropriate transport reactions. The resulting iColonEpithelium contains 6551 metabolic reactions, 4072 metabolites, and 1954 genes. The metabolic reconstruction can use short chain fatty acids, such as acetate and butyrate, to maintain biomass, which is in accordance with our targeted metabolomics data from cell culture. We tested the predictive power of the iColonEpithelium model by integrating colonocyte-specific single cell RNA seq data from Crohn's disease (CD) and Ulcerative colitis (UC) samples. The iColonEpithelium GEM predicted increased metabolic flux of GABA catabolism in UC colonocytes, and increased uptake of hydroxy metabolites in CD colonocytes. Our ongoing work on integration of iColonEpithelium GEM with MCMM models built using MICOM (in collaboration with the Gibbons Lab) will capture essential metabolic activities between the host and the gut microbiota, including the production of short chain fatty acids (SCFAs) from the MCMMs and the utilization of SCFAs by the iColonEpithelium GEM as the main energy source. Our work has the potential to predict personalized host-microbiome interactions in response to dietary intake.

## 55: Psychological stress and dietary fiber regulate markers of inflammation and anxiety

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INTRODUCTION: Prolonged psychological stress exposure compromises intestinal barrier integrity, changing interactions with gut-associated microorganisms that influence inflammation. Translocation of microorganisms into host circulation stimulates the release of proinflammatory molecules such as chemokine CCL-2 and LBP both locally and systemically. These immune responses can trigger neuroinflammation, a major contributor to the development of psychopathological conditions including depression and anxiety. Conversely, via understudied mechanisms, prebiotic dietary fiber restores intestinal barrier function and ameliorates anxiety, suggesting that it may be a promising therapeutic for neuroinflammation-driven psychopathological conditions. Therefore, the purpose of this study was to examine the effects of psychological stress and prebiotic fiber supplementation on serum CCL-2, LBP, and anxiety-like behavior. We hypothesize that stress induces anxiety-like behavior and elevates serum inflammatory markers, which are normalized by dietary fiber consumption.

METHODS: Male and female C57BL/6 mice (age 6-8 weeks, n=6, N=96) were randomized into sixteen groups with factors of sex (male/female), two weeks of diet (1. Fiber-free diet [FFD] (base diet), 2. FFD + 20% cellulose [CELL] [non-prebiotic fiber control], 3. FFD + 10% cellulose + 10% short-chain fructooligosaccharide [scFOS] [short-chain prebiotic fiber], or 4. FFD + 10% cellulose + 10% inulin [INU] [long-chain prebiotic fiber]), and two hours of daily restraint stress exposure during the second week on diet (stressed [S] vs non-stressed [NS]). On day one of restraint, blood was collected at 0, 30, 60, 90, 120 min, and one-hour post-stress (180 min) for quantification of CCL-2 and LBP via ELISA. Mice underwent open field and nest building tests to assess behavior.

RESULTS: S mice had higher nestlet scores than NS controls (p=0.0062). Anxiety-like behavior z-scores did not differ by stress or diet, but females had significantly lower z-scores than males (p=0.0043). Serum [MCP-1] did not differ by restraint stress but increased from the 0-minute timepoint in all diets (p<0.0001). scFOS and INU had higher [MCP-1] than FFD and CELL at 180 minutes (p=0.0409). Serum [LBP] did not differ by restraint stress but increased from the 0-minute timepoint in all diets (p<0.0001). scFOS and INU had lower [LBP] than FFD and CELL at the 0-minute timepoint (p=0.0415).

CONCLUSIONS: Repeated sampling stress, but not restraint stress, enhances markers of inflammation in a diet-dependent manner. Anxiety-like behavioral responses were variable, but nestlet test outcomes indicate involvement of stress-responsive regions of the brain. Future work will examine how stress-induced intestinal barrier dysfunction and related host-microbiota interactions influence neuroinflammation in stress-responsive centers of the brain.

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## 56: The Impact of Anorexia Nervosa on the Gut Microbiota

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Anorexia nervosa (AN) is an eating disorder with a high prevalence of gastrointestinal dysfunction. The activity-based anorexia (ABA) is a well-established rodent model that resembles the psychological behavior of AN along with intestinal dysfunction. We hypothesize that gut microbiota changes will occur in adolescence and persist to adulthood in this model. Adolescent female rats were assigned to one of four treatment groups. The first group was allowed access to running wheels and ad libitum (unrestricted) access to food (AL+). ABA group had access to wheels with restricted access to food (2h/day) for four days. Ad libitum access to food was returned at the end of the four-day ABA period, and wheels were locked. The third group did not have access to wheels and had ad libitum access to food (AL). The final group did not have access to wheels and had the same time restriction on food as the ABA group (TR). Rats were euthanized after two bouts of ABA. We found that ABA and TR groups gained less weight than AL and AL+ groups. Shifts in the gut microbial community were observed. Interestingly, the gut microbiota of ABA and TR groups were similar to each other, and distinct from the AL and AL+ groups, suggesting that the gut microbiota is sensitive to the feeding paradigm. Our data suggests that the ABA paradigm impacts the gut microbiome in adolescent rats. Another cohort of rats will be euthanized in adulthood to assess the long-term impact of AN in the gut microbiome.

## 57: Exploring the role of intraperitoneal immune cells in the communication between the gut microbiome and the brain

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Over 500,000 patients receive chemotherapy treatment every year. Chemotherapy-treated patients commonly report brain-mediated side effects (e.g., cognitive decline, fatigue, anxiety) that lessen their quality of life. Both preclinical and clinical studies have identified these brain-mediated side effects are associated with neuroinflammation and systemic inflammation, indicating that immune activation is an underlying mechanism. Our published work demonstrates that chemotherapy induces gut microbiome alterations, and that the transfer of gut microbes from chemotherapy-treated mice alone is sufficient to induce peripheral and central inflammation in germ-free mice. The mechanisms mediating this gut microbiome-induced inflammation remain unknown. Despite the presence of patrolling immune cells in the peritoneal cavity (i.e., intraperitoneal immune system [IpIS]) and their proximal distance to the intestines, the relationship between the gut microbiome and the IpIS has not been elucidated. Mounting evidence indicates that alterations in the gut microbiome affect the proportion and/or functionality of large peritoneal macrophages (LPMs) and B cells - the main immune populations in the IpIS. This strongly suggests a gut microbiome-IpIS communication axis. However, few studies indicate a relationship among the IpIS and peripheral/central inflammation. Our overall hypothesis is that the IpIS participates in the propagation of pro-inflammatory signaling via the gut microbiome-blood-brain axis. As a first step, here we focused on the effects of gut microbiome manipulations on the IpIS (e.g., relative abundance of different immune cell populations and inflammatory activity) and inflammatory signaling in the circulation.

Young (8-9 weeks) and retired breeders (14-15 months) c57BL6 female mice were used for these studies. First, we evaluated the effects of chemotherapy administration on the IpIS. Next, to elucidate if observed changes were due to the direct chemotherapy administration or to the chemotherapy-related gut microbiome alterations, we manipulated the gut microbiome by using oral antibiotic administration (known to reduce gut microbiome alpha diversity) and evaluated: 1) relative abundance of the main immune cells populations (flow cytometry), 2) gene expression of inflammatory markers (RT-qPCR), and, 3) release of inflammatory cytokines (multiplex immunoassays), in the IpIS and in blood. We included a preliminary study on antibiotic-treated aged mice, known to have reduced gut diversity, to address potential age-dependent effects.

Our results demonstrate that direct chemotherapy administration decreases the relative abundance of LPMs and B cells in the IpIS, while increasing the relative abundance of neutrophils. We also demonstrate that antibiotic-induced gut microbiome alterations modestly increase the inflammatory state of the IpIS cells, although the presence of cytokines is decreased in the peritoneal fluid and tend to decrease in the blood. Lastly, although we did not observed changes in the immunoprofile of the IpIS in young antibiotic-treated mice, antibiotic-treated aged mice display a significant reduction of B1-cells compared to vehicle-treated mice, suggesting that changes in the IpIS due to the gut microbiome might differ in an age-dependent manner.

Overall, our results suggest that the IpIS participates in the gut microbiome-blood axis communication, implying that this route may be a potential therapeutic target by which to modulate neuroinflammation.

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## 58: Harnessing Microbiome Associated Phenotypes for Sustainable Nutrient Retention in Agroecosystems

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Rhizosphere plant-microbe interactions govern availability of nutrients and are a target for improving crop sustainability. However, domestication and breeding practices focused on aboveground traits have inadvertently altered the rhizosphere microbiome and microbial functions that contribute to sustainability and environmental quality (nutrient acquisition, nutrient retention, and GHG production). We hypothesized that distinct plant genotypes possess varying abilities to recruit microbial functional groups, and ultimately that microbiome function can be treated as a selectable plant phenotype and optimized through plant breeding.

Our previous work showed distinct N cycling microbial communities between maize genotypes representing the endpoints of directed evolution, and also among germplasm selected under different levels of fertilization. Our results showed significantly lower rates of nitrification and denitrification in ancestral lineages of maize (teosinte). Maize-teosinte near-isogenic lines were used to narrow down the genetic region associated with these nutrient retention traits to explore the mechanistic basis for these microbiome-associated phenotypes.

We generated experimental hybrids possessing the biological nitrification inhibition (BNI) trait and demonstrated enhanced N accumulation with no yield penalty. We paired these hybrids with N-fixing inoculants, seeking to optimize the sustainability of N provisioning and N retention. Our results indicate that the BNI phenotype is associated with enhanced N accumulation when paired with an N fixing inoculant, offering support for a chain of events where diazotrophs add ammonium to the system and the BNI phenotype aids in retention and plant accumulation. This is a valuable synergy, as our results also suggest that N fixing inoculants can elevate nitrogen losses through nitrification.

We also evaluated the potential for biological denitrification inhibition (BDI) to reduce N losses and GHG production at the ecosystem level. In addition, we explored synergy between N-fixing inoculants and N retention mechanisms, potentially reducing the need for fertilizer N addition to fields. Though the 2023 drought generally reduced microbial activity, we observed suppression of denitrification by BDI maize lines at later growth stages. This resulted in reduced N2O production and increased plant N accumulation (in conjunction with a diazotroph inoculant and a supplemental carbon source), and suggests that we are realizing some synergy between the BDI trait and N-fixing inoculants.

Our results link host-associated microbiome and ecosystem function, and demonstrate the genetic capacity to optimize recruitment of N-cycling microbes to improve crop sustainability. Identifying microbiome-associated phenotypes will allow breeders and ecosystem scientists to select crop cultivars that improve the efficiency and sustainability of agriculture and protect environmental quality.

## 59: Characterization of the Gut Microbiome in Sri Lankan Asian Elephants (Elephas maximus): A Comparative Study

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The gut microbiome is a diverse community of bacteria and fungi living within the gastrointestinal tract of animals, serving a variety of important functions related to digestion and immunity. Across the animal kingdom, its composition has been shown to be impacted by many different variables including diet and environment. However, many species, such as the Asian elephant (Elephas maximus) have gone understudied in gut microbiome research as the location of field work and collection poses unique challenges for researchers. In this project, I extracted DNA from 118 fecal samples for characterization of the gut microbial communities in wild Asian elephants from two locations in Sri Lanka. DNA extractions were performed using the Qiagen DNeasy PowerSoil kit, and PCR (Polymerase Chain Reaction) was performed on products to amplify the V4 region of the 16S rRNA gene. Gel electrophoresis confirmed that DNA was extracted from each sample, as well as confirmed that the negative controls showed no contamination. PCR products were then sent to Rush University Genomics and Microbiome Core Facility for sequencing. Sequencing results were analyzed using the program QIIME2 (Quantitative Insights Into Microbial Ecology, v2022.8), and the DADA2 plugin was used to filter quality, denoise, and replicate the sequences. ASVs (Amplicon Sequence Variants) were generated and used to create a phylogenetic tree, and taxonomy was assigned at the phylum and genus level. An alpha-rarefaction command was run and beta diversity, including Weighted UniFrac, UnWeighted UniFrac, Bray-Curtis, and PCoA plots were generated along with alpha diversity, including Shannon, Faith's PD, and Observed OTU's. This data was then imported to R to generate figures for data visualization. This included running a permutation test for adonis under reduced model (PERMANOVA) as well as a pairwise adonis test for each of the variables tested. The metadata about each elephant's age, sex, body condition score (BCS), and location were provided to allow for comparison across these variables. I hypothesized that the gut microbiome makeup in these elephants would differ across age groups, as younger elephants are more likely to rely on a milk-based diet, which has been shown to differ from plant-based diets. Researchers have had conflicting findings as to the impact of sex on microbial composition, so I hypothesized that there would not be a significant difference in composition between males and females. I also predicted that there would be little to no difference between locations, as both groups of elephants have comparable

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environments. Additionally, the BCS scores do not vary greatly from elephant to elephant, so I predicted that this variable would most likely have a negligible impact on gut microbiome composition. Results indicated that age and location had no impact on microbiome composition, but sex showed a significant weak association with gut microbiome composition and BCS had a possible association with microbiome composition. The results from this study may be used to illustrate how factors such as sex and BCS impact microbial composition, and contribute to a greater understanding of the gut microbiome makeup in understudied Sri Lankan Asian elephants.

### *60: Shotgun Metagenomic Analysis of Schizaphis microbiome in response to Biotype, Carried CYDV and Time*

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Reads from a prior RNAseq study of gene expression in greenbug aphid (Schizaphis graminum (Rondani)) were used for shotgun metagenomic analysis in relation to two aphid biotypes, presence or absence of carried cereal yellow dwarf virus (CYDV), and five timepoints from zero to 20 days post infestation. There were three biological replicates per condition. Reads were aligned with bwa mem to reference or representative genomes of 35980 bacterial, 531 archaeal, 10692 viral, 435 fungal, and 94 protozoan taxa, plus greenbug and wheat, and each read was credited to the highest scoring taxon. Read counts by taxon were imported into QIIME2 for statistical analysis and display. There were 105635 to 5938545 microbial reads per sample. The reads matched 3348 genera. The ratio of total microbial counts to total greenbug counts peaked at day 5 and declined 50% by day 20. Barplotted relative frequencies indicated two major communities, one enriched in Shigella and Escherichia, the other depleted in Shigella and Escherichia but enriched in Acinetobacter and Gilbertella. With one exception, the depleted community was restricted to days 15 and 20 and existed in both biotypes with and without carried CYDV. CYDV was not detected in any sample, but an aphid pathogen, Rhopalosiphum padi virus, was present in five samples, of which four were enriched in Shigella and Escherichia. Two unrelated photosynthetic genera, Microcystis and Lamprocystis, were relatively abundant; the latter was positively correlated with Shigella. Oddly, Letharia (as an ascomycete) was the fifteenth most abundant hit overall, despite that Letharia itself is a lichen. Shigella-depleted communities in 24 samples were significantly more alpha-diverse than communities in the remaining samples (Shannon entropy 5.06 in depleted versus 3.84 otherwise, $t = -8.46$, $p = 1.8e-09$). Principal-components projection of Bray-Curtis dissimilarity showed two distinct clusters whose membership conformed to the two groups distinguishable in the barplotted relative frequencies. Sample B02 lay between the clusters. The first three principal components accounted for 60.54% of the variation. Permanova of Bray-Curtis distances with Adonis confirmed that only time ($p = 0.001$) and the interaction of time with biotype ($p = 0.035$) significantly affected beta diversity. In conclusion, two distinct microbiome communities existed in the aphids, where the Shigella-depleted community accompanied yellowing and death of the wheat host as it succumbed to aphid feeding and yellow dwarf disease.

### *61: In vitro degradation of wheat bran phenolic compounds by human gut microbiota*

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The composition of phenolic compounds (PCs) in cereals, particularly in wheat, varies significantly across different botanical sources and within kernel tissues. Ferulic acid emerges as the predominant PC in wheat, notably concentrated in the pericarp. Anaerobic microbial degradation of PCs can involve diverse processes, leading to the production of 3-phenylpropionic acid (3PPA).

This study aimed to explore how milling methods and wheat bran particle (WBP) size impact the fecal community's composition and biotransformation of PCs present in WBP.

For this purpose, WBPs, derived from five milling methods and varying in size from <180 to 850 µm, were inoculated with microbiota from three donors in phosphate-buffered gut mineral medium, fortified with proteinogenic amino acid and Wolfe's vitamin mix. After 24 hours of incubation at 37 ºC, cultures were transferred to new tubes, and this was repeated for 7 sequential cultures, over which gas composition and PC content were measured.

Results revealed that phenolic compounds released from wheat bran surfaces undergo biotransformation into 3PPA within 24 hours of fermentation. Subsequent cultivations halted the biotransformation process at 3-(3-hydroxyphenyl)propionic acid, a precursor of 3PPA. This suggests a significant influence of milling methods and WB particle size on fecal community dynamics and metabolic pathways.

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## 62: Prairie plant species differ in their resilience to fungicide-induced alterations of foliar endophytic mycobiomes

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Plants harbor a diverse suite of microbes that play various roles in mediating plant fitness. One such group of microbes includes endophytic fungi, which can be found inside all parts of plant tissue and can exhibit a continuum of lifestyles (e.g. mutualistic, commensal, parasitic, pathogenic). Due to the wide variety of effects these fungi have on plant hosts, it is vital to understand how human activities, such as the use of agricultural products, impacts the ecology of these fungi in off-target systems. Here we explored the effects of fungicide exposure on endophytic mycobiomes in prairie plants using a combination of culture-dependent and independent methods. We hypothesized that fungicide-exposure would lead to a decrease in fungal endophyte diversity and richness and lead to a change in endophyte composition within a non-agricultural system. To test these hypotheses, we utilized an experimental prairie system called the Philips Tract, which contains fungicide-treated and control plots of various tallgrass prairie plant species. To measure fungicidal effects on fungal composition, leaves of 5 plant species (Andropogon gerardii, Sorghastrum nutans, Monarda fistulosa, Penstemon digitalis, and Pycnanthemum virginianum) were used to culture endophytic fungi and metabarcode fungal DNA with a USEARCH pipeline. Both methods found an overall trend in decreased fungal diversity and fungal richness in fungicide-treated plots, but a principal coordinate analysis (PCoA) found no alteration in fungal composition. Furthermore, certain species showed a significant decrease in fungal richness while others remained unaltered. These results thus showcase the impacts that neighboring agricultural activity may be having on aboveground plant-fungal symbioses in nearby prairie systems. By further testing how the presence of agricultural products alters the ecology of off-target prairie systems, we can gain a deeper understanding of the stability and resilience of these affected ecosystems and improve management and restoration practices.

## 63: Experimental evolution of gut microbes on inulins varying in degree of polymerization

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With ever increasing evidence of the gut microbiome's influence on host health, scientists come to wrestle with the big question of "What defines a healthy gut microbiome?", where evidence suggests that healthy individuals have ecologically diverse but vastly different community structures. Understanding the factors and mechanisms that bring about gut microbial changes is crucial to create principles to engineer these ecosystems for healthy lifestyle and medical purposes, dietary changes and interventions are highlighted in scientific discussion. Specifically, dietary fiber plays a key role in the diversity of these ecologies. Which is why this study focuses on unraveling genetic mechanisms by which different bacterial models, isolated from gut samples, degrade inulins more efficiently. More specifically, evolutionary mechanisms within and between species is one of the main driving forces dictating community changes. In a hypothetical scenario, species with more efficient or effective inulin degradation genetic mechanisms would have the comparative advantage over species who don't. The experimental approach involves studying the evolutionary development of these genetic mechanisms in E. coli, K. pneumoniae and B. dentium by subjecting them to limiting nutrient conditions, i.e. progressively increasing inulins as a carbon source, selecting for organisms with more fit/efficient degradation mechanisms. Genomic and genetic techniques are to be used in these studies to further characterize and learn about the genetic mechanisms behind inulin degradation.

## 64: Predicting Phage Cocktail Efficacy: A Step towards Phage-based Bacterial Control for Water Security

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Water security is critical for protecting public health, safeguarding the environment, and fostering sustainable development. Phages are viruses that infect bacteria and archaea, and have all the typical characteristics of viruses, including tight host cell specificity, a tiny genome, considerable reliance on host machinery for replication, and lack of reproduction outside of the host (Strathdee et al., 2023). Phages can be used to selectively kill the target bacteria leaving the beneficial ones (i.e., phage biocontrol). There is a growing interest in harnessing phages to tackle opportunistic pathogens, antibiotic-resistant and disinfectant-tolerant strains of bacteria, as well as the bacteria that cause bulking, foaming infrastructure corrosion, and biofouling (Mathieu et al., 2019; Shivaram et al., 2023; Reisoglu & Aydin, 2023). Many studies reported the better performance of phage-cocktail to suppress the growth of host bacteria (Hu et al., 2023; Kauppinen et al., 2021; Soliman et al., 2023). But there is no recipe for an effective phage cocktail and creating effective cocktails relies on trial and error. Phage cocktail modeling can be a useful tool to determine phage-cocktail efficacy. Through the simulation of diverse environmental conditions and population dynamics, these models can fine-tune the dosage and timing of phage administration, thereby enhancing biocontrol effectiveness while reducing the potential for unintended outcomes.

Here, I will present my work developing the assays needed to generate the data for predicting phage cocktail efficacy. Understanding how phages inhibit bacterial growth is one of the first steps towards predicting phage-cocktail efficacy. I have monitored optical density (600 nm) in a 96-well microtiter plate

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to compare the changes in carrying capacity and growth rate of Mycobacterium smegmatis and Gordonia terrae when infected with or grown without the mycobacteriophage D29. The reasons behind choosing these hosts are that both of them are BSL-1. Mycobacterium smegmatis is a fast-growing, non-pathogenic model bacteria for common drinking water opportunistic pathogens and Gordonia terrae is an opportunistic pathogen and also creates bulking and foaming in activated sludge processes during wastewater treatment.

Both M. smegmatis and G. terrae show decreased carrying capacities (p-values = 0.00003 and 0.0008, respectively) when infected with D29, yet exhibit similar growth rates (p-values = 0.2863 and 0.1395, respectively). With this procedure, I will use five mycobacteriophages Giles, Hawkeye, Marvin, Jeon, and D29 to create various cocktails and check their potency in suppressing host growth. The outcomes of these experiments will help in the process of phage cocktail modeling. As a first step to understand the results of the cocktail tests, I will present my comparisons of these five phage genomes, including their length, GC content, and average nucleotide identity (ANI). By integrating computational modeling with experimental validation, it might be possible to design tailored phage cocktails capable of effectively targeting and controlling problematic bacteria in water treatment processes.

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### *65: Cereal fiber type and bran particle size differentially selects for gut microbial community composition and function under high dilution pressure*

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Wheat bran is a major source of dietary fiber in the Western diet, containing diverse components ranging from lignins, residual starch, carotenoids, proteins and other other potentially bioactive components. Dietary fiber in brans predominantly consists of non-starch polysaccharides (NSP), of which 17-33% is made of arabinoxylans (AX). Conventional practices to produce bran suitable for food applications involve milling and refining into smaller particle sizes. Consequently, these processing decisions and intrinsic fiber diversity may modify gut microbial responses to cereal substrates. We subjected three sizes of wheat bran (small: 180-250µm, medium: 300-500µm, large: 1000-1700µm) and wheat AX extracted from mixed brans to upper GI tract-simulated digestion and subsequently, a 7-day in vitro sequential batch fermentation involving successive 1:100 dilutions. Our objective was to elucidate the differences in microbial communities most efficient at fermenting the more heterogeneous wheat bran vs. wheat AX, and to observe communities responsive to varying bran particle size ranges. We used three different donor fecal samples and a fourth, pooled fecal sample. At the end of sequential batch fermentation, we measured metabolitic (SCFA; acetate, propionate and butyrate) and microbial community structure (16S rRNA amplification and sequencing of the V4-V5 region) outcomes. Alpha and beta-diversity metrics (Bray-Curtis dissimilarity index) and Linear Discriminant Analysis (LDA) were carried out on the processed sequences to identify differentially abundant taxa. Smaller-sized brans showed higher relative abundances of Prevotella in final communities; larger brans exhibited higher counts of Lachnospiraceae and Kineothrix. Bran-associated communities differed substantially from AX-fermenting communities, which showed higher counts of Bifidobacterium and Prevotella than all bran sizes. Propionate and butyrate production varied across particle sizes and fiber type in concert with community structure. Our findings highlight the differences in composition and metabolite production of differently sized bran particles and fiber types, suggesting that processing choices and fiber complexity may influence the gut microbiome. Overall, these results may serve as a framework to design precise cereal fiber structures capable of modulating gut communities to achieve desired health and physiological outcomes.

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## 66: Comparative Genomics of Shiga toxin producing E. coli (STEC) harbored in bison and its impact on intestinal microbiome

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Background
Bison share similar genetics, grazing habits, and environment as cattle. In the case of Shiga toxin-producing E. coli (STEC), cattle serve as the primary reservoir but their genetic closeness to bison can potentially lead to bison acting as alternative STEC reservoirs. STEC-infected meat consumption can lead to diarrhea or Hemolytic Uremic Syndrome (HUS) in severe cases, causing a significant healthcare burden and significant economic loss. Therefore, here we detected the presence of STEC from bison herds and performed genome sequencing for comparative genomic analysis with clinical controls (EDL933, Sakai, and Spinach) and isolates from cattle feed-lot. The study aims to identify genetic factors that may play a key role in transmission and disease.

Methods
In this study, we performed non-invasive fecal sampling from 4 bison herds over a winter sampling event in 2023. The sampling was followed by fecal enrichment and isolation for STEC using a protocol optimized for bison feces in-house. Isolated pathogens underwent whole genome sequencing using Illumina Library prep (IPB) kit and Illumina Novaseq (2X150) platform. Sequenced reads were assembled using Trimmomatric and SPADES software. To obtain sequence type and in-silico serotype information cgMLST website was used. Genomes of isolates from bison were compared with other outbreaks and beef isolates to get information on interspecific relatedness using Anvi'o version 8.

Results
Out of 75 fecal samples collected, ten samples were positive for STEC. In-silico results indicated isolation of non-O157 serotypes which have been regularly linked with foodborne illnesses in humans along with their presence in cattle. Isolation of these non-O157 STEC serotypes from bison increases the chance of outbreaks via meat or fecal contamination. Pan-genomic comparison using Anvi'o v8 revealed close relatedness between STEC from bison raised for meat to controls from outbreaks and beef-lot. Our future work will towards decoding microbiome related implications that might contribute to colonization of STEC in bison.

Conclusion
Overall, our study is important because bison can serve as an evolving reservoir for foodborne pathogens like STEC. The genetic surveillance of isolates originating from bison can help study the potential transmission of Shiga toxin-producing E. coli from bison to humans that might lead to outbreaks. Our research would contribute toward better management practices for raising bison for meat or conservation while mitigating STEC presence.

## 67: Determining the causal role of the gut microbiota in perinatal depression: A pilot study

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Perinatal depression (PND) affects 10-12% of women in the US, most of whom do not receive adequate care, putting both the mother and infant at risk. Given the high prevalence of untreated PND and its detrimental outcomes, uncovering an accessible, non-invasive therapeutic is crucial to the neurological, psychological, and gastrological health of current and future pregnant people and of future generations.

One promising route is via the gut microbiome, which has been shown to vary throughout pregnancy and has been implicated directly in major depressive disorder (MDD). Additionally, it is accessible to the external world via the mouth and anus, making it a promising target for non-invasive therapeutics. Researchers have demonstrated that transplanting stool from human participants with MDD to germ-free rats using fecal microbiota transplant (FMT), induces development of core features of MDD in the rats, including anhedonia and anxiety-like behaviors. These results demonstrate the sufficiency of the microbiota to induce depressive-like behaviors. Prior results from our lab have demonstrated an association between gut microbiome changes and depression throughout pregnancy in humans, however the causal impacts of the gut microbiota on PND remain unknown.

In this pilot study, we investigate the factors needed to design a humanized mouse model of PND for use in future studies, in which we will probe the mechanisms underlying the microbiota gut-brain axis (MGBA) in PND. Here, we present our preliminary results which aid in determining the optimal number of oral inoculations required to ensure the engraftment of maternal microbial communities into the murine gut and their capabilities of altering the depressive and anxiety-like phenotypes of the recipient mice at various timepoints post-transplant. We inoculated 6-week-old female germ-free mice with fecal matter from a participant with depression (CATDI=60.4) and without (CATDI=2.5), who were each at the beginning of their third trimester (10 mice per group). Mice were orally inoculated once weekly for a total of 3 or 4 weeks, then underwent behavioral testing (sucrose preference, open field, and tail suspension) at 10 and 14 days

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after the last inoculation. Given the potential differences underlying the biochemical mechanisms of MDD and PND and our interest in microbial metabolite, hormonal, and immune interactions; murine plasma, liver, spleen, intestinal, and brain tissue are being collected for future analysis.

Preliminary results presented here will aid in the construction of a novel mouse model of PND driven by the gut microbiota makeup. This mouse model of PND will then be utilized to better understand the complex systems that interact to drive the disorder.

### *68: HealthyHerd: A Web-Based Platform to surveil the spread of SARS-CoV-2 variants in Animal Populations*

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Native American and Alaska Native tribes, who maintain close contact with wildlife for subsistence and cultural reasons, are disproportionately impacted by zoonotic diseases such as COVID-19. However, due to outdated infrastructure and a lack of resources, 56.2 million acres of tribal land lack sufficient surveillance for zoonotic outbreaks. A similar need exists for wildlife surveyed by the Indiana Department of Natural Resources, U.S. Geological Survey, and farmed and companion animals that are under the authority of animal and public health agencies. SARS-CoV-2 continues to mutate and infect new species of animals and yet, there is a limited ability to monitor these new infections in the field.

In response to these challenges, we have developed HealthyHerd, a novel web-based platform that allows users to securely track and store animal health information with just an internet connection. Using a field-deployable paper-based test that detects recent SARS-CoV-2 variants through oronasal samples, our web platform:

1. Automatically quantifies viral load
2. Uploads data and graphical analysis for user viewing
3. Creates databases to securely store long-term and short-term animal and user information

This website allows users to test and upload data for 39 animals, including birds, wild animals, farm animals, and companion animals.

Our web-based platform will be used to track and anonymize testing results in order to get regional totals of outbreaks, while preserving the privacy and security of individual users. Additionally, our framework allows for a means by which various point-of-care tests can be analyzed, organized, stored, and electronically transmitted systematically. This allows for the reduction of virus transmission, a decrease in health disparities among rural and isolated populations, and allows for further population health studies.

### *69: Understanding the microbiome of Controlled Environment Agriculture towards the development of a microbial control*

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Controlled Environment Agriculture (CEA) is a cultivation technique that is gaining interest in the global food landscape due to its ability to provide high yields per unit area and offer higher growth rates than traditional soil-based cultivation techniques. These CEA systems utilize a nutrient rich solution that is circulated throughout the system in which plants are grown on an absorbent substrate. These systems have tightly regulated physiochemical parameters which along with the closed loop nature of the systems offer a low complexity microbial community assembly. However, due to a lack of active microbial control, and competitive inhibition in the low complexity community, these systems are susceptible to being overrun by both plant and human pathogens alike. While some progress towards the development of active microbial control has provided some microbial inoculant-based solutions, the microbial species used typically originate from soil-based cultivation techniques and are not perfectly suited to CEA systems. As a result, often repeated periodic applications are needed to ensure continued microbial control of these pathogens and proves to be an expensive solution. A solution that is truly customized to these systems is much needed. However, to develop such microbial inoculants, a thorough understanding of the microbial community assembly in these systems is needed. Here we present results from a case study of a microbial community survey conducted in a commercial hydroponics farm and contextualize this data in the broader scope of CEA systems around world through previously published and publicly available datasets. This work identifies general trends in the microbial colonization patterns in the different compartments of CEA systems from around the world and attempts to disentangle the different driving factors in the community assembly of these systems. Further we compare microbiome survey from traditional soil-based cultivation with the microbiome of CEA systems to distinguish their differences and their similarities. This comparison informs the relevance of considering soil-less cultivation systems as a proxy for soil-based cultivation systems and explores their overlap in microbial community assembly. In conclusion, this study focuses on identifying important members of CEA systems, their colonization profiles within the system, and compares them to soil-based cultivation in order to setup the possibility of engineering microbe or communities for inoculants in CEA systems.

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## *70: Three patterns emerge in the gut microbial community during in vitro fecal fermentation of dietary fibers varying in their level of compexity*

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The human lower gastrointestinal tract harbors trillions of microorganisms, and the interactions of these microorganisms with the host influences health. Although it is known that dietary fiber broadly affects the microbiome, there is a lack of quantified relationships between the physical structures of dietary fibers and the changes to the taxonomic composition of the colonic microbiome that they lead to. The overall objective of this project was to determine the impact of dietary fiber physical structure on taxonomic shifts in the colonic microbiome. The hypothesis was that more complex fibers would lead to larger changes to the relative abundance of microbial taxa as compared to more structurally simple fibers, and that the large changes to taxa would be more consistent between different donors. The rationale was that structural complex fibers can be accessed by some taxa much more effectively than other taxa, whereas structurally simple fibers provide more universal access to the substrate across all taxa. Data from two previously conducted in vitro fecal fermentation studies were retroactively analyzed. Donors provided fecal samples under established IRB protocol. Fecal samples from each donor were used for in vitro fecal fermentation for 12 hours, according to a previously described method. The relative abundances of taxa were determined using 16s sequencing (V4 region). To quantify how much each microbial taxa changed during the fermentation, the log of the final relative abundance divided by the initial relative abundance was taken. The entire dataset consisted of 20 total donors, 7 distinct dietary fibers, and a total of 240 samples. The first finding from our analysis was that large changes to the relative abundance of taxa were consistent between donors, whereas small changes were more variable between donors. This pattern was evidenced by a statistically significant (p<0.05) negative relationship between the coefficient of variation for the log change of a taxa between the different donors, and the magnitude of that change. The second finding from our analysis was that complex fibers tended to lead to large changes to the relative abundance of a small number of taxa. On the other hand, simple fibers led to small changes to a large number of taxa. This pattern was quantified by constructing an empirical cumulative distribution function for the change to the relative abundance of all taxa for a certain fiber, which revealed that simple fibers (Fructooligosaccharide, Xylooligsaccharide) achieved 100% of their cumulative change at smaller values of absolute change than more complex fibers (beta-glucan and xanthan gum). Lastly, we found that complex fibers led to changes of inherently lower alpha diversity (quantified as Shannon entropy) than more simple fibers. Although the microbiome is a complex system, it contains patterns that can be determined by analyzing changes to relative abundance using a model-free approach. These patterns could be leveraged by future researchers to take advantage of the inherent characteristics of the microbiome to design more effective prebiotic therapies for improved human or animal health.

## *71: Microbial Community Analysis on Coffee Roots under Legume Intercropping Systems as an Alternative To Nitrogen Supply in Colombia*

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Coffee crops require a substantial nitrogen supply in its fertilization regimen, posing an economic challenge for most small coffee growers in Colombia (96%), particularly amidst escalating fertilizer prices. Intercropping systems involving legumes emerge as sustainable alternatives to meet the nitrogen requirements partially. In such management strategies, it becomes imperative to discern the microbiome in the root system upon the implementation of these amendments, aiming to exploit potential synergies that facilitate nitrogen release and absorption. To address this, we analyzed the association of legumes Tephrosia (Tephrosia sp.) and Guandul (Cajanus cajan) supplemented with 50% additional nitrogen on the root microbiome of coffee plants cultivated in three experimental locations. These treatments were compared with those devoid of added nitrogen (50% and 0%) in the absence of legumes. Preliminary findings indicate significant divergence in fungal root endospheric composition in plots with Tephrosia in the Naranjal location, while significant differences were observed in intercropping with Guandul in Paraguacito. Notably, fungi belonging to the genus Xylomyces were abundant in both treatments; however, further analyses are imperative to elucidate potential functions associated with nitrogen cycling and plant uptake. Respecting the bacterial composition, despite the communities are not significantly different, the root endosphere of coffee plants grown with Tephrosia were enriched in genera Austiccacaulis and Labrys, this last linked to plant growth promotion in other plant species, whereas plants supplemented with 50% nitrogen were enriched in the genus Acidothermus and no nitrogen supplied were enriched in SBR1031 and Amycolaptosis, this last reported as plant-growth promoting in soil and rhizosphere. Moreover, field observations reveal a higher nitrogen release upon incorporation of Guandul biomass compared to Tephrosia, possibly attributed to genera significantly prevalent in intercropping with Guandul, such as Boothiomyces and Conlarium. Subsequent analyses of the bacterial community and functional abundance associated with the nitrogen cycle are relevant to understanding the beneficial associations of legume intercropping in fostering the coffee root microbiome.

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## *72: Ecological Memory of Nitrogen Fertilizers on Soil Microbiome Structure Becomes Evident at Peak Growth of Miscanthus*

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Prolonged nitrogen addition can induce legacy effects that persist in soil microbial communities, affecting ecosystem functions long after the initial nitrogen inputs have ceased, which could then create an "ecological memory" that affects how ecosystems respond to new nitrogen inputs. However, the specific contributions of historical nitrogen additions to the formation of this ecological memory in plant-associated microbial communities remain unclear. Here, we utilized a long-term Miscanthus trial with varying levels of historical nitrogen fertilization (0, 60, and 120 kg nitrogen-urea per hectare annually from 2008 to 2015), and investigated the impact of the fertilization legacy on microbial nitrogen cycling processes when nitrogen fertilizer application resumed in 2021. This experiment used a split-plot design to compare plots that remained unfertilized with fertilization rates of 0, 60, and 120 kg nitrogen-urea per hectare. Our findings reveal that, even after a six-year hiatus, historical nitrogen applications continue to influence nitrogen cycling functions and have primed certain functional groups to respond more intensely to new nitrogen inputs. We also utilized DNA sequencing to demonstrate that the legacy effect on soil microbiome structure becomes evident only during the peak growing season, when the microbial community structure is more stable compared to the early season. This stabilization and gradual clustering of microbiomes are shown to be driven by the enrichment of core taxa, which may be facilitated by the favorable environmental conditions associated with Miscanthus growth. Our findings underscore the complex ways in which nitrogen applications influence ecological memory within crop-associated microbial communities.

## *73: Deciphering Fiber Tolerance: Impact of Gut Bacterial Dysbiosis and Fiber Physicochemical Properties*

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Objectives:
Despite the known health benefits exerted by dietary fiber consumption, the prevalence of fiber intolerance has been on a continuous rise among the American population. Factors driving this intolerance are still to be determined. Fiber physicochemical properties, gut microbiome heterogeneity and gut inflammation might contribute to this condition. The objective of this study is to evaluate the influence of fiber- and -host-specific conditions in fiber tolerance and metabolism.

Methods:
In order to evaluate the fiber-specific characteristics, Resistant maltodextrins (RMD), high methoxyl pectin (HMP), chicory inulin (ChIn), and wheat bran (WB) were selected based on their differing physicochemical properties. Six-week-old IL-10 KO germ-free mice were randomly assigned to a purified control diet or 15% fiber-supplemented diet. Mice were colonized using fecal microbiota from a fiber-intolerant individual. Mice were assessed daily for weight loss, food consumption, and physical activity. To evaluate progression or severity of intolerance and/or health beneficial effects of the fibers, the relative gene expression of cytokines was measured using RT-PCR. Short-chain fatty acids (SCFAs) were measured by GC. Bacterial sequencing was done using 16S rRNA sequencing.

Results:
Within the first two weeks, only RMD increased the expression of IL-6. Short-term consumption of fibers did not increase the production of mucus or SCFAs. RMD and ChIn reduced the expression of Claudin-1, showing early signs of compromised gut barrier integrity. Beta-diversity analysis at the two-week fiber consumption period, showed that there was no indication of gut bacterial modulation. In contrast, eight-week consumption did modulate the gut bacterial community, RMD and ChIn were clustered together and differently than the rest of the fiber treatments. Gut bacterial diversity, assessed through Faith PD's index, showed that RMD and ChIn resulted in a decrease in diversity. Eight-week fiber consumption of RMD and ChIn, increased mice colon lengths and IL-6, along with reduced Muc-2 and Claudin-1 expression. Comparison of the two- and eight-week periods, showed exacerbation of inflammation symptoms and gut barrier dysfunction by RMD and ChIn. Despite higher production of SCFAs, adverse effects could not be reversed by RMD or ChIn. HMP treatment showed increased butyrate production and maintained gut barrier integrity without any intolerance symptoms.

Conclusions:
Gut bacterial dysbiosis highly influences the host's capacity to metabolize fiber and can lead to inflammation in a fiber-dependent manner regardless of its consumption duration. HMP exerted protective effects against the onset of inflammation symptoms and contributed to a stronger gut barrier integrity.

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## *74: field-deployable paper-based colorimetric LAMP biosensor for the detection of antimicrobial-resistant genes of Bovine respiratory disease*

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Introduction: Antimicrobial resistance (AMR) presents a critical and immediate global threat, poised to become a leading cause of death worldwide in the coming decades. AMR occurs when microorganisms such as viruses, bacteria, fungi, and parasites evolve over time to resist medicinal treatments, complicating infection management and enhancing disease transmission risks. Notably, antimicrobial-resistant organisms can transmit from food-producing animals to humans through direct or environmental contact.

Problem: Bovine respiratory disease (BRD) is a significant health issue in cattle, associated with high mortality rates and substantial economic losses. Key bacterial pathogens involved in BRD include Pasteurella multocida, Histophilus somni, and Mycoplasma bovis. Emerging research indicates that these pathogens are developing resistance to conventional treatments, underscoring the need for rapid and accurate detection methods at the farm level.

Approach: The paper-based colorimetric Loop-mediated Isothermal Amplification (LAMP) method offers a robust solution for onsite genetic testing. This technique utilizes specially designed primers to amplify target genes at a constant temperature of 65°. The amplification process alters the pH of the reaction mixture, which can be visually detected using a pH-sensitive dye. This enables the straightforward identification of AMR genes.

Conclusions: The LAMP biosensor provides a quick and user-friendly method to detect AMR genes associated with BRD, yielding results within 30 to 60 minutes. Its simplicity makes it suitable for use by individuals with minimal technical expertise, facilitating early detection and management of AMR in agricultural settings.

## *75: Unveiling the Impact of Nanoplastics on Plant-Rhizosphere Dynamics: A Study of Tomato and Lettuce Microbiomes*

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Nanoplastics (NPs) are pervasive organic contaminants present in soil, water, and food, arising from the breakdown of plastic. Despite their widespread presence, there is a lack of effective biological pathways for degradation, leading to their accumulation in the environment. In soil, NPs may disrupt microhabitats crucial for the microbiome or be taken up by plants through unknown mechanisms, potentially entering the food chain. However, the impact of NPs on the rhizosphere-root-microbiome interface remains poorly understood. This study proposes that NPs alter the root microbiome by modifying the root exudation profile of plants, potentially induced by stress-related exudates. To investigate this, we cultivated two crop species, tomato (Solanum lycopersicum cv. Micro-tom) and lettuce (Lactuca sativa L. cv Canasta), under three soil conditions: a control (zero-NP) and two experimental groups with NP concentrations of 20 and 200 mg/kg, within the Plant Phenotype facility at Purdue University. After the growth period, one gram of roots was preserved for DNA extraction from the rhizosphere and endosphere, along with bulk soil, followed by amplification and sequencing of 16S and ITS rRNA genes targeting bacterial and fungal communities, respectively, using MiSeq Illumina technology. Roots were then submerged in ultra-pure water for four hours to collect exudates, which were quantified using mass spectrometry. Sequence analysis was performed using the Qiime2 pipeline, and correlations with bacteria, fungi, and exudates were assessed using the mixOmics package. Results revealed that lettuce exhibited reduced biomass in the presence of NPs, while tomatoes showed no significant growth differences. Both crops displayed elevated levels of amino acids and carotenoids in their rhizosphere exudates. Notably, under the 200 mg/kg NP treatment, tomatoes exhibited distinct exudation of phytosiderophores. Beta diversity analysis indicated a lesser modulation of the soil community by NP presence, with the tomato microbiome showing a stronger response compared to lettuce, particularly in bacterial communities over fungal communities. Using a multivariate dimension reduction discriminant analysis method, the integration of the ASV for fungi and bacteria and the exudates indicate the most contributing exudates are different between the plant species, what could indicate a specific adaptation to the stress related to the NP presence, and surprinsily there was a higher number of fungi generas with higher correlation with the exudates them the bacterial ones in both species.In conclusion, this study highlights the intricate interactions between NPs, plant roots, and the rhizosphere microbiome, demonstrating their potential to alter root exudation profiles and microbial communities. Differential responses between tomato and lettuce underscore species-specific adaptations to NP exposure, with implications for plant growth and rhizosphere ecology. Understanding NP-mediated effects on plant-microbiome interactions is crucial for mitigating NP pollution and safeguarding environmental and agricultural health. Further research into long-term NP exposure effects and the roles of microbial taxa in mediating plant responses will inform sustainable management practices and ensure food security amidst emerging environmental challenges.

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## 76: Anti-colitic activity of phlobaphenes and anthocyanins in IL-10 -/- mice is dependent on the human donor microbiota

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Objective
Gut bacterial dysbiosis is a critical factor in the development of Ulcerative Colitis (UC). Flavonoid-rich food have shown protective effects on the gut barrier integrity in animal models. However, the role of bacterial dysbiosis on the anti-colitic properties of the flavonoids is not fully understood. This study aimed to assess the effect of UC-associated microbiota on the anti-colitic activity of phlobaphenes (PHLO) and/or anthocyanins (ANTH).

Methods
Six-week-old gnotobiotic IL-10 -/- mice were colonized with human fecal microbiota from three UC patients. Mice were assigned to five diets: a purified control (P), and four diets with 25% maize near-isogenic lines expressing no PHLO or ANTH (A), only PHLO (B), only ANTH (C), and both ANTH and PHLO (D). Seven weeks later, 1.5% dextran sodium sulfate (DSS) was used to induce colitis. Relative expression levels of colonic inflammatory markers were analyzed by RT-qPCR. 16S rRNA sequencing targeting the V4 region was applied to determine the gut microbiome composition, alpha and beta diversity indexes. Qiime2 was used for processing the demultiplexed sequences and the Greengenes database for the taxonomic assignment.

Results
Patterns of expression of inflammatory markers were dependent on the origin (human donor) of the recipient's microbiota. In IBD-2 donor recipients' higher relative expression of IL-1B and lower Occludin were observed with diet B compared to diet P, whereas TJP-1 was reduced with A, B and D diets. In IBD-3 donor recipients, TLR-4 expression was reduced by diets B and C, while TLR-5 was reduced by diets A and B. IL-1B was reduced but Occludin was elevated by diets C and D.

Overall results of alpha and beta diversity did not show significant differences between diets. However, comparing donor-associated responses (IBD-2 and IBD-3), diets A and B showed reduction in the Observed Features and Faith_PD for only IBD-3. Clustering patterns were observed in IBD-3 donor recipients based on treatments for beta-diversity parameters. P diet was slightly distant from the rest of the treatments in the Bray-Curtis and Unweighted Unifrac (UnUf) analysis. Diets B and C diets were distant in the UnUf. Jaccard analysis showed three potential clustering groups (A+B, C+D and P).
Results of bacterial composition (P diet as reference) at the phylum level an increase in the relative abundance of Proteobacteria after B diet, associated with active IBD. This is coherent with higher level of Enterococcaceae promoted by diet B. However, diets C and D were more effective reducing this bacteria family, associated with active IBD.

Conclusion
The anti-inflammatory activity of PHLO and ANTH is dependent on the individual's gut microbiota composition rather than the dysbiosis status. Correlation analysis is pending to suggest relationships between the anti-inflammatory outcomes and specific taxa. Also, associations with gut metabolites will be investigated. Further research will contribute to support dietary recommendations of bioactives for gut microbiota-associated chronic diseases.

Funding: USDA-NIFA award 2019-67017-29258.

## 77: Development of Nucleic Acid-based Diagnostics for the Detection of Viral Respiratory Pathogens in Humans and Animals on Paper

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Detection of pathogens implicated in respiratory disease at the point-of-need and in field-based settings remains elusive and thus, treatment typically consists of addressing symptoms rather than identifying causative pathogens and treating accordingly. This in turn can lead to blanket treatments, such as the unwarranted prescription of antibiotics that encourage the propagation and colonization of antimicrobial resistance along with a host of other medical complications. Here, we propose the usage of Loop-mediated isothermal amplification (LAMP) to detect and amplify target nucleic acids on paper and return results via simple color change from red to yellow. The device is capable of detecting several pathogens and is usable at the point-of-need and in field-based settings, be that in the field or in a user's home. Returned results can be used to guide patient treatment to optimize patient recovery and outcome.
To demonstrate utility of our device, we constructed a device to target SARS-CoV-2 with an overall accuracy of 98% when using digital image analysis, and 91% when accounting for variations in color perception. This device is readily adaptable to target other viral pathogens, including viruses causing respiratory disease in cattle (Bovine Respiratory Syncytial Virus (BRSV), Bovine Adenovirus (BAV), Bovine Viral Diarrhea Virus (BVDV), etc.) and acute respiratory infections in humans (Influenza A and B, Respiratory Syncytial Virus (RSV), etc.). We anticipate this device will provide inexpensive and scalable method for community surveillance and triage both in detecting endemic spread and in times of public health emergencies.

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## 78: Understanding the influence of houseplants on the indoor microbiome

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As we stay indoors for the majority of our daily hours, our health gets influenced by the indoor microbiome. Studies on the composition of the indoor microbiome have found diverse and complex communities of prokaryotic and eukaryotic microorganisms inhabiting these systems. But what a healthy indoor microbiome consists of is yet to be determined. To define this, connections need to be made among microbial presence and metabolism, environmental conditions, and human health effects. Metagenomic and metatranscriptomic studies on house dust are key to this assessment. The former provides the microbiome's taxonomy and metabolic potential, while the later answers which genes are being expressed. By providing insight into the metabolic state of the microbiota of the built environment, gene expression studies will help us connect indoor microbiota to occupant health.

Here, we seek to understand whether the introduction of indoor plants into office spaces induces changes in the indoor microbial community and environmental conditions. Houseplant presence has been associated with increased biodiversity in chamber experiments, varied effects on indoor air quality, and positive mental health benefits. However, their influence on indoor microbiota and human health remains unknown due to a lack of studies. I will present work from a pilot study of 7 office spaces with and without plants (4 offices with 1-4 potted plants, 3 controls). Settled dust samples were collected on petri dishes for 3 weeks with plates set out every week for eight weeks. A PurpleAir real-time air quality sensor in each room recorded abiotic data: humidity, temperature, pressure, dewpoint, and particulate matter (PM2.5). Total fungi and bacteria in each office's dust was quantified based on ITS and 16S gene copies, respectively, using droplet digital PCR. No significant influence of plants was observed for any of the biotic and abiotic factors with respect to the controls. We suspect that no signal was detected due to the low number of plants combined with regular usage of offices. The abundance data varied from one room to another despite the rooms being on the same floor of a building. DNA from each room has also been sent for amplicon sequencing to determine whether specific taxa found in the soil of the houseplants can be detected in the rooms with plants compared to the controls having no plants. Extraction of RNA from dust is still in development and will be instrumental in connecting the microbial community's metabolism back to human health. By establishing whether houseplants modify the microbial community of the built environment, this research investigates a method of great interest for improving human health indoors.

## 79: Evaluating the effect of lyoprotectants in preserving community structure and bacterial viability in the lyophilized fecal community of pigs.

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Fecal microbiota transplantation (FMT) stands as a potential method for enhancing pig health. FMT has progressed from the basic delivery of fresh stool, to lyophilized preparations which is beneficial for sample storage, but can damage bacterial cells. This has necessitated the need for lyoprotectants to preserve bacterial viability. However, the ideal lyoprotectant for retaining community structure and viability remains unclear. Furthermore, DNA-based microbial profiling methods such as 16S rRNA and metagenomics sequencing do not distinguish live and dead bacteria, prompting the need for other methods of distinguishing live and dead members of the bacterial community after lyophilization. PMA-seq (propidium monoazide treatment followed by 16S rRNA gene amplicon sequencing) has been shown to differentiate between live and dead bacteria in microbial communities. To refine FMT in pigs, we sought to ascertain the impact of four lyoprotectants on bacterial viability and fecal microbiome structure. Fecal samples from six pigs were treated with Mannitol, Maltodextrin, Trehalose, Maltodextrin-Trehalose mix (2.5% each) as lyoprotectants or left untreated. Lyophilized fecal samples underwent PMA treatment prior to 16S rRNA sequencing to capture the active community, and total and viable bacterial cells number were quantified. One-way ANOVA was used to analyzed total and viable bacterial cells, and richness, while PERMANOVA was used to compare beta diversity. Results revealed no initial differences in total, viable bacteria, or community structure (P > 0.05). However, untreated, and mannitol-treated groups showed significantly reduced total and viable bacterial cells post-lyophilization (P < 0.01), unlike the other lyoprotectants (P > 0.05). Mannitol and the untreated group also displayed decreased richness and beta diversity shifts (P < 0.05). Overall, lyophilization with mannitol or no lyoprotectant notably impacted pig fecal microbiota community structure, while maltodextrin, trehalose, and maltodextrin-trehalose effectively preserved bacterial viability and structure.

## 80: Biofilm formation of Bifidobacterium spp. in sorghum arabinoxylan fermentation

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Bifidobacteria, common in the human gut and linked to health benefits, are known for digesting dietary fibers and producing beneficial compounds. Dietary fibers are fermented in the gut by bacteria, which produce metabolites that are associated with health benefits like cardiovascular disease and obesity. The study investigates how various Bifidobacterium spp. interact with sorghum arabinoxylan (SAX), a complex polysaccharide, as a carbon source. SAX, previously studied for its potential to modulate gut microbiota, is of interest as functional food ingredient. OD measurements were taken over 24 hours using a 96-

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well plate. Bifidobacterium spp. exhibited weak initial growth and no significant growth over the 24-hour period. However, biofilm formation was observed sedimented in the vials, therefore a crystal violet biofilm assay was performed with different substrates (arabinose, xylose, inulin, and SAX). The biofilm formation in SAX and kestose fermentations was higher across all the species, possibly representing adaptation to the presence of fibers in the environment. Further research will study the interactions between these species and their biofilm formation when they interact with one another.

### *81: Carbon Source-Specific Regulation of Nitrogen Fixation in Paraburkholderia xenovorans*

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Paraburkholderia is a genus of metabolically diverse, diazotrophic (free-living, nitrogen-fixing) bacteria with potential use in the manufacture of biofertilizers due to their beneficial effects on plant growth. Previous research suggests that nitrogen fixation is dependent on carbon source, with aromatics, like plant-produced monophenols, producing higher rates of nitrogen fixation than carbohydrates. The aim of this study was to determine the effect of carbon source, nitrogen level, and oxygen concentration on the nitrogen fixing capability of Paraburkholderia xenovorans str. 4B. Herein, we report evidence of the ability of P. xenovorans str. 4B to fix nitrogen using a monophenol (p-hydroxybenzoic acid—pHBA) as a sole carbon source. P. xenovorans str. 4B exhibited higher rates of respiration when grown on pHBA without nitrogen, implicating an increase in the energy-intensive process of nitrogen fixation. Moving forward, we expect these methods will be used to evaluate the relationship between monophenolic exudate profile in various plant species and their influence on microbial gene regulation, with a focus on nitrogen fixing genes. This work can be used to develop effective biofertilizers and to provide insights into co-evolution of symbioses between plants and bacteria.

### *82: Whole Food Fibers For Support Of Key Gut Bacteria For Human Health*

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The modern Western diet, distinguished by low intake of whole food fibers and a high consumption of processed foods, is often deficient in the types of dietary fibers that support mucosal butyrogenic Clostridia, important for gut health and anti-inflammatory effects. This study aims to evaluate the efficacy of whole food fibers over isolated fibers like inulin in supporting butyrogenic Clostridium clusters and improving markers of gut barrier function and systemic inflammation in humans.

Our research involves two main objectives: 1) To assess and compare the effects of whole food fibers and inulin on the promotion of butyrogenic Clostridium clusters IV and XIVa, and on butyrate production in vitro. 2) To conduct a human clinical trial to compare the effects of a whole food fiber mixture and inulin on gut and systemic health markers.

We will employ a conjunct approach to assess the effects of whole food fibers versus inulin on gut microbiota and health effects. Initially, we will isolate fibers (soluble and insoluble) from whole foods and design a fiber mixture to be assessed in vitro for the purpose of comparing the fiber mixture and inulin in the promotion of butyrogenic Clostridium clusters IV and XIVa and butyrate production. We will perform methods for microbial analysis through 16S rRNA gene sequencing and SCFA analysis. Subsequently, a human clinical trial involving overweight, prediabetic subjects that will undergo treatment with whole food fibers and inulin finalized with the measurement of changes in microbiota composition, butyrate levels, and health biomarkers. These biomarkers include gut barrier integrity, systemic inflammation, and metabolic function.

We anticipate that whole food fibers, which naturally contain both soluble and insoluble components entrapped in a plant cell wall matrix, will more effectively promote the growth of butyrogenic Clostridium clusters and increase butyrate production compared to inulin. This should correlate with improvements in markers of gut barrier function and a reduction in systemic inflammation markers.

This study expects to demonstrate that whole food fibers are superior to isolated fiber supplements like inulin in supporting beneficial gut microbiota and enhancing gut and systemic health. The results could lead to dietary recommendations that emphasize the consumption of whole foods for their fiber content to prevent or mitigate chronic inflammation-related diseases prevalent in Western societies.

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## 83: A research proposal: root microbiomes and aphid resistance

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Triticum aestivum L. (wheat) is the second most important cereal grain globally in terms of production and is considered crucial for food security. Herbivorous insect pests are a major threat to wheat production. Aphids, in particular, pose significant in-field management challenges, as less than 25 aphids per grain head lead to economic injury thus reducing yields. Additionally, aphids vector many devastating diseases of wheat and other cereal crops. Identifying aphid resistance traits is inherently difficult, given that T. aestivum L. is a hexaploid containing three separate ancestral genomes. Current management techniques rely primarily on the use of pesticides, although some aphid resistant cultivars have been identified. However, plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) provide an alternative management option that could improve wheat resistance to aphids. Studies on PGPR and AMF demonstrate both insecticidal activity as well as improved yields and overall biomass. How inoculation with PGPR and AMF affects wheat hormonal pathways involved in defense against aphids, as well as impacts on aphid survival and fecundity, remains unknown. Additionally, it is unclear whether these tri-trophic interactions also affect pathogen resistance or aphid vector competence. We propose to examine the relationship between wheat root microbiomes and aphid resistance using four species of cereal aphids that span a range of specialists to generalists, and include emerging pests. We propose to inoculate susceptible and resistant/tolerant wheat cultivars with beneficial PGPRs and AMF and measure the impacts on aphid performance and wheat hormonal pathways involved in defense. We predict an increase tolerance to aphid herbivory with T. aestivum L. inoculated with AMF, in which the presence of aphids minimally decreases plant biomass and yield. Additionally, we predict aphid resistance in wheat inoculated with both PGPR and AMF where T. aestivum L. yield and biomass increases while aphid population declines. As global climate change threatens to undermine current cereal grain management strategies, we aim to understand how PGPR and AMF inoculation affect wheat hormonal defense pathways to bolster sustainable cereal grain management and ultimately control cereal gain pests.

## 84: The Molecular Ecology of Drinking Water in Building Plumbing as a Function of Stagnation

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The effects of water stagnation on the drinking water microbiome inside building plumbing is largely unknown, despite efforts to study this during COVID-19 building shutdowns. Examining how the water quality and the microbiome change during stagnation and water quality recovery efforts will lead to more effective management strategies for this water during long absences. Four mock plumbing walls were flushed at different intervals (daily, weekly, biweekly, and monthly) for 6 months. Once a month, water samples were collected from each wall and analyzed for basic water quality parameters, metals concentrations, and bacterial quantification with flow cytometry. DNA samples extracted from the water were also analyzed using 16S amplicon sequencing.

Each stagnation regime affected the water quality uniquely, even though incoming water and wall construction was identical. Most notably, bacteria utilizing sulfur metabolisms were more prevalent in samples with longer stagnation times. Other potential nitrogen reducing bacteria were more present in samples with shorter stagnation times. Daily and monthly flushing samples had the highest alpha diversity after the influent samples. Alpha diversity also varied widely with different plumbing materials within the same stagnation regime. Common drinking water pathogens were rarely detected with 16S amplicon data. The dominance of the sulfur metabolizing bacteria with stagnation, combined with the decrease in sulphate concentrations may indicate that in this groundwater fed system, controlling sulphate concentrations is the best way to control microbial growth.

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